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Seasonal and fine-scale spatial variations in egg production and triacylglycerol content of the copepod *Acartia tonsa* in a river-dominated estuary and its coastal plume

George B.McManus[1] and C.Alan Foster[2]

[1]*Department of Marine Sciences, University of Connecticut, Groton, CT 06340 and* [2]*Dauphin Island Sea Lab., Box 369, Dauphin Island, AL 36528, USA*

Abstract. We measured egg production rates of the estuarine calanoid copepod *Acartia tonsa* in Mobile Bay, an estuary in the northern Gulf of Mexico. Two stations were sampled approximately monthly, one at the mouth of the bay and the other just beyond the mouth in the salinity front between bay and coastal waters. Over the whole year, temperature was the most important environmental variable controlling egg production. Rates increased with temperature up to 30°C and 140 eggs female^{-1} day^{-1}. We found no evidence of food limitation. There was no correlation between egg production and phytoplankton abundance, nor increased egg production in response to supplements of phytoplankton added to natural food, suggesting that non-phytoplankton food was important in the diet. At the highest egg production rates, the amount of the storage lipid triacylglycerol (TAG) in adult females was greatly diminished, to <50 ng female^{-1}. This suggests that lipids in the diet can be very tightly coupled to egg production. Both egg production and TAG content of females showed significant variability on spatial scales of 3–15 km, especially in relation to the salinity front separating water outwelling from the bay from open coastal water. For organisms that are using copepods and their eggs as food, this variability would result in a heterogeneous food environment, both in terms of the abundance of food and its nutritional content.

Introduction

Estuarine copepods often experience large variations in both abiotic and biotic conditions. Temperature, salinity, dissolved oxygen concentration, food availability, and predation all vary substantially on both seasonal time scales and on time scales commensurate with the generation times of copepods. Spatial variability can also be great, especially that associated with strong salinity gradients. Although the food environment in eutrophic estuaries may be rich, it can also be highly variable due to changes in freshwater flow. High detrital and sediment loads may mean that food quality is low, even when quantity is high. In addition, losses due to variable flushing of populations out into coastal waters where food may be unsuitable or in low supply can be a significant source of mortality, especially in shallow, river-dominated estuaries having a small tidal range. For these reasons, estuarine copepod assemblages are often characterized by low diversity. Among the copepods that have adapted successfully to the variable estuarine environment, *Acartia tonsa* (*sensu latu*) is one of the most cosmopolitan and abundant species, occurring in temperate and subtropical waters circumglobally. In estuaries of North America, it is the dominant component of the summer/fall assemblage in temperate climates, and is present year round in warm-temperate and subtropical regions, including the entire Gulf coast (Conover, 1956; Booker, 1980). Many studies have been published about its ecology, including measurements of diet, *in situ* egg production, and egg viability (e.g. Ambler, 1985, 1986; Cervetto *et al.*, 1993;

Heinle, 1969; Paffenhofer and Stearns, 1988; Stearns *et al.*, 1989; Durbin *et al.*, 1990; Dam *et al.*, 1994; Miller and Marcus, 1994; Diaz-Zaballa and Gaudy, 1996).

Despite the many published reports on *A.tonsa*, little is known about spatial variations in its egg production over scales appropriate to the estuarine and coastal environments in which it is found. This organism is distributed across strong gradients in salinity, for example, which are maintained by freshwater inflow but subject to modification by tides, wind and other factors that cause mixing in the nearshore zone. We studied the ecology of egg production by *A.tonsa* in Mobile Bay, a shallow, river-dominated estuary on the US Gulf coast, concentrating on variations in egg production at the bay mouth and in its adjacent outflow plume on seasonal time scales. We also studied variations in egg production across the salinity front that bounds the plume to the east, where bay outflow and coastal waters of the Gulf of Mexico mix. In addition to measuring environmental variables that possibly control egg production in *A.tonsa* (temperature, salinity, food concentration), we evaluated several morphometric and biochemical condition indices for their ability to predict short-term and small spatial-scale variability in egg production. In the outflow plume, we also performed several food addition and reduction experiments to evaluate food limitation of production across the salinity gradient in the near-shelf environment.

Method

Study site

Mobile Bay is a shallow, river-dominated estuary on the US Gulf of Mexico coast (30°14′N, 88°03′W). Its average depth is 3 m except for a 13 m dredged navigation channel. It has a diurnal tide, with ~0.5 m range in water height, and an average freshwater discharge of 2245 $m^3 s^{-1}$, with high flow periods exceeding 7000 $m^3 s^{-1}$ (Pennock *et al.*, 1994). Two stations were located near the main pass (Figure 1). The Bay Mouth station was located in shallow water (3 m) just east of Dauphin Island; the Channel station was located in the navigation channel (13 m), just west of the Fort Morgan peninsula, and just outside the mouth of the bay. Since the bay's outflow plume tends to flow westward along the Dauphin Island shore, the Channel station was usually located in or near the salinity front separating the outwelling bay water from coastal Gulf of Mexico water.

Plankton collections

Plankton samples were collected at the two stations between 08:00 and 11:00 h on each of 13 sampling dates between January 1994 and April 1995. Copepods were collected by towing a 0.75 m diameter, 153 μm mesh net just below the sea surface for 5 min at ~0.5 m s^{-1}. Cod end contents were gently transferred to clean insulated containers and diluted with surface seawater for transport back to the laboratory. Five, 2.3 l polycarbonate bottles were filled with surface water at each station. This water was used in subsequent egg production incubations and for phytoplankton pigment samples. All samples were returned to the shore laboratory within an hour of collection. Temperature and salinity of the surface water

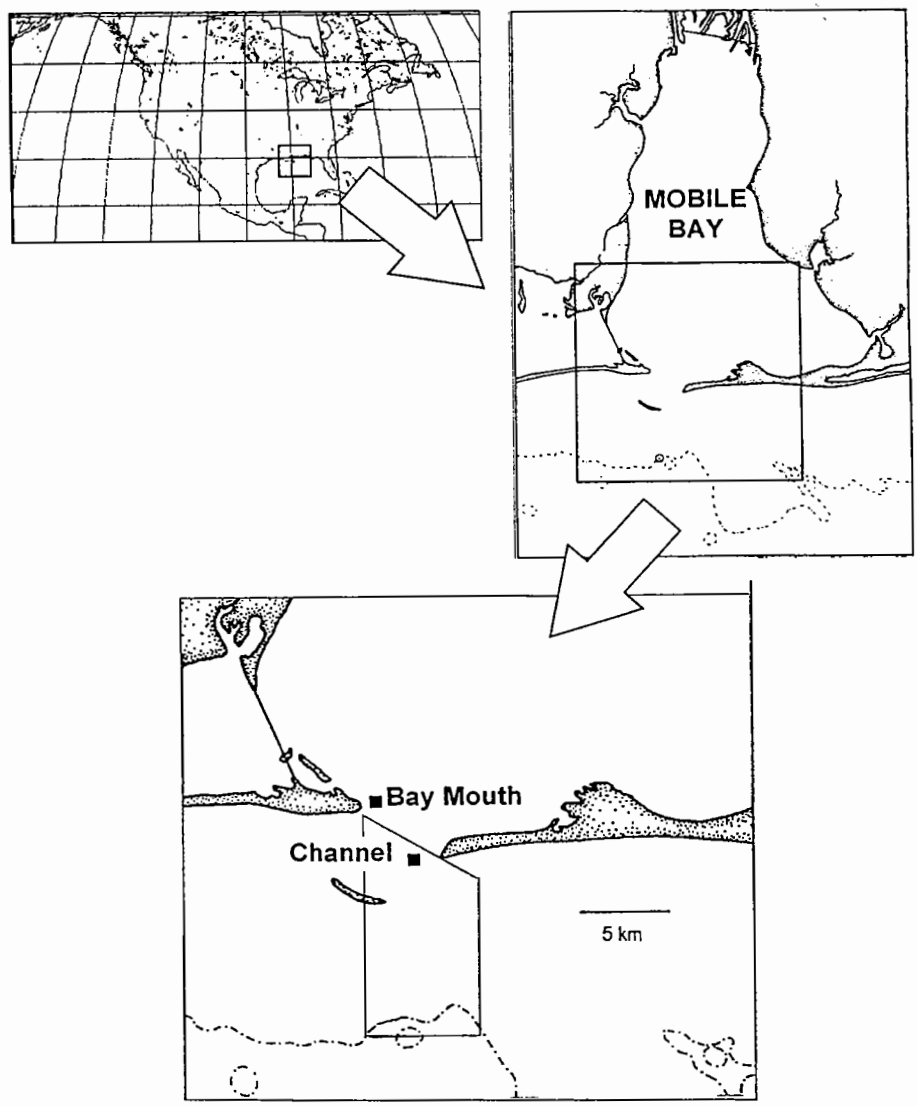

Fig. 1. Chart of sampling area, showing monthly stations (Bay Mouth and Channel), and outlining the general area of the plume cruises. Dashed line is the 20 m depth contour.

at each station were measured with bulb thermometer and temperature-corrected refractometer or inductive salinometer.

Egg production incubations

In the laboratory, subsamples of copepods were narcotized with carbonated, filtered seawater. Five adult female *A.tonsa* were separated from each subsample,

allowed to recover from narcotization, and placed in 2.3 l polycarbonate bottles containing surface seawater that had been filtered through a 64 μm sieve to remove larger zooplankton and eggs, while allowing most phytoplankton to pass. At this low density of copepods, we estimate that <20% of the bottle volume had been cleared of food by the end of the experiment, ensuring that the food environment would not be altered drastically during the incubations (Saiz *et al.*, 1997). The containers were placed in a temperature- and light-controlled incubator with side illumination of 100–200 μmol m^{-2} s^{-1} for 24 h on a 14:10 light–dark cycle at *in situ* temperature (averaged between stations when there was a difference). We used 3–5 replicate bottles per station. After 24 h, copepods and eggs were gently collected on a 64 μm mesh and placed in shallow dishes for enumeration. Egg production was calculated as total eggs (plus any hatched nauplii) produced during the incubation, divided by the number of females alive at the end of the incubation (Bellantoni and Peterson, 1987). Mortality in the incubations was <4% over all experiments.

Lipid analysis

After the incubations were under way, batches of 50 adult female copepods were separated and placed in filtered seawater for 1 h to clear their guts of food, then transferred to 40 ml test tubes containing 5 ml each of dichloromethane, methanol, and deionized water for lipid extraction. Except for the first four sampling dates, when only one batch of 50 animals was extracted, all extractions were done in triplicate for each station. Samples were sonicated and extracted three times with dichloromethane to separate completely lipid from water-soluble material. The extract was evaporated to dryness under nitrogen, and redissolved in 50 μl of dichloromethane.

Intact lipid classes were quantified by thin-layer chromatography with flame-ionization detection (TLC/FID) (Volkman *et al.*, 1986), using the procedures described in Ederington *et al.* (1995). The instrument was an Iatroscan Mark V, calibrated with triolein and cholesterol for triacylglycerol (TAG) and sterol (ST), respectively. These measurements were used to calculate a condition index (TAG:ST; Fraser, 1989; Hakanson, 1993).

C:N and morphometric analyses

Additional batches of 50 copepods were picked for length, dry weight, and C:N measurements. Prosome length of adult females was measured at 25× with a calibrated reticule. C:N was measured, after drying and weighing, on a Carlo-Erba NA1500 CNS analyzer. As with lipids, all measurements were made in triplicate for each station, except on the first four sampling dates. Length and weight measurements were used to calculate a morphometric condition index (0.1 × dry weight/length^3; Durbin *et al.*, 1983).

Pigments

For phytoplankton biomass, water samples of 500 ml were collected on glass fiber filters (Whatman GF/C) and extraced in acetone. Chlorophyll *a* in the extracts was measured by high performance liquid chromatography, as described in (VanHeukelem *et al.*, 1994). The system was calibrated with pure chlorophyll *a* (Sigma).

Plume cruises

Four cruises were made in the bay's outflow plume, with transects sampled across the front at its eastern edge. On each cruise, we measured egg production rates at 3–6 stations. Animals were picked and incubations set up at sea and bottles were stored in the dark in insulated coolers before being placed in the laboratory incubators upon return to shore at the end of the day.

During two of the plume cruises (3 March and 15 May, 1995), we evaluated how reduced or enhanced food concentrations would affect egg production at three stations, selected from surface salinity distributions to represent 'plume', 'frontal' and 'coastal' water (lowest salinity, maximum salinity gradient, and highest salinity water, respectively). We conducted egg production incubations in duplicate, as described above, but including, in addition to *in situ* food concentrations, two reduced-food treatments (50% and 100% filtered seawater from the same station), and an enhanced food treatment (whole water supplemented with cultures of the diatom *Thalassiosira weissflogii* added to bring the total chlorophyll *a* concentrations up to 2–6 × *in situ* levels).

Results

Mobile Bay is a river-dominated estuary, with large seasonal variations in river flow driving a salinity gradient between the bay mouth and coastal waters that can vary from almost zero to >10 (practical salinity scale) over a very short distance (Figure 2). Our Bay Mouth and Channel stations were only ~3 km apart, but showed distinct salinity differences through most of the year. Although the entire salinity field was not mapped on each sampling date, most of the time the Channel station was probably in or near the salinity front that separates the clear coastal water of the Gulf of Mexico from that of the bay and its coastal plume. The annual surface temperature ranges at the two stations were similar, with values of <10°C in winter, up to nearly 30°C in summer (Figure 2). Water at the shallow Bay Mouth station was cooler than that at the Channel station during the second winter of the study period.

Chlorophyll *a* concentrations were mostly <10 μg l^{-1}, and did not vary consistently between stations (Figure 2). Mobile Bay is relatively pristine, with low levels of anthropogenic nutrient inputs and strong intermittent flushing by storms and freshwater flow, compared to deeper systems in areas of greater coastal development (cf. Chesapeake Bay, Delaware Bay; Boynton *et al.*, 1982; Pennock *et al.*, 1994).

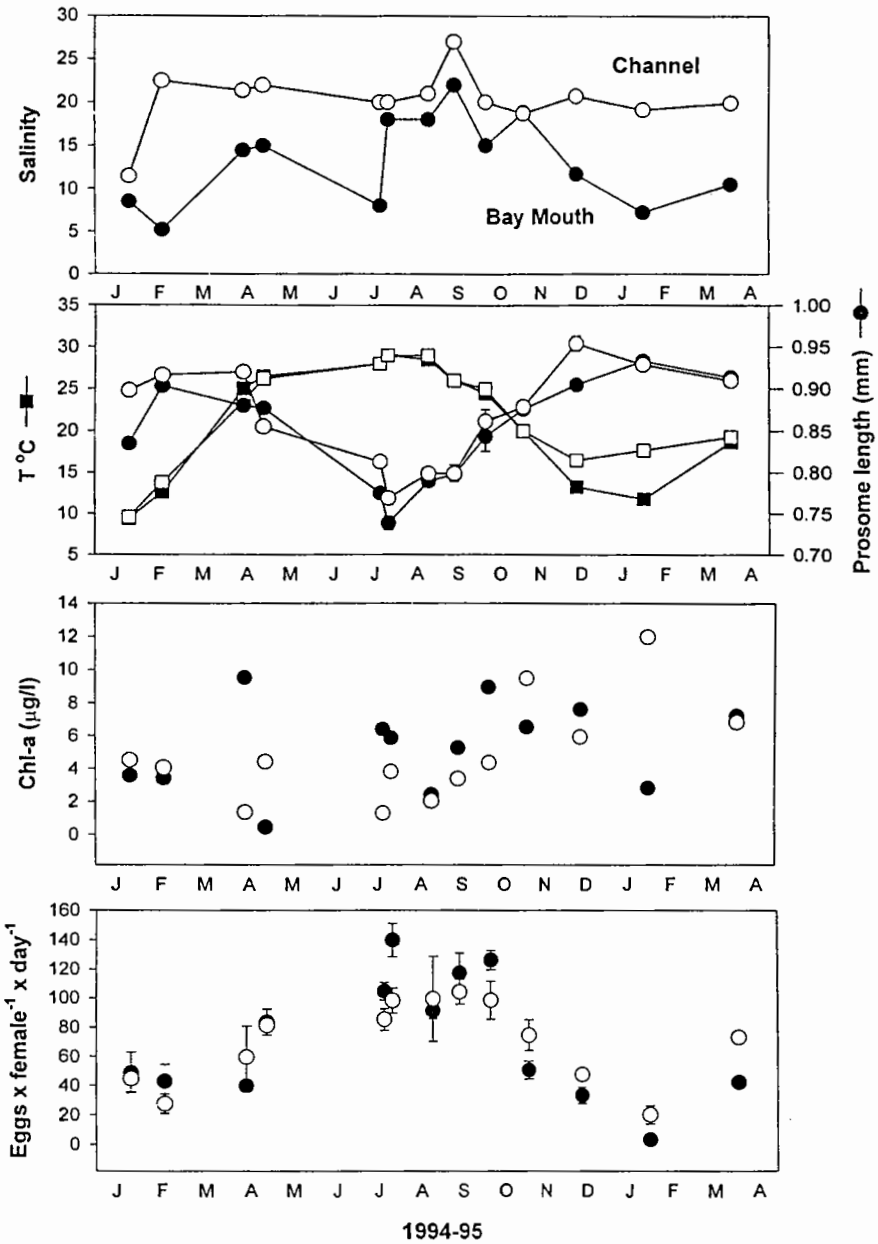

Fig. 2. Salinity (practical salinity scale), temperature, length, chlorophyll *a*, and egg production variations over the course of the study at the Bay Mouth (closed symbols) and Channel (open symbols) stations. Error bars for egg production and prosome length are standard deviations, which were sometimes smaller than the symbols. Salinity, temperature, and chl *a* measurements were not replicated.

Prosome length of adult females was inversely related to temperature, ranging from 0.738 ± 0.021 mm to 0.954 ± 0.022 mm (means, with 95% confidence intervals; Figure 2). Length increased with decreasing temperature, leveling off or declining slightly at temperatures below 15°C. Regression of prosome length on temperature (>15°C) had a slope (–0.0105 mm $°C^{-1}$) similar to those of Heinle (1969) and Ambler (1985) (–0.0116 and –0.0120, respectively). Our animals were slightly larger at all temperatures, however, resulting in differences in elevation for the regressions from the three studies (1.10 mm, 1.045 mm, and 1.014 mm for the present study, Heinle, and Ambler, respectively).

Rates of egg production tracked temperature closely, with maxima at both stations occurring simultaneouly with temperature maxima, and minima in winter (Figure 2). Greater cooling at the Bay Mouth station during the second winter was clearly reflected in lower egg production, compared to the Channel station. We observed rates of >140 eggs $female^{-1}$ day^{-1} during summer, among the highest ever observed, to our knowledge, surpassing the maximum values (~100 eggs $female^{-1}$ day^{-1}) observed by Ambler (1985) in another Gulf of Mexico estuary at a similar latitude to that of Mobile Bay.

Product-moment correlation coefficients for egg production versus chlorophyll, temperature, and salinity were evaluated. Of these, only the correlation with temperature was significant ($r = 0.79$; $P < 0.0001$).

Relationships between egg production and various indices of condition are illustrated in Figure 3. Only the morphometric condition index explained a significant amount of the variance. This reflects an increase in weight through the late fall, accompanied by a more gradual increase in length. A sharp drop in dry weight between the November and January sampling dates, while length increased slightly, resulted in a much lower value for this index during the period of low productivity in the colder months. Prosome length alone was a good predictor of egg production rates over the entire study (egg production = 487 – 483 × length; $n = 26$; $R^2 = 0.67$; $P < 0.0001$), probably due mostly to its strong correlation with temperature.

Copepod C:N did not exhibit a great enough range to be of much use as a predictor of egg production, while the TAG:ST index was weakly negatively correlated with egg production. Since most of the variability in this measurement is associated with the levels of TAG in the copepods, it is instructive to examine how this direct estimate of lipid reserves varies with egg production rates. Although *A.tonsa* does not produce an oil sac, its TAG content can show significant variation over seasonal and shorter time scales, and its boreal congeners have a pronounced seasonal cycle in fatty acid content (Norrbin *et al.*, 1990). It was this variability that encouraged us to evaluate TAG as an indicator of short-term (days) nutritional condition, and hence a possible predictor of egg production rates. TAG levels in adult females ranged from 7.3 to 224.0 ng $copepod^{-1}$. Copepods collected from the Channel station generally had higher levels of TAG, although the overall means were not significantly different between the two stations (70.8 ± 33.8 and 44.4 ± 21.4 ng $copepod^{-1}$ for the Channel and Bay Mouth copepods, respectively; means ± 95% confidence interval; $P > 0.05$ by t-test). Perhaps most surprising is the degree of variability between stations on a given

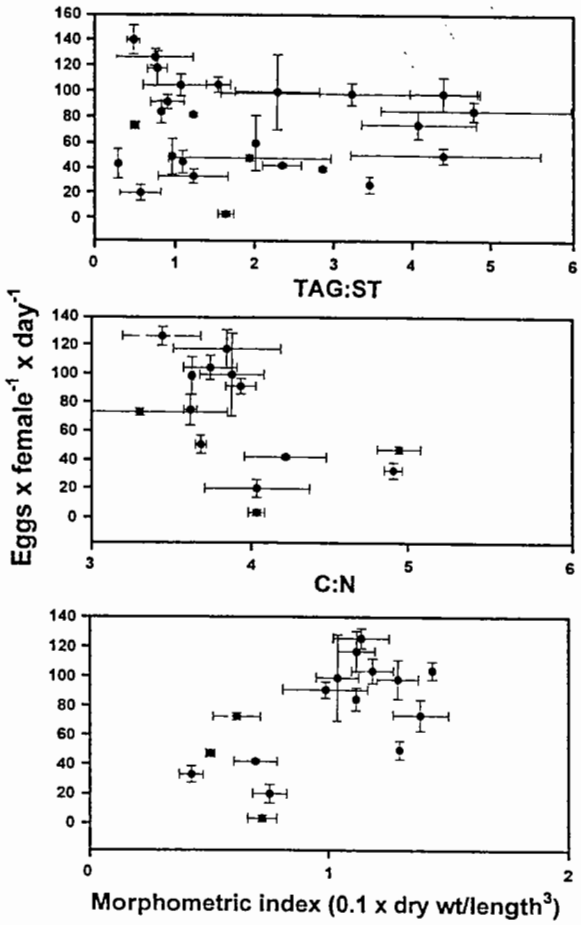

Fig. 3. Relationships between egg production rates and indices of copepod condition (means, with standard deviations). TAG:ST is the ratio of triacylglycerol to sterol content of copepods (w/w). C:N is the ratio of carbon to nitrogen in copepods (w/w). Morphometric condition index = 0.1 × dry weight/length^3.

sampling date. On six of the nine occasions for which triplicate batches of copepods from each station were analyzed, TAG levels differed significantly between stations ($P < 0.05$; t-test), indicating strong variability in nutritional quality of copepods over very small spatial scales. By comparison, sterol, which as a cell membrane component does not vary as much as other lipid components (Ederington *et al.*, 1995), differed significantly between stations on only two dates.

Although overall TAG or TAG:ST did not help predict egg production rates on short temporal or spatial scales, one clear pattern emerged when just the data for samples taken at temperatures >20°C were considered (Figure 4). The copepods with the highest egg production rates, at the higher temperatures, contained the lowest levels of TAG we observed. From 21 July through 6 October, copepods

Table I. Egg production rates (E_p; eggs female^{-1} day^{-1}) measured during the plume cruises

Date	Station	Salinity	T°C	E_p (SD)	ANOVA result[a]
24 May 94	MP1-1	18.9	24.6	61.6 (13.0)	$P < 0.05$
	MP1-2	25.9	25.1	32.0 (5.7)	
	MP1-3	24.1	25.9	28.7 (13.7)	
	MP1-4	17.3	25.7	43.9 (4.2)	
	MP1-5	19.3	25.7	36.9 (3.3)	
13 Dec 94	MP2-1	11.1	13.3	25.9 (5.2)	$P < 0.001$
	MP2-2	11.7	13.3	33.1 (5.6)	
	MP2-3	33.2	17.2	23.7 (0.6)	
	MP2-4	34.0	17.7	17.2 (1.9)	
	MP2-5	30.4	17.8	79.7 (24.3)	
	MP2-6	20.7	16.5	77.9 (2.8)	
3 Mar 95	MP3-1 (plume)	7.7	13.0	15.3 (0.5)	$P < 0.001$
	MP3-4 (front)	24.2	15.7	31.5 (1.4)	
	MP3-3 (coastal)	33.9	15.2	21.7 (0.5)	
15 May 95	MP4-1 (plume)	23.4	25.6	143.3 (15.9)	$P < 0.05$
	MP4-5 (front)	23.9	27.8	102.9 (10.7)	
	MP4-3 (coastal)	26.0	25.9	78.3 (3.9)	

[a]ANOVA results = the probability of no differences in egg production among stations.

at both stations were producing 100 or more eggs per day, and TAG levels were <50 ng per copepod. The initial cooling of the water column from 25 to 20°C between 6 October and 3 November was accompanied by a drop in egg production to 51 eggs female^{-1} day^{-1} and a sharp increase in TAG from <20 ng copepod^{-1} to 146 ng copepod^{-1} at the Bay Mouth station. Egg production also declined simultaneously with an increase in TAG for copepods at the Channel station at that time. For the remaining three sampling dates of the study, through the winter, egg production remained low and TAG declined again. This suggests some degree of lipid storage occurs in *A.tonsa* toward the end of the productive season. This

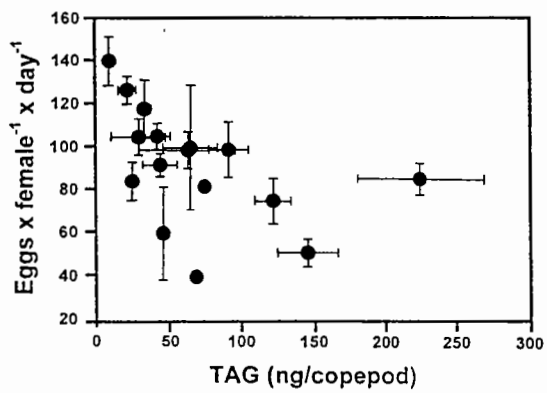

Fig. 4. Relationship between triacylglycerol content and egg production rates for all samples taken at T°C > 20 (means, with standard deviations).

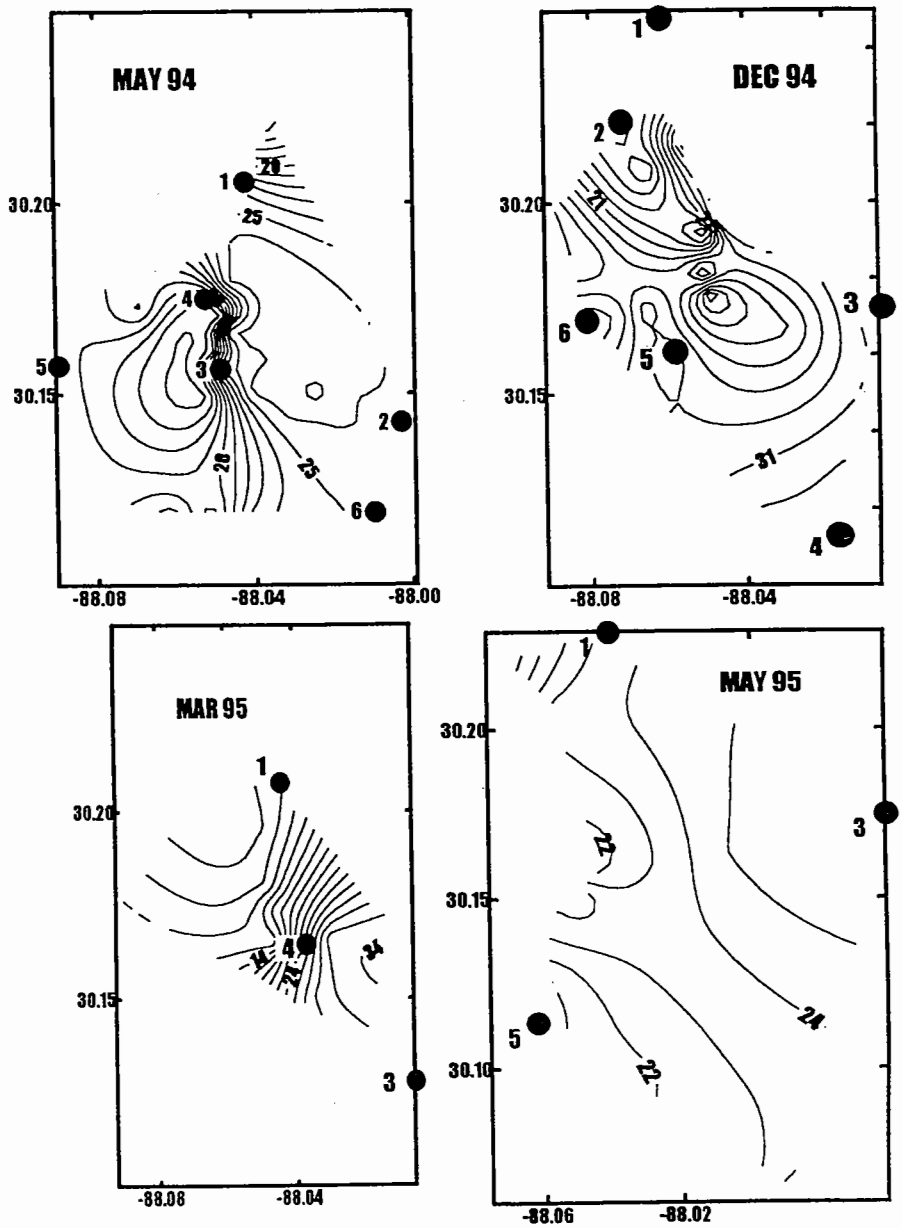

Fig. 5. Salinity contours for the plume cruises. March and December were high flow periods, as indicated by the salinity gradients. Station numbers refer to designations in Table I.

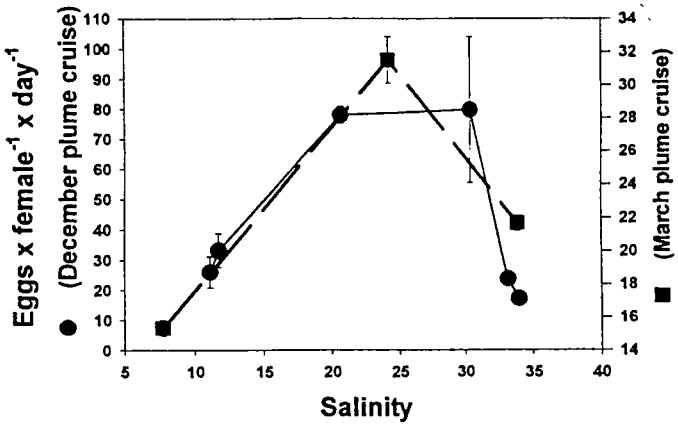

Fig. 6. Egg production was highest at intermediate salinities (frontal stations) on both of the high flow plume cruises (means, with standard deviations).

may be the direct result of lipid accumulation as egg production declines due to lowered temperature. At a TAG level of 20 ng egg^{-1} (Kiørboe *et al.*, 1985; H.R.Harvey and McManus, unpublished results), females producing 100 eggs per day in fall had ~2.5 eggs-worth of TAG in their bodies. This suggests extremely tight coupling between ingestion of food and egg production (though not necessarily food limitation of egg production). Decreasing egg production would presumably free up ingested lipid for storage.

On the plume cruises, we examined more closely the spatial variation in egg production rates across a range of salinities associated with the mouth of the bay and the plume of lower salinity water outwelling onto the coast. These cruises took place during both high flow (13 December 1994 and 3 March 1995) and low flow periods (24 May 1994 and 15 May 1995), resulting in observations of strong and weak salinity gradients, respectively (Figure 5). ANOVA was performed to test the effect of station location on egg production. In all four cases, egg production varied significantly over this small spatial scale of <15 km (Table I). For the two high flow periods, where we sampled a salinity gradient of >20, egg production was clearly lower at both high and low salinities, suggesting enhancement of the food environment at the plume front (Figure 6). For the two low flow periods, higher egg production was not related to salinity in any obvious way.

On the last two plume cruises (3 March and 15 May 1995), we performed food addition/removal experiments at three stations to evaluate the degree of food limitation of egg production *in situ*. In each case, treatments of diluted natural food from the same stations were accompanied by an undiluted treatment and one with added food (saturating concentrations of the diatom *Thalassiosira weissflogii*, grown in the laboratory). In all cases, egg production declined substantially for copepods incubated with no food (filtered seawater). In four of six cases, egg production declined at 1:1 filtered seawater:whole water from the same station; and in none of the six cases did egg production increase with added

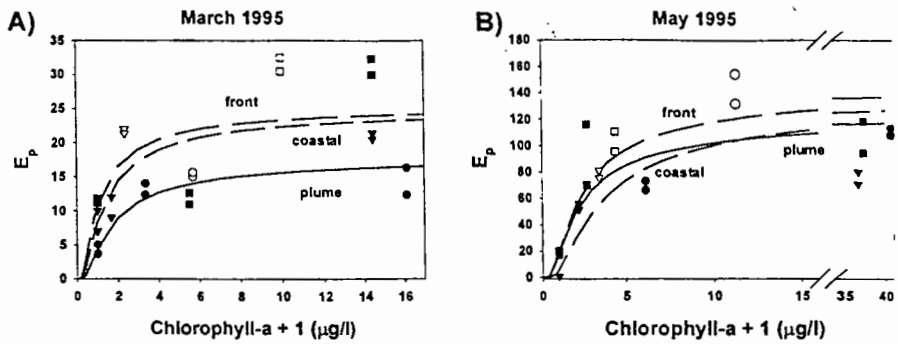

Fig. 7. Relationship between egg production and chlorophyll *a* concentrations during the food addition/reduction experiments. Curves are fit to the saturation equation, with parameters estimated by least-squares fit after linearization, and with 1 added to chlorophyll values to avoid division by zero (see text). Separate fits were made for plume (circles), frontal (squares) and coastal (inverted triangles) stations. Open symbols indicate *in situ* food treatment (no dilution or addition).

food, even though not all stations showed the same egg production at *in situ* food concentrations (Figure 7).

To compare the egg production responses to food concentration at the different stations, we fit the data from the food addition/removal experiments to a saturation model of egg production versus food concentration as proposed by (Kiørboe *et al.*, 1985):

$$E_p = E_{p\,max} e^{-b/C}$$

where E_p is egg production rate, $E_{p\,max}$ is its maximum, C is food concentration, and b is a constant. We used chlorophyll *a* as a surrogate for total food concentration, and added 1 to all values of C to avoid division by zero. Although there were evident differences in egg production among the stations, analysis of covariance (ANCOVA) failed to show differences in the shapes of the curves generated by the experimental treatments (Figure 7).

Discussion

There have been many observations published on the nature and extent of food limitation of copepod egg production. For *A.tonsa* in particular, Beckman and Peterson (1986), Bellantoni and Peterson (1987), Kleppel (1992), Kiørboe and Johansen (1986), and Kiørboe *et al.* (1988) all observed correlations between egg production rates and phytoplankton biomass *in situ*, and inferred food limitation. Durbin *et al.* (1983) found consistent increases in egg production during incubations of field-caught *A.tonsa* when the natural food was supplemented with cultured phytoplankton. White and Roman (1992) and Purcell *et al.* (1994), on the other hand, suggested that this copepod was rarely, if ever, food limited in Chesapeake Bay, and the observations of Bellantoni and Peterson (1987) suggest that food limitation in Long Island Sound only occurs at chlorophyll concentrations

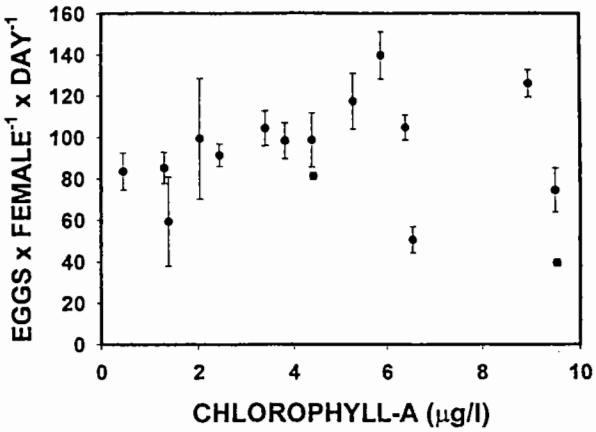

Fig. 8. Relationship of egg production to chlorophyll *a* concentration for the seasonal study (means, with standard deviations), with only the data for samples taken when surface water was ≥20°C shown (Bay Mouth and Channel stations together).

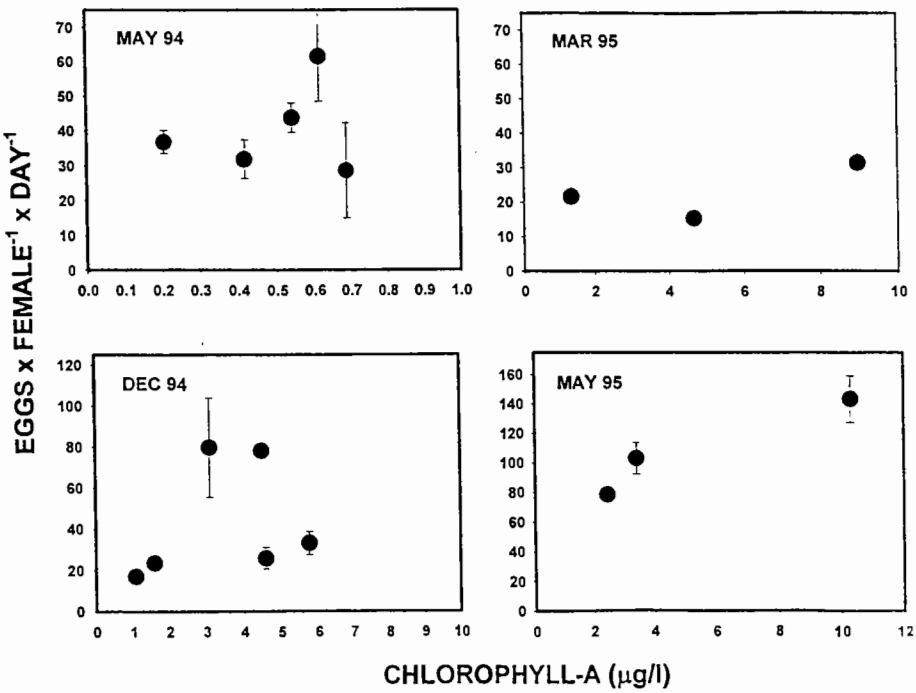

Fig. 9. Relationship between chlorophyll *a* and egg production rates for the plume cruises (means, with standard deviations, except where smaller than symbol).

substantially below 5 μg l^{-1}, or when most of the phytoplankton biomass is in the form of particles <10 μm in size. Others have suggested that chemical composition of available food, or other aspects of food quality, are most often limiting in nature (Ambler, 1985, 1986; Jonasdottir, 1994). It has been suggested that food limitation could occur below a chlorophyll concentration of 5 μg l^{-1} (Ambler, 1986), an observation generally confirmed in laboratory studies with *A.tonsa* (e.g. Berggreen *et al.*, 1988; Støttrup and Jensen, 1990). We found no evidence of food limitation in Mobile Bay or its outflow plume. Seasonally, egg production is obviously controlled by temperature, as observed by others. However, even during warmer months, no correlation between egg production and chlorophyll concentration was present, even though concentrations were below 5 μg l^{-1} more than half of the time (Figure 8). During the individual plume cruises, on each of which we observed a wide range of chlorophyll concentrations, no clear relationships between phytoplankton abundance and egg production rates were seen, except perhaps during the May 1995 cruise (Figure 9).

Correlations with phytoplankton abundance may not be reliable estimators of food limitation, since detritus and microzooplankton may also be important in the diet (Conover, 1956; Lonsdale *et al.*, 1979; Roman, 1984; Stoecker and Egloff, 1987; Gifford and Dagg, 1988; Dolan, 1991; White and Roman, 1992). The lack of any response to food addition during the March and May, 1995, plume cruises, even when *in situ* chlorophyll *a* concentrations were well below 5 μg l^{-1}, suggests that ample non-phytoplankton food was available. Maximum egg production rates were observed at concentrations of chlorophyll *a* below 2 μg l^{-1} (March, coastal station). We did observe reductions in egg production at 50% dilution in four of six cases, suggesting that the copepods are not far along the saturation curve. Although our short incubations would not have provided enough time for the copepods to equilibrate egg production with higher food levels in the addition treatments, they were long enough to have seen some response to added food (Kiørboe *et al.*, 1985; Dagg, 1988), so we would have expected some response to our treatment had food been limiting. Probably, *A.tonsa* becomes food limited regularly near the northern limit of its range (i.e. Baltic Sea, Long Island Sound, Narragansett Bay), but not in warm-temperate or subtropical regions (Chesapeake Bay, Mobile Bay, East Lagoon, TX).

Among a large number of laboratory and field studies on the egg production of *A.tonsa*, our maximum rates are the highest published to date. For temperatures of ~20°C, the previously reported maxima are in the range of 60–70 eggs $female^{-1}$ day^{-1} (Wilson and Parrish, 1971; Durbin *et al.*, 1983; Beckman and Peterson, 1986; Bellantoni and Peterson, 1987), similar to what we found. At temperatures above 25°C, however, we consistently observed rates in excess of 100 eggs $female^{-1}$ day^{-1}, up to 140 eggs $female^{-1}$ day^{-1} (e.g. at a station in the outflow plume in May, 1995 at 25.6°C), with no indication of temperature inhibition or deceleration of the relationship.

Kiørboe *et al.* (1985) suggest that egg production in *A.tonsa* asymptotically reaches a maximum value of 64% of body carbon per day. Based on our measured carbon contents for females in each experiment, and average carbon and nitrogen contents of *A.tonsa* eggs of 30.5 ng and 7 ng, respectively (Ambler, 1985), we

observed specific production rates in excess of 64% day^{-1} in 11 of 16 cases for the seasonal data set. In July, at the Bay Mouth station, at 28°C, copepods were producing 160% and 140% of their bodily C and N content, respectively, per day. At a gross growth efficiency of 0.4 (Dam *et al.*, 1994), these females would have to be ingesting 3.5–4 × their body weight per day, much higher than most laboratory-observed ingestion maxima (Kiørboe *et al.*, 1985; Houde and Roman, 1987; Berggreen *et al.*, 1988; Støttrup and Jensen, 1990). The apparent reduction in stored lipids in the most fecund females suggests that some production may be fueled by reserves, but this possibility would seem to be limited because the warm-season animals have extremely low TAG stores to begin with, only enough for less than an hour's worth of egg production. Therefore, laboratory estimates of either growth efficiency or maximum ingestion rates must not be applicable to the animals in Mobile Bay.

Increased egg production in response to higher N-content of food in other studies suggests that copepod egg production can be limited by nitrogen (Checkley, 1980a,b; Kiørboe, 1989). Copepods can store carbon and energy for egg production in the form of TAG or wax esters (e.g. Hakanson, 1984), so carbon is not likely to be limiting in copepods with lipid reserves. For small copepods like *A.tonsa*, however, which do not carry significant lipid reserves, energy and carbon in the food are translated directly into eggs. Tester and Turner (1990) showed that radioactive carbon introduced in food could be measured in newly produced eggs in <10 h in *A.tonsa*, suggesting very tight coupling between feeding and egg production. In addition, in some cases egg production may be limited by specific biochemicals in the diet, even when not limited by bulk carbon or nitrogen. Jonasdottir (1994) and Jonasdottir *et al.* (1995), for example, found that the kinds and amounts of essential polyunsaturated fatty acids in the diet were correlated with maximum egg production. Although copepods can utilize body protein and sterol for egg production when food is of poor quality (Durbin *et al.*, 1983; Ederington *et al.*, 1995), our observations of very low lipid stores, and no increase in copepod C:N at the highest fecundities (hence, presumably, undiminished protein content) suggest that lipids could potentially become limiting at the highest egg production rates we observed. Although we did not observe this directly, the close coupling between ingested lipids and those in the eggs (inferred from lack of lipid reserves in females that were still producing eggs) implies that any decrease in food quality (e.g. from higher detrital or sediment loading) would decrease lipid ingestion and hence egg production, especially at the highest egg production rates.

Perhaps the most interesting part of our study was the high degree of spatial variability we observed in both the egg production rates of *A.tonsa* and in its lipid content. Between the two stations in the seasonal study (Bay Mouth and Channel), egg production was significantly different on 8 out of 13 dates ($P < 0.05$; *t*-test), even though these stations were only about 3 km apart. Likewise TAG content was significantly different between stations two-thirds of the time (6 of 9 dates for which triplicate batches of copepods were extracted). For the plume cruises, we always observed significant variation in fecundity among stations across the front separating plume waters from those of the open coast. For

samples taken during high flow, this variation was clearly related qualitatively to variations in the salinity field in and around the bay mouth and the plume. This relationship may be due in part to a direct effect of salinity causing energy reserves to be used in maintaining osmotic balance. It is also likely that this variability is related to hydrographic factors, including rates of mixing, salinity stratification, frontal convergence, and upwelling. The high variability on small spatial scales that we observed in both copepod egg production and TAG content at the Bay Mouth and Channel stations implies that the food environment experienced by larval fish is variable on the same scales.

Because egg production rates cannot be measured instantaneously, and require sorting of animals and counting of eggs produced during incubations, it is difficult to obtain data at the high resolution needed to discern correlations between production and hydrography on small spatial scales. Thus, there are few studies available for comparison with ours. Floodgate *et al.* (1981) observed higher zooplankton biomass in a tidal-mixing front in Liverpool Bay, but did not measure egg production. In the North Sea, Kiørboe and Johansen (1986) found higher egg production rates in the transition (frontal) region between well-mixed and stratified waters, and strong evidence for food limitation of egg production. In the Skagerrak, Peterson *et al.* (1991) found that egg production rates could not be related to frontal or other hydrographic features, and evidence of food limitation was weak. Kiørboe *et al.* (1986) argued that persistence of fronts on a time scale commensurate with the generation times of the copepods would be necessary if these features were to become consistent areas of higher egg production or copepod abundance. In fact, they found decreased correlation between hydrography and abundances of increasingly advanced stages of copepods, suggesting that the enhanced productivity signal generated by ephemeral fronts would be blurred as a cohort of copepods matures.

Higher predation in frontal areas would also alter any distribution involving higher abundances of copepods or eggs at a front (Peterson and Kimmerer, 1994). For this reason, the direct measurement of egg production provides the best opportunity to observe enhancement of zooplankton production in relation to hydrographic features. Fronts associated with the outflow plume of Mobile Bay can persist over time scales of several tidal cycles to several days, depending on shelf surface currents and local wind (Abston *et al.*, 1987; Dinnel *et al.*, 1990), long enough to support enhanced egg production, if food conditions are favorable, but short enough to disperse populations of copepodites and adults during development.

The enhanced production of eggs near fronts associated with estuarine salinity gradients may have implications for feeding and growth of larval fish, which are often found in higher abundance near fronts (e.g. Govoni and Grimes, 1992). Because larval fish grow faster in response to higher concentrations of food, rather than higher production of it, knowing the persistence of these features on time scales relevant to the motility and development of larval fish is critical to understanding their role in supporting higher fish production. Thus the fate and significance of the high egg production we observed in this study depend

ultimately on the nature of horizontal mixing of bay waters with those of the coast and on factors that sustain and enhance the salinity gradient.

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