Long-term experimental in situ farming of *Crambe crambe* (Demospongiae: Poecilosclerida)

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ABSTRACT

**Background.** The marine sponge *Crambe crambe* was chosen as an experimental model of sustainable shallow-water mariculture in the Sardinian Sea (Western Mediterranean) to provide biomass with high potential in applied research.

**Methods.** Explants were cultured in four long-term experiments (19 and 31 months at ca. 2.5 m depth), to determine the suitability of new culture techniques by testing substrata and seeding time (season), and monitoring survival and growth. Explants were excised and grown in an experimental plant close to the wild donor sponge population. Percentage growth rate (GR%) was measured in terms of surface cover area, and explant survival was monitored in situ by means of a digital photo camera.

**Results.** Explant survival was high throughout the trial, ranging from 78.57% to 92.85% on travertine tiles and from 50% to 71.42% on oyster shells. A few instances of sponge regression were observed. Explant cover area correlated positively with season on two substrata, i.e., tiles and shells. The surface cover area and GR% of explants were measured in the starting phase and monitored up to the end of the trial. High GR% values were observed both on tiles (>21%) and on oyster shells (>15%).

**Discussion.** The data on the behaviour and life-style of cultured fragments, together with an increase >2,400% in cover area, demonstrate that in situ aquaculture is a viable and sustainable method for the shallow-water biomass supply of *Crambe crambe*.

INTRODUCTION

The sustainable exploitation of marine organisms is a key issue for the supply of biomass as a source of bioactive compounds, e.g., in the case of sponges (see Pérez-López et al., 2017). Sponges are key invertebrates in maintaining the biodiversity of benthic communities. The overexploitation of sponge populations could have wide-ranging negative impacts on ecosystems, e.g., biotope architecture and landscape, biodiversity, and trophic and
symbiotic relationships (Pronzato, 1999; Wulff, 2006; Bell, 2008; Bell et al., 2015; Wulff, 2017; and references therein).

Several biotechnological approaches have been developed for the production of valuable marine sponge products, including ex situ culture (Sipkema et al., 2005) primmorph (Müller et al., 2000; Le Pennec et al., 2003; Valisano et al., 2006; and references therein), and cell and fragment culture (Nickel et al., 2001; Nickel & Brümmer, 2003; Pérez-López et al., 2014; and references therein). Although laboratory experiments on explants are essential to the thorough investigation of sponge biology (e.g., the existence of a developmental growth program and the role of collagen in guiding axial growth; Wanick et al. 2017), ex situ culture has not been considered a feasible means of producing large amounts of biomass (Belarbi et al., 2003; Koopmans, Martens & Wijffels, 2009).

In strategic conservation plans to maintain marine biodiversity, farming sponge explants in situ is suggested as one of the most cost-effective and sustainable approaches to producing large amounts of bioactive metabolites (Duckworth & Battershill, 2003a; Page et al., 2005; Pronzato & Manconi, 2008; Murray et al., 2013; Pérez-López et al., 2017; Ternon et al., 2017) and biomaterials (Pronzato et al., 1999; Hoffmann et al., 2003).

Approximately 25 bioactive compounds of sponges and alkaloids from Crambe crambe (Schmidt, 1862) were involved in worldwide preclinical pharmacological research conducted in 2012–2013 (Rubíolo et al., 2013; Mayer et al., 2017).

Although protocols have been developed for the short- or medium-term cultivation of several Mediterranean sponge species (Pronzato et al., 1999; Corriero et al., 2004; Ferretti et al., 2009; Osinga et al., 2010; Ledda, Pronzato & Manconi, 2014), few experiments involving the in situ and ex situ culture of C. crambe have been performed (Cebrian et al., 2003; Garcia Camacho et al., 2006; De Caralt et al., 2007; Pérez-López et al., 2014; Ternon et al., 2016; Ternon et al., 2017).

In the present study, C. crambe was chosen as a mariculture experimental model for the supply of sponge biomass, owing to its high content of specialized metabolites. New protocols were developed in order to improve sustainable culture techniques of this species in very shallow water (see Pérez-López et al., 2017). Short-, medium-, and long-term experiments focused on constraints such as substrata suitability and thermal stress, which can have positive or negative effects on biomass production and morphofunctional performances under farming conditions. Observations on sponge acclimation, health, survival, growth dynamics, suitable substrates, thermal stress, behaviour, morphotraits and life-style in “captivity” are provided.

**MATERIALS & METHODS**

**Study area**

All experiments were carried out in the Porto Conte Bay, a pristine area of the Northern Sardinian Sea in the C zone of the Capo Caccia–Isola Piana Marine Protected Area (MPA) (Western Mediterranean Sea) (Fig. 1). The sponge-farming plant was opportunistically located in a small marina in Tramariglio Cove (NW Porto Conte Bay, 40°35′33″N, 08°10′12″E), a few kilometres from the sampling site (SE Porto Conte Bay, 40°36′12.61″N,
Figure 1  Study area in the Sardinian Sea (Capo Caccia–Isola Piana Marine Protected Area, Western Mediterranean Sea). (A) Sardinia Island (grey) in the Western Mediterranean Sea. (B) Capo Caccia–Isola Piana MPA (grey area within red circle). (C) Sponge culture plant site in Tramariglio Cove (black triangle) and nearby collection site of sponge donors (black star) in Porto Conte Bay. (D) Aerial view of Tramariglio Cove, showing the pier (black circle) to which the sponge culture plant is anchored. Photo credit: the authors.
8°13'6.77''E) on suitable pre-existing submerged man-made structures. The plant modules were anchored to the underwater structure of a pier in very shallow water (2–3 m depth), partially shaded by the pier and sheltered from the prevailing North–Western wind, but occasionally exposed to gales from the South–West.

Prairies of *Posidonia oceanica* (Linnaeus) Delile, 1813 harbouring diversified benthic assemblages are dominant over large extensions of the mainly sandy-silty seabed surrounding the plant in very shallow water (max depth ca. 2.5 m), where patch meadows of the invasive *Caulerpa cylindracea* Sonder, 1845 are also present ([Chessa et al., 1989](#); [Gambi et al., 1989](#); [Maj & Taramelli, 1989](#); [Russo et al., 1991](#); [Barberi, Baroli & Cossu, 1995](#); [Gambi et al., 1995](#)). The hydrological characteristics (temperature, salinity) and primary productivity (Chlorophyll a) of the Sardinian Sea in the Alghero-Provençal Basin had previously been investigated by [Bosc, Bricaud & Antoine (2004)](#) and Olita et al. (2011).

**Experimental design**

Our target was to optimize *C. crambe* cultivation by identifying suitable conditions: site, depth, method, and water temperature. Four experiments were planned in order to monitor the temporal dynamics of survival, growth form, cover area, and growth rate. To test seasonal thermal stress, the timing of explants—winter (February) vs. summer (June) seeding—was scheduled in order to identify the most suitable seeding time (Fig. 2).

All sponge explants were obtained from 10 wild donors (*n* = 5 summer seeding; *n* = 5 winter seeding), fragmented into 28 explants for each season. For each of the four plant
modules, 14 explants of *C. crambe* were seeded in very shallow water (1.5–2.5 m depth) (Fig. 2). Donors were identified following current light-microscopy analysis of the skeletal spicular complement. Taxonomic status was validated on the basis of the description of the family Crambeidae in Systema Porifera (Van Soest, 2002) and the World Porifera Database (Van Soest et al., 2017). Moreover, the metabolome of specimens from the same sampling site was previously analysed (Ternon et al., 2017). Consequently, all explants can be considered to belong to a homogeneous wild population.

**Target species**

*Crambe crambe* (Demospongiae: Poecilosclerida: Crambeidae) was selected on account of its ability to produce bioactive compounds, although it is difficult to farm because it needs solid substrata for settlement, owing to its soft, fragile consistency and encrusting growth form. This red sponge species is common and widespread in the entire Mediterranean Sea (Boury-Esnault, 1971; Pulitzer-Finali, 1983; Uriz, Rosell & Martín, 1992; Pansini & Longo, 2008; Van Soest et al., 2017) and the Macaronesian archipelagos (Duran, Giribet & Turon, 2004).

Sexual reproduction in *C. crambe* occurs through internal fertilisation and brooding (viviparity) of lecithotrophic, swimming larvae (large parenchymellas) then released into the water column during July–August in the western Mediterranean populations (Uriz et al., 1998; Uriz, Becerro & Turon, 2001). A high fission rate during asexual reproduction of this species in the wild enhances its rate of spatial expansion (Garrabou & Zabala, 2001).

Toxic compounds are concentrated in the periphery of the sponge body (spherulous cells), protecting *C. crambe* against potential epibionts, endobionts, predators, and competitive neighbours (Uriz et al., 1996), like a chemical shield (see Ternon et al., 2016).

**Sponge sampling**

Explants of *C. crambe* were collected from donor specimens by means of SCUBA diving and/or snorkelling at 2–4 m depth in the south-eastern area of the Porto Conte Bay near the farming site (Fig. 1). A significant portion of the wild sponges (donors) were left on their substrata, in order to favour natural regenerative processes.

*C. crambe* was scraped from substrata (calcareous rocks, *Spondylus gaederopus* Linnaeus, 1758, and *Arca noae* Linnaeus, 1758) and immediately transferred to the plant. Each sample was cut with scalpels into small replicates of similar size: 3–4 cm in diameter (~8 cm²), thickness <5 mm. Explants were fixed onto two different hard natural substrates. All substrates were suspended in plant modules in accordance with USAMA® patented systems (Pronzato, Manconi & Corriero, 2006).

**Abiotic parameters**

The light intensity and water temperature were recorded every 6 h from 2012 to 2014 by means of an underwater HOBO® Data Logger (Onset, MA, USA) installed in the plant. Monthly average values were then calculated.

During the study period, a series of periodic controls in the water column were conducted by means of a multiparametric probe (YSI 6600 V2) to characterize the site. Temperature,
Table 1  Environmental variables of shallow water in Tramariglio Cove (Capo Caccia –Isola Piana MPA, Sardinian Sea). Mean values recorded in the water column by multiparametric probe (YSI 6600 V2).

| Month  | Temperature °C | Salinity PSU | Dissolved oxygen % | pH  | Chlorophyll a µg l⁻¹ |
|--------|----------------|--------------|---------------------|-----|----------------------|
| May 2013 | 17.8           | 38.3         | 111.6               | 8.22| 0.14                 |
| Oct 2013  | 22.5           | 37.8         | 92.8                | 8.18| 0.42                 |
| May 2014  | 16.5           | 38.7         | 127.1               | 8.18| 0.00                 |
| Nov 2014  | 19.7           | 38.7         | 98.1                | 8.08| 0.09                 |

pH, salinity, dissolved oxygen and chlorophyll a were assessed. Controls were conducted in pre-summer (May 2013 and May 2014) and autumn (October 2013 and November 2014) (Table 1).

Substrata tested and seeding seasons
To test potentially suitable substrates for *Crambe crambe* settlement, preliminarily experiments were carried out on various kinds of material: simple pockets of soft plastic net, plastic cups, square transparent Perspex/Plexiglas plates, natural stone plates (travertine tiles), and marine biogenic carbonate substrata (oyster shells) (Fig. 2). From among the substrata tested, two natural carbonate substrata were chosen: square travertine tiles and bivalve (oyster) shells (Fig. 3).

Four plant modules (Mod), two for each seeding season, were set up; each module consisted of a square PVC frame, inside which either 14 travertine tiles or 14 oyster shells were suspended; the explants were then seeded onto these substrata (Fig. 3). A code was assigned to each of the four modules (Mod) involved in the four experiments (Exp), i.e., Mod1TT (Exp 1), Mod2TT (Exp 2), Mod1OS (Exp 3), and Mod2OS (Exp 4), denoting both the seeding season (cold-winter = 1; warm-summer = 2) and substrate (Travertine Tile = TT; Oyster Shell = OS) (Fig. 2). The modules were vertically orientated in the water column and anchored to the pier. Each explant was photographed on each occasion of seasonal monitoring.

Empty shells of the commercial oyster *Magallana gigas* (Thunberg, 1793) (previously *Crassostrea gigas*) reared in a Sardinian coastal basin (San Teodoro Lagoon) were recycled. Before being used, the shells were sterilised and maintained in seawater for 48 h.

Plant modules
Standard USAMA® square modules (60 × 60 cm) made of PVC tubes connected by means of L-shaped joints were used to support the two different natural substrata (TT and OS) for the adhesion and settlement of *C. crambe* explants (Figs. 3A and 3D). Biogenic marine substrates, i.e., Oyster Shells (OS) enclosed singly within a soft net, were fixed at their upper and lower ends to support ropes, which were separated by plastic spacers. Each explant was secured to the shell by means of cotton laces (Figs. 3D–3F). Travertine Tiles (TT) 10 × 10 × 0.5 were anchored to the support ropes by plastic ties threaded through holes drilled in the four corners of each tile (Figs. 3A–3C). Each explant was secured by cotton laces and was partly covered with a fine net to prevent detachment.
Survival and health

Sponge explants were monitored periodically (3–5 months) to evaluate settlement and adhesion to the substrate, survival, size, growth, and health (presence/absence of necrotic areas); the typical characteristics of the species, i.e., colour, growth form, consistency, and surface traits, were observed in each explant.
Table 2  
*Crambe crambe* *in situ* culture in the Sardinian Sea (Tramariglio Cove, Capo Caccia–Isola Piana Marine Protected Area, Western Mediterranean Sea). Dataset of four experiments started in wintertime (February 2012) vs summertime (July 2013). Winter experiments lasted 31 months (Exp 1; Exp 2). Summer experiments lasted 19 months (Exp 3; Exp 4). The area increase value was calculated in relation to AVG cover area at seeding time (winter vs summer). Acclimation Phase (lasting 4 months after seeding) is reported as AP. Months = m. Minimum and Maximum values of GR cover area increase (see Figs. 5 and 6).

| Experiment code | Survival % | AVG cover area cm² | Area increase % | Growth rate % |
|-----------------|------------|-------------------|----------------|--------------|
|                 | AP        | 12 m | 24 m | End  | Start | 12 m | 24 m | End  | AP    | 12 m | 24 m | End  | AP | Min | Max |
| **Winter**      |           |      |      |      |       |      |      |      |       |       |      |      |      |    |      |     |
| Exp 1 Mod1TT    | 100       | 92.85| 92.85| 92.85| 8.10  | 11.02| 73.13| 173.35| 202.80| 36.00 | 802.50| 2,039.40| 5.94| 0.36 | 21.52 |
| Exp 2 Mod1OS    | 92.85     | 92.85| 78.57| 78.57| 7.80  | 8.24 | 20.51| 48.43 | 58.66 | 5.05  | 161.15| 648.02 | 25.03| 0.69 | 8.70  |
| **Summer**      |           |      |      |      |       |      |      |      |       |       |      |      |      |    |      |     |
| Exp 3 Mod2TT    | 85.71     | 57.14| –    | 50.00| 9.47  | 31.45| 48.14| 232.09| 408.34| –    | 400.65| 22.98 | 2.52 | 22.98 |
| Exp 4 Mod2OS    | 78.57     | 71.42| –    | 71.42| 6.91  | 16.10| 34.16| 132.75| 393.88| –    | 603.04| 15.41 | 0.95 | 15.41 |

Growth rate

Each explant was photographed alongside a ruler at 3–5-month intervals with a Canon Powershot G-10 camera equipped with a waterproof case. The images were then digitalized to trace the outline of each sponge, and the area in cm² was calculated by means of the software ImageJ 1.47t (National Institutes of Health, Bethesda, MD, USA).

Considering that the encrusting habitus of *C. crambe* shows scant growth in height, sponge growth was monitored in two dimensions as the increase in the covered area of the substrate. The percentage growth rate (GR%) of each explant (used for statistical analyses) was calculated by applying the following formula, adapted for encrusting growth forms from Duckworth & Battershill (2001):

\[
GR\% = \left\{ \frac{\left( A_m - A_{m-1} \right)}{A_{m-1}} \right\} \times 100
\]

where \( A_m \) = sponge area measured at month \( m \), \( A_{m-1} \) = sponge area measured on the previous occasion, and \( n \) = number of months between one measurement and the next (3–4 months).

GR% was measured during the acclimation phase (four months) and up to the end of experimental period. Data were compared among sponge explants that had settled on the same type of substrate with regard to the seeding season, the time elapsed and the water temperature. The percentage increase was calculated in relation to the area covered at seeding time (Table 2).

Statistical analyses

Repeated-measures analysis of variance (rANOVA) was performed in order to assess the significance of the effect of seeding time (winter vs summer) by comparing cover area and growth rate between pairs of experiments with the same substrate (i.e., travertine tiles
Exp 1 vs Exp 3, and oyster shells Exp 2 vs Exp 4) in five successive controls 4/5 months apart. Effects were considered significant for values \( p < 0.05 \). All data were logarithmically [\( \ln(x + 1) \)] transformed to comply with the assumptions of ANOVA: normal distribution (Shapiro–Wilk test) and homogeneity of variance (Levene’s test). All statistical analyses were performed by means of XLSTAT software (Addinsoft, 2010).

**RESULTS**

All the explants responded positively to the plant types and to the new micro-habitat, displaying high survival values and a positive trend in growth rate on both substratum types and in both thermal conditions of seeding. All values related to the acclimation phase refer to 4 months after seeding.

**Growth and survival**

**Acclimation phase**

Sponge seeding in the two seasons resulted in different growth rate percentages during the 4-month acclimation phases. Regarding the winter seeding, GR% ranged from ca. 5.95% on travertine tiles (Exp 1; Mod1TT) to ca. 25% on shells (Exp 2; Mod1OS) (Figs. 4A and 5A; Table 2). As for the summer seeding, GR% ranged from ca. 22.98% on tiles (Exp 3; Mod2TT) to ca. 15.40% on shells (Exp 4; Mod2OS) (Figs. 4B and 5B; Table 2).

**Winter seeding**

The highest GR% values recorded in experiments on winter seeding were 21.5% (June–September 2012) in Mod1TT (Exp 1) and 8.7% (September 2012–January 2013) in Mod1OS (Exp 2). The lowest values were 0.36% in January–May 2014, after 28 months, in Mod1TT, and 0.69% in May–September 2014, after 31 months, in Mod1OS (Figs. 4A and 5A; Table 2).

**Summer seeding**

Concerning summer seeding, the highest GR% values were recorded in both experiments during the acclimation phase (June –September 2013): 22.98% in Mod2TT (Exp 3) and 15.40% in Mod2OS. The lowest values were 2.52% in Mod2TT in January–May 2014, after 15 months, and 0.95% in May–September 2014, after 19 months, in Mod2OS (Figs. 4B and 5B; Table 2).

**Long-term dynamics**

After 31 months, the average (AVG) cover area had increased by ca. 2,403%, from 8.10 cm\(^2\) to 202.8 cm\(^2\), in experiment 1 on tiles (Mod1TT). In experiment 2, by contrast, the AVG cover area had increased by ca. 648%, from 7.8 cm\(^2\) to 58.66 cm\(^2\), on shells (Mod1OS) (Table 2).

In experiments 1 and 2, *C. crambe* began to colonize the backside of the substrata after 24 months; both the front and back cover area values were therefore considered for each explant for the last year (2014). In experiments 3 and 4, after 19 months the AVG cover area had increased by ca. 400%, from 9.47 cm\(^2\) to 47.41 cm\(^2\), on tiles (Exp 3; Mod2TT) and by ca. 603%, from 6.91 to 48.63 cm\(^2\), on shells (Exp 4; Mod2OS).
High survival values were recorded in sponges seeded in winter in Mod1TT (Exp 1); after the acclimation phase, survival was 100%, declining to 92.85% after 31 months. In Mod1OS (Exp 2), survival after the acclimation phase was 92.85%, and declined to 78.57% after 31 months (Fig. 6A; Table 2). By contrast, in sponges seeded in summer, survival in Mod2TT was 85.71% during the acclimation phase and 50% after 19 months; in Mod2OS, survival was 78.57% during the acclimation phase and 71.42% after 19 months (Fig. 6B; Table 2).

**Seeding season**

No significant effect of the seeding season (winter vs summer acclimation) was observed on considering cover area data in experiments conducted both on travertine tiles (Exp 1 vs Exp 3; rANOVA, $F = 2.672, p = 0.114$) and on oyster shells (Exp 2 vs Exp 4; rANOVA, $F = 0.003, p = 0.960$).

Regarding growth rate data, a significant effect of the seeding season was observed only in experiments on travertine tiles (Exp 1 vs Exp 3; rANOVA, $F = 7.761, p = 0.10$). No
significant effects were seen in experiments on oyster shells (Exp 2 vs Exp 4; rANOVA, $F = 0.736, p = 0.401\%)$.

**Morphofunctional traits and behaviour**

Light microscopy analysis of skeletal morphotraits of sponge samples revealed a spicular complement consisting exclusively of two categories of styles, namely tylostyles and subtylostyles of $230–280 \times 3.5–5 \, \mu m$. This dimensional range matches those reported in the literature (see Rützler, 1965). By contrast the wild population of *C. crambe* at Porto Conte Bay and the cultured sponges at Tramariglio Cove do not have chelae as microscleres.

The reproductive timing of sponges in our experiments was synchronous with that of wild populations, as suggested by the presence of brooded, subspherical, orange larvae in the choanosome during the late spring; this is also in agreement with Becerro, Uriz & Turon (1997).

**Abiotic parameters and site characterisation**

The water temperature was calculated by averaging the measurements recorded at the fixed station (1.5 m depth), and varied from a minimum of ca. 14 $^\circ$C in February to ca. 24 $^\circ$C from August to October. A minimum value of 7 $^\circ$C was registered in June 2013 at 6.00 a.m. and a maximum value of ca. 27 $^\circ$C in August 2013 at 6.00 p.m.
Figure 6   Survival trends in *Crambe crambe* shallow-water mariculture on Travertine Tiles (TT) vs Oyster Shells (OS) (Capo Caccia–Isola Piana MPA, Sardinian Sea). Survival of sponge explants and water temperature trend compared in all four experiments. (A) Experiments 1–2 (winter seeding, cold water); (B) Experiments 3–4 summer seeding (warm water). Seeding time is indicated by stars.

Light intensity recorded at 12 noon throughout the year at 1.5 m depth showed a monthly mean range from ca. 152 to ca. 951 lux. The lowest values were recorded from July to September, probably because the sensor was partly obscured by the flourishing growth of algal mucilage.

The periodic control of the water column parameters (from the surface to ca. 2.50 m depth) highlighted the pristine conditions of the site (Table 1). Water temperature was in line with the seasonality, with no differences along the water column. The high values of salinity (37.8 to 38.6 PSU) indicate the scarcity of continental water inputs and negligible related nutrient loads. pH normally ranged between 8.05 and 8.22. Dissolved Oxygen was markedly above the saturation level on pre-summer control and slightly lower than this threshold during the autumn months. Chlorophyll *a* (*<0.5 *µ*g l<sup>−1</sup>), in association with high transparency of the water, was typical of oligotrophic conditions, with low productivity values, in agreement with Olita et al. (2011).

Water monitoring (Directive 2006/7/EC) performed by the Sardinian Environmental Protection Agency (ARPAS) in the four-year period 2013–2016 confirmed that the water in the study area within the Porto Conte Bay in the Marine Protected Area Capo Caccia—Isola Piana was of excellent quality. The ARPAS assessment was based primarily on microbiological parameters and on the evaluation of presence/absence of bituminous residues, glass, plastic or other wastes, phytoplankton blooms, and macro-algae proliferation.
DISCUSSION
Substrata for settlement
Substrata were selected after preliminary experiments to identify suitable and/or sustainable materials. Nylon line, which has been used in the farming of other sponge species (Pronzato & Manconi, 2008; Pérez-López et al., 2017) is unsuitable for *C. crambe*, which has a soft, fragile and encrusting growth form. The plastic cups used in preliminary experiments were also deemed unsuitable, as the excessive accumulation of silt clogged the aquiferous system of the explants, causing high mortality. Similarly, transparent Perspex/Plexiglas squares also proved unsuitable (high mortality, low or negative growth), probably because too much light passed through the substratum. Conversely, travertine tiles and oyster shells proved to be optimal for survival and growth, as shown by the present data.

Behaviour and lifestyle
Field observations confirmed that the explants of *C. crambe* adapted well to their new habitat. Indeed, larval production was seen to be synchronous with that of the wild population of *C. crambe* in the study area; larvae were produced in late spring, in accordance with Becerro, Uriz & Turon (1997).

*C. crambe* seems not to be vulnerable to stress caused by manipulation, as suggested by survival and growth values during acclimation. The sponge displayed marked resilience in response to experimental fragmentation, which is consistent with the processes of fission and fusion that take place during asexual reproduction in the wild, as reported by Garrabou & Zabala (2001).

Allocating the sponge-farming facility to an area close to the wild donor populations enhanced the ability of explants to acclimate rapidly and to re-grow after fragmentation in the new habitat, where the substrata were suspended in the water column and partly shaded by the pier.

With regard to the substrata tested, oyster shells, being natural biogenic marine materials, fit perfectly with the behaviour of *C. crambe* in the Porto Conte Bay. Indeed, the sponge preferentially selects calcareous substrata for larval settlement, such as the surfaces of shells of bivalves (*S. gaederopus*, *A. noae*, *Pinna nobilis* Linnaeus, 1758), and of gastropods (*Hexaplex* spp.), together with coralline algae and crab carapaces (Rützler, 1965; Corriero, Pronzato & Sarà, 1991; R Manconi, pers. obs., 2011). Unfortunately, we were unable to use the shells of these native bivalves as a substrate, notwithstanding their optimal morphotraits (see Marin & López Belluga, 2005), for several reasons: (i) *A. noae* shells are unsuitably small; (ii) these molluscs are not commercialized in Sardinia; (iii) these shells are rarely stranded along the coast; (iv) *P. nobilis*, which has with a suitably large, almost flat shell, is a protected species (2006/105/CE Directive); (v) the low abundance of their populations after several mass mortalities in the past. By contrast, travertine tiles, although man-made, closely mimic natural rocky calcareous marine substrata in terms of both structure and composition.

A peculiar behaviour was displayed by *C. crambe* in the plastic cups tested. Indeed, during the experiments, the sponge explants moved from the inside of the cup (through holes of a few mm in diameter pierced in the bottom) to the outside, where they actively
grew on the outer wall until the entire external surface of the cup was encrusted. This ability of sponges to escape unsuitable farming conditions by actively moving on the substratum fits in with the behaviour previously reported by Pronzato (2004) for *Chondrilla nucula* Schmidt, 1862, in similar experimental farming conditions.

**Morphological traits**

With regard to growth form and morphofunctional traits, *C. crambe* explants promptly displayed a tendency to assume the typical habitus of wild sponges. Just after fragmentation, the explants had a more or less square flat shape. Subsequently, however, during the early growth phase, a very thin encrusting patina (0.5–1 mm in thickness) expanded from the margins of the explants to colonise the surfaces of both substrata (TT and OS); the body then spread in all directions, reaching a thickness of 0.7–10 mm after some time (e.g., Figs. 3C and 3F). This behaviour was very similar to that seen in the wild population, which usually encrusts and adheres tightly to the irregular surfaces of the shells of living molluscs throughout the *Posidonia* meadows of the bay.

Our cultured sponges were characterised by a spicular complement consisting exclusively of two categories of styles; these were the same as those observed in the wild population of *C. crambe* in the bay, and fit the description provided by Van Soest (2002). In ex situ experiments, Maldonado et al. (1999) suggested that *C. crambe* is genetically capable of producing spicule types that are not normally found in all natural populations (i.e., microscleres such as aster-like desmas).

**Thermal acclimation and survival**

In all four experiments, after the 4-month acclimation phase, survival was notably high in cold-water seeding conditions (13 °C to 14 °C). The sponges underwent initial stress due to transplantation and seeding in warmer-water seeding conditions (20 °C to 24 °C). Survival data showed that the sponges were still subject to mortality even after the acclimation phase, independently of the seeding season or the substrata. The lower survival values in the explants seeded during the warm season indicate that *C. crambe* seems to be sensitive to warm water during transplantation and acclimation, in agreement with Turon, Becerro & Uriz (1996).

**Cover area and growth dynamics**

Our experiments showed that the growth of *C. crambe* on tiles was notably high over the two years up to January 2014 (Fig. 5; Table 2). The growth dynamics was similar in all experiments; initially, a thin film colonised the substratum along the border of each explant, gradually covering the entire available surface of substratum (on both sides) and subsequently increasing in thickness. This phase of colonisation was followed by a phase in which the sponges extended along the support ropes and plastic spacers of the modular plants. Our data show a higher growth rate in the explants seeded on travertine tiles than in those seeded on oysters shells (the highest value was recorded in the acclimation phase on Mod2TT, seeded in summer); this is probably due to the affinity of these encrusting red sponges for relatively corrugated surfaces and porous substrata, rather than the pearly and concave surfaces of shells.
The seeding season seems particularly to influence growth rate; indeed, (rANOVA) a statistically significant association was observed only in experiments on travertine tiles (Exp 1 vs Exp 3), and not on oyster shells. In contrast, no significant relationship (rANOVA) was detected between the cover area and the seeding time in experiments on either tiles or shells, suggesting that the cover area is only constrained by seasonal cycles and time-frame.

In agreement with Turon, Becerro & Uriz (1996), our results show that farmed C. crambe explants follow a seasonal trend, i.e., sponges grow faster during the late spring and summer, concomitantly with larval release, while growth is slower but constant in winter, until all the available substratum is covered. These findings are in agreement with data recorded in this species under natural conditions (Turon, Tarjuelo & Uriz, 1998; Garrabou & Zabala, 2001), or in farming experiments involving other species (Duckworth & Battershill, 2003b). Seasonal growth differences have been reported for sponge typically dwelling in shallow water characterised by fluctuating conditions (Lewandrowski & Fell, 1981; Barthel, 1986).

The two-year growth rate of C. crambe recorded in the wild by Turon, Tarjuelo & Uriz (1998) was of an average size increase of about 2/5 times in 26 months, whereas in our farming experiments mean size increased about 25 times in 31 months (starting from already-settled fragments). Indeed, it is well known that wild sponges are constrained by substrate competition with other benthic species inhabiting hard substrata (Rützler, 1970). The suitability of the substrata selected and the scant spatial competition contributed to the high growth rate in the Sardinian plant.

High intra-population (farmed sponges) variability in growth rate percentage was observed during our study. Indeed, explants displayed a GR% range from ca. −6% to ca. 29% in September 2012 on Mod1TT; this pattern is reported to be typical of sponges (Ayling, 1983; Todd & Turner, 1988; Stocker, 1991; Turon & Becerro, 1992; Turon, Tarjuelo & Uriz, 1998; Ferretti et al., 2009), which display a wide range of non-synchronous behaviour, i.e., precocious or tardy growth. In our experiments, the data on body size increase were in accordance with high weight increase values (higher than 1000% over the initial weight in ca. 22/45 days), as also reported for ex situ explants of C. crambe (Belarbi et al., 2003).

**CONCLUSIONS**

Sponge mariculture and biomass production constitutes a living laboratory for the rational management, conservation and monitoring of marine benthic bioresources. Our long-term in situ cultivation experiments supported investigations into the behaviour and strategies of adaptation of C. crambe to seasonal, climatic and ecological fluctuations in the pluri-annual cycle by observing quite “pure phenomena” while avoiding intra- and inter-species competition. Technical approaches were improved in order to fit the morpho-, eco- and etho-logical traits of this target species, e.g., the availability of a suitable, natural, hard biogenic substratum for sponges that display a typical thin, fragile, encrusting habitus. The Tramariglio Cove within the Capo Caccia—Isola Piana MPA is an ideal environment for sustainable sponge farming. Indeed, the sustainability of sea-based sponge culture at Tramariglio (with Sarcotragus spinosulus as target species) was recently tested by the life-cycle assessment (LCA) approach, which utilises a systematic set of
procedures for compiling and examining the inputs and outputs of materials and energy and the associated environmental impacts directly attributable to the functioning of a product or service system throughout its life cycle (see Pérez-López et al., 2017).

Explant survival is usually high, and in most cases a long-term healthy growth phase occurs, during which both sexual and asexual reproductive phases can be observed. Moreover, *C. crambe* is particularly adapted to farming in very shallow water (i.e., ca. 2.5 m depth) (Fig. 7).

All present low-tech experiments that use recycled and/or natural substrata for sponge settling are in agreement with sustainable approaches (see Pérez-López et al., 2017). They also avoid potential constraints imposed by artificial materials used in farming, i.e., an aggressive response to chemicals in the materials (Duckworth & Battershill, 2003b). An added value is that these filter feeders intensively farmed in situ are able to retain and recycle particulate and dissolved organic matter in the water column (see Ledda, Pronzato & Manconi, 2014).
The occurrence of sexual reproduction also highlights the potential of conserving *C. crambe* by restocking coastal populations with released larvae and asexual propagules from farmed sponges, as previously suggested for other sponge species (Pronzato, 1999; Pronzato et al., 1999; Scalera Liaci et al., 1999; Corriero et al., 2004; Mercurio et al., 2004; Pronzato, 2004; Pronzato & Manconi, 2008). This is particularly true if we consider that one of the preferred substrata of *C. crambe* in the shallow waters of the western Mediterranean consists of shells of living bivalve molluscs, e.g., *S. gaederopus*, *A. noae*, and *P. nobilis*, which have been hit by massive mortality and the disappearance of many populations in recent decades (Meinesz & Mercier, 1983).

Our results are in agreement with those of several authors, who have claimed that the success of farming *in situ* is affected by the seeding season, hydrological conditions, depth, light and location (Wilkinson & Vacelet, 1979; Duckworth, Battershill & Bergquist, 1997; Turon, Tarjuelo & Uriz, 1998; Van Treeck et al., 2003; Duckworth, Battershill & Schiel, 2004). However, Ternon et al. (2017) demonstrated that the production of guanidine alkaloids by *C. crambe* is not constrained by *in situ* farming conditions.

The Sardinian pilot plant was of small size, as the main aim of our study was to identify suitable substrata. The next step will be to increase the size and number of modular structures and calcareous substrata, in order to assess the feasibility of the large-scale biomass production of *C. crambe* for commercial purposes. Indeed, our experiments show that it is possible to renew sponge biomass production in an annual cycle by means of new seeding through the fragmentation of explants from the crop of the same farming plant.

Farming *C. crambe* as a source of bioactive compounds will probably support the supply of marine pharmaceuticals (Mayer et al., 2017) with potential applications for the therapy of cancer and other diseases (El-Demerdash et al., 2018). Moreover, it has a low environmental impact and increases ecosystem services without affecting wild populations (Pronzato & Manconi, 2008). Indeed, the life-cycle assessment previously performed on models of sponge mariculture in the Tramariglio plant for the production of bioactive compounds revealed that the preparation of the crude extract was the main contributor (85–99%) to the environmental burden (Pérez-López et al., 2017). *In situ* sponge culture enables sponges to be grown continuously. Moreover, input requirements are relatively low, as the sponges consume nutrients available in the water column, without raw materials having to be added. At the same time, the bioremediation potential of sponges (filtering capacity; removal of bacterial and organic material) deducts around 5% of the total environmental impact.

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Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Andrea Padiglia conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
• Fabio D. Ledda conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, approved the final draft.
• Bachisio M. Padedda performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, authored or reviewed drafts of the paper, approved the final draft.
• Roberto Pronzato and Renata Manconi conceived and designed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.

Data Availability
The following information was supplied regarding data availability:
The raw data are provided in the Supplemental File.

Supplemental Information
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REFERENCES
Addinsoft SARL. 2010. Addinsoft SARL XLSTAT software. Version 10. Paris: Addinsoft Inc.
Ayling AL. 1983. Factors affecting the spatial distributions of thinly encrusting sponges from temperate waters. Oecologia 60:412–418 DOI 10.1007/BF00376861.
Barberi R, Baroli M, Cossu A. 1995. Indagini fenologiche e lepidocronologiche finalizzate alla stima della produttività primaria della prateria a Posidonia oceanica (L.) Delile nella baia di Porto Conte (NW Sardegna). Biologia Marina Mediterranea 2:347–349.
Barthel D. 1986. On the ecophysiology of the sponge Halichondria panicea in Kiel Bight. I. Substrate specificity, growth and reproduction. Marine Ecology Progress Series 32:291–298 DOI 10.3354/meps032291.

Becerro MA, Uriz MJ, Turon X. 1997. Chemically-mediated interactions in benthic organisms: the chemical ecology of Crambe crambe (Porifera, Poecilosclerida). Interactions and adaptation strategies of marine organisms. Developments in Hydrobiology 121:77–89 DOI 10.1007/978-94-017-1907-0_9.

Belarbi EH, Gomez AC, Chisti Y, García F, Grima EM. 2003. Producing drugs from marine sponges. Biotechnology Advances 21:585–598 DOI 10.1016/S0734-9750(03)00100-9.

Bell JJ. 2008. The functional roles of marine sponges. Estuarine, Coastal and Shelf Science 79:341–353 DOI 10.1016/j.ecss.2008.05.002.

Bell JJ, McGrath E, Biggerstaff A, Bates T, Cárdenas CA, Bennett H. 2015. Global conservation status of sponges. Conservation Biology 29:42–53 DOI 10.1111/cobi.12447.

Bosc E, Bricaud A, Antoine D. 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from four years of SeaWiFS observations. Global Biogeochemical Cycles 18(1):Article GB1005 DOI 10.1029/2003GB002034.

Boury-Esnault N. 1971. Spongiaires de la zone rocheuse littorale de Banyuls-sur-Mer. I. Ecologie et répartition. Vie et milieu Océanographie 22:159–192.

Cebrian E, Martí R, Uriz JM, Turon X. 2003. Sublethal effects of contamination on the Mediterranean sponge Crambe crambe: metal accumulation and biological responses. Marine Pollution Bulletin 46:1273–1284 DOI 10.1016/S0025-326X(03)00190-5.

Chessa L, Bionda G, Buia MC, Gambi MC, Lorenti M, Maj R, Manconi R, Martinelli M, Pintus MG, Russo GF, Scipione MB, Taramelli E. 1989. Indagini su Posidonia oceanica nella rada di Porto Conte (Sardegna nord-occidentale): caratteristiche della prateria e fauna vagile. Oebalia 15:79–104.

Corriero G, Longo C, Mercurio M, Marzano CN, Lembo G, Spedicato MT. 2004. Rearing performance of Spongia officinalis on suspended ropes off the Southern Italian Coast (Central Mediterranean Sea). Aquaculture 238:195–205 DOI 10.1016/j.aquaculture.2004.04.030.

Corriero G, Pronzato R, Sarà M. 1991. The sponge fauna associated with Arca noae L. (Mollusca, Bivalvia). In: Reitner J, Keupp H, eds. Fossil and recent sponges. Berlin: Springer, 395–403.

De Caralt S, Otjens H, Uriz MJ, Wijffels RH. 2007. Cultivation of sponge larvae: settlement, survival, and growth of juveniles. Marine Biotechnology 9:592–605 DOI 10.1007/s10126-007-0913-5.

Duckworth AR, Battershill CN. 2001. Population dynamics and chemical ecology of New Zealand Demospongiae Latrunculia sp. nov. and Polymastia croceus (Poecilosclerida: Latrunculidae: Polymastiidae). New Zealand Journal of Marine and Freshwater Research 35:935–949 DOI 10.1080/00288330.2001.9517055.
Duckworth AR, Battershill CN. 2003a. Sponge aquaculture for the production of biologically active metabolites: the influence of farming protocols and environment. Aquaculture 221:311–329 DOI 10.1016/S0044-8486(03)00070-X.

Duckworth AR, Battershill CN. 2003b. Developing farming structures for production of biologically active sponge metabolites. Aquaculture 217:139–156 DOI 10.1016/S0044-8486(02)00038-8.

Duckworth AR, Battershill CN, Bergquist PR. 1997. Influence of explant procedures and environmental factors on culture success of three sponges. Aquaculture 156:251–267 DOI 10.1016/S0044-8486(97)00131-2.

Duckworth AR, Battershill CN, Schiel DR. 2004. Effects of depth and water flow on growth, survival and bioactivity of two temperate sponges cultured in different seasons. Aquaculture 242:237–250 DOI 10.1016/j.aquaculture.2004.08.046.

Duran S, Giribet G, Turon X. 2004. Phylogeographical history of the sponge Crambe crambe (Porifera, Poecilosclerida): range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. Molecular Ecology 13:109–122 DOI 10.1046/j.1365-294X.2003.02022.x.

El-Demerdash A, Atanasov AG, Bishayee A, Abdel-Mogib M, Hooper JN, Al-Mourabit A. 2018. Batzella, Crambe and Monanchora: highly prolific marine sponge genera yielding compounds with potential applications for cancer and other therapeutic areas. Nutrients 10(1):Article 33 DOI 10.3390/nu10010033.

Ferretti C, Vacca S, De Ciucis C, Marengo B, Duckworth AR, Manconi R, Pronzato R, Domenicotti C. 2009. Growth dynamics and bioactivity variation of the Mediterranean demosponges Agelas oroides (Agelasida, Agelasidae) and Petrosia ficipiformis (Haplosclerida, Petrosiidae). Marine Ecology 30:327–336 DOI 10.1111/j.1439-0485.2008.00278.x.

Gambi MC, Giangrande A, Chessa LA, Manconi R, Scardi M. 1989. Distribution and ecology of polychaetes in the foliar stratum of a Posidonia oceanica bed in the bay of Porto Conte (N.W. Sardinia). In: Boudouresque CF, Meinesz A, Fresi E, Gravez V, eds. International workshop on posidonia beds. Vol. 2. France: GIS Posidonie Publication, 175–187.

Gambi MC, Giangrande A, Martinelli M, Chessa LA. 1995. Polychaetes of a Posidonia oceanica bed off Sardinia (Italy): spatio-temporal distribution and feeding guild analysis. Scientia Marina 59:129–141 DOI 10.3989/scimar.1998.62n1-21.

Garcia Camacho F, Chileh T, Ceron Garcia MC, Sanchez Miròn A, Belarbi EH, Contreras Gómez A, Molina Grima E. 2006. Sustained growth of explants from Mediterranean sponge Crambe crambe cultured in vitro with enriched RPMI 1640. Biotechnology Progress 22:781–790 DOI 10.1021/bp050341m.

Garrabou J, Zabala M. 2001. Growth dynamics in four Mediterranean demosponges. Estuarine, Coastal and Shelf Science 52:293–303 DOI 10.1006/ecss.2000.0699.

Hoffmann F, Rapp HT, Zoller T, Reitner J. 2003. Growth and regeneration in cultivated fragments of the boreal deep water sponge Geodia barretti Bowerbank, 1858 (Geodiidae, Tetractinellida, Demospongiae). Journal of Biotechnology 100:109–118 DOI 10.1016/S0168-1656(02)00258-4.
Koopmans M, Martens D, Wijffels RH. 2009. Towards commercial production of sponge medicines. Marine Drugs 7:787–802 DOI 10.3390/md7040787.

Ledda FD, Pronzato R, Manconi R. 2014. Mariculture for bacterial and organic waste removal: a field study of sponge filtering activity in experimental farming. Aquaculture Research 45:1389–1401 DOI 10.1111/are.12084.

Le Pennec G, Perovic S, Ammar MSA, Grebenjuk VA, Steffen R, Brümmer F, Müller WE. 2003. Cultivation of primmorphs from the marine sponge Suberites domuncula: morphogenetic potential of silicon and iron. Journal of Biotechnology 100:93–108 DOI 10.1016/S0168-1656(02)00259-6.

Lewandrowski KB, Fell PE. 1981. Sequential reproduction by different types of specimens of the estuarine sponge, Halichondria sp., with an emphasis on reproduction of postlarval specimens. International Journal of Invertebrate Reproduction 3:227–236 DOI 10.1080/01651269.1981.10553398.

Maj RLC, Taramelli E. 1989. Mysidacea of Posidonia oceanica (L.) Delile beds in Torvaldiga (Latium) and Porto Conte (Sardinia). In: Boudouresque CF, Meinesz A, Fresi E, Gravez V, eds. International workshop on Posidonia beds. Vol. 2. France: GIS Posidonie Publication, 203–206.

Maldonado M, Carmona MC, Uriz MJ, Cruzado A. 1999. Decline in Mesozoic reef-building sponges explained by silicon limitation. Nature 401:785–788 DOI 10.1038/44560.

Marin A, López Belluga MD. 2005. Sponge coating decreases predation on the bivalve Arca noae. Journal of Molluscan Studies 71:1–6 DOI 10.1093/mollus/eyh045.

Mayer A, Rodríguez AD, Taglialetela-Scafati O, Fusetani N. 2017. Marine pharmacology in 2012–2013: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiproteinase, antituberculosis, and antiviral activities affecting the immune and nervous systems, and other miscellaneous mechanisms of action. Marine Drugs 15:Article 273 DOI 10.3390/md15090273.

Meinesz A, Mercier D. 1983. Sur les fortes mortalités de Spondyles (Spondylus gaederopus Linné) observées sur les côtes de Méditerranée. Travaux Scientifiques du Parc National de Port-Cros 9:89–95.

Mercurio M, Longo C, Nonnis Marzano C, Scaleria Liaci L, Corriero G. 2004. Demosponges from Mediterranean lagoons. Biologia Marina Mediterranea 11:444–447.

Müller WE, Böhm M, Batel R, De Rosa S, Tommonaro G, Müller IM, Schröder HC. 2000. Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from Dysidea avara. Journal of Natural Products 63:1077–1081 DOI 10.1021/np000003p.

Murray PM, Moane S, Collins C, Beletskaya T, Thomas OP, Duarte AWF, Nobre FS, Owoyemi IO, Pagnocca FC, Sette LD, McHugh E, Causse E, Pérez-López P, Feijoo G, Moreira MT, Rubiolo J, Leirós M, Botana LM, Pinto S, Alves C, Horta A, Pedrosa R, Jeffries C, Agathos SN, Allewaert C, Verween A, Vyverman W, Laptev I, Sineoky S, Bisio A, Manconi R, Ledda F, Marchi M, Pronzato R, Walsh DJ. 2013. Sustainable production of biologically active molecules of marine based origin. New Biotechnology 30:839–850 DOI 10.1016/j.nbt.2013.03.006.
Nickel M, Brümmer F. 2003. *In vitro* sponge fragment culture of *Chondrosia reniformis* (Nardo, 1847). *Journal of Biotechnology* 100:147–159 DOI 10.1016/S0168-1656(02)00256-0.

Nickel M, Leininger S, Proll G, Brümmer F. 2001. Comparative studies on two potential methods for the biotechnological production of sponge biomass. *Journal of Biotechnology* 92:169–178 DOI 10.1016/S0168-1656(01)00357-1.

Olita A, Ribotti A, Sorgente R, Fazioli L, Perilli A. 2011. SLA–chlorophyll-a variability and covariability in the Algero-Provençal Basin (1997–2007) through combined use of EOF and wavelet analysis of satellite data. *Ocean Dynamics* 61:89–102 DOI 10.1007/s10236-010-0344-9.

Osinga R, Sidri M, Cerig E, Gokalp SZ, Gokalp M. 2010. Sponge aquaculture trials in the East-Mediterranean Sea: new approaches to earlier ideas. *The Open Marine Biology Journal* 4:74–81 DOI 10.2174/1874450801004010074.

Page MJ, Northcote PT, Webb VL, Mackey S, Handley SJ. 2005. Aquaculture trials for the production of biologically active metabolites in the New Zealand sponge *Mycale hentscheli* (Demospongiae: Poecilosclerida). *Aquaculture* 250:256–269 DOI 10.1016/j.aquaculture.2005.04.069.

Pansini M, Longo C. 2008. Checklist della flora e della fauna dei mari italiani (Parte I). *Biologia Marina Mediterranea* 15(1):42–66.

Pérez-López P, Ledda FD, Bisio A, Feijoo G, Perino E, Pronzato R, Manconi R, Moreira MT. 2017. Life cycle assessment of in situ mariculture in the Mediterranean Sea for the production of bioactive compounds from the sponge *Sarcotragus spinosulus*. *Journal of Cleaner Production* 142:4356–4368 DOI 10.1016/j.jclepro.2016.11.137.

Pérez-López P, Ternon E, González-García S, Genta-Jouve G, Feijoo G, Thomas OP, Moreira MT. 2014. Environmental solutions for the sustainable production of bioactive natural products from the marine sponge *Crambe crambe*. *Science of the Total Environment* 475:71–82 DOI 10.1016/j.scitotenv.2013.12.068.

Pronzato R. 1999. Sponge-fishing, disease and farming in the Mediterranean Sea. *Aquatic Conservation: Marine and Freshwater Ecosystems* 9:485–493 DOI 10.1002/(SICI)1099-0755(199909/10)9:5<485::AID-AQC362>3.0.CO;2-N.

Pronzato R. 2004. A climber sponge. *Bollettino dei Musei e degli Istituti Biologici dell’Università di Genova* 68:549–552.

Pronzato R, Bavestrello G, Cerrano C, Magnino G, Manconi R, Pantelis J, Sarà A, Sidri M. 1999. Sponge farming in the Mediterranean Sea: new perspectives. Origin and Outlook. *Memories of the Queensland Museum* 44:485–491.

Pronzato R, Manconi R. 2008. Mediterranean commercial sponges: over 5000 years of natural history and cultural heritage. *Marine Ecology* 29:146–166 DOI 10.1111/j.1439-0485.2008.00235.x.

Pronzato R, Manconi R, Corriero G. 2006. *Tipologie di Impianto Modulare per la Spongicoltura Subacquea anche in Policultura U.S.A.M.A. (Underwater Sponge Aquaculture Modular System)* Brevetto per Invenzione Industriale, n. 0001334230, Ufficio Italiano Brevetti e Marchi. Roma: Ministero delle Attività Produttive.
Pulitzer-Finali G. 1983. A collection of Mediterranean demosponges (Porifera) with, in appendix, a list of the Demospongiae hitherto recorded from the Mediterranean Sea. 
*Annali del Museo Civico di Storia Naturale di Genova* 84:445–621.

Rubiolo JA, Ternon E, Lopez-Alonso H, Thomas OP, Vega FV, Veytes MR, Botana LM. 2013. Crambescidin-816 acts as a fungicidal with more potency than crambescidin-800 and -830, inducing cell cycle arrest, increased cell size and apoptosis in *Saccharomyces cerevisiae*. *Marine Drugs* 11:441–443 DOI 10.3390/md11114419.

Russo GF, Chessa LA, Vinci D, Fresi E. 1991. Molluscs of *Posidonia oceanica* beds in the bay of Porto Conte (North-Western Sardinia): zonation pattern, seasonal variability and geographical comparison. *Posidonia Newsletter* 4:5–14.

Rützler K. 1965. Systematik und ökologie der poriferen aus litoral-schattengebieten der Nord Adria. *Zoomorphology* 55:1–82.

Rützler K. 1970. Spatial competition among Porifera: solution by epizoism. *Oecologia* 5:85–95 DOI 10.1007/BF00347624.

Scalera Liaci L, Mercurio M, Palladino F, Massari S, Correro G. 1999. L’allevamento di spugne commerciali nella Riserva Marina di Porto Cesarea (LE). *Biologia Marina Mediterranea* 6:110–118.

Sipkema D, Yosef N, Adamczewski M, Osinga R, Mendola R, Tramper J, Wijffels RH. 2005. Large-scale production of pharmaceuticals by marine sponges: sea, cell, or synthesis? *Biotechnology and Bioengineering* 90:201–222 DOI 10.1002/bit.20404.

Stocker LJ. 1991. Effects of size and shape of colony on rates of fusion, growth and mortality in a subtidal invertebrate. *Journal of Experimental Marine Biology and Ecology* 12:161–175.

Ternon E, Perino E, Manconi R, Pronzato R, Thomas OP. 2017. How environmental factors affect the production of guanidine alkaloids by the mediterranean sponge *Crambe crambe*. *Marine Drugs* 15:Article 181 DOI 10.3390/md15060181.

Ternon E, Zarate I, Chenesseau S, Croué J, Dumollard R, Suzuki MT, Thomas OP. 2016. Spherulization as a process for the exudation of chemical cues by the encrusting sponge *C. crambe*. *Scientific Reports* 6:29474 DOI 10.1038/srep29474.

Todd CD, Turner SJ. 1988. Ecology of intertidal and sublittoral cryptic epifaunal assemblages. II. Nonlethal overgrowth of encrusting bryozoans by colonial ascidians. *Journal of Experimental Marine Biology and Ecology* 115:113–126 DOI 10.1016/0022-0981(88)90097-4.

Turon X, Becerro MA. 1992. Growth and survival of several ascidian species from the northwestern Mediterranean. *Marine Ecology Progress Series* 12:235–247.

Turon X, Becerro MA, Uriz MJ. 1996. Seasonal patterns of toxicity in benthic invertebrates: the encrusting sponge *Crambe crambe* (Poecilosclerida). *Oikos* 75:33–40 DOI 10.2307/3546318.

Turon X, Tarjuelo I, Uriz MJ. 1998. Growth dynamics and mortality of the encrusting sponge *Crambe crambe* (Poecilosclerida) in contrasting habitats: correlation with population structure and investment in defence. *Functional Ecology* 12:631–639 DOI 10.1046/j.1365-2435.1998.00225.x.
Uriz MJ, Becerro MA, Tur JM, Turon X. 1996. Location of toxicity within the Mediterranean sponge Crambe crambe (Demospongiae: Poecilosclerida). *Marine Biology* **124**:583–590 DOI 10.1007/BF00351039.

Uriz MJ, Becerro MA, Turon X. 2001. Morphology and ultrastructure of the swimming larvae of Crambe crambe (Demospongiae, Poecilosclerida). *Invertebrate Biology* **120**:295–307 DOI 10.1111/j.1744-7410.2001.tb00039.x.

Uriz MJ, Maldonado M, Turon X, Martí R. 1998. How do reproductive output, larval behaviour, and recruitment contribute to adult spatial patterns in Mediterranean encrusting sponges? *Marine Ecology Progress Series* **167**:137–148 DOI 10.3354/meps167137.

Uriz MJ, Rosell D, Martin D. 1992. The sponge population of the Cabrera Archipelago (Balearic Islands): characteristics, distribution, and abundance of the most representative species. *Marine Ecology* **13**:101–117 DOI 10.1111/j.1439-0485.1992.tb00343.x.

Valisano L, Bavestrello G, Giovine M, Arillo A, Cerrano C. 2006. Seasonal production of primmorphs from the marine sponge Petrosia ficiformis (Poiret, 1789) and new culturing approaches. *Journal of Experimental Marine Biology and Ecology* **337**:171–177 DOI 10.1016/j.jembe.2006.06.030.

Van Soest RWM. 2002. Family Crambeidae Lévi, 1963. In: Hooper JNA, Van Soest RWM, eds. *Systema Porifera: a guide to the classification of sponges*. Vol. 1. New York: Kluwer Academic/Plenum Publishers, 547–555 DOI 10.1007/978-1-4615-0747-5_1.

Van Soest RWM, Boury-Esnault N, Hooper JNA, Rützler K, De Voogd NJ, Alvarez B, Hajdu E, Piser A, Manconi R, Schönberg C, Klautau M, Picton B, Kelly M, Vacelet J, Dohrmann M, Diaz MC, Cárdenas P, Carballo JL, Rios P, Downey R. 2017. World Porifera database. Available at http://www.marinespecies.org/porifera (accessed on 22 November 2017).

Van Treeck P, Eisinger M, Müller J, Paster M, Schuhmacher H. 2003. Mariculture trials with Mediterranean sponge species: the exploitation of an old natural resource with sustainable and novel methods. *Aquaculture* **218**:439–455 DOI 10.1016/S0044-8486(03)00010-3.

Wanick R, Mermelstein C, Andrade IR, Santelli RE, Paranhos RP, Coutinho CC. 2017. Distinct histomorphology for growth arrest and digitate outgrowth in cultivated Haliclona sp. (Porifera: Demospongiae). *Journal of Morphology* **278**:1682–1688 DOI 10.1002/jmor.20741.

Wilkinson CR, Vacelet J. 1979. Transplantation of marine sponges to different conditions of light and current. *Journal of Experimental Marine Biology and Ecology* **37**:91–104 DOI 10.1016/0022-0987(79)90028-5.

Wulff JL. 2006. Ecological interactions of marine sponges. *Canadian Journal of Zoology* **84**:146–166 DOI 10.1139/Z06-019.

Wulff JL. 2017. Bottom-up and top-down controls on coral reef sponges: disentangling within-habitat and between-habitat processes. *Ecology* **98**:1130–1139 DOI 10.1002/ecy.1754.