ORIGINAL RESEARCH

Evaluation of microalgal and formulated diets for the culture of the New Zealand pipi clam *Paphies australis*

Nawwar Z. Mamat · Andrea C. Alfaro

Abstract   A set of feeding trials was carried out for different microalgal species and processed diets for the culture of the New Zealand pipi, *Paphies australis*. Five microalgal species (*Isochrysis galbana* clone T-ISO, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and *Thalassiosira pseudonana* clone 3H) and three formulated diets (baker's yeast, wheat flour, and corn flour) were fed to spat, juvenile, and adult pipi for 21 days. Unfed pipi were used in the control group. The spat and juvenile pipi showed the major shell increase with *I. galbana*, while the greatest wet weight increase was obtained with *P. lutheri*. The shells of adult pipi grew better with corn flour and *P. lutheri*-fed group obtained the greatest wet weight. Results of proximate analysis in adult pipi revealed that proteins and lipids were accumulated in the tissue for all fed groups, while carbohydrate levels depleted in all treatments including the control group. It is suggested that the gonads have developed during the experiment.

Keywords   Clam aquaculture · Microalgae diets · Formulated diets · *Paphies australis* · Pipi

Introduction

One of the main constraints in bivalve rearing for aquaculture purposes is the production of appropriate diets, including live (microalgae) and formulated feeds. The high variability in nutritional requirements among bivalve species, even within the same genus has made it difficult to generalise diet formulation techniques across species (Coutteau and Sorgeloos 1992). Thus, new cultured species require considerable background and experimental research to identify the best diets for cost-effective stock production.

Numerous studies have investigated the nutritional value and feeding applications (e.g., ration size, cell concentration) of a number of microalgal species (Webb and Chu 1983; Hooker 1995; Galley et al. 2010). Many of these microalgal species are regularly used in hatchery production, including *Tetraselmis suecica*, *T. chuii*, *Nannochloropsis atomus*, *N. oculata*, *Isochrysis galbana* (T-ISO clone), *Pavlova lutheri*, *P. salina*,

N. Z. Mamat (✉)
Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor, Malaysia
e-mail: nawwar353@perlis.uitm.edu.my

N. Z. Mamat
Faculty of Applied Sciences, Universiti Teknologi MARA Perlis, 02600 Arau, Malaysia

A. C. Alfaro
School of Applied Sciences, Faculty of Health and Environmental Sciences, Auckland University of Technology,
Private Bag 92006, Auckland 1142, New Zealand

Chaetoceros calcitrans forma pumilus, *Chaetoceros muelleri*, *Skeletonema costatum*, and *Thalassiosira pseudonana*. Depending on their nutritional contents, a combination of microalgal species often is used to provide a balanced diet for larvae and juvenile bivalves (Hooker 1995; Ceron-Ortiz et al. 2009). For example, in the lion-paw scallop *Lyropecten subnodosus*, spat fed *P. lutheri*/*C. muelleri* mixture diet recorded the major increases in growth (Ceron-Ortiz et al. 2009). Similarly, growth of the blue mussel *Mytilus eduli*s veliger larvae fed mixed diets outperformed single species diets (Galley et al. 2010). However, the cultivation of microalgae is labour extensive and expensive. Thus, the replacement of live microalgae with low-cost alternative diets has been used for a number of bivalve species (Perez-Camacho et al. 1998; Pales-Espinosa and Allam 2006; Mazon-Suastegui et al. 2008; Nevejan et al. 2008).

Substitutions of microalgae with processed diets, such as yeast and plant-based flours, have been shown to be effective for the cultivation of *Tapes philippinarum* (Caers et al. 1999), *Ruditapes decussatus* (Albentosa et al. 1999), and *Mercenaria mercenaria* (Epifanio 1979). Albentosa et al. (1999) in their study with *T. philippinarum* spat have successfully replaced up to 50% of the daily food ration with wheat germ flour. Within the 6 weeks of culture period, spat fed 50:50 *I. galbana* and wheat germ flour mixed diet obtained a final dry weight of 12.61 mg ind^{-1}, showing no significant difference ($p > 0.05$) with spat fed exclusively *I. galbana* (14.09 mg ind^{-1}). While these studies provide good information regarding the potential uses of various live and formulated diets for bivalve rearing, experimental studies on the effects of specific diets on the growth of new aquaculture species are needed to elucidate the most appropriate and cost-effective diets.

The pipi clam, *Paphies australis*, is one of the most widely distributed and common infaunal clams in New Zealand. This species is endemic to New Zealand, and constitutes a thriving fisheries industry for the local market. A number of studies have been focused mainly on biological and ecological aspects of wild populations, including the reproduction and larval development (Hooker 1995), population dynamics (Hooker1995; Hewitt et al. 1997; McLeod and Wing 2008), and burrowing behaviour (Hull et al. 1998). While *P. australis* has one of the best aquaculture potentials of any New Zealand clam, there is a significant lack of information regarding its nutritional requirements and the hatchery conditions necessary for its cultivation. Thus, the purpose of this study is to assess the survival and growth performance of spat, juvenile and adult pipi clams fed a range of microalgal and formulated diets in the laboratory.

Materials and methods

Experimental setup

Pipi clams of a wide range of sizes were collected from Waiwera Beach, Northern Auckland (−35.550°N 174.717°W). The animals were taken to the Auckland University of Technology (AUT) aquaculture laboratory, where they were acclimatised for 3 days before the start of the experiment. Three clam size classes were selected as: spat (0–10.0 mm in shell length), juveniles (15.0–25.0 mm in shell length), and adults (30.0–40.0 mm in shell length). Three replicate tanks (2-L tanks 178 mm length × 130 mm width × 120 mm height) were used for each feeding treatment and for each of the three clam size classes. Each tank contained 30 spat, 20 juveniles, or 10 adults per tank. All individuals were marked with a number on both valves so that individual shell lengths and weights could be recorded at the start and at the end of the experiment. Maximum shell lengths were measured with a vernier calliper to the nearest millimetre. Wet weights were obtained while individuals were submerged in the water within each experimental container. A tray connected to a bottom hook on a balance supported above each experimental container was placed underwater within each tank. After zeroing the balance, each animal was placed on the submerged tray to obtain its wet weight. Accurate wet weights were achieved with this method, since the clams were not removed from the water, thus avoiding water content biases.

The feeding treatments included five microalgal species (T-ISO clone *Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros muelleri*, and 3-H clone *Thalassiosira pseudonana*), three formulated diets (yeast, wheat flour and corn flour), and a starved group for each of the three size classes. The feeding rations were 2, 4, and 6 % (dry food weight/live animal weight/tank) for spat, juvenile and adult clams, respectively. Feeding was conducted daily after a full water exchange for an experimental period of 21 days. For all tanks, the water salinity was maintained at 35 ppt and the temperature at 17–18 °C throughout the experiment.

Constant aeration was maintained with individual airflow lines to each tank. The tanks were cleaned daily to remove solid waste products.

Experimental diets

Microalgal stocks were obtained from CSIRO Microalgae Research Centre, Australia and grown in 15 L carboys at a constant temperature of 18 °C and exposed to a 12L:12D illumination cycles. The cultures were enriched with f/2 medium as described by Guillard (1975). The baker's yeast granules were previously ground to a powder form and sieved through a 20-μm sieve. The baker's yeast (Edmonds Ltd.), corn flour (Edmonds Ltd.), and wheat flour (Woolworths Ltd.) were suspended in seawater before being poured into the tanks to avoid lumping.

The dry weight of microalgal cells was determined by filtering the culture through 0.45-μm pore-size membrane filters. The filters were then rinsed with 0.5 M ammonium formate to eliminate salt residues (Epifanio 1979), and dried for 12 h at 100 °C (Table 1).

Proximate analysis

Proximate analysis (proteins, lipids, carbohydrates, ash and moisture) was performed in a group of adult clams at the start of the experiment (initial condition) and at the end of the experiment (final condition). Spat and juveniles provided insufficient materials to carry out the proximate analysis; therefore, only the adult clams were analysed for evaluating the nutritional benefit of each diet on the growth and body content of pipi. Three replicate samples of 1.0 g of dry tissue were used for each analysis.

Before the start of the feeding trial, 30 adult clams were frozen (−20 °C) to determine the initial proximate composition after 3 days acclimatisation. On day 21, following the growth measurements, the remaining adult clams were killed and the soft tissues were separated from the shell. The frozen tissues from the initial samples were thawed. The tissues (initial and final samples) were carefully blotted with absorbent papers to remove excess wetness prior to oven drying.

Moisture contents were determined by weight difference before and after drying the samples at 80 °C for 24 h, and ash contents were obtained by burning the dry samples in a furnace at 600 °C for 2 h and cooled to room temperature in a desiccator. The differential weights were determined to be the ash content. Analyses of proteins were conducted following the Kjeldahl method as described by AOAC (2005). Lipids were quantified by a modification of Bligh and Dyer method (1959). Total carbohydrate contents were estimated by the difference between 100 and the sum of protein, lipid, and ash contents (FAO 2003). The same analysis was performed for three replicate samples of 1.0 g for each food type (five microalgae and three formulated feeds).

Statistical analyses

The SPSS version 15.0 (SPSS Inc.) statistical software was used to analyse the results. Growth (shell length and wet weight) and proximate composition (animals and feeds) data were analysed with parametric statistics. The proportions of shell lengths, wet weights, and proximate composition were transformed by arcsine square root transformation. All data met parametric assumptions after transformation. One-way analyses of variances (ANOVAs), followed by Tukey's HSD *post hoc* tests were conducted to determine the differences between the means of all treatments.

Table 1 Dry weight of the microalgal diets used in the experiment

Species	Dry weight (pg cell^{-1})
Isochrysis galbana (T-ISO)	31.64 ± 0.01
Pavlova lutheri	110 ± 0.02
Tetraselmis suecica	172.81 ± 0.03
Chaetoceros muelleri	73.38 ± 0.01
Thalassiosira pseudonana (3H)	29.67 ± 0.02

Results

Growth parameters

Clams within all three clam size classes increased in shell length and wet weight after being exposed to all treatments, except for those which were starved (Fig. 1). For all three size classes, shell lengths were significantly smaller for starved individuals, and significant weight losses were observed in all starved clams.

For the spat size class, the greatest length increase was for those fed on *I. galbana* (1.21 ± 0.10 mm/ind.) (Fig. 1a). However, this increase was not significantly different to the increase in spat fed *P. lutheri* (0.91 ± 0.09 mm/ind.), *C. muelleri* (0.99 ± 0.04 mm/ind.), and yeast (1.00 ± 0.18 mm/ind.) (ANOVA, $p < 0.05$). Wet weight gains were greatest for spat fed *P. lutheri* (125.14 ± 8.69 mg/ind.), which was significantly different from the other treatments ($p > 0.05$) (Fig. 1b).

Juveniles had the greatest shell length increase when they were fed *I. galbana* (1.72 ± 0.18 mm/ind.) (Fig. 1c). However, this increase did not differ significantly from the increments obtained by juveniles fed *P. lutheri* (1.34 ± 0.16 mm/ind.) and *T. suecica* (1.31 ± 0.44 mm/ind.) ($p > 0.05$). Tukey's test showed that juveniles fed *T. pseudonana*, yeast, wheat flour and corn flour were not significantly different to those fed with *C. muelleri* ($p > 0.05$). The greatest wet weight increase in juvenile pipi was recorded in animals fed *P. lutheri* (177.94 ± 8.12 mg/ind.) ($p > 0.05$) (Fig. 1d).

For adult pipi, the greatest shell increase was observed in individuals fed corn flour (0.79 ± 0.29 mm/ind.), which was not significantly different to adults fed *P. lutheri* (0.53 ± 0.09 mm/ind.), yeast (0.51 ± 0.15 mm/ind.), and wheat flour (0.59 ± 0.10 mm/ind.) ($p < 0.05$) (Fig. 1e). Adults fed *P. lutheri* (167.74 ± 22.34 mg/ind.) achieved the greatest weight, but this value was not significantly different from those fed *C. muelleri* (150.71 ± 8.11 mm/ind), yeast (131.75 ± 7.89 mm/ind.), and corn flour (145.88 ± 12.09 mm/ind.) ($p > 0.05$) (Fig. 1f).

Mortality

Relatively low mortality was observed after the 21-day experiment across all treatments for spat, juvenile, and adult pipi. Spat that was starved displayed the highest percent mortality at 22.22 % ± 3.85 SD, followed by spat fed yeast (18.89 % ± 3.85 SD). Spat fed *I. galbana*, *T. suecica*, *C. muelleri*, *T. pseudonana* and yeast treatments had pipi mortalities of 16.67 % ± 3.33 SD, 17.78 % ± 8.39 SD, 13.33 % ± 3.33 SD, 15.56 % ± 5.09 SD, and 14.44 % ± 1.92 SD, respectively. The lowest mortality was found in spat fed *P. lutheri* (12.22 % ± 3.85 SD) and wheat flour (12.22 % ± 1.92 SD) (Fig. 2a).

The highest mortality for juvenile pipi was found in animals fed on yeast (33.33 % ± 2.89 SD), followed by *I. galbana* (25.00 % ± 10.00 SD) and wheat flour (25.00 % ± 5.00 SD) (Fig. 2b). Juveniles fed *T. suecica*, *C. Muelleri* and *T. pseudonana* had the same percent mortality at 21.67 %. Meanwhile, juveniles fed *C. muelleri* and corn flour had a similar percent mortality at 20.00 % ± 5.00 SD. The lowest mortality was found in juveniles fed on *P. lutheri* (18.33 % ± 2.89 SD).

For adult pipi, the highest mortality was for pipi fed yeast (33.33 % ± 5.77 SD), followed by adults fed wheat flour (30.00 % ± 10.00 SD), and unfed (30.00 % ± 10.00 SD). Adults fed *T. suecica* displayed a mortality of 26.67 % ± 5.77 SD. Juveniles fed *P. lutheri*, *C. muelleri* and *T. pseudonana* showed the same mortality at 23.33 % ± 5.77 SD. The lowest mortality was found in adults fed corn flour (16.67 % ± 5.77 SD) (Fig. 2c).

Proximate composition of diets

The proximate composition data of all experimental diets (five microalgal and three formulated diets) are presented in Table 2.

In general, microalgal diets (*I. galbana*, *P. lutheri*, *T. suecica*, *C. muelleri*, and *T. pseudonana*) contained high protein and lipid contents, and low carbohydrate contents. In contrast, the formulated diets (baker's yeast, corn flour, and wheat flour) were low in protein and lipid contents, but high in carbohydrate contents. The highest protein content (33.54 ± 1.54 %) among all diets was observed in *T. pseudonana* (3H), and the lowest protein content was observed in baker's yeast (5.36 ± 0.12 %). The microalga *P. lutheri* contained the highest
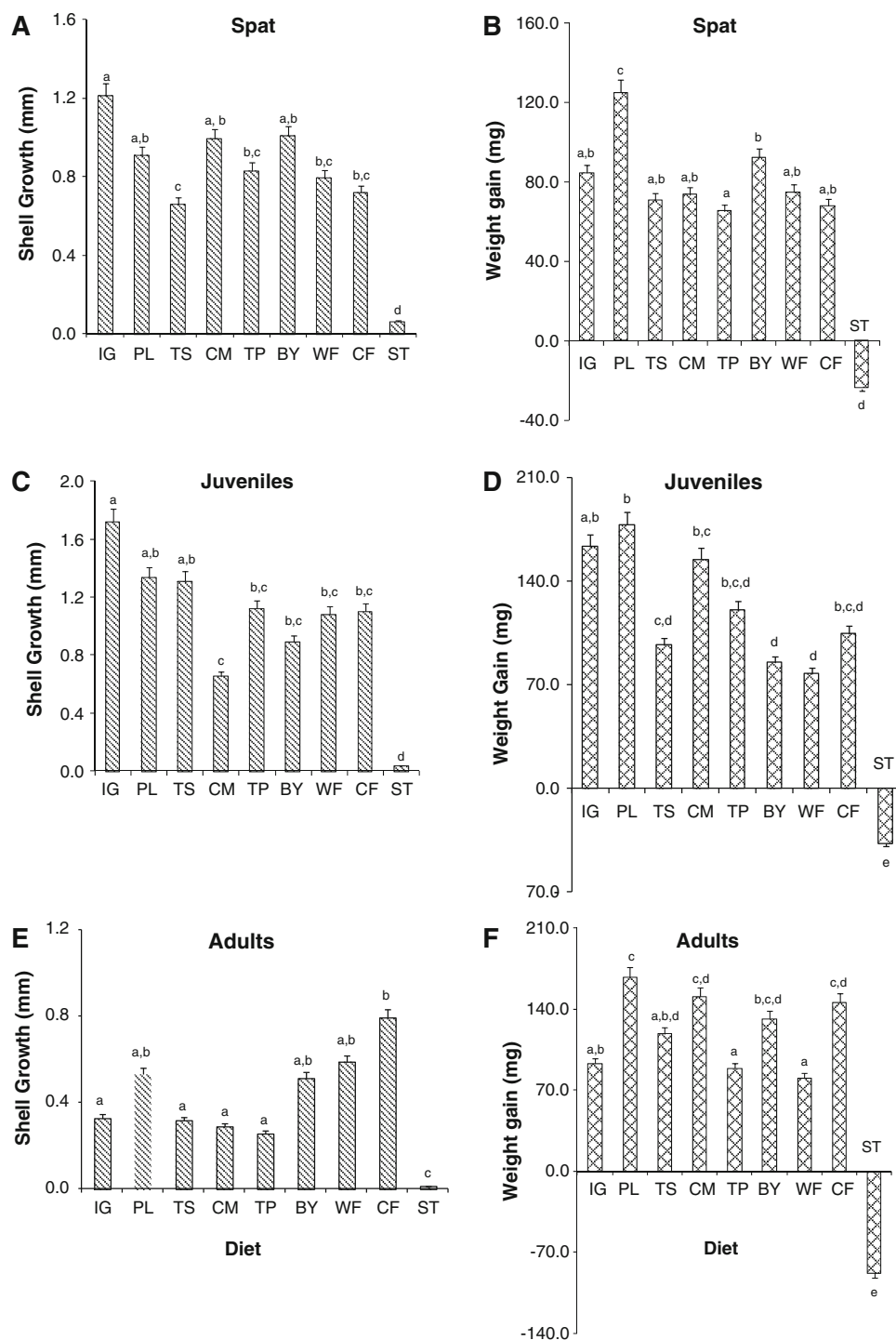
Fig. 1 Shell growth (mm) and weight gain (mg) for *P. australis* spat (**a**, **b**), juveniles (**c**, **d**), and adults (**e**, **f**) at the start and at the end of the 21-day feeding experiment. Data are for all treatment diets [*I. galbana* (IG), *P. lutheri* (PL), *T. suecica* (TS), *C. muelleri* (CM), *T. pseudonana* (TP), Baker's yeast (BY), wheat flour (WF), corn flour (CF) and starved (ST)]. The *same letters* over the *error bars* indicate non-statistical differences

proportion of lipids (23.21 ± 2.93 %), while wheat flour had the lowest lipid content (1.57 ± 0.38 %). Wheat flower had the highest amount of carbohydrates (80.18 ± 2.81 %), and *P. lutheri* had the lowest carbohydrate content (9.44 ± 3.29 %). Moisture and ash contents were highest for *P. lutheri* (34.98 ± 2.63 %) and
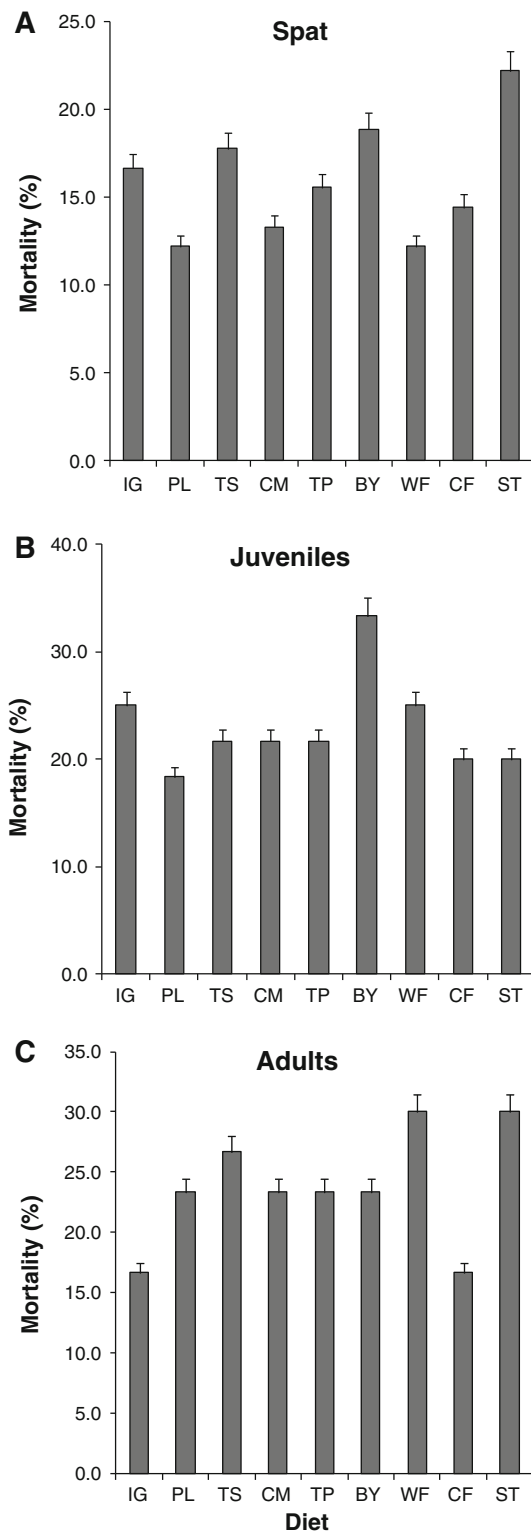
Fig. 2 Mortality of spat (**a**), juvenile (**b**), and adult (**c**) pipi during the 21-day feeding experiment. Clams were fed IG, *I. galbana*; PL, *P. lutheri*; TS, *T. suecica*; CM, *C. muelleri*; TP, *T. pseudonana*; Y, yeast; WF, wheat flour and CF, corn flour. Starved pipi (ST) was the control group. No statistical differences were observed among all treatments for spat, juvenile, and adult pipi. *Error bars* represent 95 % confidence interval of the mean

I. galbana (10.85 ± 0.30 %), respectively, and lowest for corn flour (8.49 ± 1.47 %) and wheat flour (0.39 ± 0.04 %), respectively.

Proximate composition of animals

The proximate composition data of adult clams at the start and at the end of the experiment are shown in Table 3.

Protein and lipid contents were higher for the animals within all treatments at the end of the experiment, except the starved animals (ANOVA, $p < 0.05$). The largest proportion of total protein content was found in adults fed *P. lutheri* (33.75 ± 0.87 %). However, the protein content did not differ from that of those fed yeast ($p > 0.05$). All treatments, including the starved group, had significantly higher protein contents than the initial value.

Lipid contents were highest in adult pipi fed *I. galbana* (6.13 ± 0.53 %), but the lipid content was not significantly different from those fed *P. lutheri* (5.41 ± 0.45 %) and corn flour (4.46 ± 0.36 %) ($p > 0.05$). The starved group lost 11.8 % of its lipid reserves, and that value was not significantly different from the initial lipid content ($p > 0.05$).

Carbohydrate contents were lower for all animals after the experimental period (including starved animals) compared with the initial values. However, these differences were not significant between the initial values and those of the animals fed *T. suecica*, *C. muelleri*, wheat flour, and the starved group ($p < 0.05$).

Table 2 Proximate composition (%) of the microalgal and formulated diets (mean ± SD)

Diet	Protein	Total lipids	Total carbohydrates	Moisture	Ash
Isochrysis galbana (T-ISO)	21.30 ± 0.59[a]	19.06 ± 0.65[a]	19.59 ± 1.86[a]	29.19 ± 2.51[a]	10.85 ± 0.30[a]
Pavlova lutheri	27.23 ± 1.69[b,c]	23.21 ± 2.93[b]	9.44 ± 3.29[b]	34.98 ± 2.63[b,c]	5.14 ± 0.90[b]
Tetraselmis suecica	29.74 ± 1.51[c,d]	10.90 ± 0.60[c]	18.68 ± 3.49[a,c,d]	33.71 ± 1.67[b,c]	6.97 ± 0.32[c]
Chaetoceros muelleri	31.79 ± 0.48[c,d,e]	15.63 ± 1.37[d]	20.34 ± 3.02[c,d]	23.80 ± 2.01[d]	8.45 ± 0.67[d]
Thalassiosira pseudonana (3H)	33.54 ± 1.54[d,e]	18.86 ± 1.55[e]	13.56 ± 5.31[e]	28.19 ± 4.49[e]	5.86 ± 0.58[e]
Yeast	5.36 ± 0.12[f]	1.80 ± 0.24[f,g]	77.19 ± 1.66[f,g,h]	14.35 ± 2.04[f]	1.30 ± 0.94[f]
Wheat flour	8.34 ± 0.16[g,h]	1.57 ± 0.38[f,g]	80.18 ± 2.81[f,g,h]	9.51 ± 1.47[g,h]	0.39 ± 0.08[g]
Corn flour	8.39 ± 1.01[g,h]	5.56 ± 0.68[h]	76.79 ± 0.47[f,g,h]	8.49 ± 2.74[g,h]	0.77 ± 0.04[h]

The same superscripts after the values indicate no significant difference

Table 3 Proximate composition (%) of adult *P. australis* at the start and at the end of the experimental period (mean ± SD)

Treatment	Protein	Total Lipids	Total Carbohydrates	Moisture	Ash
Initial	6.99 ± 0.43[a]	1.02 ± 0.15[a]	17.20 ± 3.47[c]	74.04 ± 3.48[b]	0.75 ± 0.10[a,b,c]
Isochrysis galbana (T-ISO)	24.27 ± 0.88[e]	6.13 ± 0.53[e]	7.03 ± 0.94[a,b]	62.02 ± 1.40[a]	0.56 ± 0.05[d,e]
Pavlova lutheri	33.75 ± 0.87[f]	5.41 ± 0.45[d,e]	4.04 ± 0.20[a]	56.10 ± 0.95[c]	0.71 ± 0.35[a,b,c,f,g,h,e]
Tetraselmis suecica	10.77 ± 0.44[b]	2.67 ± 0.81[b]	16.32 ± 2.38[c]	69.58 ± 2.01[b]	0.66 ± 0.16[a,b,f,c]
Chaetoceros muelleri	14.06 ± 0.39[c]	2.92 ± 0.27[b,c]	10.28 ± 2.37[b,c]	71.75 ± 2.30[b]	1.00 ± 0.25[b,f,c,g,h,j]
Thalassiosira pseudonana (3H)	18.19 ± 1.60[d]	3.33 ± 0.54[b,c]	7.53 ± 1.52[a,b]	70.00 ± 0.29[b,d]	0.94 ± 0.4[b,c,g,h,j]
Yeast	23.47 ± 1.43[e]	4.28 ± 0.28[c,d]	7.98 ± 1.58[a,b]	62.89 ± 0.75[a]	1.37 ± 0.42[i]
Wheat flour	18.86 ± 0.28[d]	2.08 ± 0.70[b]	13.31 ± 4.08[b,c]	65.00 ± 3.92[a]	0.75 ± 0.28[a,b,d]
Corn flour	18.92 ± 0.39[d]	4.46 ± 0.36[c,d,e]	7.90 ± 1.49[a,b]	67.83 ± 1.49[a]	0.89 ± 0.32[g,h,j]
Starved	7.53 ± 0.28[b]	0.90 ± 0.14[a]	12.18 ± 3.97[b,c]	78.78 ± 4.04[a,e]	0.62 ± 0.08[c,d,j]

The same superscripts after the values indicate no significant difference

The moisture content in starved clams was the highest in the initial value (82.33 ± 0.86 %) and the moisture decreased for all treatment groups during the final 21st day of the feeding trial. Ash content in clams fed *C. muelleri* was 1.00 ± 0.32 %, and did not differ significantly from the values obtained in clams fed *T. pseudonana* (0.94 ± 0.48 %) and corn flour (0.89 ± 0.32 %) ($p > 0.05$).

Discussion

Effects of the different diets on growth and mortality

Results of these experiments demonstrate that the processed diets (yeast, wheat flour, and corn flour) are potential substitutes for laboratory-grown pipi. The use of yeast for spat pipi, for example, showed similar shell growth as those fed *I. galbana* ($p > 0.05$). Spat fed wheat and corn flours showed no differences in shell growth when compared to those fed the microalgal diets, except for *I. galbana* ($p < 0.05$). For juveniles, the corn flour fed animals had similar weight gain with *I. galbana* ($p > 0.05$). Microalga *I. galbana* was the main source of energy for a great weight gain in juvenile pipi (Fig. 1d). All fed clams, regardless of size class, grew relatively better than the no-food control. It was apparent that *P. lutheri* microalga mostly supported weight gains for all size classes.

In general, spat and juvenile pipi grew faster than adults. During the earlier life stages (larvae, spat, and juveniles), nutrients derived from diets are catabolised to provide energy for growth. The same growth pattern has been observed in other bivalve species such as in the rock-boring clam *Penitella penita* (Evans 1968), the fingernail clam *Sphaerium striatinum* (Hornbach et al. 1983), the Asiatic clam *Corbicula fluminea* (Welch and Joy 1984), the hard clam *Mercenaria mercenaria* (Lorio and Malone 1995), the Antarctic clam *Laternula elliptica* (Ahn and Shim 1998), the black clam *Villorita cyprinoides* (Arun 2009).

The uses of yeast, wheat flour, and corn flour have been reported as potential substitutes for microalgae in other bivalve species. For example, Perez-Camacho et al. (1998) suggested the use of corn-based diet to substitute 50 % of the 2 % phytoplankton daily ration in the little neck clam, *Ruditapes decussatus*. In another study, Albentosa et al. (2002) found no significant difference between spat fed 100 % *I. galbana* and a mixed diet consisting of 50 % *I. galbana* and 50 % wheat germ flour, in the Manila clam (*Ruditapes philippinarum*). In respect to substituting yeast for microalgal diets, many studies have shown positive results for growth in *R. philippinarum* (Coutteau and Sorgeloos 1992), the hard clam *M. mercenaria* (Epifanio 1979; Coutteau et al. 1994), the blue mussel *Mytilus edulis* (Epifanio 1979), the bay scallop *Argopecten irradians* (Epifanio 1979), the Pacific oyster *Crassostrea gigas* (Coutteau et al. 1993), and the Sydney rock oyster *Saccostrea commercialis* (Brown et al. 1996). However, growth in the soft tissue of *C. gigas* oysters decreased with the increasing amount of yeast in the diet (Epifanio 1979).

Effect of dietary contents of the diets on growth performance

High protein contents in dietary microalgae were reported to enhance growth performance in the short-neck clam *Tapes japonica* (Gallager and Mann 1981), the Pacific oyster *Crassostrea virginica* (Webb and Chu 1983), the European flat oyster *Ostrea edulis* (Enright et al. 1986), and the Mediterranean mussel *Mytilus galloprovincialis* (Langdon and Onal 1999) juveniles. On the other hand, Uriarte and Farias (1999), working with the scallop *Argopecten purpuratus*, and Ceron-Ortiz et al. (2009) with *Lyropecten subnodosus*, found that spat of both species grew relatively better with higher lipid levels in the diets. On the basis of proximate composition of the experimental diets used in this study (Table 2), it is speculated that lipids play a major role in determining weight gain in spat and juveniles. In support to this, growth of *Ostrea edulis* spat also was influenced by the amount of lipids in larval tissues during metamorphosis and in the diets (Laing and Millican 1986). Similarly, a significant correlation between growth and dietary lipid content was observed in *C. virginica* spat (Wikfors et al. 1984). However, Waldock and Nascimento (1979) and Langdon and Waldock (1981) found that level of lipids in diets was negatively correlated with growth rates of *C. gigas* larvae and spat.

Conversely, adult pipi obtained higher growth with diet containing high carbohydrate level, particularly corn flour. These positive results propose the commercial application of corn flour in diets of pipi, mainly for adults. Carbohydrates are important to balance the utilisation of proteins and lipids in catabolic pathways for

energy production (Whyte et al. 1989) and also regarded as the principal energy reserve for adult marine bivalves; both for gametogenesis and during food deficiency especially in winter time (Reid 1969).

Effect of the diets on body composition of adult pipi

Proximate analysis of adult pipi revealed that proteins and lipids were accumulated in the tissue of the fed groups, while carbohydrate levels depleted in all treatments including the control group. The concomitant increase in the levels of proteins and lipids and carbohydrate depletions, were profoundly due to accumulation of mature oocytes. These results suggest that the adults may have initiated their gonadal development. This conforms with other studies where accumulations of proteins in bivalve tissue are simultaneously an indicator to maturation of gonads (Devi et al. 1985; Kreeger 1993; Rivonker and Parulekar 1995), and as a reserve prior to spawning activity (Nagabhushnam and Mane 1978; Gabbott and Peek 1991). Proteins and lipids were continuously metabolised to support energy demand whenever needed (food scarcity or insufficient carbohydrates).

Meanwhile, carbohydrates in adult tissue were rapidly catabolised to meet the energy requirements for reproduction. Similar observations were made by Rodriguez et al. (1993) who regarded carbohydrates as the main energy source for gametogenesis in clams *Tapes decussatus* and *R. philippinarum*. For adult oysters, cornstarch feeds have been used to complement natural microalgae to improve condition index of the animals (Haven 1965; Ingle 1967; Dunathan et al. 1969). These findings emphasised the importance of carbohydrates accumulation as energy reserves in the form of glucogen in gametogenesis (Gabbott 1975). Starvation in adult pipi resulted to expenditures of lipid and carbohydrate resources. This is explained by the functions of lipids and carbohydrates during food scarcity (Whyte et al. 1990).

Microalgal and substitute diets

In recent years, there have been numerous studies on microalgal replacements for bivalve cultures. Among these, partial substitutions with alternative diets have been studied in other clam species such as *R. philippinarum* (Albentosa et al. 1989, 2002), *R. decussatus* (Albentosa et al. 1999), *C. fluminea* (Foe and Knight 1986), and *M. mercenaria* (Coutteau et al. 1991). The use of low-cost processed diets used in the present study would allow an increase in pipi production in hatcheries by reducing the operational costs.

In conclusion, microalgae are still the main nutrient sources for pipi, particularly for spat and juveniles. It is also suggested that corn flour should be considered in the formulation of diets for laboratory-grown pipi. The outcomes of this study are useful in providing better understandings on nutrients required by pipi and proposing the use of processed diets in their feeding.

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