



Microalgae *Ankistrodesmus gracilis* as feed ingredient for ornamental fish *Xiphophorus maculatus*

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Received: 17 August 2018 / Accepted: 19 March 2019 / Published online: 30 March 2019
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Abstract A trial evaluated the effectiveness of macrophytes, *Azolla caroliniana* (AAC) and *Lemna minor* (ALM) as a culture media for microalgae *Ankistrodesmus gracilis*, and their possible use as feed ingredient for ornamental fish. Water quality in all aquariums was adequate for the growth of *Xiphophorus maculatus*, whilst pH acidity in inert diet (ID) treatment and high content of P in AAC diet treatment did not interfere in fish development. Growth performance parameters were higher ($P < 0.05$) in ALM diet treatment, except condition factor and feed conversion ratio, with similar rates ($P > 0.05$) in all diet treatments. Survival was above 75% and total length was similar ($P > 0.05$) for ID and AAC diet treatments. Lipid levels ranged between 7 and 11%, and was enough to maintain high survival rates ($> 80%$) and weight gain above 0.42 g to mixed diet (AAC and ALM) and 0.27 g to inert diet. *Ankistrodesmus gracilis* and macrophytes as a culture media are useful as functional ingredients, because biomass may be incorporated with the food supplement for *X. maculatus* to enhance nutrition quality and to maintain high growth performance. Although *A. caroliniana* and *L. minor* may be used with culture media, *L. minor* provided the best results to microalgae growth biomass and ornamental fish.

Keywords Culture media · *Azolla caroliniana* · *Lemna minor* · Nutritional values · Growth performance

Introduction

Trade in ornamental fish has been on the increase during the last decades. The color of the animal is the main purchase factor, since it is associated with the quality of feed for the increase of growth performance. Microalgae are known as excellent live feed due to their pigments that improve color, survival, and growth rates. Its potential as feed supplements is due to high levels of fatty acids, polysaccharides, vitamins, minerals, and essential amino acids and pigments (such as chlorophyll and carotenoids). They may also be used as partial replacement for conventional proteins (Madeira et al. 2017).

Fish may be maintained and developed by live feed through large-scale production of microalgae at high costs, especially with regard to culture media. Several research works have underscored the use of alternative culture media, such as inorganic fertilizers (El-Nabris 2012; Ramaraj et al. 2015) or the employment of organic wastes retrieved from aquaculture, such as macrophytes (Sipaúba-Tavares et al. 2009, 2018).

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The above-mentioned alternative culture media decrease production costs by maintaining the nutritional biomass of algal biomass. The latter is a crucial factor for the production of high-quality fish feed. Employing certain species of microalgae in fish diet, Sathasivam et al. (2017) reported price reduction and increase of nutritional value in fish. Although several species of microalgae are used in aquaculture, a few studies deal with *Ankistrodesmus gracilis*. Microalga is well accepted by fish larvae, and may be easily grown in the laboratory due to size, shape, thickness of cell walls, nutritional value, and fast growth (Sipaúba-Tavares and Rocha 1993).

Macrophytes, such as microalgae, are primary producers and remove from water and sediment (rooted macrophytes), the nutrients necessary for their development, mainly nitrogen and phosphorus. They are rich in minerals, fibers, and amino acids. *Lemna* and *Azolla* are macrophytes that occupy an important place as food components in fish feeding due to their highly protein value around 20.4% and 21.9%, respectively (Mandal et al. 2010).

High fiber contents of the whole macrophyte meal have put great limitations to fish food despite their high nutritive value (Sotolu and Sule 2011). However, aquatic plants may be used through technologies that make possible their rich potential in nitrogen and phosphorus, basic nutrients for the growth of microalgae. Aquatic plants may be duly employed as culture medium for the growth of *A. gracilis*. Consequently, the joint use of the two components (microalgae and macrophytes) provides the production of a proper feed. In fact, both have large profiles of macro- and micro-nutrients, vitamins, antioxidants, and essential acids that improve growth, weight gain, and immune response of some aquatic organisms.

The use of macrophyte as culture medium for microalga production will play an important role as food and will directly influence the growth of ornamental fish. Platy fish are omnivorous: they mainly eat plants, algae, insects, and small crustaceans. They are popular aquarium fish, subject to important ornamental fisheries in aquaculture, and have been used as research models as early as the 1930s (Pitts and Volkoff 2017). Most successfully reared ornamental fish are omnivores. In fact, the species have best adapted to captivity conditions, including available nutrition (Abowei and Ekubo 2011).

Current study evaluates the effectiveness of macrophytes, *Azolla caroliniana* and *Lemna minor* as culture media for microalga *Ankistrodesmus gracilis* production, and their potential use as feed ingredients for ornamental fish.

Materials and methods

Microalgae strain and culture conditions

Strain (005CH) of microalgae *Ankistrodesmus gracilis* was batch-cultured at 22 ± 2 °C and exposed to light at $60 \mu\text{mol m}^{-2} \text{s}^{-1}$, on a 24-h light cycle. Macrophytes *Azolla caroliniana* (AAC) and *Lemna minor* (ALM) were used as culture media. Culture medium with different macrophytes has already been discussed by Sipaúba-Tavares et al. (2009, 2018). The macrophytes were chosen due to their great availability and abundance on the site. The effects of macrophyte culture media on the growth of *A. gracilis* were simultaneously investigated during a 28-day cultivation period, by experiments under similar growth conditions. Approximately 5-kg wet weight of each plant was washed gently under tap water to remove detritus and epiphytes. The material was sun-dried and then dried in an oven at 60°C, overnight. Dried plants were homogenized in a grinder and boiled in distilled water for 1 h. The hot extract was filtered and autoclaved at 120 °C during 20 min. A 70 mL sample was collected, cooled, and diluted with distilled water up to 1.4 L, when 2.5 mL NPK were added. The experiment started with 10 mL at a density of 0.75×10^5 cells mL^{-1} containing CHU₁₂ medium. When cultures reached the last exponential growth phase (7th day), approximately 250 mL, at a density of 2×10^5 cells mL^{-1} , were added to the 2 L NPK medium. After the 7 day exponential growth phase, the culture at a density of 4.4×10^5 cells mL^{-1} was transferred to sterilized 2-L recipients containing the two different macrophytes media (AAC = *Azolla caroliniana* and ALM = *Lemna minor*). Vitamin B complex was added to the alternative culture media at the rate of 0.02 g L^{-1} (7 mg—B1; 7 mg—B2; 5 mg—B6; 33 μg —B12) plus biotin (0.1 mg L^{-1}) to induce microalga growth. Continuous illumination was applied and the macrophytes media were employed to grow the microalgae cultures in 2 L. Cell growth in all treatments was evaluated in triplicate ($n = 3$) for 28 days.



Fish-rearing conditions and experimental diets

Assays were performed with *Xiphophorus maculatus* (GUNTHER 1866) juveniles (45 days old) commonly known as platy fish, which were acquired from the Ornamental Fish Laboratory (21°15'19"S and 48°19'21"W), Brazil. Prior to the start of the experiment, fish were fed on *Artemia* for 2 weeks to acclimatize them to experimental facilities. The fish were randomly divided into 15 experimental aquariums (three treatments and five replicates) with 15 fish stocked in each experimental unit. The experiment was carried out during 90 days. Initial weight of fish in all treatments was 0.30 ± 0.004 g, with initial length at 2.7 ± 0.1 cm. Mean fish weight at the start and end of the experiment was determined. Weight gain and total length of fish were measured one by one.

The following formulae were used to assess growth performance: condition factor (k) = $100 \times (W/L^3)$ where: W = live body weight (g) and L = total body length (cm); specific growth rate (SGR%) was calculated by $([\ln(\text{final weight}) - \ln(\text{initial weight})]/\text{number of days}) \times 100$, following Kestmons and Stalmans (1992); survival (%) = $[\text{number of surviving fish}/\text{total number of fish stocked}] \times 100$; weight gain (g) = final mean fish weight – initial mean fish weight and feed conversion ratio (FCA) = amount of feed supplied/weight gain.

Feed treatments

A control, inert diet (ID) was formulated to contain 32% crude protein, featuring $20,362 \text{ J g}^{-1}$ gross energy based on feed stuff rates reported by Tamaru and Ako (1999). Microalgae biomass in each culture medium designates as: AAC with *Ankistrodesmus gracilis* microalgae cultured in *Azolla caroliniana* medium and ALM with *A. gracilis* microalgae cultured in *Lemna minor* medium. Two different formulated diets added dried microalgae biomass culture in macrophytes (AAC and ALM) culture media to the inert diet containing 47.06 g kg^{-1} (AAC) and 45.27 g kg^{-1} (ALM). Fishmeal was included in diets to ensure palatability. Table 1 shows formulation of the diets. The ingredients tested were ground in a hammer mill, mixed, moistened, and pelleted in a meat grinder. The pellets were dried for 24 h, and fractionated and sieved to obtain a diameter of 0.005–0.05 mm.

Analytical methods

Triplicate 1-mL aliquots were removed daily from the microalgae culture and $2 \times 1 \mu\text{L}$ sub-samples were used for cell quantification by a Neubauer hemocytometer. Growth rate (k) was calculated by formula $k = (3.322/t_2 - t_1 \times \log N_2/N_1)$ (t = time; N = number of cells; subscripts denote rates at different times) (Guillard 1973). Doubling time was calculated from results obtained from growth rate, with formula $T_d = 1/k$ (T_d = duplication time, $1/k$ = days per division) (Guillard 1973). Microalgae biomass was harvested, centrifuged, and lyophilized for the analysis of proteins and lipids (AOAC 1990). Chlorophyll-*a* was extracted with alcohol 90% and quantified at 663 nm and 750 nm (Nusch 1980). Analyses of macro-nutrients (N, P, K, Ca, and Mg) of culture media (without microalgae) and plants followed methodology by Bataglia et al. (1983). The ornamental fish water parameters of each experimental aquarium were performed weekly at 08:00 h, during 90 days. Water temperature, dissolved oxygen, conductivity, and pH were measured in situ with a multi-sensor Horiba U-10. Total phosphorus and total inorganic nitrogen were quantified by spectrophotometry following Golterman et al. (1978) and Koroleff (1976). The analysis contents of diet treatments were dry matter, ash, crude protein, crude fiber, ether extract, nitrogen-free extract, and gross energy (AOAC 1990).

Data analysis

All data underwent one-way analysis of variance (ANOVA) with Statistica 8.0 package, to test the effects of culture media and experimental diets (Statsoft 2007). Tukey's test was applied when differences between culture media occurred. Differences were considered significant at $P < 0.05$. All experiments were carried out in triplicate for microalgae and in five replicate for fish.

Table 1 Ingredients and formulation of diet treatments (ID inert diet (control); AAC inert diet with dried microalga *Ankistrodesmus gracilis* biomass cultured in *Azolla caroliniana* medium; ALM = inert diet with dried microalgae *Ankistrodesmus gracilis* biomass cultured in *Lemna minor* medium)

Ingredients (g kg ⁻¹)	Inert diet	Mixed diet	
	ID	AAC ^f	ALM ^f
Corn ^a	275	246.7	262.71
Fish meal ^a	250	264	250
Soybean meal ^a	216	150	150
Wheat meal ^a	143.7	140	140
Soybean Oil ^a	78.7	64	60
Mineral–vitamin mix ^b	5	5	5
Dicalcium phosphate ^d	2.5	2	3
Antifungic ^e	3	3	3
BHT ^c	0.3	0.3	0.3
Sodium chloride	5	5	5
Lysine	10	10	10
Methionine	5	5	5
Limestone	6	5	6
AAC ^f	–	100	–
ALM ^f	–	–	100

^aFish meal, corn, soybean meal, wheat meal, and soybean oil obtained locally. The approximate composition of ingredients was fish meal: 91.7% dry weight, 61.4% crude protein, 0.3% crude fiber, 20.5% ash, 17,552 J gross energy and 5.9% ether extract; corn: 87% dry weight, 7.59% crude protein, 20% crude fiber, 4.1% ash, 3,002 J gross energy and 3.7% ether extract; soybean meal: 88.8% dry weight, 44.2% crude protein, 5.3% crude fiber, 6.0% ash, 17,096 J gross energy and 1.7% ether extract; wheat meal: 88.4% dry weight, 15.6% crude protein, 9.5% crude fiber, 16,360 J gross energy and 3.5% ether extract; soybean oil: 1.0% dry weight, 6.0% ash, 40,893 J gross energy, and 99% ether extract

^bMineral–vitamin mix guaranteed levels per kg of diet: vit A = 500,000 IU; vit D3 = 200,000; vit E = 5000 IU; K3 = 1000 mg; vit. C = 1500 mg; vit. B1 = 1500 mg; vit. B2 = 1500 mg; vit. B6 = 1500 mg; biotin = 50 mg; B12 = 4000 mg; folic acid = 500 mg; pantothenic acid = 4000 mg; choline = 40 g; Co = 10 mg; Cu = 500 mg; Fe = 5000 mg; I = 50 mg; Mn = 1500 mg; Se = 10 mg; Zn = 5000 mg

^cBHT: butyl-hydroxytoluene or butylated hydroxytoluene, is a lipophilic organic compound, antioxidant properties

^dCaHPO₄·2H₂O: calcium supplement

^eIt has synergistic action of organic and inorganic acids for prevention and treatment of fungal contamination and yeasts in raw materials, food and feed

^fMicroalgae (*Ankistrodesmus gracilis*) and macrophytes *Azolla caroliniana* (AC) and *Lemna minor* (LM) obtained locally; – = not analyzed

Results

Microalgae growth and culture media

Cell growth rate of *Ankistrodesmus gracilis* was higher in LM culture medium and reached maximum cell density, at 473×10^5 cells mL⁻¹, on the 25th day. In the case of AAC culture medium, maximum cell density was almost half, with 292×10^5 cells mL⁻¹, on the 23th day. *Ankistrodesmus gracilis* on AAC culture medium featured lower cell density as from the 7th day when compared to ALM culture medium (Fig. 1).

Best results for production parameters for *A. gracilis* culture in macrophytes media were obtained in ALM culture medium, except chlorophyll-*a* and protein with similar density ($P > 0.05$) in the two macrophyte media. The fast doubling time in ALM culture medium (5.4 days) may be due to higher growth rate ($k = 0.26$) when compared to that of the other culture medium. Lipid levels were below 13% of dry biomass and were higher ($P < 0.05$) in ALM culture medium (Table 2).



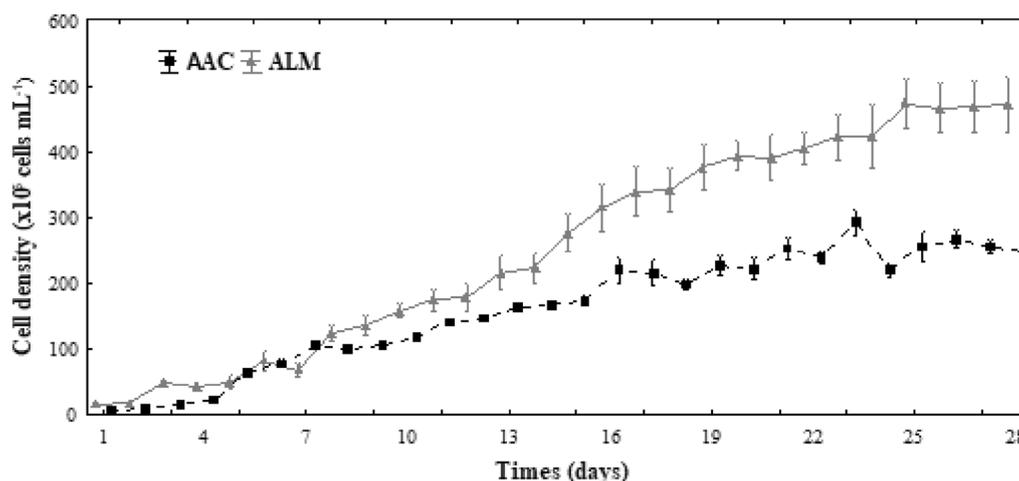


Fig. 1 Daily concentration of *Ankistrodesmus gracilis* cultured in two macrophyte media *Azolla caroliniana* (AAC) and *Lemna minor* (ALM). Data represent mean \pm SD of three replicates per medium

Table 2 Production parameters of *Ankistrodesmus gracilis* cultured in macrophytes media of *Azolla caroliniana* (AAC) and *Lemna minor* (ALM)

Parameters	Culture media	
	AAC*	ALM*
Doubling time (days)	6.8	5.4
Growth rate (k)	0.19	0.26
Mean cell density ($\times 10^5$ mL $^{-1}$)	161.8 \pm 86 ^b	253.7 \pm 160 ^a
Maximum cell density ($\times 10^5$ mL $^{-1}$)	292 ^b	473 ^a
Chlorophyll- a (g L $^{-1}$)	4.0 \pm 1.4 ^a	3.9 \pm 0.5 ^a
Protein (% dry biomass)	30 \pm 11 ^a	29 \pm 6 ^a
Lipid (% dry biomass)	7 \pm 2 ^b	11 \pm 2 ^a

The values are the mean of three replications and variation (\pm) is standard deviation

*Microalgae and macrophytes obtained locally. Values in the same row with different superscripts are significantly different ($P < 0.05$)

Plant nutrients

The two plants feature high macro-nutrients rates. Nitrogen and P were higher in *L. minor* in the culture medium and plant, whilst K and Ca were higher in *A. caroliniana* plant and culture medium. Magnesium was higher in *A. caroliniana* plant and *L. minor* in the culture medium. All nutrients in the culture media were lower than in the plant (Fig. 2).

Fish-growing responses

Relative concentrations of dry matter in inert diet treatment and mixed diet treatments were above 94%. Ether extract and gross energy were similar in all diet treatments. Crude fiber, crude protein, and nitrogen-free extract were higher in ALM and AAC, while ash content was high in ID treatment (Table 3).

Growth performance parameters were higher ($P < 0.05$) in ALM diet treatment, except condition factor and feed conversion ratio, with similar ($P > 0.05$) rates in all diet treatments. Survival was above 75% and total length was similar ($P > 0.05$) for ID and AAC diet treatments. The use of dried microalgae biomass cultured in macrophyte culture media as food supplement for *X. maculatus* showed positive results, since the growth performance, in general, was higher ($P < 0.05$) in these treatments (Table 4).

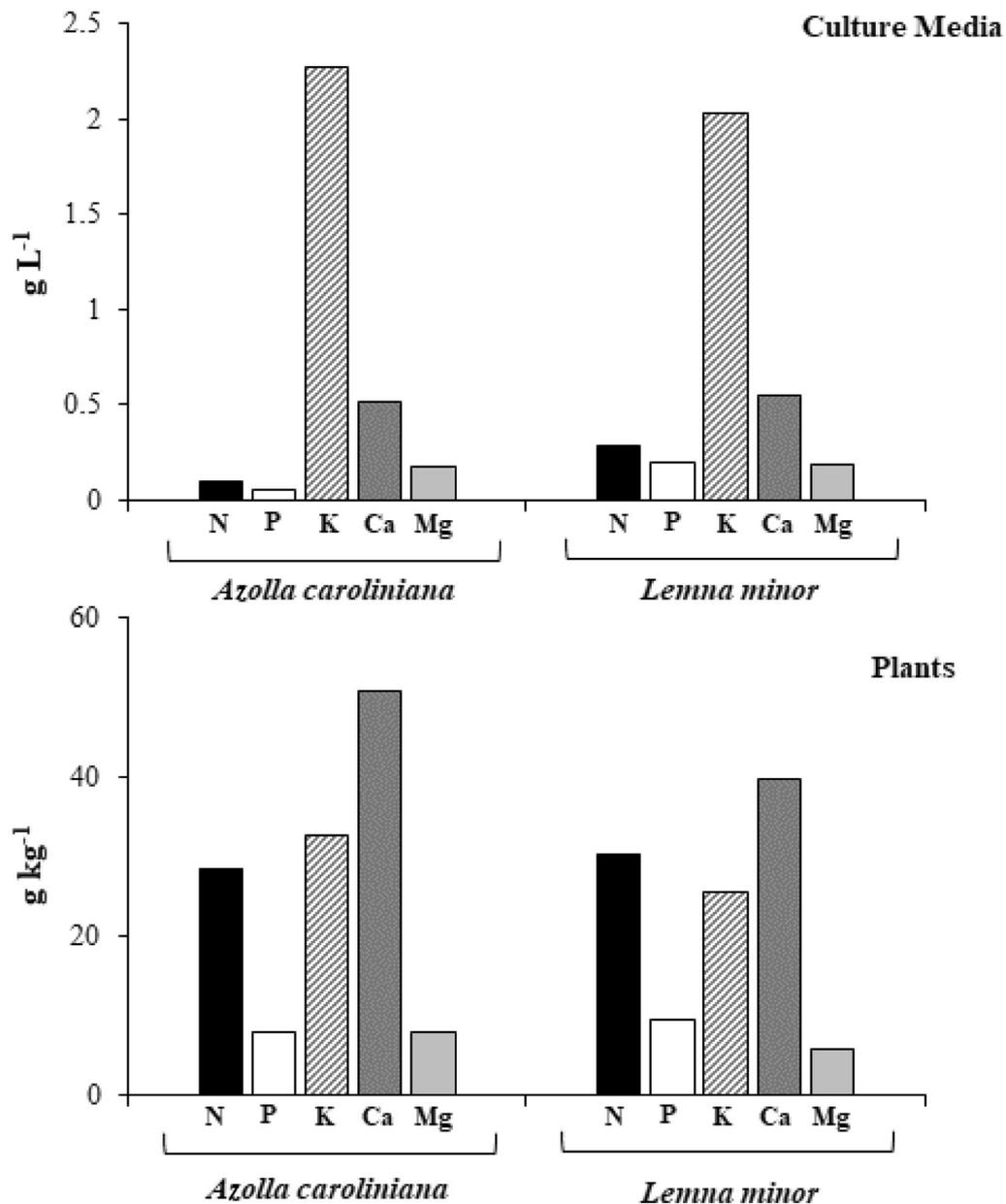


Fig. 2 Nutrient composition of macrophyte culture media (g L^{-1}) and plants (g kg^{-1}) *Azolla caroliniana* and *Lemna minor*

Controlled conditions in the laboratory were appropriate for the assay, since results of water-quality variables were similar ($P > 0.05$) in the different diet treatments, except pH which was acidic in the ID diet treatment, and P, which was higher in AAC diet treatment. Total inorganic nitrogen contents were higher ($> 1.2 \text{ g L}^{-1}$) and total phosphorus ranged between 6.7 and 19.2 mg L^{-1} in all diet treatments. Due to constant oxygenation in the aquariums, dissolved oxygen was above 4 mg L^{-1} , whilst conductivity was below 61.5 $\mu\text{S cm}^{-1}$ (Table 5).

Discussion

Mixed diet treatments (AAC and ALM) supplemented by algae influenced the growth rates of *X. maculatus*. Diet treatment with ALM provided the best results in fish growth. However, when compared to ID diet

Table 3 Proximate composition of the formulated diet treatments: inert diet (ID) and mixed diets (AAC and ALM—inert diet with dried microalgae biomass cultured in macrophytes media and dry weight basis) for rearing *Xiphophorus maculatus* juveniles in a 90-day experiment

Proximate composition (dry weight)	Inert diet		Mixed diet	
	ID		AAC ^a	ALM ^a
Ash (%)	24.8		17.8	21.1
Crude protein (%)	32		33.1	32.3
Crude fiber (%)	4.4		4.9	6.1
Dry matter (%)	95.0		94.8	94.6
Ether extract (%)	13.3		13.2	12.5
Nitrogen-free extract (%)	20.5		25.8	22.6
Gross energy (J g ⁻¹) ^b	20,362		20,600	20,463

^aMicroalgae and macrophytes obtained locally^bGross energy: calculated by oxygen bomb calorimeter**Table 4** Biological indexes for *Xiphophorus maculatus* inert diet (ID—control) and mixed diet (AAC = inert diet with dried microalga *Ankistrodesmus gracilis* biomass cultured in *Azolla caroliniana* medium; ALM = inert diet with dried microalgae *Ankistrodesmus gracilis* biomass cultured in *Lemna minor* medium)

Growth performance Parameters	Inert diet		Mixed diet	
	ID		AAC*	ALM*
Final weight (g)	0.58 ± 0.1 ^c		0.72 ± 0.1 ^b	0.88 ± 0.1 ^a
Weight gain (g)	0.28 ± 0.04 ^c		0.43 ± 0.1 ^b	0.57 ± 0.1 ^a
Final length (cm)	3.0 ± 0.4 ^{ab}		3.3 ± 0.4 ^{ab}	3.5 ± 0.6 ^a
Specific growth rate (% day ⁻¹)	0.73 ± 0.1 ^c		1.0 ± 0.1 ^b	1.2 ± 0.2 ^a
Condition factor (k)	2.08 ± 0.3 ^a		2.02 ± 0.1 ^a	1.99 ± 0.1 ^a
Feed conversion ratio (FCR)	4.0 ± 0.4 ^a		3.5 ± 0.3 ^a	3.5 ± 0.8 ^a
Survival (%)	80 ± 4.7 ^b		89 ± 10.1 ^{ab}	92 ± 7.3 ^a

*Microalgae and macrophytes obtained locally. Values in the same row with different superscripts are significantly different ($P < 0.05$)**Table 5** Water variables for different diet treatments of *Xiphophorus maculatus*, where: ID = inert diet (control); AAC = inert diet with dried microalga *Ankistrodesmus gracilis* biomass cultured in *Azolla caroliniana* medium; ALM = inert diet with dried microalgae *Ankistrodesmus gracilis* biomass cultured in *Lemna minor* medium

Parameters	Inert diet		Mixed diet	
	ID		AAC*	ALM*
Water temperature (°C)	28 ± 0.7 ^a		28 ± 0.7 ^a	28 ± 0.8 ^a
pH	6.7 ± 0.3 ^b		7.1 ± 0.3 ^a	7.2 ± 0.1 ^a
Dissolved oxygen (mg L ⁻¹)	5.5 ± 0.7 ^a		5 ± 1 ^a	5 ± 1 ^a
Conductivity (µS cm ⁻¹)	37 ± 5 ^a		38 ± 7 ^a	36 ± 1 ^a
Total inorganic nitrogen (g L ⁻¹)	2.13 ± 0.7 ^a		2.8 ± 0.9 ^a	2.5 ± 0.8 ^a
Total phosphorus (mg L ⁻¹)	8.9 ± 5.7 ^{ab}		12.3 ± 3.7 ^a	9.1 ± 3.9 ^{ab}

The values are the mean of five replications and variation (±) is standard deviation

*Microalgae and macrophytes obtained locally. Values in the same row with different superscripts are significantly different ($P < 0.05$)

treatments with mixed diet, results for the growth performance of *X. maculatus* were better in the diet supplemented by algae (macrophytes medium). Mandal et al. (2010) showed that 20% of *Salvinia cucullata* power resulted in better growth of rohu (*Labeo rohita*) than 20–80% of conventional feedstuff.



Protein levels of *A. gracilis* in the two macrophyte media ranged between 22 and 48% dry biomass. However, *A. gracilis* contained all essential amino acids, particularly leucine, valine, and isoleucine (Sipaúba-Tavares and Pereira 2008). The protein levels found in current study were within the range established for microalgae due to the high nitrogen levels that improve protein levels and decrease lipid levels. The nutrition value of microalgae is highly variable. In current study, the protein levels in the culture media were enough to maintain *X. maculatus*. Chong et al. (2004) observed that feeding of female *X. helleri* with 20% dietary protein might cause a lower growth rate. Regarding AAC and ALM diets for *X. maculatus*, the specific growth rate was higher in these dietary treatments than in inert diet. The same occurred with nitrogen-free extract and protein, mainly in AAC dietary treatment. Mixed diets promote high food consumption and growth due to protein and nitrogen-free extract components that trigger growth in *X. helleri* (James and Sampath 2004). Poor-specific growth rate in fish fed on ID diet treatment suggests that non-conventional food, such as microalgae culture with macrophyte media (AAC and ALM), is suitable for *X. maculatus* juveniles.

Microalgae use lipids as energy source or in the formation of cell membranes. The percentage of lipid content per dry biomass of microalgae may range between 1.55 and 75%, depending on the culture medium (D'Alessandro and Antoniosi-Filho 2016). In current study, *A. gracilis* cultured in macrophytes media lies within this range and it is similar to inert diet. Interactions between dietary protein and lipid influence specific growth rate. Excess dietary lipid may decrease growth rate due to high levels of dietary energy (Ling et al. 2006). Lipid levels in current study ranged between 7 and 11%, which is sufficient for high survival rate (> 80%) and weighs gain above 0.42 g for mixed diet (AAC and ALM) and 0.27 g for inert diet. At present, scanty information on lipid requirements for *X. maculatus* is available. However, 8–16% dietary lipids levels with 20% and 30% protein levels diets have significantly improved *X. helleri* growth performance (Ling et al. 2006).

Nutrients had higher rates in *L. minor* plant and culture medium, except K. However, N and P in the plants were higher in LM than in AC. The above may have occurred, because *A. gracilis* had the best cell growth in ALM culture medium when compared to AAC medium. Potassium is an important nutrient for the metabolism of microalgae and probably influenced the growth performance of *X. maculatus*. In fact, final weight, weight gain, specific growth rate, and survival were higher in the dietary treatment with microalgae (AAC and ALM).

High levels of Ca in plants and K in culture media improved the growth of *A. gracilis*. Similar levels of chlorophyll-*a* in both culture media were influenced by Ca, Mg and N in plants and culture media. Concentrations of N and P sources are very important, and affect other factors in the growth of aquatic organism. Phosphorus is commonly supplemented in the diet feed, since it is essential in growth, bone mineralization, and carbohydrate metabolism (Velasco-Santamaría and Corredor-Santamaría 2011).

Microalgae are not considered an essential feed source, since great amounts of biomass are required. However, even in small amounts, they have improved the weight, number of eggs, and reproductive performance of fish (Kovac et al. 2013). Water quality in all aquariums was adequate for the growth of *X. maculatus*, and pH acidity in ID diet treatment and high content of P in AAC diet treatment did not interfere in fish development.

Ground or diluted macrophytes in diets as culture medium for microalgae improved the nutritional conditions of diet provided to fish. A better digestibility occurred, since fiber rates in aquatic plants were dissolved in the culture medium. The procedure aggregated the nutritional values of the two components as primary producers that retrieve from the environment the nutrients required for their development. In fact, they trigger adequate growth performance and high survival rates for *X. maculatus*. Natural components in fish diet are a tool that should be employed in aquaculture. Besides reducing feed costs, it provides protein with high nutritional rates involving carotenoids, vitamins, minerals, energy, carbohydrates, proteins, lipids, and others. Consequently, the employment of organic items, such as macrophytes, which are actually biological residues and develop intensely in sub-tropical fish farms, is highly effective in the feed of ornamental fish such as *X. maculatus*.

Conclusions

Ankistrodesmus gracilis and macrophytes as a culture media are useful as functional ingredients, since the biomass may be incorporated with food supplement for *X. maculatus* to enhance nutrition quality and the



maintenance of high growth performance. Although *A. caroliniana* and *L. minor* may be used with culture media, *L. minor* provided best results for microalgae growth biomass and ornamental fish. Diet treatment with microalgae demonstrated that these mixed diets might be used as a new tool in the feeding of *X. maculatus*. Since no difference in feed conversion ratio has been observed between diet treatments, the above indicated that mixed and inert diets are similarly employed by fish to increase biomass. The production of low-cost food supplement from natural origin such as microalgae and macrophytes enhances future prospects capable of sustaining stocking density and high growth performance.

Acknowledgements The authors would like to thank FAPESP for funding (14/24697-3). Thanks are also due to CNPq for the scholarship awarded to the first (PQ II 305343/2014-9) and third (134543/2016-5) authors. The authors would like to thank the colleagues of the Ornamental Fish Laboratory.

Compliance with ethical standards

Conflict of interest There is no conflict of interest between authors in the publication of this paper.

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References

- Abowei JFN, Ekubo AT (2011) A review of conventional and unconventional feeds in fish nutrition. *Br J Pharmacol Toxicol* 2:179–191
- AOAC (1990) Association of Official Analytical Chemists Official methods of analysis, 15th edn. AOAC, Washington
- Bataglia OC, Furlani AMC, Teixeira JPF, Furlani PR, Gallo JR (1983) Chemical analysis methods for plants. *Boletim Técnico: Instituto Agronômico*, 78
- Chong ASC, Ishak SD, Osman Z, Hashim R (2004) Effect of dietary protein level on the reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquaculture* 234:381–392. <https://doi.org/10.1016/j.aquaculture.2003.12.003>
- D'Alessandro EB, Antoniosi-Filho NR (2016) Concepts and studies on lipid and pigments of microalgae: a review. *Renew Sustain Energy Rev* 58:832–841. <https://doi.org/10.1016/j.rser.2015.12.162>
- El-Nabris KJA (2012) Development of cheap and simple culture medium for the microalgae *Nannochloropsis* sp. based on agricultural grade fertilizers available in the local market of Gaza stripe (Palestine). *J Al Azhar Univ Gaza (Nat Sci)* 14:61–76. <https://doi.org/10.2139/ssrn.2045661>
- Golterman HL, Clymo RS, Ohmstad MAM (1978) Methods for physical and chemical analysis of fresh water, 2nd edn. Blackwell Scientific Publications, Oxford (**IBP**, 8)
- Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycolgical methods. Culture methods and growth measurements. Cambridge University Press, London, pp 289–311
- James R, Sampath K (2004) Effect of feed type on growth and fertility in ornamental fish, *Xiphophorus hellerii*. *Isr J Aquac Bamidgeh* 56:264–273
- Kestmons P, Stalmans JM (1992) Initial feeding of European minnow larvae *Phoxinus phoxinus* L. 1. Influence of diet and feeding level. *Aquaculture* 104:327–340. <https://doi.org/10.1111/arc.12756>
- Koroleff F (1976) Determination of nutrients. In: Grashof E, Kremling E (eds) Methods of seawater analysis. Verlag Chemie Weinheim, German, pp 126–133
- Kovac DJ, Simeunovic JB, Babic OB, Misan AC, Milovanovic IL (2013) Algae in food and feed. *Food Feed Res* 40:21–31
- Ling S, Hashim R, Kolkovski S, Shu-Chien AC (2006) Effect of varying dietary lipid and protein levels on growth and reproductive performance of female swordtails *Xiphophorus helleri* (Poeciliidae). *Aquac Res* 37:1267–1275. <https://doi.org/10.1111/j.1365-2109.2006.01554.x>
- Madeira MS, Cardoso C, Lopes PA, Coelho D, Afonso C, Bandarra NM, Prates JAM (2017) Microalgae as feed ingredients for livestock production and meat quality: a review. *Livest Sci* 205:111–121. <https://doi.org/10.1016/j.livsci.2017.09.020>
- Mandal RN, Datta AK, Sarangi N, Mukhopadhyay PK (2010) Diversity of aquatic macrophytes as food and feed components to herbivorous fish—a review. *Indian J Fish* 57:65–73
- Nusch EA (1980) Comparison of different methods for chlorophyll and phaeopigments determination. *Archives für Hydrobiologie* 14:4–36
- Pitts PM, Volkoff H (2017) Characterization of appetite-regulating factors in platyfish *Xiphophorus maculatus* (Cyprinodontiformes Poeciliidae). *Comp Biochem Physiol Part A* 208:80–88. <https://doi.org/10.1016/j.cbpa.2017.03.018>
- Ramaraj R, Tsai DDW, Chen PH (2015) Carbon dioxide fixation of freshwater microalgae growth on natural water medium. *Ecol Eng* 75:86–92. <https://doi.org/10.1016/j.ecoleng.2014.11.033>
- Sathasivam R, Radhakrishnan R, Hashem A, Abd-Allah EF (2017) Microalgae metabolites: a rich source for food and medicine. *Saudi J Biol Sci* 24:1–14. <https://doi.org/10.1016/j.sjbs.2017.11.003>



- Sipaúba-Tavares LH, Pereira AML (2008) Large scale laboratory cultures of *Ankistrodesmus gracilis* (Reisch) Korsikov (Chlorophyta) and *Diaphanosoma birgei* Korinek (Cladocera). *Braz J Biol* 68:875–883
- Sipaúba-Tavares LH, Rocha O (1993) Large-scale cultivation of plankton for feeding larvae and fish fry: I—algae green. *Biotemas*. 6:93–106. <https://doi.org/10.5007/2175-7925>
- Sipaúba-Tavares LH, Ibarra LC, Fioresi TB (2009) *Ankistrodesmus gracilis* (Reinsch) Korshikov (Chlorophyta) laboratory cultured in CHU₁₂ and macrophyte with NPK media. *Boletim do Instituto de Pesca* 35:111–118. <https://doi.org/10.20950/1678-2305>
- Sipaúba-Tavares LH, Florêncio T, Scardoelli-Truzzi B (2018) Aquaculture biological waste as culture medium to cultivation of *Ankistrodesmus gracilis* (Reinsch) Korshikov. *Braz J Biol* 78:579–587. <https://doi.org/10.1590/1519-6984.175432>
- Sotolu AO, Sule SO (2011) Digestibility and performance of water hyacinth meal in the diets of African catfish (*Clarias gariepinus*; Burchell, 1822). *Trop Subtropical Agroecosyst* 14:245–250
- Statsoft Inc (2007) Statistica (data analysis software system), version 8
- Tamaru CS, Ako H (1999) Using commercial feeds for the culture of freshwater ornamental fishes in Hawaii. *Tech Rep* 28:109–119
- Velasco-Santamaría Y, Corredor- Santamaría W (2011) Nutritional requirements of freshwater ornamental fish: a review. *Res MVZ Córdoba* 1:2456–2469

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