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Effect of strong winds on the nutritional condition of anchovy (Engraulis encrasicolus L.) larvae in the Bay of Biscay, Northeast Atlantic, as inferred from an early field application of the DNA/C index

Jean-Pierre Bergeron

Laboratoire Ecologie Halieutique, Direction des Ressources Vivantes, IFREMER, Centre de Nantes, B.P. 21105, 44311, Nantes cedex 03, France

Tél.: 33 2 40374162; fax: 33 2 40374075; e-mail: jean.pierre.bergeron@ifremer.fr.

Abstract:

A new biochemical index was used to assess larval fish nutritional condition during a survey cruise in the Bay of Biscay in early June 1993. This period corresponds to the breeding season of the European anchovy (*Engraulis encrasicolus* L.). The analysis of samples taken at grid points indicated a clear spatial pattern in nutritional condition, which increased from the middle shelf to the shelf edge. A relatively large fraction of the larval population proved to be in poor condition. Strong winds with speeds of 20–30 knots occurred during 3 d after the first survey and affected the vertical hydrological structure. During a subsequent survey, samples taken at approximately the same locations showed that anchovy larvae in all areas were in very poor condition. These results support the notion that some environmental stability is required for good feeding conditions. Strong winds are not uncommon in the Bay of Biscay and their potential effects on anchovy recruitment variability are discussed.

Keywords: Bay of Biscay, DNA/C, European anchovy, fish larvae, nutritional condition, windstress

Introduction

Whether starvation-induced larval fish mortality plays a major role in determining recruitment fluctuations remains an unsolved question (Heath, 1992) and still continues to sustain keen debates (Leggett and DeBlois, 1994, and comments from several authors in Mar. Ecol. Prog. Ser., vol. 128, 305-310, 1995). Nevertheless, it is widely recognized that high mortality during the larval stages occurs and may be due directly to starvation or to poor feeding conditions, which reduce larval growth rate and increase the duration of exposure to potential predators. The search for reliable and accurate indices of larval fish nutritional condition has been the focus of a steadily growing number of studies (Ferron and Leggett, 1994). Biochemical tools have been the object of most recent developments: they are based on determination of lipids (Fraser *et al*., 1987; Håkanson, 1989), nucleic acids (Buckley, 1979 ; Clemmesen, 1987), digestive enzymes (Hjelmeland *et al*., 1984) or "metabolic enzymes" (Clarke *et al*., 1992) activities. Much progress has been made in improving the sensitivity of analytical methods, which allow measures at the individual level (Clemmesen, 1988; Ueberschär, 1988).

The use of nucleic acids-based indices has been strongly advocated during the past few years (Bergeron, 1997) with a special attention to the RNA/DNA ratio, which proves to be clearly affected by starvation. However, this index shows also a high variability in well-fed fish larvae reared in the laboratory (Bergeron and Boulhic, 1994 ; Clemmesen, 1994). This unexplained variability makes the technique not totally suitable for certain field applications. The relative larval DNA content based on dry weight (DNA/DW) has been suggested as an alternative index by Bergeron *et al*. (1991). This index has been technically improved (Bergeron *et al*., 1997) by using the carbon content (DNA/C) instead of dry weight. Early laboratory experiments demonstrated three main attributes of the DNA/C index: variability in control fed larvae is very low (Bergeron *et al*., 1991; Bergeron and Person-Le Ruyet, 1997); estimates are independent of temperature conditions (Bergeron *et al*., 1997); starvation leads to a fast and sharp increase, especially in early larval stages (Bergeron *et al*., 1991; Bergeron and Person-Le Ruyet, 1997).

A field application of the DNA/C index is presented for larvae of the European anchovy (Engraulis encrasicolus L.) in the Bay of Biscay, along the French Atlantic coast. Studying recruitment of this population is of special interest because the catches of the French fishery have been varying between 5000 and 85 000 tons per year over the past 50 years and because one-year old individuals represent 50 to 90 % of the catches. Anchovy being a short-lived species, advances in the knowledge of environmental factors affecting recruitment are crucial for managing the fishery. We try to answer the question whether

starvation of larval stages plays a determining role among the numerous biological and ecological processes involved in recruitment regulation of the anchovy population in the Bay of Biscay.

Physical processes and their effect on hydrological structures of the region have been reviewed recently by Koutsikopoulos and Le Cann (1996). Currents are rather weak and a general trend from south to north characterizes the residual circulation over the shelf. A major freshwater inflow, originating from the Gironde river in the north, results in important coastal low salinity water masses, the extent of which is obviously regulated by the discharge rates of the river but is also driven by winds (Lazure and Jegou, 1998). Winds are generally rather strong in the Bay of Biscay, commonly around 5-10 knots.

Materials and methods

The ERAG ("Ecologie et Recrutement de l'Anchois du golfe de Gascogne") cruise was carried out in 1993 (5-21 June) on R.V. Thalassa. Anchovy larvae were collected by double oblique tows between bottom and surface with a "carré net" (one square meter mouth opening; 500µm mesh size) along west-east transects spaced 30 nautical miles apart (Fig. 1). The larvae were quickly sorted, after roughly estimating standard length to the nearest mm, frozen at -40°C, and subsequently stored at -30°C until analysis in the laboratory.

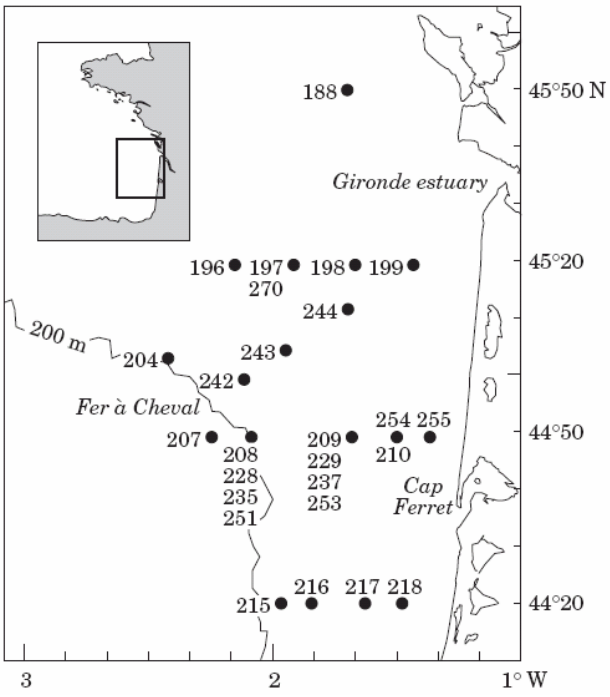

Figure 1. Location of sampling stations in the Bay of Biscay (insert). Multiple station numbers refer to samples taken at subsequent dates.

After thawing, larvae were individually ground in varying volumes (0.8 to 3.7 ml according to size) of cold distilled water (4°C) with a Potter Elvehjem homogenizer. An aliquot was rapidly picked up and placed in a tin capsule for later carbon analysis. Then the sample was immediately processed for DNA determination by the fluorometric method initially suggested by Le Pecq and Paoletti (1966) and modified according to Karsten and Wollenberger (1972, 1977), with type I DNA (Sigma) from calf thymus as standard. The sample for carbon determination was gently dried (60°C) in an oven and then processed in a Perkin Elmer CHNS/O 2400 analyzer.

Accurate measurement of standard length (L) is rather difficult on board and lengthens processing time of the samples. Therefore, it was decided to roughly assign each larva to a size class and then to refer to a DNA weight. The relationship between DNA content (in µg) and L (between 6 mm, i.e. after yolk resorption, and 14 mm) has been established as:

$$\ln \text{DNA} = 0.215\ L + 0.243 \qquad (r^2 = 0.76).$$

The conceptual basis for the use of DNA/C rests on potentially larger variations in carbon content, which is illustrated by the lower r^2 (0.59) for the correlation between ln C and L. The flexion of the notochord, a most important step in larval fish development, occurs at 9-10 mm in length (Ré, 1994), which corresponds to a DNA content within the range of about 9-11 µg (ln DNA = 2.18 to 2.39).

A problem in interpreting the significance of the data might be in the lack of laboratory calibration of the DNA/C index for the species studied. Preliminary rearing experiments have been carried out on Dover sole *Solea solea* L. (Bergeron *et al*., 1991, 1997) and the European sea bass *Dicentrarchus labrax* L. (Bergeron and Person-Le Ruyet, 1997). Despite slight differences in the timing of the measurements relative to larval ontogenesis, the values obtained proved to be fairly close. Data available on relative DNA content of larval fish (Table 1) suggest small differences between species, except for the results of Mathers *et al*. (1994) for herring larvae which have been questioned before by Folkvord and Moksness (1995). It should be noted that the variability among different species diminishes if timing during larval development is taken into account: relative DNA content clearly decreases from yolk-sac stage to the late larval stage (Bergeron and Person-Le Ruyet, 1997). The carbon-dry weight fraction has been defined for Dover sole larvae as 0.37 (Bergeron *et al*., 1997), very similar to *Sprattus sprattus* (Håkanson *et al*., 1994) and slightly lower than the value of 0.43 reported for plaice *Pleuronectes platessa* (Ehrlich, 1974) and walleye pollock *Theragra chalcogramma* (Harris *et al*., 1986).

Overall, one may reasonably assume that DNA/C values within the range of 40-60 µg DNA.mg C^{-1} should characterize good nutritional condition for larvae before the notochord flexion and values of 30-50 µg DNA.mg C^{-1} thereafter. Higher values would reflect a poorer condition.

Table 1. Relative DNA content (in µg.mg^{-1} dry weight) measured in fed larval fish of different species.

Species	DNA/DW	References
Gadus morhua	10-17	Buckley, 1979
Gadus morhua	15-32	Grønkjær *et al*., 1995
Solea solea	10-28	Bergeron *et al*., 1991
Scophthalmus maximus	10-20	Clemmesen, 1987
Clupea harengus	10-16	Clemmesen, 1987
Clupea harengus	3-5	Mathers *et al*., 1994

Results

Overall, the set of indices obtained for 189 individuals provides a somewhat scattered picture (Fig.2a): values range from 25 to 125 µgDNA.mgC^{-1}, mostly (82 %) varying between 40 and 100 (9% <40; 9% >100). The estimated larval size from the ln transformed DNA content has no apparent effect on the distribution of the index (r^2 = 0.0004). The relatively high variability appears to be partly caused by spatial differences in mean values (Table 2). A first group of lower DNA/C values (i.e. in principle signifying a better nutritional condition) observed among the different locations, which were covered within 55 hours during the first period (stations 188 to 218), is made up by several stations along the shelf edge. Another low mean value was obtained at station 188 close to the mouth of the Gironde estuary where a low surface salinity indicated contact with the freshwater outflow from the river.

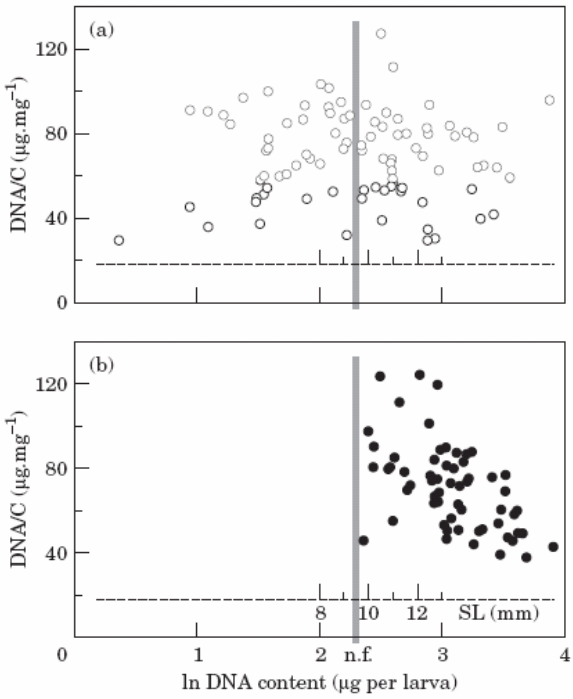

Figure 2. Distribution of individual DNA/C values (µg mg^{-1}) as function of ln transformed DNA content (µg; as estimator of larval size; equivalent standard length in mm on secondary scale) of all larvae collected a) during the first survey of the sampling grid (stations 188 to 218) and b) at the end of the cruise (stations 242 to 270). Shaded vertical mark indicates the beginning of notochord flexion (n.f.).

Table 2. Mean DNA/C (in µg.mg^{-1}) of anchovy larvae and standard deviations for each sampling station (n is number of individuals).

Station	Location	Mean	S.D.	n
188	Gironde Plume	54	6	7
196	Middle Shelf	77	18	4
197	Middle Shelf	66	6	5
198	Middle Shelf	76	15	8
199	Halfway	64	13	8
204	Shelf-edge	47	11	9
207	Shelf-edge	50	13	9
208	Shelf-edge	50	9	8
209	Middle Shelf	93	28	3
210	Middle Shelf	74	28	5
215	Shelf-edge	47	18	9
216	Middle Shelf	69	22	10
217	Middle Shelf	73	13	9
218	Middle Shelf	78	15	8
----------*Wind event* ----------				
228	Shelf-edge	49	11	9
229	Middle Shelf	105	11	5
235	Shelf-edge	91	20	7
237	Middle Shelf	91	15	5
242	Halfway	65	16	9
243	Middle Shelf	84	17	7
244	Middle Shelf	85	20	7
251	Shelf-edge	63	18	8
253	Middle Shelf	86	8	6
254	Middle Shelf	64	28	8
255	Coastal	58	12	9
270	Middle Shelf	90	15	6

Examining individual DNA/C values showed that most of the larvae sampled at station 199 south of the Gironde estuary, another location obviously influenced by freshwater discharge, also appear to be in good condition. However, this sample revealed more heterogeneity, with some individuals in good condition and others in markedly bad condition. A similar variability is observed along the southernmost transect (44°20): good condition was found in larvae along the shelf edge (station 215) with a gradual reduction when moving closer to the coast (stations 216 to 218). Almost all other higher values of DNA/C seem to characterize larvae sampled over the middle continental shelf. Comparison of the mean values and standard deviations obtained from locations of the middle shelf and shelf edge (Table 2) indicates highly significant differences during the first period. A good illustration of this contrast is given by stations 208 and 209 along the transect located at 44°50, which were less than 18 nautical miles apart but revealed pronounced differences in DNA/C.

These two stations were sampled four times during the cruise, which permitted us to try to confirm these differences over a 8 day period (Fig. 3). However, after the first coverage a special wind event occurred, with speeds of 20-30 knots during three days, which affected the vertical structure of the water column, as indicated by the variations of both surface temperature and location of the thermocline (Fig. 4). By accident, this allowed an interesting observation of temporal variation in the index. The larvae collected during the second sampling (approximately thirty hours after the wind fell) did not show any significant change in the index (Fig. 3). In contrast, DNA/C had strongly increased in larvae collected on the shelf edge one day later. Finally, the fourth and last sampling two days later provided an intermediate picture, i.e. some larvae returned to good condition while others remained in poor condition. Wind stress did not have an apparent effect on the larvae of the middle shelf location during the same eight day period.

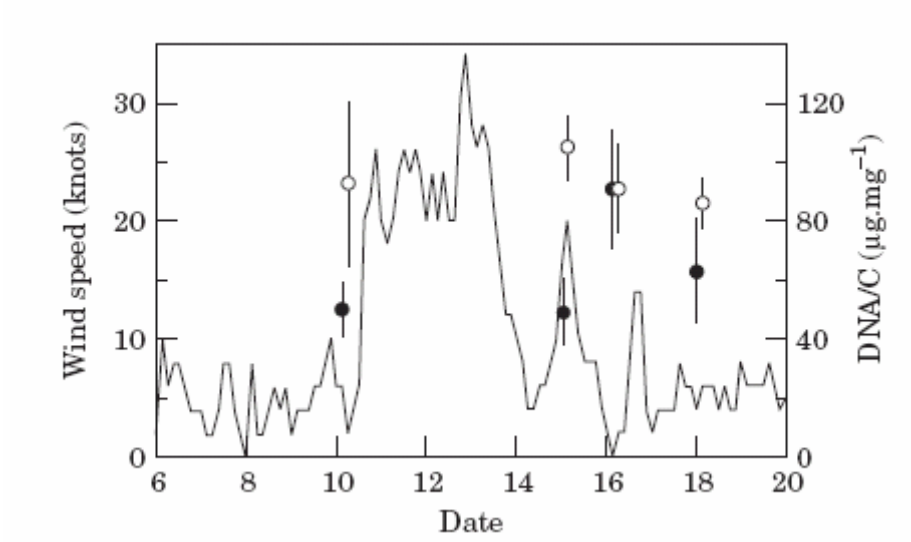

Figure 3. Variation in wind speeds recorded at the Cap Ferret semaphore (Météo France data: mean values in knots by three hours intervals) during the cruise and mean values and standard deviations of DNA/C for two locations sampled on four occasions (cf. Figure 1; filled circles: shelf edge - stations 208 … 251; open circles: middle shelf - stations 209 … 253).

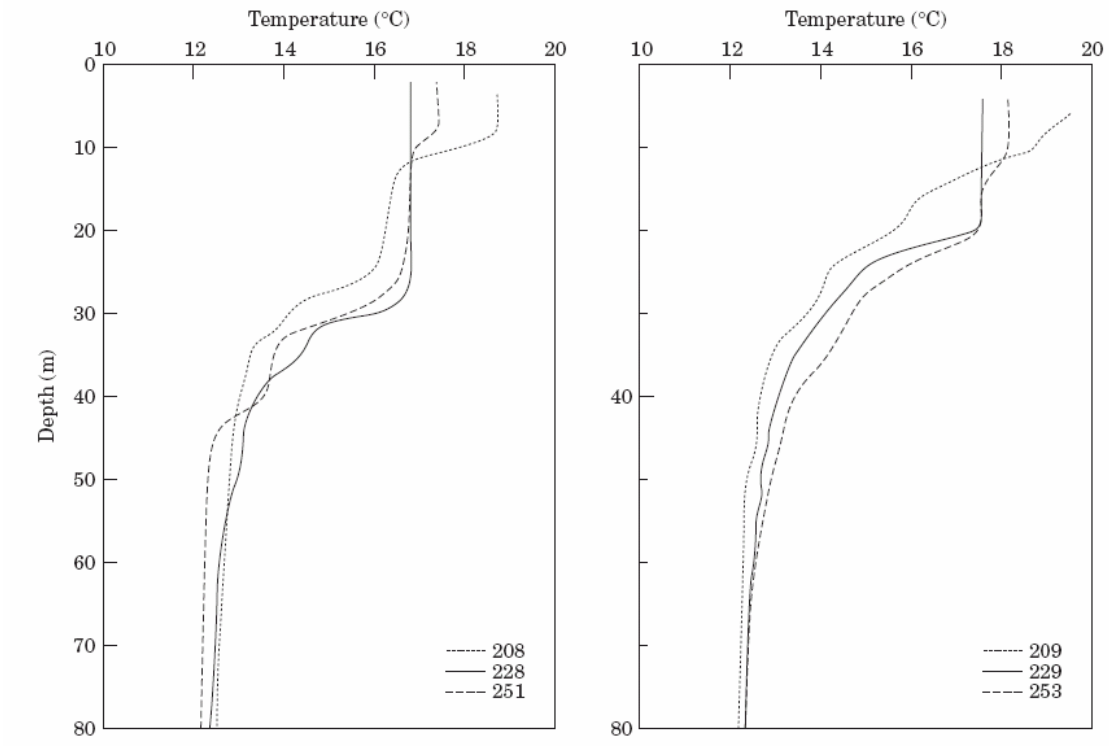

Figure 4. Vertical temperature profiles recorded at the two locations identified in Figure 3 (left panel: shelf edge; right panel: middle shelf; for clarity 235 and 237 have been omitted)

Other samples at the same (197-270) or nearby (196, 198 and 243, 244) stations before and after the wind event also revealed a highly significant increase in the index. Thus, the momentary mixing of initially well-stratified waters appears to have caused an overall worsening in the nutritional condition of anchovy larvae. It should be noted that only large-sized larvae could be sampled at these stations towards the end of the cruise, reflecting the development of the larval population. All larvae collected after 17 June (stations 242 to 270) had DNA contents higher than 10 µg, indicating a developmental stage well beyond the notochord flexion phase (Fig. 2b). The distribution of these DNA/C values in relation to size clearly shows clearly a decreasing trend, suggesting that the smaller larvae were much more affected than the larger ones.

Discussion

This first attempt at field application of DNA/C has been carried out without prior laboratory calibration on the target species. Clemmesen *et al*. (1997) recently followed the same approach with the RNA/DNA ratio on another *Engraulis* species. Ferron and Leggett (1994) noted that nucleic acid-based indices have relatively low species-specificity and the use of values determined for different species in rearing experiments seems justified (Clemmesen *et al*. 1997). Values of the DNA/C index in well-fed individuals of *Solea solea* (Bergeron *et al*., 1997) and *Dicentrarchus labrax* (Bergeron and Person-Le Ruyet, 1997) were indeed very close. However, in contrast to the RNA/DNA ratio for which Clemmesen (1994) define a "mean starvation line", it seems more appropriate to establish levels of DNA/C that characterize well-fed larvae. Higher values indicate poor nutritional condition (Bergeron, 1997). With very few exceptions, it should be noted that the lowest DNA/C values measured in anchovy larvae are very close to those found in well-fed *Dicentrarchus labrax* larvae (Bergeron and Person-Le Ruyet, 1997). Thus, the global patterns of DNA/C variations in larvae of both species appear quite similar.

DNA/C values were not randomly distributed during this survey. First, it seems logical that river plumes represent favourable places where nutritional condition of anchovy larvae may be strongly enhanced, because these waters are responsible for enrichment of coastal areas (Yin *et al*., 1996). Determinants of good condition over the continental slope are less clear. Just a little more to the NW of the Fer à Cheval area, sea surface temperatures obtained from satellites indicate the occurrence of up-welled waters. From the south of the Bay of Biscay large anticyclonic oceanic eddies of slope water ("SWODDIES" ; Pingree and Le Cann, 1992) are formed. These processes may provide retention areas in

the inner part, but also produce turbulent effects in the outer part with potentially important biological implications (Pingree and Le Cann, 1992). Whatever type of physical forcing, conditions along the shelf break appear to be favourable (Motos *et al*., 1996). In these areas, as well as in the river plumes, biological productivity is markedly enhanced, which probably determines spawning concentrations of anchovies, as indicated by the spatial distribution of their eggs (Motos *et al*., 1996). Year-to-year changes in the distribution are relatively small and obviously influenced by river plumes (Adour in the south, Gironde in the north) and shelf break waters (Fer à Cheval).

The effect of wind stress on larval nutritional condition conforms to the "stable ocean" concept proposed by Lasker (1975), which stipulates that survival of fish larvae requires a minimum stability of the water column, because fine-scale food aggregations are destroyed - and larvae may die of starvation - beyond certain turbulence thresholds. Several studies have illustrated the effects of wind-induced turbulence. Yin *et al*. (1996) demonstrated turbulence effects on fundamental production processes and Peterman and Bradford (1987) found a significant correlation between larval mortality rates of the Northern anchovy *Engraulis mordax* and frequency of low wind speed periods during the spawning season. Recently, Borja *et al*. (1996) showed the potential effect of different wind regimes on recruitment of the anchovy population of the Bay of Biscay. Bailey *et al*. (1995) reported that, based on two contrasting years, lower food abundance as well as poorer nutritional condition as estimated by RNA content and higher mortality of *Theragra chalcogramma* larvae were linked to strong winds. In a study of distribution, feeding and condition of larvae on Georges Bank, Lough *et al*. (1996) also encountered a one-day strong wind event and found no difference in the RNA/DNA ratio of larval cod sampled on the following day, whereas the ratio had significantly increased in larval haddock. One hypothesis proposed by the authors was an increase in prey availability, but this does not seem plausible because they argue that at least 2-3 days are required to assess a feeding change in the RNA/DNA ratio value.

The data presented provide an interesting insight in the time scale of the realization of such processes. At the shelf edge location sampled consecutively on four occasions, a very clear effect of wind mixing was revealed by the change in vertical hydrological structure at the time of the second sampling, while no change could be observed in DNA/C values. The change in the latter occurred one day later, about two days and a half after the end of the wind event, which is quite consistent with the assumption of Lough *et al*. (1996) and experimental results by Clemmesen (1994) for herring. Another important point is that, if the anchovy larvae appear to be in poor condition at the third sampling, 5 out of 8 seem to be

recovering two days later at the fourth one. This may be found surprising in comparison with experimental observations (Bergeron *et al*., 1991; Bergeron and Person-Le Ruyet, 1997), especially for larger specimens (after notochord flexion) in which DNA/C did not increase as strongly in response to starvation as in smaller ones. This may be ascribed to general laboratory/field differences underlined by several authors (quoted by Ferron and Leggett, 1994) and supports the idea that a natural diet in the field results in better condition. Large individuals would also have greater robustness. The apparent recovery of larvae in the last sample suggests that the observed wind event has been strong enough to affect the hydrological structure and the nutritional condition but not strong or long enough to cause starvation. Consequently, it seems quite possible that such an event has no long term effect on the survival of the larvae within this size range. Smaller individuals were not found during the last days of the cruise, although they were fairly numerous before the wind event. Anchovy spawning is a continuous process during the breeding season (Motos, 1996) and a different fate cannot be excluded for first-feeding larvae, which are widely recognized as more sensitive to their feeding environment (Hewit *et al*., 1985; Heath, 1992). Blaxter (1988) stressed the importance of the notochord flexion as a fundamental step in the larval fish ontogenetic development. Our results strongly suggest that anchovy larvae are more robust beyond this stage.

The spatial distribution of DNA/C shows that a notable fraction of the larval population is in poor condition. This is likely to have an effect on growth rates and may lengthen the period of larval development. Therefore, the duration of exposure to potential predation might be increased for this fraction. If environmental conditions on the middle shelf are less favourable for primary productivity, this might have important consequences when anchovy larvae are advected to these areas under certain wind regimes. And if such winds predominate during a breeding season, the effects on recruitment could be dramatic. However, in general the potential effect of wind stress on recruitment is probably moderate because such meteorological events last mostly for short periods relative to the duration of the breeding season. An examination of time series data from Météo France (Cap Ferret semaphore) reveals that daily mean wind speeds over 20 knots rarely last more than one or two days in May or June. Nevertheless, the frequency of rather high wind speeds, with monthly mean values generally around 8-10 knots in May-July, has an overall effect on the depth of the thermocline.

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