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Patterns of primary growth increments in otoliths of *Sparus aurata* larvae in relation to water temperature and food consumption*

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SUMMARY: *Sparus aurata* larvae reared under controlled water-temperature conditions during the first 24 days after hatching displayed a linear relationship between age (t) and standard length (SL): SL = 2.68 + 0.19 t (r^2 = 0.911). Increments were laid down in the sagittae with daily periodicity starting on day of hatching. Standard length (SL) and sagittae radius (OR) were correlated: SL(mm) = 2.65 + 0.012 OR(mm). The series of measurements of daily growth increment widths (DWI), food density and water temperature were analyzed by means of time series analysis. The DWI series were strongly autocorrelated, the growth on any one day was dependent upon growth on the previous day. Time series of water temperatures showed, as expected, a random pattern of variation, while food consumed daily was a function of food consumed the two previous days. The DWI series and the food density were correlated positively at lags 1 and 2. The results provided evidence of the importance of food intake upon the sagittae growth when temperature is optimal (20 °C). Sagittae growth was correlated with growth on the previous day, so this should be taken into account when fish growth is derived from sagittae growth rates.

Key words: daily growth patterns, time series, *Sparus aurata*.

RESUMEN: PAUTAS DE INCREMENTOS EN EL CRECIMIENTO PRIMARIO DE OTOLITOS DE LARVAS DE *SPARUS AURATA* EN RELACIÓN CON LA TEMPERATURA DEL AGUA Y EL CONSUMO DE ALIMENTO.– Las larvas de *Sparus aurata* criadas en cautividad en condiciones de temperatura controlada durante los 24 primeros días de vida, muestran una relación lineal entre la edad (t) y la talla estandard (SL): SL = 2.68 + 0.19 t (r^2 = 0.911). Se formaron incrementos en las sagittae con periodicidad diaria a partir del día de eclosión. La talla estandard (SL) y el radio de las sagittae (OR) están correlacionados: SL (mm) = 2.65 + 0.012 OR (mm). Las series de medidas del grosor de los incrementos de crecimiento diario (DWI), densidad del alimento y temperatura del agua fueron analizados por medio de análisis de series temporales. Las series de DWI están fuertemente autocorrelacionadas, el crecimiento de un día dependía del crecimiento del día anterior. Las series temporales de temperatura del agua mostraron un patrón de variación al azar como era de esperar. Mientras que la cantidad de alimento consumido diariamente era función del consumido los dos días anteriores. Las series de DWI y de densidad del alimento estaban correlacionadas positivamente con un lag de 1 y 2. Los resultados aportan evidencia de la importancia del alimento consumido en el crecimiento del otolito cuando la temperatura es óptima (20 °C). El crecimiento de la sagitta estaba correlacionado con el crecimiento del día previo, esta propiedad debe tenerse en cuenta al calcular el crecimiento diario a partir de las tasas de crecimiento del otolito.

Palabras clave: pautas de crecimiento diario, series temporales, *Sparus aurata*.

INTRODUCTION

The gilthead seabream, *Sparus aurata* L. (Pisces: Sparidae) is an important component of the littoral ecosystems throughout the Mediterranean, also having a high commercial value. It is abundant in waters shallower than 60 m and in brackish water lagoons. *S. aurata* is found from England to Senegal, but it is not commonly observed in Atlantic coastal waters.

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Sagittae microstructure has become accepted as a valuable tool for estimating larval age and for back-calculating birth dates and daily growth rates in length and weight (CAMPANA and NEILSON 1985). Temperature is one of the main factors regulating both fish and otolith growth (BROTHERS 1987). GUTIÉRREZ and MORALES-NIN (1986) suggested that sagittae otolith growth in sea bass is a conservative process that reflects the effect of water temperature. Temperature may have a positive or negative influence on juvenile flounder sagittae growth, depending on the degree (MAY and JENKINS, 1992). Other factors regulating sagittae growth are food (KARAKIRI *et al.* 1989), environmental stress (MAILLET and CHECKLEY 1991), and photoperiod (WRIGHT, 1991; WRIGHT *et al.* 1992), or combinations of these factors (BERGHAHN and KARAKIRI 1990, BROTHERS and MACFARLAND 1981).

Here we present the results of the first study of sagittae growth in *Sparus aurata* (Pisces:Sparidae) larvae. Larvae were reared at a constant optimal temperature of 20 ºC and fed according to the results of QUILLET and CAMARET (1982). The experiment was designed to study the periodicity of the sagittae increments under controlled conditions to gain a better understanding of factors that may influence sagittae growth. Although sample size was small, the analysis of temperature and food in relation to sagittae growth and fish growth might give some insights into processess of somatic fish growth.

MATERIALS AND METHODS

Experimental procedures

Larvae were obtained from naturally spawning adult *S. aurata* kept at the "Es Murterar" Aquaculture Station under photoperiod conditions appropriate to stimulate spawning in December. Fertilized eggs were placed in incubation boxes, and larvae hatched after 40h at 19–20 ºC. All the larvae hatched in 2h. Larvae were reared in a 18 m^3 tank with recirculated sea water at a concentration of 40 larvae/l. During the first 5 days, two-thirds of the total tank capacity was replaced with sea water, and from day 12, 100% of tank capacity was replaced daily.

Water temperature was recorded daily and photopheriod was regulated at 14 light hours: 10 dark hours (14L: 10D). The feeding was adapted to optimal larval development at 20 ºC according to QUILLET and CAMARET (1982). The rotifer, *Brachionus plicatilis*, was provided at a density of 10–20 rotifers/ml from day 3. *Artemia spp* nauplii were provided from day 14 at a density of 8–10 *Artemia*/ml. The clorophiceae algae, *Tetraselmis* sp., was added from day three to maintain good water quality. The amount of food available in the tank was determined at 3h intervals. When the prey item density was lower than the optimum level (see above), food was supplied until the optimum level was reached again. The amount of food consumed daily was calculated as the total number of prey items given each day divided by the tank volume.

Three larvae of *S. aurata* were collected every two days and fixed in buffered formalin of pH=8, in order to preserve them for measurements. The experiment ended when the larvae were transferred to a larger tank, 24 days after hatching.

Sagittae and body growth measurements

Standard lengths (SL) of preserved specimens were measured to the nearest 0.1 mm and ranged from 2.7 to 7.5 mm. Lengths were not corrected for shrinkage due to preservation. Sagittae (N = 36) were disected out using probes, washed with distillated water and allowed to dry for 2 h, and then mounted sulcal side down in Eukitt (PALOMERA *et al.* 1988).

The sagittae were very fragile, 28% of sagittae fractured when touched or presented abnormal morphology. This might have been due to formalin preservation, even at pH=8, or might have been related to deficiencies in our rearing conditions because malformations in juvenile fishes were observed. The total number of sagittae mounted and studied were 26.

The sagittae were examined using a compound microscope with a 100X immersion objective and polarized light. To examine the sagittae microstructure we used a video-camera connected to the microscope. The image obtained was displayed and digitised on a microcomputer. The width of the distance between the end of the growth of the previous day and the growth of the following day was measured along a consistent radius for all specimens considered from sagittae core to sagittae dorsal edge. Growth increments of the sagittae were recorded and averaged (Fig. 1c).

The number of sagittae primary increments counted from each larvae were related to actual larval age for validation of daily periodicity.

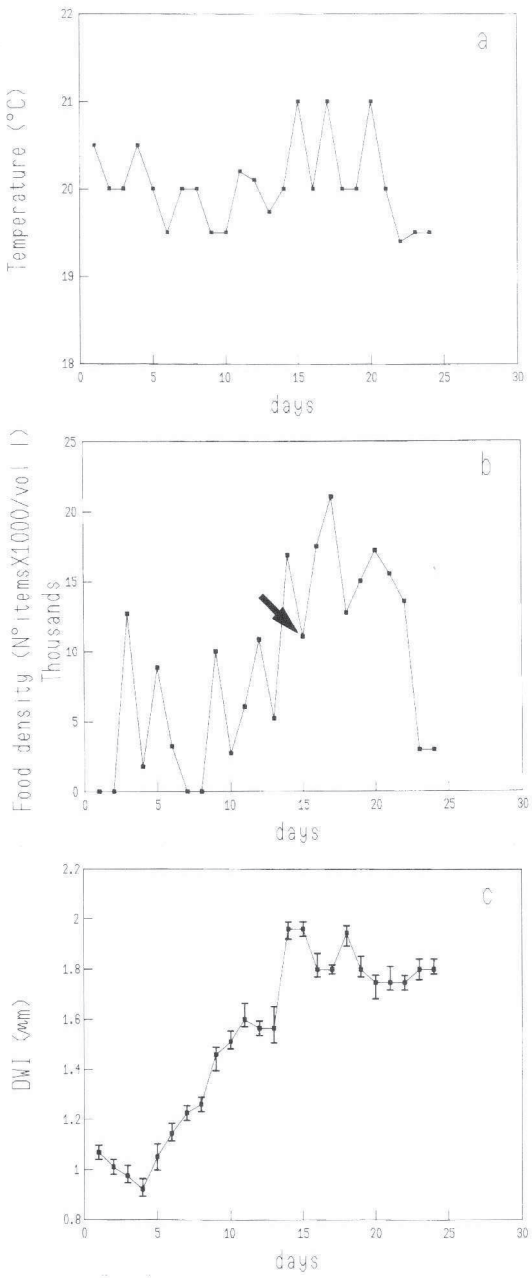

FIG. 1. – Time series of the studied variables during the experimental period, a: water temperature, b: daily food intake (N prey items/tank volume l) arrow indicates *Artemia* incorporation into the diet, c: *Sparus aurata* otolith daily growth increments (DWI) (Error bars = 1 SD).

Statistical procedure

Variations in temperature experienced were in the range of 1.6 ºC in spite of our attempts to maintain a constant environment of 20 ºC (Fig. 1a) and therefore we believed this to be important enough to be included in our analysis. The series of measurements of temperature (Fig. 1a), food availability (Fig. 1b), and sagittae increment widths (Fig.1c) were analyzed independently of each other in order to determine what kind of pattern they exhibited over time. Water temperature was analyzed in relation to DIW in order to examine the effects of small variations in temperature might have on the sagittae growth. Their relationships were determined using cross-correlation functions.

The analysis was carried out using time series methodology in order to explore the dynamic nature of processes that regulate sagittae growth (CHATFIELD 1975; BOX and JENKINS, 1976; GUTIÉRREZ and MORALES-NIN, 1986). The most basic and common tools used for analysing the structure of a time series are auto-correlation, partial autocorrelation functions, and cross-correlation functions in order to determine the relationship among time series.

The auto-correlation function (ACF) is the result of computing the autocorrelation coefficients from one to a prescribed number of lags and then plotting these coefficients as a function of the lags:

$$r_k = c_k / c_0$$

$$c_k = 1/N \sum_{k+1}^{N} (z_i - \bar{z})(z_{i-k} - \bar{z})^2$$

where: $z_i = z_1, \ldots, z_N$ is the stationary time series; $k = 0,1,\ldots$ are the lags; z is the mean of the series, c_0 is the variance of the series; N being the number of observations. Obviously z_k cannot be calculated for $k > N-1$, and in practice it should not be calculated for $k > N/4$. The partial auto-correlation function (PACF) is the set of partial auto-correlations coeffients at various lags k. The standard errors for each coefficient of the ACF are computed as the square root of the variance and for the PACF as the inverse of the square root of N. The influence of water temperature and food availability on growth increments were analyzed by means of cross-correlation functions (CCF) (BOX and JENKINS, 1976).

Relationships between body measurements and between age and number of increments, and age and SL were determined by means of linear relationships fitted by least-squares techniques. The significance of the linear relationships was determined by means of t-tests with a probability of 0.05. All the calculations were made using the PC-SCA statistical system.

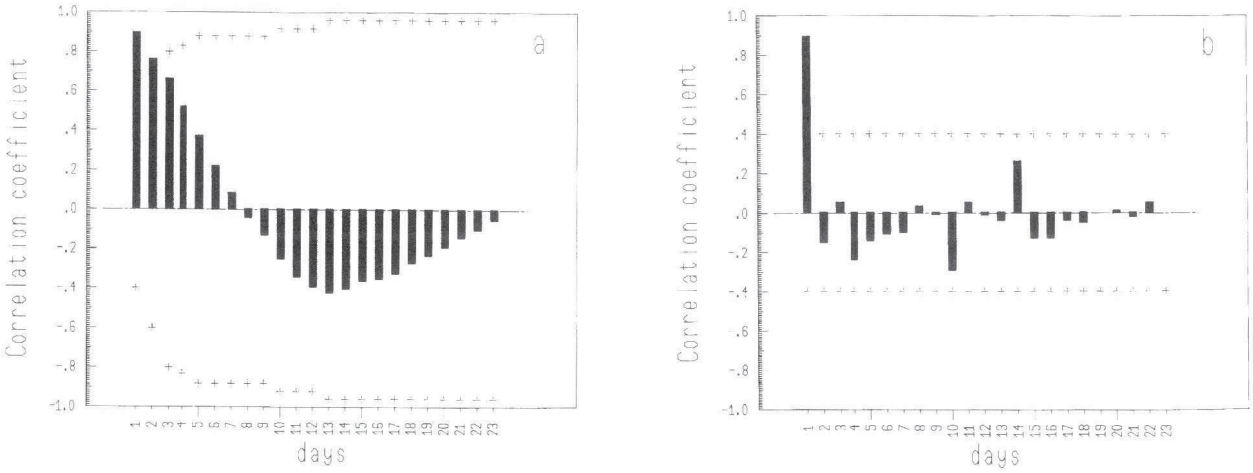

FIG. 2. – Estimated functions for *Sparus aurata* otolith daily growth increments (DWI), a: autocorrelation function (ACF) and b: partial autocorrelation function (PACF). Dotted lines represent significance limits (2 standard errors from zero).

RESULTS

Larvae development

The larvae had functional stomachs at the end of day 1 as well as intestines, and vents by day 2. They commenced feeding on day 3. Remnants of the yolk sac were present through the first 4–6 days of life. Larvae mortality was 20% in the first week and 39.32% over the entire period of the experiment.

For the specimens studied under our conditions, the relationship between standard body length (SL) and age (t) in days was found to be linear over the entire size range. This relationship is expressed numerically as: SL = 2.68 + 0.19 t (r^2 = 0.911 & N = 26, $p < 0.05$). This suggests an overall growth rate of approximately 0.19 mm d^{-1}.

Periodicity of sagittae growth increments

At hatching, the sagittaes of each larvae consisted of a lenticular core that had a mean radius of 8.36 µm. Some (3-4) poorly-defined increments were visible within the sagittae core. Concentric increments consisting of an adjacent dark and a light zone were laid down around the core, corresponding to the structures described as occurring daily in other fishes (PANNELLA, 1971).

The correlation between number of increments and actual age was high and significant (increment = – 0.139 + 0.7682t, r^2 = 0.9851 & N = 18, $p < 0.05$). The intercept of the regression line with the x-axis was negative but statistically not significantly different from 0 ($p < 0.05$, t-test). The slope of the regression was 0.9682 increments d^{-1}, which was not significantly different from 1 ($p < 0.05$, t-test). The age determined from counts indicated daily increment formation. Thus we conclude that the increases in sagittae growth occured daily, and incremental growth was recorded from the day of hatching.

Standard length and sagittae radius relationship

The relation of standard length (SL) to sagittae radius (OR) was linear: SL (mm) = 2.65 + 0.012 OR (µm) (r^2 = 0.951, N = 25).

Daily patterns of sagittae growth and rearing factors

The time series analysis of growth increments (Fig. 1c) revealed a non-randomness in the sagittae growth process of larvae. The autocorrelation function (ACF) (Fig. 2a) showed a strong positive autocorrelation with an exponential decay, and the partial autocorrelation function (PACF) revealed that there was a statistically significant dependence upon growth on one day (t) with respect to the growth of the previous day (t–1) (Fig. 2b). This type of structure in the ACF suggests an autoregressive model of order 1 (AR1) (BOX and JENKINS, 1976), which means that the actual growth is influenced by previous growth.

The series of water temperature (Fig. 1a) and of food density (Fig. 1b) were also analyzed by means

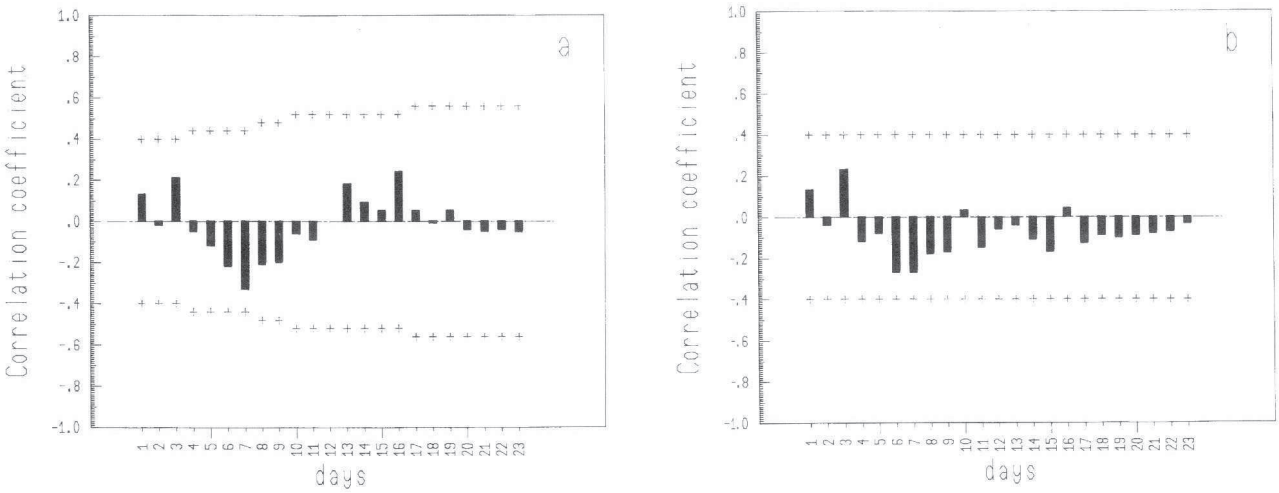

FIG. 3. – Estimated functions for daily water temperature, a: autocorrelation function (ACF) and b: partial autocorrelation function (PACF). Dotted lines represent significance limits (2 standard errors from zero).

of ACF and PACF. The temporal pattern of water temperature did not reveal any significant structure, and the small variations of water temperature can be considered random variations (Fig. 3a and 3b). The temporal structure of food density showed significant positive correlations for ACF at lags k=1 and k=2, and a negative correlation for PACF at k=7. The consumed food was a function of the food ingested the two previous days. There was also a significant weak effect of food provided at weekly intervals (Fig. 4a and 4b).

Sagittae daily growth in relation to temperature and food

It has been suggested that small variations in water temperature can affect sagittae growth (PANNELLA, 1980; BROTHERS, 1987). However, the CCF between water temperature and daily growth increments revealed no significant relationship ($p<0.05$) (Fig. 5a).

The CCF between daily consumed food density and daily sagittae growth series yielded statistically

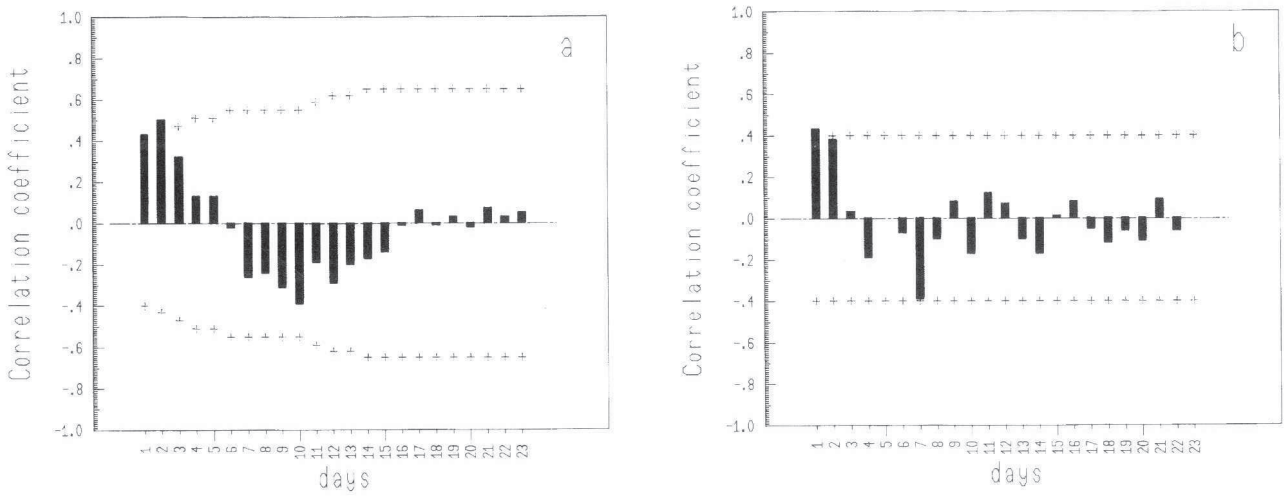

FIG. 4. – Estimated functions for daily food ration, a: autocorrelation function (ACF) and b: partial autocorrelation function (PACF). Dotted lines represent significance limits (2 standard errors from zero).

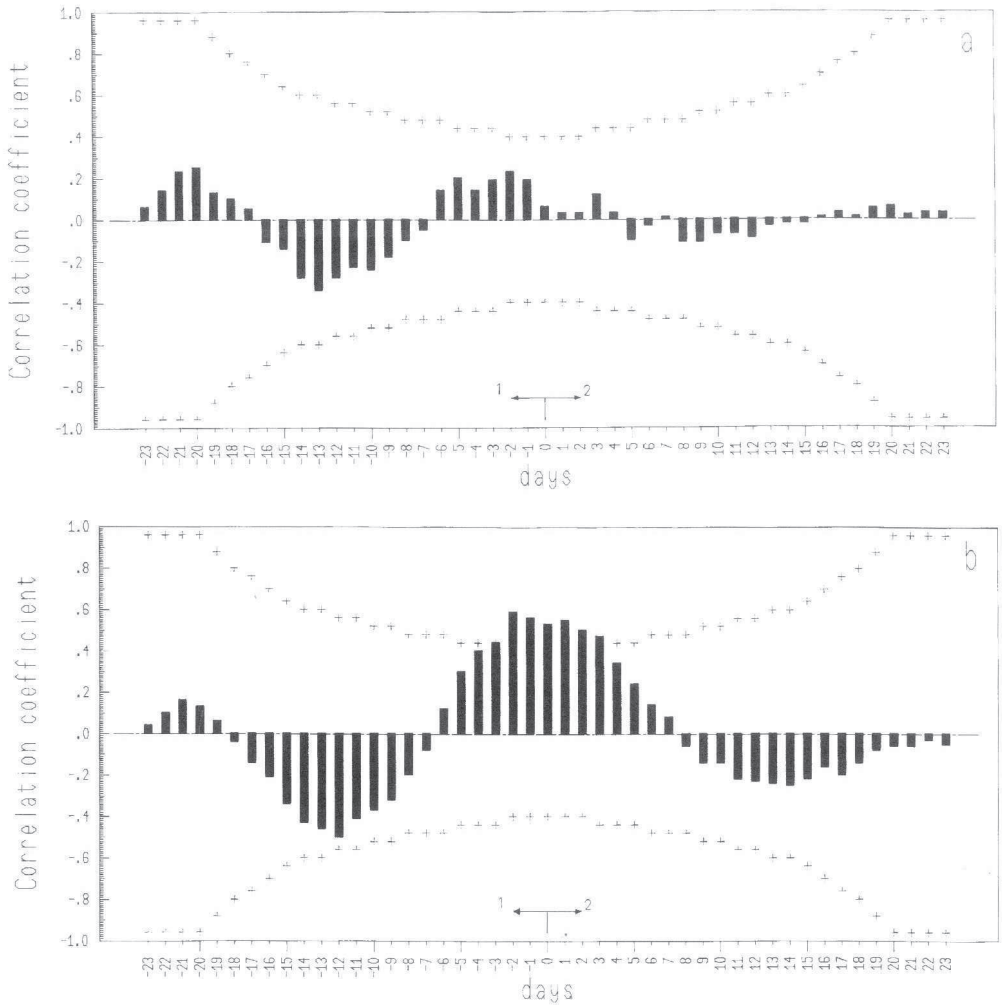

FIG. 5. – Cross-correlation functions between daily sagittae increment width and (a) daily water temperature series, (b) daily food ration. Dotted lines represent significance limits (2 standard errors from zero). (1) dependent variable series lagged behind the independent variable series and (2) the lagging of the independent variable.

significant relationships when food density was held fixed and DWI was lagged. The coefficients at time lags k=0, k=1, and k=2 were found to be statistically significant ($p < 0.05$) (Fig. 5b).

DISCUSSION

The sagittae of reared gilthead seabream presented growth structures similar to those on sagittae of fish living free in the natural environment (MORALES-NIN, unpubl. dat.), though somewhat less well-defined. The increments were laid down in *S.aurata* sagittae with daily periodicity starting the day of hatching. Increment formation may start during the embryonic period, on the day of hatching, at first feeding, etc. depending on the species (RADTKE, 1989). *S.aurata* started feeding at age 3, thus the start of increment formation was dependent on activity and physiological changes related to hatching.

Fish growth in length over time was linear, however for many organisms this kind of relationship is allometric following a Gompertz growth curve (PALOMERA *et al.*, 1988). The fact that we obtained a linear relationship, may be due to the short time span of the study and therein giving us a short age span within which we made our observations.

The daily pattern of microscopic growth increments in the otoliths is an evidence of clock-like mechanisms of deposition driven by at least two diurnal endogenous processes, calcium mobilisation (MUGIYA, 1987) and peptide secretion (ZANG,

1992). Although some fish respond to stress by depositing more or less than one growth increment per day in their otoliths, other species have otolith microincrement formation processes that are resistant to physiological stress (CAMPANA and NEILSON, 1985). Sagittae growth in *S.aurata* under the experimental conditions, keeps daily clock-like increment periodicity. Moreover, the growth rate (reflected as increment width) is a conservative process depending on previous growth and food conditions. When the range of water temperature variability was narrow, random and kept at optimal levels for the development of the larvae (QUILLET and CAMARET, 1982), it did not significantly influence sagittae growth. The conservative nature of sagittae formation has also been reported for juvenile flounder (MAY and JENKINS, 1992) and sea bass (GUTIÉRREZ and MORALES-NIN, 1986).

A number of studies have documented the influence of temperature on the width of daily growth increments (e.g., MARSHALL and PARKER, 1982; NEILSON and GEEN, 1982; GUTIÉRREZ and MORALES-NIN, 1986; MAILLET and CHECKLEY JR., 1991; MAY and JENKINS, 1992). The lag between temperature change and its effect on growth over a number of days has been reported in juvenile sea bass (GUTIÉRREZ and MORALES-NIN, 1986), and in the early stages there is no memory. These same findings have been reported for winter cohorts of juvenile flounder, in which high temperatures have a negative effect on growth (MAY and JENKINS, 1992), a trend that also appears in gilthead bream during the first 10 days of life. Temperature and food availability have been shown to be determinant on larval Atlantic menhaden growth and survival (MAILLET and CHECKLEY, 1991).

The ability to estimate individual growth histories from the correspondence between increment widths and somatic growth in length or weight provides the fundamental tool for demographic analysis of fish populations. However,sagittae growth and fish growth, although related, can be uncoupled in certain circumstances (MOSEGAARD *et al.* 1988; BRADFORD and GEEN 1987; 1992). Also, the otolith growth autocorrelation found in *S.aurata* and its dependence of the previous otolith growth, showed a certain independence from the daily growth rate variations. Thus, when sagittae and fish growth are studied, the conservative nature of sagittae growth should be considered before fish growth variations are inferred from the increment width.

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