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Replacement of dried freshwater alga *Arthrospira maxima* with marine diatom *Schizochytrium limacinum* in a diet of freshwater mussel *Unio crassus* (Philipsson, 1788)

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Abstract
The present study was conducted to determine the possible usage of dried *Schizochytrium limacinum* as a replacement of *Arthrospira maxima* in a ration of the adult freshwater mussel (*Unio crassus*). The experimental diets were prepared with the different concentrations of protein-rich green freshwater algae *A. maxima* and docosahexaenoic acid (DHA)-rich diatom *S. limacinum*. Combinations were done by replacing *A. maxima* with 0%, 30%, 70%, and 100% of dried *S. limacinum* then named AM100, AM70, AM30, and AM0, respectively. These diets were used to feed 180 individuals (15 mussels/tank) of *U. crassus* in 12 independent glass (4 groups and 3 replicates) tanks. Live weight gain, shell dimensions, biochemical compositions, and fatty acid contents of *U. crassus* from whole tissue were examined before and after 30 days of feeding trial. The live weight gain was significantly higher in the AM100 (1.66 ± 1.32 g, $5.00 \pm 3.95\%$) and AM70 (1.32 ± 0.83 g, $4.05 \pm 2.56\%$) groups than in the (0.06 ± 1.91 g, $0.31 \pm 5.73\%$) and AM0 (-0.17 ± 0.61 g, $-0.52 \pm 1.84\%$) groups. We were able to detect increasing levels of DHA in the *U. crassus* tissues, while the given ratios of *S. limacinum* were increased in the diet. The results showed that *S. limacinum* was digested by adult *U. crassus* to some extent and DHA was retained in the mussel tissue. These results showed that the dependence on live algae can be decreased in the conservation effort of *U. crassus* by using *S. limacinum* in a ration.

Keywords Docosahexaenoic acid · Marine diatom · Freshwater algae · Conservation · Unionidae

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Introduction

Unio crassus (Philipsson, 1788) is a freshwater mussel under the family "Unionidae," and it has been considered as an endangered species, due to habitat loss by anthropogenic activities since the 1990s (Gillis and Mackie 1994; Taeubert et al. 2012; Gillis et al. 2017; Sousa et al. 2021). Initially, the populations of *U. crassus* do not seem to be endangered in Turkey (Ercan and Tarkan. 2014; Serdar et al. 2019). However, the extinction risks of these freshwater mussels were observed after industrialization, which strongly affected the freshwater systems through anthropogenic activities in Europe. Status of *U. crassus* was updated to be endangered at IUCN red list recently by Lopes-Lima et al. (2014, 2017), and regulations of EU Habitat Directives have been applied since 1992 (Bouchet et al. 1999; Zettler and Jueg 2007). To mitigate the effects of human activities on the freshwater mussels, governments and non-profit organizations should act as controllers of the sanction on the harvesting of this endangered species.

Recently, our scientific knowledge has been expanded to hatchery culture of freshwater mussels to prevent their extinction and protect the remaining healthy populations, and the scientists have focused on various methods for the conservation of freshwater mussels (Inoue et al. 2017; Dobler et al. 2019; Douda et al. 2021). Culturing of freshwater mussels in laboratory conditions and releasing them into protected areas is the artificial method of conservation as reported by Eybe et al. (2013). This method consists of the hatchery culture of mussels to the metamorphosis and releasing them into the conserved areas.

According to the literatures, researchers has focused focusing on glochidia culture and survival rates of freshwater mussels after metamorphosis, and they were successful with in vitro and in vivo cultures (Gąsienica-Staszeczek et al. 2017; Ma et al. 2018; Douda et al. 2021). Adult mussels can be kept in captivity for these types of laboratory experiments or in hatchery when well-balanced algal diets are provided to the mussels (Galley et al. 2009; Pettersen et al. 2010). Live microalgal diets were usually applied to juvenile mussels in a hatchery. However, these algal feeding stages have various problems. Hatchery feeding should provide a well-balanced diet (by maintaining protein levels, lipid ratios, etc.) for a long time by culturing or purchasing two or more species of live algae (Gatenby et al. 2003). Production of live-algae comes with various problems such as bacterial contaminations, fluctuating food values and rapid die-offs at any time. Producing and storing of live algae are also creating a need for large-scale culture and storage areas, which affects hatchery expenses (Bleakley and Hayes 2017). For the sustainable conservation of the endangered species, governments and non-profit organizations need to keep operation costs as low as possible by manipulating the conservation or culture methods. Keeping conservation costs low would be possible with the use of dried algae, especially heterotrophic algae, instead of live algae. Heterotrophically grown algae produced in a dried form would be beneficial as it has a low-cost of production, requires a small storage area, and is a sustainable operation. In a hatchery, such production creates the opportunity to manipulate the food value as desired according to the nutritional requirements of cultured mussel species (Barclay et al. 1994; Önal et al. 2005).

Various alternative diets were studied for many bivalve species by replacing the live algae with the dried algal products. Boeing (1997) has successfully shown the partial replacement of live algae with the *Schizochytrium* sp. in the ration of *Tapes semidecussata*. Langdon and Önal (1999) were successful in the culture of *Mytilus galloprovincialis* diet with full replacement of live algae in the ration with dried algae (*Schizochytrium* sp., *Arthrospira platensis*). Önal et al. (2005) has partially replaced the live algal diet with the

dried algal diet of the *Tapes phillippinarum*. Arney et al. (2015) did not recommend dried *Spirulina* sp. or *Schizochytrium* sp. as a diet for the geoduck clam (*Panopea generosa*). These studies were carried out on marine bivalves, which were fed with the marine algae species. The success of the alternative diet seems to be species dependent.

Keeping adult individuals in captivity for lifelong would be beneficial for conservation efforts by providing easy access to *U. crassus* for experimental purposes and possible restocking in recovered habitats. In addition, researchers could change the biochemical composition of the *S. limacinum* by growing it in the bioreactor with the specifically designed broth (Patel et al. 2020). We could alter the biochemical composition of *U. crassus* with this diatom for the studies on fecundity, immune system activation, improved meat quality for human consumption, or survival rate for conservation efforts.

In this study, we have focused on docosahexaenoic acid (DHA) and followed it to the tissue level to test the hypothesis that the *U. crassus* can digest *S. limacinum* and retain DHA in the tissue. To achieve this, feed mixture was evaluated, using alone or in combinations with paper dried freshwater algae *Arthrospira maxima* and heterotrophically grown spray-dried marine diatom *Schizochytrium limacinum*.

Materials and methods

Collection and identification of mussel

All experiments were conducted at the Laboratories of Fisheries Engineering Faculty, Mugla Sitki Kocman University in Mugla, Turkey. In total, 240 individuals of freshwater mussel were collected from the Tersakan stream at Dalaman, Mugla, Turkey (36° 45.874′ N, 028° 49.352′ E) in September 2017. The collected mussels were separated according to their shell structures to distinguish the mussel species and 228 mussels were identified as *U. crassus* (Killeen et al. 2004). Another separation was made according to their live weights and shell dimensions to decrease the standard deviations among the experimental groups (smallest and biggest mussels were not used). Totally, 210 individuals of *U. crassus* were chosen, within which 180 individuals of *U. crassus* were used for feeding trials, while the remaining 30 were used to determine the initial proximate composition and fatty acid content of *U. crassus* tissue. Shell length, width, and height measurements were made with the digital caliper (Mitutoyo ABSOLUTE, 0.001 mm). Initial mean shell length (5.913 ± 0.204 mm), shell height (2.351 ± 0.077 mm) and shell width (4.189 ± 0.188 mm) were recorded. Initial live weights were measured with a digital balance (± 0.01 g), and mean live weights were calculated as 33.27 ± 2.01 g for all collected individuals of *U. crassus*.

Experimental design

Twelve glass aquariums ($70 \times 35 \times 30$ cm each) were filled with 50 L of filtered water with reverse osmosis (RO) treatment systems to decrease any bacterial or algal contamination from the tap water. Mussel survival was recorded as 100% with RO water in adaptation period. Water quality parameters were measured daily with a handheld device (HANNA HI 98,194, Italy). Water temperature, pH and dissolved oxygen levels were 14–17 °C, 8.50–8.70, and 5–6 $mg{\cdot}L^{-1}$, respectively. The light regime was adjusted to 16:8 (L:D) until the end of the experiment. The water was changed daily with 10–15% of filtered water. The

system was aerated with atmospheric air using stones, which were connected to a blower. Air flows were adjusted according to decrease precipitation rate of dried diets using screw valves.

Mussels ($n=180$) were divided into 4 feeding groups with 45 mussels in each group. Each feeding group was divided into 3 glass aquariums with 15 mussels for each. All the mussels were marked with a hand tool on the shell with the numbers from 1 to 15 in each aquarium. Mussels from each aquarium were taken out from the water and dried with paper towel and then measured immediately. Initial and final measurements of each feeding group were recorded and presented as mean values in Table 2.

Feeding groups were named to represent the replacement ratios of freshwater algae (*A. maxima*) with marine algae (*S. limacinum*) as AM100, AM70, AM30, and AM0. AM100 was the 100% of *Arthrospira maxima*, while the AM70, AM30, and AM0 were replaced with *S. limacinum* with ratios of 30%, 70%, and 100%, respectively.

Feed preparation and feeding trials

All the algal products used in the experiments were purchased from the local companies in a dried form. *Arthrospira maxima* were cultured with bag culture method and dried on a drying paper at room temperature by the company, HANA Spirulina in Turkey. *S. limacinum* was cultured under heterotrophic conditions in the bioreactor (fermenter) and then spray dried by the company, MarinBio, Turkey.

Proximate compositions of algal products were supplied by the companies, MarinBio and HANA Spirulina (Table 1). Dried algae were weighed before making a suspension to reach a final concentration of 0.01 $g.L^{-1}$ in 50 L of filtered tap water. The algal mixtures were prepared by suspending the dried products in a 500 ml beaker with the IKA T18 UltraTurrax at 6000 RPM and supplied in the tanks twice a day and feeding trials were carried out for 30 days. Prepared algal suspensions were continuously mixed with an MTOPS

Table 1 Proximate compositions (%), docosahexaenoic acid (DHA) contents (%) of *U. crassus* tissues, and compositions (%) of experimental diets. AM, *A. maxima*; SL, *S. limacinum;* N/A, not available; INITIAL, (0 h) samplings which represents the natural population

		INITIAL (%)	AM0(%)	AM30(%)	AM70(%)	AM100(%)
Ratios	AM:SL	N/A	0:100	30:70	70:30	100:0
Protein	Tissue	6.35	7.53	9.27	12.32	9.32
	Diet	N/A	15.00	30.90	52.10	68.00
Lipid	Tissue	0.37	0.87	0.47	0.52	0.57
	Diet	N/A	40.00	31.45	20.05	11.50
Carbohydrates	Tissue	11.60	8.25	7.39	1.68	4.98
	Diet	N/A	18.00	12.90	7.05	2.55
Ash	Tissue	1.64	1.86	2.23	2.33	2.14
	Diet	N/A	22.00	18.70	14.30	11.00
Moisture	Tissue	80.04	81.49	80.53	83.34	82.90
	Diet	N/A	5.00	5.45	6.05	6.50
DHA	Tissue	2.09	6.45	5.29	4.13	3.27
	Diet	N/A	13.55	7.71	2.42	0.31

MS300HS magnetic mixer to prevent the loss of homogeneity until the time they were used. The proportions of algal mixture were poured into aquariums from the glass measuring cylinder (Table 1).

Proximate composition analysis

All the proximate composition analysis of the *U. crassus* tissues were done in the Seafood Processing Laboratories of Fisheries Engineering Faculty of Muğla Sıtkı Koçman University. Sampling pool was made from fifteen *U. crassus* inner body tissues at the beginning of the study to determine initial proximate composition. At the end of the 30 days of feeding trial, fifteen *U. crassus* were sampled from each group by randomly taking five individuals from each aquarium, to make a sampling pool. Mussel tissues were collected for proximate composition analysis at the end of the study. The fifteen *U. crassus* tissues were mixed and homogenized by IKA T18 UltraTurrax in a glass beaker from each feeding group. Analyses of the homogenized tissue samples were done with the following techniques: proximate proportion of protein (AOAC 2002), lipid (Bligh and Dyer 1959), ash (AOAC 1990), moisture (AOAC 1995), and total carbohydrate (Merrill and Watt 1974). These results were compared with those of the initial proximate analysis.

Fatty acid analysis

Methyl esters were prepared from the collected lipid samples for the proximate composition analysis by transmethylation using 2 M KOH in methanol and isooctane according to the method described by Ichihara et al. (1996) with minor modification. In total, 25 mg of extracted oil was dissolved in 2 ml isooctane, followed by 4 ml of 2 M methanolic KOH. The tube was then vortexed for 2 min at room temperature. After centrifugation at 4000 rpm for 10 min, the isooctane layer was taken for GC analyses. GC analysis was done according to the AOAC (2001).

Statistical analysis

Statistical significance at the 95% confidence with Bonferroni correction was determined by one-way ANOVA with post hoc *t* test to identify the effects of *S. limacinum* ration on growth parameters and biochemical compositions of the *U. crassus*. Simple linear regression with least- squares method was used to identify any significant pattern that would occur in proximate composition values and between the DHA levels of the feeding groups which were fed with an increased level of DHA content in their ration.

Results

The experimental design was appropriate to adjust the level of algae and stocking density in the tanks. We were able to observe the filtration rate of the mussels and adjust the algal concentration by decreasing the stock concentration of algae since it was important to maintain good water quality in the closed glass tank system. Precipitation of the dried algae was observed at a high feeding concentration of the ration in tanks. Therefore, precipitation was avoided as much as possible by aeration. The rest of the precipitated algae

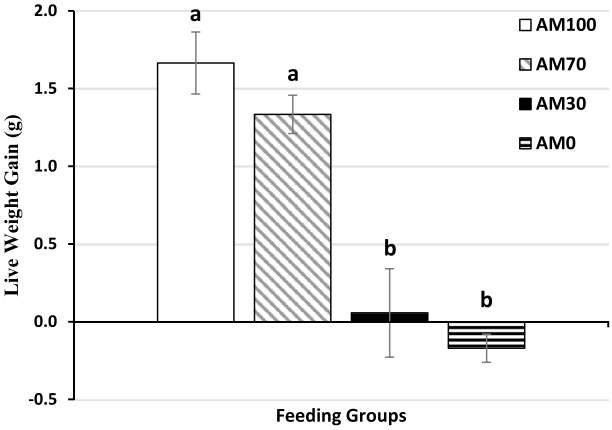

Fig. 1 Live weight gain (g) of *Unio crassus* for each treatment. AM100 = 100% of *A. maxima*. AM70 = 70% *A. maxima* and 30% *S. limacinum*. AM30 = 30% *A. maxima* and 70% *S. limacinum*. AM0 = 100% *S. limacinum*. Averages followed by different letters are statistically different ($p < 0.05$). Lines on bars show standard errors between individuals

Table 2 Biometric measurements of the mussels ($p > 0.05$). Means (± standard deviation) of the shell length (mm), width (mm), height (mm), and weight (g) of *Unio crassus*

	AM100	AM70	AM30	AM0
Initial length	5.92 ± 0.211	5.93 ± 0.161	5.91 ± 0.187	5.88 ± 0.243
Final length	5.93 ± 0.214	5.94 ± 0.163	5.94 ± 0.181	5.91 ± 0.206
Initial width	4.05 ± 0.207	4.25 ± 0.142	4.25 ± 0.164	4.19 ± 0.160
Final width	4.13 ± 0.152	4.15 ± 0.148	4.13 ± 0.172	4.09 ± 0.151
Initial height	2.36 ± 0.077	2.34 ± 0.069	2.34 ± 0.078	2.34 ± 0.083
Final height	2.41 ± 0.063	2.40 ± 0.071	2.36 ± 0.083	2.34 ± 0.080
Initial weight	33.47 ± 1.906	33.28 ± 1.888	33.15 ± 1.918	33.20 ± 2.302
Final weight	35.14 ± 2.272	34.61 ± 1.909	33.21 ± 2.070	33.03 ± 2.413

was removed by daily siphoning with an aquarium hose. No mortality of the mussels was observed during the 30 days of feeding trials in all groups, and there were no statistically significant changes found in any groups despite small changes occurred in shell dimensions of the mussels ($p > 0.05$).

Final measurements of the live weight of the mussels showed that the live weight gain was significantly higher in the AM100 (5.00%) and AM70 (4.05%) groups than in the AM30 (0.31%) and AM0 (−0.52%) groups ($p < 0.05$, Fig. 1). Individual weight loss instead of a weight gain or no change was also observed in the AM30 (0.31%) and AM0 (−0.52%) groups (Table 2). Mussel tissues were analyzed for their proximate composition to identify any effects other than the biometric effects of the marine algae *S. limacinum* replacements in the ration. There was no significant difference found in the proximate composition of tissues by ANOVA ($p > 0.05$, Table 1).

We checked DHA content in the mussel tissues along with the non-significant proximate composition results. We analyzed the DHA content using the fatty acid methyl esters (FAME) levels in the tissues for all groups with the expectation of increased DHA levels as the proportion of the *S. limacinum* increased in the feed. FAME analysis showed that the DHA contents of the AM100, AM70, AM30, and AM0 rations were 2.72%, 12.07%, 24.53%, and 33.87%, respectively (Table 1). FAME analysis of the *U. crassus* tissues showed the similar increasing patterns on DHA contents as 3.27%, 4.13%, 5.29%, and

6.45%, respectively, while the initial group from the natural habitat was 2.09% ($p < 0.05$, Table 1). Linear regression (least-squares methods) results showed a positive relationship between the DHA content of rations and tissues ($R^2 = 0.9847$, Fig. 2).

Discussion

Use of dried algae in mussel culture for feeding purposes has been done for many species (Langdon and Önal 1999; Önal et al. 2005; Arney et al. 2015). However, no research has been conducted on feeding freshwater mussel with marine algae. Hence, we focused on the use of dried marine algae (*S. limacinum*) instead of dried freshwater algae as a food for the culture of freshwater mussel culture (*U. crassus*).

The duration of the feeding trials was not enough to yield statistically significant data on the shell dimensions of the adult *U. crassus* due to their slow growth rates, which correlates with their age and shell dimensions (Neves and Widlak 1987; O'Beirn et al. 1998; Karayücel et al. 2003; Yalçın 2006). Therefore, collection of measurable and comparable data was difficult for the short-term feeding studies on adult freshwater mussels (Nobles and Zhang 2011). Ercan and Tarkan (2014) observed that the annual growth rate of the shell length of *U. crassus* as well as the shell height and width was not statistically comparable. We suggest that shell dimensions are not useful parameters to assess the use of *S. limacinum* by the adult *U. crassus*.

The live weight gain of the *U. crassus* individuals was significantly higher at the AM100 and AM70 than the other groups, while no significant differences were observed between the AM100 and AM70 or AM30 and AM0 groups. Ercan and Tarkan (2014), found around 19% increase of the annual live weight in the natural populations, whereas 4.55% of live weight gain was obtained in *U. crassus* after 4 weeks of the captive feeding trial. These results indicate that the potential for captive culture of *U. crassus* with the diet based on dried algal products. Additionally, freshwater mussels are known to hold water in their mantle, which could affect the live weights, and differences at the live weights may not be a certain explanation of the efficient usage of *S. limacinum* by adult *U. crassus* whether it is significant or not. A deeper investigation was needed to identify the usage of *S. limacinum* as a food source. Therefore, proximate composition of adult *U. crassus* was analyzed to do more target specific observation on the

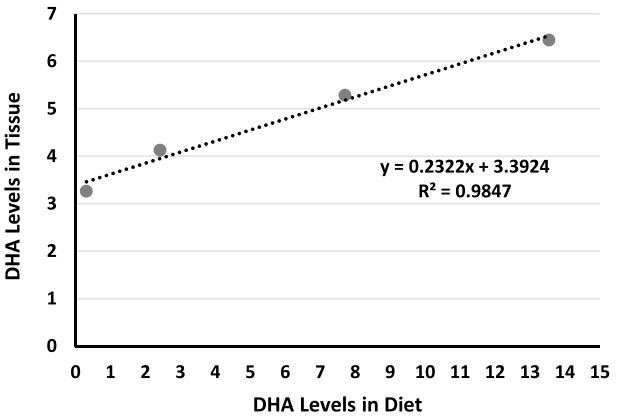

Fig. 2 Simple linear regression between DHA levels in *Unio crassus* tissue and DHA levels in diet for groups that were fed using different diets. AM100 = 100% of *A. maxima*. AM70 = 70% *A. maxima* and 30% *S. limacinum*. AM30 = 30% *A. maxima* and 70% *S. limacinum*. AM0 = 100% *S. limacinum* (1, AM100; 3, AM70; 4, AM30; 5, AM0)

proteins and lipids, where protein-rich (*A. maxima*) and lipid-rich (*S. limacinum*) microalgae were used for feeding. Decreasing protein levels and increasing lipid levels were expected in *U. crassus* tissue as the proportion of *A. maxima* decreased and *S. limacinum* increased. It is puzzling that there was no statistically significant difference or significant pattern observed due to changes in the proportion of microalgae in ration. There have been several possible explanations for these non-significant changes. First, carbohydrate is the main energy sources in higher vertebrates, and lipids are secondary, while the proteins are third. As Carvalho et al. (2007) stated, the stabilized lipid regulatory mechanism of bivalves can be a possible explanation for the non-significant differences of the lipid composition of mussel tissue in the present study. Fearman et al. (2009) also reported that the lipids are used as energy sources in the mollusks, especially in the absence of carbohydrates during the winter months and in the gonadal development stage although they are not the main energy reserves. In the case of mussels, lipids are correlated with the growth and reproduction in algae-based diets (Gatenby et al. 1997, Marshall et al. 2010, Lazzara et al. 2012). Furthermore, a few studies have shown that lipids play an important role in the survival of aquatic animals including the mollusks, and they are important sources of basal metabolism to meet energy demand (Abad et al. 1995; Pazos et al. 1997).

In the present study, use of proteins as energy sources might be possible when there were insufficient levels of carbohydrates and lipids as primary and secondary energy sources for the experimental group AM100, while the individuals at AM70 showed the higher protein levels, which could be caused by sufficient level of lipids and carbohydrates. Protein, carbohydrate, and lipid ratios for *U. crassus* tissue and rations showed how balances of biochemical compositions changed in the tissue and ration.

DHA (docosahexaenoic acid, 22:6n-3) was reported as important nutrients for growth and development for mussels (Pettersen et al. 2010). Long-chain polyunsaturated fatty acids were found to be stored in a bivalve larvae tissue (Pettersen et al. 2010). Thus, examining the lipid content in the mussel's tissue can lead the researchers to know about the comparable and reliable data for short-term feeding experiments as mussels generally take nutritional fatty acids from algae and metabolize it for energy needs or convert them into various forms to store (Ackman 1983; Pazos et al. 1997). Spray-dried *S. limacinum* product, containing 33.87% DHA of total lipid, was used in this study. This level of DHA in the ration enabled us to follow DHA in the tissue as the mussels can store specific types of fatty acids. DHA levels in all experimental diets were estimated using FAME analysis as was done on *A. maxima* and *S. limacinum* (Table 3). Statistical differences among the experimental groups showed the positive relationship of DHA content of ration and *U. crassus* tissue. These results suggested that the adult *U. crassus* individuals were able to digest at some levels and used marine algae *S. limacinum* in a spray-dried form as a source of food.

The results of this study showed that the 30% of dried *S. limacinum* could be used as a nutritional source for adult individuals of endangered *U. crassus* and weight losses might be prevented. In addition, our findings revealed that *S. limacinum* could be used as a food for the *U. crassus* in hatchery culture. Hence, other culture studies could help conservation methods to protect freshwater mussel populations.

Table 3 Fatty acid composition of the two main rations used in the feeding experiments: *S. limacinum* (AM0) and *A. maxima* (AM100)

Fatty acids		*S. limacinum (%)*	*A. maxima (%)*
Lauric acid	C12:0	0.27	0.00
Tridecylic acid	C13:0	0.07	0.00
Myristic acid	C14:0	4.10	1.27
Pentadecylic acid	C15:0	3.64	1.53
Palmitic acid	C16:0	40.43	33.46
Margaric acid	C17:0	1.11	1.22
Stearic acid	C18:0	1.80	5.80
Arachidic acid	C20:0	0.24	14.41
Heneicosylic acid	C21:0	0.00	0.00
Behenic acid	C22:0	0.08	0.91
Tricosylic acid	C23:0	0.00	0.00
Lignoceric acid	C24:0	0.08	0.98
Total saturated fatty acid		43.74	59.57
Myristoleic acid	C14:1	0.26	0.00
Cis-10-Pentadecenoic acid	C15:1	0.02	0.00
Palmitoleic acid	C16:1	0.24	3.64
Cis-10-Heptadecenoic acid	C17:1	0.04	1.20
Trans-Oleic acid	C18:1n9t	0.19	0.96
Oleic acid	C18:1n9c	0.57	5.87
Cis-11-Eicosenoic acid	C20:1n9	9.00	1.87
Erucic acid	C22:1n9	0.00	2.08
Total monounsaturated fatty acid		10.32	15.60
Trans-linoleic acid	C18:2n6t	0.01	1.28
Linoleic acid	C18:2n6c	2.46	10.95
Gamma-linoleic acid	C18:3n6	0.00	3.18
Alfa-linoleic acid	C18:3n3	0.00	0.59
Cis-11–14-Eicosadienoic acid	C20:2	0.09	0.90
Cis-8–11-14-Eicosatrienoic acid	C20:3n6	0.10	0.88
Cis-11–14-17-Eicosatrienoic acid	C20:3n3	0.00	0.00
Arachidonic acid	C20:4n6	0.60	0.00
Cis-13–16-Docosadienoic acid	C22:2	0.51	0.00
Cis-5–8-11–14-17-Eicosapentaenoic acid (EPA)	C20:5n3	6.24	1.67
Docosahexaenoic acid (DHA)	C22:6n3	33.87	2.72
Total poly-unsaturated fatty acid		43.88	22.16
Total n3		40.11	4.98
Total n6		3.17	16.29
n3/n6		12.66	0.31
N/A		2.06	2.68
n6/n3		0.08	3.27
DHA/EPA		5.43	1.63

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Data availability Research data will be made available if it is requested by an editor of the journal.

Declarations

Ethics approval Animals used in the study are invertebrates, which are exempted from the animal ethics directives.

Conflict of interest The authors declare no competing interest.

References

Abad M, Ruiz C, Martinez D, Mosquera G, Sánchez JL (1995) Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibran (Galicia, Spain). Comp Biochem Physiol 110C:109–118. https://doi.org/10.1016/0742-8413(95)00006-A

Ackman RG (1983) Fatty acid metabolism of bivalves. In: Bio-chemical and Physiological Approaches to Shellfish Nutrition. Proc. 2nd International Conference Aquaculture Nutrition, World Mariculture Society Specific Publication No. 2. Louisiana State University Louisiana, 358–376. https://doi.org/10.1016/0044-8486(84)90293-X

AOAC (1990) Official methods of analysis (13th Ed.) Association of Official Analytical Chemists, Official method 950.46, Washington, D.C., USA

AOAC (1995) Official methods of analysis, (14th. Ed.) Association of Official Analytical Chemists, Washington, DC., USA

AOAC (2001) Official method 996.06 fat (total, saturated and unsaturated) in foods, hydrolytic extraction gas chromatographic method, First Action 1996 Revised 2001

AOAC (2002) Protein content in meat. 928.08. Official Method of Analysis, 17th edn. Association of Official Analytical Chemists, Gaithersburg

Arney B, Liu W, Falconer I, McKinley RS, Pearce CM (2015) Feasibility of dietary substitution of live microalgae with spray-dried *Schizochytrium* sp. or *Spirulina* in the hatchery culture of juveniles of the Pacific geoduck clam (*Panopea Generosa*). Aquaculture 444:117–133. https://doi.org/10.1016/j.aquaculture.2015.02.014

Barclay WR, Meager KM, Abril JR (1994) Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. J Appl Phycol 6:123–129. https://doi.org/10.1007/BF02186066

Bleakley S, Hayes M (2017) Algal proteins: extraction, application, and challenges concerning production. Foods 6(5):33. https://doi.org/10.3390/foods6050033

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099

Boeing P (1997) Use of spray-dried *Schizochytrium* sp. as a partial algal replacement for juvenile bivalves. J Shellfish Res 16:284

Bouchet P, Falkner G, Seddon M (1999) Listing of protected land and freshwater molluscs in the Bern Convention and European Habitats Directive: are they relevant to conservation? Biol Conserv 90:21–31. https://doi.org/10.1016/S0006-3207(99)00009-9

Carvalho F, Lima P, Goncalves F, Russel-Pinto F, Machado J (2007) Development of suitable maintenance diet for *Anodonta cygnea*. J World Aquac Soc 35:189–198. https://doi.org/10.1111/j.1749-7345.2004.tb01074.x

Dobler AH, Geist J, Stoeckl K, Inoue K (2019) A spatially explicit approach to prioritize protection areas for endangered freshwater mussels. Aquat Conserv Mar Freshw Ecosyst 29:12–23. https://doi.org/10.1002/aqc.2993

Douda K, Haag WR, Escobar-Calderón F, Vodáková B, Reichard M, Chen X, McGregor M, Yang J, Lopes-Lima M (2021) Effects of in vitro metamorphosis on survival, growth, and reproductive success of freshwater mussels. Biol Conserv 254:108964. https://doi.org/10.1016/j.biocon.2021.108964

Ercan E, Tarkan AS (2014) Effect of salinity on the growth and survival of the freshwater mussel, *Unio Crassus*, in an environmentally disturbed river. Pak J Zool 46:1399–1406

Eybe T, Thielen F, Bohn T, Sures B (2013) The first millimetre-rearing juvenile freshwater pearl mussels (*Margaritifera margaritifera* L.) in plastic boxes. Aquat Conserv Mar Freshw Ecosyst 23(6):964–75. https://doi.org/10.1002/aqc.2384

Fearman JA, Bolch CJS, Moltschaniwskyj NA (2009) Energy storage and reproduction in mussels, *Mytilus galloprovincialis*: the influence of diet quality. J Shellfish Res 28(2):305–312. https://doi.org/10.2983/035.028.0212

Galley TH, Batista FM, Braithwaite R, King J, Beaumont AR (2009) Optimization of larval culture of the mussel *Mytilus edulis* (L.). Aquac Int 18:315–325. https://doi.org/10.1007/s10499-009-9245-7

Gąsienica-Staszeczek M, Zając K, Zając T, Olejniczak P (2017) In vitro culture of glochidia of the threatened freshwater mussel *Unio crassus* Philipsson 1788 the dilution problem. Invertebr Reprod Dev 62:1–9. https://doi.org/10.1080/07924259.2017.1362482

Gatenby CM, Parker BC, Neves RJ (1997) Growth and survival of juvenile rainbow mussels, *Villosa iris* (Lea, 1829) (Bivalvia: Unionidae), reared on algal diets and sediment. Am Malacol Bull 14:57–66

Gatenby CM, Orcutt DM, Kreeger DA, Parker BC, Jones VA, Neves RJ (2003) Biochemical composition of three algal species proposed as food for captive freshwater mussels. J Appl Phycol 15:1–11. https://doi.org/10.1023/A:1022929423011

Gillis PL, Mackie GL (1994) Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair Can J Zool 72:1260–1271. https://doi.org/10.1139/z94-168

Gillis PL, McInnis R, Salerno J, de Solla SR, Servos MR, Leonard EM (2017) Freshwater mussels in an urban watershed: impacts of anthropogenic inputs and habitat alterations on populations. Sci Total Environ 574:671–679. https://doi.org/10.1016/j.scitotenv.2016.09.110

Ichihara K, Shibahara A, Yamamoto K, Nakayama T (1996) An improved method for rapid analysis of the fatty acids of glycerolipids. Lipids 31:535–539. https://doi.org/10.1007/BF02522986

Inoue K, Stoeckl K, Geist J (2017) Joint species models reveal the effects of environment on community assemblage of freshwater mussels and fishes in European rivers. Divers Distrib 23:284–296. https://doi.org/10.1111/ddi.12520

Karayücel S, Kaya Y, Karayücel İ (2003) Sinop bölgesinde akdeniz midyesinin (*Mytilus gallaprovincialis* Lamarck, 1819) kondisyon faktörü ve biyokimyasal kompozisyonu üzerine çevresel faktörlerin etkisi. Turkish J Vet Anim Sci 27:1391–1396

Killeen ID, Aldridge C, Oliver G (2004) Freshwater bivalves of Britain and Ireland. FSC Publications, Shrewsbury (**ISBN: 9781851538928**)

Langdon CJ, Önal E (1999) Replacement of living microalgae with spray-dried diets for the marine mussel *Mytilus galloprovincialis*. Aquaculture 180:283–294. https://doi.org/10.1016/S0044-8486(99)00197-0

Lazzara R, Fernandes D, Faria M, López JF, Tauler R, Porte C (2012) Changes in lipid content and fatty acid composition along the reproductive cycle of the freshwater mussel *Dreissena polymorpha*: its modulation by clofibrate exposure. Sci Total Environ 432:195–201. https://doi.org/10.1016/j.scitotenv.2012.05.094

Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, Bespalaya Y, Bodis E, Burlakova L et al (2017) Conservation status of freshwater mussels in Europe: state of the art and future challenges. Biol Rev 92:572–607. https://doi.org/10.1111/brv.12244

Lopes-Lima M, Kebapçı U, Van Damme D (2014) *Unio crassus*. The IUCN Red List of Threatened Species e.T22736A42465628. https://doi.org/10.2305/IUCN.UK.2014-1.RLTS.T22736A42465628.en

Ma XY, Wen HB, Zou J, Jin W, Hua D, Gu RB, Xu P (2018) An improved method for in vitro culture of glochidia in freshwater mussel *Cristaria plicata* (Mollusca, Bivalvia). Hydrobiologia 810:133–144. https://doi.org/10.1007/s10750-016-3012-3

Marshall R, McKinley S, Pearce CM (2010) Effects of nutrition on larval growth and survival in bivalves. Rev Aquac 2:33–55. https://doi.org/10.1111/j.1753-5131.2010.01022.x

Merrill AL, Watt BK (1974) Energy value of foods-basis and derivations. USDA Agriculture Handbook 74:2-3 Washington DC. https://www.ars.usda.gov/ARSUserFiles/80400525/Data/Classics/ah74.pdf. Accessed 09 Sept 2021

Neves RJ, Widlak J (1987) Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. Am Malacol Bull 5:1–7

Nobles T, Zhang Y (2011) Biodiversity loss in freshwater mussels: importance, threats, and solutions. In: Biodiversity loss in a Changing Planet., InTechOpen; Rijeka, Croatia. pp. 137–162. https://doi.org/10.5772/25102

O'Beirn FX, Neves RJ, Steg MB (1998) Survival and growth of juvenile freshwater mussels (Unionidae) in a recirculating aquaculture system. Am Malacol Bull 14:165–171

Önal E, Langdon C, Önal U (2005) The evaluation of spray-dried microalgae in diets for juvenile manila clam. Tapes Philippinarum. J Shellfish Res 24:1061–1065. https://doi.org/10.2983/0730-8000(2005)24[1061:TEOSMI]2.0.CO;2

Patel A, Liefeldt S, Rova R, Christakopoulos P, Matsakas L (2020) Co-production of DHA and squalene by thraustochytrid from forest biomass. Sci Rep 10:1992. https://doi.org/10.1038/s41598-020-58728-7

Pazos AJ, Román G, Acosta CP, Sánchez JL, Abad M (1997) Lipid classes and fatty acid composition in the female gonad of *Pecten maximus* in relation to reproductive cycle and environmental variables. Comp Biochem Physiol 117B:393–402. https://doi.org/10.1016/S0305-0491(97)00135-1

Pettersen AK, Turchini GM, Jahangard S, Ingram BA, Sherman CDH (2010) Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus Galloprovincialis*) larvae. Aquaculture 309:115–124. https://doi.org/10.1016/j.aquaculture.2010.09.024

Serdar S, Bulut H, Eden M, Özdemir Y (2019) Determining bioecological and biometric properties of freshwater mussels (*Unio crassus* Philipsson, 1788). Pol J Environ Stud 28:1917–1924. https://doi.org/10.15244/pjoes/90621

Sousa R, Halabowski D, Labecka AM, Douda K, Aksenova O, Bespalaya Y, Bolotov I, Geist J, Jones HA et al (2021) The role of anthropogenic habitats in freshwater mussel conservation. Glob Chang Biol 27:2298–2314. https://doi.org/10.1111/gcb.15549

Taeubert JE, Gum B, Geist J (2012) Host-Specificity of the endangered thick-shelled river mussel (*Unio Crassus*, Philipsson 1788) and implications for conservation. Aquat Conserv: Mar Freshw Ecosyst 22:36–46. https://doi.org/10.1002/aqc.1245

Yalçın M (2006) Tatlısu midyesi (*Unio pictorum* LİNNAEUS, 1758)'nin büyüme, kondisyon ve biyokimyasal parametreleri üzerine çevresel faktörlerin etkisi. Msc Thesis, *Ondokuz Mayıs Üniversitesi*

Zettler ML, Jueg U (2007) The situation of the freshwater mussel *Unio Crassus* (Philipsson, 1788) in North-East Germany and its monitoring in terms of the EC Habitats Directive. Mollusca 25:165–174