

Evaluating food quality of *Arthrospira platensis* **for culturing** *Daphnia magna*

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Abstract

Arthrospira is a flamentous cyanobacterium, rich in proteins and bioactive compounds, which is a dietary supplement for humans and animals. However, the contribution of this "superfood" on the life history traits of zooplankton is poorly addressed. We conducted *Daphnia magna* growth experiment using *Arthrospira platensis* comparing it with *Chlamydomonas reinhardtii* as quality food, to evaluate the nutritional adequacy of *A. platensis* for rearing daphnids. The trichomes of *A. platensis* was fragmented using a bead crusher for easier ingestion by the daphnids. The growth experiments revealed that *A. platensis* alone did not support both survival and growth of *D. magna*, but the daphnid survival increased after the addition of 20% *C. reinhardtii.* When *A. platensis* was supplemented with 50% and more of *C. reinhardtii*, however, growth was better than to those daphnids exclusively fed on *C. reinhardtii*. This suggests that *A. platensis* have some nutritional limitations essential for daphnids survival and growth instead of less digestibility or toxicity. Carbon (C) to Phosphorus (P) ratio (C:P=46) of *A. platensis* was far below the threshold for daphnid growth (C:P=116). This implies that P limitation is unlikely in *A. platensis* as feed for daphnids. Although the lack of some essential biochemicals such as sterols and polyunsaturated fatty acids may be a more plausible explanation, a small addition of *A. platensis* to standard microalgal foods would result in better growth of zooplankton, including *D. magna*.

Keywords *Arthrospira platensis* · *Daphnia magna* · Essential biochemicals · Food quality

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Introduction

Daphnia is a keystone zooplankton, transferring energy and carbon (C) from primary producers to higher trophic levels (Urabe et al. [1997](#page-11-0); Martin-Creuzburg et al. [2005;](#page-10-0) Freese and Martin-Creuzburg [2013](#page-10-1)). It is a non-selective suspension feeder which consumes various phytoplankton including cyanobacteria and bacteria regardless of their nutritional quality (Fink et al. [2011](#page-10-2); Freese and Martin-Creuzburg [2013;](#page-10-1) Bednarska et al. [2014;](#page-9-0) Wenzel et al. [2021\)](#page-11-1). Previous studies have revealed the inadequate food quality of cyanobacteria for supporting the growth and reproduction of crustacean zooplankton because of their poor manageability, toxicity, and lack of or an insufficient quantity of essential dietary materials such as polyunsaturated fatty acids (PUFAs) and sterols (von Elert [2002](#page-11-2); Wacker and Martin-Creuzburg [2012](#page-11-3); Thomas et al. [2022](#page-11-4)).

Cyanobacteria is poorly ingested by zooplankton because of the long trichome or colonial nature of their cells (von Elert [2002;](#page-11-2) Bednarska et al. [2014](#page-9-0); Thomas et al. [2022](#page-11-4)). *Daphnia* efficiently consumes food particles in the intermediate size range of $0.3-40 \mu m$, but not those over ca. 50 μm (Geller and Müller [1981;](#page-10-3) Porter and McDonough [1984;](#page-11-5) Urabe et al. [1996\)](#page-11-6). Long flamentous algae are too large for daphnids to ingest, and such large food items interfere with the collection of other foods, and consequently cause loss of ftness (Bednarska et al. [2014\)](#page-9-0).

On the other hand, C transfer from cyanobacteria to daphnids can be constrained by elemental limitations; for example, shortage of phosphorus (P) for daphnid growth happens when the C to P ratio in the diet exceeds 300 (Urabe et al. [1997,](#page-11-0) [2018;](#page-11-7) Gulati and Demott [1997\)](#page-10-4). Higher P allocation into ribosomal RNA is required to support fast protein synthesis, which explains the direct relationship between growth and P availability in the food (Elser et al. [2003](#page-10-5); Bukovinszky et al. [2012](#page-9-1)). The efect of P limitation in the life history traits of zooplankton depends on the developmental stages and taxa (Thomas et al. [2022](#page-11-4)). In *Daphnia*, P limitation is pronounced at both growth and reproduction stages (Hartnett [2019\)](#page-10-6), which makes it a crucial element in the food. In addition, the availability of P afects the relative importance of fatty acids in the diet (Gulati and Demott [1997;](#page-10-4) Thomas et al. [2022\)](#page-11-4).

Even though most cyanobacteria show such food quality constraints in supporting zooplankton life history traits, some cyanobacterial species have outstanding nutritional profles and provide substantial benefts for humans and animals. *Arthrospira* is a flamentous cyanobacteria that is rich in proteins and bioactive compounds (Martins et al. [2021;](#page-10-7) Spínola et al. [2022\)](#page-11-8). Its nutritional quality has been widely investigated and used as a food supplement for humans and animals, and in pharmaceutical products (Gentscheva et al. [2023\)](#page-10-8). Its high levels of proteins (60–70%), essential amino acids, fatty acids, and carbohydrates have attracted attention to it as a sustainable food source for humans (Wang et al. [2021\)](#page-11-9) and animals (Altmann and Rosenau [2022\)](#page-9-2). It also has anticancer, antibacterial, antifungal, and antiviral activities because of its phycocyanin, phycocyanobilin, and allophycocyanin production (Nuhu [2013](#page-10-9); Wang et al. [2021\)](#page-11-9).

Arthrospira can be cultured using the anaerobically digested effluent (ADE) of organic wastes (Dunn et al. [2013](#page-10-10); Hultberg et al. [2017;](#page-10-11) Matos et al. [2021\)](#page-10-12) and aquatic weeds (O'Sullivan et al. [2010;](#page-10-13) Kimura et al. [2019](#page-10-14)). Water hyacinth (*Eichhornia crassipes*), for example, is an invasive aquatic weed that has deleterious efects on various aquatic ecosystems worldwide (Williams [2004](#page-11-10); Villamagna and Murphy [2010](#page-11-11)). In our ongoing project, *Arthrospira* is cultured using ADE produced from over-growing water hyacinth in Lake Tana, Ethiopia (Dersseh et al. [2019;](#page-10-15) Damtie et al. [2022a](#page-9-3), [b](#page-9-4)). Utilizing the *Arthrospira*

produced from the ADE of water hyacinth to culture zooplankton, and then to culture fsh using the zooplankton as feed, may be an appropriate way to maximize use of water hyacinth. However, there is limited information on the efects of *Arthrospira* on the growth and reproduction of zooplankton including daphnids. In this study, we conducted a *D. magna* growth experiment using *A. platensis* as a single food and in combination with *C. reinhardtii* to evaluate the food quality of *A. platensis* to rear *D.magna*.

Methods

Microalgae cultivation

A. platensis (NIES-39) and *C. reinhardtii* (NIES-2235) were cultured using SOT medium (Ogawa and Terui [1970\)](#page-11-12) and C medium (Ichimura [1971\)](#page-10-16), respectively (Table [1](#page-2-0)). Both *A. platensis* and *C. reinhardtii* were grown in 1-L fasks under a photoperiod of 12 D:12 L with light intensity of ca. 440 µmol photons $m^{-2} s^{-1}$ at 25 °C and ca. 180 µmol photons m^{-2} s⁻¹ at 20 °C, respectively. The algae were harvested at the exponential growth phase and concentrated through centrifugation $(4200 g)$. An aliquot of the algal suspension was collected from the well-mixed algal culture, and the absorbance of the algal suspension was measured with a spectrophotometer (SP-300, OPTIMA®, Tokyo, Japan) at a wavelength of 680 nm. The carbon concentration of the algal suspension was estimated from a regression equation between absorbance and the carbon mass of the cells established before the experiment. A new inoculation for the microalgal culture was made every 5 days for *A. platensis* and every 7 days for *C. reinhardtii* to provide a constant supply of fresh algal food throughout the experiment.

SOT medium		C medium		
Chemicals	Concentration (mg/L)	Chemicals	Concentration (mg/L)	
NaHCO ₃	16,800	Ca(NO ₃), 4H ₂ O	150	
K_2HPO4	500	KNO ₃	100	
NaNO ₃	2500	β -Na ₂ glycerophosphate•5H ₂ O	50	
K_2SO_4	1000	$MgSO_4$ -7H ₂ O	40	
NaCl	1000	Vitamin B_{12}	0.0001	
MgSO ₄ ·7H ₂ O	200	Biotin	0.0001	
CaCl ₂	40	Thiamine HCl	0.01	
FeSO ₄ ·7H ₂ O	10	Tris (hydroxymethyl) aminomethane	500	
Na ₂ EDTA.2H ₂ O	80	Na ₂ EDTA·2H ₂ O	3	
H_3BO_3	2.86	FeCl ₃ ·6H ₂ O	0.588	
MnSO _A ·7H ₂ O	2.5	MnCl ₂ ·4H ₂ O	0.108	
ZnSO ₄ ·7H ₂ O	0.222	ZnSO _A ·7H ₂ O	0.066	
CuSO ₄ ·5H ₂ O	0.079	CoCl ₂ ·6H ₂ O	0.012	
$Na2MoO4·2H2O$	0.021	$Na2MoO4·2H2O$	0.0075	

Table 1 Chemical compositions of SOT and C media

Fragmentation of *A. platensis* **trichome**

The size of untreated *A. platensis* trichomes ranges from 50 to 500 μm (Wan et al. [2021](#page-11-13)), which is too large for daphnids to ingest (Geller and Müller [1981;](#page-10-3) Wenzel et al. [2012;](#page-11-14) Bednarska et al. [2014\)](#page-9-0). To be easily ingested by the daphnids, *A. platensis* trichomes were fragmented with a bead crusher $(\mu T-01, TAITEC)$. A 2-mL conical screw cap microtube (1392-200-C, WATSON) and zirconia beads (CZS0060, AS ONE Corporation) with a diameter of 0.4–0.6 mm were used to fragment the trichomes. Approximately one-third of the volume of the microtube was flled with zirconia beads, and the remaining volume was flled with an *A. platensis* suspension with concentration of 0.5–0.8 mg C mL−1. The shaking speed of the bead crusher was set at 4600 rpm for 5–25 s. The fragmented *A. platensis* cells were checked by a microscope every 5 s, and the sizes of the trichomes were measured with a digital micrometer (Wraycam, NF500) under inverted microscope (Olympus, IX70) at a magnifcation of 20×.

Growth experiment in *D. magna*

A stock culture of *D. magna* was maintained in aged and fltered tap water (ATW) with a glass fiber filter (Whatman, GF/F, diameter, 47 mm) at 20 $^{\circ}$ C, photoperiod of 12 L:12 D and light intensity of ca. 180 µmol photons $m^{-2} s^{-1}$ with sufficient amounts of *C. reinhardtii* (> 10^5 cells mL⁻¹). For each food treatment, arbitrarily sorted five neonates born within 12 h from a stock culture were transferred to a 200-mL glass jar flled with ATW and an algal food suspension. The food treatments were conducted at fve diferent ratios of fragmented *A. platensis*:*C. reinhardtii* (100:0, 80:20, 50:50, 20:80, and 0:100) at a food concentration of 4 mg C L−1 (Table [2](#page-3-0)). The *A. platensis* trichomes used for this experiment was fragmented for 25 s. Control treatments exclusively using *C. reinhardtii* as the food alga were conducted at [2](#page-3-0)0%, 50%, and 80% of 4 mg C L^{-1} (Table 2).

The experimental animals were transferred to new bottle containing fresh food and culturing water every day, and dead animals were recorded. All treatments were made in triplicate and lasted for 6 days. At the end of the experiment, the surviving animals from each treatment were collected from the jar, washed with ATW, transferred to a tin capsule, dried in an oven (DV-600, YAMATO) for 24 h, and then weighed on a digital balance with a minimum weighing value of 1 μ g (AD-4212D-32, A&D Instruments

Food treatment (AP:CR)	Carbon mass of food (mg CL^{-1})		
A. <i>platensis</i> alone (100:0)	4		
80% A. platensis + 20% C. reinhardtii (80:20)	$3.2 + 0.8$		
50% A. platensis $+50\%$ C. reinhardtii (50:50)	$2 + 2$		
20% A. platensis + 80% C. reinhardtii (20:80)	$0.8 + 3.2$		
C. reinhardtii alone (0:100)	4		
C. reinhardtii alone (0:80)	3.2		
C. reinhardtii alone (0:50)	2		
C. reinhardtii alone (0:20)	0.8		
No food	$\mathbf{0}$		

Table 2 Food treatments at diferent supply ratios of *Arthrospira platensis* to *Chlamydomonas reinhardtii* (AP:CR) for *Daphnia magna* growth experiments at food concentration of 4 mg C L−1

Ltd). In addition, 30 neonates born within 12 h were arbitrarily sorted from a stock culture, and the same procedure described above was carried out to determine the initial dry weight of the animals. Somatic growth rates (*g*) of the animals were calculated as the increment of the dry mass between newborn (M_0) and 6-day-old animals (M_6) using Eq. (1) (1) :

$$
g = \frac{lnM_6 - lnM_0}{6} \tag{1}
$$

Elemental analysis of microalgae

To determine the elemental composition of C, nitrogen (N), and P of the microalgae, we took an aliquot of approximately 10–15 mL of the fresh algal suspension, equivalent to a dry weight of 9.1 ± 0.4 mg for *A. platensis* and 2.9 ± 0.16 mg for *C. reinhardtii* and filtered with a pre-combusted and pre-weighed glass fber flter (GF/F, diameter, 47 mm, Whatman). The fltered microalgae were dried in an oven (DV-600, YAMATO) at 60 °C for 24 h and weighed on an electronic balance (AW220, SHIMADZU). C and N contents of the algal cells were determined with an NC analyzer (NCH-22 A, SUMIGRAPH). The P contents were determined with an autoanalyzer (Bran+Luebbe, AACS II, Norderstedt, Germany) after degrading the sample with potassium peroxodisulfate at a maximum absorbance wavelength of 880 nm, based on the phosphomolybdenum blue method (Murphy and Riley [1962\)](#page-10-17).

Statistical analysis

Diferences in the somatic growth rates of daphnids among the food treatments were tested with one-way analysis of variance (ANOVA). Treatments with no surviving animal per beaker were excluded from the ANOVA. When the ANOVA showed a signifcant diference, Tukey's honestly significant difference (HSD) test was conducted at $p < 0.05$. All statistical analyses were made using R statistical software (R Core Team [2022](#page-11-15)).

Results

Trichome sizes of *A. platensis*

Trichome sizes of untreated *A. platensis* ranged from 64 to 595 μm with an average size of 363μ m. These are too large for daphnid ingestion. To ease consumption, the trichomes were fragmented with a bead crusher at the speed of 4600 rpm for diferent lengths of time (Figs. [1](#page-5-0) and [2\)](#page-5-1). This efficiently reduced the trichome to 50 μ m on average in 5 s. The fragmented *A. platensis* trichomes were further crushed for up to 25 s, resulting in an average size of 15.6 μm. This is a similar size range to *C. reinhardtii*, 10 μm on average, and can be easily ingested by daphnids. Therefore, we used this fragmented *A. platensis* for the following experiments.

Fig. 1 Size distributions of *Arthrospira platensis* trichomes fragmented with a bead crusher at 0, 5, 10, 15, 20, and 25 s represented as a, b, c, d, e, and f, respectively. Blue vertical lines represent median values

Fig. 2 Average trichome sizes of *Arthrospira platensis* at diferent times of crushing. Vertical bars represent standard deviation

Elemental composition of the two microalgae

The C, N, and P composition of *A. platensis* and *C. reinhardtii* cells is shown in Table [3](#page-6-0). P contents in *A. platensis* and *C. reinhardtii* were 10.3 ± 0.6 and 15.4 ± 0.8 mg g dry weight−1, respectively. The C:P ratios in *A. platensis* and *C. reinhardtii* were 46.3 and 35.5 dry weight, while C:N ratios were 3.9 and 4.8 dry weight, respectively. There were no noticeable diferences in the composition of the elements between the two microalgae.

Survival and growth of *D. magna*

All experimental animals fed exclusively on *A. platensis*, and those without food (starvation) died within 5 days (Fig. [3\)](#page-6-1). On the other hand, animals fed on *A. platensis* supplemented with at least 20% *C. reinhardtii* survived longer than 5 days. The survival rates increased with increasing supplementation of *C. reinhardtii.* Although more than 50% of daphnids fed on 80% *C. reinhardtii* alone died within 6 days old, the remaining animals produced eggs and neonates.

Growth rates of the [da](#page-7-0)phnids increased from 0.25 ± 0.031 day ⁻¹ to 0.37 ± 0.041 day ⁻¹ with increasing proportions of *C. reinhardtii* in the mixtures from 20 to 80% (Fig. 4). The diferences among the treatments were statistically signifcant (Table [4](#page-7-1)). The growth rate in the treatment of 80:20 of AP:CR was lower than that in 0:20, while that in $20:80$ was higher than that in $0:80$ (Fig. [4\)](#page-7-0).

Fig. 3 Survival rates of *Daphnia magna* at diferent supply ratios of *Arthrospira platensis* to *Chlamydomonas reinhardtii* (AP:CR) as food during the study period

Fig. 4 Average specifc growth rates of *Daphnia magna* reared with a mixture of *Arthrospira platensis* (AP) and *Chlamydomonas reinhardtii* (CR) at diferent ratios from 100:0, 80:20, 50:50, 20:80, and 0:100 of AP:CR, and exclusively on CR at 20%, 50%, and 80% of 4 mg C L^{-1} . Vertical bars denote standard deviation. Diferent letters above each column indicate signifcant diferences by post hoc test using Tukey's HSD

Table 4 Results of one-way analysis of variance and post hoc Tukey's HSD test for the efects of food treatments on growth of *Daphnia magna. D f*, degrees of freedom; *SS*, sum of square; *MS*, mean square; *F*, F value; and *p*, p value

	Df	SS	МS		
Between groups		0.03048	0.005080	4.899	0.00793
Within group	13	0.01348	0.001037		
Bartlett's K-squared = 1.6583, df = 6, $p = 0.9483$					

Discussion

Poor manageability of some cyanobacteria owing to their long chain, spiral, or colonial forms is one of the major barriers in ingestion and trophic transfer of energy and C to zooplankton (Bednarska et al. [2014](#page-9-0)). Mechanical interference with the fltering process is one reason for the inferior assimilation of cyanobacterial C to zooplankton (Porter and McDonough [1984](#page-11-5)). Although the trichome size of *A. platensis* (ca. 363 μm on average) was too large for daphnids, ingestion was easy when the size was reduced to $\langle 20 \mu m \rangle$ after fragmentation. We found, however, that daphnids could not survive when fed on such fragmented *A. platensis* alone. This implies that *A. platensis* lacks some essential nutrients mandatory to the survival and growth of daphnids.

Supplementation of *A. platensis* by just 20% of *C. reinhardtii* to total amount of food enhanced the survival and growth rates of daphnids, but these rates were lower than daphnids exclusively fed on *C. reinhardtii* at 20% supply. This suggests that the shortage of some essential elements in *A. platensis* for daphnid growth cannot be improved by a small supplementation of *C. reinhardtii*. Growth improved when the daphnids were fed on >50% supplementation of *C. reinhardtii*. In 80% supplementation of *C.*

reinhardtii, the growth rate was higher than those daphnids fed on *C. reinhardtii* alone. This suggests that daphnids may be able to digest *A. platensis* and that it can contribute more efectively to growth once the essential nutrients are compensated by addition of a suitable alga such as *C. reinhardtii*. Similar studies have also indicated that daphnids in cyanobacterial food needs at least 50% eukaryotic microalgae to meet the nutritional limitations of cyanobacteria (Martin-Creuzburg et al. [2008](#page-10-18); Pietrzak et al. [2010;](#page-11-16) Wenzel et al. [2012\)](#page-11-14). The use of cyanobacteria mixed with other better-quality microalgae has been found to enhance daphnid growth (Gulati and Demott [1997;](#page-10-4) Urabe and Waki [2009](#page-11-17); Bednarska et al. [2014\)](#page-9-0). This clearly explains that, even though cyanobacteria alone cannot sustain zooplankton life history traits, they can contribute to better growth and reproduction once their limitations are relaxed.

The poor nutritional quality of food algae for rearing daphnids is probably related to the limitation of essential nutrients. The trophic transfer of C and energy is limited by a small subset of elements and their proportions in the diet (Demott et al. [1991](#page-9-5); Gulati and Demott [1997\)](#page-10-4). Models of nutrient recycling and energy fow in zooplankton have revealed that the C:P ratio of zooplankton species is relatively more constant than that of their phytoplankton food. Stoichiometric variation in the algal food, mainly the low availability of P, afects zooplankton by direct elemental limitations, reducing digestibility and declining PUFA content in the diet (Müller‐Navarra[1995](#page-10-19); Urabe et al. [1997;](#page-11-0) Gulati and Demott [1997](#page-10-4)). In this study, the composition of elements in *A. platensis* appeared similar to that of *C. reinhardtii*, suggesting the availability of sufficient *C*, N, and P to rear daphnids. The C:P dry weight ratios of *A. platensis* (46.3) were well below the threshold of 116 for daphnid growth (Urabe et al. [1997](#page-11-0), [2018](#page-11-7)). This demonstrates that elemental limitation is an unlikely explanation for the poor quality of *A. platensis* to rear daphnids.

Another reason for the poor quality of *A. platensis* for supporting daphnid growth might be the absence or insufficient availability of dietary lipids, especially PUFA and sterols. Previous studies have shown that 74% of lipid content of *C. reinhardtii* is unsaturated fatty acids (USFA) of which 48% are omega-3 fatty acids, while in *Spirulina* (in commercial *Arthrospira* powder), 38.6% of the lipid content was USFA with <1.5% omega-3 fatty acid. In addition, alpha-linolenic acid, an important PUFA for daphnid growth, was found to be 42.4% in *C. reinhardtii* and 0.12% in *Spirulina* (Dar-wish et al. [2020\)](#page-9-6). The most biologically important PUFAs for daphnid growth and reproduction such as alpha-linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid are found in smaller quantities in *Spirulina platensis* (=*A. plat-*ensis) (Tokuşoglu and Unal [2003](#page-11-18)). This implies that insufficient availability of biologically important PUFAs in *Arthrospira* may contribute to its inferior performance in supporting daphnid growth.

Previous studies have clearly demonstrated that the poor quality of cyanobacteria for zooplankton is due to lack of sterols, which are important lipids responsible for cell membrane fuidity and are precursors of steroid hormones (Elert et al. [2003](#page-10-20); Martin-Creuzburg and Elert [2004](#page-10-21); Martin-Creuzburg et al. [2005,](#page-10-0) [2008](#page-10-18), [2009\)](#page-10-22). Crustaceans convert phytosterols to cholesterol (Teshima [1971](#page-11-19)). It has been shown that sterols are primarily limiting elements for daphnid growth and play an important role in juvenile development (Martin-Creuzburg et al. [2011](#page-10-23)). Daphnid growth was initially limited by sterol availability but switched to PUFA once sterols shortages are compensated (von Elert [2002](#page-11-2); Martin-Creuzburg et al. [2005,](#page-10-0) [2008\)](#page-10-18). The premature death of daphnids when they fed on *A. platensis* in this study might be associated with lack of sterols.

Conclusions

Although *A. platensis* was ingestible by *D. magna* after appropriate fragmentation, it does not support survival and somatic growth of daphnids. *A. platensis* supplemented with *C. reinhardtii* as a standard algal food enhanced the survival and growth of the daphnids, suggesting the limitation of essential nutrients in *A. platensis* to support daphnid's life history traits. Although such insufficiency might be the cause of the poor nutritional quality of *A. platensis* for rearing *D. magna*, a small addition of *A. platensis* to standard microalgal foods would contribute to better daphnid growth than when standard foods are fed exclusively.

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Author contribution All authors contributed to the study idea and experimental design. Conducting the experiment, data analysis, and frst draft manuscript preparation were performed by A.M. and revised by S.B., X.L., and M.M. All authors commented on the manuscript and read and approved the fnal manuscript.

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Data availability All data generated or analyzed during this study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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