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Research Article

Effect of alternative mediums on production and proximate composition of the microalgae *Chaetoceros muelleri* as food in culture of the copepod *Acartia* sp.

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ABSTRACT. Microalgae *Chaetoceros muelleri* was cultured in three different mediums consisting on an agricultural fertilizer (Agr-F), aquacultural fertilizer (Aq-F) and a conventional medium (F/2, control). These microalgae were later used as natural food to culture the copepod *Acartia* sp. The productive response and chemical proximate composition of microalgae and copepods were monitored. Growth rate and final cell concentration were higher in microalgae cultured in Agr-F compared to the control. In addition, the final biomass and cellular concentration were also the highest in Agr-F. Microalgae from Agr-F and Aq-F had higher carbohydrate and lower protein contents than those in the control. No differences in lipid and ash contents were observed. Regarding copepod production, higher densities and fecundity indexes were observed for those fed with microalgae previously cultured in Agr-F and Aq-F, compared to the control. The adult-nauplii ratio was also higher in copepods fed on microalgae from Agr-F compared to Aq-F and control. Copepods fed on Agr-F and Aq-F microalgae, had higher protein content compared to those fed on control microalgae; carbohydrates were higher in copepods fed on Agr-F as compared to Aq-F microalgae. No differences in lipid and ash contents were registered. Agr-F and Aq-F were adequate alternative mediums to produce *C. muelleri*, which produced higher quality microalgae that increased the copepod production.

Keywords: agricultural fertilizer, aquacultural fertilizer, microalgae quality, productive response, proximate composition, copepod production.

Efecto de medios alternativos sobre la producción y composición proximal de la microalga *Chaetoceros muelleri* como alimento en cultivo del copépodo *Acarti*a sp.

RESUMEN. La microalga *Chaetoceros muelleri* fue cultivada en tres medios diferentes basados en un fertilizante agrícola (Agr-F), un fertilizante acuícola (Aq-F) y un medio convencional (F/2, control). Éstas microalgas fueron posteriormente utilizadas como alimento natural para cultivar el copépodo *Acartia* sp. La respuesta productiva y la composición proximal de las microalgas y copépodos fueron monitoreadas. La tasa de crecimiento y concentración final de células fueron mayores en la microalga cultivada en Agr-F, comparada con el control. La biomasa y concentración celular finales también fueron más altas en Agr-F. Las microalgas de Agr-F y Aq-F tuvieron mayor contenido de carbohidratos y menor contenido de proteína en comparación con el control. No se observaron diferencias en los contenidos de lípidos y cenizas. Respecto a la producción de copépodos, las mayores densidades e índices de fecundidad se observaron en los organismos alimentados con microalgas producidas en Agr-F y Aq-F, en comparación con el control. La proporción adulto-nauplio también fue mayor en copépodos alimentados con microalga de Agr-F comparada con Aq-F y el control. Los copépodos alimentados con microalgas del Agr-F y Aq-F, tuvieron un mayor contenido de proteínas que el control; la cantidad de carbohidratos fue mayor en copépodos alimentados con microalga del Agr-F comparada con Aq-F. No se observaron diferencias en los contenidos de lípidos y cenizas. Agr-F y Aq-F fueron medios alternativos adecuados para producir *C. muelleri*, los cuales produjeron microalgas de alta calidad que incrementaron la producción de copépodos.

Palabras clave: fertilizante agrícola, fertilizante acuícola, calidad de microalga, respuesta productiva, composición proximal, producción de copépodos.

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INTRODUCTION

One of the aquaculture resources that are mainly fed during nursery and larval phases of cultured fish and crustacean is the zooplankton; moreover, recent reports propose the use of natural feed in pre-grow and/or grow-out phases of shrimp culture (Jory, 2000; Campaña-Torres *et al*., 2009, 2010).

Nonetheless, the production of natural food such as microalgae and zooplankton species is commonly accompanied by high economical investments, often attributed to the high cost of culturing mediums used for the production of microalgae; additionally, these mediums are highly specialized and sometimes difficult to obtain in local markets. Furthermore, such microalgae is used to produce *Artemia* larvae, which are also expensive and sometimes scarce (Lin *et al*., 2009; González *et al*., 2010). It is therefore necessary to study alternative strategies aimed at decreasing costs without affecting the production. For instance, some efforts have been made in order to find alternative mediums for microalgae culture; also, different organisms such as copepods, rotifers and cladocerans have been proposed to replace *Artemia* (Støttrup, 2000; Voltolina & Lopez-Elías, 2002; Martin *et al*., 2006; Campaña-Torres *et al*., 2009; Farhadian *et al*., 2009). From these experiments, some promising results have emerged.

Although several experiments have been performed in order to analyze the effect of different media on the productive response and proximate composition of microalgae, most of those studies do not include the subsequent effect of such microalgae on the productive response of zooplankton species (Wikfors, 1986; Fidalgo *et al*., 1998; Toyub *et al*., 2007). The quantity, but also the quality of the zooplankton consumed by fish or crustacean, may have an impact on their productive response. Gusmão & McKinnon (2009) demonstrated that the production and nutritional composition of copepods was related to the type of feed they consumed.

The calanoid copepod *Acartia* sp., can be used as an alternative natural food for diverse aquacultural species, since these organisms can be massively produced in laboratory and have demonstrated to be widely consumed by aquatic species (Schipp *et al*., 1999); additionally, they can be found in commercial laboratories and/or a wide diversity of ecosystems. Støttrup (2000) remarked the great potential of copepods as live feed in marine aquaculture. Microalgae such as *Chaetoceros muelleri* has been widely used in aquaculture due to their rapid growth rate, resistance to adverse conditions and nutritional quality; in particular, it has been used to feed *Acartia* sp. (Gusmão & McKinnon, 2009).

The aim of this study was to analyze the effect of two alternative media on the productive response and proximate composition of microalgae *C. muelleri*, and the subsequent effect of such microalgae on the productive response and proximate composition of the copepod *Acartia* sp.

MATERIALS AND METHODS

Experiment 1- Microalgae culture

Two alternative media based on an agricultural and aquacultural fertilizers, and a special medium for microalgae production, were used to culture the microalgae *C. muelleri*. A simple experimental design was performed with four replicates per treatment. Microalgae inoculums were obtained from a commercial laboratory (Maricultura del Pacifico S.A.) at Sonora, México. Guillard F/2 media was used to maintain the inoculums alive.

The control was based on the commercial medium Guillard F/2 (Guillard, 1975; ProLine® F/2 Algae Feed, Aquatic Ecosystems, Florida). The treatment 1 (Agr-F), was based on an agricultural fertilizer (Monoammonium Phosphate; EuroChem, Moscow) prepared as follows: 137 g of the fertilizer +30 g of sodium metasilicate diluted in 1 L of distilled and sterile water. Treatment 2 (Aq-F) was based on an aquacultural fertilizer (Nutrilake®), enriched with vitamins (Bedoyecta®) (Table 1). The three different media were added to each treatment at the beginning of the trial (1 mL L^{-1}).

A multi-step system was performed to increase microalgae volume. Thereafter, the microalgae were distributed into 12 fiberglass columns (80-L; 4 columns/treatment) at an initial concentration of 2 x 10^5 cells mL^{-1}. Treatments were maintained under the same following conditions for four days: light 140 µmL m^{-2} s^{-1}, temperature 21-23ºC, pH 8.2-8.5,

Table 1. Chemical proximate composition of the fertilizers used to culture of microalgae *C. muelleri*.
Tabla 1. Composición química proximal de los fertilizantes utilizados para cultivar la microalga *C. muelleri*.

Component	Agricultural fertilizer (%)	Aquacultural fertilizer (%)	F/2 Medium (%)
Nitrogen as NH_4	13	-	-
Nitrogen as NO_3	-	15	11
Phosphorus pentoxide	52	6	-
Monosodium phosphate	-	-	4.6
Silicates	-	3.5	35.3
Sodium	-	23.2	27.1
Minerals	-	10	15
Minerals and metasilicates	33	-	

*The percentages are presented considering the manufacturer specifications and the additives to improve the formulation.
Note: The rest of the components to achieve the 100% were not reported by the manufacturers.

salinity 35 psu and constant aeration, as suggested by Lopez-Elías *et al*. (2004).

Microalgae response

The productive response of *C. muelleri* was evaluated during and at the end of the bioassay; the cellular density was measured using a Neubauer® chamber (VWR Scientific, Los Angeles, CA., USA) and an optical microscope (Ward´s®, Rochester, N.Y., USA). Growth rate was estimated as $\mu = \frac{(\ln B_n - \ln B_o)}{(t_n - t_o)}$, where μ was considered as the growth rate, B_0 microalgae density (cells mL^{-1}) at the beginning, Bn microalgae density at any time, and t_n-t_0 the period of microalgae culture since inoculation. The generation time (gt) was estimated as $gt = \left(\frac{1}{\mu}\right)(24)$. Dry biomass was estimated after filtration with a 0.2 μm membrane average pore and dried in an oven (70ºC) until constant weight.

To analyze microalgae proximate composition (protein, lipid, carbohydrate and ash), samples were filtered using fiberglass filters (47 mm; Whatman® Maidstone UK) previously dried. The protein content was measured following the methodology described by Lowry *et al*. (1951), and modified by López-Elías & Voltolina (1993). The carbohydrate concentration was estimated by the "phenol-sulfuric acid" method reported by Dubois *et al*. (1956). The lipid content was calculated by a colorimetric method described by Pande *et al*. (1963) and modified by Bustillos-Hurtado & López-Elías (1994). Ash was estimated by incineration at 550ºC (AOAC 1995). Thereafter, the percentage of the organic fraction was calculated for microalgae from each treatment.

Experiment 2- copepod culture

Microalgae cultured in the different media (control, Agr-F and Aq-F) were thereafter used to culture the copepod *Acartia* sp. for 15 days. The experimental design was similar to that for microalgae, with four repetitions per treatment; the experimental units consisted on 10-L tanks provided with constant aeration (≥ 6 mg L^{-1}), temperature 28ºC, salinity 35 psu and pH 8.0-8.3. The copepod cultures were initiated with 2400 organisms per experimental unit, and a microalgae density of 3.0 x 10^4 cells mL^{-1} was maintained within each tank; the microalgae density was estimated and readjusted twice a day (06:00 and 18:00 h) for each experimental unit.

At the end of the culture, copepods were harvested with a net (70 mesh screen [212 μm]), and copepod density, nauplii-adult ratio and fecundity index were estimated; subsamples (10 x 20 mL) were collected from each unit and counted by stereoscopic microscopy (40x) (Del Valls *et al*., 1996). Fecundity index was estimated following the methodology proposed by Ramírez-Sevilla *et al*. (1991), considering the number of eggs and the female number.

The proximate composition on dry basis (DB) of copepods was estimated only for those adult organisms; a special net (50 mesh screen [300 μm]) was used to separate adult copepods. Protein, carbohydrate, lipid and ash content of copepods were estimated by using the standard methods of the Association of Official Analytical Chemists (AOAC).

Statistical analysis

Production parameters of microalgae and copepods were analyzed by a one-way ANOVA and a *posteriori* comparison test (Tukey). If data did not comply with normality (Shapiro-Wilk) and homoscedastic (Bar-

tlett) tests, a non-parametric test was used (Kruskall-Wallis). Proximate composition of microalgae and copepods was analyzed by a chi-square test. A level of $\alpha = 0.05$ was considered significant.

RESULTS

The culture mediums had an effect on the productive response of microalgae (Table 2). Growth rate was 19% higher in microalgae cultured with Agr-F compared to those cultured in Aq-F; however, such difference was not reflected in the generation time, which was lower than Aq-F (24.7 *vs* 27.9 h) but not statistically different. The increases in cellular concentration and biomass are shown in Fig. 1. The highest final cellular concentration was achieved in Agr-F (3.75×10^6 cells mL^{-1}), followed by control (2.94) and Aq-F (2.28) respectively. The highest biomass was also found in Agr-F (0.34 g L^{-1}), while no differences were observed among Aq-F (0.25 g L^{-1}) and the control (0.26 g L^{-1}) (Table 2).

The carbohydrate level was significantly higher (> 21%) in microalgae cultured in Agr-F and Aq-F compared to those cultured in the control (< 19%), (Table 3). In the case of protein level, microalgae from the control had ~3% more protein content, compared to both treatments. No statistical differences were observed among treatments for lipid or ash content (Table 3). The same tendencies were observed for protein, lipid and carbohydrate proportion when the organic fraction was considered (Fig. 2).

The productive responses and proximate composition of copepods were affected by the consumption of microalgae from the different treatments (Table 4). Fecundity index was significantly higher in copepods fed on microalgae produced in Agr-F and Aq-F (> 0.7) compared to those fed on control microalgae (< 0.5). The nauplii-adult ratio was higher in copepods from Agr-F compared to the, other treatments. The final copepod density was not different among both treatments (> 2.3 copepods· mL^{-1}), but the density of copepods fed on Aq-F microalgae was significantly higher than that observed in the control-m (1.97 copepods mL^{-1}) (Table 4).

The carbohydrate content of copepods fed on Agr-F microalgae (15.7%), was significantly higher those fed on Aq-F (12.8%), while no differences were found among Agr-F and the control (Table 5). No statistical differences were observed among treatments regarding lipid (21-23%) or ash content (~5.5%). The protein content was higher in those copepods fed on control microalgae (> 58%) compared to those fed on Agr-F and Aq-F (< 55%).

DISCUSSION

Microalgae cultured in the agricultural fertilizer (Agr-F) had a similar productive response to that observed in the control; both results, were similar to those reported as successful for *C. muelleri* using conventional mediums (Medina-Reyna & Cordero-Esquivel, 1998). Pacheco-Vega *et al*. (2010) cultured *C. muelleri* in different mediums, not finding any significant difference in growth response among those microalgae cultured in F/2 and those produced in a medium based on an agricultural fertilizer; moreover, the biomass and cells production with Agr-F was higher than that reported by Becerra-Dórame *et al*. (2010) using an alternative system to increase their productions of *C. muelleri* cultured outdoor. These results may be explained by the slight decrease in generation time of Agr-F, which indicates that these microalgae duplicate their cell number in less time. Thus, the agricultural fertilizer seems to be an adequate alternative to replace the conventional mediums. Microalgae cultured in the aquacultural fertilizer (Aq-F) had poorest lower productive response; however, the results were within the range

Table 2. Production parameters of *C. muelleri* cultured in different media.

Tabla 2. Parámetros de producción de *C. muelleri* cultivada en diferentes medios.

Treatment	Culture medium	Growth rate (μ)	Generation time (h)	Final concentration (cells mL^{-1} x 10^6)	Final biomass (g L^{-1})
Agr-F	Monoammonuim Phosphate	0.97 ± 0 .14b	24.7 ± 4.2a	3.75 ± 0.44b	0.34 ± 0.05b
Aq-F	Nutrilake	0.82 ± 0.10a	27.9 ± 3.8a	2.28 ± 0.55a	0.25 ± 0.04a
Control	Guillard F/2	0.90 ± 0.16ab	26.6 ± 4.5a	2.94 ± 0.49ab	0.26 ± 0.04ab

*Different letters in the same column indicate significant differences (One way analysis of variance, $P < 0.05$ and Kruskall-Wallis for generation time).

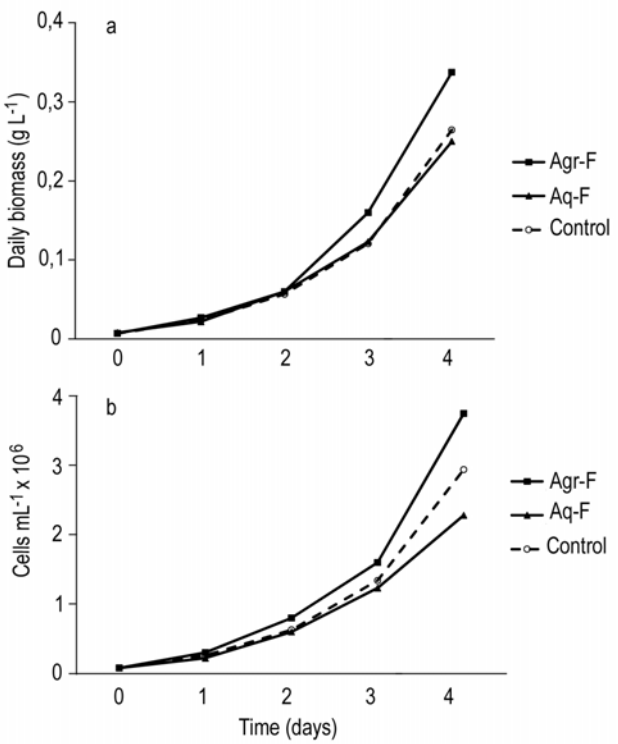

Figure 1. a) Daily biomass, b) cellular concentration *Chaetoceros muelleri* cultured in the commercial medium F/2 (control), an aquacultural fertilizer (Aq-F) and an agricultural fertilizer (Agr-F).

Figura 1. a) Biomasa diaria, b) concentración celular *Chaetoceros muelleri* cultivadas en el medio comercial F/2 (control), un fertilizante acuícola (Aq-F) y uno agrícola (Agr-F).

reported for commercial laboratories specialized on shrimp larvae production (López-Elías *et al*., 2003). In particular, Aq-F had the same efficiency than the conventional F/2 media, in terms of biomass, which support the use of Aq-F as a reliable strategy for microalgae production.

The proximate composition of diatoms varies widely, because they are affected by several factors. However, the results of the three treatments were similar (except for protein) to those reported by Medina-Reyna & Cordero-Esquivel (1998) for *C. muelleri* cultured in Guillard F/2 medium (carbohydrate 28.7%, lipid 21.3%, protein 30.2% and ash 44.8%) at seven days of culture. The higher protein content observed in microalgae cultured with the conventional medium (control) may be associated to the higher content of total nitrogen in F/2 (12.4 g L^{-1}) prepared medium, compared to that of the agricultural and aquacultural fertilizers (≤ 10 g L^{-1}). For carbohydrate concentration, Pacheco-Vega *et al*. (2010) reported a slight increase in carbohydrate content of microalgae cultured with an agricultural fertilizer, compared to those cultured in F/2, which was a similar response than the observed in this experiment. Valenzuela-Espinoza *et al*. (2002) explained that factors influencing the carbon fixation of microalgae may result in modifications of their carbohydrate content; however, specific studies have to be done to elucidate if the mediums used affected the carbon fixation of *C. muelleri*.

The microalgae cultured in different media, had a subsequent effect on the productive response and proximate composition of copepods. The copepod production in terms of ind L^{-1}, was lower than that reported in other experiments (Hernández-Molejón & Alvarez-Lajonchére, 2003), probably because a mixture of microalgae species are commonly used to feed the copepods, in order to have a more complete nutrient profile, whereas in this experiment, a single microalgae species was used. Knuckey *et al*. (2005) observed that copepods (*Acartia sinjiensis*) feed with mono-algal diets had a lower level of development; herein, diets containing a mixture of different microalgae may have a more complete aminoacid and lipid profile than mono-algal diets.

Despite the productive responses of microalgae cultured in the conventional medium (F/2) and Agr-F were statistically similar, the lower productive response of copepods was achieved when they were fed on control microalgae; thus, the high protein content, was not reflected in high copepod production; it is possible that amino acid, fatty acid and sugar profiles from control microalgae were not adequate for culturing copepods. Contrarily, Agr-F and Aq-F microalgae had a positive effect on the productive performance of copepods, which suggests that the quality of microalgae cultured in those mediums was more adequate for the species. The higher proportion of adults and higher fecundity index of copepods fed on Agr-F microalgae may provide a better production. Regarding to fecundity index, the higher index found in both alternative mediums compared to the control, could be associated to the higher carbohydrate content of microalgae from Agr-F and Aq-F. Herein, Guisande & Harris (1995) implicated carbohydrates as a source of metabolic energy for embryogenesis and thus it is possible that carbohydrate reserves are also an energy source during embryogenesis.

In general, copepod proximate composition of the different treatments had a similar composition than that reported for *A. southwelli* (Protein 63.8%, lipid 16.9%, carbohydrate 10.5% and ash 5.1%) and *A. centrura* (Protein 63.9%, lipid 17.0%, carbohydrate 10.5% and ash 5.3%) (Vengadeshperumal *et al*.,

Table 3. Chemical proximate composition (dry basis) of *C. muelleri* cultured in three different mediums.

Tabla 3. Composición química proximal (base seca) de *C. muelleri* cultivada en tres medios diferentes.

Treatments	Carbohydrate (%)	Lipid (%)	Protein (%)	Ash (%)
Agr-F	22.75 ± 1.19b	26.35 ± 1.52a	14,62 ± 1.95a	35.5 ± 0.4a
Aq-F	21.97 ± 0.96b	27.33 ± 0.50a	15.48 ± 0.72a	35.8 ± 0.5a
Control	18.39 ± 0.96a	27.34 ± 2.02a	18.06 ± 1.07b	34.9 ± 0.7a

*Different letters in the same column indicate significant differences (Chi square test, $P < 0.05$).

Table 4. Production parameters of the copepod *Acartia* sp., fed with microalgae *C. muelleri* previously cultured in different mediums.

Tabla 4. Parámetros de producción del copépodo *Acartia* sp., alimentado con la microalga *C. muelleri* previamente cultivada en diferentes medios.

Treatments (Feed source)	Copepods mL^{-1}	Adult-nauplii ratio	Fecundity index
Agr-F microalgae	2.31 ± 0.49ab	2.6:1	0.74 ± 0.09b
Aq-F microalgae	2.52 ± 0.22b	2.1:1	0.76 ± 0.07b
Control microalgae	1.97 ± 0.40a	2.2:1	0.49 ± 0.11a

*Different letters in the same column indicate significant differences (One way analysis of variance, $P < 0.05$).

Table 5. Chemical proximate composition (DB) of copepod *Acartia* sp., fed on *C. muelleri* previously cultured in different mediums.

Tabla 5. Composición química proximal (BS) del copépodo *Acartia* sp., alimentado con *C. muelleri* previamente cultivada en diferentes medios.

Treatments (Feed source)	Carbohydrate (%)	Lipid (%)	Protein (%)	Ash (%)
Agr-F microalgae	15.71 ± 1.37b	22.63 ± 2.40a	52.02 ± 1.31a	5.5 ± 0.2
Aq-F microalgae	12.78 ± 0.76a	22.59 ± 1.11a	54.88 ± 1.52a	5.7 ± 0.3
Control microalgae	13.20 ± 2.20ab	21.75 ± 0.48a	58.52 ± 1.83b	5.4 ± 0.3

*Different letters in the same column indicate significant differences (Chi square test, $P < 0.05$).

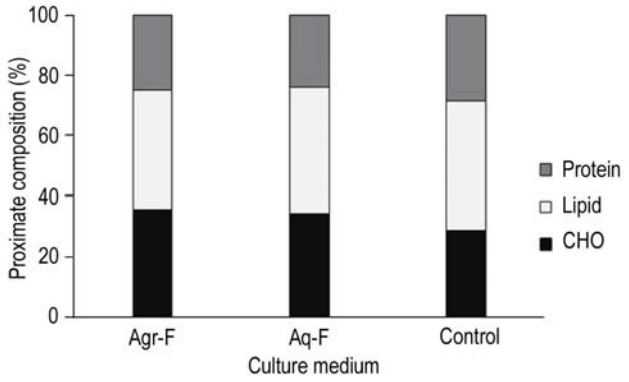

Figure 2. Proximate composition percentage of the organic fraction of *C. muelleri* cultured in different mediums. Agr-F (medium based on an agricultural fertilizer), Aq-F (aquacultural fertilizer) and control (conventional medium F/2).

Figura 2. Porcentaje de la composición proximal de la fracción orgánica de *C. muelleri* cultivada en diferentes medios. Agr-F (medio basado en un fertilizante agrícola), Aq-F (fertilizante-acuícola) y control (medio convencional F/2).

2010). However, the microalgae consumption had an effect on the proximate composition of copepods; some of the changes were related to the proximate composition of the microalgae, for instance, copepods fed on control microalgae which had the highest protein content, showed a higher protein percentage in their proximate composition. A similar tendency was observed for carbohydrate content. It is important though, to study the proximate composition from a deeper perspective in further experiments, considering the amino acid, fatty acid and sugar profiles of microalgae and copepods. Nevertheless, the results indicate that the quality of copepods have a dependence on the medium used to culture the microalgae they consume.

From the results, it can be concluded that both fertilizers (Agr-F and Aq-F) can be adequate alternatives to be used for *C. muelleri* culture. In particular, Agr-F can achieve higher microalgae productions than F/2 medium. Microalgae cultured in the conventional medium F/2, are not adequate for copepod rearing; rather, the use of Agr-F or Aq-F are more recommendable if the microalgae will be used for copepods. The productive response and the quality of copepod as feed source for aquaculture purposes, depend not only upon the microalgae species they consume, but also on the nutritional quality of such species.

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