

## *Elysia subornata* (Mollusca) a potential control agent of the alga *Caulerpa taxifolia* (Chlorophyta) in the Mediterranean Sea

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The biological characteristics of *Elysia subornata* (Mollusca: Opisthobranchia) were studied in an aquarium to assess its risks and chance of success as a potential biological control agent against the invasive alga *Caulerpa taxifolia* (Chlorophyta) in the Mediterranean Sea. This species feeds only on *Caulerpa* and has benthic larval development. Dietary switching is possible on some Mediterranean caulerpales but feeding on other algae and sea grass is unlikely. The main limiting factor for the success of studied Caribbean strain of *E. subornata* are the Mediterranean winter temperatures which are lethal for that species. For the five months of the year which are favourable for feeding, growth and reproduction, the tested strain of *E. subornata* cannot reach a population density capable of controlling *C. taxifolia*.

### INTRODUCTION

Since 1984, the introduced green tropical alga *Caulerpa taxifolia* (Vahl) C. Agardh, originating from aquariums, spread along the Mediterranean coast (Meinesz & Hesse, 1991; Jousson et al., 1998). By 2000, a total of 6000 hectares of sea bottom had been colonized (according to the definition of Vaugelas et al., 1999) by this alga, in more than 100 independent sites in six countries (Croatia, France, Italy, Monaco, Spain and recently Tunisia (Langar et al., 2000)). This alga exhibits characteristics which differentiate it from the tropical strain: resistance to low temperatures, gigantism of the thallus, high growth rate and high levels of repellent toxins, mainly caulerpenyne (Meinesz et al., 1995; Komatsu et al., 1997; Amade & Lemée, 1998). *Caulerpa taxifolia* occurs along exposed and sheltered areas and is able to colonize most of the substrates including silt, rock, sands and sea grass beds between 0 and 50 m depth (Meinesz et al., 1993).

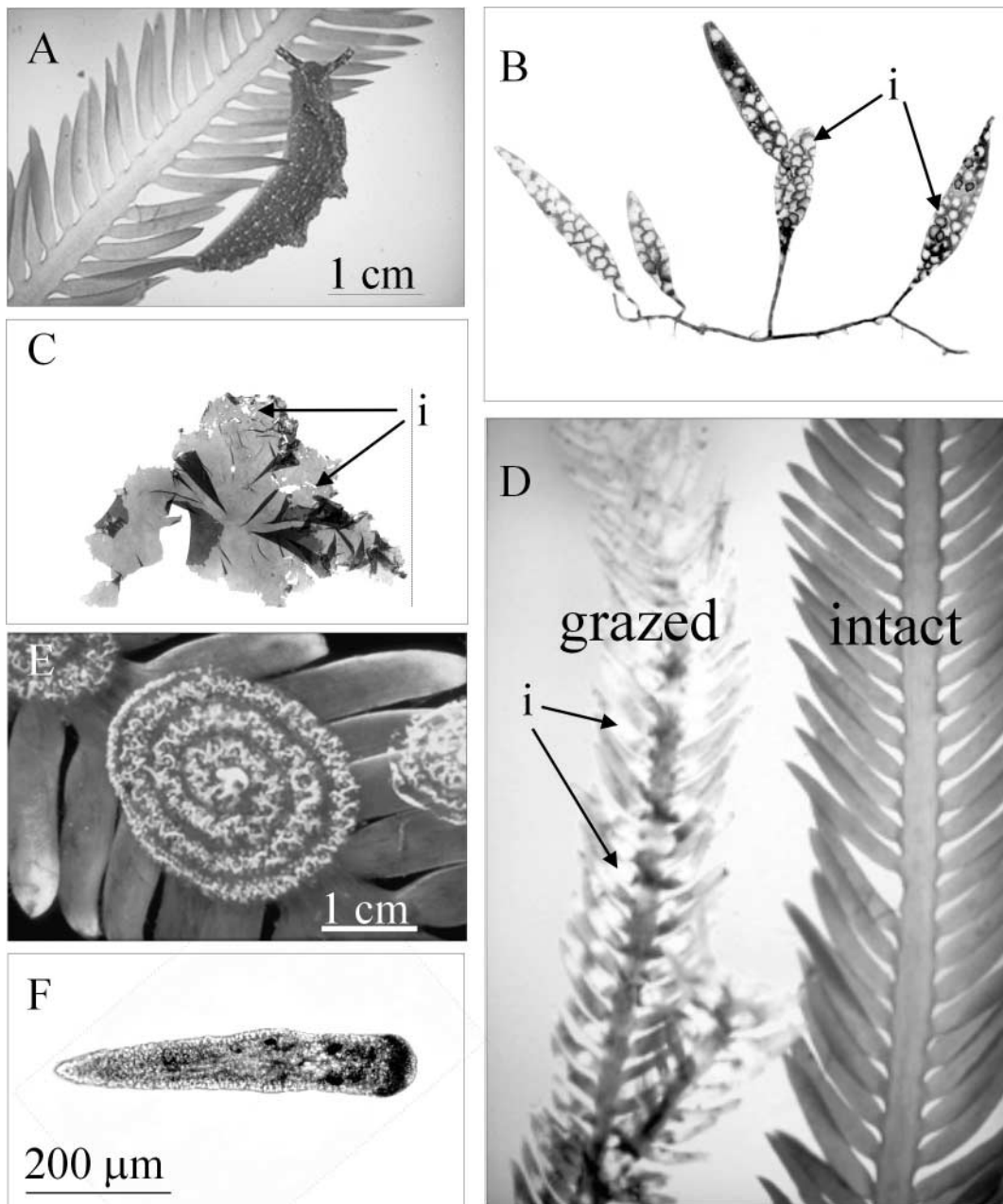
The invading, dominant and persistent characteristics of *C. taxifolia* has led to the disturbance of ecosystem that it colonizes (Boudouresque et al., 1995; Francour et al., 1995).

Since 1992, many attempts have been made to control this alga including the use of physical (Robert & Gavez 1998) and chemical techniques (Jaffrenou & Odonne, 1994; Escoubet et al., 1998; Gavach et al., 1998). These techniques, however, are only appropriate when dealing with small areas (<1 ha). With the greatly increased number of marine biological invasions occurring globally it is inevitable that attention would turn to marine biocontrol (Carlton, 1997). Since 1994, the potential use of four ascoglossans (Mollusca: Opisthobranchia) as biological control agents against *C. taxifolia* has been

examined. Preliminary studies concern two tropical species with a benthic larval development, (*Elysia subornata* Verrill, 1901 (Figure 1A) and *Oxynoe azuropunctata* Jensen, 1980), and two Mediterranean species with a pelagic larval development (*Oxynoe olivacea*, Rafinesque 1814 and *Lobiger serradifalci*, Calcara 1840 (Meinesz et al., 1996; Thibaut et al., 1998; Thibaut & Meinesz, 2000)). As recommended by the International Council for the Exploration of the Sea (ICES) and the Food Administration Organization's (FAO) guidelines on biological control or the introduction of species (FAO, 1997; ICES, 1997) this study on non-native ascoglossans from the Mediterranean Sea could not be undertaken in open sea. Therefore, as a first step toward the evaluation of using *E. subornata* as a biocontrol agent against *C. taxifolia*, the adaptation of the individuals to the Mediterranean temperatures (feeding, reproduction, survival and growth rates) were assessed as the evaluation of one risk linked to the eventual use of the tropical *E. subornata* (dietary switching).

### MATERIALS AND METHODS

This work was carried out in a closed water circuit at the University of Nice-Sophia Antipolis (3 km from the sea) to avoid any risks of introduction of the tropical *Elysia subornata* in the Mediterranean. Since February 1994, *E. subornata* from the Martinique Island in the western tropical Atlantic have been raised in 1 m<sup>3</sup> aquaria and fed exclusively with *Caulerpa taxifolia* from the Mediterranean. All the experiments in this study were performed in 5 l Plexiglas tanks filled with filtered aerated seawater. In order to mimic the natural Mediterranean conditions, the tanks were placed in temperature-controlled incubators (17, 21 and 25°C



**Figure 1.** (A) *Elysia subornata* on *Caulerpa taxifolia*; (B) *Caulerpa prolifera*; and (C) *Ulva* sp. grazed by *E. subornata*; (D) *Caulerpa taxifolia* frond intact and grazed by *E. subornata*; (E) post-metamorphic larva of *E. subornata*. i, incision.

(Bioblock Scientific LMS cooled incubators) with a PAR (photosynthetically active radiation) of  $150 \mu\text{E m}^{-2} \text{s}^{-1}$  and a photoperiod of 14:10 h (light:dark). Salinity was maintained at 38 psu.

#### *Consumption of different marine plants*

To test whether *E. subornata* is able to feed on other plants, the asoglossans were offered different common Mediterranean algae and sea grass susceptible to grazing by *E. subornata* (Table 1). These included seven species of Chlorophyceae, of which five belong to the Bryopsidales and two to the Ulvales, two Phaeophyceae (Dictyotales) and the sea grass *Posidonia oceanica* (L.) Delile. For each plant tested between 7 and 20 *E. subornata* were

distributed in a series of 5 l tanks (1–3 ind per tank). Plant food was provided *ad libitum* for each experiment. When the plants were not grazed the experiment ended with the death of all the individuals. For each tested plant, some traces of incision were recorded. A referent tank with five *E. subornata* without food was set up in order to compare the survival times.

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#### *Consumption of C. taxifolia*

Because grazed *C. taxifolia* rotted rapidly measuring the weight was difficult, according to Thibaut & Meinesz (2000), the feeding rate was estimated after 24 h by counting the number of incisions. Ten *E. subornata* were distributed in each of the five tanks and given 23 fresh

**Table 1.** Mediterranean algae and phanerogam species tested as a potential diet for *Elysia subornata*

Species tested	Traces of incision	% of surviving after 60 d	Maximum time of surviving (d)	N
<b>Chlorophyceae</b>				
Caulerpales				
<i>Caulerpa prolifera</i> (Forsskål) Lamouroux	+	30	>60	13
<i>Caulerpa racemosa</i> (Forsskål) J. Agardh	+	100	>60	10
<i>Codium coralloides</i> (Kützting) P.C. Silva	–	0	20	10
<i>Halimeda tuna</i> (Ellis et Sollander) Lamouroux	–	0	25	20
<i>Flabellia petiolata</i> (Turra) Nizamuddin	–	0	20	20
Ulvaes				
<i>Enteromorpha compressa</i> (L.) Nees	+	0	40	7
<i>Ulva</i> sp.	+	0	32	10
<b>Phaeophyceae</b>				
Dictyotales				
<i>Dilophus spiralis</i> (Montagne) Hamel	–	0	24	7
<i>Taonia atomaria</i> (Woodward) J. Agardh	–	0	24	7
<b>Phanerogam</b>				
<i>Posidonia oceanica</i> (L.) Delile	–	0	20	10
Starved <i>Elysia subornata</i>		0	30	5

+ / – alga incised or non-incised by *E. subornata*; N, number.

*C. taxifolia* fronds. This experiment was conducted at 17, 21 and 25°C which accounts for the temperature range of the northern Mediterranean between May and October.

Because data at 17°C were not normally distributed (Kolmogorov–Smirnov test,  $P < 0.01$ ), and as there was non homogeneity of the variances (Levene test,  $P < 0.05$ ), a non parametric test (Kruskal–Wallis test) was performed in order to determine if water temperature influenced mean grazing rate. A non parametric Student–Newman–Keuls (SNK) comparison was then used to identify at which temperatures the mean grazing rates were different.

#### Reproduction

In order to study the spawning characteristics of *E. subornata* exclusively fed with *C. taxifolia*, the number of eggs per spawn was estimated, based on data from 35 individual spawns for which the eggs were counted either directly using a binocular microscope or indirectly from enlarged photographs. The spawning frequency for *E. subornata* was determined based on 20 individuals placed in 10 tanks at 17 and 21°C for a period of 91 d and at 25°C for 170 d. In addition, a group of 24 individuals kept at 25°C was used to evaluate the evolution in spawning frequency every 10 d up to a total of 170 d.

#### Survival of juveniles

In order to observe if a monospecific diet could influence the survival of *E. subornata*, the hatching and development of juveniles was observed for the first 30 d of their life in an aquarium kept at 25°C. Furthermore, survival of embryos and post-metamorphic larvae coming from five egg masses was assessed over a 240 d period at 25°C. To collect and study the egg masses without damaging

the eggs, transparent plastic wrap were placed along the walls of the rearing tanks (where *E. subornata* used to spawn). The survival curve of *E. subornata* is estimated by the equation:

$$N = N_0 e^{-m(\Delta t)} \quad (1)$$

where  $\Delta t = t - t_0$ , N = number of individuals at time  $t$ ,  $N_0$  = number of individuals at time  $t_0$  and  $m$  = instantaneous mortality rate (Frontier & Pichod-Viale, 1993).

#### Growth

Growth of *E. subornata* fed with *C. taxifolia* at 17, 21 and 25°C was estimated by regularly measuring the length of 20 individuals distributed into 10 tanks for each experimental temperature. For each of the temperatures examined, the size–age curves were drawn to assess the minimal size at reproduction.

#### Identification and quantification of caulerpenyne, main toxin of *C. taxifolia*, stocked in *E. subornata* fed with this alga

Four drops of  $C_6H_6$  were applied to five *E. subornata* to allow the mucus and body to be separated. The weight of each asoglossan was taken prior to separation by blotting on an absorbent paper and then the separated body and mucus were weighed. Each sample was extracted with 5 ml of methanol (MeOH). 500  $\mu$ l of both separate body and mucus methanolic extracts were added to 600 mg Sep-Pak Waters™ silica columns, and the resulting fluid was dried by nitrogen flux. The column was eluted twice with 5 ml hexane-ethyl acetate 95:5, resulting in the collection of samples V1 and V2. The solvent was evaporated from each sample using a vacuum with a nitrogen flux, and the dried extracts were re-dissolved in 5 ml hexane-ethyl acetate 95:5. High pressure liquid

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chromatography (HPLC) analyses of V1 and V2 samples were carried out on a Varian 54 Vista HPLC using a Chrompack™ silica column (5 µm, 100×3 mm) eluted with the mixture of solvents C<sub>6</sub>H<sub>14</sub>-EtOAc 95:5. The results were analysed using a Varian UV detector and a Pye Unicam Diode Array Detector (DAD) in conjunction with a Dell computer and compared with results using a previously prepared caulerpenyne standard. Caulerpenyne quantification was carried out according to Amade & Lemée (1998).

Prior to HPLC quantification, the presence of caulerpenyne in body and mucus of *E. subornata* extracts was ascertained by thin layer chromatography (TLC) (R<sub>f</sub>=0.35 on silica plate eluted with hexane-ethyl acetate 80:20) mass spectrometry coupled with HPLC (ESI, MS-MS, Finnigan).

To evaluate the relationship between the caulerpenyne contents in algae and ascoglossans, 21 *E. subornata* pooled in three samples were blotted on absorbent paper, weighed, and placed in three tanks. An additional tank with only *C. taxifolia* was maintained as a control to quantify the caulerpenyne in the alga. The tanks were maintained at 25°C, 38 psu salinity and 5 or 10 g of fresh frond *C. taxifolia* from Cap Martin was added every 3–5 d. Every 2 weeks, all of the living *E. subornata* were removed from one of the tanks, as were 5 g of *C. taxifolia* from the control tank, and these were blotted on absorbent paper, weighed then extracted to allow the caulerpenyne quantification in HPLC.

## RESULTS

### *Consumption of different marine plants*

*Elysia subornata* incised four of the 10 taxa of the marine plants tested (Table 1). But evidence of feeding was only detected for two species: *Caulerpa racemosa* (Forsskål) J. Agardh and *Caulerpa prolifera* (Forsskål) Lamouroux (Figure 1B, Table 1). Only 30% of the *E. subornata* individuals tested survived when fed a diet made up of *C. prolifera* exclusively, whereas 100% of individuals tested survived when fed only *C. racemosa*. Incisions were observed on the Ulvaceae, *Enteromorpha compressa* (Linnaeus) Nees and *Ulva* sp. (Figure 1C), but the ascoglossans were unable to survive beyond 40 d when exclusively fed these algae. In the presence of the non-incised algae, *E. subornata* only survived 25 d (Table 1). Deprived of all food sources, *E. subornata* survived up to 30 d at 25°C (Table 1).

### *Consumption of C. taxifolia*

*Elysia subornata* makes incisions on all parts of *C. taxifolia*. It perforates the cell wall with its uniserial radula and sucks up a small portion of the algal contents, leaving light coloured markings on the alga. On this portion, necrosis rapidly follows (Figure 1D).

The grazing rates correspond to the destruction of 5–6 cm frond.d<sup>-1</sup> at 21°C (56.44 2.90 incisions ind<sup>-1</sup> d<sup>-1</sup>) and a 8–9 cm frond d<sup>-1</sup> at 25°C (81.50 3.60 incisions ind<sup>-1</sup> d<sup>-1</sup>) (Table 2A). Temperature influences the grazing activity (Kruskal–Wallis test, Table 2B) and temperatures have different effects on the grazing (Table 2C).

### *Life history*

The main events of the life cycle of *Elysia subornata* are summarized in the Figure 2.

### *Reproduction*

*Elysia subornata* laid its egg masses as well on the tank wall as on the *C. taxifolia* frond (Figure 1E). The mean number of eggs per egg mass was in the order of 385 ± 48 (N=35). *Elysia subornata* does not spawn at 17°C but releases 0.7 and 1.5 spawns per week at 21 and 25°C, respectively (1 spawning event every 2–12 d). At 25°C, spawning begins 75–80 d (when *E. subornata* reaches 25 mm in length) after hatching until the death of the individuals (240 d: maximum life observed). The frequency of egg mass deposition varies with the age of the animal, from 0.3 to 1.5 egg mass per week between 75 and 125 d after hatching. Then, the frequency of egg deposition reaches a maximum between 126 and 175 d (1.6–2.1 egg masses per week) and decreases between 176 and 240 d (from 1.7 to 0.1 egg masses per week). The first sterile spawns appear after the 145 d. Four individuals, isolated just after a mating period, continued to spawn for three months.

### *Hatching and survival of juveniles*

Hatching of *E. subornata* began 13 d after a spawning event and lasted between 3 and 19 d. For all of the 20 spawning events examined, the appearance of eyes always occurred between 6 and 8 d. By day 17, two-thirds of the

**Table 2.** Grazing rate of *Elysia subornata* on *Caulerpa taxifolia* at different temperatures

#### A. Numbers of incisions (ind<sup>-1</sup> d<sup>-1</sup>)

Temperature (°C)	Mean	SE	Range	N
17	17.83	1.70	0–86	115
21	56.44	2.90	0–136	115
25	81.50	3.60	3–208	115

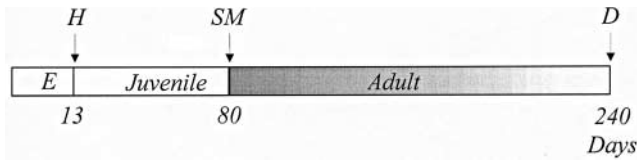
#### B. Results of the non parametric Kruskal–Wallis test

Temperature (°C)	Average rank
17	9253.0
21	22219.5
25	28212.5
H-statistic	164.2
P	0.0

#### C. Results of the SNK test on the comparison

	17°C	21°C
21°C	***	
25°C	***	**

N, number; SE, standard error; \*\* and \*\*\*, denotes difference at  $P < 0.005$  and  $P < 0.001$



**Figure 2.** Life cycle of *Elysia subornata*. E, embryonic phase; H, hatching; SM, sexual maturity; D, death.

individuals had hatched. Generally, the juveniles hatched but remained within the spawning envelope, which they subsequently pierced starting from the external side. Occasionally, the membrane was accidentally ripped leading to the release of a large number of juveniles. Juveniles located towards the middle of the sac experience greater difficulty in escaping and sometimes become trapped in the structure. Upon release, the post-hatched benthic juveniles (Figure 1F), shaped like adults, move directly towards *C. taxifolia*. After 3 or 4 d of existence among *C. taxifolia*, the juveniles take on a green colour

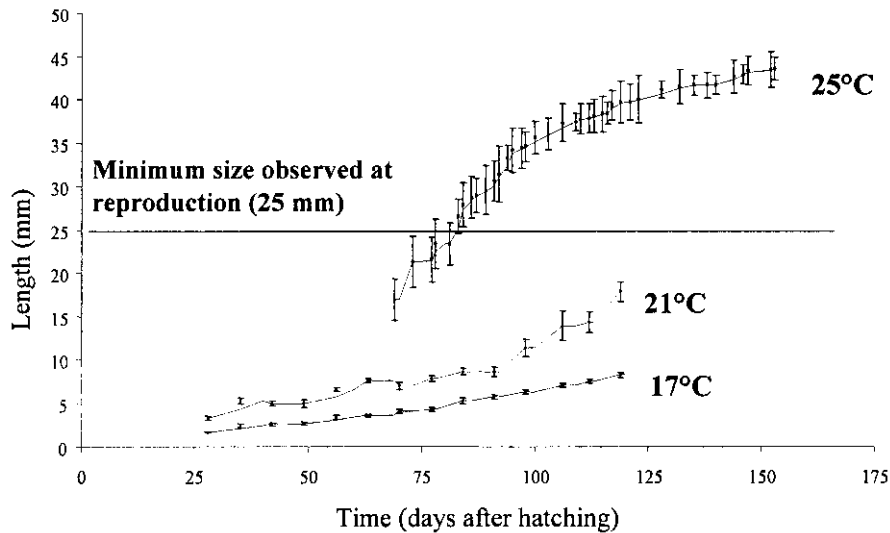
indicating the beginning of plastid retention. At 21°C, the incubation period of eggs is slightly longer than that observed at 25°C (hatching occurs at around 16 d). In the aquarium experiments, 58% of eggs generated living post-hatched larvae but only 50% of these were still alive 20 d later (Figure 3).

*Growth*

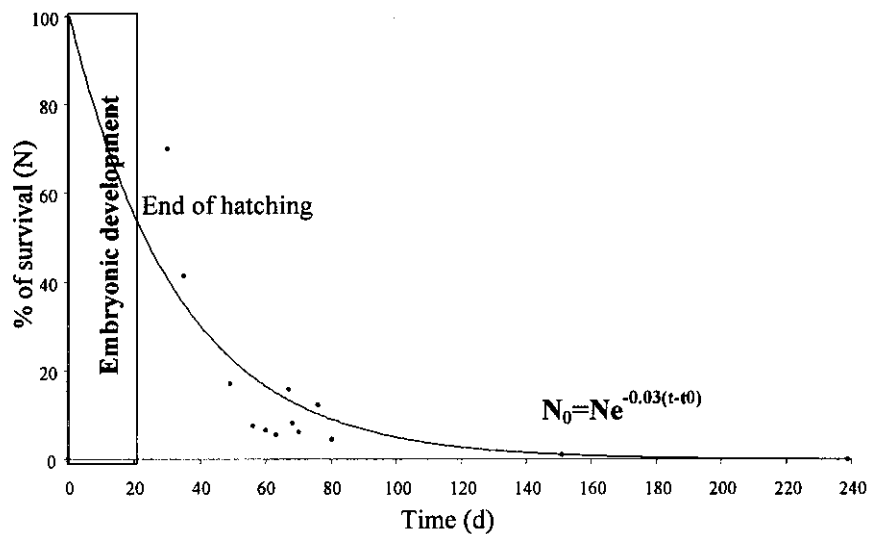
Temperatures of 15°C and lower are lethal for *E. subornata*. Growth was monitored at 17, 21 and 25°C. (Figure 4). Because of technical problems the survey of the growth at 17 and 21°C was stopped at 120 d. At 17 and 21°C reproduction was never observed.

*Identification and quantification of caulerpenyne*

The identification of caulerpenyne in the mollusc extracts was carried out by comparing the spectra



**Figure 3.** Percentage of survival of *Elysia subornata*.



**Figure 4.** Growth curves of *Elysia subornata* from hatching at 17, 21 and 25°C.

obtained in the corresponding analysis (HPLC, MS-MS and TLC) with pure caulerpenyne and based on previously published data (Guerrero et al., 1992). HPLC quantification in the mucus and body extracts of five molluscs indicated that more caulerpenyne was present in the mucus ( $1.13 \pm 0.27$ ,  $N=5$ ), than in the body ( $0.26 \pm 0.09$ ,  $N=5$ ). The wet weight of mucus represented one quarter of the total animal wet weight (total animal =  $308.2 \pm 102.8$  mg ww,  $N=5$ ; body =  $221.0 \pm 76.4$  mg ww,  $N=5$ ; mucus =  $76.8 \pm 21.4$  mg ww,  $N=5$ ).

In feeding experiments, the caulerpenyne amounts in the algae were always found higher than ones in *E. subornata* with a mean ratio of  $2.06 \pm 0.41$  ( $N=3$ ).

## DISCUSSION

### *Feeding, growth, reproduction and survival*

Our studies show that the main limiting factor of the feeding, growth, reproduction and survival of the Caribbean *Elysia subornata* is closely related to water temperature. At  $17^\circ\text{C}$ , *E. subornata* no longer reproduces and at  $15^\circ\text{C}$  it dies. This means it would not survive the winter period in the Mediterranean Sea where the seawater temperature is under  $15^\circ\text{C}$ . In addition, feeding and reproduction are only significant above  $20^\circ\text{C}$ . In the northern Mediterranean Sea the temperature is only above  $20^\circ\text{C}$  for five months (from May to October). This period is too short to obtain a sustainable population from only a few individuals or spawns introduced at the onset of the favourable season. It would therefore be of interest to study other populations of *E. subornata* which are more resistant to the winter temperatures observed in the northern Mediterranean (the strain used in the present study originated from Martinique, a region where water temperatures range from  $27.1$ – $32.8^\circ\text{C}$ ).

All the previous data on the biological characteristics of *E. subornata* deal with individuals living in Florida and fed in aquaria with other *Caulerpa* species than *C. taxifolia*. Thus, a sexually mature Floridean *E. subornata* (25 mm long) can deposit daily around 500 eggs per spawning, whose post-metamorphic larvae hatch around 14 d later (Clark et al., 1979; De Freese, 1988). And although, De Freese (1988) observed that the slightest change in the environmental conditions (food quality, water quality, rearing tank size, etc.) can substantially influence the development of *E. subornata*. So, our laboratory results are in accordance with the previous ones and show the excellent adaptation of *E. subornata* to its new exclusive diet: the aquarium strain of *C. taxifolia* developing in the Mediterranean.

### *Risks assessment*

As far as the risks associated with the introduction of *E. subornata*, four of these are identified below: (i) dietary switching to non-target species; (ii) introduction of pathogens; (iii) competition with the indigenous Mediterranean ascoglossan; (iv) spreading over the Mediterranean.

### *Dietary switching*

The possible use of *E. subornata* in the biological control of *C. taxifolia* in the Mediterranean raises the problem of

dietary switching of *E. subornata* to non-target diet species. Our laboratory studies show that *E. subornata* preferentially feed on coenocytic algae and appears unable to survive on alga other than *Caulerpa* spp. although as already observed with another *Elysia* spp. (*E. halimeda* Macnae) used to feeding exclusively on *Halimeda maculosa* Decaisne (Paul & Van Alstyne, 1988), *E. subornata* is able to graze for a short time on *Enteromorpha compressa* (Linnaeus) Nees but will not survive with it as a main diet.

Within its natural biotope, the diet of *E. subornata* consists of: *C. cupressoides* (Vahl) C. Agardh, *C. mexicana* Kützinger ex Sonder, *C. racemosa* (Forsskål) J. Agardh, *C. paspaloides* (Bory) Greville, *C. sertularioides* (Gmelin) Howe, *C. verticillata* J. Agardh and *C. ashmeadii* Harvey, and an isolated population was observed feeding on *Penicillus dumetosus* (Lamouroux) Blainville (Udoteaceae-Caulerpales) in an area where an acceptable amount of *Caulerpa* spp. was rare (Clark & Bussaca, 1978; Jensen, 1980; De Freese & Clark, 1991).

The shape of the radular teeth of *E. subornata* prevents this organism from piercing either calcified algae, such as the coenocytic Mediterranean Udoteaceae *Halimeda*, or those algae possessing a thick cell wall (Jensen, 1993).

Most ascoglossans need to inquest secondary metabolites from its diet for its own defence (Paul & Hay, 1986) and results show that *E. subornata* can store and use caulerpenyne from *C. taxifolia* as a feeding deterrent. Furthermore, the need to ingest and store resistant chloroplasts to allow individuals to survive if food is lacking (Hinde & Smith, 1974), explain the high degree of stenotrophy of *E. subornata* towards *Caulerpa* sp.

Thus, the possible dietary switches once ascoglossans have been introduced to the Mediterranean should be limited to Mediterranean Caulerpales. Among this family, *C. racemosa* var. *occidentalis* is also considered to be invasive to the Mediterranean (Verlaque et al., 2000). The possibility that *E. subornata* could overgraze the Mediterranean populations of *C. prolifera*, *C. mexicana*, *C. racemosa* var. *lamourouxii* (Turner) Weber van Bosse f. *requienii* (Montagne), *C. racemosa* var. *turbinata* (J. Agardh) Eubank–wifera (C. Agardh) J. Agardh and *C. scalpelliformis* (R. Brown) C. Agardh (these last three species are solely observed along the coasts of a few Mediterranean countries located between Turkey and Tunisia (Meinesz & Hesse, 1991; Verlaque et al., 2000)) remains to be assessed.

### *Competition*

Three ascoglossan species inhabit the *C. prolifera* meadows of the Mediterranean: *Oxynoe olivacea*, *Lobiger serradifalci* and *Cylindrobulla fragilis* Jeffreys 1858 (Murillo et al., 1986). *Elysia subornata* can compete for food with these three species, as two of these, *O. olivacea* and *L. serradifalci*, are known to eat *C. taxifolia* (Thibaut & Meinesz, 2000). It would therefore appear that only total control of the *Caulerpa* spp. present in the Mediterranean by *E. subornata* would endanger the native ascoglossan populations. Total eradication of all the *Caulerpa* spp. by biocontrol is not ecologically possible according to the optimal foraging theory (Hugues, 1980) where *E. subornata* would leave patches of *Caulerpa* before complete depletion.

### Pathogen introduction

The possibility of introducing a pathogen to the Mediterranean species of the *Elysia* genus (*E. timida* Risso, *E. viridis* Montagu=*E. translucens* Pruvot-Fol, *E. flava* Verrill and *E. gordanae* Thompson & Jaklin) should be considered. Although various intracellular symbionts were observed (McLean, 1978) and that an endogenous viral expression in *E. chlorotica* Gould leading to an annual mass mortality of this species (Pierce et al., 1999), no pathogen transmission between species was recorded. To minimise this risk, specimens of *E. subornata* were obtained from eggs reared for two generations in an axenic medium (20 mg l<sup>-1</sup> of Polymixin B) with the destruction of all preceding generations. This rearing protocol is in accordance with the ICES guidelines pertaining to the introduction of an exotic species (ICES, 1997). In addition, rearing of several generations in an aquarium over an extended period of time (more than one year) acts as a period of quarantine to eliminate all potential parasites.

### Spreading

The risk of a man-mediated displacement of *E. subornata* populations from the Mediterranean towards the Red Sea should be considered (competition with Indo-Pacific ascoglossan species which feed on other species of *Caulerpa*).

## CONCLUSION

There are no universal and reliable rules which can predict whether a biocontrol organism will be effective (Carlton, 1997). However, fundamental guidelines for marine biocontrol programs can ensure the quality of data for both the biocontrol and target species. Following this principle, we have collected data showing that *Elysia subornata* could be a promising biological agent, if a suitable cold resistant strain is found, in the struggle to control the expansion of *Caulerpa taxifolia* in the Mediterranean Sea. To our knowledge, this species, along with *Oxynoe azuropunctata*, is the only ascoglossan to feed exclusively on caulerpales while at the same time lacking a pelagic larval phase (brevipelagic development for *O. azuropunctata* and direct development for *E. subornata* (Clark et al., 1979; Clark & Jensen, 1981). This type of development should allow for a rapid increase in population densities following the release of only a few individuals or spawn onto a *C. taxifolia* meadow. In addition, this form of benthic development allows *E. subornata* to be reared fairly easily and at a low cost. The quantities of *C. taxifolia* eaten by *E. subornata* are 2–11 times higher than those recorded for the Mediterranean ascoglossan species (Thibaut & Meinesz, 2000) and 5–8 times greater than for *O. azuropunctata* (Meinesz et al., 1996). The risks involved following the introduction of this species into the Mediterranean Sea seem to be low. Additional behavioural studies involving large mesocosms coupled with computer modelling (Hill et al., 2000) can detect some artefacts of the results found in small aquaria and should allow a more accurate assessment of the probability of success of a cold resistant strain of *E. subornata* as a biological control agent

before authorized field experiments. The true risks associated with the continued spread of *C. taxifolia* throughout the Mediterranean must also be considered in the decision to proceed with *in situ* trials in the Mediterranean. According to the recommendations of the Working Group on Introduction and Transfers of Marine Organisms, which is dependent ICES, a working group on this subject should be set up in order to advise on the feasibility of such a biological control (ICES, 1997).

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