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Investigating environmental influence and temporal changes in sole (*Solea solea*) larvae condition using histology

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Abstract :

In the eastern part of the English Channel, common sole (Solea solea) has strong interests in fisheries research. Low recruitment along with a decline in spawning stock biomass have been observed for several years. According to the recruitment hypotheses, larval survival may play an important role that needs to be considered. The fish larval condition can be assessed using histology which has been recognised as the most appropriate method to provide a reliable index of the nutritional status. Based on this approach, this study aimed to identify critical periods of wild-collected sole larvae and to determine sources of variations of their condition between two periods separated by more than 20 years. In line with other studies, the transition from endogenous to exogenous feeding was identified as the most critical period with the lowest proportion of healthy larvae observed. During this first feeding stage, good larval conditions were located in sampling stations close to the coast and at the end of the spring season, in relation to higher temperature and fluorescence values. This highlights the need for sole larvae survival to cross the coastal front, which splits the central and coastal waters, to reach more stable and productive areas. This coastal migration pattern was consistent between 1995 and 2017, with however significantly lower larval abundances in the recent period. Multivariate analyses showed that the spring environmental conditions of 1995, characterised with lower temperature and higher fluorescence values were more favourable to larval condition, compared to the spring in 2017. Areas providing suitable environmental conditions in 2017 were more restricted and limited to sampling stations in front of estuaries. Since small differences in larval survival can lead to large fluctuations in recruitment, the larval condition should be studied in a more long-term approach. This would provide a better understanding of the environmental influence on larval survival and recruitment success.

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Highlights

► First feeding stage corresponds to the critical most period during sole larval ontogeny. ► Good larval condition was related to estuarine areas in the Eastern English Channel. ► Lower sole larval abundances in spring 2017 compared to spring 1995. ► Environment suitable for a good sole larvae condition was more limited in 2017.

Keywords : Eastern English channel, Critical period, Starvation, Fish larval condition, WitOMI

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1 Introduction

In the eastern English Channel (EEC), common sole (*Solea solea*) spawning occurs from February to June close to the coasts (Eastwood et al., 2001). The pelagic phase lasts about six weeks (Vaz et al., 2019). Dispersion, nychthemeral and tidal migrations drive larval settlement in the coastal and estuarine nursery grounds during metamorphosis (Grioche et al., 2000; Grioche et al., 2001; Koutsikopoulos et al., 1989; Rochette et al., 2012). There is some evidence that the population is supplied by a pool of three distinct nurseries: along the English coast, the Seine Bay area, and the nurseries along the south-east coasts of the EEC including the three estuaries Somme, Authie and Canche (Du Pontavice et al., 2018; Rochette et al., 2013). Juveniles settle down for two years before recruiting into the adult population, with very low connectivity between the different nursery pools mentioned above (Le Pape and Cognez, 2016). This strong spatial structuration seems to persist during the adult phase (Du Pontavice et al., 2018; Lecomte et al., 2019; Randon et al., 2018; 2020). However, the EEC sole stock remains assessed and managed as a single, spatially homogeneous population (ICES division 107D).

The ECC common sole is a stock of high economic value in the area (Gibson et al., 2014). Since many fleets rely on it, the stock has been the subject of particular attention for several years. Low recruitment along with a decline in spawning stock biomass, which is now around Blim (i.e stock size below which there is a high risk of reduced recruitment), have been observed since 2011 (ICES, 2018) despite the stock management being close to Maximum Sustainable Yield (MSY). Likewise, the potential role of the larval phase, especially its survival rate, remains misunderstood and needs to be considered.

Many hypotheses for recruitment success rely on larval survival which requires favourable transport as well as a spatial and temporal coincidence of fish larvae with their trophic resources (Somarakis et al., 2017). Since the number of offspring recruiting in the adult population is not necessarily proportional to the spawning biomass (Anderson, 1988; Houde, 2008), larval starvation and predation have been accepted as major sources of variability in larval survival and recruitment (Peck et al., 2012).

Food deprivation in fish larvae can be assessed using condition indices (Ferron and Leggett, 1994). Many indices are available to highlight the effects of starvation

on growth and nutritional condition (Buckley, 1979; Clemmesen, 1994; Diaz et al., 2018), energy reserves (Fraser, 1989; Giraldo et al., 2011) or tissue integrity (Diaz et al., 2013; O'Connell, 1976; Theilacker, 1978). The latter is an integrative approach of the level of starvation and can be evaluated using histology. Histological-based observations have been recognised as the most appropriate method to provide a reliable index of the larval nutritional status (Di Pane et al., 2019; Ferron and Leggett, 1994; Gisbert et al., 2008). It informs on the direct effects of starvation on the organs state, especially those related to nutrition (*e.g* guts, liver, and pancreas). Indeed, food deprivation leads to abnormal development and degeneration of cells and tissues regardless of stages or species. (McFadzen et al., 1997; O'Connell, 1976; Sieg, 1998).

The present work aims to evaluate the sole larval condition on the French side of the EEC. Using a histological condition index developed and calibrated experimentally on sole larvae, the objectives are (1) to evaluate larval condition and identify the critical period(s); (2) to determine environmental sources of variation in the condition; and (3) to study temporal changes in these factors and their impact on the larval condition between two periods more than 20 years apart. In line with the recruitment assumptions, we expect a higher starvation incidence for first feeding larvae. Moreover, in a context of changing environment and low recruitment observed for sole since 2011, we expect individuals collected in 2017 to display poorer condition and/or lower abundances than cohorts from two decades ago.

2 Materials and methods

2.1 Data origins

Data used in this study come from oceanographic surveys conducted in spring 1995 and 2017 (Figure 1). Histological data on sole larvae captured during spring 1995 are based on work carried out by Grioche (1998). The same methodology for sampling and larval condition analyses was used for the two periods to ensure comparability.

2.2 Surveys

Data come from five ichthyoplanktonic surveys that were conducted in the EEC between March and May in 1995 and 2017 (Table 1, see Grioche et al. (2001) and Di

Pane et al. (2020a) for more details). Coastal-offshore samplings were performed between the Bay of Seine and Belgium on the French side and between the Isle of Wight and the Thames estuary on the English side (Figure 1). At each of the 169 stations, environmental variables were recorded and fish larvae were sampled.

Table 1: Sampling period of the surveys used in the present study

Survey	Year	Start	End	Number of stations
REISE1	1995	11/04	13/04	45
REISE2		02/05	04/05	48
REIVE1	2017	23/03	30/03	26
PHYCO		21/04	29/04	24
REIVE2		05/05	12/05	26

Fish larvae were sampled using a bongo net (60 cm diameter) fitted with two 500 µm mesh-size nets. The nets were deployed through a double-oblique tow between the surface and five meters above the seabed during 10 minutes at a speed of 2 knots. The filtered volume of seawater was calculated using a digital flowmeter fixed at the entry of each net. The content of the first net was dedicated to calculate fish larval abundances and was fixed in a buffered-formalin seawater solution (Di Pane et al., 2020a). Fish larvae from the second net were fixed in Bouin-Hollande solution for two days and then stored in ethanol 70° for two months before histological analyses. Sole larvae were sorted out from the samples under a stereomicroscope and determined following Russell (1976). A developmental stage, ranging from 1 to 5, was attributed to each individual according to Ryland (1966): stage 1- yolk-sac larvae; stage 2- yolk sac exhausted and notochord straight; stage 3- caudal extremity of notochord bent and eyes symmetrical; stage 4: eyes start to be asymmetrical indicating the beginning of metamorphosis; stage 5- the left eye on or beyond the edge of the head. Abundances (ind.100 m^{-3}) of each stage were calculated using the filtered volume of seawater of the first net.

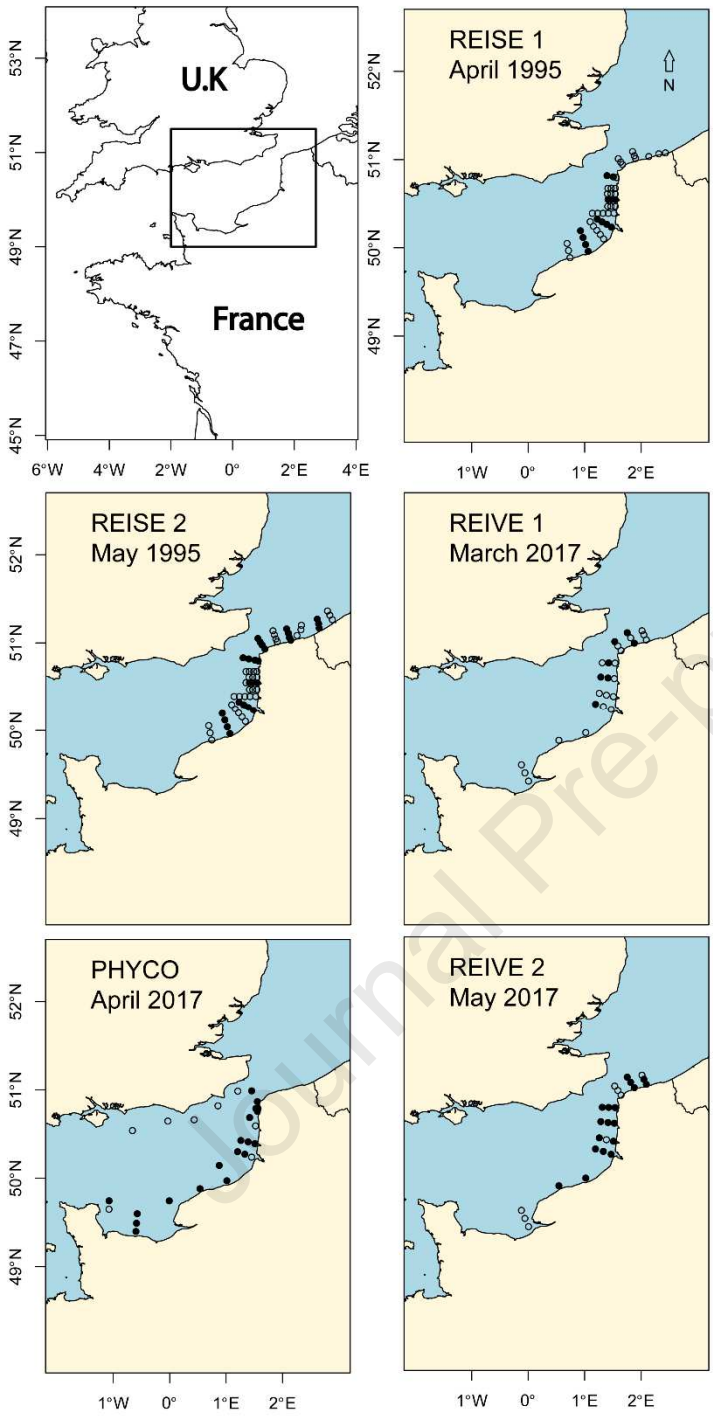

Figure 1 | Maps of the study area and sampling stations for each survey. Black dots correspond to stations where sole larvae were sorted for histological analyses.

2.3 Environmental variables

At each sampling station, vertical profiles of temperature (°C), salinity (Practical Salinity Scale) and fluorescence (mg Chla.l^{-1}) were recorded using a Seabird CTD (Conductivity Temperature Depth) profiler and averaged over the water column.

147 Mesozooplankton was collected through vertical hauls using a WP2 net (200 µm
148 mesh size). Total mesozooplankton abundance (ind.m^{-3}) was considered as a proxy
149 of planktonic production and food availability. Geographical factors such as distance
150 to the coast (degrees), bedstress (N.m^{-2}), and depth (m) were post-calculated with a
151 geographic information system (ArcMap 9.2 from ESRI).

152 2.4 Larval condition scoring

153 Histological preparation was performed following Di Pane et al. (2020b). Larval
154 condition was evaluated by optical microscopic observation of the liver, the pancreas
155 and the gastrointestinal tract (foregut, midgut, and hindgut; Figure 2). For each larva,
156 several histological sections were examined. Qualitative assessment was based on
157 the histological grades defined by Boulhic (1991) which were determined at the larval
158 scale.

159 **Grade° 6**: Larva is in good health related to a good nutritional state. Reserves of
160 glycogen and lipids in the form of vacuoles are visible in the liver hepatocytes. The
161 acini of the pancreas are well structured with a large amount of zymogen. Intestinal
162 cells are high.

163 **Grade° 5**: Larva is in good health. Organs are in good condition but the liver has
164 fewer vacuoles indicating lower levels of glycogen. Vacuoles are present in the
165 intestine.

166 **Grade° 4**: The beginning of starvation is noted. An organ is altered, often the
167 posterior intestine. Few vacuoles are visible in the intestine.

168 **Grade° 3**: Advanced starving or food recovery. The digestive tract and the liver
169 are altered, intercellular spaces can be observed, but cells show some signs of
170 absorption (vacuoles). In the case of refeeding after a starving phase, goblet cells are
171 present in the oesophagus.

172 **Grade° 2**: Severe starvation. Organs such as the liver, pancreas, and digestive
173 tract are altered. Very weak cellular cohesion. Hepatocytes are small.

174 **Grade° 1**: Larva is at the point of no return. It is an irreversible state leading to
175 death. All organs are altered. The pancreas is no longer structured. There is neither
176 zymogen in the pancreas, nor glycogen in the liver. Some nuclei are pycnotic.
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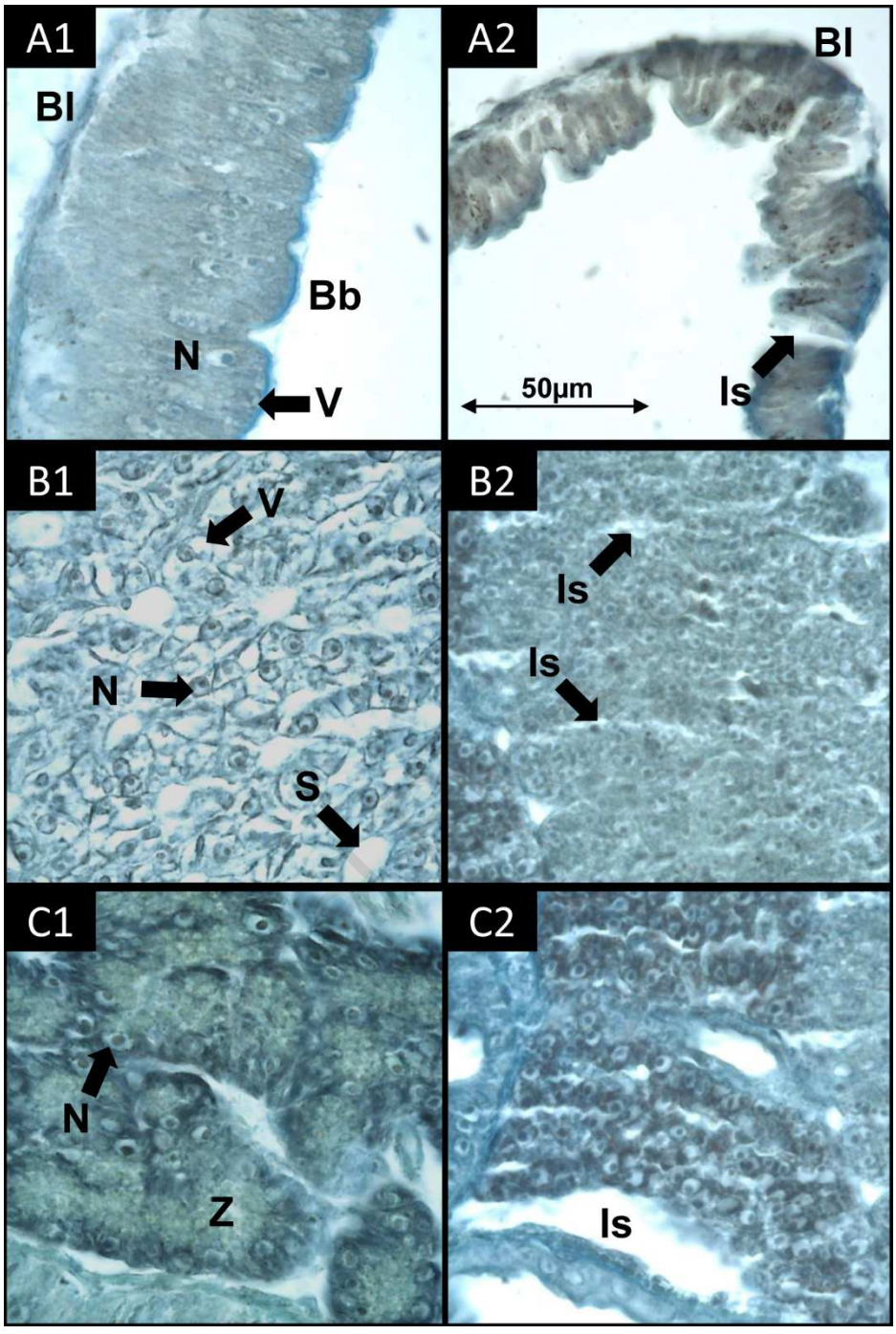

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179 Figure 2 | **Sagittal histological section of stage 2 sole larvae.** (**A**) Intestine, (**B**)
180 liver, and (**C**) pancreas. Magnification X1000; oil immersion. In good condition (left, **1**)
181 intestinal cells are high with a visible brush border. In the liver, vacuoles are
182 numerous and wide. Hepatocytes are large and distinct. Nucleus is lateral with
183 reduced and distinct nucleoli. The acini of the pancreas are well structured with a
184 large amount of zymogen. In case of poor condition (right, **2**) organs are altered
185 presenting intercellular spaces. Epithelial cells are small with a weak cohesion.
186 Hepatocytes are small with small and mostly indistinct nuclei. There are no more
187 vacuoles in the liver or zymogen in the pancreas. **Bb**: brush border; **Bl**: basal lamina;
188 **Is**: intercellular space; **N**: nucleus; **S**: sinusoid; **V**: vacuole; **Z**: zymogen.

2.5 Statistical analyses

Analyses were applied under the R software (R Core Team, 2020).

2.5.1 Sole larvae abundances

Sole larvae abundances and proportions of the five developmental stages were calculated for each of the stations. Total abundances per station where sole larvae were present were compared between surveys by ANOVA followed by a post-hoc Tukey test.

2.5.2 Environmental influence on sole larvae during the critical period

The aim was to define environmental and geographic characteristics of the area suitable for good larval condition during the critical period. Only larvae of stage 2 were considered and the area was restricted to southern coastal nurseries of the EEC where sampling was conducted both in 1995 and 2017. Environmental and geographic variables with a significant effect on condition grades were selected using a non-parametric Kruskal-Wallis test ($P < 0.05$). The values of each retained explanatory variables (i.e temperature, fluorescence, distance to the coast and bedstress) were then divided into four equifrequent classes based on quartiles (Table 2).

Table 2 | Ranges of values for the four environmental variables classes. For a given variable, the same number of sampling stations is present in each class. Q25: 0-25%; Q50: 25-50%; Q75: 50-75%; Q100: 75-100%.

	Q25	Q50	Q75	Q100
Temperature (°C)	[9.17_9.38]	(9.38_10.1]	(10.1_10.6]	(10.6_12.6]
Fluorescence (mg Chla.l^{-1})	[0.52_4.89]	(4.89_13.6]	(13.6_21.6]	(21.6_36.3]
Coast dist. (degrees)	[0.01_0.05]	(0.05_0.13]	(0.13_0.2]	(0.2_0.3]

Bedstress ($m.N^{-1}$)	[0.48_0.61]	(0.61_0.96]	(0.96_1.1]	(1.1_3]

The number of individuals of the six grades in each environmental class was then calculated. A Correspondence Analysis (CA) was performed on a contingency table composed of the environmental classes in columns (*n* columns = *n* selected variables * 4) and the grades in rows (*n* = 6) in order to relate the environmental and geographical classes to the larval condition. A cluster analysis was then performed on the coordinates of the different environmental classes on the selected CA axes which cumulate more than 80% of the variation. An Euclidean distance matrix was calculated from these coordinates and the Ward's aggregation criterion was used to group the quantiles of variables according to their similarity in the axis system of the CA. The number of groups was selected using the "GAP statistics" method (Tibshirani et al., 2001). Indicator Values (IndVals, Dufrêne and Legendre, 1997) were then calculated to determine to which group of environmental variables the different grades were related to (*labdsv* package; Roberts, 2019). IndVals range from 0 to 1, depending on the level of association between grades and groups of environmental variables. The significance of the IndVals was tested using a permutation test (999 permutations) to identify significant grade/group associations. Only significant IndVals (significance threshold set at 10% for this analysis) and greater than 0.3 (Dufrêne and Legendre, 1997; Legendre and Legendre, 2012) of each grade according to the group to which they belonged, as defined by the cluster, were considered.

2.5.3 Differences between 1995 and 2017

The Within Outlying Mean Indexes (WitOMI) were used to study changes in the environmental conditions in relation to the grades of condition grades between 1995 and 2017 (*subniche* package; Karasiewicz et al., 2017). The WitOMI calculates additional parameters based on the Outlying Mean Index (OMI) analysis (Dolédec et al., 2000) allowing to study the effect of environmental changes of an entire community at different temporal or spatial scales. The method provides the marginality of the different grades of condition in relation to the environmental

envelope of the sampled area. First, the WitOMI splits the total environmental envelope (E) into sub-envelopes (E_K) according to a temporal and/or spatial factor. In our case, the environmental envelope E corresponded to the range of environmental conditions encountered in the analysed stations. On the other hand, the sub-envelopes E_K, are the range of environmental conditions (called subsets in this study) of the stations in 1995 and in 2017 (hereafter named E_1995 and E_2017 respectively). This analysis provides valued parameters for each grade in each subset, which are calculated from the origin (G) or from the subsets' origin (G_K). The origin G represents the mean environmental conditions of E whereas G_K (G_1995 and G_2017) is the representation of the average environmental conditions encountered in each subset, E_K (E_1995 and E_2017). In both cases, the parameters obtained are the marginality (WitOMIG and WitOMIG_K), the tolerance (TolG and TolG_K) and the residual tolerance. The values obtained give for each grade its level of marginality and tolerance. The higher the marginality value is, the higher the singularity of the environmental conditions is for the grade. Therefore, the grade will be considered as being in an unusual environment in comparison to G or G_K. In addition, the higher the tolerance value is, the greater the environmental range within which the grade can be found is, and can be considered as ''generalist''. Inversely, the grades are considered as ''specialist'' when they have a low tolerance value. Finally, this analysis also provides a residual tolerance value, where the higher the residual tolerance value is, the weaker the relationship is between the grade distribution and environmental parameters.

Only individuals caught and analysed between the bay of Seine and the Belgium border in April and May have been considered in order to have the same spatio-temporal coverage between years. Only non-permanent variables (that may have changed over time) were considered: temperature and fluorescence, abundance of stage 2 larvae (as a proxy for intraspecific competition) and total mesozooplankton abundance (as a proxy for food availability). A Principal Component Analysis (PCA) was performed on the selected variables. The number of each grade per station was weighted by the abundances of stage 2 larvae encountered at the station. An OMI analysis (package *ade4;* Dray and Dufour, 2007) was then carried out to link PCA data to the grade abundances (log[X+1] transformed) at each station. A Monte Carlo permutation test (999 permutations) was performed to determine the significance of the OMI. Finally, the WitOMI was performed and the significance of the variables

influencing the grades marginality within the two subsets (G_1995 and G_2017) was tested by a permutation test (999 permutations). The marginality of grades within each subset were represented.

3 Results

3.1 Sole larvae abundances

Abundances and proportions of the different developmental stages of sole larvae were calculated and compared between surveys. An effect of the survey on abundances was observed (ANOVA: $F=13.27$; $df = 4$; $P < 0.01$). The results of the differences highlighted by the Tukey post-hoc test are shown in Appendix A.1. Maps of the different developmental stages proportions are also given in appendix (Appendix A.2).

In April 1995 (REISE 1), no stage 1 larvae were captured and stage 2 larvae were largely dominant (94.1% Appendix A.1). In May 1995 (REISE 2), stage 2 larvae were more abundant than in April and still dominant (63.6%), but older stages were also observed in greater proportion, especially at the more coastal stations (Appendix A.1). It is during this survey that sole larvae were the most abundant in all our samples. Abundances in March 2017 were low and only yolk sac (stage 1, 25%), first-feeding (stage 2, 69.8%) and a few flexion (stage 3, 5.2%) stages were captured, mainly at the most offshore stations. In April 2017, abundances were higher and few stage 4 (postflexion, 8.7%) individuals were captured. The highest abundances were found near the Somme and Authie estuaries where stage 2 larvae were dominant (Appendix A.2). Sole larvae were also present along the English coast, in relatively low abundance. In May 2017, overall abundances were higher than in April and stage 5 larvae were caught. The highest abundances observed were at the most coastal stations in front of the three estuaries (Appendix A.2). Few larvae were captured in the bay of Seine.

3.2 Ontogenetic variation of the sole larval condition

Proportions of the six grades of condition per developmental stage for the 559 sole larvae analysed are shown in Figure 3. Overall, an improvement of the condition was observed from stage 2 to 4, followed by a re-increase in the proportion of larvae in poor condition for stage 5 in May 2017 (REIVE 2).

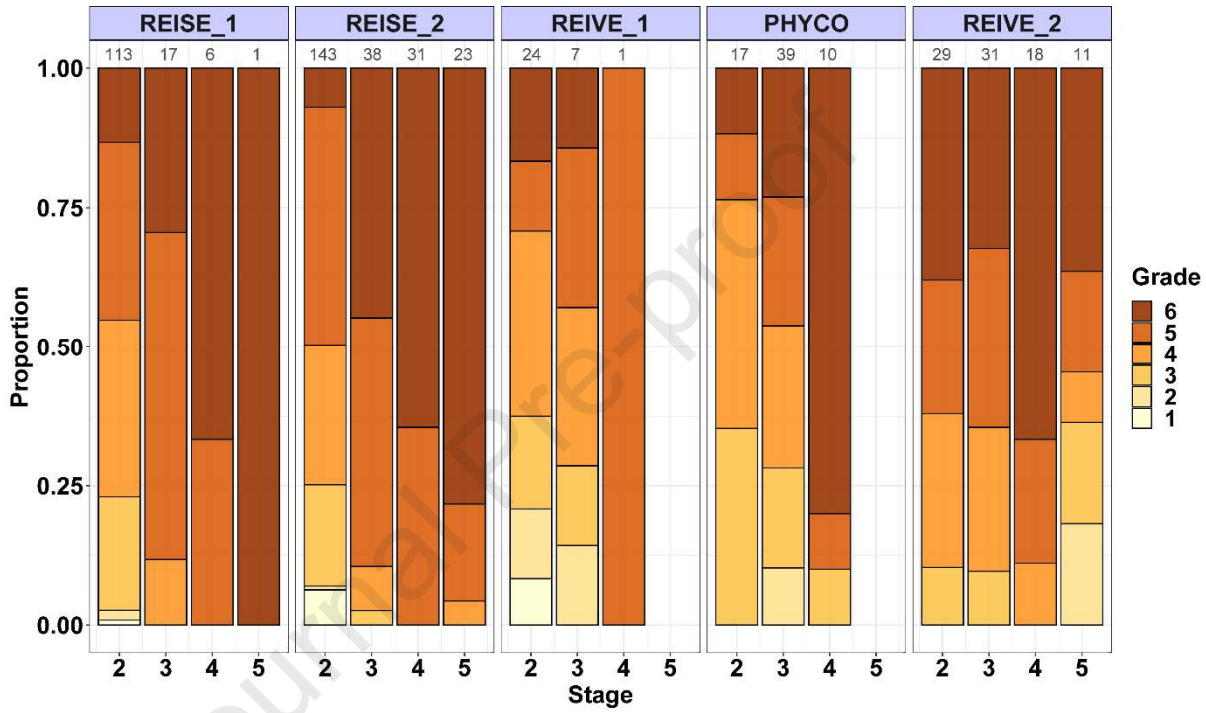

Figure 3 | Proportion of the six grades of condition according to the stage of development for each survey. The number of larvae analysed is indicated.

In all surveys combined, stage 2 larvae had 3.7% of grade 1 individuals, 1.8% grade 2, 19% grade 3, 29.1% grade 4, 33.4% grade 5 and 12.9% grade 6. For stage 3 larvae, no grade 1 individuals were found. Grade 2 represented 3.8%, grade 3 9.1%, grade 4 18.9%, grade 5 36.4% and grade 6 a proportion of 31.8%. For stage 4 larvae, no individuals of grades 1 and 2 were captured, 1.5% were grade 3, 3% were grade 4, 28.8% were grade 5 and 66.7% were grade 6. For stage 5, no larvae of grade 1 were observed. Grades 2, 3 and 4 represented 5.7% of the larvae analysed while grade 5 represented 17.1% and grade 6 represented 65.7%.

3.3 Histological condition variation of first-feeding sole larval stage

3.3.1 Environmental influence

The effect of environmental and geographical variables on the proportion of grades was tested by a Kruskal-Wallis test. For the environmental variables, temperature and fluorescence showed a significant effect on the grades (P = 0.03 and 0.04 respectively). No significant effect of salinity on condition was found (P = 0.19). Among the geographical variables, distance from the coast (P = 0.02) and bedstress (P < 0.01) showed a significant effect on grade, in contrast to depth (P = 0.67). The variables that showed a significant effect on grades were divided into four classes before conducting a CA (Table 2).

The variables that showed a significant effect on grades were divided into four classes before conducting a CA. In order to associate environmental classes with the different grades, a cluster analysis was carried out on a matrix of Euclidean distance of the coordinates of the classes on the first three axes of the CA (83% of variance explained). Three groups of environmental classes were selected. The CA is represented in Figure 4. The first two axes account for 67% of the variance observed.

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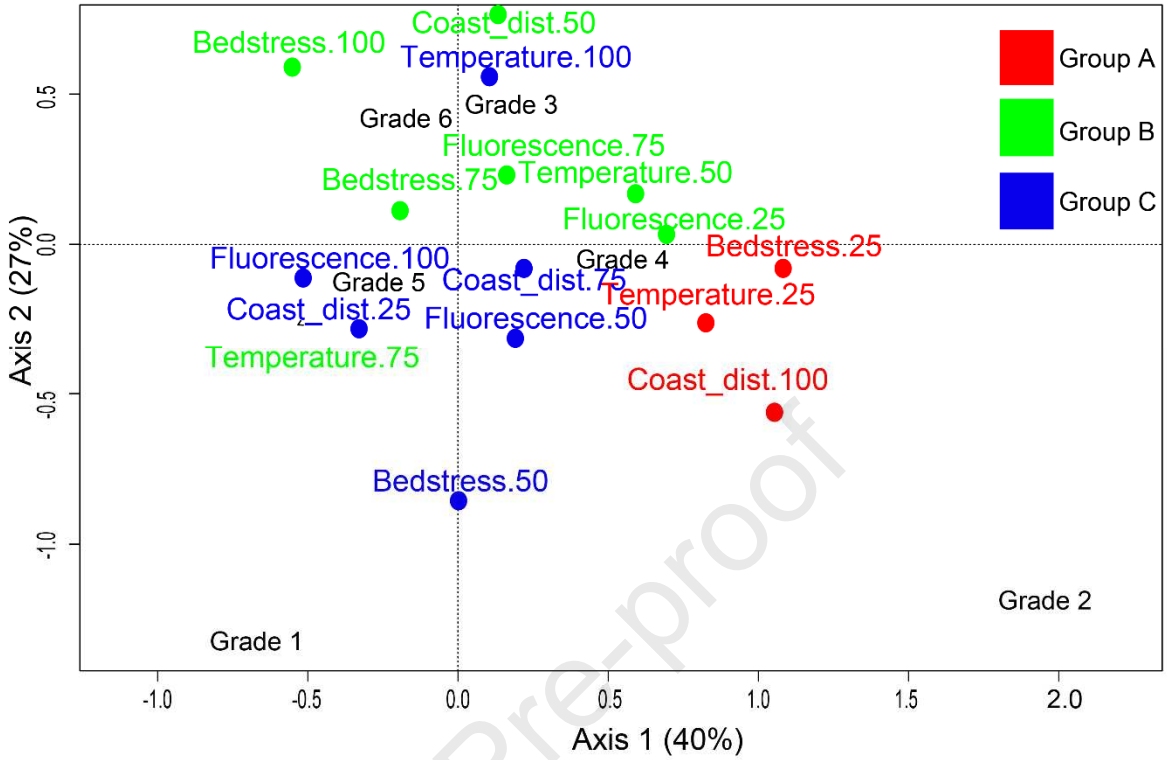

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Figure 4 | Scattered plot of the Correspondence Analysis results with the grade number and each class of environmental variables. The colouring of the classes was carried out according to the results of the clustering.

334 Group A gathered stations furthest from the coast, with low temperature and
335 bedstress values (Figure 4). The grades significantly associated with this group were
336 grades 2 and 4 (IndVals = 0.61 and 0.44; P = 0.04 and 0.01 respectively). Group B
337 gathered stations with low to intermediate temperatures and fluorescence values. It
338 also included high bedstress and intermediate distance from the coast values. Grade
339 3 individuals were significantly linked to this group (Indval = 0.45; P = 0.01). Group C
340 had the highest temperature and fluorescence values and low to intermediate
341 bedstress and distance to the coast values. Grades 5 and 6 were significantly
342 associated with Group C (IndVals = 0.38 and 0.41; P = 0.05 and 0.09 respectively).
343 Grade 1 was not significantly associated with any of the groups.

344 *3.3.2 Comparison between 1995 and 2017*

345 Sole larvae of stage 2 caught along the south east coast of the EEC in April
346 and May 1995 and 2017 were selected. The proportion of different grades by year is
347 shown in Figure 5. A Chi-square test of independence showed a significant
348 difference in the proportion of different grades according to the year ($P < 0.05$).

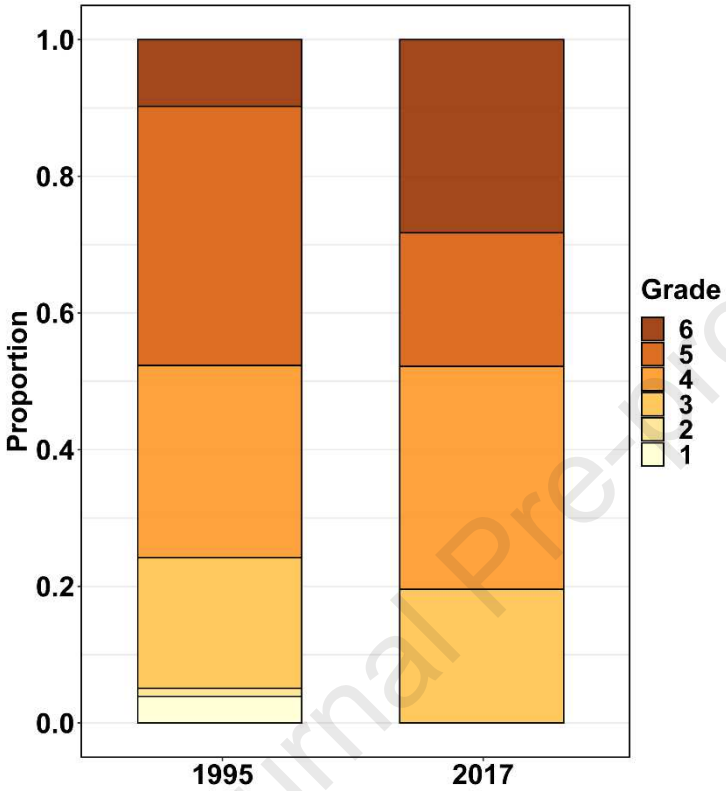

Figure 5 |Proportion of the six condition grades by year for stage 2 sole larvae caught in April and May.

349 In 1995, the proportion of each grade from 1 to 6 present in the analyzed stage
350 2 larvae were the following: 3.9%, 1.2%, 19.1%, 28.1%, 37.9% and 9.8% from grade
351 1 to 6 respectively. In 2017, no larvae of grades 1 and 2 were caught during April and
352 May. Grade 3 represented a proportion of 19.6%, grade 4 32.6%, grade 5 a
353 proportion of 19.6% and grade 6 represented 28.3% of the stage 2 larvae analysed.

354 The WitOMI indexes calculated after the OMI analysis has been performed to
355 study the influence of environmental differences on the grade of condition between
356 1995 and 2017. The grades 1 and 2 have been merged (Grade 1.2) as they both
357 correspond to poor condition with respectively low abundances. The OMI analysis

was significant (Monte Carlo permutation test; 999 permutations; $P < 0.01$). For all grades, at the exception of grade 3, their respective marginality was significant and suggests an influence of environment (set of variables used) on condition (Appendix A.3). Among the total inertia explained by OMI (46%), the first two axes explained 91% of the projected inertia (74% and 17% respectively). The positioning of grade 1.2 was characterised by the highest marginality (i.e. strong deviation from the origin) and tolerance (i.e. dispersion). Grade 1.2 also had the lowest percentage of residual tolerance, suggesting a strong link between the environment and the distribution of the grade. Conversely, grades 3, 4, 5 and 6 were more found in more common environmental conditions (lower marginality indices) and in narrower range conditions (lower tolerance). Furthermore, the grades 3, 4, 5 and 6 were characterized by high residual tolerance indices (>50%) which indicate a weaker link between the distribution of these grades and the environmental variables used

In order to compare the distribution of the grades between the two periods of interest (i.e 1995 vs. 2017), subsets were defined considering the environmental conditions of the two years (Figure 6). On the four variables used, only fluorescence and temperature significantly influenced the separation (or position) of the two subsets (Monte-Carlo tests; 999 permutations; $P < 0.01$). The number of sole larvae and the total mesozooplankton abundance (Ab_Plankton) did not significantly participate in the distinction of the two subsets ($P = 0.34$ and 0.88 respectively). In other words, they were not significantly different from the overall mean value. For both periods, the mean environmental condition of the subset (defined by G_K) was significantly different from G, i.e the overall mean conditions ($P < 0.01$). Environmental envelopes were also statistically different from each other, leading to a low overlap (Figure 6).

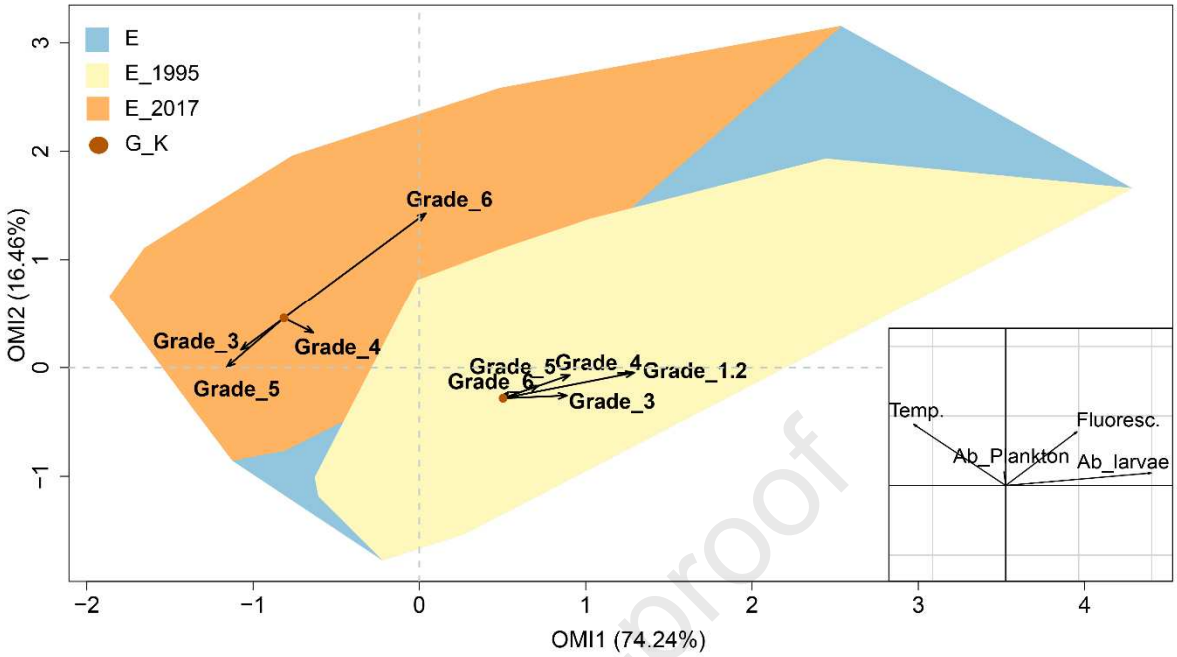

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Figure 6 | WitOMI analysis. The subsets for both years were represented (E_1995 and E_2017) within the total environment (E). For each subset the centre of gravity of the distribution is indicated (G_K). For each grade within a subset, the length of the arrows corresponds to its marginality with respect to G_K. Canonical weights of environmental variables calculated from the OMI have been added.

384 The 2017 subset was significantly different from the 1995 subset, by higher
385 temperatures (E_2017: 11.4 ± 0.5°C; E_1995: 9.9 ± 0.5°C) and lower fluorescence
386 values (E_2017: 8.2 ± 10.7 mg Chla.l^{-1}; E_1995: 15 ± 8.1 mg Chla.l^{-1}). All grades
387 within both subsets were significant (Monte Carlo permutation test; 999 permutations;
388 $P < 0.01$). The data provided by WitOMI have been compiled in Table 3. The
389 marginality of the grades (WitOMIG_K) with respect to the mean environmental
390 conditions of the subsets (G_K) indicated variations in habitat preference between
391 years. In 1995, the grade with the highest marginality (WitOMIG_K) was the grade
392 1.2, while in 2017 it was grades 5 and 6. Tolerance values within each subset
393 (TolG_K) showed variations in dispersion between grades within a subset but also
394 between the same grades in the two subsets. For 1995, it is the grade 1.2 that had
395 the highest tolerance, while for the 2017 subset it was the grade 6 that had the
396 highest dispersion.

Table 3 | Marginality (WitOMIG_K) and tolerance (TolG_K) parameters obtained by the WitOMI analysis for the different grades according to the two years.

Year	Parameter	Grade 1.2	Grade 3	Grade 4	Grade 5	Grade 6
1995	WitOMIG_K	1.04	0.18	0.24	0.06	0.1
	TolG_K	4.19	1.75	2.19	1.46	2.12
2017	WitOMIG_K	-	0.4	0.14	0.7	1.78
	TolG_K		0.31	0.77	0.88	3.37

4 Discussion

In this study, abundances and the influence of the environment on the nutritional condition of sole larvae during the spring were investigated. Differences between 1995 and 2017 were studied. According to our hypothesis, we observed lower larval abundances in 2017. A higher starvation incidence for first feeding larvae was also highlighted. This poor condition was even more pronounced in 2017 compared to 1995 due to a lower availability of suitable area.

4.1 Sole larvae abundances

The abundances found in May 1995 (REISE 2) were two to three times higher than those found in all other surveys, especially those in 2017. These higher abundances encountered in 1995 compared to 2017 are in line with the results of Di Pane et al. (2020a) where this difference was observed for several species of the area. The month of May (1995 and 2017) appeared to be the month when the highest abundance of sole larvae was encountered. So, despite lower abundances in 2017, the peak period in larval abundance was then similar between the two years, indicating no significant phenological shift.

4.2 Histological condition indices for field studies

Histology was used to assess sole larvae condition at different development stages. Histological gradation is based on a general pattern of tissue degradation that is relatively independent upon size and species (Ferron and Leggett, 1994). However, histology-based evaluation of condition for wild caught fish larvae has some limitations. Indeed, some degradation levels of larval condition described from starvation experiments might not be observed in the field because of non-linear and unaccounted for processes in controlled conditions. In the wild, larvae undergo stochastic feeding constraints alternating phases of good nutrition and starvation. Hence, intermediate conditions (grades 3 and 4) may be the result of starvation for some time, or a relatively recent resumption of food after a long period of starvation. Larvae in the field are also subject to additional biotic pressures other than starvation. The higher the level of starvation, the more sensitive the larvae are to other sources (e.g. predation, disease) of mortality (Hare and Cowen, 1997; Leggett and Deblois, 1994; Purcell et al., 1987; Rosenthal and Alderdice, 1976). This results in the lowest grades to be less observed in the field.

Another possible approach is the use of digital analyses to provide quantitative measurements such as cellular volume, diameter or intercellular space. Whereas quantitative measurements were believed to be less subjective than qualitative ones (Oozeki et al., 1989), Catalán (2003) found that, except regarding muscle, qualitative measurements (i.e grading) were as powerful, if not more, to correctly classify individuals whatever the stage of development. Author states this is due to the higher number of cell and tissular features observable when performing qualitative analyses.

4.3 Ontogenetic variation in sole larval condition

Stage 2 larvae were the most abundant during all surveys. This stage corresponds to the exogenous first feeding stage and showed the highest proportion of individuals in poor condition. This result illustrates the "critical period" paradigm (Hjort,1914) which states that recruitment variability is determined by the feeding success of the larvae shortly after yolk sac resorption. Because of their low energy reserves (Ehrlich, 1974; Di Pane et al., 2019), these young larvae are very sensitive to starvation and will experience high mortality over a short period of time (Houde, 1987). The proportion of healthy individuals was higher for later stages 3 and

448 especially 4 and decreased for stages 5 where a higher proportion of unhealthy
449 individuals was found in 2017.

450 The ontogenetic variability in the condition using a biochemical approach was
451 also demonstrated *in-situ* for a benthopelagic species (*Merluccius hubbsi*) by Diaz et
452 al. (2014). The authors found an ontogenetic pattern of condition similar to our study
453 with an increase in the proportion of larvae in good condition after the pre-flexion
454 stage (stage 2) to the post-flexion stage (stage 4), followed by a decrease of
455 condition at the end of the post-flexion stage and at the beginning of metamorphosis.

456 For flatfish, metamorphosis lasts from stage 4 to the beginning of the juvenile
457 stage (Christensen and Korsgaard, 1999; Geffen et al., 2007). During this phase,
458 flatfish will undergo major changes in morphology, behaviour and habitat in order to
459 switch from a bilaterally symmetric pelagic larva to a benthic asymmetric juvenile
460 (Ahlstrom et al., 1984). Di Pane et al. (2019) showed that there are also changes in
461 the energy allocation strategy during metamorphosis where individuals favour energy
462 storage over growth. Despite the maintenance of feeding activity for sole larvae
463 (Lagardère et al., 1999), metamorphosis of flatfish is an energy-intensive process
464 that takes place during a period of behavioural and physiological rearrangement that
465 can lead to feeding difficulties (Gwak et al., 2003; Keefe and Able, 1993). Also, at
466 stage 5, larvae start to be present closer to the ground (Grioche et al., 2000) and
467 pass from a planktonic diet to a more benthic one (Grioche, 1998). This may require
468 sole larvae some time to adapt to their new feeding habits and can lead to feeding
469 difficulties as depicted by the higher number of individuals in poorer condition. Hence,
470 stage 5 may potentially represent another critical period during the larval
471 development. The later statement will need more explorations since only a few stage
472 5 individuals were captured in the present study. Later, metamorphosing sole larvae
473 settling in the coastal nurseries may experience density dependence effects due to
474 strong intraspecific competition (Day et al., 2020; Le Pape and Bonhommeau, 2015
475 and references cited) related to food availability.

476 4.4 Environmental influence during the critical period

477 Identification of factors influencing the variation in larval condition and the
478 estimation of their optimal environmental values during the critical period at stage 2
479 has been little studied by histological approach (Catalán et al., 2006; Diaz et al.,
480 2011; Oozeki et al., 1989; Sieg, 1998).

In the southeast part of the EEC, temperature and planktonic productivity increase along the spring season and follow a central-coastal gradient (Brylinski and Lagadeuc, 1990). The boundary between these two adjacent water masses is a frontal zone isolating the coastal water mass characterised by higher turbidity, lower salinity, higher production and higher temperature. This front plays an important role on ichthyoplanktonic assemblages which, depending on the vertical configuration of the front, are either accumulated or separated into coastal and offshore assemblages (Grioche and Koubbi, 1997; Grioche et al., 1999). There are situations where this frontal zone disappears, reducing its influence. During low tide, the water is stratified and the interfaces between water masses are thus multiplied, favouring exchanges between coastal and central waters (Dupont et al., 1991).

Among the variables used in the study, temperature, fluorescence, distance to the coast and bedstress were significantly linked to sole larvae condition during the critical period. Results of the CA highlighted three groups of environmental variable classes associated with different grades of condition. Based on these results, the coastal area, especially close to estuaries where high temperature and high phytoplankton production were observed, are favourable for good nutritional condition of sole larvae during the critical period of first feeding stage. Higher temperature and fluorescence being characteristic of the coastal waters, larval survival success, or at least good condition, would depend in part on the ability of post-yolk individuals to cross the frontal zone and reach the coastal flow, where productivity and hydrological stability are higher (Brylinski and Lagadeuc, 1990; Dupont et al., 1991).

The direct effect of temperature on larval growth is a well-known phenomenon (Pepin, 1991). It positively influences hatching size (Pepin et al., 1997) as well as the efficiency of yolk sac use, which is one of the factors affecting larval size at the end of endogenous feeding (Peck et al., 2012). Regarding the high proportions of unhealthy larvae found in March 2017, a lower temperature may have a deleterious effect on growth and thus on condition with a greater vulnerability of post-yolk sac larvae to starvation (Garrido et al., 2015).

In situ fluorometry provides estimates of phytoplankton biomass, and appears also related positively to sole condition. Previous studies have shown that the condition of fish larvae in the natural environment was better in non-stratified areas (Catalán et al., 2006) along the Spanish Catalan coasts and in the frontal zone along

515 the northern Patagonian coasts (Diaz et al., 2014). The authors highlight the
516 productive character of these areas in terms of chlorophyll-a and microzooplankton,
517 leading to greater food availability and therefore greater feeding efficiency of fish
518 larvae by reducing the foraging energy cost. Sieg (1992) compared by histological
519 observations the nutritional condition of stage 2 fish larvae between contrasting
520 environments in terms of primary production and food density. The author also found
521 out that larvae caught in less productive area had a higher proportion of individuals in
522 poor condition.

523 Larvae in poor and intermediate condition were linked to areas further
524 offshore, especially at the beginning of the season. This result is in line with the study
525 of Sieg (1998) who also showed that anchovy larvae in poor condition were mostly
526 located at the furthest offshore stations. However, we had more difficulties to define
527 specific environmental parameters for these levels of condition because of the
528 reasons outlined in the section 4.2., i.e. only few individuals in the poorest conditions
529 were observed.

530 4.5 Inter-annual difference during the critical period

531 Influence of environmental drivers of condition was also examined between
532 two contrasting years, 1995 and 2017. From the WitOMI, the variables used
533 appeared clearly different between the two years, April and May 2017 having higher
534 temperatures and lower fluorescence values compared to April and May 1995. In
535 1995, grade 6 had a low marginality and high tolerance with respect to the mean
536 environment of that year while in 2017, this grade of good condition appeared to be
537 the most marginal. This result reflects a variation in the response of stage 2 sole
538 larvae condition to differences in temperature and fluorescence between the two
539 years. In 2017, there were less suitable areas for sole larvae and these were
540 restricted to stations located close to estuaries. This could seem contradictory with
541 the higher proportion of grade 6 encountered in 2017. However, in 2017, low larval
542 abundances have been found compared to 1995. Hence, the majority of individuals
543 analysed during this year come from the sampling stations at the estuary's mouth.
544 The samples collected at these stations could lead to an overestimation of the good
545 condition at the scale of the entire study area. The use of multivariate statistics such
546 the WitOMI allowed us to remove this sampling artefact by obtaining the marginality
547 of these good condition grade.

548 Regarding poor condition, in 2017 no individuals of grade 1 or 2 were found in
549 April and May, while in 1995 few individuals in poor condition were captured, forcing
550 us to group them for statistical robustness. However, the high marginality of these
551 poor condition grades in 1995 reinforces the idea that spring 1995 provided good
552 environmental parameters for sole larval condition.

553 This study is the first comparing larval condition between two contrasted
554 period in terms of contrasted environment and recruitment. However, larval condition
555 is directly related to larval survival and other studies looked at the relationship
556 between this larval survival success and recruitment rates in a context of changing
557 environment (see Somarakis et al., 2017). Indeed, a slight shift in survival rates of
558 early-life history stages affects significantly recruitment success (Houde, 1987).
559 Larval survival rate is believed to be dependent on a spatio-temporal match between
560 early-life stages and their prey (Brosset et al., 2020). However, it is now well known
561 that at the end of the nineties the north Atlantic experienced an increase of
562 temperature due to a shift from a cold to a warm phase of the Atlantic multidecadal
563 oscillations (Drinkwater et al., 2014), accompanied by regime shifts in planktonic
564 (Alvarez Fernandez et al., 2012; Boersma et al., 2015; Edwards et al., 2013) and fish
565 compartments (Auber et al., 2015, 2017; McLean et al., 2018). In response to a low
566 recruitment observed since 2005, Tiedemann et al. (2020) studied the environmental
567 influences on Norwegian spring-spawning herring larvae. Authors found that weak
568 recruitment occurred the years where larvae experienced a positive phase of the
569 Atlantic multidecadal oscillations. As a consequence, the environmental changes that
570 have taken place accompanied by those of the lower trophic levels could have
571 decreased food availability for fish larvae. It shows a negative indirect effect of
572 temperature on fish larval nutrition. Thus, sole larval feeding success, condition and
573 therefore recruitment rate could have been negatively impacted between 1995 and
574 2017.

575 **5 Conclusion**

576 The present study confirmed that transition from endogenous to exogenous
577 feeding corresponded to a critical step. Also, transition from pelagic to benthic life
578 appeared to be potentially another major critical period in early-life history of flatfish.
579 These two transitional stages could represent bottlenecks in larval survival and, as a
580 consequence, to the number of fishes recruiting in the adult population. This study
581 also provided a methodological example of how the larval condition determined on

582 wild collected larvae can be used to identify suitable environmental factors for larval
583 development. Differences in sole larvae abundances, condition and environmental
584 preferences between two contrasted periods is a first step to explain low recruitments
585 observed in recent years with respect to lower larval survival success due to a
586 changing environment.

587 Only two years were compared thus, in a context of climate change, we
588 suggest as future direction the study of the larval condition in a more long-term
589 approach. Time series of fish larval condition, focusing on critical period stages, have
590 the potential to provide clues of basic necessities on the effects of direct (e.g. food
591 resources) or indirect (e.g. temperature) environmental variations on the future status
592 of fish stocks.

593

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601

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- First feeding stage corresponds to the critical most period during sole larval ontogeny
- Good larval condition was related to estuarine areas in the Eastern English Channel
- Lower sole larval abundances in spring 2017 compared to spring 1995
- Environment suitable for a good sole larvae condition was more limited in 2017

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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