

Diet is correlated with otolith shape in marine fish

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ABSTRACT: Previous studies have shown that the amount of food influences fish otolith structure, opacity and shape and that diet composition has an effect on otolith chemical composition. This study investigated the potential correlation between diet and otolith shape in 5 wild marine fish species by addressing 4 complementary questions. First, is there a global relationship between diet and otolith shape? Second, which prey categories are involved in this relationship? Third, what are the respective contributions of food quantity and relative composition to diet–otolith shape co-variation? Fourth, is diet energetic composition related to otolith shape? For each species, we investigated how otolith shape varies with diet. These questions were tackled by describing diet in the analysis in 4 different ways, while also including individual-state variables to remove potential confounding effects. First, besides the strong effect of individual-state, a global relationship between diet and otolith shape was detected for 4 out of 5 fish species. Second, both main and secondary prey categories were related to variability in otolith shape, and otolith outline reconstructions revealed that both otolith global shape and its finer details co-varied with these prey categories. Third, the contribution of relative diet composition to diet–otolith shape co-variation was much higher than that of ingested food quantity. Fourth, the energetic composition of diet was related to otolith shape of only 1 species. These results suggest that diet in marine fish species may influence the quantity and composition of saccular endolymph proteins which play an important role in otolith biomineralization and their resulting 3D structure.

KEY WORDS: Fourier analysis · English Channel · Interspecific · Morphometric analysis · Otolith growth · Saccular otolith · Stomach contents

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INTRODUCTION

Otoliths are calcified structures found in the inner ear of teleostean fish. They are organised into 3 pairs and assist with auditory and balance functions of fish. They are critical tools in fisheries science and management. Their structure allows aging of individual fish and the determination of population age structure for stock assessment (e.g. Worthington et al. 1995, Caldwell & Wellington 2003). Their morphology can be used for individual assignment to (sub-)populations and to infer population structure (e.g. Castonguay et al. 1991, Stransky et al. 2008). The sagit-

tae (the most studied otolith pair because of their large size) are mainly composed of calcium carbonate in aragonite form deposited on an organic matrix which represents 0.1 to 10% of total material (Degens et al. 1969). The organic matrix, although present in small to minute amounts in otoliths, is thought to play a key role in its formation as in all biomineralization processes (Nagasawa 2013). Biomineralization of the sagittal otolith (referred to hereafter as 'otolith') is an acellular process that takes place in the saccule (otic sac). Otoliths grow by accretion and precipitation of organic and ionic precursors contained in the saccular endolymph in which they

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are bathing. Otolith biomineralization is therefore totally dependent on the endolymph composition and precursors that are either synthesized (organic) or transported (ionic) by secretory cells and ionocytes, respectively, belonging to the saccular epithelium (Payan et al. 2004). Moreover, the spatial distribution of these cells in the saccular epithelium induces concentration gradients of both ions and organic precursors in the endolymph that are involved in otolith biomineralization processes (Pisam et al. 1998, Payan et al. 1999, Borelli et al. 2001).

Otolith biomineralization results from multi-causal processes due to the interaction of many internal (physiological) and external (environmental) factors (Allemand et al. 2007), which generate high morphological variability in otolith shape at both intra- and inter-specific level. First, otolith shape is species-specific (Tuset et al. 2006), reflecting genetic determinism (L'Abée-Lund 1988, Vignon & Morat 2010). Second, factors or processes acting on fish metabolism and physiology have an impact on otolith morphology, such as ontogenetic development (size: Hüsey, 2008; age: Castonguay et al. 1991; sexual maturation Mérigot et al. 2007; sex: Castonguay et al. 1991, Bolles & Begg 2000). Third, environmental factors such as water temperature produce otolith growth variation, and thus shape variability (Cardinale et al. 2004). Food quantity can also impact otolith shape both directly and indirectly. It has an indirect effect on global otolith shape through its effect on otolith growth and a direct effect on otolith crenation (Gagliano & McCormick 2004, Cardinale et al. 2004, Hüsey 2008).

Consequences of fish nutrition on otolith structure and growth, especially the impact of starvation or food restriction and satiation, have been well-studied (Molony & Choat 1990, Molony & Sheaves 1998, Hüsey & Mosegaard 2004, Fernandez-Jover & Sanchez-Jerez 2015). A decrease in the otolith increment widths and thus in otolith growth was observed after reduced feeding periods (Massou et al. 2002). The deposited material is more translucent in response to severe (long period and low ration) food restriction, which can lead to otolith structural discontinuities that do not conform to the seasonal opaque and translucent layers of annuli (Høie et al. 2008), referred to as 'checks' (Panfili et al. 2002). Such changes in opacity are the consequences of variation in the composition of inorganic and organic otolith compounds (Jolivet et al. 2013) and precursors. A starvation period leads to change in blood plasma composition, which generates a decrease in the acid–base equilibrium in the saccular endolymph

and thus induces a reduction of aragonite precipitation rate. As a consequence, a reduction of daily growth rate due to starvation could be observed even if calcium concentration was not affected (Payan et al. 1998). Concerning the organic precursors, only the protein 'factor retarding crystallization' (FRC) concentration decreases during starvation periods, especially in the proximal zone (Guibbolini et al. 2006). This change may play a key role in the intensity of aragonite deposition and thus otolith growth. In conclusion, food amounts may affect otolith growth, opacity (or structure) and biomineralization.

Several papers have also documented a link between energy metabolism and otolith growth. Otolith growth is closely related to standard metabolic rate (Mosegaard et al. 1988, Fablet et al. 2011) and otolith accretion appears to be regulated by feeding-induced thermogenesis (Huuskonen & Karjalainen 1998). Otolith growth in larvae and juveniles is also related to individual condition index, estimated from fish lipid composition (Amara et al. 2007), which in turn depends on zooplankton biomass (Suthers et al. 1992). Besides the fact that lipid quantity such as triacylglycerol content can be used as a condition index for fish (Fraser 1989), taken together, these results suggest that lipid content in diet and energy metabolism may influence otolith growth. Given that lipid content of prey is the primary determinant of their energy density (Anthony et al. 2000, Spitz et al. 2010), diet energy content or composition in terms of energetic prey categories is a good candidate for encompassing both effects.

Along with food abundance and energy content, diet composition can also affect otoliths, especially their chemical composition (Sanchez-Jerez et al. 2002). For instance, Barium (Ba) and strontium (Sr) concentrations in *Pomatomus saltatrix* otoliths were related to the concentration of these elements in their prey (Buckel et al. 2004). Here, the authors assumed the diet effect on Ba and Sr concentration in otoliths could be either direct or indirect through diet-based growth rate changes that induce element incorporation rate variation in otoliths. Even if around 80% of Sr and Ba in otoliths comes from water (Walther & Thorrold 2006) and not from diet, a trophic transfer may be considered as a potential source of element accumulation in fish otoliths. The concentration of manganese (Mn) in the habitat and prey items was also related to its accumulation in otoliths (Sanchez-Jerez et al. 2002). The fact that the Mn:Ca ratio in otoliths is not correlated to the same ratio in water suggests a trophic transfer of metallic elements, such as Mn (Thorrold et al. 1997). Moreover, variations in

$\delta^{13}\text{C}$ otolith values were observed to correlate with variations in muscular $\delta^{13}\text{C}$ values among diet treatment (Elsdon et al. 2010).

In summary, previous experimental work has revealed that the food ration level affects otolith structure, opacity and shape. Studies in the laboratory and *in situ* have shown that metabolic rate and food energy content influence otolith growth, and that diet composition affects otolith chemical composition. In the present study, we investigated the potential relationship between diet, described as a combination of both food composition and quantity, and the otolith shape at the intra-population level in 5 marine fish species, including 3 roundfishes and 2 flatfishes sampled in the wild. More specifically, we addressed 4 related questions. We first tested for a global relationship between diet (represented by the weight of each taxonomic prey category) and otolith shape. Second, in case of significant diet effect, taxonomic prey categories involved in the relationship with otolith shape were identified. Third, we quantified the respective contributions of food quantity and taxonomic composition to diet-

otolith shape co-variation. Fourth, we tested for the relationship between diet composition in terms of energetic prey categories and otolith shape. For all questions, the effect of potential confounding factors, i.e. individual-state variables (age, length, sex and maturity status), on otolith shape was quantified and removed to obtain unbiased estimates of food effects.

MATERIALS AND METHODS

Sample collection

Five marine fish, 3 roundfishes and 2 dextral flatfishes, were sampled in the eastern English Channel (Fig. 1): 47 striped red mullets *Mullus surmuletus*, 28 tub gurnards *Chelidonichthys lucerna*, 32 red gurnards *C. cuculus*, 42 European plaices *Pleuronectes platessa* and 36 common soles *Solea solea* (Table 1). These species were chosen because of their commercial significance, the fact that they represent a combination of round and flat fish, and that

they are among the most abundant species in the area. For each species, individuals sampled belonged to a single population. All fish were caught during the annual Channel Ground Fish Survey (CGFS) operated on board the RV 'Gwen Drez' in October 2009. The fishing gear was a Grande Ouverture Verticale bottom trawl, with a 10 mm stretched mesh size in the codend, that was towed for 30 min at an average speed of 3.5 knots (Coppin et al. 2002). Following their capture, fish were identified at species level and sampled individuals were frozen in liquid nitrogen on board.

Back in the laboratory, individuals were defrosted, measured (total length, L_T) to the nearest cm, and sex and maturity status were determined by macroscopic observation of gonads ac-

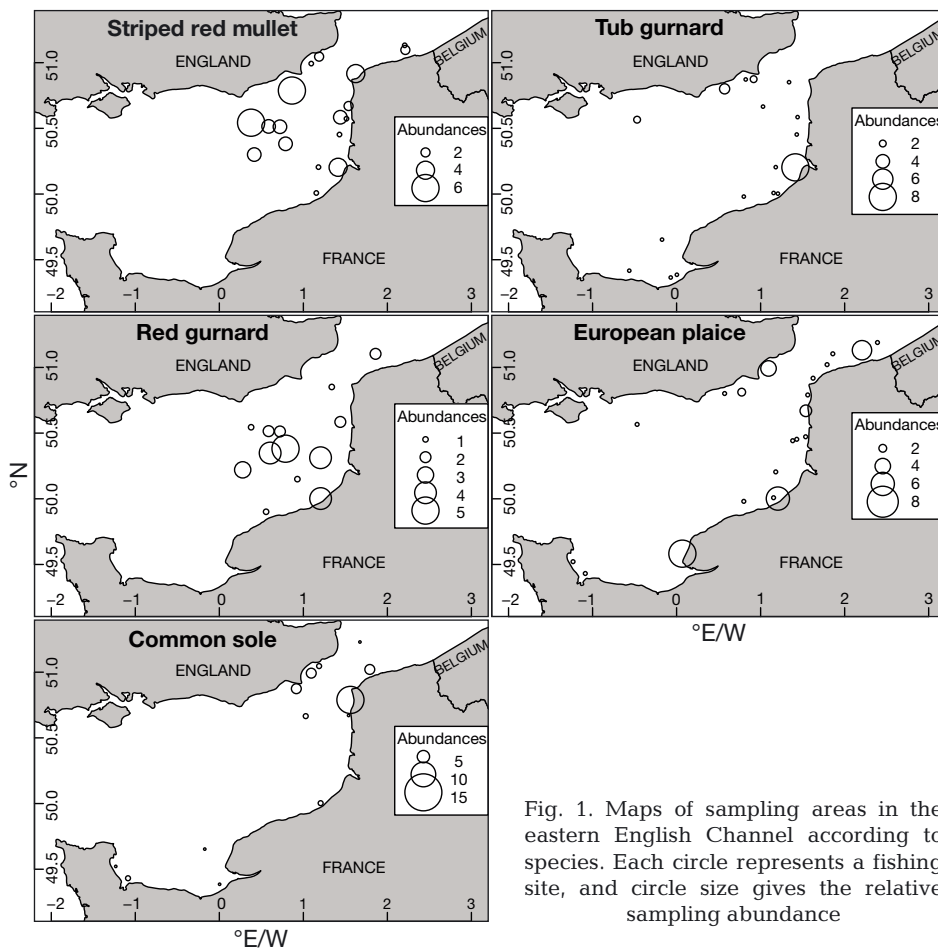


Fig. 1. Maps of sampling areas in the eastern English Channel according to species. Each circle represents a fishing site, and circle size gives the relative sampling abundance

Table 1. Characteristics of the samples studied. Number of samples analyzed (N), number of females (N_F), number of males (N_M), number of individuals with undetermined sex (N_U), proportion of mature individuals (% Mat), age and total length distributions of the samples

Species	N	N _F /N _M /N _U	% Mat	Age (yr)	Total length (cm)
				Mean ± SD Range	Mean ± SD Range
Striped red mullet	47	08/16/23	48.94	0.62 ± 0.79 0–3	16.51 ± 5.60 9–32
Tub gurnard	28	10/18/00	32.14	1.75 ± 0.84 0–3	25.86 ± 6.61 16–40
Red gurnard	32	12/19/01	37.5	2.69 ± 0.74 1–4	24.91 ± 2.85 20–31
European plaice	42	26/14/02	76.19	1.81 ± 1.38 0–7	27.33 ± 7.45 9–43
Common sole	36	18/17/01	91.67	2.44 ± 1.61 1–6	25.94 ± 5.81 17–38

according to the recommendations of international expert groups (ICES 2014). The digestive tract was extracted and its contents removed and stored in a Petri-dish for analysis. Sagittal otoliths were also removed from each individual, one of them being used to estimate the individual's age by interpreting macrostructures according to accepted standard ageing protocols (ICES 2010, 2012) and the second one for shape analysis, each of them coming always from the same side. For striped red mullet, age was estimated by reading macrostructures on the sagittal otolith pair. The left otolith was read under transmitted light while the right one was read under reflected light before being burned to confirm the age estimation done previously (ICES 2012). The left otolith (not burned) was then used for shape analysis. Even though recommendations from ICES expert groups do not exist for tub and red gurnards, the same methods were effective when applied to these species. For European plaice, the entire left otolith was used to estimate age as well as for shape analysis (ICES 2010), whereas, for common sole, a transversal section of the left otolith was necessary for age reading (Mahé et al. 2012) so that the right otolith was used for shape analysis.

Otolith shape analysis

Each otolith was cleaned in an ultrasonic bath in water at room temperature for a duration of 10 min, then brushed to remove residual tissues and stored dry in tubes. Batches of otoliths were automatically digitized using orthogonal projection at a high

resolution (3200 dpi) using a scanner EPSON V750 and individual images were extracted. An Elliptical Fourier analysis was performed on each otolith contour delineated and extracted after image binarization. This method reconstructs any type of shape with a closed 2-dimensional contour (Kuhl & Giardina 1982) using ellipses named harmonics. Each harmonic (H_i) is characterized by 4 coefficients (A_i , B_i , C_i and D_i), called elliptic Fourier descriptors (EFDs), which correspond to the parameters of the trigonometric equations describing the corresponding ellipse. The number of harmonics n used to reconstruct each otolith outline in the sample was determined as follows using the cumulated Fourier power ($P_F(n_k)$). This parameter was calculated for each otolith k as the sum of the proportion of variation in contour coordinates accounted for by each harmonic; this is equal to:

$$P_F(n_k) = \sum_{i=1}^{n_k} \frac{A_i^2 + B_i^2 + C_i^2 + D_i^2}{2} \quad (1)$$

The number of harmonics n_k was then chosen such that $P_F(n_k)$ reaches 99.99% of variation in contour coordinates or, in other words, such that shape is reconstructed at 99.99% (Lestrel 2008). A majority of studies (e.g. Mérigot et al. 2007, Lestrel 2008) compute the cumulated Fourier power P_F using EFDs averaged across the full sample or part of it, so that the selected harmonics describe the average otolith shape. In this study, $P_F(n_k)$ and n_k were calculated for each individual otolith k in order to ensure that each individual otolith in the sample was reconstructed with a precision of 99.99%. The maximum number of harmonics $n = \max_k(n_k)$ across all otoliths was then used to reconstruct each individual otolith of the sample.

After extracting the n harmonics for each individual otolith, their EFDs were normalized by the first harmonic providing EFDs invariant with respect to size, rotation and starting point (Kuhl & Giardina 1982), and resulting in the degeneration of the first 3 EFDs (A_i , B_i and C_i), respectively equal to 1, ≈ 0 and ≈ 0 for each individual. EFDs were then gathered in a matrix F with EFDs as columns and individuals as rows.

All otolith images and EFDs were obtained using the software TNPC 7.0 (www.tnpc.fr).

Diet analysis

For each fish, taxonomic identification of prey items in the stomach content was carried out using a binocular loupe. Prey items were identified to the lowest possible taxonomic level before being weighed (wet weight, g). In view of their high diversity, prey were grouped into 22 taxonomic categories (Table S1 in the Supplement at www.int-res.com/articles/suppl/m555p167_supp.pdf) based mainly on their main taxonomic level. Prey were categorized at least to phylum-level in the taxonomic hierarchy (e.g. Annelida, Cnidaria). If further taxonomic determination was possible, taxonomic prey categories were based on class (e.g. Cephalopoda, Gastropoda), order (e.g. Amphipoda, Isopoda) or infra-order (e.g. Brachyura, Anomura). Teleosts were split into 2 taxonomic prey categories depending on their energetic value. The energy content of each fish prey (found at www.nutraqua.com/) was plotted. Fish species gathered in 2 main groups (fat and lean) were separated by a threshold of 1 kcal g⁻¹.

Alternatively, prey were regrouped into 3 categories based on their energetic content (low/medium/high; Table S1 and Fig. S3 in the Supplement) estimated from appropriate literature (Norrbin & Båmstedt 1984, Steimle & Terranova 1985, Dauvin & Joncourt 1989, Spitz et al. 2010) and following the 3 energetic categories proposed by Spitz et al. (2010).

For each studied species, stomach content data were grouped in a diet composition matrix in terms of weight, based either on taxonomic W_t or energetic prey categories W_e , with each cell corresponding to $w_{t,ij}$ or $w_{e,ij}$ of prey category j (columns) in the digestive tract of individual i (rows). In addition, the relative contribution of taxonomic prey categories to diet composition by weight ($\%W_{t,j} = \sum_i w_{t,ij} / \sum_i \sum_j w_{t,ij}$) and the relative frequency of occurrence ($\%F_{t,j}$) of each taxonomic prey category (Godfriaux 1969), were computed for each studied species (Fig. 2).

Statistical analyses

A principal component analysis (PCA) combined with broken stick principal component selection (Borcard et al. 2011) was performed on the EFDs matrix \mathbf{F} . The aim was to decrease the number of dimensions used to describe otolith shape variability while avoiding collinearity between them and ensuring that the main sources of shape variation were kept (Rohlf & Archie 1984). The selected principal

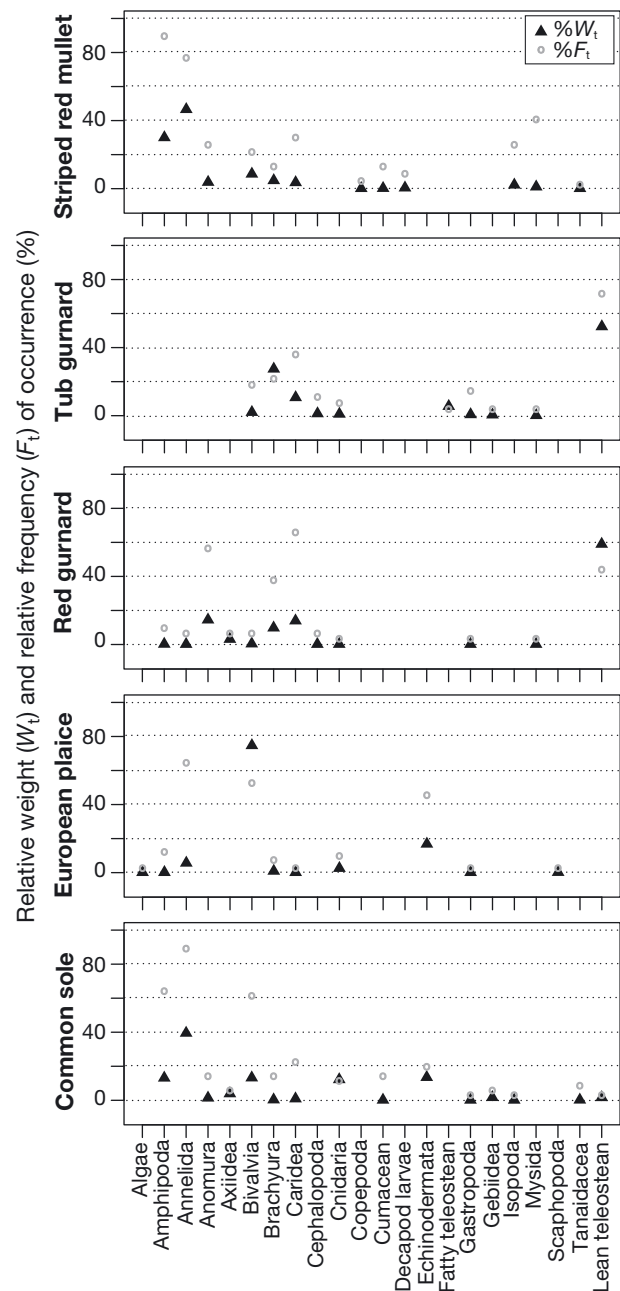


Fig. 2. Contribution of taxonomic prey categories to species specific diet, measured in terms of relative weight and relative frequency of occurrence

components were gathered to construct the otolith shape matrix \mathbf{S} with principal components of EFDs as columns and individuals as rows.

The otolith shape matrix \mathbf{S} was modelled using redundancy analysis (RDA), depending on 3 explanatory matrices: an individual matrix \mathbf{I} grouping individual-state variables and a diet matrix \mathbf{D} derived from the diet composition:

$$\mathbf{S} \sim \mathbf{I} + \mathbf{D} \quad (2a)$$

The matrix **I** was included in the model to disentangle and remove the effect of individual-state as possible confounding factors on otolith shape. It was composed of fish age *A* as a factor and total length L_T as a continuous effect to represent the ontogenetic effect on otolith shape, sex *Se* and maturity status *M* of the individual as factors potentially affecting fish physiology and metabolism, and thus indirectly otolith biomineralization. The resulting model was:

$$\mathbf{S} \sim A + L_T + Se + M + \mathbf{D} \quad (3a)$$

Alternatively, the potential confounding effect of environmental factors was also accounted for by including an environmental matrix **E** grouping external environmental factors in model 2:

$$\mathbf{S} \sim \mathbf{I} + \mathbf{E} + \mathbf{D} \quad (2b)$$

Matrix **E** contained 4 variables to describe environmental conditions that may also affect otolith biomineralization: temperature *T* and salinity *Sa* that were extracted from the hydrodynamic model MARS 3D (Lazure & Dumas 2008) and averaged over the month of October 2009, depth *Dp* that was measured at each sampling station during the survey, and longitude and latitude ($Lo \times La$) of the sampling station. The resulting model was:

$$\mathbf{S} \sim A + L_T + Se + M + T + Sa + Dp + Lo + La \quad (3b)$$

The complete model described by Eq. (3) (Eq. 3a and Eq. 3b for the model without and with the environmental variables, respectively) was reduced by stepwise selection based on significance (p-values) of the effects determined by permutation tests (Borcard et al. 2011). Potential collinearity between explanatory variables was checked by computing their variance inflation factors (VIF) before and after model reduction (Borcard et al. 2011). No strong collinearity (VIF < 10) was detected after model reduction. Then, a variation partitioning was performed to estimate the percent contribution of the two (**I** and **D**) or three (**I**, **E** and **D**) reduced matrices to otolith shape variation. The strict contribution of each reduced matrix to variation was tested using partial redundancy analysis (pRDA) followed by a permutation tests, with the matrix for which the contribution was estimated as an explanatory matrix and the other matrix or matrices depending on model version (3a or 3b) as covariables. SD was computed for all fractions of variation by bootstrapping. Five hundred bootstrap samples (random sampling with replacement) were enough

to obtain stable standard deviation estimates in all cases.

In order to answer the 4 main questions of the study, the previously described analysis was performed with the diet matrix **D** constructed in 4 different ways (Fig. 3) as described below.

Global relationship between diet and otolith shape (model 1, Fig. 3A)

In order to estimate the potential global relationship between diet and otolith shape, matrix **D** was composed of a number of selected correspondence axes resulting from a correspondence analysis (CA) applied to the diet composition matrix based on taxonomic prey categories \mathbf{W}_i . Correspondence axes were selected according to the broken stick method. As for the PCA applied to EFDs, this analysis was chosen to decrease the number of dimensions used to describe fish diet variation and to remove collinearity between prey categories. Moreover, CA is a method adapted to the analysis of species abundance data without pre-transformation because abundance data within taxonomic prey categories are not normally distributed (Borcard et al. 2011). Model reduction was performed while considering variables of matrix **I** (and **E**) separately and matrix **D** as a whole. Hence, matrix **I** (and **E**) used in variation partitioning was (were) reduced, while matrix **D** was not when kept in the reduced model.

Prey categories involved in the relationship between diet and otolith shape (model 2, Fig. 3B)

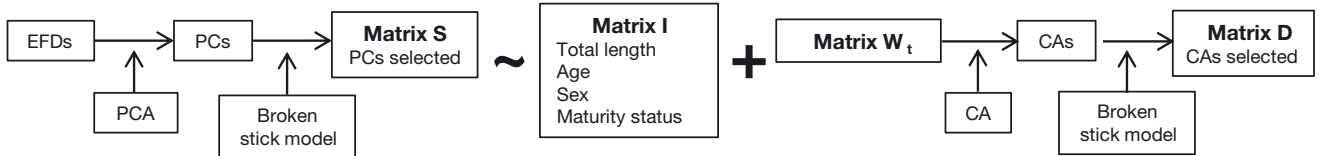
In this analysis, matrix **D** was simply set equal to the diet composition matrix based on taxonomic prey categories \mathbf{W}_i and model reduction was directly performed on Eq. (3). Hence, matrices **I**, (**E**) and **D** used in variation partitioning were all reduced. In order to identify the main prey categories involved in diet–otolith shape co-variation, permutation tests were performed for each selected prey category to test their significance. Moreover, to illustrate the relationship between the prey categories selected and otolith shape, 8 predicted otolith outlines were produced for each species in the following way: a pRDA was performed on the otolith shape matrix **S** with the selected prey categories as explanatory variables and the selected individual-state (and environmental variables) as covariables. From this pRDA, 8 sets of coordinates in the matrix **S** space

were predicted at the 8 combinations of ± 1 SD along the 2 first axes of the pRDA $\{(+SD_1, 0), (-SD_1, 0), (+SD_1, +SD_2), (-SD_1, +SD_2), (+SD_1, -SD_2), (-SD_1, -SD_2), (0, +SD_2), (0, -SD_2)\}$ representing variation in linear combinations of the selected prey categories. Predictions in the matrix **S** space were then projected back to the matrix **F** space to produce predicted EFDs that were then used to draw predicted otolith shapes on the pRDA biplot.

Contribution of diet relative composition vs. food quantity to diet–otolith shape co-variation (model 3, Fig. 3C)

In this analysis, matrix **D** was decomposed into a matrix representing the relative diet composition based on taxonomic prey categories **C** and a vector representing food quantity **Q**. The relative diet composition matrix **C** was obtained by performing

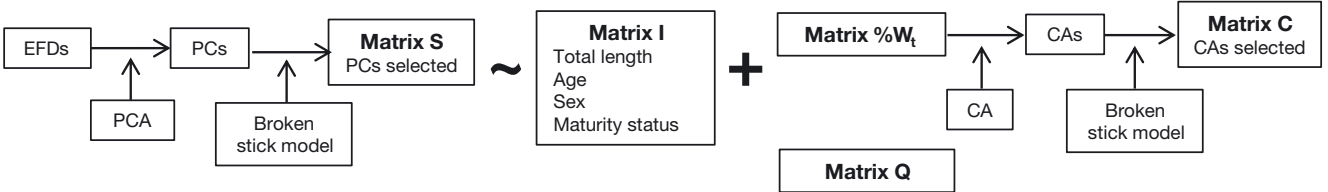
A. Global relationship between diet and otolith shape (model 1)



B. Taxonomic prey categories involved in the relationship between diet and otolith shape (model 2)



C. Contribution of diet relative composition vs. food quantity to diet–otolith shape co-variation (model 3)



D. Relationship between diet energy composition and otolith shape (model 4)

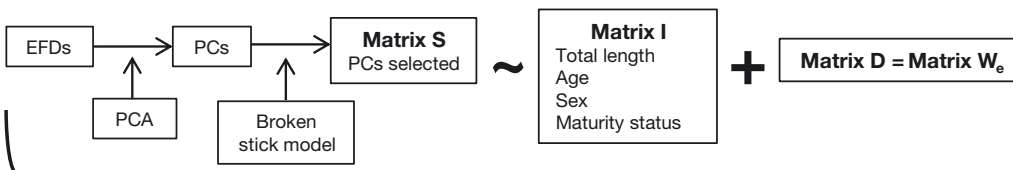
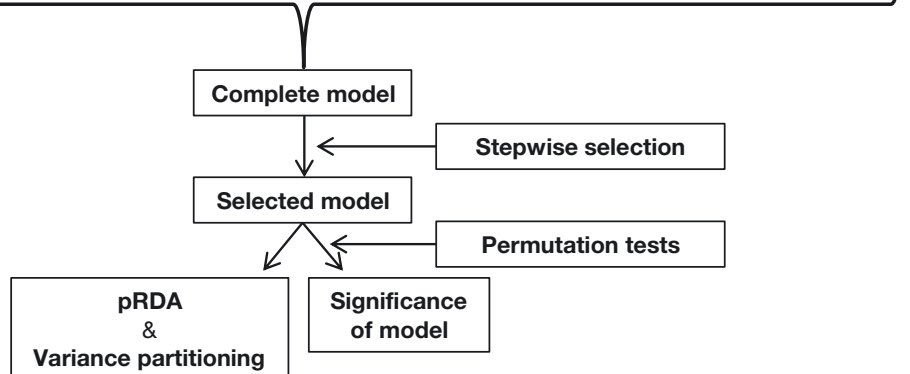


Fig. 3. Schematic representation of the sequential steps of the 4 RDA statistical analyses performed to investigate the 4 questions of the study: (A) the global relationship between diet and otolith shape (model 1), (B) the prey categories involved in this relationship (model 2), (C) the relative contributions of diet relative composition and quantity to diet–otolith shape co-variation (model 3) and (D) the relationship between diet energy composition and otolith shape (model 4)



a CA on the matrix of relative contribution of taxonomic prey categories to diet composition $\%W_{t,j}$ and selecting correspondence axes according to the broken stick method. The vector **Q** gathered the total weight of the stomach content of each individual, in other words the sums along rows $\sum_j W_{t,ij}$ of the diet composition matrix **W_t**. In order to ensure that matrices **C** and **Q** were kept in the reduced model (with the ultimate aim to estimate their relative contribution to otolith shape variation), model reduction was based on a pRDA where the otolith shape matrix **S** was explained by matrix **I** (and **E**) while matrices **C** and **Q** were considered as covariables. Hence, matrix **I** (and **E**) used in variation partitioning were reduced, while matrices **C** and **Q** were not.

Relationship between diet energy composition and otolith shape (model 4, Fig. 3D)

In this last analysis, matrix **D** was set equal to the diet composition matrix based on energetic prey categories **W_e**. Model reduction and variation partitioning were then performed as for model 1.

All statistical analyses were performed using the package *vegan* (Oksanen et al. 2013) in the statistical environment R.3.1.1 (R Core Team 2014). The R codes used in this study are available from the authors upon request.

RESULTS

Only results based on models without environmental confounding factors (Eq. 3a) are presented in details in this section. The rationale is that considering environmental variables at sampling site as related to otolith shape implies assuming that these environmental conditions are representative of those experienced by individuals during a substantial part of their life, which is subject to controversy for mobile organisms such as fish (see 'Discussion: Limitations of the study'). Only important differences between the results without and with environmental factors are highlighted here. Detailed results when accounting for environmental factors (Eq. 3b) can be found in Tables S2, S4 & S5 and Figs. S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m555p167_supp.pdf. In addition, the effects of individual-state variables on otolith shape have already been studied in detail (Hüssy 2008, Capoccioni et al. 2011). They were accounted

for in the analyses to avoid potential confounding effects but were not the main focus of this study. Consequently, their effects are not detailed here but can be found in Tables S3 & S4.

Global relationship between diet and otolith shape (model 1)

The reduced models explained between 11 and 26% of otolith shape variability for roundfish species. For flatfish species, percentages of explained variation were higher: 14 and 38% for plaice and sole, respectively (Table 2). Variation partitioning revealed that the individual matrix **I** explained the greatest part of variation in otolith shape for all species, between 19 and 27%, except for red gurnard and European plaice for which it was not significant (Fig. 4, first column). For all species, a significant diet contribution was detected at an alpha threshold of 5%, except for tub gurnard and European plaice for which it was significant at an alpha threshold of 10% only. Matrix **D** explained between 10 and 16% of otolith shape variability (Fig. 4). When including the environmental matrix **E** in the analysis, a significant diet contribution was detected at an alpha threshold of 5% for European plaice that accounted for 13% of variation (Fig. S1, first column, & Table S2 in the Supplement).

Prey categories involved in the relationship between diet and otolith shape (model 2)

Between 2 and 7 taxonomic prey categories were selected in the reduced model according to species (Table 2), except for tub gurnard for which no taxonomic prey category was selected and hence no reduced model was tested. Explained variation by model 2 varied between 25 and 35% according to species. As in model 1, the individual matrix **I** had a significant effect on otolith shape for all species (Fig. 4, second column). It explained between 14 and 23% of shape variability. Concerning taxonomic prey categories, a significant contribution to otolith shape variation was detected for all species except one, tub gurnard. The selected categories explained between 11 and 201% of otolith shape variability, which was slightly higher than the global effect of diet in model 1. According to species, the taxonomic prey categories contributing significantly differed in terms of relative frequency of occurrence $\%F_j$ and relative contribution of prey

Table 2. Results of the 3 RDA models (as detailed in Fig. 3) for the 5 studied fish species. 'Otolith shape' gives the number of principal components (N PCs) in the response matrix **S** used to describe otolith shape and the percentage of variance explained (%) in elliptical Fourier descriptors. 'Individual' and 'Diet' correspond to explanatory matrices **I**, and **D** in reduced models. More precisely, 'Individual' gives the selected individual-state variables. 'Diet' indicates the variables matrix **D**, i.e. the number of correspondence axes (N CAs) and the percentage of variance they explain (%) in diet composition W_i and relative diet composition $\%W_{t,j}$ in models 1 and 3, respectively, and the selected prey categories in models 2 and 4. 'Model selected' gives the degrees of freedom (df), the *F*-statistic, the corresponding p-value and the percentage of variation explained (%) by the reduced model

Species	Otolith shape S		Individual I	Diet D		Model selected			
	N PCs	%		N CAs	%	df	<i>F</i>	p	%
Global relationship between diet and otolith shape (model 1)				N CAs	%				
Striped red mullet	4	77.00	Size, Sex	10	99.85	13	2.22	0.002	25.61
Tub gurnard	2	70.18	Age, Sex	4	90.59	8	2.06	0.016	23.95
Red gurnard	3	77.84	–	4	94.01	4	1.97	0.039	11.11
European plaice	3	74.02	Maturity	4	93.53	5	2.29	0.011	13.56
Common sole	3	78.23	Age, Size, Sex	8	95.32	16	2.31	0.003	37.50
Taxonomic prey categories involved in the relationship between diet and otolith shape (model 2)				Taxonomic prey category					
Striped red mullet	4	77.00	Size, Sex	Annelida, Anomura, Bivalvia, Brachyura, Caridea, Decapod larvae, Isopoda		10	3.49	0.001	35.11
Tub gurnard	2	70.18	–	–		–	–	–	–
Red gurnard	3	77.84	Age, Maturity	Amphipoda, Gastropoda, Caridea		7	2.51	0.003	25.40
European plaice	3	74.02	Age	Amphipoda, Echinodermata		8	2.94	0.004	27.51
Common sole	3	78.23	Age, Size, Sex	Anomura, Cnidaria, Gebiidea		11	2.64	0.002	34.05
Contribution of diet relative composition vs. food quantity to diet-otolith shape co-variation (model 3)				N CAs	%				
Striped red mullet	4	77.00	Size, Sex	10	99.87	14	2.19	0.001	26.66
Tub gurnard	2	70.18	–	–	–	–	–	–	–
Red gurnard	3	77.84	–	4	94.23	5	1.61	0.012	9.02
European plaice	3	74.02	Age	4	92.64	16	2.12	0.005	30.44
Common sole	3	78.23	Age, Size, Sex	9	96.78	18	2.12	0.01	36.54
Relationship between diet energy composition and otolith shape (model 4)				Energetic prey category					
Striped red mullet	4	77.00	Size, Sex	–		3	3.94	0.001	16.09
Tub gurnard	2	70.18	Age, Size	Low/medium/high		7	2.53	0.003	28.45
Red gurnard	3	77.84	Size	–		1	2.53	0.047	4.70
European plaice	3	74.02	Age	Low/medium/high		9	2.27	0.014	21.76
Common sole	3	78.23	Age	–		5	2.38	0.007	16.43

categories to diet $\%W_j$. For striped red mullet, the significant taxonomic prey categories (Fig. 5) were either primary ones, i.e. characterized by a high $\%F_{t,j}$ and high $\%W_{t,j}$ such as Annelida, or intermediary ones, i.e. characterized by a small $\%W_j$ with respect to $\%F_j$ such as Caridea, or secondary ones, i.e. with a low $\%F_j$ and small $\%W_j$ such as Bivalvia, Brachyura, and Decapoda larvae (Fig. 2). Similarly for red gurnard, influential taxonomic prey categories were either primary ones such as Caridea or secondary ones such as Gastropoda.

For European plaice, only a primary taxonomic prey category was related to otolith shape, namely Echinodermata, contrary to common sole, for which only a secondary taxonomic prey category, Cnidaria, was linked to otolith shape. When including the environmental matrix **E** in the analysis, the contribution of taxonomic prey categories to otolith shape variation increased to 40% for red gurnard but was relatively stable for the other species (Fig. S1, second column, & Table S2 in the Supplement).

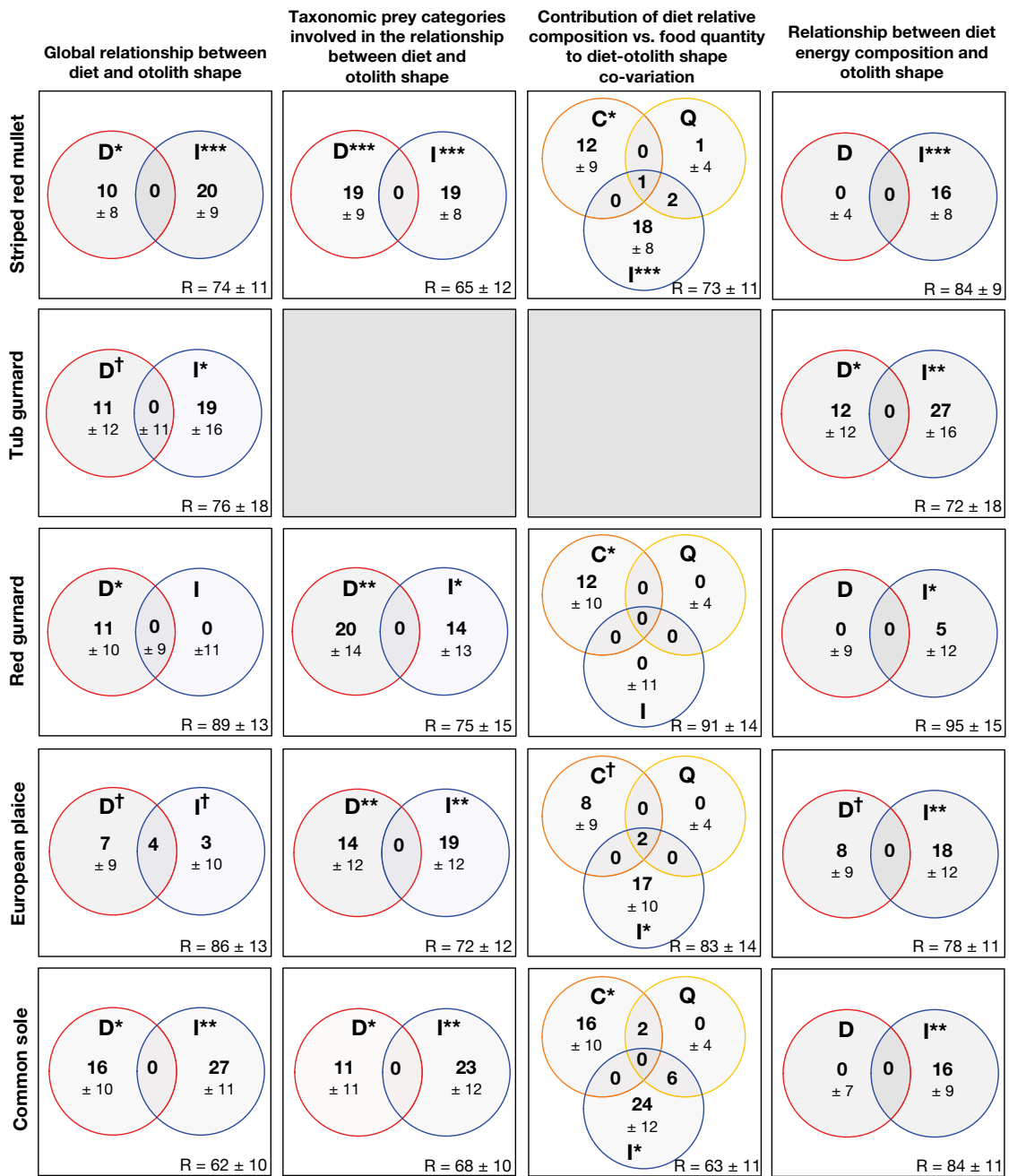


Fig. 4. Variation partitioning between diet and individual-state for the 4 questions investigated for each species. Each circle corresponds to one matrix: diet matrix **D**, individual-state variable matrix **I**, diet relative composition matrix **C**, and food quantity **Q**. The number in the non-overlapping part of each circle represents the unique contribution in percentage of variation of the corresponding matrix. The numbers in the overlapping parts of the circles represent the joint contribution in percentage of variation of the corresponding matrices. Standard deviations obtained by bootstrapping are given for unique contributions only for ease of reading. All contributions and their standard deviations are given in Table 3 for more details. p-values of contribution fractions are indicated by the following symbols: †p < 0.1, *p < 0.05, **p < 0.01, ***p < 0.001

Predicted otolith shapes as reconstructed in Fig. 5 revealed that diet was related to global otolith shape through the length/width ratio and thus otoliths' ellipticity, but also to finer details. Variations occurred in the

otolith crenations, the width of the *excisura major* (the indentation between the rostrum and the antirostrum; Panfili et al. 2002), the length of the rostrum and the antirostrum, and the posterior part of the otolith shape.

Contribution of relative diet composition vs. food quantity to diet-otolith shape co-variation (model 3)

For tub gurnard, model 3 was not estimated given the absence of diet effect in model 1 (and subsequently model 2). For the other species, model 3 explained between 9 and 37% of otolith shape variability (Table 2). Variation partitioning gave similar results in terms of individual effects to those obtained with model 1, except for European plaice for which the explained variation by the individual matrix **I** increased strongly (Fig. 4, first and third columns,

fourth line). Regarding diet, relative composition **C** contributed significantly to otolith shape variation for striped red mullet, red gurnard and common sole. Its effect explained 12 to 16% of variation. No significant contribution of **C** was found for European plaice even if variation partitioning attributed 8% of otolith shape variation to this matrix. In contrast, when the environmental matrix **E** was added in the model, a significant contribution of **C** was detected for European plaice while significance disappeared for common sole, despite the slight decrease in percentage of variation explained (Fig. S1, third column, &

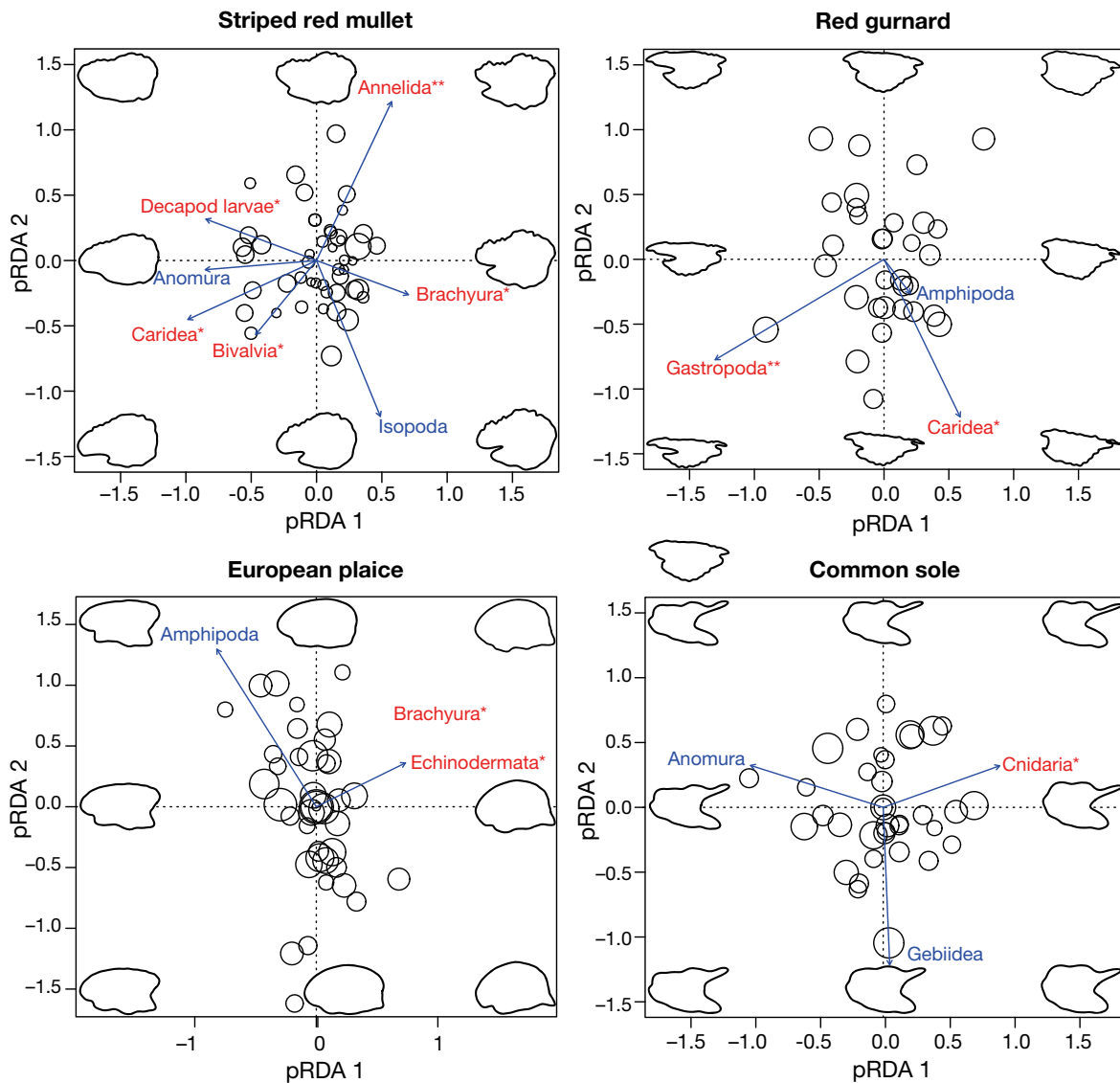


Fig. 5. pRDA biplot of otolith shape constrained by selected taxonomic prey categories and conditioned by selected individual variables (model 3) according to species. Explanatory variables with a significant effect (permutation test) on otolith shape are in red, the corresponding p-value being indicated by the following symbols: * $p < 0.05$, ** $p < 0.01$. Each circle represents an individual and its size represents the individual total length. For each species, 8 otolith shapes have been reconstructed from model predictions illustrating the relationship between diet and otolith shape

Table S2). Contrary to diet relative composition **C**, food quantity **Q** did not contribute significantly to otolith shape variation whatever the species, including and excluding the environmental matrix **E** in the model.

Relationship between diet energy composition and otolith shape (model 4)

The reduced models explained between 5 and 28% of otolith shape variation (Table 2). Variation partitioning revealed that the individual matrix **I** explained the greatest part of variation in otolith shape for all species, between 5% and 27 (Fig. 4, fourth column). A significant contribution of diet energetic composition was detected for tub gurnard and European plaice only and at an alpha threshold of 5 and 10%, respectively. Matrix **D** explained 12 and 8% of otolith shape variation, respectively (Fig. 4, fourth column).

DISCUSSION

First, in this study, we found that individual-state variables contributed the largest fraction of otolith shape variation in most cases. This result was expected, given the already well described effect of individual-state variables on otolith shape (see Cardinale et al. 2004, Hüsey 2008, Capoccioni et al. 2011 for further discussion). Besides this known effect, we showed an intra-population relationship between diet and otolith shape for all fish species studied, although the relationship was less robust for tub gurnard. For the latter, only the relationship between diet energetic composition and otolith shape was significant at an alpha threshold of 5%, the global relationship between diet weight composition and otolith shape being significant at an alpha threshold of 10% only. A small sample size of this species compared to the others may explain a lower power of signal detection. Second, we were able to relate either primary, intermediary or secondary prey categories to otolith shape variations. Moreover, otolith reconstructions suggest that these variations could affect both global shape and its finer details. Third, by comparing the contributions of food composition and quantity, we showed that food composition on the whole contributed more to otolith shape variation than the quantity of food ingested by fish. For 2 species, a dietary influence based on energetic content categories was detected to be significant.

The role of organic matrix composition in otolith biomineralization

Although the organic matrix of sagittal otoliths represents a minor fraction of the total material (Carlström 1963, Degens et al. 1969), it plays an important role in otolith formation. It controls the nucleation, the crystallization, the orientation and the morphology as well as the polymorphism of crystal units the otolith is composed of (Nagasawa 2013). The organic matrix is mainly composed of proteins, amino acids (AAs), collagens and proteoglycans, of which precursors are secreted by the saccular epithelium in the endolymph (Payan et al. 2004). However, only 3 major proteins are present both in the endolymph and the otolith. This suggests that the organic matrix is not only directly composed of compounds present in endolymph, but also of proteins derived from the modification of precursors during their deposition into the otolith (Borelli et al. 2001). McMahon et al. (2010) observed that AA $\delta^{13}\text{C}$ values in fish muscle and in their diet co-varied, with significant differences among diet treatments. Moreover, they showed that AA $\delta^{13}\text{C}$ values in muscles and in otoliths were correlated with a slope of around 1, and thus displayed identical dietary information (McMahon et al. 2011). This suggests that the AAs found in otolith proteins come from the food consumed by fish.

Otolith shape is determined by its crystalline architecture (calcium carbonate, CaCO_3). Several proteins are known to control CaCO_3 polymorphism (aragonite, calcite or vaterite) and the morphology of its crystal units. Starmaker (Söllner et al. 2003) and otolith matrix macromolecule-64 (OMM-64) (Tohse et al. 2009) are both water-soluble and acidic (due to a calcium-binding region rich in glutamate) glycoproteins involved in the control of crystal polymorphism (Nagasawa 2013). The otolith matrix protein-1 (OMP-1) is another water-soluble protein required for normal otolith growth and for the deposition of another otolith protein, otolin-1. The latter is a collagenous protein that makes up the structural network for subsequent calcification, and thus stabilizes otolith mineral and organic fractions and insures the correct arrangement of otoliths on the sensory epithelium (Murayama et al. 2005).

Potential mechanisms underlying the relationship between diet composition and otolith shape

In the present study, a significant relationship between diet taxonomic composition and otolith shape

was detected for all species except for one. According to the taxonomic prey categories consumed, otolith shape presented some variations in both global shape, such as the degree of ellipticity, and finer details, such as otolith crenation or the width of the *excisura major*. Two hypotheses, or a combination of both, could explain the correlation between otolith shape and diet taxonomic composition. First, the total quantity of proteins in the saccular endolymph could vary according to the quantity of proteins in the prey consumed, which would affect the rate of organic matrix synthesis and thus CaCO_3 deposition and ultimately otolith growth. Protein consumption has been known to have the highest regulatory impact on protein synthesis (Houlihan et al. 1988). Consequently, diet taxonomic composition could influence 'global' otolith shape through effects on otolith growth. Secondly, the proteic composition of the organic matrix, i.e. the relative quantity of water-soluble, water-insoluble and insoluble proteins, may change according to food composition, which would impact the crystal structure (orientation, morphology, and polymorphism) of precipitated CaCO_3 and, thus, otolith shape. More precisely, food composition varies in terms of proteins or even AAs, whether essential (e.g. leucine) or not (e.g. glutamic acid), that are necessary for the synthesis of some otolith matrix proteins involved in the control of crystal structure (Asano & Mugiya 1993, Davis et al. 1995, Sasagawa & Mugiya 1996, Nagasawa 2013). Consequently, food proteic composition could have a direct contribution to variations in otolith crenation or/and an indirect contribution through its effect on otolith growth, which affects global otolith shape. In addition, some proteoglycans and polysaccharides are present in both the saccular endolymph and the organic matrix (Murayama et al. 2005). Even if their role in otolith biomineralization is unknown, variability in their quantity and composition in prey could also affect otolith shape in the same way as variability in proteins.

Diet energetic composition was significantly related to otolith shape in 2 species only. Contrary to proteins and glucids, lipids are not components of the otolith organic matrix, which could explain the absence of a strong relationship between the diet energetic composition and otolith shape. Lipids are indeed the main determinant of prey energetic content as energy per unit of mass in prey is positively correlated to their lipid content and generally negatively correlated to their protein content (Spitz et al. 2010). The fact that diet taxonomic composition was better correlated with otolith shape variation than diet energetic composition suggests that prey lipid versus protein

content is less related to otolith shape variation than prey composition in terms of proteins and carbonates. This result seems rather logical given the composition of the otolith and its precursors, when thinking about a direct effect on otolith shape through its organic matrix. In contrast, when a lipid-otolith-shape relationship exists, this may be through an indirect effect on otolith shape via otolith growth. High dietary lipid levels can improve body size growth (Vergara et al. 1999, Boujard et al. 2004). Diet lipid content is thus likely to be related to otolith growth and thus shape. The lack of a strong relationship could result from several aspects. First, high dietary protein levels also favour faster growth. Given that lipid and protein levels in diet are oppositely correlated to diet energy content (Spitz et al. 2010), the 2 effects could cancel each other out when considering the effect of diet energetic composition. Second, some of the reduced models included a size effect that could absorb the indirect effect of diet energetic composition through growth. Third, diet energetic composition was based on 3 qualitative, relatively coarse, energetic prey categories, which may not be precise enough to detect a relationship. Studies based on a proper quantification of diet energy content, through bomb calorimetry of stomach contents for instance, or on the lipid composition of prey, would allow further investigation of the potential relationship between diet energy content and otolith shape.

In this study, locations of otolith shape variation related to food composition were identified from reconstructed shapes. Although these reconstructions were 'caricatures' predicted from a statistical model limited to individuals from the eastern English Channel and to our observations in terms of individual-state, they highlighted the large number of otolith shape areas co-varying with food composition, suggesting the importance of understanding otolith biomineralization 3D processes. Otolith shape variation could be also explained by variations in spatial precursor distribution, due to physical constraints on the saccule which would impact the otolith shape. However, the current lack of knowledge regarding such processes prevents clear evaluation of the likelihood of this hypothesis.

Absence of a relationship between food quantity and otolith shape

No significant relationship between food quantity and otolith shape was detected in this study. This result contrasts with several works that showed experi-

mentally that food quantity impacted otolith shape both indirectly via variation in otolith growth creating variation in 'global' otolith shape, and directly on otolith crenations (Gagliano & McCormick 2004, Cardinale et al. 2004, Hüsey 2008). A possible explanation is that in the present study, the quantity of ingested food did not differ sufficiently between individuals to be able to observe a significant influence on otolith shape. Likewise, Hüsey et al. (2004) did not observe any effect of food quantity on otolith opacity and on the ratio between water-soluble and water-insoluble proteins in the organic matrix, whereas, under more severe food restriction for a longer period, Høie et al. (2008) observed that more translucent otolith material was deposited. Such apparent discrepancies are well reconciled in the light of temperature and food effect interactions (either synergetic or antagonistic) on otolith opacity (Fablet et al. 2011). Moreover, here food quantity was measured as the sum of the weights of all prey items found in an individual's stomach. However, prey items in stomach contents are digested at varying degrees according to individuals, which can introduce a bias in the estimation of inter-individual differences in ingested food quantity (Gannon 1976).

Limitations of the study

The imprecision of food quantity measures highlights a potential, more general, limitation of stomach content analysis, in that it only provides a snapshot of a fishes' diet (and in this precise case, for a single season, since all fish in this study were sampled in October). The interpretation of the results in the present study rely on the assumption that observed inter-individual differences in diet are consistent over a sufficiently long time period to be able to relate this to inter-individual otolith shape differences. The assumption is concerned with the representativeness of inter-individual differences, i.e. an individuals' specialization, and not of an individuals' diet itself. In other words, the assumption is that inter-individual differences in diet at a given time gives an index of dietary specialization even though an individuals' diet may vary through time. To our knowledge, such an assumption has never been directly confirmed nor invalidated in fish, given that no longitudinal study on fish diet, i.e. with repeated observations of prey selectivity or stomach content on the same individuals, was performed for testing. Although the possibility that this hypothesis does not hold cannot be totally ruled out, several arguments can be brought in its support.

There is ample literature on the importance and prevalence of individual diet specialization (see reviews in Bolnick et al. 2003; Araújo et al. 2011), notably long-term trophic specialization in freshwater and marine vertebrates (e.g. Bearhop et al. 2006, Newsome et al. 2009, Hückstädt et al. 2012, Rosenblatt et al. 2015) including fish (e.g. Beaudoin et al. 1999, Svanbäck & Persson 2004, Matich et al. 2011), that support this assumption based on isotopic data. Consistent inter-individual differences in several behavioural traits that may affect diet have also been documented in fish (see review in Mittelbach et al. 2014) such as habitat use and movements (e.g. Matich & Heithaus 2015) or boldness (e.g. Ward et al. 2004, Harcourt et al. 2009). A more technical argument is that the presence of multiple prey items per stomach ensures that cross-sectional samples of an individuals' diet are relevant to estimating individual diet specialization (Araújo et al. 2011). An additional argument in support of our assumption comes from the relative stability of our results across the 3 different analyses based on taxonomic prey categories for each species, and the relatively low SDs of fractions in variation partitioning obtained from bootstrapping analyses (Table 3, Table S5 in the Supplement). It should also be noted that, despite its limitations, stomach content analysis is the only way to obtain an indication of ingested food quantity in natural conditions. In contrast, carbon stable isotope ratios could provide a temporally integrated view of an individuals' diet composition and account for seasonal changes in diet, but without allowing the quantification of the amount of food ingested. Additionally, the precise identification of the consumed prey items from carbon stable isotope ratios by using so-called mixing models requires knowledge of the isotopic ratios of all potential preys and of the isotopic fractionation between preys and consumers (Post 2002, Fry 2007). Still, variation of carbon stable isotope ratio (in muscles and/or otoliths) across individuals could be used to describe variability in individual diet composition with the aim of linking it to the otolith shape variability. This would complement the results obtained in this study.

Likewise, the environmental variables considered in our supplementary analyses (see Figs. S1 & S2 and Tables S2 & S4 in the Supplement), i.e. temperature, depth, salinity, longitude and latitude, were a snapshot of the environment experienced by individuals as they were measured at sampling site, and averaged over a single month. Similar to stomach contents, their use in the analyses (Eqs. 2b & 3b) relies on the assumption that they are representative of inter-individual differences in the environment experi-

enced over a sufficiently long time period to be related to otolith shape. Such an assumption may seem unlikely for a majority of fish given their mobility, which could explain the fact that for a majority of the studied species, the environmental matrix was not significantly related to otolith shape (Fig. S1). However, for European plaice, the inclusion of the environmental matrix in the analysis has enabled the description of a supplementary part of otolith shape variation that, it seems, was obscuring the diet signal since the diet matrix became significant with the addition of the environmental variables in the model. This result may be linked to the supposedly lower mobility of benthic flatfish such as plaice. In order to have a temporally integrated view of the environment experienced by individuals, possibly also accounting for seasonality, otolith chemistry (such as the variation of oxygen isotopic ratios as an index for temperature [Kalish 1991], or the Sr:Ca ratio as an index for the salinity [Secor 1992]) could be used in future studies. Moreover, all fish in this study were sampled in October, i.e. during a single season. The influence of seasonality on the relationship between

diet and environment on the one hand and otolith shape on the other should also be studied.

In summary, an intra-population relationship between diet and otolith shape was detected for some common roundfish and flatfish species from the eastern English Channel. Detailed analyses revealed that both main and secondary prey categories were involved in this relationship, and that variations influenced both otolith global shape and some finer details. The contribution of relative diet taxonomic composition to otolith shape variation was much higher than that of ingested food quantity represented by the weight of prey items. Finally, diet energetic composition was correlated with otolith shape of only one species and marginally for another. Gagliano & McCormick (2004) suggest that otolith shape could be used to discriminate fine-scale events, such as the magnitude and periodicity of feeding in wild fish populations, in addition to discrimination between stocks and populations based on coarser aspects such as differences in life-history. The present study shows that diet composition may also be a source of otolith shape variability within populations through direct and/or

Table 3. Percent contribution with bootstrapped standard deviation of the diet matrix (**D**), the individual matrix (**I**), the food composition matrix (**C**), the food quantity matrix (**Q**) and residuals (**Res**), obtained from variation partitioning performed on the reduced model for the 4 questions investigated and each studied species

Global relationship between diet and otolith shape (model 1)										
	D		D & I		I		Res			
Striped red mullet	10 ± 8.29		0 ± 7.31		20 ± 8.51		74 ± 10.94			
Tub gurnard	11 ± 11.73		0 ± 8.66		19 ± 15.72		76 ± 17.54			
Red gurnard	11 ± 9.71		0 ± 9.44		0 ± 11.30		89 ± 13.26			
European plaice	7 ± 9.09		4 ± 9.54		3 ± 10.18		86 ± 12.95			
Common sole	16 ± 10.28		0 ± 11.37		27 ± 11.26		62 ± 9.69			
Taxonomic prey categories involved in the relationship between diet and otolith shape (model 2)										
	D		D & I		I		Res			
Striped red mullet	19 ± 9.30		0 ± 7.00		19 ± 8.05		65 ± 11.64			
Tub gurnard										
Red gurnard	20 ± 13.98		0 ± 15.25		14 ± 12.76		75 ± 14.52			
European plaice	14 ± 12.41		0 ± 10.29		19 ± 12.20		72 ± 11.95			
Common sole	11 ± 11.30		0 ± 14.15		23 ± 11.93		68 ± 10.12			
Contribution of diet relative composition vs. food quantity to diet-otolith shape co-variation (model 3)										
	C		Q	I		C & Q	Q & I	C & I	C & Q & I	Res
Striped red mullet	12 ± 9.46		1 ± 3.54	18 ± 7.78		0 ± 3.44	2 ± 4.89	0 ± 7.07	1 ± 5.54	73 ± 11.39
Tub gurnard										
Red gurnard	12 ± 10.04		0 ± 4.05	0 ± 11.39		0 ± 3.88	0 ± 4.31	0 ± 10.09	0 ± 4.20	91 ± 13.72
European plaice	8 ± 8.68		0 ± 3.86	17 ± 10.47		0 ± 4.45	0 ± 3.93	0 ± 10.12	2 ± 4.75	83 ± 13.53
Common sole	16 ± 10.50		0 ± 4.31	24 ± 12.10		2 ± 5.34	6 ± 6.55	0 ± 11.90	0 ± 7.05	63 ± 11.49
Relationship between diet energy composition and otolith shape (model 4)										
	D		D & I		I		Res			
Striped red mullet	0 ± 4.34		0 ± 2.87		16 ± 7.67		84 ± 8.84			
Tub gurnard	12 ± 11.73		0 ± 8.66		27 ± 15.72		72 ± 17.54			
Red gurnard	0 ± 9.18		0 ± 7.30		5 ± 11.78		95 ± 15.01			
European plaice	8 ± 9.12		0 ± 7.68		18 ± 11.54		78 ± 11.10			
Common sole	0 ± 6.76		0 ± 5.96		16 ± 9.00		84 ± 11.08			

indirect (via otolith growth) processes. This introduces a novel potential interpretation of 3 classically known effects on otolith shape. First, otolith shape variation across age and size is generally assigned to ontogenetic changes in metabolism and physiology (Campana & Casselman 1993, Mérigot et al. 2007). Ontogenetic changes in diet composition could also contribute directly to otolith shape variation, thereby acting as a confounding factor (Morat et al. 2012, Vignon 2012). Likewise, sexual dimorphism in otolith shape is generally attributed to physiological differences between sexes. However, sexual dimorphism in diet composition, especially at the time of mating, has been documented in several fish species (Casselman & Schulte-Hostedde 2004, Tsuboi et al. 2012) and could thus also explain otolith shape dimorphism. Finally, environmental abiotic factors, such as temperature and salinity, are also known to influence otolith shape (Lombarte & Leonart 1993) and spatial variations in otolith shape are often interpreted as resulting from habitat differentiation (Morat et al. 2012). However, such variation in abiotic factors is generally related to differences in prey categories available to an individual predator, such that geographical variations in diet composition could also generate geographical variations in otolith shape (Vignon 2012). Such consequences call for further investigations of the sources of otolith shape variation and their mechanistic effect on biomineralization, notably those related to diet.

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