



Growing or reproducing in a temperate sea: optimization of resource allocation in a colonial ascidian

Susanna López-Legentil,^{1,a} Patrick M. Erwin,¹ Marta Velasco,¹ and Xavier Turon²

¹ Department of Animal Biology, University of Barcelona, Barcelona 08028, Spain

² Center for Advanced Studies of Blanes (CEAB-CSIC), Blanes 17300, Girona, Spain

Abstract. Relatively little is known about the life cycles of ascidians in temperate seas. Here, we investigated the biological cycle of the colonial ascidian *Didemnum fulgens*, a dominant species in some shallow localities of the NW Mediterranean Sea. Growth rates and frequencies of fission/fusion events were calculated over a period of 13 months, and the reproductive cycle determined after 32 months of observation. For analyses of reproduction, zooids were dissected in the laboratory and classified into five reproductive categories; these data were used to calculate a maturity index. For growth analyses, underwater photographs of marked colonies were used to estimate the surface area of *D. fulgens* colonies, calculate monthly growth rates, and document fusion and fission events. Clear seasonal patterns in reproduction and growth were observed, with distinct periods of investment into each function. Gonad maturation started in winter and larval release occurred in early summer, just before maximal sea temperatures were reached. After reproducing, colonies shrank and aestivated during the warmer summer months. Growth occurred during the cooler months, with maximal and minimal growth rates observed in winter and summer, respectively. Fusions and fissions occurred year-round, although fissions were more frequent in fall (coincident with high growth rates) and fusions in spring (coincident with reproduction). These results add to the mounting evidence that ascidian life cycles in temperate seas are characterized by a trade-off between investment in reproduction and growth, triggered by seasonal temperature shifts and constrained by resource availability during summer.

Additional key words: growth, maturity, Mediterranean, temperature, tunicate

Ascidians or sea squirts (Chordata: Tunicata) are sessile, filter-feeding organisms that occur in benthic ecosystems across a broad range of depths and latitudes (Lambert 2005). These animals are notorious for their detrimental effects on aquaculture facilities, as fouling organisms attached to ship hulls (Turner et al. 1997; Rodriguez & Ibarra-Obando 2008; Hewitt et al. 2009; Carman et al. 2010), and as introduced and invasive species (e.g., *Didemnum vexillum* KOTT 2002; Lambert 2009; Stefaniak et al. 2009, 2012). In addition, ascidians have been used as bioindicators of pollution (Agell et al. 2004; Carman 2007; Azumi et al. 2008) and as a source of bioactive secondary metabolites for anti-cancer drug research (Erwin et al. 2010). While their ecological and biotechnological importance is well known, compara-

tively little is known about their biology and, in particular, their growth and reproductive strategies.

Ascidians are hermaphroditic and mostly reproduce through sexual cross-fertilization, resulting in the production of free-living, non-feeding larvae that must find a suitable substrate on which to settle and metamorphose in a matter of hours to days (Svane & Young 1989; Rius et al. 2010). Solitary ascidians are normally oviparous, while colonial ascidians usually brood their larvae. Colonial ascidians can also experience fission and fusion events. Fission is a phenomenon in which an existing colony divides to generate two or more smaller colonies; it is considered an asexual method of colony proliferation. In contrast, fusion is the union of two or more colonies, resulting in rapid growth. In didemnid ascidians, colonies appear to fuse indiscriminately (Bishop & Sommerfeldt 1999), whereas in botryllids, fusion is regulated by genetic histocompatibility (Oka &

^aAuthor for correspondence.
E-mail: slopez@ub.edu

Watanabe 1957; Sabaddin 1962; Saito et al. 1994). Within the colonial ascidians, most of the available information on reproductive ecology and colony dynamics concerns botryllid ascidians (e.g., Newlon et al. 2003; Bates 2005; Rinkevich 2005; Marshall et al. 2006; Shenkar et al. 2008; Westerman et al. 2009). In contrast, much less is known about aplousobranch ascidians like didemnids (e.g., Bak et al. 1981; Bishop & Sommerfeldt 1999; Dias et al. 2008).

In general, ascidians that live in warmer waters reproduce and grow throughout the year (Duyl et al. 1981), whereas those that live in temperate and subtropical seas tend to exhibit seasonal life cycles (e.g., Gotelli 1987; Davis 1989; Hirose et al. 2005, 2006, 2007; Fukuda & Hirose 2008; Panagiotou et al. 2008; Shenkar & Loya 2008; Vafidis et al. 2008; Ritzmann et al. 2009). Exceptions to this trend have been reported for some invasive and introduced species, which appear to be able to reproduce year-round in some of their introduced areas (Bourque et al. 2007; Valentine et al. 2007; Wong et al. 2011; Pineda et al. 2013). In the Mediterranean, most colonial ascidians reproduce before maximal sea temperatures are reached in summer (Turon 1988; Turon & Becerro 1992; Molin et al. 2003; López-Legentil et al. 2005), whereas a few species do so during the cooler months of the year (e.g., *Aplidium* spp., Turon 1988; Turon & Becerro 1992). Similarly, most of the colonial ascidians studied in the Mediterranean experience positive growth during fall and winter (Turon & Becerro 1992; López-Legentil et al. 2005; Pérez-Portela et al. 2007), and no species has been observed to actively grow during the summer months. Millar (1971) proposed temperature as the main regulator of the life cycles of temperate ascidians, but other factors, such as resource availability (Bates 2005; Lambert 2005), turbidity (Millar 1974), salinity (Hirose et al. 2007), currents and wave exposure (Shenkar & Loya 2008), or competing invertebrate species (Stocker & Underwood 1991; Dias et al. 2008) may also influence their life cycles. A trade-off between reproduction and growth has also been observed in some ascidians, suggesting a partitioning of resources to specific biological functions (López-Legentil et al. 2005; Pérez-Portela et al. 2007). In fact, López-Legentil et al. (2007) showed that reproduction was the main factor driving both growth and defense production (secondary metabolites and ash content) in a temperate ascidian.

Resting stages are also common in ascidians living in temperate seas, and normally occur during the unfavorable season. For most Mediterranean filter-

feeders, the resting period occurs during summer and is called aestivation (Coma et al. 2000). The function of aestivation is not well understood in ascidians and several hypotheses have been suggested for different species. Berrill (1951) described a regression period after reproduction that involved tissue reorganization. Burighel et al. (1976) suggested an excretory role of degenerative residues. Turon (1992) proposed a “rejuvenative” process that involved the regression of old thoraxes and budding of new ones, and Molin et al. (2003) suggested a propagative budding to generate new zooids after reproduction. Although the reason for aestivation and resting is not clear, this phenomenon appears to be widespread among ascidians in the Order Aplousobranchia, and is found in many species from several different families (e.g., *Polysyncrator lacazei* [GIARD 1872], Didemnidae, Turon 1992; *Pseudodistoma crucigaster* GAILL 1972, Pseudodistomidae, Turon & Becerro 1992; *Polycitor adriaticus* [VON DRASCHE 1883], Polycitoridae, Molin et al. 2003; and *Pycnoclavella* spp., Clavelinidae, Pérez-Portela et al. 2007).

Didemnid ascidians can be dominant organisms in sublittoral communities (e.g., Bak et al. 1981; Ryland et al. 1984; Stocker 1991), especially when they become invasive (e.g., Lambert 2009; Kremer et al. 2010; Smale & Childs 2012). Thus, the study of their population dynamics and life cycles is useful to explain processes such as space occupation and community diversity patterns (Dijkstra et al. 2007; Dijkstra & Harris 2009; Fiore & Cox Jutte 2010). The colonial ascidian *Didemnum fulgens* (MILNE-EDWARDS 1841) was first described in the Celtic Sea (Atlantic, NW France), and is widely distributed in the Mediterranean (Lafargue & Wahl 1987; Koukouras et al. 1995). During the last decade, populations of *D. fulgens* have increased in number and in size in the NE coast of Spain. Formerly, individuals of this species were rarely found in this region, but currently *D. fulgens* is a common ascidian in some rocky infralittoral communities (authors' personal observation) and on the rhizomes of the seagrass *Posidonia oceanica* (LINNAEUS) DELILE 1813 (Balata et al. 2007). The recent expansion of *D. fulgens*, in terms of population numbers and sizes, raises questions about the environmental shifts that may have facilitated the spread of this species and the biological characteristics that have allowed it to outcompete resident sessile organisms. The goal of this study was to determine the seasonal cycles of reproduction and growth in *D. fulgens* and assess whether a trade-off in resource allocation exists

between these two biological functions. To address these questions, we assessed the reproductive cycle of *D. fulgens* by characterizing reproductive status monthly for over 2.5 years, and determined the growth rate and number of fission/fusion events monthly for 1 year using underwater transects and digital photography.

Methods

Study site and species identification

This study was conducted at a coastal site in the Mediterranean Sea located between L'Illa Mateua and Cala Montgó in L'Escala (NE Spain, 42°7'29" N, 3°7'57"E). Since previous studies conducted in this area during the early 2000s, we have observed the proliferation of an orange-pinkish didemnid that has rapidly become a conspicuous species in shallow rocky communities, and has reached densities of 12.93 colonies/m² (± 0.64 SE).

The species was identified following the descriptions of Lafargue (1976) and Lafargue & Wahl (1987). In short, *Didemnum fulgens* is characterized by bright orange colonies, dense calcareous spicules (up to 50 μ m in diameter) with short, pointed rays (~ 20 visible), zooids with small thoracic organs in the posterior part of the thorax, a sperm-duct with 7–9 coils, and larvae with two adhesive papillae and 4–6 pairs of epidermal ampullae.

For barcoding, one colony was preserved in 100% ethanol and stored at -20°C until processed. Zooids were carefully separated from the tunic while being observed with a stereomicroscope, and DNA was extracted using the DNeasy Blood & Tissue kit (Qiagen). The primer set Tun_forward and Tun_reverse2 described by Stefaniak et al. (2009) was used to amplify a fragment of the Cytochrome *c* Oxidase subunit I (COI) mitochondrial gene. Total PCR reaction volume was 25 μ L, including 5 pmol of each primer, 5 nmol of each dNTP, 1 \times reaction buffer (Ecogen), and 2.5 units of BIOTAQ polymerase (Ecogen). The PCR program included initial denaturing at 94°C for 5 min, followed by 40 amplification cycles of 94°C for 30 s; annealing at 40°C for 30 s; and extension at 72°C for 1 min. After the 40 amplification cycles, a final extension was carried out at 72°C for 10 min. Cleaning and sequencing reactions were carried out by Macrogen, Inc. (Seoul, Korea). The resulting sequence was deposited in the GenBank database (accession #JX846617). BLAST searches revealed another didemnid species as best match (*D. perlucidum* MONNIOT 1983, JQ731735, 88% similarity).

Sample collection and monitoring

To determine the reproductive cycle of *D. fulgens*, five colonies were collected and immediately fixed in 10% formaldehyde every month from June 2009 to May 2012 by SCUBA divers. Sampled colonies were larger than 3 cm² (to ensure that they were not too small to reproduce) and separated by at least 5 m from each other (to avoid sampling clones). Collection depth ranged from 4–10.5 m. From April 2011 to May 2012, a permanent 3.5-m horizontal transect at 7 m depth was established along a vertical wall and the position of each colony of *D. fulgens* sketched underwater on a plastic slate. Each colony was revisited monthly and photographed with an Olympus C-7070 camera equipped with an underwater PT-027 housing fixed to an aluminum frame (18.7 \times 13 cm) to keep a constant focal distance. Seawater temperature was calculated each sampling day using either a HOBO[®] Pendant Temperature Data Logger (Onset Computer Corporation) located at 14 m depth, or a diving computer during the periods in which no datalogger was deployed. No sampling could be performed in December 2009, April 2010, September 2010, November 2010, and February 2012 because of unfavorable diving conditions. For these months, seawater temperatures were calculated as an average of the previous and following months' temperatures.

Reproductive cycle

Once in the laboratory, colonies fixed in 10% formaldehyde were dissected while being observed with a stereomicroscope. Ten randomly selected zooids per colony were examined to determine their reproductive status. Each zooid was categorized as (1) immature, (2) testes present, (3) testes and oocytes present, or (4) oocytes present. A maturity index (MI, López-Legentil et al. 2005) was calculated for each colony by averaging the category number of the zooids examined, except when larvae were observed in the basal tunic. Colonies with mature larvae (not brooding) were scored with a MI value of 5 as larval production represents the culmination of reproduction and zooids regress to immature status during the brooding period. Monthly means were calculated by averaging the values of the colonies investigated that month.

Growth cycle

As colonies of *D. fulgens* have a thin and encrusting morphology, changes in the surface area over

time were calculated to estimate growth. The surface area of each colony was measured using the image analysis software ImageJ (v. 1.45s). Fission and fusion events were identified by comparing pictures of the current and previous months. Whenever a “new” colony was located precisely at the same location where a colony was photographed the previous month, the “new” colony was considered the result of a fission event. The nature of these events, whether they occur among colonies with the same genotype or different genotypes (chimeras), is unknown for *D. fulgens*. In this study, we grouped together colonies resulting from a fusion or fission event into 33 groups or “growth units.” When fusion occurred, the areas of the previous colonies were added together to obtain a single value for each unit. When fission occurred, areas of the resulting colonies were added (Turon & Becerro 1992). Monthly growth rates (GR) were calculated as follows:

$$GR_m = (A_m - A_{m-1})/A_{m-1}$$

where A_m and A_{m-1} are the areas at month m and at the previous month ($m-1$), respectively. These values were then smoothed to avoid noisy fluctuations using a weighed moving average: the value of the current month (GR_m) multiplied by 0.5, plus values of the previous (GR_{m-1}) and subsequent (GR_{m+1}) months multiplied by 0.25.

Cross-correlation analysis

Cross-correlation analyses were used to establish relationships among growth rate, maturity index, and temperature over time. In cross-correlations,

two series of data are compared as a function of a time lag (n), using the Pearson correlation coefficient to measure relationships between values of the first data series and values of the second series n months earlier (in negative lags) or later (in positive lags). Correlation coefficient values at lag 0 are equivalent to the standard Pearson correlation. We used Systat v. 12.02 for the analyses.

Results

Reproductive cycle

Reproductive cycle analyses (Fig. 1) showed a marked seasonal fluctuation in reproduction in *Didemnum fulgens*, with larval release occurring mostly in spring and early summer. Immature zooids were recorded all year round, but were more abundant from July/August to December in the three investigated years. Zooids with testes were present from January to April, zooids with both testes and oocytes were found from March to May, and zooids with only oocytes were present from May to June. Colonies with mature larvae were observed from May to June; these colonies contained either immature zooids, or zooids with oocytes. Only in May 2011 all the colonies observed did have larvae. Larval release occurred in early summer before the warmest sea temperatures were reached, and was followed by 3–4 months during which only immature zooids were recorded. Years with sharp increases in temperature coincided with a shorter larval release period (e.g., one month in 2010) while more gradual increases of temperature were accompanied by longer spawning events (e.g., three months in 2011).

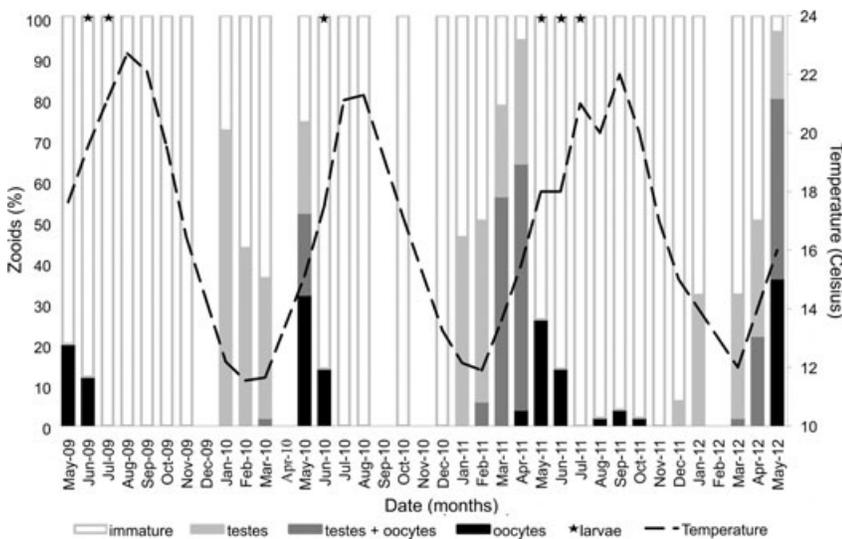


Fig. 1. Zooid reproductive status in *Didemnum fulgens* over the monitoring period. The percentage of zooids in different reproductive stages (mean of ten zooids per colony, five colonies per month) are shown for each month; stars indicate the presence of larvae in the colonies. Empty columns correspond to months without data. Temperature data (dashed line) are superimposed.

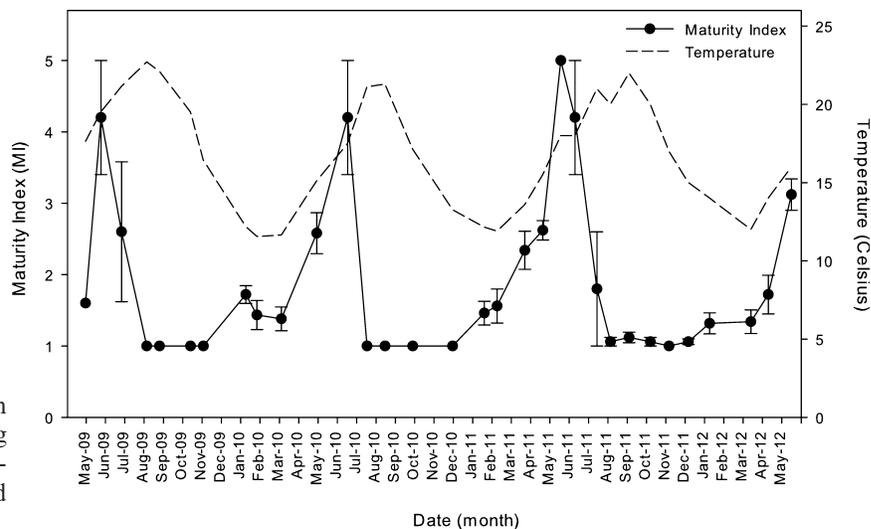


Fig. 2. Mean maturity index (MI) in *Didemnum fulgens* over the monitoring period. Error bars represent ± 1 standard error. Temperature data (dashed line) are superimposed.

The maturity index (MI) increased in winter and achieved its maximum around June each year, coinciding with the brooding period (Fig. 2). After June, MI decreased abruptly before seawater temperature reached its maximum, and maintained low values until the following winter. Cross-correlation analysis showed a significant negative correlation between the maturity index and the water temperature measured a few months before (Fig. 3). An increase in MI was therefore preceded by a temperature minimum 1–4 months beforehand (time lag -1 to -4). The wave-like form of the cross-correlation plot, with peaks separated by ~ 6 months, reflected the seasonal nature of the changes in both temperature and MI.

Most colonies entered into a resting state after reproduction that was maintained during the warmer months of the year (June, July, and August). The resting form was characterized by a closure of all incurrent siphons and a “leather-like” appearance

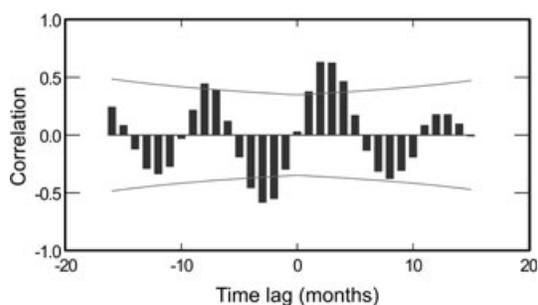


Fig. 3. Cross-correlation analysis between the maturity index and temperature, with time lag in months. Bars that reach or pass the curved lines have significant positive (upper) and negative (lower) correlation values.

of the colonies (Fig. 4). Most colonies resumed filtering (i.e., opened incurrent siphons) in September, and by October, all colonies were actively feeding.

Growth cycle

Overall, the total area of the colonies surveyed increased 261% (± 406.74 SD) during the study period (Fig. 5). Growth rates showed a clear seasonal trend, with lower values in spring and summer and higher values in fall and winter (Fig. 6). Cross-correlation results indicated that growth was significantly correlated with temperature, as growth rates increased in the colder months and decreased in the warmer months (Fig. 7A). There was a negative and significant correlation at time lags -1 , 0 , 1 , and 2 (Fig. 7A). Thus, growth rates were negatively correlated with the current water temperature and the temperature in the previous month. Growth rates tended to be higher for smaller colonies than for larger ones, as there was a marginally significant negative correlation between the initial size of the growth units and their overall growth rate (Pearson's $r = -0.376$, $p = 0.064$).

Relationships between growth and reproduction were investigated to look for potential trade-offs in the allocation of energy resources. Our results showed opposite trends for the two variables: when the maturity index increased, growth rates decreased (Fig. 7B). Cross-correlation analyses further confirmed this observation, revealing a significant negative correlation between growth rate and the maturity index at time lags -1 and -2 , indicating that higher investment in reproduction in the previous 1 and 2 months was related with lower current growth (Fig. 7B).

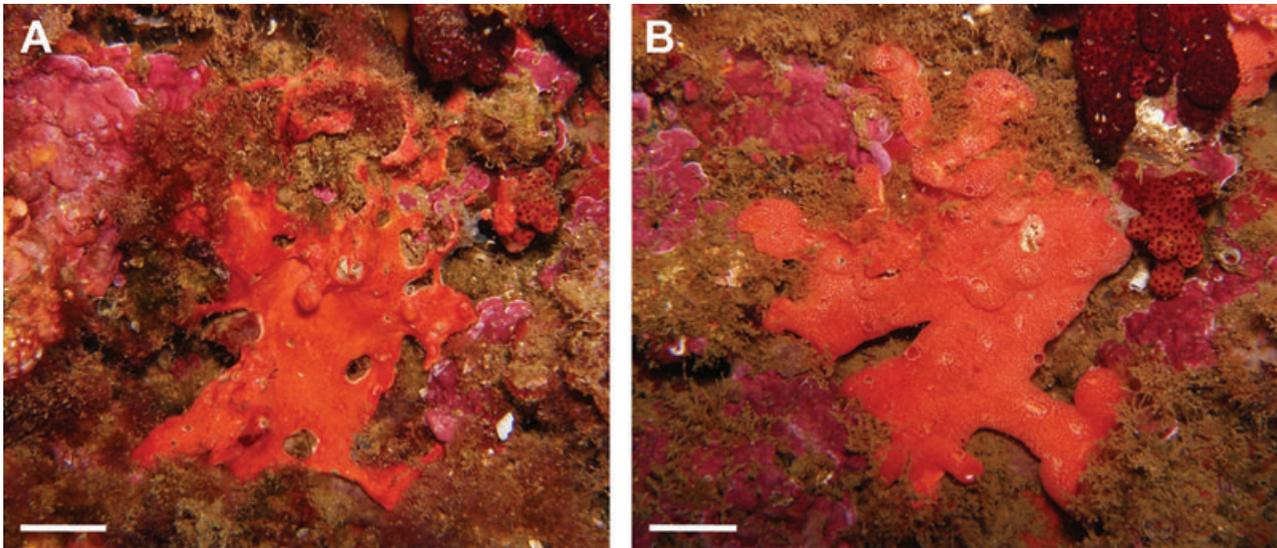


Fig. 4. *In situ* photographs of a colony of *Didemnum fulgens* monitored for growth. **A.** Resting form, July 2011. **B.** The same colony actively filtering in January 2012. Scale bars=2 cm.

A total of 18 fusions and 24 fissions occurred in 51% of the colonies surveyed (Fig. 8). Fusions and fissions were recorded throughout the year, although fusions were more abundant in spring and fissions in fall (Fig. 8). Fusions mainly occurred in March and April around the time most of the colonies reached their maximal growth rate. Fusion was related to marked area increases, as the sizes of the resulting colonies were larger ($57.77 \pm 10.48\%$, mean \pm SE) than the sum of the area of the colonies that merged. Fused colonies grew $\sim 40\%$ more than the mean colony growth recorded for that given month. On the other hand, most fission events occurred

from September to January, corresponding with reactivation after summer dormancy. Fission did not involve net growth, and the total area of the resulting colonies was similar ($0.8 \pm 15\%$, mean \pm SE) to that of the original colony. In fact, the colonies that underwent fission grew $\sim 10\%$ less than the mean growth recorded for all colonies during that given month.

Discussion

At our study site, the colonial ascidian *Didemnum fulgens* had a seasonal life cycle alternating between reproduction and growth. Maturation of the gonads started in winter and culminated with larvae release in early summer, just before maximal sea temperatures were reached. After spawning, colonies went into aestivation coinciding with the warmer months of the year. Growth started in fall and continued during winter, with a decrease in spring at the time of the rise of the maturity index. Reproduction was positively correlated with seawater temperature in the previous months, while growth rates were negatively correlated with temperature in the current and previous month and with investment in reproduction during the preceding months. A relationship between growth and size was also recovered, with smaller colonies tending to exhibit higher growth rates, as observed for other colonial ascidians (e.g., Stocker 1991; Turon & Becerro 1992; López-Legentil et al. 2005).

Temperature has often been reported as the main abiotic factor driving ascidian life cycles in temperate

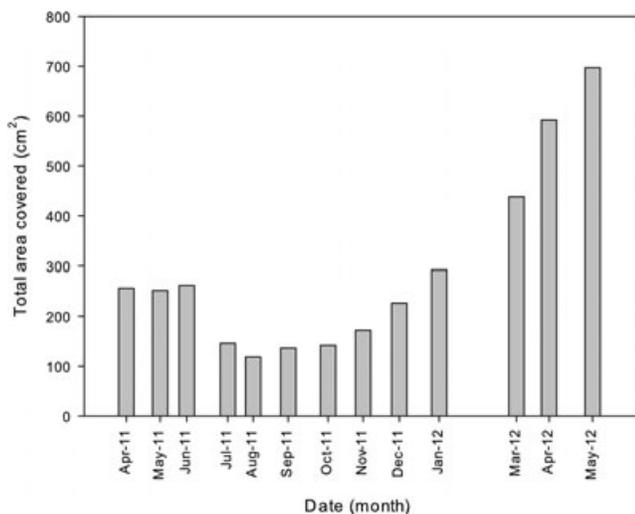


Fig. 5. Total area covered by the studied colonies of *Didemnum fulgens* over time in the surveyed area.

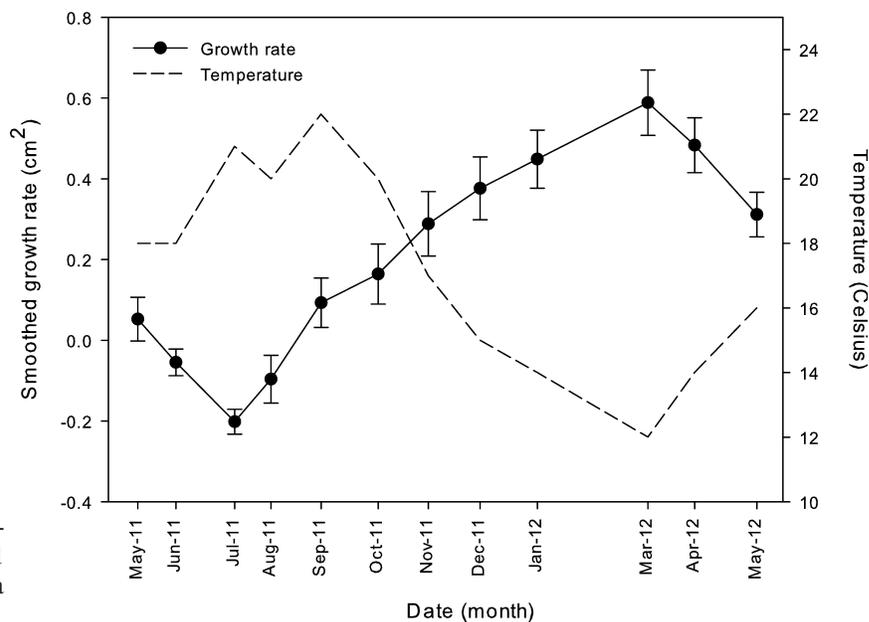


Fig. 6. Monthly growth rates of *Didemnum fulgens*. Error bars represent ± 1 standard error. Temperature data (dashed line) are superimposed.

seas (e.g., de Caralt et al. 2002; López-Legentil et al. 2005; Pérez-Portela et al. 2007; Valentine et al. 2007). Both the reproduction and growth cycles of *D. fulgens* were tightly correlated with temperature shifts over time. In the Mediterranean, ascidians reproduce either before or after maximal seawater temperatures are reached, with reproductive timing related to the biogeographical distribution of each ascidian species. Atlanto-Mediterranean species that have cold-water affinities reproduce during winter,

while endemic or Senegalian species reproduce in spring–early summer (Turon 1988). Moreover, the duration of the reproductive period in ascidians may also be related to the rate at which maximal seawater temperatures are reached each year. For instance, we found that years with sharp increases in temperature coincided with a shorter larval release period (e.g., 2010) while more gradual increases of temperature were accompanied by longer spawning events (e.g., 2011). Similarly, Pérez-Portela et al. (2007) showed that when seawater temperature increased sharply, colonies of *Pycnoclavella aurilucens* GARSTANG 1891 incubated larva for two months, while incubation could last up to four months when maximal temperatures were reached more progressively. The apparent influence of temperature on the length of the brooding period may be an indirect effect, with factors related to temperature (e.g., food availability; Coma et al. 2000) explaining the observed pattern. In addition, longer monitoring periods are necessary to confirm the observed trend. To date, there are only a handful studies that examine ascidian life cycles over more than two years. Although long-term monitoring studies are labor-intensive and time consuming, they remain necessary to acquire a thorough understanding of the factors that influence ascidian life cycles.

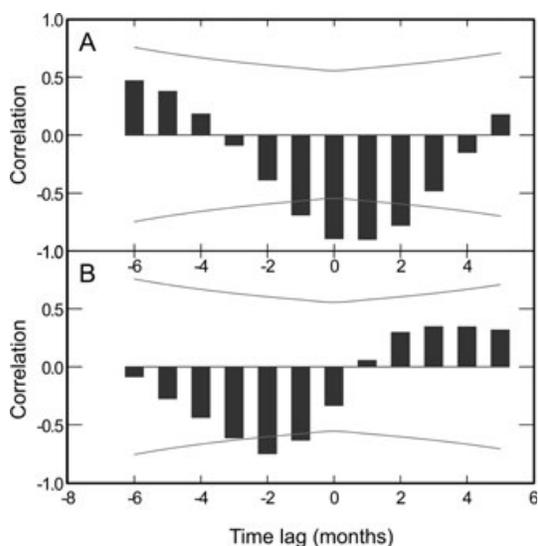


Fig. 7. Cross-correlation analyses. **A.** Growth rate and temperature. **B.** Maturity index and growth rate. Time lags are in months. Bars that reach or pass the curved lines have significant positive (upper) and negative (lower) correlation values.

Aestivation periods and resting forms are common in Mediterranean ascidians and have been ascribed to unfavorable conditions, such as high seawater temperatures and decreases in food availability for filter-feeding organisms (Coma et al. 2000;

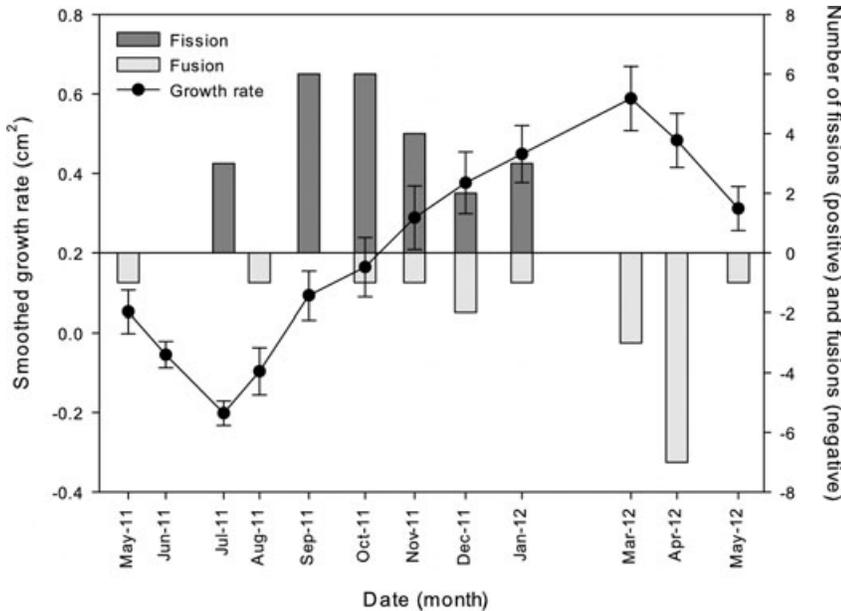


Fig. 8. Number of fusion (light gray bars) and fission (dark gray bars) events recorded during the monitoring period, with monthly growth rates (line) superimposed. Error bars represent ± 1 standard error.

Coma & Ribes 2003). Colonies of *D. fulgens* were observed to acquire a leather-like appearance following reproduction events, and zooids stopped filtering throughout the summer. Similarly, most Mediterranean ascidians with resting forms reproduce before summer (e.g., *Polysyncraton lacazei* TURON & BECERRO 1992; *Clavelina lepadiformis* (MÜLLER 1776), de Caralt et al. 2002; *Cystodytes dellechiajei* (DELLA VALLE 1877), López-Legentil et al. 2005; *Pycnoclavella brava* PÉREZ-PORTELA, DURAN, & TURON 2007, Pérez-Portela et al. 2007), while ascidians reproducing in winter correspond to Atlanto-Mediterranean species (e.g., *Applidium* aff. *conicum* TURON & BECERRO 1992; *Pycnoclavella communis* PÉREZ-PORTELA, DURAN, & TURON 2007, Pérez-Portela et al. 2007). What happens while a colony is resting is unknown in most cases and depends on the species (Burighel et al. 1976; Turon 1992). For the only didemnid ascidian investigated in the Mediterranean, *P. lacazei* TURON (1992) suggested that a “rejuvenative” process involving the regression of old thoraces and budding of new ones took place during the resting period. Further studies are needed to establish whether the same phenomenon occurs in *D. fulgens*. A thorough understanding of the physiological processes involved in the development of resting forms will cast light on the implications of these processes for ascidian health, long-term survival, and space occupation and maintenance.

Colonial ascidians are characterized by their capacity to divide and form smaller colonies (fission)

and to grow rapidly by merging previously independent units (fusion). Fusion and fission are frequent in some tropical and temperate didemnids (Bak et al. 1981; Ryland et al. 1984; Stocker 1991). In *D. fulgens*, these processes occurred at moderate frequencies, although they occurred all year round with most fissions recorded in fall, and fusions in spring. In other Mediterranean ascidians, clear seasonal patterns in the occurrence of fission and fusion events were not observed (e.g., *C. dellechiajei*, López-Legentil et al. 2005). Fusion has been reported to be a mechanism by which some ascidian species achieve the minimal colony size necessary for reproduction, which in didemnid ascidians ranges from ~ 25.4 – 32.4 mm² (Bak et al. 1981; Duyl et al. 1981; Ritzmann et al. 2009). This may not be the case in *D. fulgens*, as fusions were more common in spring when gametogenesis had already started. On the other hand, we found that fusion was linked to higher growth rates of the resulting colonies compared with those that did not undergo fusion, representing a synergistic rather than additive effect of colony merging. This process, therefore, may be a short-term strategy to accelerate growth rates and facilitate substrate occupation.

In contrast, fission of colonies of *D. fulgens* did not result in increased growth rates of the resulting units; rather, the new colonies created by fission events grew slightly more slowly during the first month than colonies that did not divide. In this case, fission may be linked to the observed partial colony regression during the unfavorable season

(summer) and the development of resting forms, with smaller colonies achieving faster colony reorganization. As small colonies grow faster than larger ones, in subsequent months, these colonies could increase in size more rapidly and colonize space left over by other regressing sessile invertebrates during summer, thus representing an additional strategy for overall growth and substrate occupation. In fact, the capacity for fusion and fission has been linked to the long-term success of genets (clones) in colonial ascidians (e.g., Ryland et al. 1984; Stoner 1989; Valentine et al. 2007; Morris & Carman 2012). Although we cannot ascertain the precise relevance of these mechanisms in *D. fulgens*, it is likely that the ability to carry out fusion and fission has conferred to this species an important competitive edge over other sessile species, as shown by the overall increase in total surface area observed during the study period and the proliferation of this species during the last decade.

Resource allocation to reproduction or growth depends on the requirements of an organism in a given environment and its energy budget within evolutionary and ecological constraints (Cohen 1971; Kozłowski 1992). Trade-offs between growth and reproduction have been described for a number of taxa, including sponges (Leong & Pawlik 2010), corals (Rinkevich 1996), and fish (Siems & Sikes 1998). Mounting evidence indicates that seasonal partitioning of available resources for either growth or reproduction is also a common strategy among colonial ascidians in temperate (Davis 1989; López-Legentil et al. 2005; Pérez-Portela et al. 2007) and sub-tropical seas (Ritzmann et al. 2009). These trade-offs in resource allocation may be linked to temperature changes, nutrient availability, or “sexual exhaustion” after larval brooding has drained all nutrient reserves (Berrill 1935; López-Legentil et al. 2007; Ritzmann et al. 2009). Brooding strategies in colonial ascidians range from the production of a few large and complex larvae per zooid to many small and simple larvae (i.e., larvae with undifferentiated tissues). Reproductive investment per zooid has been shown to be significantly higher in species that produced few well-developed larvae, especially members of the family Didemnidae (Tarjuelo & Turon 2004). As *D. fulgens* produces a single larva per zooid, we hypothesize that investment in reproduction for this species is high and that little to no energy is available for other biological functions (i.e., growth) during its reproductive period. A temporal trade-off between growth and reproduction, where the latter acted as the major energy sink, was previously described for another colonial ascidian in

the Mediterranean Sea (López-Legentil et al. 2007), and may be a common strategy among species with high reproductive investment.

In this study, we provided the first data on reproductive and growth cycles of a proliferating didemnid ascidian in the Western Mediterranean. As reported for other ascidian species, the life cycle of *D. fulgens* presented a marked seasonality and suggested resource partitioning between reproduction and growth. Seasonal patterns in fusion and fission events were coincident with higher growth rates and the reactivation after summer dormancy, respectively, and may contribute to the optimization of resource allocation and ecological success of *D. fulgens*. Overall, colonies increased in surface area during the study period, continuing the expansion observed for this species during the last decade, and probably displacing other common species in the rocky infralittoral. There is a lack of information on ascidian life cycles and their response to current and future fluctuations in abiotic factors. Although primarily descriptive, this information is critical to understand how further natural and anthropological perturbations may affect the distribution and abundance of these animals and the consequences of species expansions on benthic community diversity.

Acknowledgments. We thank Iosune Uriz, Sonia de Caralt, and Janina González (CEAB-CSIC) for providing some of the samples, and three anonymous reviewers for their helpful comments. This research was funded by the Marie Curie International Reintegration Grant FP7-PEOPLE-2010-RG 277038 within the 7th European Community Framework Program, the Spanish Government projects CTM2010-17755 and CTM2010-22218, and the Catalan Government grant 2009SGR-484 for Consolidated Research Groups.

References

- Agell G, Turon X, De Caralt S, López-Legentil S & Uriz MJ 2004. Molecular and organism biomarkers of copper pollution in the ascidian *Pseudodistoma crucigaster*. *Mar. Pollut. Bull.* 48: 759–767.
- Azumi K, Amano S, Sabau SV, Kamimura A, Satoh N & Koyanagi R 2008. Application of ascidian DNA microarray analysis for risk assessment of marine chemical pollutants. In: *Interdisciplinary studies on environmental chemistry - Biological responses to chemical pollutants*. Murakami Y, Nakayama K, Kitamura S-I, Iwata H & Tanabe S, eds., pp. 107–110. Terrapub, Tokyo.
- Bak RPM, Sybesma J & van Duyl FC 1981. The ecology of the tropical compound ascidian *Trididemnum solidum* II. Abundance, growth and survival. *Mar. Ecol. Prog. Ser.* 6: 43–52.

- Balata D, Nesti U, Piazzì L & Cinelli F 2007. Patterns of spatial variability of seagrass epiphytes in the north-west Mediterranean Sea. *Mar. Biol.* 151: 2025–2035.
- Bates WR 2005. Environmental factors affecting reproduction and development in ascidians and other protochordates. *Can. J. Zool.* 83: 51–61.
- Berrill NJ 1935. Studies in tunicate development Part III. Differential retardation and acceleration. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* 225: 255–326.
- 1951. Regeneration and budding in tunicates. *Biol. Rev.* 26: 456–476.
- Bishop DD & Sommerfeldt AD 1999. Not like *Botryllus*: indiscriminate post-metamorphic fusion in a compound ascidian. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* 266: 241–248.
- Bourque D, Davidson J, MacNair NG, Arsenault G, LeBlanc AR, Landry T & Miron G 2007. Reproduction and early life history of an invasive ascidian *Styela clava* Herdman in Prince Edward Island, Canada. *J. Exp. Mar. Biol. Ecol.* 342: 78–84.
- Burighel P, Brunetti R & Zaniolo G 1976. Hibernation of the colonial ascidian *Botrylloides leachi* (Savigny): histological observations. *Boll. Zool.* 43: 293–301.
- de Caralt S, López-Legentil S, Tarjuelo I, Uriz JM & Turon X 2002. Contrasting biological traits of *Clavelina lepadiformis* (Asciacea) populations from inside and outside harbours in the western Mediterranean. *Mar. Ecol. Prog. Ser.* 244: 125–137.
- Carman MR 2007. Water quality, nitrogen pollution, and ascidian diversity in coastal waters of southern Massachusetts, USA. *J. Exp. Mar. Biol. Ecol.* 342: 175–178.
- Carman MR, Morris JA, Karney RC & Grunden DW 2010. An initial assessment of native and invasive tunicates in shellfish aquaculture of the North American east coast. *J. Appl. Ichthyol.* 26: 8–11.
- Cohen D 1971. Maximizing final yield when growth is limited by time or by limiting resources. *J. Theor. Biol.* 33: 299–307.
- Coma R & Ribes M 2003. Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. *Oikos* 101: 205–215.
- Coma R, Ribes M, Gili J-M & Zabala M 2000. Seasonality in coastal benthic ecosystems. *Trends Ecol. Evol.* 15: 448–453.
- Davis AR 1989. Contrasting population dynamics and life histories in two populations of the colonial subtidal ascidian *Podoclavella moluccensis*. *Mar. Ecol. Prog. Ser.* 51: 107–119.
- Dias GM, Delboni CG & Duarte L 2008. Effects of competition on sexual and clonal reproduction of a tunicate: the importance of competitor identity. *Mar. Ecol. Prog. Ser.* 362: 149–156.
- Dijkstra JA & Harris LG 2009. Maintenance of diversity altered by a shift in dominant species: implications for species coexistence. *Mar. Ecol. Prog. Ser.* 387: 71–80.
- Dijkstra J, Sherman H & Harris LG 2007. The role of colonial ascidians in altering biodiversity in marine fouling communities. *J. Exp. Mar. Biol. Ecol.* 342: 169–171.
- Duyl FC, Bak RPM & Sybesma J 1981. Ecology of the tropical compound ascidian *Trididemnum solidum* I. Reproductive strategy and larval behavior. *Mar. Ecol. Prog. Ser.* 6: 35–42.
- Erwin PM, López-Legentil S & Schuhmann PW 2010. The pharmaceutical value of marine biodiversity for anti-cancer drug discovery. *Ecol. Econ.* 70: 445–451.
- Fiore CL & Cox Jutte P 2010. Characterization of macrofaunal assemblages associated with sponges and tunicates collected off the southeastern United States. *Invertebr. Biol.* 129: 105–120.
- Fukuda T & Hirose E 2008. Differences in associated crustacean fauna and seasonality of sexual reproduction between two color morphs of the photosymbiotic ascidian *Didemnum molle* (Asciacea: Didemnidae). *Pac. Sci.* 62: 309–316.
- Gotelli NJ 1987. Spatial and temporal patterns of reproduction, larval settlement, and recruitment of the compound ascidian *Aplidium stellatum*. *Mar. Biol.* 94: 45–51.
- Hewitt CL, Gollasch S & Minchin D 2009. The vessel as a vector - Biofouling, ballast water and sediments. In: *Biological invasions in marine ecosystems*. Rilov GR & Crooks JA, eds., pp. 117–131. Springer-Verlag, Berlin Heidelberg.
- Hirose E, Oka AT & Akahori M 2005. Sexual reproduction of the photosymbiotic ascidian *Diplosoma virens* in the Ryukyu Archipelago, Japan: vertical transmission, seasonal change, and possible impact of parasitic copepods. *Mar. Biol.* 146: 677–682.
- Hirose E, Adachi R & Kuze K 2006. Sexual reproduction of the *Prochloron*-bearing ascidians *Trididemnum cyclops* and *Lissoclinum bistratum*, in subtropical waters: seasonality and vertical transmission of photosymbionts. *J. Mar. Biol. Assoc. U.K.* 86: 175–179.
- Hirose E, Kojima A, Nogami J & Teruya K 2007. Seasonality of sexual reproduction in three photosymbiotic *Trididemnum* species (Didemnidae: Asciacea: Tunicata) in a subtropical sea grass bed. *J. Mar. Biol. Assoc. U.K.* 87: 979–982.
- Koukouras A, Voultziadou-Koukoura E, Kebrekidis T & Vafidis D 1995. Ascidian fauna of the Aegean Sea with a check list of the Eastern Mediterranean and Black Sea species. *Ann. Inst. Oceanogr. Paris (Nouv. Ser.)* 71: 19–34.
- Kozłowski J 1992. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends Ecol. Evol.* 7: 15–19.
- Kremer LP, Rocha RM & Roper JJ 2010. An experimental test of colonization ability in the potentially invasive *Didemnum perlucidum* (Tunicata, Asciacea). *Biol. Invasions* 12: 1581–1590.
- Lafargue F 1976. Révision taxonomique des Didemnidae des côtes de France (Ascidies composées) Les espèces de Banyuls-Sur-Mer. *Genre Didemnum*. Deuxième

- partie: Larves a deux ventouses. Ann. Inst. Oceanogr. Paris (Nouv. Ser.) 52: 259–281.
- Lafargue F & Wahl M 1987. The didemnid ascidian fauna of France. Ann. Inst. Oceanogr. Paris (Nouv. Ser.) 63: 1–46.
- Lambert CC 2005. Historical introduction, overview, and reproductive biology of the protochordates. Can. J. Zool. 83: 1–7.
- Lambert G 2009. Adventures of a sea squirt sleuth: unraveling the identity of *Didemnum vexillum*, a global ascidian invader. Aquat. Invasions 4: 5–28.
- Leong W & Pawlik JR 2010. Fragments or propagules? Reproductive tradeoffs among *Callyspongia* spp. from Florida coral reefs. Oikos 119: 1417–1422.
- López-Legentil S, Ruchty M, Domenech A & Turon X 2005. Life cycles and growth rates of two morphotypes of *Cystodytes* (Asciacea) in the western Mediterranean. Mar. Ecol. Prog. Ser. 296: 219–228.
- López-Legentil S, Bontemps-Subielos N, Turon X & Banaigs B 2007. Secondary metabolite and inorganic contents in *Cystodytes* sp (Asciacea): temporal patterns and association with reproduction and growth. Mar. Biol. 151: 293–299.
- Marshall DJ, Cook CN & Emler RB 2006. Offspring size effects mediate competitive interactions in a colonial marine invertebrate. Ecology 87: 214–225.
- Millar RH 1971. The biology of ascidians. Adv. Mar. Biol. 9: 1–100.
- 1974. A note on the breeding season of three ascidians on coral reefs at Galeta in the Caribbean Sea. Mar. Biol. 28: 127–129.
- Molin E, Gabriele M & Brunetti R 2003. Further news on hard substrate communities of the northern Adriatic Sea with data on growth and reproduction in *Polycitor adriaticus* (Von Drasche, 1883). Boll. Mus. Civ. St. Nat. Venezia. 54: 19–28.
- Morris JA & Carman MR 2012. Fragment reattachment, reproductive status, and health indicators of the invasive colonial tunicate *Didemnum vexillum* with implications for dispersal. Biol. Invasions 14: 2133–2140.
- Newlon AW III, Yund PO & Stewart-Savage J 2003. Phenotypic plasticity of reproductive effort in a colonial ascidian, *Botryllus schlosseri*. J. Exp. Zool. 297: 180–188.
- Oka H & Watanabe H 1957. Colony-specificity in compound ascidians. Bull. Mar. Biol. Stn. Asamushi. 10: 153–155.
- Panagiotou M, Antoniadou C & Chintiroglou C 2008. Population dynamics and reproductive status of *Microcosmus savignyi* Monniot, 1962 (Thermaikos Gulf, Eastern Mediterranean): a preliminary assessment. J. Nat. Hist. 42: 545–558.
- Pérez-Portela R, Palacín C, Duran S & Turon X 2007. Biological traits of three closely related species of *Pyrocyclavella* (Asciacea) in the Western Mediterranean. Mar. Biol. 152: 1031–1038.
- Pineda MC, López-Legentil S & Turon X 2013. Year-round reproduction in a seasonal sea: Biological cycle of the introduced ascidian *Styela plicata* in the Western Mediterranean. Mar. Biol. 160: 221–230.
- Rinkevich B 1996. Do reproduction and regeneration in damaged corals compete for energy allocation?. Mar. Ecol. Prog. Ser. 143: 297–302.
- 2005. Natural chimerism in colonial urochordates. J. Exp. Mar. Biol. Ecol. 322: 93–109.
- Ritzmann NF, da Rocha R & Roper JJ 2009. Sexual and asexual reproduction in *Didemnum rodriguezi* (Asciacea, Didemnidae). Iheringia, Ser. Zool. 99: 106–110.
- Rius M, Turon X, Dias GM & Marshall DJ 2010. Propagule size effects across multiple life-history stages in a marine invertebrate. Funct. Ecol. 24: 685–693.
- Rodriguez LF & Ibarra-Obando SE 2008. Cover and colonization of commercial oyster (*Crassostrea gigas*) shells by fouling organisms in San Quintin Bay, Mexico. J. Shellfish Res. 27: 337–343.
- Ryland JS, Wigley RA & Muirhead A 1984. Ecology and colonial dynamics of some Pacific reef flat Didemnidae (Asciacea). Zool. J. Linn. Soc. 80: 261–282.
- Sabaddin A 1962. Le basi genetiche della capacità di fusione fra colonie in *Botryllus schlosseri* (Asciacea). Rend. Accad. Naz. Lincei 32: 1031–1035.
- Saito Y, Hirose E & Watanabe H 1994. Allorecognition in compound ascidians. Int. J. Dev. Biol. 38: 237–247.
- Shenkar N & Loya Y 2008. The solitary ascidian *Herdmania momus*: native (Red Sea) versus non-indigenous (Mediterranean) populations. Biol. Invasions 10: 1431–1439.
- Shenkar N, Bronstein O & Loya Y 2008. Population dynamics of a coral reef ascidian in a deteriorating environment. Mar. Ecol. Prog. Ser. 367: 163–171.
- Siems DP & Sikes RS 1998. Tradeoffs between growth and reproduction in response to temporal variation in food supply. Environ. Biol. Fishes 53: 319–329.
- Smale DA & Childs S 2012. The occurrence of a widespread marine invader, *Didemnum perucidum* (Tunicata, Asciacea) in Western Australia. Biol. Invasions 14: 1325–1330.
- Stefaniak L, Lambert G, Gittenberger A, Zhang H, Lin S & Whitlatch RB 2009. Genetic conspecificity of the worldwide populations of *Didemnum vexillum* Kott, 2002. Aquat. Invasions 4: 29–44.
- Stefaniak L, Zhang H, Gittenberger A, Smith K, Holsinger K, Lin S & Whitlatch RB 2012. Determining the native region of the putatively invasive ascidian *Didemnum vexillum* Kott, 2002. J. Exp. Mar. Biol. Ecol. 422–423: 64–71.
- Stocker LJ 1991. Effects of size and shape of colony in rates of fission, fusion, growth and mortality in a subtidal invertebrate. J. Exp. Mar. Biol. Ecol. 149: 161–175.
- Stocker LJ & Underwood AJ 1991. The relationship between the presence of neighbours and rates of sexual and asexual reproduction in a colonial invertebrate. J. Exp. Mar. Biol. Ecol. 149: 191–205.

- Stoner DS 1989. Fragmentation: a mechanism for the stimulation of genet growth rates in an encrusting colonial ascidian. *Bull. Mar. Sci.* 45: 277–287.
- Svane IB & Young CM 1989. The ecology and behaviour of ascidian larvae. *Oceanogr. Mar. Biol. Annu. Rev.* 27: 45–90.
- Tarjuelo I & Turon X 2004. Resource allocation in ascidians: reproductive investment vs. other life-history traits. *Invertebr. Biol.* 123: 168–180.
- Turner SJ, Thrush SF, Cummings VJ, Hewitt JE, Wilkinson MR, Williamson RB & Lee DJ 1997. Changes in epifaunal assemblages in response to marina operations and boating activities. *Mar. Environ. Res.* 43: 181–199.
- Turon X 1988. The ascidians of Tossa de Mar (NE Spain) II.- Biological cycles of the colonial species. *Cah. Biol. Mar.* 29: 407–418.
- 1992. Periods of non-feeding in *Polysyncraton lacazei* (Ascidiacea: Didemnidae): a rejuvenative process? *Mar. Biol.* 112: 647–655.
- Turon X & Becerro MA 1992. Growth and survival of several ascidian species from the northwestern Mediterranean. *Mar. Ecol. Prog. Ser.* 82: 235–247.
- Vafidis D, Antoniadou C & Chintiroglou C 2008. Population dynamics, allometric relationships and reproductive status of *Microcosmus sabatieri* (Tunicata: Ascidiacea) in the Aegean Sea. *J. Mar. Biol. Assoc. U. K.* 88: 1043–1051.
- Valentine PC, Carman MR, Blackwood DS & Heffron EJ 2007. Ecological observations on the colonial ascidian *Didemnum* sp. in a New England tide pool habitat. *J. Exp. Mar. Biol. Ecol.* 342: 109–121.
- Westerman EL, Dijkstra JA & Harris LG 2009. High natural fusion rates in a botryllid ascidian. *Mar. Biol.* 156: 2613–2619.
- Wong NA, McClary D & Sewell MA 2011. The reproductive ecology of the invasive ascidian, *Styela clava*, in Auckland Harbour, New Zealand. *Mar. Biol.* 158: 2775–2785.