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Does the nutritional condition limit survival potential of sardine *Sardina pilchardus* (Walbaum, 1792) larvae off the north coast of Spain? RNA/DNA ratios and their variability

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Abstract

The aim of this study was to determine the nutritional condition of *Sardina pilchardus* larvae and the percentage of larvae that were starving at the time of capture. The survey was conducted, during the spawning season, in April and May of 1991 and 1992, off the northern coast of Spain. An accepted fluorimetric technique was used to determine the concentrations of both RNA and DNA and to calculate RNA/DNA ratio for each larva. RNA/DNA ratios were related to the zooplankton biomass (>53 μm). Low percentages of starving larvae (RNA/DNA ratio less than 1.3) were registered, ranging from 0% to 2.5%. Results based on the mean and variance of individual larval growth rates showed that predation pressure was not increasing from 1991 to 1992. Based on the presence of sardine larvae in good condition together with low predation, a high recruitment was expected. However, the 1993 recruitment, from 1992 spring spawning, was very poor. This was associated mainly with unfavourable advection from the nursery area. Moreover, even low levels of starvation, such as registered, operating over long time periods could have considerable consequences for larval mortality.

Keywords: Fish larvae; Nutritional condition; *Sardina pilchardus*; RNA/DNA ratio

1. Introduction

The annual recruitment to sardine (*Sardina pilchardus*) stocks, the basis of an important pelagic fishery on the Atlantic coast of the Iberian Peninsula, shows a high variability (Pestana, 1989). That may be reflected in overall stock abundance, thus affecting the fishery (Porteiro et al., 1986; Robles et al., 1992). The spawning activity of sardine seems to be linked to upwelling (Lopéz-Jamar et al., 1995; Chícharo, 1996), which in the Atlantic coast of the Iberian Peninsula is seasonal (April–September) (Fiúza, 1980, 1983; Cabanas et al., 1992). Along the north Spanish coast and in the Cantabrian Sea area, *Sardina pilchardus* spawns in May and off San Sebastian (Garcia-Soto et al., 1991),

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but along the northwest Portuguese coast, this species is a winter spawning (Ré et al., 1990). This spawning strategy could be understood as an adaptive mechanism to avoid offshore transport in spring upwelling areas and associated loss of larvae from the coastal habitat (Parrish et al., 1981; Sinclair, 1988). However, the spring coastal current (E–W) may aid the transport of small larvae hatched in the Cantabrian area, to the upwelling areas along the south Cape Ortegal. According to Dias et al. (1989), 0-group fish (recruits) are found mainly on the west coast of the Iberian Peninsula between Cape Ortegal and Cape Roca.

It is commonly assumed that to understand the recruitment variability it is necessary to study the factors which determine survival during the planktonic early life history stages. Several hypotheses – (e.g. Hjort, 1914) ''critical period'' hypothesis, (Cushing, 1975, 1990) ''match–mismatch'' hypothesis, and (Lasker, 1975) ''stable ocean'' hypothesis – link low fish larvae survival (and future recruitment) with starvation during larval stage.

Many studies have confirmed that food availability is a limiting factor for survival during these early phases (Setzler-Hamilton et al., 1987). Yet, Cushing (1995) noted that demonstration of a relationship between food level and survival, often, is equivocal. Survival of larval fish may potentially be estimated from indices of larval condition, on the assumption that larvae in poor condition grow slower and are subject to the cumulative effects of starvation, predation or disease (Suthers, 1992). Larval food and predator abundance are difficult to monitor and are often highly correlated with other environmental factors that may also influence larval fish survival. According to Pepin (1989) the mean and variance in individual growth rates appear to be a useful tool to determine whether changes in predator or food abundance influence larval fish survival.

RNA/DNA ratios have been used to assess the nutritional condition and growth rates of a wide range of marine organisms, especially of fish larvae (Buckley, 1984; Robinson and Ware, 1988; Bailey et al., 1995), and they appear to give a consistent relationship across species (Suthers, 1992). Larvae in good condition tend to have higher RNA/DNA ratios than those in poorer condition (e.g. Robinson and Ware, 1988; Clemmesen, 1994).

The present study forms part of a joint European SARP (Sardine Anchovy Recruitment Project) initiative in which larval abundance and condition are compared with subsequent juvenile recruitment and related to environmental parameters (Lopéz-Jamar et al., 1995). The aim of this study was to analyse the nutritional condition and the growth rates, based on RNA/DNA ratios, of sardine larvae off the north coast of Spain, during the spawning season. The incidence of starvation at the time of capture in sardine larvae and the influence of a measure of prey availability on larval condition were also analysed.

2. Materials and methods

2.1. Field study

Hydrographic and plankton sampling were carried out during three research cruises on Spain's northern continental shelf (45–42°N, 10–2°W) aboard the B/O ''Cornide de Saavedra'' (17 April–13 May 1991; 1–13 April 1992) and R.V. ''Valdivia'' (4–24 May 1992). The survey grid was divided into three areas: 1 – from the boundary with French territorial waters to near Gijón; 2 – from the boundary of area 1 to Cape Ortegal; and area 3 – from Cape Ortegal to Cape Finisterre (Fig. 1). In situ temperature determinations were made by CTD cast at selected stations. Plankton was sampled at each station by double oblique Bongo net tows at a ship's speed of 2–3 knots. On the cruise in 1991, the gear used was a 50 cm diameter Bongo net with a mesh size of 280 μm on both sides and mounted above the main frame, a paired 10 cm diameter Bongo-style frame fitted with 53 μm mesh net. In 1992 the gear used was similar, but the mesh size used was 200 μm (50 cm diameter). General Oceanic flowmeters were fitted to one side of each of the frames. Sampling was to 100 m depth or to within 10 m of the bottom where the depth was less, depth gauges being used to record the maximum depth sampled. The contents of cod ends of the 200 or 280 μm nets were sorted on board for sardine larvae, which were then preserved in liquid nitrogen (−196°C) for later RNA/DNA analysis. The remaining portion of the sample was preserved in 4% buffered formaldehyde solution for further ichthyoplankton studies. For the 53 μm nets the contents of

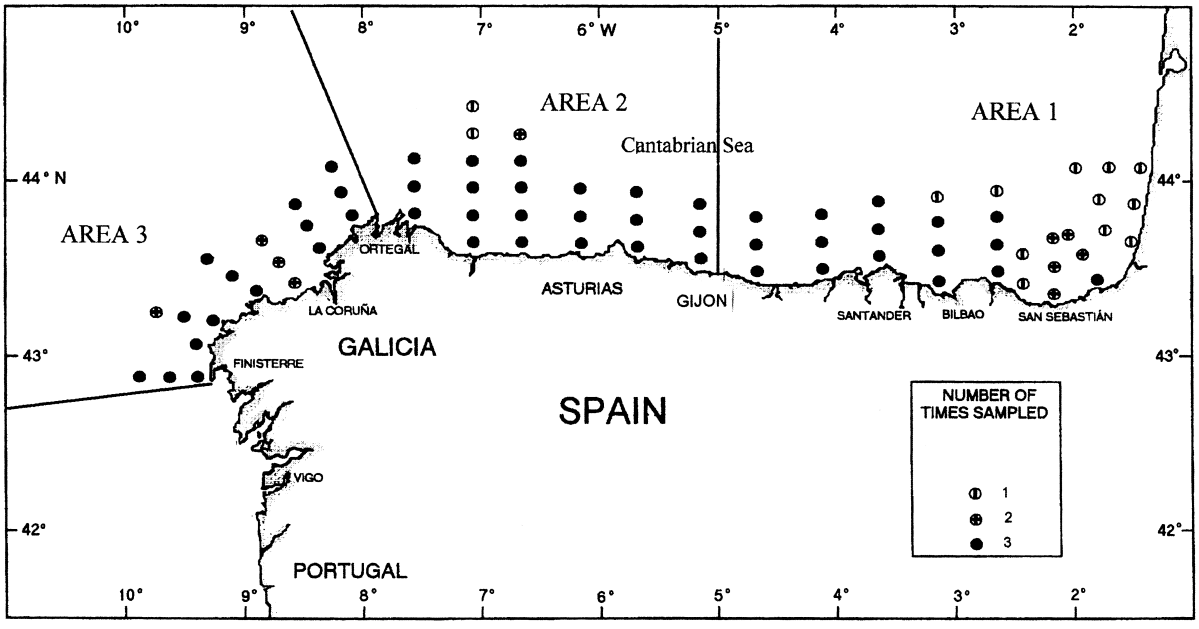

Fig. 1. Locations of sampling areas and stations, and showing the number of cruises in which the stations were sampled.

one cod end were preserved in 4% buffered formaldehyde for subsequent analysis of abundance of food organisms for sardine larvae, the other was frozen (−20°C) for biomass estimation.

2.2. Starvation field-experiment

Larvae collected during the cruises with RNA/DNA at or below 1.3 were classified as starving. This value is the mean of RNA/DNA ratio of sardine larvae obtained from an in situ experiment conducted off southern Portugal during 1992 (Chícharo, 1996, 1997), in which larvae were deprived of food inside net containers of 10 μm mesh size, for 2–6 days. In this experiment *Sardina pilchardus* larvae ranged from 5.5 to 12.3 mm and during the experiment the mean daily water temperature was 15.3°C.

2.3. Laboratory procedures

To determine the zooplankton biomass (>53 μm), samples were rinsed with an isotonic ammonium formate solution and heat dried to a constant weight in an electric oven at 60°C. The results were expressed as dry weight (mg DW/m^3). Before the nucleic acids were determined, standard lengths of thawed sardine larvae were measured (standard length) to the nearest 0.1 mm under a dissecting microscope using an ocular micrometer.

In this study, nucleic acids were analysed from whole body of *S. pilchardus*. Using a sonicator the larvae were homogenised for 1 min in 600 μl of ice-cold tris buffer containing sodium dodecyl sulphate (SDS-final concentration 1%). A highly sensitive fluorimetric method for RNA/DNA quantification in individual organisms was applied.

Our analytical procedure was adapted from the methodology of Clemmesen (1988, 1990) for fish larvae, which allows individual larval analysis. It involves purification of tissue homogenates and subsequent fluorescence-photometric measurements using ethidium bromide (EB), a specific nucleic acids fluorochrome dye. The fluorescence due to total RNA (mainly ribosomal) can then be calculated as the difference between total fluorescence (RNA and DNA) and the fluorescence subsequent to ribonuclease A (type II-A) treatment, which is assumed to be due to DNA. The fluorescence was determined by exciting at

365 nm and reading at 590 nm with a spectrofluorometer (Hitachi model 650-10). Concentrations of nucleic acids were determined by running standard curves of DNA and RNA with EB every day, with known concentrations of calf thymus DNA and yeast RNA, in the appropriate range of values. Average recovery of added calf thymus DNA to larval samples (DNA spike) was 92.1±4.6% and average recovery of added yeast RNA (RNA-spike) was 95.3±3.4%. Total amounts of DNA and RNA in the post-larvae were corrected based on these average recovery efficiencies. The limit of detection (the analyte concentration giving a signal equal to the blank signal plus two standard deviations of the blank) was 0.1 μg/ml for DNA and 0.4 μg/ml for RNA. The coefficient of variability (sample standard deviation as percentage of the mean) was 4% for DNA and 10% for RNA when 10 aliquots of tissue homogenate were measured.

2.4. Growth rates

Individual protein growth rates (Gpi), in %/day, were calculated based on larval RNA/DNA ratios and water temperature (T), according to Buckley (1984):

$$\text{Gpi} = 0.93T + 4.75\text{RNA/DNA} - 18.18.$$

2.5. Analysis

The relationship between parameters was analysed by Pearson's correlation. To avoid assuming a significant correlation due to random processes, the Bonferroni inequalities (Snedecor and Cochran, 1989) were used in the data analysis. The value of $t_{0.05}$ used was corrected to $t_{0.05/n'}$ (n'-number of pair of correlations in the matrix) and only after applying this correction did we verify if a correlation was significant. To the variables with higher significant correlations, a multiple regression was applied. The relationship between the independent variables (larval length and zooplankton biomass) and a dependent variable (RNA/DNA ratio) was investigated. The partial correlations, between the respective variable and the dependent variable, after controlling for the other independent variable in equation, were calculated.

3. Results

3.1. Hydrographic conditions

Surface (5 m) water temperature was higher in 1992 than in 1991. During April/May in 1991 the highest surface temperature occurred in Area 1 (off San Sebastián). In April 1992 a slight temperature gradient was present from east to west, with cooler (<12.5°C) waters in the east (Area 1) and warmer water in the west (Areas 2 and 3), where values were higher than 13°C. In May (''Valdivia'' cruise), surface water temperature was higher than 14°C over most of the areas except the coastal zone, halfway between Finisterre and Ortegal (Area 3), where surface temperatures were near 13°C (Lopéz-Jamar et al., 1995). The lower surface temperature in this area results from upwelling of deep cooler water, which starts at about this time of year and usually continues until the end of the summer (Robles et al., 1992).

Surface water salinity (5 m) in the study area generally ranged between 34.8 and 36.2 PSU, with lower values in the more eastern area (Area 1). Stratification values ($\Delta\sigma_t$ 0–50 m) were weak in April 1992, indicating a well-mixed water column in the entire area, at least down to a depth of 50 m. By April/May 1991 and May 1992 stratification was relatively pronounced in the eastern area (Area 1), mainly in the more inshore region. The remanding areas showed moderate stratification except off Northwest Galicia, where a well mixed water column was present, indicative of strong upwelling (Lopéz-Jamar et al., 1995).

3.2. Zooplankton biomass and abundance, distribution and length of sardine larvae

The mean zooplankton biomass of the stations where sardine larvae were analysed biochemically, revealed higher values during the 1991 cruises (April and May) than 1992 cruise (April/May) (Table 1).

Although sardine larvae can be found throughout the whole year in the study, peak spawning in Galician and Cantabrian waters takes place in the period April–May (Garcia-Soto et al., 1991). Thus, the cruises covered most of the significant spawning season. *S. pilchardus* were caught almost throughout the whole

Table 1
Sampling dates and areas, superficial water temperature, variation of sardine larvae abundance, zooplankton biomass, growth rates and starvation percentages

Vessel	Dates	Areas	No. of stations	Temperature (°C)	Sardine larvae abundance (no./m^2)	Zooplankton biomass (DW/m^3)	Protein growth rate (%/d)	Starvation percentage (%)
Cornide	17 April–11 May 1991	2	7	12.3	0 to >20	16.33±7.58	9.98±6.36	0
		3	6	12.5	0 to >10	28±9.02	12.59±6.71	0
Cornide	1–13 April 1992	1	3	12.0	5 to >80	15.76±6.9	6.15±3.28	2.5 (1(8 mm)/40)
		2	2	12.5	0–80	16.01±6.93	13.05±5.41	0
Valdivia	10–21 May 1992	1	8	14.5	0–80	13.51±5.89	13.96±7.47	0
		2	23	14.5	2.5–80	16.63±9.4	11.06±5.45	1.61 (2(7.17 mm)/124)
		3	4	14.0	0–20	11.59±4.74	15.31±7.9	0

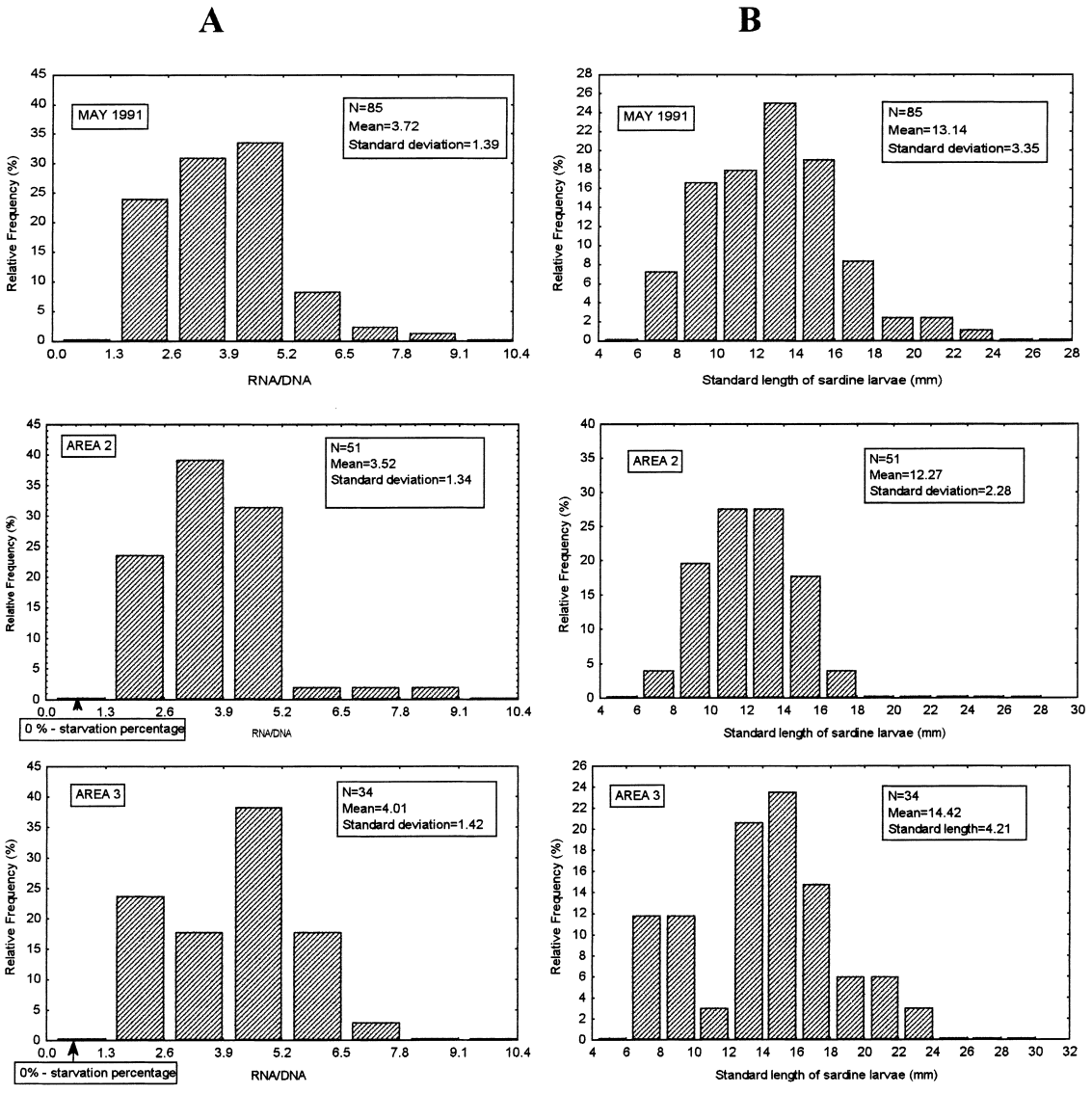

Fig. 2. Frequency distributions of (A) RNA/DNA ratios and (B) average standard lengths of *S. pilchardus* larvae in the 1991 cruise (April/May).

area under investigation. In April/May 1991, the abundance of larvae was considerably lower than that for the corresponding period of 1992. The highest concentrations were distributed along the northern coast of Spain to the east of Cape Ortegal (Areas 1 and 2) (Table 1). Abundance of sardine larvae decreased drastically along the west coast of Spain (Area 3) where frequently no larvae were collected, especially in the outer shelf stations (Lopéz-Jamar et al., 1995).

The standard length of field-caught larvae that were biochemically analysed ranged between 4 and 24 mm. Bigger larvae were found mostly in Areas 2 and 3. The distribution of length in area 3 was usually bimodal. The smallest larvae were collected at Area 1 (Figs. 2–4).

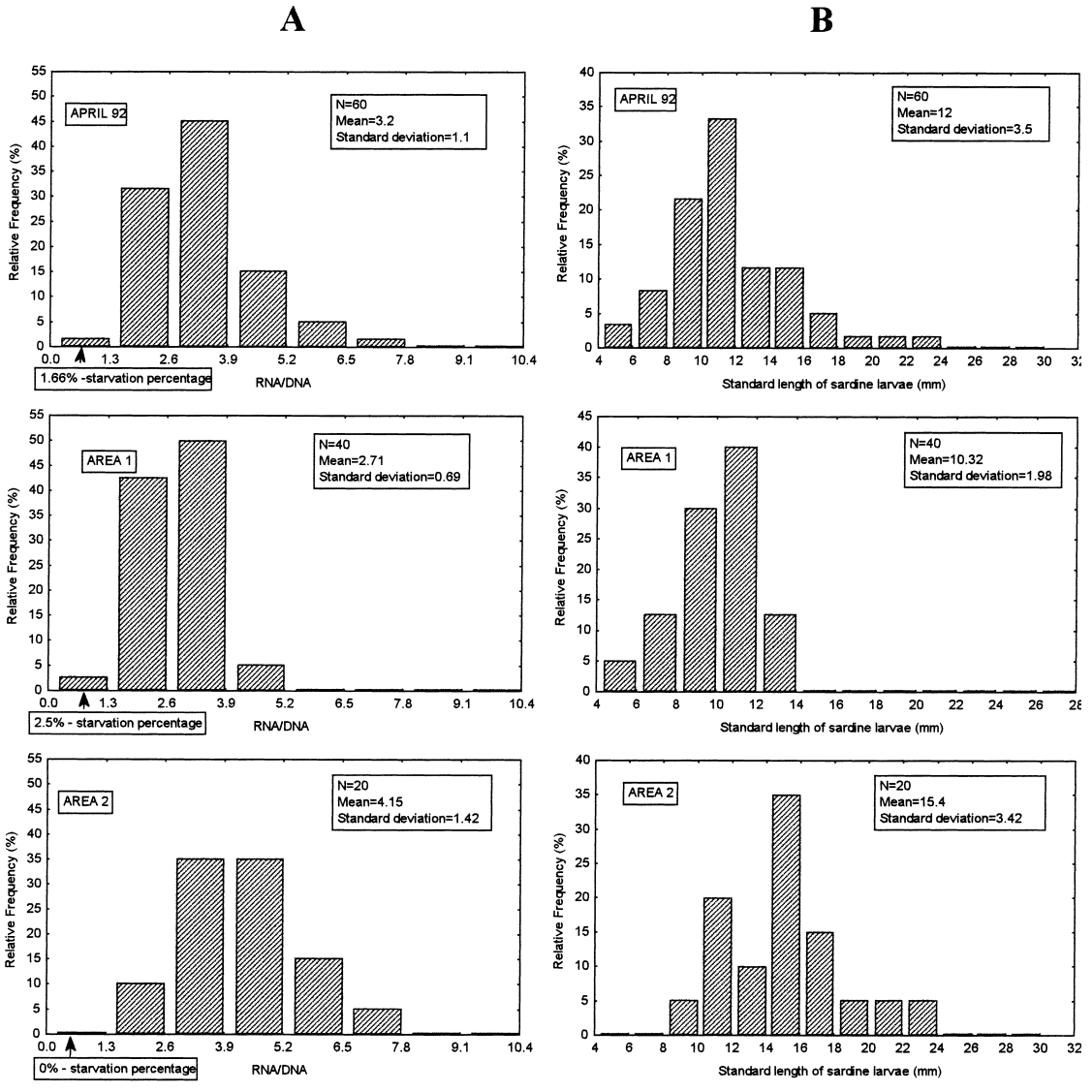

Fig. 3. Frequency distributions of (A) RNA/DNA ratios and (B) average standard lengths of *S. pilchardus* larvae in the April 1992 cruise.

3.3. Nutritional condition, growth rates and starvation percentages

The average values of RNA/DNA ratio were relatively high, but during May 1992 and in Area 3, the mean values of these indices were the highest (Table 1, Fig. 4). Also during 1991, high values of RNA/DNA ratios were more frequent in Area 3 (Table 1, Fig. 2). Low values of RNA/DNA were found especially at Area 2 and Area 1, during 1991 and 1992, respectively (Table 1, Figs. 2–4).

The general correlation between the RNA/DNA ratio and measured parameters revealed significant correlations only with standard length and zooplankton biomass (Table 2). A low and non-significant correlation with temperature was registered. The multiple regression, where RNA/DNA ratio was the dependent variable and larval standard length (SL)

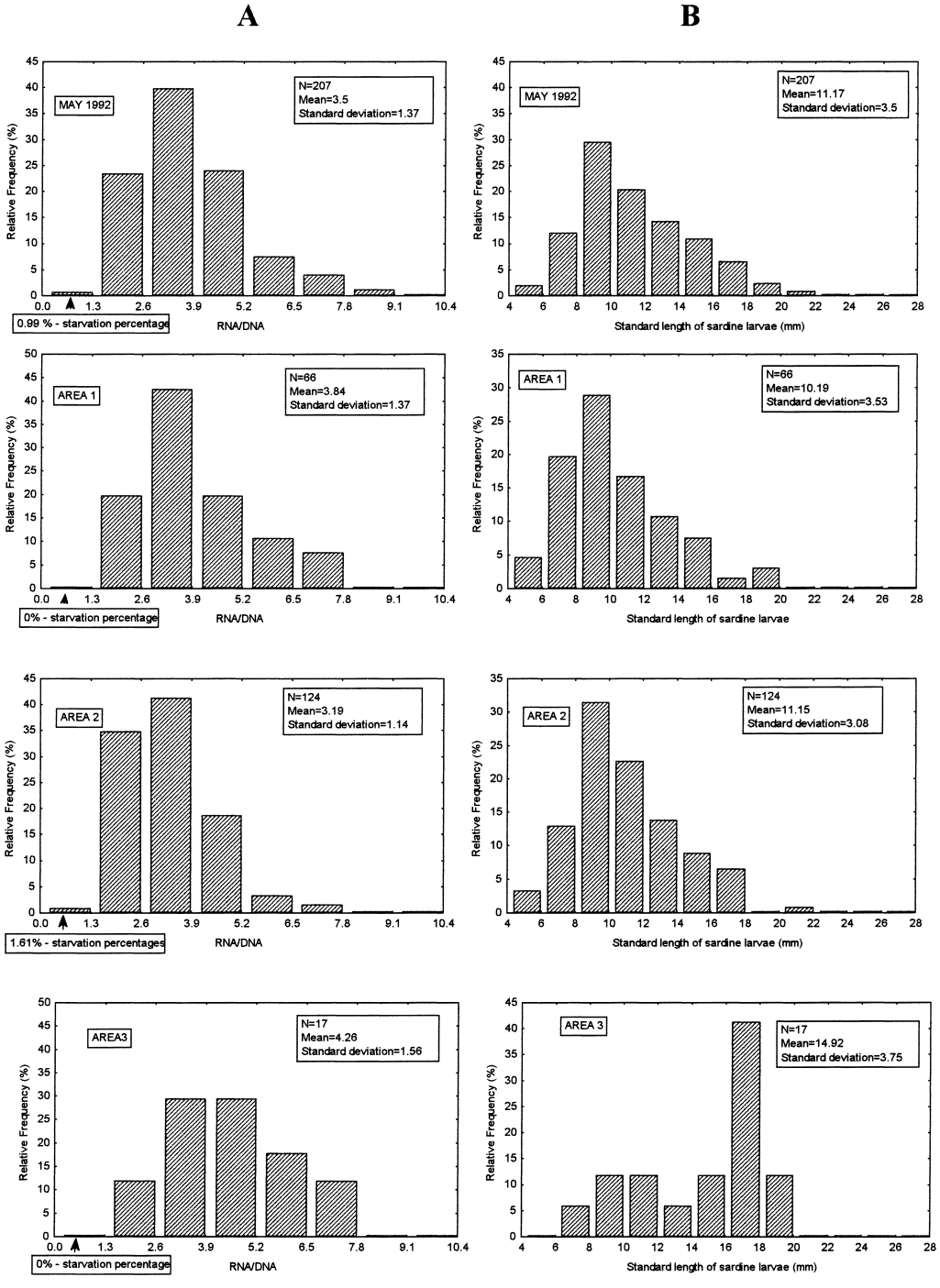

Fig. 4. Frequency distributions of (A) RNA/DNA ratios and (B) average standard lengths of *S. pilchardus* larvae in the 1992 May cruise.

Table 2
Spearman correlations among the studied environmental parameters

	TEMP	ZB	SL	RNA/DNA
TEMP	1.000			
ZB	0.254	1.000		
SL	−0.322	−0.175	1.000	
RNA/DNA	0.001	0.205[a]	0.402[a]	1.000

n=52, TEMP=temperature (°C), SL=standard length (mm), and ZB=zooplankton biomass.
[a] p<0.05 with Bonferroni correction.

Table 3
Summary of a multiple regression for dependent variable RNA/DNA

Independent variable	Partial correlation	B	SE B	t(49)	p-Level
Intercept		1.40	0.58	2.42	0.019
ZB	0.31	0.08	0.03	2.24	0.029
SL	0.45	0.15	0.04	3.57	0.001

SL=standard length (mm), ZB=zooplankton biomass, B=slope, and SE=standard error.

and zooplankton biomass (ZB) were the independent variables, revealed that 24% of the variation was explained by these two variables (R^2=0.24, n=52, p<0.001, Table 3). This relation was described by the equation

$$\mathrm{RNA/DNA} = 1.4 + 0.085\mathrm{ZB} + 0.15\mathrm{SL}.$$

The standard error of estimate was 0.73. Zooplankton biomass was significantly correlated with total RNA/DNA ratios, although only a small percentage of the variation in individually RNA/DNA ratios was explained by this parameter (partial correlation r=0.31, p<0.029). Larval length seemed to contribute more to this variation with a partial correlation of 0.45 (p<0.001) (Table 3). A plot of the residuals versus the predicted value of RNA/DNA ratio illustrated that the points form a horizontal band around zero, then no inadequacies of the assumed relation were detected.

Similarly to the RNA/DNA ratios the growth rate were higher in 1992 and especially in Area 3 (Table 1). The standard deviation regressed against the mean growth rate shows that the standard deviation was higher, for a given mean, during 1992 than 1991. Also a positive relation between mean growth rate and standard deviation was found only during 1992 (Fig. 5).

The starvation percentages were generally low. The highest value, 2.5%, was observed during the earliest cruises in April 1992 (Table 1). Values near zero were found frequently in most of the areas (Table 1).

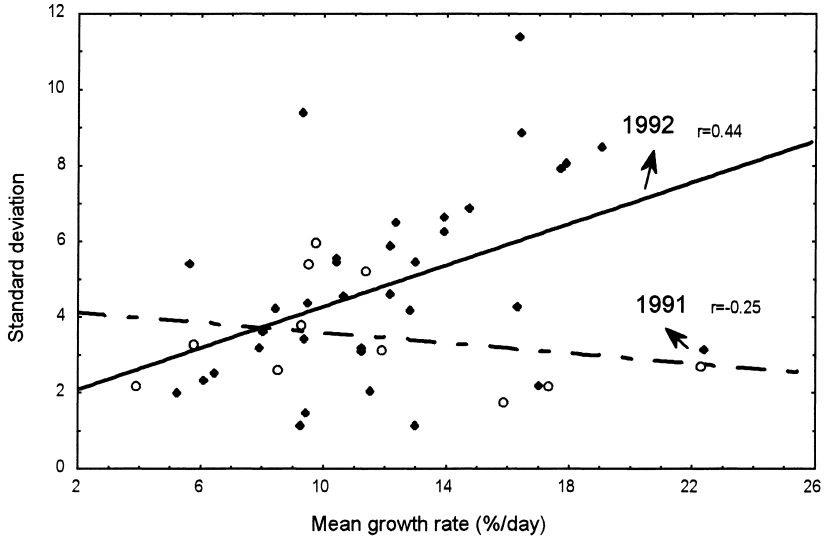

Fig. 5. Standard deviation plotted against the mean growth rate of sardine larvae caught during 1991 and 1992. (Full circles and solid line are data from 1992, open circles and dashed line are data from 1991. The regression for 1992 is: y=1.53+0.274x, r^2=0.19, p<0.008 and the regression for 1991 is: y=4.26−0.67x, r^2=0.06, p<0.117).

4. Discussion

One way to assess starvation on a field-caught larva is to determine the RNA/DNA ratio, below which larvae will be classified as starving. The idea of the ''critical ratio'' was originally discussed by Robinson and Ware (1988) and is based on a model of the general relationship between RNA/DNA ratio, temperature and protein growth rate determined and reported by Buckley (1984). Robinson and Ware (1988) defined the ''critical ratio'' as the RNA/DNA ratio of an animal when larval protein growth rate is zero. Rooker and Holt (1996) suggested that caution be used when applying the ''critical ratio'' to new species in field studies due to the inherent developmental variation seen in RNA/DNA ratios of many species of marine teleost larvae.

A solution to this problem is to calculate for each species, under controlled conditions, the mean RNA/DNA ratios of larvae deprived of food (''minimum ratio''). Until now, this kind of calibration was done only with fed and starved laboratory reared larvae (Buckley, 1984; Clemmesen, 1987; Robinson and Ware, 1988; Pittman, 1991; Chícharo, 1993; Chícharo and Chícharo, 1995). The results of such studies should be regarded with caution as laboratory conditions hardly simulate natural conditions (Blaxter, 1975; Theilacker, 1980; Mackenzie et al., 1990; Folkvord and Moksness, 1995). With the studies of Chícharo (1996, 1997) it was possible to access from a field experiment the level of RNA/DNA ratio indicative of starvation in *Sardina pilchardus* (RNA/DNA ratios less than the value of 1.3). However the range of length studied was 5.5–12.3 mm and bigger larvae are found in the sea. If this ''minimum ratio'' increases with age (length) (Clemmesen, 1994; Rooker and Holt, 1996) we could be stating that some starved larvae are in good condition. Nevertheless, if we increase this ratio to 1.7 (according to Clemmesen (1994), this is the ratio which is applied to a starved herring larvae of 23 mm), the same starvation percentages are obtained.

At most stations there were no larvae below the ''minimum ratio'' suggesting, according to Bailey and Houde (1989); Leggett and DeBlas (1994), that starving larvae might have been lost to predation, or that their feeding abilities were such that they could meet their basic nutritional requirements (Robinson and Ware, 1988). In fact, only 0.85% (3/352) of sardine larvae collected during the cruises were classified as starving.

The relatively high zooplankton biomass in 1991 may be reflected in starvation percentages, which were zero. The low water temperature during 1991 could have increased the duration of planktonic life, and predation could have had more time to eliminate weak and slow growing larvae (Bailey and Houde, 1989). Also, the decrease in variance of growth rates during 1991 suggests that predation pressure was greater in 1991. In both years, the smallest larvae were collected at Area 1 and the biggest at Areas 2 and 3, probably because of the E–W transport of larvae from spawning to nursery zone (Cabanas et al., 1992). High values of RNA/DNA were more frequent in Areas 2 and 3 during 1991 and 1992. The bimodal frequency distribution of length in Area 3 could be related to the winter spawning off the northwest Portuguese coast (Ré et al., 1990). This was probably responsible for the larger larvae and the well fed ones in Area 3. Also the multiple regression revealed that part of the variation of RNA/DNA was explained by larval length and zooplankton biomass.

Like other studies, in different geographic areas (Canino et al., 1991; McGurk et al., 1992) the great majority of the larvae collected in the Cantabrian Sea had relatively high RNA/DNA ratios and were judged to be in good condition.

The good condition of the larvae in this area (the Cantabrian Sea) indicates that potential recruitment is high, if predation or abiotic factors are not adverse. Robles et al. (1992) suggested that sardine recruitment in Galicia mainly originates from spring spawning in the Cantabrian Sea. However, observations on the birth-date distribution of sardine juvenile, integrated in the same SARP project, taken during the autumn of 1992 and winter 1992/1993 at the main nursery grounds off Galicia, found negligible survival of larvae from spring spawning (Lopéz-Jamar et al., 1995; McFadzen et al., 1997). The conclusion was that the surviving juveniles had recruited from winter spawning in Portuguese waters, further to the south.

We excluded the hypothesis of high predation pressure because, according to Pepin (1989), the increase of variability of growth rates, such as detected during 1992, reflects a potential decrease of predators abundance. Therefore, the negligible survival of larvae

from spring spawning (when this study was carried out), could be explained mainly by unfavourable advection. According to Lopéz-Jamar et al., 1995 upwelling may determine survival of these larvae through advection. Under conditions of strong upwelling off Galicia (Area 3), such as those registered, water is advected offshore with entrained larvae being transported to less productive oceanic regions away from their required nursery grounds. Condition indices cannot account for unfavourable advection, which could operate regardless of larval condition (Suthers, 1992). This offshore dispersion is also evident in the measurements of zooplankton biomass during May 1992 in Area 3. Besides that, the lower temperature in the upwelling area probably increased larval life duration. Then even a small percentage of starvation such as was detected, can have considerable consequences in larval mortality. The duration of research cruises is usually restricted and estimated starvation percentages correspond to a short period. If the spawning season is extended for several weeks, the reduction in larval numbers due to starvation might be more important (D. Houlihan, personal communication, 1997).

Therefore these related factors, starvation and strong upwelling, would contribute to the low recruitment from spring spawning. In other years, when moderate winds in the Cantabrian Sea during April–May would cause a weakening of the front off the northwest corner and a moderate upwelling off Galicia, sardine larvae in good nutritional condition would contribute more to survival and enhanced recruitment from spring spawning.

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