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ORIGINAL PAPER

Ontogenetic changes in the larval condition of Downs herring: use of a multi-index approach at an individual scale

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Abstract Evaluating fish larval condition in terms of nutrition and growth is essential as it will influence their development and survival capacity. The present study aims to investigate larval condition of Downs herring (*Clupea harengus* L.) during winter in the Eastern English Channel and Southern Bight of the North Sea. Four condition indices including ingestion rate based on gut fluorescence, instantaneous growth based on RNA/DNA, DNA/C ratios, and otolith microstructure were combined at an individual scale on herring larvae collected during the 2015 International Bottom Trawl Survey—MIK sampling. The four indices demonstrated a clear shift in the larval condition occurring at a larval size of 13 mm. While smaller larvae were shown to feed and grow, larger larvae exhibited a slower growth rate though actively feeding. This suggests that 13 mm could be a critical size for Downs herring larvae. This ontogenetic shift in the larval condition is discussed regarding environmental conditions, diet shift, and growth strategies. It is concluded that the shift from an omnivorous to a carnivorous diet constitutes an additional critical step besides such as the shift from endogenous to exogenous nutrition.

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Introduction

Survival rate during the larval stage is a major factor affecting year class strength in marine fish populations and mainly depends upon feeding conditions encountered during the larval development phase. While abiotic factors could play an important role (Anderson 1988; Bakun 1996; Houde 2008), biotic factors such as prey availability (Cushing 1990; Payne et al. 2009) and trophic competition (Harden-Jones 1968; Houde 1987) could also affect larval condition (Grioche 1998; Harlay et al. 2001; Koubbi et al. 2007; Giraldo 2012), survival, and growth (Hufnagl et al. 2015; Bils et al. 2016). Hence, most fish reproduce during spring and autumn (Russell 1976; Munk and Nielsen 2005) to maximize the temporal match between the larval occurrence and the plankton blooms (Cushing 1969).

Downs herring, however, are a North Sea herring subpopulation which reproduces during winter in the Eastern English Channel (EEC) and Southern Bight of the North Sea (SBNS; Maucorps 1969; Corten 1986; Heath et al. 1997). With a spawning stock biomass (SSB) estimated at approximately 2 million tonnes since 2012, North Sea herring has been one of the largest Northeast Atlantic fish stock in recent years (ICES 2015). Yet, despite sustainable levels of SSB and fishing pressure, the recruitment of the North Sea herring stock has been below average over a 10-year period (2003–2013; ICES 2015). Increased predation by adult herring and poor hatching conditions (Corten 2013; ICES 2015) shifts in the spatial–temporal distribution of North Sea plankton communities (Beaugrand et al.

2003; Payne et al. 2009; Alvarez-Fernandez et al. 2012) and higher larval mortality induced by increasing seawater temperature (Hufnagl and Peck 2011; Fässler et al. 2011; Petitgas et al. 2013) have all been suggested as potential drivers of this relatively low recruitment. Whereas the biomass and contribution of the Downs herring component to North Sea herring recently increased (ICES 2015), it still remains understudied compared to the other sub-populations. Recently, Denis et al. (2016) studied the feeding strategy of young larval Downs herring in a qualitative approach over a six-year period, and showed an ontogenetic shift in prey composition when larvae reached a size of 13 mm.

So far, fish larval condition has been evaluated using multiple methods (Ferron and Leggett 1994; Theilacker et al. 1996; Catalán 2003) including otolith microstructure (Pannella 1971, 1974; Folkvord et al. 2000), molecular (Clemmesen 1994, 1996; Chícharo and Chícharo 2008), biochemical (Bergeron 1997; Bergeron et al. 1997; Giraldo et al. 2016), and histological indices (Grioche 1998; Koubbi et al. 2007; Cohen et al. 2013). These indices have contrasting integration times, i.e., they depict the condition experienced by fish larvae for time scales ranging from several hours (gut contents) or days (molecular, biochemical, and histological indices), up to months (otoliths) before their capture. The RNA/DNA ratio is used as a proxy of recent growth rate, i.e., of the relative activation of protein synthesis (Bulow 1970; Bergeron 1997; Buckley et al. 2008), and shows measurable responses within 3–4 days of a dietary shift (Clemmesen 1987). The DNA/C ratio was developed by Bergeron et al. (1991) and Bergeron (1997) as an alternative to RNA/DNA ratio dedicated more specifically to young larvae. It is also a short-time response index (1–3 days; Bergeron 2000) which is used as a proxy of nutritional condition. Otoliths are the only known structures that consistently record daily events in early life stages of fish. Micro-increments provide measures of daily growth inferred from otolith and allow the assessment of the impact of larval feeding through assimilation efficiency and metabolic rate (Kiørboe et al. 1987; Mosegaard et al. 1988; Secor et al. 1993; Fablet et al. 2011).

Besides limited experimental studies (e.g., Peck et al. 2015), the different indices are typically estimated from different individuals, making results difficult to interpret, where large inter-individual variation occurs. Hence, Clemmesen and Doan (1996) recommended that researcher should measure several indices on the same individuals to see if they provide similar results. In this study, we measured four independent condition indices of different nature and contrasted integration time on each herring larva collected in winter 2015 in the English Channel and Southern North Sea. Ingestion rate was determined from the gut fluorescence method as a quantitative estimate of Downs larvae ingestion over a short-time scale. The main objectives were (1) to characterize larval condition of Downs larvae during the first stages, (2) to compare the results obtained from different indices considering their different response time, and (3) to identify among environmental, spatial, and ontogenetic factors, those that influenced larval condition.

Materials and methods

Field sampling

Sampling was performed in winter 2015 (January–February) during the French part of the International Bottom Trawl Survey (IBTS), in the EEC and the SBNS (Fig. 1). The sampling strategy of the IBTS (ICES 2015) is stratified according to statistical rectangles of 1° longitude × 0.5° latitude. Each rectangle is sampled at night, either twice (SBNS) or four times (EEC) during the whole sea cruise to collect hydrological parameters (temperature, salinity, chlorophyll *a,* and phaeopigments concentrations), mesozooplankton, and fish larvae. Temperature and salinity were continuously measured at 3–5 m below the sea surface using an SBE 21 SeaCAT thermosalinograph.

Chlorophyll concentration

Seawater samples were collected at 1 m depth using a 5 L Niskin bottle. Two replicates (0.5–1 L) seawater were

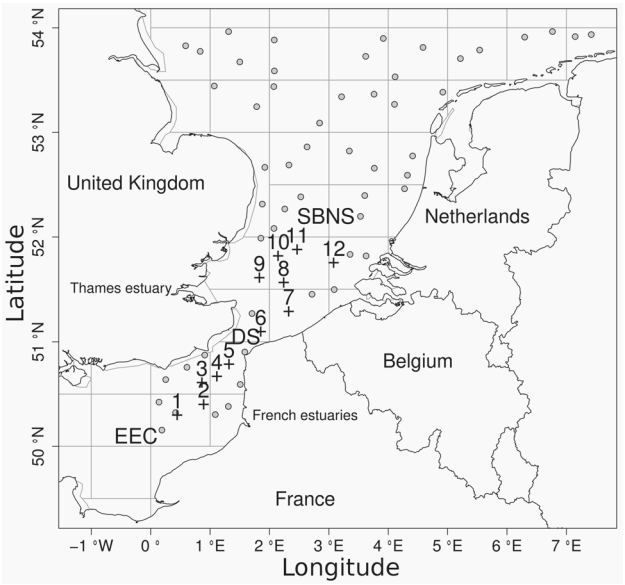

Fig. 1 Sampling location (*open circles*) of hydro-biological parameters, mesozooplankton and herring larvae in the Eastern English Channel (EEC), Dover Strait (DS), and the Southern Bight of the North Sea (SBNS) during the French IBTS in winter 2015 (January–February). Crosses with associated numbers stand for stations, where the larval condition was analysed. ICES statistical rectangles are also depicted

immediately filtered on glass-fibre filters of 47 mm diameter and 1.2 µm mesh size (Whatman GF/C) and frozen at −20 °C. In situ chlorophyll *a* (chl *a*) and phaeopigments concentrations (T_{pig}, µg L^{-1}) were estimated using the spectrochromatic monochromatic method (Lorenzen 1967; Aminot and Kérouel 2004).

Mesozooplankton sampling and identification

Mesozooplankton samples were collected through oblique hauls using a WP2 net (200 µm mesh size; Tranter and Smith 1996) deployed from 3 m above the seabed to the surface at 0.75 m s^{-1}. Net contents were preserved in a 0.9% buffered (sodium glycerophosphate) formalin seawater solution (Mastail and Battaglia 1978 modified by Bigot 1979; Lelièvre et al. 2010).

Total mesozooplankton abundance was determined in the laboratory using the ZooScan system (Grosjean et al. 2004; Gorsky et al. 2010). Prior to scanning, samples were first separated into two size-fractions (>500 and 200–500 µm) to prevent misrepresentation of large organisms. Each fraction was then divided with a Motoda splitter (Motoda 1959) until the subsample was diluted enough to contain about 1000–2000 objects. The subsamples were poured onto the scanning cell (11 × 24 cm) and organisms were manually separated to minimize overlap. Image acquisition and processing were carried out following Lelièvre et al. (2012). Automated recognition of objects was made using a classification model (classifier) built with the Random Forest supervised learning algorithm (Breiman 2001), available in the Plankton Identifier free software (Gasparini and Antajan 2013; Version 1.3.4), and a learning set (a representative subset of objects classified manually into taxon categories or groups) dedicated to winter EEC and SBNS zooplankton. To correct for the residual error associated with the misclassification among groups, each sample was manually validated by sorting misidentified objects into the right categories. Results from the two size-fractions were summed to obtain total mesozooplankton abundance (ind m^{-3}).

Downs herring larvae

Sampling and identification

Fish larvae were sampled during the night using a Midwater Ring Net (13 m long, 2 m diameter, 1.6 mm mesh size with a 500 µm cod end, ICES 2015). The ring net was deployed obliquely from 5 m above the seabed to the surface for at least 10 min. A flowmeter was installed inside the net mouth opening to measure the filtered volume. In this study, only herring larvae located south of 54°N were considered as they were assumed to belong to the Downs herring sub-population (ICES 2015). At each of the 12 stations, approximately 30 herring larvae were visually sorted onboard and frozen in liquid nitrogen for condition analyses. The remainder of the sample was preserved in a 0.9% buffered formalin seawater solution at room temperature for subsequent estimation of abundance.

Larval abundance and size distribution

Herring larvae abundance (ind m^{-3}) was estimated from subsamples (from 1/2 to 1/256 of the original sample) using a Motoda splitter. A minimum of one hundred larvae per subsample was counted (Motoda 1959), from which at least 50 were individually measured (standard length, SL ± 1 mm). SL was corrected for potential shrinkage due to preservation using a linear model (ANOVA, $P < 0.05$) taking into account the length before (SL) and after preservation (Ls) in either formalin solution ($SL = 1.2064 \times Ls - 1.1224$) or liquid nitrogen ($SL = 0.9588 \times Ls + 0.892$). Counts and measurements in the subsamples were estimated for the total sample and divided by the filtered volume.

Condition

For each of the 12 stations (Fig. 1), 15 frozen larvae were placed in Petri dishes filled with milliQ water and examined on ice under cool light stereomicroscopy (×10 magnification). They were measured ±0.1 mm (Campana 1990) and grouped into three size classes: 8–12, 13–14, and 15–18 mm, following Denis et al. (2016) who showed a diet shift occurring at 13 mm, and to have at least five individuals per size class. For each larva, the gut was removed and transferred into glass tubes with 4 ml of 90% acetone for gut fluorescence measurement, and the head was removed and preserved in 95% ethanol for otolith extraction. The remainder of the sample was preserved at −80 °C for biochemical analyses (RNA/DNA and DNA/C ratios).

Gut fluorescence Gut fluorescence was measured following a method used for herbivorous copepods (Mackas and Bohrer 1976) adapted to herring larvae and used as a proxy of larval ingestion rate. Briefly, dissected guts were acetone extracted for 6 h at 4 °C in the dark. Fluorescence was measured before and after acidification with 10% HCl (Parsons et al. 1984) using a Trilogy Laboratory Fluorometer (Turner Designs EPA 445). "Blank guts" (B_{gut}) were set at each station by emptying the guts of five randomly selected larvae with dissecting forceps. Larval gut content (G_{fish}, ng chl *a* eq ind^{-1}) was estimated from the total amount of pigments (T_{pig}) recovered in the gut content after subtracting B_{gut}. Ingestion rate (I_{fish}, ng chl *a* eq ind^{-1} $days^{-1}$) was estimated

from G_{fish} using the gut evacuation rate value of 40 min^{-1} for herring larvae (Pedersen 1984).

RNA/DNA and DNA/C ratios For each larva, body muscle was crushed in cold distilled water (4 °C) with a glass rod and samples were prepared for quantification of nucleic acid and elemental carbon concentrations. Total RNA and DNA were extracted following Yandi and Altinok (2015), and their concentration was measured with QUBIT, using the RNA DNA HS assay Kits (Invitrogen, Life Technologies). For determination of % carbon, a fraction of the crushed larval tissue was placed into a tin capsule, dried in an oven (48 h at 60 °C), and subsequently processed using an elemental analyser (Thermo Finnigan Flash EA 1112). The multi-species larval fish growth model of Buckley et al. (2008) was used to calculate the instantaneous growth rate (G_i, $days^{-1}$) accounting for spatial variation in seawater temperature (Eq. 1):

$$G_i = 0.0145 \times RD + 0.0044 \times (sRD \times T) - 0.078 \quad (1)$$

where sRD in the standardised RNA/DNA ratio following Caldarone et al. (2006) and T is the temperature (°C) at the sampling station. In this study, we used RD which is the non-standardised ratio instead of sRD, because the measurement protocol of Yandi and Altinok (2015) based on QUBIT differs from those of Caldarone et al. (2006) and the results obtained cannot be standardised following Caldarone et al. (2006). Instantaneous growth rate values of 0 refer to no growth, while values of 1 reflect a doubling of larval mass per day. For the DNA/C ratio, larval starvation was determined based on a threshold derived from anchovy larvae in the Bay of Biscay for DNA/C (Bergeron 2000). Here, the lower the value of the ratio, the better the nutritional condition of a given larva (Bergeron 2000).

Otolith microstructures Micro-growth increments were assessed from sagittal otoliths extracted using fine needles. They were then examined under a microscope equipped with a polarized light and mounted on slides with Crystal Bond® thermoplastic cement. After polishing with 0.05–3 µm micro-abrasive discs (LP Unalon®), otoliths were examined by microscopy at ×126 magnification (oil-immersion, Olympus BX51). The location of the check corresponding to the complete absorption of the yolk sac (Geffen 1982; Høie et al. 1999; Fox et al. 2003) was located as a darker increment at the center of the otolith. Otolith diameter (D, µm), increment number (N_{inc}), and mean increment width (MIW, µm) from the central zone (*nucleus*) to the edge of the otolith along the longest radius (Campana et al. 1987) were measured using TNPC 7.0 package (www.tnpc.fr). MIW was calculated for each individual to estimate the individual growth from the check to the edge. Growth rate (mm $days^{-1}$) was estimated by a linear regression between larval length (TL) and N_{inc}. Micro-increments smaller than 1 µm were used to identify slow-growing periods (Campana et al. 1987; Folkvord et al. 2000; Feet et al. 2002).

Mapping and statistical analyses

Larval distribution, ingestion rate, instantaneous growth rate, DNA/C ratio, and MIW were mapped using the *mapplots* package of the R software (R Development Core Team 2005). Normality and homoscedasticity of these data were assessed using a Shapiro–Wilk test ($P < 0.05$) and a Levene's F test ($P < 0.05$), respectively. Parametric tests (ANOVA, HSD Tukey) were then used to assess spatial differences in larval distribution, ingestion rate, instantaneous growth rates, DNA/C ratio, and MIW. Parametric tests were performed using the *Stats* package in R.

The gradient of the larval conditions matrix was determined as to be linear using a Detrended Correspondence Analysis (DCA; Legendre and Legendre 2012). A Redundancy Analysis (RDA) was performed as a constrained ordination technique to determine how much the amount of the larval condition variability could be explained by environmental, spatial, and biological factors. Amongst the 180 larvae analysed, 15 were discarded from the analyses as they were either vateritic or had crystalline otoliths. Therefore, analyses were carried out on a matrix of four condition indices × 165 observations. Eight co-variables were used as environmental (seawater temperature, salinity, and in situ chlorophyll *a* and phaeopigments concentrations), spatial (latitude and longitude), and biological (larval and mesozooplankton abundance, larval size) factors. The data were centered and reduced before analyses. Significant co-variables were selected through forward selection using a Monte Carlo permutations test ($n = 999$; Borcard et al. 2011). Contribution of each selected co-variable to larval condition variation was finally assessed using a variance partitioning analysis and a permutation test (Borcard et al. 2011).

A Hierarchical Classification Analysis (HCA) based on the first two RDA axes (explaining at least 60% of the total inertia) was finally performed to identify groups of individuals with similar larval condition. Euclidean distance was used and the individuals were grouped according to the Ward criterion. The number of significant groups was determined as the one leading to the highest correlation (Spearman coefficient) between the original distance matrix and the binary matrix calculated for each cutting level of the dendrogram (Borcard et al. 2011).

The DCA, RDA, variation partitioning, and HCA were performed using the *vegan* (Oksanen et al. 2013) and *FactoMineR* (Lê et al. 2008) packages of the R software.

Results

Environmental conditions, mesozooplankton, and herring larvae distribution

In 2015, the spatial distributions of temperature, salinity, in situ chl *a* and phaeopigment concentrations, and abundance of mesozooplankton were highly structured in the EEC and the SBNS (Table 1). Winter temperature and salinity were higher in the EEC (between 10.3 and 10.9 °C and 35.2 and 35.3, respectively) and lower in the SBNS (between 6.7 and10.2 °C and 34.7 and 35.2, respectively). *In situ* chl *a* and phaeopigment concentrations followed a reverse pattern with twice higher values in the SBNS (0.56–1.12 µg L^{-1}, stations 6–12) compared to the EEC (0.41–0.58 µg L^{-1}; stations 1–5).

Mesozooplankton abundance distribution and in situ chlorophyll *a* and phaeopigments concentrations were significantly correlated (Spearman rank correlation, $rs = 0.85$, $N = 12$, $P < 0.001$) with values ranging from less than 200 (EEC) to above 5500 ind m^{-3} (SBNS; Table 1). Markedly high abundance values (between 2095 and 5542 ind m^{-3}) of mesozooplankton were recorded in the SBNS (stations 6–12).

Downs herring larvae showed a clear and significant southwestern–northeastern distribution gradient coinciding with an increase in larval size and a decrease in larval abundance [ANOVA, $F(11,56) = 2.180$, $P = 0.02869$; Fig. 2]. Smaller larvae (8–12 mm) were distributed overall the study area though more abundant in the EEC (between 5500 and 31,562 ind m^{-3}), whereas larger larvae were restricted to the SBNS.

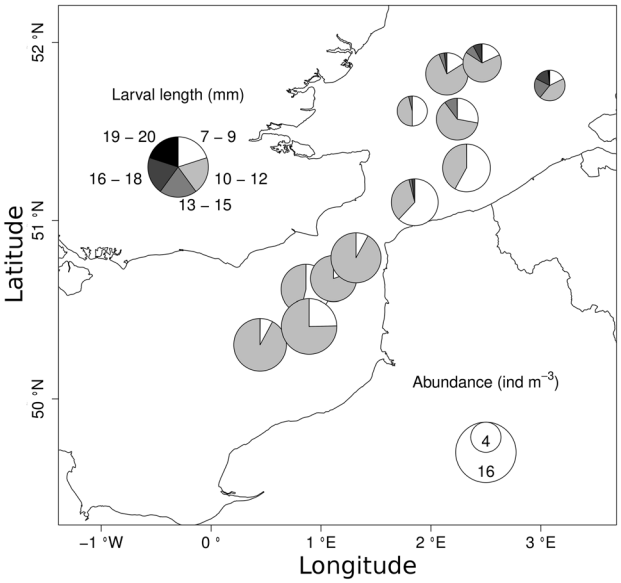

Fig. 2 Size and abundance (ind m^{-3}) distribution of Downs herring larvae during winter 2015 (January–February) in the Eastern English Channel, Dover Strait, and the Southern Bight of the North Sea. Only stations, where the larval condition was analysed, are depicted

Ingestion rate

For 8–18 mm larvae, ingestion rates varied significantly with size [ANOVA, $F(8,171) = 1.999$, $P = 0.09248$; Fig. 3a]. The ingestion rate decreased from 8–9 to 10 mm from 22.5 to 18.6 ng chl *a* eq ind^{-1} $days^{-1}$, then remained almost constant at 19.5–20.1 ng chl *a* eq ind^{-1} $days^{-1}$ until 12 mm, with the lowest value being displayed by 13 mm individuals. For the largest larvae (13–18 mm), ingestion rate increased and was twice as high, reaching a maximum value of 26.9 ng chl *a* eq ind^{-1} $days^{-1}$. Regarding spatial pattern of the size class 8–12 mm, the center SBNS (stations 7–9) was characterized by larvae with lower ingestion rates (7.7–18 ng chl *a* eq ind^{-1} $days^{-1}$; Fig. 3b) compared to the rest of the study area (19–46.6 ng chl *a* eq ind^{-1} $days^{-1}$).

Table 1 Temperature (°C), salinity, in situ chlorophyll *a*, and phaeopigment concentrations (µg L^{-1}) and mesozooplankton abundance (ind m^{-3}) during winter 2015 (January–February) in the Eastern English Channel (EEC), Dover Strait (DS), and the Southern Bight of the North Sea (SBNS)

Areas	Stations	Temperature	Salinity	Chlorophyll *a*	Phaeopigment	Mezooplankton
EEC	1	10.9	35.2	0.32	0.19	192
	2	10.3	35.2	0.36	0.20	1179
	3	10.7	35.3	0.32	0.09	1153
	4	10.5	35.3	0.28	0.17	614
DS	5	10.5	35.3	0.33	0.25	1865
	6	9.2	34.9	0.52	0.07	1609
SBNS	7	9.1	34.8	0.44	0.49	2451
	8	9.8	35.1	0.48	0.11	3205
	9	10.2	35.1	0.44	0.12	2095
	10	9.9	35.2	0.52	0.18	3913
	11	7.8	35.2	0.88	0.24	2508
	12	6.7	34.7	0.84	0.20	5542

See Fig. 1 for stations' location

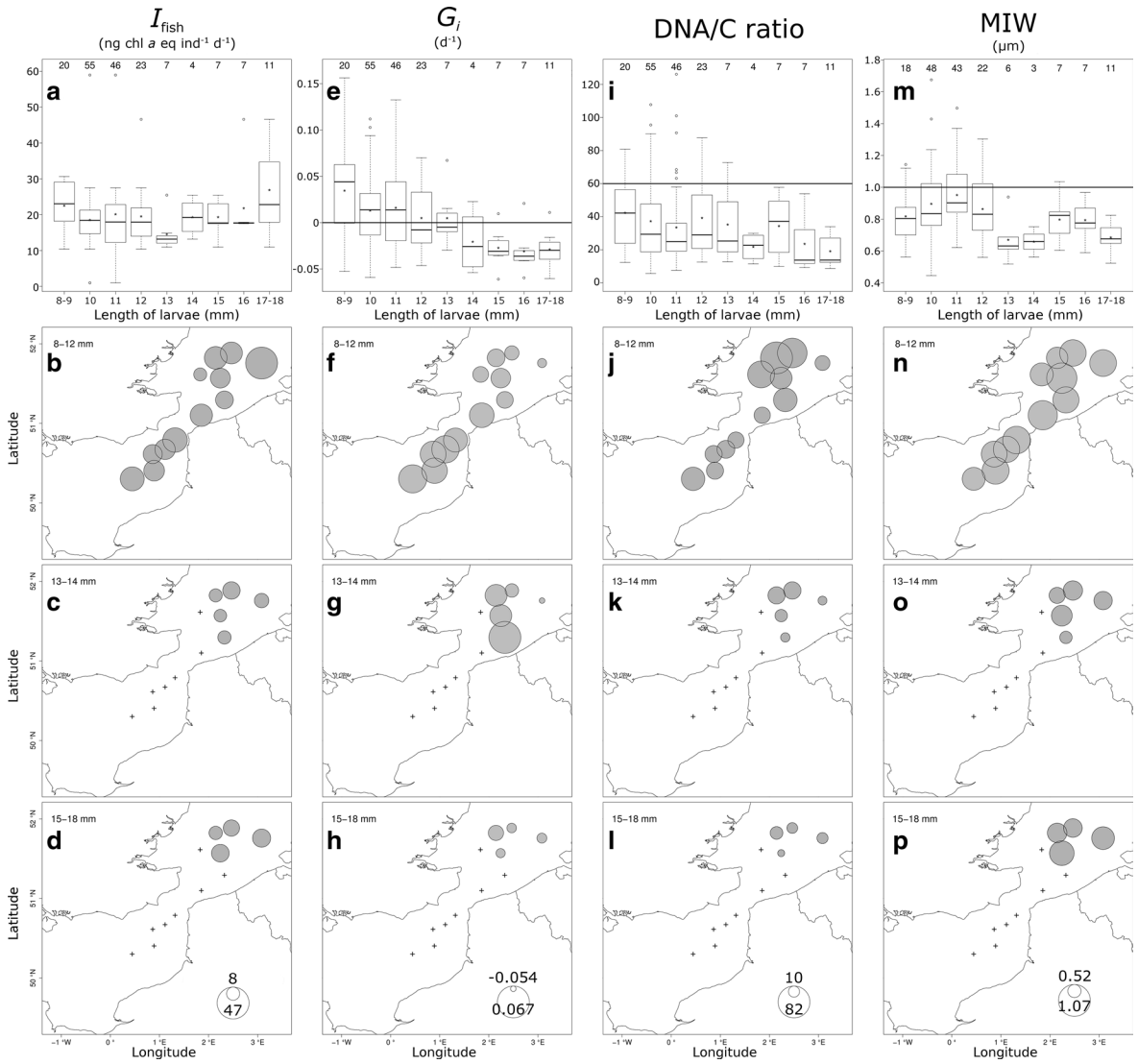

Fig. 3 Larval condition analysis of Downs herring larvae in the Eastern English Channel, Dover Strait, and the Southern Bight of the North Sea during winter 2015 (January–February). **a**–**d** Ingestion rate (I_{fish}, ng chl *a* eq ind^{-1} days^{-1}), **b**–**h** instantaneous growth rate (G_i, days^{-1}), **i**–**l** DNA/C ratio, and **m**–**p** mean increment width (MIW; µm). **a**, **e**, **i**, **m**, *Boxplots* which represent the minimum, first quartile, median, third quartile, and maximum of the four condition indices according to the larval size. *Stars* represent mean values. The number of larvae analysed for each size class is provided on the upper *X* axis. *Horizontal lines* (**e**, **i**, **m**) depict the thresholds used to determine starving (**i**; Bergeron 2000) and slow-growing (**e**, **m**; Campana et al. 1987; Folkvord et al. 2000; Feet et al. 2002) larvae. *Crosses* on the map indicated the absence of larval size class for the station

Instantaneous growth rate and DNA/C ratios

Instantaneous growth rate was significantly different between size classes (ANOVA, $F(8,171) = 4.901$, $P = 1.83e^{-5}$; Fig. 3e). It decreased from 0.005 ± 0.031 days^{-1} for smaller larvae (8–13 mm), down to −0.031 ± 0.025 days^{-1} for larger ones. From 14 mm onwards, the median and mean of the index were below zero. Instantaneous growth rate indicated that 57% of 8–12 mm larvae efficiently grew, even though they exhibited high inter-individual variability (from −0.059 to 0.156 days^{-1}). In contrast, only 19% of larger larvae (14–18 mm) were shown to be in growing condition. Individuals of the size class 8–12 mm from the EEC and Dover Strait (DS) had a significantly higher instantaneous growth rate (0.022–0.052 days^{-1}) than those from the SBNS (−0.004 to 0.046 days^{-1}; ANOVA, $F(1,142) = 100.8$, $P = 2e^{-16}$; Fig. 3f).

DNA/C ratio decreased with larval size [ANOVA, $F(8,171) = 2.498$, $P = 0.013692$; Fig. 3i] and, on average, was higher for smaller larvae (8–13 mm) compared to larger individuals, despite showing strong inter-individual variation (from 6 to 126). Around 83% of smaller (8–13) and 100% of larger larvae appeared to be in feeding

condition (Fig. 3i). Individuals of the size class 8–12 mm had a higher DNA/C in the SBNS than in the EEC (Fig. 3j).

Otolith microstructure

An average growth rate of 0.26 mm days^{-1} [Linear regression, $r2 = 0.88$, $F(1,163) = 1195.7638$, $P < 0.001$] was estimated for 8–18 mm larvae (Fig. 4a). The highest increment widths were recorded for the first three increments with mean values of 1.4–1.6 µm. A linear decrease from 1.6 to 0.9 µm was observed between the first and 35th increments (Fig. 4b). Increment width showed high inter-individual variation between the 7th and 11th increments and lower ones between the 11th and the 35th. Thereafter, from the 35th to 43rd increments, corresponding to 16–18 mm larvae, increment width increased linearly to reach 1 µm. Beyond the 43rd increments, results were not interpretable due to the low number of larvae having a high number of increments.

The highest MIW (0.82 and 0.95 µm) was recorded for 8–12 mm larvae amongst which 67% were below the threshold and could be considered in a slow-growing state (Fig. 3m). For larger larvae, MIW were lower, ranging from 0.66 to 0.80 µm and 96% of these larvae were below the threshold corresponding to slow-growing state. There was no clear pattern in the spatial distribution of increments width (Fig. 3n–p).

Redundancy analysis

Within the eight co-variables, seven (temperature, salinity, in situ chl *a* and phaeopigments concentrations, mesozooplankton abundance, latitude, longitude, and larval size) were finally determined as significant and selected (Fig. 5). Three groups of individuals were obtained from the HCA and distributed along the two first axes of the RDA (61.42% of the variation, Fig. 5). The adjusted *r2* (variance explained by the selected co-variables) was of 32%. The first group of individuals was associated with high DNA/C ratio and mainly included small larvae from the SBNS. The second group of individuals was associated with high instantaneous growth rate and mean increment widths as well as high temperature and salinity. It included smaller larvae (8–12 mm) belonging to the EEC and DS stations. The third group was associated with high ingestion rate and included most of larger larvae (13–18 mm) belonging mainly to DS and SBNS stations. The first and the third groups were also associated with high in situ concentrations of chl *a* and phaeopigments and mesozooplankton abundance.

Overall, the variance partitioning analysis showed the main contribution of spatial variables (11%) to the explained variation of Downs larval condition (32%), followed by biochemical variables (7%) and larval length (5%). Biochemical and spatial variables shared 6% of the explained variation.

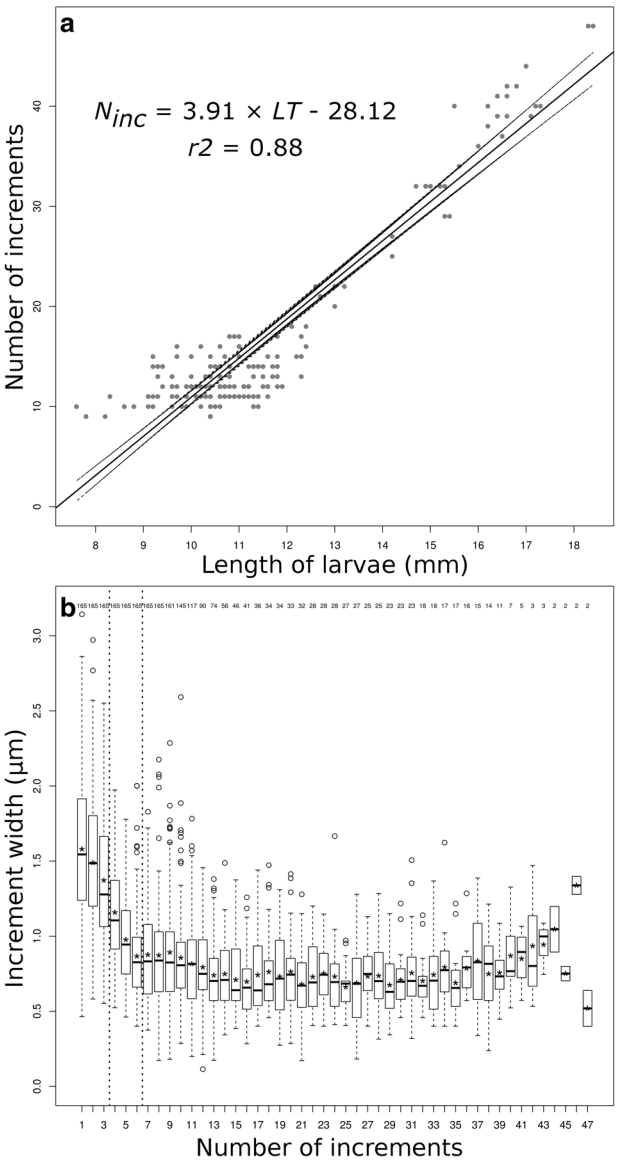

Fig. 4 Otolith micro-increment analysis of Downs herring larvae in the Eastern English Channel, Dover Strait and the Southern Bight of the North Sea during winter 2015 (January–February). **a** Number of increments according to the larval size. Fitted linear regression and confidence interval (95%) are also indicated. **b** *Boxplots* which represent the minimum, first quartile, median, third quartile, and maximum of micro-increment width according to the increments number. *Stars* show mean values and the *two vertical dotted lines* indicate the check location of the complete yolk-sac absorption (Geffen 1982; Høie et al. 1997; Fox et al. 2003). The number of larvae analysed for each number of increments is indicated on the *top* (**b**)

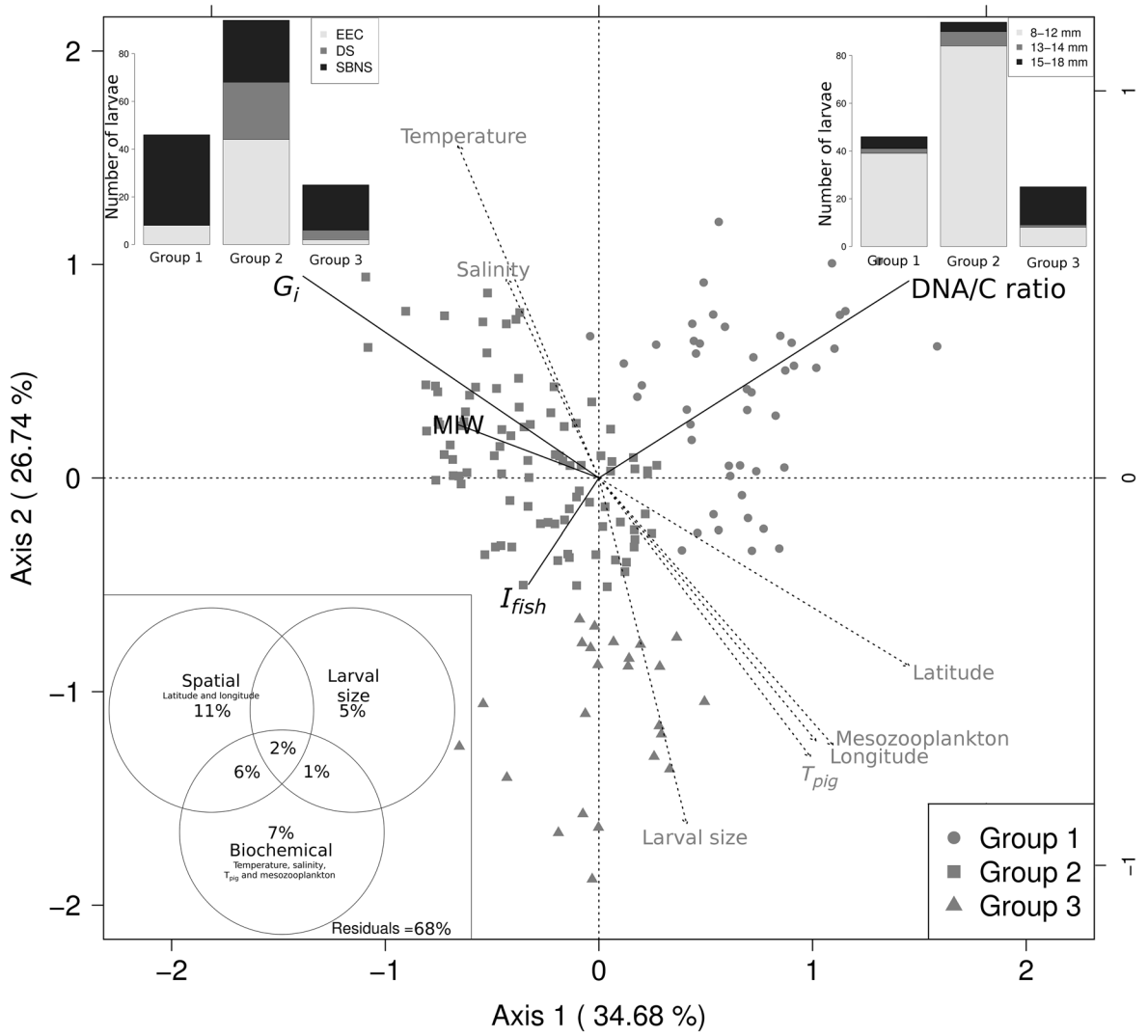

Fig. 5 Redundancy and variance partitioning (*bottom left*) analyses of the larval condition [ingestion rate (I_{fish}), instantaneous growth rate (G_i), DNA/C ratio, and mean increment width (MIW)] of Downs herring larvae in the Eastern English Channel (EEC), Dover Strait (DS), and the Southern Bight of the North Sea (SBNS) during winter 2015 (January–February) constrained by selected biochemical (temperature, salinity, in situ chlorophyll *a* and phaeopigment concentrations (T_{pig}), and mesozooplankton abundance), spatial (latitude and longitude), and larval size variables. *Bars* (*top left* and *top right*) give, for each of the three identified groups of the HCA (*bottom right*), the number of individuals belonging to the three areas (*top left*, see Table 1) and size classes (*top right*). Numbers in the *circles* (*bottom left*) represent the proportion of variance explained by each variable

Discussion

The present study combines a multi-index approach employed at the scale of individual fish to evaluate the condition of Downs herring larvae in EEC and SBNS. Our results clearly showed that (1) in spite of their contrasting nature and integration time, the different indices led to a clear pattern in the larval condition according to the ontogeny, (2) a change in Downs larval condition occurred at a size of 13–14 mm both in terms of nutrition and growth, (3) smaller larvae (8–12 mm) fed and grew, 13 mm larvae had the poorest condition and larger larvae (14–18 mm) fed, but did not grow, and (4) EEC seemed to provide a better habitat for smaller larvae to feed and grow than SBNS.

Robustness of the larval condition indices

The gut fluorescence method used to quantify prey ingestion is sensitive to potential sampling bias induced by gut content evacuation during larval capture or fixation (Lebour 1924; Bjørke 1976; Hay 1981) as well as sampling periods (day vs. night; Munk et al. 1989; Haslob et al. 2009). As our larval sampling started after sunset, some larvae could have already fed several hours before (Blaxter 1965; Fossum and

Johannessen 1979; Pedersen 1984). Since nothing can be done to prevent gut evacuation and considering these two potential sources of bias, ingestion rate values still remain comparable between larval size classes when considered as relative and minimum values of feeding activity.

While the RNA/DNA ratio has been widely used as a measure of recent growth and condition of fish larvae (Buckley et al. 1999), potential sources of variation have been reported when comparing different larval lengths in contrasting environmental conditions. RNA/DNA ratios could be influenced by ontogenetic (Foley et al. 2016) and day–night differences in feeding habits and/or activity of the endocrine systems induced by the light/dark regime (Rooker and Holt 1996; Chícharo et al. 1998; Ching et al. 2012). In our case, these effects were likely negligible as the RNA/DNA ratio was measured on larvae collected during the night, i.e., when the ratios were supposed to be the highest (Chícharo et al. 1998). The DNA/C ratio was shown to be a temperature-independent index and better adapted for small larvae (Bergeron et al. 1997). During a starvation period, carbon concentrations decrease, while DNA concentration remains constant, which leads to a rapid and sharp increase of the DNA/C ratio (Bergeron 2000). Observed DNA/C values from the present study were in accordance with other species (Bergeron 2000, 2009). We used a threshold value of 60 for the DNA/C ratio to determine poorly-feeding larvae. Although this value, initially developed on anchovy of the Bay of Biscay (Bergeron 2000), might not be directly relevant for herring, it is the only value available in the literature, and highlights the pressing need to empirically estimate threshold values for herring larvae.

The use of otolith micro-increments as a condition index assumes a daily deposition rate. However, several studies have stressed that non-daily deposition rates (growth rates of less than 0.4 mm days^{-1}) can occur under sub-optimal conditions (McGurk 1984; Moksness et al. 1987; Folkvord et al. 2000). Campana et al. (1987) argued that daily deposition rate can be assumed if micro-increments of less than 1 µm could be detected. This cannot always be achieved with optical microscopy (Campana et al. 1987; Radtke et al. 1990; Feet et al. 2002; Fox et al. 2003), although Fox et al. (2003) suggest a resolution limit around 0.3 µm. In our study, since micro-increments smaller than 0.12 µm have been observed, a daily deposition rate was assumed to start after yolk-sac absorption (Campana and Neilson 1985; Moksness 1992; Arrhenius and Hansson 1996). Yolk-sac absorption is thought to be completed at 4–5 days at 10 °C (Lough et al. 1982). In our study, the check was observed at 4–6 micro-increments (i.e., 4–6 day old larvae), which also supported the existence of a daily deposition rate.

The growth rate of Downs herring larvae observed in the present study was high and comparable (0.26 mm days^{-1}) to previous studies at the same period either in the same area (0.165 mm days^{-1}; Hempel 1960), during autumn in the central of North Sea (0.13–0.24 mm days^{-1}; Kiørboe et al. 1988) or during spring in the West of Scotland (0.17 mm days^{-1}; Checkley 1984, 0.22 mm days^{-1}; Campana and Moksness 1991). It is also comparable to field studies in other areas such as spring in the Baltic Sea (0.13–0.26 mm days^{-1}; Weber 1971, 0.21–0.29 mm days^{-1}; Waldman 1961), and in the Clyde (0.33 mm days^{-1}; Geffen 1986). This potentially suggests that these larvae were not more limited by winter conditions than autumn and spring larvae as already observed by Denis et al. (2016) regarding vacuity rates. Less suitable conditions in winter linked to lower food availability could be counterbalanced by a lower larval fish diversity and mesozooplankton abundance. In this sense, winter spawning could be an advantage for Downs herring larvae as it leads to less competition with other fish larvae and mesozooplankton. The other explanation is that under sub-optimal trophic conditions like those found in winter, only fast growing individuals survived, leading to an observational bias. This was shown for juveniles by Le Pape and Bonhommeau (2015), but could occur with larval fish too. Still, we are quite confident that micro-increments width could also be used for Downs larvae as a larval condition index as previously stated for other spring and autumn species (Geffen 1982; McGurk 1984; Suthers 1998; Folkvord et al. 2000; Fox et al. 2003).

Ontogenetic shift in the larval condition

Despite their different integration time, three of the four indices (ingestion rate, G_i and MIW) clearly showed a change in larval condition at a size of 13–14 mm. Under a size threshold of 13 mm, Downs herring larvae appeared to feed and grow quite normally. Between 7 and 12 mm, MIW increased with size which is in accordance with previous studies (Campana et al. 1987; Folkvord et al. 1997, 2000). This increase corresponded to the end of the yolk-sac stage at 3–6 micro-increments and the transition to exogenous feeding. At 13 mm, larval condition exhibited a sharp decrease, particularly in ingestion rate and increment width, indicating difficulties in feeding and a reduction in growth rate. After 13 mm, larval ingestion rate started to increase and DNA/C ratio was lower, indicating the recovery of a better nutritional status. Feeding activity for these larvae was even better than for smallest larvae. However, it appeared that this recovery was not sufficient to ensure larval growth as displayed by instantaneous growth rate and mean increment width which were still largely under the thresholds. This was also observed by Mathers et al. (1994) on experimental herring larvae, while most of the studies rather showed an increase of condition with size (Pepin et al. 1999; Kimura 2000; Clemmesen et al. 2003).

Explaining factors of the ontogenetic shift

Both RDA and variance partitioning indicated that variability in the larval condition could be related to space, abiotic (temperature and salinity) and biotic parameters (phytoplankton and mesozooplankton), and larval length. Spatial variability was clearly showed from instantaneous growth rate and DNA/C ratio highlighting, respectively, higher and lower values in the EEC compared to the SBNS. Hence, with regard to feeding activity and growth, the EEC appeared as a more favourable environment for small larvae compared to SBNS. This spatial pattern resulted from the cross effect of the southwest–northeast gradient in the larval size distribution with the ontogenetic variations in their condition.

Environmental conditions (temperature and prey concentration) were also determined as to be significant in the RDA. They are usually considered as the two most important factors that strongly impact larval condition (Radtke and Fey 1996; John et al. 2001; Oeberst et al. 2009). Higher temperatures increase larval ingestion (Kiørboe et al. 1982; Irigoien et al. 2008) and otolith growth of herring was described to be proportionally faster at higher temperatures (Campana and Hurley 1989; Wright 1991; Hoff and Fuiman 1995). High prey density was reported to increase larval ingestion and assimilation (Boehlert and Yolklavich 1984; Pasternak 1994; Fiksen and Folkvord 1999). It is unlikely that lower temperature in the SBNS could explain the spatial difference in terms of larval ingestion and growth we observed, as temperature differences were typically low (0.1–1 °C) (except for two stations) between EEC and SBNS. For prey density, our results are contradictory with previous studies, since we observed lower ingestion rates (8–12 mm larvae) and growth (8–18 mm larvae) in the SBNS, whereas prey density was higher compared to the EEC. Hence, we argue that spatial variation in environmental conditions could not explain on their own the ontogenetic shift in larval condition observed at 13 mm. It is more probable that their significant effect in the RDA has more to do with their spatial-covariation with the larval condition than with their direct impact on it.

Size was also detected by the RDA as to have a significant effect on larval condition. We argue that the ontogenetic shift in larval condition observed at 13 mm has to be related to a diet shift occurring at this size. Indeed, Denis et al. (2016) found that, contrary to larger larvae which fed mostly on bigger and less diverse zooplanktonic prey, small herring larvae fed on a high diversity of small prey, including a large quantity of protists. While they hypothesized that this also explained the higher vacuity rate observed for 13 mm larvae, the present study tends to confirm that the more diversified diet of small larvae promotes their feeding activity and growth. Since mortality of early life stages of fish was determined to be size specific (McGurk 1986), a rapid increase in larval size of Downs herring can greatly reduce their mortality and predation pressure (McGurk 1986; Bailey and Houde 1989; Houde 1997). A larval size of 13–14 mm also corresponds to the differentiation of the dorsal fin (Doyle 1977; Paulsen et al. 2016) which could quickly improve their capacity to feed on larger prey by increasing their swimming capacity (Checkley 1982; Kiørboe et al. 1985; Munk and Kiørboe 1985). Finally, it would reduce their trophic competition with copepods for phytoplankton resource as larvae greater than 13 mm are essentially carnivorous (Denis et al. 2016). However, the shift from an omnivorous to a carnivorous diet occurring at 13 mm seems to have a negative impact on their short-term feeding efficiency and is clearly made at the expense of larval growth. The rapid increase of ingestion rate after 13 mm could suggest that Downs herring larvae start to improve their feeding activity through quick adaptation to their new diet. Indeed, it has been shown recently that the early stages of seabass (*Dicentrarchus labrax*) larvae are able to modulate their enzymatic synthesis according to the composition and quantity of ingested prey (Cahu and Zambonino 2007). Pepin et al. (2015) showed that high feeding success and growth at a given time led to higher probabilities of maintaining fast growth throughout larval life. In our case, since this was not reflected in terms of larval growth, it might also suggest that Downs larvae shifted to a more storage-oriented strategy of energy allocation once they had reached a sufficient size to increase their feeding success and reduce the trophic competition and predation. This shift in the energy allocation strategy was also observed for larvae of *Pleuragramma antarcticum* (Giraldo et al. 2015), the herring-equivalent species in the Southern Ocean, and also for the icefish *Chionodraco hamatus* (Giraldo et al. 2016).

Conclusion

The multi-index approach used in the present study showed that the four indices, although of different nature and integration time, led to the same conclusive pattern that a shift in the larval condition occurred at a size of 13–14 mm. This shift corresponds to another major change displayed by Downs larvae when they shifted from an omnivorous to a carnivorous diet, potentially enhanced by the development of dorsal fins. We argue that this shift in terms of prey preferences and swimming capabilities constitutes another critical period for Downs larvae beyond the shift from endogenous to exogenous nutrition. A complementary approach based in lipid contents could be used to test for the hypothesis of a shift in energy allocation towards storage after 13 mm. Downs larval condition should also be studied for

several years to detect the impact of inter-annual variation in environmental conditions during the critical period. Our results suggest that two of the four indices used might be sufficient to characterize larval condition, one reflecting nutrition and another growth. In this context, the ease and speed of estimating DNA/C and RNA/DNA ratios represent excellent options for the purpose of a multi-annual study.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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