

Toxic phytoplankton response to warming in two Mediterranean bays of the Ebro Delta.

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#### ABSTRACT

The analysis of the phytoplankton and environmental parameters of the time series in Alfacs and Fangar bays (north western Mediterranean) from 1990 to 2009 shows some trends. There is an increase in the average water column temperature, 0.11, 0.01, 0.80 and 0.23 °C for spring, summer, fall and winter respectively in Alfacs Bay and 1.76, 0.71, 1.33, 0.89 °C for spring, summer, fall and winter in Fangar Bay. The trends in phytoplankton populations show a shift in the timing of occurrence of *Karlodinium* spp. blooms and an increase of the *Pseudo-nitzschia* spp. abundances. There is a lack of correlation between the average seasonal temperatures and the toxic phytoplankton abundances.

#### INTRODUCTION

The linear warming trend of global surface temperature over the 50 years from 1956 to 2005 is 0.13 [0.10 to 0.16] °C per decade, (IPCC, 2007). In the Western Mediterranean, during the same period of time the linear trend is 0.002 - 0.009 °C per year, 0.025 °C per year for the period 1973-2005 at the station placed in Estartit in the Catalan coast

(Vargas-Yáñez et al 2008). In other areas Peperzak (2003) suggests an increase in toxic phytoplankton blooms due to climate change as a conclusion of different laboratory studies. The analysis of the data from the Continuous Plankton Recorder in the northeast Atlantic shown that climate oscillations and warming play an important role in governing fluctuations of some HABs from exceptional blooms to long-term decadal trends (Edwards et al 2006).

To study the response of the phytoplankton to climate change the increase in temperature is a factor to consider affecting the seasonal composition of phytoplankton and the position of biogeographic boundaries, each species has a temperature window for its survival and its optimal growth, although we know that many phytoplankton species from coastal regions tolerate changes in temperature. Other factors such as stratification, upwelling, freshwater run-off from land, and cloud cover, feedback mechanisms need to be considered as indirect effects of climatic variations and these factors may be important than only the increase in temperature to explain the response of phytoplankton to climate change (Dale et al 2006). Other factor to consider is the pH, the increase in atmospheric CO<sub>2</sub> from a pre-industrial level of 280 µatm to the present level of 370 µatm has decreased surface ocean pH values by ~ 0.12 units to a pH value of 8.2 (Hays et al 2005).

Time series of environmental parameters and phytoplankton abundances are needed to asses the trends in phytoplankton populations. Unfortunately most of the toxic phytoplankton time series in the Mediterranean have started only recently, since the 1990s, associated to the European legislation on shellfish growing areas; and longer series, more than 30 years are convenient to reveal convincing relationships between climate and harmful algal blooms (Dale et al 2006).

Alfacs Bay is located in the south of the Ebro delta, it has a surface of 50 km<sup>2</sup> and its maximum depth is 6m, Fangar Bay is located in the north of the Ebro delta, it has a surface of 12 km<sup>2</sup> and its maximum depth is 4m (Camp and Delgado 1987). Both are important aquaculture areas and receive freshwater through different irrigation channels used for the culture of rice. First studies of phytoplankton populations in the Ebro delta area (figure 1) outside the bays were Herrera and Margalef 1963, Margalef and Herrera 1964, and later Estrada 1972, López and Arté 1973 in Fangar Bay; and Delgado 1987

for Alfacs Bay and Fangar Bay. In August 1987 a program was established to monitor the quality of the shellfish growing areas in Catalonia, since then phytoplankton populations and oceanographic parameters were monitored in a weekly frequency. Toxic events in these bays are related to the presence of *Alexandrium minutum*, *Alexandrium catenella*, *Dinophysis sacculus*, *Dinophysis caudata*, *Protoceratium reticulatum*, *Karlodinium veneficum* and *Karlodinium armiger*, and *Pseudo-nitzschia* spp. (Delgado 2003, Delgado et al., 1996, 2000, 2004, Diogène et al. 2008, Fernández-Tejedor et al 2004, 2008, 2009, Garcés et al 2006). The species of the genus *Pseudo-nitzschia* found in both bays are *P. calliantha*, *P. delicatissima*, *P. fraudulenta* and *P. pungens* (Quijano-Scheggia et al 2008). There is seasonality in the bloom period for the different phytoplankton species (Fernández-Tejedor et al 2008). Moreover shellfish mortalities occur in both bays associated to warm events in summer (Lletí et al. 1995, Ramón et al. 2007). Water discolorations due to *Noctiluca scintillans* were observed in the area in 1971 (López and Arté 1971) but toxic phytoplankton events were not reported before 1989. The first PSP (Paralytic Shellfish Poisoning) event in the Ebro Delta area occurred in May 1989 (Delgado et al 1990) associated to the bloom of *Alexandrium minutum*, the genus *Alexandrium* was known to be linked to PSP since 1927 (Sommer and Meyer 1937) and the species *A. minutum* was described from samples taken at the Alexandria Harbour in 1956 (Halim 1960). The first PSP event in the Mediterranean coast of Spain occurred in 1987 (Bravo 1993), PSP was not reported in the Mediterranean before 1970 (Hallegraeff 2003). DSP (Diarrhetic Shellfish Poisoning) is known since 1978 (Yasumoto et al 1978) and it was associated to *Dinophysis* in 1980 (Yasumoto et al 1980). The first DSP event in the Spanish catalan coast occurred the year 1998 in Alfacs Bay, it was reported in the French catalan coast in the year 1987 (Belin and Raffin 1998). *Dinophysis caudata* was abundant in the western Mediterranean during the Thor cruises in 1909-1910 (Navarro and Bellón Uriarte 1945) but only after 1980 it was known that certain cases of diarrhea in consumers of molluscs were not due to bacteria or virus, but to biointoxication by DSP like in the Adriatic (Ciminiello et al 2003).

The aim of this study is to analyze the phytoplankton and environmental parameters of the time series in Alfacs and Fangar bays (north western Mediterranean) from 1990 to 2009.

## METHODS

In Alfacs Bay and Fangar Bay water temperature, salinity and oxygen are measured every week since May 1990 at surface and bottom in one station placed at the center of each bay. Since January 1992 these parameters are also measured at every meter in the water column, in June 1992 more sampling stations were added to the weekly monitoring. Since May 1990 phytoplankton species composition is determined every week at the same stations. The depths for phytoplankton samples are surface, bottom and integrated water column for the central station, only surface for the rest, in 2006 surface samples were replaced for integrated water column samples to adapt the sampling scheme to the European legislation (EC 2004). Phytoplankton samples are preserved using formalin and Utermöhl is used for identification and quantification using the magnifications 100x, 200x and 400x (Hasle 1978). The main references used for the identification are Tomas 1997, Throndsen et al 2007 and the IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (Moestrup et al 2009). Since October 2000 chlorophyll is measured in duplicates of the phytoplankton samples, not preserved maintained in dark until *in vivo* fluorescence is measured using a TURNER fluorometer (Lorenzen 1966, Jeffrey and Welschmeyer 1997).

The average temperature of the water column at the central stations of each bay (Alfacs n=8-24, Fangar n= 6-18, per week, 8/6 depths per sampling day and 1-3 sampling days per week) was calculated for every week and used to calculate the seasonal average for every year between 1990 and 2009, 6762 and 4661 temperature measurements in total for Alfacs and Fangar bays respectively. The linear regression was calculated to evaluate the increase or decrease in the seasonal average temperature. Winter average is referred to the year of the season initiation.

Water temperature anomalies were calculated for every week and every depth, the water column average of the anomalies was calculated for every week and used for the seasonal anomalies averages of every year. The mean of all the standard deviations for every week and every depth per season was used to determine which years were different from the average due to the lack of significant differences between different years for each season. The Kruskal-Wallis One Way Analysis of Variance on Ranks

result was  $H = 18$  with 18 degrees of freedom ( $P = 0.456$ ) for spring, fall and winter;  $H = 19$  with 19 degrees of freedom ( $P = 0.457$ ) for summer.

The temperature and salinity measurements were used to calculate sigma-t for every week and every depth. The difference between sigma-t at 6m and at 0.5 m depth was used as a stratification index. The average stratification index was calculated for every season.

The average phytoplankton species abundances of the surface and bottom weekly samples at the central station of each bay were used to calculate the seasonal average per year.

The Spearman Rank Order Correlation was employed to test the correlation between the seasonal temperature and species abundances or the seasonal stratification index and species abundances.

## RESULTS

### Temperature

In Alfacs Bay, average seasonal temperatures for the period summer 1990- summer 2009 were  $18.9 \pm 0.5$ ,  $26.1 \pm 0.6$ ,  $17.4 \pm 1.1$ ,  $11.3 \pm 1.1$  °C (mean  $\pm$  SD, n=19-20 years) for spring, summer, fall and winter respectively. In Alfacs Bay the linear trend from spring 1990 to spring 2009 shows a non significant increase of 0.006, 0.0003, 0.042, 0.012 °C per year ( $R^2 = 0.00, 0.00, 0.05, 0.00$ ) for spring, summer, fall and winter respectively (figure 2). In Fangar Bay, average seasonal temperatures were  $18.3 \pm 0.9$ ,  $25.3 \pm 0.7$ ,  $16.6 \pm 1.4$ ,  $10.9 \pm 1.1$  °C (mean  $\pm$  SD, n=19-20 years). The linear trend shows a non significant increase of 0.093, 0.037, 0.070, 0.047 °C per year ( $R^2 = 0.33, 0.10, 0.08, 0.06$ ) for spring, summer, fall and winter respectively (figure 3). In these 19 years, from 1990 to 2009, the seasonal water column temperature increased 0.11, 0.01, 0.80 and 0.23 °C for spring, summer, fall and winter respectively in Alfacs Bay. In Fangar Bay the increase was 1.76, 0.71, 1.33, 0.89 °C for spring, summer, fall and winter. The mean of the seasonal water temperature standard deviations were 1.8, 1.4, 1.3, 1.9 in Alfacs Bay and 1.8, 1.8, 2.4, 2.1 in Fangar Bay for spring, summer, fall and

winter respectively, the means were used to determine the years with positive and negative seasonal temperature anomalies. From summer 1990 to summer 2009, in Alfacs Bay positive water temperature anomalies occurred in fall 1995 and 2006 and negative temperature anomalies occurred in fall 1993 and 1999 and winter 2004-2005 (figures 4-7). Positive temperature anomalies also occurred in Fangar Bay during winter 1996-1997 and fall 2006 (figures 6-7). The average water column temperature was higher than 28°C during 10 weeks for the decade 1990-1999 and 17 weeks for the decade 2000-2009.

### **Salinity**

For the period 1990-2009, salinity in Alfacs Bay has increased 0.019, 0.030, 0.010 units per year, for spring, summer and fall and decreased -0.014 units for winter, this negative value of the linear trend in winter is due to de very low salinities in winter 2008-2009 while the linear trend for winter is positive, 0,039 units for the period 1990-2007 (figure 8).

### **Stratification**

In Alfacs Bay mean seasonal stratification indexes were  $1.83 \pm 0.44$ ,  $2.06 \pm 0.36$ ,  $1.83 \pm 0.43$ ,  $1.16 \pm 0.58$  for spring, summer, fall and winter (figure 9). The maximum was measured in spring 2003, stratification was also strong in winter 1996, summer and fall 1992 (3.2, 3.1, 2.9, 3.0 respectively).

### **Toxic phytoplankton**

In Alfacs Bay there is a shift in the time of occurrence of *Karlodinium* spp. blooms, from winter in 1994-1999 to spring in 2000 and spring-summer in 2003-2009 (figure 10). Positive correlation exists between *Karlodinium* spp. cell abundances and the stratification index in spring (Spearman rank 0,682 P= 0,003). There is a trend to an increase in the cell abundances of *Pseudo-nitzschia* spp. (figure 11) more important in summer and fall seasons. *D. sacculus* (figure 12) and *A. minutum* (figure 13) are more abundant in spring and winter. There is a lack of correlation for the averaged temperatures of each season per year and the phytoplankton cell abundances. In Fangar

Bay there is a decrease in *D. sacculus* abundance during all seasons of the year, while *D. caudata* increases the abundance during the summer season (figures 14-15). In Fangar Bay *Pseudo-nitzschia* spp. blooms do not show the increase in abundance observed in Alfacs Bay (figure 16).

## DISCUSSION

Long-term changes related to global warming are usually statistically modeled by means of linear regression, Vargas-Yáñez et al (2009) showed that the results reported in different studies on the trends for salinity and temperature in the western Mediterranean depend on the period of time considered and the data analysis methods used. The linear regression model was not significant to describe the increase of temperature for the 1990-2009 time series in Alfacs and Fangar bays.

Warming is more intense in Fangar Bay than in Alfacs Bay for all seasons of the year, especially in summer and spring, 109 and 16 times more intense respectively. In the Thau lagoon, Collos et al. 2009 calculated the increase in water temperature 0.091, 0.061, 0.052 °C per year for spring, summer and fall for the time series from 1972 to 2006. The increase in temperature in the Thau lagoon for spring and fall are similar to the increase in Fangar Bay but the summer increase in water temperature in Fangar Bay is half of the increase in Thau.

Average seasonal water column temperatures for the period 1990-2009 were 18.3, 25.3, 16.6, 10.9 °C for spring, summer, fall and winter in Fangar Bay. For comparison purposes we present here the data of López and Arté (1973), who measured the water column temperature at the same station during the years 1968-1971. The average seasonal water column temperatures calculated from their measurements during the years 1968-1971 were 17.9, 24.5, 14.2, 10.7 °C for spring, summer, fall and winter. The comparison between the average for the period 1968-71 and the period 1990-2009 shows an increase of 0.5, 0.8, 2.4, 0.1 °C for spring, summer, fall and winter respectively (figure 17).

Positive thermal anomalies even of short duration may represent an important physiological change for some organisms as *Mytilus galloprovincialis*. During warm

events a reduction in grazing together with an increase in nutrients could conduct to the high phytoplankton abundances as observed in 2003 in Fangar Bay (Ramón et al. 2007). The effect of water temperature on mussel (*M. galloprovincialis*) mortality was studied in laboratory experiments by Anestis et al (2007), they showed that animals started to die as water temperature reached 26°C and when temperature exceeds 25°C filtration falls significantly. In Alfacs and Fangar bays, summer shellfish mortalities occur due to high water temperatures (Lletí et al. 1995, Ramón et al. 2007). The average water column temperature in summer for the period 1990-2009 for Alfacs and Fangar bays were  $26.1 \pm 0.6$  and  $25.3 \pm 0.7$  (mean  $\pm$  SD) exceeding the threshold for filtration rate in both bays and for mortality in Alfacs Bay.

There is a lack of correlation for the averaged temperatures of each season per year and the toxic phytoplankton cell abundances. The trends in the abundances of the toxic phytoplankton species present in Alfacs and Fangar show a shift in the bloom period for the ichthyotoxic species of the genus *Karlodinium* and an increase in *Pseudo-nitzschia* spp. cell abundances in the last 7 years. Spring *Karlodinium* blooms are correlated to stratification. The main factor controlling stratification in Alfacs Bay is the status of the irrigation channels discharging into the bay (Solé et al 2009). The management of the channels is controlled by the associations of rice producers and shellfish producers generating two main periods, an 'open channel' period (more stratified water column) and a 'closed channel' one (less stratified water column).

Figure 1: Map of the Mediterranean Sea, the grey circle shows the location of the Ebro delta.

Figure 2: Average seasonal temperature for the period spring 1990-summer 2009 in Alfacs Bay (n=12-14 weeks).

Figure 3: Average seasonal temperature for the period spring1990-summer 2009 in Fangar Bay (n=12-14 weeks).

Figure 4: Temperature anomalies in Alfacs and Fangar bays in spring for the period 1990-2009 (n=12-14 weeks).

Figure 5: Temperature anomalies in Alfacs and Fangar bays in summer for the period 1990-2009 (n=12-14 weeks).

Figure 6: Temperature anomalies in Alfacs and Fangar bays in fall for the period 1990-2009 (n=12-14 weeks).

Figure 7: Temperature anomalies in Alfacs and Fangar bays in winter for the period 1990-2009 (n=12-14 weeks).

Figure 8: Average seasonal salinity in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 9: Average seasonal stratification index in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 10: Seasonal *Karlodinium* spp. cell abundances in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 11: Seasonal *Pseudo-nitzschia* spp. cell abundances in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 12: Seasonal *Dinophysis sacculus* cell abundances in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 13: Seasonal *Alexandrium minutum* cell abundances in Alfacs Bay for the period 1990-2009 (n=12-14 weeks).

Figure 14: Seasonal *Dinophysis sacculus* cell abundances in Fangar Bay for the period 1990-2009 (n=12-14 weeks).

Figure 15: Seasonal *Dinophysis caudata* cell abundances in Fangar Bay for the period 1990-2009 (n=12-14 weeks).

Figure 16: Seasonal *Pseudo-nitzschia* spp. cell abundances in Fangar Bay for the period 1990-2009 (n=12-14 weeks).

Figure 17: Average seasonal temperature in Fangar Bay. The average values for the period 1968-1971 are shown in the grey bars, the black bars show the average for the period 1990-2009. The frequency of the measurements was once a month for the year 1968 and every 2 months for the years 1969-71, in total 27 days of measurements in 4 years.

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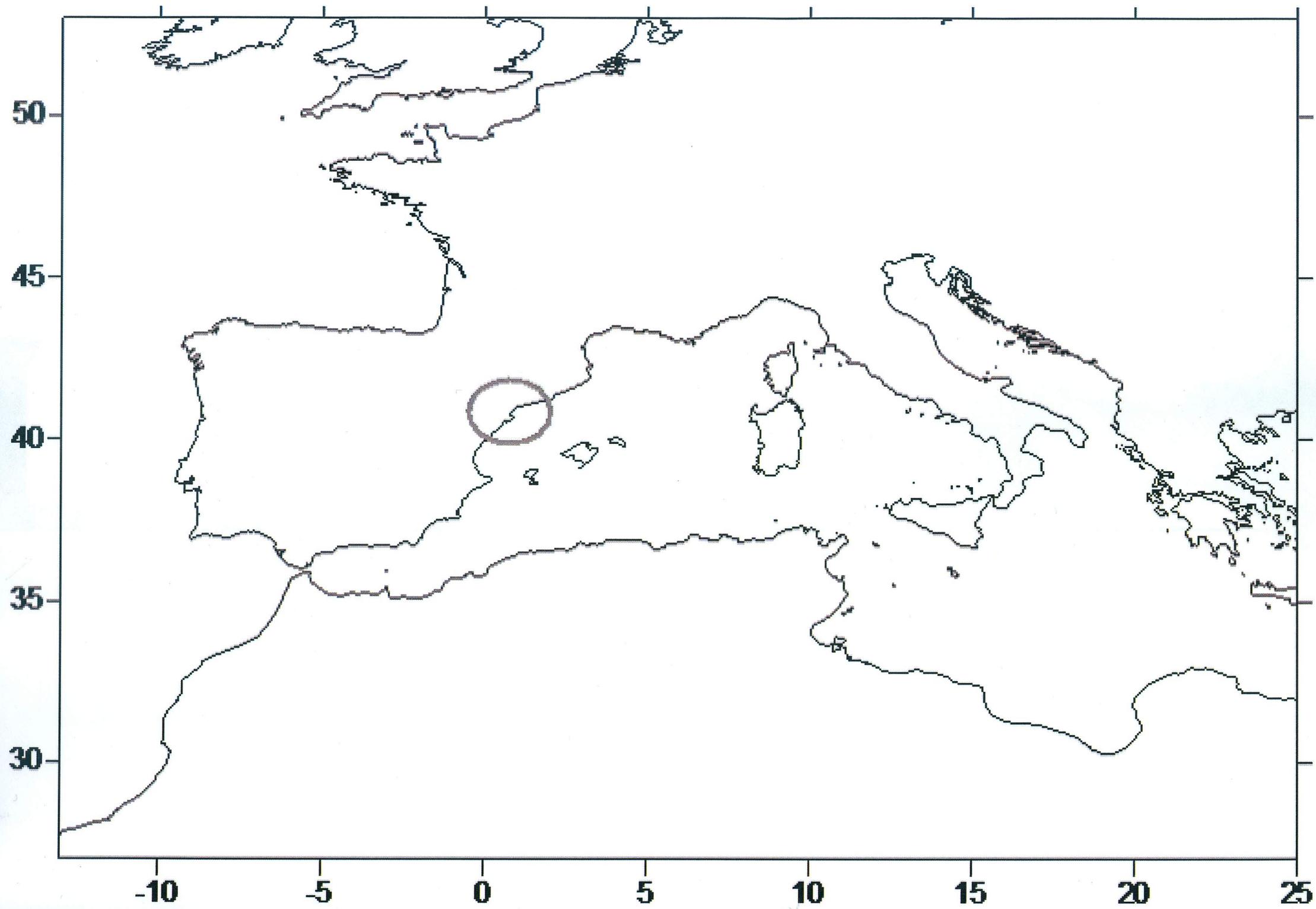
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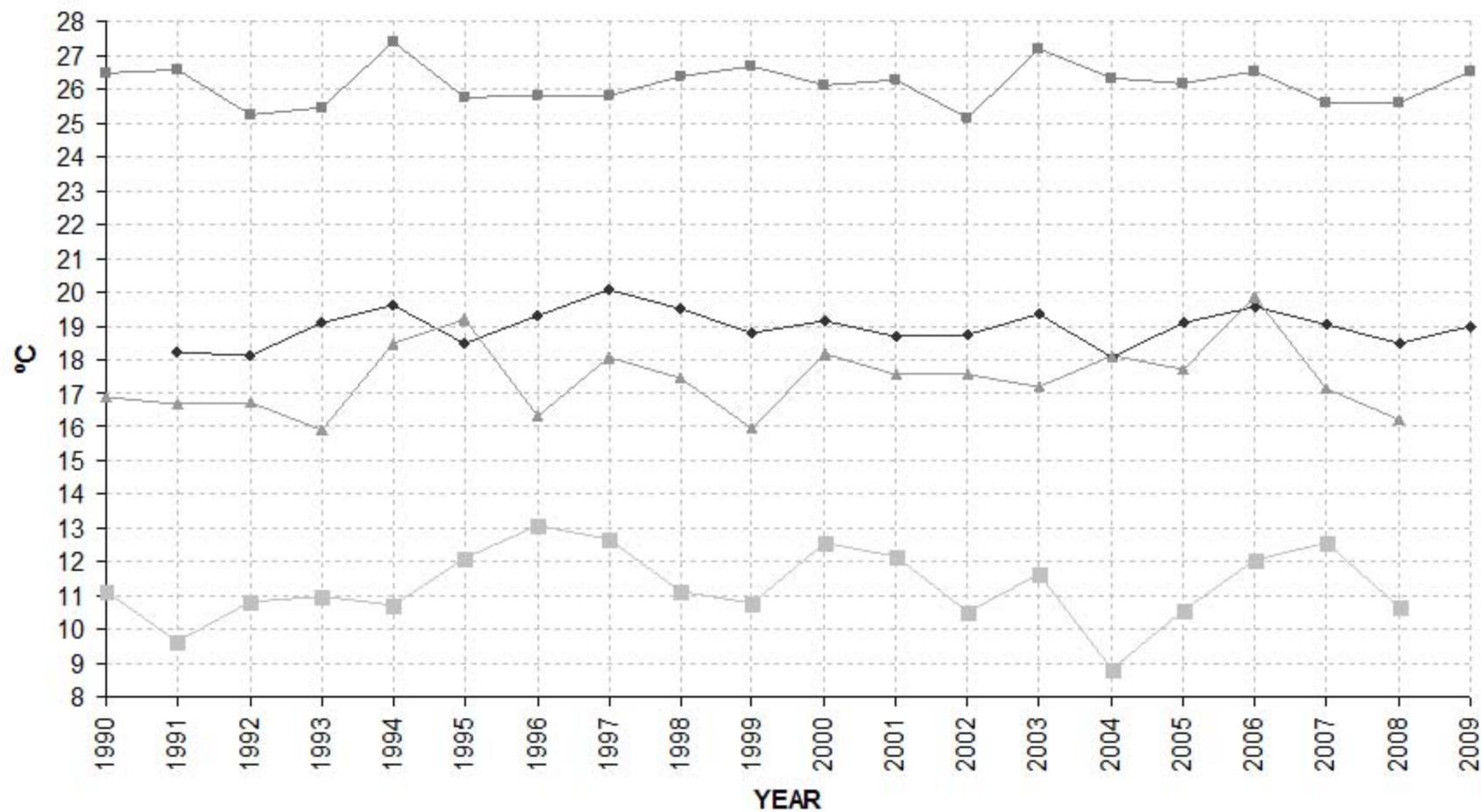
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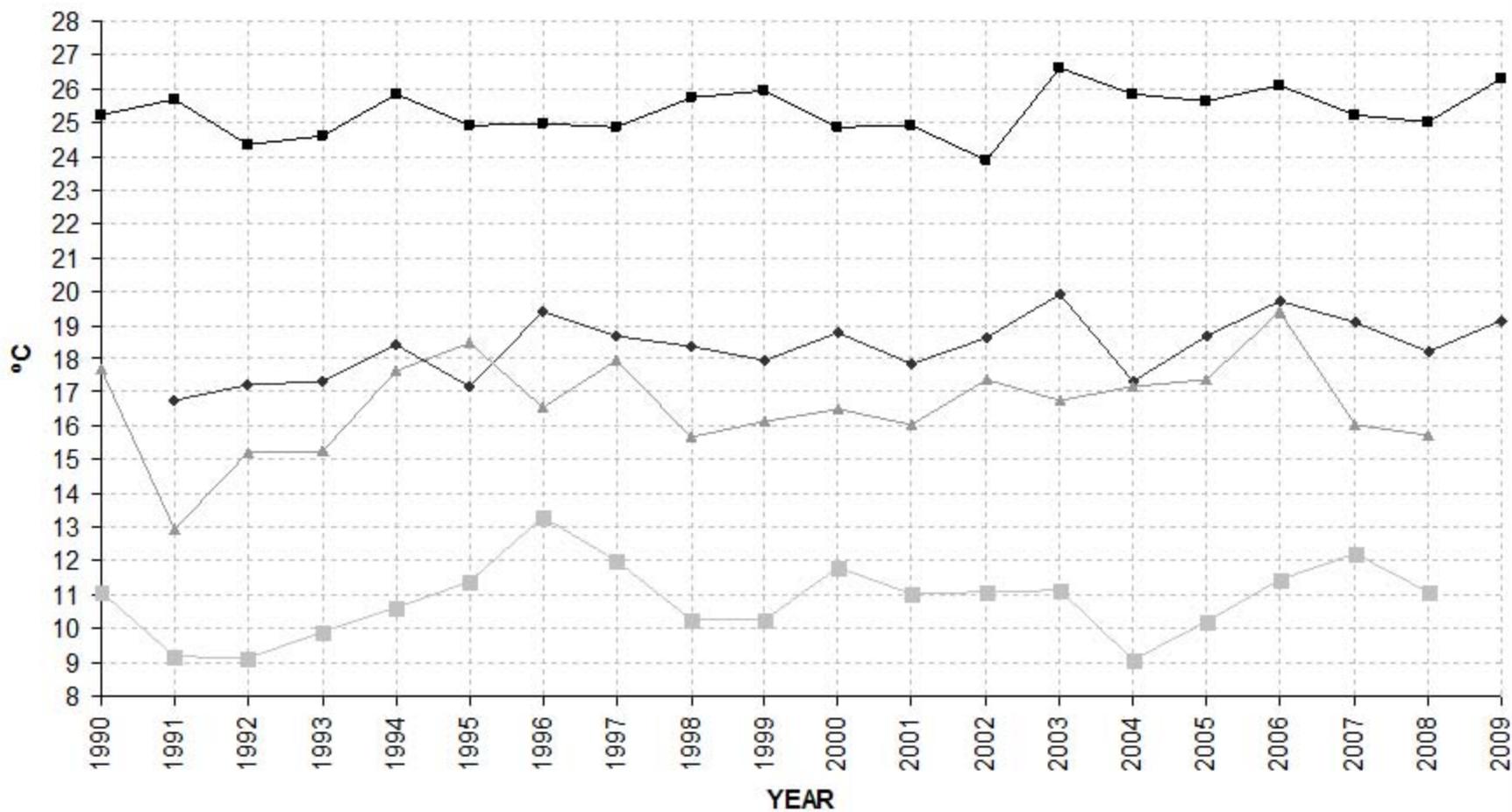
# ALFACS: AVERAGE SEASONAL TEMPERATURE

—◆— SPRING    —■— SUMMER    —▲— FALL    —■— WINTER

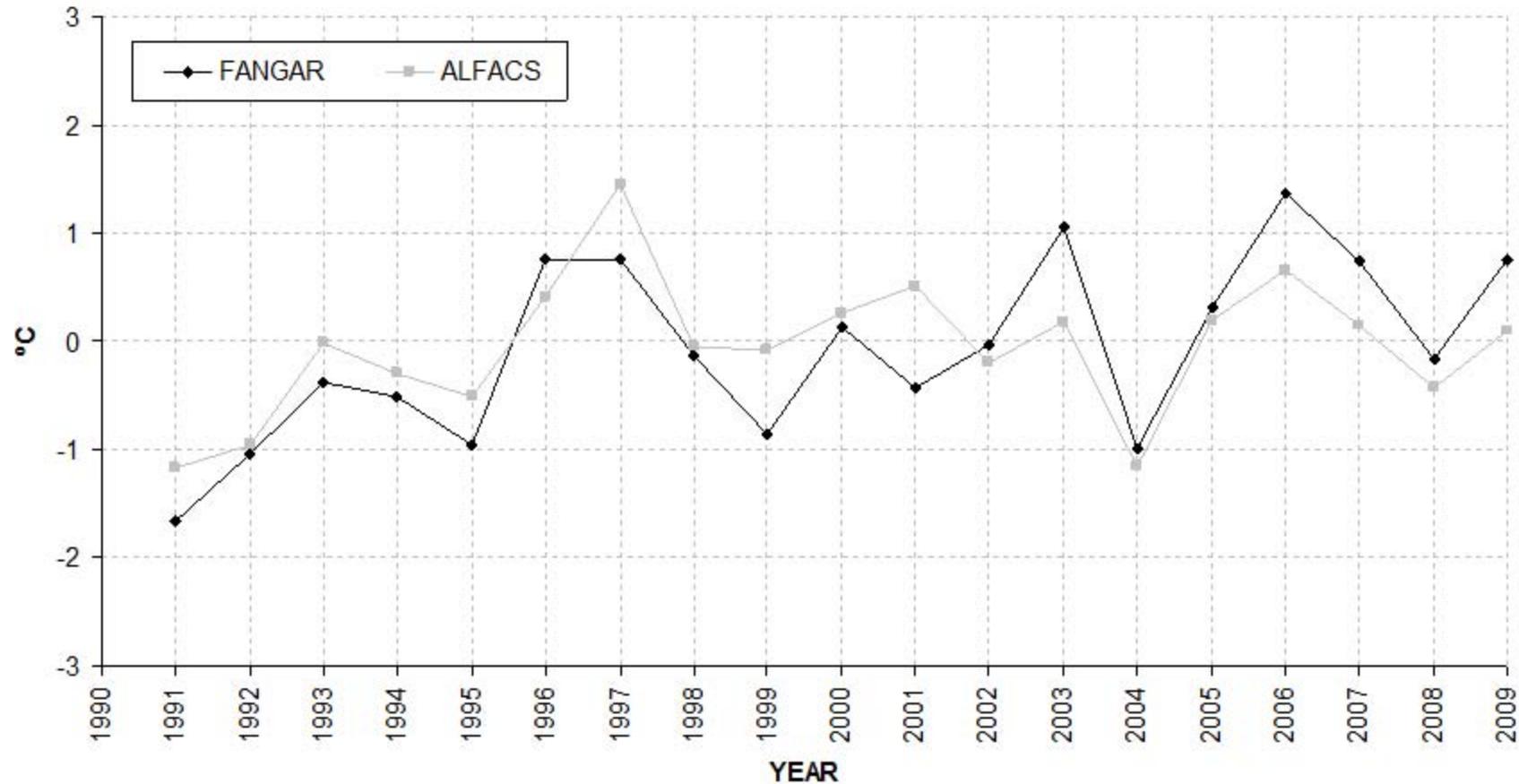


# FANGAR: AVERAGE SEASONAL TEMPERATURE

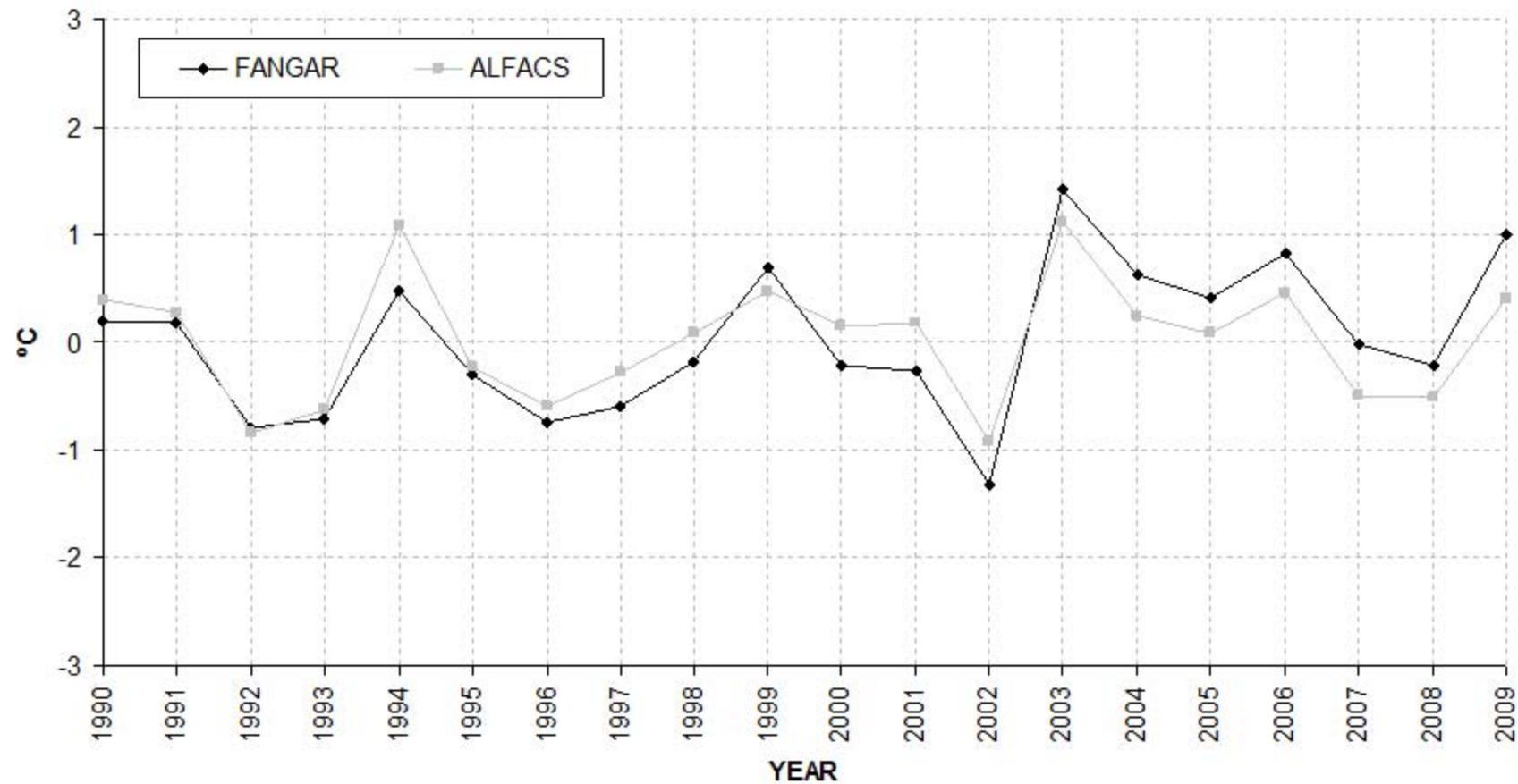
—◆— SPRING    —■— SUMMER    —▲— FALL    —■— WINTER



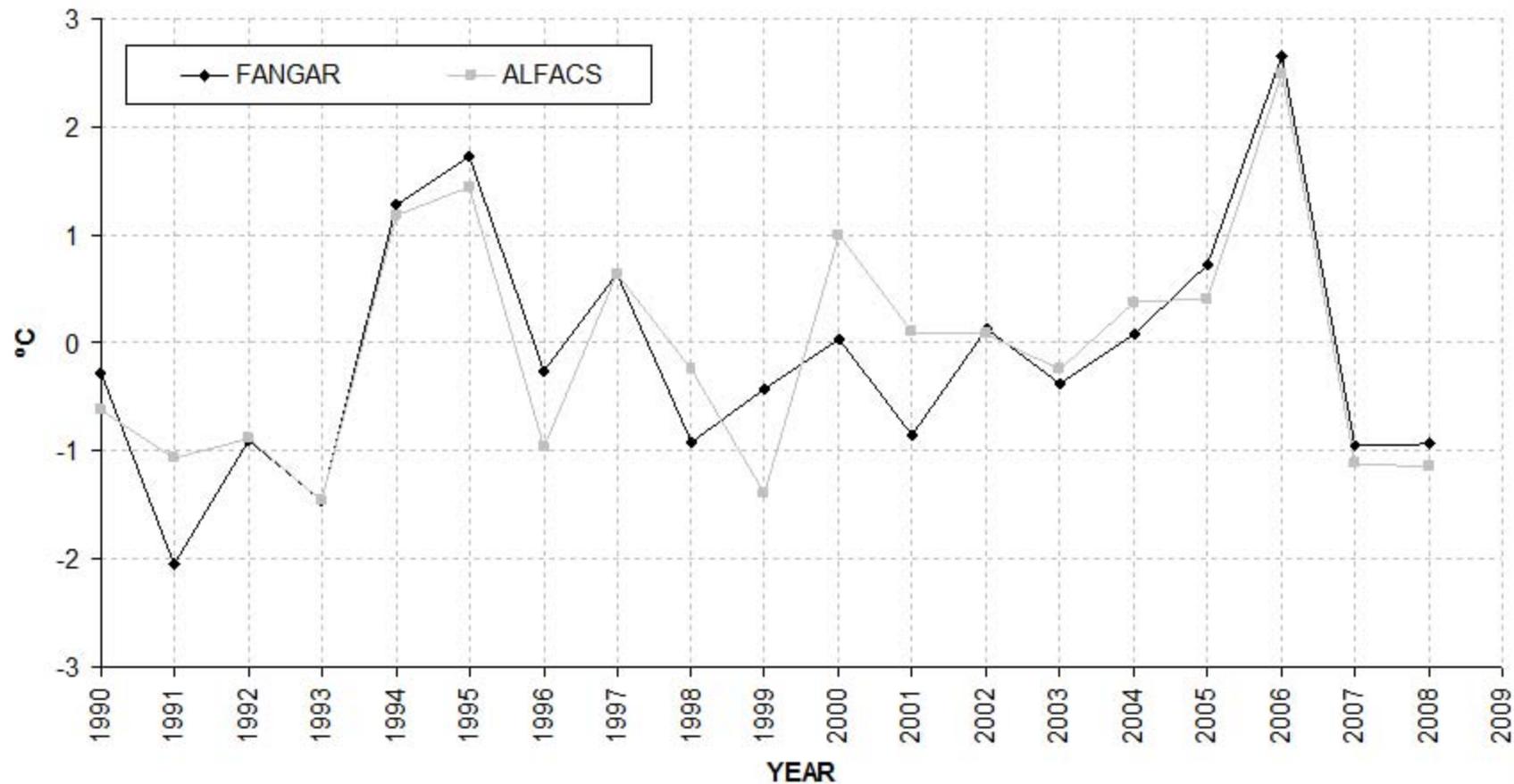
### AVERAGE SEASONAL TEMPERATURE ANOMALIES: SPRING



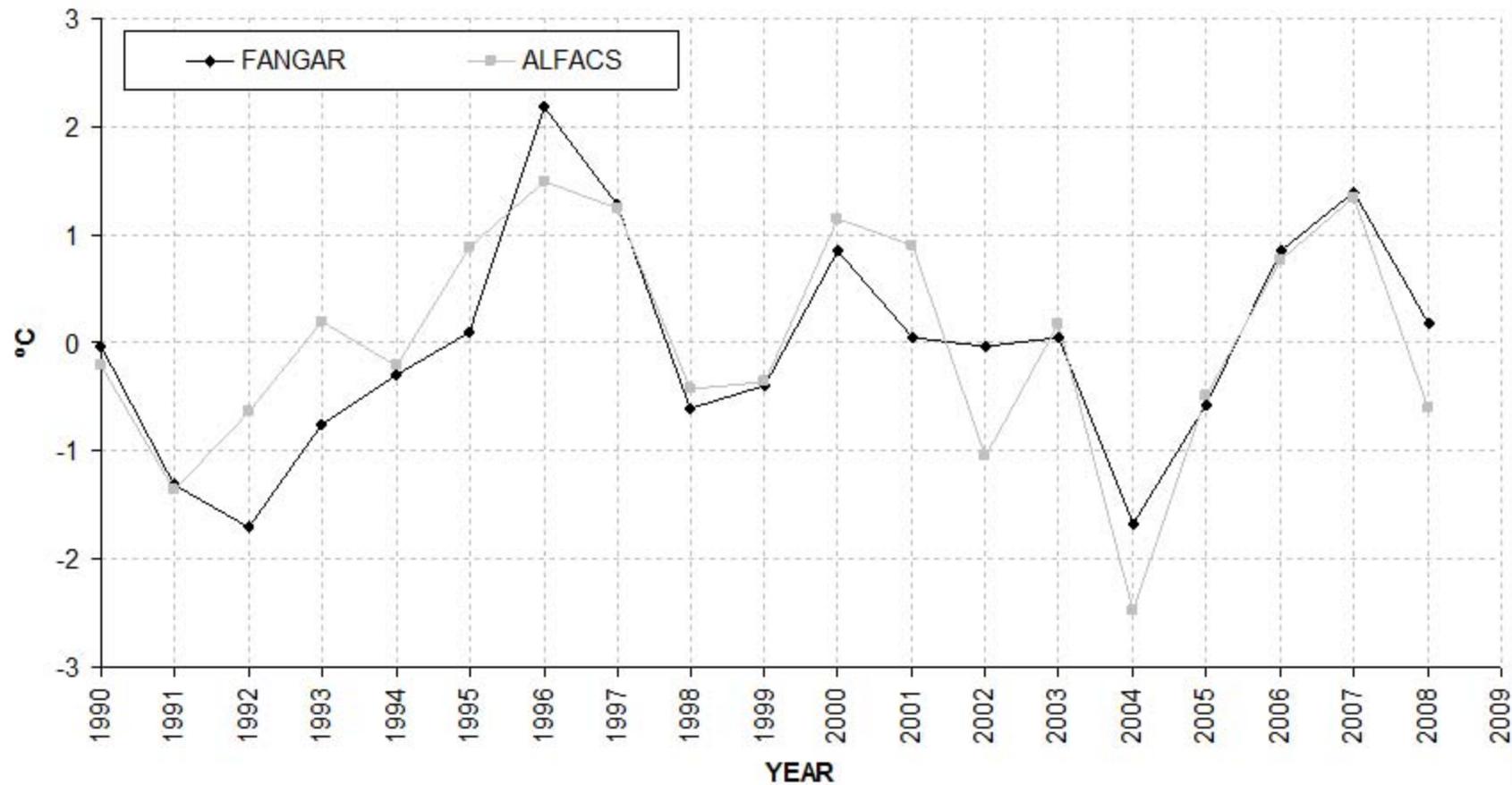
### AVERAGE SEASONAL TEMPERATURE ANOMALIES: SUMMER



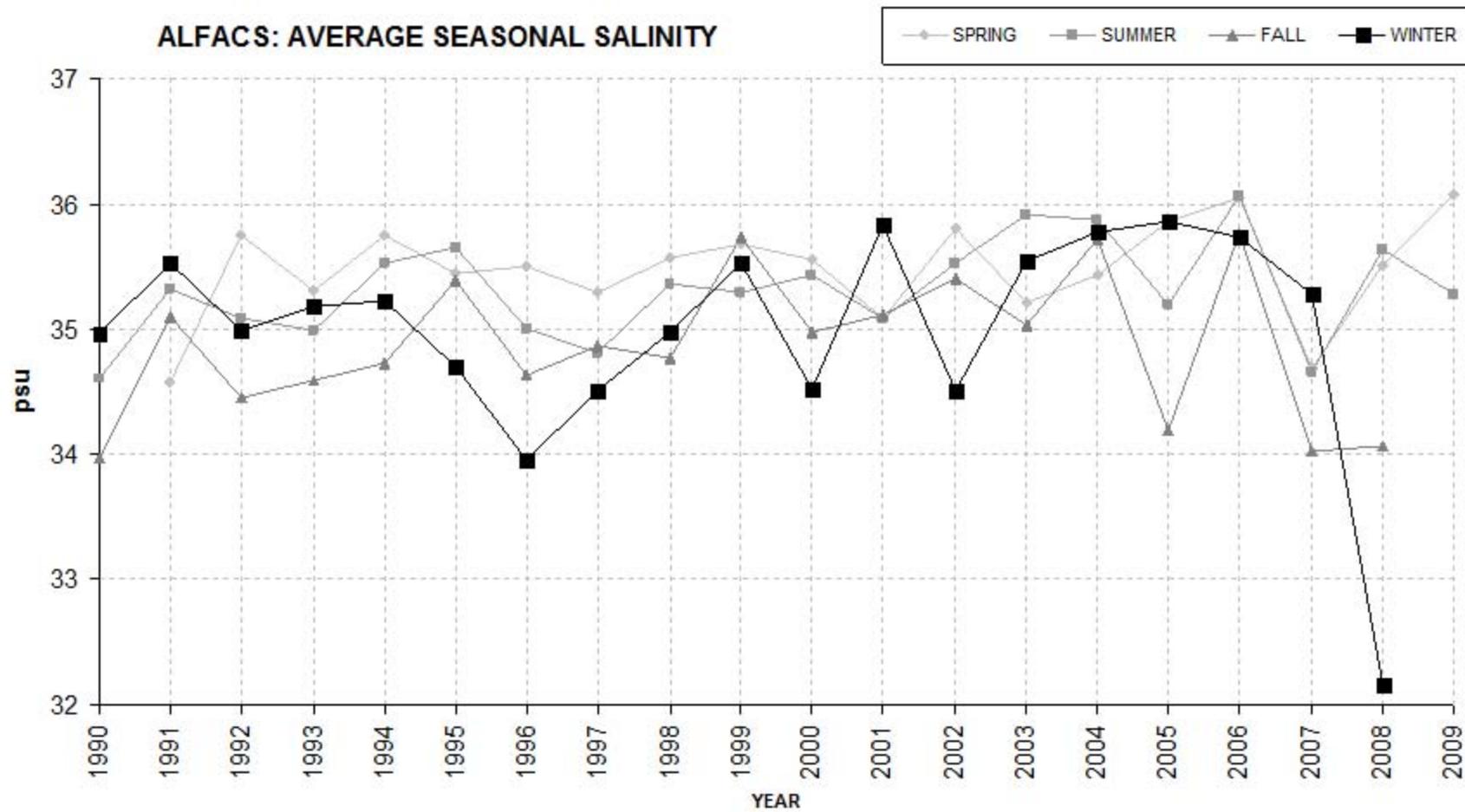
# AVERAGE SEASONAL TEMPERATURE ANOMALIES: FALL



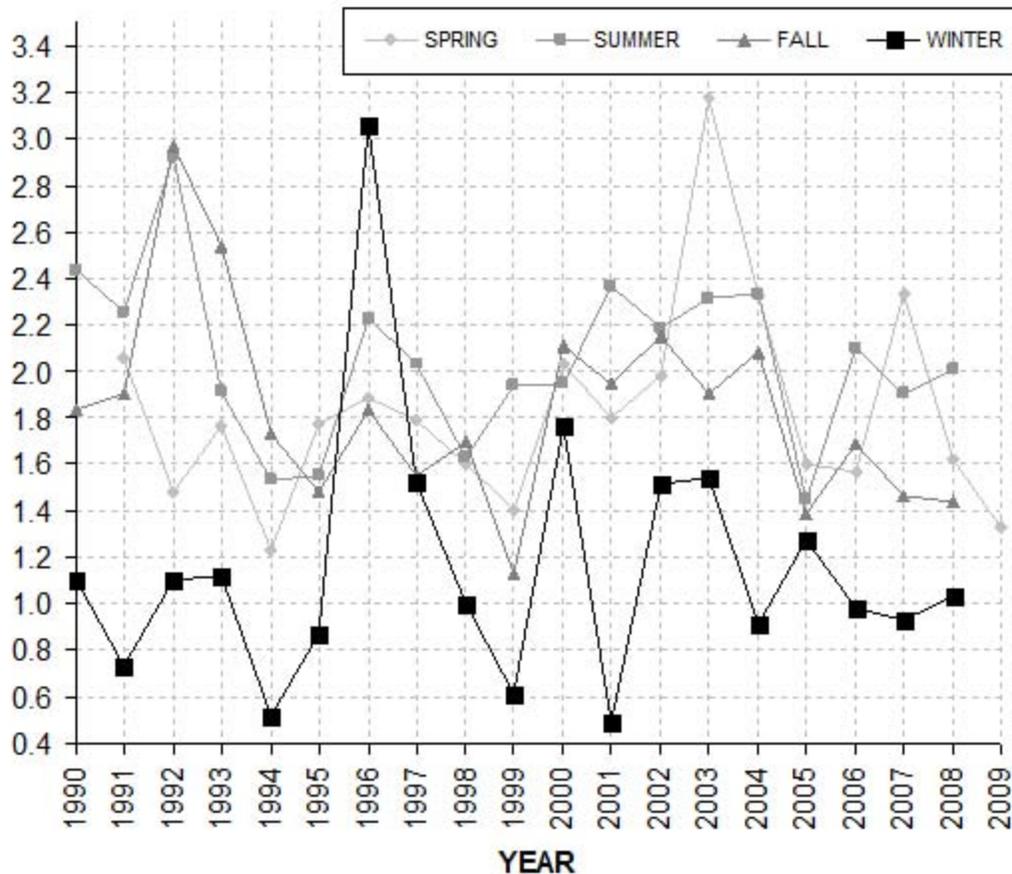
# AVERAGE SEASONAL TEMPERATURE ANOMALIES: WINTER



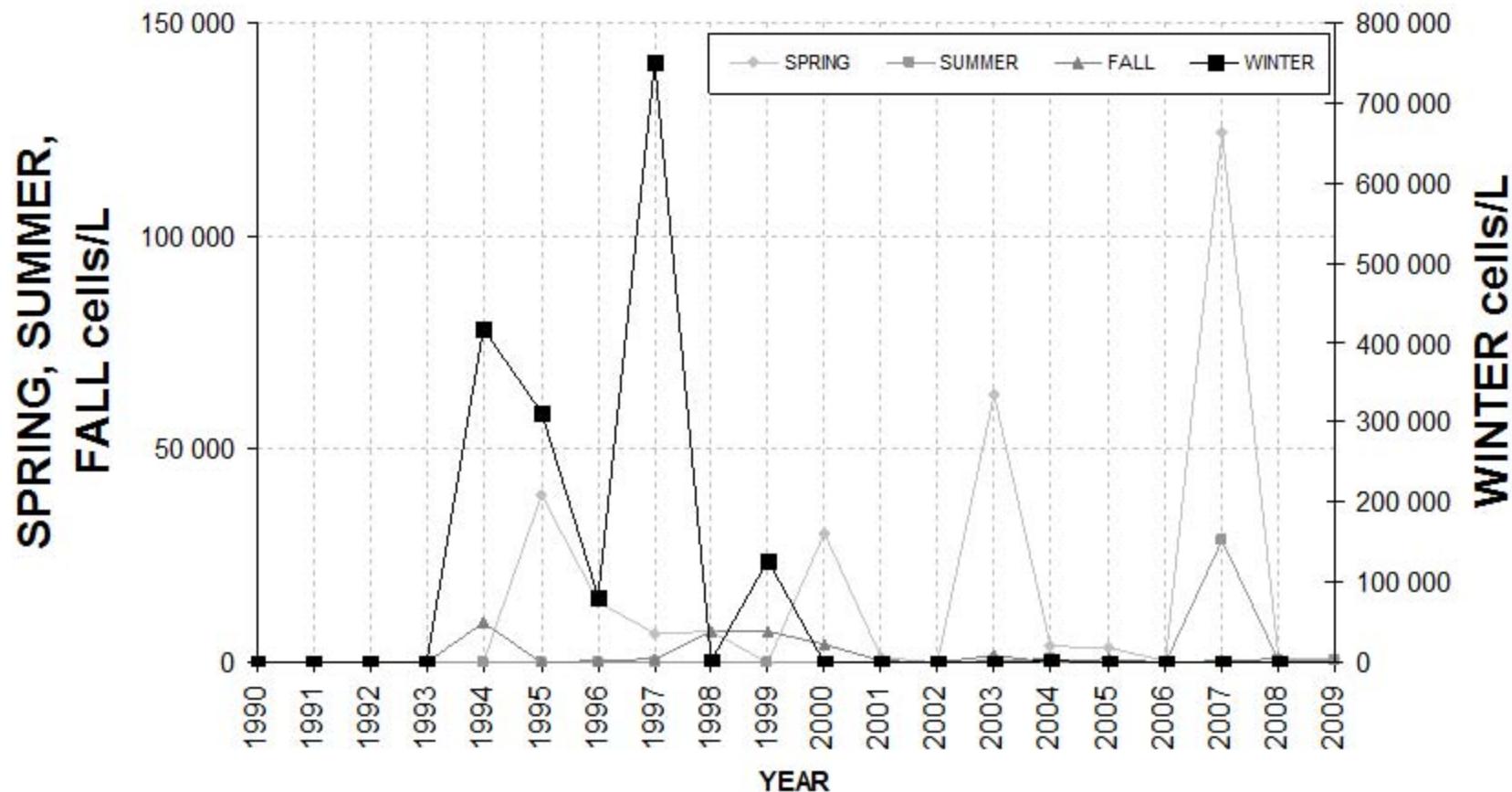
# ALFACS: AVERAGE SEASONAL SALINITY



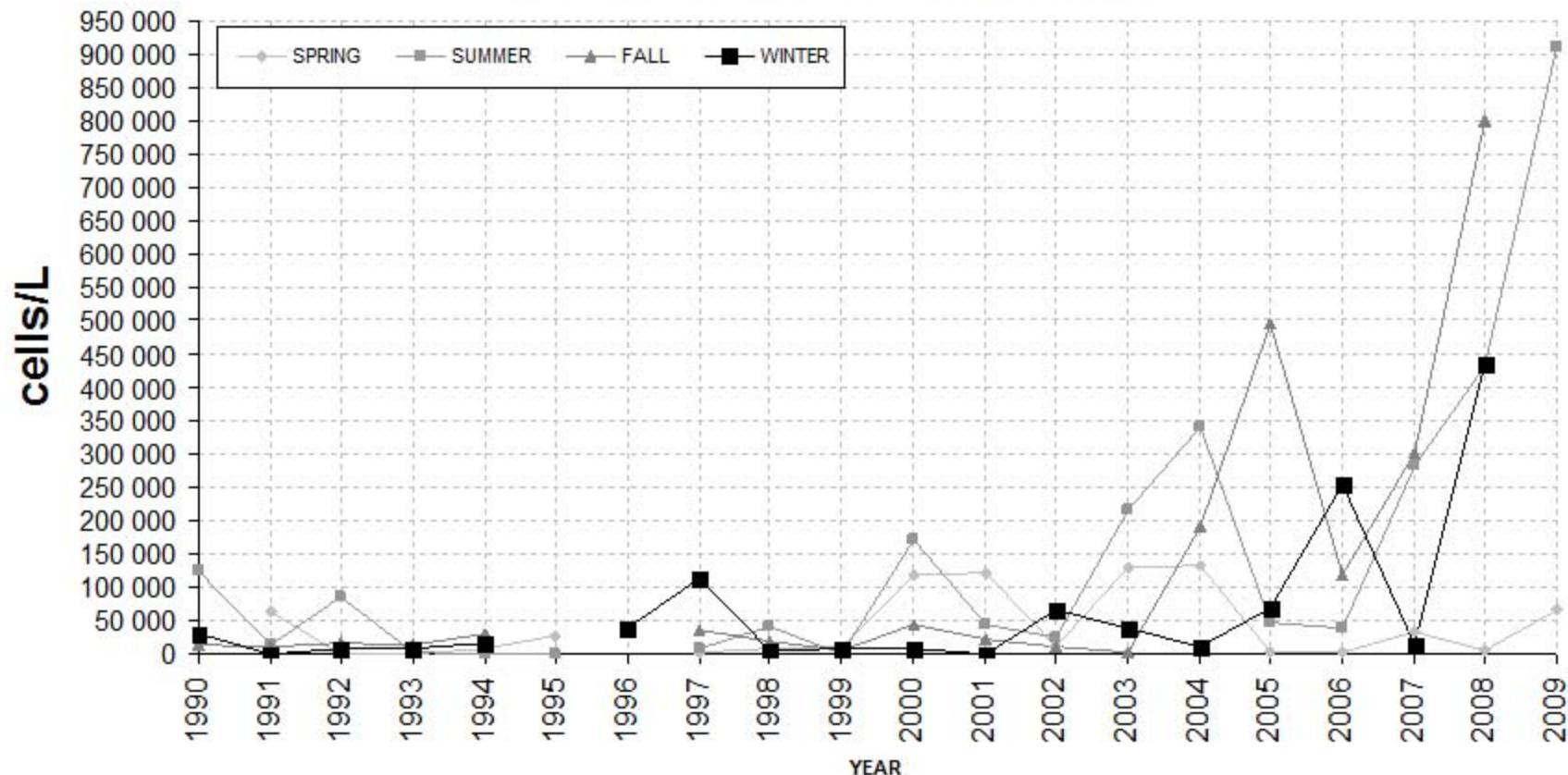
# ALFACS: AVERAGE SEASONAL STRATIFICATION INDEX



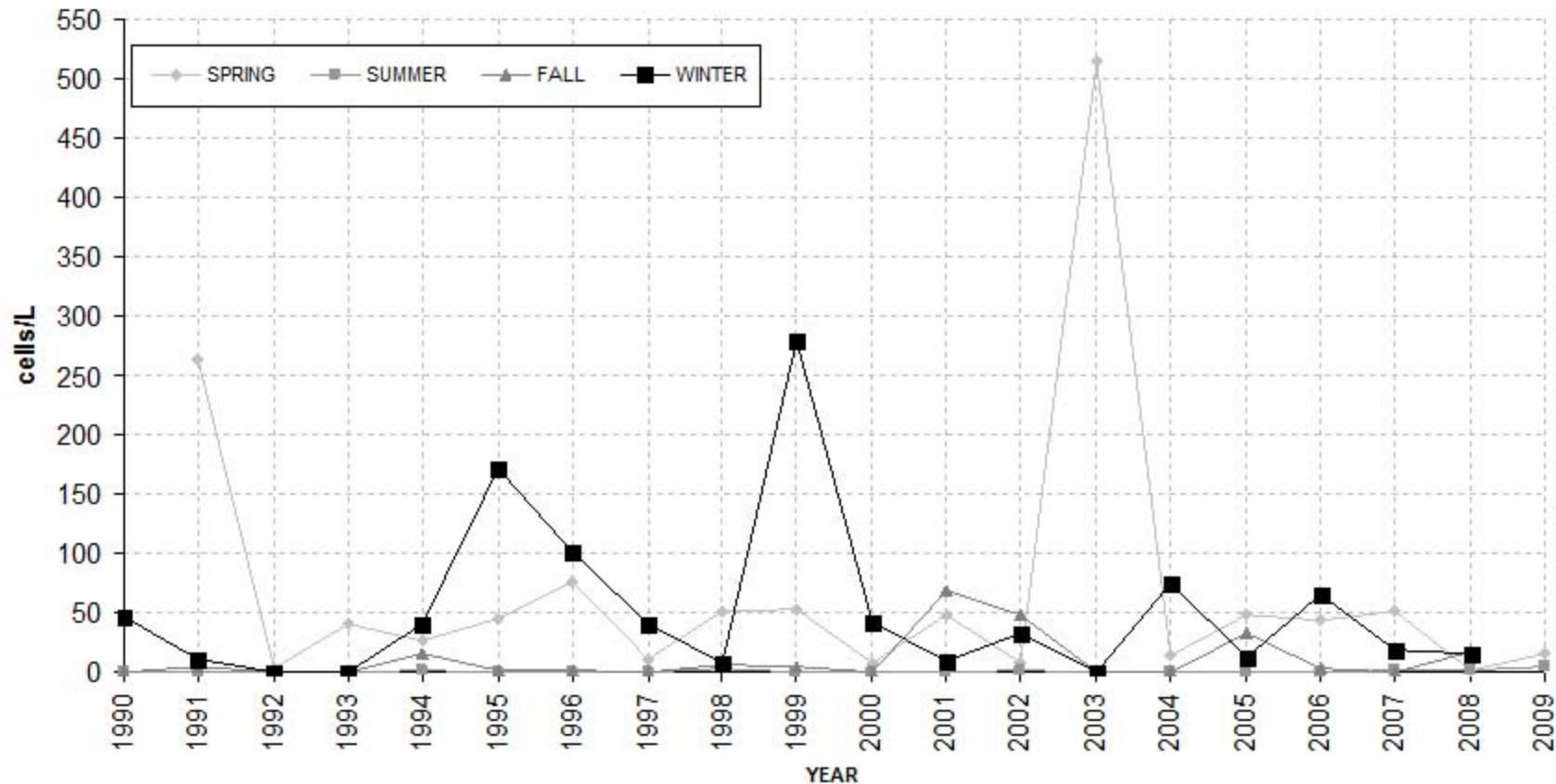
# ALFACS: SEASONAL *Karlodinium* spp. ABUNDANCE



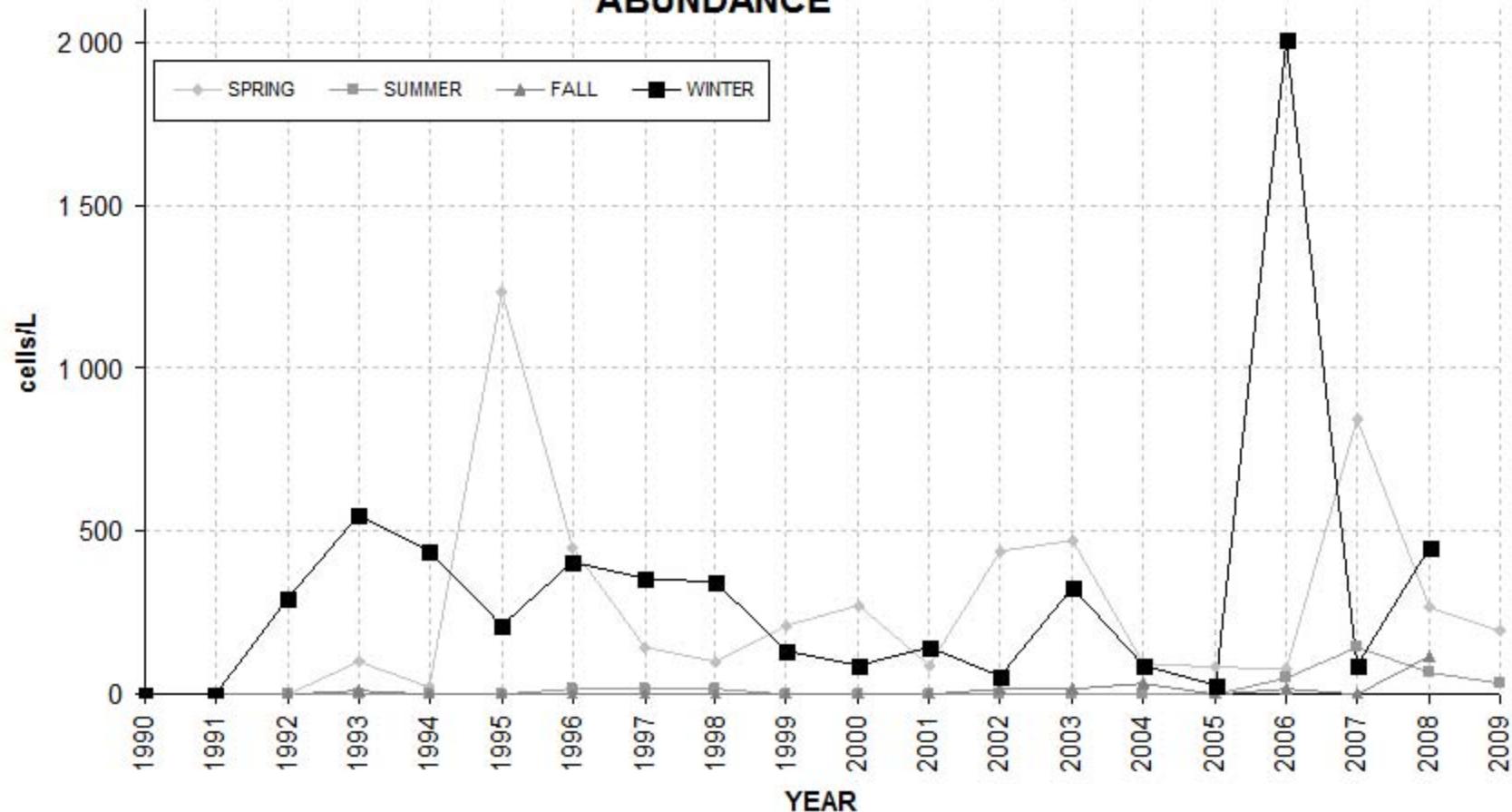
# ALFACS: AVERAGE SEASONAL *Pseudo-nitzschia* spp. ABUNDANCE



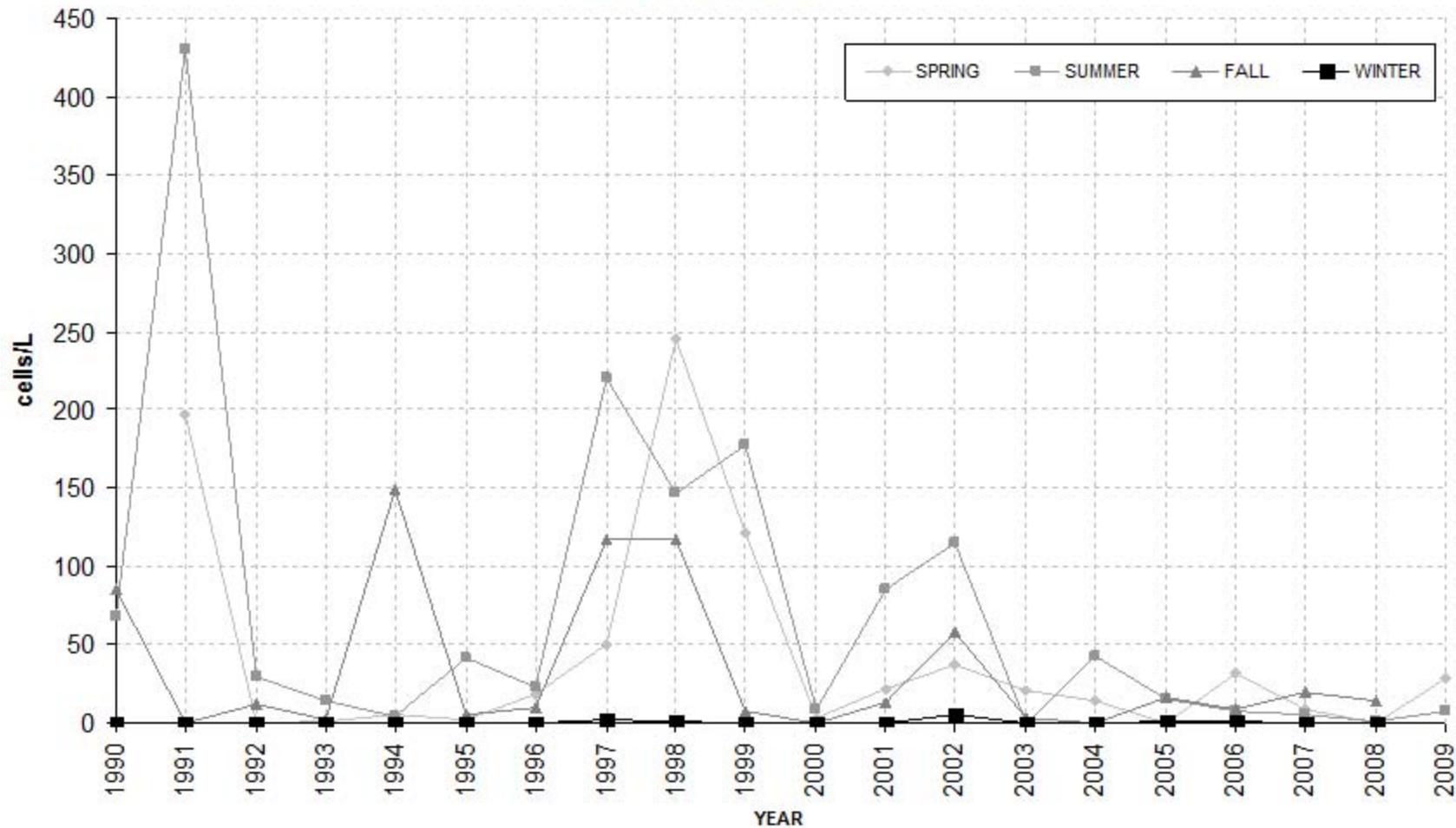
# ALFACS: AVERAGE SEASONAL *Dinophysis sacculus* ABUNDANCE



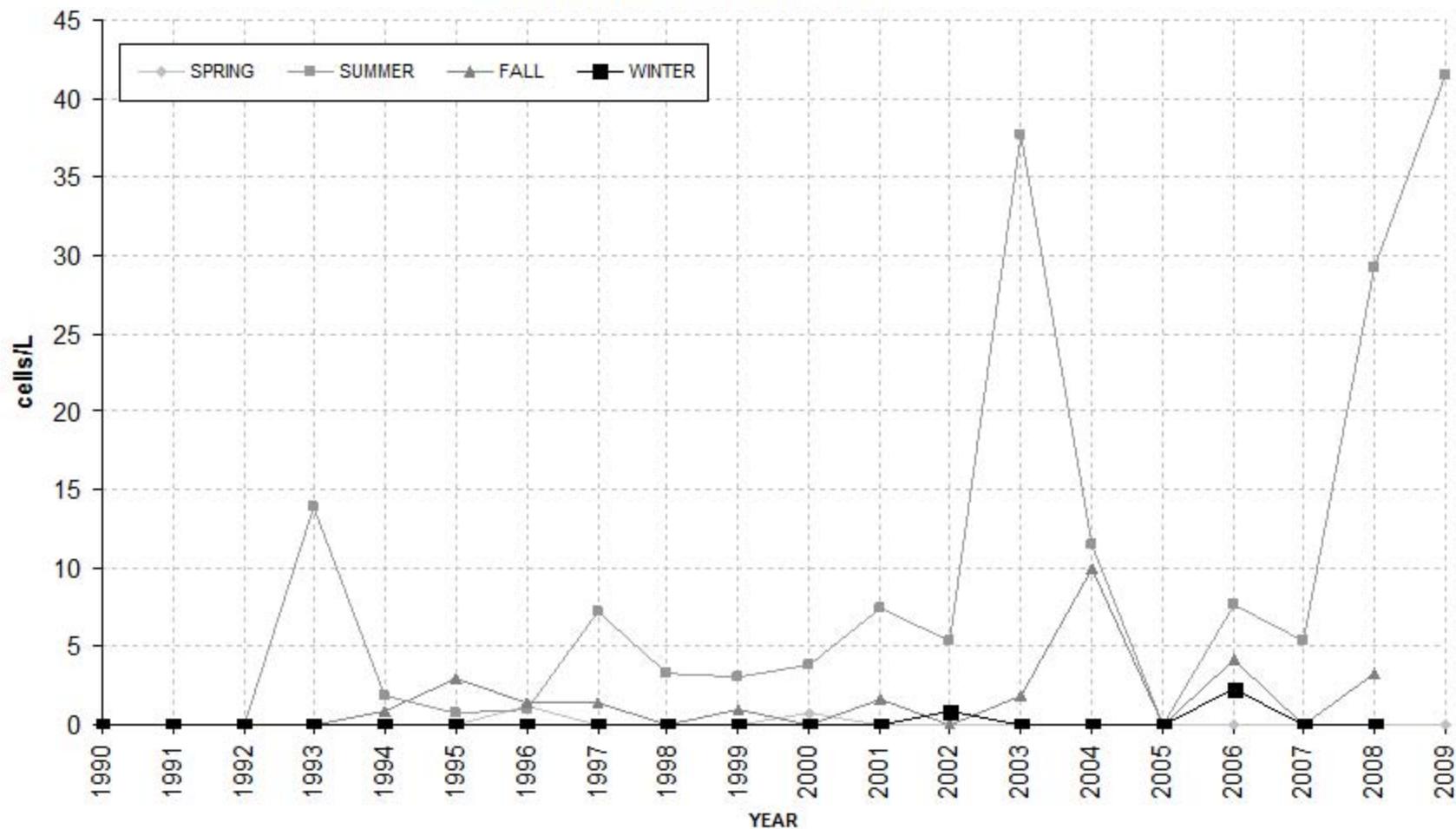
# ALFACS: AVERAGE SEASONAL *Alexandrium minutum* ABUNDANCE



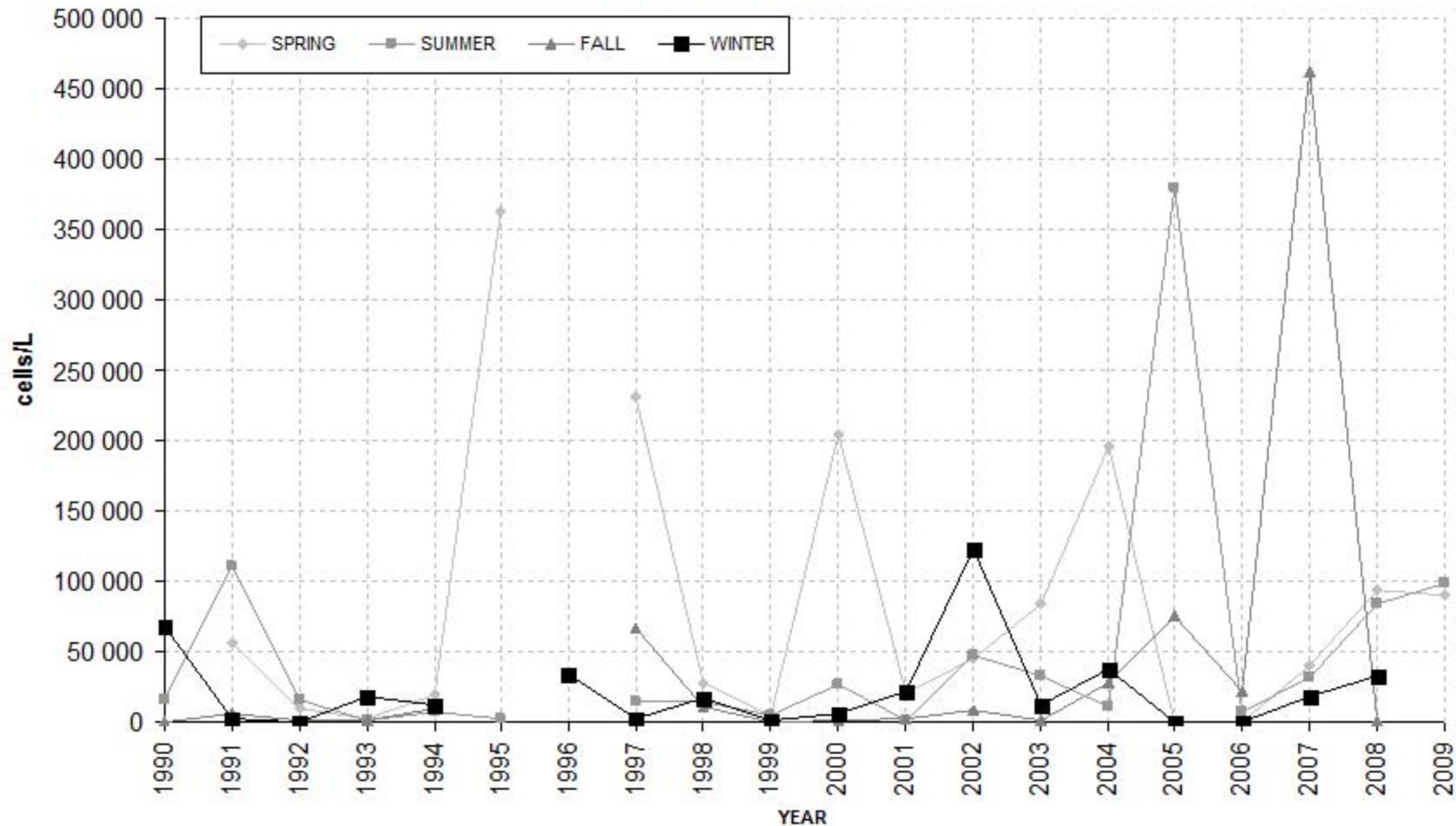
# FANGAR BAY: AVERAGE SEASONAL *Dinophysis sacculus* ABUNDANCE



# FANGAR BAY: AVERAGE SEASONAL *Dinophysis caudata* ABUNDANCE



# FANGAR BAY: AVERAGE SEASONAL *Pseudo-nitzschia* spp. ABUNDANCE



# FANGAR BAY: AVERAGE SEASONAL TEMPERATURE

