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Application of a digital pattern recognition system to *Dinophysis acuminata* and *D. sacculus* complexes

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Abstract

An image discrimination technique was developed to improve specific identification of some toxic *Dinophysis* cells (marine dinoflagellates involved in diarrhoeic shellfish poisoning), especially the "*acuminata*" and "*sacculus*" groups, which can be present at different ratios in natural sea-water samples collected during toxic episodes. This work was performed with image analysis software SAMBA (TITN Alcatel) using preserved cells directly observed through an inverted microscope and recorded with a video camera before further processing. All morphometric parameters used for discrimination of 7 different species or morphotypes of *Dinophysis* were tested with discriminant analysis. This study indicates that *Dinophysis* sp. and *D. pavillardi* seem well classified at the species level, whereas *D.* cf. *acuminata* and *D. sacculus* appear to be morphotypes of *D. acuminata*.

Keywords: *Dinophysis* sp., image analysis, taxonomy, phytoplankton.

Utilisation d'un système d'analyse d'image numérique pour l'étude des complexes Dinophysis acuminata *et* D. sacculus.

Résumé

Une technique d'analyse d'image a été utilisée pour améliorer l'identification spécifique de quelques *Dinophysis* toxiques (dinoflagellés marins impliqués dans les intoxications diarrhéiques par les coquillages), en particulier les groupes « *acuminata* » et « *sacculus* », qui peuvent se présenter dans des proportions variables dans les échantillons prélevés lors d'épisodes toxiques. Ce travail a été réalisé avec le logiciel d'analyse d'image SAMBA (TITN Alcatel) en utilisant des cellules fixées observées directement par microscopie inversée et enregistrées avec une caméra vidéo avant traitement. Tous les paramètres morphométriques utilisés pour différencier sept espèces ou morphotypes de *Dinophysis* ont été testés par analyse discriminante. Cette étude montre que *Dinophysis* sp. et *D. pavillardi* semblent bien classés au niveau spécifique, tandis que *D.* cf. *acuminata* et *D. sacculus* apparaissent comme des morphotypes de *D. acuminata*.

Mots-clés : *Dinophysis* sp., analyse d'image, taxonomie, phytoplancton.

INTRODUCTION

Outbreaks of food poisoning due to shellfish consumption have occurred in Europe since 1960 (Korringa and Roskam, 1961; Kat, 1984; Fraga *et al.*, 1984; Dahl and Yndestad, 1985) in conjunction with summer proliferations of the genus *Dinophysis* (Dinophyceae) in coastal plankton. To reduce consumer risk of diarrhoeic shellfish poisoning (DSP), monitoring networks have gradually been set up in several countries, including Spain, France, the Netherlands and Norway. Daily examination of water samples collected in the summer months from shellfish farming areas is the main method employed to detect toxinogenic species. As soon as a "suspect" concentration threshold is reached (200 to 1 000 cells.l^{-1} for the French network), diarrhoeic toxin detection tests are carried out on shellfish.

Despite the large number of samples inspected in coastal stations and laboratories, it is difficult to determine exactly which species is responsible in every case. All *Dinophysis* species are therefore considered potentially DSP-toxic, and this assumption is respected in consumer protection practices.

As identification at species-level requires a high degree of taxonomic expertise which cannot be used to practical advantage for efficient network operation, image analysis techniques seem to provide a promising alternative. Several attempts to date have involved a small number of phytoplankton species, generally restricted to dinoflagellates with flat cell shapes (*Prorocentrum, Dinophysis, Ceratium*) which are easier to analyze in terms of contour (Steidinger *et al.*, 1989, 1990; Ishizuka *et al.*, 1986; Simpson *et al.*, 1992, 1993). When cell shape is more rounded (*Gyrodinium*), size criteria such as area, length, width and volume seem to prevail over other parameters (Estep and Macintyre, 1989).

At least three approaches have been attempted for image analysis of the genus *Dinophysis*, which is of particular interest because of the great number of species producing diarrhoeic toxins: 1) recognition of *Dinophysis* cells among other dinoflagellates; 2) discrimination between different *Dinophysis* species or varieties, and 3) a combination of both approaches.

Crochemore (1988) compared the results of discriminant and structural analysis for automatic detection of *Dinophysis*. Structural analysis allowed discrimination of 95% of the *Dinophysis* species counted.

Culverhouse (1995) applied neural network techniques for the recognition of algae and other marine organisms. His results suggest that specimens of *D. acuta, D. acuminata* and *D. sacculus* can be categorised by a back-propagation network (BPN) system, with respectively 80, 79 and 100% successful recognition scores.

Ishizuka *et al.* (1986) described an automatic image analysis system applied to *D. fortii* and *D. acuminata*. These authors initially undertook a structural analysis on a binary image produced by thresholding and filling in of the contours and clear areas of the object in order to differentiate, *Dinophysis* from otther dinoflagellates. Subsequent analysis of the dorsal curve of the hypotheca by means of modified Fourier descriptors enabled *D. fortii* to be differentiated from *D. acuminata*.

The purpose of the present study was not to discriminate *Dinophysis* sp. from other objects but, after preliminary selection, to differentiate species belonging to this genus. Since the morphological parameters customarily used by taxonomists are inapplicable to image analysis, other types of morphological criteria were investigated, *i.e.* replacing thecal ornamentation patterns (sulcal list length, spacing between the three sulcal spines, cell-wall porulation, sulcal platelet arrangement, etc.) with more "global" parameters (convex surface, bending energy, form factor, orthogonal projections, etc.). In this first attempt, we focused on two ambiguous groups of DSP-producing species, the *D. acuminata* and *D. sacculus* groups responsible for diarrhoeic outbreaks every summer along French coasts (Lassus *et al.*, 1985; Lassus and Bardouil, 1991).

METHODS

Microscopy and the digital pattern recognition system

An Olympus IMT 2 microscope equipped with a video viewer was used. Samples were placed in standard 10 or 25 ml counting chambers. During preliminary trials, different magnifications were used, the best results being obtained with ×100. About 50 water samples from French and foreign coastal areas obtained between 1984 and 1994 were used to provide a standard material with roughly equivalent proportions of each *Dinophysis* species. This material was subsequently used for morphometric studies of 7 *Dinophysis* species and morphotypes.

The image analysis system consisted of a camera, a central processing unit, an input image monitor, an output image monitor and a printer. The central processing unit was equipped with a set of cards for image acquisition, digitizaton and processing. Samba was used as the software base (TITN-Alcatel), running in Microsoft MS-DOS Windows. The system included operational libraries organized in hierarchical menus to simplify the use of major software modules and optimize the speed of executing applications. The major modules were used to acquire and process images, (data acquisition in square pixels) extract the pertinent descriptors, exploit the results in statistical and graphic form and then create the user's own applications.

The sensor used in this study was a KY 15 model JVC Tri CCD (Charge Coupled Devices) camera used

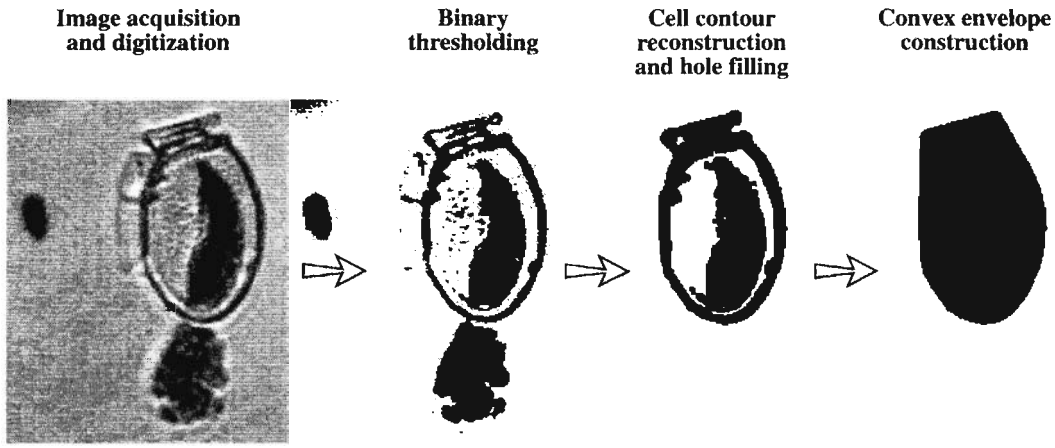

Figure 1. – Image processing: digitization, binary thresholding, cell contour reconstruction and hole filling (all parameters measured at this step, except convex area), convex envelope construction (convex area estimated during this final step).

for a previous application (Le Déan and Gauthier, 1991) and set for monochrome images.

Image recording and processing

Recordings of objects were performed directly using the microscope at magnification ×100. After settling on the counting chamber bottom, cells to be recorded were randomly selected according to optimal observation conditions, *i.e.* those selected lay flat on the bottom rather than side by side with or covered by other particles, showed no morphometric defects and had a well-contrasted cell contour.

Contrary to the ideal configuration displayed in taxonomy gide-books (fig. 2h), the sulcal list of most of the recorded cells was poorly contrasted against the tank bottom in any lighting condition used, whereas the cingular list was relatively well contrasted. To facilitate contour studies, sulcal lists were deleted systematically on the images. The binary threshold used to differentiate the cells from other objects and the bottom varied for each image, depending on lighting conditions and the quantity of objects present (other cells and debris).

The morphometric study was carried out according to the following basic output parameters: 1) length: number of pixels in the contour; 2) perimeter: length with corrections assigned to pixel values according to the type of connection (horizontally, vertically or diagonally connected pixels); 3) area within the contour and convex area; 4) form factor: $(\text{perimeter})^2/4\times\pi\times\text{area}$; 5) bending energy, or an integration of bending calculated for each pixel of the contour; 6) minimum (DMIN) and maximum (DMAX) diameters or, respectively, the width and the length of the longest rectangle circumscribed to the object. A gain in accuracy was also obtained by using indirect parameters issued from secondary calculations (e.g.: DMIN/DMAX).

The *Dinophysis* discrimination algorithm was as follows :

Image digitization in grey levels
↓
Image contrast correction
↓
Thresholding and binary imaging
↓
Image labeling
↓
Image sorting by surface test
↓
Manual correction of the contour
↓
Parameter calculation
↓
Convex area construction and calculation
↓
Data recording

All parameters were tested with discriminant analysis ("STATISTICA" software), a powerful technique for multivariate analysis (Culverhouse, 1995) used to separate the different *Dinophysis* groups, e.g. well-defined species and morphotypes.

Different *Dinophysis* cells were recorded and processed according to the Samba software procedure: 1) image digitization; 2) binary thresholding (the most contrasted black and white image was kept); 3) cell contour reconstruction and cell hole filling, and 4) filling in external concavities to obtain a convex envelope (fig. 1). The parameters listed above were measured at the third step, except the convex envelope

which was measured at the last step of processing. Between 28 and 52 cells were recorded for each species and morphotype.

For this study, two groups of toxic *Dinophysis* out of 16 different species and morphotypes were more accurately analysed owing to marked similarities between cell contours: an "*acuminata*" group (*D. acuminata* from Norway and Korea, *D.* cf. *acuminata* and *D.* cf. *norvegica* from French West Atlantic coasts) and a "*sacculus*" group (*D. pavillardi*, *D. sacculus* from French Mediterranean coasts and *Dinophysis* sp., a new, as yet undetermined, toxic species 70-75 μm long, discovered in Urbino Lagoon (Corsica) (fig. 2). The morphotypes *D.* cf. *acuminata* and *D.* cf. *norvegica*, associated with diarrhoeic outbreaks on French coasts, have been described by Lassus and Bardouil (1991).

For both groups, *D. acuminata* from Norway and Korea were used as reference materials since this species is assumed to display the typical taxonomical morphology of European and Asiatic *D. acuminata* (Balech, 1976; Solum, 1962; Abé, 1967; Fukuyo *et al.*, 1990). In addition, this species is considered to produce diarrhoeic shellfish toxins (DST), at least in Northern and Southern European waters (Kat, 1984; Dahl and Yndestad, 1985), and thus requires special attention.

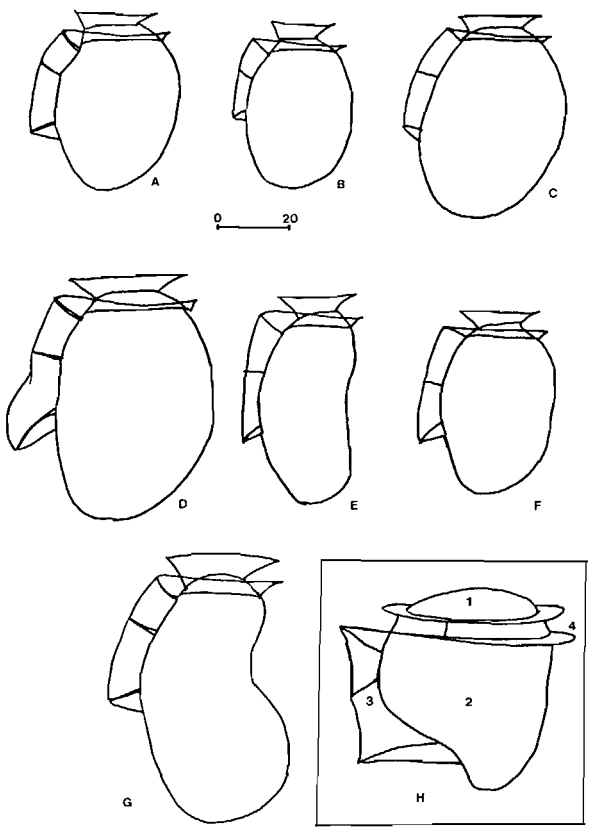

Figure 2. – Side views (left side) of different species and morphotypes of *Dinophysis* analysed with the SAMBA system. a: *D. acuminata* (Norway), b: *D. acuminata* (Korea), c: *D.* cf. *acuminata* (France), d: *D.* cf. *norvegica* (France), e: *D. pavillardi* (France), f: *D. sacculus* (France), g: *Dinophysis* sp. (Corsica), h: diagrammatic left side view of a basic *Dinophysis* cell showing epitheca (1), hypotheca (2), left sulcal list (3) and cingular lists (4) [in: Sournia, 1986]. Scale bar: 20 μm.

RESULTS

Discriminant analysis was performed for all species composing the "*acuminata*" and "*sacculus*" study groups (a total of 229 individuals), using nine explicative variables in the following order: length, perimeter, cell area, form factor, bending energy, DMIN, DMAX, convex area and diameter ratio. As a consequence, the set of variables was defined *a priori* and no step-by-step procedure was performed before analysis. Table 1 shows that most of these variables contributed to the first two canonical roots (1 and 2). In fact, the cumulative proportions of explained variance accounted for by each function already reached 82% for roots 1 and 2 (statistically significant). As a result, these canonical roots constitute the main projection plane. In particular, there was a marked influence of DMAX (var 1) on root 1 and of variables 3, 6, 8 and 9 (respectively cell area, DMIN, convex area and diameter ratio) on root 2. Moroever, perimeter, cell area, DMAX and convex area made a significant contribution to root 3. It may be considered that the most discriminant parameters were cell area and size. The morphometric criteria relating to convexity (form factor, bending energy) were only slightly implicated.

Table 1. – Factor structure matrix (1: length, 2: perimeter, 3: cell area, 4: form factor, 5: bending energy, 6: DMIN, 7: DMAX, 8: convex area, 9: diameter ratio), Eigenvalues and cumulative proportion (percentages) of explained variance.

Correlation variables	Canonical roots					
	Root 1	Root 2	Root 3	Root 4	Root 5	Root 6
1	0.402	0.252	0.405	-0.031	0.266	0.195
2	0.415	0.293	0.468	0.008	0.280	0.380
3	0.280	0.633	0.670	0.052	0.134	0.086
4	0.277	-0.217	0.028	-0.030	0.135	0.420
5	0.154	0.073	0.180	-0.028	0.183	0.733
6	0.130	0.856	0.352	0.186	0.130	0.174
7	0.513	0.297	0.747	0.071	0.270	-0.083
8	0.397	0.615	0.623	0.061	0.148	0.128
9	0.289	-0.803	0.317	0.004	0.077	-0.266
Eigenvalues (%)	11.57	3.61	2.17	0.67	0.28	0.04
Cumulative proportion (%)	63	82	94	98	99	100

The correlation matrix for all variables (Table 2) indicates that some morphometric parameters displayed tight affinities, especially length, perimeter, conv. area and DMAX. Conversely, form factor, bending energy, diameter ratio, and to some extent DMIN were very

Table 2. – Correlation matrix of all morphometric variables used in discriminant analysis.

Variables	Length	Perimeter	Cell area	Form factor	Bend. energy	DMIN	DMAX	Conv. area	Diam. ratio
Length	1.00	0.98	0.80	0.66	0.70	0.1	0.89	0.87	0.18
Perimeter	0.98	1.00	0.83	0.66	0.75	0.65	0.92	0.90	0.16
Cell area	0.80	0.83	1.00	0.14	0.47	0.89	0.87	0.98	-0.15
Form factor	0.66	0.66	0.14	1.00	0.71	-0.05	0.45	0.28	0.50
Bending energy	0.70	0.75	0.47	0.71	1.00	0.42	0.54	0.53	0.06
DMIN	0.61	0.65	0.89	-0.05	0.42	1.00	0.60	0.84	-0.56
DMAX	0.89	0.92	0.87	0.45	0.54	0.60	1.00	0.93	0.32
Convex area	0.87	0.90	0.98	0.28	0.53	0.84	0.93	1.00	-0.05
Diameter ratio	0.18	0.16	-0.15	0.50	0.06	-0.56	0.32	-0.05	1.00

poorly related to each other, thus showing no overlap in shape discrimination.

From discriminant functions, it is possible to develop a classification process and then to estimate the morphological group to which each observed cell belongs. The classification matrix provides the good fitting level between observed and estimated classifications. The higher the percentage of correct classification, the better the discrimination with the other groups.

The classification matrix (Table 3) allows certain species and morphotypes to be identified and the respective percentages of correct classifications to be determined. It is apparent that species 7 (*Dinophysis* sp.) differs significantly (100% correct classification) from the other morphological types. It is thus likely that the *Dinophysis* cells isolated from Urbino Lagoon were not a variety of *D. pavillardi* or *D. sacculus* but probably a new endemic species (Fig. 3). If correct classifications above 80% are considered for the other morphological types, *D. acuminata* (Norway), *D. acuminata* (Korea) *D. pavillardi* and *D.* cf. *norvegica* differ significantly on the basis of the morphometric criteria used.

Although these results are hardly surprising for species other than *D. acuminata,* it is curious to note that what was considered until now as an Asiatic morphotype of *D. acuminata* is rather

Table 3. – Classification martrix 1: *D. acuminata* (Norway), 2: *D. acuminata* (Korea), 3: *D. pavillardi*, 4: *D. sacculus*, 5: *D.* cf. *acuminata*, 6: *D.* cf. *norvegica*, 7: *Dinophysis* sp.

		Percent. correct	Predicted classification						
			1	2	3	4	5	6	7
Observed classification	1	95.0	38	2	0	0	0	0	0
	2	80.6	3	25	1	1	1	0	0
	3	89.7	0	1	35	3	0	0	0
	4	64.7	1	4	3	22	4	0	0
	5	51.4	3	1	1	6	18	6	0
	6	83.3	0	0	0	0	4	20	0
	7	100.0	0	0	0	0	0	0	26
	Total	80.3	45	33	40	32	27	26	26

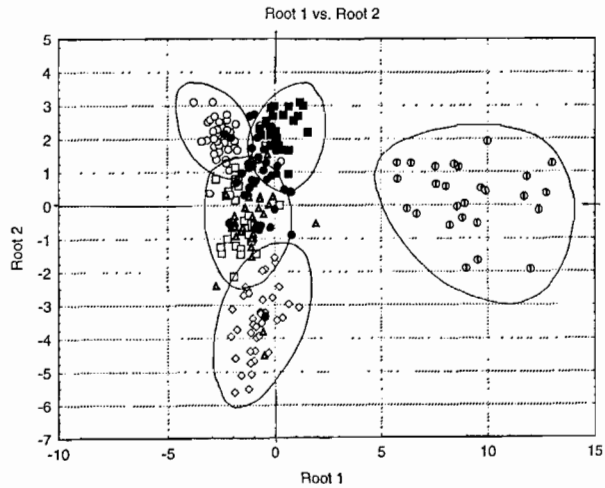

Figure 3. – Discriminant analysis plot for roots (canonical axes) 1 and 2. ○ *D. acuminata* (Norway); □ *D. acuminata* (Korea); ◊ *D. pavillardi*; △ *D. sacculus*; • *D.* cf. *acuminata*; ■ *D.* cf. *norvegica*; ⓘ *Dinophysis* sp.

clearly discriminated by the analysis. Species 4 and 6, representing respectively 64 and 51% correct classifications, cannot be easily categorized as significantly different. The overlapping of *D. sacculus* and *D. acuminata* (Korea) is too great, and *D.* cf. *acuminata* was identified erroneously among all species studied except *Dinophysis* sp.

DISCUSSION AND CONCLUSION

The morphometric parameters used appear to be helpful in discriminating between several *Dinophysis* species. A sorting operation based on these parameters and discriminant analysis was used to differentiate recognized toxic species, as well as those of uncertain toxicity, on the basis of size and morphological affinity. However, the use of this shape-recognition tool encountered a basic difficulty, namely the intraspecific morphological variations characteristic of tiny toxinogenic species designated as "*sacculus*" (Mediterranean affinities) and "*acuminata*" (Northern European affinities). By adopting *D. acuminata* from

Norway as a reference, it was possible to determine certain parameters which seem more pertinent than others for each of these groups, especially DMAX, DMIN, their ratio, cell area and convex area. Factors related bo bending of the hypotheca seem less relevant. This allowed us to distinguish rather clearly among some of the species of *D. sacculus*, *D.* cf. *norvegica*, *D. pavillardi*, *D.* cf. *acuminata* and *D. acuminata* from Korea.

Nevertheless, the use of all parameters in a descriminant analysis appears to be a necessary and complementary tool in order to obtain a good level of discrimination. Such analysis strongly suggested that *Dinophysis* sp. from Corsica is a potentially new species and that there is hardly any difference between the *acuminata* and *sacculus* groups.

Moreover, *D. pavillardi* was recognized and classified as significantly different from *D. sacculus*, according to previous descriptions by Balech (1976), *D. acuminata* varieties from Norway and Korea appeared to be well discriminated, as well as *D.* cf. *norvegica*; whereas *D.* cf. *acuminata* seemed to be only a morphotype of *D. acuminata*.

The shape-recognition system described here is certainly not valid alone as a diagnostic method. Nevertheless, it provides the specialist with an additional tool to classical taxonomical parameters (e.g., plate pattern, sulcal list extention, etc.). Thus, it is possible to use the system efficiently in its present form, especially for assistance in classifying "problematic" *Dinophysis*.

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