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Changements ontogéniques de la condition larvaire chez une espèce de poisson à reproduction hivernale, le hareng des Downs

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Résumé

La condition des larves de hareng des Downs durant la période critique a été caractérisée entre 2008 et 2015, en Manche orientale et dans la Baie sud de la mer du Nord à partir des données collectées, durant la campagne International Bottom Trawl Surveys (IBTS). Tout d'abord, l'étude de la stratégie alimentaire, à partir de l'observation des proies dans les contenus digestifs par deux méthodes complémentaires, (microscopie électronique et mesure de la fluorescence) a montré un changement de régime en fonction de la taille. Les larves inférieures à 13 mm avaient un régime omnivore très diversifié et composé de protistes et de petites proies zooplanctoniques, tandis que les larves plus grandes avaient un régime moins diversifié et composé principalement de plus grandes proies zooplanctoniques. En parallèle du changement de régime alimentaire, la combinaison de quatre indices de condition (taux d'ingestion, rapports ARN/ADN et ADN/C et otolithes) à l'échelle individuelle a également révélé un changement de l'état nutritionnel et de la croissance de ces larves. Les larves inférieures à 13 mm avaient une nutrition et une croissance soutenues, tandis que la croissance des larves plus grandes était faible. Ceci peut traduire un changement dans la stratégie d'allocation de l'énergie à partir de 13 mm, qui serait plus orientée vers du stockage que vers de la croissance. Ces changements ontogéniques dans la condition larvaire suggèrent que le changement de régime alimentaire qui s'opère à partir de 13 mm, pourrait constituer le cœur de la période critique pour les larves de hareng des Downs.

Mots clés: Hareng des Downs, condition larvaire, nutrition, croissance, période critique, Manche-mer du Nord.

Abstract

The larval condition of Downs herring during the critical period was investigated between 2008 and 2015 in the eastern English Channel and Southern Bight of North Sea from data collected during the International Bottom Trawl Survey (IBTS). First, the study of the feeding strategy from gut contents analysis using two complementary approaches (electronical microscopy and measure of fluorescence) revealed a shift in the feeding diet occurring at a larval size of 13 mm. Smaller larvae had an omnivorous and a more diversified diet composed of numerous protist and small zooplanktonic preys whereas bigger larvae had a less diversified diet composed mainly of bigger zooplanktonic prey. Along with the diet shift, combination of four condition indices (ingestion rate, RNA/DNA and DNA/C ratios and otoliths) also revealed important changes in the nutritional status and growth of these larvae. Larvae smaller than 13 mm had a sustainable nutrition and growth whereas larger larvae depicted low growth rate. This could reflect a change in the energy-allocation strategy from a growth-oriented strategy towards a more storage-oriented strategy. These ontogenetic changes in the larval condition suggest that the diet shift occurring at 13 mm could constitute the core of the critical period for Downs herring larvae.

Keywords: Downs herring, larval condition, feeding strategy, growth, critical period, English Channel-North Sea.

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« Plus la bataille est difficile, plus la victoire est belle »

Proverbe chinois

« Il faut d'abord savoir ce que l'on veut,
il faut ensuite avoir le courage de le dire,
il faut ensuite l'énergie de le faire. »
Georges Clémenceau, 1841-1929.

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Bgut : Blank guts

CA : Correspondence Analysis

CCA : Canonical Correspondence Analysis

chl *a* : Chlorophyll *a*

D : Electivity index

DCA : Detrended Correspondence Analysis

DO : Otolith Diameter

DR : Daily Rations

DS : Dover Strait

EEC : Eastern English Channel

F : Occurrence

G : Gut content

GER : Gut Evacuation Rate

Gfish : Larval gut content

$\mathbf{G_i}$: Instantaneous growth rate

HCA : Hierarchical Classification Analysis

IBM : Modèles individus centrés

IBTS : International Bottom Trawl Survey

ICES : International Council for the Exploration of the Sea

Ifish : Ingestion rates

$\mathbf{Ifish_C}$: Ingestion rate in carbon

IGA : Impact des Grands Aménagements

L : Length before preservation

Ls : Length after preservation

LT : Standard length

MEB : Microscopie Electronique à Balayage

MWI : Mean Micro-increments Width

Ninc : Micro-increments number

$\mathbf{O_i}$: Occurrence of prey

P : Specific abundance

PCA : Principal Component Analysis

RDA : Redundancy Analysis

Rfish : Respiratory carbon

SBNS : Southern Bight of the North Sea

SD : Standard Deviation

SEM : Scanning Electron Microscopy

$\mathbf{T_{pig}}$: *In situ* chlorophyll *a* and phaeopigments concentrations

V : Vacuity rate

INTRODUCTION

1. La Manche-Mer du Nord

La Manche et la mer du Nord sont des mers épicontinentales (maximum 200 m de profondeur) soumises à un fort hydrodynamisme du fait de leur régime mégatidal et des épisodes de vents violents qui peuvent s'y manifester. Les zones les plus profondes sont rencontrées au centre de la Manche et au nord de la mer du Nord (Figure 1). D'un point de vue hydrologique, elles sont influencées par les eaux atlantiques qui rentrent au niveau de la Manche occidentale et par le nord de la mer du Nord ainsi que par les eaux plus froides et moins salées de la mer Baltique arrivant par le Skagerrak (Turrell et al., 1996). Le courant résiduel de marée créé par le régime mégatidal est orienté du sud-ouest vers le nord-est et permet les échanges entre les deux bassins (Hill et al., 1993).

Ces mers abritent de nombreuses espèces de poissons d'intérêt halieutique (Carpentier et al., 2009) qui y effectuent l'ensemble de leur cycle de vie selon le triangle de migration de Harden-Jones (Harden-Jones, 1968). Les zones de reproduction des adultes appelées frayères sont généralement distribuées au large tandis que les zones de nourriceries où grandissent les juvéniles sont le plus souvent situées à la côte. La phase larvaire, de par son caractère planctonique, assure le bouclage du cycle de vie, les larves étant transportées par les courants depuis les frayères jusqu'aux nourriceries. Les derniers travaux sur les larves de poissons en Manche remontent aux études de Grioche (Grioche, 1998; Grioche et al., 2001, 2000, 1999; Grioche and Koubbi, 1997), Harlay (Harlay, 2001; Harlay et al., 2001), et Koubbi (Koubbi et al., 2006), qui se sont principalement intéressés à des espèces à reproduction printanière. Ces travaux ont étudié l'influence des conditions physico-chimiques et trophiques, et des structures hydrologiques (front tidal, fleuve côtier et gyre) présentes au printemps sur la répartition des assemblages ichtyoplanctoniques, la distribution ontogénique et le comportement de quelques espèces clés comme la sole, le flet et la limande. Ces travaux ont également montré l'impact du bloom zooplanctonique sur la nutrition et la condition pour la sole et le flet.

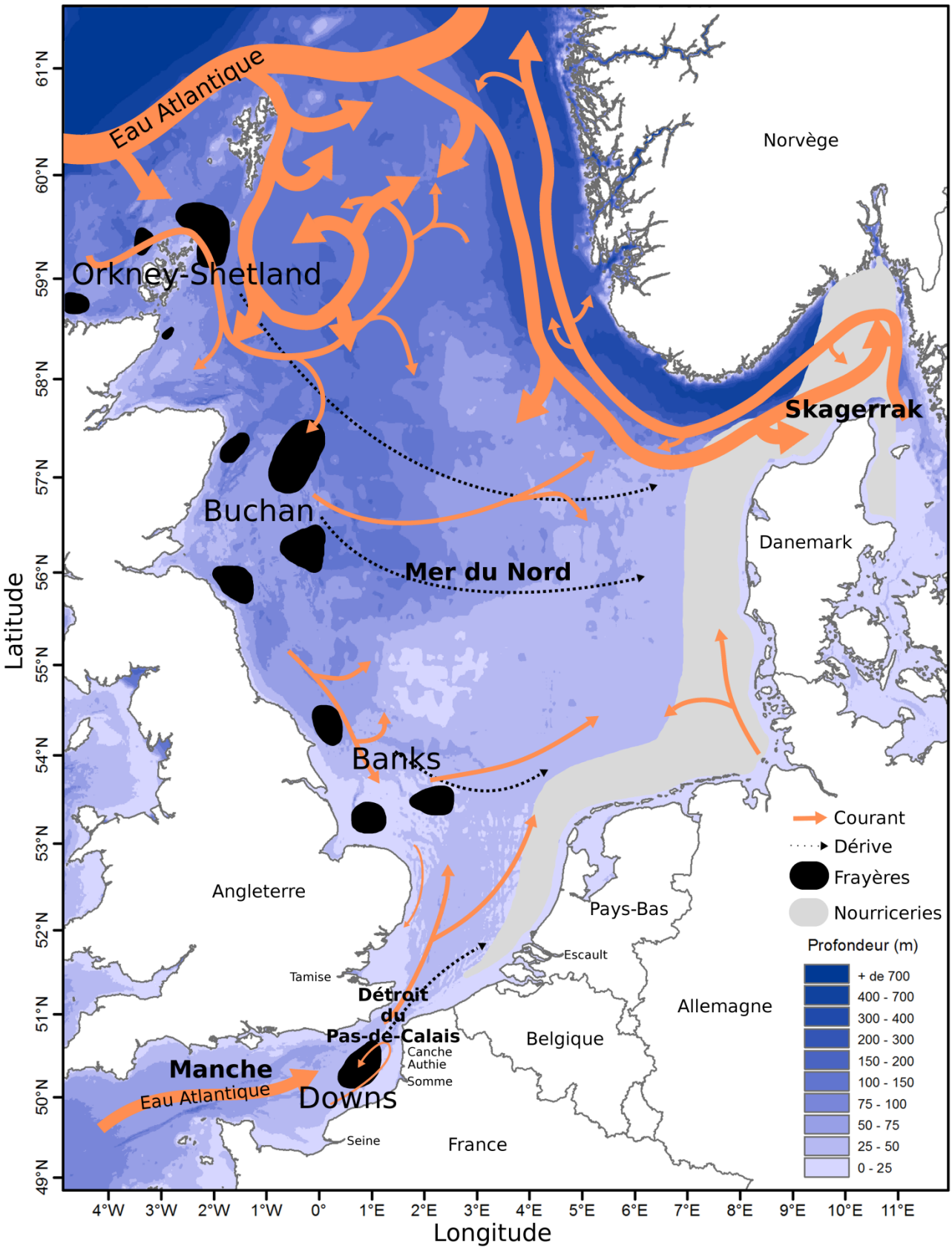

Figure 1: Schéma représentant la circulation générale des courants d'après Turrell et al. (1996), la profondeur (m), ainsi que les zones de frayère et de nourricerie du hareng de mer du Nord (modifié de Corten (2013)). La dérive des larves (flèches pointillées) assure la connexion entre les frayères des quatre sous-populations et les nourriceries.

2. **Le cas du hareng des Downs**

Le hareng (*Clupea harengus* L.) est une espèce de poisson pélagique de la famille des clupéidés. Il est présent dans les mers froides de l'Atlantique, de la Baltique, de la mer du Nord, de la mer de Norvège et du Pacifique. Chaque population est constituée de plusieurs sous-populations qui se distinguent au moment de la reproduction. Le hareng se déplace en bancs suivant des schémas de migration entre les zones de frai et d'hivernage situées pour la plupart le long des zones côtières peu profondes (Maucorps, 1969). Les sous-populations s'individualisent lorsqu'elles migrent vers les frayères. Le hareng ne se reproduit qu'une fois par an sur une période relativement courte. Les œufs démersaux sont pondus sur des substrats de fucus ou de graviers et éclosent au bout de quelques jours (Maucorps, 1969; Parrish et al., 1959) jusqu'à 3 semaines en fonction de la température (ICES, 2015). Après l'éclosion, les larves sont pélagiques et dérivent passivement via les courants marins vers les zones de nourriceries côtières. Les juvéniles se trouvent près des côtes jusqu'à 2 ans tandis que les adultes se trouvent plus au large. D'une longévité de 10 ans environ, le hareng acquiert sa maturité sexuelle vers l'âge de 2-3 ans. Sa taille varie selon la population, le hareng de la Baltique étant le plus petit de tous alors que le hareng norvégien peut dépasser 40 cm. Au cours de leurs migrations, les harengs adultes ne restent pas toujours en sous-population bien distinctes et des mélanges peuvent se produire. Les adultes vivent en profondeur le jour et se rapprochent de la surface la nuit pour se nourrir de petits crustacés planctoniques et de larves de mollusques (ICES, 2015).

Le hareng des Downs appartient au stock du hareng de la mer du Nord qui est une espèce emblématique à la fois d'un point de vue écologique et économique. D'un point de vue écologique, le hareng de la mer du Nord a fait l'objet d'une attention considérable au cours du siècle dernier (Hjort, 1914) et représente le premier modèle écologique pour les théories développées sur les larves de poissons (Cushing, 1969; Hjort, 1914; Houde, 2008; Iles and Sinclair, 1982). Il est également l'une des deux espèces pélagiques majeures de la mer du Nord (avec le sprat) et exerce un contrôle de type « wasp-waist » (Duarte and García, 2004; Fauchald et al., 2011) sur les niveaux trophiques inférieurs comme le zooplancton (contrôle top-down) et sur les niveaux supérieurs (contrôle bottom-up) tels que les prédateurs piscivores.

D'un point de vue économique, il représente la moitié des débarquements de poissons pélagiques (en moyenne 417 140 tonnes) dans la zone depuis les années 1950 (ICES, 2015). Depuis 2002, le stock connaît un faible recrutement malgré une forte biomasse de poissons

adultes et une faible mortalité par pêche (Figure 2). Afin d'expliquer ce faible recrutement, plusieurs hypothèses ont été discutées par Corten (2013), parmi lesquelles: des difficultés d'alimentation pour les larves, une augmentation de la prédation par les adultes de hareng, des conditions d'éclosion peu favorables, un changement de régime dans les communautés planctoniques de la mer du Nord (Alvarez-Fernandez et al., 2012; Beaugrand et al., 2003) et une augmentation de la mortalité des larves résultant de la récente augmentation des températures de l'eau (Becker and Pauly, 1996; Fässler et al., 2011; Hufnagl and Peck, 2011; Petitgas et al., 2013). Corten (2013) suggère également que ces changements peuvent avoir favorisé la sous-population des Downs dont la contribution à la population totale a été récemment supérieure à celle de chacune des trois autres sous-populations (ICES, 2015).

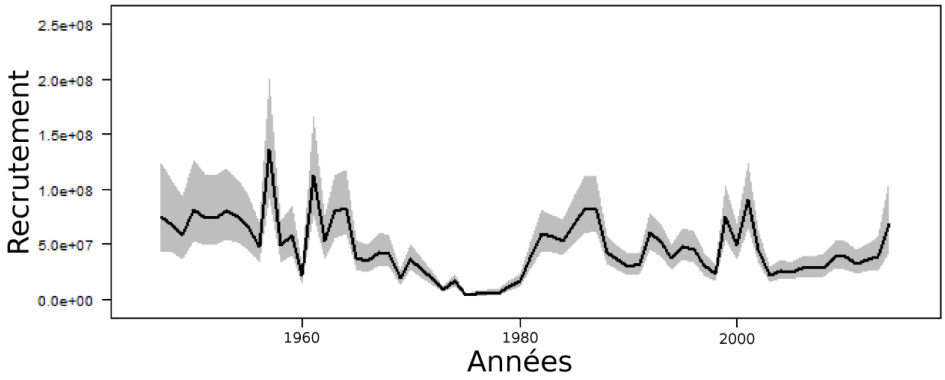

Figure 2: Evolution du recrutement (en tonnes) du hareng de Mer du Nord de 1950 à 2014, modifié d'ICES (2015). En gris est représentée l'incertitude.

Contrairement aux trois autres sous-populations qui se reproduisent à l'automne (Septembre-Octobre) le long des côtes anglaises de la mer du Nord, le hareng des Downs vient se reproduire en hiver (décembre-janvier) en Manche orientale et dans la baie sud de la mer du Nord (Maucorps, 1969; Figure 1).

Au cours de leur dérive de quelques mois, les larves des Downs vont subir d'importants changements morpho-anatomiques qui peuvent être regroupés au sein de quatre stades de développement (Figure 3) définis par Russell (1976). Le stade vitellin regroupe les larves de 8 à 9 mm qui se nourrissent sur leurs réserves vitellines. Le stade pré-flexion rassemble les larves de 10 à 17 mm et correspond à la première nutrition exogène des larves, au début de la flexion de la notochorde et du développement de la nageoire dorsale. Le stade flexion correspond à une flexion totale de la notochorde des larves de 17-21 mm. Le stade post-flexion réunit les larves de 21-40 mm, dont les nageoires anales et caudales sont développées.

Les larves du hareng des Downs en janvier-février, traversent le détroit du Pas-de-Calais pour atteindre les nourriceries côtières le long des côtes Belges, Hollandaises, Allemandes et Danoises de la Mer du Nord.

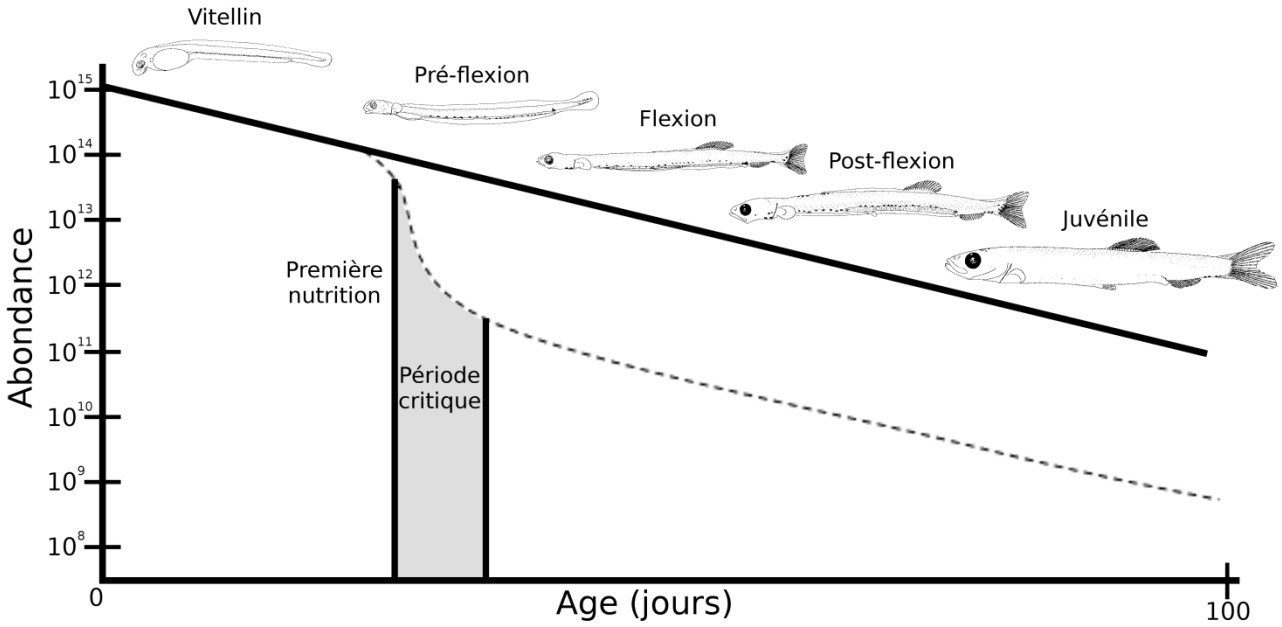

Figure 3: Hypothèse de la « période critique » de Hjort (1914) modifiée par Houde (2008).

3. **Problématique**

Le passage du stade vitellin au stade pré-flexion constitue selon Hjort (1914) la période critique pour les larves. Durant cette période, le passage d'une nutrition endogène à une nutrition exogène conduit à un taux de mortalité élevé et à une chute brutale de l'abondance larvaire (Figure 3). Cette hypothèse implique que le taux de mortalité larvaire dépend de la réussite de la première nutrition des larves qui dépend à son tour de la disponibilité des proies planctoniques dans le milieu. Cushing (1990, 1974) et Bakun (1996) suggèrent que le succès de la nutrition larvaire dépend de la coïncidence temporelle (théorie du match-mismatch) et spatiale (Triade de Bakun) entre les larves et leurs proies. Si la reproduction printanière d'une majorité des espèces en Manche permet aux larves de bénéficier du bloom planctonique et des structures hydrologiques nécessaires à leur survie (Grioche, 1998), la stratégie de reproduction du hareng des Downs conduit à une apparition de ces larves pendant l'hiver dans des conditions qui paraissent moins favorables d'un point de vue trophique. Toutefois, la persistance de cette espèce à se reproduire durant l'hiver ainsi que sa contribution élevée et récente à la population totale supposent une adaptation aux conditions hivernales. On peut

alors s'interroger sur la stratégie adoptée par ces larves pour se nourrir et se développer pendant la période critique.

L'étude de la condition larvaire est un moyen d'étudier l'état des larves du point de vue de leur nutrition et de leur croissance, qui vont toutes deux influencer le développement et la capacité de survie de ces larves. La condition larvaire est essentielle dans la détermination de la stratégie de survie d'une espèce comme le hareng des Downs, compte tenu des conditions environnementales particulières que ces larves vont rencontrer durant l'hiver. Plusieurs indices ont été proposés (Catalán, 2003; Ferron and Leggett, 1994; Theilacker et al., 1996) pour caractériser la condition larvaire des poissons. Ces indices diffèrent selon leur nature (morphologique, biochimique, histologique...), les temps d'intégration des changements de la condition (court, moyen et long terme), le type de condition qu'ils caractérisent (nutrition, croissance, stockage...) et le coût engendré par leur utilisation.

La nutrition et l'état nutritionnel des larves à court terme peuvent être estimés à partir de méthodes directes basées sur l'analyse de leurs contenus digestifs. Jusqu'à présent, l'identification visuelle des proies ingérées s'est faite majoritairement à l'aide de la microscopie optique (Arula et al., 2012; Blaxter, 1965; Catalán et al., 2010; Davis et al., 2012; Landaeta et al., 2014; Robert et al., 2011). L'utilisation récente de la microscopie électronique à balayage (MEB) a permis d'observer une diversité plus importante de proies, notamment des proies phytoplanctoniques, dans le régime alimentaire des larves (Giraldo et al., 2011; Koubbi et al., 2007; Vallet et al., 2011). Ces observations suggèrent un régime alimentaire omnivore, alors que les larves de poissons étaient jusque-là considérées purement carnivores et ciblant des proies zooplanctoniques comme les copépodes (Giraldo et al., 2011; Koubbi et al., 2007). Cependant, du fait du coût engendré par l'analyse des contenus digestifs au MEB, le régime alimentaire des larves n'a pu être considéré que d'un point de vue qualitatif (présence-absence, diversité, sélectivité). Depuis les années 1970, le taux d'ingestion du zooplancton est estimé de manière quantitative par la fluorescence du tube digestif (Mackas and Bohrer, 1976). Cette méthode rapide et facile à mettre en place a été utilisée sur une grande variété d'organismes planctoniques comme les copépodes, les salpes et le krill (López et al., 2007; Pakhomov et al., 1996; Perissinotto and Pakhomov, 1998) mais rarement sur les larves de poissons (Conway et al., 1996; Otake et al., 1990).

Des méthodes indirectes comme le rapport ADN/C peuvent également être utilisées pour étudier l'état nutritionnel des larves. Le rapport ADN/C a été développé par Bergeron et al. (1991) comme une alternative au rapport ARN/ADN, plus adapté aux larves en phase de première nutrition. Le rapport ADN/C caractérise l'état nutritionnel des larves à l'échelle de 1

à 3 jours, car la concentration en carbone (quantité de molécules organiques) diminue durant une période de jeûne alors que la concentration d'ADN reste constante. De fortes valeurs de ce taux indiquent ainsi une moins bonne condition des larves (Bergeron, 2000).

Parmi les indices disponibles pour étudier la croissance larvaire, les indices moléculaires et la microstructure des otolithes fournissent une évaluation robuste de la condition larvaire à moyen et à long terme (Bergeron, 1997; Clemmesen, 1994; Pannella, 1974, 1971). Le rapport ARN/ADN a souvent été utilisé pour caractériser la croissance des larves de poissons (Bang et al., 2006; Bergeron et al., 1997; Clemmesen, 1996, 1994; Folkvord et al., 1996; García et al., 1998; Yandi and Altinok, 2015). Il est une approximation du taux de croissance récente estimé, lié à la synthèse de nouvelles protéines (Bergeron, 1997; Buckley et al., 2008; Bulow, 1970). La teneur en ARN larvaire varie en fonction de la vitesse de synthèse des protéines alors que la teneur en ADN est approximativement constante et dépend principalement de la taille de la larve (Bergeron, 2009, 1997). En laboratoire, le rapport ARN/ADN répond dans les 3-4 jours à un changement de régime alimentaire (Clemmesen, 1987) et de fortes valeurs de ce ratio indiquent une bonne croissance.

Les otolithes sont des pièces calcifiées de l'oreille interne qui enregistrent la croissance somatique tout au long de la vie du poisson (Campana, 1984; Mosegaard et al., 1988). L'analyse de la microstructure des otolithes est l'approche la plus utilisée pour évaluer la croissance (Folkvord et al., 2000; Fox et al., 2003; Geffen, 1982; McGurk, 1984).

A l'exception de rares études expérimentales (Peck et al., 2015), les indices sont généralement mesurés sur des individus différents, ce qui rend les résultats difficiles à interpréter du fait de la variabilité individuelle qui peut exister et de l'échelle de temps contrastée de ces indices qui peut être de quelques heures (contenus digestifs), quelques jours (rapports ARN/ADN, ADN/C) ou quelques mois (otolithes). Clemmesen and Doan (1996) recommandent ainsi de combiner différents indices de condition à l'échelle du même individu afin de déterminer si les résultats obtenus sur la condition larvaire sont comparables entre les indices.

OBJECTIFS ET PLAN DE LA THÈSE

La phase larvaire du hareng des Downs étant de loin la moins étudiée des quatre sous-populations de la Mer du Nord (Lebour, 1924, 1921), ce travail de thèse vise à développer les connaissances sur la condition, notamment la nutrition et la croissance de ces larves pendant leur période critique en Manche orientale et dans la baie sud de la mer du Nord. Plus précisément, il s'agit de déterminer comment varient leur stratégie alimentaire, leur état nutritionnel et leur croissance au cours de leur développement ontogénique. Les résultats permettront de mieux comprendre pourquoi ces larves apparaissent en hiver, dans des conditions trophiques peu favorables, dans la mesure où ce déterminisme demeure encore un mystère.

Le manuscrit de thèse est divisé en six chapitres dont les chapitres III à V sont des articles publiés, soumis et en préparation.

Le chapitre I décrit le matériel et les méthodes utilisés dans les chapitres II à V. Il présente la campagne scientifique IBTS qui a permis la collecte des échantillons et des données, supports de cette thèse. Il décrit les méthodes utilisées pour l'analyse qualitative et quantitative du régime alimentaire et pour l'étude de la condition larvaire. Enfin, il présente les analyses statistiques utilisées afin d'analyser et cartographier les résultats obtenus.

Le chapitre II a pour objectif de présenter la distribution spatio-temporelle de l'environnement abiotique et biotique et la distribution en taille des larves de hareng des Downs entre 2008 et 2015. La relation entre l'environnement et la distribution des larves est également étudiée grâce à des analyses multi-variées.

Le chapitre III est tiré d'un article publié dans Journal of Sea Research (Denis et al., 2016). Il présente et discute les résultats concernant l'étude qualitative des contenus digestifs des larves de hareng de 8 à 19 mm entre 2008 et 2014 à l'aide de la microscopie électronique. Plus précisément, il caractérise le comportement alimentaire des larves, y compris leurs taux de vacuité, leurs préférences alimentaires, leur niche trophique et la sélectivité des proies ingérées.

Le chapitre IV est tiré d'un article en préparation (Denis et al., in prep) pour Marine Ecology Progress Series. Dans ce chapitre, la méthode d'analyse des contenus digestifs par fluorescence a été adaptée dans le cadre de cette thèse aux larves de 8 à 13 mm afin d'estimer d'un point de vue quantitatif la contribution du phytoplancton au régime alimentaire de ces larves. L'ingestion de phytoplancton par les larves de hareng a été comparée à celle mesurée

pour les six espèces de copépodes herbivores rencontrées dans la zone et aux estimations des besoins métaboliques journaliers de base pour les larves de hareng des Downs.

Le chapitre V est tiré d'un article soumis dans Marine Biology (Denis et al., submitted). Il présente et discute les résultats de l'approche multi-indices (taux d'ingestion, rapports ARN/ADN et ADN/C et microstructures des otolithes) utilisée à l'échelle individuelle en 2015 afin de caractériser l'état nutritionnel et la croissance des larves de 8 à 18 mm. Les objectifs étaient de comparer les réponses obtenues par les différents indices, dont les temps d'intégration sont différents, et de déterminer par une approche intégrée les facteurs spatiaux, environnementaux et ontogénétiques influençant la condition des larves.

Le chapitre VI consiste en une synthèse générale des principales conclusions obtenues. Tout d'abord, les apports et limites des méthodes utilisées pour étudier la condition trophique des larves de hareng des Downs en hiver sont discutés. Puis les résultats obtenus sur la condition des larves du hareng des Downs sont discutés dans le contexte plus général de l'écologie des larves en période critique. Enfin, l'utilisation des résultats obtenus à des fins appliquées et les perspectives envisagées pour compléter les connaissances acquises au cours de ce travail sont discutées.

CHAPITRE I
Material and methods

1. Sampling

1.1. The International Bottom Trawl Survey (IBTS)

The French part of the IBTS from 2008 to 2015 was the sampling support to collect both environmental and biological data used throughout the present work (Table 1).

The IBTS is an international stock assessment campaign coordinated by the International Council for the Exploration of the Sea (ICES) since 1980. It takes place during the first and third quarters (January-March and August-September) and eight countries (Denmark, England, France, Germany, Norway, Scotland, Sweden and the Netherlands) are involved. France is only involved in the first quarter on board of the N/O Thalassa and covers the eastern English Channel (EEC) and southern part of the North Sea. From 2008 to 2015, the French part of the IBTS took place between mid-January and mid-February except for 2008 where it started a bit later (Table 1). The main objective is to collect data for stock assessment of the main commercial fish species exploited in the English Channel and North Sea (whiting, cod, haddock, Norway pout, herring, sprat, mackerel, plaice...). Both fish adults and larvae are collected during the survey.

Table 1: Time period of the French part of the International Bottom Trawl Survey (IBTS) between 2008 and 2015 and number of sampling stations available.

Year	**Time of the IBTS**		**Stations**
	Start	End	
2008	25/1	22/2	128
2009	13/1	13/2	115
2010	12/1	19/2	123
2011	13/1	14/2	125
2012	13/1	14/2	129
2013	15/1	14/2	83
2014	13/1	14/2	114
2015	12/1	10/2	117

Fish larvae are collected only at night following a stratified sampling based on statistics rectangles of 1° longitude × 0.5° latitude (Figure 4; ICES, 2015). Each rectangle is sampled either twice (southern Bight of the North Sea; SBNS) or four times (EEC). Hydro-biological parameters, protists, and metazooplankton are also collected.

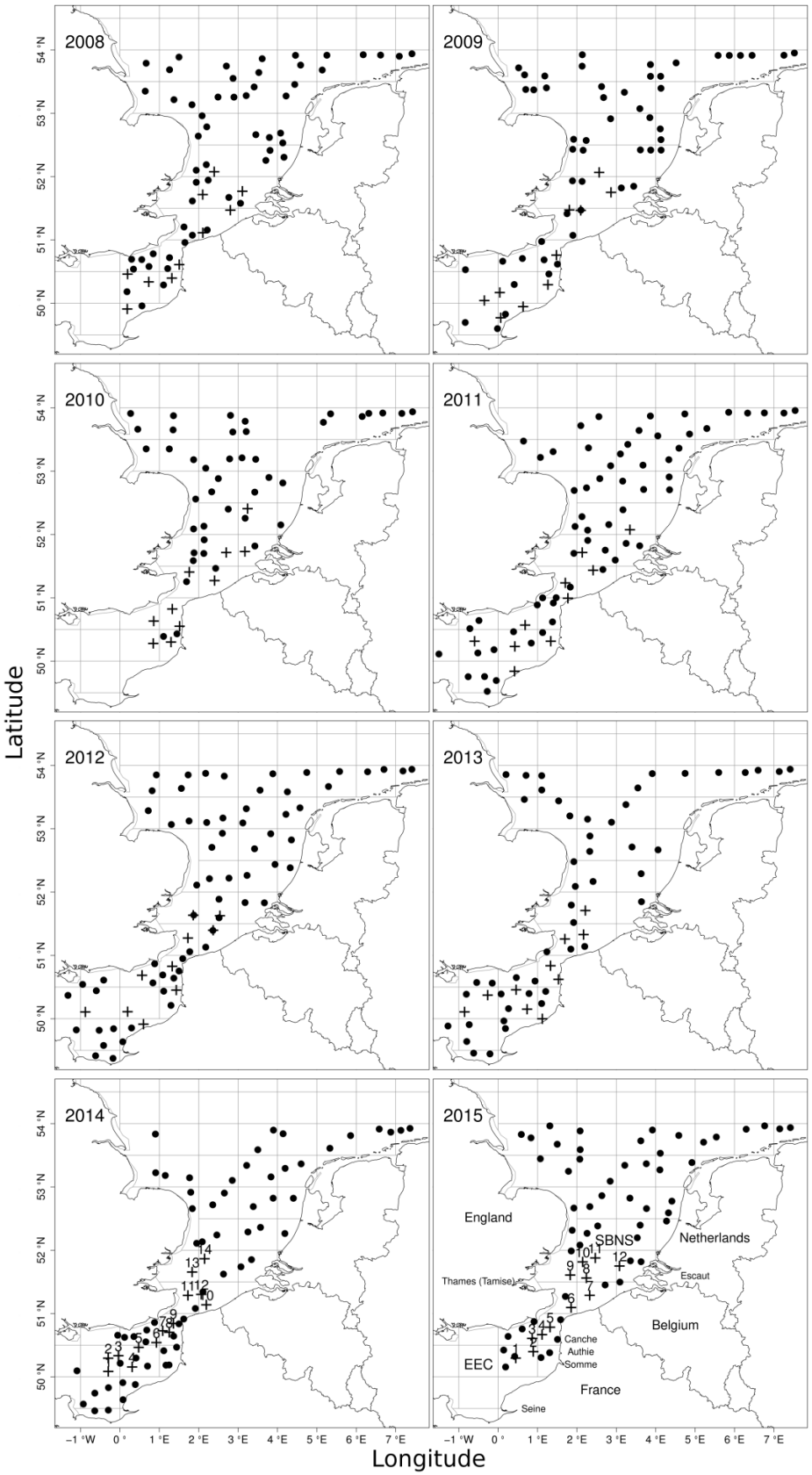

Figure 4: Location of larval fish sampling stations (dots) during the first quarter of the French part of the International Bottom Trawl Survey (IBTS) in the English Channel (EEC), Dover Strait (DS) and the Southern Bight of North Sea (SBNS) from 2008-2015. Crosses stand for stations where the protists, metazooplankton and larvae were analysed. ICES statistical rectangles are indicated.

1.2.Hydrobiological parameters and protists

Height parameters were used in the present study: sea surface temperature and salinity, fluorescence, bathymetry, sea-bed stress, chlorophyll concentration, suspended matter and nutrients. Temperature (°C), salinity and fluorescence (µg L^{-1}) were continuously measured at 3-5 m below the sea surface using a SBE 21 SeaCAT thermosalinograph and a fluorometer and the bathymetry (m) was measured by a sonar. Sea-bed stress (N m^{-2}) is a measure of the shear friction of water on the seabed due to the tidal currents. It was estimated using a 2D hydrodynamic model (Aldridge and Davies, 1993), originally developed at the Proudman Oceanographic Laboratory.

Seawater samples were collected in order to estimate chlorophyll concentration, suspended matter, nutrients and protists. They were collected at 1 m depth using a 5 L Niskin bottle.

Two replicates of 500 to 1000 ml seawater were immediately filtered on glass-fibre filters of 47 mm diameter and 1.2 µm mesh size (Whatman GF/C) and frozen at -20°C for chlorophyll concentration. *In situ* chlorophyll *a* (chl *a*) and phaeopigments concentrations (T_{pig}, µg L^{-1}) were estimated using the spectrochromatic monochromatic method (Aminot and Kérouel, 2004; Lorenzen, 1967) after grinding the glass-fibre filters and extracting the chlorophyll pigments in 90% acetone at 4°C in the dark.

About 1000 ml seawater was filtered on glass-fibre filters and frozen at -20°C for suspended matter. Total suspended matter (organic and inorganic) was obtained according to the protocol established by Aminot and Chaussepied (1983). Briefly, the method consists in measuring a weight of dry matter after drying the glass-fibre filters in the oven at 70°C for 2 hours.

Dissolved nutrients (ammonium, nitrate, nitrogen oxide, phosphates and silanol) were measured from seawater filtered on a 50 µm mesh net by automated colorimetric method with the auto-analyzer of nutrients (Alliance Instruments) according to the method of Aminot and Kerouel (2007).

Around of 250 mL were kept in a Lugol acid solution (4% final concentration) for protists (auto- and heterotroph unicellular organisms) identification.

1.3.Metazooplankton

Metazooplankton samples were collected through oblique hauls using a WP2 net (200 µm mesh size; Tranter and Smith, 1996), except in 2014 where a double WP2 net was used. The

net was deployed from 3 m above the bottom up to the surface at 0.75 m s^{-1}. For zooplankton taxonomy and abundance, content net (the first one in 2014) was preserved in a 0.9% buffered (sodium glycerophosphate) formalin seawater solution (Lelièvre et al., 2010; Mastail and Battaglia, 1978 modified by Bigot, 1979) for later identification. In 2014, content of the second net was deposited on Whatman shark-skin filters (8-12 µm, 125 mm diameter). The filters were folded in a cryotube and immediately frozen in liquid nitrogen for subsequent gut content analyses.

1.4.Herring larvae

Herring larvae were sampled along with the other larval species using a Mid-water Ring Net of 13 m long, 2 m in diameter and 1.6 mm mesh size except for the last metre which is 500 µm (ICES, 2015). The ring net was deployed obliquely for at least 10 min from 5 m above the bottom up to the surface. Only herring larvae of less than 20 mm and located south of 54°N were considered as they were assumed to belong to the Downs herring sub-population (ICES, 2015). These larvae were easily recognizable from other larval species (Russell, 1976) because of their smaller size and their high abundance in the samples. Sub-samples of 15-30 individuals were sieved on board on a 500 µm mesh net, visually sorted and were either frozen in liquid nitrogen or fixed in 95% ethanol for gut contents and condition analyses. The rest of the sample was preserved in the same formalin solution as for copepods for later identification and qualitative analysis of gut contents by Scanning Electron Microscopy (SEM) (Figure 5).

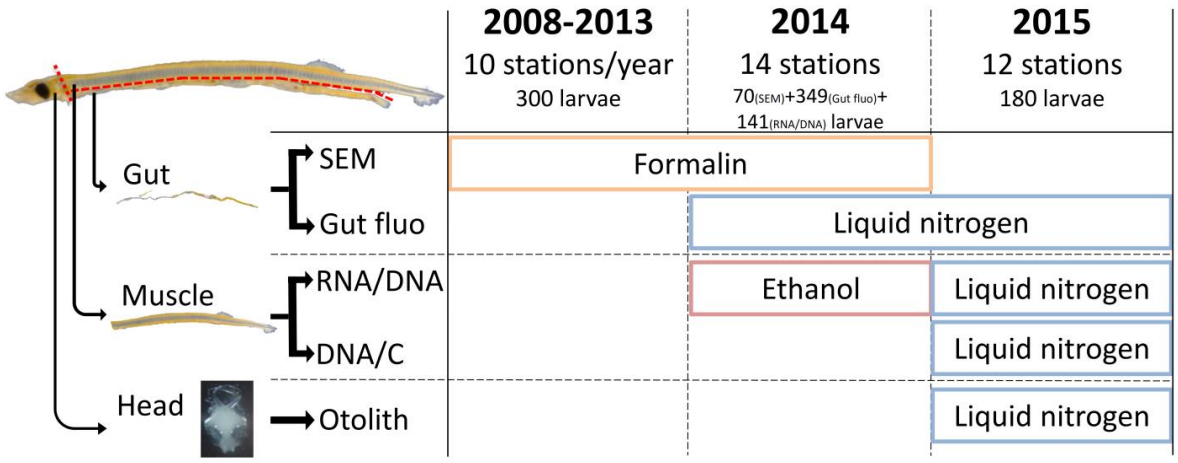

Figure 5: Summary of the methods, number of stations and larvae and type of conservative (formalin, liquid nitrogen and ethanol) used for gut content and condition index analyses of Downs herring larvae from 2008 to 2015. SEM: gut contents analysis by Scanning Electron Microscope, Gut fluo: gut fluorescence, RNA/DNA: RNA/DNA ratio analysis, DNA/C: DNA/C ratio analysis and Otolith: otolith microstructure analysis.

2. **Identification**

2.1.Protists

Protists from 2008 to 2010 samples were identified using an inverted microscope (Utermöhl, 1958). The number of cells for each species (or taxon) was counted in a sedimentation chamber and the data obtained was then recoded in term of presence-absence. Protists from 2011 to 2015 samples were analysed using SEM (LEO 438VP; modified from Paerl and Shimp, 1973). For each sample, 30 ml were filtered on a 0.4 µm polycarbonate filter deposited on a carbon tape glued to a metal stub of 10 mm diameter. Stubs were dried during 24 h under a laminar flow hood and metallized with a thin gold-palladium layer (Polaron SC7620). For each sample, the stub was totally scanned at a magnification of 3.4 × 4.6 mm and all visible species were identified. The stub was then partially scanned (one horizontal line and one vertical through the middle of the stub) at a magnification of 0.456 × 0.306 mm in order to identify very small species (Vallet et al., 2011). Each species identified was determined as present or absent. Taxonomic data obtained with SEM was finally aggregated to the same level as the one obtained from inverted microscopy so that the data were comparable between the two periods.

2.2.Metazooplankton

Metazooplankton from 2008 to 2015 samples were identified back at laboratory using the ZooScan system (Gorsky et al., 2010; Grosjean et al., 2004). Prior to scanning, samples were first separated in two size-fraction (> 500 µm and 200-500 µm) to prevent misrepresentation of large organisms. Each fraction was then split with a Motoda splitter (Motoda, 1959) until the subsample was diluted enough to contain about 1000-2000 objects. The subsamples were deposited onto the scanning cell (11 × 24 cm) and organisms were manually separated to minimize overlapping. Image acquisition and processing were done according to 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 an already existing 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 the abundance (ind m^{-3}) of each

species in the whole zooplankton sample. Abundances were finally reclassified in terms of presence-absence for later calculation of prey selectivity.

2.3.Downs herring larvae

Abundance (ind 5000 m^3) and size class distribution of Downs herring larvae from 2008 to 2015 were determined from formalin samples. First, samples were splitted into subsamples of 1/2 to 1/256 using a Motoda splitter. A minimum of one hundred individuals per subsample was counted (Motoda, 1959). Of these larvae, 50 were measured individually (standard length, SL) to the lowest millimetre. Standard lengths were corrected for potential shrinkage due to preservation according to the linear models (ANOVA, $P < 0.05$) relating length before (L) and after preservation (Ls) in either formalin solution ($L = 1.2064 \times Ls - 1.1224$), 95% ethanol ($L = 1.01172 \times Ls + 1.0155$) and liquid nitrogen ($L = 0.9588 \times Ls + 0.892$).

3. **Gut contents analysis**

3.1.Copepods

Gut content of the six dominant copepod species (*Calanus* spp., *Euterpina acutifrons, Oncaea* spp., *Paracalanus parvus, Pseudocalanus elongatus* and *Temora longicornis*) was determined quantitatively by fluorometry (Mackas and Bohrer, 1976). Depending on species abundance, 1-3 replicates of 1-2 individuals for *Calanus* spp. and 15-35 individuals for the other species per station on a total of 14 stations in 2014 (Figure 4) were analysed. Copepods were individually sorted on ice and picked from frozen shark-skin samples under a cool light stereomicroscope. Individuals were rinsed in filtered 0.2 µm seawater to eliminate phytoplankton cells stuck to feeding appendages and were then transferred into 4 ml acetone 90%, ground and extraction was carried out in the dark at 4°C for 6 h. Fluorescence of the extract (chl *a* and phaeopigments pigments) was measured before and after acidification with 10% HCl (Parsons et al., 1984) using a Trilogy Laboratory Fluorometer (Turner Designs EPA 445). Gut content (G, ng chl *a* eq ind^{-1}) was obtained from the addition of chl *a* and phaeopigments concentrations. Values were not corrected for pigment degradation on the recommendation of Durbin and Campbell (2007). Because total copepod gut content cannot be considered to equal the sum of gut content obtained for each of the six species studies, range of gut content values will be presented.

3.2.Downs herring larvae

Gut contents of Downs herring larvae were analysed by two complementary approaches, a qualitative approach of prey identification using SEM performed on 2008-2014 samples and a quantitative approach of larval ingestion rate based on fluorometry measurement as for the six dominant copepods (see chap. I section 3.1) for 2014 and 2015 (Figure 5). A total of 10 stations per year from 2008 to 2013, 14 stations in 2014 and 12 stations in 2015 were analysed (Figure 4 and 5).

3.2.1. Qualitative analysis

The qualitative analysis of larval gut contents was performed following Vallet et al. (2011). Each year, five larvae per station were analysed. For each larva, the gut was removed and opened in deionized water (Milli-Q) and its content was resuspended to facilitate optical examination (×10 magnification). Larger prey (> 50 µm) were identified to the finest possible taxonomic level depending upon their digestion state under a stereomicroscope. For smaller prey, gut contents were then filtered on a 0.4 µm polycarbonate filter deposited on a carbon tape (Agar Scientific) glued to an aluminium stub of 10 mm diameter. Stubs were dried during 24 h under a laminar flow hood and metallized under argon flow with Au/Pd for 90s (Polaron SC 7620). Smaller prey were identified following the same methodology as the one used for protists samples in 2011-2014. The stub was totally scanned at a magnification of 3.4 × 4.6 mm and partially scanned (one horizontal line and one vertical through the middle of the stub) at a magnification of 0.456 × 0.306 mm. Detected prey were identified in term of presence-absence to the lowest taxonomic level depending upon their digestion state and measured (diameter and/or width) using an image analyzer (LEO 32). They were finally classified into ten prey groups (Table 3).

The larval gut contents analyses were also performed quantitatively by SEM to compare analysis duration and information provided. Gut content of five larvae were prepared in the same way as for qualitative method with a totally scanned at a magnification of 0.456 × 0.306 mm. Detected prey were counted to estimate the number of prey in each gut contents.

Vacuity rate, feeding strategy and prey selectivity were derived from prey identification in the gut contents and in the water (from protists and metazooplankton samples, see chap. I section 2.1 and 2.2).

Larval vacuity rate (V; Berg, 1979) was calculated by station as the percentage of larvae at each station without any prey in their gut and by larval length as the percentage of larvae at each larval length group without any prey in their gut (Eq.1).

$$V_s = n_s / N_s \times 100 \quad \text{Eq.1}$$

where V_s is the vacuity rate, n_s is the number of larvae without prey in the gut by station or larval length and N_s is the total number of larvae analysed by station or larval length.

Larval feeding strategy was determined based on the modified Costello graphical method (Amundsen et al., 1996) using the specific abundance (P) and occurrence (F) of the different prey in the gut contents.

The calculation of specific abundance (P) was adapted to presence-absence data by Vallet et al. (2011) following Eq.2:

$$P_i = n_i / N_i \times 100 \quad \text{Eq.2}$$

where P_i is the specific abundance of prey i, n_i is the number of guts where the prey i was present and N_i is the total number of prey within all the guts containing prey i.

Occurrence (F) was calculated for each year following Eq.3:

$$F_i = n_i / N_f \quad \text{Eq.3}$$

where F_i is the occurrence of prey i, n_i is the number of guts where the prey i was present and N_f is the total number of larvae containing prey in the guts.

The diagram of the mean specific abundance versus the mean occurrence was then plotted and the size of the trophic niche was interpreted following Amundsen et al. (1996).

Larval feeding selectivity was determined for each prey found in the larval guts using the modified Ivlev's Electivity Index (D; Jacobs, 1974) and adapted to presence-absence data by Vallet et al. (2011). It is based on the proportion of prey in the guts (r) and in the water (p) and was calculated following Eq.4:

$$D_i = r_i - p_i / r_i + p_i - 2 r_i p_i \quad \text{Eq.4}$$

where r_i is the ratio of prey i (actually 1) over the total number of prey in the gut and p_i is the ratio of prey i (actually 1) over the total number of prey in the water. It was calculated by station, then averaged by year and finally averaged overall the years.

The selectivity index varies from -1 to 0 for a negative selectivity and from 0 to 1 for a positive selectivity.

The selectivity index was plotted according to the occurrence (O_i) of prey in the water calculated following Eq.5:

$$O_i = n_i / N_f \times 100 \qquad \text{Eq.5}$$

where n_i is the number of station where the prey i was present and N_f is the total number of station analysed.

3.2.2. Quantitative analysis

The quantitative analysis of larval gut contents was performed by adapting the fluorimetric method used for herbivorous copepods (see chap. I section 3.1).

Each larva was measured and its gut was removed and transferred into glass tubes with 4 ml of 90% acetone. Several trials (not presented) were performed to determine the optimal extraction time and the minimum number of larvae required for signal detection. Larval gut fluorescence was first measured every 2h over a 12h period. This allowed setting the optimal extraction time at 6h as no significant difference (Kruskall-Wallis test, $p > 0.05$) was observed thereafter. Fluorescence measurement on an increasing number (from 2 to 16) of larval guts allowed defining the optimal number of 10 larvae per replicate. Frozen larvae were placed in petri dishes filled with milliQ water and examined on ice under cool light stereomicroscopy (×10 magnification). Extraction was performed for 6 h at 4°C in the dark. Gut fluorescence was then measured in the same way as for copepods. At each station, "Blank guts" (Bgut) were set by emptying the guts of ten randomly selected larvae with a dissecting forceps. Larval gut content (Gfish, ng chl *a* eq ind^{-1}) was estimated from the total amount of pigments (chl *a* and phaeopigments) in the gut after subtracting Bgut.

Ingestion rates (Ifish, ng chl *a* eq ind^{-1} d^{-1}) were calculated from Gfish according to the Eq.6:

$$Ifish = Gfish \times GER \qquad \text{Eq.6}$$

where GER is gut evacuation rate (d^{-1}). Given the difficulty to maintain larval herring onboard after collection, we did not directly measure GER. Instead, we used a GER value of 40 min^{-1} obtained from the conversion of hourly values of 0.667 h^{-1} (Fossum, 1983) and 0.706 h^{-1} (Pedersen, 1984) reported for herring larvae (8 to 40 mm) feeding on zooplankton prey at temperature ranging from 6-9°C.

In 2014, daily ration and respiration rate were calculated to evaluate their daily metabolic needs of Downs herring larvae.

Ingestion rate was converted to carbon ($Ifish_C$, µgC ind^{-1} d^{-1}) using a C/chl *a* ratio of 50 (Banse, 1977). Larval body carbon content (µgC ind^{-1}) was estimated from the length-weight specific relationship of Hufnagl and Peck (2011) and converted to carbon assuming that carbon is 44.5% of dry weight (Arrhenius and Hansson, 1996). Larval daily rations (DR, % body C d^{-1}) was estimated from the ratio of carbon ingestion to larval body carbon content. Ingestion rates were compared to estimates of basic respiratory requirements for fish based on the empirical relationship linking dry weight to respiration rates (De Silva and Tytler, 1973). Oxygen values were converted into respiratory carbon (Rfish, µgC ind^{-1} d^{-1}) after calculating respiration rates (µLO_2 ind^{-1} d^{-1}) and assuming a respiratory quotient of 0.8 (Tytler and Calow, 2012).

4. **Larval condition**

Four indices were used to characterise the larval condition of Downs herring larvae: feeding indice based on ingestion rate (see chap. I section 3.2.2), molecular and biochemical indices (RNA/DNA and DNA/C ratios) and a growth index derived from otolith microstructure analysis (Figure 5).

4.1.RNA/DNA and DNA/C ratios

RNA/DNA ratio was measured in 2014 (14 stations) on larvae kept in ethanol and 2015 (12 stations) on larvae kept in liquid nitrogen and DNA/C ratio was measured in 2015 (12 stations) on larvae kept in liquid nitrogen (Figure 4 and 5). 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 were 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 (48h 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, d^{-1}) accounting for spatial variation in seawater temperature (Eq. 7):

$$G_i = 0.0145 \times RD + 0.0044 \times (sRD \times T) - 0.078 \quad \text{Eq.7}$$

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).

4.2.Otolith microstructure

Sagittal otoliths of larvae kept in liquid nitrogen were used for the analysis of micro-growth increments on 12 stations in 2015 (Figure 4 and 5). They were extracted using fine needles and a microscope equipped with polarized light and then mounted in Crystal Bond® thermoplastic cement on slides. The otoliths were then polished using a 0.05-3 µm micro-abrasive discs (LP Unalon®). They were examined from oil-immersion at magnification of 126 using an optical microscope (Olympus BX51). Otolith diameter (DO, µm) micro-increments number (Ninc) and mean micro-increments width (MWI, µm) from the central zone (*nucleus*) to the edge of the otolith along the longest *radius* (Campana et al., 1987; Figure 6) were performed using the TNPC 7.0 software (www.tnpc.fr). Position of the check corresponding to the complete absorption of the yolk-sac (Fox et al., 2003; Geffen, 1982; Høie et al., 1997; Figure 6) was determined on the *radius* from the *nucleus*. MWI was calculated for each larva as a proxy of individual growth from the check to the otolith edge. Growth rate (mm d^{-1}) was estimated by a linear regression between larval length (TS) and Ninc. Micro-increments which were smaller than 1 µm were used to identify slow-growing larvae (Feet et al., 2002; Folkvord et al., 2000).

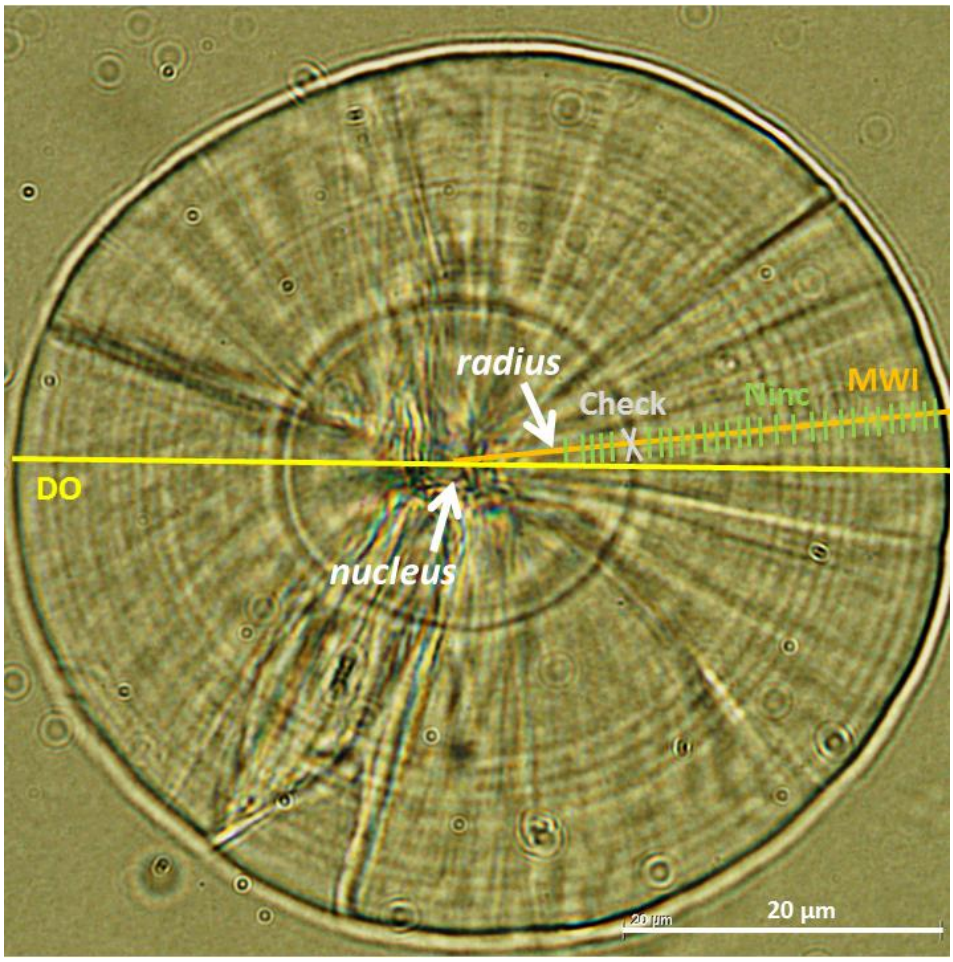

Figure 6: Optical photomicrograph of a sagittal otolith from a 29 days-old Downs herring larva. Otolith diameter (DO, µm) and location of the check, micro-increments (Ninc) and mean increments width (MWI, µm) from the central zone (*nucleus*) to the edge of the otolith along the longest *radius* are represented.

5. **Database**

Data coming from the gut contents and larval condition analyses were stored in the access database dedicated to the hydrological part of the IBTS. Data were uploaded into multiple tables using appropriate forms that were created during this PhD work. These tables were connected using primary keys (Annexe I) and crossed data were extracted using adapted requests. These data were finally exported in statistical softwares for mapping and data analysis.

6. **Spatial distribution mapping**

Length distribution, diet composition, vacuity and ingestion rates and feeding condition indices (growth rates, instantaneous growth rate and DNA/C ratios) of herring larvae, the

protists groups and of the dominant zooplankton were mapped using the *mapplots* package as there was no sufficient data for kriging interpolation.

Hydro-biological parameters (seawater temperature, salinity, bathymetry, sea-bed stress, total suspended matter, nutrients and chl *a* and phaeopigment concentrations) were mapped for each year using geostatistics (Legendre and Legendre, 2012) of the *gstat* (Pebesma, 2004) package of the R software.

Geostatistics include several methods for analysing spatial data using a correlogram or a variogram. The variogram (Figure 7) is a structural function that summarizes how the variance of a variable changes according to the distance between measurement points of this variable. Spatially-structured data have a variance that increases with greater distance separation. The range is the limit of the spatial dependency hence the distance after which data are spatially independent. At this point, the variogram reaches a maximum called the sill. Positive intersection of the variogram on the y-axis, called the nugget, represents the small-scale variance and can be due to measurement errors and variation on a scale smaller than the sampling scale.

The experimental variogram (y(d)) was calculated from the data (y(d); Legendre and Legendre, 2012) using the following Eq.8:

$$y(d) = \frac{1}{2\,W(d)} \sum_{i=1}^{W(d)} (y_i - y_{i+d})^2 \qquad \text{Eq.8}$$

where d is the distance measurement between two points taken in a specified direction only, i is the coordinates vector and W(d) is the class of distance d.

A model of variogram (called the theoretical variogram) was then calculated and adjusted automatically to the experimental variogram.

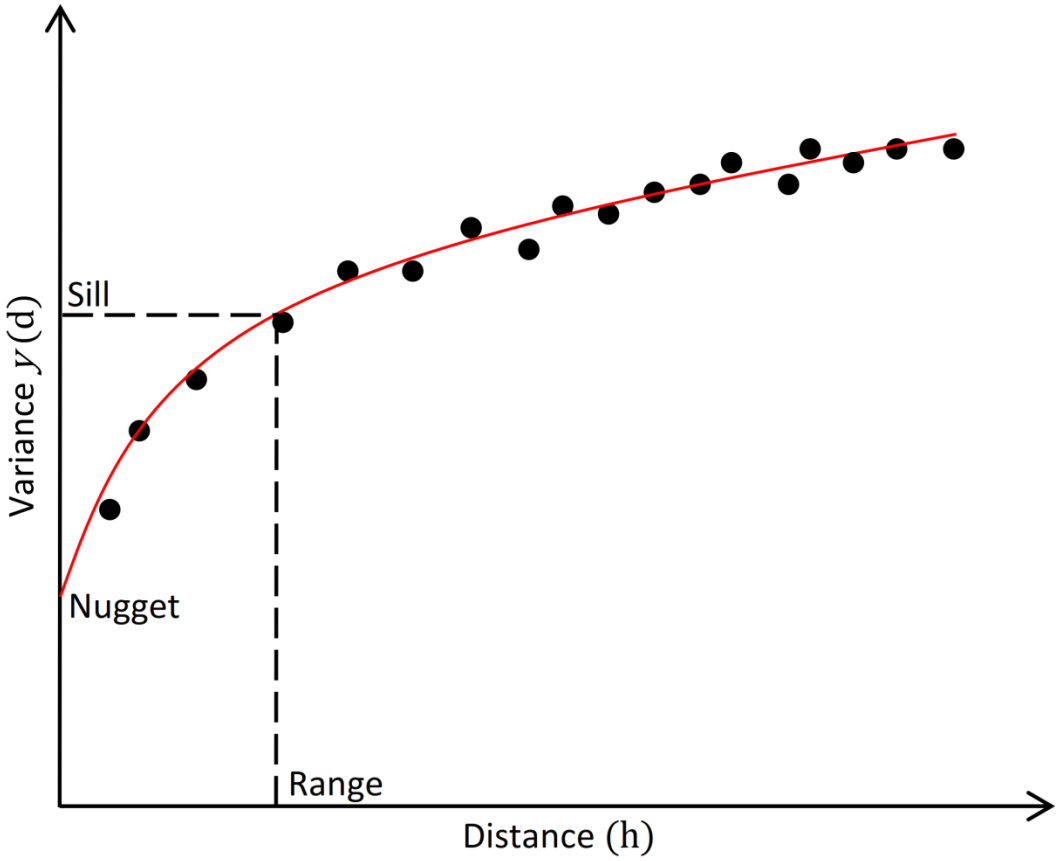

Figure 7: Representation of the experimental (black) and theoretical (red) variograms and associated parameters modified from Legendre and Legendre (2012).

The theoretical spherical model was adjusted using the least-square regression method (Webster and Oliver, 2007). This theoretical variogram was then used to estimate z(x), i.e. abundance at the location x, on the mesh of a regular grid by using the ordinary kriging interpolation method. This is a weighted average method based on the variogram model to predict the value of the variable studied at unsampled points (Legendre and Legendre, 2012). A mesh size of 0.15 decimal degrees was chosen for the interpolation grid.

7. **Statistical analyses**

7.1. Univariate analyses

Normality and homoscedasticity of the environmental and biological 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 and CHI^2 tests) were then used to assess spatial differences in larval distribution, growth rates, instantaneous growth rate and DNA/C ratios and ingestion rates. The statistical analyses were performed using the *Stats* package.

7.2.Multivariate analyses

7.2.1. Unconstrained analyses

Unconstrained analyses belong to gradient analysis methods where a matrix of explanatory variables (X) is linked to the response matrix (Y) by projecting X in the space of Y. Detrended Correspondence Analysis (DCA), Principal Component Analysis (PCA) and Correspondence Analysis (CA) are unconstrained analyses.

A DCA was used to measure the length of the gradient in the ecological data in order to determine which type of analysis among PCA and CA should be applied. The principle of the DCA is to separate the first axis in several segments in the same scale as the standard deviation (SD), the maximum length of the DCA gradient is then an approximation of the length of the ecological gradient. It is recognized that for short gradients (< 3 SD) the linear model is a good approximation and a PCA or its constrained version (Redundancy Analysis, RDA) should be used, whereas along larger gradients (> 4 SD), the unimodal model is better suited (Legendre and Legendre, 1998; Ter Braak, 1986) and then a correspondence analysis or its constrained version (Canonical Correspondence Analysis, CCA) (Ter Braak, 1994; Ter Braak and Smilauer, 1998) should be used.

A PCA was performed on the hydro-biological parameters (temperature, salinity, bathymetry, sea-bed stress, chlorophyll concentration, suspended matter and nutrients) to study how the environmental conditions of the English Channel and North Sea varied in time and space. Hydro-biological parameters were used as the variables (columns, n = 7) and stations from 2008 to 2015 were used as the observations (rows, n = 534). In PCA, the variables are summarized in main components. The relationship between two variables is measured by the linear correlation coefficient (Pearson). A classification of observations is carried out on the principle that two observations are alike if they possess close values for all variables. The distance between two observations is based on the Euclidean metric. The role of variables and observations in structuring axes is determined with the relative contribution to the total inertia of the axes (%), the cosine-square values (between 0 and 1) and r correlation coefficients. The data of hydro-biological parameters were log-transformed (log + 1) to reduce the asymmetry of the distribution and centered and reduced per years.

A Hierarchical Classification Analysis (HCA) was finally performed on the first two axes (explaining at least 60% of total inertia) of the PCA performed on the hydro-biological parameters to determine areas with similar conditions. The Euclidean distance was used for the HCA and the stations were grouped according to the Ward criterion. A spearman

correlation coefficient was used to determine the number of significant groups, by comparing the original matrix of the distances to the binary matrix calculated from the different dendrogram levels. The number of groups was selected from the highest correlation (Borcard et al., 2011).

A CA was used to study the variability of diet composition across the years (2008-2014) according to the larval size. The CA was applied on the occurrence of the different prey found in the larval guts for all years between 2008 and 2014. Larval lengths were used as the descriptors (columns, n = 14) and prey were used as the individuals (rows, n = 39). The CA uses the chi-square metric that weights each gap between rows and columns taking into account the total number of columns and rows. The role of descriptors and individuals in structuring the axes is determined by analysis of the relative contributions to the total inertia of the axes (%), the cosine-square values (between 0 and 1) and r correlation coefficients.

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

7.2.2. Constrained analyses

In constrained analyses, Y is constrained by X in order to determine how much of the variability in Y can be explained by X. This is performed by firstly regressing Y on X and then by performing an unconstrained analysis of the regressed response (Ter Braak and Smilauer, 1998). RDA and CCA are the constrained version of PCA and CA.

A CCA was performed to explore the relationship between herring larval length assemblages and hydro-biological, spatial and temporal parameters. The CCA is the equivalent of the RDA when the gradient is unimodal. The CCA was performed on a matrix of 13 columns corresponding to the various larval lengths (7-20 mm) and 297 rows corresponding to the stations. Twelves co-variables including hydro-biological (seawater temperature, salinity, bathymetry, total suspended matter, dissolved nutrients, chl *a* and phaeopigment concentrations and sea-bed stress), spatial (latitude, longitude, distance to the spawning ground and to the coast) and temporal (years) parameters were used. A HCA based on the first two CCA axes (explaining at least 40% of total inertia) was finally performed to identify groups of larvae with similar condition. The co-variables were centred and reduced before analyses. Significant co-variables were selected through forward selection using a Monte-Carlo permutations test (n=999; Borcard et al., 2011).

A RDA was performed to explore how much of the variability in the larval condition could be explained by environmental, spatial and biological factors. On the 180 larvae

analysed, otolith reading was not possible for 15 because of malformed otoliths, 165 larvae for which the four indices were available. A matrix of four columns corresponding to the four larval condition indices and 165 rows corresponding to the analysed larvae was used. Eight co-variables were used as environmental (seawater temperature, salinity and chl *a* and phaeopigment concentrations), spatial (latitude and longitude) and biological (larval and metazooplankton abundance and larval length) factors. A HCA based on the first two RDA axes (explaining at least 60% of total inertia) was finally performed to identify groups of larvae with similar condition. The data and co-variables were centred and reduced before analyses. Significant co-variables were selected through forward selection using a Monte-Carlo permutations test (n=999; Borcard et al., 2011). Once significant co-variables have been selected, a variation partitioning analysis followed by a permutations test (Legendre et al., 2011) were performed to estimate the contribution of each co-variable to the total variation.

The CCA, RDA and variation partitioning were performed using FactoMineR (Lê et al., 2008) packages of the R software.

CHAPITRE II
Caractérisation environnementale et géographique de la distribution spatiale des larves de hareng des Downs en hiver

L'objectif de ce chapitre est de présenter les conditions abiotiques (température et salinité de surface, tension de cisaillement des fonds marins, matière en suspension, sels nutritifs et la concentration en pigments chlorophylliens) et biotiques (protistes et métazooplancton) dans lesquelles vivent les larves du hareng des Downs en Manche-Mer du Nord pendant la période hivernale. Pour cela, la distribution spatio-temporelle des conditions environnementales et des larves de hareng entre 2008 à 2015 a été cartographiée (voir chap. I, section 6 pour la méthode) à partir des données de la campagne IBTS (voir chap. I, section 1) et les relations entre l'environnement et les larves ont été étudiées à partir d'analyses multi-variées (voir chap. I, section 7.2).

1. Résultats

1.1. Caractérisation hydrobiologique de la Manche-Mer du Nord

L'analyse en composantes principales (ACP) et la classification ascendante hiérarchique (CAH) réalisées sur les conditions environnementales abiotiques ont permis d'identifier deux groupes de stations distribuées le long de l'axe I de l'ACP (Figure 8A et B). Le premier groupe, situé sur la partie positive de l'axe I (39,65%), englobait les stations principalement localisées au large de la zone d'étude (Figure 8C) et caractérisées par des valeurs plus élevées (Figure 8B) de température de surface (3,5 à 11,1°C; r = 0,77), de salinité (30,7 à 35,4; r = 0,85) et de profondeur (20 à 100 m; r = 0,75). Le deuxième groupe, situé sur la partie négative de l'axe II, était constitué des stations situées le long des côtes et influencées par les principaux estuaires (Baie de Seine, Trois Estuaires, Escaut, Tamise). Ces stations étaient caractérisées par des valeurs de pigments chlorophylliens (16,7 à 106,8 $\mu g.L^{-1}$; r = -0.44), de matière en suspension (22,2 à 82,8 $\mu mol.L^{-1}$; r = -0,31) et de sels nutritifs (1,1 à 14,3 $\mu g.L^{-1}$; r = -0.71) plus élevées (Figure 8B). L'axe II (17,29%) de l'ACP séparait dans sa partie positive la Manche Orientale et la baie sud de la Mer du Nord et dans sa partie négative la partie Nord du Sud de la Mer du Nord avec la tension de cisaillement (r = 0,88) corrélé positivement à l'axe II.

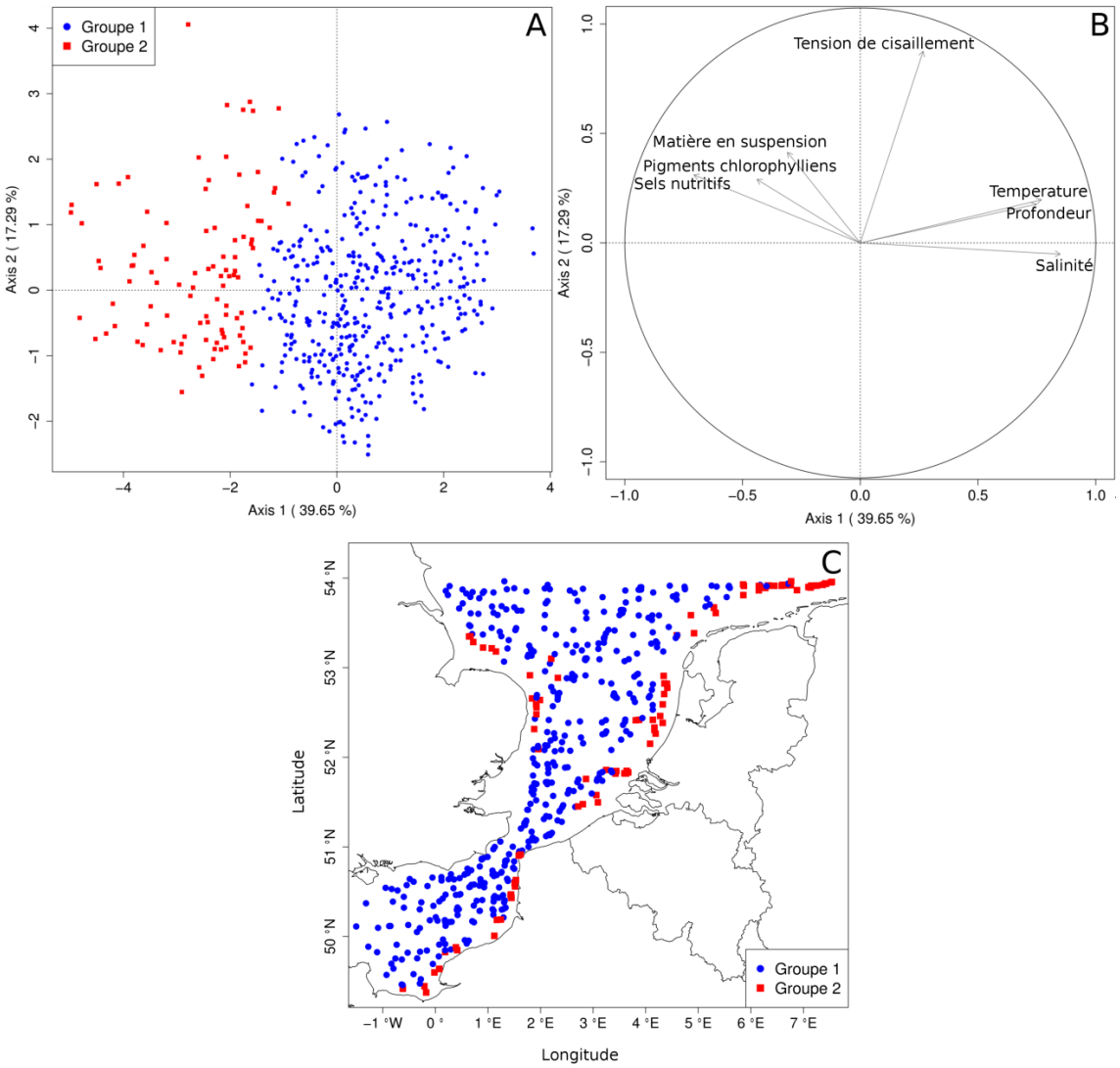

Figure 8: ACP et CAH réalisées sur les paramètres hydrobiologique (température, salinité, tension de cisaillement, profondeur, matière en suspension, sels nutritifs et pigments chlorophylliens) en hiver (mi-janvier-mi-février) des années 2008 à 2015. A) Nuage des observations (stations) dans le plan des deux premiers axes de l'ACP. Les deux groupes de stations obtenus par la CAH sont indiqués. B) Cercle des corrélations des deux premiers axes de l'ACP. C) Répartition géographique des deux groupes de stations obtenus par la CAH.

Le modèle de distribution des paramètres hydrobiologiques au sein des deux groupes de stations identifiés se retrouvait en général chaque année (Figure 9). Les valeurs de matière en suspension, de sels nutritifs et de pigments chlorophylliens étaient les paramètres qui variaient le plus d'un point de vue inter-annuel. Les années 2009, 2014 et 2015 étaient marquées par des valeurs en matière en suspension plus élevées au niveau de l'estuaire de la Tamise. Des concentrations plus élevées (4,1-95,4 µmol.L^{-1}) en sels nutritifs ont été observées de 2011 à 2014 depuis la Baie de Seine jusqu'aux côtes hollandaises. Enfin, les années 2008 à 2010 et l'année 2014 montraient des concentrations plus élevées (de 0,23-14,29 µg.L^{-1}) en pigment chlorophylliens au niveau de l'estuaire de l'Escaut.

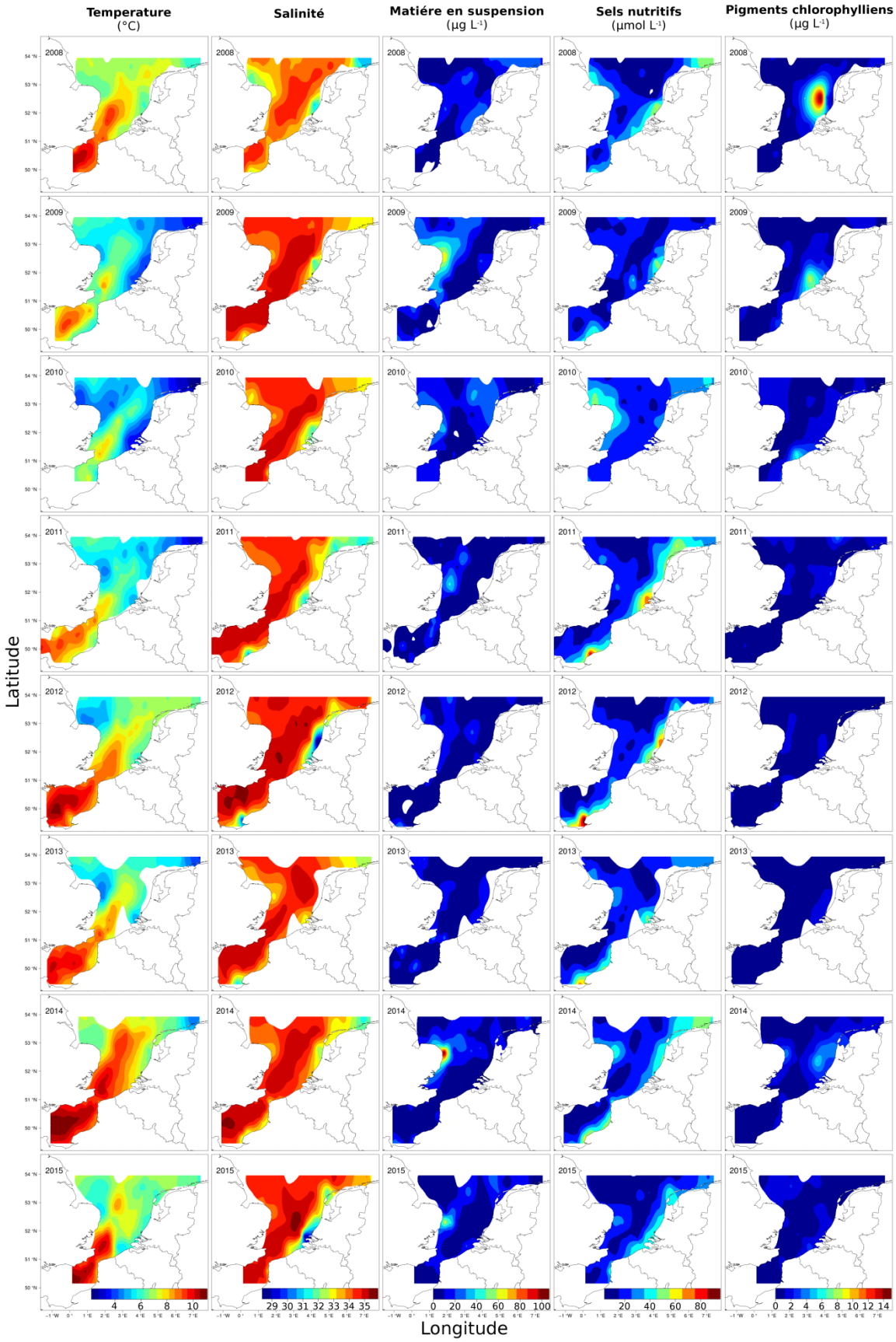

Figure 9: Distribution spatiale des paramètres hydrobiologiques (température et salinité de surface, matière en suspension, sels nutritifs et pigments chlorophylliens) pendant l'hiver (mi-janvier-mi-février) dans la Manche-Mer du Nord de 2008 à 2015.

1.2.Distribution spatio-temporelle des protistes et du métazooplancton

Entre 2008 et 2015, les diatomées dominaient en termes de nombre d'espèces sur l'ensemble des stations (Figure 10). Les silicoflagellés, les flagellés et les dinoflagellés étaient également présents dans la plupart des stations, surtout de 2008 à 2010 et en 2013. La répartition spatiale variait peu entre les années et montrait un nombre d'espèces compris entre 7 et 17 par station. Seules quelques stations de 2009 à 2013 présentaient un faible nombre d'espèces (entre 7 et 10 espèces), et étaient localisées le long des côtes près des estuaires de la Seine, de la Tamise et de l'Escaut.

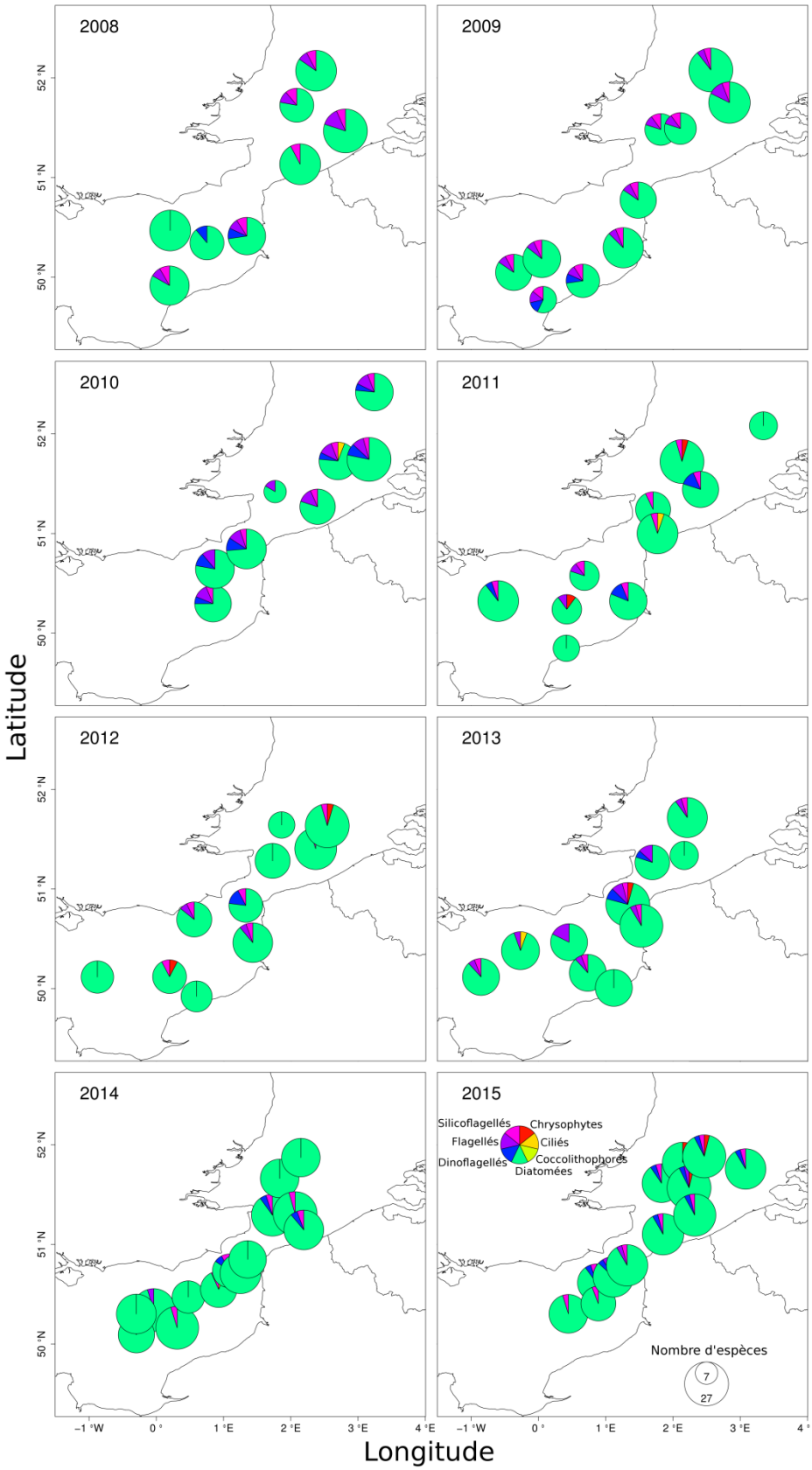

Figure 10: Distribution spatiale des différents groupes de protistes pendant l'hiver (mi-janvier-mi-février) dans la Manche-Mer du Nord de 2008 à 2015. Les camemberts représentent la composition des protistes selon les différents groupes et la taille des cercles est reliée au nombre d'espèces identifiées au sein de chacun des groupes à chaque station.

Entre 2008 et 2015, la répartition spatiale de l'abondance totale du métazooplancton était très structurée aussi bien en Manche Orientale qu'en baie sud de la mer du Nord (Figure 11). L'abondance du métazooplancton était plus élevée (entre 1200 à 5542 ind.m^{-3}) le long des côtes près des estuaires français (Somme, Authie et Canche), de la Tamise et de l'Escaut. La distribution du métazooplancton était caractérisée par des valeurs d'abondances particulièrement plus élevée dans la Mer du Nord (1928 à 5542 ind.m^{-3}). De manière générale, le métazooplancton montrait une abondance totale et une diversité plus élevée en 2014 et 2015 (3634 à 5542 ind.m^{-3}).

Le zooplancton en Manche Orientale et en baie sud de la mer du Nord était principalement constitué de copépodes. Les copépodes *Paracalanus parvus*, *Pseudocalanus elongatus* et *Temora longicornis* étaient les trois espèces de copépodes dominantes en matière d'abondance. *P. elongatus* était plus abondant au large et *T. longicornis* plus abondant à la côte (Figure 11). Les nauplii de copépodes et de cirripèdes étaient plus abondants en 2010 et le copépode *Euterpina acutifrons* était plus abondant en 2014.

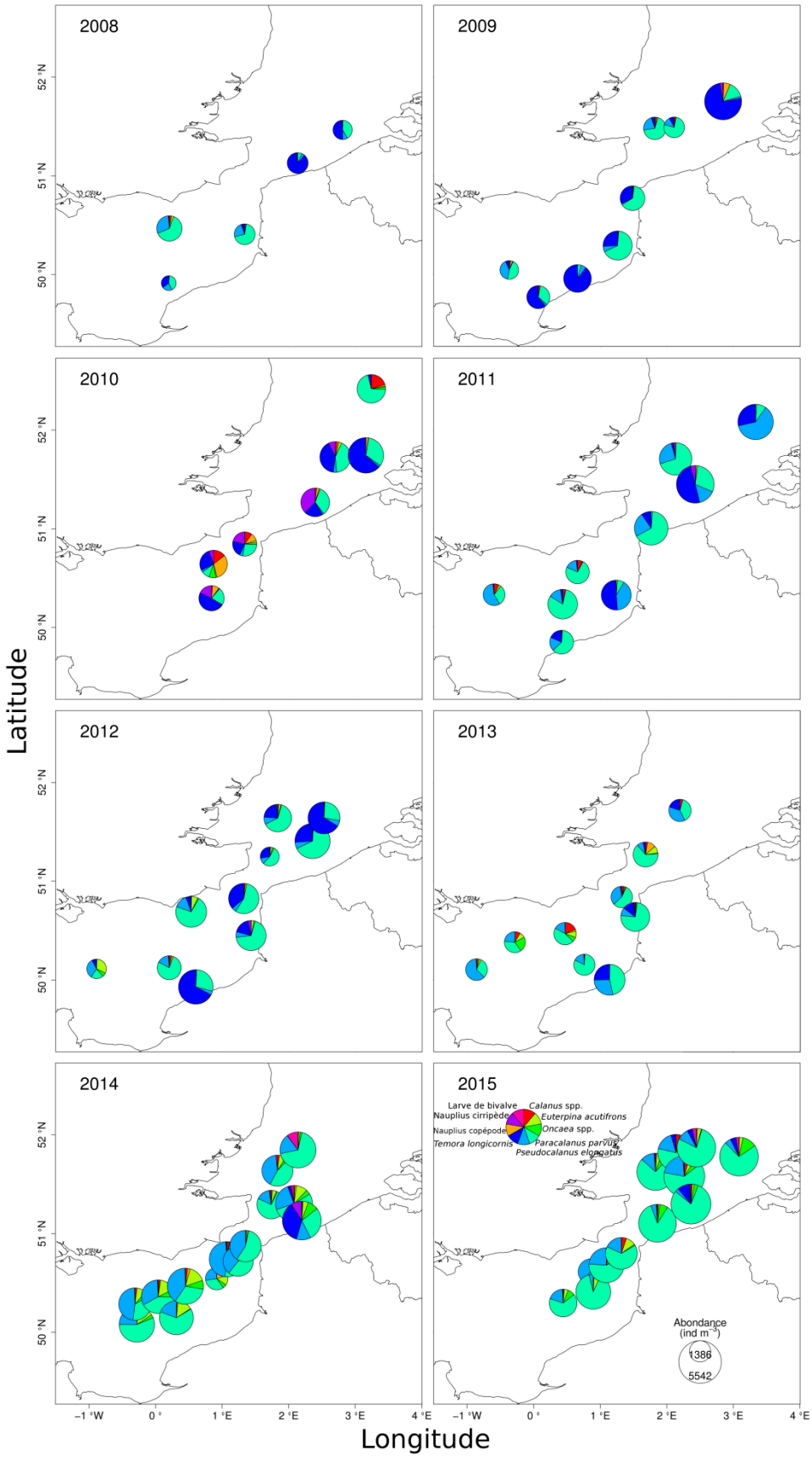

Figure 11: Distribution spatiale de l'abondance (ind.m^{-3}) des principales espèces zooplanctoniques pendant l'hiver (mi-janvier-mi-février) dans la Manche-Mer du Nord de 2008 à 2015. Les camemberts représentent la composition du zooplancton et la taille des cercles est reliée à l'abondance pour chaque station.

1.3.Distribution spatio-temporelle des larves de hareng

Pour l'ensemble des huit années, les larves de hareng de 7 à 12 mm étaient les plus abondantes (Figure 12). Les larves de 7 mm ont été capturées uniquement en 2008 et de 2011 à 2014.

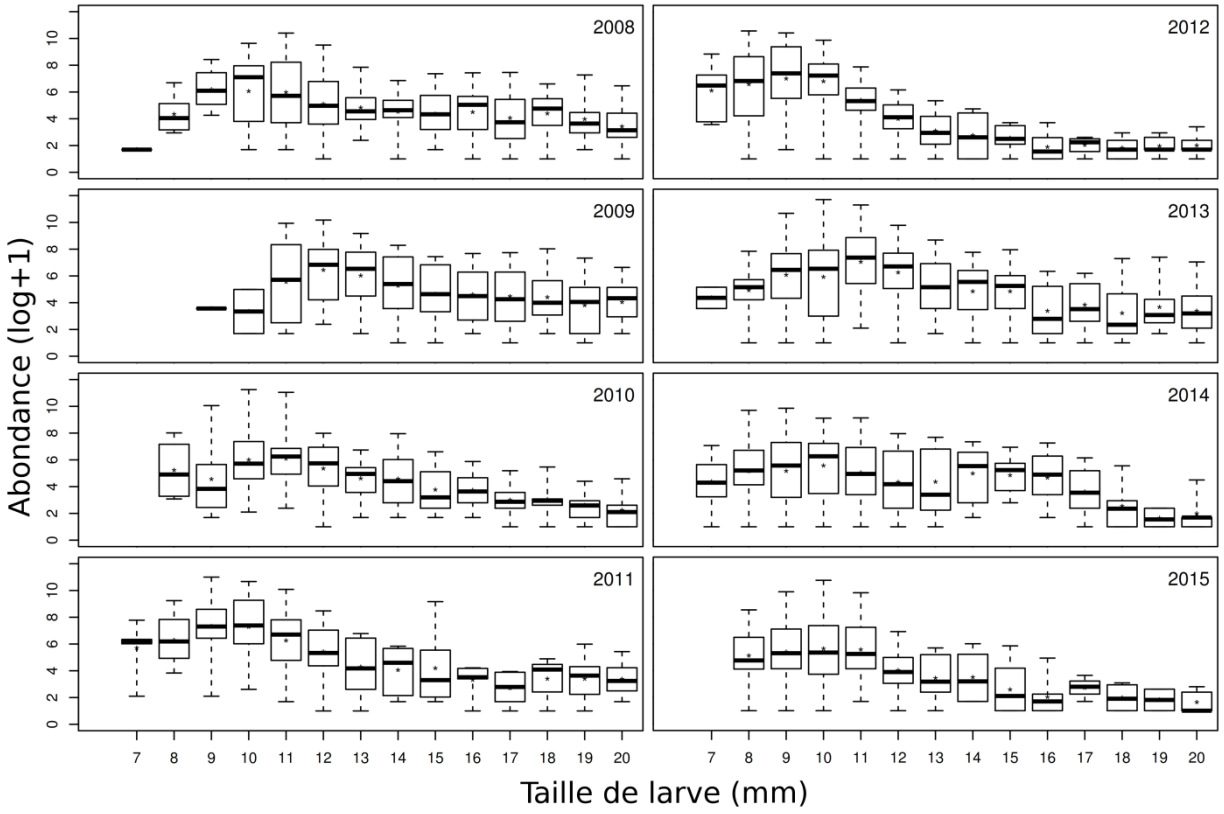

Figure 12: Distributions des abondances (5000.ind^{-1}), selon la taille, des larves de hareng des Downs entre 7 et 20 mm collectées en hiver (mi-janvier-mi-février) entre 2008 et 2015 pendant la campagne IBTS. L'abondance est donnée en échelle logarithmique. La boite à moustache représente de haut en bas, les valeurs maximum, le quartile 75%, la médiane, le quartile 25% et la valeur minimum. Les croix représentent la moyenne.

D'un point de vue spatial, les larves étaient distribuées selon un gradient de taille croissante et d'abondance décroissante depuis la Manche orientale jusqu'à la baie sud de la mer du Nord (Figure 13). De manière générale, les larves montraient une abondance totale plus élevée au large. De 2008 à 2010, les larves étaient plus grandes et des quantités importantes se trouvaient en Manche orientale. De 2011 à 2015, des quantités plus importantes de larves de 7-12 mm ont été observées et étaient principalement distribuées en Manche Orientale et en baie sud tandis que les larves de 13 à 20 mm étaient distribuées uniquement dans la baie sud de la mer du Nord.

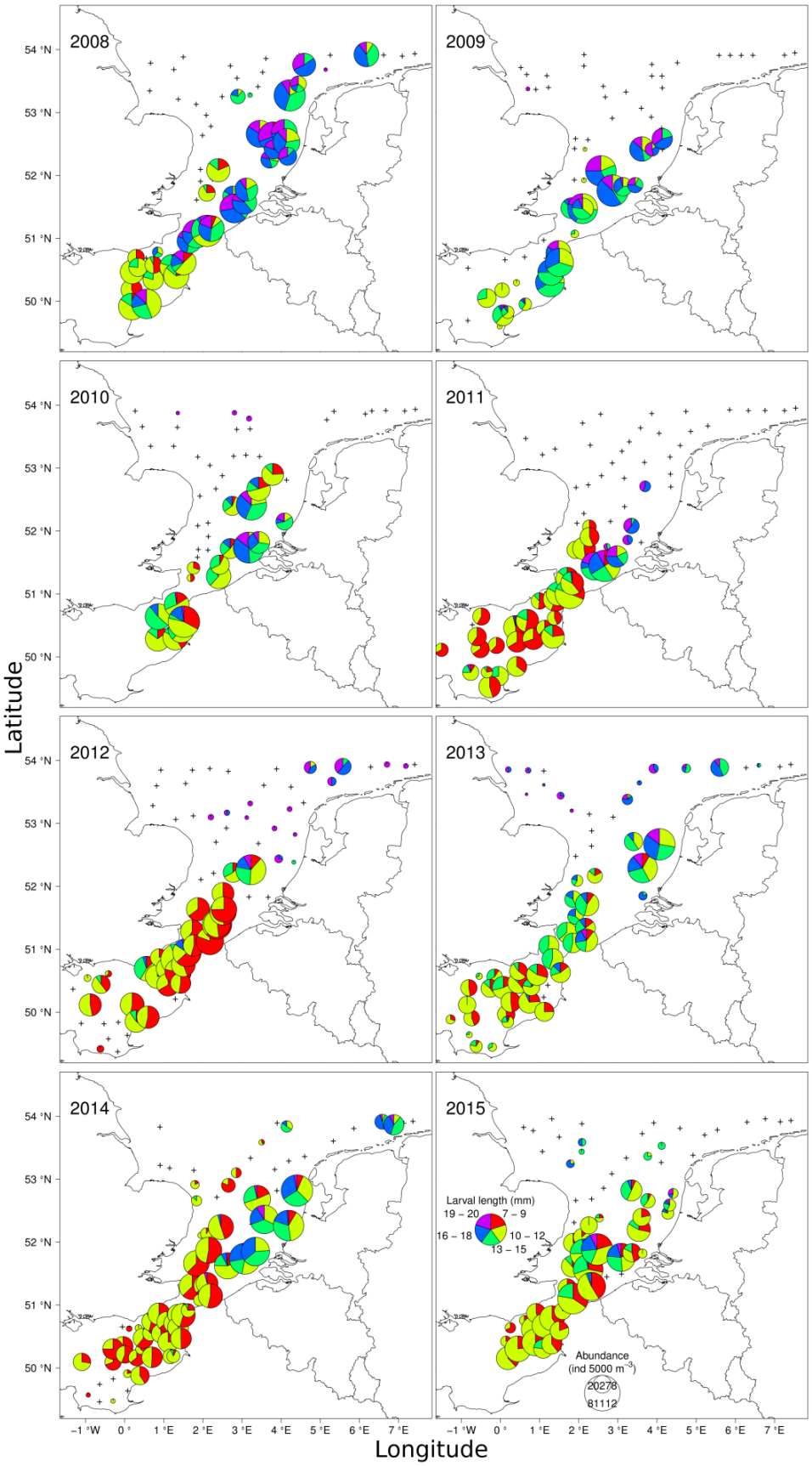

Figure 13: Distribution spatiale de l'abondance des larves de hareng des Downs en fonction de leur longueur (mm) pendant l'hiver (mi-janvier-mi-février) dans la Manche-Mer du Nord de 2008 à 2015. La taille des cercles est liée à l'abondance totale larvaire observée à chaque station. Les croix représentent les stations sans larves.

1.4. Influence environnementale et géographique sur la distribution des larves de hareng des Downs

Sur les 19 variables testées, neuf (température de surface, sels nutritifs, distance aux frayères, la tension de cisaillement et les années 2008, 2009, 2011, 2012 et 2014) ont été retenues (Figure 14) par la CCA. Trois groupes de stations ont été obtenus à partir de la CAH. Ces trois groupes étaient distribués selon la distance aux frayères et la température. Le premier groupe comprenait des stations localisées principalement en Manche Orientale et en baie sud de la mer du Nord. Ces stations étaient les plus proches des frayères et caractérisées par des températures élevées. Les larves de 7 à 12 mm étaient associées à ce groupe et étaient plus abondantes en 2008 et de 2011 à 2014. Le deuxième groupe comprenait quelques stations côtières en Manche et une majorité de stations dans la baie sud de la mer du Nord. Les larves de 13-15 mm étaient associées à ce groupe. Le troisième groupe rassemble les stations situées majoritairement au nord de la zone d'étude et caractérisées par des températures plus faibles. Les larves de 19 à 20 mm, plus abondantes en 2009, étaient associées à ces stations.

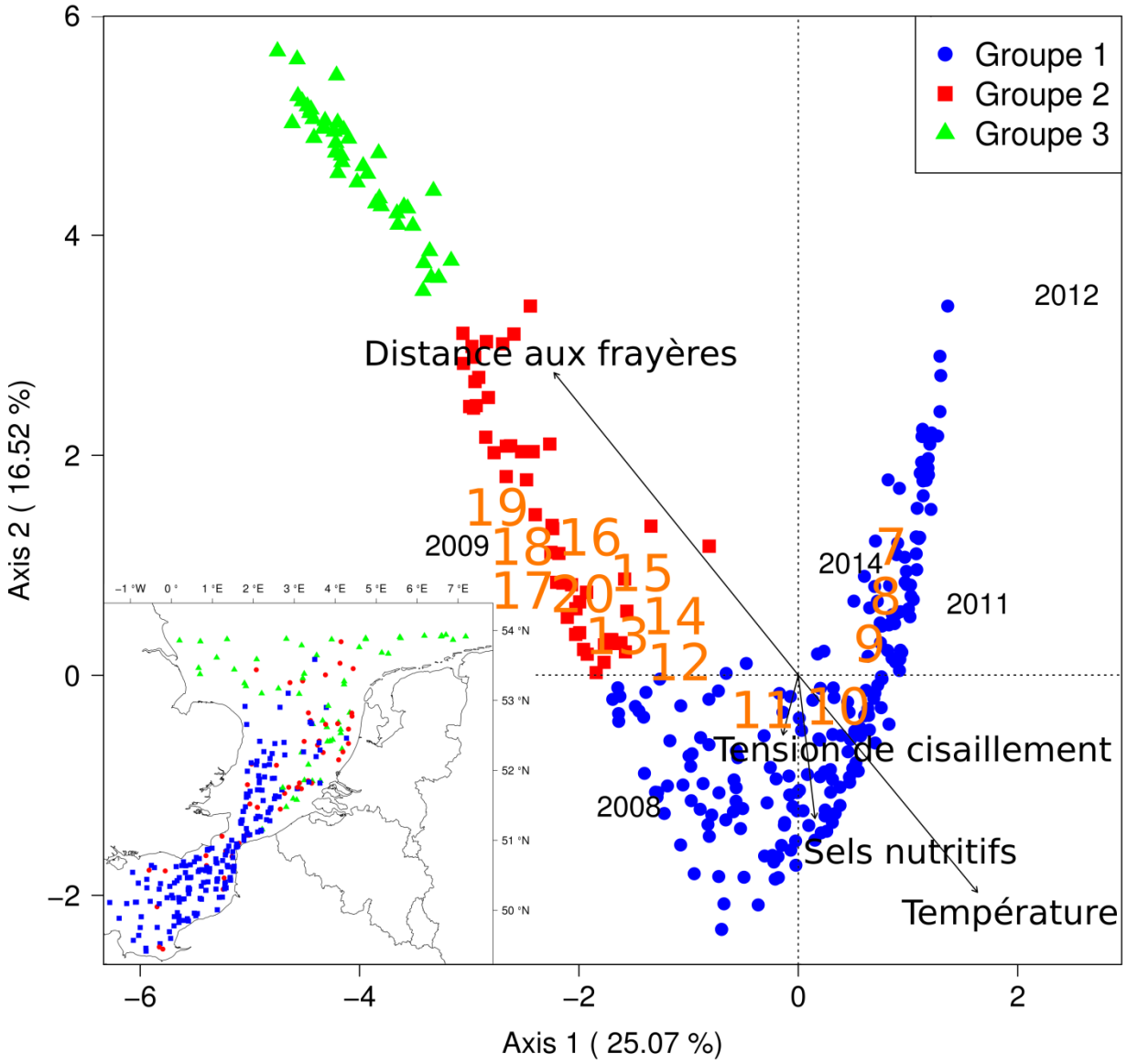

Figure 14: Représentation des deux premiers axes de la CCA réalisée sur les tailles de larves de hareng des Downs de 7 à 20 mm au cours de l'hiver (mi-janvier-mi-février) et contrainte par les paramètres hydrobiologiques, géographiques et les années. Seuls les paramètres et les années retenus sont représentés. Les groupes de stations, déterminés par la CAH, sont indiqués en haut à droite et la répartition géographique des trois groupes de stations, obtenus par la CAH, en bas à gauche.

2. Discussion

La ségrégation spatiale observée entre 2008 et 2015 pendant la période hivernale entre les eaux du large et les eaux côtières selon leurs caractéristiques hydrologiques est en accord avec les études antérieures (Laane et al., 1993; Lancelot-Van Beveren, 1980; Taylor and Stephens, 1983) et récentes (Delavenne et al., 2013). Les eaux du large sont plus chaudes et plus salées contrairement aux eaux côtières qui, elles, sont plus riches (matières en suspension, sels nutritifs, pigments chlorophylliens). Ce schéma de température est l'inverse de celui observé

pendant le printemps et l'été. L'influence marine des eaux Atlantiques sur les eaux du large explique la stabilité interannuelle du modèle de distribution de la température et de salinité observée pour ces eaux. L'influence plus terrestre des eaux côtières par le débit des fleuves au niveau des estuaires explique quant à elle la plus grande variabilité interannuelle observée pour les autres paramètres qui les caractérisent tels que les pigments chlorophylliens, la matière en suspension et les sels nutritifs (Lancelot-Van Beveren, 1980; Pätsch and Radach, 1997; Schlüter and Jerosch, 2009; Van Bennekom and Wetsteijn, 1990). Les concentrations hivernales en pigments chlorophylliens observées entre 2008 et 2015 étaient plus faibles comparées à l'été et au printemps (Delavenne et al., 2013; Lancelot-Van Beveren, 1980).

Entre 2008 et 2015, les larves de hareng des Downs de 7 à 20 mm étaient distribuées suivant un gradient spatial de taille croissante et d'abondance décroissante depuis la Manche orientale vers le sud de la mer du Nord. Ce gradient correspond au schéma de dérive du courant résiduel de marée depuis les frayères situées sur les zones de fucus et de graviers (Geffen, 2009; Maucorps, 1969) en Manche Orientale et en baie sud de la mer du Nord (Corten, 2013, 1986; Heath, 1993; Heath et al., 1997; Maucorps, 1969) jusqu'aux nourriceries côtières au sud-est de la mer du Nord (Dickey-Collas et al., 2009; Heath et al., 1989; Rockmann et al., 2010). En Manche, le courant résiduel de marée est orienté du sud-ouest vers le nord-est (Salomon and Breton, 1991) et est constant entre les années même si des inversions ponctuelles peuvent se produire en période de forts vents de nord-est (Salomon and Breton, 1991). Cette constance du courant résiduel de marée explique la faible variabilité interannuelle observée dans le modèle de distribution des larves selon leurs tailles. La seule différence notable pendant la période étudiée est la présence principalement en Manche orientale entre 2011 et 2015 d'une proportion plus importante de larves de 7 à 12 mm. Si en 2008, le décalage de 10 jours dans les dates de campagne pourrait expliquer cette différence, ce n'est pas le cas pour 2009 et 2010 dont les campagnes ont été réalisées aux mêmes dates que les années suivantes. La température a un effet sur la taille des larves à l'éclosion via l'influence du temps d'incubation des œufs, avec de grandes tailles de larves à de forte températures (Blaxter and Hempel, 1963; Brooke and Colby, 1980; Fairchild and McCormick, 1996; Hempel, 1979; Stratoudakis et al., 1998). En ce qui nous concerne, il apparaît que les années 2008 à 2010 n'étaient pas plus chaudes que les autres années. Il est plus probable que cette différence soit due à un décalage dans la période ponte, qui semble avoir été plus précoce entre 2008 et 2010. Ce décalage pourrait être expliqué par l'influence de la température subie par les adultes reproducteurs l'année précédente lors de la maturation des gonades. En effet, des températures plus élevées, augmentent la vitesse de maturation des

gonades (Winters and Wheeler, 1996) ce qui a pour effet d'avancer la date de ponte (Berenbeim et al., 1977; Messieh, 1978; Ware and Henriksen, 1978; Winters and Wheeler, 1996).

3. Conclusion

En janvier-février, les larves de hareng des Downs inférieures à 20 mm sont principalement distribuées dans les eaux centrales de la Manche orientale et de la baie sud de la mer du Nord. Ces eaux plus chaudes et plus salées offrent un environnement assez stable et reproductible entre les années d'un point de vue de la température, de la salinité et de la dominance des principaux groupes de protistes et de métazooplancton. Le maintien de ces larves dans les eaux du large ainsi que le gradient d'abondance et de taille observé dans la distribution larvaire indique une dérive purement passive suivant le courant résiduel de marée qui est à mettre en relation avec leur jeune stade de développement. Le comportement alimentaire de ces larves de hareng des Downs au cours de la période critique dans des conditions hivernales moins favorables en termes de concentration en proies potentielles va être étudié dans le chapitre suivant.

CHAPITRE III
Feeding strategy of Downs herring larvae: a qualitative approach

Conventionally, the diet of fish larvae is observed using a direct approach based on gut contents analysis (Hyslop, 1980). Whereas most of the studies relied on optical microscopy (Arula et al., 2012; Blaxter, 1965) to determine the prey composition of fish larvae, recent studies (Koubbi et al., 2007; Vallet et al., 2011) used the SEM and demonstrated that OM led to a truncated vision of small larvae (less than 20 mm) diet by ignoring the relevance of smaller prey like protists.

The aim of this chapter is to present and discuss the results obtained from the qualitative approach used to characterise the feeding strategy of Downs herring larvae. Gut contents of Downs larvae from 2008 to 2014 were analysed using SEM (see chap. I section 3.2.1 for the methodology). Diet composition, vacuity rate and prey selectivity of larvae from 8 to 19 mm were assessed from samples collected during the IBTS (see chap. I section 1.4 for the sampling).

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Feeding strategy of Downs herring larvae (*Clupea harengus* L.) in the English Channel and North Sea

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1. Results

1.1. Comparison of the qualitative and quantitative methods

The results obtained showed that the prey were distributed heterogeneously over the entire surface of the stub (Figure 15).

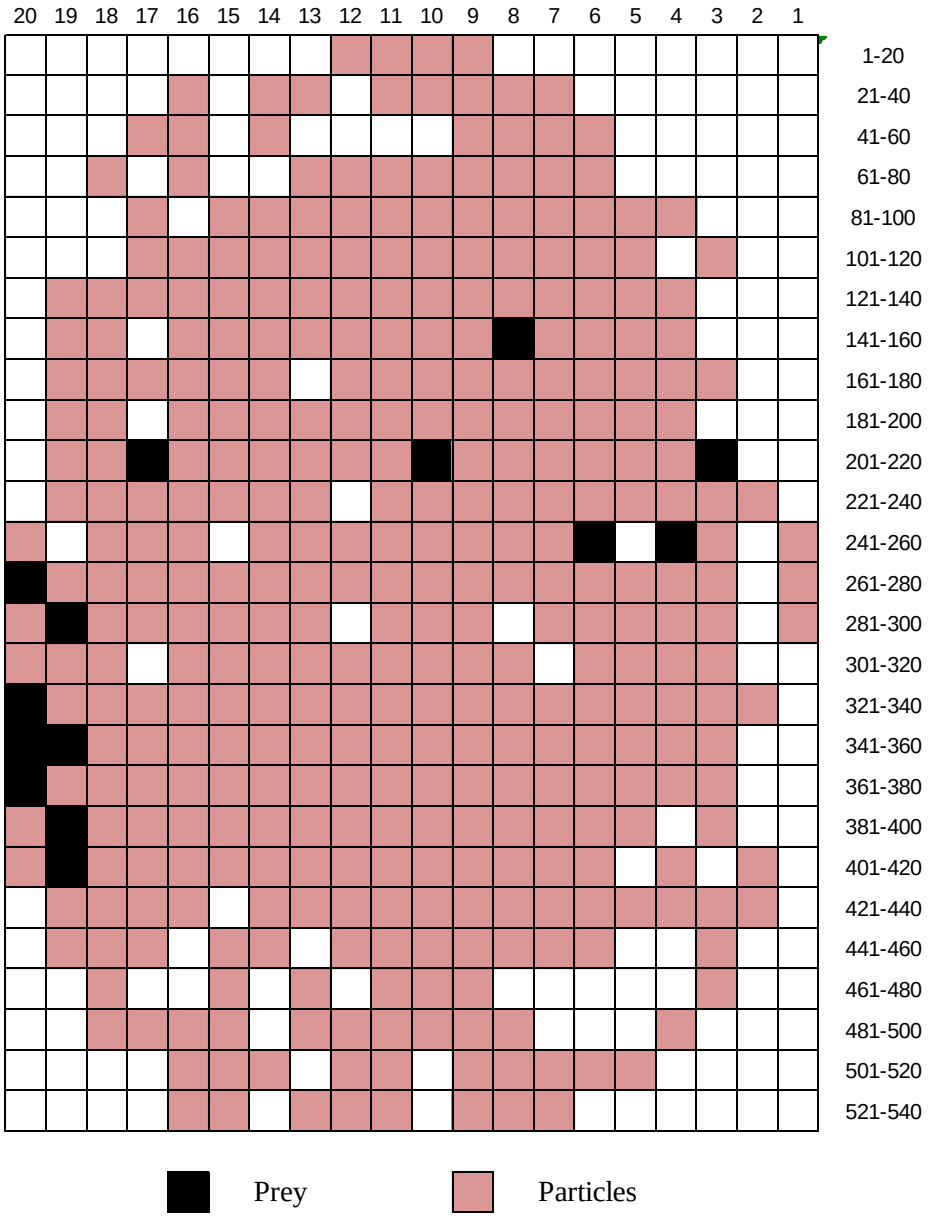

Figure 15: Distribution of prey and particle on the stub surface observed by the SEM quantitative method of the gut contents of Downs herring larvae.

The quantitative method indicated a larger number of prey than the qualitative method but an equivalent diversity. Quantitative analysis of the larval gut contents by SEM requires 3 to 4 times more than the qualitative method (Table 2).

Table 2: Comparison of the number of prey and diversity in the gut contents of Downs herring larvae observed in SEM by a quantitative and qualitative method. The time (minute) for prey observation is also indicated.

Methods	Parameters	Stub N°1	Stub N°2	Stub N°3	Stub N°4	Stub N°5	Observation time
Quantitative	**Number of prey**	3	0	4	14	3	120-180 min
	Diversity	3	0	1	1	3	
Qualitative	**Number of prey**	3	0	2	5	3	30-60 min
	Diversity	3	0	1	1	3	

1.2.Vacuity rate

Of the 375 larvae analysed, 46% were empty. The vacuity rate varied according to larval size (Figure 16). The highest values were observed for larvae of 8 and 13 mm (respectively 65 and 47%). For the other sizes, the vacuity rate was almost constant under 46%.

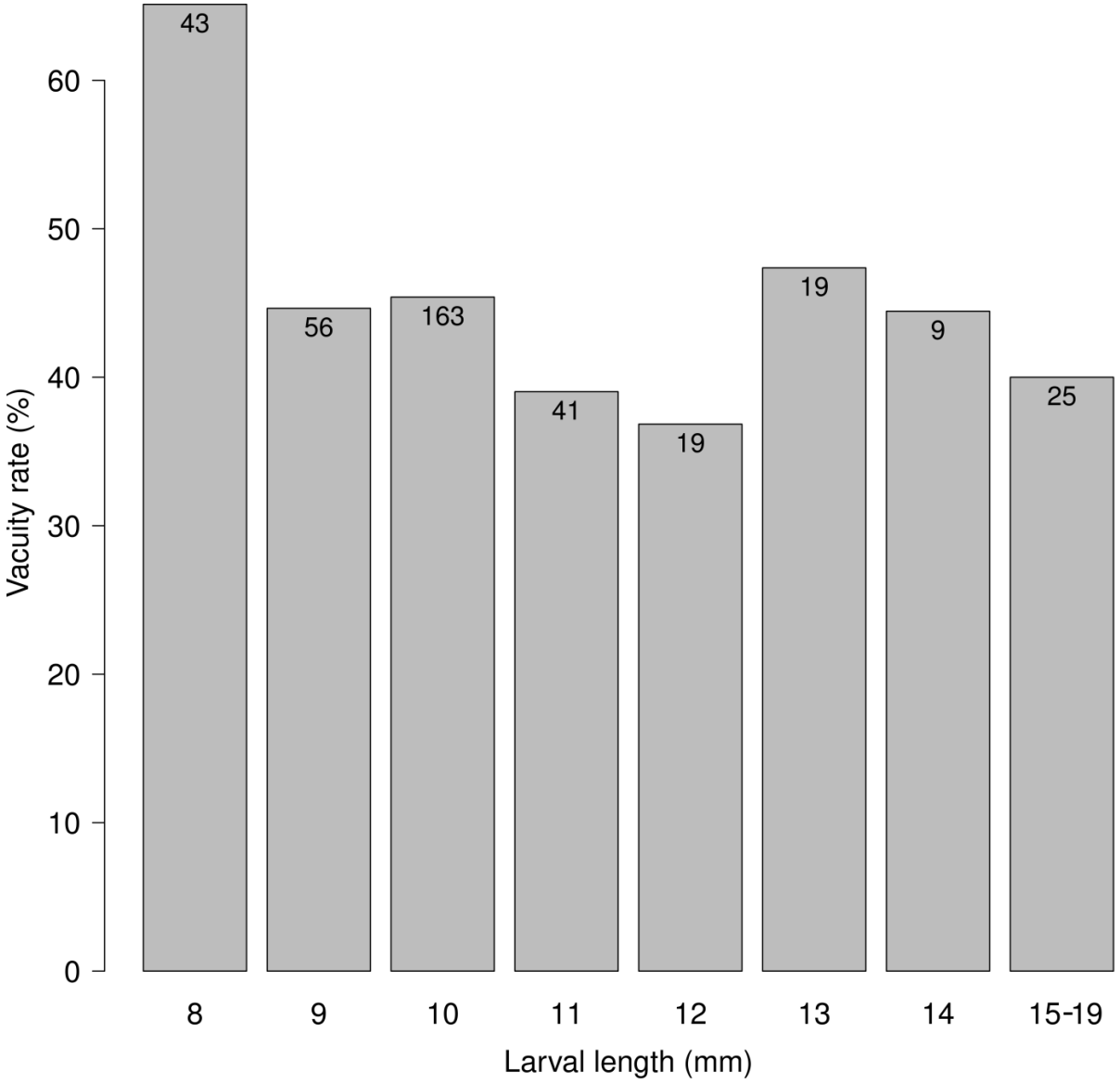

Figure 16: Vacuity rate (%) for Downs herring larvae between 8 and 19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. Number of larvae analysed for each length is also indicated.

The vacuity rate also varied in time and space (Figure 17). It was higher in 2008, 2009, 2011 and 2014 (51-66%) compared to the other years (20-44%). Three areas of low vacuity rate were identified along the three French estuaries, in the middle of the EEC and the Dover Strait (DS) (except for 2011).

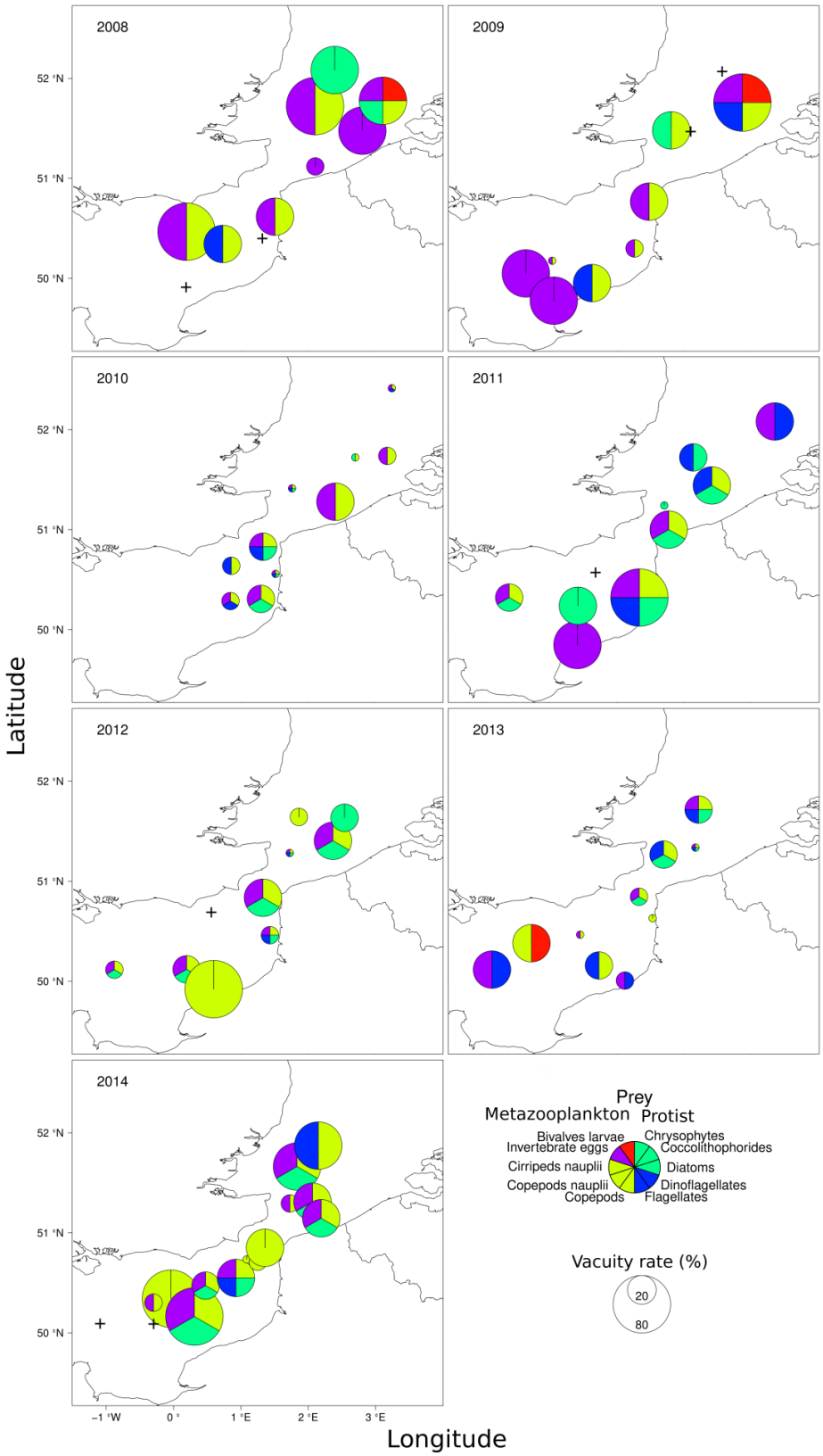

Figure 17: Spatial distribution of prey composition in the larval guts and vacuity rate (%) of Downs herring larvae from 8 to 19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. Pies represent the prey composition and pie size is related to the vacuity rate at each station. Details on each prey group are given in Table 3.

1.3.Feeding strategy

Of the 39 prey taxa identified in the gut contents, 28 were protist (auto- and heterotroph unicellular organisms) and 11 were metazooplankton (Table 3). Around 59% analysed larvae ingested exclusively zooplanktonic prey, 17% ingested protist prey and 24% ingested both. Prey size varied from 3 to 450 µm (Table 3) with the largest sizes for the zooplanktonic prey (8-450 µm) and conversely for protist prey (3-145 µm).

Table 3: Prey composition observed in the guts of Downs herring larvae between 8-19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. Prey size (µm), mean (± SD) values of prey occurrence (*F*), specific-abundance (*P*) and selectivity (*D*) are also indicated.

	Groups	Prey taxa	Abbrevations	Size (min-max)	*F* (%)	*P* (%)	*D*
Protist	Chrysophytes		Chry	9.3	0.8 ± 2.2	14.3 ± 37.8	-
	Coccolithophorides		Cocc	9.4	0.4 ± 1	14.3 ± 37.8	0.44 ± 0.19
	Diatoms	*Chaetoceros* spp.	Chae	10	1.7 ± 4.4	4.8 ± 12.6	-0.06
		Corethron criophilum	C.cri	20	0.5 ± 1.3	14.3 ± 37.8	-
		Coscinodiscus spp.	Cosc	120-144	2.3 ± 2.4	31.4 ± 37.6	0.52 ± 0.08
		Delphineis surirella	D.sur	3.7-4.8	2.4 ± 3.6	26.2 ± 38.3	-
		Minidiscus trioculatus	M.tri	3-4.8	1.9 ± 1.9	19 ± 19.7	0.16 ± 0.15
		Navicula spp.	Navi	2.7-7.7	4.2 ± 4	33.6 ± 34.7	-0.01 ± 0.43
		Nitzschia spp.	Nitz	5-5.2	1.2 ± 2.1	11.9 ± 20.9	-0.43 ± 0.03
		Paralia sulcata	P.sul	16-17.6	0.8 ± 2	4.8 ± 12.6	-0.23
		Plagiogrammopsis vanheurckii	P.van	8-25	0.9 ± 1.6	14.3 ± 24.4	0.38 ± 0.25
		Plagiotropis spp.	Plag	3.8	0.8 ± 2.2	7.1 ± 18.9	0.39
		Psammodictyon panduriformis	P.pan	4.9-13.9	5.9 ± 5.8	34.8 ± 28.9	0.23 ± 0.42
		Raphoneis amphiceros	R.amp	45-120	1.4 ± 3.6	4.1 ± 10.8	-
		Thalassiosira curviseriata	T.cur	3.9-6	2.8 ± 3	37.1 ± 38.6	0.51 ± 0.27
		Thalassiosira spp.	Thal	14.5-110	2.1 ± 3	19 ± 24.4	-0.17 ± 0.18
		Thalassiosira tenera	T.ten	6.5-8.4	2 ± 4.4	17.9 ± 37.4	-0.33 ± 0.12
		Thalassiosira levedenri	T.lev	10.9-15.9	1.3 ± 2.2	21.4 ± 39.3	-
		Unidentified diatoms	Dia.un	20	0.6 ± 1.6	14.3 ± 37.8	-
	Dinoflagellates	*Alexandrium* spp.	Alex	50-60	2.5 ± 6.6	3.6 ± 9.4	-
		Dinophysis acuminata	D.acu	57.5-100	6 ± 9.9	33 ± 37.5	-
		Diplopsalis spp.	Dipl	66-130.7	1.5 ± 2.9	7.5 ± 12.8	-
		Gonyaulax spp.	Gony	45-127	6.3 ± 6.8	17.7 ± 17.3	0.99 ± 0.01
		Prorocentrum lima	P.lim	4.5-10	0.9 ± 1.5	19 ± 37.8	0.60
		Prorocentrum micans	P.mic	30.3-73.7	3.7 ± 4.7	20.5 ± 21.1	0.66 ± 0.18
		Pyrophacus spp.	Pyro	66.5	0.8 ± 0.2	1.8 ± 4.7	0.97
		Unidentified dinoflagellates	Din.un	145	0.4 ± 1	14.3 ± 37.8	-
	Flagellates		Flag	4.5	0.4 ± 0.9	7.1 ± 18.9	-
Metazooplankton	Copepods	*Euterpina acutifrons*	E.acu	110-220	9.3 ± 6.2	42.3 ± 25.8	0.23 ± 0.36
		Oncaea spp.	Onca	74-350	10 ± 10.2	41.1 ± 32.7	0.50 ± 0.35
		Paracalanus parvus	P.par	100-400	22.6 ± 15.4	49.7 ± 10.3	0.45 ± 0.26
		Pseudocalanus elongatus	P.elo	106-370	4.6 ± 2.5	64.3 ± 39	-0.12 ± 0.23
		Temora longicornis	T.lon	200-450	3.1 ± 3.9	39.3 ± 45.3	-0.06 ± 0.46
		Unidentified calanoides	Cal.un	144-199	4.5 ± 6.6	33.1 ± 37.3	-0.05 ± 0.36
		Unidentified copepods	Cop.un	210-229	1.7 ± 2.3	28.6 ± 39.3	-
	Copepods nauplii		Cop.n	60-224.3	22.6 ± 14.7	39.8 ± 8.7	0.72 ± 0.10
	Cirripeds nauplii		Cir.n	60-200	7.7 ± 7.4	43.7 ± 27.5	0.43 ± 0.28
	Invertebrate eggs		Eggs	7.8-163	34.9 ± 14.7	54.4 ± 14.7	0.93 ± -0.04
	Bivalve larvae		Biv.l	96.2-230	1.5 ± 1.9	21.4 ± 36.6	0.27 ± 0.04

Plot of specific-abundance versus prey occurrence showed a large trophic niche indicating that different prey types were consumed (Figure 18). A high inter-individual

variability in the diets (occurrences were very low and specific abundances highly variable) was also observed. Except for several prey like the copepod *Pseudocalanus elongatus*, invertebrate eggs and the copepod *Paracalanus parvus*, it appeared that there was no dominant prey and that Downs herring larvae were mostly generalist feeders. Zooplanktonic prey had on average higher specific-abundance and prey occurrence compared to protist prey. Among the zooplanktonic prey, the copepods *P. elongatus* and *P. parvus* and invertebrate eggs had the highest specific-abundance, respectively 64 ± 39%, 50 ± 10% and 54 ± 15% (Table 2). *P. parvus* and invertebrate eggs also had the highest occurrence (0.23 ± 0.15-0.35 ± 0.15) whereas *P. elongatus* had a low one (0.05 ± 0.03). Nauplii of copepods and cirripeds as well as the copepod *Euterpina acutifrons* had specific-abundance of 40 ± 9%, 44 ± 28% and 43 ± 26%, respectively and nauplii of copepods had an occurrence around 0.23 ± 0.15. Other zooplanktonic prey had specific-abundance lower than 33% and low occurrence (<0.08). Among the protist prey, *Thalassiosira curviseriata*, *Psammodictyon panduriforme* and *Dinophysis acuminata* had the highest specific-abundance but relatively low occurrence (< 0.06). Other protist prey had low specific-abundance (< 34%) and occurrence (< 0.04).

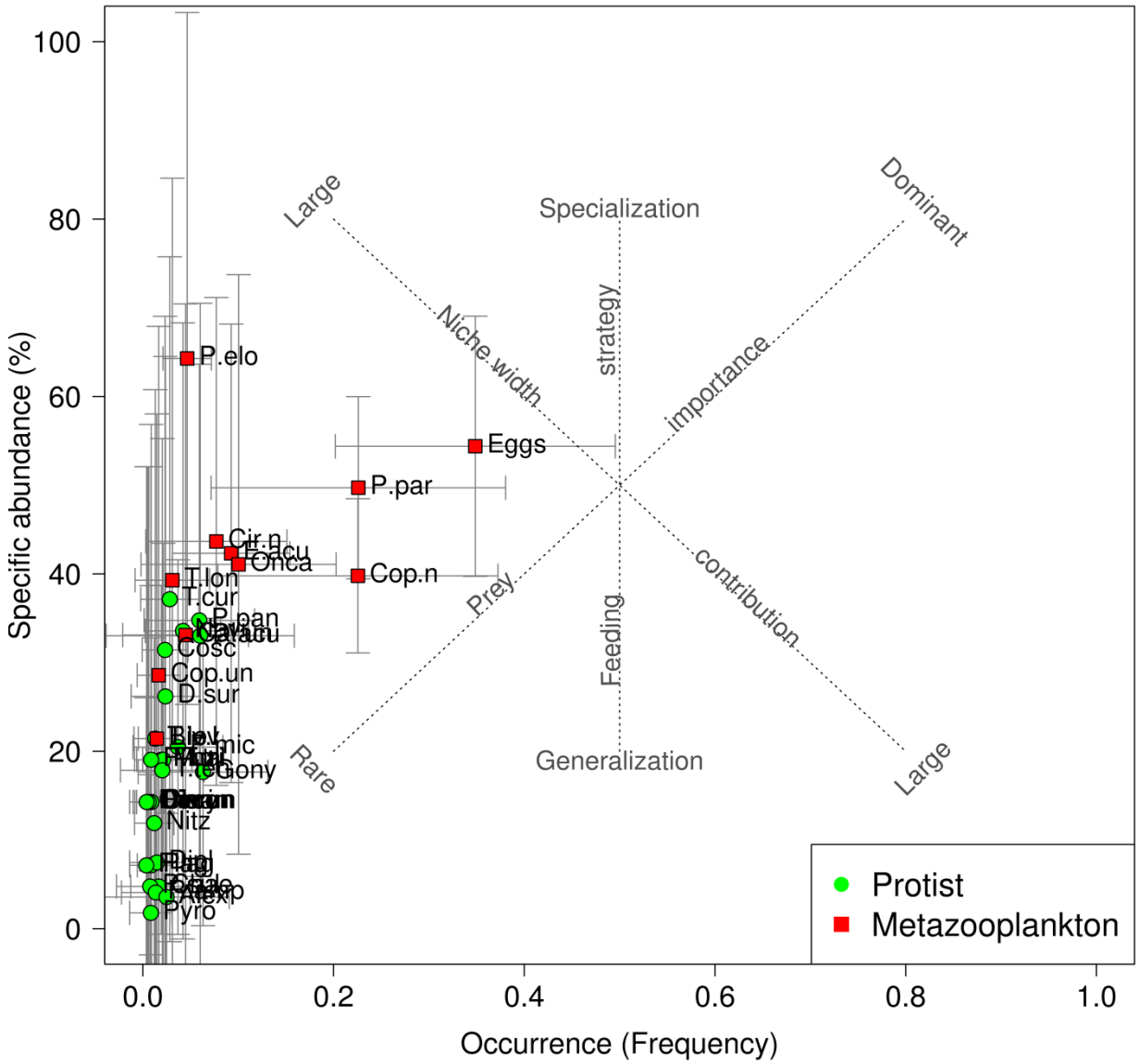

Figure 18: Feeding strategy of Downs herring larvae between 8 and 19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. Specific-abundance and occurrence of each prey observed in the larval guts are plotted as their mean (± SD) for the 2008-2014 period. See Table 3 for the prey abbreviations.

Results of the CA showed that the diet composition varied with larval length (Figure 19). The first and second axes of the CA explained respectively 26.84% and 23.15% of the total variability. The first axis clearly separated three groups of larval sizes: larvae of 8-12 mm, larvae of 13 mm and larvae of 14-19 mm. On the second axis, larvae of 13 mm were clearly separated from other larvae. These three groups of larvae were associated with different prey. Smaller larvae (8-12 mm) were close to a large number of small prey including protists like diatoms (*Coscinodiscus* spp., *Thalassiosira* spp., *Raphoneis amphiceros*, *Plagiotropis* spp. and *Delphineis surirella*), dinoflagellates (*Pyrophacus* spp., *Prorocentrum micans*,

Alexandrium spp., *Dinophysis* spp., *Diplopsalis* spp.) and zooplanktonic prey including invertebrate eggs, *E. acutifrons*, nauplii of cirripeds and copepods and bivalve larvae. Larger larvae (13-19 mm) were associated with a lower number of prey. Larvae of 13 mm were close to unidentified calanoids, *Oncaea* spp., chrysophytes and diatoms whereas larvae of 14-19 mm were close to larger copepods prey including *Temora longicornis*, *P. parvus*, unidentified copepods, *P. elongatus* (106-370 µm) but also small protists like the dinoflagellates *Gonyaulax* spp. and *Prorocentrum lima* and the diatom *T. curviseriata*.

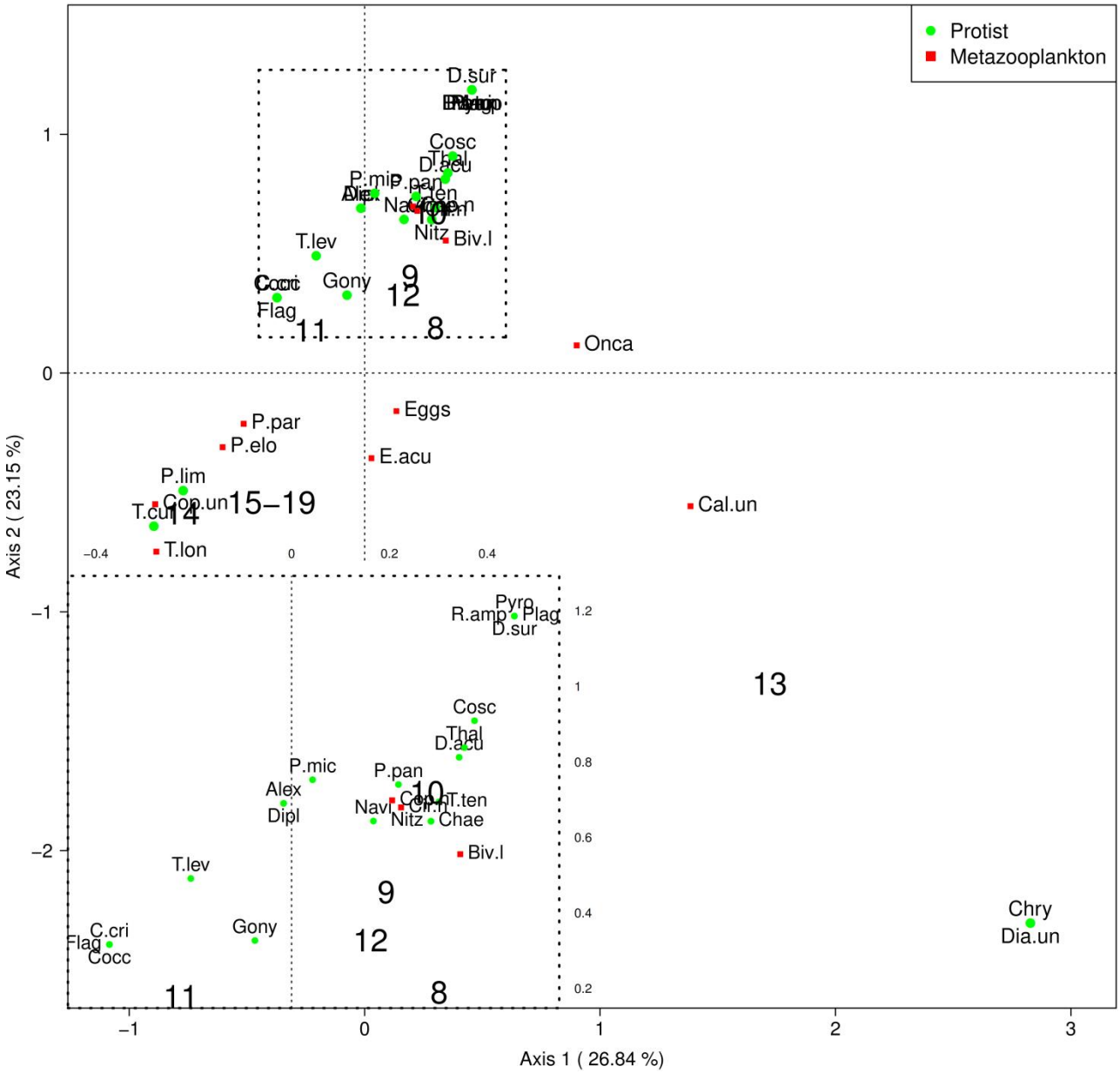

Figure 19: Plot of the first two axes of the CA performed on prey composition in the guts of larval herring from 8 to 19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. The insert in the bottom-left corner zooms on the larvae of 8-12 mm. See Table 3 for the prey abbreviations.

Among the 38 prey taxa identified in the guts, 27 were also found in the water (protist and metazooplankton) samples. Hence, the selectivity was calculated only for these 27 taxa (see Table 3). The selectivity index (*D*) showed that except for *P. parvus* and for the nauplii of copepods, there was an inverse relationship between prey selectivity and prey occurrence in the water (Figure 20). Highly selected prey (> 0.5) were *Gonyaulax* spp. (0.99 ± 0.01; Table 3), *Pyrophacus* spp. (0.97), invertebrate eggs (0.93 ± 0.04), *P. micans* (0.66 ± 0.18), nauplii of copepods (0.72 ± 0.10), *P. lima* (0.60), *Oncaea* spp. (0.50 ± 0.35) and *T. curviseriata* (0.51 ± 0.27) and conversely for *Nitzschia* spp. (-0.43 ± 0.03), *Thalassiosira tenera* (-0.33 ± 0.12), *Thalassiosira* spp. (-0.17 ± 0.18), unidentified calanoid (-0.05 ± 0.36) and *P. elongatus* (-0.12 ± 0.23; Figure 19). The selectivity index of prey varied between 2008 and 2014 mainly for prey like *T. longicornis* (-0.06 ± 0.46), *P. panduriformis* (0.23 ± 0.42), *Navicula* spp. (-0.01 ± 0.43), *Oncaea* spp. (0.50 ± 0.35) and *E. acutifrons* (0.23 ± 0.36).

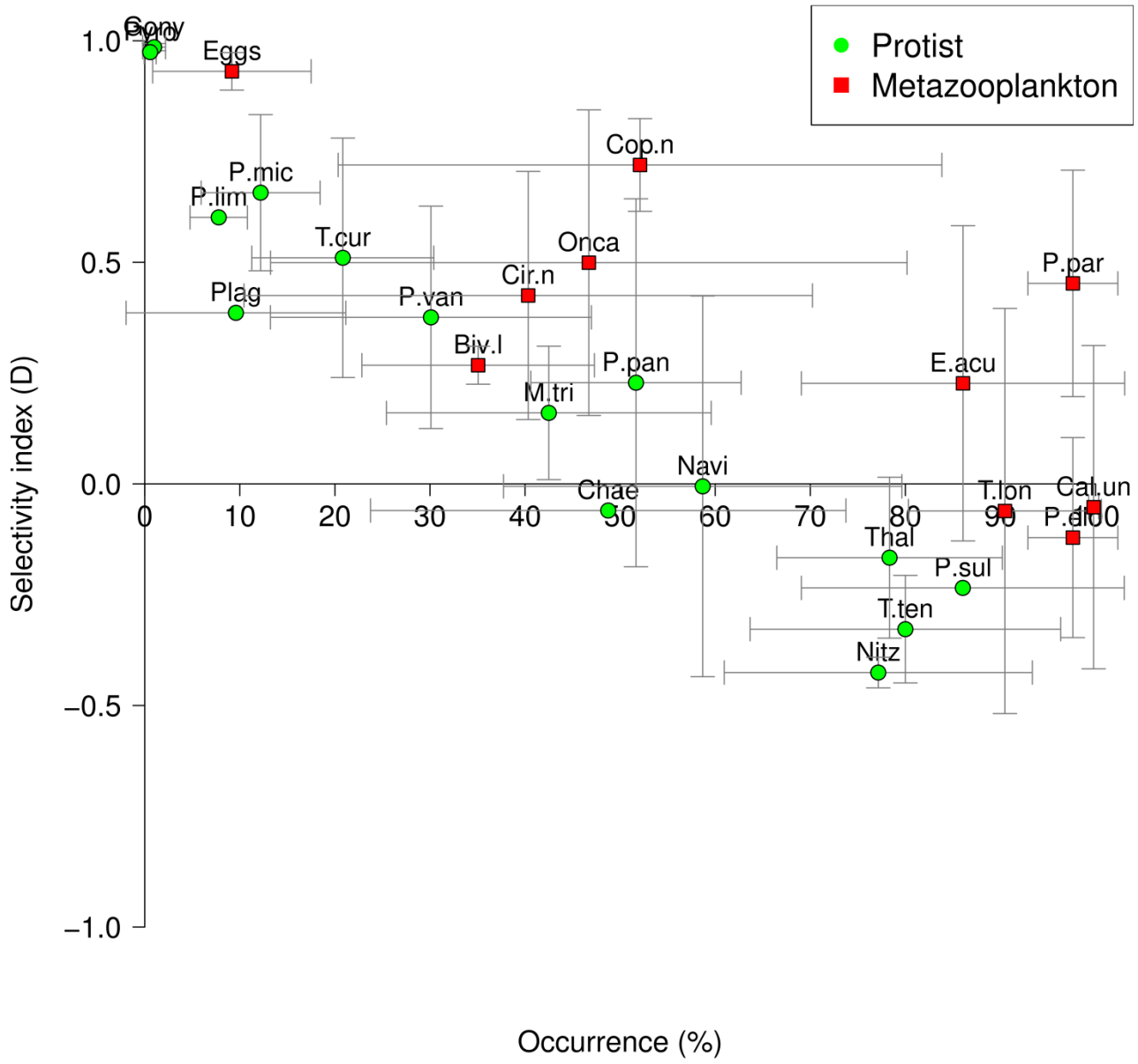

Figure 20: Selectivity and occurrence in the water of prey consumed by Downs herring larvae of 8 to 19 mm during winter (mid January-mid February) in the EEC and the SBNS from 2008-2014. Selectivity and occurrence are plotted as their mean (± SD) for the 2008-2014 periods. See Table 3 for the prey abbreviations.

2. Discussion

Several methodological aspects, in particular on the use of gut content analysis and SEM to study the diet of larval fish, need to be debated before discussing on the larval feeding of Downs herring.

2.1.Relevance of gut contents to depict larval fish diet

Gut contents analysis is by far the most used approach to study the feeding behaviour of marine organisms (Hyslop, 1980) because it is a direct approach and an easy way to quickly

assess the different prey ingested by a predator. However, several factors may affect their relevance to depict larval fish diet. Many authors stressed the potential underestimation of larval feeding (de Figueiredo et al., 2005; Pepin and Dower, 2007; Theilacker et al., 1996) due to the evacuation of gut contents during larval capture or fixation (Bjørke, 1976; Checkley, 1982; Dower et al., 1998; Hay, 1981; Lebour, 1924). Night sampling may also lead to some bias because larval feeding decreases with light intensity at nightfall (Haslob et al., 2009; Munk et al., 1989) and has a peak just after sunrise (Bjørke, 1976; Blaxter, 1965; Heath, 1993) and before sunset (Cohen and Lough, 1983; Fox et al., 1999). These two phenomenons may have some implications in the estimation of the evacuation rate which highly relies on the observed number of empty larvae. In our case, as the larval sampling extended from sunset until sunrise, some larvae could have already fed several hours before (Blaxter, 1965; Fossum and Johannessen, 1979; Pedersen, 1984). Hence, the vacuity rate obtained in the present study for Downs herring larvae is potentially overestimated and should be considered as a maximum value. However, since there is no reason why this overestimation should not be constant for the different larval sizes considered in the present study, the comparison of vacuity rate between these larval sizes remains relevant.

The other main criticism that is addressed to gut content analysis is that it provides only a momentary image of the predator diet and is largely influenced by the number of larvae analysed. In our case, it is compensated by the shorter duration of the larval phase compared to juveniles and adults. We covered the whole distribution area of 8-19 mm larvae and their potential prey by sampling both in the EEC and the SBNS. Whereas the number of larvae analysed in the present study could appear as relatively low and/or insufficient, it already represents a huge amount of sampling effort. Increasing the number of larvae analysed will lead to increase the number of prey rarely ingested but will not change the picture we have of the larval diet. We then assume that the number of larvae analysed in our study was sufficient to adequately depict the diet of Downs herring larvae.

2.2.Interest of SEM in larval gut contents analysis: pros and cons

Whereas most of the studies based on gut content analysis relied on optical microscopy (Arula et al., 2012; Blaxter, 1965; Catalán et al., 2010; Davis et al., 2012; Landaeta et al., 2014; Robert et al., 2011), recent studies by Koubbi et al. (2007), Montagnes et al. (2010), Vallet et al. (2011) recognised that SEM had strong advantages over the other methods. They proved that optical microscope led to a truncated view of first-feeding larvae diet by ignoring

the relevance of smaller protists and zooplanktonic prey. Our study confirmed the usefulness of the SEM to study the diet of small Downs herring larvae (8-15 mm) as a high quantity of prey smaller than 50 μm were detected. This would not have been possible with optical microscope alone. The other main advantage lies into the taxonomic accuracy SEM provides especially for protists identification. In our study, most of them were determined at the species level which could not have been done with inverted microscopy for instance. If one wants to calculate a selectivity index that takes into account this very high precision, determination of prey in the water should also been achieved with the same level of precision. This is not always an easy task to do, especially when dealing with small protist. In our study, we also used SEM to reach the same precision when we analysed protist samples but only for the 2011-2014 period as those from the 2008-2010 period were already analysed and discarded. This ended up with several species for which it was not possible to calculate the selectivity index, in particular those that were observed in the guts only between 2008 and 2010. For the other prey for which a selectivity was produced, the difference in the methodology of samples analysis was accounted for by decreasing the level of taxonomic determination obtained from SEM from species to genera so that it was comparable with inverted microscopy. In this way, it ended up with an unbiased selectivity index.

SEM also has several disadvantages. The first one is that it is expensive and time consuming if one wants to analyse all the stubs and identify and count all the prey. However, as stated by Baker et al. (2014), counting prey is not essential and prey abundance does not systematically bring more significant information on fish diet than prey occurrence. They argued that not only is it easier to calculate, prey occurrence is also more reliable and robust. From the several tests that we performed, we also reached the same conclusion that the benefit in term of information on the larval diet was not enough to compensate for the time spent (up to three times more) to scan all the stubs. In our study, SEM was then used in a qualitative way and prey were determined in terms of presence-absence.

The potential of SEM could be limited by the solution used to preserve the samples. While zooplankton samples are classically preserved in a formalin solution, one knows that it is not adapted to preserve soft-bodied organisms (Montagnes et al., 2010) like naked ciliates and non-armoured dinoflagellates. This could explain why none of these organisms had been found in the guts of the present study whereas their relevance for fish larvae had been underlined by several authors (Bjørke, 1976; Checkley, 1982; de Figueiredo et al., 2007, 2005; Fukami et al., 1999; Hunt von Herbing et al., 2001; Hunt von Herbing and Gallager, 2000; Lasker, 1975; Lebour, 1921; Nagano et al., 2000). Even if soft-bodied organisms are

rapidly digested and hence could be hard to detect in the gut contents (de Figueiredo et al., 2007, 2005; Fukami et al., 1999; Hunt von Herbing et al., 2001; Nagano et al., 2000; Ohman et al., 1991), their study would require the preservation the larvae in a different preservative solution like Bouin or Lugol (de Figueiredo et al., 2007, 2005).

Except for soft-bodied organisms, we assume that gut contents analysis based on SEM was a valuable way to study the feeding of Downs herring larvae.

2.3.Main feeding areas

Three main areas of low vacuity rate were identified along the three French estuaries (Somme, Authie and Canche), in the DS and in the middle of the EEC. The first two areas spatially matched with high chlorophyll *a* concentrations and high abundance of metazooplankton (see chap. II section 1.2). Larvae along the three French estuaries were usually smaller (8-12 mm) than those in the SBNS (13-19 mm, see chap. II section 1.3). The third area in the middle of the EEC did not match with any particular high prey concentration and was occupied mainly by small larvae (8-12 mm, see chap. II section 1.4). Temporal variations in the larval vacuity were shown and larvae in the years 2008, 2009 and 2011 had usually a higher vacuity rate. Because the environmental and trophic conditions showed little temporal variation in their spatial structure, these temporal variations in the vacuity rate still remain unexplained. This bears out that a relationship between nutrition success and environmental conditions cannot always be found when analysing larval gut contents due to differences in integration time (Fox et al., 1999).

2.4.Relevance of protist

Our study clearly showed that protist constitutes a relevant part of Downs herring larvae diet both in term of diversity and positive selectivity. While this was previously observed for herring from other areas by Bjørke (1976), Checkley (1982), de Figueiredo et al. (2007, 2005) and Lebour (1921), our study provides for the first time the list of species ingested along with their selectivity for the Downs component of herring larvae.

Dinoflagellates including *Alexandrium* spp., *Dinophysis acuminate*, *Prorocentrum micans*, *Pyrophacus* spp. and *Diplopsalis* spp. and most of the diatoms were positively selected. Direct or indirect consumption of protist by fish larvae is still a matter of debate (Bjørke, 1976; Checkley, 1982; de Figueiredo et al., 2007, 2005; Fukami et al., 1999; Hunt von Herbing et al., 2001; Hunt von Herbing and Gallager, 2000; Lasker, 1975; Lebour, 1921;

Nagano et al., 2000). They might not be used directly by larvae for their growth as suggested by Hjelmeland et al. (1988) because their digestive tract remains incompletely developed and limit their ability to digest and absorb microalgae. However, it has been reported that ingestion of particular phytoplanktonic species, like the diatoms *Coscinodiscus* spp., plays an essential role in the larval growth by improving the digestion efficiency of other prey like zooplankton through the increase of the enzymatic activity (Cahu et al., 1998; Hjelmeland et al., 1988; Illing et al., 2015; Reitan et al., 1997; St. John et al., 2001) and a significant contribution has an intake of lipid and free amino acids (Braven et al., 1995).

Conversely, some diatom species like *Nitzschia* spp., *Thalassiosira* spp. and *Thalassiosira tenera* were clearly negatively selected whereas they were highly abundant in the water column. This might be due to their elongated form which makes them difficult to be ingested by small larvae. Our study also revealed an efficient feeding on toxic dinoflagellates like *Alexandrium* spp. and *Gonyaulax* spp., both species being associated to low fish survival and high mortality (Gosselin et al., 1989; Huntley, 1989; Robineau et al., 1991).

2.5. Ontogenetic dietary shift

The correspondence analysis clearly showed that there was a shift in the diet of Downs herring larvae at a size of 13 mm. Change in the diet was previously observed in experimental studies by Checkley (1982) to occur at a similar size (12 mm) for the other herring populations. The diet of first feeding larvae (8-12 mm) was more diverse and composed of previously enumerated protist prey and small zooplanktonic prey like *Euterpina acutifrons*, *Oncaea* spp., copepodite stages of *Pseudocalanus elongatus* and nauplii of copepods and cirripeds. The selection of zooplankton prey by fish larvae relies on their size, their catchability, their escape capacity as well as the larval behaviour (Checkley, 1982; Heath, 1993), length and the opening of the mouth (Arula et al., 2012; Blaxter, 1965; Checkley, 1982; Cohen and Lough, 1983). Positive selection of these small copepods suggests that they were more accessible, as a result of their limited escape capacity and their smaller size (Checkley, 1982) and conversely for large zooplanktonic prey.

Larvae greater than 13 mm had a less diversified diet mainly composed of zooplanktonic prey including larger copepods like *T. longicornis* and *Paracalanus parvus* and also invertebrate eggs. Increase in the swimming capacity of fish larvae, the opening of the mouth and the width of the gut improved the success of capturing and ingesting larger prey (Checkley, 1982; Haslob et al., 2009; Kiørboe et al., 1985; Munk, 1992; Munk and Kiørboe,

1985). Ingestion of large copepods with higher carbon content (Peters, 1983) better fulfils the higher energetic requirements of larger larvae. The relevance of invertebrate eggs through their positive selection was also observed for other subpopulations (Blaxter, 1965; Cohen and Lough, 1983; Fox et al., 1999; Marshall et al., 1937) even if Conway et al. (1994) showed that fish larvae have limited digestion capacities of copepod eggs. Observed eggs can also be remaining elements of one or several ingested ovigerous copepods.

2.6.Negative effect of winter conditions on larval diet?

The vacuity rate of Downs herring larvae (46%) is relatively low compared to previous studies at the same period either in the same area, (61%; Lebour, 1924) or in other areas like in the Clyde Sea (70%; Marshall et al., 1937), Georges Bank (51%; Cohen and Lough, 1983) and Pärnu Bay (30-80%; Arula et al., 2012). Whereas larval herring from the Irish Sea had a vacuity rate of 17% (Checkley, 1982), these larvae were fed in experimental conditions which are usually more than optimal and unrealistic compared to those experienced by Downs herring larvae in winter. It is also comparable to field studies during spring (29-75%; Fox et al., 1999) and autumn in other area like Northern North Sea (55%; Heath et al., 1989) and other species like sole (42-55%; Grioche, 1998), plaice (40%; Shelbourne, 1957) and sand-eel (32-41%; Ryland, 1964). The comparable (sometimes even lower) values of vacuity rate observed for Downs herring larvae suggest that these larvae are not more limited by winter trophic conditions than spring larvae could be during the planktonic bloom. The less productive conditions of winter experienced by Downs herring larvae might be counterbalanced by a lower number of winter species larvae, hence ensuring less competition for food and an equivalent level of nutrition as the one experienced by larvae during spring. Downs herring larvae might also diversify their diet in order to support less productive conditions of winter. Arula et al. (2012) suggested that a diversified diet allows for adapting to inter-annual fluctuations of prey composition in the sea.

2.7.Identification of the critical period

During this period, the present study identified two peaks of higher vacuity rate. The peak (74%) observed for larvae of 8 mm is in agreement with their yolk-sac nutrition. The vacuity rate decreased at a size of 9 mm, which indicates an active feeding linked to the yolk sac absorption (Heath and MacLalan, 1987; Lebour, 1924). Up to 13 mm, the vacuity rate remained constant between 30 and 40%. The other peak (56%) of vacuity rate which occurs at

13 mm suggests some difficulties for these larvae to feed and corresponded to the shift observed in the prey composition. Rather than the yolk-absorption period, we assume that this ontogenetic shift in term of prey preference constitutes the critical period for Downs herring larvae. Change in their food habit will make the need some time to adapt to their new zooplanktonic prey which are potentially less abundant and more difficult to capture than protist.

3. Conclusion

The present study 1) demonstrated the usefulness of SEM to study the diet of young Downs herring larvae, 2) characterised the omnivorous diet of these larvae in term of prey composition and vacuity rate and 3) identified a potential critical period in term of feeding activity at a size of 13 mm linked to a shift in prey preferences. While SEM allowed to consider smaller size prey which were unnoticed so far, it remains a time-consuming, expensive and qualitative approach, stressing out the need to develop more quantitative methods. This quantitative approach of Downs larvae ingestion will be presented in the next chapter.

CHAPITRE IV
Quantitative assessment of Downs herring larvae herbivory

The SEM approach used in the previous chapter demonstrated the significant contribution of protists (diatoms and dinoflagellates) from a qualitative point of view to Downs herring larvae diet, particularly regarding first feeding stages. The present chapter presents and discusses the results obtained from the quantitative approach used to quantify the contribution of phytoplankton to Downs herring larvae diet, to compare it to feeding rates to those of six dominant copepods (*Calanus* spp., *Euterpina acutifrons, Oncaea* spp., *Paracalanus parvus, Pseudocalanus elongatus* and *Temora longicornis*) and to estimate the feeding activity of Downs herring larvae in the EEC and the SBNS during winter 2014. Gut contents of 8-13 mm Downs larvae collected during the 2014 IBTS (Figure 4 and 5) were analysed with the fluorimetric approach adapted from copepods (see chap. I section 3.2.2 for the methodology). The objectives were to 1) adapt and validate the quantitative approach based on the the fluorimetric method to Downs herring larvae, 2) quantify at the same time the ingestion rate of both Downs larvae and the six dominant copepods and 3) evaluate their daily metabolic needs of Downs herring larvae.

This chapter is partly based on an article in preparation for submission in Marine Ecology Progress Series:

Gut fluorescence analyses of Downs herring larvae : an approach to quantify ingestion in the English Channel and North Sea

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1. Results

1.1.Copepods ingestion

Copepod gut content varied from 0.12 to 9.02 ng chl *a* eq ind^{-1} and the highest values were observed when the largest copepods were sampled (e.g. *Calanus* spp. and *Pseudocalanus elongatus* for station 2, 7 and 9; Figure 4 for numerous of station, Table 4). On the contrary, lowest values (from 0.12 to 0.70 ng chl *a* eq ind^{-1}) were attributable to the smallest species namely *Oncaea* sp. and *Euterpina acutifrons* (station 6). Intermediate gut content values ranged from 0.7 and 5.5 ng chl *a* eq ind^{-1} and corresponded to the dominant *Paracalanus parvus* (e.g. stations 3 and 13) and *Temora longicornis* (station 10). Phaeopigment contribution to gut content was highly variable (from 5 to 100%) but most values were higher than those recorded for seawater (30-50%).

Table 4: Dominant copepod (range for the six dominant species) and Downs herring larvae (mean ± SD) gut content (ng chl *a* eq ind^{-1}) and contribution of phaeopigments (% total gut content) during winter 2014 (mid January-mid February) in the EEC and the SBNS.

Location	Stations	Copepods		Downs herring larvae	
		ng chl *a* eq ind^{-1}	% Phaeo	ng chl *a* eq ind^{-1}	% Phaeo
EEC	1	0.09 - 0.7	34 - 39	0.54 ± 0.22	72 ± 2
	2	0.12 - 4.24	50 - 97	3.8 ± 3.18	76 ± 9
	3	0.51 - 3.44	38 - 68	1.51 ± 0.84	78 ± 3
	4	0.16 - 0.62	47 - 72	1.47 ± 1.02	69 ± 2
	5	0.51 - 4.7	50 - 91	1.83 ± 0.72	75 ± 3
	6	0.13 - 2.49	34 - 98	2.17 ± 1.46	68 ± 3
	7	0.21 - 4.16	51 - 143	0.51 ± 0.17	70 ± 3
	8	0.51 - 2.79	19 - 48	1.83 ± 1.61	69 ± 1
	9	0.31 - 8.9	5 - 96	0.51 ± 0.24	76 ± 5
SBNS	10	0.08 - 2.12	21 - 94	0.4 ± 0.23	70 ± 5
	11	0.31 - 5.55	46 - 85	0.28 ± 0.13	66 ± 1
	12	0.17 - 1.84	39 - 63	0.55 ± 0	74 ± 0
	13	0.4 - 4.11	52 - 79	0.8 ± 0.1	86 ± 2
	14	0.34 - 9.02	38 - 74	1.3 ± 0.38	68 ± 1

1.2.Larval feeding

Larval gut contents were in the same range of order than values observed for copepods (Table 4). Mean values ranged between 0.28 ± 0.12 (station 11) and 4.19 ± 3.15 ng chl *a* eq ind^{-1} (station 2) and were significantly higher in the EEC compared to the SBNS (ANOVA, $p<0.05$). About 66 ± 1 to 86 ± 2% of chlorophyll concentration ingested by herring larvae were phaeopigments, a contribution markedly higher than values observed for copepods.

Larval ingestion rates on phytoplankton varied significantly with larval size (ANOVA, $p < 0.05$; Figure 21A). It corresponded to an increase of mean phytoplankton ingestion from 24.6 ± 28.7 to 52.2 ± 50.9 ng chl *a* eq ind^{-1} d^{-1} for 8-11 mm larvae (i.e. 1.1 ± 1.3 to 2.3 ± 1.9 µgC ind^{-1} d^{-1}; Table 3 and 4) while mean values slightly decreased to 43.3 ± 48.2 ng chl *a* eq ind^{-1} d^{-1} for 12-13 mm larvae (2.6 ± 2.6 µgC ind^{-1} d^{-1}). Spatial variability in mean ingestion rates is depicted in figure 21B-D. A clear distinction could be made between rather high phytoplankton ingestion rates of the EEC larvae (19.2-188.1 ng chl *a* eq ind^{-1} d^{-1}) and lower values (8.8-83.5 ng chl *a* eq ind^{-1} d^{-1}) recorded for larvae collected in both the DS and the SBNS.

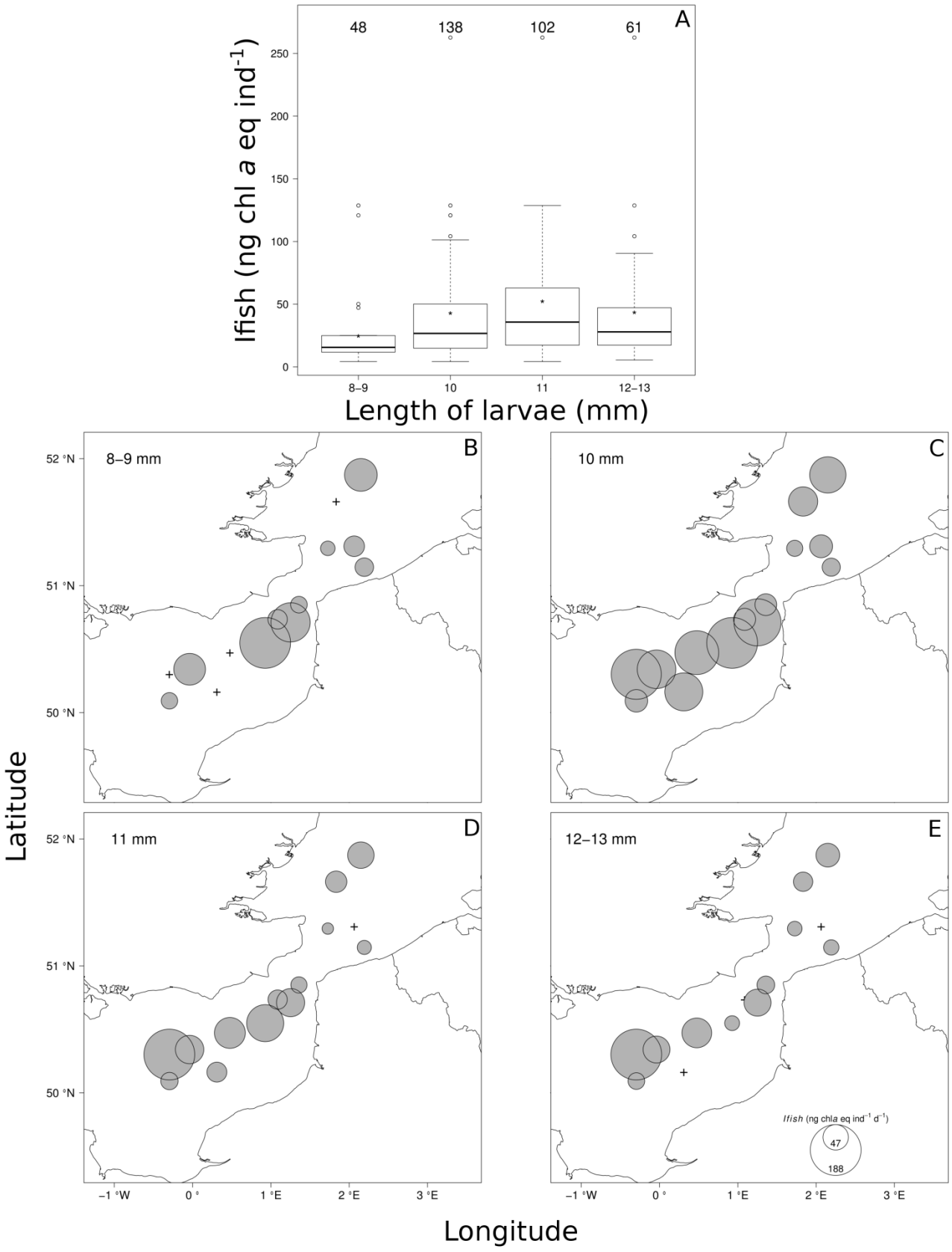

Figure 21: Ingestion rate (Ifish, ng chl *a* eq ind^{-1} d^{-1}) of Downs herring larvae during winter 2014 (mid January-mid February) by size (from 8 to 13 mm, A) and stations (B-E). The number of larvae analysed for each size class is indicated on the top of each bar (A). Stars stand for the mean (A). Crosses (B-E) stand for stations with the absence of larval size class.

Larval daily rations ranged from 2.2-3.9% body C d^{-1} (Table 5). Based on the relationship between body weight and respiration, herring larvae needed to ingest 18.7-24.4% body C d^{-1} just to balance their respiratory needs, smaller larvae (8-9 mm) having the highest daily respiratory requirements. Therefore, herring larvae from winter 2014 were in carbon deficit as only 11-18% (i.e. ratio of daily ration to respiration rate) of their metabolic requirements were covered by pigment ingestion.

Table 5: Downs herring larvae (mean ± SD) parameters derived from allometric relationships (Body carbon content, respiration rates Rfish) and gut content analyses (Carbon ingestion rates, $Ifish_C$ and Daily Ration DR) during winter 2014 (mid January-mid February) in the EEC and the SBNS.

Size class (mm)	Body Carbon Content	$Ifish_C$	Daily Ration (DR)	Rfish	
	µgC ind^{-1}	µgC ind^{-1} d^{-1}	% body C d^{-1}	µgC ind^{-1} d^{-1}	% body C d^{-1}
8 - 9	31.4 ± 9.9	1.1 ± 1.3	2.8 ± 3.3	7.6 ± 2	24.4 ± 1.4
10	57.6 ± 0	2.3 ± 2.6	3.9 ± 4.4	12.6 ± 0	21.8 ± 0
11	83.1 ± 0	2.3 ± 1.9	2.7 ± 2.3	17 ± 0	20.4 ± 0
12 - 13	137 ± 29.6	2.6 ± 2.6	2.2 ± 2.2	25.5 ± 4.6	18.7 ± 0.7

2. Discussion

2.1.Shortcomings and advantages of the quantitative approach

Although widely used to quantify herbivorous feeding of numerous zooplankton taxa amongst which copepods (Kleppel et al., 1988; López et al., 2007; Mackas and Bohrer, 1976), salps (Madin and Cetta, 1984; Perissinotto and Pakhomov, 1998), and krill (Pakhomov and Perissinotto, 1996), the quantitative approach based on the gut fluorescence method has seldom be applied to fish larvae (Conway et al., 1996; Otake et al., 1990). The method has indeed long been criticized as it suffers uncertainties regarding gut pigment destruction to non-fluorescent end-products (see review in Pasternak, 1994). Gut evacuation rates can vary greatly in relation to temperature (Irigoien et al., 2008; Kiørboe et al., 1982), food concentration (Pasternak, 1994) and initial gut content (Perissinotto and Pakhomov, 1996), thus impacting feeding estimates. In this study, gut contents were examined from animals caught at night during a 10 day sampling period in winter. As no spatial pattern in seawater temperature and pigment concentrations were observed over the sampling area (Table 1), we can consider that gut fluorescence contents reflected the size-classes and spatial differences in ingestion rates of herring larvae rather than the variability of gut evacuation rate and food concentration. The present study therefore shows that the gut fluorescence method works for herring larvae and provides a quantitative snapshot of feeding on total ingested pigments (chl

a and phaeopigments). We advise the use of this approach as an overall estimate of feeding and to simultaneously estimate chl *a*/Phaeopigment ratio to estimate the contribution of heterotrophic prey to diets.

2.2.Feeding of Downs herring larvae

As the same computation methods were used for estimating gut contents (ng chl *a* eq ind^{-1}) direct comparison could be made between larvae and copepod feeding behaviour. The gut fluorescence method demonstrated the quantitative contribution of phytoplankton to Downs herring larvae diet during winter. Gut content values for Downs herring larvae (0.3-4.2 ng chl *a* eq ind^{-1}) were within the range of those reported for species of comparable size (1.62 ± 0.63 ng chl *a* eq ind^{-1}; Conway et al., 1996; Otake et al., 1990) as well as within the range recorded for dominant copepods (Table 5). Consequently, being less food depleted, winter Downs herring larvae may exhibit nutritional levels comparable to those of spring larvae. High phaeopigments contents in the larval guts (66-86%) compared to copepods and seawater (30-50%) suggested that the pigments could have remained in the gut for a long time (Otake et al., 1990) and thus, were subjected to chemical degradation by both enzymes and acid secretion. Another possible explanation for this is that heterotrophic prey (e.g. ciliated protozoans, dinoflagellates and/or copepods) contributed highly to larval fish diet as recently demonstrated by Denis et al. (2016) (see chap. III). Small copepods, invertebrate eggs, diatoms and dinoflagellates were in fact recognized as important prey items for small larvae (8-12 mm) whereas larger larvae (13-19 mm) appeared to feed almost exclusively on copepods and heterotrophic dinoflagellates. Ingestion of soft-bodied ciliates may have occurred as demonstrated in field and experimental studies (de Figueiredo et al., 2007; Fukami et al., 1999; Hunt von Herbing and Gallager, 2000) but it could not be inferred neither from Denis et al. (2016) nor from our results. Hence, the amount of total ingested chlorophyll concentration (chl *a* and phaeopigments) measured in the gut reflects both an effective direct consumption of phytoplankton and an indirect consumption of herbivorous zooplankton (metazoans or protists).

Our results demonstrated a monotonic increase in ingestion rate with a slight decrease for the larger larvae. Smaller herring larvae (8-11 mm) ingested phytoplankton at high rates (24.6-52.2 ng chl *a* eq ind^{-1} d^{-1}) whereas lower rates (43.3 ng chl *a* eq ind^{-1} d^{-1}) were measured in larger larvae (12-13 mm). This increase combined to the spatial gradient in larval size distribution led to a clear distinction between the ECC and the SBNS. Basically, smaller

larvae collected in the EEC had a more diversified diet (i.e. including protists) and higher ingestion rates than larger ones located in the SBNS. The spatial variability of larval ingestion revealed a paradox between higher ingestion rates in the EEC where pigment concentrations were low and lower ingestions rates in the SBNS where pigment concentrations were high. This suggests that herring larvae carbon intake did not depend on *in situ* pigment concentrations, which was confirmed by the absence of significant relationship between fish ingestion and pigment concentrations ($r = -0.09$, $P > 0.05$) and metazooplankton abundance ($r = -0.21$, $P > 0.05$). The lower ingestion rate of 12-13 mm larvae could also be explained by their shift from omnivory to carnivory with decreased phytoplankton ingestion (Denis et al., 2016; see chap. III). The highly diverse omnivorous diet appears to allow small larvae to feed enough to survive in winter trophic conditions. Hence, EEC could appear as a more favourable environment than SBNS. The protists play an essential role in larval growth by improving the digestion efficiency of other prey like zooplankton by increasing enzyme activity (Cahu et al., 1998; Hjelmeland et al., 1988; Illing et al., 2015; Reitan et al., 1997; St. John et al., 2001) and contribute to the intake of lipid and free amino acids (Braven et al., 1995). Low daily rations indicate that fish larvae were not actively feeding on phytoplankton during winter 2014, thus exerting a weak top-down control on this food source. In fact, exclusive herbivory only covered 18.7 to 24.4% of fish herring metabolic requirements. Consequently, phytoplankton prey are only part of the diet of larvae and would offset the energy requirements due to the low diversity of prey in winter, particularly regarding 8-12 mm larvae with high carbon needs. Increasing of the larval gape size and maturation of the digestive tract allow growing fish larvae to feed on larger and more energetic prey such as copepods (de Figueiredo et al., 2007; Friedenberg et al., 2012; Fukami et al., 1999; Nagano et al., 2000; Reitan et al., 1997; Van Der Meeren, 1991). Shift towards copepods consumption can also be explained by the spatial co-occurrence of 12-13 mm larvae with high zooplankton abundance in the SBNS (Boehlert and Yolklavich, 1984; Nunn et al., 2012; Theilacker, 1987).

3. Conclusion

The present chapter demonstrated the validity and reliability of the gut fluorescence method to quantify herbivorous feeding of Downs herring larvae in the EEC and the SBNS. By quantifying the amount of pigment ingested, fish gut fluorescence integrates both direct ingestion of phytoplankton prey and indirect consumption of herbivores. Gut fluorescence analysis can be used as feeding index faster than SEM observation. The ingestion rate varied

according to the larval size and confirms their shift in diet composition with larval growth and development, in particular with decreased phytoplankton prey consumption. Determination of the larval feeding strategy, either from a qualitative or a quantitative point of view, does not inform on the larval condition. This is a major issue that will be addressed in the next chapter.

CHAPITRE V
Larval condition of Downs herring: use of a multi-indices approach at the individual scale

The feeding strategy of young larval Downs herring over a seven years period (see chap. III) evidenced an ontogenetic shift in prey composition at a larval size of 13 mm. The aim of this chapter was then to evaluate whether that diet shift could be associated to changes in larval condition.

Four condition indices of different nature and contrasted integration time (ingestion rate, RNA/DNA and DNA/C ratios and otoliths micro-increments) were measured on herring larvae collected in winter 2014 and 2015 during the IBTS (see chap. I section 1).

The main objectives of this chapter were 1) to characterize the larval condition of Downs larvae during the first feeding stages, 2) to compare the results obtained from the different indices considering their different response time and 3) to identify among environmental, spatial and ontogenetic factors, those that influenced the larval condition.

This chapter is partly based on an article submitted in Marine Biology:

Ontogenetic changes in the larval condition of Downs herring - Use of a multi-indices approach at the individual scale

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1. Results

1.1.Ingestion rate

For 8-18 mm larvae, ingestion rates decreased significantly with size (ANOVA, $p < 0.05$; Figure 22A). The ingestion rate decreased from 8-9 mm to 10 mm from 22.5 to 18.6 ng chl *a*

eq ind^{-1} d^{-1}, then remained almost constant at 19.5-20.1 ng chl *a* eq ind^{-1} d^{-1} until 12 mm, with 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} d^{-1}. Regarding spatial pattern of the size class 8-12 mm, the centre of the SBNS (stations 7 to 9, see Figure 4 for numerous of station) was characterized by larvae with lower ingestion rates (7.7-18 ng chl *a* eq ind^{-1} d^{-1}; Figures 22B) compared to the rest of the study area (19-46.6 ng chl *a* eq ind^{-1} d^{-1}).

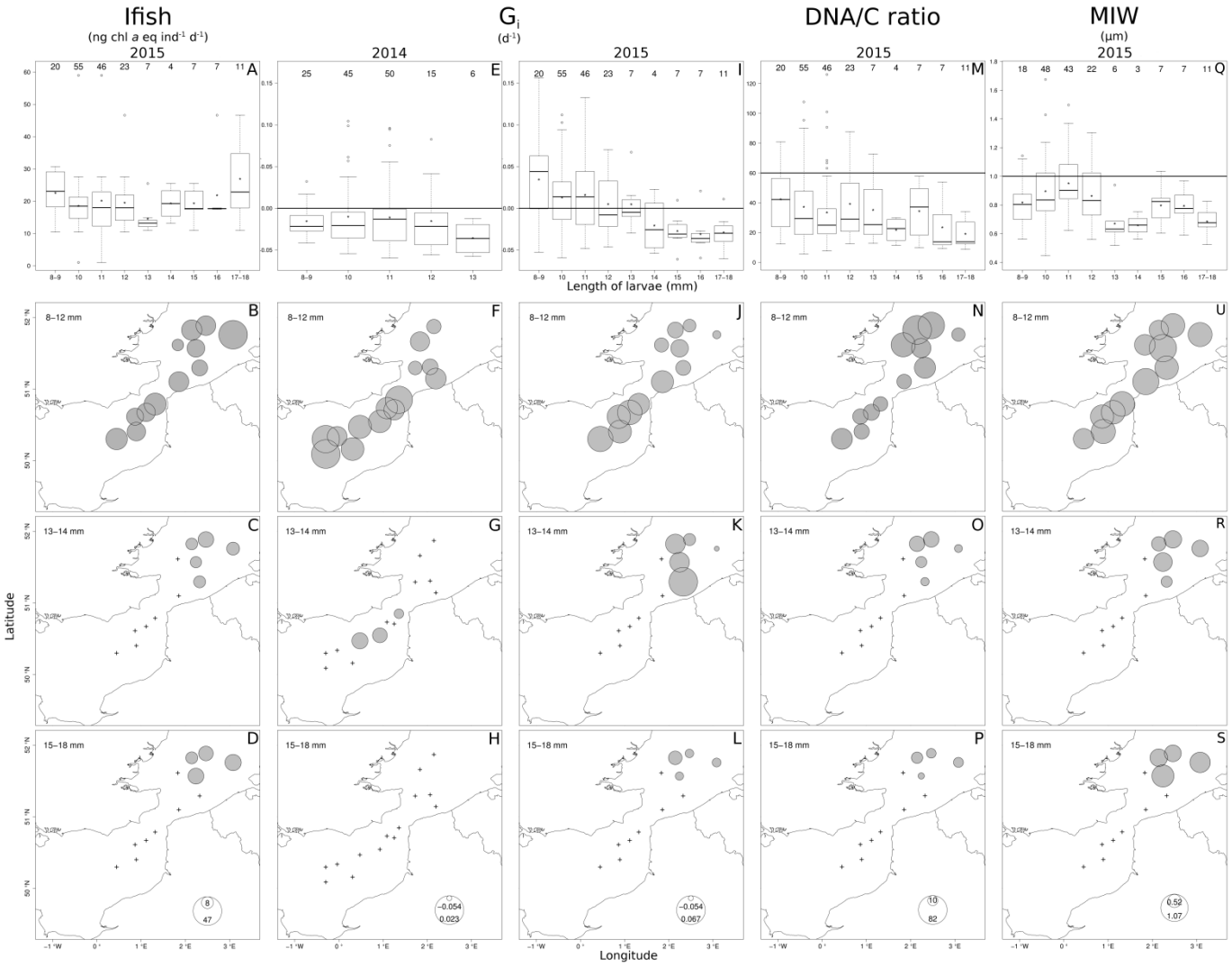

Figure 22: Larval condition analysis of Downs herring larvae in the Eastern English Channel, Dover Strait and the Southern Bight of the North Sea during winter 2014 and 2015 (mid January-mid February). (A-D) Ingestion rate (Ifish, ng chl *a* eq ind^{-1} d^{-1}), (E-H in 2014 and I-L in 2015) instantaneous growth rate (G_i, d^{-1}), (M-P) DNA/C ratio and (Q-S) mean increment width (MIW, µm). A, E, I, M, and Q boxplots 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 and Q) depict the thresholds used to determine starving

(I; Bergeron 2000) and slow-growing (E, M and Q; 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.

1.2. Instantaneous growth rate and DNA/C ratios

Instantaneous growth rate was significantly different between size larval (Figure 22E and I; ANOVA, $p < 0.05$). It decreased with larval size and ranged between -0.011 ± 0.033 d^{-1} (8-11 mm) and -0.021 ± 0.035 d^{-1} (12-13 mm, Figure 21E) in 2014, and 0.005 ± 0.031 d^{-1} for smaller larvae (8-13 mm) down to -0.031 ± 0.025 d^{-1} for larger ones (Figure 22I) in 2015. Each year, smaller larvae (8-12 mm) exhibited a higher variability of instantaneous growth rate with respective minimum and maximum values of -0.059 and 0.156 d^{-1}. In 2014, more than average of the individuals exhibited a ratio below the threshold whereas it concerned only larvae from 13 mm in 2015. In 2014 and 2015, instantaneous growth rates indicated that respectively 22% and 57% of 8-12 mm larvae efficiently grew, even though they exhibited high inter-individual variability. In contrast, 0% and 19% of larger larvae (13-18 mm) were shown to be good growth condition. For both years, 8-12 mm larvae from the EEC and the DS had a significantly higher instantaneous growth rate (-0.020-0.022 and 0.022-0.052 d^{-1}, respectively; ANOVA, $p < 0.05$; Figure 22F) than those from the SBNS (-0.016--0.037 and -0.004--0.046 d^{-1}, respectively; ANOVA, $p < 0.05$; Figure 22J).

In 2015, DNA/C ratios decreased with the larval size (Figure 22M; ANOVA, $p < 0.05$), and on average, was higher for smaller larvae (8-13 mm) compared to larger indiciduals, despite showing strong inter-individual variation (from 6 to 126). Around 83% of smaller (8-13 mm) and 100% of larger larvae appeared to be in good feeding condition (Figure 22M). Individuals of the size class 8-12 mm had a higher DNA/C in the SBNS than in the EEC (Figure 22N).

1.3.Otolith microstructure

An average growth rate of 0.26 mm d^{-1} ($r^2 = 0.88$, $p < 0.05$) was estimated for 8-18 mm larvae (Figure 23A). The highest increment widths were recorded for the first three increments with mean values of 1.4-1.6 µm. A monotonic decrease from 1.6 to 0.9 µm was observed between the first and the 35th increment (Figure 23B). Increment width showed high inter-individual variation between the 7th and the 11th increment and lower ones between the 11th and the 35th. Thereafter, from the 35th to 43rd increments, corresponding to larvae of 16-18 mm, increment width increased linearly to reach 1 µm. Beyond the 43rd

increments, results were not interpretable due to the low number of larvae having high number of increments.

Highest MIW (0.82 and 0.95 µm) were recorded for 8-12 mm larvae amongst which 67% were below the threshold and could be considered in a slow-growing state (Figure 22Q). For these larvae, MIW increased along with the size. At 13 mm, MIW exhibited an abrupt decrease. For larger larvae, MIW were much lower, ranging from 0.66 to 0.80 µm and 96% of these larvae were under the growth threshold. There was no clear pattern in the spatial distribution of increments width (Figure 22U-S).

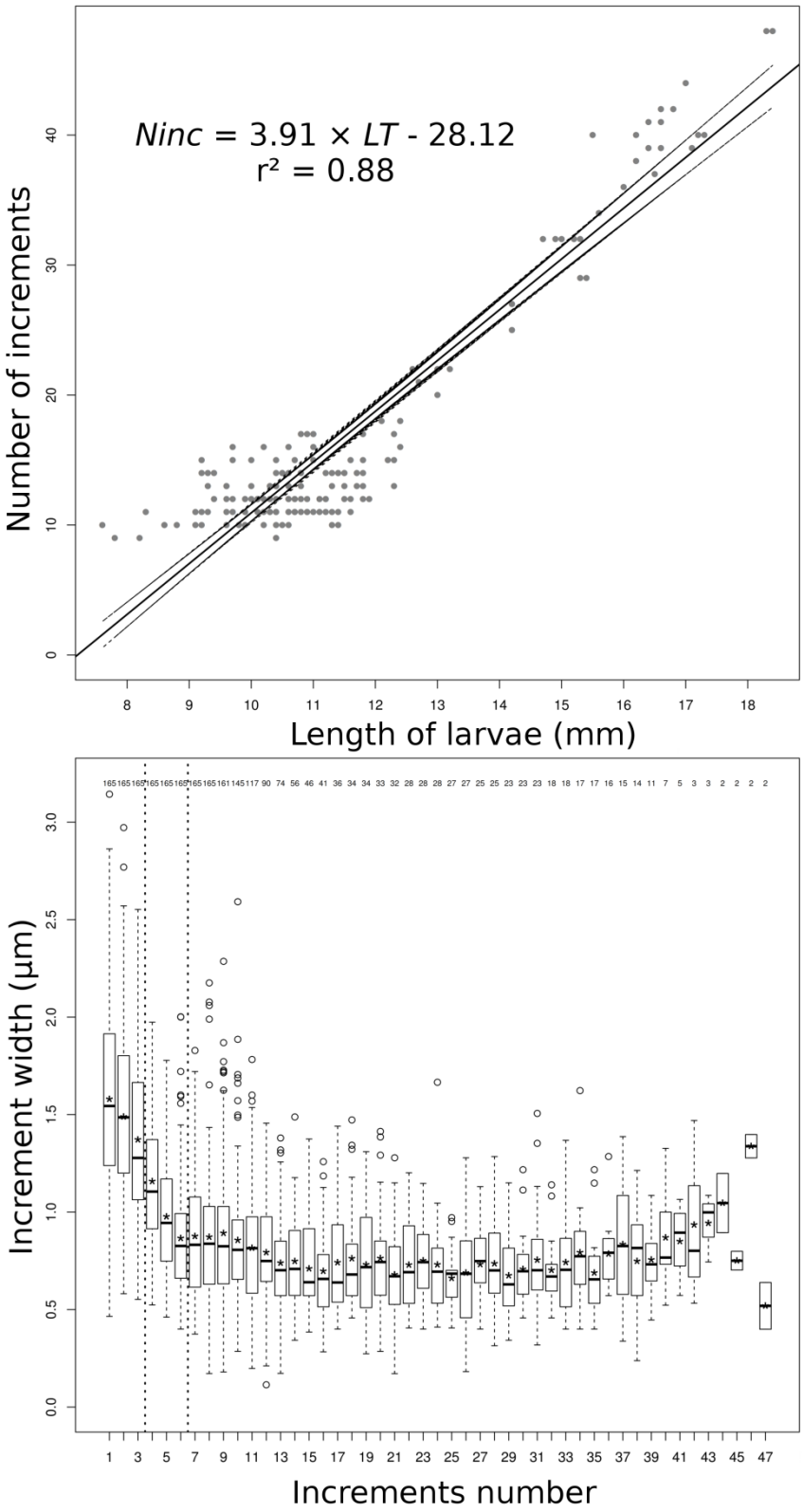

Figure 23: Otolith micro-increments analysis of Downs herring larvae in the Eastern English Channel, Dover Strait and the Southern Bight of the North Sea during winter 2015 (January-February). (A) Number of increments according to the larval size. Fitted linear regression and confidence interval (95%) are also indicated. (B) 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 (Fox et al., 2003; Geffen, 1982; Høie et al., 1997). The number of larvae analysed for each number of increments is indicated on the top (B).

1.4.Redundancy analysis

Within the eight co-variables tested, seven (temperature, salinity, *in situ* chlorophyll *a* and phaeopigments concentrations, metazooplankton abundance, latitude, longitude and larval size) were finally determined as significant and selected (Figure 24). Three groups of individuals were obtained from the HCA and distributed along the two first axes of the RDA (61.42% of the variation, Figure 24). The adjusted R^2 (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* chlorophyll *a* and phaeopigments concentrations and metazooplankton abundance.

Overall, the variance partitioning analysis evidenced 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.

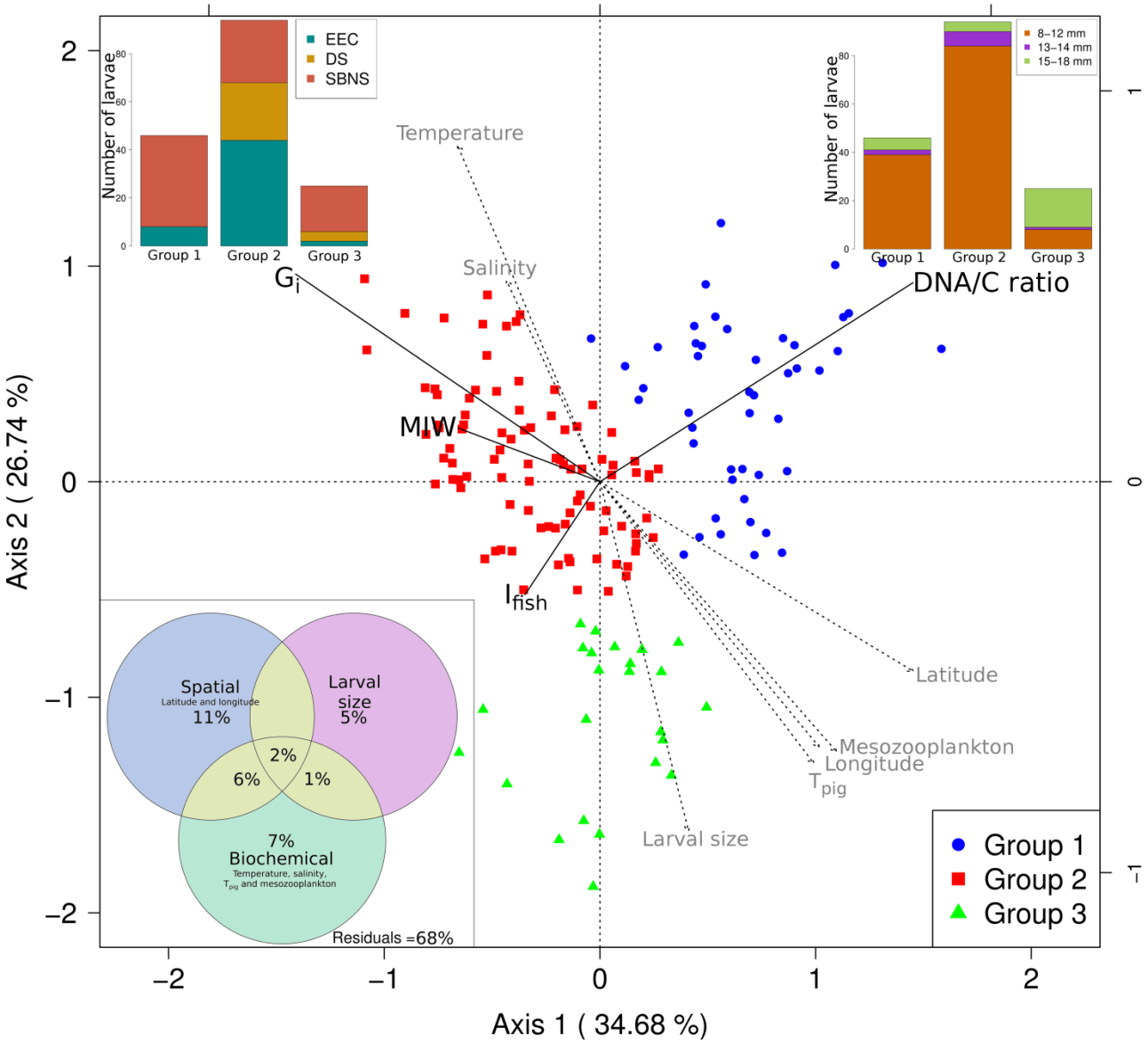

Figure 24: Redundancy and variance partitioning (bottom-left) analyses of the larval condition (Ingestion rate (I_{fish}), instantaneous growth rate (G_i), DNA/C ratio, and mean increment width (MIW) of Downs herring larvae in the Eastern English Channel (EEC), Dover Strait (DS) and the Southern Bight 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 metazooplankton 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) and size classes (top-right). Numbers in the circles (bottom-left) represent the proportion of variance explained by each variable.

2. Discussion

2.1.Robustness of the larval condition indices

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 length in contrasting environmental conditions. RNA/DNA ratios could therefore 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 (Chícharo et al., 1998; Ching et al., 2012; Rooker and Holt, 1996). 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, 2009, 2000). We used a threshold value of 60 for the DNA/C ratio in order 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 d^{-1}) can occur under sub-optimal conditions (Folkvord et al., 2000; Geffen, 1982; Lough, 1982; McGurk, 1984; Moksness et al., 1987). Campana et al. (1987) argued that daily deposition rate can be assumed if micro-increments of less than one micrometer could be detected. This cannot always be achieved with optical microscopy (Campana et al., 1987; Feet et al., 2002; Fox et al., 2003; Radtke et al., 1990) although Fox et al. (2003) suggested 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 (Arrhenius and Hansson, 1996; Campana and Neilson, 1985; Lough, 1982; McGurk, 1984; Moksness, 1992; Moksness et al., 1987). Yolk-sac absorption is thought to be completed at 4-5 d at 10°C (Lough, 1982). In our study, the check was observed at 4-6 micro-increments (i.e. 4-6 days 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 d^{-1}) to previous studies at the same period either in the same area (0.165 mm d^{-1}; Hempel, 1960), during autumn in the central of North Sea (0.13-0.24 mm d^{-1}; Kiørboe et al., 1988) or during spring in the West of Scotland (0.17 mm d^{-1}; Checkley, 1984, 0.22 mm d^{-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 d^{-1}; Weber, 1971; 0.21-0.29 mm d^{-1};

Waldman, 1961) and in the Clyde (0.33 mm d^{-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 lower larval diversity and metazooplankton abundance. In this sense, winter spawning could be an advantage for Downs herring larvae as it lead to less competition with other fish larvae and metazooplankton. The other explanation is that under suboptimal 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 in 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 (Folkvord et al., 2000; Fox et al., 2003; Geffen, 1982; McGurk, 1984; Suthers, 1998).

2.2.Ontogenetic shift in the larval condition

Despite their different integration time, three of the four indices (ingestion rate, G_i and MIW) clearly showed an abrupt 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, larval growth rate increase with size which is in accordance with previous studies (Campana et al., 1987; Folkvord et al., 2000, 1997). 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, Downs larvae condition exhibited a sharp decrease, particular 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 (Clemmesen, 1996; Clemmesen et al., 2003; Kimura, 2000; Pepin et al., 1999).

2.3. 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 metazooplankton) and larval length. Spatial variability was clearly evidenced from instantaneous growth rate and DNA/C ratios distribution highlighting respectively higher and lower values in the EEC compared to the SBNS. Hence, with regards 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 (Fey, 2000; Irigoien et al., 2008; John et al., 2001; Kiørboe et al., 1982; Moksness, 1992; Munk et al., 1991; Oeberst et al., 2009; Pasternak, 1994; Paulsen et al., 2014; Radtke and Fey, 1996). Higher temperature increases larval ingestion (Irigoien et al., 2008; Kiørboe et al., 1982) and otolith growth of herring was described to be proportionally faster at higher temperature (Campana and Hurley, 1989; Folkvord et al., 1997; Hoff and Fuiman, 1995; Wright, 1991). High prey density was reported to increase larval ingestion and assimilation (Boehlert and Yolklavich, 1984; Fiksen and Folkvord, 1999; Folkvord et al., 2000; Pasternak, 1994; Suthers, 1998; Theilacker, 1987). 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 2 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-co-variation 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 (Bailey and Houde, 1989; Houde, 1997; McGurk, 1986). A larval size of 13-14 mm also corresponds to the differentiation of the dorsal fin (Doyle, 1977; Paulsen et al., 2017) which could quickly improve their capacity to feed on larger prey by increasing their swimming capacity (Checkley, 1982; Haslob et al., 2009; Kiørboe et al., 1985; Munk, 1992; Munk and Kiørboe, 1985). Finally, it would reduce their trophic competition with copepods for phytoplankton resource (Denis et al., 2016) 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 bass 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 hamatu*s (Giraldo et al., 2016).

3. 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 in order 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 characterise 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.

CHAPITRE VI
Conclusions générales et perspectives

1. Etude de la condition larvaire

La condition des larves du hareng des Downs a été étudiée grâce à cinq indices, trois caractérisant leur nutrition (analyse qualitative et quantitative des contenus digestifs et le rapport ADN/C) et deux caractérisant leur croissance (le rapport ARN/ADN et les micro-incréments des otolithes). Le récapitulatif des différents indices utilisés est présenté dans la Table 6.

Table 6: Récapitulatif des différents indices de condition mesurés sur les larves de hareng des Downs pendant les hivers (mi-janvier-mi-février) 2008 à 2015 en Manche et Mer du Nord.

Type d'indice	Méthode	Temps d'intégration	Mesures	Avantages	Inconvénients
Nutrition	Observation des contenus digestifs par MO et MEB	Court	Identification des proies ingérées, sélectivité des proies, taux de vacuité	Détection et identification de petites proies (< 50 µm)	Chronophage et coûteux, qualitatif Biais : évacuation (effet capture et fixation, effet jour/nuit, temps variable) et niveau de dégradation des proies
Nutrition	Mesure de la fluorescence des contenus digestifs	Court	Taux d'ingestion, pression de broutage	Rapide, peu coûteux, quantitatif	Biais : évacuation (effet capture et fixation, effet jour/nuit, temps variable) et niveau de dégradation des proies, proies non pigmentées
Nutrition	Mesure du rapport ADN/C sur le muscle	Moyen	Etat de la nutrition, nombre de larves en train de se nourrir	Rapide, peu coûteux, indice réactif, indépendant de l'effet ontogénique et température	Biais : effet jour/nuit, qualité/quantité des proies
Croissance	Mesure du rapport ARN/ADN sur le muscle	Moyen	Etat de la croissance, nombre de larves en train de croître	Rapide, peu coûteux, indice réactif	Effet jour/nuit, qualité/quantité des proies, effet ontogénique, effet température
Croissance	Lecture des otolithes	Long	Relation taille/âge, taux de croissance journalier Distance inter-stries	Retrace la condition de la larve jusqu'à sa capture	Chronophage, nécessite dépôts journaliers, haute résolution de la lecture, effet température

La nutrition des larves de hareng des Downs a été étudiée à partir de deux approches directes basées sur l'analyse de leurs contenus digestifs et une approche indirecte basée sur le rapport ADN/C mesuré sur leur muscle. L'analyse des contenus digestifs a permis d'identifier les différentes espèces de proies ingérées le jour même, de déterminer la sélectivité des larves pour ces proies et de mesurer le taux de vacuité d'un point de vue qualitatif et le taux d'ingestion d'un point de vue quantitatif. L'utilisation du rapport ADN/C comme indice de

condition a permis d'évaluer l'état de nutrition des larves de hareng plusieurs jours avant leur capture.

L'utilisation du MEB pour l'identification des proies ingérées étant coûteuse en termes de temps et d'argent, la détermination des proies dans les contenus digestifs a été réalisée dans le cadre de cette thèse de manière qualitative (présence-absence des proies). Elle a révélé l'importance des petites proies (< 50 µm), notamment des protistes auto et hétérotrophes, dans le régime alimentaire des larves de hareng des Downs. Du fait de leur taille, ces proies n'auraient pu être identifiées par la microscopie optique classique, ce qui confirme la pertinence du MEB pour observer et étudier le régime alimentaire des larves de poissons. L'utilisation du MEB a également permis d'améliorer la précision des calculs de sélectivité en identifiant les proies protistes dans l'eau au même niveau taxonomique que celui obtenu pour les proies observées dans les contenus digestifs. Le taux de vacuité (nombre de larves ayant le tube digestif vide) a été calculé et les valeurs obtenues (47-65%) sont comparables à celles d'autres espèces, même printanières. Plusieurs facteurs liés à l'évacuation du contenu digestif avant l'analyse (capture, fixation, effet jour/nuit) ou à l'état de dégradation des proies peuvent conduire à sous-estimer la nutrition des larves. Les taux de vacuité obtenus doivent donc être considérés comme des valeurs minimales.

L'observation des contenus digestifs par le MEB a été complétée par une analyse quantitative des contenus digestifs par fluorimètrie. Cette approche a été adaptée pour la première fois dans le cadre de cette thèse aux larves de hareng des Downs. Elle a montré une contribution non négligeable du phytoplancton au régime alimentaire des larves de hareng. Au-delà de l'ingestion du phytoplancton, cette technique permet également d'évaluer de manière quantitative l'ingestion globale (via la consommation de proies herbivores) des larves. L'ingestion mesurée a donc été utilisée comme un indicateur de la condition et notamment de l'activité alimentaire à court terme. Comme pour l'approche qualitative, l'évacuation des proies avant l'analyse, la destruction pigmentaire et la présence de proies non pigmentées dans le tube digestif peuvent conduire à sous-estimer l'ingestion larvaire. Les taux d'ingestion obtenus doivent donc être considérés comme des valeurs minimales et relatives mais restent comparables entre les classes de tailles lorsqu'ils sont utilisés pour caractériser la condition nutritionnelle des larves.

L'utilisation du rapport ADN/C comme indice de condition a été récemment conseillé par Bergeron (1997) comme une alternative au rapport ARN/ADN, car il serait plus adapté aux premiers stades larvaires et serait indépendant de la température. Son application pour la première fois sur les larves de hareng au cours de ce travail de thèse a permis de montrer une

amélioration de l'état nutritionnel avec la taille des larves. Le calcul du pourcentage de larves en bonne ou mauvaise condition requiert l'utilisation d'une valeur limite qui correspond au seuil proposé par Bergeron (2000) pour les larves d'anchois du Golfe de Gascogne au printemps. Bien que certainement inadapté pour les larves de hareng des Downs en hiver, il est le seul disponible à l'heure actuelle pour les larves de poissons pélagiques. Les pourcentages de larves en bonne condition sont donc certainement sous-estimés et les valeurs doivent être considérées de manière relative entre les différentes classes de taille.

La croissance des larves de hareng des Downs a été étudiée à partir de deux indices, un indice à moyen terme, basé sur le rapport ARN/ADN, qui renseigne sur l'état de leur croissance quelques jours avant leur capture, et un indice à long terme, basé sur les micro-incréments des otolithes, pour mesurer le taux de croissance journalière des larves jusqu'à leur capture.

Le rapport ARN/ADN est un des indices les plus utilisés pour caractériser la condition des larves de poissons. Son application au hareng des Downs a permis de comparer les valeurs obtenues à celles d'autres espèces. Son utilisation comme indice de la croissance récente requiert de prendre en compte l'effet jour/nuit sur l'alimentation, ainsi que la variabilité liée à l'ontogénie et à la température. Dans le cadre de ce travail de thèse, son utilisation était justifiée puisqu'il a été mesuré sur un gradient de tailles de larves restreint collectées au cours de la nuit et sur un ensemble de stations où les températures étaient comparables. Ainsi, les valeurs obtenues étaient pertinentes, le signal était clair et montrait une diminution nette de la croissance avec la taille des larves.

L'utilisation des micro-incréments comme indice de condition suppose un dépôt journalier des stries de croissance. Lorsque le taux de croissance est supposé être faible comme pour le hareng des Downs, les résultats obtenus ont montré que l'utilisation d'un microscope haute résolution permettait la détection des anneaux de croissance. Le taux de croissance journalière a ainsi pu être estimé et les valeurs obtenues étaient comparables à celle des larves de hareng pour les autres saisons et pour d'autres espèces (Campana and Moksness, 1991; Checkley, 1984; Geffen, 1986; Hempel, 1960; Waldman, 1961; Weber, 1971). L'utilisation de la distance inter-stries comme indice de croissance a permis de repérer les phases critiques en termes de croissance et également d'identifier la taille des larves leur correspondant. Le signal obtenu était comparable à celui du rapport ARN/ADN, mais la diminution était beaucoup plus forte et le signal plus clair.

Les indices disponibles pour étudier la condition chez les larves de poissons sont nombreux. Cependant, leur multiplicité, leur échelle contrastée d'intégration des changements, ainsi que leur application à des espèces, des saisons, des années et des individus différents, rend difficile l'évaluation de leur efficacité pour mesurer la condition et la comparaison des résultats obtenus. Dans le cadre de cette thèse, quatre des cinq indices (le taux d'ingestion, les rapports ARN/ADN et ADN/C et la largeur des micro-incréments) ont ainsi été mesurés à l'échelle individuelle en 2015 afin de comparer un ensemble d'indicateurs intégrant les changements selon différentes échelles de temps, de plusieurs heures à plusieurs mois. Les résultats obtenus ont mené, pour chacun des deux types d'indices (nutrition et croissance), aux mêmes conclusions concernant les changements ontogéniques de la condition des larves du hareng des Downs. Ces résultats ont également montré une déconnexion entre la nutrition et la croissance des larves, ce qui prouve que la mesure de l'une ne peut pas être utilisée pour évaluer l'autre. Dans un contexte de réduction du coût des analyses pour des études futures, ces résultats soutiennent la possibilité de réduire le nombre d'indice à un par type de condition (nutrition et croissance). Du fait du temps d'analyse nécessaire, l'utilisation des otolithes n'est pas envisageable dans le cadre de l'étude de la condition en routine. Cependant, ils peuvent être utilisés dans une première approche afin de repérer les périodes de faible croissance ayant eu lieu tout au long de la vie larvaire. Ceci peut être réalisé sur des larves âgées, ce qui évite d'avoir à mesurer la condition sur toutes les tailles ou stades de développement. Une fois ces périodes détectées, on peut alors cibler les tailles de larves concernées et évaluer leur condition par des indices plus rapides et moins coûteux à mesurer. Dans ce contexte, la capacité des rapports ADN/C et ARN/ADN à évaluer rapidement la nutrition et la croissance les rend les plus pertinents pour une étude en routine des périodes difficiles subies par les larves. Le taux d'ingestion basé sur la méthode de fluorescence a un temps d'intégration trop court pour montrer l'impact d'une période de jeûne sur la condition mais peut permettre de calibrer au départ le rapport ADN/C.

2. La condition des larves de hareng des Downs durant la période critique

Entre 2008 et 2015, les larves de hareng des Downs de 8 à 20 mm étaient principalement distribuées dans les eaux centrales, plus chaudes et plus salées, de Manche orientale et de la baie sud de la mer du Nord selon un gradient orienté sud-ouest nord-est de tailles croissantes et d'abondances décroissantes. Ce gradient suit le courant résiduel de marée et traduit la dérive des larves depuis les zones de ponte situées en Manche Orientale et dans la

baie sud de la mer du Nord vers les nourriceries côtières situées le long des côtes au sud de la mer du Nord. Ce travail de thèse a montré que des changements ontogéniques en termes de régime alimentaire et de condition avaient lieu au cours de cette dérive (Figure 25).

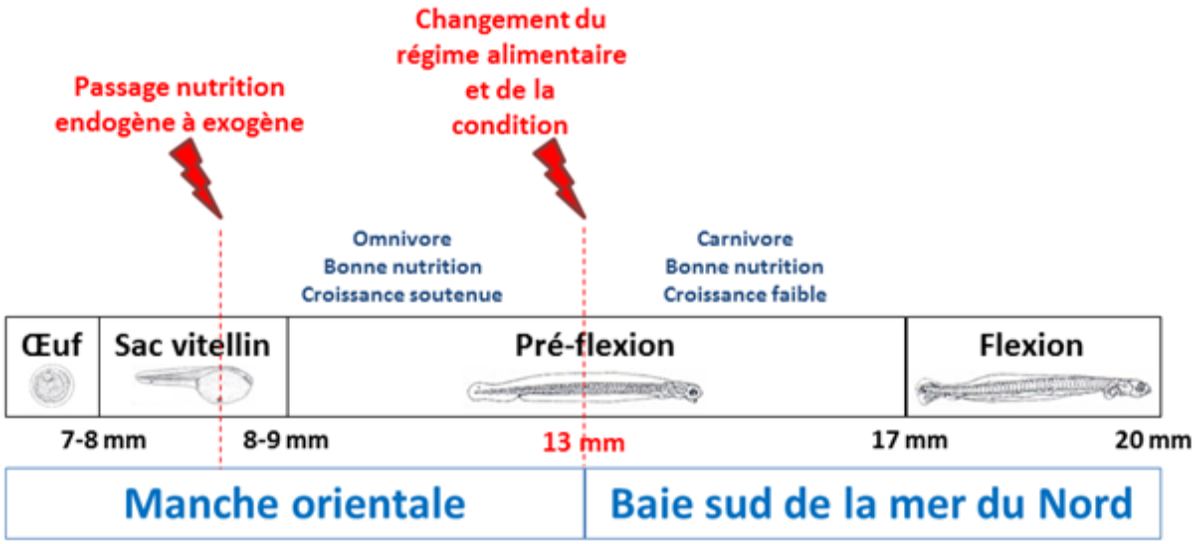

Figure 25: Schéma récapitulatif des changements ontogéniques de la condition des larves de hareng des Downs pendant les hivers (mi-janvier-mi-février) 2008 à 2015 en Manche et Mer du Nord.

2.1. Les larves de hareng de 8 à 12 mm : manger pour grandir

Entre 8 et 12 mm, les larves de hareng des Downs étaient principalement distribuées en Manche Orientale. L'analyse des contenus digestifs a mis en évidence que ces larves avaient un régime alimentaire omnivore diversifié et composé de protistes et de petites proies zooplanctoniques. Les protistes constituaient une part importante du régime alimentaire de ces larves en terme de diversité. Les dinoflagellés tels que *Alexandrium* spp., *Dinophysis acuminate*, *Pyrophacus* spp. et *Diplopsalis* spp. ainsi que plusieurs diatomées comme *Coscinodiscus* spp., *Thalassiosira* spp., *Raphoneis amphiceros*, *Plagiotropis* spp. et *Delphineis surirella* étaient sélectionnées positivement. Le régime alimentaire était dominé en terme d'occurrence par des proies zooplanctoniques de petite taille comme *Euterpina acutifrons*, *Oncaea* spp., les copépodites de *Pseudocalanus elongatus*, les œufs d'invertébrés, les larves de bivalves et les nauplii de copépodes et de cirripèdes. Les fortes variabilités de l'ingestion et du rapport ADN/C observées pour ces larves suggéraient qu'un certain nombre d'entre elles (17%) avaient des difficultés à se nourrir. Le taux de vacuité élevé des larves de 8 mm (65%) est en accord avec leur nutrition vitelline. Le taux d'ingestion augmente et la vacuité diminue pour les larves de 9 à 12 mm, ce qui indique une alimentation active liée à

l'absorption du sac vitellin. Les taux de croissance mesurés par le rapport ARN/ADN et à partir des otolithes indiquaient une croissance soutenue, majoritairement supérieure au seuil de détermination de bonne croissance pour le rapport ARN/ADN et bien supérieure à celle des larves plus grandes. Le régime alimentaire omnivore très diversifié semble permettre aux larves en première nutrition de se nourrir suffisamment pour croître. Les protistes jouent un rôle essentiel dans la croissance des larves en améliorant l'efficacité de la digestion des autres proies comme le zooplancton par l'augmentation de l'activité enzymatique (Cahu et al., 1998; Hjelmeland et al., 1988; Illing et al., 2015; Reitan et al., 1997; St. John et al., 2001) et contribuent à l'apport de lipides et d'acides aminés (Braven et al., 1995). Ces larves affectent principalement les réserves d'énergie vers la croissance somatique notamment pour diminuer le risque de prédation et de mortalité. Les larves en faible croissance restent vulnérables à la prédation pendant une période plus longue (hypothèse "stage-duration") tandis que les larves en forte croissance ont un potentiel de survie plus élevé (hypothèse "Bigger is better"; Anderson, 1988; Houde, 2008, 1987).

2.2. Une période difficile à 13 mm

Les larves de 13 mm étaient distribuées principalement dans le Détroit du Pas-de-Calais et en baie sud de la mer du Nord et montraient une diminution brutale de leur ingestion et de leur croissance. En effet, à l'exception du rapport ARN/ADN, tous les indices de condition étaient au plus bas, ce qui indique de très grandes difficultés pour ces larves à se nourrir et à croître. Ces changements en termes de nutrition et de croissance correspondent à des modifications observées dans le cadre de ce travail dans le régime alimentaire de ces larves qui s'orientent vers un régime moins diversifié, plus carnivore, et composé principalement de proies zooplanctoniques (copépodes calanoides indéterminés et de *Oncaea* spp.) et quelques espèces de chrysophytes et de diatomées indéterminées. L'augmentation de la taille des proies ingérées est à mettre en relation avec la capacité de nage, l'ouverture de la bouche et la largeur du tube digestif qui augmentent avec la taille des larves (Checkley, 1982; Haslob et al., 2009; Kiørboe et al., 1985; Munk, 1992; Munk and Kiørboe, 1985). Les larves de 13 mm deviennent ainsi capables de consommer de plus grandes proies, mais ce changement semble avoir un impact négatif sur la condition des larves et suggère que la sélection des nouvelles proies zooplanctoniques ingérées requiert un temps d'adaptation des larves pour capturer des proies plus mobiles. Selon Hjort (1914), la période critique a lieu lors du passage de la nutrition endogène à la nutrition exogène, pendant lequel de fortes mortalités larvaires

peuvent s'observer. Au vu des résultats obtenus, nous supposons que, plus que le passage à une nutrition sur le milieu, c'est le changement de régime alimentaire à partir de 13 mm qui constitue la période critique pour les larves de hareng des Downs.

2.3. A partir de 14 mm : manger pour stocker ?

L'augmentation du taux d'ingestion et la diminution du rapport ADN/C suggèrent une amélioration de l'état nutritionnel des larves à partir de 14 mm qui va jusqu'à atteindre un niveau supérieur et une variabilité moindre que ceux des larves plus petites. Ces larves ont un régime alimentaire majoritairement carnivore, moins diversifié et composé de proies plus grandes comme les copépodes *Temora longicornis*, *Paracalanus parvus* et *Pseudocalanus elongatus* et quelques protistes comme les dinoflagellés *Gonyaulax* spp. et *Prorocentrum micans* et la diatomée *Thalassiosira curviseriata*. La distribution de ces larves dans les eaux plus concentrées en pigments chlorophylliens et en abondance de métazooplancton dans la baie sud de la mer du Nord permet ainsi de soutenir la nutrition. Par ailleurs, les faibles taux de croissance mesurés à partir des otolithes et du rapport ARN/ADN suggèrent soit que la nutrition est insuffisante pour soutenir la croissance soit que les larves se sont orientées vers une stratégie de stockage de l'énergie plutôt qu'une stratégie d'utilisation de l'énergie pour la croissance. Si la nutrition est insuffisante pour soutenir la croissance, cela implique que les larves de taille supérieure à 13 mm, bien que se nourrissant, se trouvent toujours en période critique. Dans le cas contraire, les larves en s'alimentant plutôt de proies zooplanctoniques comme les copépodes s'assurent un apport plus riche en carbone qui leur permet de stocker des réserves pour faire face à des périodes de jeûne ou des conditions hydro-biologiques défavorables. En effet, lorsque l'apport d'énergie par la consommation de proies dépasse les besoins métaboliques de la larve, l'excès d'énergie peut être stocké dans les tissus sous forme de triglycérides (Lehninger, 1975). Cette stratégie ne serait pas spécifique aux larves hivernales puisqu'elle a déjà été observée pour des espèces printanières comme la sole (Grioche, 1998) et le flet (Harlay, 2001) en Manche Orientale et *Chionodraco hamatus* (Giraldo et al., 2016) dans l'Océan Austral. Dans le cas du hareng des Downs, cette stratégie de stockage pourrait permettre aux larves de supporter les conditions hivernales plus pauvres en nourriture et de survivre jusqu'au printemps où les conditions trophiques sont plus favorables.

3. Perspectives

3.1. La place des larves de hareng des Downs au sein du réseau trophique hivernal

L'analyse du régime alimentaire des larves de hareng des Downs par le MEB et la fluorescence a mis en évidence que ces larves avaient un rôle de consommateur primaire et secondaire en se nourrissant d'une diversité importante à la fois de protistes et de zooplancton. La présence ponctuelle de ces larves et dans des proportions très importantes leur confère un rôle essentiel au sein du réseau trophique planctonique hivernal qui mérite d'être étudié plus en détail.

L'identification des proies ingérées au MEB a été limitée aux organismes à "corps durs" du fait de la conservation, antérieure au début de ce travail de thèse, des échantillons de larves de hareng collectées pendant la campagne IBTS dans une solution formolée. Ce type de conservation ne permet pas de préserver les organismes à corps mous (ciliés et dinoflagellés nus) qui sont potentiellement des proies importantes pour les larves de poissons (Illing et al., 2015; Montagnes et al., 2010). L'estimation de la contribution de ces organismes au régime alimentaire des larves du hareng des Downs par le MEB nécessitera de préserver les larves dans une autre solution de conservation (bouin ou le lugol) et de les sécher au point critique plutôt qu'à la hotte sous flux laminaire (Montagnes et al., 2010).

L'identification des proies ingérées à partir de l'observation directe des contenus digestifs a également été limitée par l'état de digestion avancé de certaines proies, rendant impossible leur identification jusqu'à l'espèce. La méthode d'analyse génétique ou "barcoding" (Riemann et al., 2010) pourrait pallier ce problème, car cette technique permet d'identifier les proies dans un contenu digestif par le séquençage de leur ADN quel que soit leur état de dégradation. Comme la méthode d'analyse au MEB, il s'agit d'une méthode qualitative (détermination des proies) qui souffre des mêmes biais engendrés par l'évacuation du contenu digestif avant capture mais dont le coût moins important permettrait de traiter un plus grand nombre d'échantillons plus rapidement.

Afin de remédier au problème de l'évacuation du contenu digestif des larves avant capture, les résultats obtenus sur la nutrition récente (contenus digestifs) des larves devront nécessairement être complétés par l'utilisation d'indices permettant d'identifier les proies ingérées sur une échelle de temps plus importante. L'utilisation des isotopes stables du carbone (^{13}C) et de l'azote (^{15}N) mesurés dans les tissus des consommateurs permet de déterminer l'origine des sources de nourriture, le niveau trophique de chacune des espèces et

les interactions trophiques entre les espèces (Fry, 1988). Les acides gras accumulés dans les tissus des organismes sont caractéristiques des proies qu'ils ingèrent et sont également utilisés comme traceurs du régime alimentaire, avec un temps d'intégration variant de quelques jours à quelques semaines. La combinaison de ces deux indices (Couturier et al., 2013) mesurés sur l'ensemble des organismes planctoniques présents en hiver permettrait alors de replacer de manière plus globale les larves de hareng des Downs au sein du réseau trophique hivernal.

3.2. Approche expérimentale

La caractérisation *in situ* de la condition larvaire du hareng des Downs nécessite d'être complétée par une approche expérimentale. En effet, au cours de ce travail de thèse, le pourcentage de larves en bon état nutritionnel a été calculé, pour le rapport ADN/C, à partir d'un seuil calculé pour les larves d'anchois du Golfe de Gascogne au printemps. Une calibration en laboratoire du rapport ADN/C en élevant les larves de hareng des Downs dans des conditions connues permettrait de déterminer un seuil qui leur soit plus spécifiquement adapté, et ainsi d'améliorer l'estimation du pourcentage de larves considérées comme étant en bonne condition. Il s'agira de mesurer l'évolution du rapport ADN/C des larves de hareng des Downs en fonction de conditions d'alimentation différentes (allant d'une forte nutrition à une période de jeûne) qu'on leur aura fait subir expérimentalement.

3.3. Etude de l'habitat de bonne condition larvaire

Les résultats obtenus dans le cadre de cette étude sur la variabilité spatiale, temporelle et ontogénique de la condition des larves de hareng des Downs suggèrent l'existence de zones plus favorables que d'autres à la condition larvaire. En effet, si la condition des larves du hareng des Downs était spatialisée principalement selon la taille des larves, elle montrait également une variabilité spatiale au sein d'un même groupe de tailles. Par exemple, les quelques larves de 8 à 12 mm présentes en baie sud de la mer du Nord avaient une condition moins bonne que celles de Manche orientale. Cela signifie potentiellement que la Manche orientale est un habitat plus favorable pour les larves de cette gamme de tailles. De plus, le rapport ARN/ADN mesuré sur les larves de 2014 et 2015 montrait une fluctuation du taux de croissance avec des valeurs plus faibles en 2014. La modélisation des habitats est un des outils qui permettrait d'identifier les facteurs qui influencent la distribution spatiale et temporelle de la condition larvaire. La réalisation d'un modèle d'habitat nécessite un effort

conséquent de suivi à moyen terme (5-10 ans) de la condition larvaire afin de disposer d'un nombre suffisant d'années contrastées d'un point de vue environnemental.

3.4. Implications pour le recrutement du hareng

Actuellement, le recrutement du hareng de la mer du Nord est estimé par un indice larvaire calculé à partir des abondances de larves supérieures à 20 mm collectées au-dessus de 54°N pendant la campagne IBTS (ICES, 2015). Les larves des Downs étant exclues du calcul de cet indice du fait de l'incertitude quant à leur mortalité, il n'existe pas d'indice larvaire pour cette sous-population. Théoriquement, le calcul d'un indice larvaire pour le hareng des Downs pourrait être réalisé 1) à partir des abondances des larves ayant passé la période critique (comme cela est fait actuellement pour les trois sous-populations de la mer du Nord), et 2) à partir des abondances des jeunes larves collectées pendant la campagne IBTS à condition d'estimer la mortalité qu'elles subissent.

L'utilisation des larves ayant passé la période critique pour calculer un indice larvaire requiert en premier lieu de déterminer la durée de la période critique. D'après les résultats obtenus, nous avons supposé que la période critique démarre à 13 mm lorsque les larves changent leurs habitudes alimentaires. Après 13 mm, nous avons montré un changement en termes de croissance, ce qui pourrait indiquer une mauvaise condition de ces larves ou être lié à un changement d'allocation de l'énergie. La quantification des proportions des principales classes lipidiques (triglycérides, cires, phospholipides et cholestérols) dans les tissus larvaires permettrait de préciser la stratégie d'allocation d'énergie adoptée par ces larves (Giraldo, 2012). En particulier, la quantification du taux de triglycérides des larves permettrait d'identifier la taille larvaire marquant la fin de la période critique, c'est à dire la taille à partir de laquelle les apports énergétiques sont suffisants pour permettre aux larves de croître ou de stocker. La détermination de la durée de la période critique par les lipides nécessite d'analyser un spectre de tailles de larves plus large que celui analysé dans le cadre de cette thèse. Ceci ne peut être réalisé à partir de la campagne IBTS et nécessite de mettre en place une campagne plus tardive ou d'utiliser les larves de hareng collectées au printemps dans le cadre de suivi écologique tel que l'Impact des Grands Aménagements (IGA) des centrales nucléaires de Gravelines et de Penly.

L'utilisation des jeunes larves collectées pendant la campagne IBTS pour calculer un indice larvaire requiert d'estimer la mortalité subie par ces larves. Les indices de nutrition et de croissance utilisés au cours de cette étude ont montré qu'une partie des petites larves de

hareng des Downs avaient une nutrition faible. Mais ces indicateurs ne permettent pas d'estimer la proportion de larves qui va survivre. L'utilisation des indices histologiques, déterminés à partir de l'analyse visuelle des tissus de certains organes (Tube digestif, foie et pancréas; Grioche, 1998), permettra d'estimer une proportion de larves au point de non-retour. Le point de non-retour correspond au moment où, même alimentées, les organes des larves sont trop dégradés pour leur permettre de survivre (Blaxter and Hempel, 1963). L'estimation du nombre de larves de hareng au point de non-retour permet potentiellement d'estimer la mortalité subie par ces larves pour chaque classe de taille. Du fait de son coût non négligeable, cette approche ne peut être utilisée en routine mais peut être utilisée en complément afin de calibrer des indices, comme le rapport ARN/ADN ou le rapport triacylglycerol/cholesterol (Giraldo et al., 2016), qui sont plus rapides à mesurer.

Les résultats obtenus dans cette thèse peuvent également être utilisés afin d'améliorer les modèles mécanistiques. En particulier, les modèles individus centrés (IBM) sont devenus l'un des outils les plus importants utilisés pour identifier les processus affectant les taux de survie des jeunes stades de larves de poissons et prédire le recrutement (Hinrichsen et al., 2011; Miller, 2007). Dickey-Collas et al. (2009) ont développé un modèle pour étudier l'influence des conditions hydrodynamiques sur le recrutement du hareng. Les données d'alimentation, de croissance et de condition obtenues dans le cadre de cette thèse pourraient être utilisées afin de compléter le paramétrage de ces modèles.

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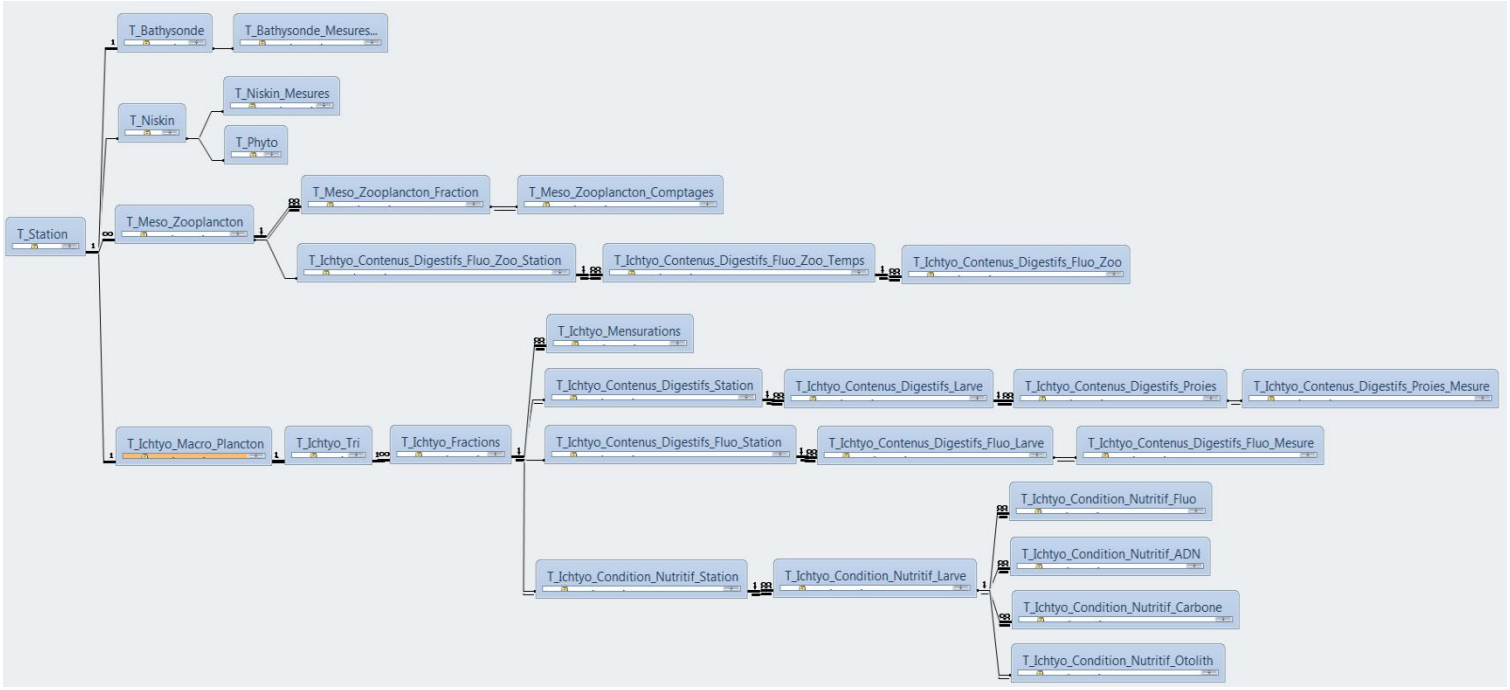
Annexe I

Base de données de la campagne IBTS complétée par les analyses de contenus digestifs et de conditions effectuées durant cette thèse



Liste des communications

Articles

Denis, J., Vallet, C., Courcot, L., Lefebvre, V., Caboche, J., Antajan, E., Marchal, P., Loots, C. Feeding strategy of Downs herring larvae (*Clupea harengus* L.) in the English Channel and North Sea. *Journal of Sea Research*. Publié.

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