Contribution to the Themed Section: Advances in Plankton Modelling and Biodiversity Evaluation

Comparison of copepod species-based and individual-size-based community structuring

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Functional trait studies are proliferating in plankton ecology, especially studies analysing body size. Yet, empirical studies comparing species versus individual-size structuring are scarce. Here, we conducted a comparison of copepod species-based and individual-size-based community structuring in the East China Sea, and found that: (i) Species, species-based nominal-size and individual-size distributions exhibit very different patterns, and that juveniles (neglected in species counts due to limitation of recognition) tend to dominate in a certain size range. (ii) Species-based structuring is more strongly shaped by physical conditions, while individual-size-based structuring is more strongly shaped by food availability. (iii) Despite these differences, the congruence (i.e. degree of match) between species-based and individual-size-based structuring remains statistically significant. Finally, we tested intrinsic factors potentially explaining the degree of mismatch (i.e. species richness and proxies for: size partitioning of species without accounting for intraspecific variability, intraspecific variability without accounting for ontogeny and ontogeny). (iv) The frequency of juveniles (proxy for ontogeny) is the only intrinsic factor significantly explaining the mismatch between species and size structuring, highlighting the problem of the classic species-based analysis that unavoidably neglects juveniles in the species counts. These results support individual-size as a useful complementary descriptor to species-based studies.

KEYWORDS: individual-size distribution; size structure; community structure; copepod; East China Sea

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INTRODUCTION

A major question in ecology is how diversity affects ecosystem functioning and how diversity itself can be maintained (Naeem *et al.*, 2012). Increasing number of studies have explored the role of functional diversity (i.e. diversity of functional traits) in these processes, considering that species have different roles in a community and thus measures based only on species numbers may be misleading due to functional redundancy (Petchey and Gaston, 2006; Cadotte *et al.*, 2011). In addition, functional trait approaches have the advantage that they incorporate intraspecific variability (Violle *et al.*, 2012). High intraspecific variability of traits and functional rates has been reported for plankton species (e.g. Narcy *et al.*, 2009; Kremp *et al.*, 2012), and in particular, ontogenetic niche shifts in metazoans are ubiquitous (Miller and Rudolf, 2011). Indeed, intraspecific variability has recently been suggested on the basis of modelling studies as a key to maintain high species diversity in plankton and thus to explain the "Paradox of the Plankton" (De Laender *et al.*, 2014; Menden-Deuer and Rowlett, 2014).

One of the important functional traits is body size. There has been a lot of research showing that body size determines various biological rates (Brown *et al.*, 2004) and to a significant extent determines predator–prey interactions (Brose *et al.*, 2006). In marine plankton, size has been suggested as a key trait for phytoplankton as well as zooplankton (Litchman and Klausmeier, 2008; Litchman *et al.*, 2013). Studies addressing the role of size in community structuring and functioning have primarily focused on unicellular organisms (reviewed by Marañón, 2015). For metazoans, studies are fewer, and these studies indicate that size scaling is not as evident as for microplankton (i.e. size–abundance relationship departs from linearity on a log-scale) (e.g. Sprules and Goyke, 1994; San Martin *et al.*, 2006; Sourisseau and Carlotti, 2006; Chang *et al.*, 2014; García-Comas *et al.*, 2014). Nevertheless, mesozooplankton size structure may play an important role in trophic transfer through size-based predator–prey interactions (Ye *et al.*, 2013; García-Comas *et al.*, 2016).

In order to better understand the role of size in community structuring and its relationship with species composition, empirical studies that compare species versus individual-size composition are needed. However, such studies are very scarce due to the "double" effort required to characterize a community (Gilljam *et al.*, 2011). Furthermore, generally in these kind of studies, either size or taxon or both are not fully resolved. That is, taxonomic categories are broader than species (e.g. genus level) or size data are derived from taxon-based nominal size (e.g. average size of adults of a species) but not individual size. Either way, if limitations of the datasets are not considered, relationships explored in such studies may be prone to erroneous conclusions. Here, we conduct a thorough comparison of species-based versus individual-size-based structuring of copepod communities. To do this, we have sorted into species and measured the individual size of copepods in the East China Sea (ECS). Copepods correspond on average to 70% of total mesozooplankton in these waters, and their identification can be made to the species level except for juveniles. Samples covered the whole ECS and covered three seasons.

Our main objectives were: (i) to compare species, species-based nominal-size and individual-size distributions; (ii) to explore the effect of environmental factors in shaping species structuring and individual-size structuring; (iii) to explore the congruence (i.e. degree of match) between species and individual-size composition of copepod communities and finally (iv) to identify possible intrinsic factors (i.e. community descriptors) affecting the degree of congruence. To achieve the fourth objective, we tested relationships of the species-individual-size congruence with several community descriptors, including species richness as well as descriptors dissecting the size-based community structuring, in order to better understand the implications of the commonly used synthetic indicators not resolved at the individual level (e.g. community size structure based on: species presence only, species nominal size assigned to individuals or actual individual size but only of adults due to problems in identification of juveniles). Specifically, (i) we tested the relationship with species richness, because species richness affects the congruence in species-size structuring via sampling effect. (ii) We examined the distribution of the nominal size of species constituting the community (presence only, disregarding their abundances). This provides information on the size partitioning of species: their potential degree of functional redundancy without accounting for intraspecific variability, which is a common practice in studies analysing the diversity of functional traits. (iii) We tested the difference between community structuring based on species nominal size (same size assumed for individuals of a given species) and that based on the actual sizes of the individuals classified into species. Because only adults are classified into species (due to problems of identification of juveniles), this difference acts as a proxy for intraspecific variability without accounting for ontogeny, which is a common case in studies based on species composition. (iv) We tested the percentage of juveniles in the community. This descriptor represents ontogeny, and thus the importance of population dynamics in community structuring.

METHOD

Study area

The ECS represents one of the largest marine shelf areas in the world. It is located in the northwest Pacific, and bounded by Mainland China, North Korea, the Kyushiu and Ryukyu Japanese Islands and Taiwan. The sampling area (25–32°N, 120–127°E) includes coastal waters shallower than 50 m depth and mid-shelf sampling sites ranging from 50 to 200 m depth (Fig. 1). Samples covered the three major water masses that result in significant hydrographic heterogeneity in the ECS. Moving from west to east, the East China Sea Coastal Current (ECSCC) influenced by high nutrient enrichment from river run-off, especially from the Yangtze River, the warm oligotrophic Taiwan Warm Current (TWC) and the Kuroshio.

Sampling of copepods

A total of 73 samples were collected from 34 stations on eight different cruises: three in spring, four in summer and one in winter (Figs 1 and 2). Copepods were sampled with an ORI net (330 μm mesh size and 1.6 m mouth diameter). Tows were oblique from 10 m above the sea floor to the surface at a speed of $0.3 \, \mathrm{m \, s^{-1}}$. Upon retrieval of the net, the contents of the cod-end were immediately preserved in 4% buffered formalin.

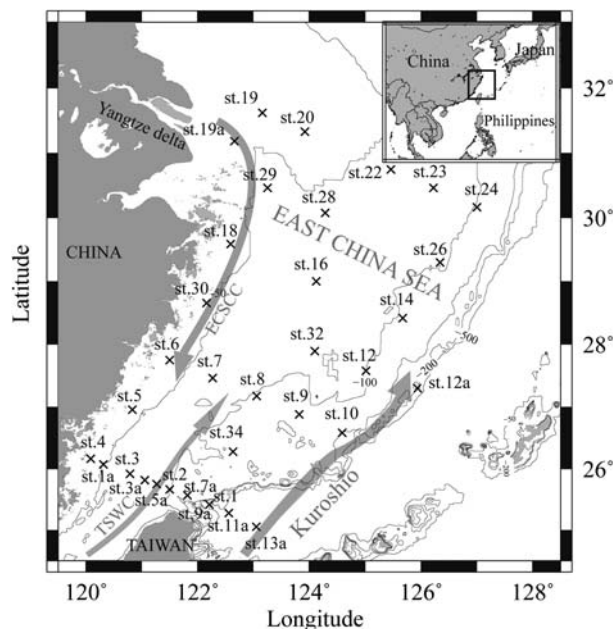**Fig. 1.** Map illustrating sampling locations in the ECS, with superimposed main currents and bathymetry.

Size and taxonomic classification of copepods

For each sample, two independent subsamples were fractionated with a Folsom Plankton Splitter and analysed with a microscope for taxonomic classification, and with a ZooSCAN (Gorsky et al., 2010) for size measurements. The use of two different instruments is justified by their respective limitations. Whereas the ZooSCAN can provide automatic measurements of the silhouette of all organisms in the subsample in about 30 minutes, the taxonomic classification of ZooSCAN images, even with human intervention (taking an additional 40 minutes), is generally limited to family or order level in subtropical environments around Taiwan. In contrast, under the microscope, taxonomic classification can reach the species level, although generally only for adults, but the procedure is much more time consuming (about 20–40 hours per subsample in this study, depending on species composition); moreover, as a trade-off, the number of organisms analysed is lower, copepodites are often not accounted for due to limitation of recognition, and size if measured is generally limited to prosome length. Analyses with the microscope and ZooSCAN were conducted on average 1 year after sampling, and thus we assume stabilization of formalin shrinking effects on size (Harris et al., 2000).

ZooSCAN data: individual-size measurements
On average, 750 copepods per sample were measured with the ZooSCAN. The subsample was scanned at 2400 dpi (pixel resolution of $10.58 \, \mu\mathrm{m}^2$) and analysed with the Zooprocess Integrated System (Gorsky et al., 2010) to obtain individual copepod 2D measurements. From the major and minor axes of the best fitting ellipse of each copepod area, we calculated the Ellipsoidal volume (EllipVol) of each copepod. The EllipVol was computed instead of the most common equivalent spherical diameter (ESD) volume because copepods have an elongated shape and thus the ESD overestimates their volume (Sprules et al., 1998). From here on, we will refer to the EllipVol obtained with the ZooSCAN as individual size.

In order to compare species versus individual-size composition, size was classified into size classes. Size classes ranged from −4.19 to $2.50 \log(\mathrm{mm}^3)$ to cover the whole size spectrum in our samples, and had equal width on the logarithmic scale to adjust the apparent variance of organisms of diverse size. Thus, the size resolution corresponded to 20 size classes, which coincides with the average number of species composing a community in our samples. We tested the appropriateness of using 20 size classes following a simulation of goodness of fit of
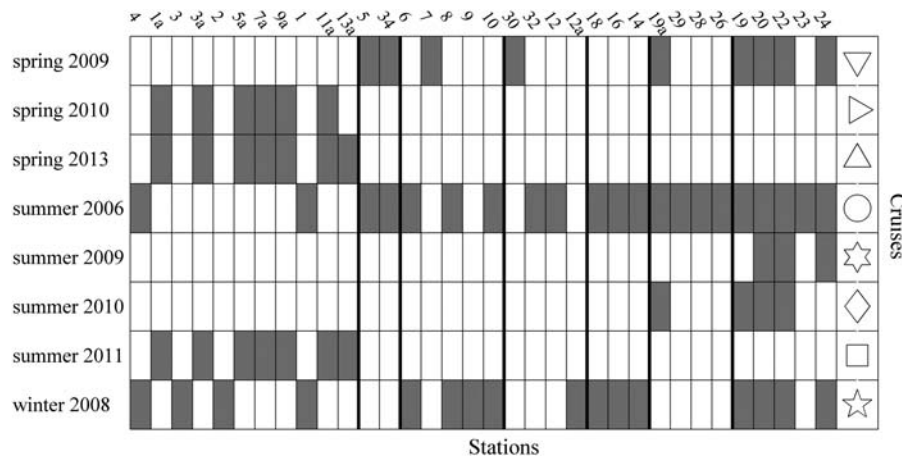
Fig. 2. Chequerboard plot indicating stations sampled during each cruise (grey squares). Thick vertical lines divide longitudinal transects (from south to north). Symbols on the right column correspond to symbols representing each cruise in subsequent figures.

individual-size composition with species composition and environmental variables depending on the resolution of size binning. Resolution of size binning affected the strength of relationships but the sign and statistical significance held (Fig. S1 of Supplementary Material).

Microscope data: species counts and species nominal size
On average, 350 copepods per sample were identified under the microscope. Adult copepods were identified into 151 species, whereas copepodites (i.e., juveniles) were classified at a range of taxonomic resolution depending on recognition feasibility (list of taxa in Table S1 of Supplementary Material). In order to have robust species counts, taxonomic classification was considered for adults only (i.e. at species level), because the taxonomic resolution for copepodites was generally broader (i.e. often at genus level but not consistently in our subtropical environment). These broader categories largely dominated the bulk abundance, and would therefore bias analyses. In addition, individual prosome lengths were measured under the microscope. The nominal size of each species was calculated as the mean prosome length of all adult individuals in the data set belonging to that species (mean size and size ranges per taxon reported in Table S1 of Supplementary Material). The species nominal size is often used to characterize the size structure of a community due to the difficulty of measuring the size of all individuals in a sample (e.g. Chiba *et al.*, 2015; Pomerleau *et al.*, 2015).

Community descriptors

Several community descriptors, used to explain the degree of mismatch between species-based and individual-size-based community structuring, were calculated from the

microscope counts: (i) species richness, (ii) nominal-size distribution descriptors (i.e. mean, range, coefficient of variation, skewness and kurtosis) of the species constituting the community (presence only, disregarding their abundance), (iii) the difference between community structuring based on species nominal size (same size assumed for individuals of a given species) versus that based on the actual size of the individuals classified into species (i.e. the residuals of a Procrustes analysis comparing the two types of size-based structuring) and (iv) the percentage of juveniles in the community.

(i) The species richness could affect the degree of mismatch between species and individual-size structuring simply by sampling effect, although the shape of the relationship is not intuitive. (ii) The nominal-size distribution descriptors represent the size partitioning of species neglecting intraspecific variability. (iii) The difference between community structuring based on species nominal size (i.e. all organisms classified into a species are assumed to have the same size) and that based on the actual sizes of the individuals classified into species acts as a proxy for the effect of intraspecific variability neglecting ontogeny (note that the species counts contain only adults, due to identification limitations for juveniles). Specifically, this index is calculated as the residuals resulting from matching by Procrustes (Gower, 1975), the community structuring based on the actual size of adult individuals with that based on species nominal size. Procrustes consists of linearly transforming a matrix-based ordination to maximize its superimposition on another matrix-based ordination. The residuals of this matching are non-directional and reflect the difference between the two ordinations, in this case, the two kinds of size-based community structuring. In order to apply Procrustes, the prosome lengths were sorted into 20 size classes of equal

width on the log-scale with limits corresponding to the shortest (420 µm) and the longest (3.14 mm) nominal prosome lengths (very few individuals had actual lengths beyond this range and were included into the limit size classes). Finally, (iv) the percentage of juveniles in the community was used as a proxy for the effect of ontogeny and thus it represents population dynamics in the community structure.

Environmental descriptors

Temperature and salinity were recorded with a SeaBird CTD profiler (SBE9/11 plus, SeaBird Inc., USA). Nitrate (NO_3), phosphate (PO_4), silicate (SiO_3) and chlorophyll *a* (chl *a*) concentrations were measured from water samples collected at discrete depths (e.g. 0, 10, 25, 50, 75, 100 m depth) with Go-Flo bottles, and stored in liquid nitrogen until analysis. Analytical methods are detailed elsewhere (Gong *et al.*, 2000).

Depth-weighted integrated average values of temperature, salinity, chl *a* and nutrient concentrations above the mixed layer depth (MLD) were used instead of surface conditions as this is the zone where copepods obtain most of their food. The MLD was calculated following Levitus thermal criteria (i.e. the depth at which ΔT with SST was 0.5°C; Levitus, 1982) and was also considered as an environmental descriptor. Statistics of environmental variables can be found in Table S2 of Supplementary Material.

Data analyses

First, (1) the patterns of species, species nominal-size and individual-size distributions were compared using histograms; while, the environmental conditions were summarized with a Principal Component Analysis (PCA) (Legendre and Legendre, 1998). Then, ordination analyses were used to analyse community structuring. Prior to ordination analyses, abundances in both species and individual-size matrices were Hellinger-transformed to down-weight the effect of rare categories (Legendre and Gallagher, 2001).

(2) The effects of environmental factors on species-based and individual-size-based community structuring were investigated with a Redundancy Analysis (RDA) (Legendre and Legendre, 1998). RDA is a multiple linear regression between response variables (i.e. transformed abundance of species or individual-size classes) and explanatory variables (i.e. environmental conditions), which is consequently represented in the space of a set of orthogonal (i.e. independent) axes. It is thus considered a constrained version of PCA, as the canonical axes built from linear combinations of the response variables also have to be linear combinations of the explanatory variables. With this analysis, in addition to depicting the proportion of variance of community structuring explained by the environment, we could elucidate, by stepwise forward selection, which factors were the most important in shaping the species-based or the individual-size-based community structuring.

(3) Species-based and individual-size-based community structuring were compared with Procrustes (Gower, 1975). The individual-size matrix was manipulated to best match the species matrix (target matrix), and the correlation-like statistic was used as a measure of congruence (i.e. match); its significance was obtained by randomization test of symmetric Procrustes (permutations = 9999). In addition, the residuals of Procrustes, which are non-directional, were used to quantify for each sample the magnitudes of departure from the overall association (Peres-Neto and Jackson, 2001) of individual-size structuring with species structuring.

Finally, (4) the residuals of Procrustes obtained from the comparison of species-based versus individual-size-based community structuring were: first, superposed on the space of the first two principal components (PCs) of the environmental PCA to visually inspect their relationships with water masses and seasons; and then, explained by the community descriptors using Pearson correlation to test the relationship of these descriptors with the mismatch between species-based and individual-size-based community structuring.

The procedure of data analyses is summarized in Fig. 3. Analyses were performed with MATLAB R2013b and the Vegan (2.2–1) (Oksanen *et al.*, 2015) package in R 3.1.1 (R Core Team, 2014). Statistical tests (i.e. Procrustes protest *r* and Pearson correlations) were computed on the whole data set, separately by season, and on a summer cruise and the winter cruise for their 12 matching sampling stations covering the ECS.

RESULTS

Species distribution versus size distribution

Classification of the copepod data set into species and size reflected different patterns (Fig. 4); this is true either through examining the whole data set or the data divided by cruise (Figs. S2–S7 of Supplementary Material). The rank-abundance distribution curve computed from the total 151 species in the samples' pool shows the ubiquitous pattern of dominance by a few species and a long tail of rare species (Fig. 4a). In contrast, copepod individual-size distribution showed a
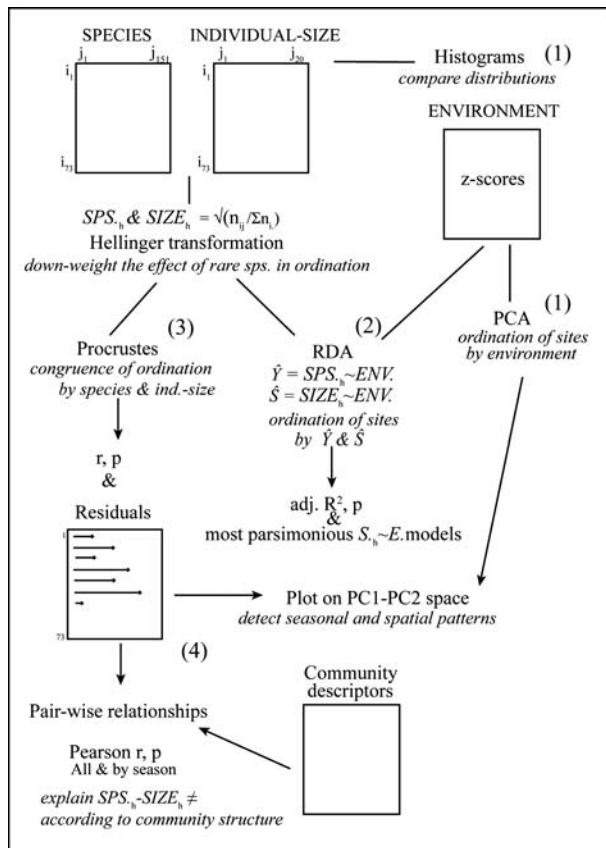
Fig. 3. Flowchart describing the procedures of data analyses: (1) to compare species, species nominal-size and individual-size distributions, as well as to summarize environmental conditions; (2) to explore the effects of environmental factors on species-based and individual-size-based community structuring; (3) to compare species-based and individual-size-based community structuring and finally, (4) to relate mismatch between species-based and individual-size-based community structuring with environmental and community descriptors.

bimodal pattern, with dominance of small organisms and a secondary mode towards larger copepods (Fig. 4b). Copepod communities consisted of an average of 20 species, and ranged from 2 to 55 species per sample (Fig. 4c). Despite the fact that small species ($< \sim 1$-mm prosome length) dominated the species pool (*x*-axis of Fig. 4d), larger species (of ~ 2-mm prosome length) were as frequent as the small ones in each sample (*y*-axis of Fig. 4d).

The pattern of size distribution changed substantially depending on the kind of size definition used. Specifically, if we used the nominal size of the species (average prosome length per species) to construct the size distribution (ignoring intraspecific variability), we obtained a pattern that is very different from that constructed from the actual individual-size of copepods constituting the community (Fig. 4e versus Fig. 4b). Even considering the actual prosome length of each copepod

measured under the microscope, the observed spectrum shows more size discontinuities and smoother modes than the one based on EllipVol from the ZooSCAN (Fig. 4f versus Fig. 4b). Furthermore, copepodites constituted 30% of the data set; thus they may substantially influence the shape of the copepod size spectra (Fig. 4f). Indeed in our data set, copepodites constituted 60% of individuals ranging from 1 to 1.5 mm (10th–14th size classes) of prosome length (Fig. 4f).

Species-based and individual-size-based community structuring in relation to environmental factors

The PCA reflected two main environmental gradients: (i) offshore–coastal gradient (PC1) with decreasing temperature and salinity, and increasing chl *a* and nutrients towards the coast and (2) summer–spring–winter gradient (PC2) with decreasing temperature and chl *a* and increasing MLD (Fig. 5a; complementary maps in Figs. S8 and S9 of Supplementary Material).

Environmental conditions played a stronger role in species structuring (adjusted $R^2 = 0.29$) than in individual-size structuring (adjusted $R^2 = 0.21$). Accordingly, in the RDA triplots, the species assemblages represented by certain species (e.g. *Paracalanus parvus*, *Calanus sinicus*, *Oncaea venusta*, *Paracalanus aculeatus*, *Temora turbinata*) exhibited discrete structuring in relation to the environmental factors (Fig. 6a), while the individual-size structure rather showed a continuum (Fig. 6b). The species assemblages were mostly structured by the physical conditions (e.g. first salinity and secondarily temperature, both alone accounting for 75% of the adjusted explained variance, followed by chl *a*, bathymetry and PO$_4$); that is, the main community structuring corresponded to coastal versus offshore communities (Fig. 6a; Fig. S10). The individual-size structuring was better explained by silicate concentration followed by bathymetry, chl *a* and NO$_3$ (Table I), and we only observed a coastal-offshore pattern in winter (Fig. 6b; Fig. S12). It is worth mentioning that the explanatory value of environmental conditions was not particularly high (i.e. 36% for species and 29% for individual-size) due to the high spatio-temporal coverage in our samples. With this coverage we aimed at exploring the general pattern of association.

Congruence between species-based and individual-size-based community structuring

In spite of species-based and individual-size-based community structuring showing different patterns, the individual-
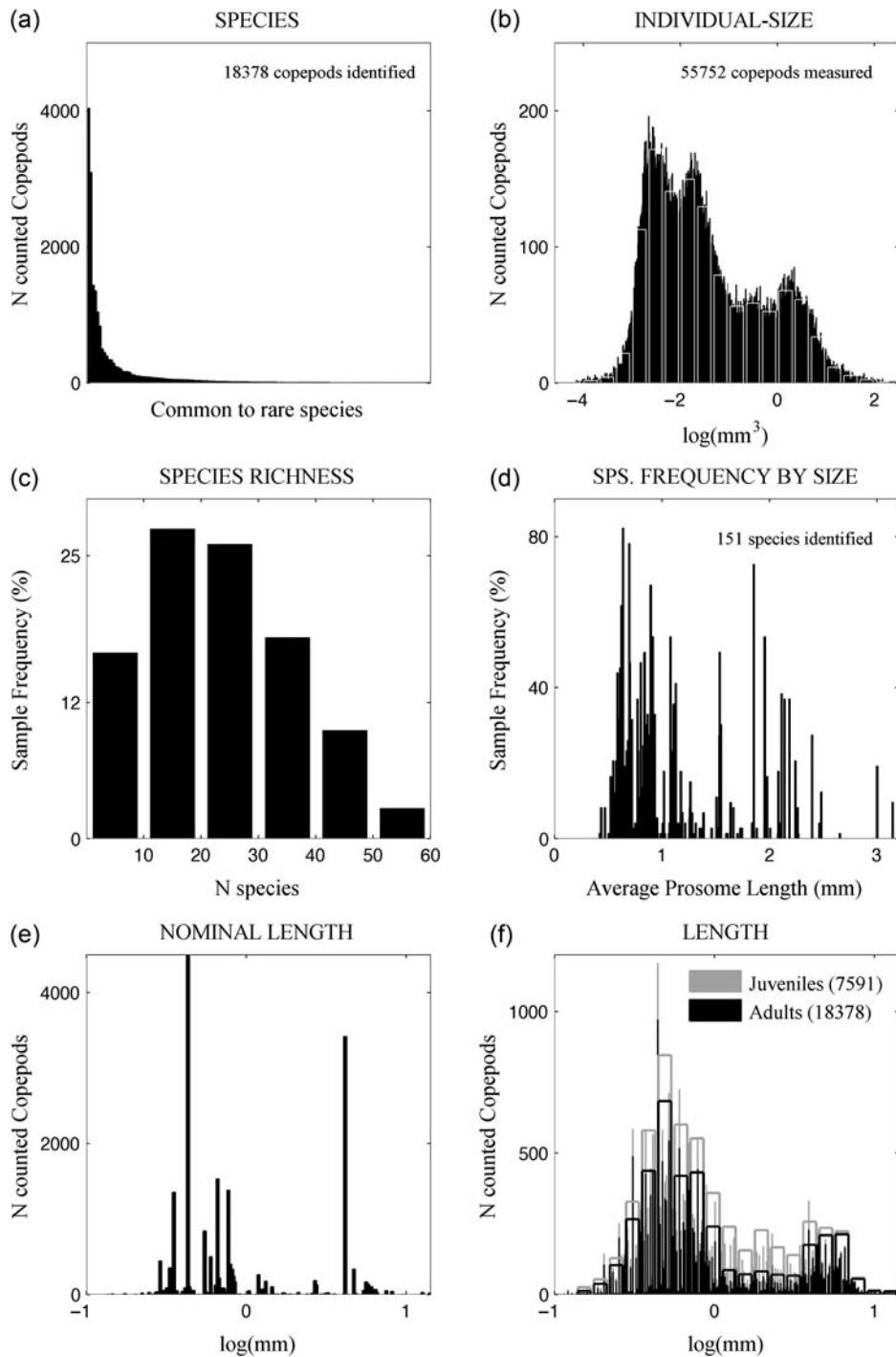
Fig. 4. Species and size distribution of copepods analysed with the microscope (panels a and c–f) and with the ZooSCAN (panel b). Panel (**a**) shows the species rank-abundance distribution of copepods (adults only). Panel (**b**) shows the individual-size distribution (EllipVol of copepod individuals, disregarding their developmental stages or species; histogram of 1000 size classes), with superposed bars corresponding to the rescaled 20 size classes into which individual-size is sorted for statistical analyses. Panel (**c**) illustrates the frequency distribution of species richness. Panel (**d**) shows the frequency of species in the samples according to their nominal size (i.e. average adult prosome length of each species measured under the microscope). Panel (**e**) shows the copepod size distribution when we consider a single size for all individuals constituting a given species (i.e. the nominal species prosome length). Panel (**f**) shows the size distribution of actual individual prosome length when including only adults (black bars) or including both adults and juveniles (grey bars) (length sorted into an histogram of 1000 size classes). Superposed bars correspond to the rescaled spectrum of 20 size classes of equal width in the log-scale.

size-based community structure still significantly matched species-based community structure (Procrustes protest $r = 0.67$, $P = 0.0001$). The residuals of Procrustes (i.e. mismatches of species-based and individual-size-based structuring) tended to be greater in summer than in spring and winter (Fig. 5b; Fig. S14 of Supplementary Material). Indeed, Procrustes analyses by season revealed that summer exhibited lower congruence of individual-size versus species structuring than spring and winter (Table II). To

account for the possibility that this finding could be due to heterogeneous sampling, we repeated the analysis for 12 common sampling locations of the winter cruise and the summer cruise with the widest spatial coverage; we found our conclusions remained valid (Table II). Regarding the spatial distribution, we did not find a pattern for the residuals, except for winter that the residuals tended to be lower in coastal waters (Fig. 5b; Fig. S14 of Supplementary Material).
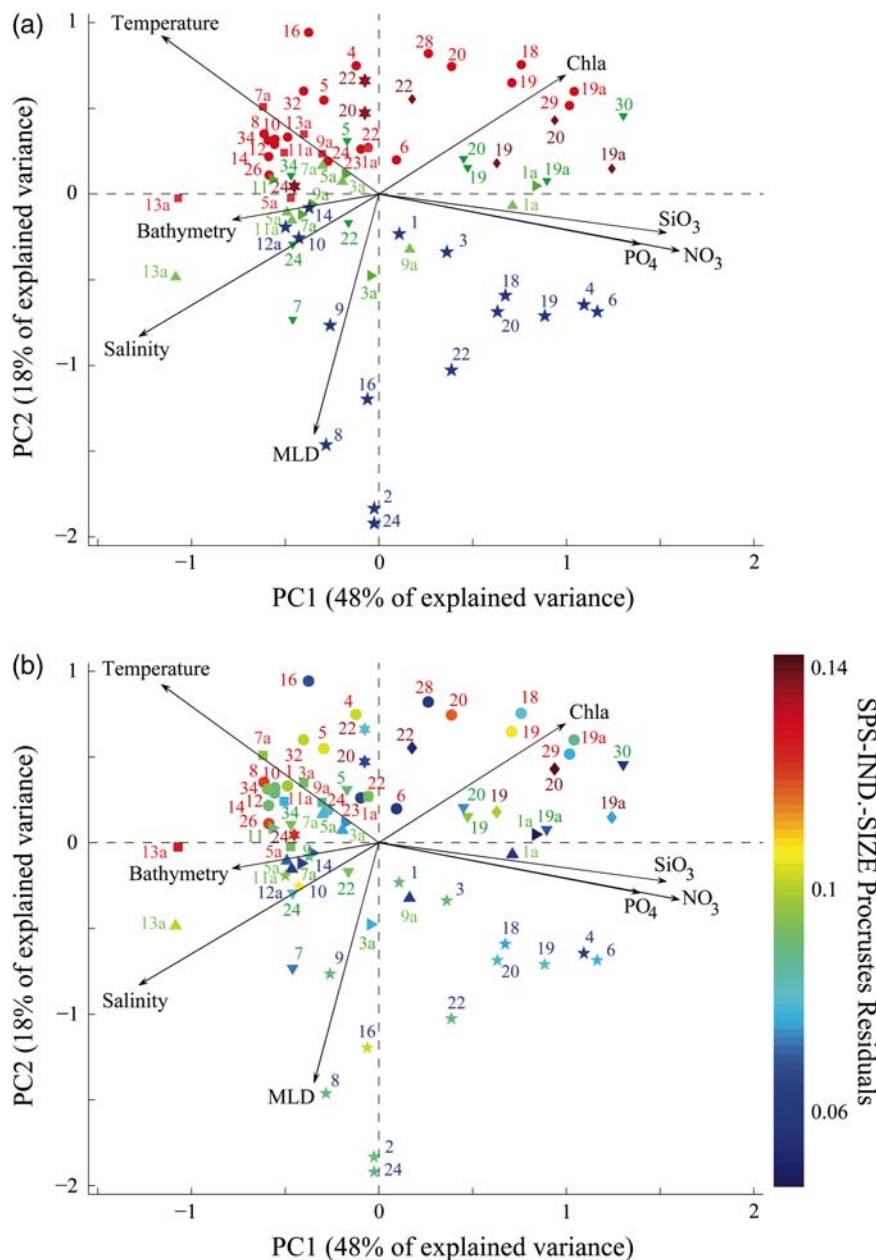
Fig. 5. PCA biplot of environmental conditions with colours corresponding to seasons (i.e. green for spring, red for summer and blue for winter), and symbols and colour tones corresponding to different cruises (a); and the same biplot with colours corresponding to residuals of the species-individual-size Procrustes (Protest's $r = 0.67$; $P = 0.0001$; $n = 73$) (b).
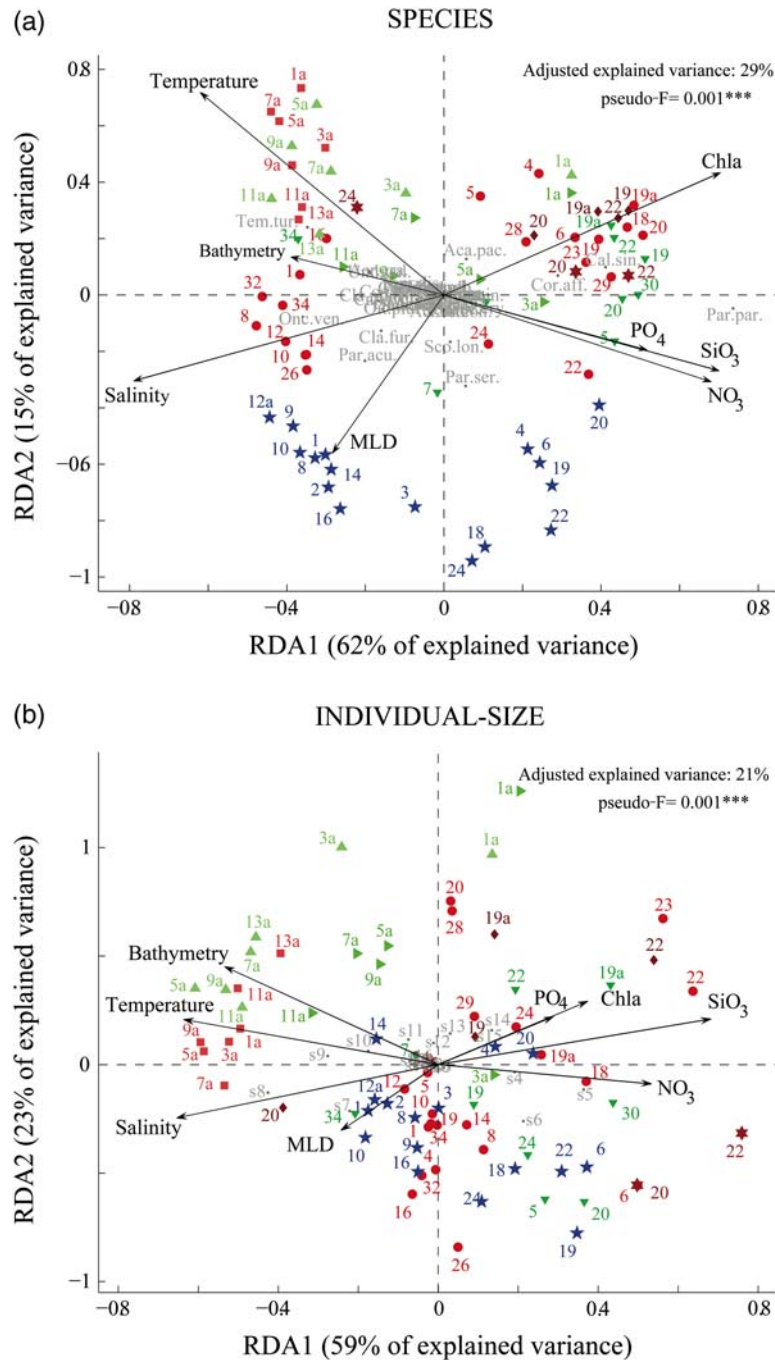
Fig. 6. Correlation triplots (scaling 2) summarizing RDA results: Ordination of sites according to associations of community structure with environmental conditions. Community structure is defined by Hellinger-transformed abundances of species (grey dots with abbreviated names corresponding to the 151 species listed in Table S1 of Supplementary Material) (a), and by Hellinger-transformed abundances of 20 size classes (grey dots order from the smallest (s1) to the largest (s20))(b). Colours correspond to seasons (i.e. green for spring, red for summer and blue for winter) and symbols and colour tones distinguish the eight cruises listed in Fig. 2.

The species versus individual-size Procrustes residuals tended to increase linearly with the proportion of juveniles in a community (Fig. 7), exhibiting a significant positive correlation except for winter (Table III). The Procrustes residuals showed no linear relationship with the index acting as proxy for intraspecific variability without accounting for ontogeny (i.e. the difference between community structuring based on species nominal size and that based

Table I: Stepwise selection of environmental variables constituting the most parsimonious RDAs with associated cumulative explained variance (i.e. adjusted R^2)

	1st.	2nd	3rd	4th	5th
Species	Sal. 14%	Temp. 22%	Chl *a* 25%	Bathy. 27%	PO$_4$ 28%
Individual-size	SiO$_3$ 7%	Bathy. 11%	Chl *a* 12%	NO$_3$ 15%	

Table II: Results of the species-individual-size Procrustes analysis computed on the whole data set, separately by season and for the same spatial coverage (i.e. same stations) in a summer cruise and the winter cruise.

	$r(\sqrt{(1 - ss^2)})$	P
All ($n = 73$)	0.67	0.0001
Spring ($n = 22$)	0.85	0.0001
Summer ($n = 35$)	0.71	0.0001
Winter ($n = 16$)	0.84	0.0001
Summer cruise ($n = 12$)	0.75	0.0001
Winter cruise ($n = 12$)	0.86	0.0001

on the actual sizes of the individuals classified into species), except for a marginal positive correlation in summer (Table III). We did not find either significant relationships of the Procrustes residuals with any of the descriptors of the species nominal-size distribution, except when considering the summer and winter cruises separately. For the summer cruise, in addition to "the positive relationship of the residuals with" the percentage of copepodites, the residuals significantly decreased with the mean nominal size of species. In winter, the residuals showed significant positive correlation with the species-size range (Table III; Fig. S16 of Supplementary Material).

DISCUSSION

Our comparison of species-based versus individual-size-based community structuring reveals: (i) different patterns in the community distribution depending on the ordination "currency" (i.e. species, species-based nominal-size, individual-size) (Fig. 4); (ii) that species structuring by environmental filtering is more related to physical conditions, while the individual-size structuring by environmental filtering is more related to food conditions (Fig. 6; Table I); (iii) despite differences in environmental effects on species-based and individual-size-based community structuring, the two types of structuring significantly matched (Table II) and (iv) the percentage of juveniles in

the community was the main intrinsic community descriptor explaining mismatch between both types of structuring (Table III; Fig. 7). These results suggest individual-size as a useful complementary descriptor in species-based studies.

Relevance of considering individual-size distribution

Our study stresses that intraspecific variability and in particular secondary production (i.e. juveniles) can be accounted for in individual-size-based analyses (Fig. 4b,f), in contrast to analyses based on species abundance (Fig. 4a) or species-based nominal size (Fig. 4e). Indeed, the frequency of juveniles in the community was the intrinsic characteristic that best explains mismatch between species-based and individual-size-based community structuring (Table III; Fig. 7). This might seem trivial, as juveniles were not accounted for in species counts due to limitations in their identification. However, the critical message is that, through studying individual-size distribution, one can examine the influences of population dynamics in structuring copepod communities. Specifically, we found that juveniles tend to dominate a certain size range (i.e. contributing to more than half of individuals sampled in the mid-size range; Fig. 4f). According to previous studies, this might imply dominance of a certain trophic niche (Rudolf and Rasmussen, 2013; Ye *et al.*, 2013). Thus, detailed examination of individual-size distribution could be a useful complementary descriptor to species-based studies because species-based studies typically neglect the contribution of juveniles to the community structure.

Indeed, ontogeny is one of the most important features of intraspecific variability and often implies niche shifts (Werner and Gilliam, 1984; Miller and Rudolf, 2011). For example, Rudolf and Rasmussen (2013) have demonstrated, through manipulation of the predatory dragonfly and beetle larvae in freshwater mesocosms, that demographic differences can be more important than interspecific differences in altering community structure and functioning. The same argument may apply in copepods. For copepods, juveniles have underdeveloped feeding appendages and smaller body size, suggesting that they have feeding strategies different from adults, although empirical studies are scarce and inconclusive (e.g. Meyer *et al.*, 2002; Brucet *et al.*, 2008). In addition, different nutrient requirements at different life stages might imply different targeted prey and assimilation rates (Hirst and Bunker, 2003) as well as different mortality rates throughout ontogeny (Ohman *et al.*, 2002). In this aspect, Woodward and Warren (2007) demonstrated that defining individuals by their
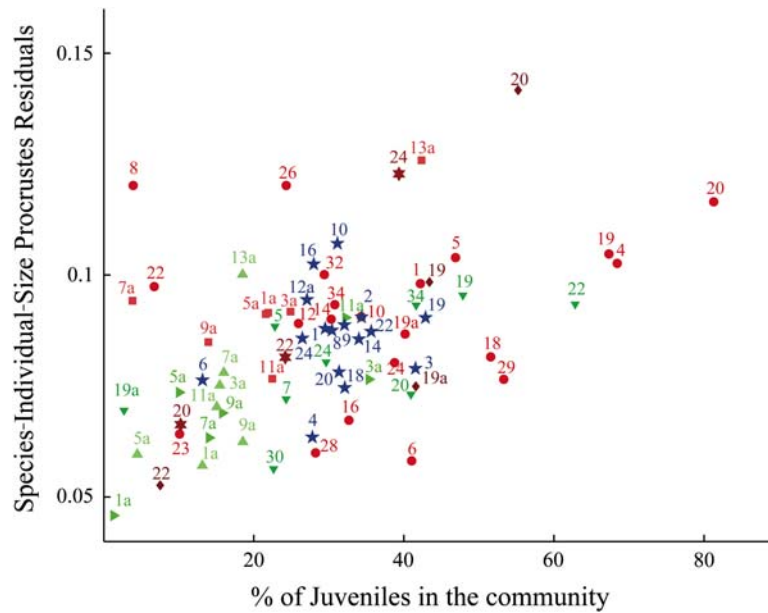
Fig. 7. Relationship of % of juveniles in the community and the residuals of the species-individual-size Procrustes ($r = 0.46$; $P \leq 0.0001$; $n = 73$).

Table III: Pearson correlations between the species-individual-size Procrustes residuals and the community descriptors (P $\leq$ 0.0001***, P $\leq$ 0.01**, P $\leq$ 0.05*, P = 0.05.) computed on the whole data set, separately by season and for the same spatial coverage (i.e. same stations) in a summer cruise and in the winter cruise

	Species richness	Nominal length C.V.	Mean nominal length	Nominal length range	Nominal length skewness	Nominal length kurtosis	% of Juveniles	Nom. length—length residuals
All ($n = 73$)	0.17	0.03	−0.02	0.11	0.08	0.07	0.46***	0.17
Spring ($n = 22$)	0.30	−0.17	−0.10	−0.04	−0.21	−0.21	0.66***	−0.06
Summer ($n = 35$)	0.11	0.0001	0.03	0.04	0.11	0.01	0.34*	0.32
Winter ($n = 16$)	0.44	0.20	0.38	0.71**	0.11	0.28	0.14	−0.21
Summer cruise ($n = 12$)	−0.17	−0.15	−0.58*	−0.39	0.17	0.11	0.67**	−0.01
Winter cruise ($n = 12$)	0.45	−0.01	0.08	0.58*	0.34	0.41	0.42	−0.46

species nominal size led to noise and underestimation of predator–prey size ratios in a freshwater trophic web. That is, the intraspecific variation of body size in a species entails that only part of its population may be predators or prey of another species (or part of their population), hence highlighting the relevance of resolving size beyond species.

Different environmental effects on species-based and individual-size-based community structuring

Species assemblages were mainly related to salinity and temperature. Salinity and temperature alone contributed

75% of the adjusted explained variance in the RDA analysis relating species assemblages with environmental factors (Table I). The importance of salinity and temperature over chl *a* for characterizing the ECS copepod communities has already been reported in a previous study (Zuo *et al.*, 2006). Indeed, copepod species assemblages are commonly associated with thermal and salinity ranges, and consequently used as indicators of water masses and/or seasonality (e.g., Beaugrand *et al.*, 2002; Mackas and Coyle, 2005; Chiba *et al.*, 2006). Our results support the use of species assemblages as indicators of water masses and seasonality in the ECS, as reflected by the dispersion of samples in the RDA space (Fig. 6, Figs. S10–S11 of Supplementary Material). Copepod species assemblages tended to be dominated by a few distinctive

species (e.g., *P. parvus, C. sinicus, O. venusta, P. aculeatus, T. turbinata*). For those interested in using species assemblages as indicators, we additionally report the five most dominant species in each sample (Table S3 of Supplementary Material).

Contrary to the species-based structuring, we show that individual-size structuring is more related to food availability than to temperature or salinity (Fig. 6, Table I), suggesting that individual-size structure could serve as an indicator for trophic status of the system. On the one hand, food affects community size structure via metabolic scaling and thus food availability to support larger organisms (Brown *et al.*, 2004); on the other hand, food fuels secondary production, as food has been reported to have a stronger effect on copepod reproduction than on somatic growth (Kiørboe, 1997). Indeed, analyses on the size diversity of zooplankton in the same communities suggest that population dynamics affect size structure more strongly than metabolic scaling (García-Comas *et al.*, 2014). Trophic status alters community individual-size structure and this, in turn, has been suggested to have an impact on trophic transfer (Ye *et al.*, 2013; García-Comas *et al.*, 2016). Therefore, bottom-up effects fuelling secondary production are better revealed when analysing individual-size structure, which incorporates information of juveniles into analyses. In contrast, species-based community analyses (relying exclusively on data of adults) do not provide information on secondary production.

Mismatch of species-based and individual-size-based community structuring

The mismatch between species-based and individual-size-based structuring was best explained by the percentage of juveniles in the community (Fig. 7). Our comparative analysis demonstrates the need to incorporate the information on juveniles to better understand copepod community dynamics in response to environmental variation. Yet, one should note that incorporating juveniles into the classic species-based community analysis is problematic, because by doing so we unavoidably have to employ broader taxonomic groups. Adding such broad taxonomic categories (i.e. adding juveniles in the taxonomic resolution of genus or order) masks the important role of species in community structuring. Thus, we suggest that species-based and individual-size-based analyses should be used in concert but not combined, as our results suggest that they provide complementary information. Although this approach cannot completely solve the problem that most juveniles cannot be identified to species, this is a step forward and can be

efficiently done with the aid of the ZooSCAN or other image-based automatic systems.

Curiously, we found that the degree of mismatch between species-based and individual-size-based structuring was weaker in winter than in summer (Table II), and that the frequency of juveniles generally enhanced the species-size mismatch except in winter (Table III). In addition, the index for intraspecific variability (not accounting for ontogeny) explained, although marginally, the mismatch of species-based versus individual-size-based structuring only in summer. These results suggest that in summer, species niches may tend to overlap through ontogeny, intraspecific variability increases due to overlapping of multiple generations, and this results in the observed species-size mismatch. Indeed in summer, both the frequency of juveniles and the intraspecific variability of adults tend to be higher than in winter (Fig. 7 and Fig. S16G of Supplementary Material). Supporting our proposed mechanism, Woodward *et al.* (2010), using a fully resolved freshwater food web, reported highest species niche overlap in summer due to ontogeny.

In winter, the only variable significantly explaining mismatch between the species and size structuring was the size range of nominal sizes (Table III). This positive correlation between the strength of mismatch versus size range goes against intuition. Intuitively, a larger size range likely entails less probability of niche overlapping. Also, as aforementioned, niche overlapping related to population dynamics is weaker in winter compared to summer. Then, how to explain this positive correlation? Perhaps, the positive correlation of size range with species-size mismatch could be a spurious correlation related to the spatial gradient of the response variable in winter. In winter, in coastal areas, species-based and individual-size-based structuring match better and those communities have narrower size ranges (Fig. 5b; Fig. S14; Fig. S16D of Supplementary Material).

Implications of our results: different data to tackle different ecological questions

Our results highlight the diverse and complementary information provided by the species-based and the individual-size-based vision of a copepod community (Fig. 4). Thus, there should be no argument against either approach. Rather, we suggest that the choice of descriptor will depend on the ecological question to tackle, and both approaches can provide complementary information. For example, the species-composition data allow exploring diversity partitioning through

environmental gradients, which can give insight on mechanisms maintaining diversity (Logue *et al.*, 2011). In addition, species composition provides useful information as habitat indicators (coastal versus offshore; latitude and season; Fig. 6a; Table S3 of Supplementary Material). This cannot be done with individual-size-composition data at the mesoscale, where effects of population dynamics confound those of species composition (Fig. 6b). In contrast, focusing on body size rather than on species is valuable in analysing important functional traits to formulate general ecological laws explaining diversity maintenance and the diversity–ecosystem functioning relationship (Petchey and Gaston, 2006; Cadotte *et al.*, 2011). Through arranging species by their nominal size (i.e. average length of adults), we confirm size as a critical organizational trait of communities and observe well-known patterns (White *et al.*, 2007): there are more small species than large ones in the 151 species pool, and the abundances of small species tend to outnumber those of large ones in the communities (Fig. 4d,e), although notice that the second most relatively abundant and frequent species has a prosome length close to 2 mm. Interestingly, we found no relationship of species frequency with their size (Fig. 4d). We encourage future studies on these contrasting patterns that point to size-based niche partitioning playing a relevant role in species distribution.

Finally, if the questions we are interested to tackle are about size-based trophic structure and functioning, then we recommend analysing the individual-size distribution (Fig. 4b) and avoid making inferences from species-centric size distribution that neglect the role of intraspecific variability (from population dynamics) on trophic interaction and functioning. Recent analyses of individual-size distributions of mesozooplankton in the ECS suggest that size diversity of zooplankton enhances top-down control of phytoplankton through diet niche partitioning (Ye *et al.*, 2013).

Consideration in methodology

We should mention some limitations in this study that are common to most mesozooplankton studies, yet it is necessary to consider them in order to emphasize future improvements. Regarding sampling coverage, we deal with limited sampling in a highly heterogeneous region that might lead to limited interpretation of results. Regarding sampling and sample treatment, two main limitations arise. First, the 330 μm mesh zooplankton net does not capture properly the smallest size fractions and thus significantly neglects the contribution of juveniles of small copepods (Fig. 4b,f). Secondly, size estimation represents a source of error. On the one hand,

microscope size measurements correspond to prosome length and therefore do not account for inter- and intraspecific shape variability. This is reflected in the higher discontinuity and smoother modes of the microscope-based size spectrum with respect to the ZooSCAN-based size spectrum (Fig. 4f versus Fig. 4b). On the other hand, regarding the ZooSCAN size measurements, part of the size variability would be due to the extrapolation of volume from area, and to the variable orientation of organisms in the scanning tray and/or state of their appendages due to sample manipulation. Nevertheless, we should bear in mind that most calanoids settle in the scanning tray on their side, and that variability in the position of settling comes mostly from among-taxa variation. It is worth noting that size binning mitigates the error associated with the precision of measurement, but still, the decision on how to bin the size data may be subjective and requires sensitivity analyses. In this study, we carried out some sensitivity analyses and found that our conclusions remained when testing finer size resolutions (Fig. S1 in Supplementary Material).

CONCLUSIONS

Our comparison between species-based and individual-size-based community structuring highlights the complementary information given by the individual size to the species composition. Individual size incorporates intraspecific variability, which is generally neglected in species-based studies. From our results, we speculate that while species-based ordination serves to define habitat indicators, individual-size-based ordination could serve as an indicator of trophic status (i.e. size structure driven by nutrient availability, especially through nutrient effects on secondary production, and potentially affecting trophic transfer to higher trophic levels). More empirical data on individual-size distributions across systems are needed in order to further test the role of body size distribution on functioning, and particularly on trophic transfer.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

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