Population dynamics and life cycle of the introduced ascidian *Microcosmus squamiger* in the Mediterranean Sea

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Abstract Marine introductions are a serious threat for biodiversity, especially in seas where shipping is intensive. *Microcosmus squamiger* is a widespread marine invader that can alter native biota and it is therefore imperative to understand its biology and ecology. We studied the population dynamics and reproductive cycles of *M. squamiger* over a 2-year period, as well as its settlement and colonization patterns, in a north-western Mediterranean (NE Spain) locality where *M. squamiger* has been introduced. All biological parameters showed a strong seasonal pattern that peaked in summer with a major spawning episode at the end of summer. Size-frequency histograms indicated a 2-year cycle. Colonization experiments suggested that *M. squamiger* recruitment mortality is high and requires a well structured community. In addition, we monitored the abundance of the native predator *Thais haemastoma*, which showed a significant positive correlation with *M. squamiger* biomass, indicating its potential usefulness as a biological control.

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Introduction

Biotic invasions are one of the major threats for the maintenance of global biodiversity (Mack and D'Antonio 1998; Mack et al. 2000). In the Mediterranean Sea, the increasing arrival of non-indigenous marine organisms through shipping, mariculture and with the opening of the Suez Canal has resulted in serious alterations of the native biota (Galil 2000; Streftaris and Zenetos 2006). Some introductions have caused loss of biodiversity and are threatening native species (Papaconstantinou 1990; Zibrowius 1991; Galil 2000; Occhipinti Ambrogi 2000; Fine et al. 2001; Piazzi et al. 2001; Boudouresque and Verlaque 2002; Galil 2007).

Ascidians have recently received great attention as marine invaders (Lambert 2007; Whitlatch and Bullard 2007). They can spread through shipping (Wasson et al. 2001) and, once established, extend their range rapidly (Bullard et al. 2007) to become the dominant species (Lambert and Lambert 2003), displacing native space occupiers (Castilla et al. 2004).

The solitary ascidian *Microcosmus squamiger* (order Stolidobranchia, family Pyuridae) is considered to be native to Australia (Michaelsen 1927;

Kott 1985; Monniot et al. 2001; Rius et al. 2008) but it has been detected worldwide (Naranjo and García-Gómez 1994; Naranjo et al. 1996; Lambert and Lambert 1998; Monniot et al. 2001; Monniot 2002; Primo and Vázquez 2004; Turon et al. 2007). In its introduced range, this species can live attached to either artificial or natural hard substrata both inside and outside of harbours (Naranjo et al. 1996). *Microcosmus squamiger* is currently considered a global marine invader, as it has established itself in many regions around the world where it alters local biota (Lambert and Lambert 1998, 2003; Turon et al. 2007). In southern California, for example, the solitary ascidian *Styela canopus* that was previously well established in harbours, is now in decline while *M. squamiger* has increased its abundance and appears to be replacing *S. canopus* (Lowe 2002). In a nearby location, Bahía Falsa, this species has been found in high abundance in oyster farms, where it poses an economic threat (Rodriguez and Ibarra-Obando 2008). Even in its native range this species has been found competing for space with oysters on the western (Kott 1985) and eastern (M.R. pers. obs.) Australian coasts, and in Tasmania, where it can be destructive to oysters (Kott 1985). As a result, this species must be considered as a potential threat for both the local biota and economies. In the Mediterranean Sea, *M. squamiger* was first recorded (as *M. exasperatus*) in the early 1960s in Bizerte, Tunisia (Monniot 1981). Since then, Mediterranean records of this species have frequently referred to it as *M. exasperatus*, but a taxonomic revision by Turon et al. (2007) has established the present range of *M. squamiger* in the Mediterranean, which covers its entire western basin (see also Mastrototaro and Dappiano 2005).

Crucial to the establishment of an alien species as an invader is its ability to overcome local control by resident species (Osman and Whitlatch 1998; Stachowicz et al. 2002), the adequacy of temperature and other parameters for its development (deRivera et al. 2007), and interactions with native predators (Noonburg and Byers 2005) and local biota (Ranasinghe et al. 2005; Rodriguez 2006). Clearly, a preliminary step in the management of any invasive species is to acquire a deep knowledge of its biology and ecology; in particular, what concerns reproductive strategies, growth rates, population dynamics, and interactions with other species. Only a few studies have focused

on the life cycles of introduced marine organisms in their new environment (e.g. Grosholz and Ruiz 1996; Fine et al. 2001; Thornber et al. 2004). In the case of ascidians, although information is accumulating on biological and ecological features of solitary and colonial species (e.g. Becerro and Turon 1992; Turon and Becerro 1992; Giangrande et al. 1994; Eckman 1996; de Caralt et al. 2002; López-Legentil et al. 2005; Pérez-Portela et al. 2007), studies focussing on introduced ascidians are rare (but see Yamaguchi 1975; Parker et al. 1999; Shenkar and Loya 2008). This type of study is crucial for understanding the colonizing processes of invasive species, as well as their establishment in the new environment and potential interactions with native species. From preliminary observations we often witnessed the muricid gastropod *Thais haemastoma* actively predating on *M. squamiger* by inserting its proboscis through the ascidian tunic.

In this work we studied the population dynamics of *M. squamiger* over a 2-year period in a location on the northeast coast of the Iberian Peninsula. *Microcosmus squamiger* is the dominant species along this stretch of coast (Turon et al. 2007), densely carpeting rocky reefs, thriving on natural and artificial rocky reefs and displacing native assemblages (authors' pers. obs.). We monitored the life cycle features of this species through analyses of its population structure and reproductive cycle, as well as its settlement and colonization patterns on bare substratum. In addition, we monitored the abundance of the predator *T. haemastoma* and tested its correlation with the abundance of the ascidian prey.

Materials and methods

Study site

The study was undertaken in the outer part of the main breakwater of Cubelles (41°11′37.2″ N, 1°39′17.46″ E), which was located in the north-western Mediterranean Sea, NE Spanish coast. This coastal defence structure (approximately 800 m long) faces southeast and is formed by concrete blocks (~ 4 m^2). The closest main harbour and recreational marina are situated in Tarragona (ca. 40 Km) and Segur de Calafell (ca. 4 Km), respectively. The sea bottom is constituted by fine sand and, as a result, the

only hard substratum available is the actual breakwater. The maximum depth is 3 m and *M. squamiger* has colonized from the bottom up to a few centimetres below the surface, covering 100% of the available space in most places. In the upper level of its distribution, *M. squamiger* shares the available space with clumps of the mussel *Mytilus galloprovincialis*. Five points separated by at least ~ 25 m were randomly selected along the coastal defence structure for survey sites.

Fieldwork

Surveys were done monthly from July 2005 until June 2007 using SCUBA. For the study of abundance and population structure we scraped the rocky surface at each sampling point ($n = 5$) using a chisel. The area scraped was 18*12 cm, and it was delimited by a metal frame randomly placed on the substratum. Samples were taken between 2 and 3 m depth at each of the five sites. Each sample was placed in a labelled plastic bag. To detect the scraped plots in subsequent surveys (see below), we marked them with a plastic label attached to a bolt with a cable tie. The bolts were driven into holes bored at one corner of the scraped area using a manual drill. The water temperature was recorded using a dive computer with 0.1°C of precision. In addition, we surveyed the abundance of *T. haemastoma* through randomly placing two 50×50 cm quadrats on the rock at each of the five sampling points and counting all *T. haemastoma* individuals in the quadrats. In order to study the reproductive cycles of *M. squamiger*, we randomly collected 25 fully grown individuals situated away from the sampling points. After each dive we transported the samples to the laboratory in a 20 l insulated container with seawater. Transport time did not exceed 45 min.

In February 2006, we studied whether depth had any effect on the length and wet weight of *M. squamiger* populations. For this, we divided the breakwater into 3 depth levels (0–1 m, 1–2 m and 2–3 m) from surface to bottom. At each of the 5 sampling points we randomly collected 5 fully grown animals per depth level.

To assess recruitment, we periodically placed settlement plates in the field. We used plastic Petri dishes (60 mm diameter, 2 dishes per site) with a hole in the centre, through which the dish was fastened to the substratum with screws. This study started in December 2005 and the dishes were removed monthly (from January to October 2006) and replaced by new ones. We systematically scrutinized each dish for the presence of *M. squamiger* recruits using a dissecting microscope.

The monthly scrapings provided experimental units for assessing colonization of bare substrata by *M. squamiger*. In March 2007 we re-scraped all sampled squares. We managed to re-scrape most squares from each sampling date, except July and November 2005 for which all labels were lost. Thus, we had a range of surfaces with different times of exposure and different seasons of initial scraping.

Laboratory techniques

Once in the laboratory the scraped material was immediately fixed using 70% ethanol. Thus, the individuals were not previously relaxed and in contact with ethanol they quickly contracted. We assumed that all individuals were similarly contracted when body measurements took place, but these do not reflect maximal sizes for this reason. During sample processing, all *M. squamiger* individuals were separated from the rest of the organisms (polychaetes, molluscs, algae and other ascidians) and sand using a 3 mm sieve under running freshwater. During the cleaning of *M. squamiger* samples, we occasionally found a similar species, *M. polymorphus*, which could be distinguished from *M. squamiger* because *M. polymorphus* is generally bigger, has a tougher tunic and a larger and tougher attachment base. In case of any doubt, specimens were dissected and identified following Monniot ([1962](#)). To obtain the abundance of *M. squamiger*, sample counts were transformed to individuals per square meter.

The tunics of *M. squamiger* individuals were carefully cleaned to remove any attached organisms. We then measured the body of the individuals (length, width, height and intersiphonal distance) to the nearest mm using a calliper. Subsequently, we blotted the animal on paper tissue and measured the wet weight of the whole organism. Afterwards, the ascidians were dissected, tunic and mantle separated, blotted dry and wet weighed. Finally we placed the mantle and the tunic at 60°C for 24 h to obtain their dry weight. After measuring and weighing many individuals (see results), we found a highly significant

positive relationship between all variables and, as the whole process was extremely time consuming, we decided to simplify the measurements of the subsequent individuals ($N = 1{,}439$) by measuring only the length and the wet weight of the entire organism. The same methodology was used for the samples taken to assess differences of *M. squamiger* populations at different depths and for the re-scraped samples.

The samples for the study of the reproductive cycles were preserved using 10% buffered formalin, to better preserve the internal organs. We dissected 5 individuals per month and separated the gonads from the mantle by carefully dissecting the gonad lobes under a dissecting microscope. Care was taken especially with the left gonad to avoid cutting away fragments of the gut. Subsequently, we measured the wet and dry weight of the gonads, mantle and tunic as described above. A gonad index was then calculated dividing the added dry weights of the two gonads by the mantle dry weight as described in Becerro and Turon (1992).

In order to analyse the state of maturity of the gonads, we analysed stained sections of the gonad. For this, we dissected at least 3 individuals per sampling date and removed a piece of the central lobe of the right gonad (ca. 0.025 cm^2). The pieces of gonad tissue were dehydrated, embedded in paraffin, sectioned and stained with haematoxylin-eosin following standard procedures.

The gonad sections were observed under a microscope equipped with a micrometer. We measured the diameter of oocytes (following Bingham 1997) sectioned through the nucleolus (100 oocytes whenever possible, and a minimum of 70). For the testes we established a categorical maturity index, following Becerro and Turon (1992) according to five (subjective) degrees of male follicle development (i.e., 1 = absent, 2 = traces, 3 = low, 4 = medium and 5 = high).

Data analysis

Morphometric relationships were tested by the Spearman Rank Correlation coefficient, and the significance level Bonferroni-adjusted for the number of pairwise comparisons between variables made.

Temporal trends of several variables were compared among them or with temperature using cross-correlation analysis. In this analysis, the standard Pearson coefficient between variables is calculated with one variable lagged with respect to the other (time lags were in months). A correlation for a negative lag indicates the correlation of the values in the first series to values in the second series that number of months earlier. The correlation at lag 0 is the usual Pearson correlation. Similarly, correlations at positive lags relate values in the first series to subsequent values in the second series.

One-way ANOVA was performed to assess the effect of depth on the length and wet weight data from the organisms collected for this purpose in February 2006. Normality and homogeneity of variance of the data were tested using Shapiro-Wilk's W test and Levene's test, respectively.

We performed the analyses using the software SYSTAT (v. 11, SPSS Inc., 2004), STATISTICA (v. 6, Statsoft Inc., 2001) and SPSS (v. 12, SPSS Inc., 2003).

Results

The patterns of abundance over the study period are shown in Fig. 1a. *Microcosmus squamiger* occurs in high numbers (from ca. 500 up to ca. 2,300 individuals/m^2) all year round. The most prominent feature observed was a decrease in abundance following the maximum temperature in July 2005 and 2006. The values of density are more erratic in the winter months, which may reflect the spatial heterogeneity of the population. Decreases in abundance were observed at the end of winter (February 2006 and 2007). Overall, an increasing trend in numbers during spring was evident in 2006, leading to a peak in July. This same increase was found in spring 2007, and the abundance values at the end of the study were the highest recorded. A cross-correlation analysis of abundance versus temperature (Fig. 2) shows that the correlation coefficient was not significant at any time lag, although the highest coefficients corresponded to a negative relationship between abundance and temperature in the preceding months (time lags of -2 and -3).

We found that all morphometric variables of the individuals measured showed a significant positive correlation when paired-comparisons were made using 522 individuals from the first 13 months (Table 1). We therefore restricted further analysis to

Fig. 1 (a) Mean density of
Microcosmus squamiger
and water temperature over
the study period. (b) Mean
length and wet weight of
Microcosmus squamiger
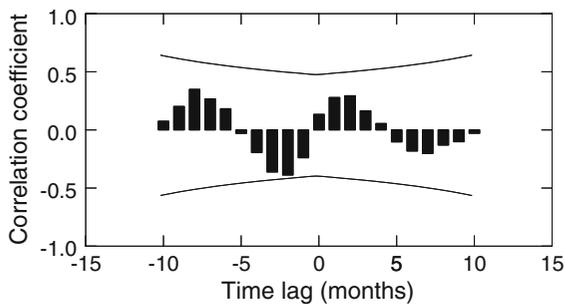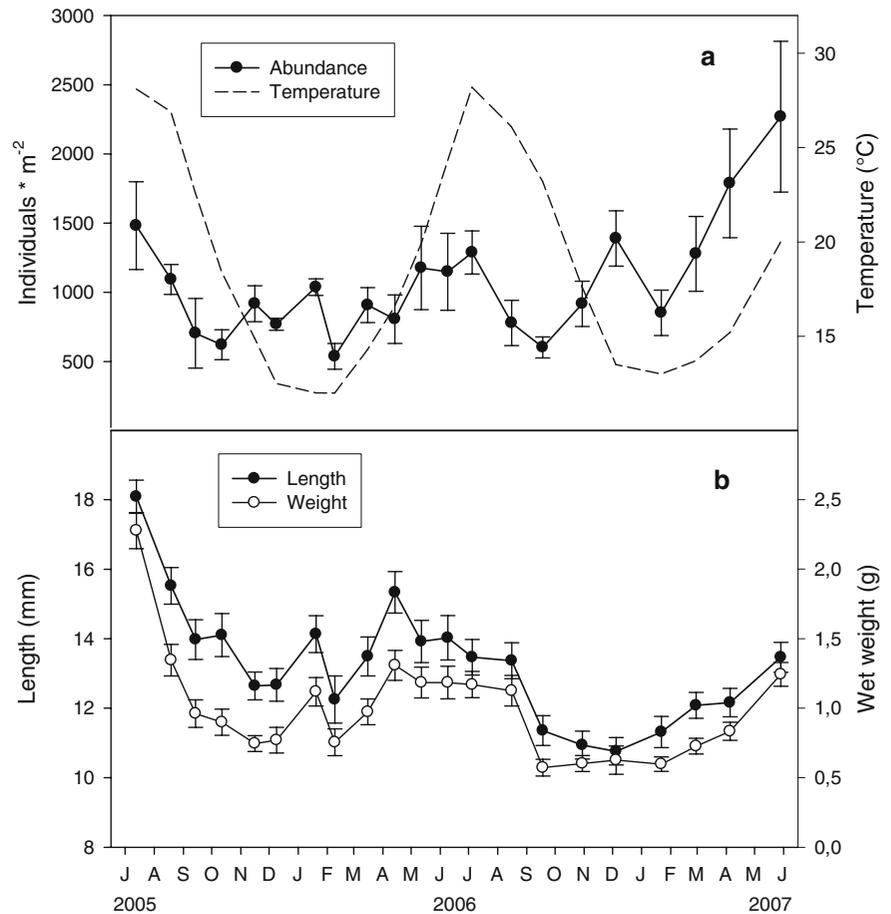over the study period.
Vertical bars denote
standard error

Fig. 2 Results of the cross-correlation analysis of *Microcosmus squamiger* abundance vs. temperature. The curved lines represent the 95% confidence interval of the correlation coefficient

only length and wet weight. When we plotted the values of these variables over the study period (Fig. 1b), a decline in both variables after summer was apparent, with the values remaining low during winter and increasing again in spring. This pattern

reflected a general decreasing trend from the beginning of the study (July 2005), when all variables showed the highest values, until the end of the study.

The mean values of size-related variables (length and weight) did not reflect the underlying cohort dynamics. We therefore plotted these variables in separate size-frequency histograms per month (Fig. 3, as weight and length provided the same information, only length is shown) to elucidate the settlement period and the longevity of *M. squamiger*. It should be noted that the smallest size classes that could be quantified accurately in our samples were of ca. 4–5 mm.

There was a bimodal distribution apparent in the months from March to July. After that, there was a decrease in numbers in August through October associated with the disappearance of the largest mode. The remaining individuals increased in size in the following months and, in February small individuals began to become abundant, with the

Table 1 Spearman Rank Order correlations among all variables measured on *Microcosmus squamiger*

Spearman correlations	Length	Width	Height	Intersiphonal distance	Wet weight	Mantle wet weight	Mantle dry weight
Length	1	0.817***	0.853***	0.903***	0.925***	0.882***	0.896***
Width		1	0.799***	0.775***	0.909***	0.877***	0.87***
Height			1	0.845***	0.921***	0.865***	0.869***
Intersiphonal distance				1	0.884***	0.826***	0.848***
Wet weight					1	0.933***	0.939***
Mantle wet weight						1	0.932***
Mantle dry weight							1

*** Indicates significant correlation after a Bonferroni correction

bimodality being restored during the following months. The overall pattern was repeated in the second year of the study. The observations are coherent with a 2-year cycle, with small individuals appearing in the samples around February-March, growing until August the following year, and disappearing in September. Each cohort coexists with the next one during some months (in its second winter), in which a bimodal distribution of size is observed. The decreasing trend observed in the mean size values (Fig. 1b) was the result of the high proportion of individuals of the younger cohort in the last months of the study. When we plotted the percentages of small (<5 mm) and big (>20 mm) individuals of each month (Fig. 4), the appearance of small recruits in autumn-winter, and the decline in big specimens at the end of summer was apparent.

The reproductive cycle of *M. squamiger* showed a clear annual pattern, with an important peak of the gonad index in summer and minimum values in winter and spring (Fig. 5a). In general, maximum values in summer were followed by an abrupt decrease. Interannual differences were also found, as in 2006 the cycle was clearly advanced with respect to 2005. The cycle of 2007 closely mirrored that of 2006. Interannual differences may be related to differences in temperature. A cross-correlation analysis of gonad index vs temperature (Fig. 6) shows a typical seasonal pattern with high similarity in the time course of both variables. Maximal correlation values were found at time lags close to 0 and negative values at time lags 5–6 months apart.

The examination of the gonad histology showed the presence of a central core of female follicles with oocytes surrounded by peripheral male follicles. The condition of the male and female gonads confirmed the time course of the reproductive cycle previously found. Both the mean oocyte diameter and the maturity state of testes (Fig. 5b) indicated a reproductive event at the end of summer, followed by a non-mature state during winter and a gradual build-up of the gonads during spring. Interestingly, after the abrupt gonad index decrease of July–August 2006, both oocyte size and the maturity state of testes pointed out that gonads still remained mature until October, which suggests that after an initial spawning episode, additional gamete release events could occur over several months.

The percent of oocyte size-categories (Fig. 7) showed that oocytes of the larger size classes (>50 μm) appeared in spring and were shed by the end of summer. Residual oocytes of the larger classes could be observed in December 2005 and November 2006, but generally during wintertime most of the oocytes belonged to the smallest size-classes (0–25 and 25–50 μm).

The measurements of length and weight of the animals collected to test the effect of depth (0–1, 1–2, and 2–3 m depth levels) on *M. squamiger* populations showed non-significant differences between depths in both variables (1-way ANOVA, $F_{2,72} = 0.217$, $P = 0.806$, for length; and $F_{2,72} = 0.026$, $P = 0.974$, for wet weight).

The surveys of the predator *T. haemastoma* showed that the mean density of the gastropod and the available biomass of *M. squamiger* (calculated as the product of density by wet weight) followed a similar trend (Fig. 8). Accordingly, the cross-correlation between both variables (Fig. 9) showed a highly significant (Pearson's $r = 0.737$, $P < 0.001$) value at time lag 0, and the second largest correlation coefficient was between abundance of *T. haemastoma* and
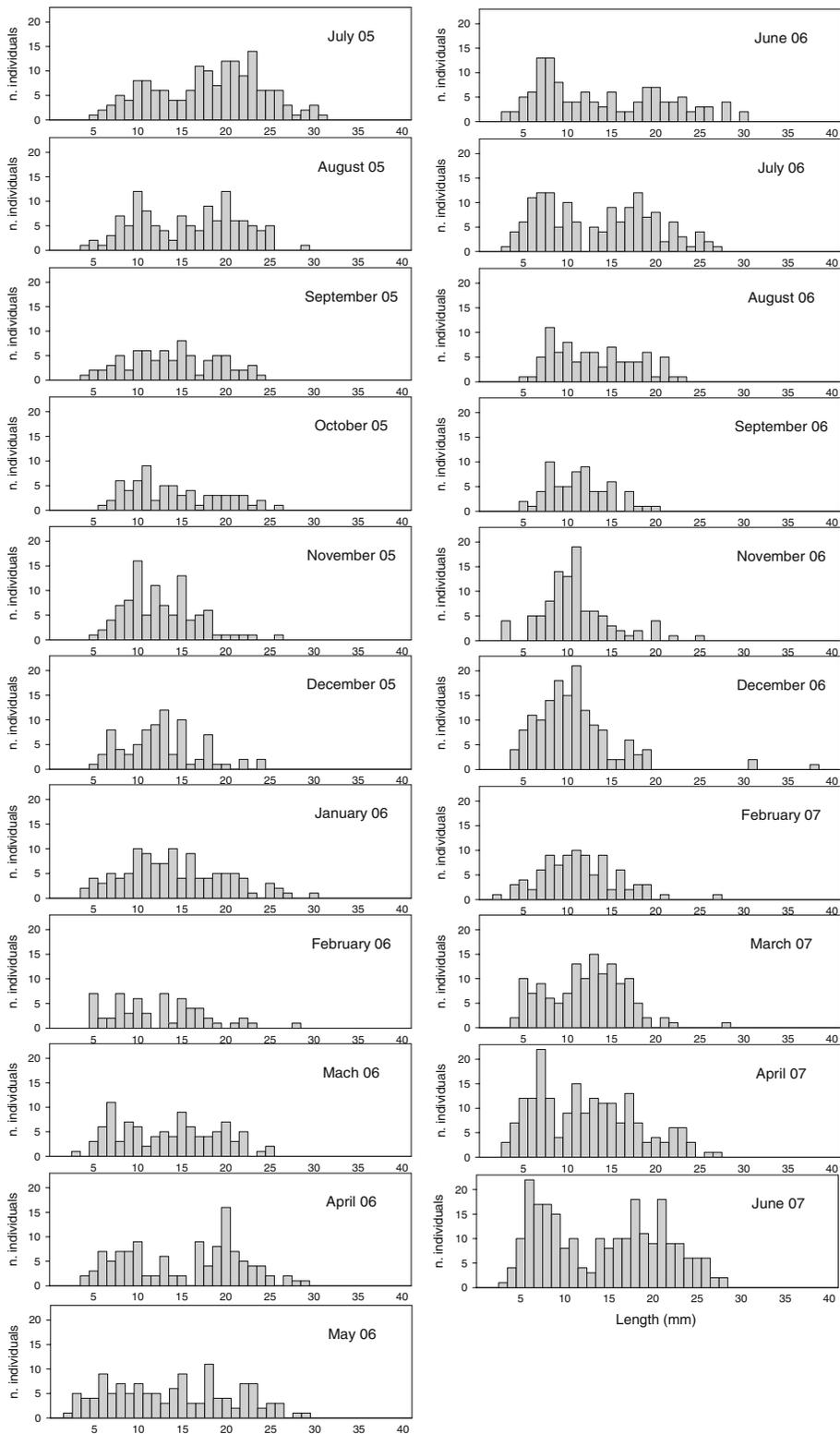
Fig. 3 Size-frequency histograms of *Microcosmus squamiger* for each sampled month

Fig. 4 Mean percentage of *Microcosmus squamiger* individuals larger than 20 mm and smaller than 7 mm in relation to water temperature over the study period
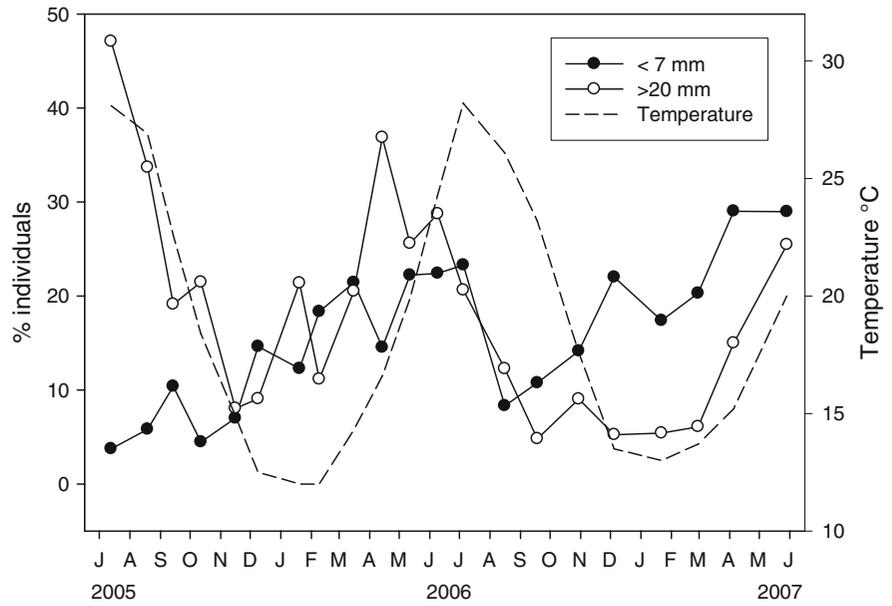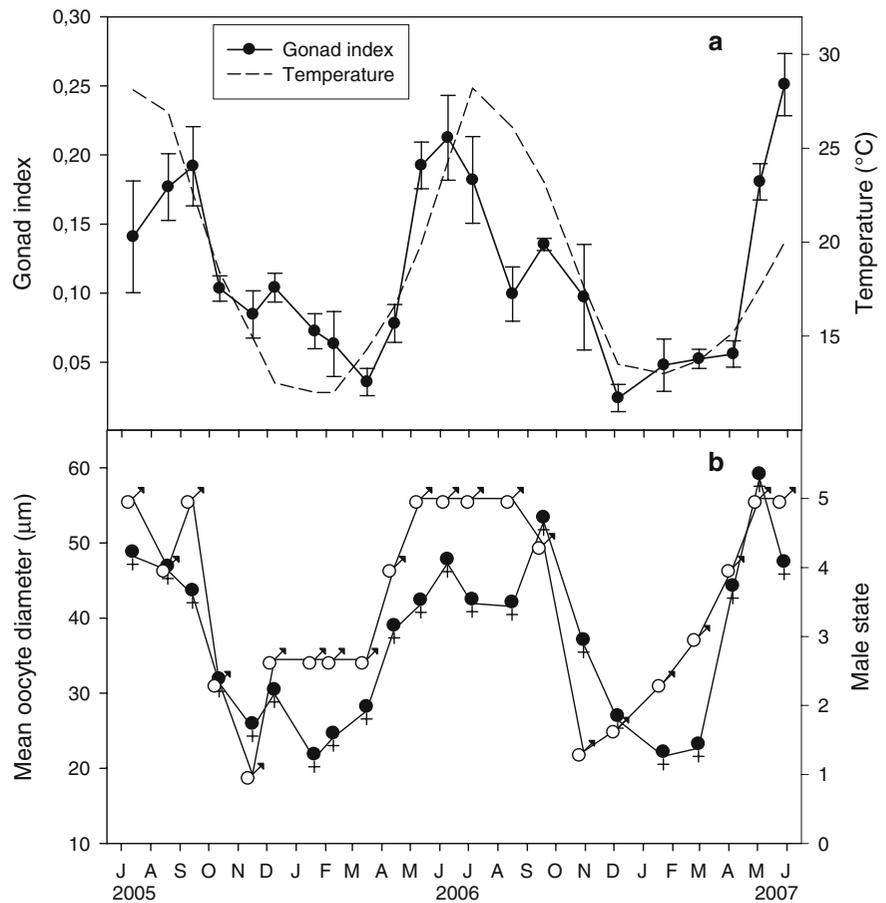

Fig. 5 Gonad cycle of *Microcosmus squamiger* during the study period. (**a**) Mean gonad index and water temperature. (**b**) Mean male maturity state (♂) and mean oocyte diameter (♀) over time
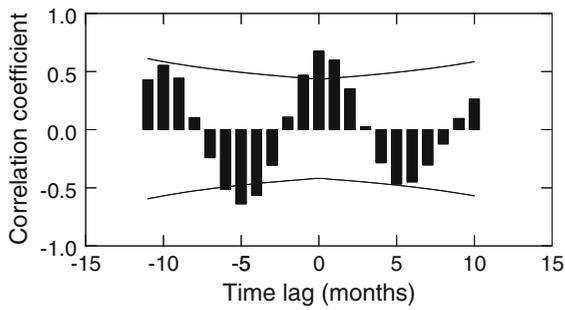
Fig. 6 Results of the cross-correlation analysis of *Microcosmus squamiger* gonad index vs. temperature. The curved lines represent the 95% confidence interval of the correlation coefficient
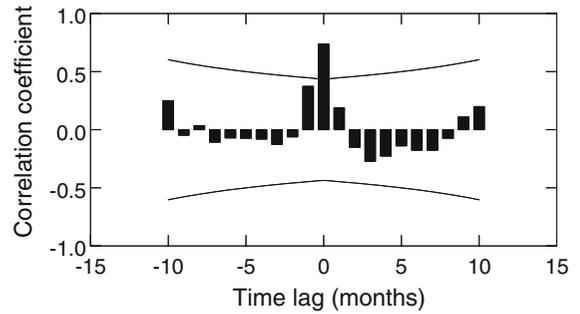

Fig. 9 Results of the cross-correlation analysis of *Thais haemastoma* abundance vs. *Microcosmus squamiger* biomass. The curved lines represent the 95% confidence interval of the correlation coefficient

Fig. 7 Percentage of each oocyte size-class of *Microcosmus squamiger* over the study period
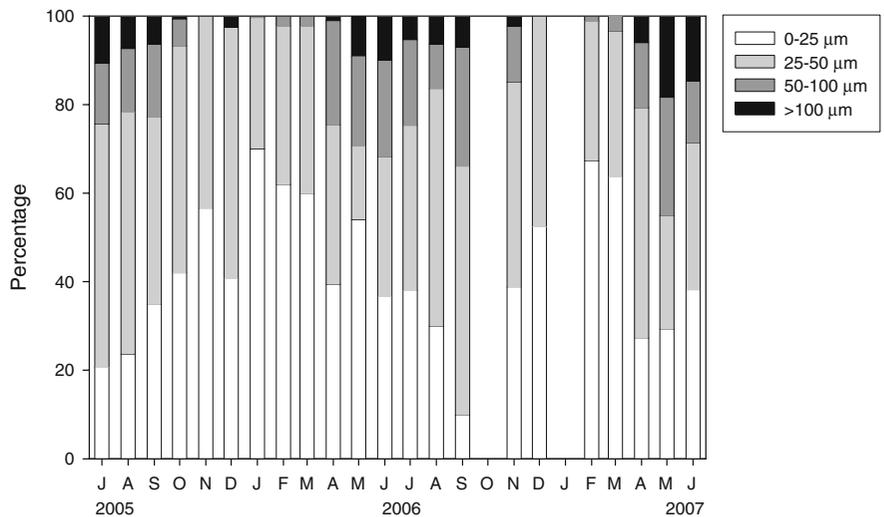

Fig. 8 Mean biomass of *Microcosmus squamiger*, number of *Thais haemastoma* and water temperature over the study period. Vertical bars denote standard error
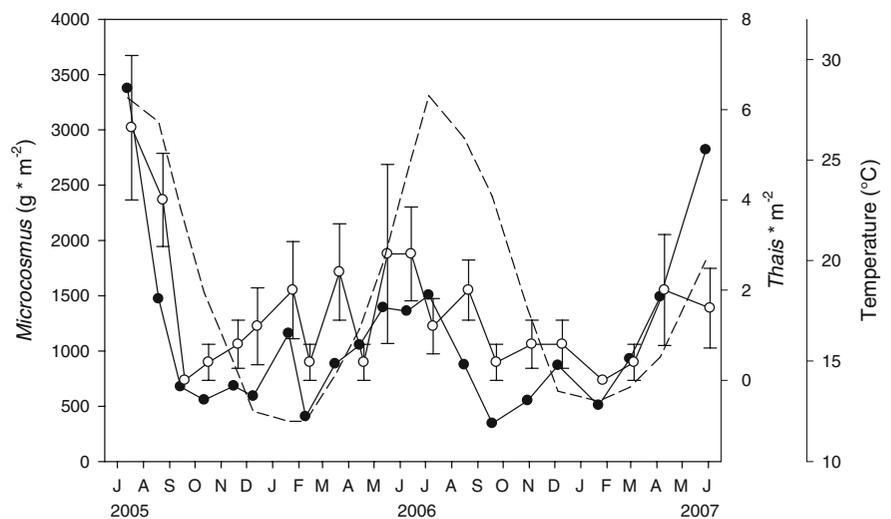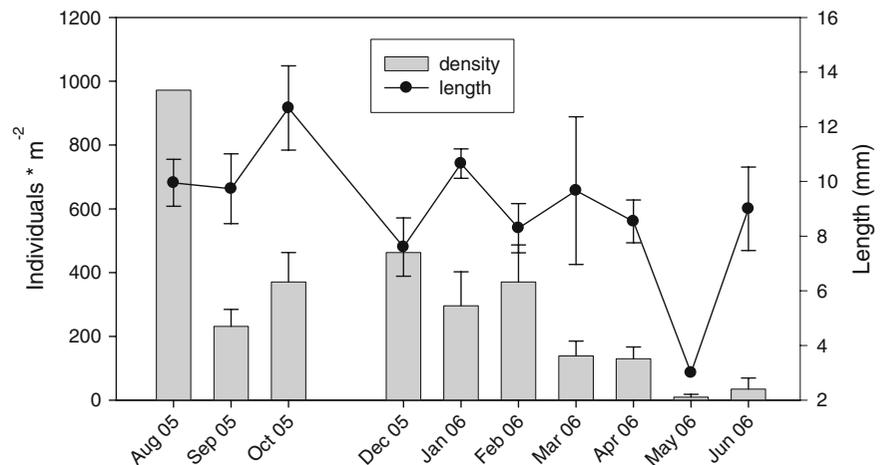
Fig. 10 Re-scrapings of
the sampled plots in March
2007. Mean density of
Microcosmus squamiger
and mean length of the
individuals for each month.
Vertical bars denote
standard error

M. squamiger biomass in the preceding month (time
lag −1).

A high number of scraped plots (ca. 70%) could be
located again and re-scraped in March 2007. These
showed no new *M. squamiger* colonizers in plots
sampled after July 2006. There was a trend of
increasing number of individuals in older plots
(Fig. 10), although the values generally remained
much lower than the mean values of abundance found
in the initial scrapings. Only in a plot scraped in
August 2005 did the recolonization value rise to
almost 90% of the original abundance. The size of the
individuals (presented as length in Fig. 10) generally
followed an increasing trend as we move backwards
in the timing of the first scraping of the plots. In spite
of this evidence of recolonization, on the settlement
plates deployed monthly we could not find any
M. squamiger recruits. The plates were colonised by
a variety of other organisms (mainly algae), but the
only ascidians present were a few specimens of
Botryllus schlosseri.

Discussion

The introduced ascidian *Microcosmus squamiger*
formed dense populations in the area studied, reach-
ing up to 2,300 ind m^{-2}. Albeit with important
density fluctuations, the species was present all year
round and constituted the major structure-forming
organism in the shallow sublittoral of this zone.
Moreover, this species was found colonizing nearby
natural rocky habitats in this area, although with

lower densities (authors' pers. obs.). Its presence,
therefore, has strong implications in the structure and
functioning of native assemblages. In a phylogeo-
graphic study of *M. squamiger*, Rius et al. (2008)
found that introduced populations of this species have
a high genetic variability (possibly as a result of
recurrent introductions). This species, therefore, has
the potential to further expand its presence in
Mediterranean sublittoral communities. The syner-
gism arising from the combination of ecological and
genetic perspectives may be crucial for the develop-
ment of efficient measures to control the threats
posed by invasive species (Sakai et al. 2001).

The studied species displayed a strong seasonal
pattern in the biological parameters analysed. *Micro-
cosmus squamiger* abundance peaked in summer
months, when reproduction occurs. Old individuals
died soon after and the stock was replenished by the
growth of a new cohort during winter. Size and
weight similarly showed a minimum after summer.
The cycle of *M. squamiger*, therefore, appeared to be
coupled to the seasonal variations in temperature and
associated parameters (e.g. food availability, see
Coma and Ribes 2003). Interannual differences were
also observed, with more individuals in 2007 than in
2005, but smaller. The size frequency histograms
showed that this was due to the greater strength of the
smallest cohort of individuals. The reason for these
variations is unknown; perhaps it was related to a
trend of increasing temperatures or inscribed in a
longer cycle of changes.

Although we could not monitor individual speci-
mens over time, the size-frequency histograms

indicated a 2-year cycle. In this cycle individuals settled as a result of the summer reproduction, grew during the next winter and spring and remained in the population until summer of the following year, disappearing thereafter. Consequently, the size structure of the population showed a bimodal distribution during some months, when the two coexisting cohorts could be discerned. This was in accordance with what Panagiotou et al. (2007) found with the congener *Microcosmus savignyi* in the Mediterranean Sea. As we have used only fully grown individuals in the gonad analyses, we cannot assess whether they were one- or two-years old. However, it seems likely that a given cohort can reproduce in the first and the second summer of its life-span. The suggested life-span of two years falls within the range indicated for other solitary ascidians, although in general life-spans can vary according to the latitudinal range in which the species are found (Millar 1971; Svane 1983). Other species of the same family (Pyuridae) can have a remarkable longevity in cold waters, e.g. *Pyura tessellata* on the Swedish coast (Svane and Lundalv 1982).

The mortality of *M. squamiger* observed after summer suggests that this season was the unfavourable one, as found in other Mediterranean invertebrates (Coma et al. 2000). It was common to observe underwater carcasses (in the form of empty and decomposing tunics) of dead individuals during the months following the peak temperatures (authors' pers. obs.). In a study that assessed the fertilization success of *M. squamiger* in the laboratory at different temperatures, fertilized eggs developed normally at 20 and 25°C, while at 30°C eggs failed to produce viable embryos (MR, unpubl. res.). This result and the mortality observed in the present study suggest that during extraordinary warm periods, which have been recently often observed during summer in the Mediterranean, this species' survival might be compromised.

Reproductive cycles showed a clear trend: the mean gonad weight was the highest in summer, followed by an abrupt decrease. These results are similar to those found for *Halocynthia papillosa* in the Mediterranean by Becerro and Turon (1992), while the same study found that *Microcosmus sabatieri* reproduced later, from autumn until the beginning of winter. These results indicate that all these species have seasonal reproductive patterns.

Interestingly, another pyurid species introduced in the Mediterranean Sea, *Herdmania momus*, has continuous breeding in its native range (the Red Sea) whereas in the Mediterranean this species appears to have two short reproductive periods in spring and autumn (Shenkar and Loya 2008).

The histological analysis of the gonads was coherent with the gonad index results and showed a single spawning season per year, although mature oocytes and sperm were found during several months (from May to September). This suggests that, although the steep decrease in gonad index clearly indicates the main gamete release episode, smaller spawning events could occur over subsequent months. Alternatively, those gametes could be simply non-spawned remains of the reproductive season to be gradually resorbed. The mean oocyte diameter and the testes' development followed the same trend, with the highest values during summer months and minimum values between November and February. *Microcosmus squamiger* can be considered a simultaneous hermaphrodite since both sperm and oocyte maturation and release takes place synchronously, as has been reported in many other solitary ascidians (Millar 1971; Becerro and Turon 1992; Marshall 2002). Nevertheless, testes seemed to recover faster than oocytes after the minima in November, which indicated a slight protandry in gonad development. Some degree of protandry was also observed in a Brazilian population of *Phallusia nigra* (da Rocha et al. 1999).

It is surprising that we could not detect any settlers or recruits on the Petri dishes placed in the field. In a separate study, *M. squamiger* settlement was studied in the laboratory (MR unpubl. res.) using the same type of Petri dishes. This surface was therefore suitable for settlement and we have extensive experience with observations of both settlers and recruits of this species, so could not possibly have overlooked them. Our own data on settler growth indicate that they ranged in length from ca. 0.2 mm the first week to ca. 2 mm after 5 weeks, which should be clearly detectable. One possible explanation would be that the plastic surfaces are not preferred for settlement when larvae have a choice of where to settle (unlike the situation in a laboratory). Alternatively, high mortality during the vulnerable first weeks of life could have resulted in the loss of all settlers before we could examine them.

The recolonization experiment (through re-scrapings) is consistent with high recruit mortality. The percent of recolonization was low even for plots that were scraped more than one year before the re-scraping. Significantly, plots scraped from December 2005 to October 2006 and re-scraped in March 2007 could only receive recruits from the 2006 reproduction event, yet the older plots had more recruits than the more recently scraped, and we did not detect any recruits in plots scraped from July 2006 onwards. This suggests that some level of community development is necessary for *M. squamiger* settlement or, alternatively, for avoidance of predation of juvenile specimens (the more recent plots were still essentially bare rock with filamentous algae). This, together with the lack of recruitment in the petri dishes suggests that *M. squamiger* is a secondary colonizer that needs a well structured community in order to ensure successful establishment.

We found a strong correlation between the biomass of *M. squamiger* and the density of the whelk *T. haemastoma*, which has already been identified as a predator of other pyurids such as *Pyura praeputialis* in Chile (Castilla et al. 2004). Whether this relationship responds to both species having a similar seasonal cycle or whether the abundance of *M. squamiger* enhances the population of the predator needs to be addressed with experimental work. Notwithstanding, the clear correlation found points to a potential role of the native *T. haemastoma* as a biological control of the introduced ascidian (see Lafferty and Kuris 1996). *Thais haemastoma* is known to shift feeding habits when novel types of prey are available (Rilov et al. 2002). During the surveys, we frequently observed *T. haemastoma* laying eggs in between crevices around where *M. squamiger* dominated the substrata. The gastropod itself is appreciated as a delicacy and harvested in the Spanish Mediterranean, which may make its use in pest control profitable, although clearly more research is needed about the specificity and consumption rates of the predator. Another approach could be to develop a commercial fishery of *M. squamiger* outside harbour areas. Some species of the genus *Microcosmus* are harvested as food in France (Lambert and Lambert 1998), and related species of the genus *Pyura* are used as fish bait or as food in distant regions of the world (Kyle et al. 1997; Monteiro et al. 2002; Castilla et al. 2004).

The study of life cycles is particularly relevant for species that are widely distributed as a result of multiple introductions. It has been noted that such species adapt their growth and reproduction features to local conditions (particularly temperature). Several ascidian species, such as *Ciona intestinalis*, *Styela plicata* or *Herdmania momus*, provide clear instances of such adaptive plasticity (Yamaguchi 1975; Shenkar and Loya 2008; see Millar 1971 for a review of earlier literature). Thus, it is not possible to extrapolate results across different parts of the distributional range, which suggests that an effective management would include the acquisition of knowledge about the biology of the organism in the geographical area concerned. Further studies are necessary to compare life history traits of *M. squamiger* in the native and introduced distributional ranges.

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