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Egg production of the copepod *Acartia tonsa* in Florida Bay: role of fatty acids in the nutritional composition of the food environment

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ABSTRACT: The relationship between copepod egg production (EP) and nutritional constituents of the seston was investigated in a variety of environments in Florida Bay. Seston samples were collected for analyses of protein, carbohydrate, lipid, and specific fatty acid concentrations, and egg production rates of the planktonic calanoid copepod *Acartia tonsa* were measured during 'non-bloom' conditions in January and May of 1998 near Rankin Key, during a diatom-dominated bloom in January of 1999 near Flamingo, and during a flagellate-dominated bloom in October of 1999 near Rabbit Key. Fatty acid concentrations were also measured in copepod adults and eggs off Rabbit Key. Egg production rates from Rankin Key were low (2.7 to 2.8 eggs female^{-1} d^{-1}) compared with rates from the 'bloom' sites (25.0 to 56.3 eggs female^{-1} d^{-1}). Concentrations of nutritional constituents in the seston were low off both Rankin Key and Flamingo, but significantly higher off Rabbit Key. Adult copepods contained high proportions of omega-3 fatty acids (18:3ω-3, 20:5ω-3, and 22:6ω-3), but of these only 18:3ω-3 was present in the eggs. Although EP varied independently of the concentrations of proteins, carbohydrates, lipids, and most fatty acids, it varied directly with the concentration of the 18:3ω-3 fatty acid.

KEY WORDS: *Acartia tonsa* · Nutritional ecology · Fatty acids · Proteins · Lipids · Carbohydrates

INTRODUCTION

Copepod egg production is affected by both food concentration and food quality (Kleppel 1993). Extensive research has been conducted on the effects of food concentration on egg production, but comparatively little is known about the effects of food quality, particularly nutritional composition, on egg production (Roman 1984, 1991, Kleppel & Burkart 1995). A poor quality diet may result in food limitation even when food concentrations are high (Durbin et al. 1983, Kleppel & Burkart 1995, Burkart 1998). Changes in food quality can influence the assimilation efficiency (Angel 1984), which, in turn, may affect energy allocation for metabolism, somatic growth and reproduction. Because adult copepods exhibit almost no somatic growth, the assimilated energy budgeted to growth is expressed predominantly as egg production (Kiørboe et al. 1985). Copepod egg production can thus reveal a great deal about the nutritional quality of the food environment (Støttrup & Jensen 1990).

Florida Bay is a mangrove-dominated estuary at the southern tip of the Florida peninsula, centered at 25.00° N, 80.75° W. The bay is comprised of a network of shallow and well-mixed basins, with minimal vertical variability in temperature and food distributions (Fourqurean et al. 1992). The phytoplankton assemblage varies between basins, allowing them to be compared as distinct 'microcosms' in studies of trophic dynamics (Burkart 1998, Kleppel et al. 1998b). However, Florida Bay is also a perturbed system, where urban and agricultural development on the mainland over the past 50 yr have severely altered the bay's hydrology and chemistry. Since 1987, large areas of sea grass in the bay have died, and persistent blooms of cyanobacteria have occurred (Fourqurean et al. 1992).

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Acartia tonsa is the biomass-dominant calanoid copepod in Florida Bay (Ortner et al. 1998). It readily adapts to a range of changes in the food environment, and can reproduce at relatively low food concentrations (Kiørboe et al. 1985). In Florida Bay, *A. tonsa* consistently consumes phytoplankton at rates that are higher than typical for this species (Kleppel & Hazzard 2000, G. S. Kleppel unpubl. data). Kleppel et al. (1996) found particle consumption to be non-selective in >80% of experiments conducted with *A. tonsa*. It responds rapidly (<24 h) to the food environment (Tester & Turner 1990), making this an appropriate species for studying the relationship between diet and egg production (Jónasdóttir et al. 1995).

Although copepod egg production is strongly influenced by the lipid content of the diet (Gatten et al. 1980, Støttrup & Jensen 1990), correlations have not previously been found between *Acartia tonsa* egg production and total lipid content of the seston in Florida Bay (Kleppel & Hazzard 2000). However, egg production (EP) may be better correlated with specific lipid components, such as certain fatty acids and sterols, rather than total lipids (Ederington et al. 1995). For instance, the 20:5ω-3 and 22:6ω-3 fatty acids are essential for growth in many species (Støttrup & Jensen 1990, Mueller-Navarra et al. 2000), and are vital to the physiological functions of cell membranes (Pond et al. 1996). Egg production by copepods, including *A. tonsa*, has been found to be correlated with the ratio of 20:5ω-3 to 22:6ω-3 (Støttrup & Jensen 1990, Jónasdóttir 1994, Jónasdóttir et al. 1995, Kleppel & Burkart 1995), although reasons for this correlation are not completely understood.

Kleppel et al. (1998b) found egg production rates by *Acartia tonsa* in Florida Bay to be low (5.8 to 14.2 eggs female^{-1} d^{-1}) relative to the performance of this species at other locations. Considering the exceptionally high feeding rates observed during this study (>100% body C d^{-1}), higher rates of egg production would be expected (Kleppel et al. 1998b, Kleppel & Hazzard 2000). Temperature-dependent models predict that, at typical Florida Bay temperatures, copepods will produce at least 20 eggs female^{-1} d^{-1} under nutritionally replete conditions (Kleppel et al. 1996, Burkart 1998). It has been suggested that reduced crustacean production in Florida Bay may be partially due to food composition (Kleppel 1992) or possibly chemical toxicity (Scott et al. 2002).

In this study, we examined the relationship between copepod egg production and the nutritional quality of the seston food environment. Egg production rates of *Acartia tonsa* in Florida Bay were measured during various types of phytoplankton blooms, including diatom-dominated, flagellate-dominated, and 'non-bloom' conditions. Proteins, carbohydrates, lipids and specific fatty acid concentrations were analyzed in the seston food environment. Correlations between egg production and concentrations of nutritional constituents were ascertained.

MATERIALS AND METHODS

Sampling. To assess a variety of nutritional environments, copepod egg production was measured under both 'bloom' and 'non-bloom' conditions in Florida Bay (Fig. 1). Seston samples were collected off Rankin Key during non-bloom conditions on January 20, 1998, and May 27, 1998. Protein, carbohydrate, and lipid concentrations and egg production data from Rankin Key have been published previously (Kleppel & Hazzard 2000), but fatty acid composition of Florida Bay seston was assessed for the first time in the present study. Samples were also collected near Flamingo during a diatom-dominated bloom on January 6 and 9, 1999, and off Rabbit Key during a flagellate-dominated bloom on October 20 and 23, 1999. Seston samples were collected for analysis of nutritional composition by filling 20 l acid-washed plastic jugs with water from just below the sea's surface. Zooplankton, for egg production measurements, were collected from a small boat towing a 202 µm Nitex-mesh ring net, 0.5 m in diameter with a solid cod end, for 5 min through the shallow-water column (2 to 3 m). Replicate samples were taken on each date (Table 1).

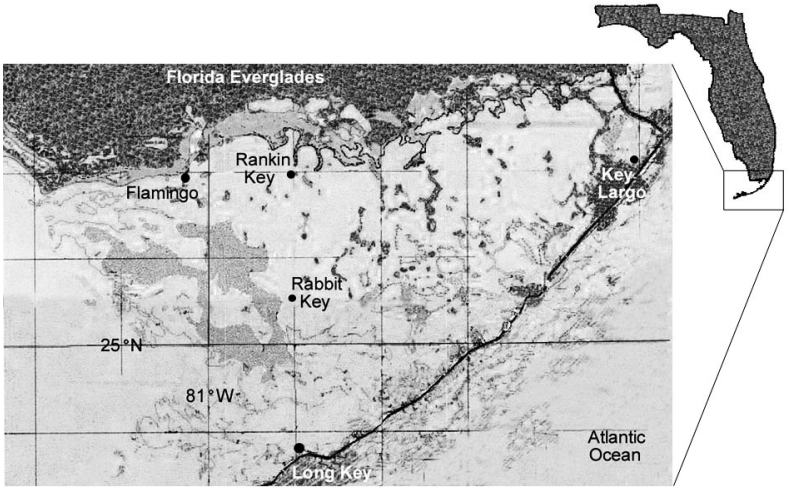

Fig. 1. Map of study sites in Florida Bay. Seston samples were collected off Rankin Key on January 20 and May 27, 1998, during 'non-bloom' conditions. Off Flamingo, samples were collected on January 6 and 9, 1999, during a diatom-dominated bloom, and off Rabbit Key, samples were collected on October 20 and 23, 1999, during a flagellate-dominated bloom

Table 1. *Acartia tonsa*. Experimental conditions of *A. tonsa* egg production

	Rankin Key		Flamingo		Rabbit Key	
	Jan 20, 1998	May 27, 1998	Jan 6, 1999	Jan 9, 1999	Oct 20, 1999	Oct 23, 1999
Condition	Non-bloom	Non-bloom	Diatom-dominated bloom	Diatom-dominated bloom	Flagellate-dominated bloom	Flagellate-dominated bloom
Seston lipid, carbohydrate, protein, and fatty acid analyses	Mean concentration for each constituent of replicate seston samples collected once a day		Mean concentration for each constituent of replicate seston samples collected every 6 h for 24 h		Mean concentration for each constituent of replicate seston samples collected every 6 h for 24 h	
	(n = 2)	(n = 2)	(n = 8)	(n = 8)	(n = 8)	(n = 8)
Egg production	Mean daily rate of replicate 24 h incubations		Mean daily rate of replicate 24 h incubations		Mean daily rate of replicate 24 h incubations	
	(n = 3)	(n = 3)	(n = 3)	(n = 3)	(n = 3)	(n = 3)

Egg production. Water from each station was passed through a 63 µm mesh Nitex screen to remove any copepod eggs prior to the experiments. Three wide-mouth polycarbonate bottles were each filled with 1 l of the prescreened water. Between 8 and 10 ovigerous adult female *Acartia tonsa* were pipetted into each bottle. Eggs were not separated from adult copepods because cannibalism of eggs is rare in this species (Dagg 1977). In previous studies, *A. tonsa* consumed <3% of their eggs (Kleppel 1992, Kleppel et al. 1998b). The bottles were incubated *in situ* for 24 h in translucent plastic boxes suspended just below the water's surface. The boxes allowed ~5% incident light penetration, ensuring a natural photoperiod, while minimizing photo-oxidative tissue damage (Krinsky 1971, Kleppel et al. 1988). Incubating *in situ* utilized the natural motion of the water column to keep the seston particles in suspension (Kleppel 1992). The 24 h incubation was adequate for ingested food to be converted to egg biomass (Tester & Turner 1990, Burkart 1998). After incubation, the contents of the bottles were gently filtered through a 35 µm mesh screen to collect eggs. The material on the mesh was preserved with acid Lugol's iodine for egg counting (100×). Hatching rate was not measured in this study, but Burkart (1998) found the hatching rate of *A. tonsa* eggs in Florida Bay to be >80%.

Nutritional analysis. Water samples were filtered through a 63 µm mesh Nitex screen. Aliquots of 300 to 1000 ml of the prescreened water from each station were passed through Whatman GF/C filters under low vacuum and frozen in liquid nitrogen prior to processing (Kleppel & Hazzard 2000). GF/C rather than GF/F filters were used to capture particles within the size range (≥2 µm) of the diet of *Acartia tonsa* (Paffenhöfer 1988). Lipids were extracted and quantified by the chloroform-methanol method described by Barnes & Blackstock (1973), as modified by Carter (1995). Carbohydrates were analyzed by the method of Dubois et al. (1956), as modified by Kochert (1978). Proteins were analyzed by the method of Lowry et al. (1951), as modified by Clayton et al. (1988). Absorbence was measured on an IBM Instruments Model 9410 UV-visible scanning spectrophotometer.

Lipids for fatty acid analysis were extracted by the method of Barnes & Blackstock (1973) from seston frozen on GF/C filters as described above. Extracts were purified by the method of Folch et al. (1957). A 21:0 fatty acid standard was added after extraction to assess fatty acid recovery efficiency. Extracted fatty acids and the fatty acid standard were trans-methylated to fatty acid methyl esters (FAMEs) with boron trifluoride (Morrison & Smith 1964, Wakeham & Canuel 1990, Jónasdóttir 1992). A 19:0 FAME was added as an internal standard after trans-methylation for quantification of fatty acids. Butylated hydroxytoluene (BHT) was added to each sample as an antioxidant. The FAME content was analyzed by flame ionization gas chromatography (Hewlett-Packard 5898 Series II+ GC-FID), using a Restek RTX-5 GC column (5% phenyl, 95% methyl polysiloxane, 30 m × 0.25 mm i.d., 0.25 µm film thickness). The injection port temperature was set to 250°C with a 13 ml min^{-1} split, and the initial column temperature was 70°C, with a 10°C min^{-1} ramp to 300°C.

Fatty acid transfer. The fatty acid composition of copepod adults and eggs was assessed on October 23, 1999, off Rabbit Key. Four 1 l wide-mouth polycarbonate bottles were filled with prescreened seawater (63 µm mesh). One hundred adult female *Acartia tonsa* were pipetted into each bottle and incubated *in situ* for 24 h in translucent plastic boxes suspended just below the water's surface. After incubation, the copepods were collected on a 100 µm mesh. The contents of the mesh were rinsed onto a GF/C filter and frozen for fatty acid analysis. The eggs produced during the incubation were collected on

a 63 µm mesh, rinsed onto GF/C filters, and frozen for fatty acid analysis. The fatty acid composition of an individual egg was calculated based upon the October 23, 1999, egg production rate. The concentration of the ingested fatty acids was calculated by multiplying the seston fatty acid concentration by the ratio of carbon ingested (4.2 µg C ind.$^{-1}$ d^{-1}) to the carbon content of the seston (280 µg C l^{-1}) (G. S. Kleppel unpubl. data).

RESULTS

Egg production

A 20-fold difference in rates of EP was observed during this study (Fig. 2). EP off Rankin Key (2.8 ± 1.9 and 2.7 ± 2.4 eggs female^{-1} d^{-1}) was significantly lower (Table 2) than off both Flamingo (34.3 ± 6.7 and 25.0 ± 7.0 eggs female^{-1} d^{-1}) and Rabbit Key (29.7 ± 8.6 and 56.3 ± 14.6 eggs female^{-1} d^{-1}). The differences in EP rates off Flamingo and Rabbit Key were not significant. At all of the study sites, differences in EP rates measured on different days were not significant. Considering all experiments, EP was correlated with neither temperature (r^2 = 0.02) nor salinity (r^2 = 0.23).

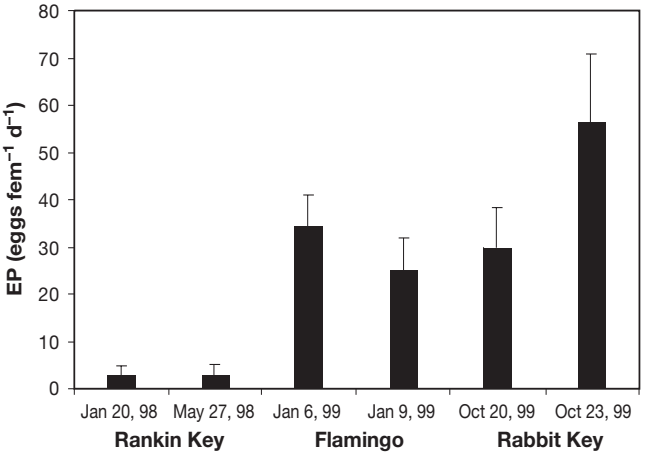

Fig. 2. *Acartia tonsa.* Egg production (EP; eggs female^{-1} d^{-1}) results, calculated as means of 3 simultaneous 24 h incubation experiments. Error bars represent +1 SD

Nutritional constituents

Lipid, carbohydrate, and protein concentrations were lowest off Rankin Key on May 27, 1998, and highest off Rabbit Key on October 23, 1999 (Table 3). In January 1998, anomalously high lipid concentrations (810.1 ± 568.2 µg l^{-1}) were removed from the data due to high error. Protein concentrations off Flamingo on January 6, 1999 were double those off Rankin Key, while protein concentrations measured 3 d later were comparable to Rankin Key concentrations. During the January 1999 Flamingo experiments, both protein and lipid concentrations differed significantly from one experiment to the next (Table 2), demonstrating the rapidity with which nutritional conditions can change in the basins of Florida Bay.

Fatty acids can be divided into 3 groups by concentration. The 14:0, 16:1, and 16:0 fatty acids were present at relatively high concentrations, up to 25 µg l^{-1}. Moderate (0.1 to 10.0 µg l^{-1}) concentrations of 18:3ω-3, 18:1 and 18:2, 18:0, 20:5ω-3, and 22:6ω-3 comprised the second group. The remaining fatty acids were scarce in Florida Bay seston. The 20:0 and 24:0 moieties were detected at trace levels (<0.1 µg l^{-1}). The 20:1 and 22:1 fatty acids were not detected in Florida Bay, although they have been reported in other estuaries (Mayzaud et al. 1989, Jónasdóttir et al. 1995). Methods of fatty acid determination recovered 83.7 ± 10.7% of the 21:0 fatty acid standard added before trans-methylation.

Table 2. *Acartia tonsa*. Statistical analysis. Mann-Whitney *U*-tests identified between and within station differences in concentrations of seston nutritional constituents and *A. tonsa* egg production (EP). Best-fit correlation coefficients assessed the relationship between each nutritional constituent and EP. Significant values ($p < 0.05$) are in **bold** type

	Mann-Whitney *U*-tests												**Correlation coefficients**	
	Between sites						Within sites						EP vs nutritional constituents	
	Rankin vs Flamingo		Rankin vs Rabbit		Rabbit vs Flamingo		Rankin		Flamingo		Rabbit			
	U	p	*U*	p	*U*	p	*U*	p	*U*	p	*U*	p	r^2	n
Lipid	4	0.134	**1**	**0.024**	**4**	**0.002**	–	–	**0**	**0.028**	4	0.342	0.750	5
Carbohydrate	20	1.056	**0**	**0.002**	**0**	**0.001**	0	0.200	1	0.058	1	0.058	0.574	6
Protein	9	0.128	**0**	**0.002**	**0**	**0.001**	1	0.400	**0**	**0.028**	5	0.486	0.642	6
18:3ω-3	**0**	**0.004**	**0**	**0.004**	**0**	**0.001**	–	–	6	0.686	7	0.886	**0.826**	6
20:5ω-3	14	0.808	**0**	**0.004**	**4**	**0.002**	–	–	1	0.058	6	0.686	0.376	6
22:6ω-3	**2**	**0.016**	**0**	**0.004**	**0**	**0.001**	–	–	7	0.886	2	0.114	0.260	6
EP	**0**	**0.002**	**0**	**0.002**	11	0.310	4	1.000	2	0.400	0	0.100	–	–

Table 3. *Acartia tonsa.* Food environment. Concentrations of nutritional constituents (mg l^{-1}) in the seston at 3 stations in Florida Bay during non-bloom (Rankin Key), diatom-dominated bloom (Flamingo), and flagellate-dominated bloom (Rabbit Key) conditions. Values in parentheses represent ±1 SD. nd: not detected

	Rankin Key		Flamingo		Rabbit Key	
	Jan 20, 1998	May 27, 1998	Jan 6, 1999	Jan 9, 1999	Oct 20, 1999	Oct 23, 1999
Lipid	nd	20.14 (23.28)	53.79 (3.77)	29.18 (2.31)	112.96 (50.66)	157.84 (26.18)
Carbohydrate	63.57 (9.35)	17.43 (3.30)	19.58 (5.62)	32.43 (8.73)	335.82 (81.39)	586.95 (179.23)
Protein	186.37 (90.15)	90.93 (100.00)	424.20 (82.16)	111.14 (17.01)	851.50 (113.78)	939.46 (105.76)
14:0	0.75 (0.16)	1.00 (0.10)	1.76 (0.48)	1.44 (0.30)	22.91 (1.56)	25.57 (3.90)
16:1	1.14 (0.09)	2.45 (0.04)	2.79 (0.69)	1.66 (0.54)	20.55 (1.51)	25.80 (3.23)
16:0	4.06 (0.09)	3.81 (0.08)	5.96 (1.52)	5.09 (1.76)	26.64 (1.97)	25.34 (5.41)
18:3ω-3	0.31 (0.04)	0.22 (0.00)	1.05 (0.25)	0.88 (0.31)	2.18 (0.70)	2.37 (1.08)
18:1 and 18:2	1.07 (0.07)	1.05 (0.00)	0.67 (0.18)	0.49 (0.10)	9.40 (0.64)	8.33 (1.37)
18:0	nd	nd	1.42 (0.13)	1.15 (0.23)	1.87 (0.35)	1.21 (0.47)
20:5ω-3	0.46 (0.10)	1.03 (0.06)	1.00 (0.40)	0.47 (0.31)	1.65 (0.20)	1.56 (0.44)
20:0	nd	nd	0.12 (0.04)	0.05 (0.05)	0.17 (0.06)	0.19 (0.06)
22:6ω-3	0.24 (0.01)	0.22 (0.04)	0.10 (0.07)	0.09 (0.09)	1.31 (0.11)	0.98 (0.26)
24:0	0.06 (0.00)	0.05 (0.00)	0.13 (0.03)	0.10 (0.03)	0.06 (0.00)	nd

Fatty acid transfer

The fatty acids in the ingested seston were predominantly the 14:0, 16:1, and 16:0 moieties. Each of these comprised ~28% of the fatty acids in the diet (Table 4). By comparison, the omega-3 fatty acids, 18:3ω-3, 20:5ω-3, and 22:6ω-3, were scarce in the diet (<3% of the total). Adult *Acartia tonsa* were composed mainly of 16:0 fatty acids (~40% of the total). The omega-3 fatty acids were present in larger proportions in the adults than in the ingested seston (~4 to 13% of the total), as 40 to 60% of the ingested omega-3 fatty acids were transferred to the adults. *A. tonsa* eggs maintained a high 16:0 content, but 20:5ω-3 and 22:6ω-3 were not detected in the eggs. 18:3ω-3 and 20:0 were transferred most efficiently from the ingested concentrations to the eggs, at 0.48 and 0.59% of each fatty acid ingested respectively.

Egg production and the nutritional environment

The nutritional composition of the food environment off Rabbit Key was unlike that of the other sites (Table 2). Seston concentrations of proteins, carbohydrates, lipids and key fatty acid (18:3ω-3, 20:5ω-3, and 22:6ω-3) were significantly ($p < 0.05$) higher off Rabbit Key than off Flamingo or Rankin Key. The food environments off Flamingo and Rankin Key differed significantly only with respect to 18:3ω-3 and 22:6ω-3 fatty acids. However, 22:6ω-3 concentrations decreased between Rankin Key and Flamingo (Table 3), while EP increased (Fig. 2).

EP rates were correlated with seston concentrations of 18:3ω-3 fatty acid ($r^2 = 0.826$; Fig. 3a), but not with any other nutritional constituent (Table 2). Correlations of EP with seston lipid and protein, though explicative of 75 and 64% of the variability in the data

Table 4. *Acartia tonsa.* Fatty acid transfer from seston, to *A. tonsa* adults, and to *A. tonsa* eggs. Each fatty acid was expressed as a concentration (±1 SD), as a proportion of the total fatty acid concentration, and as a percentage transferred from the diet

Fatty acid (FA)	Ingested (ng ind.$^{-1}$ d^{-1})	SD	% total FA ingested	Adult (ng ind.$^{-1}$)	SD	% total FA in adult	% ingested FA in adult	Egg (ng egg^{-1})	SD	% total FA in egg	% ingested FA in egg
14:0	383.60	58.50	28.0	13.04	7.93	7.7	3.4	0.11	0.07	9.7	0.03
16:1	386.97	48.45	28.2	24.44	17.94	14.4	6.3	0.31	0.22	26.6	0.08
16:0	380.07	81.15	27.7	71.49	45.01	42.1	18.8	0.47	0.40	40.4	0.12
18:3ω-3	35.51	16.20	2.6	22.20	12.45	13.1	62.5	0.17	0.08	14.7	0.48
18:1 and 18:2	124.90	20.55	9.1	14.47	11.65	8.5	11.6	0.06	0.01	5.0	0.05
18:0	18.12	7.05	1.3	nd	nd	0.0	0.0	nd	nd	0.0	0.00
20:5ω-3	23.44	6.60	1.7	13.00	18.53	7.7	55.5	nd	nd	0.0	0.00
20:1	nd	nd	0.0	1.31	0.49	0.8	0.0	nd	nd	0.0	0.00
20:0	2.88	0.90	0.2	1.55	0.41	0.9	54.0	0.02	0.00	1.5	0.59
22:6ω-3	14.76	3.90	1.1	6.25	3.95	3.7	42.3	nd	nd	0.0	0.00
24:0	nd	nd	0.0	2.17	0.54	1.3	0.0	0.02	0.01	2.2	0.00
Total FA	1370.26		100.0	169.91		100.0		1.16		100.0	

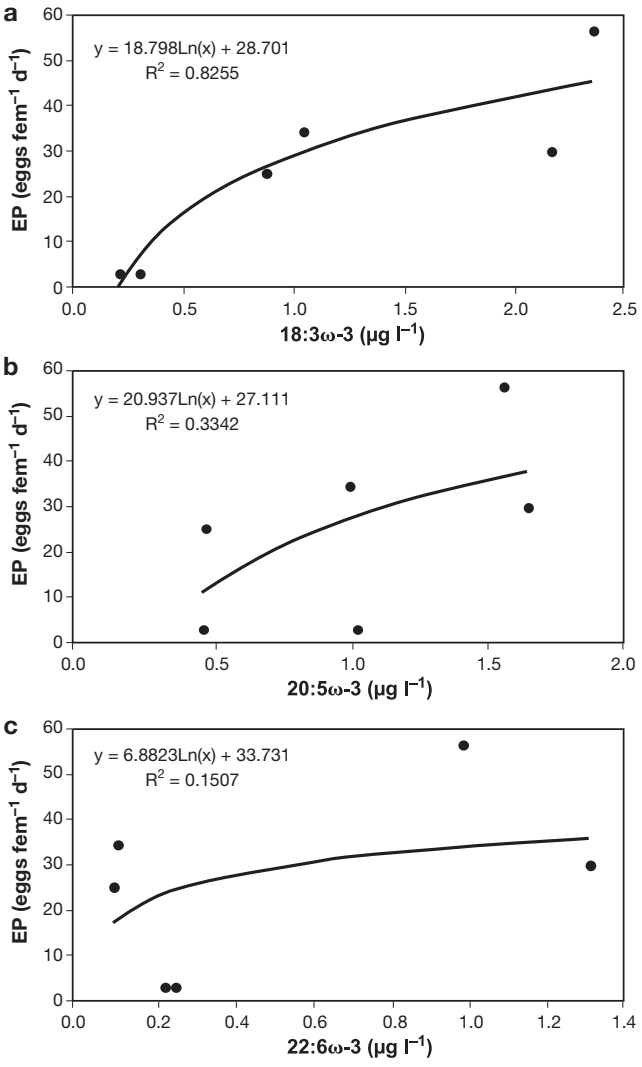

Fig. 3. *Acartia tonsa*. Egg production (EP) vs fatty acid concentration. Mean EP rates (eggs female^{-1} d^{-1}) of *A. tonsa* were logarithmically correlated with the mean concentration of (a) 18:3ω-3 fatty acid (µg l^{-1}) in the seston at 3 study sites in Florida Bay, but not with (b) 20:5ω-3 fatty acid (µg l^{-1}) or (c) 22:6ω-3 fatty acid (µg l^{-1})

respectively, were not significant due to the small sample size (Downie & Heath 1970). The fatty acid ratio of 20:5ω-3 to 22:6ω-3, which has been found to be associated with copepod egg production (Støttrup & Jensen 1990, Jónasdóttir 1994, Jónasdóttir et al. 1995, Kleppel & Burkart 1995), was also analyzed. EP was not correlated with the fatty acid ratio of 20:5ω-3 to 22:6ω-3.

DISCUSSION

The nutritional composition of Florida Bay seston, compared to results from other estuarine studies (Mayzaud et al. 1989, Jónasdóttir et al. 1995), reveals inherent variability of the food environment. Generally, proteins were the most abundant constituents of the Florida Bay seston, followed by carbohydrates, then lipids (Table 3). Hitchcock (1982) reported similar nutritional trends in cultured diatoms and dinoflagellates, while lipids were the dominant constituent in laboratory studies by Houde & Roman (1987). Limitation of dissolved inorganic nutrients in the water column can affect the composition of nutritional constituents in the food environment, causing cellular carbohydrate and lipid concentrations to increase (for energy storage), and protein content to decrease (Moal et al. 1987).

The 14:0, 16:0, and 16:1 fatty acids were the most abundant moieties in this study. Other studies (Støttrup & Jensen 1990, Jónasdóttir et al. 1995, Pond et al. 1996) include 20:5ω-3 and 22:6ω-3 among the most abundant fatty acids. However, the 20:5ω-3 and 22:6ω-3 fatty acids were scarce in Florida Bay seston (Table 3). Our analytical protocol yielded nearly 100% recovery of a 20:5ω-3 standard (Hazzard 2001). Therefore, low observed concentrations of these fatty acids are not attributed to methodological flaws, but to the nutritional variability between food environments.

Egg production by *Acartia tonsa* at Rankin Key during 'non-bloom' conditions was significantly lower than rates during both blooms (Table 2). Previous studies of other Florida Bay sites during 'non-bloom' conditions have found EP rates to be similarly low (Kleppel et al. 1998b, Kleppel & Hazzard 2000). At both bloom sites, EP by *A. tonsa* was within the range typical for this species, and the rate off Rabbit Key of 56.3 ± 14.6 eggs female^{-1} d^{-1} was within the range typical during bloom conditions (see Kleppel et al. 1998b, their Table 2). Over the course of the 6 experiments, egg production by *Acartia tonsa* appears to be associated with variability in a single key fatty acid, the 18:3ω-3 moiety (Fig. 3). Jónasdóttir (1994) also found copepod egg production to be correlated with 18:3ω-3 fatty acid concentration, along with several other fatty acids.

The importance of dietary 18:3ω-3 in egg production by *Acartia tonsa* in Florida Bay may be explained, in part, by the transfer of fatty acids from the diet to the eggs (Table 4). The 16:0 fatty acids were present in greatest proportion in the eggs, and they were readily available in the diet. On the other hand, 18:3ω-3 fatty acid comprised a moderate proportion of the fatty acid content of the eggs, but were scarce in the diet, explaining why it may limit production (Liebig 1840). Furthermore, while the 20:0 moiety was transferred most efficiently to the eggs, 18:3ω-3 levels were 8 times higher than 20:0 concentrations in the eggs. Among the omega-3 fatty acids, only 18:3ω-3 was transferred efficiently from the diet to the eggs, whereas the 20:5ω-3 and 22:6ω-3 moieties were not detected in the eggs.

Long-chain polyunsaturated fatty acids are important to embryonic development (Sargent & Henderson 1986), and the absence of the 20:5ω-3 and 22:6ω-3 fatty acids in *Acartia tonsa* eggs is significant. Marine animals are unable to synthesize 20:5ω-3 and 22:6ω-3 de novo, and a deficiency of these fatty acids in the diet may limit growth (Langdon & Waldock 1981). However, Pond et al. (1996) reported that the 20:5ω-3 and 22:6ω-3 fatty acids were not correlated with *Calanus helgolandicus* egg viability, suggesting that copepod eggs can develop normally with low concentrations of these fatty acids. Normal growth rates may be achieved by utilizing the 18:3ω-3 fatty acid (Viso & Marty 1993). It has been suggested that some copepods may be able to transform 18:3ω-3 into 20:5ω-3, but evidence of this is limited (Sargent & Henderson 1986, Jónasdóttir 1994, Kleppel et al. 1998a). Kanazawa et al. (1979) proposed the bioconversion of 18:3ω-3 to longer carbon chain omega-3 fatty acids in other marine organisms. The biochemical pathway for this transformation includes the desaturation of 18:3ω-3 to 18:4ω-3, followed by chain elongation to 20:4ω-3 and desaturation to 20:5ω-3, which can then be elongated and desaturated to 22:6ω-3 (Mead et al. 1986).

While factors other than poor food quality can influence copepod egg production, neither temperature nor salinity were correlated with nutritional variability or with egg production. The effect of poor food quality on egg production by *Acartia tonsa* off Rankin Key may be exacerbated by proximity to sources of agriculturally derived toxic contaminants, which have been shown to affect reproduction in benthic herpacticoids (Chandler & Green 1996). The arthropod endocrine disrupter endosulfan has been detected at this site (G. Scott pers. comm.). Endosulfan affects fatty acid metabolism and reproduction in grass shrimp *Palaemonetes pugio* (Wirth et al. 1999).

Although the exact function of specific fatty acids in copepod egg production is uncertain, it is apparent that specific fatty acids are influencing the productivity of *Acartiatonsa* in Florida Bay. Copepods must obtain all of the nutrients required for egg production from a diversity of phytoplankton, microzooplankton, and detrital particles in the food environment. Ultimately, some compounds may be in short supply (Liebig 1840), setting limits to egg production (assuming bottom-up control). The variety of nutritional constituents that can potentially limit production in estuarine food webs is enormous, and different constituents may be important in different places or at different times. However, our results, as well as results of other recent studies (Kleppel et al. 1998b), suggest that, in fact, only a few nutritional constituents in the food environment may drive production in a particular system.

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