Larval ecology of an ascidian tropical population in a Mediterranean enclosed ecosystem

José Luis Carballo*

Instituto de Ciencias del Mar y Limnología, UNAM, Estación Mazatlán, Apartado Postal 811, Mazatlán 82000, Mexico

ABSTRACT: The larval ecology of a population of the tropical tunicate *Ecteinascidia turbinata* was studied in a semi-enclosed lagoon on the island of Formentera (Mediterranean Sea). Due to the lagoon's isolation and processes of extinction/regeneration of the population, research was carried out to evaluate some aspects of the species' ecology which permit it to survive in this habitat. Settlement and recruitment were found to be intimately related to the period of sexual reproduction, and determined by the local production of larvae. In this sense, the population's spawning potential (larvae m^{-2}) matched a perfect linear relationship ($R^2 = 0.9496$, $p < 0.05$) with the settlement and recruitment registered on experimental settlement surfaces. Of the larvae produced by the population 29% settled locally, contributing to the consolidation of populations already established. Only 1.3% of the larval production theoretically calculated developed into colonies at a local level. To determine how settlement site might contribute to the colonies' survival and thus to population distribution, zooids were transplanted from an appropriate zone to a stressful zone, which showed that there is a risk of inappropriate habitat selection for settlement. The behaviour of the larvae (philopatry) and gregarious settlement in unpredictable environments (spatio-temporally variable) seems advantageous to prevent advection to inappropriate sites. *E. turbinata* ensures local recruitment and renewal of the established population, but it seems that there is partial dispersal of larvae beyond the parent population. The study shows that there is sufficient recruitment from within the population to maintain it and the timing of larval production and recruitment strongly suggest that recruits are from the population itself.

KEY WORDS: *Ecteinascidia turbinata* · Larval dispersal · Self-sustaining · Recruitment · Gregariousness · Philopatry · Spatial heterogeneity · Habitat selection

INTRODUCTION

Spatio-temporal variation in ascidians is influenced by a variety of factors, many of which are related to their larval stage: mode of development (Turon & Vazquez 1995), mortality (Davis & Butler 1989), environmental factors such as hydrodynamics (Havenhand & Svane 1991), the presence or absence of predators (Petersen & Svane 1995), inhibition of settlement by natural products (Davis & Wright 1990), dispersal (Bingham & Young 1991), influence of resident adults (Osman & Whitlatch 1995), etc. Dispersal capacity allows a species to colonize new habitats to expand the population; however, colonial ascidians with lecitho-trophic larvae frequently have limited larvae dispersal that often leads to aggregated spatial patterns (Young 1986, Bingham & Young 1991). Nevertheless, the capacity of invertebrate larvae to select a habitat that is appropriate for settlement should be considered as well, and although selective settlement in a suitable habitat may be advantageous for the survival of the species, the current evidence for its importance is in many cases indirect.

Ecteinascidia turbinata is an ovoviparous colonial ascidian which is a simultaneous hemaphrodite with short-lived lecithotrophic larvae (Svane & Young 1989). A large population of this tunicate is distributed throughout a lagoon on the island of Formentera (Mediterranean Sea). Although the lagoon has a narrow opening to sea, the outside water temperature for most of the year does not allow a self-sustaining popu-

*E-mail: carballo@ola.icmyl.unam.mx

lation to be maintained outside the lagoon (pseudo-population sensu Young 1989) and only scattered colonies appear occasionally. In the Mediterranean, consolidated populations of *E. turbinata* can be found only in enclosed or semi-enclosed habitats where water temperature is maintained a few degrees higher than seawater temperature. To date, substantial populations of this species exist in similar Mediterranean areas (Alicante, Mallorca, Menorca, Tunisia, etc.) more than 100 km away from our area of study (unpubl. data).

This population in the Mediterranean, unlike the one found in the Caribbean, fluctuates greatly on a temporal scale (yearly), and its distribution is patchy with dense aggregations at the bottom of the pond. Temporal variation can be observed as variation in abundance and distribution. A previous study of the sediments showed that the populations preferred to settle on coarse sandy and clay-sand substrates with a low content of organic material, while being totally absent from clay-mud areas with a high organic material content. They also grow frequently on dry seagrass (*Posidonia oceanica*, *Cymodocea* sp.), *Caulerpa prolifera* leaves, and other surfaces like plastic, rope, etc. However in the Caribbean and the Gulf of Mexico the species prefers mangrove roots for settling (Young 1986, Bingham & Young 1991, Carballo et al. 1999).

The life history of *Ecteinascidia turbinata* on the island of Formentera is very complex, and it is related to aspects like distribution, fecundity, mortality of adults, larval dispersal capacity, appropriate habitat selection, survival of the settlers, etc. It is a population characteristic of warmer waters which has found environmental conditions (especially temperature and salinity) adequate for maintaining its development in a practically closed environment. Moreover, it has been proven that when the water temperature is below 17 to 18°C (at the end of autumn), the population starts to disappear completely and the colony is reduced to the mere stolon until the following spring, when the rise in water temperature allows for a new generation of zooids (Carballo et al. 1997). Consequently, the larval phase and recruitment success play a decisive role because it must be able to detect an appropriate habitat, settle, grow and develop adequately into a colony, so that once winter is over the stolon can generate the colony again in an appropriate place.

In this ecosystem, population size, individual sexual maturity, settlement and recruitment can be measured over a complete reproductive season. In this situation, the origin of the larval supply is always autochthonous, and therefore settlement must be intimately related to the period of sexual reproduction, and completely determined by the local production of larvae. In this way, a perfect relationship between fecundity and the rate of recruitment should be expected. Low levels of correlation could reflect pre-recruitment losses or widespread larval dispersal. The goal of this study was to assess the reproductive potential in a semi-enclosed *Ecteinascidia turbinata* population, to estimate the number of larvae that settle locally within it, the capacity for larval dispersal, and the post-recruitment mortality. We also set out to determine how settlement site, as influenced by dispersal, might contribute to the survival of colonies given that local patterns of mortality can result in non-recruitment even when there is high larval production.

MATERIAL AND METHODS

Study area. Formentera Island is part of the Balearic Islands archipelago near the Spanish Mediterranean coast (Fig. 1). The Estany des Peix is at its north end, a shallow lagoon no more than 4 to 5 m deep, with an approximate surface of 100 ha. The lagoon has a northern opening out to sea about 20 m wide. Temperature (maximum-minimum), and salinity were checked daily. A series of selective samples were also taken to study granulometry and organic matter content at the bottom (Bouyoucos 1934).
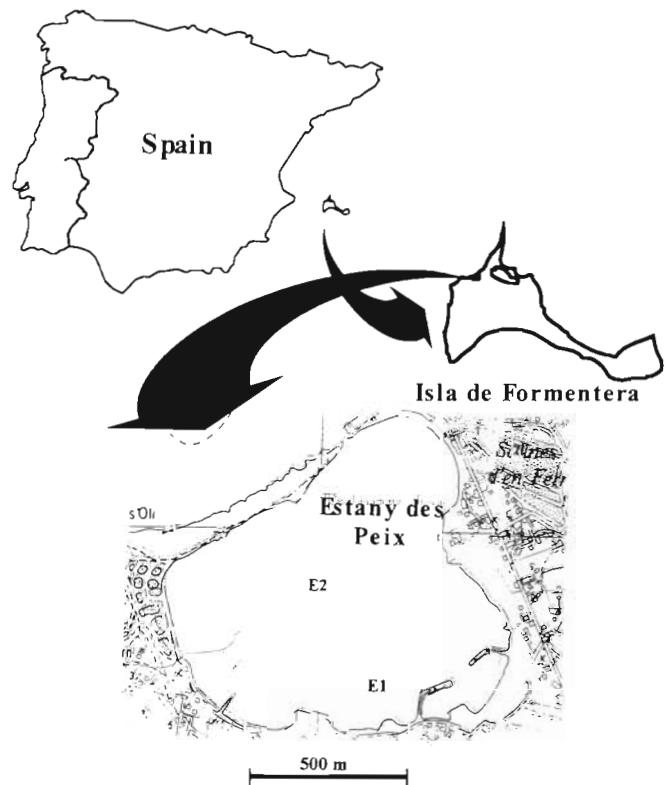

Fig. 1. Location of the study area in Isla de Formentera showing sampling stations

Population density. A general overview of the area was first taken with an underwater glider pulled by a boat. In order to estimate density, sampling was carried out in the following manner: a random series of nautical bearings were established from 1 reference buoy. Within each bearing, different sampling points were also selected at random depths (Carballo et al. 1997). The bearings selected were 0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270° and 300°, and the depths were <1, 1 to 2, 2 to 3, 3 to 4 and >4 m. A total of 60 sampling points (bearing, depth) were established. At each point, density was calculated by dropping a 1 m^2 quadrant from the surface. Because it was a difficult process, population density was carried out only in June and August.

Colony output. Seven colonies representative of the population were collected underwater on a weekly basis. Fifteen zooids were selected from different parts of each colony. This amount was considered the minimum sample size in previous research (Luján-Feliu unpubl. data). In order to assess asexual reproduction the weight of each colony and the number and size of all the zooids per colony were registered. Sexual reproduction was measured by registering the number of zooids with larvae/colony, the degree of maturation and the number of larvae for each zooid. The reproductive cycle was studied in a zone representative of a coarse sandy substrate (Stn E1).

Population spawning potential. The population spawning potential (larvae m^{-2}) was calculated in June and August in the following manner: no. of colonies m^{-2} × average no. of zooids with larvae/colony × average no. of larvae/zooid. In the rest of the samples, the population spawning potential was calculated based on a standardized unit of effort (% of mature colonies from the total colonies sampled × average no. of zooids with larvae/colonies × average no. of larvae/zooid). This way, the population spawning potential would have a relative value, but it would be perfectly valid for making spatio-temporal comparisons or for relating it to the number of larvae that had settled.

Dispersal, settlement and recruitment. In order to study dispersal capacity, settlement and recruitment, we used plastic surfaces of 10 m^2 (10 m long × 1 m high) with small air pockets to ease vertical flotation that were anchored at the bottom of the lagoon at Stn E1 (Fig. 2). In order to study dispersal and habitat selection capacities an area devoid of colonies was selected which was also representative of the center of the lagoon (clay-mud areas) (Stn E2). A few days before the expected larval release, 8 plastic lines were installed at Stn E1 which represented a settlement sur-
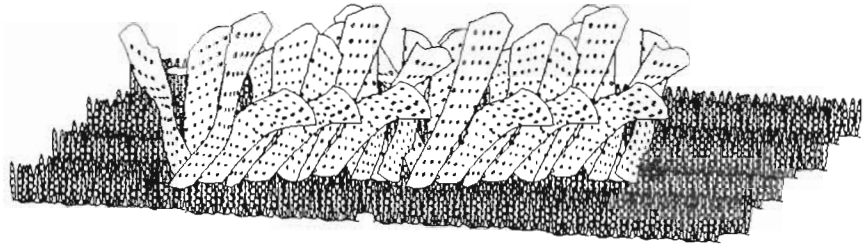

Fig. 2. Plastic screen for larvae settlement

face of 160 m^2 (8 strips × 10 m × 2 faces), and 4 plastic strips were set up at Stn E2, representing a surface area of 80 m^2; these surfaces were considered large enough to permit spatial variability in the settlers. Settlement of ascidian larvae is influenced by light, so the surfaces were placed on an east-west axis and sampling was carried out on the northern face. At each of the experimental surfaces the number of larvae m^{-2}, the number of zooids m^{-2} and the number of colonies m^{-2} were registered *in situ* (underwater) on a weekly basis. This was done by placing small metallic quadrants (25 cm side^{-1}, 0.062 m^2 total surface) approximately every other meter so that for each strip we had a sampling area of 0.62 m^2 (10 sampling units).

On settlement surfaces larvae m^{-2} are considered as those individuals recently settled that are in the process of metamorphosis; the amount of zooids m^{-2} include the single individuals recruited in the sampling period that were not asexually produced, and colonies m^{-2} are those zooids which had started to form a stolon and had generated at least 1 zooid by budding next to the parental zooid.

In order to determine significant differences between larvae, zooids and colonies m^{-2} on the plastic strips of Stn E1 versus Stn E2, a 1-way ANOVA was used following a previous log transformation of data. The significance of the differences between the treatments was tested using the Tukey test (Sokal & Rohlf 1981).

The metamorphosis from larva to zooid lasts approximately 6 to 12 h (at 24 to 26°C) (Iglesias et al. 1995), and the larva formation process is not instantaneous, since the duration of the process from the time the eggs are fertilized until the larvae are released lasts 7 to 9 d, and the zooids later die off after spawning (semelparous) (Svane & Young 1989). Therefore the population spawning potential at Stn E1 was related to the number of larvae and zooids m^{-2} (considering both jointly) registered on the experimental surfaces on the days following the calculation of said index. In this way we used more real information, since what the population spawning potential indicates is the reproductive potential of the population before larval release.

Estimated settlement and recruitment. In order to estimate the number of larvae that remain locally, the population spawning potential (as larvae m^{-2}) was related to the number of fixed larvae (settlers) and zooids (recruits) on the experimental surfaces. On the other hand, in order to calculate the number of larvae that survive until generating a colony, the total number of settlers registered on the Stn E1 surfaces were counted and compared to the number of colonies obtained at the end of the study. We are aware this estimate could be misleading, because a weekly sample only detects larvae corresponding to the day of sampling, so settlers on other days of the week are probably not being registered. However, given the reduced period of larval production that the species has in this habitat, we consider the data to be close to the real one and interesting for further comparisons.

Transplant survival. The distribution of *Ecteinascidia turbinata* at the bottom of the lagoon implied that appropriate habitats were patchily distributed, and therefore the larvae play a very important role when colonizing new habitats due to the risk of not finding adequate conditions for settling. On 9 July, 4 of the 8 settlement surfaces with developed zooids were moved from Stn E1 to Stn E2 in order to study survival in an area with no *E. turbinata* population. Although we did not have a control to estimate mortality attributed to the transplantation procedure, to avoid stress in the zooid population, the experimental surfaces were moved slowly and completely submerged using SCUBA diving.

RESULTS

Physico-chemical variables

Salinity was constant between 39 and 40‰ and the only parameter that behaved somewhat irregularly was water temperature, which dropped below 22°C during July (Fig. 3). The granulometric study and the organic material content showed that the edges along the bottom are composed mainly of coarse sand and clay-sand with a moderate organic content (2.72 to 4.12%), whereas the center is composed of muddy clay-sand with a high organic content (>13%) (Table 1). The rest of the physico-chemical variables registered

Table 1. Values for type of sediment and organic content for different parts of Estany des Peix

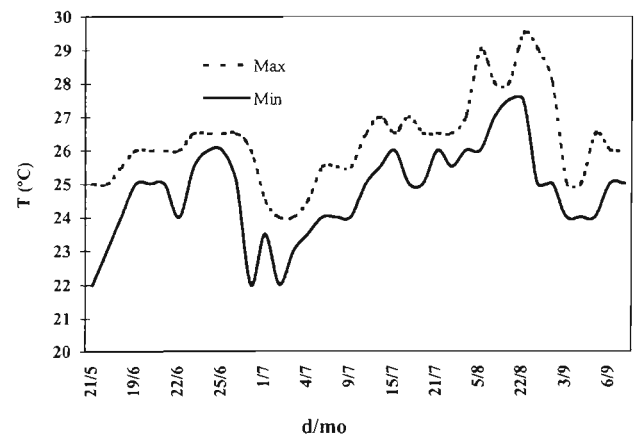|	Type of sediment	Organic content (%)
Edge	Sand	2.862
Edge	Sand	2.728
Center	Sand/silt/clay	13.448

Fig. 3. Minimum and maximum water temperatures at the bottom

basically normal levels and have been described in Carballo et al. (1997).

Reproductive output

The sexual maturation of *Ecteinascidia turbinata* started in the beginning of June with the sequential development of the testicle and ovary, followed by the formation of the embryos and larvae. Two periods of larval production were observed, one from the end of June until mid-July and a shorter one during the last 2 wk of August. Sexual maturation was almost complete by the end of summer, and after 6 September the zooids developed only the testicle, which they probably reabsorbed later to continue developing as asexual zooids (Fig. 4). On the other hand, although asexual growth and sexual reproduction occurred simultaneously, the proportion of asexual zooids versus sexual zooids was greater at the beginning and at the end of the population's life-cycle than at its middle (Fig. 5). Due to the asexual generation of zooids from the stolon, there was large variation in the number of zooids in the colony, oscillating between 54 on 25 July and 157 on 16 August, with a reduction in the number of zooids after each larval production period. In the same way, the variations in average size are interpreted in part because of the recruitment of new zooids developed from the stolon (reduction in average size), and partly because of sexual maturation (increase in average size).

Dispersal, larval recruitment and settlement

Two periods of settlement were detected at Stn E1, the first one from 22 June to 25 July and the second one from 20 August to 10 September. These coincide with
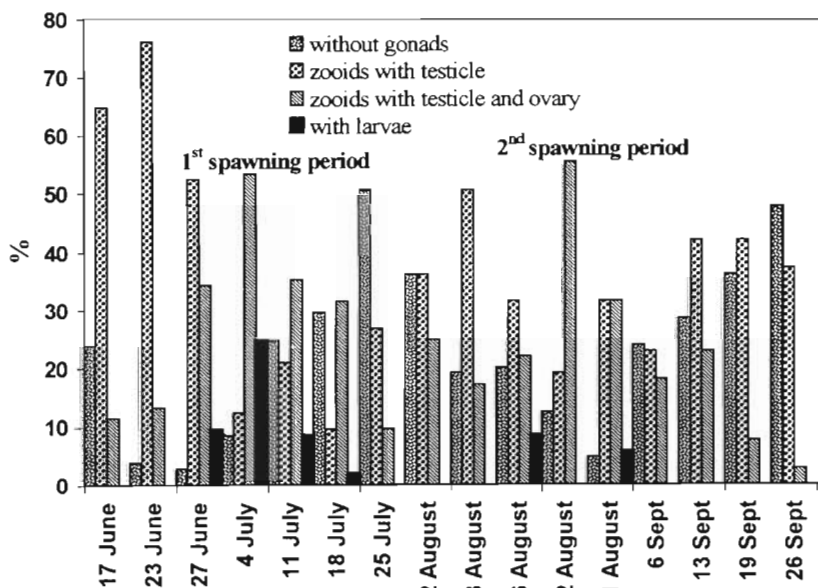
Fig. 4. Timing of gonadal and larval development

tion spawning potential. During the first period of larval settlement (from June to July), the population spawning potential and the number of larvae and zooids m^{-2} on the experimental surfaces matched a linear relationship ($R^2 = 0.9496$, $p < 0.05$; $y = 24.37 + 3.62x$) (Table 5). There was not enough data during the second period to perform the calculation.

Estimated settlement and recruitment

On 21 June a population spawning potential of 616 larvae m^{-2} (Table 4) was calculated, and on 25 June 140 larvae and 40 zooids were registered per m^2 (Table 2). If we assume that all the larvae were released from the zooids from 21 to 25 June, this value means that at least 29% of the larvae estimated in the

the 2 periods of maximum larval production previously discussed. The highest values were registered at the end of June, followed by an increase in the number of zooids, which reached a maximum of 175 zooids m^{-2} (Fig. 6). After this date the number of zooids dropped as a direct consequence of the drop in the number of larvae that had settled. No more settlers were subsequently detected until 30 August (5 larvae m^{-2}), a process which probably started a few days before sampling because of the number of zooids observed (22 zooids m^{-2}). Larval settlement was maintained until 10 September, and no more settlers on the experimental surfaces were observed after that date. As far as the appearance of colonies, there was a first peak observed on 24 July (20 colonies m^{-2}) (Table 2).

On the surfaces installed at Stn E2 larval settlement was not as great when compared to that at Stn E1, although some occasional settlement occurred at the end of July (Fig. 7). The analysis of variance showed significant differences between the 2 areas for the settled larvae, zooids and colonies (Table 3). Nevertheless, although settlement was low, a small population of zooids developed and were able to bud before dying. Juvenile colonies were found during August. Table 4 summarizes the data used for calculating the popula-

Table 2. Number of larvae, zooids and colonies m^{-2} (average values of the different settlement surfaces) at Stns E1 and E2 (see 'Materials and methods' for explanation)

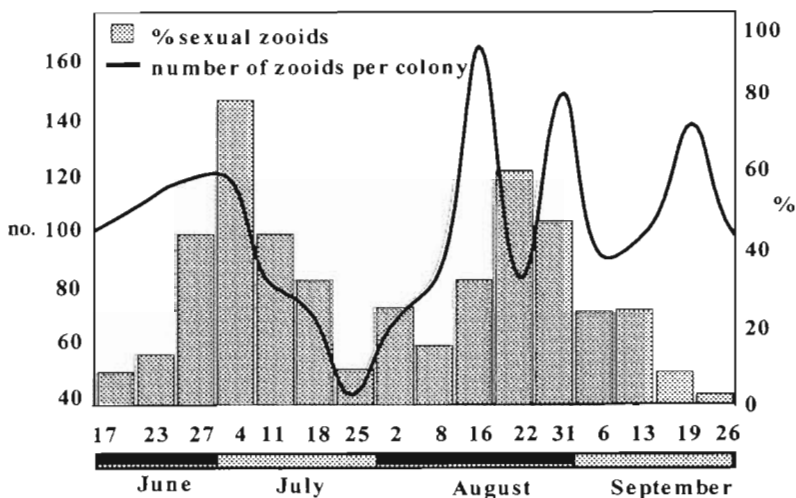|	Jun		Jul					Aug				Sep		
	22	25	3	9	16	24	30	5	13	20	30	5	10	19
Stn E1														
Larvae m^{-2}	4	140	95	31	25	0	0	0	0	5	4	3	1	0
Zooids m^{-2}	0	40	102	175	82	10	7	3	4	22	18	27	10	5
Colonies m^{-2}	0	0	0	3	11	20	4	3	11	26	16	15	24	14
Stn E2														
Larvae m^{-2}	0	0	0	0	0	4	1	0	0	0	0	0	0	0
Zooids m^{-2}	0	0	0	0	0	30	42	14	4	2	1	0	0	0
Colonies m^{-2}	0	0	0	0	0	0	0	2	2	1	1	0	0	0

Fig. 5. Bars indicate the percentage of sexual zooids (scale on the right axis). (———) Number of zooids per colony (scale on the left axis)
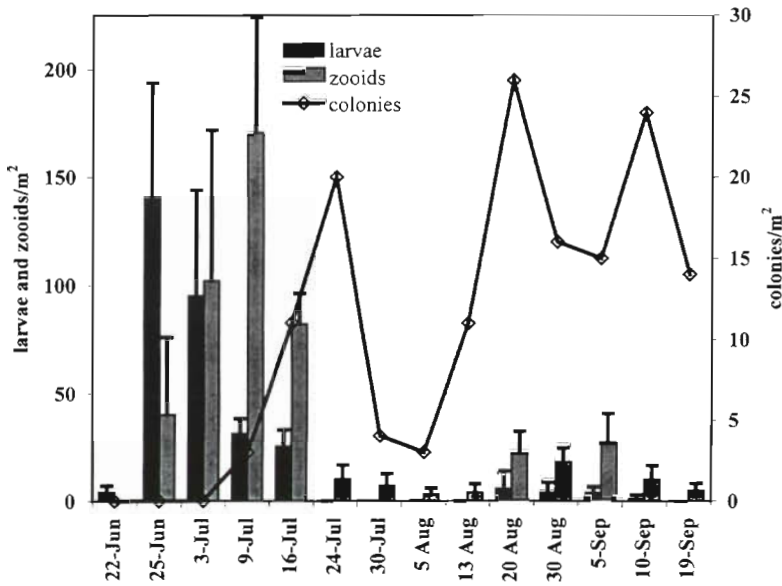
Fig. 6. Larvae, zooids and colonies m^{-2} (average values) on settlement surfaces at Stn E1. Vertical lines indicate standard deviation
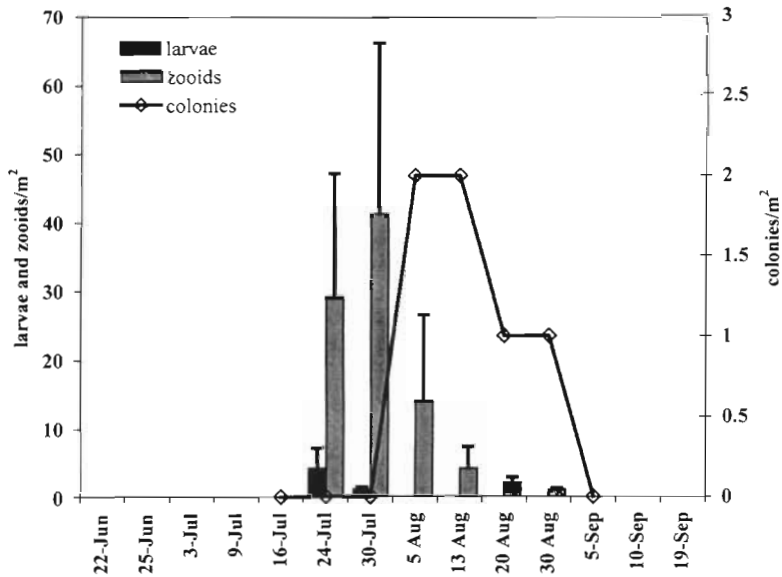

Fig. 7. Larvae, zooids and colonies m^{-2} (average values) on settlement surfaces at Stn E2. Vertical lines indicate standard deviation

Table 3. One-way ANOVA for larva, zooid and colony (factor: Stns E1 and E2). F-statistic: $^{***}p < 0.001$; $^{****}p < 0.0001$

	Source of variation	Sum of squares	df	Mean square	F-ratio
Larva	Between groups	6.8	1	6.8	16.14***
	Within groups	39.6	94	0.42	
Zooid	Between groups	9.92	1	9.92	19.56****
	Within groups	57.81	114	0.5	
Colony	Between groups	8.89	1	8.89	34.89****
	Within groups	20.9	82	0.25	

population settled locally within the population. On 20 August a production of 288 larvae m^{-2} was estimated and on 30 August 4 larvae m^{-2} + 18 zooids m^{-2} + 16 colonies m^{-2} were registered (Table 2). In this case we were unable to estimate the percentage of locally settled larvae because on the day that the population spawning potential was calculated there were already 22 zooids m^{-2} and 26 colonies m^{-2}, so we could not be certain which of the zooids registered on 30 August came from the larvae calculated by the population spawning potential or from the previous zooids which had not yet generated colonies. The survival of the colonies also varied throughout the study, with a decrease occurring from 30 July to 20 August. At the end of the study, a total of 14 colonies m^{-2} were counted on the experimental surfaces, meaning there was a 4.5% colony survival developed from the total number of settlers (308 larvae m^{-2}), and therefore only 1.3% of the population's larval potential calculated using the population spawning potential transformed into colonies at a local level.

Transplants

The transplant of settlers from Stn E1 to Stn E2 (Table 6) seems to indicate that conditions were not optimum for the survival of adult zooids in some central areas of the lagoon. At the beginning of August, we saw that in spite of the number of zooids increasing as a consequence of the sporadic settlement previously mentioned, there was a significant decrease in the number of zooids as well as in the number of colonies, which finally disappeared completely in late August-early September.

DISCUSSION

Larval ecology

Our results indicate that *Ecteinascidia turbinata* reproduces sexually only during a few weeks in the summer when the water temperature increases, and that during this time asexual growth occurs simultaneously with gametogenesis. These 2 types of reproduction follow 2 different objectives: develop-

Table 4. Population values used for calculating population's spawning potential. This was calculated as larvae m^{-2} only for 21 June and 20 August in order to estimate the percentage of larvae that settle locally in relation to theoretical production of larvae from the population. For the rest of the samples it was calculated on the basis of the proportion of colonies that had zooids with larvae per each unit of effort (see 'Materials and methods' for explanation)

Date	Colonies m^{-2} (proportion of colonies with zooids with larvae)	Average no. of zooids with larvae/colony	Average no. of larvae/zooid	Population spawning potential
21 Jun	7 colonies m^{-2}	11	8	616 larvae m^{-2}
27 Jun	(57)	11	8	49.6
4 Jul	(29)	25	7	50.4
11 Jul	(43)	7	5	15
18 Jul	(14)	2	6	1.68
16 Aug	(43)	15	7	44.8
20 Aug	4 colonies m^{-2}	12	6	288 larvae m^{-2}
31 Aug	29	21	9	54

ment of the colony (asexual phase), and dispersal and colonization of new habitats (sexual phase), and both phases in turn contribute to the consolidation of populations already established. The timing of larval production and recruitment strongly suggest that recruits are from the population itself, since the first settlement on the experimental surfaces coincided with the moment when the greatest percentage of zooids with embryos and larvae in the population were registered.

The positive linear relationship of the population spawning potential versus settlers on the experimental surfaces at Stn E1 show the self-recruitment capacity of the populations of *Ecteinascidia turbinata*. Similar results have been found in other colonial ascidians such as *Didemnum candidum*, where the diel timing of release, planktonic abundance and settlement were significantly correlated with each other: release-plankton ($r = 0.873$); release-settlement ($r = 0.864$); plankton-settlement ($r = 0.941$) (Hurlbut 1992). It seems that *E. turbinata* ensures local recruitment and renewal of the established population, but the settlement at Stn E2, although occasional and scarce, suggests that the larva is able to disperse a few hundred meters beyond the parent. In this sense, Young (1986) and Bingham &

Young (1991) found that dispersal time was much greater for larvae generated from colonies growing on seagrass and sand than for those proceeding from mangrove roots, indicating the larvae's preference for that habitat.

The pre-recruitment and post-recruitment survival percentages are generally quite low in other species. Davis (1987, 1988) carried out underwater observations and estimated that out of 6430 larvae produced per m^2, only 6.1% settled and only 63% of the settlers survived the first month, and of these only 14% survived to adulthood. Therefore, only 0.56% of all the larvae produced reached maturity. The values registered in our study were somewhat higher than the ones previously discussed; 29% of larvae settle locally versus 6.1%, 1.3% of larvae that transformed into colonies versus 0.56%. Our results could be higher than those obtained by Davis because *Ecteinascidia turbinata* is probably not subject to specific predation on the island of Formentera, as it is in Caribbean populations (Bingham & Young 1995), and the larvae also have a chemical defense (Young & Bingham 1987). On this matter, Olson & Macpherson (1987) observed *in situ* that a large amount of the larvae of *Lissoclinum patella* were consumed by predators (47%); Davis & Butler (1989) reported that 5% of the larvae of the ascidian *Podoclavella moluccensis* followed in the field were consumed before they could settle, and Stoner (1990) observed that 29% of the larvae of the ascidian *Diplosoma similis* were eaten.

Table 5. Data used in the population spawning potential—larvae + zooids m^{-2} relationships, on experimental surfaces at Stn E1 Data for larvae and zooids m^{-2} correspond to average values

Population spawning potential (date)	Larvae m^{-2} (date)	Zooids m^{-2} (date)	Total (larvae + zooids)
49.6 (27 Jun)	95 (3 Jul)	102 (3 Jul)	197
50.4 (4 Jul)	31 (9 Jul)	175 (9 Jul)	206
15 (11 Jul)	25 (16 Jul)	82 (16 Jul)	107
1.68 (18 Jul)	0 (24 Jul)	10 (24 Jul)	10
44.8 (16 Aug)	5 (20 Aug)	22 (20 Aug)	27
54 (31 Aug)	3 (5 Sep)	27 (5 Sep)	30

Table 6. Zooids and colonies m^{-2} (average values) on settlement surfaces at Stn E2 transplanted from Stn E1

	Jul			Aug				Sep
	16	24	30	5	13	20	30	5
Zooids m^{-2}	24	35	27	6	0	1	1	0
Colonies m^{-2}	5	9	6	1	1	1	1	0

Although using large settlement surfaces and extensive sampling site ensures our estimates to be accurate to a certain degree, we must consider the difficulty of working in the natural environment, and it is very possible that some larvae may have settled in other places that were overlooked during sampling. Moreover, we cannot completely disregard that some larvae from other areas of the lagoon may have settled on the surfaces and were counted with the others.

Like other semelparous species, *Ecteinascidia turbinata* (Svane & Havenhand 1993) has a mode of reproduction which ensures local recruitment and renewal of the established population. We feel that this self-recruitment capacity is related to the type of development, since this in turn is related to the dispersal capacity of the larval stages. Thus ovoviviparous species with lecithotrophic larvae such as *E. turbinata* have little time to find an adequate substrate to settle on, because metamorphosis commences shortly after the larvae are released. And, according to the results, the self-recruitment capacity could also serve to ensure an ideal habitat for colonization.

Effect of habitat selection on species survival

The Estany des Peix is a shallow lagoon, and finer particles with a high organic matter content seem to settle at its center more frequently (clay). They resuspend easily and seem to impede the normal development of the larvae that have settled, thus affecting species survival. The transplantation of developed zooids from Stn E1 to Stn E2 suggests there is a risk of inappropriate habitat selection for settlement. Nevertheless, although the transplanting procedure was carried out very carefully and the zooids were always kept underwater, the mortality at Stn E2 could be attributed to either the transplantation procedure or to the conditions at Stn E2.

In this sense, Svane (1987) transplanted juveniles from the species *Ascidia mentula* and found that habitat selection (different inclinations and positions with respect to a vertical subtidal rock) was essential in preventing predation and/or silting. The experiments carried out by Young (1986) and Bingham & Young (1991) also show the importance of habitat selection for species survival.

Consequently, the risk of dispersal may be especially high in semelparous species such as *Ecteinascidia turbinata*, since if a parent population survives to reproduce in a favourable habitat, it may be an unsafe investment on a short-term scale to risk dispersal to 'unknown' and possibly unfavourable habitats (Svane & Havenhand 1993). Gregarious settlement is advantageous in unpredictable environments (spatio-tempo-

rally variable) because it ensures greater stability on a small scale due to the risk of selecting an inappropriate substrate for the survival of the stolon during the month when the adult phase disappears. The *E. turbinata* population disappears at the end of October but remains latent until it grows again from the stolon in mid-May, when the stolon generates a first blastozooid population which reproduces asexually first, and later sexually (Carballo et al. 1997). Hibernation is of great evolutionary importance as well, especially in a habitat like the Estany des Peix, given that without at least 1 phase of asexual development, *E. turbinata* would not propagate sexually. Hibernation is also known in *Botrylloides leachi* and bud formation is known in some colonial ascidians, such as *Stolonica socialis*, that form survival buds which survive the disappearance of the parent in late summer and develop by the following spring (Satoh 1994).

The *Ecteinascidia turbinata* population is local, appearing and disappearing every year (extinction and establishment of new populations), and is confined to this lagoon (isolation). Thus, the behaviour of the larvae may represent a local adaptation to prevent advection to inappropriate sites. In evolutionary terms, the benefits of aggregation must exceed the cost, and the results suggest that the evolution of settlement is linked to the risk of settling in a stressful environment.

LITERATURE CITED

Bingham BL, Young CM (1991) Larval behavior of the ascidian *Ecteinascidia turbinata* Herdman; an *in situ* experimental study of the effects of swimming on dispersal. J Exp Mar Biol Ecol 145:189–204

Bingham BL, Young CM (1995) Stochastic events and dynamics of a mangrove root epifaunal community. PSZN I: Mar Ecol 16:145–163

Bouyoucos GJ (1934) The hydrometer method for making mechanical analysis of soils. Soil Science 38:335–343

Carballo JL, Naranjo S, Hernández-Zanuy A, Kukurtzü B (1997) Estudio de la población de *Ecteinascidia turbinata* Herdman 1880 (Ascidiacea) en la isla de Formentera (Mediterráneo. España): Distribución, densidad y crecimiento. Ciencias del Mar UAS 15:7–15

Carballo JL, Hernández-Zanuy A, Naranjo S, Kukurtzü B, García-Cagide A (1999) Recovery of *Ecteinascidia turbinata* Herman 1880 (Ascidiacea: Perophoridae) populations after different levels of harvesting on a sustainable basis. Bull Mar Sci 65(3):755–760

Davis AR (1987) Variation in recruitment of the subtidal colonial ascidian *Podoclavella cylindrica* Quoy & Gaimard: the

role of substratum choice and early survival. J Exp Mar Biol Ecol 106:57–71

Davis AR (1988) Effects of variation in initial settlement on distribution and abundance of *Podoclavella moluccensis*. J Exp Mar Biol Ecol 117:157–167

Davis AR, Butler AJ (1989) Direct observations of larval dispersal in the colonial ascidian *Podoclavella moluccensis* Sluiter: evidence for closed populations. J Exp Mar Biol Ecol 127:189–203

Davis AR, Wright AE (1990) Inhibition of larval settlement by natural products from the ascidian, *Eudistoma olivaceum* (Van Name). J Chem Ecol 16:1349–1357

Havenhand JN, Svane IB (1991) Roles of hydrodynamics and larval behaviour in determining spatial aggregation in the tunicate *Ciona intestinalis*. Mar Ecol Prog Ser 68:271–276

Hurlbut CJ (1992) Larval release and supply predict temporal variation in settlement of a colonial ascidian. Mar Ecol Prog Ser 80:215–219

Iglesias C, Herraez P, Guinea J (1995) Aquaculture of tunicates producing anticancer compounds: reproduction and larval development of *Ecteinascida turbinata*. Proceedings of the International Aquaculture Symposium, San Diego. Univ California, San Diego

Olson RR, Macpherson R (1987) Potential vs realized larval dispersal: fish predation on larvae of the ascidian *Lissoclinum patella* (Gottschadt). J Exp Mar Biol Ecol 110:245–256

Osman RW, Whitlatch RB (1995) The influence of resident adults on larval settlement: experiments with four species of ascidians. J Exp Mar Biol Ecol 190:199–220

Petersen J, Svane IB (1995) Larval dispersal in the ascidian *Ciona intestinalis* (L.). Evidence for a closed population. J Exp Mar Biol Ecol 186:89–102

Satoh N (1994) Developmental biology of ascidians. Cambridge University Press, Cambridge

Sokal RR, Rohlf JJ (1981) Biometry. WF Freeman and Co, San Francisco

Stoner DS (1990) Recruitment of a tropical colonial ascidian: relative importance of pre-settlement vs post-settlement processes. Ecology 71:1682–1690

Svane IB (1987) On larval behaviour and post-metamorphic mortality of *Ascidia mentula* O.F. Müller. Ophelia 27:87–100

Svane IB, Havenhand JN (1993) Spawning and dispersal in *Ciona intestinalis* (L.). PSZN I: Mar Ecol 14:53–66

Svane IB, Young CM (1989) The ecology and behaviour of ascidian larvae. Oceanogr Mar Biol Annu Rev 27:45–90

Turon X, Vazquez E (1995) A new larval type in ascidians: *Protostyela longicauda*, morphological traits and adaptive value. Larval Biology Meetings, 16–19 August 1995, Ft. Pierce, Florida. Harbor Branch Oceanographic Institution, Ft. Pierce

Young CM (1986) Direct observations of field swimming behavior in larvae of the colonial ascidian *Ecteinascidia turbinata*. Bull Mar Sci 39(2):279–289

Young CM (1989) Distribution and dynamics of an intertidal ascidian pseudopopulation. Bull Mar Sci 45(2):288–303

Young CM, Bingham BL (1987) Chemical defense and aposematic coloration in larvae of the ascidian *Ecteinascidia turbinata*. Mar Biol 96:539–544