high field ^{13}C-NMR-data: UV (CH_3OH) λ_{max} (ε) 242(10, 200), 311 nm (158); $[\alpha]_\lambda^{20}$ (CH_3OH), c0.014) − 128.3° (λ365), 189.4° (λ435), 84.0° (λ546), 71.2° (λ577), 66.8° (λ589 nm); ^{13}C-NMR[8] (pyridine-d_5) δ(multiplicities and/or assignments are given when unambigously attributable) 203.44 (s, C-6), 166.05 (s, C-8), 121.69 (d, C-7), 84.22 (s, C-14), 77.57 (d, C-22), 76.89 (s, C-20), 69.59 (s, C-25), 68.15 (d, C-2 or C-3), 68.07 (d, C-2 or C-3), 51.40, 50.15, 48.14 (s, C-13), 42.63, 38.69 (s, C-10), 38.02, 34.49, 32.43, 32.04, 31.78, 30.11, 30.01, 27.48, 24.47, 21.68, 21.50, 21.16, 17.90.

These findings pose a number of intriguing questions about the origin and the role of ecdysterone in *G. savaglia* and about the relationship, if any, with the same steroid in marine crustacea. Regarding the origin of the steroid, it is relevant to note that *G. savaglia*, after it had been kept for 15 months in our aquarium[6], still gave ecdysterone in roughly the same large amounts as immediately after its collection.

It may be that part of the ecdysterone found in the zoanthid kept in our aquarium has a dietary origin, e.g. from copepods and plancton[6]. However, we could not detect ecdysterone in the diet[6] by HPLC-UV on examination of diet amounts sufficient to the zoanthid for months. This agrees with the fact that crustaceans may normally contain ecdysterone in amounts not larger than a few mg per ton[5a]. Therefore, unless ecdysterone was either not used, nor given to the surroundings by the zoanthid kept in the aquarium, the above findings show that the zoanthid cannot have received all its ecdysterone from the diet alone. It cannot then be ruled out that ecdysterone is synthetized within the zoanthid from, possibly, dietary cholesterol. These facts urge both the examination of *G. savaglia* from different marine areas and a careful examination of the zoanthid for microbial symbionts, which could well be the producers of ecdysterone. Although the presence of zoochlorellae seems to be excluded owing to the pale yellow color of the zoanthid, which was not altered in the aquarium conditions, nothing is known about other symbionts for *G. savaglia*. Ultimately, if no symbionts are found, a study of the biosynthesis of ecdysterone by the zoanthid could be attempted.

The role of ecdysterone as a hormone in the zoanthid is ruled out because of the high concentration of the steroid. We may consider a defensive role, as has been suggested, but never substantiated, for ecdysteroids accumulated by plants[5]. This stimulates the study of predators of *G. savaglia*, about which nothing is known. Nudibranchs are possible candidates, because they are known to feed on coelenterates (e.g. on hydroids), thereby accumulating their steroids[9]. Crustaceans are not predators of *G. savaglia*, perhaps because crustaceans are affected by ecdysterone[5].

We conclude that it is likely that ecdysteroids will prove to be much more widely distributed in the marine environment than was thought, unless *G. savaglia* possesses it uniquely. The recent finding that pinnasterol, an ecdysteroid-like sterol, is a constituent of the red alga *Laurencia pinnata* Yamada[10] might support this hypothesis.

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2 Laboratorio di Chimica.
3 Stazione Sperimentale Agraria.
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Marine diatoms affecting the stability of oil-in-water emulsions and hydrocarbon distribution in sea water

M. Karydis

Department of Biology, Nuclear Research Center 'Democritos', Aghia Paraskevi, Attikis, Athens (Greece), 5 March 1982

Summary. The importance of phytoplankton in stabilizing oil-in-water emulsion and affecting hydrocarbon distribution in the sea was studied by using marine diatoms. All microalgae tested increased emulsion stability and favored the presence of polycyclic aromatics in the sea water.

The formation of oil-in-water emulsions in the sea after an oil spill, extensively discussed over the last few years[1,2], has mainly been described as the result of physical and chemical processes[3]. However, laboratory work on hydrocarbon uptake by a marine diatom showed that algal cultures could support higher hydrocarbon concentrations than sterile media[4]. The subsequent aim was therefore to examine by standard emulsification procedures[5] whether marine diatoms, the most important group of a phytoplankton community, could also be considered as factors increasing the stability of oil emulsions. In addition, the distribution of the oil components into the water phase and emulsion was studied fluorimetrically, because of the ecological significance of the aromatic compounds[2].

Experimental part. Four marine diatom species, *Skeletonema costatum* (Grev.) Cleve, *Cyclotella cryptica*, Reinan, J. Lewin and Guillard, *Nitzschia closterium* (Ehr.) W.Sm. and *Chaetoceros affinis* Laud., were used as test organisms. Culture media and conditions have been described elsewhere[6]. The stability of the emulsion was tested by the

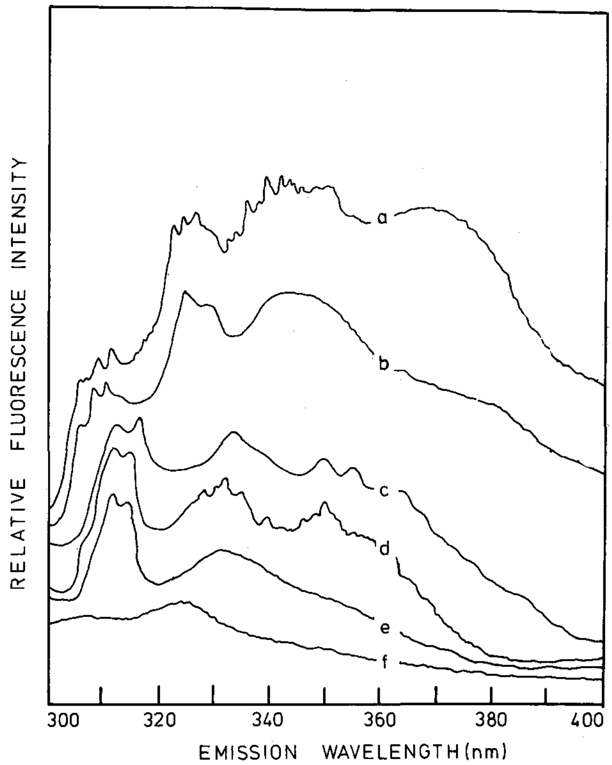
Deemulsification time of the diatom cultures/crude oil system. The final volumes of oil, water (culture) and emulsion remaining after 1 h are also given. The numbers are the average of 3 samples

Treatment	Time (min)	Oil phase (ml)	Water (ml)	Emulsion (ml)
Sea water	10	40	40	0
Filtrate	10	40	40	0
Skeletonema costatum	30	40	40	0
Cyclotella cryptica	30	40	38	2
Chaetoceros affinis	60	38	32	10
Nitzschia closterium	15	40	37	3
Mixed culture	30	40	37	3

standard ASTM method[5] measuring the ability of petroleum to separate from water (algal cultures in the present case). A 40-ml algal culture (cell density 1.5×10^5 cells/ml) and 40 ml of Tunisian crude oil were stirred for 24 h at 3000 rpm and 20 °C. The time required for the separation of the emulsion thus formed was recorded; if complete separation of the 2 phases did not occur after standing for 1 h, the volumes of water, oil and emulsion were reported. UV-fluorescence spectroscopy was applied by synchronously scanning excitation and emission monochromators[7,8] in a MPF-3L Perkin Elmer Spectrofluorimeter, to identify any differences in aromatic composition among the various oil extracts.

Results and discussion. The deemulsification times are shown in the table. Sea water takes 10 min for complete separation from the crude oil. Filtrate from *Sk. costatum* culture (mainly composed of extracellular products) also separates within the same period of time (table). This indicates that organic compounds do not seem to contribute significantly to the stability of the emulsion. On the contrary the diatoms prolong the separation time, *C. affinis* being the most effective in forming stable emulsions. The mixed algal suspension did not show any particular tendency towards emulsion stability which indicates that there is no diatom interaction that increases the stability. The results suggest that diatoms do act as stability factors in the oil-in-water emulsion formation and this may be explained on account of their cell wall composition; the diatom frustule, composed of silica and covered by a mucilage sheath, is known for its absorptive properties[9]. However, their actual capacity to stabilize the emulsion was found to be a species-specific process. The external morphology of the diatoms could possibly help to explain the species specificity of the emulsifying ability of the algae; for example, the 'spines' of the silica skeleton of *C. affinis* provide a good support for the oil globules by surface phenomena, which was confirmed by microscopical examination.

The distribution of aromatic compounds in the different oil extracts is shown in the figure. The main peakes in the synchronous spectra are identified according to the number of fused aromatic rings present in the sample determining the wavelength at which maximum emission occurs[8]. Naphthalenes emit about 310 nm whereas aromatics having 3 or 4 rings generally emit between 340 and 380 nm; substances composed of more than 5 rings emit between 360 and about 400 nm. Hydrocarbons extracted from sea water (figure, e) seem to contain mainly naphthalene, a water soluble hydrocarbon. On the contrary the water phase of the culture, containing oil globules and forming an oil-in-water emulsion (figure, c) is slightly enriched in



Synchronous fluorescence spectra of aromatic hydrocarbons in hexane. The difference between emission and excitation wavelength was 30 nm. *a* crude oil; *b* hydrocarbons extracted from the emulsified layer of a *Skeletonema costatum* culture; *c* hydrocarbons extracted from the water phase of the culture; *d* hydrocarbons extracted from filtrate; *e* hydrocarbons extracted from sea water and *f* hexane solvent.

hydrocarbons with more than 2 fused rings compared to the profile of the oil extracted from the water phase (figure, e). Oil extracted from the emulsified layer (figure, b) showed very high absorption bands in the region of 320-400 nm, the profile of the spectrum being almost similar to the crude oil profile (figure, a). This indicates the ecological significance of the emulsification process on the distribution of the oil components. Very toxic polynuclear aromatics, largely water insoluble and consequently not available to planktonic organisms, can be stabilized in the water column in the form of an oil-in-water emulsion. High densities of suspended particles and planktonic organisms in the marine environment, which is the case in eutrophic waters, might therefore affect the distribution of polynuclear compounds in the sea water, increasing the ecological impact of oil on the marine life.

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