ADVANCES IN EARLY LIFE HISTORY STUDY OF FISH
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Co-occurrence of European sardine (*Sardina pilchardus*), anchovy (*Engraulis encrasicolus*) and sprat (*Sprattus sprattus*) larvae in southern North Sea habitats: Abundance, distribution and biochemical-based condition

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SUMMARY: Spawning populations of European sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*) have become re-established in the southern North Sea after a ~30-year absence and now co-occur with sprat (*Sprattus sprattus*). Consequently, little is known concerning potential interactions among these three species in this region. Based upon parallel cruises conducted in June/July 2005, we compared the larval abundance, size-distributions and biochemical-based condition of these species among 1) nearshore (Wadden Sea) areas and offshore (German Bight) areas that were 2) vertically mixed, 3) frontal zones, or 4) stratified waters. In general terms, larval condition (*RNA*:*DNA*) was relatively high at all stations. Although fronts clearly acted to concentrate larvae, larval condition was not necessarily higher. For example 9% of sardines captured at the tidal mixing front were categorised as starving, while no starving larvae were sampled in the stratified water masses. Habitats of sardine and sprat larvae were similar, whereas anchovies were primarily restricted to nearshore areas. This is the first study examining the potential role of near- and offshore habitats as nursery areas and the extent to which resource (habitat) partitioning exists among the larvae of sprat and newly established anchovy and sardine in the North Sea.

Keywords: clupeiform fish, larvae, habitats, RNA:DNA, growth.

RESUMEN: CO-APARICIÓN DE LAS LARVAS DE SARDINA EUROPEA (*SARDINA PILCHARDUS*), ANCHOA (*ENGRAULIS ENCRASICOLUS*) Y ESPADÍN (*SPRATTUS SPRATTUS*) EN HÁBITATS DEL SUR DEL MAR DEL NORTE: ABUNDANCIA, DISTRIBUCIÓN Y CONDICIÓN BIOQUÍMICA. – La puesta de la sardina (*Sardina pilchardus*) y de la anchoa (*Engraulis encarsicolus)* se han restablecido en el sur del mar del Norte después de casi 30 años de ausencia, apareciendo ahora juntamente con el espadín (*Spattus sprattus)*. Consecuentemente, poco se conoce de las interacciones entre estas tres especies en esta región. Se ha llevado a cabo dos campañas entre Junio y Julio 2005, comparando la abundancia larvaria, distribución de tallas y la relación ARN:ADN de estas tres especies de clupeidos entre: 1) zonas costeras (Mar Wadden) y mar abierto (Bahía Alemana), 2) mezcla vertical, 3) zonas frontales y 4) masas de aguas estratificadas. En términos generales, la condición larvaria (relación ARN:ADN) fue relativamente alta en todas las estaciones. Aunque las zonas frontales actúan claramente como concentradoras de larvas, la condición larvaria no fue necesariamente más alta allí. Por ejemplo, el 9% de las larvas de sardinas capturadas en la zona de mezcla de marea fue clasificada en estado de inanición, mientras que las larvas de sardinas en estado de no inanición fueron capturadas en zonas de masas de aguas estratificadas. El hábitat de las larvas de sardina y de las larvas de espadín fueron más similares entre sí, mientras que las larvas de anchoas fueron encontradas en zonas costeras. Este estudio es el primero en investigar la importancia del hábitat de estas especies en zonas costeras y mar abierto como áreas de cría, así como determinar hasta que punto existe una partición en los recursos (hábitat) entre las larvas de espadín y las de las especies nuevamente establecidas, sardina y anchoa, en el sur del mar de Norte.

Palabras clave: peces clupeiformes, larvas, hábitat, ARN:ADN, crecimiento.

INTRODUCTION

Small pelagic fish species are one of the most sensitive bio-indicators of climate change on regional and basin scales due to their short life spans, high intrinsic growth rates (r), and tight coupling to mesoscale physical processes linked to climate processes (e.g. Cury and Roy, 1989; Borja *et al*., 1998; Roy *et al*., 2007). Survey data indicate that European sardine (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) dramatically increased in the southern North Sea in the mid-1990s (Beare *et al*., 2004) and the first evidence of spawning activity of sardine and anchovy in the North Sea was detected during the cruises made in 2003-2004 as part of the German GLOBEC programme (J. Alheit, IOW, Warnemünde Germany, pers. com.). However, episodes of increased abundance of anchovy and sardine in the North Sea were previously documented in 1948-1952 and 1958-1960 (Aurich, 1954; Postuma, 1978). Thus, the current (re-) immigration of these warm-water clupeid species into the North Sea is probably related to changes in water temperatures and circulation patterns (Corten and van de Kamp, 1996) that may be driven by relatively long-term climate cycles and/or climate change.

The impact that sardine and anchovy will have on populations of "resident" North Sea clupeid species such as sprat (*Sprattus sprattus*) and Atlantic herring (*Clupea harengus*) is currently an area of active research (e.g. EU 7th Framework project "FACTS", 2010-2013) due to both the potential ecological and the economic consequences. North Sea populations of sprat, sardine and anchovy may exhibit resource competition and the degree of habitat partitioning among species is unclear. From an early life stage perspective, all three species spawn in the late spring and/or early summer (Alheit *et al*., 1987). During this period a variety of different habitats exist for eggs and larvae, including: 1) nearshore environments that are high-energy, tidally dominated shallow-water areas (Wadden Sea) and offshore areas that are 2) permanently-mixed water masses, 3) frontal zones generated by tidal mixing and river plumes, or 4) stratified water bodies. The latter habitat is an example of a mesoscale hydrographic feature playing a major role in determining the patterns of larval fish abundance, distribution and sometimes growth rates in many areas (Nakata and Zenitani, 1996; Sabatés and Olivar, 1996) including the North Sea (Munk, 1993; Valenzuela and Vargas, 2002). The extent to which larval sardine, anchovy and sprat use these different North Sea habitats could have important implications for competition and relative inter-specific productivity.

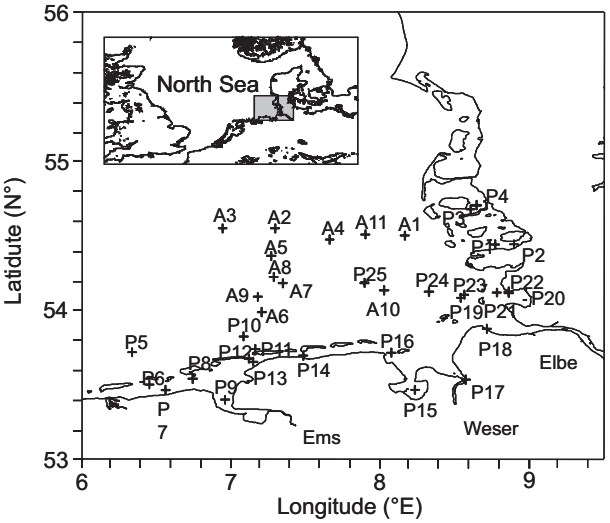

FIG. 1. – Map showing positions of Alkor (A) and Prandtl (P) stations sampled in June/July 2005 and the position of the sampling area relative to the North Sea (insert).

The objective of the present study was to examine the abundance, length-distribution and biochemical condition (*RNA:DNA*) of sprat, sardine and anchovy among offshore areas (German Bight) that were either well-mixed, stratified or frontal zones and shallow nearshore areas (Wadden Sea). Environmental data included both physical (temperature, salinity) and biological (zooplankton) characteristics to help us interpret any potential differences in distribution, abundance and condition among the three species in the four different areas. Despite decades of research on larval fish in the southern North Sea, the sampling scheme employed here (parallel cruises in near- and offshore areas) was the first to allow direct comparisons among these different habitats, and the first investigation of possible competition and habitat partitioning among the three co-occurring clupeiform species.

MATERIAL AND METHODS

Field investigation

The study area was located in the German Bight in the southern North Sea (Fig. 1). Samples were taken between 28 June and 13 July 2005 as part of the GLOBEC-Germany and NUTEX Projects. In these surveys, RV *Alkor* (cruise AL 260) conducted

TABLE 1. – Stations sampled for clupeiform larvae within the southern North Sea during June / July 2005. The 36 stations were separated into four categories: Mixed (M), Wadden Sea (W), Stratified (S), and Tidal Mixing Front (F).

Station (ID)	Date (Day-Month)	Time (Hour)	Location Lat. (°N)	Long. (°E)	Depth (m)	Category	SW sampled (m^{-3})	Additional Data Coll. Copepods	CTD
A1	28-jun	4:30	54.52	8.16	16	M	206.0	x	x
A2	28-jun	12:31	54.55	7.29	32	S	130.1	x	x
A3	28-jun	15:37	54.55	6.94	37	S	114.8	x	x
A4	28-jun	13:04	54.47	7.66	25	S	250.0	x	x
P5	29-jun	15:30	53.72	6.33	22	M	63.1	x	x
P6	29-jun	10:41	53.50	6.45	2	W	23.7		
P7	29-jun	11:50	53.46	6.56	3	W	22.9		
P10	30-jun	15:36	53.82	7.08	20	M	26.7		x
P8	30-jun	7:56	53.54	6.74	12	W	26.8	x	x
P9	30-jun	9:56	53.40	6.96	11	W	28.5		
A5	01-jul	16:13	54.36	7.27	43	S	211.4	x	x
A6	01-jul	11:54	53.99	7.20	28	S	199.8	x	x
P11	01-jul	9:52	53.74	7.16	5	W	45.4	x	x
P12	01-jul	10:53	53.67	7.12	7	W	30.6		
P13	01-jul	11:20	53.65	7.15	2	W	33.5		
A7	02-jul	14:31	54.18	7.34	38	M	157.1		x
A8	02-jul	16:29	54.22	7.29	38	F	57.2	x	x
A9	02-jul	8:19	54.09	7.18	36	S	63.8	x	x
A10	04-jul	14:17	54.13	8.02	28	F	82.9	x	x
P14	04-jul	12:40	53.69	7.48	10	W	41.3	x	
P15	05-jul	17:17	53.47	8.23	18	W	10.2		x
A11	06-jul	8:44	54.51	7.90	19	S	157.9	x	x
P16	06-jul	8:22	53.71	8.07	13	W	7.5		
P17	06-jul	16:24	53.53	8.57	11	W	10.9		
P18	07-jul	15:14	53.87	8.71	18	W	16.9	x	
P19	08-jul	11:07	54.08	8.54	9	M	10.8		x
P20	08-jul	14:41	54.12	8.86	8	W	58.7		
P21	08-jul	15:22	54.12	8.78	8	W	25.6	x	
P22	08-jul	15:46	54.11	8.86	8	W	44.0		x
P23	09-jul	10:41	54.10	8.56	9	M	48.8		
P24	09-jul	11:50	54.12	8.32	10	S	27.8		x
P25	09-jul	15:15	54.18	7.89	40	S	41.1	x	x
P1	10-jul	16:01	54.44	8.77	11	W	29.8	x	
P2	11-jul	13:03	54.44	8.89	3	W	31.3		x
P3	12-jul	10:52	54.68	8.60	11	W	31.3	x	
P4	12-jul	11:38	54.70	8.64	9	W	39.4		

11 hauls in the offshore area and RV *Ludwig Prandtl* conducted 25 hauls in the nearshore area using two bongo nets with 500 μm mesh-size and a diameter of 40 cm in a double oblique tow. Bongo nets from the *Ludwig Prandtl* were towed for 1 to 3 minutes at 0.5-3.5 knots and those on the "Alkor" were towed for 4 to 8 minutes at 2-3 knots. An estimate of the volume of water filtered was obtained using internal flowmeters mounted in the mouth of each net. The whole water column was sampled. The sample from one of the bongo nets was preserved in 4% buffered formalin. In the second net sample, clupeiform larvae were removed and immediately frozen at –80°C prior to preserving the net sample in 4% formalin.

In the Alkor 260 transects, the hydrographic situation in the sampling area was determined with a video plankton recorder (Seascan Inc.) equipped with a CTD recording temperature, salinity, fluorescence and density. The *Ludwig Prandtl* used a "Ferry Box" to record hydrographic data and additionally deployed a CTD at 10 stations (Table 1).

Sampling stations were partitioned into four different categories: 1) offshore vertically mixed, 2) tidal mixing zone (TMF), 3) stratified water and 4) Wadden Sea vertically mixed. The first three categories were based on the stratification variable (*f*) derived from vertical density profiles r(z) (Simpson *et al*., 1979). The value of *f* represents a measure of the amount of energy required to mix the water column and thus increases with increasing water column stratification (mixed waters <10 J m^{-3}, frontal waters 10-20 J m^{-3} and stratified waters >20 J m^{-3}) (Lee *et al*., 2007). The Wadden Sea is a flat high-energy system separated from the North Sea by a row of barrier islands and sandbanks. Deep tidal channels form the connection between the North Sea and Wadden Sea. Within the Wadden Sea these channels branch into numerous gullies and creeks. During ebb tides, vast areas of tidal flats emerge and, at low tide about two third of the bottom of the Wadden Sea is exposed. The limits of the Wadden Sea were defined by the 10-m water depth profile along the German coast offshore of the islands.

Species identification

In order to limit degradation processes of the frozen larvae, the vials were stored on ice and the following work was done as rapidly as possible. After defrosting, larvae and seawater were gently emptied into a small petri dish which was also placed on ice for cooling. Formalin-preserved larvae were processed without cooling. Larvae were identified according to published keys (Halbeisen, 1988; Munk and Nielsen, 2005). Because of the close resemblance in terms of body shape and pigmentation of sprat and sardine, the identification was difficult for these two species, especially in cases in which the larva suffered damage during capture. The most important characteristics used to distinguish sprat and sardine larvae were myomere counts (all numbers relate to preanal counts from first neck myomere to anus): 36-38 myomeres for sprat and 40-42 myomeres for sardine in the earliest stages. After the flexion stage, starting at approximately 9 mm S_L, the difference is 31-35 versus 36-41 myomere (Halbeisen, 1988). Identification by myomere count was impossible when the gut was detached from the anus region of the larval body. It was not possible to identify 56% of all clupeid larvae to species: net damage made it impossible to distinguish between sprat and sardine, especially in relatively small (4 to 8 mm S_L) larvae. These potential mixtures of sprat and sardine larvae were placed within "unidentified clupeids".

Anchovy larvae were easier to distinguish from sprat and sardine due to differences in pigmentation patterns (a few groups of melanophores versus rows of melanophores) and in the proportion of pre-anal to post-anal length (ca. 3:1 versus >4:1). In later stages, European anchovy develops a different position of the lower jaw and a more posterior position of the dorsal fin respective to the anal fin (Munk and Nielsen, 2005).

After identification, the larvae were digitally photographed using a Leica DC 300 digital video camera connected to a Leica MZ 16 stereomicroscope. Larvae for *RNA:DNA* measurements were refrozen in individual vials at –80°C. Standard length (S_L, ±0.05 mm) was measured with an image analysis system (Optimas 6.5). A preservation length correction was applied for specimen preserved in formalin based on a formula by Fey (2002): $SL_{live} = 0.910\ SL_{preserved} + 2.695$. To establish an index of secondary production, copepods in the preserved bongo net samples of 19 stations were counted and abundances per square metre were calculated (Table 1, Fig. 2).

Extraction and quantification of nucleic acids

Analysis of whole larval RNA and DNA concentrations was performed by a modification of a protocol published by Caldarone *et al.* (2001). Larval samples were freeze-dried to constant weight (12 h, Christ Alpha 1-4 freeze drier, –51°C). The freeze-dried larvae were rehydrated in 150 ml 1% Sarcosil TRIS-EDTA buffer (STEP) and vortexed at high speed for 15 min. The larval tissues were homogenised by sonification on ice for 12 min, using an ultrasonic bath (Bransonic 221). Larvae >12 mm S_L were additionally homogenised by an ultrasonic pulse instrument (Sonoplus) before quantification of nucleic acid concentrations.

Total nucleic acid concentrations (RNA and DNA) were determined fluorimetrically in a microtiter fluorescence reader (Spectrofluorometer Safas flx-Xenius) (excitation: 520 nm, emission 605 nm) using specific dye for nucleic acids (ethidium bromide). Estimates of the DNA and RNA content of each larva were taken from calibration curves established using standard DNA (calf thymus) and RNA (yeast). To evaluate reproducibility, a control homogenate (larval fish tissue) was added and processed in each microplate. To establish an inter-laboratory *RNA:DNA* comparison, all *RNA:DNA* values were standardised according to the procedure described in Caldarone *et al*. (2006) using 2.4 as the reference slope-ratio value. Growth rates (G, % day^{-1}) were then calculated from *RNA*:*DNA* and water temperature (T, °C) using the equation published by Buckley *et al*. (2008):

$$G\ (d^{-1}) = 0.0145\ (RNA{:}DNA) + \\ +0.0044\ (RNA{:}DNA)^{*}\ T - 0.078.$$

Data analysis

We employed both multivariate (all stations) and univariate (four station categories) statistics. In terms of multivariate statistics, non-parametric regression models (i.e. GAMs) were used to identify factors affecting larval abundances in the field. A GAM is a statistical model blending properties of multiple regressions (a special case of a general linear model) with additive models. In a GAM, the parameter terms bi and xi of multiple regression are replaced with functions f(xi): The functions f(xi) are arbitrary and often non-parametric, thus providing the potential for better fits to data than other methods. In the

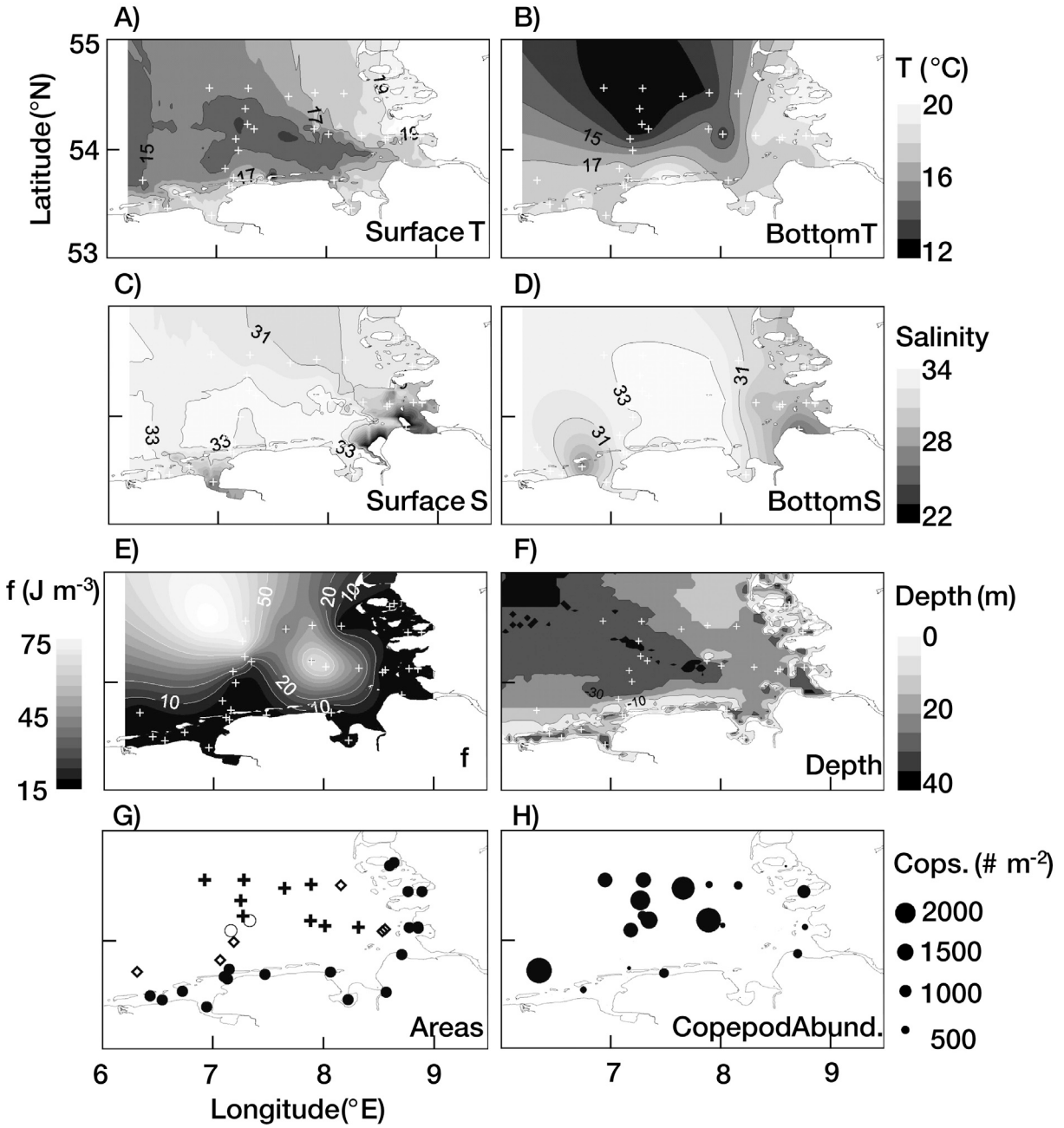

FIG. 2. – Hydrographic situation within the sampling region during June/July 2005. Panels A - F display surface (ST) and bottom (BT) temperature (C°), surface (SS) and bottom (BS) salinity (psu), stratification index (*f*) and depth (m), respectively. Note that within panel A-E, kriged values are shown. Panel G indicates the four station categories (nearshore/Wadden Sea [filled circles], well-mixed water masses [unfilled diamonds], tidal mixing front [unfilled circles] and stratified water masses [crosses]), and finally, the abundance of copepods (individuals m^{-2}) captured using 500-µm Bongo gear is indicated in Panel H. Sampling locations are indicated by small white crosses.

first step, we used GAMs with single predictors to identify the relationships between individual hydrographic factors and the probability of larvae presence. Each predictor can be analysed with regard to the percent deviance explained, and its generalised cross-validation (GCV) score. The GCV is a measure of predictive error of the model and thus takes into account not only the fit, but also the model complexity. In the second step, after the key environmental variables affecting larval abundance were identified, it was tested whether the inclusion of interaction between key variables increased the percentage of the explained deviance and reduced the GCV.

In terms of univariate statistics, larval abundance, standard length and biochemical condition were compared among the four habitat categories using

one-way analysis of variance (ANOVA). When significant differences were detected with an ANOVA, pairwise comparisons were made using Tukey tests. T-tests (assuming unequal variance) were also employed. Differences were considered significant at the alpha = 0.05 level. ANOVAs were followed by Tukey tests. A Bonferroni correction was used to adjust the level of significance to account for the effects of multiple comparisons.

RESULTS

Hydrography

The hydrographic situation encountered during the cruises was in accordance with the typical summer conditions in the southern North Sea (Fig. 2). A well-pronounced seasonal thermocline was found in the open areas of the German Bight at water depths of 10 to 15 m that separated 16°C surface water from a deeper, colder (12°C) water layer. In the southern Bight the tidal mixing front was well developed. A belt of cold surface water separated the well-mixed coastal regions from the thermally stratified offshore areas. In contrast, the conditions in the eastern German Bight were most influenced by the plume of the Elbe River, characterised by sharp horizontal gradients in temperature and salinity. The surface temperature ranged between 21°C in the Wadden Sea and 15°C in the colder water belt. Salinity decreased from 33.5 psu offshore to 27 psu in near-shore areas. The Wadden Sea included 18 stations with a stratification *f*-value from 0.1 to 4.3 J m^{-3}, the mixed water area contained 6 stations with a stratification *f*-value of 0.3 to 9.4 J m^{-3}. Two stations were categorised as frontal areas with stratification *f*-values of 11.4 and 17.9 J m^{-3}, whereas nine stations were considered stratified (*f* = 21.3 to 72.1 J m^{-3}).

Tidal currents were the most dominant current signal with up to 50 cm s^{-1}. Residual currents were approximately 4 to 5 cm s^{-1}, an order of magnitude less than the tidal signal. During the two-week sampling campaign, the net transport was eastward in surface waters, northward in the midwater depths and westward near the bottom. Hydrographic model runs (HAMSOM, T. Pohlmann, IfM, Univ. Hamburg, data not shown) indicated that the likelihood of sampling the same water masses on subsequent days at different stations was relatively low.

The concentrations of copepods (determined from samples taken with 500 μm nets) were highest at the stratified stations (2000 to 5500 copepods m^{-2}), intermediate in the TMF (560 to 780 copepods m^{-2}) and vertically mixed waters (190 to 1650 copepods m^{-2}) and relatively low in the Wadden Sea (14 to 450 copepods m^{-2}). A relatively large size fraction of copepods was captured in the Bongo nets, but it did not cover the entire prey size spectrum of the captured larvae. Thus, the concentrations of copepods reported here serve more as an index of secondary production rather than a quantity representing the total suitable prey abundance.

Abundance and distribution of larvae

A total of 1355 formalin-preserved larvae from 24 sampling stations were analysed. At stations where larvae occurred (66%), the minimum and maximum abundance of clupeid larvae, including sprat, sardine and unidentified clupeids, were 0.17 and 163.5 individuals m^{-2} (Fig. 3). Station features could be ranked

TABLE 2. – Results of Generalised Additive Model (GAM) analysis indicating the most relevent environmental factors that describe the abundance of all clupeids, sardine and sprat at different stations within the southern North Sea. Both the percent explained deviance (ED %) and the Generalised Cross Validation score (GCV) are provided. Single-factor and multiple-factor analyses were conducted.

Parameter	All Clupeids		Sardine (*Sardina pilchardus*)		Sprat (*Sprattus sprattus*)	
	ED	GCV	ED	GCV	ED	GCV
Single Factors						
Depth	58.7	0.91	70.2	0.04	47.6	0.02
Bottom Temperature (BT)	32.1	1.50	41.9	0.09	32.7	0.02
Surface Temperature (ST)	29.7	1.55	40.8	0.04	31.4	0.02
Bottom Salinity (BS)	16.8	1.84	20.0	0.12	14.5	0.03
Surface Salinity (SS)	14.1	1.90	17.1	0.12	12.6	0.03
Copepod Index (CI)	23.3	2.61	15.3	0.18	4.2	0.05
Multiple Factors						
Depth +BT	59.4	0.95	70.4	0.047	47.7	0.018
Depth +BT+ST	63.3	0.92	76.5	0.040	51.6	0.017
Depth +BT+ST+SS	63.9	0.96	77.0	0.042	51.9	0.019
Depth +BT+ST+SS+BS	64.4	1.01	77.3	0.043	55.4	0.018
Depth +BT+ST+SS+BS+CI	68.4	2.16	83.5	0.070	55.8	0.047

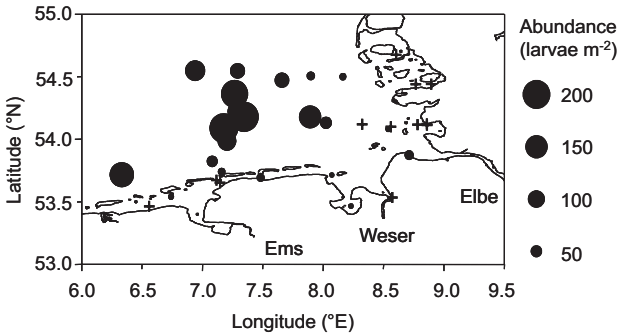

FIG. 3. – Abundance (circles, individuals m^{-2}) of clupeiform larvae, including unidentified clupeids, sardine, sprat and anchovy, at different sampling stations in the German Bight. Crosses indicate stations where clupeiform larvae were not captured.

according to the percentage of deviance explained and GCV scores for clupeid abundance. For all clupeids (containing unidentified clupeids, sprat and sardines), water depth was the best predictor of larval abundance (Table 2). The lowest-ranking predictors were bottom temperature, surface temperature, surface salinity and bottom salinity. Zooplankton abundance was a poor indicator of sprat and sardine larval abundance. No GAM analyses could be made for anchovy due to the low number of stations where this species was captured.

On the basis of this predictor ranking, a series of GAMs of an increasing number of predictors (1-5) was conducted. For each level, all possible arrangements of predictors were tested and only the combination with the lowest GCV score was retained. The best predictive model for sardine abundance included all environmental factors (83.5% variance explained; 0.070 GCV). The best model for clupeids incuded depth, surface and bottom temperature and copepod abundance (68.4% explained variance; 2.16 GCV). The best model for sprat included depth, surface temperature and bottom temperature (51.6% variance explained; 0.017 GCV).

In terms of univariate (station category) analyses, the abundance of clupeiform larvae was significantly different among the four habitats (ANOVA, df =3, 32, F=10.95, p<0.0001), with significantly greater concentrations of clupeiform larvae at frontal stations (f = 10-20 J m^{-3}) than at mixed (p<00.5), stratified (p<0.01) and Wadden Sea stations (p<0.001) (Tukey HSD, p<0.05). The same significant differences in abundance among the different areas were detected for sardine (ANOVA, df =3, 32, F=18.99, p<0.0001) (Fig. 4). Sprat also diplayed an uneven distribution among habitats (ANOVA, df =3, 32, F=11.63, p<0.0001), with higher abundances in the TMF than in mixed water areas (p<0.01) or the Wadden Sea (p<0.05), but

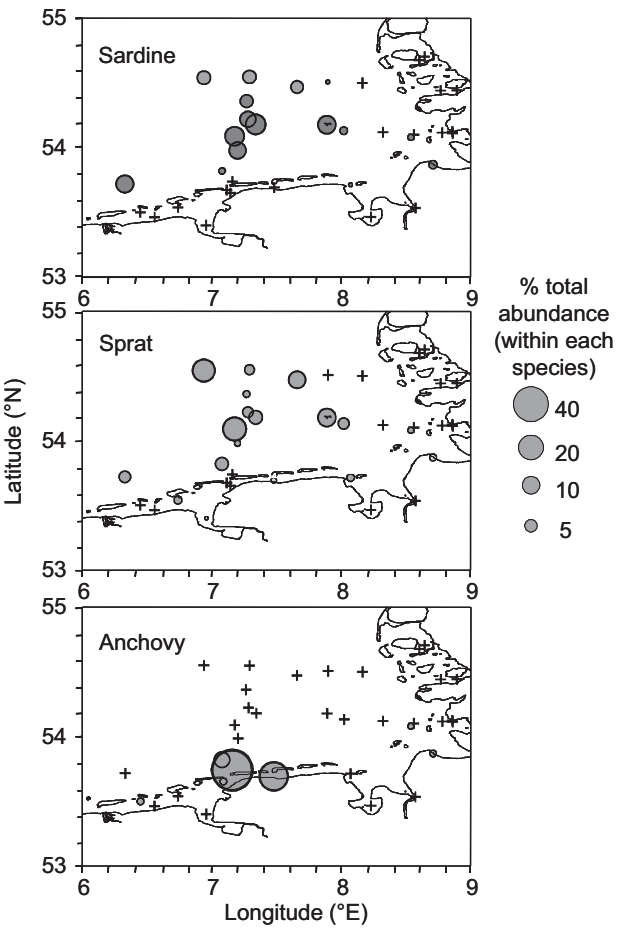

FIG. 4. – Relative abundance of clupeiform larvae within each species in the German Bight, circles indicate percentage of total abundance, crosses indicate stations without larvae (Panel A) sardine, abundance ranged from 0.6 to 45.3 larvae m^{-2}; (Panel B) sprat, abundance ranged from 0.2 to 24.7 larvae m^{-2}; (Panel C) anchovy, abundance ranged from 0.02 to 5.8 larvae m^{-2}.

no significant difference between TMF and stratified water masses. In the Wadden Sea, anchovy larvae were the most abundant clupeiform species. Sprat was found in low concentrations and sardines were completely absent except at station P18. No anchovy larvae were found offshore in the stratified and frontal areas (Fig. 4). The abundance of anchovy in mixed and Wadden Sea areas was relatively low (between 0.05 and 5.61 larvae m^{-2}) compared to the abundance of clupeids in offshore areas.

In terms of length distributions, unidentified clupeids, sardine, sprat and anchovy were 4.5 to 20.3 mm, 4.6 to 20.7 mm, 6.9 to 17.6 mm, and 5.6 to 10.3 mm S_L (after correction for shrinkage) (Fig. 5). Larvae >16 mm S_L were excluded from further analysis due to possible net avoidance by larvae in these length classes. Compared to those captured in mixed waters, clupeid larvae found in stratified and frontal areas were significantly larger (unpaired

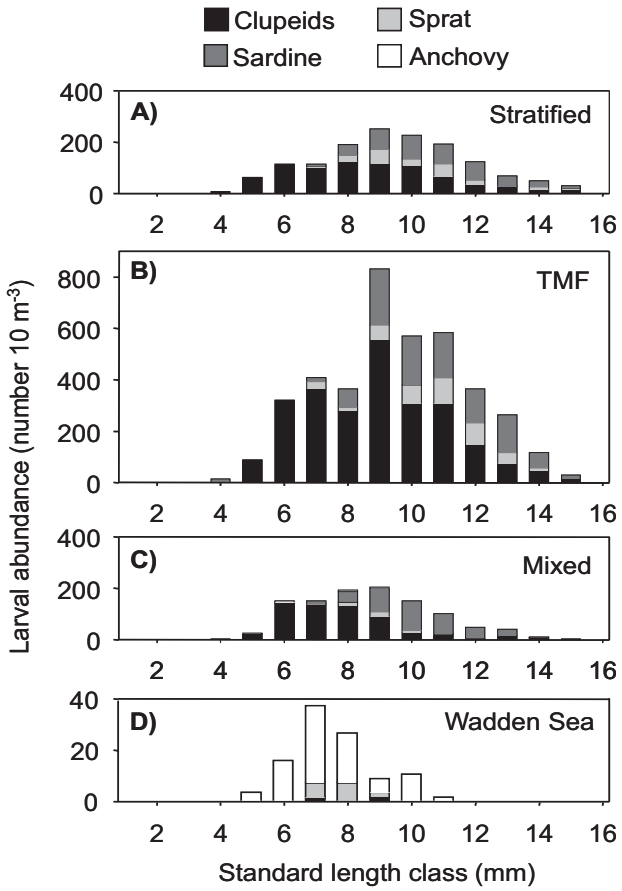

FIG. 5. – Standard length (mm) abundance distribution of clupeiform larvae sampled in the tidal mixing front (TMF) (n=272), stratified areas (n=668), well-mixed water masses (n=354) and Wadden Sea (n=55). Note the smaller x-scale in Wadden Sea box .

t-test, t=4.524, df=573, p<0.0001; t=5.804, df= 378, p<0.0001). Sprat larvae were larger in stratified and frontal areas than in the vertically mixed zone and the Wadden Sea (ANOVA, df = 161, F= 9.004, p<0.0001). There was no significant difference in S_L of sardines (ANOVA, df = 450, F=0.46, p>0.05) among the different areas. In anchovy no size differences were observed between mixed water masses and the Wadden Sea either.

Spatial variability in condition

A total of 263 undamaged larvae were available for measurements of biochemical-based condition. No size correction for shrinkage due to preservation at –80°C was applied. *RNA*:*DNA* values for clupeid larvae captured within the study area were highly variable (Fig. 6) and ranged from 0.73 to 7.32, 0.59 to 6.72, and 0.89 to 6.43 in sardine, sprat and anchovy, respectively. A correlation between *RNA*:*DNA* (ln-transformed) and S_L was observed for sprat (Pearson correlation, n=163, Pearson r= 0.34, p<0.001) but not for sardine (n=71, Pearson r= 0.11) or anchovy (n=29, Pearson r= -0.12). Due to the low numbers of undamaged larvae, *RNA*:*DNA* values were pooled in a reasonable way to make inter-specific / within-habitat comparisons as well as intra-specific / among-habitat comparisons. Larvae of the same species that were sampled at similar station categories (e.g. Wadden Sea, TMF, etc.) were combined in two length categories (5 to 10 mm and 10 to 15 mm S_L) based on the development of the caudal fin (flexion stage) and behavioural changes (e.g. schooling) occurring at ~10 mm S_L in sprat, sardine and anchovy. No significant differences in mean *RNA*:*DNA* were found in different water masses for sardine or sprat (Fig. 6), nor were there differences between pre-and post-flexion sprat and sardine larvae in the same water masses. Significant differences between anchovy and sprat *RNA*:*DNA* (p=0.006, t=3.722, df=40) and *G* (p=0.0001, t=4.234, df=48) were detected in the Wadden Sea samples, in which anchovy displayed higher values than sprat.

Growth rates in the frontal and stratified stations were calculated in two different steps. Since fish larvae collected during double oblique tows of Bongo gear, their vertical distribution was unknown. To account for potential differences in temperature, maximum and minimum *G* values were calculated for each larva using sea-surface temperature and bottom temperature, respectively. *G* was generally positive, with mean values of 16.2 (max) and 14.5 (min) % d^{-1} for sprat, 19.2 (max) and 15.5 (min) % d^{-1} for sardine and 27.2% d^{-1} for anchovy. Nevertheless, some individuals had negative *G* values based upon Buckley's (2008) interspecific equation. The total percentage of very slow-growing larvae (G<0% d^{-1}) sampled were 2% (maximum *G*) and 5% (minimum *G*) for sardine and <1% for sprat larvae. There were some differences in the percentage of starving larvae detected among the areas. The highest proportions of larvae with zero growth were sardines at the frontal stations (9% starving larvae; stations A6, A9) and on the stratified side close to the river plume of the Elbe River (7.1% starving larvae; station P25). For larval sprat, average proportions of very slow growing larvae were 4.3% in the stratified area and 3.2% in the Wadden Sea. Larval nutritional condition was not correlated to any environmental factors including depth, surface and bottom temperature, surface and bottom salinity and copepod abundance.

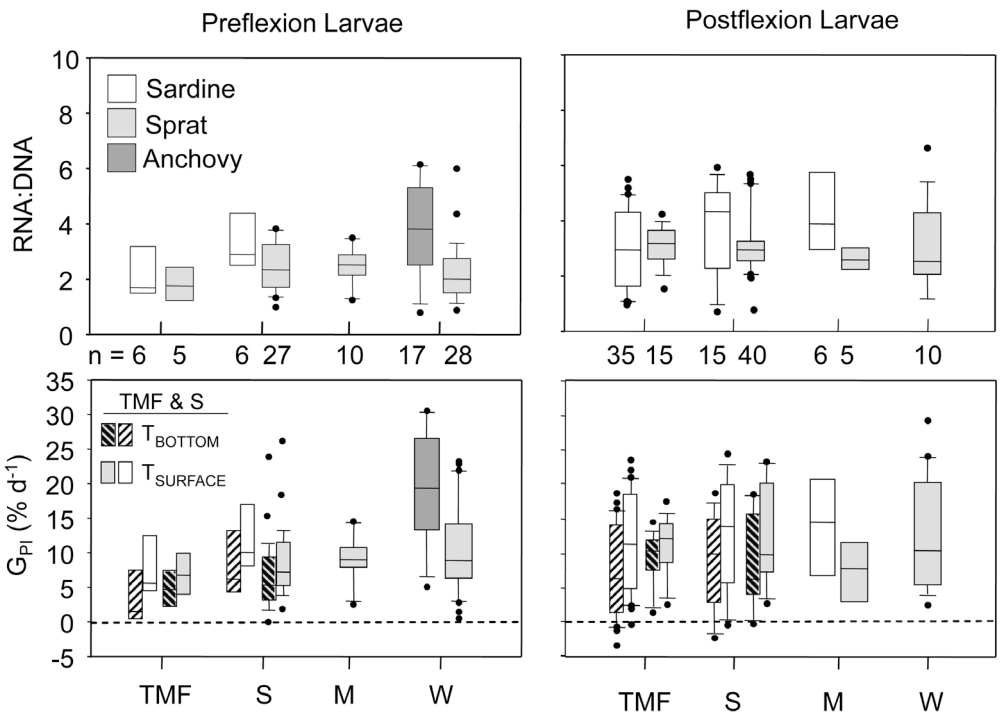

FIG. 6. – Box and whisker plots of values of *RNA:DNA* and protein-specific growth rate (G_{PI}) for pre-and post-flexion clupeiform larvae collected in each of four different habitats (tidal mixing front (TMF), stratified (Strat.), mixed (Mixed), and Wadden Sea. For sprat and sardine sampled in frontal and stratified areas, minimum (light grey) and maximum G_{PI} (calculated based upon minimum and maximum water temperatures that could have been experienced at each station) are shown (sprat, white background; sardine, grey background). The median, 10th, 25th 75th and 90th percentiles and outliers are indicated. The number of larvae analysed is shown between top and bottom panels.

DISCUSSION

Our results confirm the re-establishment of spawning populations of anchovy and sardine within the southern North Sea. While water circulation in the North Sea can transport eggs and foraging larvae from large catchment areas (including the English Channel) into the German Bight (Bartsch and Knust, 1994), the abundances of extremely young (preflexion) anchovy and sardine larvae obtained in the present study indicate adult spawning in the region.

Spawning events of sardines and anchovies in the German Bight were described by Aurich (1954) during the period 1948-1952 but were not described in the following 50 years. The first finding of early stages of sardines in the German Bight in recent years was recorded in 2003 (Huwer, 2004; Malzahn and Boersma, 2007). Aurich (1954) discussed climate change and above-average summer temperatures in relation to this phenomenon—a situation similar to that of 2003 and 2005, when summer temperatures in the North Sea were well above the long-term average (2003 was + 2 to 3°C; 2005 was + 1.0 to 2.5°C). It seems reasonable that warm water temperatures are at least a precondition for anchovy and sardine spawning. The recent changes in phytoplankton and zooplankton species composition of the North Sea (Reid *et al*., 1998, Alheit *et al.,* 2005) might also be favourable for sardine and anchovy. The increase in abundances of warm-water species such as *Calanus helgolandicus* a predominantly Mediterranean species, might offer more suitable food for anchovy and sardine.

The present study was the first to synchronously sample the larval fish community in both nearshore (Wadden Sea) and offshore areas in the southern German Bight. In offshore areas, the highest abundance of larval sprat and sardine were found at frontal stations, whereas lower abundances were found in the well- mixed and strongly stratified areas. These findings agree well with studies examining the distribution of sprat in the same area (Munk, 1993; Valenzuela and Vargas*,* 2002). Advection and retention processes in different frontal areas have been described in a number of studies (e.g. Fortier and Gagne, 1990; Munk, 1993) and may have important consequences for the distribution and abundance of larvae in this study. Stratified and frontal areas contained significantly larger larvae than well-mixed areas, suggesting either an offshore movement of

sprat and sardine with development or that the latter areas supported lower rates of growth and survival. In contrast to offshore areas, near-shore and/or coastal well-mixed habitats (such as the Wadden Sea) do not appear to be an important foraging area for the larvae of sprat (low abundance, 3.6% starving) and sardine (one station, low abundance). These coastal areas, however, appear to be very important for anchovy larvae, which were absent at offshore stations. Aurich (1954) described a similar distribution pattern for anchovy larvae in the same area.

Although our GAM analyses indicated that stations supporting high abundances of sprat and sardine could not be easily separated (e.g. depth, surface and bottom temperature, surface and bottom salinity were associated with the abundance of both species), temperature probably plays an important role in habitat partitioning among the larvae of these three clupeid species. For example, maximum spawning activity for sprat, sardine and anchovy in European waters occurs at 6-12°C, 14-16°C, and 14-19°C, respectively (de Silva 1973; Ré, 1990; Sola *et al*., 1990; Motos *et al*., 1996). These temperature preferences lead to seasonal habitat partitioning in the Mediterranean Sea, where anchovy spawns in summer and sprat and sardine spawn during the winter (Olivar *et al*., 2001). Planque *et al*. (2007) suggested that bottom water temperature was the strongest predictor for potential spawning habitat in anchovy (threshold >12°C), whereas sardine appears to have a greater tolerance than anchovy for low bottom temperature (Planque *et al*., 2007). The role of salinity for sprat, sardine and anchovy larvae is not fully understood. Larval sprat can tolerate low salinity in the Baltic Sea (8 psu, Voss and Hinrichsen, 2003) but sprat eggs in the North Sea are distributed mainly in waters with a salinity of 30-33 psu (Moksness and Torstensen, 1985), while larvae are likely to occur over a similar or slightly higher range of salinities (Moksness and Torstensen 1985; this study). Reid (1966) argued that anchovy (and other engraulids) have an affinity towards estuaries and other coastal areas, but not necessarily towards waters having a specific range in salinities. Whereas river plumes (i.e. low salinity) appear to be recurrent, preferential areas for spawning, anchovy also spawns in other areas such as slope water eddies or the shelf break, which are characterised by high salinity throughout the water column (Motos *et al*., 1996). Little is known about the impact of salinity on spawning and the larvae of sardine (Planque *et al*., 2007).

No data are yet available concerning adult spawning stock sizes of sardine and anchovy in the North Sea. We suggest that the relatively low abundance of anchovy (six inshore stations) compared to sprat and sardine (offshore areas) in our survey was probably due to a better match with the spawning period of sprat and sardine compared with that for anchovy in the southern North Sea. Latitudinal gradients in seasonal water temperatures will shift spawning seasons in different areas (Stratoudakis *et al*., 2004). Peak spawning by anchovy occurs between May and June in the Bay of Biscay (Planque *et al*., 2007) but probably occurs later (July/August) in the North Sea at the northernmost limit of the latitudinal range of this species. However, it should be noted that abundances of clupeiform larvae in the northeastern inshore area of the German Bight found in the present study were generally lower than those previously observed for sprat (Alshuth, 1988), sardine (Huwer, 2004) and anchovy (Aurich, 1954) in this region. These differences might be explained by different currents and drift patterns among the sampling months and years.

In most marine fish species, the probability of survival during early life is thought to be positively correlated with growth rate as faster-growing individuals spend less time in stages particularly vulnerable to starvation and predation: larger larvae have enhanced ability to feed and avoid predators (Cushing, 1974; Rice *et al*., 1993). The hypothesis that growth of larvae might be promoted in frontal zones (Munk, 1991; Nakata and Zenitani, 1996; Lee *et al*., 2007) was not unconditionally supported in the present study. Values of *RNA*:*DNA* for sprat and sardine were generally high (albeit quite variable) and appeared unrelated to hydrographic conditions in the southern North Sea in June/July 2005. However, the finding that 9% of sardine at frontal stations appeared to be starving suggests that competition for zooplankton could have been acting in these waters at this time period.

The finding in the present study that anchovy had significant higher condition (*RNA*:*DNA*) and growth rate (*G*) than sprat in the Wadden Sea was unexpected. Sprat and anchovy consume similar prey items during the pre-flexion stage, mainly copepod nauplii (Dickmann *et al*., 2007; Rossi *et al*., 2005), although there is some evidence that anchovy larvae are opportunistic feeders that can consume protozoans (Rossi *et al*., 2005). We speculate that anchovy larvae may be able to better exploit prey resources in the Wadden Sea. On the other hand, otolith studies

suggest that growth potential of anchovy and sprat are slightly different, making it difficult to compare the "suitability" of the same habitat based solely on growth rates. Larval sprat growth rates between 0.30 and 0.36 mm d^{-1} were reported at 15°C in the North Sea (Alshuth, 1988) and rates of 0.40 to 0.42 mm d^{-1} were reported in January in the Adriatic Sea at surface water temperatures between 10 and 11.5°C (Dulčić, 1998). Anchovy can reach higher growth rates in the same size class (6-20 mm), ranging from 0.5 mm d^{-1} at 16-18°C (Garcia *et al*., 1998; Somarakis and Nikolioudakis, 2007) to 0.9 mm d^{-1} at 20°C (Palomera *et al*., 1988). These examples for sprat and anchovy also suggest that the larvae of the latter species might cope better with the relatively high temperatures (17.0-20.5°C) associated with nearshore areas such as the Wadden Sea.

In conclusion, we provide a snapshot of the distribution, abundance and biochemical-based condition and growth of sprat, anchovy and sardine larvae in both near- and offshore habitats of the southern North Sea. Naturally, temporal patterns may differ due to changes in extrinsic (environmental) factors and intrinsic factors (timing of life-history events such as adult spawning and migrations) factors. Nevertheless, the present study suggests that larvae of these three species generally have high growth rates in the areas where they were found, but can potentially compete for prey resources in specific habitats (fronts and stratified waters, sardine and sprat). The three species exhibited a degree of habitat partitioning both spatially (anchovy occur in nearshore areas, sardine do not) and perhaps temporally (peak anchovy spawning will probably occur later than sprat). Ontogenetic changes are known to occur in habitat requirements and utilisation: for example, post-larval and juvenile stages of sprat are known to actively migrate to inshore areas for foraging (Beyst *et al*., 1999). Accordingly, it will be important to continue to examine the inter-relationships between sardine, sprat and anchovy throughout their first year of life, since factors affecting recruitment are not only acting during the larval period but also during the early juvenile period in many fish species (Sogard, 1997).

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REFERENCES

Alheit, J., E. Wahl, and B. Cibangir. – 1987. Distribution, abundance, development rates, production and mortality of sprat eggs. *ICES CM/H*:45

Alheit, J., C. Möllmann, J. Dutz, G. Kornilovs, P. Loewe, V. Mohrholz and N. Wasmund. –2005. Synchronous ecological regime shifts in the central Baltic and the North Sea in the late 1980s. *ICES J. Mar. Sci*., 62(Suppl. 7): 1205-1215.

Alshuth, S. – 1988. *Bestimmung von Alter und Wachstumsraten bei Larven und Juvenilen der Sprotte,* Sprattus sprattus *L.* Ph. D. thesis. AWI (Alfred-Wegener-Institut für Polar- und Meeresforschung), Bremerhaven.

Aurich, H.J. – 1954. Verbreitung und Laichverhältnisse von Sardelle und Sardine in der südöstlichen Nordsee und ihre Veränderungen als Folge der Klimaänderung. *Helgol. Wiss. Meeresunters*, 4: 175-204.

Bartsch, J., and R. Knust. – 1994. Predicting the dispersion of sprat larvae (*Sprattus sprattus* (L.)) in the German Bight. *Fish. Oceanogr*., 3(Suppl. 4): 292-296.

Beare, D., F. Burns, E. Jones, K. Peach, E. Portilla, T. Greig, E. McKenzie and D. Reid. – 2004. An increase in the abundance of anchovies and sardines in the north-western North Sea since 1995. *Glob. Change Biol*., 10(Suppl. 7): 1209-1213.

Beyst, B., J. Mees and A. Catrtrijsse. – 1999. Early postlarval fish in the hyperbenthos of the Dutch Delta (south-west Netherlands). *J. Mar. Biol. Assoc. U.K*., 79(Suppl. 4): 709-724.

Borja, A., A. Uriarte, J. Egaña, L. Motos and V. Valencia. – 1998. Relationships between anchovy (*Engraulis encrasicolus*) recruitment and environment in the Bay of Biscay (1967-1996). *Fish. Oceanogr.,* 7(Suppl. 2): 375-380.

Buckley, L.J., E.M. Caldarone and C. Clemmesen. – 2008. Multispecies larval fish growth model based on temperature and fluorimetrically derived RNA/DNA ratios: results from a meta analysis. *Mar. Ecol. Prog. Ser.*, 371: 221–232

Caldarone, E.M., C. Clemmesen, E. Berdalet, T.J. Miller, A. Folkvord, G.J. Holt, M.P. Olivar, M.P. and I.M. Suthers. – 2006. Intercalibration of four spectrofluorometric protocols for measuring RNA/DNA ratios in larval and juvenile fish. *Limnol. Oceanogr, Methods,* 4: 153-163

Caldarone, E.M., M. Wagner, J. St. Onge-Burns and J.L. Buckley. – 2001. Protocol and Guide for Estimating Nucleic Acids in Larval Fish Using a Fluorescence Microplate Reader, *NEFSC, Reference Document* 01-11.

Corten, A. and G. van de Kamp. –1996. Variation in the abundance of southern fish species in the southern North Sea in relation to hydrography and wind. *ICES J. Mar. Sci*., 53(Suppl. 6): 1113-1119.

Cury, P. and C. Roy. –1989. Optimal environmental window and pelagic fish recruitment success in upwelling areas. *Can. J. Fish. Aquat. Sci*., 46(Suppl. 4): 670-680.

Cushing D.H. – 1974. The natural regulation of fish populations. In: F.E. Harden Jones (eds.), *Sea fisheries research,* pp 399-412. Elek. Science, London.

de Silva, S.S. – 1973. Aspects of the reproductive biology of sprat, *Sprattus sprattus* (L.) in inshore waters of the west coast of Scotland. *J. Fish. Biol*., 5(Suppl. 6): 689-705.

Dickmann, M., C. Möllmann and R. Voss. – 2007. Feeding ecology of Central Baltic sprat *Sprattus sprattus* larvae in relation to zooplankton dynamics: implications for survival. *Mar. Ecol. Prog. Ser*., 342: 277–289.

Dulčić, J. – 1998. Larval growth of sprat, *Sprattus sprattus phalericus*, larvae in the northern *Adriatic. Fish. Res*., 36: 117-126.

Fey, D.P. – 2002. Formation of daily increments in otoliths of larval and juvenile herring (*Clupea harengus* L.) and early juvenile smelt (*Osmerus eperlanus* L.) in low-food conditions. *Arch. Fish. Mar. Res*., 49(Suppl. 3): 189-197.

Fortier, L. and J.A. Gagne. – 1990. Larval herring (*Clupea harengus*) dispersion, growth, and survival in the St. Lawrence Estuary: Match/mismatch or membership/vagrancy? *Can. J. Fish. Aquat. Sci*., 47(Suppl. 10): 1898-1912.

Garcia, A., D. Cortes and T. Ramirez. –1998. Daily larval growth and RNA and DNA content of the NW Mediterranean anchovy (*Engraulis encrasicolus*) and their relations to the environment. *Mar. Ecol. Prog. Ser*., 166: 237-245.

Halbeisen, H.W. – 1988. Bestimmungsschlüssel für Fischlarven der Nordsee und angrenzender Gebiete. *Ber. Inst. Meeresk. Nr.* 178.

Huwer, B. – 2004. *Larval growth of* Sardina pilchardus *and* Sprattus sprattus *in relation to frontal systems in the German Bight.* Diploma thesis, Univ. Kiel.

Lee, O., B.S. Danilowicz and R.D.M. Nash. – 2007. Small scale variability in growth and condition of dab *Limanda limanda* (L.) larvae in relation to an Irish Sea tidal-mixing front off the west coast of the Isle of Man. *J. Fish Biol*., 71(Suppl. 4): 1056-1068.

Malzahn, A.M. and M. Boersma. – 2007. Year-to-year variation in larval fish assemblages of the Southern North Sea. *Helgol. Mar. Res.* 61: 117-126.

Moksness, E. and E. Torstensen. – 1985. The buoyancy of sprat (*Sprattus sprattus*) eggs and larvae in the Skagerrak area. *ICES CM/L:6*.

Motos, L., A. Uriarte and V. Valencia. – 1996. The spawning environment of the Bay of Biscay anchovy (*Engraulis encrasicolus* L.). *Sci. Mar*., 60(Suppl. 2): 117-140.

Munk, P. – 1991. Changes in mean size and distribution of juvenile North Sea sprat (*Sprattus sprattus* L.) in the period 1976-1990. *ICES CM/H:*41.

Munk, P. – 1993. Differential growth of larval sprat *Sprattus sprattus* across a tidal front in the eastern North Sea. *Mar. Ecol. Prog. Ser*., 99: 17-29.

Munk, P. and J.G. Nielsen. – 2005. *Eggs and larvae of North Sea fishes*. Biofolia, Frederiksberg, Denmark.

Nakata, H. and H. Zenitani. – 1996. Did offshore shift of the main spawning ground of Japanese sardine affect the availability of food for the larvae? In: Y. Watanabe, Y. Yamashita and Y. Ooseki (eds.), *Survival strategies in Early Life Stages of Marine Resources,* pp. 115-123. A.A. Balkema, Rotterdam.

Olivar, M.P., J. Salat and I. Palomera. – 2001. Comparative study of spatial distribution patterns of the early stages of anchovy and pilchard in the NW Mediterranean Sea. *Mar. Ecol. Prog. Ser*., 217: 111-120.

Palomera, I., B. Morales-Nin and J. Lleonart. – 1988. Larval growth of anchovy, *Engraulis encrasicolus*, in the western Mediterranean Sea. *Mar. Biol*. 99(Suppl. 2): 283-291.

Planque, B., E. Bellier and P. Lazure. – 2007. Modelling potential spawning habitat of sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) in the Bay of Biscay. *Fish. Oceanogr*., 6(Suppl. 1): 16-30.

Postuma, K.H. – 1978. Immigration of southern fish into the North Sea. *Rapp. P.-V. Reun. Cons. Int. Explor. Mer.*, 172: 225-229.

Ré, P. – 1990. Tidal transport and retention of anchovy eggs and larvae within Mira Estuary (Portugal). *Port. Zool*., 1(Suppl. 2): 7-13.

Reid, J.L. –1966. Oceanic environment of the genus *Engraulis* around the world. *CalCOFI Rep.*, 11: 29-33.

Reid, P.C., B. Planque and M. Edwards. – 1988. Is observed variability in the long-term results of the Continuous Plankton Recorder survey a response to climate change? *Fish. Oceanogr.*, 7(Suppl. 3): 282-288.

Rice, J.A., L.B. Crowder and K.A. Rose. – 1993. Interactions between size-structured predator and prey populations: Experimental test and model comparison. Trans. *Am. Fish. Soc*., 122(Suppl. 3): 481-491.

Rossi, S., A. Sabates, M. Latasa and E. Reyes. – 2006. Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*) larvae in the NW Mediterranean. *J. Plankton Res*., 28: 551-562.

Roy, C., C.D. van der Lingen, J.C. Coetzee and J.R.E. Lutjeharms. – 2007. Abrupt environmental shift associated with changes in the distribution of Cape anchovy *Engraulis encrasicolus* spawners in the southern Benguela. *Afr. J. Mar. Sci*., 29(Suppl. 3): 309-319.

Sabatés, A. and M.P. Olivar. – 1996. Variation of larval fish distributions associated with variability in the location of a shelf-slope front. *Mar. Ecol. Prog. Ser*., 135: 11-20.

Simpson, J.H., D.J. Edelsten, A. Edwards, N.C.G. Morris and P.B. Tett. – 1979. The Islay front: physical structure and phytoplankton distribution. *Estuar. Coast. Mar. Sci*., 9: 713-726.

Sogard, S.M. – 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull. Mar. Sci*., 60(Suppl. 3): 1129-1157.

Sola, A., L. Motos, C. Franco and A. Lago de Lanzos. – 1990. Seasonal occurrence of pelagic fish eggs and larvae in the Cantabrian Sea (VIIIc) and Galicia (IXa) from 1987 to 1989. *ICES CM 1990/H*:25:14.

Somarakis, S. and N. Nikolioudakis. – 2007. Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolus*) in the northern Aegean Sea (eastern Mediterranean). *Mar. Biol*., 152(Suppl. 5): 1143-1158.

Stratoudakis, Y., S.H. Coombs, T. Smyth, J. Costas, C. Franco, A. Lago de Lanzós, A. Silva, M.B. Santos, P. Alvarez and M. Santos. – 2004. Sardine (*Sardina pilchardus*) spawning season in the North East Atlantic and relationships with sea surface temperature. *ICES CM 2004/Q:*19:19.

Valenzuela, G.S. and C.A. Vargas. – 2002. Comparative larval growth rate of *Sprattus sprattus* in relation to physical and biological oceanographic features in the North Sea. *Arch. Fish. Mar. Res*., 49(Suppl. 3): 213-230.

Voss, R. and H.-H. Hinrichsen. – 2003. Sources of uncertainty in ichthyoplankton surveys: modelling the influence of wind forcing and survey strategy on abundance estimates. *J. Mar. Sys.*, 43(Suppl. 1): 87-103.

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