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EFFECT OF DIATOM AND DINOFLAGELLATE DIETS ON EGG PRODUCTION AND INGESTION RATE OF *Centropages furcatus* (COPEPODA: CALANOIDA) FROM A SUBTROPICAL BAY (BAHÍA DE LA PAZ, GULF OF CALIFORNIA)

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ABSTRACT. This study experimentally determined the role of local diatom and dinoflagellate diets and their fatty acid composition on the survival, ingestion, and egg production rates of the copepod *Centropages furcatus* from Bahía de La Paz. The fatty acid profiles of the diatoms *Odontella longicruris* and *Chaetoceros* sp*.,* and of the dinoflagellates *Scrippsiella* sp*., Gyrodinium* sp*.,* and *Prorocentrum micans* were determined. After incubating at 24 °C in darkness during 24 h, survival within all phytoplankton diets was > 90%. Dinoflagellate diets provided higher egg production (>25 eggs female^{-1} day^{-1}) than diatom diets (<10 eggs female^{-1} day^{-1}). No significant differences were observed in the ingestion rates when fed dinoflagellates or diatoms, which varied between 400 and 900 ng C copepod^{-1} h^{-1}. Higher egg production with dinoflagellate diets suggests better food quality, which may be attributed to higher proportions of the fatty acids 18:4 (n-3) and 22:6 (n-3). These results suggest that when *C. furcatus* predominantly graze on dinoflagellates egg production will increase. Higher abundances of dinoflagellates in the La Paz bay could be coupled with higher egg production of the copepod *C. furcatus*.

Keywords: *Centropages furcatus*, egg production, fatty acids, Bahía de La Paz, phytoplankton.

Efecto de dietas con diatomeas y dinoflagelados en la producción de huevos y tasas de ingestión de *Centropages furcatus* (Copepoda:Clanoidea) de una bahía subtropical (Bahía de La Paz, Golfo de California)

RESUMEN. El objetivo del presente trabajo fue estimar bajo condiciones de laboratorio el efecto de dietas de diatomeas y dinoflagelados en las tasas de sobrevivencia, ingestión y producción de huevos del copépodo *Centropages furcatus* recolectado en la Bahía de La Paz. Se determinó el perfil de ácidos grasos de las diatomeas *Odontella longicruris* y *Chaetoceros* sp. y de los dinoflagelados *Scrippsiella* sp*., Gyrodinium* sp. y *Prorocentrum micans*, suministrados como alimento a *C. furcatus*. Después de incubar a 24 °C en oscuridad durante 24 h, la sobrevivencia de las hembras en todas las dietas fue > 90%. Las dietas de dinoflagelados favorecieron una mayor producción de huevos (>25 huevos hembra^{-1} día^{-1}) con respecto a las diatomeas (<10 huevos hembra^{-1} día^{-1}). No se observaron diferencias significativas en las tasas de ingesta al alimentarse con dinoflagelados o diatomeas, que variaron entre 400 y 900 ng C copépodos^{-1} h^{-1}. Se obtuvo una mayor producción de huevos al utilizar dinoflagelados como alimento, sugiriendo una mayor calidad nutricional que se pudiera atribuir en parte a la mayor proporción de los ácidos grasos 18:4 (n-3) y 22:6 (n-3). Es posible que una mayor abundancia de dinoflagelados en la Bahía de La Paz pudieran relacionarse con una mayor producción de huevos de *C. furcatus.*

Palabras clave: *Centropages furcatus,* producción de huevos, ácidos grasos, Bahía de La Paz, fitoplancton.

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INTRODUCTION

There is often a clear functional relationship between annual cycles of zooplankton and phytoplankton productivity. In general, in polar and temperate ecosystems, the former lags the second by two months, approximately but there are different cycles in different latitudes (Fernández-Álamo & Färber-Lorda, 2006). There is only one annual peak of phytoplankton and zooplankton stocks in polar oceans, due to the brief period of light, while in the North Atlantic and other temperate waters there are typically two peaks in the annual cycle, one during spring and a smaller one during autumn (Fernández-Álamo & Färber-Lorda, 2006). In tropical and subtropical latitudes, there are not always such obvious seasonal changes, but a succession of small pulses of increases and decreases in phytoplankton and zooplankton stocks, largely modulated by local weather conditions and the movement of water masses (Sournia, 1969).

Most of the studies on egg production and ingestion rates in copepods are restricted to temperate ecosystems. Several studies have demonstrated correlations between copepod production and food quality and quantity (Roman, 1984; Kleppel, 1993), which ultimately affect production at higher trophic levels (Smith & Eppley, 1982, in Kleppel 1993).

Recently the nutritional value of diatoms for copepods has been challenged since high ingestion rates are frequently followed by low egg production and hatching success rates, including abnormal eggs and nauplii (Poulet *et al*., 1994; Laabir *et al*., 1995; Hyung-Ku & Poulet, 2000). Other studies demonstrated that some diatom species produce toxic, unsaturated aldehydes that affect normal embryogenesis (Pohnert *et al*., 2002; Ceballos & Ianora, 2003) or deform nauplii (Ianora *et al*., 2004). In several ecosystems dinoflagellates species are a major component of copepod diets (Kleppel *et al*., 1991; Cottonec *et al*., 2001), but their role has been rarely quantified and seems to depend upon the copepod species under study (Morey-Gaines, 1982) and the nutritive value of dinoflagellates (Cottonec *et al*., 2001).

Particular relationships have been found between polyunsaturated fatty acids (PUFA) and zooplankton growth and egg production, particularly 20:5 (n-3) (eicosapentaenoic acid, EPA) and 22:6 (n-3) (docosahexaneoic acid, DHA) (Jónasdóttir, 1994; Müller-Navarra *et al*., 2000; Anderson & Pond, 2000). Specific fatty acid requirements for egg production occur in different *Acartia* species (Jónasdóttir, 1994). Diatoms are typically rich in EPA, but deficient in DHA, whereas dinoflagellates contain high DHA and low EPA (Anderson & Pond, 2000). Several studies have demonstrated that egg production rates in copepods in polar and temperate ecosystems can be a biological index to estimate feeding conditions of females (Dagg, 1977; Saiz *et al*., 1993), but few studies have been done in subtropical zones to support this hypothesis.

Copepod secondary production has been extensively studied (Runge & Roff, 2000). Planktonic copepods play a key role in the transfer of material and energy between primary producers and higher trophic levels (Cotonnec *et al*., 2001; Hirst & Bunker, 2003). To assess secondary production of copepods in natural habitats, where several environmental factors have influence, the effect of each environmental factor should be studied independently maintaining the rest of the variables constant (Shin *et al*., 2003). Several studies have already considered these aspects in different copepod species of *Calanus*, *Temora*, and *Acartia* (Mauchline, 1998). Although *Centropages furcatus* Dana 1894 is numerically abundant in coastal waters of subtropical regions, such has Bahía de La Paz (Palomares-García, 1996; Lavaniegos & González-Navarro, 1999). Gómez-Gutiérrez *et al*. (1999) where the first in estimate the egg production of *C. furcatus* but few studies have dealt with this species.

The determination of grazing rates and egg production of copepods fed with different local phytoplankton species can allow a better understanding of the environmental factors that define the ecological niches of the copepods, leading the way to a description of environmental controls on community composition and on food web structure. This cannot be attained easily from field studies and requires experimental laboratory designed studies to test such trophic effects.

The goal of this study was to determine the role of dinoflagellate and diatom diets isolated from Bahía de La Paz and their fatty acid composition, on survival, ingestion rate, and egg production of the tropical copepod *C. furcatus* collected in Bahía de La Paz, under laboratory conditions.

MATERIALS AND METHODS

Cultures of *Odontella longicruris* (Greville) Hoban 1983, *Chaetoceros* sp., and *Prorocentrum micans* Ehrenberg 1833 collected from Bahía de La Paz, located on the western side of the Gulf of California and *Scrippsiella* sp. and *Gyrodinium* sp. collected from Bahía de Topolobampo on the eastern side of the Gulf of California were cloned under laboratory conditions (Table 1, Fig. 1). Vegetative cells of these species were collected by vertical tows with a 20-µm phytoplankton net. The phytoplankton was sieved through a 60-µm mesh screen to eliminate larger organisms. It was then placed in a 250-mL culture container filled with filtered seawater. In the laboratory, phytoplankton vegetative cells were isolated with micro-pipettes under an inverted microscope. Single cells and chains were transferred to 96-well plates with modified f/2 medium (Anderson *et al*., 1984) and maintained at 24 ± 1°C with 150 µE m^{-2} s overhead illumination supplied with cool-white fluorescent lights.

Culture media were prepared with seawater obtained from the Ensenada de La Paz, a lagoon located at the southern part of Bahía de La Paz. Seawater was filtered through GF/F filters and sterilized in an autoclave at 121 °C and 1.1 kg cm^{-2} for 20 min. Cultures from wells were transferred to 50-mL culture tubes for maintaining the strains.

Dinoflagellate strains where grown in modified f/2 medium (Anderson *et al*., 1984) and silica was added for diatom strains. Batch cultures where cultivated in 1-L polycarbonate

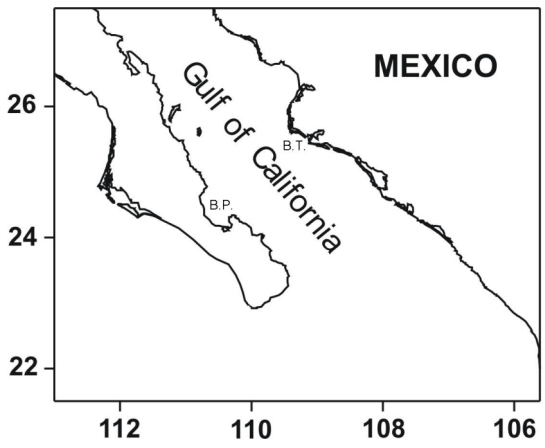

Figure 1. Location of Bahía de La Paz (B.P) and Bahía Topolobampo (B.T.) on opposite sides of the Gulf of California.

vials and maintained under temperature and light conditions described previously.

Phytoplankton carbon content was estimated from cell volume, based on length and width measurements of 30 cells from each strain (Strathmann, 1967) (Table 1).

Copepods were collected from Bahía de La Paz near the surface with a 333-µm plankton mesh net (Fig. 1). Plankton samples were transferred to the laboratory in iceboxes filled with *in situ* surface seawater. In the laboratory, adult females of *C. furcatus* where separated manually using a stereoscopic microscope and acclimated for two hours in filtered seawater at 24 °C and salinity of ~35 psu. Additionally 80 adult females of *C. furcatus* were separated, stored at -20 ºC for analysis of fatty acids.

For each phytoplankton diet tested, 30 adult females were transferred to 1 L plastic flasks with 500-mL filtered seawater passed through GF/F filters and incubated in darkness at a temperature of 24 °C, salinity of 35 psu during 24 h. There were three replicates of each treatment within each trial. To determine the phytoplankton growth rate, two flasks without copepods where incubated under previously described conditions. At the beginning and the end of each trial, two mL subsamples of phytoplankton where fixed in Lugol´s iodine solution (Throndsen, 1978). At least 400 cells where counted on 1 mL Sedgwick-Rafter counting slides. Cell density was used to calculate exponential growth rates according to Guillard (1973) and female ingestion rates according to the equation of Frost (1972): $I = ([V \times g]/N) \times C$, $g = (\ln(Ci) - \ln(Cf))/(t + k)$, where, V = volume of cell suspension in each flask (mL), g = grazing coefficient, N = number of copepods in each flask, C = cell concentration (cells mL^{-1}), Ci = initial cell concentration (cells mL^{-1}), Cf = final cell concentration (cells mL^{-1}), t = time (h), and k = phytoplankton growth rate h^{-1}. Copepods incubated in filtered seawater without phytoplankton represented the initial reproductive condition of females.

After incubation for 24 h, 30 adult females in each bottle were gently separated through a 200-µm mesh screen and eggs and nauplii were collected in a 50-µm mesh screen. Surviving females, eggs, and nauplii where counted. Surviving females were pooled to complete at least 1mg of dry biomass for fatty acid analysis.

Table 1. Isolation date, geographical origin, cell volume, carbon content, and initial carbon concentration of different phytoplankton, and survival outcome of the copepod *Centropages furcatus* fed a diet composed of each phytoplankton species

Phytoplankton diets	Isolation date	Source	Cell volume (μm^3)	Carbon content ($pg\ cell^{-1}$)	Initial concentration ($\mu g\ C\ L^{-1}$)	Survival (%)
Odontella longicruris	February, 2004	Bahía de La Paz	18,536	650	1000	91.8 ± 2.1
Chaetoceros sp.			31	650	1000	90.1 ± 10
Scrippsiella sp.	November, 2004	Bahía de Topolobampo	2,478	352	1000	96.9 ± 4.4
Gyrodinium sp.			486	83	400	100 ± 0
Prorocentrum micans	May, 2004	Bahía de La Paz	6,898	873	800	100 ± 0
No food (control)						97.2 ± 3.0

Dry biomass of microalgae and copepods were obtained by centrifugation and washed with 0.5 M ammonium formate before lyophilization (Virtis, SL, Gardiner, NY). Extraction and methanolysis of fatty acids where carried out by direct trans-esterification (Lepage & Roy, 1984, 1986; Barnung & Grahl-Nielsen, 1987). Samples were placed in thick-walled glass tubes adding 500 L dry methanolic HCl, 2 N (Supelco, Bellefonte, PA) tightly closed with teflon-lined caps, sonicated for 20 min and heated at 90 ºC for 2 h in a water bath (Terlab, Guadalajara, Mexico). Excess of hydrochloric acid was removed under a nitrogen stream. The remaining solution was mixed with 4 mL hexane and 0.5 mL distilled water, mixed for 1 min with a vortex and centrifuged for 5 min at 10°C at 2000 g. The upper phase of hexane with fatty acids was separated and 10 L of buthylated hydroxytoluene (BHT 1%) was added to prevent oxidation.

Methyl esters were analyzed by gas chromatography with a 30 m × 0.25 mm fused silica capillary column (Omegawax, Supelco, Bellefonte, PA), with polyethylene glycol as the stationary phase with a thickness of 0.25 mm and helium as the carrier gas. The column was mounted in a gas chromatograph coupled to a mass spectrometer detector (GCD 1800B, Hewlett-Packard, Palo Alto, CA).

The chromatographic conditions were: helium flow of 0.9 mL min^{-1} and injector temperature 250 °C. After injection, the temperature of the column was subjected to the following sequence: 110°C for three min, increased to 165 °C at a rate of 30 °C min^{-1}, maintained at 165 °C for two min, increased to 209 °C at a rate of 2.2 °C min^{-1}, and maintained at 220 °C for 35 min. The detector temperature was set at 260 °C and the ion source was set at 70 eV.

Fatty acid identification was based on the interpretation of their mass spectra and compared with the mass spectra generated from commercial standards of 30 fatty acid methyl esters (FAMEs) commonly reported in marine organisms (Sigma, St. Louis, USA). When fatty acid isomers where found, retention time of at least one of the isomers in commercial standards allowed double bond positioning of the other isomers because the isomers with double bonds closer to the ester group elute earlier than isomers with more remote double bonds. Differences among fatty acid detector responses were calculated by plotting five different concentrations of FAMEs, ranging from 20 to 100 µg mL^{-1} on the x-axis against their peak areas on the y-axis. Simple linear regression models of each plot estimated the response factor for each fatty acid, and its concentration (ng/µg DW-dry weight) was calculated with the formula: $F_i=[(A_i/R_i) \times V]/DW$ where, F_i = concentration of each fatty acid (ng µg DW^{-1}); A_i = peak area of each fatty acid (area units); R_i = response factor of each fatty acid (area units × µL μg^{-1}); V = hexane volume of sample (µL); and DW = sample's dry weight (µg). Fatty acid percentages were further calculated as $F_i \times 100 / F_i$ for each sample.

Carbon ingestion rates (ng C $copepod^{-1}$ h^{-1}) were transformed to daily dry mass ingestion rates (µg DW $copepod^{-1}$ day^{-1}) as follow: $DW = [100 \times I_c \times 24] / [\{(P*C_P)+ (L*C_L)+ (C*C_C)\}/100]$, where, I_c = carbon ingestion rate, P = protein, L = lipid, C = carbohydrate percentages of diatoms (Rivero-Rodríguez *et al*., 2007) and dinoflagellates (Cabell & Alatalo, 1992), and C_P, C_L, and C_C = the relative carbon content in proteins (53.06 %), lipids (77.63 %), and carbohydrates (44.44 %) respectively (Postel *et al*., 2000).

Daily fatty-acid ingestion rate of *C. furcatus* fed with diatom or dinoflagellate diets (ng copepod^{-1} day^{-1}) was calculated by multiplying the amount of each fatty acid on each phytoplankton diet (ng µg DW^{-1}) with the daily dry mass ingestion rates.

The percentage of the fatty acid concentration data were arcsine-transformed, and the ingestion and egg production rates were log-transformed prior to statistical analyses. A one-way ANOVA test was applied ($p < 0.05$), followed by Tukey's *post hoc* analyses. The relative contribution of each fatty acid to the overall fatty acid composition between diatoms and dinoflagellates diets and composition of copepods where analyzed using a cluster analysis technique estimated with single linkage and Euclidean distance. Statistical analyses were performed with Statistica v.6 software (StatSoft).

RESULTS

Characteristics of phytoplankton diets: Isolation date, source, cell volume, and carbon content of phytoplankton diets are presented in Table 1. Species volume varied from 31 to 18536 µm^3. Initial carbon concentration ranged from 400 to 1000 µg C L^{-1}.

After 24 h incubation, no significant differences were detected in the survival of adult females of *C. furcatus*. Survival rates with the different phytoplankton diets was >90.1% (Table 1). When *C. furcatus* was fed *Gyrodinium* sp. and *P. micans* no mortality was observed. Without food (control) survival of *C. furcatus* was also high (97.2%).

Egg production rates in *C. furcatus* was different with each unialgal diet (Fig. 2). Significantly higher egg production rates were found in dinoflagellate diets (>30 eggs female^{-1} day^{-1}) with the exception of the *Scrippsiella* sp. based diet. When *C. furcatus* were fed diatoms, egg production rates were below 10 eggs female^{-1} day^{-1}. No significant differences were observed in the ingestion rates in *C. furcatus* when fed dinoflagellates or diatoms (Fig. 2). Ingestion rates varied between 400 and 900 ng C copepod^{-1} h^{-1}.

Fatty acid composition of *C. furcatus* females and their different phytoplankton diets are shown in Table 2 and Figure 3. Common fatty acids in all samples were 14:0, 16:0, 18:0, 18:1 (n-9), 18:1 (n-7), 18:2 (n-6), 18:4 (n-3), 20:5 (n-3) (except in *Gyrodinium* sp.), and 22:6 (n-3). Some saturated and monounsaturated fatty acids observed in *C. furcatus* were not present in each phytoplankton diet, such as iso 18:0, 19:0, 17:0, 17:1, ante iso 17:0, ante iso 16:0, 20:1 (n-9), 22:1 (n-9), and 23:0. Higher 16:1 (n-7)/C18 PUFA and 20:5 (n-3)/22:6 (n-3) ratios, and C16 PUFAs were common in diatom diets, whereas high proportions of C18 PUFA and 22:6 (n-3) were common in dinoflagellate diets.

The biomass and fatty acid consumption per day indicate that when females of *C. furcatus* are fed with diatoms, the ingestion rate is higher (Fig. 4a). The ingestion of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) varies between each diet offered. However, the proportion of DHA is higher with dinoflagellate diets (Fig. 4b).

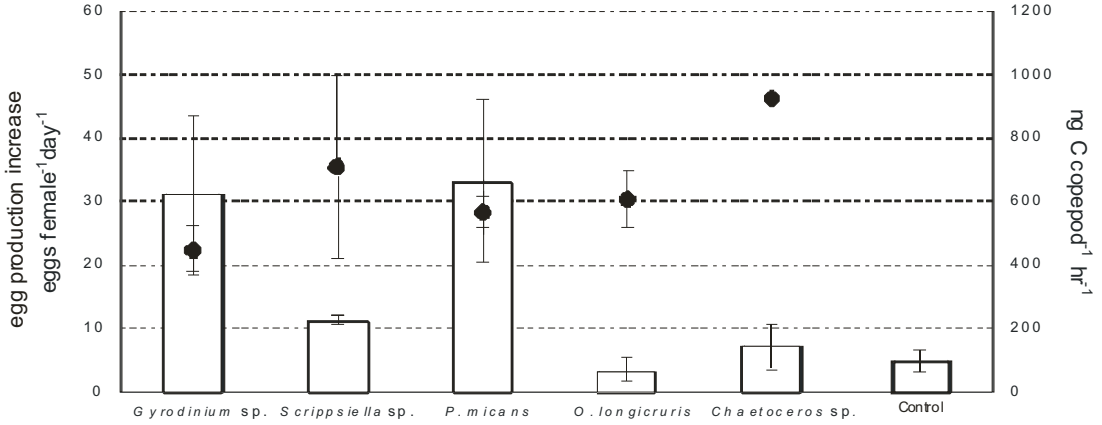

Figure. 2. Average egg production (bars, eggs/female day) and ingestion rates (circles, ng C/copepod hr) of *Centropages furcatus* fed different phytoplankton diets. Vertical whisker lines = SD.

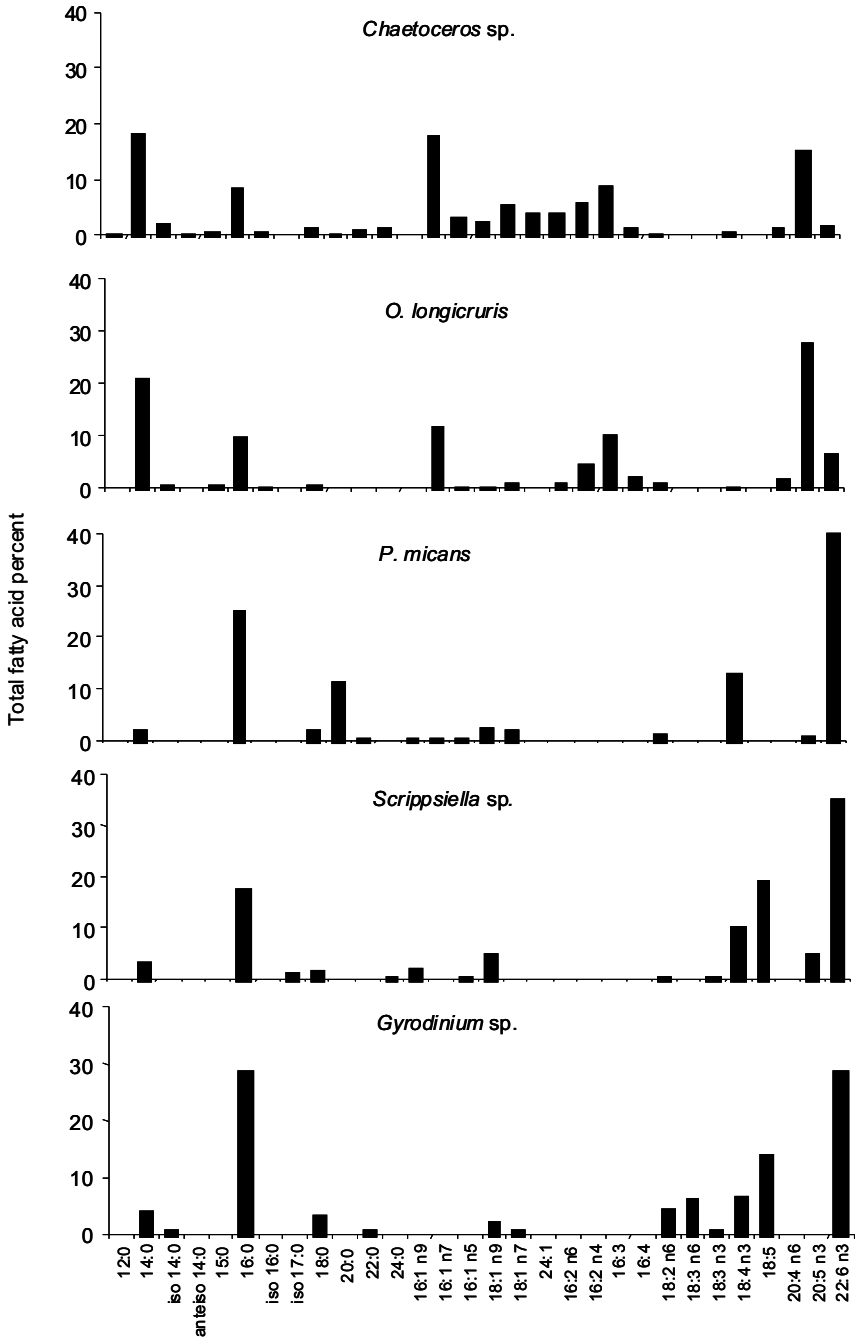

Figure. 3. Fatty acid profile (%) of diatom and dinoflagellate diets.

Greater similarities between the fatty acid profiles of *C. furcatus* collected from the field and dinoflagellate diets were confirmed by cluster analysis (Fig. 5). *C. furcatus, Gyrodinium* sp., and *Scrippsiella* sp. were grouped at a Euclidean distance of 23, whereas *P. micans* was grouped at a Euclidean distance of 24. The diatoms *Chaetoceros* sp. and *O. longicruris* formed a separated group at a Euclidean distance of 37 from *C. furcatus*.

DISCUSSION

Under our incubation conditions different phytoplankton diets (diatoms *vs*. dinoflagella-

Table 2. Comparison of percentages (%) of fatty acids in *C. furcatus,* diatoms, and dinoflagellates

	DIATOMS		DINOFLAGELLATES			
FAME	*C. furcatus*	*Chaetoceros* sp.	*O. longicruris*	*P. micans*	*Scrippsiella* sp.	*Gyrodinium* sp.
12:0	0.2	0.2	-	-	-	-
14:0	5.6	17.5	21.2	1.9	3.2	3.9
iso 14:0	0.3	1.9	0.6	-	-	0.4
ante iso 14:0	0.2	0.1	-	-	-	-
15:0	1.0	0.4	0.4	-	-	0.1
16:0	22.3	8.0	9.5	24.9	17.4	28.6
iso 16:0	0.2	0.3	0.2	-	-	-
ante iso 16:0	0.2	-	-	-	-	-
17:0	1.8	-	-	-	-	-
iso 17:0	-	-	-	-	0.8	-
ante iso 17:0	0.3	-	-	-	-	-
18:0	12.4	1.2	0.5	1.8	1.2	3.2
iso 18:0	0.1	-	-	-	-	-
19:0	0.2	-	-	-	-	-
20:0	0.4	0.1	-	11.2	-	-
22:0	1.1	0.8	-	0.3	-	0.6
23:0	0.5	-	-	-	-	-
24:0	1.5	1.1	-	-	0.5	-
SFAS	48.2	31.6	32.4	40.1	23.1	36.8
16:1 (n-9)	0.2	-	-	0.2	2.1	-
16:1 (n-7)	3.3	17.0	11.8	0.3	-	-
16:1 (n-5)	0.1	3.0	0.3	0.1	0.1	-
17:1	0.8	-	-	-	-	-
ha18:1 (n-9)	2.2	2.3	0.3	2.5	4.9	2.2
18:1 (n-7)	2.2	5.1	1.3	1.9	0.1	0.4
20:1 (n-9)	0.1	-	-	-	-	-
22:1 (n-9)	0.4	-	-	-	-	-
24:1 (n-9)	2.2	3.7	-	-	-	-
MUFAS	11.5	31.1	13.7	5.0	7.2	2.6
16:2 (n-6)	-	3.7	0.8	-	-	-
16:2 (n-4)	-	5.7	4.2	-	-	-
16:3 (n3)	-	8.5	10.2	-	-	-
16:4	-	1.4	1.9	-	-	-
18:2 (n-6)	1.0	0.3	0.9	1.2	0.3	4.5
18:3 (n-6)	-	-	-	-	-	6.3
18:3 (n-3)	0.5	-	-	-	0.2	0.4
18:4 (n-3)	0.2	0.7	0.2	12.9	10.3	6.7
18:5 (n3)	-	-	-	-	19.0	14.0
20:4 (n-6)	2.3	1.0	1.7	-	-	-
20:5 (n-3)	6.9	14.5	27.6	0.7	5.0	-
22:5 (n-3)	0.7	-	-	-	-	-
22:6 (n-3)	28.7	1.5	6.4	40.1	34.9	28.7
PUFAS	40.3	37.3	53.9	54.9	69.7	60.6
C16 PUFA	0.0	19.3	17.0	0	0	0
C18 PUFA	1.8	1.1	1.1	14.1	29.8	31.8
16:1(n-7)/C18 PUFA	1.9	15.8	10.6	0	0	0
18:1 (n-9)/18:1 (n-7)	1.0	0.5	4.0	0.8	0	0.2
20:5 (n-3)/22:6 (n-3)	0.2	9.7	4.3	0	0.1	0

tes) did not affect survival nor ingestion rates of the copepod *C. furcatus*. However, dinoflagellate diets favored higher egg production rates (>25 eggs female^{-1} day^{-1}) than diatom diets (<10 eggs female^{-1} day^{-1}), this could be explained in part by the higher ingestion of DHA with dinoflagellate diets.

The survival of *C. furcatus* was not affected when fed with *Gyrodinium* sp.; in contrast, *Acartia lilljeborgii* Giesbrecht 1889 and *A. clausi* Giesbrecht 1892 from Bahía de La Paz, had lower survival rates, 89.6 and 44.5%, when fed with *Gyrodinium* sp. (Band-Schmidt

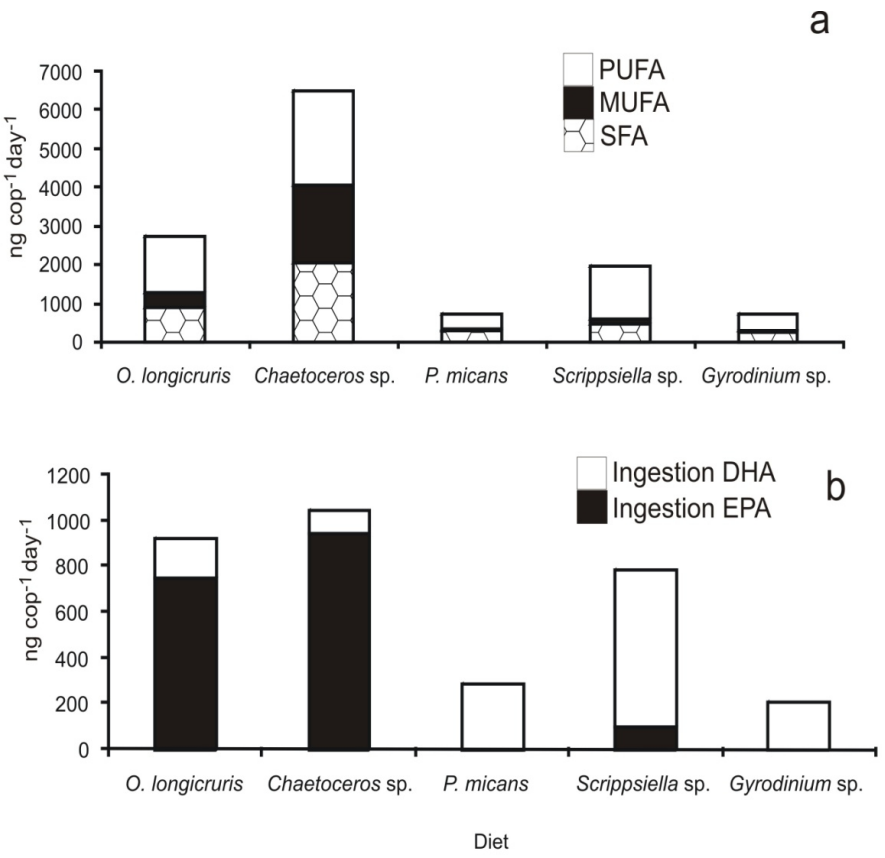

Figure 4. Daily ingestion of fatty acids of *C. furcatus* fed different phytoplankton diets.

et al. 2008). Although the possible toxicity of our *Gyrodinium* strain requires further research, results suggest that *Acartia* copepod species are probably more sensitive than *C. furcatus* to *Gyrodinium* sp. toxic metabolites. Based on egg production rates, single-diatom diets were inadequate for *C. furcatus*. When *A. clausi* and *A. lilljeborgii* were fed with diatom diets *Dytilum brightwelli* (West) Grun 1860, *Cylindrotheca closterium* (Ehrenberg) Reimann & Lewin 1964, and *Odontella longicruris* (Greville) Hobban 1983) low egg production were observed; only *Chaetoceros* sp. supported high egg production rates (Band-Schmidt *et al*., 2008). Several studies have shown that ingestion of diatoms at high concentrations (=10^3 cells mL^{-1}) are deleterious for copepod reproduction (Laabir *et al*., 1995; Ban *et al*., 1997), as was also demonstrated by low egg production and low hatching success rates, including abnormal egg and nauplii development (Poulet *et al*., 1994; Hyung-Ku & Poulet, 2000). Other studies demonstrated that some diatom species produce toxic, unsaturated aldehydes that block embryogenesis (Ceballos & Ianora, 2003) or deform nauplii (Ianora *et al*., 2004). Daily ingestion rates of fatty acids indicate that when fed diatom diets DHA ingestion is relatively low compared to dinoflagellates.

Low egg production rates occurred with diatom diets containing low proportions of 22:6 (n-3), despite the high proportions of 20:5 (n-3) (EPA) in *C. furcatus.* Diets composed of dinoflagellates yield higher egg production rates, perhaps caused by a higher content of 18:4 (n-3) and 22:6 (n-3) (DHA); similar high and low egg productions occurred in *Acartia omorii* Bradford 1976 with the same combinations of fatty acids (Kyoungsoon *et al*., 2003). Polyunsaturated fatty acids (PUFAs) particularly 20:4 (n-6), 20:5 (n-3), and 22:6 (n-3) play an important role in reproduction, 20:4 (n-6) and 20:5 (n-3) are precursors of prostaglandins, hormones regulating ion fluxes, oocyte maturation, egg production, and hatching in marine invertebrates (Stanley-Samuelson, 1987, 1994). The fatty acid 22:6 (n-3) was strongly linked

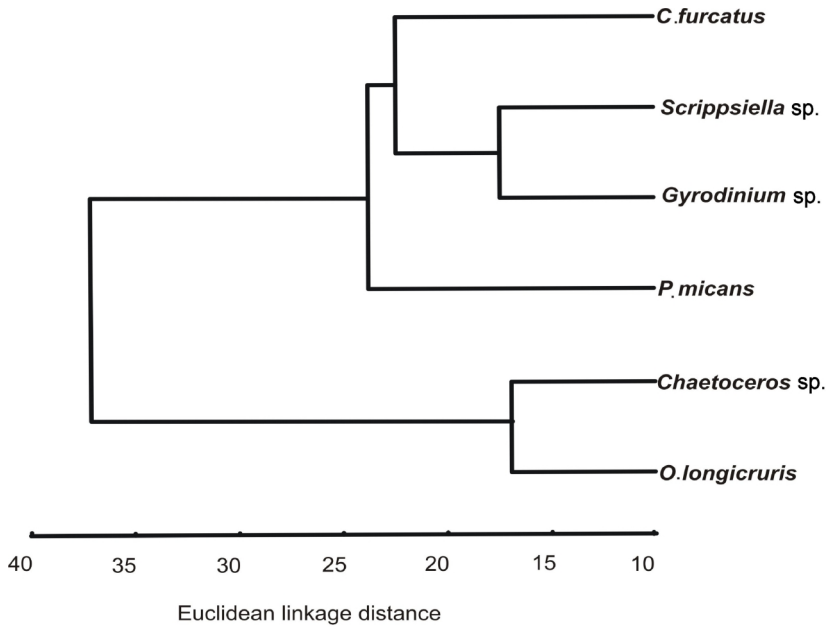

Figure 5. Euclidean distance between the fatty acid profile of the copepod *C. furcatus* and its various phytoplankton diets.

with development of neural and visual functions in fish larvae (Bell *et al.*, 1995). Several authors report that copepods require 20:5 (n-3) and 22:6 (n-3) in their diets for egg production rates. Theoretically, in omnivorous copepods, 20:5 (n-3) limits egg production when the diatom fraction is low, and 22:6 (n-3) when the dinoflagellate fraction is less abundant or as a lower contribution. However, Anderson & Pond (2000) suggest that the lack of PUFA synthesis in calanoid copepods occurs by stoichiometric limitation in carbon and nitrogen, rather than by lack of the required enzymes, and 22:6 (n-3) when the dinoflagellate fraction is low. In our study, dinoflagellates contributed with a low proportion of 20:5 (n-3), but a higher proportion of their precursor 18:4 (n-3). Limitations of zooplankton production by essential PUFAs is low if they actively synthesize them (Anderson & Pond, 2000), or if copepods can do retro-conversion of 22:6 (n-3) to shorter chains, such as 20:5 (n-3), as has been demonstrated in brine shrimp (Navarro *et al*., 1999). In this sense, dinoflagellates not only would provide essential PUFA to *C. furcatus*, but higher concentrations of carbon and nitrogen than other particles of similar size because they provide between 2 to 6 times more protein, 2.5 to 3.5 times more carbohydrate, and 1.1 to 3.0 times more lipid than diatoms of equivalent volume (Kleppel, 1993). This result is consistent with a higher response of egg production to dinoflagellate diets in *C. furcatus*. Calbet & Alcaraz (1996) suggested that a close association exists between food availability and egg production in copepods. Higher egg production using dinoflagellate diets suggests that these diets could have a higher food quality for copepods, as these values were even higher than the mean egg production of *C. furcatus* in Bahía de La Paz under satiated food conditions in laboratory studies (23 eggs female^{-1} day^{-1}) (Palomares-García *et al*., 2003). However these egg production values are much lower than those reported in other regions for *C. furcatus*, such has Bahía Magdalena with maximum values of 54 eggs female^{-1} day^{-1} (Gómez-Gutiérrez *et al*., 1999) and the Gulf of Mexico with 120 eggs female^{-1} day^{-1} (Checkley *et al*., 1992).

Different responses in egg production rates have been observed among copepod species when fed dinoflagellate diets. For instance, adult female *Centropages hamatus* Lilljeborg 1853 fed with *P. micans* and *Scrippsiella trochoidea* (Stein) Loeblich 1976 did not produce eggs, whereas *Lingulodinium polyedrum* (Stein) Dodge1989 and *G. sanguineum* Hirasaka 1922 provided the nutrients necessary for egg production (Murray & Marcus, 2002). *P. minimum* proved to be a good diet for *Temora stylifera* Dana 1849, promoting moderate egg production and excellent egg viability

(Ceballos & Ianora, 2003). Hatching success and nauplii production of *A. clausi* decreased after ingestion of a progressively high number of toxic *Alexandrium minutum* Halim cells (Frangópulos *et al*., 2000), but the ingestion of the toxic dinoflagellate *Gymnodinium catenatum* Graham apparently had no adverse effects. The high ingestion and egg production rates of *C. furcatus* suggest that these dinoflagellates have higher nutritional value than natural phytoplankton assemblages (Palomares-García *et al*., 2003).

The lack of significant differences in ingestion rates between diets of dinoflagellates or diatoms might indicate that *C. furcatus* is a less selective feeder than other copepod species, such as *Acartia lilljeborgii* and *A. clausi,* that exhibit significant differences in ingestion rates between single-celled algae diets (Band-Schmidt *et al*., 2008).

A more diverse diet might increase the probability that *C. furcatus* will obtain a nutritionally-complete ration under different environmental food conditions. *C. furcatus* also feeds heavily on micro-zooplankton (Kleppel, 1993), reflecting its dual herbivorous and carnivorous foraging. The ratio between 18:1 (n-9) and 18:1 (n-7) fatty acid isomers has been proposed as a relative measure of carnivorous habits in several groups of marine invertebrates (Graeve *et al*., 1997; Falk-Petersen *et al*., 2000). Although *C. furcatus* can feed heavily on microzooplankton (Kleppel, 1993), a ratio of 1.0 suggests a higher phytoplankton contribution to its diet than for euphausiids from Bahía de La Paz, with a ratio of 3.1 (Del Angel-Rodríguez *et al*., 2008), or for carnivorous amphipods, with ratios ranging from 3.2 to 4.9 (Auel *et al*., 2002). The quotients between 16:1 (n-7) *versus* C18 PUFAs and 20:5 (n-3) versus 22:6 (n-3) have been proposed as indicators of diatom consumption (Graeve *et al*., 1997). Fatty acid composition of *C. furcatus* collected from the sea showed a low ratio (1.9%) of 16:1 (n-7)/C18 PUFA and a low ratio (0.2%) of 20:5 (n-3)/22:6 (n-3), suggesting low diatom consumption. Also, *C. furcatus* collected from Bahía de La Paz showed a higher proportion (29.4%) of 22:6 (n-3), suggesting higher dinoflagellate consumption at the time of collection.

Our study suggests that, under natural conditions, *C. furcatus* predominantly graze on dinoflagellate species. However, a more diverse diet may increase the probability of success in *C. furcatus* to attain the adequate fatty acid composition to fuel its reproduction under different environmental conditions. Grazing on diatoms may provide enough food for survival, but egg production will probably decrease. It is quite possible that higher concentrations of dinoflagellates provide higher biomass production of *C. furcatus* in Bahía de La Paz .

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