IDENTIFICATION OF SPECIES OF CALANOID COPEPODS USING A NEW INARIANT CORRELATION ALGORITHM

BY

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ABSTRACT

Digital images of Calanus pacificus, Rhincalanus nasutus, Pleuromamma gracilis, Temora discaudata, and Acartia tonsa were processed to obtain their diffraction pattern. For this analysis an algorithm using the square module of the fast Fourier transform was used. The diffraction patterns of these copepod species were correlated with phase-only filters in an invariant way to discriminate between each species and sex. Results indicate that this method is promising and that in a relatively short time an automated system for plankton identification could be developed.

RÉSUMÉ

Des images digitalisées de Calanus pacificus, Rhincalanus nasutus, Pleuromamma gracilis, Temora discaudata et Acartia tonsa ont été traitées pour obtenir leur modèle de diffraction. Pour cette analyse, un algorithme utilisant le module carré de la transformation rapide de Fourier a été utilisé. Les modèles de diffraction de ces espèces de copépodes ont été corrélés avec des filtres uniphases de façon invariante pour discriminer entre chaque espèce et sexe. Les résultats indiquent que cette méthode est prometteuse et que dans un temps relativement court, un système automatique pour l’identification du plancton pourrait être développé.

INTRODUCTION

Copepods are the dominant group in the marine zooplankton, making up at least 70% of the planktonic fauna (Raymont, 1983). This group of organisms has a great
diversity. There are about 11,500 species of copepods described (Humes, 1994) and the number is still steadily increasing with the description of new species, such as those from anchialine caves (Fosshagen & Iliffe, 1991), hydrothermal vents (Humes, 1991), and those previously reported under the name of another species (Bradford, 1976; Soh & Suh, 2000).

Copepods are of prime importance in marine ecosystems. The majority of copepods feed on phytoplankton, forming a direct link between primary production and commercially important fish, such as sardine, herring, and pilchard. Copepods are also the main food source for a great variety of invertebrates. Studies on copepod abundance and species composition are particularly relevant, because most larvae of commercial fish feed on copepods. Hence, changes in the abundance of these plankters from year to year, may determine interannual population fluctuations of the commercially exploited fish stocks in a particular region. The importance of copepods is not only embodied in the role they play in the transfer of energy from primary production to higher trophic levels (Mauchline, 1998). Also, some species may be used as water mass indicators (Longhurst, 1967; Dawson & Knatz, 1980; Cross & Small, 1967), or as bio-indicators for chemical contamination and/or eutrophication (Dawson & Knatz, 1980).

Despite the importance of studies on copepod abundance and species composition, zooplankton communities that are continuously monitored are only few. One of the reasons for the lack of continuous sampling, is the time and experience needed to analyse the samples. Therefore, it has become necessary to seek the application of new techniques for the development of an automated system for plankton identification. Zooplankton assessments have been made earlier through silhouette photography (Ortner et al., 1979) and video camera imaging (Latrous, 1984; Rolke & Lenz, 1984). However, the orientation of the organism or the resolution of the images were limiting factors for a complete taxonomic classification. Recently, the use of the diffraction pattern has been reported to be useful as a tool for taxonomic identification of plankton species. Zavala-Hamz et al. (1996) were able to discriminate between three copepod species, using digital binary contours of the organisms. Pech-Pacheco et al. (1999) worked with phytoplankton species and demonstrated that the use of diffraction patterns gave reliable results to discriminate between species. In this study, the diffraction pattern is used to discriminate between five species of copepods as well as between female and male of each species. In contrast with previous studies it is of special significance that our analysis has been carried out with non-modified digital images.

METHOD

The copepods used in this study are some of the most frequently occurring species in the California Current region (Fleminger, 1967). Adult stages of the
copepods *Calanus pacificus* Brodskii, 1948, *Rhincalanus nasutus* Giesbrecht, 1888, *Pleuromamma gracilis* Claus, 1863, *Temora discaudata* Giesbrecht, 1892, and *Acartia tonsa* Dana, 1849, were separated from several plankton samples. Thirty males and thirty females of each species were chosen for study. The specimens were observed under a light microscope and their images were digitally captured using a Charge Coupled Device camera (CCD). The image of each individual was captured in dorsal view, except in the case of *C. pacificus*, which was usually found in the plankton chamber in lateral view, whence the images of fifteen organisms were taken in left view and fifteen in right view. The images were 256 × 256 pixels in size and a total of 300 images was used for the analysis.

A numerical simulation was performed in order to correlate diffraction patterns (DP) of copepod species with phase-only filters (Horner & Gianino, 1984). All steps were developed digitally. The present approach uses both the Fourier transform and the Mellin transform. In this method the introduction of a scale-factor $\sqrt{r}$ ($r$ is the radial spatial frequency, the origin of which lies at zero frequency in the optical representation of the Fourier-spectrum; Pech-Pacheco et al., 2001) is associated with the modulus of the Fourier transforms of our candidate-images, with the purpose of obtaining the scale-transform described by Cohen (1993, 1995). The introduction of this scale-factor converts the Mellin-transform into a scale-transform. Also, the characteristic drop-off of the modulus with high frequencies is compensated applying an according mask proportional to $1/r$. This results in an enhancement of the modulus at high frequencies (high-pass filtering effect was enhanced applying a parabolic function of the modulus of the fast Fourier transform (FFT); in this way, low frequencies are attenuated and high frequencies are enhanced in proportion of $w_x^2$, $w_y^2$). Consequently, the effective window applied is $1/\sqrt{r}$, and by using this we obtained maximum reliability in the classification procedure.

In fig. 1 the method is presented graphically, using a general example, in which we have selected, as an initial image, the image of the alphabet letter “E” (fig. 1a). The first step is to obtain the square modulus of the Fourier transform of the initial image, which is shown in fig. 1b. Then, the high frequencies are enhanced using a parabolic function as the one shown in fig. 1c. Fig. 1d shows the enhanced image, in which the high frequencies are now observed in more detail. This better definition in the high frequencies will help, by consequence, to obtain a good identification of the object to be recognized. Then, the scale factor $\sqrt{r}$ (fig. 1e) is applied to the image of fig. 1d in order to obtain fig. 1f. As mentioned above, this process is what differentiates the scale transform from the Mellin-transform. After these steps, the Cartesian coordinates were mapped (fig. 1f) to polar coordinates (fig. 1g). In this step a bilinear interpolation of the first data of coordinate conversion is introduced (Pech-Pacheco et al., 2001). This is done to
Fig. 1. Images of the invariant method: a, input image; b, square modulus of the Fourier transform of the image; c, parabolic-mask; d, modulus with parabolic-effect; e, scale factor; f, image (d) with scale factor; g, polar mapping of (f); h, log-polar mapping of (g), this image is used for the calculation of the correlation invariant to translation, rotation, and scale.

minimize sampling errors, which in one way or another affect the identification of the objects. Figure 1h shows the image in polar coordinates, but with a logarithm in the part of $r$, which is going to facilitate the identification invariant to scale. The resulting image (fig. 1h) is the one that is going to be correlated with the phase-only filters (filters which have information about species to be recognized). Steps from fig. 1c to 1h are necessary to ensure that the information contained in the diffraction pattern is invariant to rotation, scale, and position, thus obtaining correlation invariant of the diffraction patterns.
To discriminate between copepod species and sex, all diffraction patterns were transformed like in fig. 1b-h, and after this process they were correlated with different filters. The filters were selected choosing one from the diffraction patterns corresponding to male and female of each species, i.e., to discriminate *C. pacificus* females from the rest of the organisms, the diffraction pattern of one female of *C. pacificus* was chosen as a filter, therefore a total of 10 filters was used.

**RESULTS AND DISCUSSION**

In fig. 2, the five species of copepods used in this study and their respective diffraction patterns are shown. The original diffraction patterns were modified only in this figure for a better visualization, they were obtained in log_{10} and the results binarized. (The mean was obtained and multiplied by a factor of 1.5. Binary values larger than 1.5 were 255 and less than 1.5 were zero.)

The correlation values obtained for each filter against all other diffraction patterns were box plotted and for each group of organisms the mean and standard error (SE) is represented (fig. 3a-j), correlation values are non-normalized. Each copepod species is represented by three letters, the first letter represents the genus (C = *Calanus*, R = *Rhincalanus*, P = *Pleuromamma*, T = *Temora*, and A = *Acartia*), the second letter represents the species (p = *pacificus*, n = *nasutus*, g = *gracilis*, d = *discaudata*, and t = *tonsa*) and the third letter represents the sex (F = female, M = male).

For *C. pacificus* females a mean value of 6.64 ± 0.04 (table I) was obtained when the filter *CpF* was correlated with all *C. pacificus* females DP. This value was separated from the correlation values obtained for males (6.18 ± 0.04) (fig. 3a). Then, it can be observed that correlation values for the other species were also separated from the group of *C. pacificus* females. In fig. 3b the correlation values obtained for *C. pacificus* males with the *CpM* filter are represented, a mean value of 6.2 ± 0.04 for males was obtained, which value is completely separated from the values for females (6.63 ± 0.04) of the same species and from all other species as well. The correlation values obtained for *R. nasutus* females with *RnF* filter gave a mean of 6.76 ± 0.1, a value that was completely separated from the mean obtained for *R. nasutus* males (6.22 ± 0.09) and from all other species (fig. 3c). The mean value for *R. nasutus* males with *RnM* filter gave a value of 6.32 ± 0.09, which was also separated from the mean value (6.82 ± 0.09) obtained for females of the same species (fig. 3d). In the case of *P. gracilis* it was also possible to discriminate between female and male, correlation values for females with *PgF* filter gave a mean of 4.89 ± 0.03, this value was separated from the correlation mean obtained for males (4.87 ± 0.03) and both values were separated from the other species
### Table I

Mean correlation values (relative units) from correlations of copepod diffraction patterns with the following filters: (CpF) *Calanus pacificus* Brodskii, female; (CpM) *Calanus pacificus*, male; (RnF) *Rhincalanus nasutus* Giesbrecht, female; (RnM) *Rhincalanus nasutus*, male; (PgF) *Pleuromamma gracilis* Claus, female; (PgM) *Pleuromamma gracilis*, male; (TdF) *Temora discoidata* Giesbrecht, female; (TdM) *Temora discoidata*, male; (AtF) *Acartia tonsa* Dana, female; (AtM) *Acartia tonsa*, male

<table>
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<tr>
<th>Diffraction patterns of:</th>
<th>Filters used (Mean ±1SE)</th>
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<tr>
<td></td>
<td>CpF</td>
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<tr>
<td>CpF</td>
<td>6.64 ± 0.04</td>
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<tr>
<td>CpM</td>
<td>6.18 ± 0.04</td>
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<tr>
<td>RnF</td>
<td>4.11 ± 0.09</td>
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<tr>
<td>RnM</td>
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<td>PgM</td>
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<tr>
<td>TdF</td>
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<tr>
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<td>AtF</td>
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<td>AtM</td>
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Fig. 3. Mean correlation values of copepod diffraction patterns using the following filters: a, Calanus pacificus Brodskii, 1948, female; b, Calanus pacificus, male; c, Rhincalanus nasutus Giesbrecht, 1888, female; d, Rhincalanus nasutus, male; e, Pleuromamma gracilis Claus, 1863, female; f, Pleuromamma gracilis, male; g, Temora discaduta Giesbrecht, 1892, female; h, Temora discaduta, male; i, Acartia tonsa, Dana, 1849, female; j, Acartia tonsa, male. Arrows indicate the filter used in each correlation set.

The correlation values for P. gracilis males with PgM filter gave a mean of 4.64 ± 0.01, which was different from the mean obtained for females (4.62 ± 0.01) (fig. 3f). The mean correlation value for females of T. discaduta with the TdF filter was 5.39 ± 0.04 and for males 5.38 ± 0.04 (fig. 3g) and for T. discaduta
Fig. 3. (Continued).

males with TdM filter gave a mean of $5.28 \pm 0.02$ for males and $5.26 \pm 0.02$ for females (fig. 3h). Although the mean correlation values for females and males of *T. discaudata* were different, the values overlapped slightly at 1.96 SE, but at 1 SE it is still possible to separate the sexes. The correlation values for *A. tonsa* females with AtF filter gave a mean of $4.35 \pm 0.01$ and $4.30 \pm 0.01$ for males. The mean correlation value for *A. tonsa* males with AtM filter was $4.24 \pm 0.01$ for males and $4.29 \pm 0.0$ for females. In both cases *A. tonsa* male and female were separated from all other species (fig. 3i-j) and despite the close mean values between females and males, discrimination was still possible.

The results obtained are very good, considering the fact that in previous studies for zooplankton identification, i.e., the silhouette photographs (Ortner et al., 1979; Jeffries et al., 1984) and the digital binary contours (Zavala-Hamz et al., 1996; Zavala-Hamz & Alvarez-Borrego, 1997) mentioned above, have been used instead of real images. Also, it is important to note that despite the similarity in body shape between the males and females of all species of copepods included in this
study, it was still possible to discriminate between sexes. In summary, an adequate method for the discrimination between species of copepods has now been developed. However, the generation of an image catalogue is necessary, which should include the digital images of all copepod species from several geographic regions in order to apply the technique and to continue with this research. This work is a basis from which new techniques can be applied for the development of an automated system for the identification of copepods.

REFERENCES


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