Marine Biology (1998) 131: 639–645

A. Marin · M. D. López · M. A. Esteban
J. Meseguer · J. Muñoz · A. Fontana

Anatomical and ultrastructural studies of chemical defence in the sponge *Dysidea fragilis*

Received: 4 June 1997 / Accepted: 28 January 1998

Abstract The marine sponge *Dysidea fragilis* from El Mar Menor, a hypersaline coastal lagoon (Murcia, Spain), contains the furanosesquiterpenoid *ent*-furodysinin as the major secondary metabolite. *D. fragilis* emits a defensive white fluid when it is disturbed. Electron micrographs of this fluid revealed intact vesiculated cells together with other amorphous material. Dissociated cells are more rounded in shape but maintain the same ultrastructural features as cells observed in ultrathin sections of the whole sponge. The defensive secretion is composed mainly of sponge cells with abundant light vesicles. Sometimes these light vesicles appear to open into the intercellular space; this correlates with surface blebs on these cells observed under scanning electron microscopy. The intracellular location of *ent*-furodysinin was confirmed by Erlich staining. In laboratory assays, we examined the role of *ent*-furodysinin as a feeding deterrent to generalist fish predators. It was isolated from *D. fragilis* and incorporated into a carrageenan-based artificial diet. The addition of *ent*-furodysinin to the artificial diet reduced feeding by the fish *Thalassoma pavo*. Similarly, fish did not feed on artificial diet above which defensive secretion of *D. fragilis* had been ejected with a small syringe.

Communicated by A. Rodríguez, Puerto Real

A. Marin (✉) · M.D. López
Departamento de Ecología e Hidrología,
Facultad de Biología, Universidad de Murcia,
E-30100 Murcia, Spain

M.A. Esteban · J. Meseguer · J. Muñoz
Departamento de Citología e Histología,
Facultad de Biología, Universidad de Murcia,
E-30100 Murcia, Spain

A. Fontana
Istituto per la Chimica di Molecole di Interesse Biologico,
CNR, via Toiano 6, I-80072 Arco Felice,
Naples, Italy

Introduction

The phylum Porifera, the most primitive of modern animals, represents a level of organization somewhere between a colony of cells and a true multicellular organism. Sponges possess remarkably high tissue-plasticity, which is probably not exceeded by any other metazoan group. The natural disappearance of choanocyte chambers from sponges has been recorded in a number of earlier works (e.g. Bidder 1933). The spontaneous disorganization and reorganization of the canal system has been described in the marine demosponges *Ficulina ficus* and *Suberites massa*. Such remodelling is related to maximizing canal-system efficiency, re-establishing canal- and water-pumping following sexual reproduction, and producing new growth (Fauré-Frémiet 1932; Diaz 1979). When exposed to changes in salinity, the euryhaline sponge *Microciona prolifera* loses oscula, subdermal spaces, canals and choanocyte chambers (Knight and Fell 1987; Fell et al. 1989). Tissue regression appears to be an alternative process to a gemmule reproduction strategy in response to lower temperature and other factors. Furthermore, it may be a transitory phenomenon set in motion by the widespread changes that occur during gametogenesis and larval development. In general, the tissue loss in sponge (buds, gemmules, tissue regression), which is usually seasonal, has been described as being accomplished by asexual or sexual developmental processes (e.g. Simpson 1984).

The occurrence of symbiotic bacteria, fungi and algae has been described in numerous sponges (Vacelet and Donadey 1977). The biosynthetic origin of some secondary metabolites isolated from sponges has often been ascribed to these prokaryotic endobionts. Marine sponges of the family Dysideidae contain sesterpenoids, diterpenoids and, most commonly, sesquiterpenoids of a wide variety of skeletal types (Guella et al. 1985b; Faulkner 1986, 1987, 1988, 1990, 1992). Recently, Unson and Faulkner (1993) reported that cyanobacterial symbionts biosynthesized chlorinated metabolites contained

in *Dysidea herbacea*, whereas the accompanying sesquiterpenoids were found only in the sponge cells. Symbionts are difficult to grow in axenic cultures, and there is no guarantee that a symbiont in pure culture will produce the secondary metabolites that they would normally produce when associated with the sponge. To elucidate how symbiotic prokaryotes contribute to the production of secondary metabolites in the sponges, Unson et al. (1994) isolated representative cell types. They assumed that a compound localized exclusively in a single cell type had probably been produced by that cell.

Chemical variability has been observed in natural products isolated from *Dysidea fragilis,* which contains several sesquiterpenoids such as longiforin and *ent*-furodysinin (Guella et al. 1985a) and, in some coastal areas, the azacyclopropene dysidazirine (Molinski and Ireland 1988). Also, intraspecific variation in the sterol composition has been reported in Mediterranean populations of *D. fragilis* (Aiello et al. 1995). We studied a chemotype located in El Mar Menor lagoon that was characterized by the presence of *ent*-furodysinin (Fontana et al. 1994).

The present study investigated the occurrence of tissue fragmentation, the cellular location and the defensive role of the furanosesquiterpenoid *ent*-furodysinin in the marine sponge *Dysidea fragilis*.

Materials and methods

Collection

Dysidea fragilis (variety Mar Menor), a greyish-blue massive sponge, was collected by hand at 1 m depth in El Mar Menor, a hypersaline coastal lagoon. Sponges were removed from shaded rocky bottoms, and freshly collected specimens were placed in aquaria containing sea water with a salinity of 38‰ and a temperature of 15 °C.

Microscopy

For electron microscopy, freshly collected pieces of sponge were immediately fixed in 2.5% Milloning's phosphate-buffered glutaraldehyde (pH 7.2 to 8.2) for 1 h. Pieces were then washed in 2.5% $NaHCO_3$ in distilled water (60 min at 25 °C), postfixed in 2% osmium tetroxide in 1.25% $NaHCO_3$ for 1 h, dehydrated through an ethanol series, and then embedded in Epon. Ultra-thin sections were cut with glass or diamond knives, and stained with uranyl acetate and lead citrate. Fixation of cell suspensions was performed as above, except that at each step the cells were centrifuged.

The intracellular location of *ent*-furodysinin was studied by light microscopy. Crude and isolated cell suspensions were cytocentrifuged for 10 min. Cells settled on a microscope slide were air-dehydrated and examined under light microscopy using transmitted or polarized light. The dried cells were stained with Ehrlich reagent and monitored constantly by light microscopy.

The morphological structure of sponge tissues and cells was observed using a scanning electron microscope. Some fragments of sponge were fixed in 2.5% gluteraldehyde, pH 7.2, for 2 h at 4 °C, washed in buffer, dehydrated in acetone, critical point-dried, sputter-coated with gold and studied with a Jeol JSM T-300 scanning electron microscope.

Dissociation and isolation of sponge cells

Small blocks of fresh tissue (5 × 5 mm) were cut from the sponge and placed in a flask containing artificial sea water. Blocks were stirred at low velocity using a magnetic stir-bar for 10 min at 20 °C. The dissociated cellular material was filtered onto nylon mesh (mesh size 102 μm) to separate sponge homogenates from the spongin skeleton. The cells were washed twice and centrifuged at 400 ×*g* for 10 min; then the sponge homogenates were purified by isopycnic centrifugation. Isotonic gradients were prepared by layering 9 ml of Percoll (Pharmacia; diluted 9:1 in artificial sea water) aliquots prepared by dilution with artificial sea water (80:60:54% Percoll solutions). Artificial sea water was prepared from double-distillate water and contained 462 m*M* NaCl, 8.4 m*M* Na_2SO_4, 11 m*M* KCl and 2.1 m*M* $NaHCO_3$. Percoll gradients were prepared just before use and kept at 5 to 10 °C. Continuous density gradients were monitored using coloured beads (Pharmacia) with calibrated density values. Crude cell-suspensions were layered onto the Percoll gradients and centrifuged at 30 000 ×*g* for 30 min in a refrigerated (4 °C) centrifuge. Sample bands that formed at the separate interfaces were removed by Pasteur pipette and examined under light and electron microscopes. The cells were classified according to size and morphology.

Chemical analysis

A specimen of *Dysidea fragilis* was extracted with acetone. The filtered acetone solution was concentrated, diluted with water, and extracted with diethyl ether. Evaporation of the solvent under reduced pressure produced an oily residue that was chromatographed on a silica-gel column with petroleum ether as eluant, to give a mixture of Ehrlich-positive sesquiterpenoids containing mainly *ent*-furodysinin. Further chromatography on 8% $AgNO_3$–SiO_2 yielded pure *ent*-furodysinin (Fontana et al. 1994), which was then used as the reference standard for the chemical analyses.

Each Percoll cell fraction was separately diluted by distilled water (2 ml) and extracted with diethyl ether (3 × 1.5 ml). The crude ether extracts from the Percoll fraction (212 mg) were fractionated on a SiO_2 column using petroleum ether to give a mixture of furanosesquiterpenes, with *ent*-furodysinin as the major constituent. Gas chromatographic examination was carried out on a Carlo Erba OV-1 capillary column at 130 °C. Cell extracts (0.2 mg) were dissolved in diethyl ether (0.4 ml). Each injection was 6 μl. The mixture of furanosesquiterpenoids from the sponge (1 mg) was dissolved in diethyl ether (1 ml). Each injection comprised 2 μl.

Palatability of *Dysidea fragilis*

Palatability assays were conducted using the wrasse *Thalassoma pavo* in an aquarium. Fishes were conditioned for 10 d and accustomed to a carrageenan-based food (2% carrageenan supplemented with freeze-dried powdered sardines) (Harvell et al. 1988). The secondary metabolite *ent*-furodysinin was incorporated into the artificial diet (10% of dry mass). The diet was cut into 1 × 0.5 × 2 cm strips. In order to test for the continued presence of *ent*-furodysinin in the artificial diet throughout the assays, 20 pieces of artificial diet were placed in an aquarium without fishes during the experiment. The compound remained on the artificial diet throughout the assays as confirmed by thin-layer chromatography (TLC). In the laboratory, individual wrasses were each offered one piece of artificial food containing *ent*-furodysinin and one control pellet treated with solvent only. For defensive-secretion assays, pieces of artificial food were attached to a plastic strip on the side of the tank. A piece of polystyrene tubing (500 mm long, 0.5 mm diam) was attached to a small syringe containing defensive secretion. The tubing ended immediately above the piece of food, enabling the secretion to be ejected into the water (Fig. 1B). Defensive secretion was obtained by disturbing the sponge with a magnetic stir-bar for 5 min, thus inducing spontaneous tissue-fragmentation. The ejected fluid was diluted 1:1 in sea water, and 1 ml was injected into each piece of the artificial diet. Paired controls were assayed using 1 ml sea water. When all defensive fluid or control fluid had been ejected from the syringe (estimated time = 60 s) the amount of food consumed was recorded. The amounts of the two diets consumed were estimated visually with a

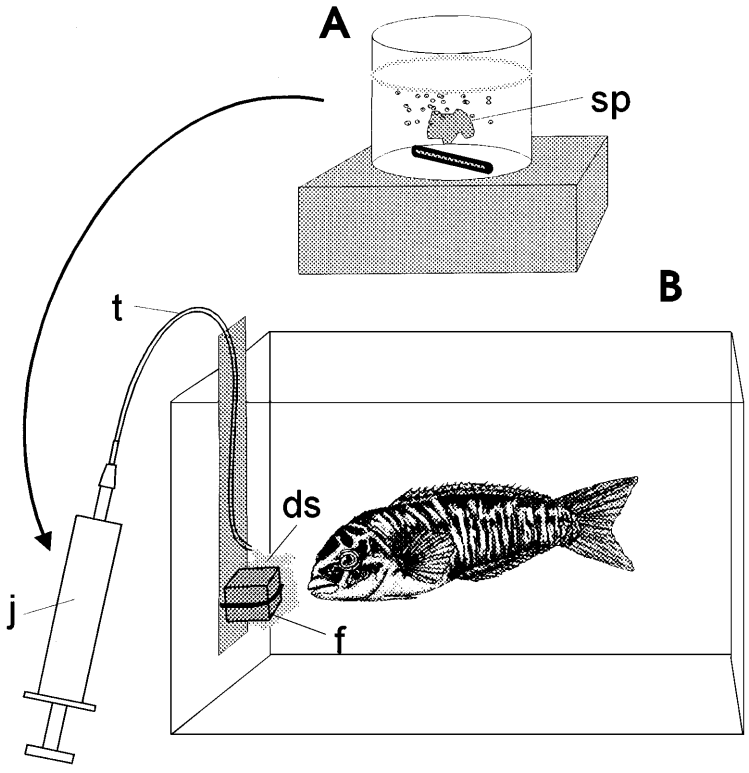

Fig. 1 Diagram of experimental set-up for secretion assays. **A** tissue disintegration of sponge *Dysidea fragilis* into magnetic stirrer for palatability assays; **B** for defensive secretion assays with wrasse *Thalassoma pavo*, sponge fluid was introduced into sea water immediately above pieces of artificial diet (*ds* defensive fluid; *f* artificial diet; *j* syringe; *sp* sponge, *t* polystyrene tubing)

glass reticule. Comparisons of the amounts of the two diets consumed in the laboratory test with *T. pavo* were analyzed by a Student's paired *t*-test.

Results

Description of *Dysidea fragilis*

The external surface of the sponge *Dysidea fragilis* was conulose due to the presence of conical or pyramidal elevations, as observed by scanning electron microscopy. This surface bore randomly distributed open pores (Fig. 2a). The outer surface of the sponge was covered by a simple thin layer of pinacocytes. Choanocyte chambers were large, and round to oval (Fig. 2b). The fragile spongin fibres contained entrapped foreign particles. It was not possible to distinguish between the primary and secondary fibres recorded for other horny sponges. The principal fibres extended to the surface conules.

The mesohyl of *Dysidea fragilis* was composed of several different cell types surrounded by an amorphous intercellular matrix that included collagen fibrils. Most were discoidal and elliptical cells disposed in a coarse parallel arrangement. No contact between these cells was apparent, as each cell was visibly surrounded by the intercellular matrix. The spherular cells (Type I) had a large, oval to round, centrally located nucleus with a small rim of heterochromatin and one or two eccentric nucleoli (Fig. 2d). The cytoplasm was heterogeneous in appearance due to the presence of abundant organelles. A well-developed juxtanuclear Golgi apparatus with several dictyosomes was present in spherular cells. Near to the prominent Golgi apparatus, small granules with an extremely electro-dense core and surrounded by a light halo were observed (Fig. 2d). Numerous light vesicles of different sizes and shapes were seen. They contained a light granular material and usually appeared to open into the intercellular space. Round mitochondria, flattened cisternae of rough endoplasmic reticulum, and free ribosomes were also seen.

Other sponge mesohyl cells (spherular cells Type II) contained a small, irregularly outlined, centrally located or slightly eccentric nucleus. Most of the cytoplasm of these cells was filled by large spherical or ovoid vesicles with extremely electro-dense contents. These electro-dense contents were surrounded by a light halo (Fig. 2e). No cyanobacteria were observed in the mesohyl. The absence of cyanobacteria was confirmed by examining the sponge tissue under UV light microscopy.

Defensive tissue-fragmentation

Dysidea fragilis was reduced to its spongin skeleton by means of light mechanical disturbance with a magnetic stir-bar. The supernatant consisted of a cell suspension mixed with cellular organelles. Electron micrographs of such suspension revealed intact vesiculated cells and other amorphous material. Dissociated cells were more rounded in shape but maintained the same ultrastructural features as cells observed in ultra-thin sections of the whole sponge (Fig. 2f).

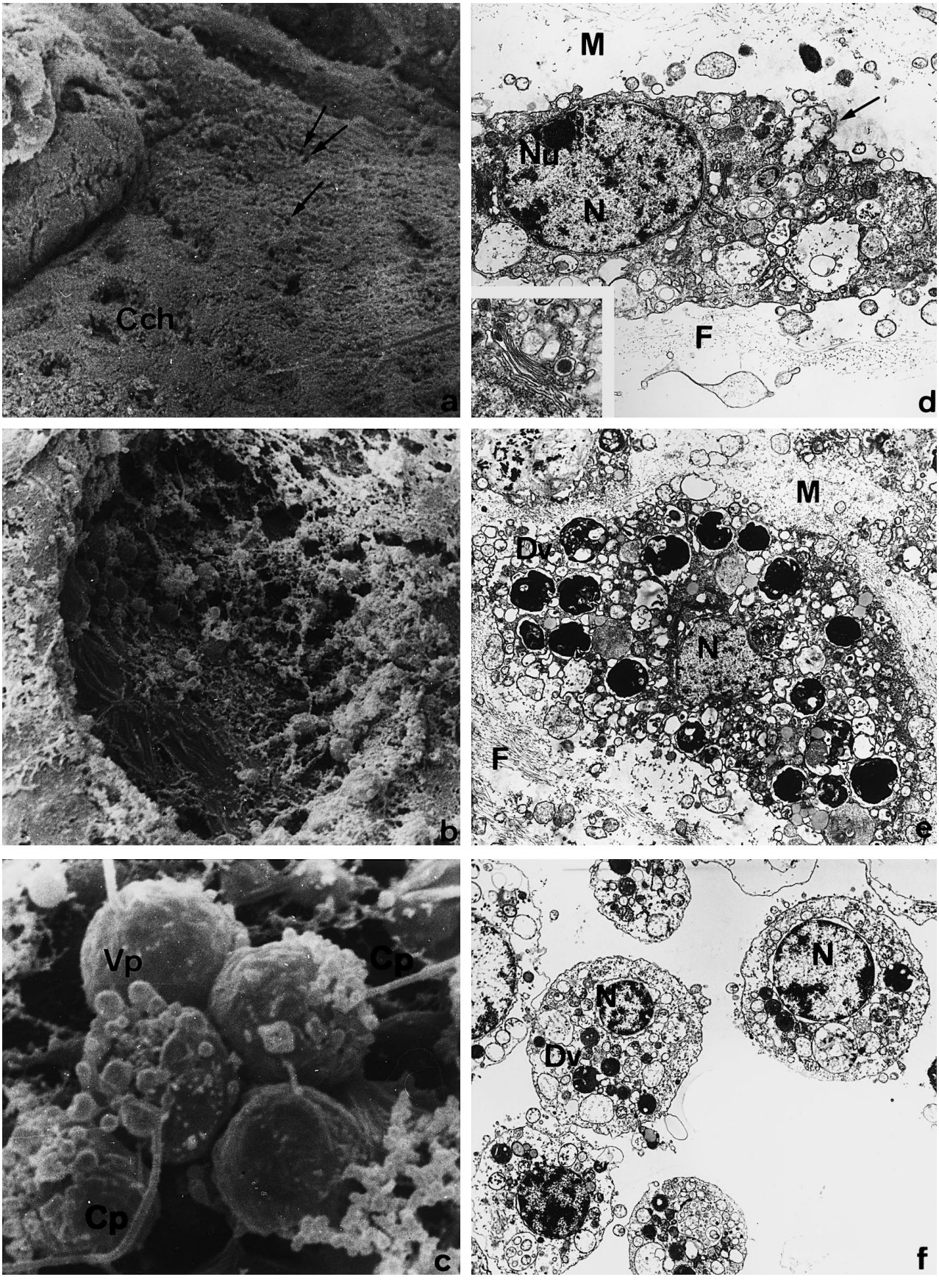
Cch
a
M
Nu
N
F
d
b
M
Dv
N
F
e
Vp
Cp
Cp
c
N
N
Dv
f

Fig. 2 *Dysidea fragilis*. Scanning electron micrographs of **a** external surface (*arrows* indicate pores; ×50), **b** oval choanocyte chamber (×500), **c** spherular cells with rugged surface and vesicular processes (×3500); and transmission electron micrographs of: **d** spherular cell Type I [note presence of light cytoplasmic vesicles opening into intracellular space (*arrowed*) (×8400) and electro-dense granules related to a dictyosome of Golgi apparatus (*inset*; ×12 000)], **e** spherular cell containing cytoplasm filled with large vesicles with extremely electro-dense contents (×6000), **f** spherular cells from Fraction 3 of Percoll gradient (*Cch* choanocyte chambers; *Cp* cell processes; *Dv* electro-dense vesicles; *F* fibres; *M* matrix; *N* nucleus; *Nu* nucleolus; *Vp* vesicular processes)

◀

Under the scanning electron microscope, spherular cells displayed a rugged surface with large protrusions and long cell processes (Fig. 2c). Some of these spherular cells had vesicular processes, giving them a wavy outline.

Cellular location of chemical defence

The cellular material from the defensive fluid ejected by *Dysidea fragilis* was separated using Percoll gradients. Four cellular fractions were obtained from the Percoll gradients. The lightest fraction (Fraction 1) contained bacteria, but TLC analysis of this fraction did not detect *ent*-furodysinin in these cells. Fraction 2 contained small sponge cells (61 to 72% pinacocytes, 20 to 28% choanocytes and 11 to 18% small archeocytes and cell fragments). This fraction contained a low concentration of *ent*-furodysinin. Fraction 3 contained mainly large spherular cells (74 to 86% of Type I and 26 to 14% of Type II) and also other small archeocytes. TLC analysis of the organic soluble fraction revealed the presence of Ehrlich-positive compounds in Fraction 2, which was characterized by the presence of spherular cells with a diffuse vacuole system (Fig. 3). Moreover, the gas-chromatographic comparison of this ether-soluble fraction with the ether extract from the whole sponge revealed the same furanosesquiterpenes pattern, mainly consisting (as already previously shown Fontana et al. 1994) of *ent*-furodysinin. This demonstrated the compartmentalization of *ent*-furodysinin exclusively into the spherular cells. The remaining fraction (Fraction 4) contained amorphous material and cell fragments; chemical analysis did not indicate the presence of Ehrlich-positive compounds in this last fraction.

The intracellular location of *ent*-furodysinin was confirmed by Ehrlich staining. The spherular cells settled on the microscope slide changed colour immediately upon contact with Ehrlich's reagent. Light microscopy revealed that this colour change was due to the presence of numerous cytoplasmic blue vesicles. Both size classes of spherular cells contained numerous Ehrlich-positive vesicles. Careful observation under the light microscope established that these vesicles change colour only upon contact with Ehrlich's reagent.

Palatability assays

At a concentration of 4%, the furanosesquiterpene *ent*-furodysinin, the major secondary metabolite of *Dysidea fragilis* from El Mar Menor lagoon, significantly deterred feeding by *Thalassoma pavo* (Student's paired *t*-test, $P < 0.001$) (Fig. 4). This indicates that the sponge allomone deters potential fish predators which bite the sponge tissues.

The defensive-secretion assays demonstrated that the effect of ejecting sponge secretion immediately above the artificial diet was similar to that in feeding assays where the metabolite *ent*-furodysinin was incorporated into the food. The sponge fluid containing *ent*-furodysinin

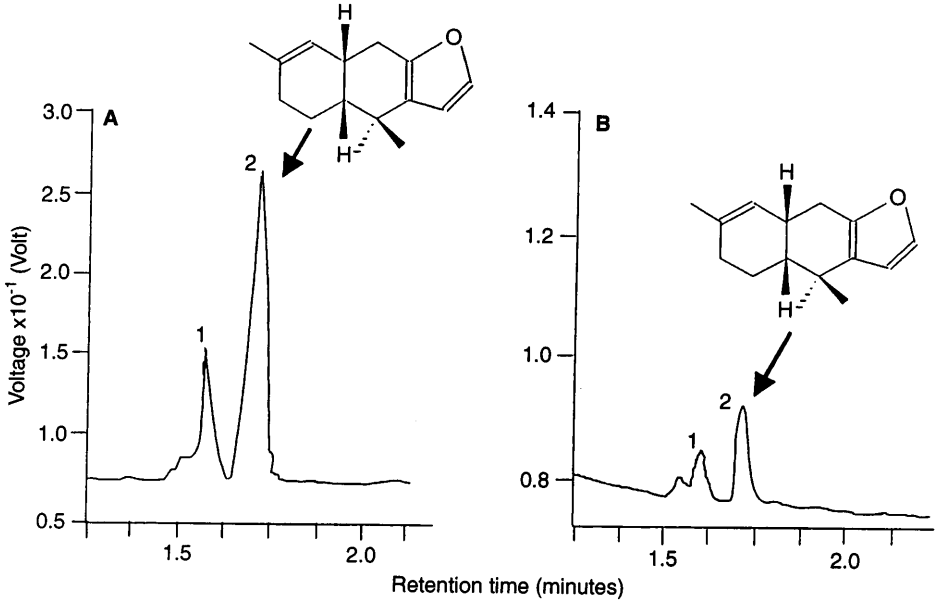

Fig. 3 *Dysidea fragilis*. Gas-chromatography profiles of furanosesquiterpenoid fraction from diethyl ether extract of whole sponge (**A**), and from Fraction 3 of Percoll gradients obtained from sponge defensive-secretion (**B**). Retention times shown for unresolved mixture of unidentified furanosesquiterpenes (*1*), and *ent*-furodysinin (*2*) (nature of Peak 2 was ascertained by comparison with pure *ent*-furodysinin)

▶

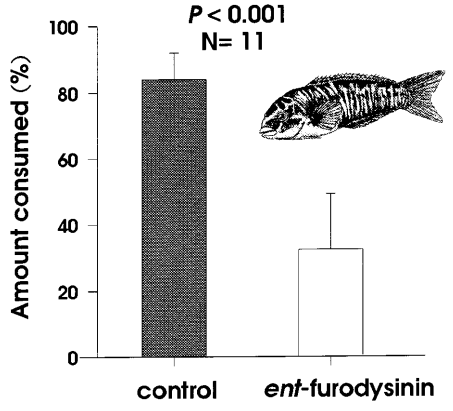

Fig. 4 *Thalassoma pavo*. Effect of secondary metabolite *ent*-furodysinin of *Dysidea fragilis* (tested at a concentration comprising 4% of the dry artificial diet) on feeding by the wrasse. Amounts of artificial diet consumed (%) are means ± SE. Sample size and *P*-value are shown

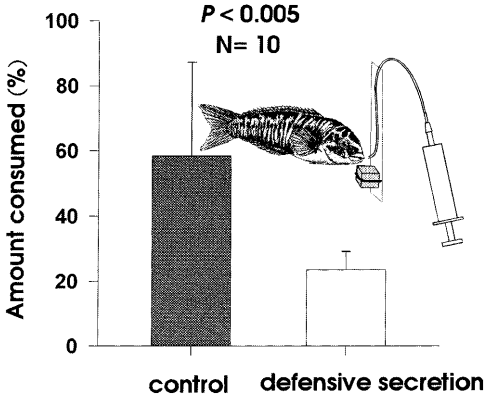

Fig. 5 *Thalassoma pavo*. Consumption of artificial diet when defensive secretion of sponge *Dysidea fragilis* was ejected into seawater immediately above food (*vertical lines* ± 1 SE). Sample size and *P* value of Student's paired *t*-test are shown

significantly deterred fish feeding in the paired assays (Student's paired *t*-test, $P < 0.005$) (Fig. 5).

Discussion

Ultrastructural features of sponge mesohyl cells have been little studied (De Vos et al. 1991). Our study demonstrates that *Dysidea fragilis* spherular cells (Types I and II) contain a well-developed Golgi apparatus as well as many cisternae comprised of rough endoplasmic reticulum. The marked development of both these organelles suggests their importance in the production of secretory materials (Weiss 1988). Electro-dense granules and abundant light vesicles were associated with the Golgi region. Sometimes these light vesicles opened into the intercellular space; this correlates with the surface blebs observed under scanning electron microscopy.

Chemical analysis of the whole sponge and the Percoll gradient fractions containing the spherular cells Types I and II (Band 3) revealed the presence of Ehrlich-positive furanosesquiterpenoids consisting mainly of *ent*-furodysinin. TLC of pure *ent*-furodysinin revealed a spot typically positive to Ehrlich's reagent, and the microscope slides containing the spherular cells displayed the same positive reaction when sprayed with Ehrlich's reagent. When the dried spherular cells were bathed in Ehrlich's reagent and examined under the light microscope, abundant Ehrlich-positive vesicles were observed, often opening into the intercellular space. Furthermore, it is reasonable to assume that since *ent*-furodysinin is soluble in ethanol (the solvent used in the dehydration protocol of electron microscopy), this metabolite is removed in the process and the space previously occupied will appear light in the micrographs. The numerous light vesicles observed in the spherular cells suggest that these vesicles may be storage sites for the defensive metabolite. The chemical analysis, the Ehrlich staining technique and the ultrastructural morphology of the spherular cells (Types I and II) all indicate that these cells of the sponge mesohyl are involved in the production and secretion of the secondary metabolite *ent*- furodysinin.

In addition, some archeocytes (which also possessed a well-developed secretory apparatus) contained abundant vesicles with an extremely electro-dense material in their cytoplasm. These cells may be involved in the secretion and accumulation of various secretion products. Thus, sponge mesohyl cell vesicles could be involved in intracellular transport, synthesis and secretion of various materials, and in membrane regeneration.

To demonstrate that the accumulation of furanoterpenes is restricted to a specific class of sponge cells, the sedimented bands were separately extracted with diethyl ether. TLC of the organic ether-soluble fractions from Band 3 revealed Ehrlich-positive compounds. Gas-chromatographic comparison of the ether-soluble fraction from Band 3 with the extract from the whole sponge revealed the same furanosesquiterpene pattern, mainly consisting (as already previously reported: Fontana et al. 1994) of *ent*-furodysinin. This demonstrated the compartmentalization of the toxins exclusively into the spherular cells.

Earlier studies have shown that sponge metabolites can be localized in specific cell types. The brominated metabolites aerothionin and homoaerothionin have been localized within the spherular cells of the Pacific sponge *Aplysina fistularis*. The bromine content of these natural products permitted detection by energy-dispersive X-ray microanalysis at the fine structural level (Thompson et al. 1983). There is chemical and biological evidence that *A. fistularis* exudes aerothionin and homoaerothionin into the surrounding sea water (Thompson 1985; Walker et al. 1985). The sponge *Dysidea avara* contains the antibacterial sesquiterpenoid hydroquinone avarol. Light and transmission electron-microscopic studies on *D. avara* have revealed that avarol is probably stored only in choanocyte cells (Müller et al. 1986; Uriz et al. 1996). Microbial symbionts have also been isolated from the cell fractions and

grown in culture (Müller et al. 1986; Uriz et al. 1996). The microorganisms did not contain furanoterpenes, suggesting that the allomones were synthesized by the sponge.

Dysidea fragilis does not contain an established bacterial flora, as do other demosponges of high tissue-density. In well-irrigated demosponges with low tissue-density, the bacteria are monospecific and scarce (Vacelet and Donadey 1977). Bacteria are very numerous in massive sponges with high tissue-density, and can constitute 38% of the tissue volume in *Verongia* species (Vacelet 1975). In *Chondrosia reniformis*, bacterial flora is transmitted via the larva to the newly metamorphosing sponge (Levi and Levi 1976). The isolation of sponge cells and symbiont microorganisms in continuous density gradients depends on cellular density. Density gradients are effective when cellular components have different densities (e.g. bacterial flora of smaller size and lower density than sponge cells), but are inefficient when the cellular components are of similar density (e.g. cyanobacterial symbionts and some sponge cells). Unson et al. (1994) used flow-cytometry to separate glutaraldehyde-fixed cyanobacterial and sponge cells from *D. herbacea* on the basis of phycoerythrin fluorescence. The brominated biphenyl ethers found in *D. herbacea* are associated with the symbiotic cyanobacterium and probably biosynthesized by the blue-green alga. However, the terpenes reported from this sponge have been confirmed to be true sponge metabolites (Unson and Faulkner 1993). The use of a different variety of *D. fragilis* that has no algal symbiotic relationship would remove any doubts as to the biosynthetic origin of *ent*-furodysinin.

Acknowledgements We are grateful to the staff of the Servicio de Microscopia of the University of Murcia for providing facilities and technical assistance. We thank D.J. Faulkner for comments on drafts of this manuscript.

References

Aiello A, Fattorusso E, Menna M, Carnuccio R, Iuvone T (1995) New cytotoxic steroids from the marine sponge *Dysidea fragilis* coming from the lagoon of Venice. Steroids 60: 666–673

Bidder GP (1933) Sponges without collared cells. Nature Lond, 132: p. 441

De Vos L, Rützler K, Boury-Esnault N, Donadey C, Vacelet J (1991) Atlas of sponge morphology. Smithsonian Institution Press, Washington

Diaz JP (1979) La degenerescence ovocytaire chez la demosponge *Suberites massa*. Colloques int Cent natn Rech scient 291: 79–86

Faulkner DJ (1986) Marine natural products. Nat Product Rep 3: 1–33

Faulkner DJ (1987) Marine natural products. Nat Product Rep 4: 539–576

Faulkner DJ (1988) Marine natural products. Nat Product Rep 5: 613–663

Faulkner DJ (1990) Marine natural products. Nat Product Rep 7: 269–309

Faulkner DJ (1992) Marine natural products. Nat Product Rep 9: 323–364

Fauré-Frémiet E (1932) Involution expérimentale et tension de structure dans les cultures de *Ficulina ficus*. Archs Anat microsc 28: 121–157

Fell PE, Knight P, Rieders W (1989) Low-salinity tolerance of and salinity-induced dormancy in the estuarine sponge *Microciona prolifera* (Ellis et Solander) under long-term laboratory culture. J exp mar Biol Ecol 133: 195–211

Fontana A, Giménez F, Marin A, Mollo E, Cimino G (1994) Transfer of secondary metabolites from the sponges *Dysidea fragilis* and *Pleraplysilla spinifera* to the mantle dermal formations (MDFs) of the nudibranch *Hypselodoris webbi*. Experientia 50: 510–516

Guella G, Guerriero A, Pietra F (1985a) Sesquiterpenoids of the sponge *Dysidea fragilis* of the North-Brittany Sea. Helv chim Acta 68: 39–48

Guella G, Guerriero A, Pietra F, Traldi P, Pietra F (1985b) Penlanfuran, a new furano sesquiterpene from the sponge *Dysidea fragilis* (Mont.) of Brittany, a striking difference with the same Hawaiian species. Tetrahedron Lett 24: 3897–3898

Harvell CD, Fenical W, Greene CH (1988) Chemical and structural defenses of Caribbean gorgonians (*Pseudopterogorgonia* spp.). I. Development of an in situ feeding assay. Mar Ecol Prog Ser 49: 287–294

Knight P, Fell PE (1987) Low salinity induces reversible tissue regression in the estuarine sponge *Microciona prolifera* (Ellis & Solander). J exp mar Biol Ecol 107: 253–261

Lévi C, Lévi P (1976) Embryogenèse de *Chondrosia reniformis* (Nardo), démosponge ovipare, et transmission des bactéries symbiotiques. Annls Sci nat (sér Zool) 18: 367–380

Molinski TF, Ireland CM (1988) Dysidazirine, a cytotoxic Azacyclopropene from the marine sponge *Dysidea fragilis*. J org Chem 53: 2103–2105

Müller WEG, Diehl-Seifert B, Sobel C, Bechtold A, Kljajic Z, Dorn A (1986) Sponge secondary metabolites: biochemical and ultrastructural localization of the antimitotic agent avarol in *Dysidea avara*. J Histochem Cytochem 34: 1687–1690

Simpson TL (1984) The cell biology of sponges. Springer-Verlag, New York

Thompson JE (1985) Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. I. Biological evidence. Mar Biol 88: 23–26

Thompson JE, Barrow KD, Faulkner DJ (1983) Localization of two brominated metabolites, aerothionin and homoaerothionin, in spherular cells of the marine sponge *Aplysina fistularis* (= *Verongia thiona*). Acta zool, Stock 64: 199–210

Unson MD, Faulkner DJ (1993) Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). Experientia 49: 349–353

Unson MD, Holland ND, Faulkner DJ (1994) A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. Mar Biol 119: 1–11

Uriz MJ, Turon X, Galera J, Tur JM (1996) New light on the cell location of avarol within the sponge *Dysidea avara* (Dendroceratida). Cell Tissue Res 285: 519–527

Vacelet J (1975) Étude en microscopie électronique de l' association entre bactéries et spongiaires du genre *Verongia* (Dictyoceratida). J Microscopie 23: 271–288

Vacelet J, Donadey C (1977) Electron microscope study of the association between some sponges and bacteria. J exp mar Biol Ecol 30: 301–314

Walker RP, Thompson JE, Faulkner DJ (1985) Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. II. Chemical evidence. Mar Biol 88: 27–32

Weiss L (1988) Cell and tissue biology. Urban & Schwarzenberg. München, Germany