**How does drought disrupt the transfer of essential omega-3 fatty acids from freshwater to terrestrial ecosystems?**

Madeline Pratt Hannappel[1,2,+], Robert Joseph Fournier[1], Adrianne P Smits[4], Lauren Esposito[3] & Albert Ruhí[1]

[1] Department of Environmental Science, Policy, and Management, University of California Berkeley, Berkeley, CA 94720, USA.
[2] Institute for Biodiversity Science and Sustainability, California Academy of Sciences, San Francisco, CA 94118, USA.
[3] Islands & Seas, San Francisco, CA 94124, USA.
[4] Department of Environmental Science & Policy, University of California Davis, Davis, CA 95616, USA.
+ To whom correspondence should be addressed: mphannappel@gmail.com; mphannappel@berkeley.edu

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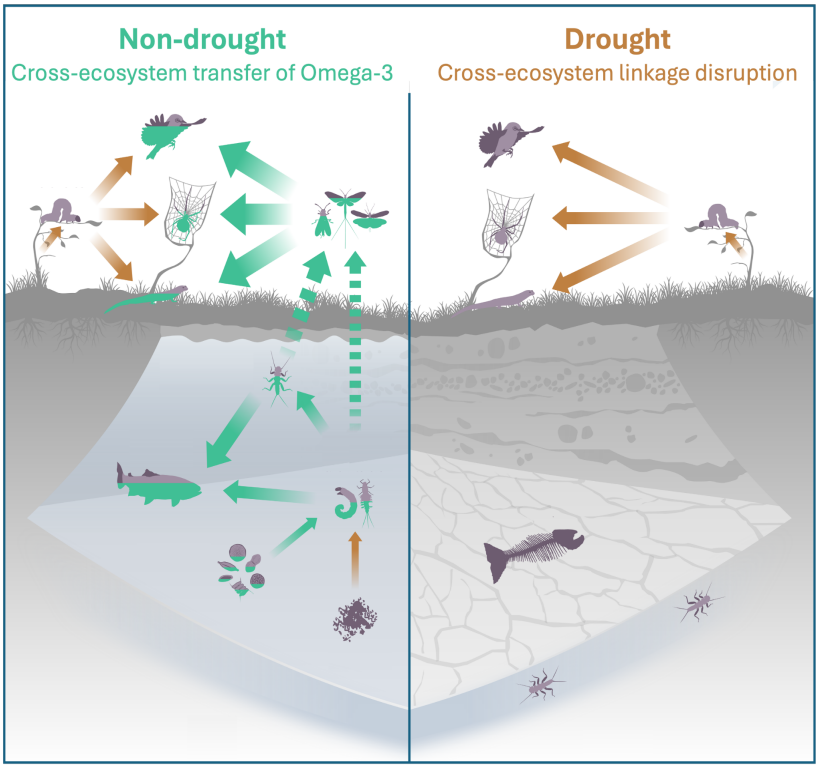
Graphical abstract:




Drought disrupts aquatic–terrestrial food web linkages by altering the production, trophic transfer, and export of essential fatty acids from freshwaters to the terrestrial environment. We synthesized existing evidence, identified knowledge gaps, and proposed a 6-point research agenda to advance nutritional ecology in the context of increasingly dry futures.

**Abstract**

Freshwater and terrestrial food webs are connected via cross-ecosystem subsidies, including essential nutrients like omega-3 fatty acids that originate in the aquatic environment (microalgae) but support terrestrial consumers. Although global change stressors could be disrupting these linkages, most research to date has focused on subsidy quantity rather than quality. Here, we reviewed the literature on omega-3 long-chain polyunsaturated fatty acids (ω3 LC-PUFA) in fresh waters, and we examined how drought-related stress may influence their production, accumulation, and trophic transfer. Existing studies indicate that warming and drying-induced shifts in basal resource composition, altered consumer production and emergence phenology, and reduced capacity for ω3 LC-PUFA bioconversion are poised to disrupt ω3 LC-PUFA fluxes from the aquatic to the terrestrial environment. Accumulation of ω3 LC-PUFA in organisms is strongly influenced by life-history traits, including drought adaptations (e.g., resistance and resilience strategies), temporal integration of diet, and feeding ecology. We confirmed and illustrated these mechanisms via a case study on intermittent stream invertebrates at Pinnacles National Park, California. Finally, we identified key challenges and recommendations for advancing ω3 LC-PUFA research. These include (i) explicitly considering organismal life histories; (ii) improving experimental design to strengthen inference; (iii) considering underground ecological processes; (iv) advancing research on consumer fitness responses to ω3 LC-PUFA limitation and on 'upgrading'; (v) measuring rates and fluxes; and (vi) scaling up and forecasting fatty acid flux under increasingly dry futures by integrating models of hydrology, organismal biology, and community dynamics. We contend that the disruption of cross-ecosystem linkages, not just in resource magnitude but also in quality, is a "blind spot" of global change ecology that demands immediate attention.

**Keywords:** compound-specific stable isotopes, cross-ecosystem linkages, food webs, global change ecology, nutritional ecology, omega-3, Polyunsaturated Fatty Acids (PUFA).

## 1. Cross-ecosystem linkages: both quantity and quality matter

Cross-ecosystem subsidies, or movements of organisms and materials across ecosystem boundaries, are ubiquitous in nature and considered foundational to ecosystem structure and stability (Nakano & Murakami, 2001; Polis et al., 1997). Freshwater ecosystems have long been recognized to “fertilize” the watersheds they are embedded in, often by providing aquatic prey to terrestrial and riparian predators. Fluxes of flying insects that developed in-stream (e.g., mayflies, stoneflies, midges) represent key subsidies to riparian consumers including spiders, lizards, and birds (Baxter et al., 2005; Sabo & Power, 2002; Schürings & Olden, 2026). However, much of the research on these cross-ecosystem subsidies has focused on the quantity or magnitude of flux transferred from the aquatic to the terrestrial system, frequently overlooking the nutritional quality of those fluxes (Marcarelli et al., 2011; Marczak et al., 2007; Pilecky et al., 2021a). There is increasing recognition that ecologically important compounds beyond bulk carbon and nitrogen (Baxter et al., 2005) are also transported across aquatic-terrestrial ecosystem boundaries (Burdon et al., 2025; Hixson et al., 2015a; Twining et al., 2019, 2025). Omega-3 long-chain polyunsaturated fatty acids (hereafter, ω3 LC-PUFA) have emerged as particularly important aquatic-derived subsidies (Pilecky et al., 2021b; Twining et al., 2019; Yan et al., 2024). Whether and how these critical nutrients respond to global change stressors remains an important and unresolved question.

In contrast to terrestrial ecosystems, where primary production is dominated by vascular plants, freshwater production is largely driven by microscopic algae (periphyton in streams; phytoplankton in lakes and rivers; Hixson et al., 2015a; Power, 1992). Consequently, changes in aquatic basal production or grazer communities can rapidly propagate through the food web and alter cross-ecosystem fluxes of biomass and associated macronutrients (Marcarelli et al., 2011; Twining et al., 2019). In most freshwater ecosystems, emergent aquatic insects transport energy and essential macronutrients, including physiologically important fatty acids (FA; Shipley et al., 2024). Omega-3 LC PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic

acid (DHA; Table 1), are widely considered as essential for growth and development (Parmar et al., 2022; Shipley et al., 2024). Due to limited biosynthetic capacity, most animals rely on dietary sources of ω3 LC-PUFA and selectively retain these compounds in tissues (Twining et al. 2016a). Because these ω3 LC-PUFAs are virtually absent in terrestrial primary producers, aquatic sources that are comparatively rich in ω3 LC-PUFAs provide important nutritional subsidies to both aquatic and terrestrial consumers, with well-documented benefits for growth, immune function, and survival (Kowarik et al., 2023; Twining et al. 2016a). For example, brown trout that consumed more terrestrial prey had significantly lower muscle DHA and EPA content, and smaller brains, than trout feeding on more aquatic prey rich in ω3 LC-PUFA (Závorka et al., 2021). Similarly, dietary intake of aquatic-derived ω3 LC-PUFA (specifically, EPA) enhanced immunocompetence in riparian birds (Twining et al. 2016a) and spiders (Fritz et al., 2017; Kirschman et al., 2024). As primary sources of essential ω3 LC-PUFA, aquatic systems play a disproportionate role in supporting riparian food webs, making their nutritional subsidies particularly sensitive to environmental change.

There is increasing evidence that global change stressors (e.g., altered regimes of temperature, nutrients, or light) can decrease the production of ω3 LC-PUFA in aquatic systems and disrupt these important subsidies to terrestrial systems (Yan et al., 2024; Yoon et al., 2022). River drying is widespread globally (Messager et al., 2021) and is increasingly a hallmark of hydroclimate ‘whiplash’ (Carlson et al., 2024). Because drought alters food-web structure via changes in community composition, phenology, and energy flows (Lake, 2011; Leathers et al., 2024; Ledger et al., 2013), there is good reason to believe that drought could impact both the synthesis of ω3 LC-PUFA within aquatic systems, and its cross-system transfer to the riparian environment. However, research on ω3 LC-PUFA synthesis and transfer under drought conditions remains fragmented, with studies addressing individual components or drought-associated conditions rather than drought as an integrated process. Here, we review and synthesize current knowledge on this topic, identify key knowledge gaps, and highlight

opportunities to improve our understanding of how drought in freshwater ecosystems affects the movement of ω3 LC-PUFAs from aquatic to adjacent terrestrial ecosystems.

## 2. Mechanisms of PUFA synthesis and transfer between freshwater and terrestrial ecosystems

We synthesized the different mechanisms connecting drought-induced stress to PUFA synthesis and transfer, type of support (observational, experimental, computational), and strength of evidence for each mechanism, in Table 2.

We found that environmental conditions (e.g., light, temperature, and nutrients) interact with periphyton community composition to shape the cumulative amount of PUFA available to higher consumers (Galloway & Winder, 2015; Hixson & Arts, 2016; Keva et al., 2021; Paerl & Paul, 2012; Strandberg et al., 2022; Taipale et al., 2016; Table 2). Multiple studies also reported that algal community composition explains more variation in the ω3 LC-PUFA levels in a periphyton assemblage than environmental conditions alone (Galloway & Winder, 2015; Keva et al., 2021; Strandberg et al., 2022; Taipale et al., 2016). These patterns suggest that environmental change can affect ω3 LC-PUFA production both by altering the physiology of individual algal taxa and by shifting the dominance of high- *vs.* low-PUFA producers within the assemblage (Strandberg et al., 2022; Twining et al., 2020; Table 2). Specifically, under eutrophic or warmed conditions, periphyton assemblages tend to shift from diatom dominance toward cyanobacteria and green algae, groups that generally produce little to no ω3 LC-PUFA (Hixson & Arts, 2016; Paerl & Paul, 2012; Taipale et al., 2013, 2016). For example, lower temperatures favor higher ω3 LC-PUFA production as an adaptation to maintain appropriate membrane fluidity (“homeoviscous adaptation”; Hixson & Arts, 2016; Hu & Gao, 2006). Additionally, algal ω3 LC-PUFA production tends to be greatest under high nutrient availability that supports algal growth (Guo et al., 2015; Taipale et al., 2019), and under lower light and cooler temperatures that enhance ω3 LC-PUFA synthesis (Fournier & Magoulick, 2022; Guo et

al., 2015, 2016a; Hu & Gao, 2006; Keva et al., 2021; Piepho et al., 2012; Strandberg et al., 2022; Zhou et al., 2026; Table 2). However, we note that warming can offset the positive effects of elevated nutrients on periphyton ω3 LC-PUFA, such that the combination of high nutrients and high temperature results in lower ω3 LC-PUFA than nutrients alone (Zhou et al., 2026). This result highlights the potential interactions of drought-associated stressors, which are sometimes hypothesized but rarely assessed empirically. Nevertheless, ω3 LC-PUFA of aquatic consumers, specifically grazing insects, has been shown to differ systematically among ecosystems with different conditions (e.g., warmer or colder stream temperatures), suggesting that environmental variation exerts a first-order control on ω3 LC-PUFA synthesis of producers, ultimately influencing ω3 LC-PUFA availability to the higher trophic levels (Smits et al., 2015).

Within aquatic food webs, consumers can vary substantially in ω3 LC-PUFA composition and content, which is typically related to differences in behavior, diet, physiology, and environmental conditions (Guo et al., 2018; Smits et al., 2015; Twining et al. 2021a). Unsurprisingly, biofilm grazers (e.g., *Baetis* mayflies) consuming diatom-rich periphyton tend to accumulate more EPA than shredders feeding primarily on terrestrial leaf litter and associated microbial production (Guo et al., 2018; Yan et al., 2024). Yet, even shredders can accumulate ω3 LC-PUFA, suggesting they selectively assimilate algal-derived fatty acids from mixed diets or from seeking out algae on epiphytic biofilms (Guo et al., 2018; Kühmayer et al., 2020).

Notably, some consumers can also synthesize ω3 LC-PUFA from precursor compounds, including the short-chain ω3 PUFA alpha-linoleic acid (ALA). However, this ability to ‘upgrade’ can be metabolically costly (e.g., in Daphnia; Martin-Creuzburg & Von Elert, 2009), and seems to vary widely among taxa and with temperature (Sperfeld & Wacker, 2012; Twining et al. 2021b; Yoon et al., 2022). For example, *Daphnia* growth was more strongly EPA-limited at colder temperatures, indicating a greater structural requirement for ω3 PUFA at low temperatures despite higher dietary EPA assimilation (Sperfeld & Wacker, 2012); and fish selectively retain higher proportions of ω3 LC-PUFA at lower temperatures (Tadesse et al.,

2003). Similarly, the ability of chironomids to elongate and desaturate precursor fatty acids into longer-chain products is enhanced at colder temperatures (Strandberg et al., 2021). This finding is ecologically relevant, considering chironomids ('midges') frequently dominate freshwater insect assemblages and can account for a large proportion of secondary production and cross-ecosystem flux in streams (Berg & Hellenthal, 1992; Leathers et al., 2024; Mathieu-Resuge et al., 2021). Because only a small number of taxa have been directly assessed for this biosynthetic capacity (Twining et al. 2021a), we lack a clear understanding of which taxa are capable of meaningful ω3 LC-PUFA upgrading, and under what environmental conditions this capacity is promoted or constrained.

Moving up the food chain, primary consumers feeding on resources high in ω3 LC-PUFA become high-quality food for predators, and can export ω3 LC-PUFA out of the aquatic ecosystem. For example, emergent aquatic insects serve as ω3 LC-PUFA-rich prey that supports the breeding success of aerial insectivores such as tree swallows (Shipley et al., 2022; Twining et al., 2018), as well as the nutritional condition of riparian spiders (Kowarik et al., 2021). Predators, whether riparian or aquatic, tend to have ω3 LC-PUFA profiles that reflect those of their prey (e.g., macroinvertebrates: Guo et al., 2018; fish: Taipale et al., 2016). With increasing trophic levels, ω3 LC-PUFA signatures typically persist and their concentrations increase with trophic position, a pattern known as biomagnification (Guo et al., 2018, 2022; Strandberg et al., 2015). Predatory insects like phantom midges (Chaoboridae) have higher concentrations than their prey or other organisms occupying lower-trophic positions in the food web (Martin-Creuzburg et al., 2017). Freshwater insects, therefore, represent a significant source of ω3 LC-PUFA to terrestrial insectivores, especially compared to terrestrial insects, which accumulate negligible amounts of ω3 LC-PUFA compared to their freshwater counterparts (Hixson et al., 2015b; Kowarik et al., 2021; Parmar et al., 2022; Twining et al. 2021b). Once aquatic insects emerge, they constitute primary vectors of ω3 LC-PUFA movement (Martin-Creuzburg et al., 2017; Moyo et al., 2017), and riparian insectivores (e.g.,

179 spiders, birds, and lizards) can consume significant amounts of aquatic-derived ω3 LC-PUFA
180 via emergent insects (Kowarik et al., 2021; Landaverde et al., 2025; Twining et al. 2016a;
181 Twining et al., 2019). Thus, the ultimate flux of ω3 LC-PUFA from aquatic to terrestrial systems,
182 and the benefit to terrestrial consumers, is shaped by a complex interaction of basal production,
183 consumer uptake and bioconversion, export via insect emergence, and the effects of
184 environmental variation on each of these processes.
185
186 **3. How does drought impact ω3 LC-PUFA fluxes?**
187 *Drought and drying as a complex disruptor of* ω3 *LC-PUFA flux*
188 Drought, defined as a period of deficient precipitation and streamflow, is a multi-faceted
189 stressor that alters many abiotic conditions in freshwater ecosystems, including temperature,
190 light availability, and nutrient concentrations (Lake, 2003). River networks globally are naturally
191 dominated by low-order (i.e. headwater) stream and river segments that dry for at least part of
192 the year (Messager et al., 2021). Despite the ubiquity and increasing pervasiveness of drying,
193 flow intermittency has been rarely examined in the context of ω3 LC-PUFA (but see: Kowarik et
194 al., 2026; Sampera-Calbet et al., 2017).
195 The anticipated impact of complete drying on the ω3 LC-PUFA production and trophic
196 transfer within a waterbody is likely difficult to overstate: without water to support the
197 photosynthetic algae that synthesize ω3 LC-PUFA, or the aquatic consumers that accumulate
198 ω3 LC-PUFA and transport them to terrestrial ecosystems, aquatic-terrestrial ω3 LC-PUFA flux
199 may effectively halt. The abiotic changes that occur during drying strongly influence community
200 composition at all trophic levels (with implications for accumulation and biomagnification) and
201 likely alter magnitude and phenology of insect emergence to terrestrial systems (with
202 implications for its cross-ecosystem transfer; Figure 1).
203

### ω3 *LC-PUFA during the drying phase of intermittent streams*

In the early drying phases, reduced streamflow leads to the loss of shallow microhabitats such as riffles and runs (Figure 1). Water temperature rises, and standing water conditions often result in short-term increases in algal biomass, and shifts in periphyton community composition, losing high-quality ω3 LC-PUFA-producing diatoms (Fournier & Magoulick, 2022; Hixson & Arts, 2016; Paerl & Paul, 2012; Piepho et al., 2012; Yan et al., 2024). As the drying phase progresses, thermal stress and night-time hypoxia also lead to shifts in aquatic consumer communities. At the community level, flow cessation is associated with major reductions in aquatic consumer abundances, richness, and community composition (Chanut et al., 2023; Siebers et al., 2020; Figure 1). For example, rheophilic macroinvertebrates (e.g., many Ephemeroptera, Plecoptera, and Trichoptera) are often the most sensitive to intermittent flow conditions (Siebers et al., 2020). Notably, several of these flow-dependent taxa (e.g., Ephemeroptera and Trichoptera) are consistently the strongest accumulators of ω3 LC-PUFA within a stream (Martin-Creuzburg et al., 2017; Parmar et al., 2022; Smits et al., 2015; Strandberg et al., 2023), and their loss may exacerbate the decline in trophic transfer of ω3 LC-PUFA within the food web and export to terrestrial systems. Additionally, warmer water temperatures accelerate stream insect growth rates but often result in reduced size at emergence (Dallas & Ross-Gillespie, 2015; Leathers et al., 2024), a key influence of flux as predators would need to handle higher numbers of prey to achieve similar nutritional benefits.

Omega-3 LC-PUFA transfer and its sensitivity to drought is a highly dynamic process. Early in the drying process, emergent aquatic insect flux of ω3 LC-PUFA to terrestrial systems may only be slightly altered, even increased due to higher biomass emerging from warmer waters despite potential lower ω3 LC-PUFA content within individual insects (Fehlinger et al., 2025). Similar patterns have been observed in lentic systems across a productivity gradient: biomass-specific ω3 LC-PUFA content of individual insects (Chironomidae) declined at higher nutrients levels (total phosphorus), but the increase in total biomass emerging resulted in higher

total export of ω3 LC-PUFA to the terrestrial system (Scharnweber et al., 2020). However, in more ephemeral systems, shortened hydroperiods (e.g., contracting the aquatic phase to only a few months or weeks) may prevent semi-voltine taxa from completing their life cycles, and will likely influence the timing of emergence for insects that can emerge (Bogan & Lytle, 2011; Carey et al., 2021). In turn, decreased availability of ω3 LC-PUFA to both aquatic and riparian predators and increased competition among consumers for increasingly scarce resources may result in reduced consumer growth, fitness, and reproduction (Shipley et al., 2022; Taipale et al., 2018). Overall, significant changes to ω3 LC-PUFA flux are to be expected during drying. Not only are both the biomass and nutritional quality of emerging insects expected to decline, but warmer temperatures may compress and alter the timing of emergence windows, ultimately resulting in phenological mismatches with riparian consumers (Shipley et al., 2022).

*Disruption of LC-PUFA synthesis and transfer during the dry phase of intermittent streams*

After the recession of surface flow, photosynthetic production and synthesis of ω3 LC-PUFA ceases. Most aquatic consumers, especially those occupying higher trophic-levels and sensitive to low-flow conditions such as fish, are also unlikely to persist under complete drying, effectively shortening stream food chains (Datry et al., 2026; Sabo et al., 2010; Figure 1). Still, many invertebrate taxa can survive drought through resistance strategies, including finding refuge in the hyporheic zone (Fournier et al., 2023; Lake, 2000). It is unlikely that the hyporheic zone can support significant production of ω3 LC-PUFA by photosynthetic algae, especially diatoms, as it is typically dominated by detrital pathways (Brunke & Gonser, 1997; DelVecchia et al., 2016). However, almost nothing is known about the ω3 LC-PUFA synthesis and cycling in the hyporheic. Other taxa (primarily amphibiotic insects) can disperse to perennial reaches or nearby water bodies as a resilience strategy, returning to the stream and recolonizing when rewetting occurs. The timing and duration of intermittency are major influences on the recovery of aquatic communities and cross-system subsidy transfer (Chanut et al., 2023; Kowarik et al.,

2026). For example, ω3 LC-PUFA content of riparian spiders significantly decreased as stream intermittency increased, suggesting that flow intermittency reduces the transfer of ω3 LC-PUFA to terrestrial consumers (Kowarik et al., 2026). As flow intermittency is expected to increase, the total availability of ω3 LC-PUFA would decrease and the window of availability would narrow, resulting in riparian consumers relying on higher proportions of nutritionally-poorer terrestrial prey (Shipley et al., 2022; Figure 1). Overall, complete drying is expected to halt the aquatic-terrestrial ω3 LC-PUFA flux until water returns and primary production is restored.

*Effects of rewetting on LC-PUFA flux*

Flow resumption is expected to strongly influence ω3 LC-PUFA fluxes through increased dispersal of algae and consumers ('mass effects'; Ruhí et al., 2017), successional change, and the recovery of trophic interactions that disappear or remain underground during the dry phase (Datry et al., 2026; Yan et al., 2024). Importantly, rewetting is not simply a mirrored reversal of the drying and dry phases, and specific outcomes are highly dependent on the severity and duration of drying, the timing and magnitude of rewetting, and the characteristics of the individual system (Datry, 2017; Datry et al., 2026; Fournier et al., 2023). While algal biomass and gross primary productivity can increase rapidly, sometimes within days to a week of rewetting (Acuña et al., 2005; Timoner et al., 2012), community composition of periphyton may remain altered (Sabater et al., 2016). Studies suggest that the drying-rewetting cycle can shift basal communities toward desiccation-tolerant cyanobacteria or green algae over ω3 LC-PUFA-rich diatoms (Yan et al., 2024). The structural habitat provided by filamentous green algae for epiphytic diatoms may also facilitate recovery toward higher-quality communities (Marks et al., 2025; Power et al., 2008). However, the mechanisms by which food webs reassemble and shape the eventual recovery of ω3 LC-PUFA flux may be highly variable over space and time, even at a given site (e.g., between growing seasons of wet *vs.* dry winters; Power et al., 2008).

## 4. Technical advances for the study of ω3 LC-PUFA fluxes

Omega-3 LC-PUFA are not only indicators of nutritional quality, but also powerful tools for understanding how food webs function and respond to environmental change. Fatty acid profiles hold potential as metrics for trophic recovery or remediation success in freshwater systems, as algal fatty acid content has been shown to respond rapidly to agricultural best-management practices (Whorley & Wehr, 2018) and PUFA concentrations in periphyton and consumers vary with pollution gradients (Mahboob et al., 2019). However, using FA profiles as biomarkers of impact or recovery remains challenging: it would likely require sampling before and after disturbance events to determine change and "healthy" baselines. Because ω3 LC-PUFA are both ecologically essential nutrients and strongly linked to diet (i.e. can function as dietary tracers), they provide a unique lens through which to examine trophic pathways, resource use, and the life histories of consumers themselves.

Emerging approaches, such as compound-specific stable isotope analysis (CSIA) of fatty acids, are expected to expand the types of inferences that can be made, allowing researchers to distinguish between dietary acquisition and endogenous processing based on isotopic signatures of carbon or hydrogen (i.e., $\delta^{13}C$ or $\delta^{2}H$) measured in individual fatty acids, including ω3 LC-PUFA (e.g., $\delta^{13}C$ or $\delta^{2}H$ of EPA; Twining et al. 2020). In doing so, these methods offer the possibility to trace the origins of ω3 LC-PUFA within consumers, including whether they were derived from local production, transported from other water bodies, or synthesized from precursor compounds. Additionally, they can provide detailed information about the trophic transfer of organic matter more generally, with the potential to differentiate/account for the contribution of autochthonous and allochthonous sources to aquatic consumers. As these tools continue to develop, they offer unique opportunities to better understand how ω3 LC-PUFA move through aquatic food webs and how these dynamics are altered under changing environmental conditions, including drought and drought-related adaptations in freshwater organisms.

## 5. Key challenges and recommendations for understanding current and future impacts of drought on ω3 LC-PUFA production and transfer

In reviewing the literature on both ω3 LC-PUFA dynamics and drought-driven ecological changes in freshwater ecosystems (Table 2), we found that key components of these systems have been studied in isolation, but rarely integrated. Current evidence of change in ω3 LC-PUFA synthesis under environmental conditions commonly associated with drought supports the expectation that increased drying frequency may diminish, restructure, and alter the timing of ω3 LC-PUFA fluxes in both aquatic and riparian food webs. The fragmented state of the scientific literature limits our ability to understand how drought reshapes ω3 LC-PUFA production, accumulation, and fluxes. Here, we identify major challenges and recommendations for advancing the field, including (i) better accounting for organismal life histories, (ii) strengthening experimental design, (iii) including ecological processes that occur underground, (iv) quantifying organismal fitness responses and ability for fatty acid elongation or 'upgrading', (v) shifting the focus from quantifying fatty acid stocks to process rates; and (vi) building a quantitative framework that allows for scaling up and forecasting fatty acid flux under changing hydroclimates via assimilation of data and models.

### *Recommendation 1: Explicitly consider organismal life histories*

Life histories need to be explicitly considered when interpreting an ecological community's lipidome. Notably, species-specific drought adaptations may influence how PUFA are accumulated or retained because these adaptations affect food sources, metabolic activity, and persistence through dry phases. Although a large body of work demonstrates that insect ω3 LC-PUFA profiles are strongly influenced by life-history-linked variation in diet and physiology, the extent to which drought-specific strategies may influence accumulation of ω3 LC-PUFA or complicate their interpretation remains largely unknown. In many systems, consumer ω3 LC-

PUFA closely reflects those of basal resources, indicating tight trophic coupling, but this relationship is mediated by the temporal integration of dietary signals within tissue. As a result, ω3 LC-PUFA measured in a given organism represent not just current diet, but an integration of feeding history over time, with the duration of that integration depending on traits such as lifespan, and growth rate. This means that short-lived taxa are likely to reflect recent resource conditions, whereas longer-lived consumers may retain signals from prior environmental states, including pre-drought conditions. Field studies also indicate seasonal shifts in aquatic consumer ω3 LC-PUFA are common, and often aligned with changes in basal production and life stage (Guo et al., 2018; Landaverde & Otter, 2026; Martin-Creuzburg et al., 2017), including peak accumulation prior to emergence in some taxa (Gmeiner et al., 2025). Further, for holometabolous insects (i.e., those undergoing complete metamorphosis) like chironomids, lipid composition can shift substantially during metamorphosis (Pietz et al., 2023; Pilecky et al., 2024). These results suggest that ω3 LC-PUFA profiles could largely reflect an organism's life history – a dimension that is rarely acknowledged in the literature.

As an empirical illustration of how taxon identity and ecological context influence ω3 LC-PUFA patterns, we present a case study on aquatic insects collected from perennial and intermittent stream reaches in a pristine watershed (Chalone Creek) in Pinnacles National Park, California (Box 1; methods provided in Supplementary Material S2). We hypothesized that perennial stream reaches, with longer periods of flow to support basal production of ω3 LC-PUFA, would result in insects with higher ω3 LC-PUFA levels compared to intermittent streams. Fatty acid profiles of the aquatic invertebrate community differed significantly between perennial and intermittent streams and among taxa (Box 1, Figure a; Supplementary Fig. 1). When comparing ω3 LC-PUFA concentrations, there was no significant difference at the community level (Box 1, Figure. b). We further compared ω3 LC-PUFA concentrations in two taxa that differ in their drought responses: a highly mobile resilience strategist (water strider, Gerridae: *Aquarius* sp.) and a resistance strategist that burrows into the substrate (fishfly, Corydalidae:

359 *Neohermes filicornis*). Both insects are opportunistic predators, but they differ in the position
360 and origin of their prey: *Aquarius* nymphs and adults are surface neuston predators that detect
361 and capture invertebrates, both of aquatic and terrestrial origin, trapped at the water surface,
362 whereas *Neohermes* larvae are benthic engulfer-predators feeding primarily on aquatic
363 macroinvertebrates on the streambed (Merritt & Cummins 1996). Average ω3 LC-PUFA
364 concentrations did not differ significantly between perennial and intermittent streams for
365 Gerridae, whereas Corydalidae exhibited significantly higher concentrations at intermittent
366 stream sites (Box 1, Figure. c). Interestingly, EPA concentrations in Corydalidae from
367 intermittent streams increased with insect mass, indicating accumulation of ω3 LC-PUFA over
368 the lifespan of individuals. These patterns, which conflict with our original hypothesis, likely
369 reflect reduced metabolic turnover during dry-phase dormancy: long-lived (3-6 years)
370 *Neohermes* larvae likely slow metabolic activity while burrowed in the sediment or hyporheic
371 zone, and experience reduced EPA catabolism and favoring its accumulation. Compound-
372 specific stable isotopes of EPA ($\delta^{13}$C-EPA) did not vary with insect mass, suggesting that this
373 pattern reflects growth-driven accumulation rather than a shift in dietary sources
374 (Supplementary S2). Together, these results demonstrate that different taxa at the same site
375 and under the same environmental conditions can show contrasting ω3 LC-PUFA profiles,
376 highlighting the importance of life history context when interpreting fatty acid patterns.
377
378 ***Recommendation 2: Improve experimental design to strengthen inference***
379 Future studies should span the full hydrological cycle, and be conducted at temporal
380 scales capable of capturing responses across multiple trophic levels. To our knowledge, only
381 one study examined ω3 LC-PUFA in basal resources along the drying and rewetting phases
382 (reporting reduced quality following rewetting; Sampera-Calbet et al., 2017), and none have
383 assessed post-drying recovery of ω3 LC-PUFA in consumers or fluxes, denoting a major gap.
384 Repeated sampling is still rare, which can be problematic for at least two reasons. First, ignoring

background temporal variability in ω3 LC-PUFA may conflate drought-driven changes with variation associated with seasonal or developmental processes. Second, because consumer tissues assimilate dietary ω3 LC-PUFA over time, a single snapshot may not reveal consumer responses to basal resource impoverishment. We acknowledge that financial trade-offs exist between sampling repeatedly over time *vs.* sampling more taxa in a community, or more sites. Expectedly, studies that have sampled over longer timescales (e.g., weeks to months) have typically focused on one taxon or taxonomic group (e.g., marine and freshwater phytoplankton: Galloway & Winder, 2015; tetragnathid spiders: Landaverde et al., 2025; and heptageniid mayflies: Landaverde & Otter, 2026). However, the very few studies that sampled multiple taxa over time (e.g. Shipley et al 2022) show the importance of considering community-level processes that govern ω3 LC-PUFA flux, such as variation in phenology.

Similarly, there is currently little guidance on which taxa might serve as reliable indicators of broader changes in ω3 LC-PUFA availability within aquatic food webs. Thus, it would be valuable to identify taxa whose ω3 LC-PUFA profiles consistently track ecosystem-scale changes as potential “sentinel species” for local ω3 LC-PUFA dynamics, analogously to the use of contaminant sentinels (Beeby, 2001). However, relatively few studies have compared ω3 LC-PUFA dynamics across ecosystem types, taxa, and time, leaving limited empirical basis for selecting such indicator taxa even under perennial freshwater conditions (but see Guo et al., 2018; Martin-Creuzburg et al., 2017), or for identifying taxa that are relatively insensitive to environmental change and thus could serve as baselines. Responses of ω3 LC-PUFA profiles to environmental change span timescales: some consumers preferentially retain ω3 LC-PUFA over other catabolizable fatty acids, allowing signals to persist through starvation periods associated with drying (e.g., amphipods; Taipale et al., 2021), while others shift profiles within days of altered resource conditions yet retain physiologically important ω3 LC-PUFA on lower-quality diets (e.g., *Daphnia*; Taipale et al., 2011). Until reliable indicator taxa are identified, sampling across the food web remains important, as taxa differ in both the rate and duration of

411 their ω3 LC-PUFA responses. Interpreting these patterns will require a comparative framework
412 that accounts for differences in accumulation rates and retention times, rather than relying on a
413 single taxon as a proxy for ecosystem-level change. One approach to selecting target taxa for a
414 study could be to focus sampling efforts on taxa known to be consumed by terrestrial predators,
415 as they would best represent fluxes of ω3 LC-PUFA into terrestrial food. Overall, studies that
416 prioritize frequent, multi-trophic sampling are essential to understand the decline and recovery
417 of ω3 LC-PUFA production and assimilation by consumers.
418
419 ***Recommendation 3: Consider ecological processes that occur underground***
420 The hyporheic zone, defined as the subsurface habitat where groundwater and surface
421 waters exchange, supports diverse communities of microbes, meiofauna, and macrofauna,
422 including aquatic insect larvae, and can remain biologically active even during surface drying
423 (Boulton et al. 2010). Some invertebrates inhabit the hyporheic zone for extended periods (e.g.,
424 weeks to months for certain stoneflies; Stanford et al., 2024), while many other taxa retreat
425 there as a refuge temporarily during surface flow cessation (DelVecchia et al., 2022). However,
426 the extent to which the hyporheic zone functions as a refuge versus a “graveyard” for aquatic
427 organisms is still a matter of debate, and may be highly context dependent (Kawanishi et al.,
428 2013; Vander Vorste et al., 2016). As drying intensifies, thresholds in key abiotic conditions
429 such as dissolved oxygen and resource supply are exceeded, shifting the environment from
430 supporting persistence to causing mortality (DelVecchia et al., 2022; Pařil et al., 2019). This
431 variability reflects differences across systems, taxa, and drying dynamics, but overall highlights
432 the hyporheic zone as a conditional refuge whose function may change across the drying–
433 rewetting cycle (Palmer et al., 1992; Stubbington, 2012). Yet, the nutritional dimension of the
434 hyporheic zone, including ω3 LC-PUFA availability to subsurface consumers, has received
435 essentially no attention.

Under flowing conditions, downwelling surface water can transport algal-derived organic matter as one potential ω3 LC-PUFA subsidy into shallow hyporheic sediments (Boulton et al., 2010), but this pathway is likely reduced or severed as flows decline and surface algal production decreases during drought (DelVecchia et al., 2022). Light limitation restricts algal primary production in the hyporheic zone, where heterotrophic microbial biofilms dominate organic matter processing (Brunke & Gonser, 1997) and chemolithotrophic processes, including methanogenesis, can contribute under anoxic conditions (DelVecchia et al., 2016); however, bacteria are not considered substantial producers of ω3 LC-PUFA (Gladyshev et al., 2013). This raises important questions concerning how and in what quantities invertebrates acquire ω3 LC-PUFA within the hyporheic zone. Potential pathways include reliance on surface-derived subsidies, unrecognized subsurface production, or physiological compensation via elongation and desaturation of short-chain precursors. An additional uncertainty concerns taxa that enter diapause or dormancy belowground, where metabolic depression may alter both demand for and retention of ω3 LC-PUFA.

***Recommendation 4: Advance research on consumer fitness response to ω3 LC-PUFA limitation and on 'upgrading'***

Despite increasing attention to ω3 LC-PUFA dynamics in freshwater ecosystems, we still lack a clear understanding of how variation in ω3 LC-PUFA availability translates into consumer fitness across most taxa and ecosystem contexts. Most empirical evidence linking ω3 LC-PUFA availability to fitness outcomes comes from vertebrates, especially fish and riparian birds (Taipale et al., 2018; Twining et al., 2019; Závorka et al., 2021), with comparatively limited evidence for invertebrates (but see experimental work on *Daphnia*: Kiene et al., 2023; Yan et al., 2024; and chironomids: Goedkoop et al., 2007). Emergent aquatic insects rich in EPA and DHA provide an important nutritional subsidy to riparian consumers, improving growth rate, body condition, and fledging success in insectivorous birds relative to diets dominated by

terrestrial insects that are comparatively depleted in ω3 LC-PUFA (Shipley et al., 2022; Twining et al., 2018). Despite the clear importance of these subsidies to higher consumers, the fitness consequences of ω3 LC-PUFA limitation for aquatic macroinvertebrates themselves remain poorly characterized (Yan et al., 2024), even though these taxa represent the primary conduit transferring EPA and DHA from algal producers to higher trophic levels.

A key source of uncertainty is the extent to which some consumers may be able to upgrade short-chain ω3 PUFA to ω3 LC-PUFA (i.e., “elongation”). That capacity likely varies widely across taxa, but may be notable in some invertebrates, including chironomids (Goedkoop et al., 2007; Pilecky et al., 2024; Strandberg et al., 2020, 2021) and riparian spiders (Mathieu-Resuge et al., 2022). For example, ALA (18:3n-3) can be converted to EPA and DHA via sequential desaturation and elongation steps mediated by fatty acid elongase and desaturase enzymes, yet most animals lack Δ12 and Δ15 desaturases and cannot synthesize ALA *de novo*, and even when ALA is obtained through the diet, front-end desaturase capacity to convert it to EPA and DHA is limited in many taxa, making dietary acquisition of ω3 LC-PUFA essential (Pilecky et al., 2021b; Twining et al. 2021a). However, which taxa can perform these transformations, the extent to which they contribute to consumer ω3 LC-PUFA pools, and how environmental stressors influence these pathways all remain poorly understood. Temperature, for example, may be an underappreciated regulator of ω3 LC-PUFA production and fluxes: through homeoviscous adaptation, elongation capacity is likely physiologically plastic rather than fixed. Warming may simultaneously decrease ω3 LC-PUFA synthesis within both producers and consumers, and increase consumer dependence on dietary sources of ω3 LC-PUFA. Because of these knowledge gaps, meaningful thresholds linking ω3 LC-PUFA concentrations in consumer tissues to biologically relevant fitness outcomes remain largely unresolved for most aquatic taxa (Pilecky et al., 2021b). This limitation makes it difficult to determine whether observed variation in tissue ω3 LC-PUFA reflects nutritionally limiting conditions.

***Recommendation 5: Measure rates and fluxes - not just standing stocks***

A considerable limitation in assessing the nutritional consequences of drought for freshwater food webs is that most empirical assessments of ω3 LC-PUFA dynamics in freshwater systems quantify tissue concentrations in individual organisms (e.g., μg $mg^{-1}$ C or % of total fatty acids; Guo et al., 2016b), but not rates of change or transfer per unit of time. Thus, while common measurements reflect dietary quality experienced by an individual, they are largely decoupled from the total flux of ω3 LC-PUFA available to higher trophic levels at the ecosystem level over a given timeframe (Kowarik et al., 2023). Concentration-only assessments of ω3 LC-PUFA are limited in their utility as the nutritional contribution of aquatic insects to freshwater and riparian food webs ultimately depends not only on their fatty acid composition, but also on biomass and production rates. Changes in community composition, for example, could maintain or even increase total ω3 LC-PUFA export if high-PUFA taxa compensate for the loss of others in biomass production. Conversely, increases in the biomass production of nutritionally poor taxa could reduce the overall availability of ω3 LC-PUFA despite high insect abundance. Developing approaches that integrate fatty acid profiles with secondary production estimates across emergent taxa and hydrologic conditions is critical for assessing ecosystem-scale ω3 LC-PUFA fluxes and their influence to aquatic and riparian consumers.

***Recommendation 6: Model PUFA fluxes at the watershed scale and into the future***

Translating the mechanistic understanding developed in preceding sections into quantitative predictions of ω3 LC-PUFA aquatic–to-terrestrial flux under climate change will require linking models across at least three domains: climate, hydrology, and food-web dynamics. However, two critical limitations exist. First and foremost, there is an important mismatch in spatial scales between existing ‘macroscale’ hydrologic models (e.g., Soil and Water Assessment Tool, SWAT; Variable Infiltration Capacity, VIC) and the microhabitats where ω3 LC-PUFA synthesis and accumulation by consumers actually occurs. Developing transfer

functions linking reach-scale hydrologic metrics (e.g., summer low-flow magnitude and duration) to microhabitat availability (e.g., pool area and hydroperiod length, and reach-level drying extent) represents an open but tractable challenge. Some attempts have been made to bridge spatial scales in watershed contexts (e.g., California Basin Characterization Model), enabling locally-relevant biodiversity projections across large scales (Flint et al., 2013; Flint & Flint, 2012).

A second challenge stems from the complexity of quantifying how drought-driven shifts in community composition propagate into changes in ω3 LC-PUFA flux at the watershed level. As this review documents, the aquatic-terrestrial ω3 LC-PUFA subsidy is shaped by at least three interacting components: basal production by algae, trophic bioaccumulation through invertebrate consumers, and the magnitude and phenology of adult insect emergence. Drought alters all three components simultaneously. Community composition and emergence phenology can be predicted from hydrology (e.g., Evangelista et al., 2025), and some attempts have been made to connect drought to food-web dynamics by accounting for fine-scale phenological shifts (e.g., Leathers et al., 2024). However, these predictions have not yet been coupled to ω3 LC-PUFA dynamics. Closing this gap will likely require trait-based frameworks that use organism-level attributes, such as voltinism, drought survival strategy, trophic position, and elongation capacity, as proxies for ω3 LC-PUFA accumulation potential, analogous to how trait-based flow-ecology research has developed approaches for scaling biodiversity responses to hydrological change (Poff, 2018; Sarremejane et al., 2020). Time-series based ecological forecasting frameworks (e.g., Ruhí et al., 2016; Sarremejane et al., 2021) could be used to estimate how flow regimes drive changes in community composition and emergence phenology, which in turn could be multiplied by taxon-specific ω3 LC-PUFA transfer coefficients to ultimately estimate watershed-level ω3 LC-PUFA export to terrestrial consumers.

Improving predictive capacity using ω3 LC-PUFA data will ultimately require a coordinated research agenda spanning empirical, experimental, and modeling approaches. The

most pressing empirical need is for repeated, multi-trophic field campaigns that generate the parameters needed to link hydrology to ω3 LC-PUFA flux: concurrent measurements of periphyton community composition and ω3 LC-PUFA content, invertebrate tissue ω3 LC-PUFA concentrations across functional feeding groups and life stages, and emergence trap data spanning full drying-rewetting cycles to estimate biomass flux. Compound-specific stable isotope analysis of fatty acids (CSIA-FA), as piloted in the Pinnacles case study (Box 1), offers a powerful tool for resolving the origin of ω3 LC-PUFA through food webs in ways that bulk fatty acid profiling cannot, allowing validating of model predictions. Last but not least, an important modeling challenge that will need to be confronted directly is non-stationarity, or the fact that flow-ecology relationships developed under historical conditions may not transfer reliably to the novel flow regimes projected under climate change (Tonkin et al., 2019). Process-based models that account for mechanisms and propagate uncertainty accurately will be especially important when modeling ω3 LC-PUFA flux, as the effects of the ongoing “hydroclimate whiplash” (Swain et al., 2018, 2025) may affect not only climate, hydrology, and organisms – but also the relationships between these three key components.

## 6. Concluding remarks

Nutritional subsidies of ω3 LC-PUFA from freshwater ecosystems are of increasing interest and have been shown to confer important fitness and reproductive benefits to terrestrial consumers and food webs. Our review of the existing literature suggests that drought and associated climate stressors are poised to disrupt this nutritional subsidy through multiple interacting pathways, including altered basal production, shifts in community composition, and changes in the timing and magnitude of aquatic insect emergence. Because ω3 LC-PUFA are essential for growth, reproduction, and physiological function in consumers (Shipley et al., 2024), reductions in their availability or changes in their spatiotemporal variability may have cascading effects on consumer fitness and riparian food webs. Our synthesis further reveals that current understanding of ω3 LC-PUFA dynamics remains fragmented across disciplines,

with key components often being studied in isolation. This fragmentation, combined with a lack of studies directly addressing drought, limits our ability to predict how ω3 LC-PUFA production and transfer will respond to environmental changes and how such changes will translate into biologically meaningful impacts on consumers. To address these gaps, there must be a coordinated effort to integrate the insights from physiology, population and community ecology, and ecosystem science. A priority moving forward should be to build a body of comparable ω3 LC-PUFA datasets that can support synthesis, meta-analysis, and model development and assimilation, as well as filling in critical geographic and ecological gaps across all types of freshwater systems (e.g., ponds, lakes, wetlands, and estuaries in addition to lotic systems). Such integration would help developing the predictive understanding needed to anticipate how the nutritional links between aquatic and terrestrial ecosystems will respond to an increasingly variable hydrologic future.

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**Data availability statement:**

Data and code are available upon request. Contact mphannappel@berkeley.edu

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## Figures, Boxes, and Tables

**Figure 1.**

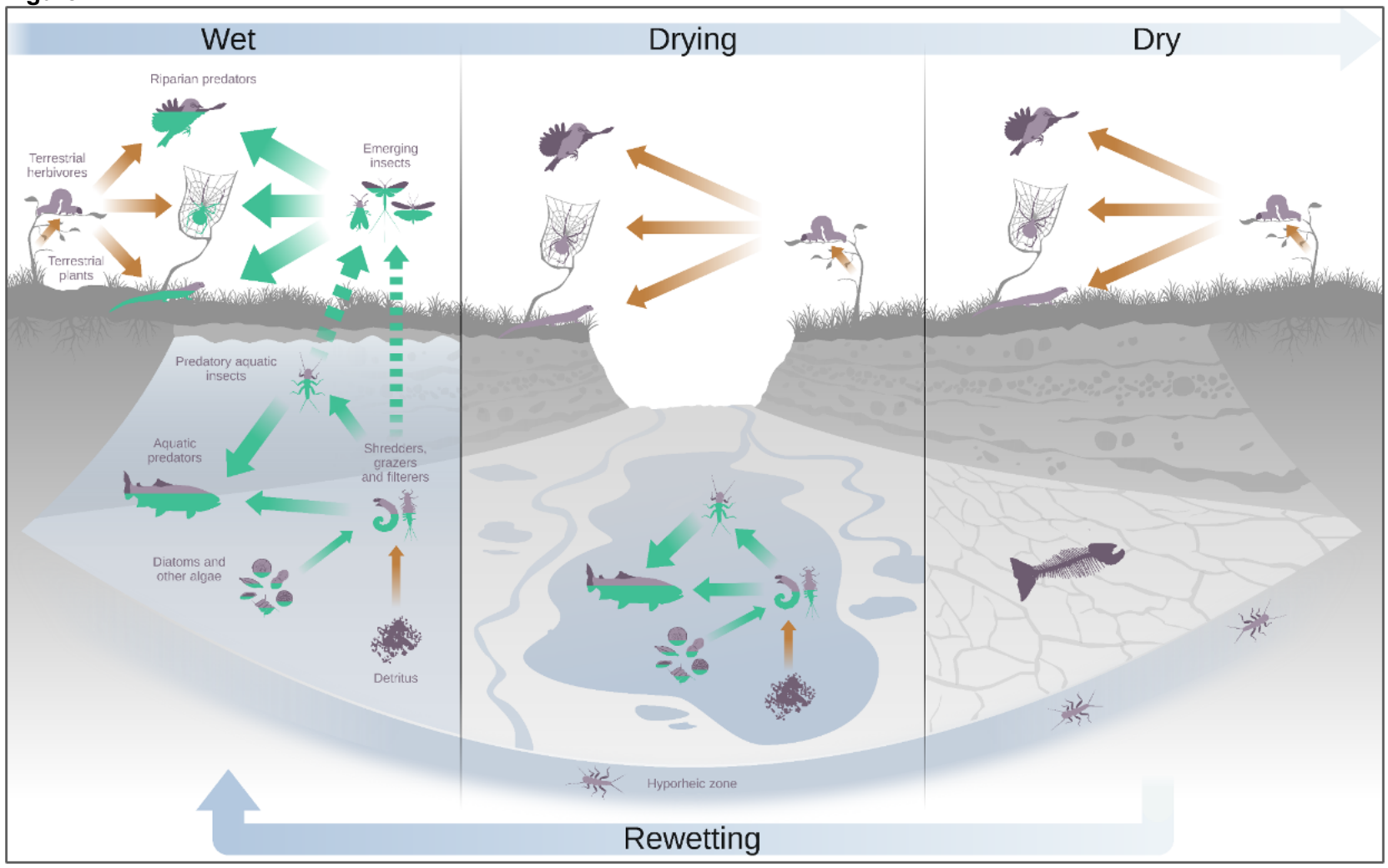
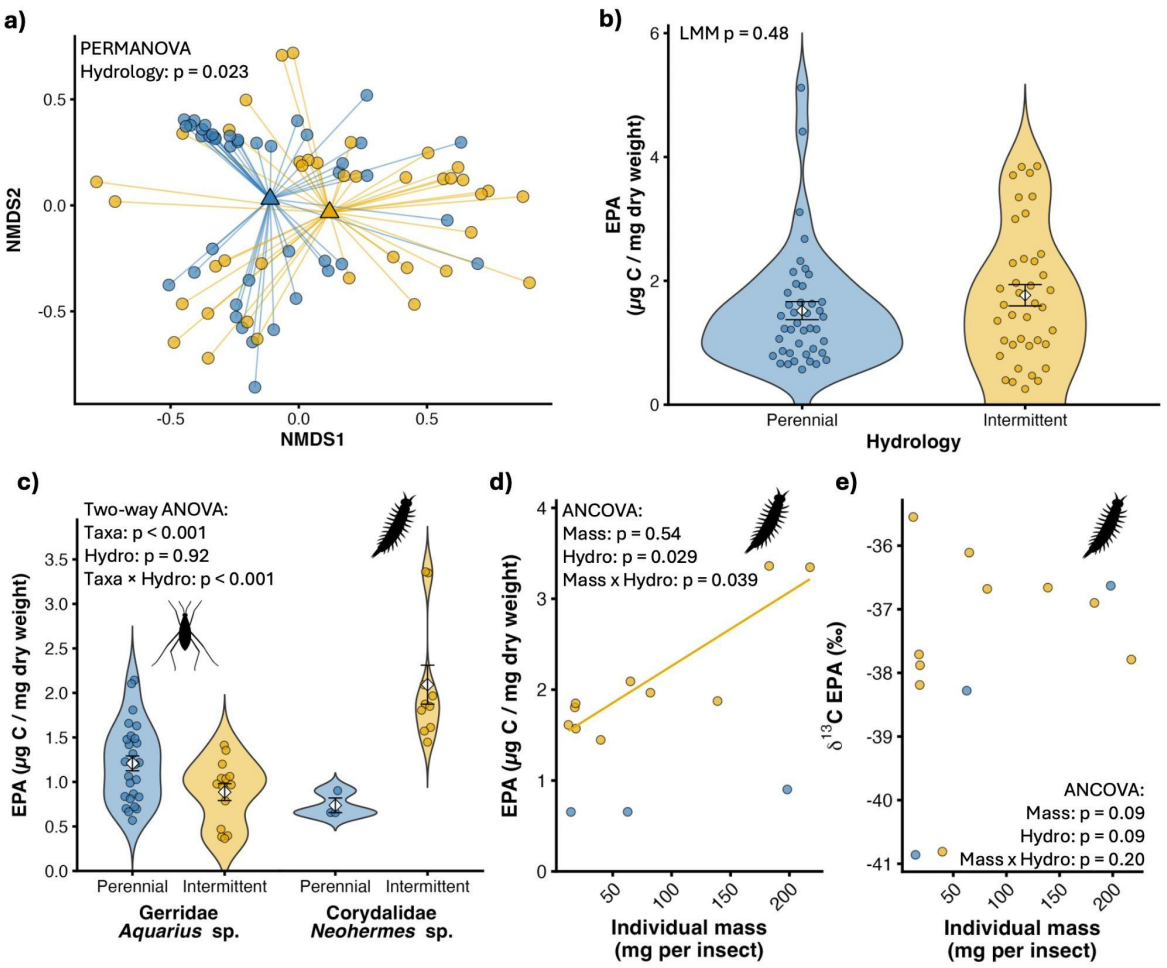


**Figure 1 (previous page). Predicted drought effects on the synthesis and transfer of ω3 LC-PUFA from freshwater to terrestrial habitats across hydrological phases:** Under wet conditions, diatoms drive ω3 LC-PUFA production, which accumulates in consumers and provides prey high in ω3 LC-PUFA to both aquatic and terrestrial predators. As water levels drop during the drying phase, larval insects may lack sufficient time to develop and emerge before pools dry, reducing transport to riparian consumers. In the dry phase, most aquatic organisms are lost, but some persist in the hyporheic zone (e.g., stoneflies), supported by heterotrophic microbes rather than algae. Arrows indicate trophic relationships, with the size reflecting relative nutritional contribution and color indicating prey ω3 LC-PUFA content (green = high, aquatic sources; brown = low, terrestrial sources); green infilling within organisms indicates consumer ω3 LC-PUFA bioaccumulation. Artwork: J. Mumbres.

**Box 1.**

**Box 1 text:**

To assess how life histories mediate fatty acid bioaccumulation across hydrologic regimes, we compared aquatic insects at perennial and intermittent stream sites (n=6) in Pinnacles National Park, California, USA. Community-level fatty acid profiles differed by hydrology (PERMANOVA, $F_{1,68} = 3.49$, $p = 0.023$) and taxon (PERMANOVA, $F_{7,68} = 4.01$, $p = 0.001$; Supplementary Fig 1), but EPA concentrations (representing 99.9% of ω3 LC-PUFA) did not differ between hydrology (LMM, $F_{1,72.5} = 0.49$, $p = 0.48$) [panels a and b]. We then compared two abundant taxa, Gerridae (Aquarius sp.) and Corydalidae (*Neohermes filicornis*), and found that EPA responses to hydrology differed between them (2-way ANOVA interaction, $F_{1,50} = 23.87$, $p < 0.001$), with no effect in Gerridae and higher EPA at intermittent sites in *Neohermes* [panel c]. In *Neohermes* from intermittent sites, EPA concentrations increased with insect mass (post-hoc slope test, $t = 5.64$, $p < 0.001$), indicating growth-driven accumulation [panel d]. This size-dependent accumulation may reflect the dormancy strategy of *Neohermes*, which are long-lived (3-6 years) and burrow into sediments to avoid drought conditions. Compound-specific stable isotopes of EPA ($\delta^{13}C$ EPA) revealed that the source of EPA to *Neohermes* did not vary with mass (ANCOVA: $F_{1,9} = 3.47$, $p = 0.096$), consistent with growth-driven rather than diet-driven accumulation [panel e]. This case study highlights the importance of life history in shaping fatty acid patterns under hydrologic stress, and underscores the risk of interpreting community-level profiles without considering taxon-specific life history strategies. See details on invertebrate sampling and fatty acid analysis in Supplementary Material S2.




**Box 1 caption: (a)** Non-metric multidimensional scaling (NMDS) plot showing differences in fatty acid profiles for different insect samples (circles) collected from perennial (blue) and intermittent (yellow) stream sites. Triangles represent the fatty acid centroid at the community level. **(b)** Insect EPA concentrations by hydrology. The data distribution is indicated by the shaded area (cloud) and points indicate individual data, and white diamonds and whiskers indicate mean ± SE. **(c)** EPA concentrations for *Aquarius* sp. (Gerridae; resilience strategist) and *Neohermes* (Corydalidae; resistance strategist). Points indicate the raw data, the shaded area indicates density of the data distribution, and the white diamonds and whiskers indicate mean ± SE. **(d-e)** Relationship between individual mass and *Neohermes* **(d)** EPA concentrations, or **(e)** $\delta^{13}C$ of EPA, collected from perennial (blue) and intermittent (yellow) stream sites. Line indicates a significant relationship with individual mass.

**Table 1.**

**Select fatty acid terminology.**

| Abbreviation | Full Name | Description/ Nomenclature |
|---|---|---|
| FA | Fatty acid | — |
| PUFA | Polyunsaturated fatty acid | FA with at least two double bonds |
| LC-PUFA | Long-chain polyunsaturated fatty acid | PUFA with at least 20 carbon atoms |
| ω3 LC-PUFA | Omega-3 long-chain polyunsaturated fatty acid | LC-PUFA with the first double bond on the third carbon atom from the terminal methyl group |
| EPA | Eicosapentaenoic acid | Specific ω3 LC-PUFA (20:5ω3) |
| DHA | Docosahexaenoic acid | Specific ω3 LC-PUFA (22:6ω3) |
| ALA | Alpha-linolenic acid | Specific ω3 short-chain PUFA (18:3ω3). Precursor to ω3 LC-PUFA |

**Table 2.**

**Synthesis of drought-induced responses on ω3 LC-PUFA production and transfer.** References prioritize primary studies directly measuring ω3 LC-PUFA where available, and broader ecological studies where such data are lacking. Evidence is summarized using two metrics based on studies explicitly measuring ω3 LC-PUFA: (1) type of support and (2) strength of evidence (1–5 scale; see table notes and Supplementary Information S1). Definitions of both metrics are provided in the table notes and detailed in Supplementary Information S1.

| Drought-induced condition | Change in ω3 LC-PUFA production & transfer | Reference | Type of support | Strength of evidence |
|---|---|---|---|---|
| Increased intermittency (low flow) | **Production:** Drying can shift basal production from autochthonous to allochthonous resources, reducing ω3 LC-PUFA concentrations in benthic resources[1]. Complete drying would halt ω3 LC-PUFA production by periphyton entirely, but very little is known about phases between wet and drying[1] or the role of the hyporheic zone. | 1 | *Obs* | ** |
| | **Aquatic consumers:** Intermittency strongly affects community composition and can decrease the abundance of benthic macroinvertebrates[2–4]. Taxa-specific effects are expected: many aquatic consumers are adapted to intermittency with resistance or resilience strategies (e.g., many benthic macroinvertebrates)[5], but other taxa are highly sensitive to flow cessation (e.g., fish). However, we did not find any studies directly assessing intermittency on aquatic consumer ω3 LC-PUFA profiles. | 2-4 | - | * |
| | **Flux to terrestrial consumers:** Increased intermittency could result in fewer total insects completing metamorphosis and emerging, reducing the transfer of ω3 LC-PUFA to riparian predators[3]. Increased intermittency may also shift emergence timing and cause phenological mismatches between insect availability and consumer demand[6] leading to fewer resources provided to terrestrial insectivores (e.g., spiders[7]). | 6-7 | *Obs* | ** |
| Increased temperature | **Production:** Decrease in algal ω3 LC-PUFA production and shift the algal community toward low-ω3 LC-PUFA cyanobacteria is typical with warmer temperatures[8–13]. | 8-13 | *Obs, Exp, MaM* | ***** |
| | **Aquatic consumers[a]:** Warmer temperatures accelerate growth rate, but often reduce size at maturity (especially for invertebrates[14]). Combined with ω3 LC-PUFA-poor diets, faster growth may decrease ω3 LC-PUFA accumulation[15,16]. Selective retention of ω3 LC-PUFA may increase at higher temperatures, while bioconversion capacity of ω3 LC-PUFA may decline[17,18]. Community composition shifts toward heat-tolerant taxa[9,19]. | 9, 15-19 | *Obs, Exp* | **** |
| | **Flux to terrestrial consumers[b]:** Rising temperatures may promote insect flux in cooler climates or seasons, while reducing biomass exports in already warm systems[20]. Despite higher biomass emerging, the ω3 LC-PUFA flux from emerging insects could decrease due to a combination of lower accumulation from ω3 LC-PUFA poor diets[21] and changes in community composition[20]. Emergence phenology may also shift, potentially decoupling insect availability from riparian consumer demand[6,14,22]. | 6, 20-21 | *Obs, Exp* | *** |

**Table 2 (cont.)**

| | | | | |
|---|---|---|---|---|
| Increased nutrients | **Production:** Nutrients (e.g., phosphorus) can limit primary producer growth and ω3 LC-PUFA synthesis at low concentrations[23,24]. Elevated nutrient levels, especially eutrophication, reduce the nutritional quality of basal resources primarily by favoring phytoplankton and benthic algal assemblages dominated by low-ω3 LC-PUFA taxa (e.g., cyanobacteria) and loss of ω3 LC-PUFA producing taxa such as diatoms[23–26]. Nutrient enrichment may act synergistically with temperature and light: high nutrients in combination with high temp, or light conditions result in lower ω3 LC-PUFA production[9,11,27,28]. | 9,11,23-28 | *Obs, Exp* | **** |
| | **Aquatic consumers:** Eutrophic conditions are consistently associated with reduced ω3 LC-PUFA content in aquatic consumers, including zooplankton, aquatic macroinvertebrates, and fish[9,24–26,28,29]. Declines in consumer ω3 LC-PUFA content are primarily attributed to bottom-up effects driven by reduced ω3 LC-PUFA synthesis at the base of the aquatic food web[28,30], and impaired trophic transfer from basal resources to herbivores: "edibility barrier due to taxonomic shift in phytoplankton (e.g., colonial, or filamentous algae) toward inedible taxa for herbivorous (e.g., zooplankton)[29]. Although eutrophication can increase food availability and consumer biomass, it often coincides with reduced food quality, leading to lower ω3 LC-PUFA concentrations in consumer tissues. However, fish ω3 LC-PUFA concentrations do not always change with nutrient status of a waterbody[9,29]. | 9, 24-26, 28- 30 | *Obs, Exp* | *** |
| | **Flux to terrestrial consumers:** Total biomass emerging typically increases with water nutrient levels[31], but individual insect quality concerning ω3 LC-PUFA concentrations may decrease under eutrophication[20,31,32]. The increase in biomass exported may result in higher total ω3 LC-PUFA subsidy to terrestrial environments compared to nutrient poor systems[20,31,32]. | 20, 31, 32 | *Obs, Exp* | *** |
| Increased light | **Production:** Algal ω3 LC-PUFA production decreases with increased light intensity provided to a waterbody (primarily measured as canopy cover: lower canopy cover results in decreased ω3 LC-PUFA synthesis)[10,27,28]. | 10, 27, 28 | *Obs, Exp* | *** |
| | **Aquatic consumers & flux to terrestrial consumers:** Benthic macroinvertebrates (grazers) from shaded streams grew larger and were in higher densities than those from streams with open canopies, with differences associated with the nutritional quality of periphyton[28]. | 28 | *Exp* | ** |

**Table notes:**

[a]Aquatic consumers: Primarily aquatic insects during larval stages, given their role in cross-ecosystem transfer; other examples include fish.

[b]Flux to terrestrial consumers: Primarily emergent aquatic insects (adult stage), including effects on ω3 LC-PUFA content, biomass, and composition; studies on terrestrial consumers may also be included.

Support type: Describes the types of studies that assessed ω3 LC-PUFA directly using field observation ("Obs"), experimental ("Exp"), or Meta-analyses/modeling ("MaM").

Strength of support: Semi-quantitative score (1 to 5 stars) reflecting the level of empirical support for each predicted response, based on studies explicitly measuring ω3 LC-PUFA. Supporting ecological studies that do not measure ω3 LC-PUFA are included for context but not used in scoring (see Supplementary Information S1 for full definitions).

**1.** Sampera-Calbet et al. 2017; **2.** Siebers et al. 2020; **3.** Leathers et al. 2024; **4.** Chanut et al. 2023; **5.** Lake 2000; **6.** Shipley et al. 2022; **7.** Kowarik et al. 2026; **8.** Hixson & Arts 2016; **9.** Keva et al. 2021; **10.** Piepho et al. 2012; **11.** Zhou et al. 2026; **12.** Strandberg et al. 2022; **13.** Galloway & Winder 2015; **14.** Dallas & Ross-Gillespie 2015; **15.** Strandberg et al. 2020; **16.** Sperfeld & Wacker 2012; **17.** Strandberg et al. 2021; **18.** Tadesse et al. 2003; **19.** Gladyshev et al. 2011; **20.** Fehlinger et al. 2025; **21.** Goedkoop et al. 2007; **22.** Walters et al. 2018; **23.** Taipale et al. 2019; **24.** Taipale et al. 2016; **25.** Yan et al. 2024; **26.** Luo et al. 2024; **27.** Guo et al. 2015; **28.** Guo et al. 2016; **29.** Calderini et al. 2023; **30.** Taipale et al. 2018; **31.** Scharnweber et al. 2020; **32.** Fehlinger et al. 202

**Supplementary Material - How does drought disrupt the transfer of essential omega-3 fatty acids from freshwater to terrestrial ecosystems?**

## S1. Drought-related effects on ω3 LC-PUFA production and transfer table Supplementary material

**Definition of "strength of support":** Ratings reflect the weight of evidence from studies that directly measure ω3 LC-PUFA within freshwater food webs. Broader ecological theory may inform hypotheses, but only direct measurements are used to assign support levels in Table 2.

**Table S1:** Description of "strength of support" ratings used to evaluate evidence for ω3 LC-PUFA patterns in freshwater food webs

| Support rating | Description *(level of support)* | Definition |
|---|---|---|
| 1 | Literature gap *(No direct evidence)* | Effect predicted or hypothesized based on ecological literature, but no studies have directly measured ω3 LC-PUFA in appropriate taxa to evaluate the relationship in freshwater systems |
| 2 | Addressed by few studies *(Limited evidence)* | Effect suggested by a small number of studies with primarily observational evidence (1-3 studies). Evidence is restricted to single-system or a small number of taxa and lacks support from both experimental and field-based approaches. |
| 3 | Moderate evidence, limited scope *(Moderate evidence)* | Effect supported by multiple studies reporting similar patterns in ω3 LC-PUFA. Evidence includes at least one experimental study in addition to observational studies, but remains limited in taxonomic breadth, specific freshwater system type, or geographic representation. General pattern established, but only within constrained contexts. |
| 4 | Strong evidence, some generality *(Strong evidence)* | Effect supported by numerous studies across freshwater taxa and system types, incorporating both field and experimental approaches. Patterns are widely recognized and have been demonstrated in different ecological or geographic contexts. |
| 5 | Broad consensus *(Consensus evidence)* | Effect consistently observed across freshwater systems and taxa with support from multiple approaches: field, experimental, meta-analyses/modeling. General patterns are widely recognized rather than context-specific. Includes at least one review or synthesis study. |

**S2. Pinnacles case study methods:**

**S2A. Study site & sampling:**

Aquatic insects were sampled from six stream habitats within Pinnacles National Park, California, USA. Sites were classified by flow regime, with three perennial streams and three intermittent streams included in the study (Table S2). Sampling was conducted using D-frame nets following standardized kick-sampling procedures across representative microhabitats at each site. Collected specimens encompassed multiple aquatic insect orders, including Coleoptera, Diptera, Ephemeroptera, Hemiptera, Megaloptera, Odonata, Plecoptera, and Trichoptera. All samples were immediately placed on ice in the field and transported to the laboratory, where they were stored at −20 °C prior to processing.

**Table S2:** Sampling sites including site code, description, coordinates, and hydrology type.

| Site code | Site description | Hydrology | Lat | Long |
|---|---|---|---|---|
| WCCH | West Fork Chalone Creek at Chaparral | Intermittent | 36°29'35.57"N | 121°12'33.98"W |
| WCAC | West Fork Chalone Creek Above Caves | Intermittent | 36°29'41.33"N | 121°12'22.62"W |
| WCUC | West Fork Chalone Creek Upper Caves | Perennial | 36°29'50.28"N | 121°12'11.09"W |
| WCCV | West Fork Chalone Creek Balconies Caves (lower cave) | Perennial | 36°29'59.18"N | 121°12'4.86"W |
| WCBA | West Fork Chalone Creek Below Balconies Caves | Perennial | 36°30'6.58"N | 121°12'0.82"W |
| WCNF | West Fork - North Fork Chalone Creek confluence | Intermittent | 36°30'6.90"N | 121°11'0.93"W |

**S2B. Bulk fatty acid analysis and compound specific isotope analysis (*δ13C - FA*)**

*Sample preparation, fatty acid extraction and derivatization*

Insect samples were freeze dried, homogenized, and weighed prior to fatty acid analysis. Fatty acid extraction, derivatization, and analysis was performed at the UC Davis Stable Isotope Facility according to their protocols [1]. Fatty acids were extracted as described in Budge et al. (2006; Appendix 1), but using methanol without BHT. Following extraction, fatty acids were derivatized as fatty acid methyl esters (FAMEs) (Eder. 1995, Meier-Augenstein. 2002) alongside a δ13C- and δ2H-calibrated mixture of fatty acids, as well the two quality assurance reference material extracts. Briefly, fatty acid extracts in chloroform were combined with methylation reagent (concentrated sulfuric acid in anhydrous methanol), 20μL of c19:0 acid internal standard, and heptane to achieve a final volume of 1 mL within vials. Vials were vortexed, and heated at 100ºC for 1 hour. After allowing vials to cool, 300 μL of 1M NaCl and 300 μL of heptane was added, vials vortexed, phases separated, and the upper organic phase transferred to an amber GC vial. Extraction with heptane, was repeated twice and the heptane fractions were pooled. Samples are stored at -20ºC until analysis.

*Identification of FAMEs and compound-specific stable isotope analysis (δ13C - FA)*

The resulting FAME samples were dissolved in heptane and injected at 220 ºC (splitless, 1 min), and separated on an Agilent DB-FATWAX UI capillary column (30 m x 0.25 mm ID x 0.25 mm film thickness) at constant flow rate of 1.2 mL/min under the following temperature program: 60 °C (hold 1 min); 100 °C (5 °C/min); 175 °C (2 °C/min; hold 10 min.); 220 °C (2 °C/min; hold 20 min.). GC-C-P-IRMS is performed on a Thermo Trace GC 1310 gas chromatograph coupled to a Thermo Finnigan MAT 253 isotope-ratio mass spectrometer via a GC IsoLink II combustion interface. For 13C analysis, individual FAMEs are converted to CO2 within a combustion reactor composed of a NiO tube containing CuO/NiO wires maintained at

1000 °C. Water is subsequently removed through a nafion dryer and the analyte gases transferred to the IRMS.

In all, 46 unique FA were identified, and all data were mass corrected as well as converted to the concentration of individual fatty acids or fatty acid group ([g FA methyl ester/g insect]).

*QAQC*

One of every five samples were analyzed in duplicate. Replicates of the quality control and assessment materials were measured every 5 samples. Quality control and assessment mixtures were composed of pure FAMEs that have been calibrated separately by EA- and TC/EA-IRMS using certified reference materials (e.g. NBS-22, IAEA-CH-7) distributed by NIST (Gaithersburg, MD U.S.A.) or the USGS (Reston, VA U.S.A.) and were directly traceable to the primary isotopic reference material for each element (δ13C: V-PDB; δ2H: V-SMOW).

*References for FAME analysis:*

Budge, S.M., Iverson, S.J. and Koopman, H.N., 2006. Studying trophic ecology in marine ecosystems using fatty acids: a primer on analysis and interpretation. Marine Mammal Science, 22(4). doi: 10.1111/j.1748-7692.2006.00079

Eder, K., 1995. Gas chromatographic analysis of fatty acid methyl esters. Journal of Chromatography B: Biomedical Sciences and Applications, 671(1-2), pp.113-131. doi: 10.1016/0378-4347(95)00142-6

Meier-Augenstein, W., 2002. Stable isotope analysis of fatty acids by gas chromatography–isotope ratio mass spectrometry. Analytica Chimica Acta, 465(1-2), pp.63-79.doi: 10.1016/S0003-2670(02)00194-0

UC Davis SIF Extraction Description (February 24, 2022): https://stableisotopefacility.ucdavis.edu/sample-preparation-fatty-acid-methyl-ester-fame

UC Davis SIF Analysis Description (February 24, 2022):

https://stableisotopefacility.ucdavis.edu/fatty-acid-methyl-ester-fame-analysis

**S2C. Statistical analyses:**

To evaluate differences in fatty acid concentrations (μg C $mg^{-1}$ dry weight) among taxa and between flow regimes (intermittent vs. perennial), we conducted non-metric multidimensional scaling (NMDS) using the vegan package in R (Oksanen et al. 2007). We selected individual fatty acids of interest and groups of fatty acids with ecological relevance to include in our analyses: LC PUFA (EPA [20:5w3], DPA [22:5w3], and ARA [20:4w6]), essential fatty acids (LIN [18:2w6] and ALA [18:3w3]), diatom-associated fatty acids (16:4w1, and 16:2w4), and bacterial-associated FA (11:0, 17:0, 15:0, 21:0, 17:1w7, 15:1w5). Our samples included 8 taxonomic groups: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Megaloptera, Odonata, Plecoptera, and Trichoptera. We constructed Bray-Curtis dissimilarity matrices and performed ordinations to visualize multivariate differences in fatty acid profiles (Figures Box 1-a, and S1). We conducted a permutational multivariate analysis of variance (PERMANOVA) test to assess differences among taxa and flow regime.

We evaluated whether EPA concentrations differed between hydrological regimes using a linear mixed-effects model implemented with the lme4 and lmerTest packages in R. EPA concentration (μg C $mg^{-1}$ dry weight) was modeled as a function of hydrology (perennial vs. intermittent) as a fixed effect. Taxa at the order-level was included as a random intercept to account for potential non-independence among samples originating from the same taxonomic group. Models were fit using restricted maximum likelihood (REML). Significance of fixed effects was assessed using Type III analysis of variance with Satterthwaite's approximation for denominator degrees of freedom.

To assess differences in EPA concentration among taxa and hydrology types, we used a two-way analysis of variance (ANOVA) with taxon and hydrology as fixed factors, including their

interaction. Model assumptions were evaluated using visual inspection of diagnostic plots and formal tests of normality and homogeneity of variance.

We evaluated the relationship between insect body size and EPA concentration within each taxon (Corydalidae and Gerridae) using analysis of covariance (ANCOVA). EPA concentration (μg C $mg^{-1}$ dry weight) was modeled as a function of hydrology (perennial vs. intermittent) and individual mass (mg), including their interaction. Hydrology was treated as a categorical fixed effect and individual mass as a continuous covariate. Models were fit separately for each taxon using linear models in R. Significance of main effects and interactions was assessed using Type III analysis of variance. To further interpret significant interactions, we conducted post hoc analyses to estimate the slope of the relationship between body size and EPA concentration within each hydrological regime. We applied the same modeling framework to assess patterns in $\delta^{13}C$ of EPA

Multivariate analyses were performed using R version 4.2.3 (NMDS and PERMANOVA) and all other statistical analyses and graphs were produced using R version 4.5.0.

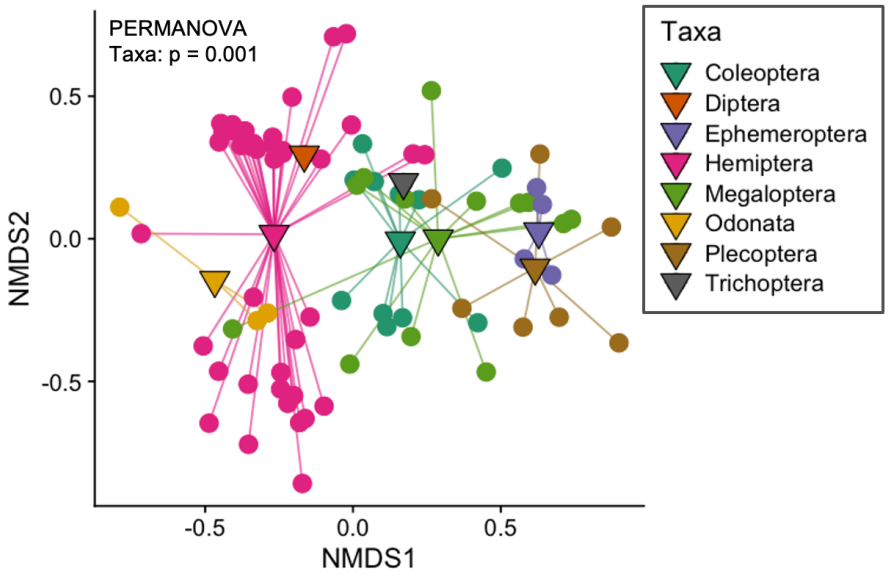
*References for statistical analyses:*

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M. H. H., Oksanen, M. J., & Suggests, M. A. S. S. (2007). The vegan package. Community ecology package, 10(631-637), 719.

**S2D. Results & Supplementary figures:**

Here we describe results from our case study not explicitly discussed in the body of the manuscript.

*Results: Effect of taxa on ω3 LC-PUFA profiles of aquatic insects*




**Supplementary figure 1:** Non-metric multidimensional scaling (NMDS) plot showing differences in fatty acid profiles for different insect samples for the insect taxa collected (indicated by color). Centroids (denoted by triangles) represent the fatty acid centroid at the taxa-level (PERMANOVA, $F_{7,68} = 4.88$, $p = 0.001$)

*Results effect of hydrology on EPA concentrations among all taxa:*

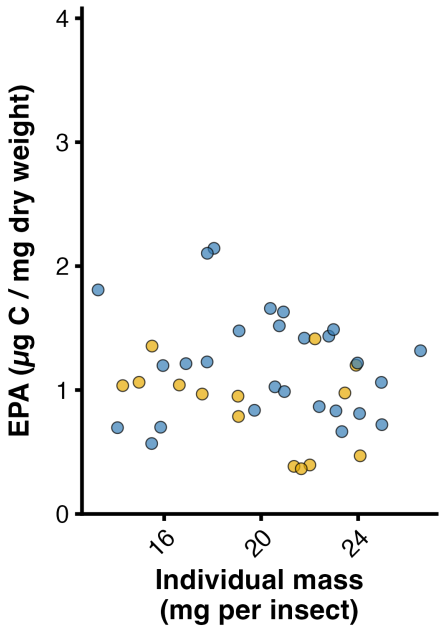
Hydrology did not significantly influence EPA concentrations (Type III ANOVA: $F_{1,72.5} = 0.49$, $p = 0.487$). The estimated difference between intermittent and perennial systems was small and not statistically significant ($\beta = 0.10 \pm 0.15$ SE, $t_{72.5} = 0.70$, $p = 0.487$). The model intercept indicated a mean EPA concentration of $2.14 \pm 0.54$ (SE) for the reference category (perennial streams). Random variation among sample types contributed substantially to overall variability in EPA concentrations (variance = 2.16, SD = 1.47), compared with residual variance within sample types (variance = 0.35, SD = 0.60). In total, the model included 81 observations across 8 taxa.

*Results effect of hydrology on EPA concentrations between two focal taxa:*

A two-way ANOVA showed a significant effect of taxon on EPA concentration ($F_{1,50}$ = 21.07, $p < 0.001$), no significant main effect of hydrology ($F_{1,50} = 0.01$, $p = 0.922$), and a significant Taxon × Hydrology interaction ($F_{1,50}$= 23.87, $p < 0.001$). Residuals showed a deviation from normality (Shapiro–Wilk test: $W = 0.93$, $p = 0.004$), but Q–Q plots indicated only minor departures. Homogeneity of variance was supported by Levene's test ($F_{3,50} = 1.13$, $p = 0.345$), and no influential observations were detected. Given the robustness of ANOVA to moderate deviations from normality, the model was considered appropriate. EPA concentrations responded differently to hydrology across taxa, with Corydalidae showing reduced EPA under intermittent conditions, while Gerridae remained relatively unchanged.

*Results EPA concentration and body size:*

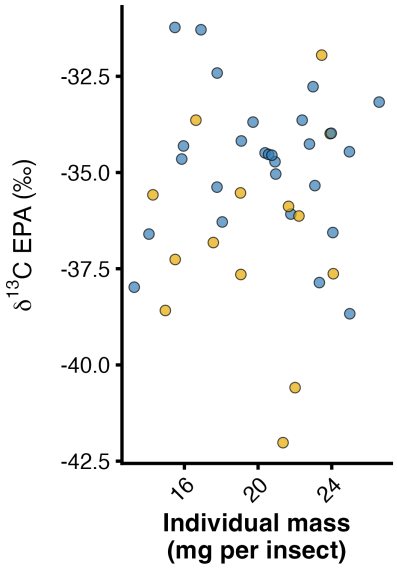
For Corydalidae, the relationship between body size and EPA concentration differed by hydrology (interaction: $F_{1,9}$=5.66, p=0.041). Post hoc tests showed that EPA concentration increased with body size in intermittent systems (β = 0.0081 ± 0.0014 SE, p = 0.0003), but not in perennial systems (β = 0.0015 ± 0.0024 SE, p = 0.536; Box figure "d" in manuscript). In contrast, EPA concentration in Gerridae was not influenced by body size, hydrology, or their interaction (interaction: $F_{1,37}$=0.33, p = 0.570). Neither body size ($F_{1,37}$=0.54, p = 0.468) nor hydrology ($F_{1,37}$=0.02, p=0.883) had significant effects (Figure S2).




**Supplementary figure 2:** Relationship between EPA concentration and insect mass for Gerridae. Blue dots indicate perennial sites; yellow dots indicate intermittent sites.

*Results d13C of EPA:*

Neither taxon showed significant effects of body size or hydrology on $\delta^{13}C$ of EPA. For Corydalidae, $\delta^{13}C$ of EPA was not influenced by the interaction between body size and hydrology (Type III ANOVA: $F_{1,9} = 1.95$, $p = 0.196$), and there were no significant effects of body size ($F_{1,9} = 3.47$, $p = 0.096$) or hydrology ($F_{1,9} = 3.56$, $p = 0.092$; Box figure "e" in manuscript). For Gerridae, $\delta^{13}C$ of EPA was not influenced by body size, hydrology, or their interaction (Type III ANOVA: interaction: $F_{1,37} = 0.15$, $p = 0.696$; body size: $F_{1,37} = 0.13$, $p = 0.723$; hydrology: $F_{1,37} = 0.69$, $p = 0.410$; Figure S3).




**Supplementary figure 3s:** Relationship between δ¹³C of EPA and insect mass for Gerridae. Blue dots indicate perennial sites; yellow dots indicate intermittent sites.

**S2E - % EPA of total n3 LC PUFA:**

Considering all samples (n = 81), we calculated the percentage of EPA comprising the total ω3 LC-PUFA in each sample. The mean %EPA was 99.9% (± 0.36 SD).