

# The paradox between the long-term decrease of egg mass size of the calanoid copepod *Eurytemora affinis* and its long-term constant abundance in a highly turbid estuary (Gironde estuary, France)

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*Eurytemora affinis* egg mass size—a commonly used demographic parameter—was surveyed in the Gironde estuary. Its decrease between 1978 and 2003 contrasted with the stability of population density during this long-term period. Different hypotheses were tested to explain this paradox. (i) Upstream shift of the population: Even though the population of *E. affinis* shifted upstream due to a greater penetration of marine water, no relationship between the population shift and demographic parameters was observed: the egg mass size exhibited the same evolution upstream. (ii) Water warming: No early egg production and no change of female size that could metabolically limit the egg mass size was observed. (iii) Food limitation: The change in food quality observed did not result in any change in total copepod abundance. (iv) Faster renewal of egg masses: The paradox cannot be explained by this factor and the number of adults observed nowadays is higher than the number of eggs produced by females, whereas fecundity rates strongly increased. The paradox is probably explained by the expulsion of viable subitaneous eggs. This may represent a recent behaviour adaptation of the copepod to environmental changes.

## INTRODUCTION

*Eurytemora affinis* is one of the most abundant zooplankton species in the brackish part of Northern Hemisphere estuaries, usually localized around the Maximum Turbidity Zone (Soetaert and Van Rijswijk, 1993; MTZ; Sautour and Castel, 1995a; Mouny and

Dauvin, 2002). Consequently, this species plays a significant role in estuarine food webs as important food supply for many fishes, shrimps and mysids (Sorbe, 1983; Fockedey and Mees, 1999; Pasquaud *et al.*, 2004; David *et al.*, 2006).

*E. affinis* females carry their eggs in egg sacs until hatching. *In situ* fecundity rates of egg-carrying copepods

(number of eggs produced per day and per egg-carrying female) are often estimated from egg mass size (number of eggs observed in each egg mass per egg-carrying female) because this is relatively easy to measure on a routine basis (Castel and Feurtet, 1989; Chow-Fraser and Maly, 1991; Hirche, 1992). Egg mass size is thus an indirect measurement of egg production (Hirche, 1992), being a compromise between fecundity rates, hatching rates (number of eggs resulting in nauplii after hatching per day) and loss rates (number of eggs lost from egg mass per day due to non-viable and resting eggs; Fig. 1). At a seasonal scale, egg mass size is usually directly or indirectly correlated to temperature and/or food availability (Hirche; 1992; Ban, 1994), as observed for egg production of *E. affinis* (Castel and Feurtet, 1989).

The Gironde estuary is an area of important economical interest. It is characterized by high turbidity limiting autochthonous primary production (Castaing *et al.*, 1984; Irigoien and Castel, 1997). *E. affinis* dominates zooplankton in winter and spring in the oligo-mesohaline area of the estuary (Castel and Feurtet, 1989). Its egg production is strongly affected by high suspended particulate matter (SPM) concentration which limits feeding on phytoplankton (Gasparini *et al.*, 1999; Tackx *et al.*, 2003).

In this estuary, egg mass size of *E. affinis* decreased sharply since 1978 at a fixed station localized in the oligo-mesohaline area (Castel, 1995). In previous studies, this decreasing trend was correlated to increasing water temperature and salinity (Castel, 1995). Environmental parameters had indeed significantly changed since 1978 at this station (David *et al.*, 2007): water temperature increased (+2°C), salinity (+3 psu) increased because of a greater penetration of marine water and SPM concentration and the vegetal particulate organic matter quality decreased. Conversely, the abundance of *E. affinis* did not significantly decrease

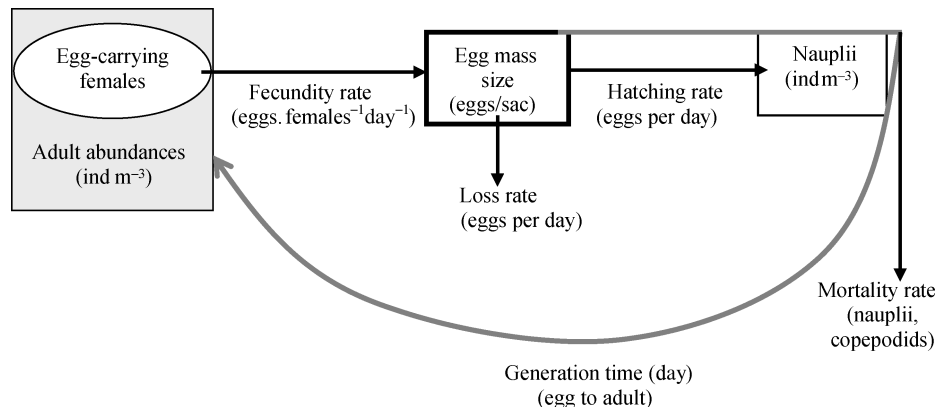
over the period (David *et al.*, 2007). The aim of this study was thus (i) to determine which environmental parameters could explain the decrease of the egg mass size and (ii) to understand how the present small egg mass size carried by females in the oligo-mesohaline area of the Gironde estuary can generate similar total *E. affinis* abundance than before. Different hypotheses were tested in this work.

**Hypothesis 1: Upstream shift of the population**

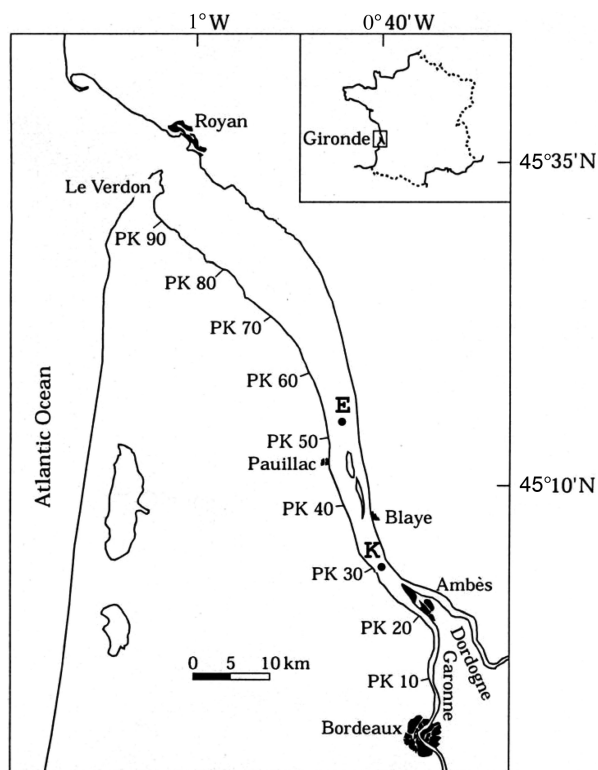
An upstream shift of the population due to the greater penetration of marine water could result in a shift of the population’s demographic characteristics observed at a given fixed station. The healthiest animals within a population are indeed likely to be mostly concentrated in the region that harbours the majority of the population. In the Gironde estuary, this was station E during the 80s (Castel and Feurtet, 1989; Castel, 1995; Fig. 2). If the bulk of the population has since moved upstream because of stronger marine water penetration, it could be that, since the change, this station harbours the ‘outliers’ of the population, possibly containing less fit animals (females with smaller egg masses).

**Hypothesis 2: Water warming**

The significant increase of water temperature could have a direct and/or an indirect impact on egg mass size. Water temperature is an important factor controlling the seasonal production of *E. affinis*: the highest fecundity rate and consequently the largest egg mass size occur at spring temperatures of ca. 15°C (Poli, 1981; Castel *et al.*, 1983). An increase of spring temperature may generate early optimal conditions for egg-production (e.g. in late winter). Warmer water could



**Fig. 1.** Schematic figure of the different metabolic rates occurring during the life cycle of *E. affinis*.



**Fig. 2.** Map of the Gironde estuary showing the sampling stations: E (PK 52) and K (PK 30). PK, distance in kilometers from the city of Bordeaux.

also indirectly act on egg mass size by reducing female size (Ban, 1994) and consequently depleting the number of eggs produced by females—egg production is metabolically limited for smaller compared with larger females (Hirche, 1992; Ban, 1994).

### Hypothesis 3: Food limitation

Small egg mass size is often attributed to food limitation (Hirche, 1992; Ban, 1994). A previous study has shown a significant decrease of the active Chl *a* [Chl *a*/(Chl *a* + 1.51 × Pheopigments)]—usually used as an indicator of the vegetal particulate organic matter quality—between 1978 and 2003 (David *et al.*, 2005, 2007). This decrease has been attributed to a larger contribution of detrital matter to seston. As egg production of *E. affinis* is known to be limited when feeding on detritus (Heinle *et al.*, 1977), the decrease of egg mass size may be due to the decrease in food quality in the Gironde estuary.

### Hypothesis 4: Faster renewal of egg masses

The paradox between the decreasing egg mass size and the constant abundance of the population could be

due to a faster renewal of egg masses. Environmental changes can modify metabolic rates acting on the production of *E. affinis* (Fig. 1). For instance, the fecundity rate is known to change as a function of temperature and SPM concentrations (Gasparini *et al.*, 1999); the hatching rate, the generation time between eggs and adults and the number of sacs produced per female vary as a function of temperature (Poli, 1981; Castel *et al.*, 1983; Andersen and Nielsen, 1997). Both water temperature and SPM concentrations had significantly changed in the Gironde estuary over the period (David *et al.*, 2007). This could have accelerated the renewal of eggs by increasing the number of eggs produced per female, and thus accelerated the development of eggs to adults.

## METHODS

### Study area

The Gironde estuary (Lat. 45°20'N, Long. 0°45'W; Fig. 2) is the largest estuary in France: it covers an area of 625 km<sup>2</sup> at high tide. The Gironde is the estuarine part of the Garonne and Dordogne rivers, which together have a catchment area of ~71 000 km<sup>2</sup> (Jouanneau and Latouche, 1981). The estuary is 70 km long from Bec d'Ambes where the two rivers meet to the mouth. Seasonal salinity variation is related to freshwater discharge that usually reaches a maximum in January–February (mean 1500 m<sup>3</sup>s<sup>-1</sup>) and a minimum in August–September (mean 250 m<sup>3</sup>s<sup>-1</sup>). The mean upstream limit of the saline intrusion (0.5 psu) is located ~75 km and 40 km from the mouth during low and high river-flows, respectively. The salinity gradient induces a residual circulation system. Using the terminology of Pritchard (1955) during high river flow the Gironde is a 'type B' estuary (intrusion of a salt wedge with tides) and a 'type C' during low river flow (partially mixed).

The Gironde is one of the most turbid estuaries in Europe: yearly mean SPM concentrations >500 mg L<sup>-1</sup> compared with Mundaka (Spain) <30 mg L<sup>-1</sup>, Westerschelde (Belgium) <100 mg L<sup>-1</sup> and Ems (Germany) 100 mg L<sup>-1</sup> (Sautour and Castel, 1995a; Burdloff *et al.*, 2002). River systems carry out between 1.5 and 3.10<sup>6</sup> t of suspended sediment to the estuary (mainly silt and clay particles, Castaing *et al.*, 1984) per year. Particulate matter is tidally resuspended and concentrations may exceed 1 g L<sup>-1</sup> at the upstream limit of the salinity intrusion (Allen *et al.*, 1974). This zone of maximum turbidity, which is due to an asymmetric tidal wave, migrates seasonally according to

river-flow and tidal cycles (Sottolichio and Castaing, 1999). As the salinity gradient limits the expulsion of suspended matter from the estuary, a particle entering the Gironde may remain in the maximum turbidity layer for one year or more before being expelled to the sea (Jouanneau and Latouche, 1981).

As a consequence of the high turbidity, primary production in the Gironde is extremely limited ( $10 \text{ gC m}^{-2} \text{ year}^{-1}$ ; H. Etcheber, Bordeaux, personal communication) compared with the primary production in the Westerschelde or the Ems ( $50\text{--}100 \text{ gC m}^{-2} \text{ year}^{-1}$ ; Heip, 1989; de Jonge *et al.*, 1993).

### Sampling methods and laboratory analysis

Samples were collected monthly, 9 months a year (March–November), at two stations (Fig. 1): station E ( $45^{\circ}14'800 \text{ N}$ ,  $0^{\circ}43'500 \text{ W}$ ) from May 1978 to November 2003 and station K, localized upstream ( $45^{\circ}04'100 \text{ N}$ ,  $0^{\circ}38'300 \text{ W}$ ), from March 1984 to November 2003. One additional winter sampling (February) was conducted per year at both stations since 1999. At each station, sampling was carried out at 3-h intervals during a tidal cycle (high and low tide, flood and ebb tide) and at two depths (1 m below the surface and 1 m above the bottom). Environmental factors (temperature, salinity, SPM concentrations, Chl *a* and pheopigment concentrations) and zooplankton were collected at each tidal state and each depth.

Water temperature and salinity were measured with a VALEPORT MK 108. SPM and pigment concentrations were analysed by spectrometry according to conventional oceanographic techniques (Aminot and Chaussepied, 1983): estuarine water samples were collected using Niskin bottles and 50–500 mL were filtered through GF/F Whatman filters. After filtration, filters were immediately dried ( $60^{\circ}\text{C}$ ; SPM) or frozen ( $-20^{\circ}\text{C}$ ; pigments) and stored until analysis. SPM concentrations were determined by filter weight differences between before and after filtration. Chlorophyll and pheopigment were measured by spectrophotometry (Aminot and Chaussepied, 1983). Active chlorophyll was calculated from pigment concentrations as:  $\text{Chl } a / (\text{Chl } a + 1.51 \times \text{pheopigments})$  and used as a marker of the quality of the vegetal particulate organic matter (Irigoiien and Castel, 1997; David *et al.*, 2005), whereas Chl *a* concentrations were used as an indicator of the vegetal biomass.

Zooplankton was collected using a standard 200- $\mu\text{m}$  WP2 net. The volume of water passing through the net was monitored with a TSK mechanical flowmeter or with a Hydrobios digital flowmeter, 4–25  $\text{m}^3$  were usually filtered. The catch was preserved in 5%

seawater/formalin until analysis. Zooplankton was sorted under a binocular microscope. Individual *E. affinis* were identified and sorted according to the following groups: copepodids, males, females and egg-carrying females. The number of eggs per egg sac was also counted.

The database analysed in our study is composed of environmental factors (temperature, salinity, SPM concentrations, Chl *a* concentrations and active Chl *a*) and biotic factors (abundance of males, non-egg-carrying females, egg-carrying females, copepodids and the egg mass size of *E. affinis*) and includes 33 760 values (10 variables and 3376 observations).

The prosome length of mature females was also measured on 30 individuals from samples taken during seasonal peaks of abundance of the species (spring abundance represents  $77 \pm 8\%$  of the total abundance observed between March and November) since 1985 at station E (no archive was available before).

### Data analysis

The database was simplified for stations E and K to provide a standardized dataset by averaging surface and bottom samples over the tidal cycle for long-term variability of biotic and environmental parameters: the resulting database had one value per month and per parameter from May 1978 to November 2003 at station E and from March 1984 to November 2003 at station K. The four assumptions were investigated independently using the same dataset, and data analysis was chosen according to the specific questions.

#### *Hypothesis 1: Upstream shift of the population*

Interannual trends were extracted by moving averages (step of 9 months; Fromentin and Ibanez, 1994) for each parameter and at each station and tested with a simple linear regression (Sokal and Rohlf, 1995): statistical significances of the regression coefficients were used to test the stability of the time series (a significant slope implies a significant increasing or decreasing trend over the time). Trends were tested at station E from 1978 to 2003 and at both stations from 1984 to 2003 in order to compare interannual trends between both stations.

#### *Hypothesis 2: Water warming*

The potential changes in the seasonal cycle of copepod abundance over the 26-year period were checked at station E considering two periods of 5 years: at the beginning (from 1979 to 1983) and at the end (from 1999 to 2003) of the time series. A two-way analysis of variance (ANOVA) (Sokal and Rohlf, 1995) was used to assess differences 'between months' (March–November)

and 'between periods' (1979–1983 and 1999–2003). Mean seasonal cycles of abundance were extracted by the CENSUS 1 method for both periods. Mean winter abundances were considered for the recent period because sampling was carried out in February only since 1999.

Variation of the prosome length of females measured during spring (seasonal peak of abundance) between 1984 and 2003 was tested using a Spearman correlation (Sokal and Rohlf, 1995) to check the possible impact of water warming on female body size.

### Hypothesis 3: Food limitation

Theoretical spring egg mass size was deduced from *in situ* prosome length measured in April from 1985 to 2003 according to equation (1) (Ban, 1994). It assumes no food limitation; egg mass size only depends on female prosome length, and thus on temperature:

$$\text{EMS} = 65.2 \times L^{3.83} \quad (1)$$

EMS is the egg mass size and  $L$  the prosome length ( $\mu\text{m}$ ). According to Ban (1994), *in situ* egg mass size smaller than theoretical egg mass size indicates food limitation. Consequently, theoretical and *in situ* egg mass sizes were compared in order to investigate potential food limitation.

### Assumption 4: Faster renewal of egg masses

The number of eggs and adults produced per cubic meter were compared at station E between (i) two periods: two annual mean cycles were identified from two different periods, at the beginning (1979–1983) and at the end (1999–2003) of the 26-year period and (ii) two seasons: March–June (highest abundances of *E. affinis*) and July–November (low abundances).

The number of egg-carrying females and the number of adults produced per cubic meter and per season were:

$$N = \text{mean abundances} \times N_D$$

with  $N_D$ , the number of generations per season deduced from the model of Poli (1981)

$$N_D = \Delta t / (1204.86 \times T^{-1.35})$$

with  $\Delta t$ , the number of days during the period and  $T$ , the mean temperature over the season ( $^{\circ}\text{C}$ )

The number of eggs produced per cubic meter over one season was then deduced from:

$$W = N_{\text{females}} \times \text{EMS} \times N_s$$

with  $N_{\text{females}}$ , the number of egg-carrying females produced per cubic meter and per season; EMS, the mean egg mass size per period and per season and  $N_s$ , the number of egg masses produced per female, in each season and each period, deduced from Castel *et al.* (1983):

$$N_s = 14.43 \exp(-0.11 \times T)$$

Fecundity and hatching rates were compared between the two periods to see if the decrease of the egg mass size could be explained by faster hatching rates.

The fecundity rate ( $F$ , number of eggs per day) was calculated from Gasparini *et al.* (1999):

$$\text{Log}F = 0.78 - 0.012(15 - T)^2 - 8.78 \times \text{SPM}$$

with SPM, mean concentration of SPM ( $\text{g L}^{-1}$ )

The hatching rate ( $H$ , number of eggs per day) was deduced from Andersen and Nielsen (1997):

$$H = 187 \times T^{-1.54}$$

## RESULTS

### Hypothesis 1: Upstream shift of the population

Temporal trends of all environmental parameters were investigated during two periods at station E: 1978–2003 and 1984–2003. Each parameter exhibited the same significant trend during both periods (Table I), with exception for salinity—the increase in salinity over time was not significant over 1984–2003, whereas it was over 1978–2003. Thus, the trends observed at station E from 1978 with 2003 were compared with the ones observed at station K from 1984 to 2003.

Water temperature and salinity increased significantly at station E from 1978 to 2003 (linear regression,  $P < 0.001$ ; Table I). These increases were not observed at the upstream station K from 1984 to 2003 (linear regression,  $P > 0.05$ ; Table I). SPM concentrations decreased significantly at station E whereas they increased significantly at station K (linear regression,  $P < 0.001$ ; Table I). Chl *a* concentrations increased

Table I: Long-term linear trends (slope expressed by month and P-value) calculated from moving average of zooplankton and environmental parameters for two periods: from 1978 to 2003 at station E and from 1984 to 2003 at stations E and K

Parameters		1978–2003		1984–2003	
		Slope (P)	Δ (séries)	Slope (P)	Δ (séries)
Egg mass size (no. eggs)	E	-0.046	-15	-0.038 (<0.001)	-9
	K	(<0.001)		-0.040 (<0.001)	
Total <i>E. affinis</i> (10 <sup>3</sup> ind m <sup>-3</sup> )	E	1190 (0.679)		-4.800 (0.133)	+5.85
	K			24.000 (<0.001)	
Copepodids (10 <sup>3</sup> ind m <sup>-3</sup> )	E	-3.150	-0.98	-4.170 (0.089)	+2.63
	K	(<0.001)		10.800 (0.005)	
Adults (10 <sup>3</sup> ind m <sup>-3</sup> )	E	3.600	+1.13	0.630 (0.524)	+3.65
	K	(<0.001)		15.000 (<0.001)	
Egg-carrying females (10 <sup>3</sup> ind m <sup>-3</sup> )	E	0.012 (0.910)		0.690 (0.591)	
	K			0.228 (0.138)	
Water temperature (°C)	E	0.006	+2.0	0.004 (<0.001)	+0.9
	K	(<0.001)		0.001 (0.075)	
Salinity	E	0.009	+2.9	0.002 (0,220)	
	K	(<0.001)		-0.001 (0.550)	
SPM concentrations (g L <sup>-1</sup> )	E	-0.003	-0.80	-0.001 (<0.001)	-0.27
	K	(<0.001)		0.002 (<0.001)	
Chl <i>a</i> concentrations (µg L <sup>1</sup> )	E	0.015	+4,74	0.018 (<0.001)	+4.38
	K	(<0.001)		-0.004 (0.604)	
Active Chl <i>a</i>	E	-3 × 10 <sup>-4</sup>	-0,09	-4 × 10 <sup>-4</sup> (<0.001)	-0.09
	K	(<0.001)		-0.007 (0.251)	

Bold values indicate significant trends. The variation over the series is indicated for significant trends.

significantly whereas active Chl *a* decreased significantly at station E (linear regression,  $P < 0.001$ ; Table I). However, no significant trend was observed for both parameters at the upstream station K (linear regression,  $P > 0.05$ ; Table I).

Whatever the period and the station, the egg mass size of *E. affinis* decreased significantly (linear regression,  $P < 0.001$ ; Figs. 3, 4 and Table I). Total abundance of *E. affinis* did not show a significant long-term trend at station E for both periods (linear regression,  $P > 0.05$ ; Fig. 3 and Table I), whereas they increased significantly at the upstream station from 1984 to 2003 (linear regression,  $P < 0.001$ ; Table I).

Even if no long-term trend was detected in total abundance, the abundance of copepodids decreased significantly and the abundance of adults increased significantly at station E from 1978 to 2003 (linear regression,  $P < 0.001$ ; Fig. 3 and Table I). In contrast, these trends were not observed for the shorter period (1984–2003; linear regression,  $P > 0.05$ ; Table I). The significant increase of total abundance observed at the upstream station K was due to the significant increase of copepodids and adults (linear regression,  $P < 0.01$ ; Table I). No long-term trend was observed for egg-carrying females (linear regression,  $P > 0.05$ ; Fig. 2 and Table I).

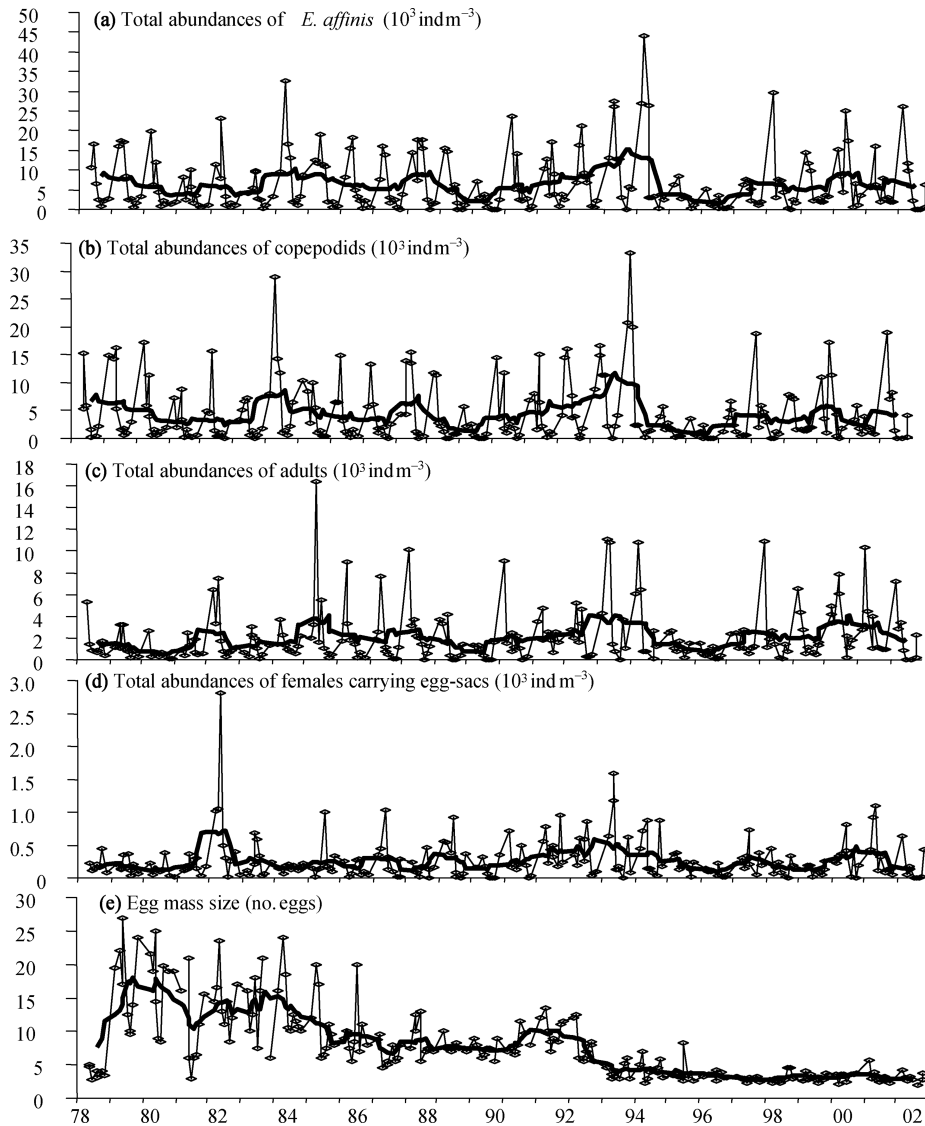
## Hypothesis 2: Water warming

### Direct impact: early seasonal peak of production

The seasonal cycle of egg mass size, total abundance of *E. affinis* and water temperature was analysed at station E for two 5-year periods: at the beginning (from 1979 to 1983) and at the end (from 1999 to 2003) of the series.

Water temperature was significantly different between periods (two-way ANOVA,  $P < 0.01$ ; factor ‘period’): it was higher during the recent period ( $17.8 \pm 0.7^\circ\text{C}$ ) than during the previous period ( $16.7 \pm 0.8^\circ\text{C}$ ). There was also a significant seasonal cycle (two-way ANOVA,  $P < 0.001$ ; factor ‘month’), which was not significantly different between periods (two-way ANOVA,  $P > 0.05$ ; factor ‘period × month’): the maximum was reached in August and the minimum in March (Fig. 5). Mean monthly temperature recorded between May and August during the first period was recorded between April and July, i.e. 1 month earlier, during the second period. Moreover, mean temperature was very low in February for the recent period ( $7.6 \pm 0.5^\circ\text{C}$ ; Fig. 5).

Mean egg mass size of *E. affinis* was significantly different between periods (two-way ANOVA,  $P < 0.001$ ; factor ‘period’): it was significantly higher for the previous period ( $14.7 \pm 0.9$  eggs) than for the recent period ( $3.1 \pm 0.1$  eggs). There was a significant seasonal cycle



**Fig. 3.** Long-term series (continuous line) and moving average (bold line) at station E from 1978 to 2003 of total abundances of *E. affinis* (a), copepodids (b), adults (c), females carrying egg sacs (d) and egg mass size (e).

in egg mass size (two-way ANOVA,  $P < 0.05$ ; factor 'month') with maximum values during spring (from March to June; Fig. 5) and lowest values during summer (July). This seasonal cycle was only observed for the first period (Fig. 5) and was not different in February from other months during the recent period (Fig. 5).

Total abundances of *E. affinis* were not significantly different between both periods at station E (two-way ANOVA,  $P > 0.05$ ; factor 'period'). There was a significant seasonal cycle (two-way ANOVA,  $P < 0.001$ ; factor 'month'), which was not significantly different between periods (two-way ANOVA,  $P > 0.05$ ; factor 'period  $\times$  month'); the maxima were reached from March to May and the minima from August to November (Fig. 5).

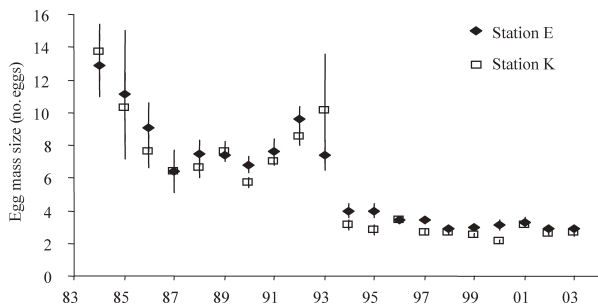
Total abundances of *E. affinis* were lower in February than during spring ( $4 \pm 1 \times 10^3$  ind m<sup>-3</sup>; Fig. 5).

#### *Indirect impact: smaller female prosome length*

Prosome length of females did not show any significant trend from 1985 to 2003 during spring (Spearman correlations,  $P > 0.05$ ). It fluctuated between  $0.73 \pm 0.01$  mm (2003) and  $0.91 \pm 0.01$  mm (1995; Fig. 6)

### **Hypothesis 3: Food limitation**

Since 1986, *in situ* egg mass size observed in spring was always lower than theoretical egg mass size deduced from female prosome length, assuming no food



**Fig. 4.** Annual mean ( $\pm$ SE) from 1984 to 2003 at stations E (PK 52) and K (PK 30) of egg mass size (no. eggs).

limitation. Moreover, *in situ* spring egg mass size was always very low and did not show any fluctuations since 1995 (Fig. 6).

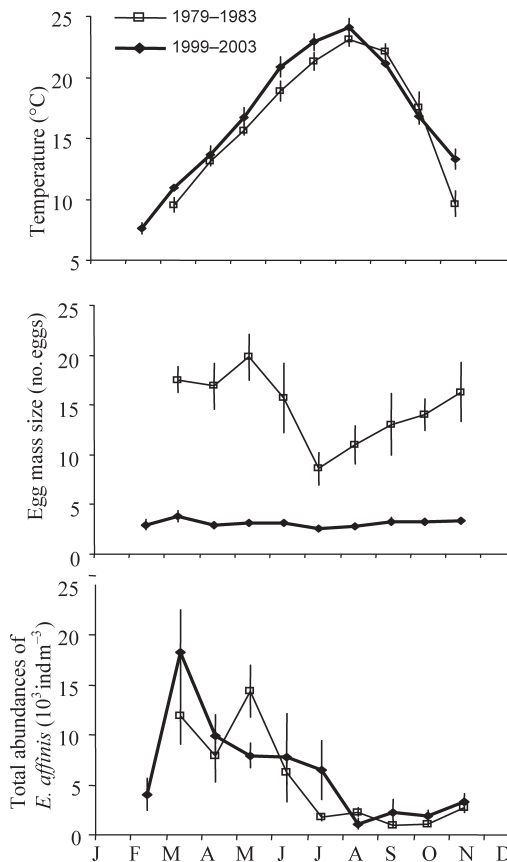
**Hypothesis 4: Faster renewal of egg masses**

The number of generations and the number of egg masses produced per female were calculated in each period (1979–1983 and 1999–2003) and each season (spring and summer–autumn). They were different between periods since the mean temperature was higher during the recent period ( $15.5 \pm 0.7^\circ\text{C}$  during spring; Table II) than during the previous period ( $14.3 \pm 0.5^\circ\text{C}$  during spring).

The number of generations was higher during the recent period than during the previous one (4.1 and 3.7, respectively, during spring; Table II), whereas the number of sacs produced per female was lower during the former than during the latter (2.80 and 3.20 during spring, respectively; Table II). Consequently, the number of egg sacs produced ( $N_D \times N_S$ ) was lower during the recent period (11.9–11.2 egg sacs  $\text{m}^{-3}$ ) than during the first period (12.2–12.5 egg sacs  $\text{m}^{-3}$ ) and the number of eggs produced per cubic meter was lower during the recent period ( $12\,796 \pm 594$  eggs  $\text{m}^{-3}$  during spring) than during the first period ( $91\,317 \pm 7038$  eggs  $\text{m}^{-3}$  during spring; Table II).

However, the number of adults per cubic meter was higher during the recent period ( $17\,814 \pm 5032$  adults  $\text{m}^{-3}$  during spring; Table II) than during the first period ( $8222 \pm 3331$  adults  $\text{m}^{-3}$  during spring). Consequently, the mean percentage of survival from egg to adult was  $\sim 9\text{--}15\%$  during the first period (Table II), whereas the number of eggs produced per cubic meter was lower than the number of adults during the recent period (Table II).

Despite the fact that hatching rates were only 10% higher, fecundity rates of *E. affinis* were four times higher for the recent period than for the first period.



**Fig. 5.** Mean monthly values ( $\pm$ SE) at station E for the period 1979–1983 (continuous line) and 1999–2003 (bold continuous line) for water temperature, egg mass size and total abundances of *E. affinis*.

**DISCUSSION**

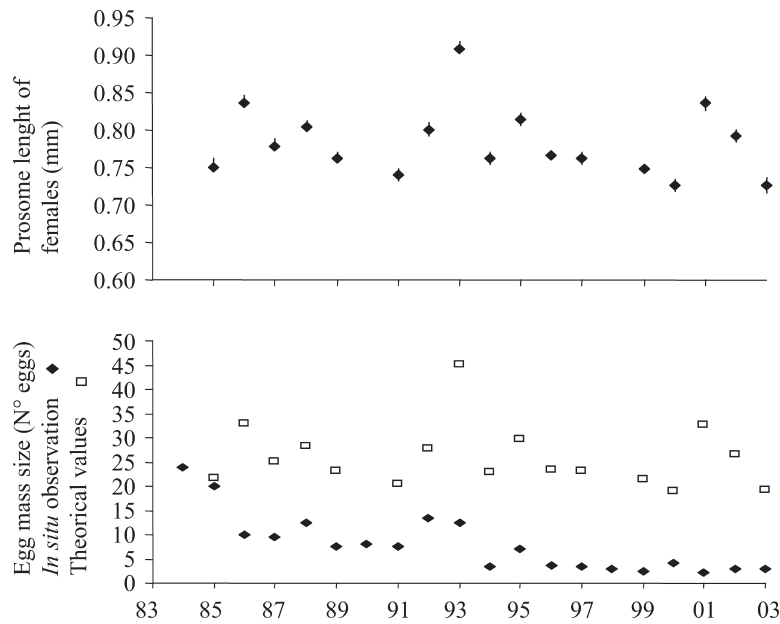
The mean annual egg mass size decreased markedly from 17 to 3 eggs per egg sac from 1978 and 2003. Moreover, the high seasonal fluctuations observed at the beginning of the survey (1979–1983) were not recorded during the recent period. The long-term decrease of egg mass size contrasts with the constant total abundance of the copepod over the 26-year period. Different hypotheses were thus tested to determine which environmental parameters could explain the decrease of the egg mass size and to understand how the present small egg mass size carried by females in the oligo-mesohaline area of the Gironde could sustain total copepod abundance, which currently remains the same as it was earlier.

**Hypothesis 1: Upstream shift of the population**

The population distribution of *E. affinis* has drastically changed since 1978 in the oligo-mesohaline area of the

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**Fig. 6.** Prosome length of females (mm) measured in April on 30 females from 1985 to 2003. Spring mean of egg mass size ( $\pm$  SE) from 1979 to 2003 (March–May) at station E, (no. eggs) and theoretical clutch size deduced from prosome length according to Ban (1994).

Gironde estuary. The total abundance of the copepod increased significantly at the upstream station K, whereas no significant change was observed at station E. The increase in total abundance at the upstream station was due to an overall increase of copepodids and adults, whereas copepodids decreased and adults increased at station E. The contrast between a decreasing trend in copepod abundance at station E and an increasing trend at the upstream station suggests that the population of *E. affinis* moved upstream. Previous studies of copepods had actually shown that older copepods exhibited a wider distribution along the salinity gradient than younger copepods (Sautour and Castel, 1995b).

The upstream transfer of the population could be attributed to environmental changes. The global

increase of salinity (+3 psu) at station E indicates a greater intrusion of marine water into the oligomesohaline area of the Gironde estuary recently. The latter was associated with an upstream movement of the MTZ since the SPM concentrations decreased and increased significantly at station E and at the upstream station K, respectively. However, *E. affinis* is a euryhaline species, well adapted to a salinity range of 0–22.5 psu (Von Vaupel-Klein and Weber, 1975), and is thus not limited by salinity at station E (<16 psu; David *et al.*, 2007). However, its distribution area is mainly restricted to low salinity (0–6 psu) in Northern European estuaries (Ems, Schelde, Seine and Gironde; Sautour and Castel, 1995a; Mouny and Dauvin, 2002; Appeltans *et al.*, 2003). It is a typical inhabitant of brackish waters, but it is known to have invaded freshwater lakes and reservoirs

*Table II: Number of generations  $N_D$  (Poli, 1981) of sacs produced per female  $N_S$  (Castel et al., 1983) depending on temperature, of eggs  $W$  and of adults  $N_{adults}$  produced per cubic meter over each season and period*

Periods	Mean temperature	$N_D$	$N_S$	$W$	$N_{adults}$	Percentage of survival
1979–1983						
March–June	14.3 $\pm$ 0.5	3.7	3.2	87617 $\pm$ 6752	8222 $\pm$ 3331	9
July–November	18.7 $\pm$ 0.7	5.6	2.2	27940 $\pm$ 1738	4093 $\pm$ 1179	15
1999–2003						
March–June	15.5 $\pm$ 0.7	4.1	2.8	12392 $\pm$ 574	17252 $\pm$ 4874	139
July–November	19.6 $\pm$ 0.6	6.1	1.8	4749 $\pm$ 182	7492 $\pm$ 2695	158

The percentage of survival from egg to adults is indicated.

within the past century (Lee, 1999). The greater penetration of marine waters, which has probably caused the MTZ upstream transfer, could thus explain the upstream shift of *E. affinis*, the copepod behaving like a passive particle (Castel and Veiga, 1990).

The upstream shift of the population could have resulted in a shift of the population's demographic characteristics observed at a given fixed station: outlier organisms of the population are present at station E (with less fit animals), whereas healthiest organisms occur at the upstream station. However, both the abundance of egg-carrying females and the egg mass size did not show a significant increasing trend at the upstream station K. Moreover, the latter decreased significantly at the upstream station in a similar way than at station E. Consequently, the paradox between the long-term constant abundance of *E. affinis* and the decreasing egg mass size over the 26-year period cannot be explained by the upstream movement of the population. Another paradoxical issue appeared: the abundance of adults increased significantly at both stations. In other words, the number of adults produced per cubic meter was higher when egg mass size was small than it was when egg mass size was greater.

### Hypothesis 2: Water warming

#### *Direct impact: early seasonal peak of production*

The long-term increase of water temperature (+2°C) at station E could have generated early optimal conditions for egg production (late winter). Maximum abundance of copepods was observed in March during the recent period in contrast to April–June during the earlier period. These results suggest a shift of the production peak from spring to winter possibly due to the global warming of the water column. The high peak of abundance observed in March may result from a high egg production that could have occurred 1 month earlier (February) since the time of development from egg to adult is about 38 days at 11°C (temperature recorded in March; Poli, 1981). However, the egg mass size was very low in February at station E during the recent period ( $2.9 \pm 0.5$  eggs). This agrees with the low temperature recorded in February ( $7.6 \pm 0.5^\circ\text{C}$ ), which contrasts with the optimal fecundity rates of *E. affinis* of  $\sim 15^\circ\text{C}$  (Poli, 1981; Castel *et al.*, 1983; Gasparini *et al.*, 1999).

It appears that no early optimal conditions for egg production in the recent period were recorded: egg mass size was not significantly higher during recent winters even if the seasonal peak of total abundance seemed to be advanced with a recent maximum in March. In addition, egg mass size showed no seasonal fluctuations during the recent period.

#### *Indirect impact: smaller female prosome length*

Female prosome length did not significantly decrease between 1985 and 2003 despite the significant increase of water temperature: the variation of the water temperature was thus insufficient to affect female size (Ban, 1994). Thus, the smaller egg mass size observed during the recent period was not due to a metabolic limitation for producing larger egg masses.

### Hypothesis 3: Food limitation

During the whole survey period, *in situ* egg mass size was lower than theoretical egg mass size when assuming temperature limitation only. This suggests that egg production was food limited (Hirche, 1992; Ban, 1994).

Chl *a* concentration increased significantly at station E between 1978 and 2003 but active Chl *a*—a marker of vegetal particulate organic matter quality (Irigoiien and Castel, 1997; David *et al.*, 2007)—significantly decreased over the period. The low quality of vegetal matter suggests the occurrence of more detrital vegetal matter during the recent period compared with the first period. Egg production of *E. affinis* is known to be limited when feeding on detritus (Heinle *et al.*, 1977). Moreover, the fecundity rate of the copepod could have been limited by high SPM concentrations (ca.  $1 \text{ g L}^{-1}$  during spring). Indeed, SPM concentrations  $>0.5 \text{ g L}^{-1}$  are known to affect the uptake of phytoplankton that limits egg production and *E. affinis* needed vegetal prey in order to obtain a minimum amount of specific components required for egg production (Hapette and Poulet, 1990; Gasparini *et al.*, 1999). Consequently, food availability could have limited egg production, either directly (food quality) or indirectly (high turbidity).

However, this hypothesis contrasts with the significant increase in adult abundance from 1978 to 2003 at station E: food limitation should reduce egg production unless the survival from eggs to adults was improved.

### Hypothesis 4: Faster renewal of egg masses

Metabolic rates acting on the production of *E. affinis* could have been affected over the period due to both increased water temperature and SPM concentration decrease at station E (Poli, 1981; Castel *et al.*, 1983; Andersen and Nielsen, 1997; Gasparini *et al.*, 1999).

These metabolic rates were calculated using literature equations for two periods (1979–1983 and 1999–2003) and two seasons: (i) March–June (highest production of *E. affinis*) and (ii) July–November. The aim of this work was first to calculate the number of eggs and adults

produced per cubic meter in each season and period and then to estimate the survival from eggs to adults in each period.

The effects of the temperature increase on the generation time (Poli, 1981) and the number of sacs produced per female (Castel *et al.*, 1983) cancel each other out. Consequently, the increasing number of adults during the recent period would not be explained by a faster renewal of egg masses but by the differences between mean abundances of adults, egg-carrying females and egg mass size between the first and the recent periods.

Whatever the season, the percentage of survival from egg to adult calculated for the first period (9–15%) was lower than that estimated from literature data based on *in situ* evaluation of mortality (29%; Castel and Feurtet, 1989), whereas total number of adults produced per cubic meter over the year was higher than the number of eggs produced from egg mass size during the recent period.

### Toward other hypotheses

At first this unexpected result can be explained by some problems in calculation. Since the survival from egg to adult agrees with literature data for the first period, the models used for calculation and estimated during the last decades in laboratory experiments can be considered valid for this period (Poli, 1981; Castel *et al.*, 1983; Gasparini *et al.*, 1999). The results obtained for the recent period on survival raise questions about the validity of the models used for the recent period. The changes of environmental parameters could have acted on the behaviour response of *E. affinis* when confronted with changes in temperature, SPM concentrations, etc. since recent studies have shown the rapid adaptation of the species in new habitats (freshwater areas; Lee and Petersen, 2002). Environmental pressure could be responsible of such adaptation: e.g. competition with a new introduced copepod species *Acartia tonsa* whose abundance has sharply increased since 1999 (David *et al.*, 2007) or predation by planktivorous species (*Syngnatus acus*, *Palaemon longirostris*) that have been increasing over the last 10 years (M. Girardin, CEMAGREF Cestas, personal communication).

Another alternative explanation is that the estimation of *in situ* fecundity rates from egg mass size is no longer applicable in the Gironde estuary. The number of adults observed recently cannot only be explained by the egg mass size. The egg mass size depends on the fecundity rates, hatching rates and loss rates (Johnson, 1980, Ban and Minoda, 1991). According to Hirche (1992), loss rates are considered to be negligible. However, hatching rates were only 10% higher, whereas

fecundity rates were four times higher during the recent period in comparison with the first period. The contrast between the high increase of fecundity rates ( $\times 4$ ) and the small increase in hatching rates (10%) could not explain the drastic decrease of the egg mass size between the first and the recent periods. Consequently, a great part of the eggs produced must be 'lost' in the environment. Lost eggs classically include non-viable and resting eggs for *E. affinis* (Hirche, 1992). The former could not explain the high number of adults, whereas the latter are normally observed during summer and autumn when environmental conditions become unfavourable (Johnson, 1980; Ban and Minoda, 1991; Hirche, 1992). Only release of viable subitaneous eggs could explain the high number of adults observed in the environment. These viable subitaneous eggs could be (i) directly spawned in the environment as for other free-spawning calanoid copepods (Kiorboe and Sabatini, 1994) or (ii) accidentally released: lost from deteriorated sacs or included in sacs detached due to the dynamic hydrographic environment (Sottolichio and Castaing, 1999). Whatever the reason, these results would imply a release of eggs in the environment all year round and thus more exposed to predation pressure: mortality in egg sac carriers is at least one order of magnitude lower than free egg spawner mortality (Kiorboe and Sabatini, 1994). This probably implies for *E. affinis* a reproductive strategy similar to free-spawning calanoid copepods and thus a progressive change in reproductive strategy over the 26 years of the survey.

This kind of reproductive strategy is conceivable since fecundity rates have increased dramatically and hatching time has decreased. Fecundity rates are usually higher for free-spawning eggs copepods (Kiorboe and Sabatini, 1994). Increasing temperature could have been a determining factor for this change in strategy since the r-strategy of free-spawning copepods is known to be more competitive during summer than K-strategy developed by egg-carrying copepods (Hirche, 1992). Moreover, this change in reproductive strategy could allow the copepod to limit the high mortality pressure on egg-carrying females more sensitive to predation than other adult copepods (Kiorboe and Sabatini, 1994) in a system where some planktivorous species have been increasing for 10 years. Finally, such results suggest that the estimation of *in situ* fecundity rates from egg mass size would no longer be applicable in the Gironde estuary.

### CONCLUSIONS

The decrease of egg mass size cannot be explained using the present knowledge on egg production and

reproductive strategy of the copepod *E. affinis*. Work on genetic evolution focused on the rapid adaptation for reproduction and laboratory experiments studying the response of demographic parameters to environmental factors could provide additional understanding of this paradox.

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