

A comparison of appendicularian seasonal cycles in four distinct European coastal environments

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ABSTRACT

The European Union project EURAPP (“Impact of appendicularians in European marine ecosystems”) represented an integrated effort to elucidate specific biological and ecological aspects of appendicularians in the marginal seas of Europe. Within EURAPP, the seasonal variation in population densities and species assemblages have been studied from March 1999 to February 2000 in four contrasting European coastal environments: the Norwegian fjords, the western English Channel, and the Cantabrian and Ligurian Seas. The seasonal succession in the structure of the appendicularian community has two distinct phases: in winter-early spring, prior to the onset of stratification or warming of the water column at the mixed water locations, the community is characterized by the presence of fritillarians while in the summer-autumn oikopleuriids dominate. This summer-autumn phase can be subdivided into two or three sub-steps depending on the dominant oikopleuriid species. There was a positive relationship between the abundance of total appendicularians and chlorophyll concentration and a strong geographical influence on species composition, which was related to temperature. Three different associations of appendicularian species were detected. The niche of each species was identified by its unimodal response to temperature. Differences in temperature and to some degree in salinity were related to the seasonal abundance and geographical distribution patterns. The close relationship between assemblages of appendicularian species and physical environmental factors suggests their potential use as indicator species of climate changes or water masses.

INTRODUCTION.

Appendicularians have received increasing attention by marine plankton ecologists due to their fast growth rates (e.g. López-Urrutia et al. 2003a), their ability to feed directly on small particles (e.g. Steinberg and Landry ; Gorsky et al. 1999), their potential contribution to the biological pump through the excretion of filter houses and fecal pellets (e.g.Sato et al. 2001) and their contribution to the diet of the larvae of some commercially important fish species (e.g. Purcell et al.). Recent studies on their ecophysiology have considerably increased our knowledge on their individual bioenergetics (e.g.Acuña et al. 1999; Bochdansky and Deibel 1999; López-Urrutia and Acuña 1999) but to translate physiological responses to population impact we need to multiply individual rates by abundance or biomass. The factors controlling their abundance and distribution are likely to be strong determinants on their role in marine ecosystems and are less well understood than the factors that regulate their physiological rates.

There is already some information on the environmental factors controlling the seasonal and vertical changes in appendicularian species abundance and composition (e.g. Fenaux 1963; Shiga 1985; Acuña and Anadón 1992; Acuña et al. 1995). Although these studies have greatly improved our understanding of the seasonal dynamics of the appendicularian community, their scope has been limited to the environmental conditions and species present in each specific area. The differences and similarities in the seasonal cycles at a range of geographical areas during the same period has never been examined. Therefore, we do not know if the patterns depicted by each study are due to particular hydrographic phenomena at each location or they are part of gradients occurring at larger scales. The comparative analysis of several time-series at distinct

biogeographical areas is a key step towards the distinction of local from broad scale changes (Duarte et al. 1992).

The European Union project EURAPP (“Impact of appendicularians in European marine ecosystems”) was organized to evaluate the role of appendicularians in the marginal seas of Europe. The EURAPP field work evaluated the seasonal cycles of appendicularian abundance at four contrasting European coastal environments: the Norwegian fjords, the western English Channel, and the Cantabrian and the Ligurian Seas. While the dynamics at each particular site have been examined separately (e.g. Båmstedt et al. ; López-Urrutia et al. 2003b), here we present an attempt to compare the changes in appendicularian seasonality and community succession from a broad scale perspective. The wide range of environments covered would allow us to determine the role of environmental variables in shaping the niches of the different species and to provide a first description of the main species associations within the appendicularian community.

MATERIALS AND METHODS

Data collection

Appendicularians were collected at four European coastal environments at a total of eight stations (Table 1): three Norwegian fjords (Herdlefjorden, Korsfjorden and Sognefjorden; Fig. 1 C), a coastal station in the western English Channel off Plymouth (L4; Fig. 1 B), three stations on a transect across the shelf in the Cantabrian Sea (E1, E2 and E3; Fig. 1 A) and a station at the entrance of the Bay of Villefranche in the Ligurian Sea (Point B; Fig. 1 D). Although there is good overlap in the periods covered at each location, the sampling schedule varied between the different sites both in temporal coverage and frequency of sampling (Table 1).

The sampling protocols also varied between sites, partly due to the need to maintain consistency with the long term plankton monitoring programmes at each location and also because the specific requirements of the physio-ecological studies conducted at each station. Appendicularian abundance and species composition were estimated from vertical hauls using a WP-2 net fitted with a 200- μ m mesh in the Cantabrian, Ligurian and English Channel stations and with a 90 μ m mesh pore size at the Norwegian fjords (Table 1). At Point B in the Ligurian Sea and the Norwegian fjords a modified large-volume non-filtering collector was used instead of the standard WP-2 codend. The vertical extent of the water column sampled at each station was also different (Table 1). At Korsfjorden and Sognefjorden samples were depth-specific consisting of a surface tow covering the upper surface layer above the pycno or thermocline and then tows at depth intervals of 200 and 400 meters respectively down to the fjord basin. Samples were preserved in buffered glutaraldehyde in the Norwegian fjords and Ligurian Sea and flash frozen into liquid nitrogen at the Cantabrian and English Channel stations. Samples were counted and species identified by different scientists at each location. Although this procedure is likely to introduce some inconsistencies in the data due to differing expertise, all the analysts were trained by Dr. Robert Fenaux during the First Workshop on the taxonomy of Appendicularia held in Villefranche during 1999.

In addition, at stations L4, E1, E2, E3, Sognefjorden and Korsfjorden, the trunk lengths (from mouth to upper gonad end) of up to 30 individuals of each species collected on a given date at a given site were measured to determine the species-specific size structure of the appendicularian population.

Water collected with 5-L Niskin bottles was filtered onto 25 mm GF/F filters, extracted in acetone and used for standard fluorometric determination of chlorophyll

concentration (U.S. Environmental Protection Agency Method 445.0). The depths sampled were: 0, 10, 20 and 30 m at the Norwegian fjords, 10 m at station L4, and 0, 10, 20, 30, 40, 50, 75 and 100 m (or down to the bottom at the shallower stations) in the Cantabrian and Ligurian seas. Vertical profiles of temperature and salinity were obtained using a Sea Bird CTD in the Cantabrian and Ligurian Sea, a CTD probe developed for the Undulating Oceanographic Recorder (Aiken and Bellan 1990) in the English Channel and a Neil-Brown miniCTD-204 in the Norwegian fjords.

Data analysis

Differences in the depths of the water column sampled and in the mesh size of the nets restrict descriptions about the relative abundance of appendicularians at each location. To mitigate these differences and because almost all species were mainly present in the surface layer (Båmstedt et al. ; López-Urrutia et al. 2003b) appendicularian counts were integrated for the water column sampled and transformed to number of individuals per square meter. Plots of seasonal changes in the abundance of the two major families of appendicularians (Oikopleuriidae and Fritillariidae) and the seasonal changes and vertical structure of temperature, salinity and chlorophyll concentration were prepared in order to depict general patterns at each location. In addition, the effect of mesh size on the estimates of appendicularian abundance was examined by comparing the size frequency distributions of the species collected.

Appendicularian species associations

A species association is a recurrent group of co-occurring species (Legendre and Legendre 1978) and represents a group of species that have similar reactions to the properties of the environment (Fager and McGowan 1963). In order to determine whether groups of appendicularians show similar distribution patterns in space and time

we used Fager 1957 non-hierarchical linkage clustering. This methodology was selected instead of the probabilistic clustering of Clifford and Goodall 1967 (see Legendre 1973 for an example) because it is based on presence and absence data, which was found more appropriate to take into account the differences in sampling protocols between sites. Krylov 1968 χ^2 probabilistic similarity index was used to calculate the level of affinity between each pair of species. The similarity between the species within a group had to be highly significant in order to accept formation of an association (χ^2 probability > 0.999). To evaluate the relationship between different environmental variables (temperature, salinity and chlorophyll concentration) and the similarities in the presence-absence patterns of the different species, Mantel statistics (as described above) were calculated between the species χ^2 similarity matrix and similarity matrices based on the temperature, salinity and chlorophyll concentrations where each species was present.

Seasonal succession of species

A chronological clustering Legendre et al. 1985 was used to describe the seasonal succession of appendicularian species at each location. Analyses were performed using the software CHRONO in the freely distributed R package (Legendre and Vaudor 1991). The output of the chronological clustering is a non-hierarchical partition of the seasonal series into non-overlapping, homogeneous groups, which can be interpreted from an ecological perspective as stages of a succession. The values for the connectedness and α -probability level used by the chronological clustering algorithm determine the number of groups of samples obtained. The connectedness is the proportion of the total number of similarities between an object or cluster and a neighbouring cluster required to evaluate whether both groups should be fused into a single cluster. The α -probability level is the probability to reject the null hypothesis that

two groups should be fused together. Legendre et al. 1985 recommend that the chronological clustering is repeated with several sets of connectedness and probability levels so the most important breakpoints in the seasonal series become obvious. For each location, nine analyses were performed at combinations of connectedness of 25, 50 and 75 % and probability levels of 0.01, 0.1 and 0.2. The major steps were depicted as those that occur at most levels of resolution.

The choice of an index of similarity is a critical step in any multivariate analysis. For the chronological clustering we used the Bray-Curtis similarity coefficient (also known as Odum's or Steinhaus' coefficient, Legendre and Legendre 1998). With this index, the same difference for either abundant or rare species contributes equally to the similarity between dates. To homogenise the variances of the different species abundances, data was squared root transformed so the less numerically dominant species still play some role in determining the similarity. The square root transformation results in a downweight of the effect of the most common species similar to, although less severe than, a log+1 transformation Clarke and Warwick 1994. After the chronological clustering was performed and the major successional steps determined, the species that characterize each stage were identified as those which together contribute more than 90% to the average similarity within each succession stage. The contribution of each species to the similarity within each succession stage was calculated using the similarity breakdown implemented in the PRIMER (Plymouth Routines In Multivariate Ecological Research) program SIMPER (similarity percentages, Clarke and Warwick 1994).

Geographical influences on appendicularian species composition.

The average number of individuals of each species was calculated for each site, square root transformed and standardized to zero mean and unit variance in order to

remove the differences between locations in the total number of individuals collected. The similarity in the species composition between each pair of locations was calculated using the Bray-Curtis similarity index. This similarity matrix was then analysed in two ways. First, a Non-Metric Multidimensional Scaling (MDS) was used to represent in a two dimensional space the distances between locations based on their respective species composition. Secondly, Mantel statistics were calculated to evaluate whether the distances between locations as determined by their species composition could be better explained by their geographic distances, by the similarity in their average temperature, salinity or chlorophyll concentration or by any combination of them. The Mantel statistic is basically a correlation coefficient between two similarity (or distance) matrices. In a similar way as a standard correlation coefficient and r^2 value give an estimate of the degree of relationship and proportion of explained variance between a dependent and independent variable, the Mantel statistics provide a measure of correlation but instead of between variables between similarity matrices. Accordingly, it is possible to select the descriptor or combination of descriptors that explain greater proportions of variance in the species composition similarity between sites.

Appendicularian species niche.

Considering a one-dimensional niche with temperature as the environmental gradient, there is usually a unimodal numerical response in the abundance of each appendicularian species (see Results and Essenberg 1922; Acuña and Anadón 1992). Therefore we plotted the average abundance of a species for different temperature intervals. The bar chart that results from this procedure resembles a probabilistic frequency distribution. This similarity between the one-dimensional niche and a probabilistic function was first used by May and MacArthur 1972, who assumed in their theoretical analysis that resource utilization is normally distributed along the resource

axis. However, a Gaussian response is not always the best approximation to the niche shape and it is often found that the response is skewed or even bimodal. Therefore, we have used Johnson 1949 systems of frequency curves to fit a curve to the abundance versus temperature “histogram” of each species. Johnson’s method is based on the fact that most probability frequency distributions can be transformed into a Gaussian distribution using the first four moments in the observed data (i.e. the mean, variance, skewness and kurtosis, for a detailed description of the method, see Johnson 1949; Elderton and Johnson 1969). Therefore the temperature niche of each species was parameterised using the mean (the optimal temperature), the variance or standard deviate (a measure of the niche breadth or eurythermality of that species) and the skewness and kurtosis (the asymmetry and peakedness of the niche shape around the optimum). The effect of other environmental variables in shaping the niche of each species was determined using this technique both on the raw data and on the residuals of the fitted temperature frequency distribution. The interaction between environmental variables in the determination of the niche was examined by two dimensional contour density plots of the appendicularian abundance as a function of combinations of environmental variables. The contour plots were calculated using a squared inverse distance interpolation.

RESULTS

Description of the environments studied.

The stations studied covered a wide range of environmental conditions from the nutrient poor and relatively warm station in the Ligurian Sea with chlorophyll concentrations lower than 1 mg m^{-3} throughout the year to the colder water stations in the Norwegian fjords where the spring phytoplankton bloom can reach chlorophyll concentrations of up to 15 mg m^{-3} .

The fjords are characterized by a strong halocline during most of the year in the upper water column related to river discharge (Fig. 2 E). In addition, a thermocline develops during spring and summer (approximately from May to November, Fig. 2 D). Sognefjorden ca. 80 km north of Bergen, is the deepest fjord in Norway, with the depth of the main basin reaching 1200-1300 m. Korsfjorden, just south of Bergen, consists of two arms, the outer one running WSW to the open sea, the inner one running SSE and connects to the outer open part of Hardangerfjorden by a sill at 450 m. The main basin is ca. 680 m deep and the sill towards the open coast in the west is 180 m. Herdlefjorden, just north of Bergen, has the shallowest sill of the fjords studied with a depth of approximately 275 m and, compared to the other two fjords, is a more enclosed system.

In contrast, tidal mixing prevents any strong stratification during most of the year at station L4 (Fig. 2 D and E), which is located in coastal waters off Plymouth in the English Channel with a bottom depth of ca. 50m. There is a strong phytoplankton bloom in April-May dominated by diatoms and flagellates with chlorophyll concentrations up to 10 mg m^{-3} (Fig. 2 C) and also a summer dinoflagellate bloom dominated by *Gyrodinium aureolum*.

Stations E1, E2 and E3 in the Cantabrian Sea are located along a coast-ocean transect across the continental shelf (Fig. 1 B). Temperatures at these stations are higher than in the fjords and station L4 while chlorophyll concentrations are lower with maximum values close to 3 mg m^{-3} (Fig. 3 D and C). Thermal stratification starts in May and is less pronounced at the more coastal station E1.

Point B in the Ligurian Sea (NW Mediterranean, Fig. 1 D) is the most oligotrophic of the stations studied with chlorophyll concentrations remaining low throughout the year with reduced seasonal variation. Temperature reaches 26°C at the surface during

the period of stratification, which lasts from March until November (Fig. 3 D), and salinities are higher than at the other stations studied (Fig. 3 E).

Total appendicularian abundance.

The number of appendicularians collected was generally higher in the Norwegian fjords and Cantabrian sea, total appendicularian abundance at station L4 and Point B was relatively low (Fig. 2 A and 3 A, note different scale axis for each station). There was no correlation between total abundance and environmental temperature (Fig. 4 A) but there was a positive relationship between the abundance of appendicularians and chlorophyll concentration ($p < 0.001$, $r^2 = 0.2$; Fig. 4 B). These statistics should be viewed with caution since they have not been corrected for the presence of autocorrelation in each individual seasonal series. The temporal autocorrelation (i.e. the correlation of a time series with itself) implies that each new value in the time series doesn't represent a full degree of freedom since samples are not statistically independent (i.e. from the previous values in the time series we already have some knowledge of what the next value is likely to be). This autocorrelation results in underestimation of the p-value and overestimation of the r^2 . Nevertheless, most of the relationship between the total abundance of appendicularians and chlorophyll concentration is due to the differences between locations (Fig 2 and 3 A and C) and therefore unaffected by autocorrelation within each time series but subject to the lack of methodological standardization in the sampling protocols.

Mesh size influence on appendicularian species size frequency distribution.

Each type of mesh was used at a different location and therefore this analysis can not provide a direct comparison on the relative collection efficiency of each net type. Nevertheless, it should provide an indication of the consequences that the lack of standardization in the sampling design could have in the comparison of abundance

estimates between locations. Both mesh sizes used resulted in an underestimation of the smaller size classes, what could explain the unimodal size frequency distribution of the appendicularian species collected (Fig. 5). If we accept that the mode in the histogram represents the minimum appendicularian size that is sampled efficiently, our data suggests that animals larger than 300 and 400 μm in trunk length were collected efficiently by the 90 (used in the Norwegian fjords) and 200 μm mesh (used at the rest of locations) respectively, although these values varied between species probably due to the different trunk length morphologies (Fig. 5).

Appendicularian species associations

Three different appendicularian species associations were detected by the non-hierarchical linkage clustering (Fig. 6). Most of the similarity in the presence-absence of the different species could be explained by temperature (44% of the variability as indicated by a Mantel statistic). The variance explained increased to 47% when including salinity and to 48% when including chlorophyll concentration. The relationship between the presence of each species and environmental temperature is summarized in Figure 6 B. The three main groups consisted of a warm water association (*F.formica*, *F.pellucida*, *O. cophocerca*, *O. fusiformis* and *O. longicauda*), a cold water association (*O.labradoriensis*, *O. parva*, *O. gorskii*, *O. polaris*, *A. sicula*, and *F. borealis*) and a group formed by *F. venusta* and *O.dioica*. The remaining species could be associated by single linkage to the “warm” water group, except *O. vanhoeffeni* that was associated to the “cold-water” association and *O.rufescens* which was associated to all groups. These species represent satellite or associate species reflecting the complexity of the biological community (sensu Venrick 1971). The seasonal changes and differences between locations in the proportion of each species association, after the abundance of each species was standardized to zero mean and unit variance, show that

the warm water group only appeared during the summer months at the northernmost locations, their presence becoming more apparent farther south (Fig. 7). An inverse trend was observed for the cold water association while the group formed by *O.dioica* and *F. venusta* did not show any clear pattern suggesting a more generalist characteristic (Fig. 7).

Seasonal succession of the appendicularian community.

The chronological clustering and similarity percentage analysis show that the appendicularian community seasonal succession can be summarized into two or three general stages. The first successional stage is characterized by the presence of fritillariids during the winter and spring months prior to the onset of stratification or warming of the water column at the mixed locations (Fig. 1 & 7). In this first stage *Fritillaria borealis* was common to all the different locations along with *F.venusta* in the fjords or *F.pellucida* at the warmer locations (Fig. 7). However, the degree of dominance of fritillariids during the winter months varied between stations and was stronger in the fjords (Fig. 6). A second successional stage is characterized by the dominance of oikopleuriids during the summer and autumn months. This main stage is subdivided into other two or three sub-stages depending on which species dominates (Fig. 7). The summer-autumn stage was dominated by *Oikopleura longicauda* and *O.fusififormis* at the more temperate stations (E3, E2, E1 and Point B) and by *O.dioica*, *O.parva* and *O.labradoriensis* in the fjords, with station L4 in an intermediate position where *O.dioica*, *O.longicauda* and *O.fusififormis* contributed more to the similarity within the autumn and summer succesional stages.

Geographical influences on appendicularian species composition.

Table 2 shows the average percentage contribution of each species to the total number of appendicularians collected at each site. The difference in species identified at

each location is reflected by the Non-metric Multidimensional Scaling (nMDS) analysis performed on the species similarity between locations that resembles the geographical position of the stations (Fig. 8). The stress value (a measure of the goodness-of-fit) was smaller than 0.01 indicating an almost perfect representation since the numerical iteration procedure used terminates when stress is reduced below this value (MDS routine in PRIMER). Since there could be some methodological error in the pattern observed reflecting the differing degree of taxonomic expertise of the analysts who counted the samples at each location, we repeated the nMDS analysis using only those species that made up more than 90 % of the total appendicularian numbers and therefore less subject to misidentification (i.e. the eight first species in table 2). The resulting nMDS representation was very similar (not shown) indicating that the pattern detected is unlikely to be a spurious result due to lack of standardization.

According to the Mantel statistics the geographical distances explained 37.6% of the similarity in the species composition between sites while temperature explained 34.5% and neither chlorophyll or salinity nor any combination of the descriptor similarity matrices improved the variance explained. A partial Mantel test (similar to partial correlation coefficients) showed a strong intercorrelation between the geographical distances and temperature indicating that the geographical component is due mainly to temperature differences between stations.

Appendicularian species niches.

Most species showed a unimodal response to temperature, with an optimum temperature (Fig. 9) reflecting the species-specific patterns detected in the previous sections. The Johnson 1949 frequency curves fitted summarize the shape of the response to temperature both in reflecting the niche optimum (mode, Fig. 9) and the eurythermality or niche breath (standard deviation, Fig. 9). However the fact that in

some cases the temperature ranges at the stations sampled were not wide enough to cover the broad niches of some species (e.g. *F. formica* as a warm water and *F. borealis* as a cold water species) forced us to assume that the distribution was symmetrical for curve fitting purposes. Despite the relationship between total appendicularian abundance and chlorophyll we could not find any correlation between chlorophyll concentration and the abundance of each individual species. However, the interpolated contour plots of appendicularian species abundance vs. salinity and temperature (Fig. 10) and histogram plots similar to those shown for temperature (using both the raw data and the residuals of the fitted temperature distributions, not shown) suggest that salinity should be possibly considered as the second most relevant variable in shaping the niche of each species.

DISCUSSION.

Since the early studies by Essenberg (Essenberg 1922; Essenberg 1926) the correlation between the number of appendicularians and habitat temperature has been described in a number of different environments (e.g. Fenaux 1963; Shiga 1985; Acuña and Anadón 1992; Acuña et al. 1995). The wide range of environments covered in our study has allowed us to provide a first description of the geographical differences and environmental effects on appendicularian species composition. At the species level appendicularian populations are mainly under thermal control, both the temperature optimum and the eurythermality are species specific. We have applied niche theory in an attempt to parameterise the patterns observed (Fig. 9). As shown by the Mantel tests and the contour plots of appendicularian species abundance vs. salinity and temperature (Fig. 10), the differences in the habitat described by temperature and to some degree salinity explained to a considerable extent the seasonal and geographical distribution patterns detected. Shiga 1985 arrived to a similar conclusion in a study of the vertical

and seasonal distribution of appendicularians in Volcano Bay in Japan. Acuña 1994 showed that the vertical distribution of the different species was also in close relationship to temperature. This thermal dependence could be a general characteristic of pelagic tunicates since it has also been reported for doliolids (Berner and Reid 1961) and to some degree in salps (Menard et al. 1994).

The realized qualitative niche of each individual species as described above represents an ecological concept that is closely linked to the successional stages within a community and the existence of recurrent groups of species (Figs 6 & 7). The latter two methods begin with the assumption that communities consist of relatively discrete entities (i.e. they are classification methods) while the theory of the realized niche is more closely related to the existence of ecological continua and gradients (ordination method, Austin 1985). Each methodological approach (i.e. successional steps, species associations and ecological niche) represents an attempt to simplify the ecological complexity of the community. They should not be considered as opposed approaches but complement one another in helping to understand the underlying structure by tackling the same question from different angles. The species that characterize each stage in the ecological succession (Fig. 7) generally co-occur and are therefore detected as associates or a recurrent group (Fig. 6). When the environmental conditions in a successional stage lie within the niche of a species it will be present and those species whose niches overlap sufficiently will form a recurrent group or species association.

Fenaux 1961 has shown that there is an ordered sequence in the numerical dominance of different species through the seasonal cycle. Acuña and Anadón 1992 suggested as a working hypothesis that the overall abundance of appendicularians is dependent on primary production, while the relative abundance of the different species depends on the temperature. This hypothesis is supported by the correlation between the

total number of appendicularians and chlorophyll concentration and the strong temperature dependent response in the abundance of each species. However the dominance, as a percentage of total individuals, is difficult to interpret when the species considered are dissimilar in biomass, activity, etc (Fager 1957). Therefore, the term “relative” should be considered with caution both in describing the seasonal sequence (e.g. Fenaux 1963) and in comparing the contribution of each species to the total number of appendicularians (e.g. our Table 2). For example, the fact that *O.labradoriensis* is generally less numerous than *O.dioica* (Table 2) does not say anything about their relative ecological relevance since their body size and therefore biomass are different (Fig. 5). Similarly the change in dominance of a particular species can be due to an increase in its abundance or a decrease in the abundance of some other species and we still know very little (if anything) about interspecific competitive exclusion processes to give dominance, even in biomass units, an ecologically sound meaning. Nevertheless, our results come to confirm the seasonal series in appendicularian species succession detected in previous studies (Fenaux 1963; Shiga 1985; Acuña and Anadón 1992; Acuña et al. 1995) and its strong relationship with temperature.

The number of species identified in samples from station L4 and the Cantabrian Sea is much lower (i.e. higher number of species with zero percentage) than in the fjords and Villefranche where the taxonomic expertise was higher (Table 2). However it should also be noted that the species not recorded at stations L4, E3, E2 and E1 are generally present in either the fjords or Villefranche but usually not at both places, and that although some species could be misidentified other are morphologically distinctive enough to at least have been recognized as different species than the ones usually present. The differences between locations in the species which characterize each

successional stage, already point to a geographical effect on the species composition. This is reflected on the non-metric Multidimensional Scaling. In the plot result of this analysis (Fig. 8), locations that appear close together have similar appendicularian species composition, while the further away two locations the appendicularian species recorded are more different. This distance between the locations according to their species composition is closely related to the geographical distances between the stations. The results of the Mantel test suggest that temperature is the main factor responsible for this geographical distinction between stations.

The development of quantitative descriptions of the species-specific response to environmental changes should not be considered only as a tool in autoecology research. Beaugrand et al. 2002 have used data collected by the Continuous Plankton Recorder (CPR) survey to show how global climate changes affect the organization of marine copepod communities in the North Atlantic. Unfortunately, appendicularians are not identified to species level by the CPR and therefore integrative studies like the EURAPP project are crucial in the quantification of appendicularian species-environment relationships. The close relationship between appendicularian species composition and physical conditions suggests that they could be greatly affected by changes in the environment and be used as indicator species of climate changes or characteristic water masses (Fenaux et al. 1998).

Fenaux et al. 1998 pointed out the difficulties in appendicularian sampling both in the use of mesh sizes and the problems concerning distribution patchiness. The observed differences in the size frequency distribution of appendicularians collected with the 200 and 90 μm mesh plankton nets are similar to those observed by Fenaux and Palazzoli 1979 (between 200 and 53 μm meshes) and Capitanio et al. 1996 (between 200 and 90 μm meshes) with the larger mesh resulting in an underestimation of the

smaller size classes. In our study, only sufficient numbers of *Oikopleura dioica* and *Fritillaria borealis* were measured with both types of meshes. At the Norwegian fjords, where temperatures are lower and therefore appendicularians larger, the 90µm mesh collected a larger proportion of smaller appendicularians although still underestimating the population. Therefore there is a general bias towards an underestimation of the total number of appendicularians, this bias being slightly less pronounced at the Norwegian fjords stations. Even with the smaller mesh there is still an underestimation suggestive of the need for new sampling approaches to improve our understanding of appendicularian population dynamics. Despite this underestimation the high appendicularian densities encountered during our study, particularly in the Norwegian fjords and Cantabrian Sea are indicative of their potential ecological relevance.

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Table 1. Summary of the sampling protocols followed

Station	Position	Bottom Depth (m)	Sampling Depth (m)	Mesh pore (μm)	Sampling frequency	Sampling period
Norwegian fjords						
Sognefjorden	61° 3' N 5° 22' E	1250	1250	90	Monthly	03/1999 – 02/2000
Korsfjorden	60° 11' N 5° 14' E	680	680	90	Biweekly	03/1999 – 02/2000
Herdlefjorden	60° 32' 49.8'' N 5° 1' 49.8'' E	275	275	90	Weekly	03/1999 – 02/2000
English Channel						
L4	50° 15' N 4° 15' W	50	50	200	Weekly	03/1999 – 02/2000
Cantabrian Sea						
E3	43° 46' N 6° 10' W	1000	100	200	Monthly	03/1999 – 05/2000
E2	43° 42' N 6° 9' W	120	100	200	Monthly	03/1999 – 05/2000
E1	43° 36' N 6° 8' W	50	50	200	Monthly	03/1999 – 05/2000
Ligurian Sea						
Point B	43° 41' 9.96'' N 7° 19' E	80	30	200	Weekly	01/1999 – 12/1999

Table 2. Percentage contribution of each species to the total number of appendicularians collected at the different locations.

	Herdle- fjorden	Sogne- fjorden	Kors- fjorden	L4	E3	E2	E1	Point B	All locations
<i>Oikopleura longicauda</i>	0	0	0	14.52	54.45	48.80	54.26	43.65	26.96
<i>Oikopleura dioica</i>	36.44	25.05	27.19	61.29	0.13	0.64	12.24	0.05	20.38
<i>Oikopleura fusiformis</i>	0	0	0	13.60	34.77	45.30	28.27	19.28	17.65
<i>Fritillaria borealis</i>	16.75	35.55	23.38	10.59	10.58	5.14	3.20	4.28	13.68
<i>Oikopleura parva</i>	9.94	3.57	19.60	0	0	0	0	0	4.14
<i>Oikopleura labradoriensis</i>	0.22	15.28	7.84	0	0	0	0	0	2.92
<i>Fritillaria venusta</i>	11.21	5.95	5.11	0	0	0	0	0	2.78
<i>Fritillaria pellucida</i>	0	0.30	0.02	0	0.07	0.11	2.04	18.02	2.57
<i>Fritillaria polaris</i>	5.23	6.02	1.62	0	0	0	0	0	1.61
<i>Oikopleura gorskyi</i>	9.31	1.15	1.84	0	0	0	0	0	1.54
<i>Fritillaria formica</i>	0	0	0	0	0	0	0	8.67	1.08
<i>Appendicularia sicula</i>	3.28	1.66	2.44	0	0	0	0	0.19	0.95
<i>Oikopleura cophocerca</i>	0	0	0	0	0	0	0	4.22	0.53
<i>Kowalevskia oceanica</i>	0	0.01	0	0	0	0	0	0.77	0.10
<i>Oikopleura albicans</i>	0	0	0	0	0	0	0	0.43	0.05
<i>Fritillaria haplostoma</i>	0	0	0	0	0	0	0	0.19	0.02
<i>Oikopleura vanhoeffeni</i>	0	0.08	0.00	0	0	0	0	0	0.01
<i>Fritillaria gracilis</i>	0	0	0	0	0	0	0	0.07	0.01
<i>Fritillaria tenella</i>	0	0	0	0	0	0	0	0.07	0.01
<i>Oikopleura rufescens</i>	0	0.01	0	0	0	0	0	0.06	0.01
<i>Fritillaria aequatorialis</i>	0	0	0	0	0	0	0	0.04	0.01

FIGURE LEGENDS.

Figure 1. Map showing the study sites. **(A)** Station L4 in coastal waters of the English Channel, approximately 55 meters water depth. **(B)** Stations E1, E2, E3 in the Cantabrian Sea (NW Spain) 50, 120 and 1000 meters deep respectively. **(C)** Stations located in the Norwegian fjords: Sognefjorden (Sf), Herdlefjorden (Hf), and Korsfjorden (Kf) with main basin depths of 1250, 680 and 275 meters. **(D)** Point B in coastal waters of the Ligurian Sea at the entrance of the Bay of Villefranche with a water column depth of approximately 80 meters.

Figure 2. Seasonal variation at the stations in the Norwegian fjords and English Channel of **(A)** appendicularian abundance, **(B)** temperature vertical contour plots, **(C)** average and maximum chlorophyll concentration over the upper 50 meters of the water column and **(D)** salinity vertical contour plots. Note different x and y axis limits for each location, December 1999 is shown as a bold D.

Figure 3. Same as Figure 2 but for the stations in the Cantabrian and Ligurian Sea.

Figure 4. Relationships between total appendicularian abundance and **(A)** temperature and **(B)** chlorophyll concentration. Appendicularian abundance values represent the vertical integrated abundance over the whole water column sampled. Temperature and chlorophyll concentration represent the average and maximum over the water column sampled. Line in B shows the log-log least squares regression line ($\log_{10}(\text{Abundance})=3.03+0.913*\log_{10}(\text{Chlorophyll})$, $n=167$; $F_{165,1}=41.5$; $p<0.001$,

$r^2=0.2$, Note that the degrees of freedom could not be corrected for the autocorrelation at each station and therefore r^2 and significance value could be overestimated).

Figure 5. Size frequency distribution of the different appendicularian species. Data were separated depending on whether samples were collected using a 200 μm (grey filled histograms, stations L4, E1, E2 and E3) or a 90 μm (white filled histograms, Korsfjorden and Sognefjorden) WP-2 net. Only the histograms for those species where at least 60 individuals were measured are shown. To calculate the histogram frequencies, data were binned into 100 μm trunk length (mouth to upper gonad end) intervals starting at 50 μm .

Figure 6. Appendicularian species associations identified using non-hierarchical complete linkage clustering. **(A)** Two dimensional principal coordinate ordination of the species, Axes I (abscissa) and II (ordinate) explain respectively 40 and 10% of the variability among species. Species names in bold represent those species grouped into an association (see B for the three species groups detected). Species names not on bold represent satellite species. Species names are abbreviated using the first letters of the genus and species name (or first two letters of the species name in case of conflict). **(B)** Species are ordered following their loadings of the first principal coordinate in A. Groups of species in bold represent recurrent associations. Horizontal lines represent the range of the temperatures where each species is present, vertical lines show the average temperature and grey rectangles the first and third quartiles calculated considering only the temperature values when that species was present. Open squares show the temperature where the maximum frequency of presences was found after the data was

binned into two degree temperature intervals. Lines on top of the panel indicate the first and third quartiles of the temperatures at each of the locations studied.

Figure 7. Appendicularian species succession at each location. **Top black and white rectangles:** interval graph representing the discontinuities in the species seasonal succession (change in colour of the rectangles) as depicted by a chronological clustering on the Bray-Curtis similarity matrix calculated on the square root transformed species abundances. Letters below each rectangle indicate those species which together contribute more than 90 % to the average similarity within each succession step (Fb, *Fritillaria borealis*; Od, *Oikopleura dioica*; Fv, *Fritillaria venusta*; Ola, *Oikopleura labradoriensis*; Op, *Oikopleura parva*; Of, *Oikopleura fusiformis*; Ol, *Oikopleura longicauda*, Fp, *Fritillaria pellucida*), **lower graph:** changes in the proportion of each species association (dark grey areas: *A.sicula*, *F.polaris*, *F. borealis*, *O.labradoriensis*, *O.parva* and *O.gorskyi* group; light grey areas: *F.venusta* and *O.dioica* association and white areas: *F.formica*, *F.pellucida*, *O.cophocerca*, *O.fusiformis* and *O.longicauda* group),

Figure 8. Non-metric Multidimensional Scaling on the Bray-Curtis similarity matrix based on the root transformed average species abundance at each location.

Figure 9. Relationship between appendicularian species abundance and environmental temperature. Species are ordered from top left to bottom right panels according to the first principal coordinate axis in Figure 4 A. Bar charts represent the mean values of the $\log_{10}+1$ -transformed abundances observed within each temperature interval. Temperature intervals were arbitrary selected as 2°C bins starting at 4°C. Only

those species with more than two presence values within at least two temperature intervals are shown. Lines represent the continuous functions fitted using Johnson's systems of frequency curves (see Methods for a detailed explanation). The optimal temperature for each species is presented as the mode of the frequency distribution and the degree of eurythermality or one-dimensional temperature niche breadth is shown in brackets as the standard deviation of the fitted distribution.

Figure 10. Relationship between temperature, salinity and appendicularian abundance. Graphs show the interpolated contour plots obtained using a squared inverse distance interpolation algorithm. Colour scales show the $\log_{10} + 1$ transformed abundance corresponding to each line in the contour plot.

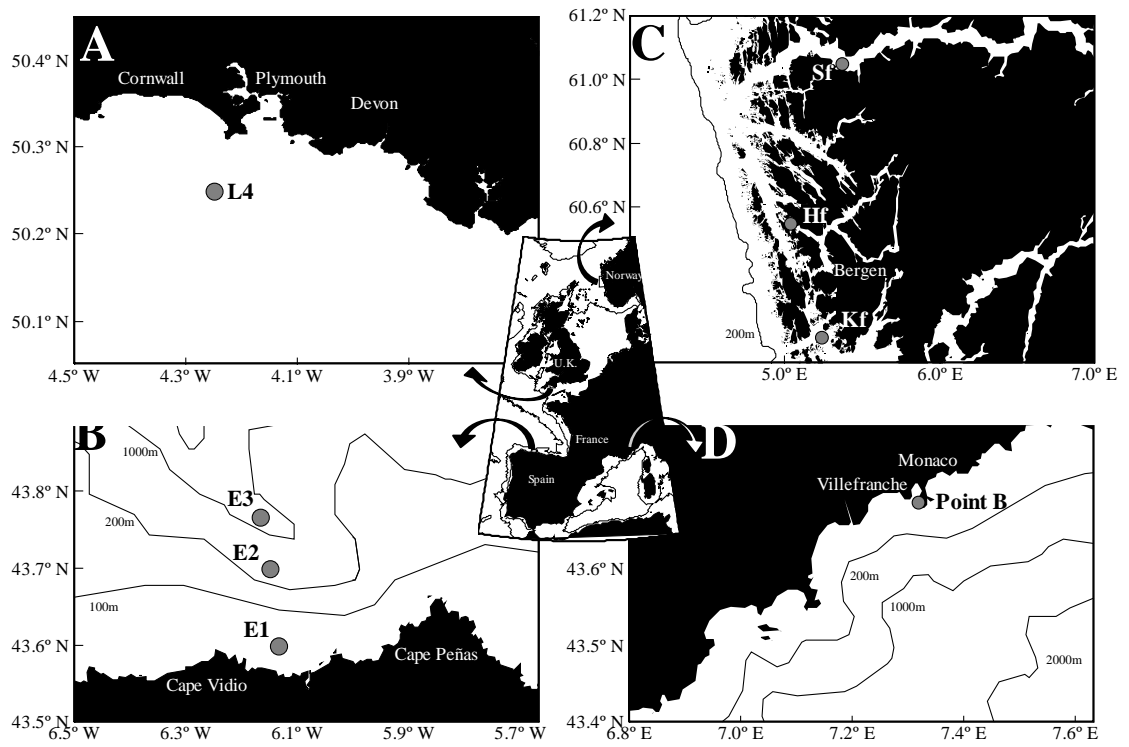


Figure 1. Lopez-Urrutia et al.

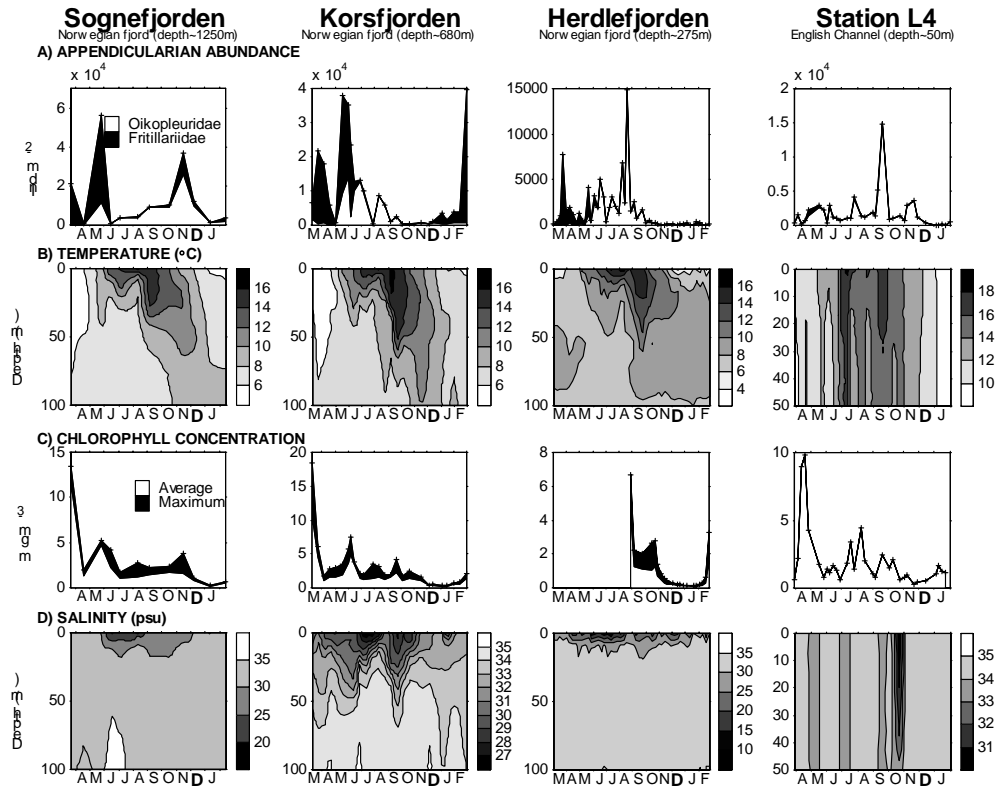


Figure 2. Lopez-Urrutia et al.

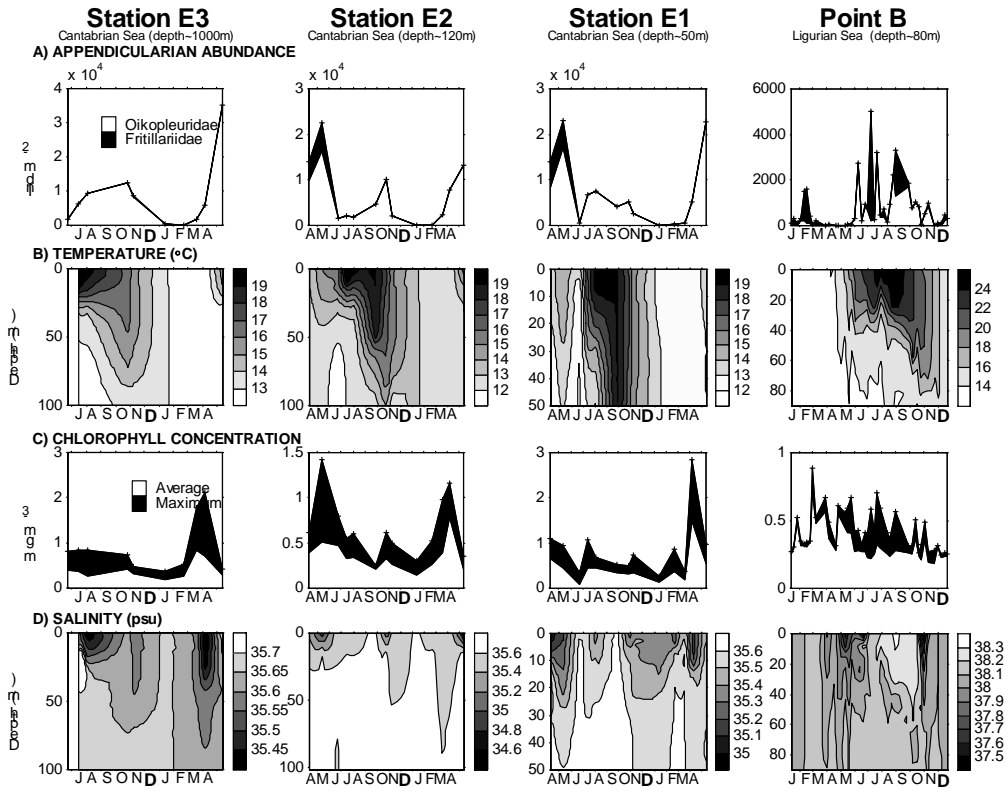


Figure 3. Lopez-Urrutia et al.

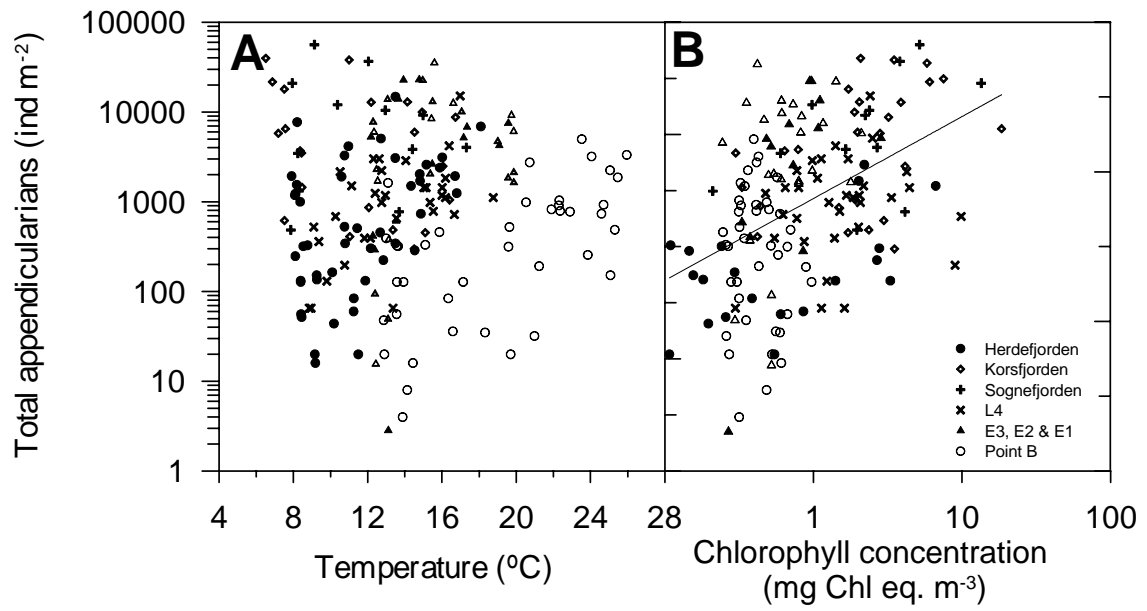


Figure 4. Lopez-Urrutia et al.

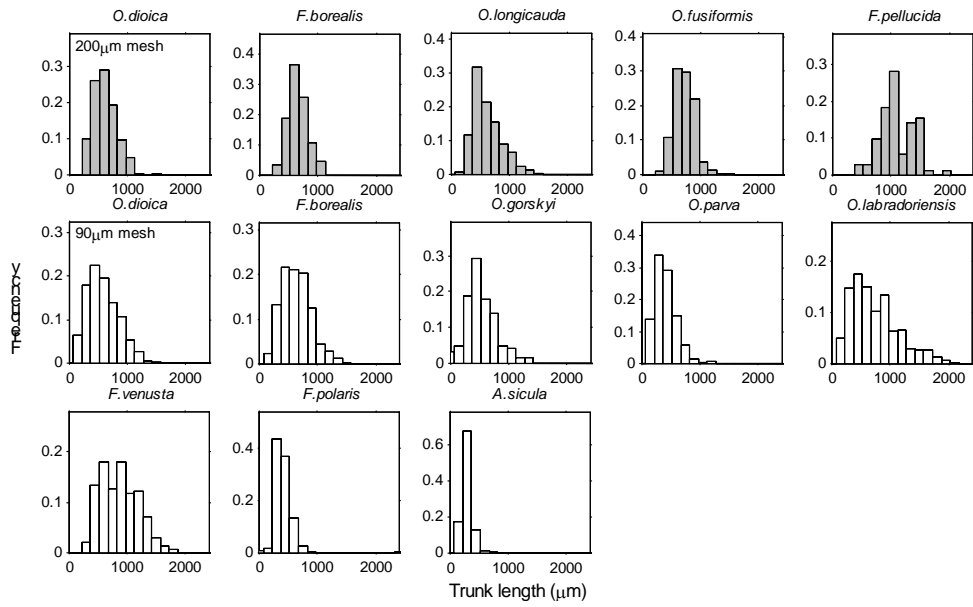


Figure 5. Lopez-Urrutia et al.

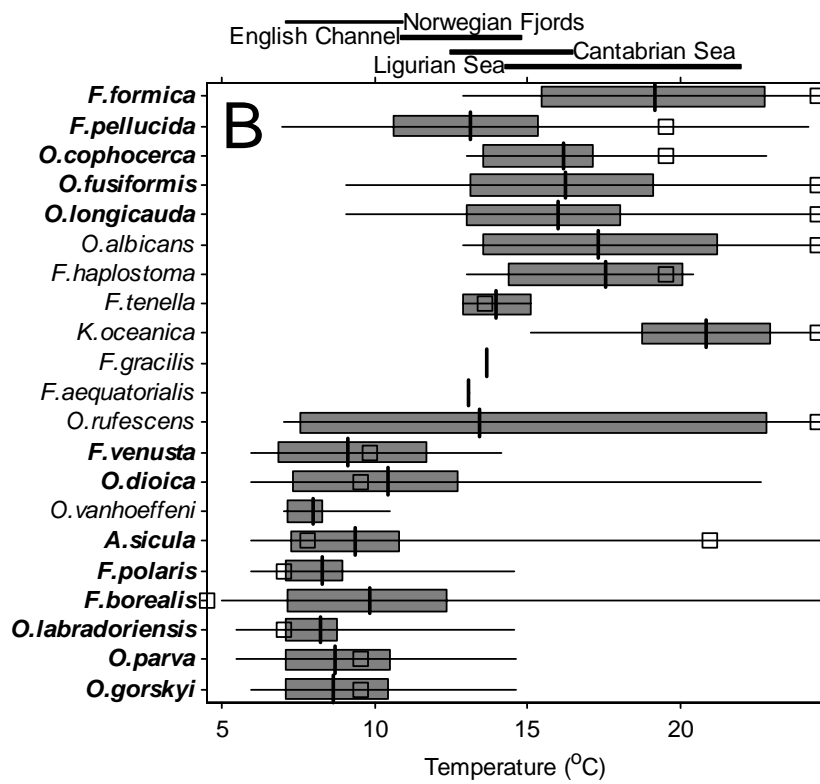
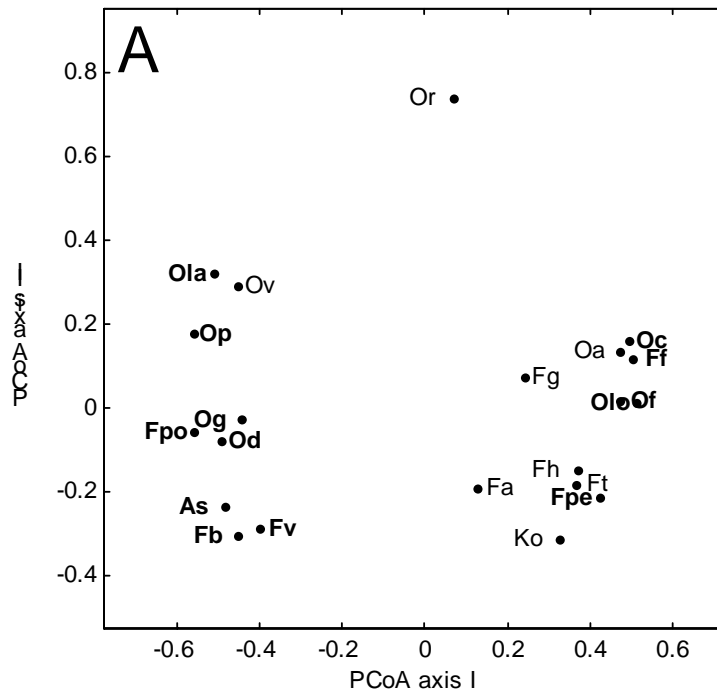


Figure 6. Lopez-Urrutia et al.

T-O-T-O-D-E-D O-T-D-U-C-T S-T-U-D-I-O-N-E-S A-R-R-I-V-E-D

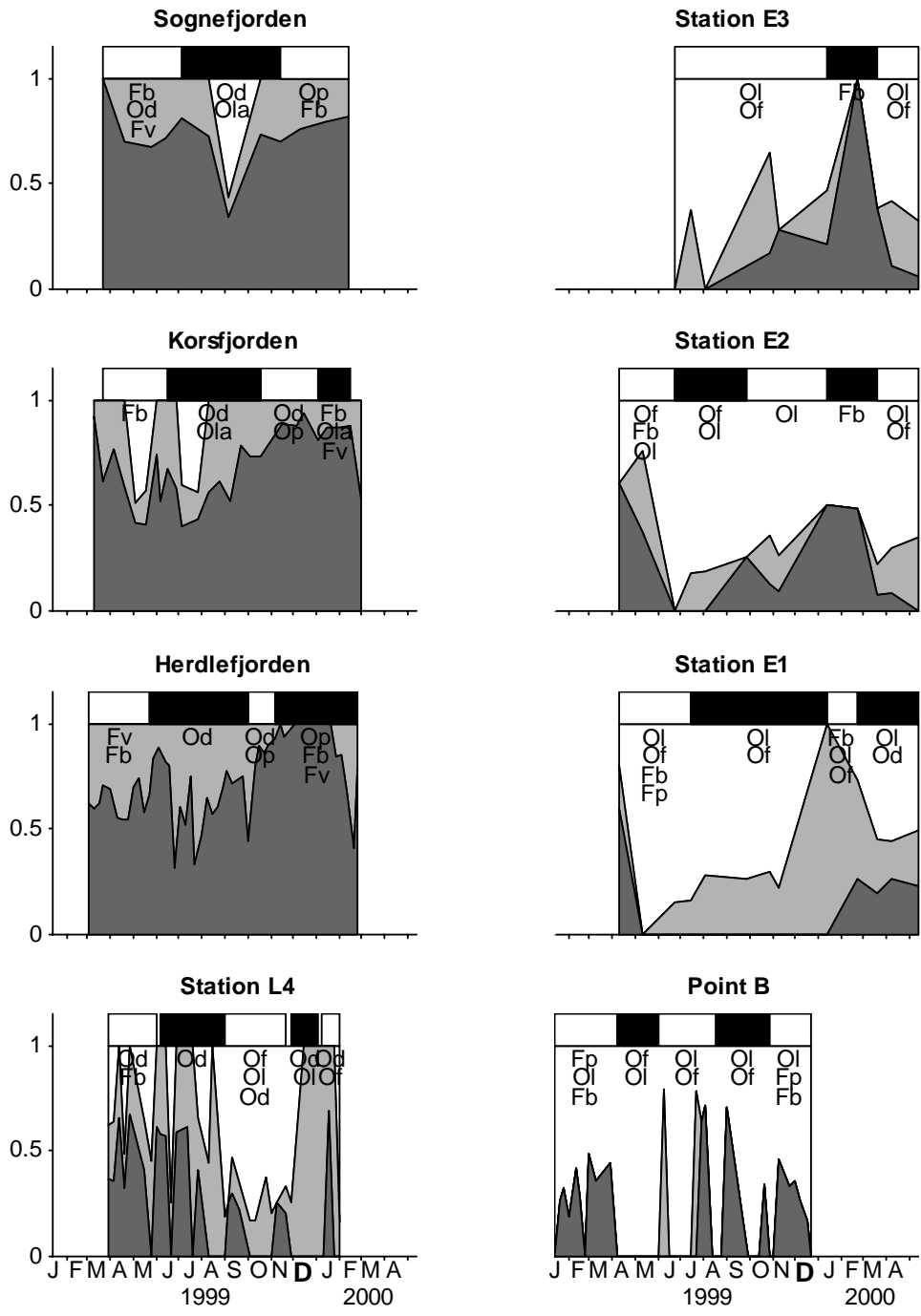


Figure 7. Lopez-Urrutia et al.

Non-metric multidimensional scaling

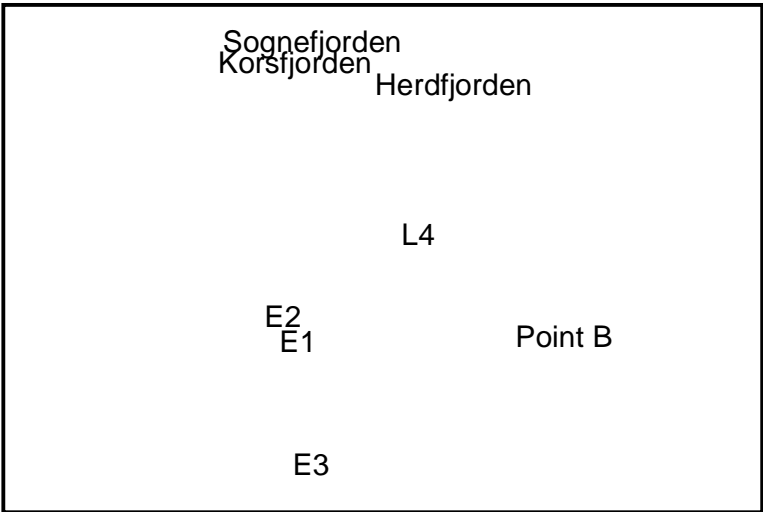


Figure 8. Lopez-Urrutia et al.

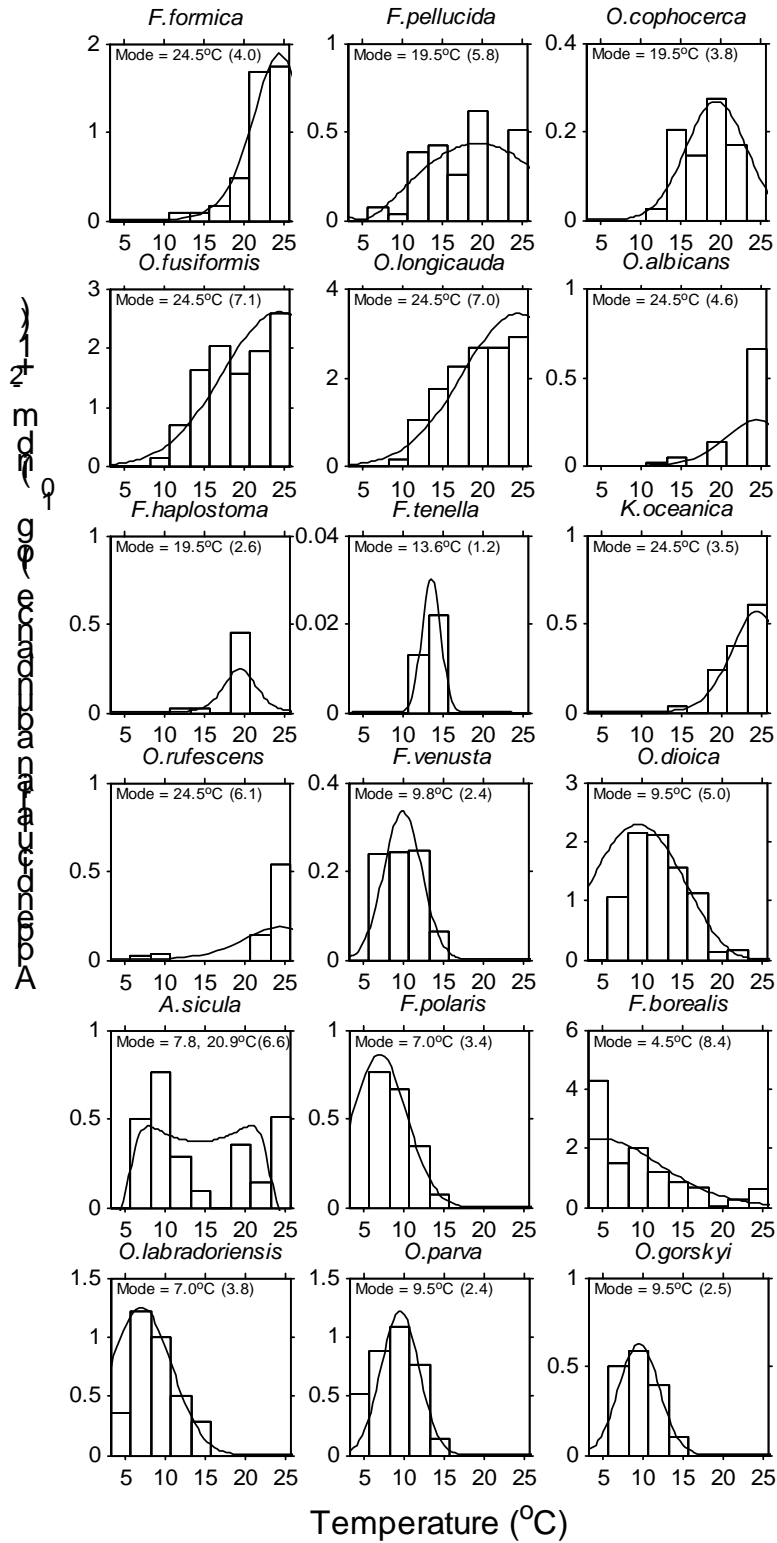


Figure 9. Lopez-Urrutia et al.

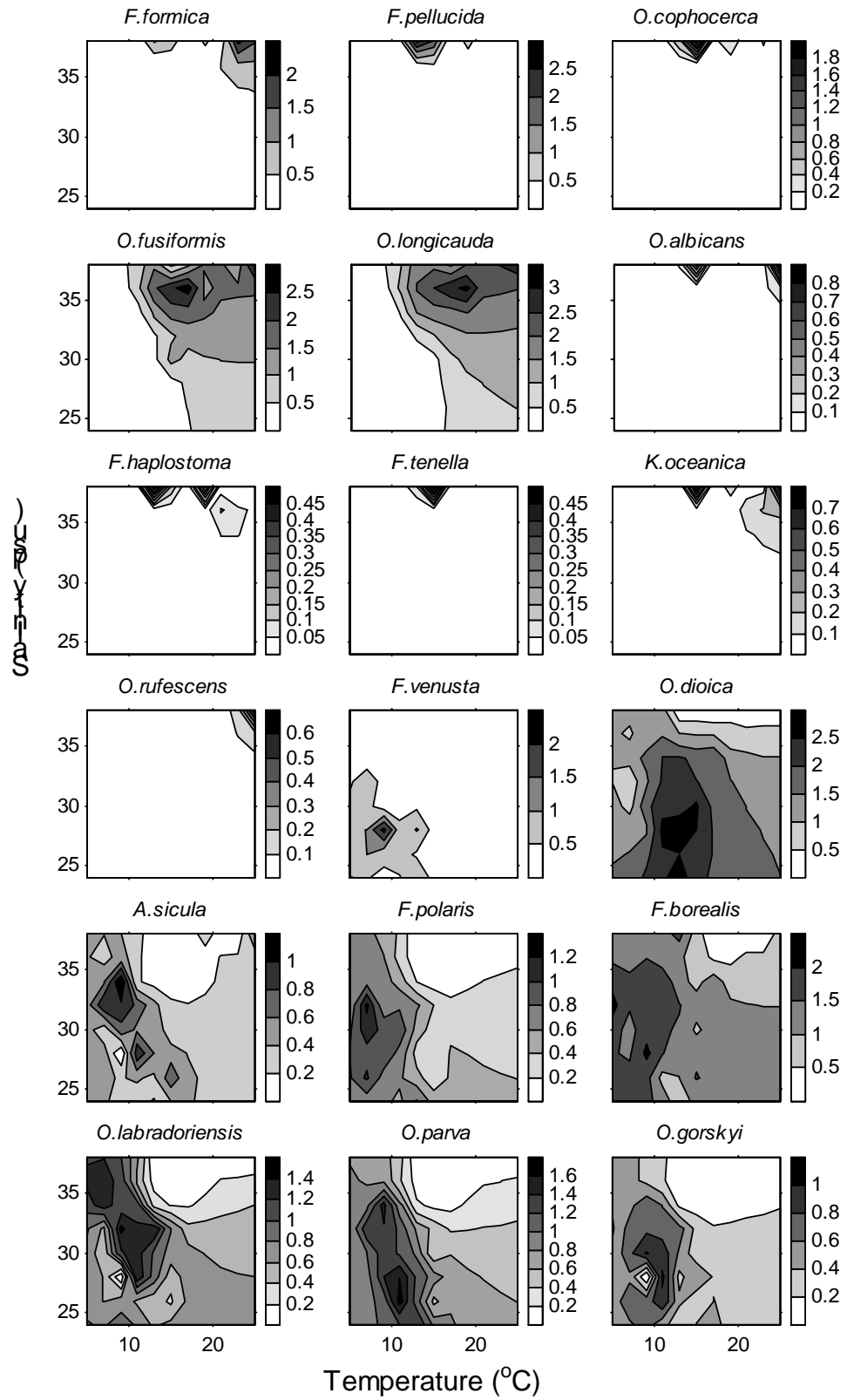


Figure 10. Lopez-Urrutia et al.