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Simulation of the mollusc Ascoglossa *Elysia subornata* population dynamics: application to the potential biocontrol of *Caulerpa taxifolia* growth in the Mediterranean Sea

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Abstract

A multi-modelling simulation was performed to assess the potentialities of a biocontrol of the alga *Caulerpa taxifolia* (Valhl) C. Agardh by the Ascoglossa *Elysia subornata* (Verrill, 1901) in the Mediterranean Sea. With this aim in view, the biological and ecological parameters considered as key factors were identified in order to present a state of the art on biological and ecological parameters related to the behaviour of *E. subornata* toward *C. taxifolia* and toward the Mediterranean conditions. To this end, growth, survival, reproduction, feeding on *C. taxifolia* and foraging of *E. subornata* are studied. Simulations, taking into account spatial effects, give encouraging results and show that additional experiments in large mesocosm may be engaged to improve the actual knowledge of *Elysia* behaviour, the impact on *C. taxifolia*, the trophic relationships, etc. The results of simulations demonstrate that the greatest impacts on *Caulerpa* are obtained using either some adults or mixing adults and juvenile slugs. In any case, better results are obtained by a scattering of slugs on isolated spots rather than on clusters (with constant surfaces). Lastly, the choice of a suitable date for scattering increases the weak consumption of *Caulerpa* resulting from the scattering of juvenile slugs.

Keywords: Multi-modelling simulation; *Elysia subornata*; *Caulerpa taxifolia*; Biological control

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1. Introduction

Four species of gastropod molluscs (Ascoglossa) are known to be specific grazers of *Caulerpa taxifolia* (Valhl) C. Agardh: *Oxynoe azuropunctata* (Jensen, 1980), *Elysia subornata*

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(Verrill, 1901), *Oxynoe olivacea* (Rafinesque, 1814) and *Lobiger serradifalci* (Calcara, 1840) (Meinesz et al., 1996; Thibaut et al., 1998). Only the unshelled species *E. subornata* is able to store the algal chloroplasts and to keep them functional (Clark et al., 1990). This phenomenon, called kleptoplasty, allows the slugs to forage on long distances (Clark, 1995). Ascoglossan species are able to concentrate secondary metabolites of *Caulerpa* species (such as caulerpenin) as repellent toxins against predators (Paul and Van Alstyne, 1988; Jensen, 1994). Among the four species, the tropical *E. subornata* seems to be the best candidate as an agent of biological control of *C. taxifolia*: indeed this species has a direct benthic development (Clark et al., 1979), allowing it to quickly establish locally dense populations, and it has the highest feeding rate on *C. taxifolia* (Thibaut et al., 1998). Lastly, *E. subornata* dies at temperatures lower than 15°C (Meinesz et al., 1996).

Since 1998, it is clear that *C. taxifolia*, a green tropical alga of aquarium origin (Jousson et al., 1998), was introduced by accident in Monaco on the French coast of the Mediterranean Sea in 1984 (Meinesz and Hesse, 1991). Sixteen years later, it has spread to six countries (France, Monaco, Italy, Spain, Croatia and Tunisia) and colonized more than 5000 ha (Meinesz et al., 1998; Langar et al., 2000) from 0 to100 m in depth (Belsher et al., 1999). This alga exhibits some particular biological and physiological features: resistance to low temperature, gigantism of the thallus, high growth rate which differentiate it from the tropical strain (Meinesz et al., 1995). The invading, dominant and tenacious characteristics of *C. taxifolia* lead to the disruption of the ecosystems that it invades (Verlaque and Fritayre, 1994; Villele and Verlaque, 1995; Poizat and Boudouresque, 1996; Villele and Verlaque, 1995; Francour et al., 1995; Bartoli and Boudouresque, 1997). The scientific community has pointed out the intense and irreversible modifications or the elimination of endogenous benthic communities and several times urged the governments of involved countries to engage additional research from the biological point of view as well as the development of methods able to control the amazing expansion and growth of the alga. In addition, social and economic repercussions of such a phenomenon cannot yet be accurately evaluated but will probably become critically important during the next decades through modifications of fishing catches and a negative effect on tourism.

Since 1994, different chemical (Escoubet and Brun, 1994; Jaffrenou and Odonne, 1996; Gavach et al., 1999) and physical (Riera et al., 1994; Avon et al., 1996; Cottalorda et al., 1996) attempts have been done to eradicate *C. taxifolia*. Unfortunately, all these techniques are only efficient on small flat surfaces. According to Lafferty and Kurris (1996), when classical approaches to eradication are unsatisfactory, the use of biological control can be considered. However, the introduction of an exotic species must only be considered after numerous and careful investigations. Before beginning any expensive experiments in a large mesocosm, we needed to assess the potentialities of this approach with computer simulations. To reach this goal, we firstly identified the biological and ecological parameters considered as key factors in order to present a state of the art on biological and ecological parameters related to the behaviour of *E. subornata* toward *C. taxifolia* and toward the Mediterranean conditions. To this end, growth, survival, reproduction, feeding on *C. taxifolia* and foraging of *E. subornata* were studied.

The main goal of the simulation model we present is to simulate over one year the impact on *Caulerpa* biomass of foraging and feeding activities of introduced populations of *E. subornata* on small surfaces (≤ 1 ha), taking into account spatial effects (migration of individuals) and several biological characteristics of this species. The results provided by simulations would help to evaluate the feasibility of *Caulerpa* growth biocontrol by this mollusc and to reach the decision to carry out additional experiments on larger scales. In this paper, we briefly present the biological and ecological results we obtained through experiments, the main characteristics of the model and some significant simulation outputs.

2. Material and methods

The design of our biocontrol simulation has been led in a hierarchical way. Sub-models were considered for individual growth, survival, reproduction, feeding rate and foraging activity of *E. subornata*. To feed such sub-models, few parameters were required (see next section) and were investigated.

To test the hypothesis of temperature-dependence of *E. subornata* growth, regular measures of the length of 20 individuals (2 individuals per tank) at 17 and 21°C were carried out during 91 days (3 months). Growth at 25°C was studied by Clark et al. (1979) and Thibaut et al., 2000. The survival of *E. subornata* was studied by us and we drew up the survival curve at 25°C from these unpublished works.

We followed the reproduction pattern at 19 and 21°C.

Previous works have demonstrated that the feeding rate is temperature dependant (Meinesz et al., 1996).

The foraging activity of *E. subornata* was studied in a large aquarium (3 m^3). Patches of 100 g of *C. taxifolia* were placed on one side of the aquarium and 15 *E. subornata* individuals were released in the middle of the aquarium 75 cm away from *C. taxifolia*. The positions of the slugs were recorded 1 and 2 h later for each test. Results were analysed by means of a chi-2 test.

The annual *Caulerpa* growth was drawn up from recent works (Meinesz et al., 1995) and from a sampling campaign achieved on several stands and for several depths in 1998 and 1999.

3. Modelling elements

In this preliminary model, we took into account the following environmental conditions: water temperature and *C. taxifolia* biomass (substrata and light will be included in further developments). Spatial effects are integrated in the model, first using maps situating *C. taxifolia* and *E. subornata* and secondly by the modelling of the individuals migration.

In summary, the main features of the simulator are the following:

1. The abstraction level of the model (= studied scale) is the population and the detail level is the *E. subornata* individual (12 states; see Section 4.1.3). However, specialized sub-models present different levels of abstraction.
2. The time step is a constant and corresponds to the main events of *E. subornata* lifecycle. We chose a time step of 20 days which represents the delay of embryonic development (De Freese, 1988). The simulation runs for 1 year and starts when water temperature is 15°C (approximately on April the 15th), lower temperatures being lethal for *E. subornata*.
3. The model runs on small maps indexed by a grid with a maximum of 100 × 100 cells, where each cell has a surface area of 1 m^2. A vector V^t_{ij}, describing the local age structure of slugs is associated to each cell (i, j). The variation of the number of individuals on a cell is computed using a Leslie matrix M_t, with the following equation:

$$V^{t+1}_{ij} = M_t \cdot V^t_{ij} \tag{1}$$

3.1. Parameters

Biological parameters were modelled either as deterministic or stochastic processes depending on their complexity.

1. Deterministic:
   - ○ Growth of *C. taxifolia*. The biomass surface ratio follows the natural annual variation (summer growth and winter degeneracy) described by Meinesz et al. (1995). However, the dispersal of *C. taxifolia* cuttings was not taken into account. Consequently, the increase of biomass induced by the first year cuttings was considered as negligible, leading to slightly overestimate the impact of slug feeding over the simulated period (1 year).
   - ○ Growth and feeding of *E. subornata* are modelled using analytic equations.

  - ○ *E. subornata* reproduction and transition from one age class to the next use a non-homogeneous Leslie matrix (with 12 states) M_t, fertilities F_i are computed at each simulation time step t as functions of temperature.

2. Stochastic:
   Although some experiments on the foraging of *E. subornata* gave significant results (a chi-2 test at a confidence level of 99.9%), reliable and satisfactory rules of migration could not be inferred about the behaviour of *E. subornata* in very large mesocosms or complex environments of natural areas (Meinesz and Thibaut, 1998). In such conditions, we adopted in a first approach a simple diffusion model in which direction and distance are randomly chosen, the foraging of adults (juveniles are unable to migrate) obeying the gaussian statistic of (m,σ) parameters. In our case, the maximum distance of migration for individuals was fixed at about 10 m ($\sigma = 3.3$ m). In the model, the success of migration depends on the following three rules:
   - ○ If a slug migrates within a radius smaller than 0.4 m, it dies, because we consider that it does not move significantly enough to survive.
   - ○ If a slug migrates out of the simulated area, the model cancels it.
   - ○ If a slug migrates to a plot and induces over-grazing or migrates to a plot which is already over-grazed, it dies.

Finally, tropical field observations showed that the maximum number of adults/m^2 is about 214 individuals (Clark and De Freese, 1987). We retained this limit as the maximum number of individuals, which could stay and feed on a plot with maximum *Caulerpa* biomass. Consequently, we retained the ratio $214/2000 \approx 0.107$ individuals. g^{-1} m^2 of *taxifolia* as the threshold value. A higher value on a spot induces the migration of slugs (randomly chosen among the adult age classes) until the ratio reaches a value inferior to 0.107.

3.2. Simulation technique and implementation choices

Models composed of other models are called multimodels (Fishwick, 1993, 1995). As exposed above, our model mixes several specialized models at different abstraction levels. In fact, multi-modelling is an extension of combined modelling, where the model combines continuous and discrete event processes (Zeigler, 1976; Kleijnen and Groenendaal, 1992; Fishwick, 1995; Hill, 1996). Lastly, because we mix deterministic and stochastic processes, the resulting process is stochastic. We retained discrete event simulation as our main implementation technique, which is the most appropriate to simulate the intrinsic parallelism between the involved biological processes as well as the discrete influence of spatial constraints in their interactions (Coquillard and Hill, 1997). To match the biological data, the kernel is clock-based, the time step being equal to 20 days.

The fact that we have a stochastic model implies the computation of numerous runs within the same experimental framework which is classically called the replication technique (Shannon, 1975; Kleijnen, 1987). Consequently, results are computed as means with confidence intervals. Geographic results (repartition of slugs and *Caulerpa* biomass) are presented as discrete spectral analysis where, for each time step and each plot, the state of a single variable is represented as a frequency computed from a large number of replicates (Hill, 1996; Hill et al., 1996, 1998). The pseudo-random generator we used is based on a generalized shift register technique, shuffled with a congruential generator (Leroudier, 1980; L'Ecuyer, 1990; Press et al., 1994).

In order to handle any littoral site of interest, the software is interfaced with a geographical information system (GIS). The software implementing the model is written with the public domain GNU C+ + compiler (2.7.2), on Unix workstation or PC running the Linux O.S. We use X-Windows graphic environment (X11R6) and a graphic user interface was built using the Gtk + library for the management of experiment files, statistical data, geographical data, results .... Fig. 1 presents a simplified chart depicting the simulation model functioning.

4. Model calibration

4.1. Study of Elysia subornata

In this section, we briefly present the results obtained in an experimental mesocosm and the equations obtained by fitting the data. These equations were incorporated in the model. Further details on these experiments can be found in Thibaut et al., (2000).

4.1.1. Growth at different temperatures

Experimental curves of *Elysia* growth demonstrate the strong influence of temperature. The curves were very well fitted by Von Bertalanffy's equation:

$$L_t = L_{\max}(1 - e^{-kt})^c \qquad (2)$$

where L_t = length (mm) at instant t, $L_{\max} = 55$ mm asymptotic size of *E. subornata*, $c = 1$, coefficient of increase in length and k = instantaneous growth rate. k is a function of temperature.

A regression curve on experimental data (Table 1) gives estimated k value at different temperatures (Fig. 2).

$$k = 10^{-5}\ e^{0.275T}\ (R^2 = 0.95) \qquad (3)$$

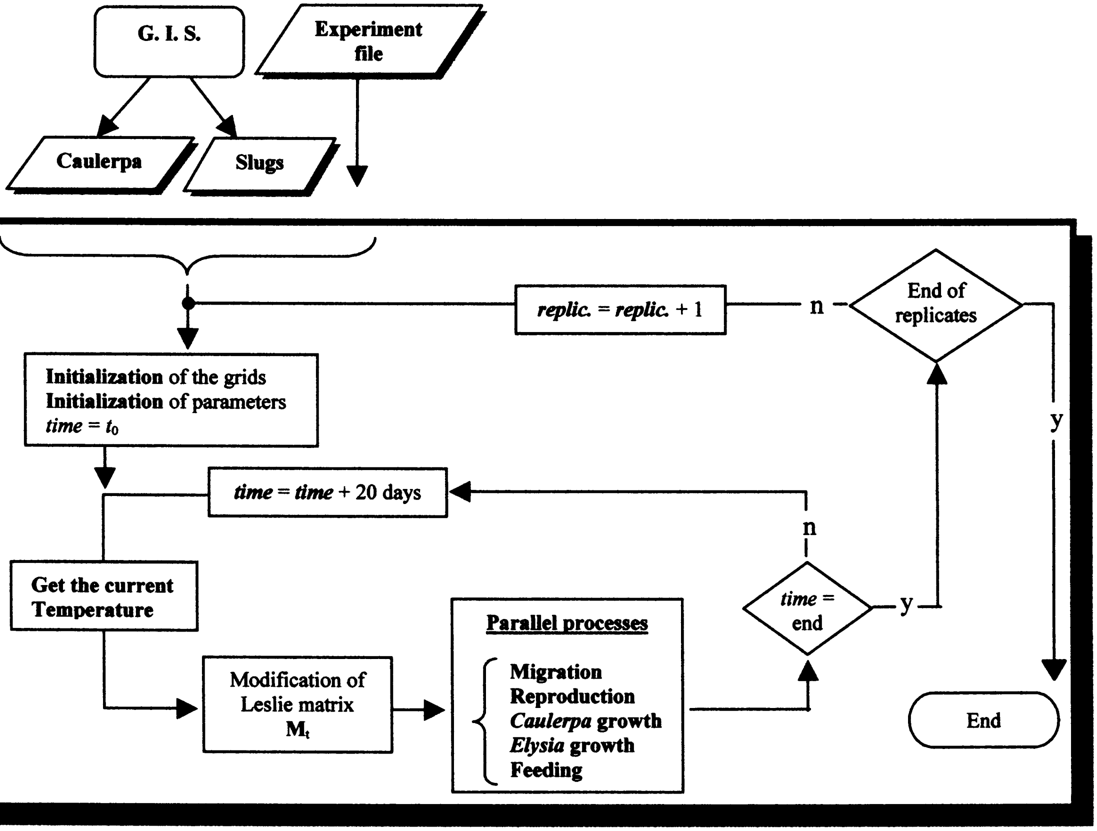

Fig. 1. Simulation functioning (simplified).

Table 1
Estimated k coefficient

t (°C)	17	18	18.5	19	21	22	23.5	25
k (estimated)	0.0011	0.0014	0.0016	0.0019	0.0032	0.0042	0.0064	0.0097

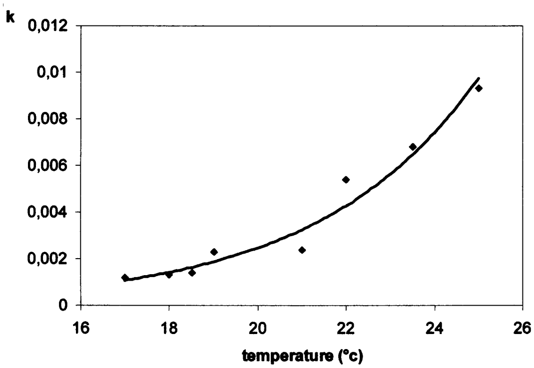

Fig. 2. Estimation of k = instantaneous growth rate from experimental data.

Finally, using Eq. (3), Eq. (2) can be written:

$$L_t = 55\,(1 - e^{-(10^{-5}e^{0.275T})t}) \tag{4}$$

The average increase in size of individuals belonging to the nth class of age over a period of 20 days is calculated from Eq. (5) (see Appendix A):

$$E[\Delta L_n] = \sum_{j=0}^{19} E[\Delta L_n^j], \tag{5}$$

where $E[\Delta L_n^j]$ is the average daily increase of individuals. As for the initial size of slugs scattered in the simulation, the values are computed on the basis of the average size reached — for each involved age class — in optimal conditions, i.e. at temperature of 25°C and non-limiting feeding resources.

4.1.2. Survival

Mortality is very high during larval development: only 48% of larvae hatched. The maximum lifespan observed is 240 days. We fitted the classical survival equation:

$$N = N_0 e^{-m(t-t_0)} \tag{6}$$

where N is the number of individuals at instant t, N_0 is the number of individuals at instant t_0 and $m = 0.04$ is the coefficient of mortality.

4.1.3. Reproduction rate

Experiments showed a strong relationship between reproduction rates and water temperature (and consequently their size). *E. subornata* laid 2.2 egg-mass per 20 days at 21°C and according to Thibaut et al. (1999), 4.2 egg-mass per 20 days at 25°C. As the population was divided in age classes, polynomial regression curves were applied to sexually mature classes in order to give the coefficients of fecundity for each class at different temperatures. Because of the hermaphrodism of *E. subornata*, each individual can reproduce. According to De Freese (1988) and Thibaut et al. (2000) who estimated the smallest size for reproduction at 25 mm, an individual of adult age class can only reproduce in favourable temperature and size conditions.

Let F_i be the fertility (expressed in number of eggs) of the i age class. We obtained from the data the following linear regressions ($R^2 = 0.98$):

$$F_5 = 17.25T - 284.46, \tag{7}$$

$$F_6 = F_7 = 24.069T - 396.9, \tag{8}$$

$$F_8 = F_{10} = 30.886T - 509.32, \tag{9}$$

$$F_9 = 32.566T - 537.02, \tag{10}$$

$$F_{11} = 15.504T - 255.66, \tag{11}$$

$$F_{12} = 8.718T - 143.76, \tag{12}$$

where T is the temperature at the instant t.

From the above results (Section 4.1.2 and Section 4.1.3), we established the Leslie matrix (Fig. 3) which was integrated in the model.

4.1.4. Feeding on C. taxifolia

Fig. 4 clearly shows the influence of temperature on the grazing activity of *E. subornata*. Impact on *C. taxifolia* would be higher during the warm season. As *E. subornata* dies at 15°C, the biological control starts in April and ends in November. Therefore, a polynomial regression curve gives the feeding rate at different temperatures.

$$C_{\mathrm{mT}} = -42.4984 + 4.19321\ T - 0.0867304\ T^2 \quad \text{with } R^2 = 0.99, \tag{13}$$

where C_{mT} is the average feeding of an adult of maximum length at temperature T, in g/20 days. From this result, the feeding of individuals of any

$$
M_t = \begin{bmatrix}
0 & 0 & 0 & 0 & F_5 & F_6 & F_7 & F_8 & F_9 & F_{10} & F_{11} & F_{12} \\
0.47 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0.43 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.45 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0.45 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.5 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0.4 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0.44 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.43 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.4 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.67 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.67 & 0
\end{bmatrix}
$$

Fig. 3. Leslie transition matrix M_t. Fertilities F_i are updated at each time step simulation using Eqs. (7)–(12). From left to right: column 1 = eggs; columns 2, 3, 4 = juveniles (larvae); columns 5–12 adults.

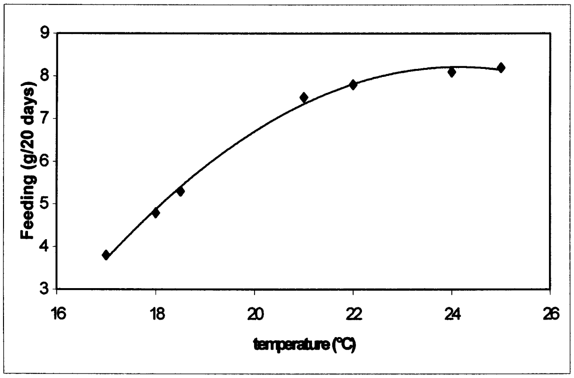

Fig. 4. Feeding of individuals of max length as a function of temperature (after Meinesz et al., 1996).

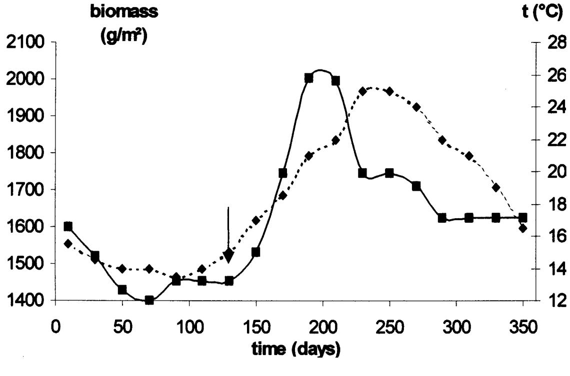

Fig. 5. Annual temperature variation (in dotted line) and annual *Caulerpa* biomass variation in g (f.w.)/m^2 (from January, the first). Temperature data gracefully provided by the Observatoire Zoologique de Villefranche-sur-mer (1995–1998). The arrow indicates the starting date of simulations.

length can be computed as a simple proportion of C_{mT}

$$C_{i\mathrm{T}} = (L_{i\mathrm{T}}/L_{\max})C_{\mathrm{mT}}, \tag{14}$$

where $C_{i\mathrm{T}}$ and $L_{i\mathrm{T}}$ are respectively the average feeding and the average length of an individual belonging to the *i* age class at temperature *T* and $L_{\max}$ the average length of individuals of maximum length.

4.2. Variation of Caulerpa biomass

The *Caulerpa* biomass is supposed to be in a steady state in the model, i.e. it has already reached the equilibrium point. However, the live biomass exhibits some seasonal temperature-dependent variations showing a winter degeneracy and a spring–summer growth. The relative variations were calculated from Meinesz et al. (1995) and applied to measurements taken in Villefranche bay (Fig. 5). The maximum biomass recorded in summer was 2000 g m^{-2} (f.w.).

5. Results

5.1. Required number of replicates

The code verification and the consistence of the conceptual model were achieved by means of numerous simulations with large numbers of replicates. No singular points or brutal bifurcation or abnormal behaviour appeared. The results we obtained by means of 350 replicates performed within a single experimental frame indicated that the *Caulerpa* biomass was estimated with some acceptable confidence intervals (Table 2).

According to Kleijnen (1987), if the number *n* of replications is great enough, the half-width of the confidence interval is approximately equal to $1.96\sigma/\sqrt{n}$ (for a confidence level of 95%). Then if we accept an uncertainty *E* of 5‰ ($\approx$ 17.68 kg) on the estimation of the residual biomass, the minimum number of replications is:

$$n = \mathrm{INT}[(1.96 \times 41.68/17.68)^2] + 1 = 22,$$

Table 2
Variation of *Caulerpa taxifolia* biomass through one simulated year (100 slugs spread)[a]

t (days)	biomass (m)	σ	σ/m	CI$_1$ $\alpha = 0.05$	CI$_2$ $\alpha = 0.01$
20	3630.00	0.00	0.00	0.00	0.00
40	3828.02	0.00	0.00	0.00	0.00
60	4356.01	0.00	0.00	0.00	0.00
80	4991.00	3.49	0.00	0.40	0.52
100	4955.71	13.16	0.00	1.49	1.96
120	4320.99	0.66	0.00	0.07	0.10
140	4308.04	21.89	0.01	2.48	3.26
160	4202.22	36.21	0.01	4.10	5.39
180	3972.54	42.32	0.01	4.79	6.29
200	3961.35	45.17	0.01	5.11	6.72
220	3956.06	46.37	0.01	5.25	6.90
240	3954.64	46.61	0.01	5.27	6.93
260	3890.34	45.86	0.01	5.19	6.82
280	3697.42	43.58	0.01	4.93	6.48
300	3472.36	40.93	0.01	4.63	6.09
320	3407.81	40.17	0.01	4.55	5.97
340	3536.42	41.68	0.01	4.72	6.20
360	3536.42	41.68	0.01	4.72	6.20

[a] CI$_1$ and CI$_2$ are radius of confidence intervals. (SD = t_0+60; see text explanations for SD). All results expressed in kg (f.w.).

where INT[x] is the integer part of a real number x.

Consequently, 22 replicates are sufficient to obtain satisfying results on biomass.

5.2. Model behaviour and sensitivity

Then, we carried out a series of simulations where the initial parameters were fixed except the date of slug scattering (SD) which appeared in preliminary simulations as one of the most sensible parameters. SDs were chosen as following: $\{t_0, t_0 + 20, t_0 + 40, t_0 + 60, t_0 + 80\}$. Slugs were scattered on 20 spots of 1 m^2 each, chosen at random, on a 50 × 50 m stand entirely colonized by *Caulerpa*. On each of the 20 spots, 100 adult slugs belonging to the 7th class age were released. In this phase, 300 replicates per experiment were done in order to obtain results as accurate as possible.

Fig. 6 depicts the variation of biomass. The best consumption of *Caulerpa* was obtained with $t_0 + 60$. Indeed, an early releasing of animals, i.e. in colder conditions (<21°C), slows down the growth of larvae and delays the reproduction which results in a weak consumption of alga. Inversely, if a later SD authorizes an immediate and high level of reproduction followed by an optimal growth of juveniles, the period of intense activity is not long enough to compensate for the delay in terms of consumption. Using the biomass results, we can calculate the consumption induced per capita of released animals (Table 3).

In a same manner, the area reached by animals considerably varies from one experiment to an-

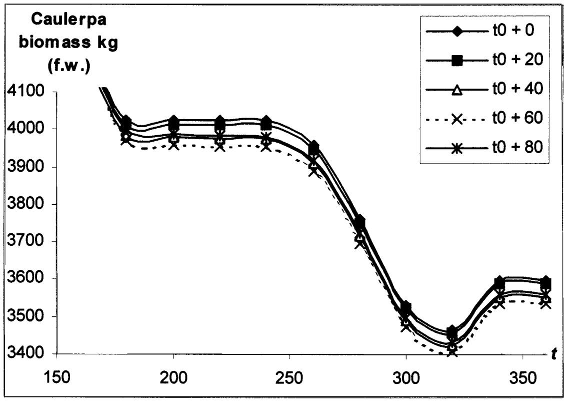

Fig. 6. Variation of *Caulerpa* biomass using several SD. Only the end of the simulation is presented (from $t = 170$ to $t = 360$).

Table 3
Absolute consumption of *Caulerpa* (kg f.w.) and consumption induced per capita (g f.w.). 2000 slugs scattered

SD	t_0	t_0+20	t_0+40	t_0+60	t_0+80
Consumption	31.66	42.47	75.65	93.58	68.58
%	0.87	1.17	2.08	2.58	1.89
Induced per capita	15.83	21.24	37.83	46.79	34.29

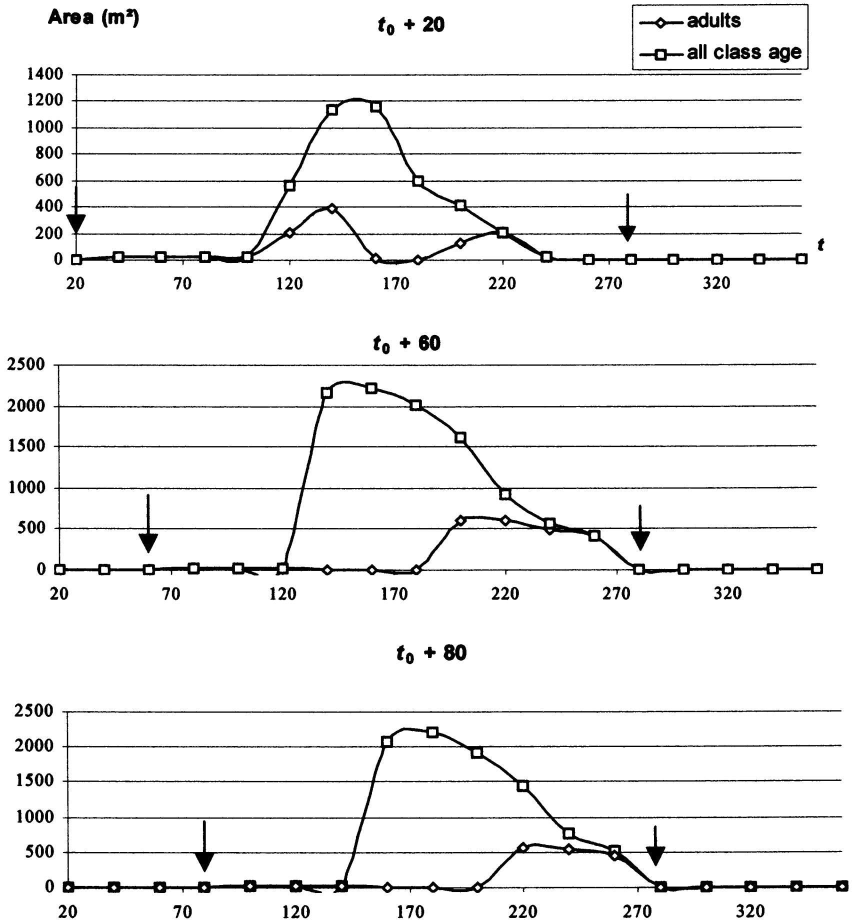

Fig. 7. Areas colonized by animals as a function of time. Arrows indicate respectively SD and the date corresponding to the lethal temperature (15°C). The 'adults' set gathers the eight oldest classes of age.

other. For instance, the maximum area colonized by animals (all class ages gathered) is equal to 416 m^2 when SD is t_0 and 2200 m^2 when SD is t_0+80 (Fig. 7). Also, the time of maximum dispersal of the animals varies as well. Experiments involving early scattering of slugs present two peaks of colonized areas due to the mortality of adults whose number diminishes during the slow growth phase of larvae. In other cases, the maximum values are later reached with successive translations along the time axis.

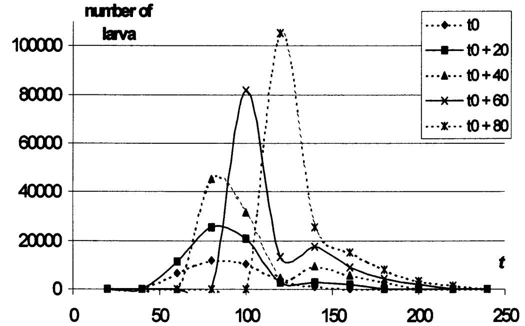

Fig. 8. Number of first class age larvae as a function of time and SD.

Examining the average number of larvae belonging to the first class age, one can see that in most cases only one laying occurs during summer in the temperature conditions of Villefranche-sur-mer. However, two secondary peaks are visible in the case of $SD = t_0 + 40$ and $SD = t_0 + 60$ at time = 140 days (9673 and 17384 new-borns respectively) (Fig. 8). Nevertheless, these new-borns cannot induce any significant impact on the *Caulerpa* biomass and we must consider that the greatest part of consumption is done by animals of the first generation.

The following of cohorts did not reveal any abnormal variation, curve slopes being parallel and obeying the mortality law we established. The number of individuals dramatically decreases, particularly the individuals of cohort 1 and 2 (see cohort 2 on Fig. 9). This is probably due to the mortality resulting in the high level of consumption (larvae cannot forage) induced by both the adults and the great number of larvae.

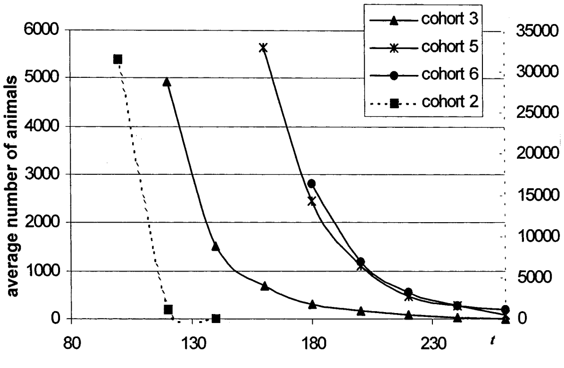

Fig. 9. Variation of the number of animals of four cohorts.

5.3. Exploration of initial conditions

The results above showed that the conceptual model was consistent and that the software was reliable enough. Thus, we designed an experimental protocol in order to explore multiple combinations of initial parameters — varying within realistic intervals — on the same stand we previously used. We retained three main parameters varying on several levels:

1. Initial repartition of slugs. 4000 slugs are scattered on spots of 1 m^2 (200 slugs per spot) using three patterns: 20 spots randomly chosen; 5 clusters of 4 m^2 each uniformly distributed; a single cluster of 20 m^2 at the centre of the stand.
2. SD. We used three dates of slug scattering: $\{t_0 + 40,\ t_0 + 60,\ t_0 + 80\}$.
3. Age of slugs. We retained four cases: all slugs belonging to class 6; all slugs belonging to class 2; slugs shared equitably between classes 2 and 6; slugs shared equitably from class 2 to class 6.

Altogether, 36 experiments of 22 replicates were done (Table 4).

Eight variables were sampled from the results of simulations. They are (1) the total grazed biomass: *Biomass*; (2) the residual numbers of larvae summed over the period 220–260 days (end of the favourable season): *Larva*; (3) the residual number of adults summed over the period 220–260 days: *Adults*; (4) the number of first generation new-born: *New-born*; (5) the maximum area colonized by adults: *AdultArea*; (6) the maximum area colonized: *TotalArea*; (7) the average area colonized over the simulation *AvAreaAll*; (8) the average area colonized by adults over the simulation *AvAreaAd*.

Then, a principal component analysis (PCA) was performed on the correlation matrix of 36 experiments × 8 variables in order to identify the correlations between variables and to estimate the best initial conditions to obtain the maximum impact on *Caulerpa*.

Table 4
Experimental protocol

Pattern	SD	Class 6	Class 2	cl.2 and cl.6	cl.2–cl.6
Random	40	1	10	19	28
	60	2	11	20	29
	80	3	12	21	30
5 × 4 spots	40	4	13	22	31
	60	5	14	23	32
	80	6	15	24	33
1 × 20 spots	40	7	16	25	34
	60	8	17	26	35
	80	9	18	27	36

Components 1 and 2 have respectively 76.5 and 13.5% of the total variance. Thus, we retained these two components for the interpretation of the analysis (Figs. 10 and 11). The correlation circle shows that variables are strongly correlated except for *Larva* and *New-born*. This can be easily interpreted as an effect of temperature which drives the development of juveniles, the grazing of *Caulerpa* increasing in the same way as larva growth (*New-born*). In addition, the number of larvae at the end of a favourable condition period (*Larva*) does not significantly influence the residual alga biomass. A hierarchical classification of second order moment based on Euclidean distance was used to group experiments in subsets. Basically, the first axis separates the experiments initialized with slugs of the second class age from other experiments and represents a positive gradient of biomass consumption. Moreover, the experiments with strong contributions on the right part of F1 were initialized with slugs spread on isolated spots or 5 × 4 clusters. The axis F2 appears as a gradient based on a mixing of age classes and date of scattering (SD). Experiments 1, 4, 19, 22, 28, 31 (negative part) were initialized with $\mathrm{SD} = t_0 + 40$ and slugs belonging to classes 6 or (2 and 6) or (2–6), whereas experiments 3, 6, 9 (positive part) where initialized with $\mathrm{SD} = t_0 + 80$ and slugs belonging only to the 6th class.

As a conclusion to PCA analysis, we can say that the greatest impacts on *Caulerpa* were obtained using either some adults or a mixing of adults and juvenile slugs. In any case, a scattering on isolated spots is preferable to a scattering on clusters. Lastly, delaying SD can compensate the weak impact obtained by the spreading of juveniles.

5.4. Simulation on Le Brusc site

Le Brusc site is a lagoon of about 1 ha which is colonized by *Caulerpa*. In this experiment, we tried to estimate the impact of releasing various quantities (from 50 to 400) of slugs belonging to the 6th class age on 40 isolated spots using $\mathrm{SD} = t_0 + 60$. In spite of the fact that temperature conditions are probably highly favourable to *Elysia* development, we choose to reuse the previous temperature histogram and consequently to run simulations with a pessimistic hypothesis. Fig. 12 depicts the variation of consumed biomass we obtained. The results follow the exponential law ($R^2 = 0.98$):

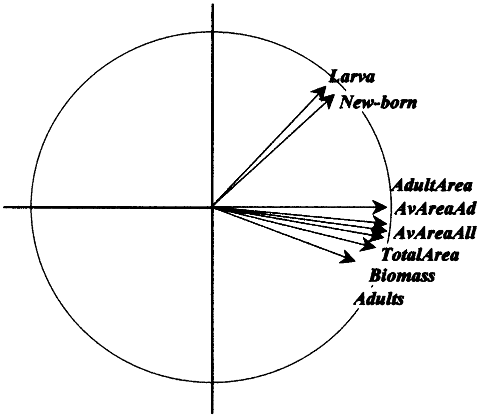

Fig. 10. Correlation circle of PCA.

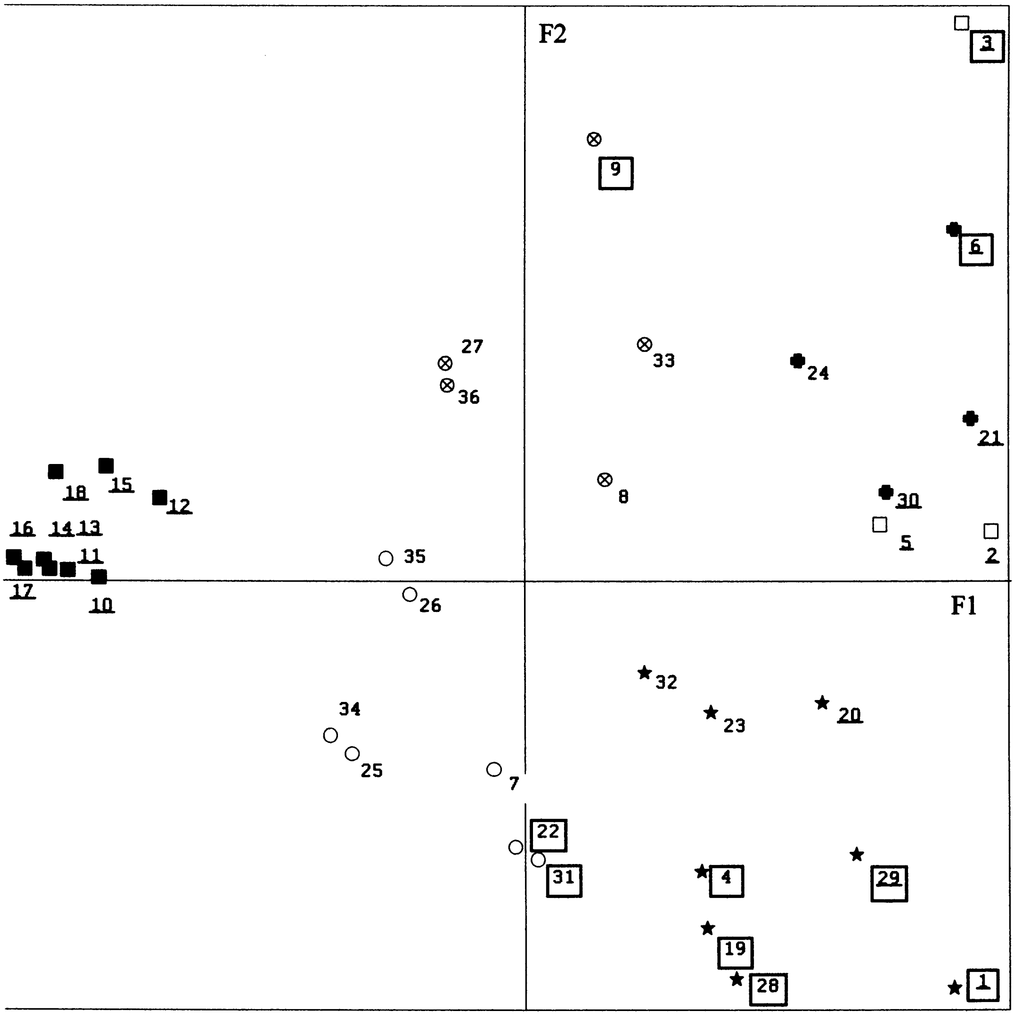

Fig. 11. PCA analysis. Projection of experiments (F1 × F2 plane). Experiments were grouped into subsets using a hierarchical classification. Heavy contributions to F1 are underlined. Heavy contributions to F2 are framed.

$$\text{Cons.} = 81.864 \quad \exp(0.0068\ s), \tag{15}$$

where s is the number of released slugs.

These results show that the releasing of 12 000 slugs (300 × 40) reduces the *Caulerpa* biomass by at least 15% (724 kg) per year. The consumption induced per capita is not a monotonous function of the initial pool of slugs, the curve exhibiting a trough (minima at 150 slugs/spot). This can be interpreted as the effect of the threshold value of the occupation ratio (0.107), which is quickly reached and provokes the migration of adults. Migration is a 'hazardous' process and many slugs can die during foraging (see Section 3.1). The weak number of released slugs cannot compensate, by reproduction, for the loss of adults. Releasing a greater number of animals re-establishes better yields. Fig. 13A–H show some 2D-screens of *Caulerpa* and *Elysia* individuals repartition of Le Brusc simulation.

6. Conclusion

The simulations of the biocontrol of *Caulerpa taxifolia* growth gave encouraging results, and we think that they are significant enough to engage in additional experiments in large mesocosms. However, the expected impacts are strongly dependant on water temperature. In fact, the tropical Ascoglossa *Elysia subomata* does not appear to be well-adapted to the Mediterranean Sea, the suitable conditions for feeding and reproduction being combined within a short summer period. Another point is the number of slugs required to obtain a significant reduction of *Caulerpa* biomass: at least 300 adults slugs/spot were needed on Le Brusc site — which represents an average number of about four adult slugs per m^2. Mixing adults and juveniles can also give similar results. Lastly, the date of animal releasing might be carefully estimated to obtain optimal impacts.

However, even if the results are satisfying on average, the extreme values of the number of individuals showed that some cohorts could disappear with a non negligible probability. Thus, one could be disappointed by some experiments in large mesocosm or in situ assays.

Consequently, the biocontrol of *Caulerpa* by means of *E. subornata* could only be reasonably envisaged to obtain significant slowing down of *Caulerpa* growth and to protect the biodiversity of specific areas (protected areas, natural parks, ...). In any case, yearly treatments might have to be engaged to maintain a sufficient level of predation on the alga and strongly reduce its biomass.

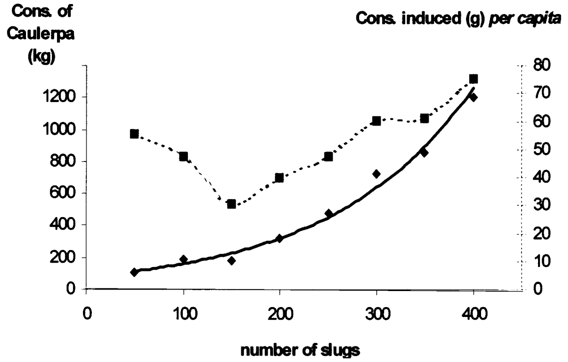

Fig. 12. Absolute consumption of *Caulerpa* (solid line) and induced consumption per capita (dotted line), as a function of the initial number of scattered slugs. Slugs released at SD $= t_0 + 60$ on 40 spots of 1 m^2.

Appendix A

Calculation of the average increase in size of the individuals. At day j, let t be the state of the random variable 'age of the individuals belonging to the nth class of age', denoted A. A is the restriction of an exponential law (see the survival equation in Section 4.1.2) on the interval:

$$[20n + j;\ 20(n + 1) + j]$$

The distribution of age over this period is thus proportional to $\lambda\,\mathrm{e}^{-\lambda t}$:$f_A(t) = k\lambda\,\mathrm{e}^{-\lambda t}$ where k is such as:

$$\int_{20n+j}^{20(n+1)+j} k\lambda \mathrm{e}^{-\lambda t}\mathrm{d}t = 1 \Rightarrow k = \frac{\mathrm{e}^{\lambda(20n+j)}}{1 - \mathrm{e}^{-20\lambda}}$$

Let L_n^j and ΔL_n^j be respectively the random variables 'length at day j of the individuals belonging to the nth class of age' and 'variation of this length': $L_n^j = L_{\max}(1 - \mathrm{e}^{-k_j A})$, so $\Delta L_n^j = k_j L_{\max}\,\Delta t \mathrm{e}^{-k_j A}$, where kj is the instantaneous growth rate at day j. Be $F_{\Delta L_n^j}$ and $f_{\Delta L_n^j}$, the repartition and density functions of ΔL_n^j, we have

$$F_{\Delta L_n^j}(\delta l) = \mathbf{P}[k_j L_{\max}\Delta t \mathrm{e}^{-k_j A} < \delta l]$$
$$= \mathbf{P}\left[A > -\frac{1}{k_j}\ln\left(\frac{\delta l}{k_j L_{\max}\Delta t}\right)\right]$$
$$= 1 - F_A\left(-\frac{1}{k_j}\ln\left(\frac{\delta l}{k_j L_{\max}\Delta t}\right)\right) \Rightarrow f_{\Delta L_n^j}(\delta l)$$
$$= \frac{1}{k_j\delta l} \times f_A\left(-\frac{1}{k_j}\ln\left(\frac{\delta l}{k_j L_{\max}\Delta t}\right)\right) = \mu(\delta l)^{\frac{\lambda}{k_j}-1},$$

where

$$\mu = \frac{\lambda \mathrm{e}^{\lambda(20n+j)}}{k_j(1 - \mathrm{e}^{-20\lambda})(k_j L_{\max}\Delta t)^{\lambda/k_j}}$$

Thus we can calculate the average size increase at day j of the nth class of age:

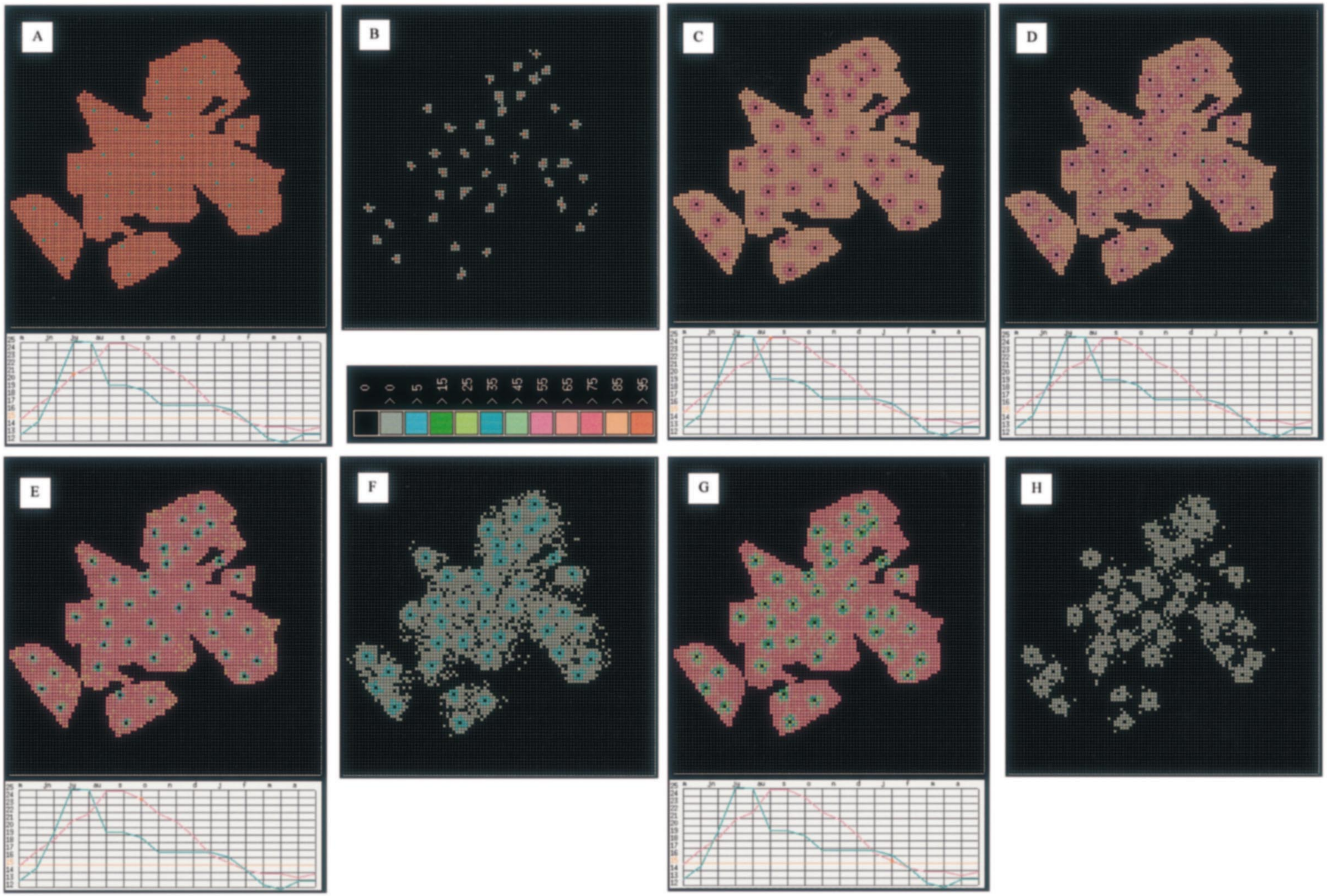

Fig. 13. Simulation on Le Brusc site. 300 adult slugs (6th class) spread per spot; 40 spots; SD = $t_0 + 60$. Curves indicate the temperature variation (in red) and the *Caulerpa* annual biomass variation (in green, arbitrary scale). The red point indicates the current time (indication of months is approximate). The legend is common for all pictures, colours indicating either the *Caulerpa* biomass or the number of slugs as a percentage of the maximum recorded over the simulation. In this case: maximum of *Caulerpa* = 2000 g/m^2, maximum of slugs = 110/m^2 (only adult slugs are represented). A, B: Beginning of the simulation; slugs have been spread, and migration has started (B); The initial spots have been completely eradicated when other spots are covered with a maximum *Caulerpa* biomass (A). C, D: Growing impact of slug feeding on *Caulerpa*. E, F: The maximum dispersal of slugs is reached (F) and the impact is visible over the entire colony of *Caulerpa* (E). G, H: End of suitable condition period for slugs which are incapable to reproduce, the feeding ratio is minimum (H) and the variation of *Caulerpa* is determined by the temperature conditions (G).

$$\mathbf{E}[\Delta L_n^j] = \int_{k_j L_{\max}\Delta t \mathrm{e}^{-k_j(20(n+1)+j)}}^{k_j L_{\max}\Delta t \mathrm{e}^{-k_j(20n+j)}} \delta l \, f_{\Delta L_n^j}(\delta l) \; \mathrm{d}(\delta l)$$

$$= \int_{k_j L_{\max}\Delta t \mathrm{e}^{-k_j(20(n+1)+j)}}^{k_j L_{\max}\Delta t \mathrm{e}^{-k_j(20n+j)}} (\delta l)^{\frac{\lambda}{k_j}} \, \mathrm{d}(\delta l)$$

$$= \frac{k_j L_{\max}\Delta t}{(1+\mathrm{k_j}/\lambda)(1-\mathrm{e}^{-20\lambda})} \mathrm{e}^{-k_j(20n+j)}(1-\mathrm{e}^{-20(\lambda+k_j)}),$$

and the average increase over the 20 days is:

$$\Delta L_n = \sum_{j=0}^{19} \Delta L_n^j \Rightarrow \mathbf{E}[\Delta L_n] = \sum_{j=0}^{19} \mathbf{E}[\Delta L_n].$$

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