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# Dietary supply with essential lipids affects growth and survival of the amphipod *Gammarus roeselii*

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ABSTRACT

Growth and survival of benthic macroinvertebrates depend on the availability and the quality of potential food sources. The significance of essential biochemical nutrients, such as sterols and polyunsaturated fatty acids (PUFAs), for benthic invertebrates has been insufficiently studied. We investigated the effects of these essential lipids on growth and survival of the benthic gammarid *Gammarus roeselii*, a widespread species in streams, rivers and lentic waters of Central Europe, in standardized feeding experiments. Juvenile gammarids were fed a mixture of three cyanobacteria with no evidence of toxin production, either unsupplemented or supplemented with cholesterol or the long-chain PUFA docosahexaenoic acid (DHA) using bovine serum albumin (BSA) to load algal or cyanobacterial cells with single lipid, and a mixture of three eukaryotic algae containing various sterols and long-chain PUFAs. Our results revealed that growth and especially survival of gammarids on the cyanobacterial diet significantly increased upon supplementation with cholesterol and DHA, indicating that the nutritional inadequacy of cyanobacteria for gammarids and potentially other benthic invertebrates is at least partially due to a deficiency in these essential lipids. We propose that the expected increase in the frequency of pelagic cyanobacterial mass developments as a consequence of global warming will also affect benthic food web processes to an as-yet-unknown magnitude.



## Introduction

Survival, growth, and reproductive success of benthic macroinvertebrates depend on the quantity and nutritional quality of their food sources (Fuller et al., 1988; Söderström, 1988; Basen et al., 2011). Numerous benthic macroinvertebrates, especially amphipods, are classified as shredders, feeding primarily on deposited leaf litter (e.g. Bärlocher and Kendrick, 1973; Anderson and Sedell, 1979). However, several studies demonstrated that amphipods prefer more valuable food sources, such as eukaryotic algae (e.g. Friberg and Jacobsen, 1994) or can prey on other invertebrates, e.g. mayflies or other amphipods, and are therefore hard to assign to one specific functional feeding group (e.g. MacNeil et al., 1997; Kelly et al., 2002). Feeding on these superior food sources, especially on animal material, results in higher survival and growth rates of amphipods (Delong et al., 1993; Gergs and Rothhaupt, 2008).

Climate scenarios which predict rising temperatures, increased atmospheric $CO_2$ supplies, and increased periods of thermal stratification are expected to favor the dominance of cyanobacteria as aquatic primary producers (Pearl and Huisman, 2009). As a consequence, significant amounts of pelagic cyanobacterial carbon will be deposited to the sediment (Nascimento et al., 2008; Suikkanen et al., 2010). Moreover, cyanobacteria also can dominate the periphyton (Poff et al., 1990; Schlagerl and Donabaum, 1998; Bourassa and Cattaneo, 2000). The use of this prokaryotic carbon pool and its significance for benthic food web processes has been poorly investigated. Recent studies showed that benthic invertebrates incorporate deposited cyanobacteria, but the nutritional value is low (Karlson et al., 2008; Nascimento et al., 2009). Cyanobacteria are of low food quality for aquatic consumers, because of morphological properties which hamper ingestion (Van Donk et al., 2011), toxicity (DeMott et al., 1991), and/or a deficiency in essential biochemical nutrients (von Elert et al., 2003). In contrast to eukaryotic algae, cyanobacteria are incapable of synthesizing sterols and long-chain polyunsaturated fatty acids (PUFAs >C18) *de novo* (Volkman, 2003; Summons et al., 2006; Martin-Creuzburg et al., 2008), both of which are essential dietary nutrients for many aquatic invertebrates. Sterols and PUFAs are necessary structural components of cell membranes and are required as precursors for other bioactive molecules, such as steroid hormones (e.g. the molt-inducing ecdysteroids in arthropods) and eicosanoids (e.g. prostaglandins),

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respectively (Goad, 1981; Grieneisen, 1994; Stanley-Samuelson, 1994; Arts et al., 2001; Martin-Creuzburg and Von Elert, 2009).

Biochemical nutrient requirements of benthic invertebrates have been poorly studied, as compared to those of pelagic invertebrates. Even though the significance of dietary PUFAs for benthic invertebrates has been recognized (Ahlgren et al., 1997; Goedkoop et al., 1998, 2000), experimental studies investigating the effect of specific nutrients on growth or survival are rare for benthic freshwater organisms (but see Wacker et al., 2002; Goedkoop et al., 2007). Recently, Basen et al. (2012) showed that somatic growth of the invasive clam *Corbicula fluminea* on cyanobacterial diets is constrained primarily by the absence of sterols. Additionally, processing and biodeposition of cyanobacteria by clams can improve the quality of cyanobacterial carbon for subsequent use by the gammarid *Gammarus roeselii* and this nutritional upgrading has been partially attributed to an enrichment of the biodeposited material with essential lipids (Basen et al., 2013). However, to assess the role of essential lipids in determining food quality for amphipods in general, supplementation experiments are required in which the availability of these nutrients is specifically manipulated. Here, we investigated the significance of essential lipids for the benthic gammarid *G. roeselii* in standardized growth and feeding experiments. Juvenile gammarids were raised on a sterol-free and PUFA-deficient (no PUFAs >C18) cyanobacterial diet supplemented with either cholesterol or the long-chain PUFA docosahexaenoic acid (DHA). For comparison, gammarids were raised on sterol- and PUFA-containing eukaryotic algae.

## Materials and methods

### *Test animals: origin and maintenance*

Adult gammarids (*G. roeselii*) were obtained from the littoral zone of the oligotrophic, prealpine Lake Constance via kick sampling at the shoreline. To hatch juveniles for the experiments, gammarids were kept in environmental chambers with a diurnal dark–light cycle of 12 h:12 h, kept at 15 °C in aerated aquaria containing lake water, gravel of different grain sizes for shelter, and dried alder leaves as a food source. To protect juveniles from potential predation by the adults, aquaria were separated by horizontal gauze passable only for juvenile gammarids (smaller 3 mm body length).

### *Food preparation*

Autotrophic food sources were cultivated semi-continuously in aerated 5 l vessels at a dilution rate of 0.2 $d^{-1}$ at 20 °C with illumination at 100–120 $\mu mol\ m^{-2}\ s^{-1}$ and harvested in the late-exponential growth phase. The coccoid cyanobacterium *Synechococcus elongatus* (SAG 89.70, Sammlung für Algenkulturen Göttingen, Germany), the filamentous cyanobacteria *Anabaena variabilis* (ATCC 29413, American Type Culture Collection, Manassas, USA) and *Aphanizomenon* sp. (CCAP 1401-1, Culture Collection of Algae and Protzoa, UK), the green alga *Scenedesmus obliquus* (SAG 276-3a), and the eustigmatophyte *Nannochloropsis limnetica* (SAG 18.99) were each grown in Cyano medium (Jüttner et al., 1983). The diatom *Cyclotella meneghiniana* (SAG 1020-1a) was grown in M3Ks medium (Nicklisch, 1992). These food organisms were used because they differ considerably in size, shape, lipid content and composition. Cyanobacteria showed no evidence for toxicity to aquatic invertebrates in former studies (e.g. von Elert et al., 2003; Martin-Creuzburg and Von Elert, 2004; Martin-Creuzburg et al., 2009; Basen et al., 2012, 2013), and cells were obtained from established reference collections which are not known to produce toxins typically produced by these genera in natural systems. Food suspensions were prepared by concentrating the cells via centrifugation (3000 × *g*, 10 min) followed by resuspension in fresh medium. Carbon concentrations of the food suspensions were estimated by photometric light extinctions (800 nm) and carbon-extinction equations determined prior to the experiment. Two different food suspensions were prepared: a mixture of the three cyanobacterial species (CM) and a mixture of the three eukaryotic algal species (AM, Table 1). Each autotrophic species represented one third of the total particulate carbon provided in each mixture.

### *Experimental setup*

Feeding experiments were conducted with juvenile gammarids (2–3 mm body length) individually placed in glass beakers filled with 100 ml of filtrated lake water (0.45 μm); a small pebble (organic matter removed using a muffle furnace) was provided as shelter in each beaker. Juvenile *G. roeselii*, which passed the gauze to separate them from the adults, were randomly transferred into the experimental beakers to avoid potential effects of post-hatch feeding. Gammarids were fed *ad libitum* individually (Gergs and Rothhaupt, 2008) or starved without adding food, and were transferred into new beakers three times a week to avoid accumulation of food, fecal pellets and biofilm. Food suspensions were added with a pipette near the bottom of the beaker resulting in fast sedimentation of food particles and therefore ensuring availability of the algae food to the amphipods.

Cyanobacteria were enriched with cholesterol (Sigma, C8667, purity 99%) or the polyunsaturated fatty acid docosahexaenoic acid (DHA; Sigma D-2534, purity ≥98%) using a modified protocol of a method originally developed by Von Elert (2002). In this method, bovine serum albumin (BSA) is used to load algal or cyanobacterial cells with single lipid compounds, i.e. the cell itself is used as a transfer vehicle to provide a consumer with the essential lipids. Cholesterol was used because is the predominant sterol in amphipods (Nelson et al., 2001) and DHA was used because amphipods have been shown to contain high proportions of this fatty acid (Nelson et al., 2001; Kolanowski et al., 2007). Cholesterol and DHA were dissolved in ethanol (2.5 $mg\ ml^{-1}$) to prepare stock solutions. For the supplementation, 40 mg of bovine serum albumin (BSA; Sigma A7906, 98%) were dissolved in 10 ml of ultrapure water and 266.7 μl of a lipid stock solution were added during gentle stirring. Subsequently, 10 ml of Cyano medium and 8 mg particulate organic carbon (POC) of the cyanobacterial mixture (CM) were added and, after 5 min of incubation, the volume was brought to 80 ml with Cyano medium. The resulting suspensions were incubated on a rotary shaker (100 rpm) for 4 h with illumination at 100 $\mu mol\ m^{-2}\ s^{-1}$. To remove excess BSA, free cholesterol, and DHA, cyanobacterial cells were concentrated by centrifugation and resuspended in fresh medium; this process was repeated twice.

The obtained cyanobacterial food suspensions ("CM + cholesterol" and "CM + DHA") were then used as food in the growth experiments (Table 1). The food suspensions CM + BSA were prepared similarly but without adding lipids. Furthermore, the unsupplemented CM and the AM were also used as food sources. Each of the five food treatments consisted of 40 replicates (i.e. individuals) and these were monitored for seven weeks from June 6th to August 1st in 2011. Body lengths of the gammarids were measured by photographing the animal once a week as described in Gergs and Rothhaupt (2008), survival was recorded three times a week.

Because not enough individuals survived the growth experiment for lipid measurements in all treatments, the sterol and fatty acid composition of gammarids for all food treatment was determined by analyzing animals from an additional experiment, which were reared on the same five food treatments as used in the growth