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Research Article

Effects of microalgal diets on growth and survival of yellow clam seeds *Amarilladesma mactroides* (Reeve, 1854)

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ABSTRACT. The effects of different proportions and biomass supply levels of three live microalgae species, *Isochrysis* aff. *galbana* (ISO), *Chaetoceros muelleri* (CM), and *Tetraselmis suecica* (TS) on the growth and survival of *Amarilladesma mactroides* seeds were evaluated in two experiments. In the first experiment, four monoalgal and three bi-algal diets, plus a control without feeding, were evaluated at a feeding level corresponding to 3% of total seed wet biomass over 28 days. Higher mass gain and shell growth were observed in seeds fed ISO and CM-based diets, particularly the monoalgal CM and bi-algal ISO/CM diets, whereas TS-based diets showed lower performance. In the second experiment, the two best-performing microalgae species (ISO and CM) were combined in different proportions (30:70, 50:50, and 70:30) and supplied at different biomass levels (3, 6, and 10% of total seed wet biomass) over 21 days. Bi-algal diets containing balanced or ISO-enriched proportions (50/50 and 70/30), particularly at 3 and 6% biomass supply levels, resulted in the highest growth performance. No significant differences in survival were observed among treatments in either experiment. These results demonstrate that diets composed of *I.* aff. *galbana* and *C. muelleri*, supplied either alone or in combination, promote improved growth performance of *A. mactroides* seeds under laboratory conditions.

Keywords: *Amarilladesma mactroides*; Mesodesmatidae; bivalve; microalgae; hatchery; surf clam

INTRODUCTION

The yellow clam *Amarilladesma mactroides* (Reeve, 1854; syn. *Mesodesma mactroides*) is a benthic bivalve mollusk distributed in Brazil, Uruguay, and Argentina (Rios 1994). Its geographical distribution center is located on the southern coast of Rio Grande do Sul, Brazil (Fiori & Defeo 2006). This species has been studied for decades due to its importance as a fishing resource (Coscarón 1959) and a source of human nutrition (Bastida et al. 1991). In addition, this species shows promising potential for aquaculture development.

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As noted, this species holds socioeconomic importance for several regions on the west coast of the South Atlantic Ocean. Therefore, studies focused on hatchery production have been conducted. These include studies evaluating the reproductive cycle and maturation of broodstock (Bernardes et al. 2023), embryonic and larval development (Santos et al. 2020b), the effect of salinity on embryonic and larval development (Santos et al. 2020a), and the effect of temperature on larval growth and survival (Santos et al. 2022).

The maintenance of bivalve mollusks in the laboratory involves feeding to meet their needs during the different stages of life. Microalgae production is an essential activity of bivalve hatcheries and production laboratories. Specifically, microalgae are the main sources of food for the maturation of broodstock (Pronker et al. 2008, González-Araya et al. 2012) and the development of larvae and seeds (Helm et al. 2004). It is estimated that the production of microalgae accounts for 30 to 50% of total seed production costs (Albentosa et al. 1997, Rivero-Rodríguez et al. 2007). As such, the diet offered must promote rapid growth and development of bivalves in order to reduce costs and enable the production of seeds in the laboratory (Rivero-Rodríguez et al. 2007).

Studies on the nutritional quality of microalgae and its effect on the cultivation of sand clam seeds in the laboratory have been successfully conducted for *Venerupis pullastra* (Albentosa et al. 1993), *Ruditapes decussatus* (Albentosa et al. 1997) and *Panopea generosa* (Ren et al. 2015). However, information regarding the nutritional requirements and laboratory cultivation of many bivalve species remains limited (Viet Le et al. 2014). Consequently, diets successfully applied to other bivalves have often been used as a reference for species with little available nutritional information (Albentosa et al. 1997, Pettersen et al. 2010). This is the case for the yellow clam *A. mactroides*, for which knowledge about the nutritional value of microalgae species and optimal dietary concentrations for seed rearing remains limited (Vieira et al. 2021). In addition, few studies have evaluated the combined effects of different microalgal proportions and biomass levels on the growth performance of this species under controlled laboratory conditions. Therefore, further studies are required to establish optimized feeding protocols for *A. mactroides* seed production.

For adult and juvenile bivalves, dietary diversity is often beneficial because it compensates for nutritional limitations of individual microalgal species (Ren et al. 2015). Nutrients, such as carbohydrates (Whyte et al. 1989) and polyunsaturated fatty acids (PUFAs), are found in different concentrations in microalgae (Langdon & Waldock 1981). These nutrients contribute to meeting the nutritional requirements of hatchery-reared bivalves (Utting & Millican 1997, Ronquillo et al. 2012, Ren et al. 2015).

However, since bivalves have limited capacity to synthesize PUFAs (Knauer & Southgate 1999), these nutrients demand special attention. Among PUFAs, the essential fatty acids eicosapentaenoic acid (EPA), arachidonic acid (ARA) and docosahexaenoic acid (DHA) are particularly important (Ronquillo et al. 2012). These fatty acids are commonly associated with diatoms microalgae, which are rich in EPA, and with flagellated microalgae, which are typically rich in DHA (Dalsgaard et al. 2003). This explains the widespread use of the genera *Isochrysis* (Prymnesiophyta), *Chaetoceros* (Bacillariophyceae) and *Tetraselmis* (Chlorophyta) in bivalve hatcheries (Coutteau & Sorgeloos 1992, Lavens & Sorgeloos 1996, Helm et al. 2004).

In hatchery conditions, the quantity of microalgae supplied to bivalve larvae and seeds is commonly expressed as a percentage of total biomass or as cell concentration per individual. Diets ranging from approximately 2 to 8% of dry microalgal biomass have been successfully applied in laboratory cultures of species such as *Crassostrea gigas* (Helm et al. 2004, Rico-Villa et al. 2006) and *Panopea generosa* (Ren et al. 2015), resulting in improved growth and survival rates. Similarly, monoalgal and mixed diets have been widely used to optimize nutrient balance and feeding efficiency in juvenile bivalves (Utting & Millican 1997, Ronquillo et al. 2012). These findings supported the selection of the microalgal biomass percentages evaluated in the present study.

The nutritional requirements of bivalves fed on microalgae vary according to life stage. Larvae generally require smaller microalgae species rich in essential fatty acids such as DHA and EPA to sustain rapid development and metamorphosis, whereas juveniles are capable of ingesting larger particles and benefit from mixed diets that enhance tissue growth and shell development (Helm et al. 2004, Rico-Villa et al. 2006). Knowledge of dietary combinations and optimal biomass supply levels can promote improved growth and survival rates under laboratory conditions (Helm et al. 2004, Rivero-Rodríguez et al. 2007, Ronquillo et al. 2012, de Oliveira et al. 2016). Thus, the objective of the present study was to determine the effect of different proportions and percentages of microalgal biomass in mono- and bi-algal diets on the growth and survival of *A. mactroides* seeds under laboratory conditions.

MATERIAL AND METHODS

Sourcing seeds

The seeds used in this study were produced at the Laboratory of Marine Mollusks, Federal University of Santa Catarina, Brazil (LMM-UFSC, by its Portuguese acronym), where microalgae culture, diet preparation, and experimental trials were also conducted.

General experimental conditions

Live microalgae cultures, *Isochrysis* aff. *galbana* (ISO), *Chaetoceros muelleri* (CM), and *Tetraselmis suecica* (TS) were maintained in semi-continuous systems using Conway medium following the compositions described by Brown (1991).

Microalgal biomass supplied (cells mL^{-1}) to the seeds was calculated according to total seed wet biomass, following the methodology adapted from Ronquillo et al. (2012). Percentages corresponded to the amount of microalgal biomass supplied relative to total seed wet biomass. Feeding rates were adjusted weekly based on updated measurements of seed wet biomass, resulting in corresponding adjustments to the microalgal cell concentrations provided to each treatment.

In both experiments, seawater was completely renewed daily, dead individuals were counted, and temperature and salinity were monitored before water exchange. Seeds were fed once daily immediately after water renewal and routine management procedures.

At the beginning and end of each experiment, seeds were photographed for shell morphometric analyses (shell height and shell length) using LAS EZ Software with the aid of a stereomicroscope (Leica LAS EZ4HD, Germany).

Experiment 1: Mono- and bi-algal diets

Seeds of *A. mactroides*, with initial mean shell height and shell length of 2.29 ± 0.2 and 3.14 ± 0.3 mm, respectively, were used. Morphometric measurements were obtained from three replicate groups of 30 seeds each (n = 90). Initial total seed wet biomass (including soft tissues and shell weight, according to Vieira et al. 2021) was 4.06 ± 0.2 mg and was determined using the same replicate groups.

Thirty seeds were stocked in each experimental unit (EU), corresponding to a moderate density commonly used in laboratory feeding trials with juvenile *A. mactroides* (Vieira et al. 2021). The EUs consisted of a 5-L plastic bucket containing 3.5 L of seawater at a salinity of 35 and 150 g of sand under constant aeration.

Eight treatments were evaluated in triplicate: seven with mono- and bi-algal diets and a control treatment without feeding (Table 1). In mono-algal treatments, a single microalgae species was supplied, whereas bi-algal treatments consisted of combinations of two species in defined proportions (e.g. 50:50 or 30:70). All treatments received microalgal biomass corresponding to 3% of total seed wet biomass, except for the treatment containing 100% TS, which was additionally evaluated at a 200% microalgal biomass supply level (Table 1). The experimental period lasted 28 days. Mean water temperature and salinity were 23.0 ± 1.2°C and 36.0 ± 0.8, respectively.

Experiment 2: Bi-algal diets with different biomass levels

The Experiment 2 was designed based on the diets that resulted in higher mass gain, growth, and survival in Experiment 1.

Seven bi-algal treatments (ISO/CM) were evaluated in triplicate using different microalgal proportions (30:70, 50:50, and 70:30) and microalgal biomass levels (3, 6, and 10% of total seed wet biomass) (Table 1).

Seeds used in this experiment presented initial mean shell height and shell length of 4.37 ± 0.4 and 6.33 ± 0.6 mm, respectively. Morphometric measurements were obtained from three replicate groups of 30 seeds each (n = 90). Initial total seed wet biomass (including soft tissues and shell weight) was 31.9 ± 1.7 mg and was determined using the same replicate groups.

Thirty seeds were allocated to each EU, which consisted of rectangular polyethylene terephthalate (PET) containers (4.5×14×12 cm), filled with sand, and maintained inside 20-L plastic buckets under constant aeration according to Vieira et al. (2021).

The experimental period lasted 21 days. Mean temperature and salinity were 21.1 ± 1.6ºC and 36.9 ± 0.8, respectively.

Statistics

Data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene tests, respectively. As the assumptions for parametric analyses were not fully met, differences in mass gain (g), shell height (mm), shell length (mm), and survival (%) among treatments were evaluated using permutation tests based on the t statistic (Neuhäuser 2017) at a 5% significance level, with p-values obtained empirically through permutations. Multiple comparisons were controlled using the Bonferroni adjustment. Statistical

Table 1. Different proportions and percentages of microalgal biomass used in the experimental stages with seeds of *Amarilladesma mactroides*. ISO: *Isochrysis* aff. *galbana*; CM: *Chaetoceros muelleri*; TS: *Tetraselmis suecica*; W/F: treatment without food. Proportions represent the relative contribution of each microalgae species within the diet mixture. The percentages in parentheses (%) indicate the amount of microalgal biomass supplied relative to total seed wet biomass.

Experiment 1 (mono- and bi-algal)			Experiment 2 (bi-algal)		
Treatment			Treatment		
1	100 ISO (3%)		**1**	30 ISO (3%)	70 CM (3%)
2	100 CM (3%)		**2**	50 ISO (3%)	50 CM (3%)
3	100 TS (3%)		**3**	70 ISO (3%)	30 CM (3%)
4	50 CM (3%)	50 TS (3%)	**4**	30 ISO (6%)	70 CM (6%)
5	50 ISO (3%)	50 TS (3%)	**5**	50 ISO (6%)	50 CM (6%)
6	50 ISO (3%)	50 CM (3%)	**6**	70 ISO (6%)	30 CM (6%)
7	100 TS (200%)		**7**	30 ISO (10%)	70 CM (10%)
8	W/F				

analyses were performed using SAS® statistical software.

RESULTS

Mass gain of *Amarilladesma mactroides* fed mono- and bi-algal diets (Experiment 1)

In the first experiment, yellow clam seeds were given dietary treatments to evaluate mass gain over a 28-day cultivation period with weekly assessments. Overall, differences among dietary treatments were observed from the first week onward, whereas the treatment without food (W/F) consistently showed no mass gain and occasional mass loss throughout the experimental period.

On day 7, the highest mass gains were observed in seeds fed ISO/CM (1.18 ± 0.7 mg) and CM (1.11 ± 0.3 mg) diets, while ISO and ISO/TS diets showed intermediate gains (0.90 ± 0.2 and 0.77 ± 0.1 mg, respectively) that did not differ from the highest-performing treatments. In contrast, TS-based diets showed lower performance (TS: 0.28 ± 0.3 mg; TS 200%: 0.16 ± 0.1 mg) and W/F presented negative mass variation (-0.15 ± 0.1 mg) (Fig. 1a).

On day 14, ISO/CM remained the treatment with the highest mass gain (4.19 ± 0.2 mg), whereas CM (2.80 ± 0.4 mg) showed relatively high values, and ISO (2.14 ± 0.2 mg) showed intermediate performance. TS-based diets, including ISO/TS (0.65 ± 0.3 mg), TS 200% (0.71 ± 0.7 mg), and TS (0.32 ± 0.2 mg), continued to present lower gains, while W/F showed no positive gain (-0.06 ± 0.1 mg) (Fig. 1b). These results reinforced the superior performance of diets containing CM, particularly when combined with ISO.

On days 21 and 28, differences among treatments became more pronounced. Seeds fed the ISO/CM diet consistently showed the highest mass gain (5.93 ± 3.4 and 7.19 ± 4.6 mg, respectively), whereas ISO (3.63 ± 0.9 and 4.40 ± 1.5 mg, on days 21 and 28, respectively) and CM (3.74 ± 0.2 and 4.24 ± 0.3 mg, respectively) presented intermediate values. In contrast, TS-based diets, including CM/TS (1.38 ± 0.2 and 1.37 ± 0.1 mg, respectively), ISO/TS (1.04 ± 0.2 and 1.13 ± 0.3 mg, respectively), TS 200% (0.20 ± 0.2 and 0.60 ± 0.5 mg, respectively) and TS (0.34 ± 0.3 and 0.40 ± 0.3 mg, respectively), resulted in lower gains (Fig. 1c-d). Throughout the experimental period, W/F seeds showed no mass gain (-0.05 ± 0.0 and -0.01 ± 0.1 mg, respectively), confirming the dependence of growth on dietary supply.

Overall, the ISO/CM diet consistently promoted the best growth performance throughout the cultivation period, whereas diets containing TS resulted in the lowest mass gains among fed treatments.

Mass gain of *Amarilladesma mactroides* fed bi-algal diets with different proportions and percentages of biomass (Experiment 2)

In the Experiment 2, mixtures containing different proportions and biomass supply levels of the microalgae that showed the best performance in experiment 1 (ISO and CM) were evaluated over 21 days. Mass gain, shell height, and shell length of *A. mactroides* seeds were assessed.

On day 7, diets containing higher proportions of ISO, particularly 70 ISO/30 CM at both biomass levels evaluated (6 and 3%), showed the highest mass gains (16.63 ± 2.4 and 16.45 ± 0.9 mg, respectively). In contrast, treatments containing higher proportions of CM, especially 30 ISO/70 CM (10%), showed the

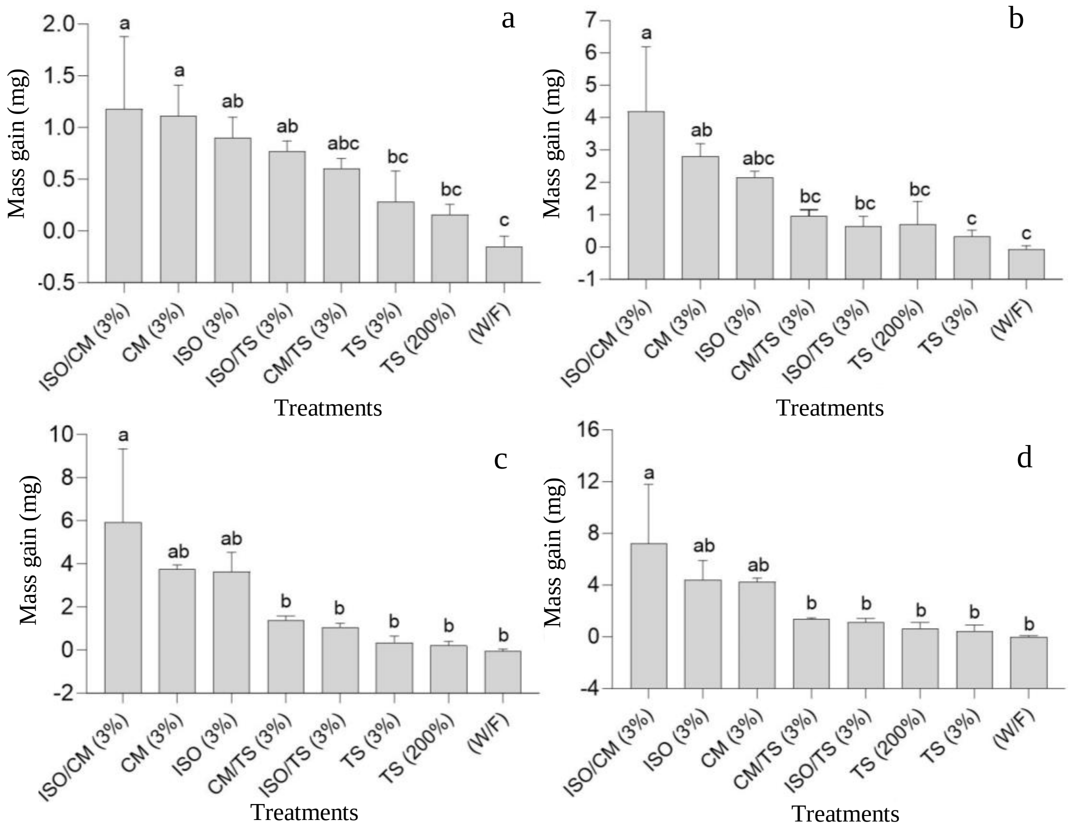

Figure 1. Means (± standard deviation) of mass gain (mg) of *Amarilladesma mactroides* seeds fed different microalgae diets for 28 days. a) Mass gain (mg) after seven days of cultivation, b) mass gain (mg) at 14 days of cultivation, c) mass gain (mg) at 21 days of cultivation, d) mass gain (mg) at 28 days of cultivation. Different letters indicate significant differences among treatments ($P < 0.05$; permutation tests based on the t statistic). ISO: *Isochrysis* aff. *galbana*, CM: *Chaetoceros muelleri*, TS: *Tetraselmis suecica*, W/F: treatment without food, (%) indicates the amount of microalgal biomass supplied relative to total seed wet biomass.

lowest gains (9.20 ± 0.5 mg), whereas balanced diets (50 ISO/50 CM, 6 and 3%) presented intermediate performance (13.32 ± 1.7 and 12.69 ± 2.4 mg, respectively) (Fig. 2a).

On day 14, differences among treatments became more pronounced, with the 70 ISO/30 CM (6%) diet showing the highest mass gain (61.52 ± 5.8 mg). This treatment resulted in significantly higher mass gain than diets with higher CM proportions, including 30 ISO/70 CM (10%) (38.55 ± 3.7 mg), 30 ISO/70 CM (3%) (35.08 ± 4.7 mg), and 30 ISO/70 CM (6%) (33.50 ± 11.3 mg) (Fig. 2b). Intermediate mass gains were observed in the 50 ISO/50 CM (6%) (52.54 ± 6.7 mg) and 70 ISO/30 CM (3%) (51.91 ± 6.8 mg) treatments.

On day 21, the superiority of the 70 ISO/30 CM (6%) diet remained evident, presenting the highest cumulative mass gain (162.61 ± 21.6 mg). However, some intermediate treatments, including 50 ISO/50 CM (6%), 70 ISO/30 CM (3%), 50 ISO/50 CM (3%), and 30 ISO/70 CM (10%), did not differ statistically from the highest-performing treatment (Fig. 2c). In contrast, diets containing higher proportions of CM at 6 and 3% biomass levels resulted in significantly lower gains.

Overall, diets enriched with higher proportions of ISO combined with moderate microalgal biomass supply (particularly 6%) promoted the best growth performance.

Shell height and shell length of *Amarilladesma mactroides* fed mono- and bi-algal diets (Experiment 1) and bi-algal diets with different proportions and percentages of biomass (Experiment 2)

At the final biometry of experiment 1 (28 days), differences in shell height were observed among dietary treatments. Seeds fed ISO/CM (2.96 ± 0.7 mm) showed the highest shell heights, whereas CM (2.86 ± 0.5 mm)

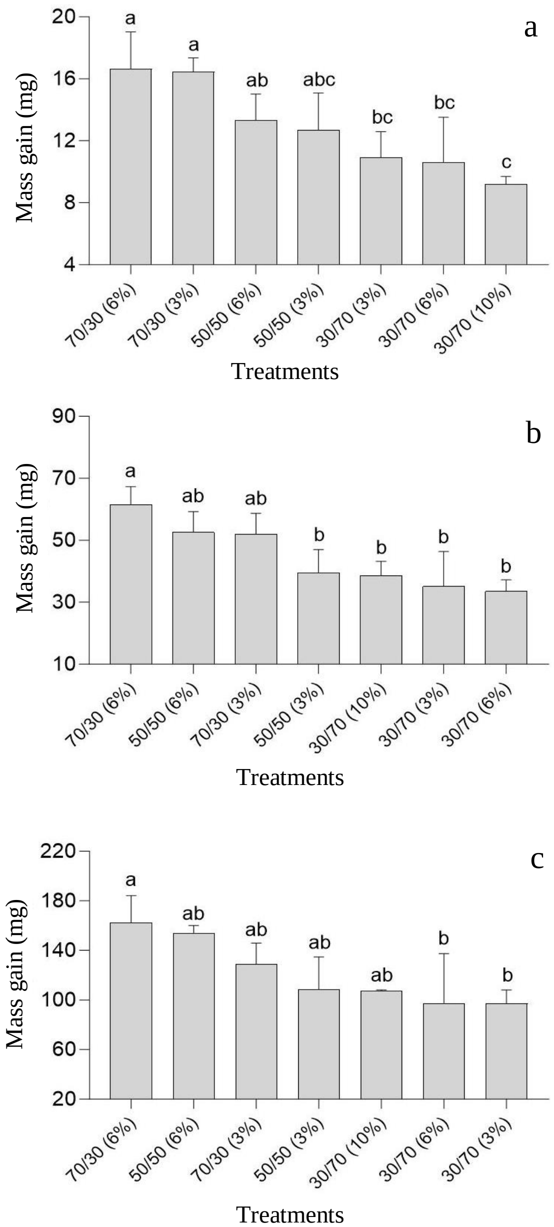

Figure 2. Means (± standard deviation) of mass gain (mg) of *Amarilladesma mactroides* seeds fed different microalgae diets for 21 days. a) Mass gain (mg) after seven days of cultivation, b) mass gain (mg) at 14 days of cultivation, c) mass gain (mg) at 21 days of cultivation. Different letters indicate significant differences among treatments ($P < 0.05$; permutation tests based on the t statistic), (%) indicates the amount of microalgal biomass supplied relative to total seed wet biomass.

and ISO (2.78 ± 0.6 mm) presented intermediate values. Treatments containing TS (CM/TS, 2.52 ± 0.3 mm; ISO/TS, 2.42 ± 0.3 mm; TS, 2.38 ± 0.2 mm; TS 200%, 2.28 ± 0.3 mm) showed lower shell heights, while W/F exhibited the lowest values (2.14 ± 0.2 mm) (Fig. 3a).

A similar pattern was observed for shell length. Seeds fed ISO/CM showed the highest values (4.07 ± 1.1 mm), followed by CM (3.78 ± 0.7 mm) and ISO (3.73 ± 0.9 mm), which presented intermediate values. In contrast, treatments containing TS (CM/TS, 3.38 ± 0.5 mm; ISO/TS, 3.27 ± 0.4 mm; TS, 3.18 ± 0.3 mm; TS 200%, 3.13 ± 0.3 mm) and W/F (2.97 ± 0.2 mm) showed lower shell lengths (Fig. 3a).

In Experiment 2, no significant differences in shell height or shell length were observed among treatments at the end of the 21-day cultivation period (Fig. 3b).

Likewise, survival rates did not differ among treatments in either experiment, indicating that dietary treatments influenced growth performance but not survival rates.

DISCUSSION

The present study demonstrated that different proportions and percentages of microalgae biomass supply influenced mass gain and shell growth of *A. mactroides* seeds under laboratory conditions, although survival was not affected by dietary treatments.

Several factors contribute to the nutritional effect of microalgae used in bivalve diets, including cell size, shape, digestibility, biochemical composition, presence of toxins, enzymatic activity, and species-specific dietary requirements (Hemaiswarya et al. 2011, Ren et al. 2015, Yang et al. 2016). Bivalve juveniles exhibit selective intake of certain species of microalgae (Ward & Shumway 2004), a factor that can vary between species, depending on ecological niche. For example, the yellow clam is a benthic species that typically inhabits sandy environments with low organic matter content (Olivier & Penchaszadeh 1971), i.e. environments that may impair its particle selection capacity. One possible factor explaining the importance of evaluating different diets and microalgal biomass proportions is the difference in cell size among the microalgae species used. TS presents larger cell dimensions (9.7 ± 1.4 and 7.1 ± 0.9 µm, respectively) compared with ISO (8.5 ± 1.6 and 5.4 ± 1.1 µm, respectively) and CM (7.4 ± 1.2 and 5.4 ± 1.1 µm, respectively) (Nell & O'Connor 1991), which may help explain the lower growth performance observed in clam seeds fed diets containing TS in the present study, since specific information regarding optimal microalgal size for *A. mactroides* is still scarce (Vieira et al. 2021).

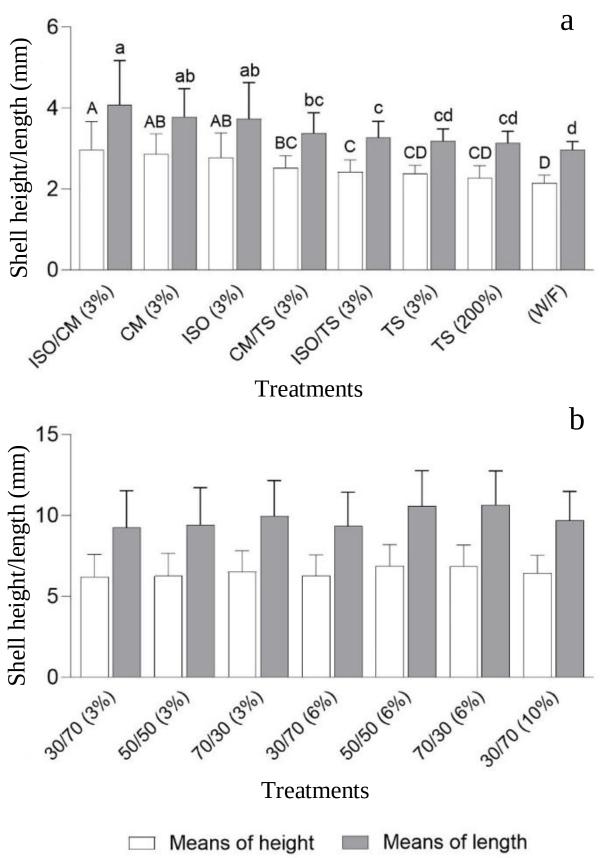

Figure 3. Means (± standard deviation) of shell height (mm) and shell length (mm) of *Amarilladesma mactroides* seeds fed different microalgae diets. a) At 28 days of cultivation (end of the first experiment), b) at 21 days of cultivation (end of the second experiment). Lowercase letters indicate significant differences among treatments for height means and uppercase letters for shell length means ($P < 0.05$; permutation tests based on the t statistic). ISO: *Isochrysis* aff. *galbana*, CM: *Chaetoceros muelleri*, TS: *Tetraselmis suecica*, W/F: treatment without food. Proportions represent the relative contribution of each microalgae species within the diet mixture and (%) indicates the amount of microalgal biomass supplied relative to total seed wet biomass.

According to Albentosa et al. (1997), TS showed superior performance for *Ruditapes decussatus* when compared to the microalgae *I. galbana* and *Phaeodactylum tricornutum*. The authors attributed this result to its carbohydrate content (97.1 pg cell^{-1}). However, the studies of *Crassostrea virginica* and *Mercenaria mercenaria* (Epifanio 1979), *C. virginica* (Romberger & Epifanio 1981), and *Venerupis pullastra* (Albentosa et al. 1993) seeds reported lower mass gain and shell growth in animals fed TS compared to other microalgae, which is consistent with the results obtained in this study. These findings suggest that the reduced performance associated with TS may be related to lower ingestion and/or digestive efficiency, although this mechanism was not evaluated in the present study.

Epifanio (1979) suggested that the lower digestibility of TS may be related to its rigid external cell structure, which can delay or limit nutrient absorption from these microalgae. This limitation may become more pronounced at elevated food concentrations, when the digestive capacity of the animal approaches saturation, potentially resulting in increased amounts of partially digested material. Importantly, the yellow clam possesses a well-developed crystalline style, a characteristic of sand-dwelling bivalves that facilitates both mechanical and enzymatic digestion in the stomach (Kristensen 1972). However, the efficiency of this structure for processing *T. suecica* at the seed stage remains unclear and was not directly evaluated in the present study. Therefore, despite the recognized nutritional quality of this microalga (Brown 1991), the lower growth performance observed in this study suggests that its assimilation may be less efficient at this developmental stage compared to other microalgae evaluated (ISO and CM), rather than indicating differences in nutritional composition alone. On the other hand, Ren et al. (2015) reported that the digestibility of nutritional components present in microalgae depends, in part, on the specific enzymatic activity of the organism. Accordingly, a possible explanation for the positive effect of diets composed of ISO and CM, whether offered separately (100 ISO, 3%; 100 CM, 3%) or mixed (50 ISO/ 50 CM, 3%), is that these diets may have stimulated digestive enzymatic activity and improved nutritional utilization by yellow clam seeds.

Furthermore, diets composed of ISO and CM are commonly used in shellfish hatcheries (Helm et al. 2004) because both microalgae have distinct characteristics in their composition. For example, ISO (flagellate) has a higher lipid content, and CM (diatom) has a higher protein content (Brown 1991). Among these components, PUFAs are classified as fundamental for most marine animals (Dunstan et al. 1994). However, their synthesis is limited in bivalves (Knauer & Southgate 1999). Langdon & Waldock (1981) attribute the synergistic effect of diets to the availability and balance of fatty acids, as a main component. Among PUFAs, DHA, ARA and EPA are considered essential fatty acids for marine bivalves (Ronquillo et al. 2012). Therefore, the presence of PUFAs in these microalgae may help explain the good performance of ISO and CM alone or in combination. For example, Langdon & Waldock (1981) reported EPA and DHA deficiency in microalgae as a limiting factor for the growth of *C. gigas* seeds, corroborating Enright et al. (1986), who reported that the nutritional needs of EPA and DHA are higher for *O. edulis* seeds. Rivero-Rodríguez et al. (2007) observed that the diet showing the highest growth for oyster *C. corteziensis* seeds was composed of *C. calcitrans*, a species rich in ARA. In addition, they noted that seeds fed with these microalgae had only 9% DHA in their tissues, while those fed with ISO had 33%, a concentration that did not result in greater growth. These findings suggest that this bivalve species may present an optimal threshold for the assimilation of these nutrients. Ren et al. (2015), in *Panopea generosa*, observed that the mixture of ISO (rich in DHA and low in EPA) and TS (rich in EPA) resulted in a greater synergistic growth than the monoalgal diet offered. The same growth was not observed with a combination of CM and TS, both with low levels of DHA, which resulted in less growth than that provided by CM alone. These findings may help explain the results of the present study, particularly the lower growth observed in seeds fed diets containing TS.

In the first experiment, the survival of *A. mactroides* seeds did not differ among treatments, even in comparison to the treatment W/F. Vieira et al. (2021) found that seeds of the bivalves *Perna pena*, *Venerupis philippinarum,* and *A. mactroides* had an average survival of 91, 84, and 98%, respectively, in the period between 21 and 35 days without feeding, corroborating the result of the present study, which lasted 28 days. It is well known that chronic food deprivation induces physiological responses such as metabolic depression and the utilization of endogenous organic osmolytes (energy reserve) until food becomes available again or energy reserves are depleted (Yan et al. 2009). This survival period may vary according to the culture system adopted and the physiology of the analyzed species.

Although ISO/CM frequently resulted in higher mean mass gain and shell growth, its performance was not consistently different from mono-algal ISO and CM diets. Nevertheless, several studies highlight the advantages of combining microalgae species in diets for aquatic organisms (Rosa et al. 2017). This strategy depends on the appropriate combination of microalgae species and nutritional components, including proteins, carbohydrates, lipids, and fatty acids, according to the developmental stage of the cultured bivalve (Cheng et al. 2020). Therefore, Experiment 2 was conducted to further evaluate the effects of different ISO/CM proportions and microalgal biomass supply levels on the growth and survival of yellow clam seeds.

The production of microalgae is considered costly in shellfish hatcheries. Access to information regarding the different proportions and percentages of microalgae offered in the diet of shellfish at different life stages may reduce production costs in the laboratory (Albentosa et al. 1997, Rivero-Rodríguez et al. 2007). The results obtained in the present study indicate that bi-algal ISO/CM diets containing balanced (50/50) or ISO-enriched (70/30) proportions, particularly at 3 and 6% microalgal biomass supply levels, promoted favorable growth performance. However, diets with higher proportions of CM in relation to ISO only led to positive effects on the mass gain of animals with a high percentage of microalgae biomass (10%) (Fig. 2c). The percentage of microalgae biomass (10%) used by Ronquillo et al. (2012) was positive for *Ostrea edulis*, but it was not as advantageous for yellow clam seeds in terms of laboratory production since the growth performance of the seeds was similar at lower biomass supply levels (3 and 6%). A lower concentration means that the need for microalgae production is reduced.

The growth responses observed among the different proportions evaluated in the present study were comparable to those reported by Ren et al. (2015) with *P. generosa*. In the present study, comparable growth performance was observed among the tested proportions (30/70, 50/50, and 70/30), indicating that, within this specific range, the proportion of ISO and CM did not markedly influence growth outcomes. According to Viet Le et al. (2014), information on diets that were successful with other species of bivalves may help when there is a lack of nutritional data on new species of bivalves with potential for cultivation, as well as assist in laboratory maintenance, regardless of the specific nutritional requirements of the species (Albentosa et al. 1997, Pettersen et al. 2010), as is the case with yellow clam.

Several studies have demonstrated positive effects of ISO/CM mixtures during larval and seed stages of bivalves (Helm et al. 2004, Matias et al. 2014, Ren et al. 2015, Cheng et al. 2020). The present study corroborates these findings. Parrish et al. (1999) reported that CM contains three times more carbohydrates per cell than ISO, which may have been an important factor for the growth of *Placopecten magellanicus* scallop larvae when fed ISO/CM. Reitan (2011) also found that 1) CM contains higher carbohydrate levels than ISO, 2) it can stimulate digestive enzymatic activity in *Pecten maximus* seeds, and 3) it may be considered more advantageous even as a monoalgal feed. However, in the present study, higher proportions of CM in the diet of yellow clam were only associated with improved performance at high biomass supply levels.

These results suggest that the observed differences in mass gain may be related not only to the proportion of microalgae, but also to differences in nutritional composition among species, including carbohydrate content, essential fatty acids (e.g. EPA and DHA), and digestibility, which are known to influence bivalve growth (Brown 1991, Dalsgaard et al. 2003, Rivero-Rodríguez et al. 2007). However, these nutritional components were not directly measured in the present study, and therefore, their specific contribution cannot be determined.

Thus, selecting diets solely based on growth performance may overlook important nutritional factors, such as biochemical composition and digestibility (Epifanio 1979, Rivero-Rodríguez et al. 2007), which may become particularly relevant for the availability and assimilation of essential nutrients (Brown 1991).

Overall, the present findings suggest that ISO/CM-based diets, particularly those enriched with ISO and supplied at moderate microalgal biomass levels, are suitable for the laboratory cultivation of *A. mactroides* seeds.

CONCLUSION

Diets composed of *Isochrysis* aff. *galbana* (ISO) and *Chaetoceros muelleri* (CM), offered alone or in combination, promoted higher mass gain and shell growth of *Amarilladesma mactroides* seeds without affecting survival. Bi-algal ISO/CM diets, particularly at balanced or ISO-enriched proportions (50/50 and 70/30) and moderate microalgal biomass supply levels (3-6%), showed the best growth performance under laboratory conditions, indicating their potential for hatchery cultivation of this species. Future studies should investigate the biochemical composition of these microalgae and the digestive physiology of *A. mactroides* to further optimize feeding protocols.

Credit author contributions

J.J. Silva Santos: methodology, formal analysis, investigation, writing-original draft; J. Portella Bernardes: methodology and data sample; J.R. Buitrago Ramírez & C.M. Rodrigues de Melo: methodology, formal analysis, writing-review and edit; L.A. Romano: conceptualization, resources, supervision and funding acquisition; C.H. Araujo de Miranda Gomes: conceptualization, supervision and data sample. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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