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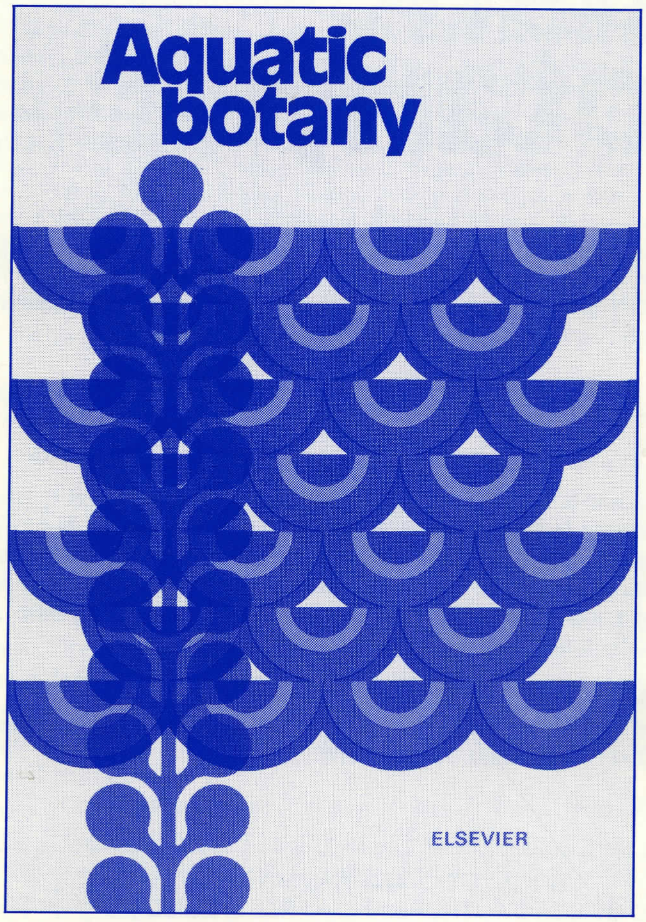

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ABSTRACT

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Seeds of *Cymodocea nodosa* (Ucria) Aschers. are abundant thoughout the year in several beds around the coasts of the western Mediterranean, but it is rare to find seedlings in situ. Experiments carried out in aquaria have shown that the higher the temperature and the lower the salinity, the faster the germination and the higher the germination rate. At high temperatures and low salinities *C. nodosa* can show more than 90% germination in 10 days, at all times of the year. Seedlings, however, show good growth only at temperatures between 17 and 25°C and when salinity is also moderately reduced. Thus, in *C. nodsa*, germination followed by seedling development requires either a reduction of salinity to between 20 and 27‰ for a prolonged period (several weeks—several months) or a more marked reduction to between 10 and 20‰ for a few days. These restrictions appear to limit the germination of *C. nodosa* in the western Mediterranean, in time to the period April—October, and in space to those seagrass beds near sources of freshwater.

INTRODUCTION

Flowering and fruiting in *Cymodocea nodosa* (Ucria) Aschers. is abundant and occurs annually at several sites investigated in the French western Mediterranean (Caye and Meinesz, 1985). Flowering occurs between 15 May and the end of June, depending on the locality and on depth. The fruit is a drupe which develops during summer with a fleshy pericarp and a hard stone. In October the fruits are ripe, they separate from the plant, the pericarp decays and the seed stays in the sediment of the seagrass bed. The seed, semicircular and laterally compressed, bears on the dorsal side three parallel more or less crenulate ridges (den Hartog, 1970). The external tegument of the seed, beige in colour, is tough, while the internal tegument is reddish brown and membranaceous.

The embryo of *C. nodosa* and its germination have been described by Bornet (1864). The voluminous embryo is without albumen and the cylindrical cotyledon occupies a kind of groove in a bulky reserve organ which

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the authors believe to be a radicle (Bornet, 1864). At the base of the cotyledon the plumule, which is protected by two small scales, will produce the first leaves and the stem. It is the cotyledon which pierces the tegument and there is no development of the primordial root in the course of this germination.

We have found *C. nodosa* seeds in the bed sediment all the year round in several localities of the Côte d'Azur, with a density of 80—220 seeds m^{-2} (Caye and Meinesz, 1985). However, during five years of investigations, we have found only two seedlings. In France, the only previous record of germination is by Bornet (1864). In southern Italy numerous seedlings have been found in situ (Pirc et al., 1983). Recent experiments on *C. nodosa* of the region of Naples (Pirc et al., 1986) show that it exhibits a distinct dormancy period of 7—8 months and starts to germinate in May when sea temperature reaches 18°C. In Yap (Micronesia) the germination of *Cymodocea rotundata* Ehrenberg et Hemprich ex Aschers. occurs throughout the year in coral sand, with high temperatures and freshwater discharges from rivulets during rains (McMillan et al., 1982).

In order to determine the factors necessary for germination in *C. nodosa*, and the reasons why seedlings have seldom been observed along the western Mediterranean coast, a study of germination under controlled conditions was carried out.

METHODS

By means of SCUBA diving, we gathered 1500 seeds at depths of 2—3 m in an extensive bed of *C. nodosa* growing on muddy sand in the bay of Golfe-Juan (Côte d'Azur, France).

Samples were taken by cutting, from the bottom, square blocks of 25×25 cm and 15 cm depth. On the beach, these blocks were broken up and sieved with a 2-mm mesh. This procedure resulted in elimination of the sediment and retention of the plants and seeds in the sieve.

In the laboratory, seed germination was investigated using 20-l aquaria, containing artificial seawater of predetermined salinities, the temperature being controlled by thermostat. In each aquarium, seeds were placed in groups of 10, in small plastic flower pots, each closed by a mesh lid. In each experiment, we used three replicates of 10 seeds. Germination rate is expressed as a percentage, each represents the mean of three replicates. We have considered as germinated those seeds in which the cotyledon had pierced through both teguments.

RESULTS

Preliminary observations and experiments

In *Cymodocea nodosa* germination, the emergence of the cotyledon from the seed occurs with the raising of the dorsal ridge. While the cotyledon is

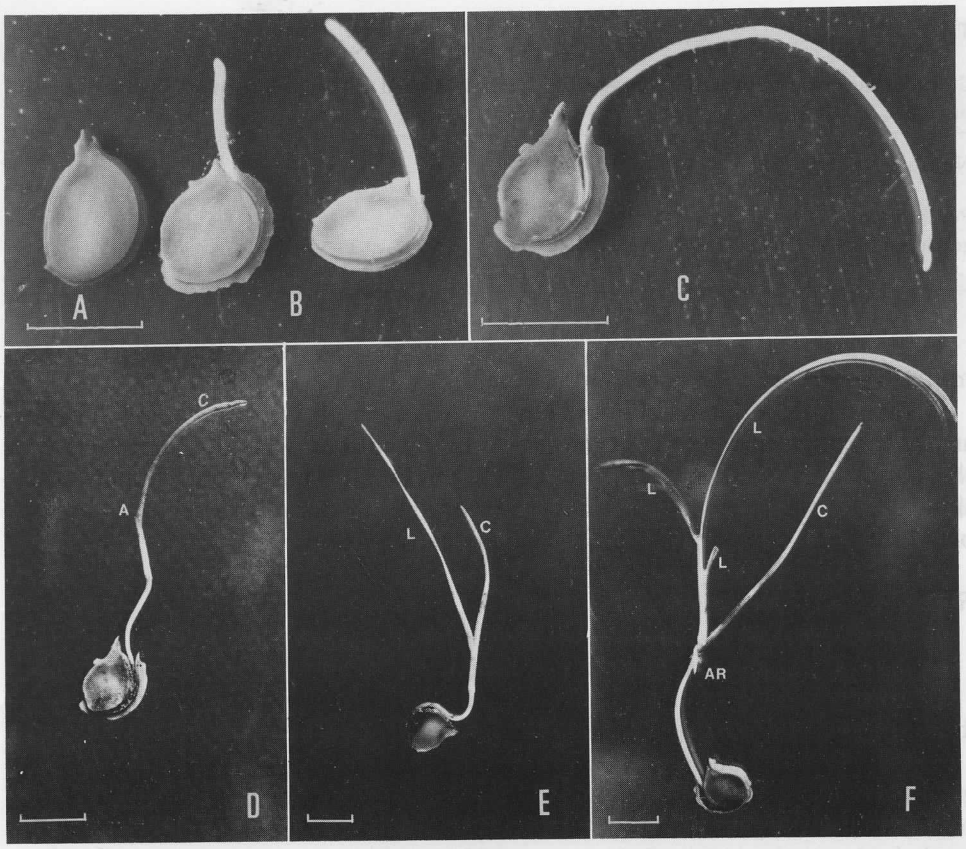

Fig. 1. The germination and seedling development of *Cymodocea nodosa*. (A) the seed; (B) and (C) elongation of cotyledon (4—8 days); (D) seedling with the young apex and the epicotyl (10—15 days); (E) seedling with the first leaf (2 weeks); (F) seedling with 3 leaves and the first adventitious root (3—4 weeks). Within (D)—(F): C = cotyledon; A = apex; L = leaf; AR = adventitious root; scale = 1 cm.

elongating and becoming green, the plumule is pushed out of the seed. The first juvenile leaf develops within the cotyledon, the latter functioning as a sheath. It is generally at the time of appearance of the 3rd juvenile leaf that the development of the first adventitious root takes place. The seedling is then aged about one month (Fig. 1).

We carried out the first experiment in December 1983 using 4 different temperature—salinity combinations

Combination	Salinity (‰)	Temperature (°C)
1	38	16
2	38	22
3	27	16
4	27	22

TABLE I

Cumulative germination percentage of *C. nodosa* seed at a salinity of 27‰ and a temperature of 22° C

Number of days	18	30	60	90	120
Percentage of germinations	7	37	47	50	60

Only seeds in combination 4 had germinated by the 18th day. The germination rate was 60% after 4 months (Table I).

This first experiment showed that two factors, temperature and salinity, are both concerned in the germination of *C. nodosa.* Even in combination 4 the conditions (salinity 27‰ at 22° C) produced a partial and late germination. Thus, we were led to investigate in greater detail the separate effects of salinity and temperature on *C. nodosa* germination.

Role of salinity

Experiment at a range of salinities (Fig. 2)

We investigated the action of freshwater and increasing salinities (5, 10, 15, 20, 27 and 38‰) at a constant temperature of 24°C.

The lower the salinity, the more rapidly the seeds germinate, and with a greater success rate. For instance in freshwater, 73% of the seeds had germinated in 2 days and 100% in 6 days; at a salinity of 5‰, 60% of the seeds had germinated in 2 days and 97% in 10 days; at a salinity of 10‰, 30% of the seeds had germinated in 2 days and 100% in 24 days. At a salinity of 27‰ germination commenced after 20 days and at 38‰ germination was not observed.

Salinity also appears to play a part in seedling development. At very low salinities, freshwater–5‰, the seedlings decay in 10 and 20 days, respectively. At a salinity of 10‰ seedling growth is slowed down and the cotyledons remain white. From salinities of 15‰ upwards, the seedlings develop well, starting with a phase in which the cotyledon lengthens and becomes green (10 and 15 days). Then the first leaf (2 weeks), the second leaf (2 to 3 weeks), the third leaf and the first adventitious root (3 to 4 weeks) appear.

Even if the seedlings are subjected to several days at a salinity of 10‰ followed by a period of increasing salinity, seedling development takes place normally.

Effect of amplitude in salinity change (Fig. 3)

In the first experiment (Fig. 3A) the germination of two groups of seeds at a salinity of 15‰ is compared. The first control group was transferred

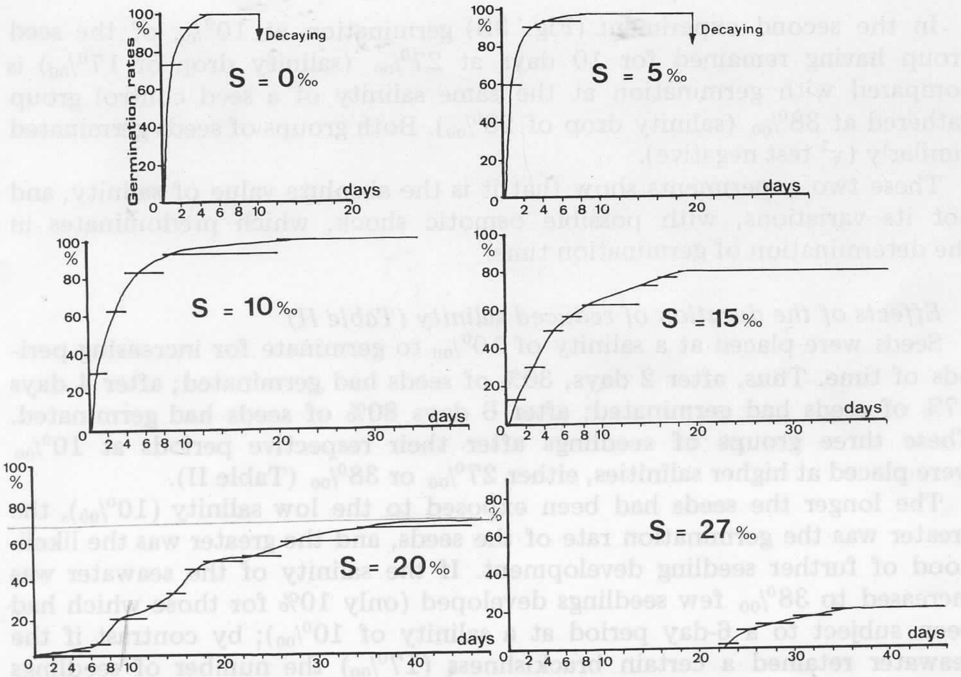

Fig. 2. Effect of salinity on germination time and success rate of *C. nodosa* seeds at a temperature of 24° C.

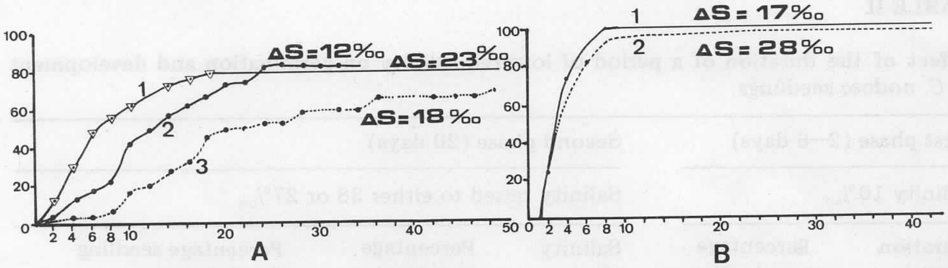

Fig. 3. Effect of salinity changes on *C. nodosa* germination. (A) seeds gathered at 38‰ germinating at 15‰: $\Delta S = 23$‰ (Curve 1), seeds gathered at 27‰ germinating at 15‰; $\Delta S = 12$‰ (Curve 2), seeds gathered at 38‰ germinating at 20‰; $\Delta S = 18$‰ (Curve 3). (B) Germination at a salinity of 10‰ of seeds having been gathered for 10 days at 27‰: $\Delta S = 17$‰ (Curve 1); and of seeds gathered at a salinity of 38‰: $\Delta S = 28$‰ (Curve 2).

from a salinity of 38‰ to one of 15‰ experiencing a drop of 23‰. The second group was made up of seeds having been kept for 6 months at 27‰ and 18° C without germination; on being transferred to 15‰, they experienced a drop of 12‰. The latter group germinated slightly slower than the control group, but much more quickly than seeds gathered at 38‰ and placed at 20‰ to germinate (salinity drop of 18‰).

In the second experiment (Fig. 3B) germination at 10‰ of the seed group having remained for 10 days at 27‰ (salinity drop of 17‰) is compared with germination at the same salinity of a seed control group gathered at 38‰ (salinity drop of 28‰). Both groups of seeds germinated similarly (χ^2 test negative).

These two experiments show that it is the absolute value of salinity, and not its variations, with possible osmotic shock, which predominates in the determination of germination time.

Effects of the duration of reduced salinity (Table II)

Seeds were placed at a salinity of 10‰ to germinate for increasing periods of time. Thus, after 2 days, 30% of seeds had germinated; after 4 days 77% of seeds had germinated; after 6 days 80% of seeds had germinated. These three groups of seedlings after their respective periods at 10‰ were placed at higher salinities, either 27‰ or 38‰ (Table II).

The longer the seeds had been exposed to the low salinity (10‰), the greater was the germination rate of the seeds, and the greater was the likelihood of further seedling development. If the salinity of the seawater was increased to 38‰ few seedlings developed (only 10% for those which had been subject to a 6-day period at a salinity of 10‰); by contrast if the seawater retained a certain brackishness (17‰) the number of seedlings produced was much higher, varying from 27 to 50%, depending on the length of the exposure to the previous low salinity.

TABLE II

Effect of the duration of a period of lowered salinity on germination and development of *C. nodosa* seedlings

First phase (2—6 days)		Second phase (20 days)		
Salinity 10‰		Salinity raised to either 38 or 27‰		
Duration (Days)	Percentage germination	Salinity (‰)	Percentage germination	Percentage seedling development
2	30	38	30	0
		27	47	27
4	77	38	77	3
		27	77	47
6	80	38	80	10
		27	83	50

Role of temperature (Fig. 4)

We investigated the effect of the following temperatures, 10, 18, 20, 24, 28 and 34°C, on percentage and speed of germination in seawater at a salinity of 10‰.

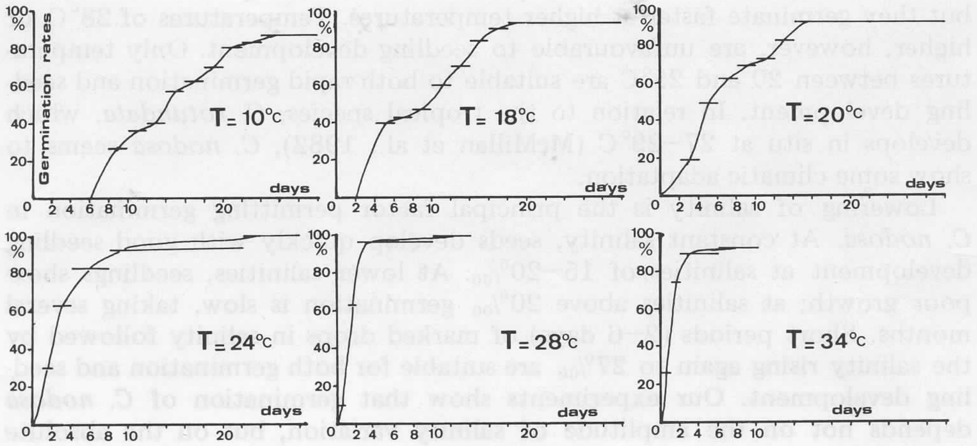

Fig. 4. Effect of temperature on germination speed and success rate in *C. nodosa* at a salinity of 10‰.

Curves of germination plotted against time for the different water temperatures, show that the higher the temperature, the faster the germination under experimental conditions (Salinity = 10‰). The temperature did not affect the final percentage of germination, which always exceeded 90%, except for the 10°C treatment in which only 87% of the seeds germinated.

However, the effect of the temperature on seedling development was different from that on seed germination. At 10°C, the cotyledon showed no growth; the seed could remain, for more than one month, at the germination stage. If the temperature then increased to 17—18°C the cotyledon resumed growth and the seedling developed. Between 18 and 24°C, growth and good development of seedlings were observed. At higher temperatures (28°C and higher), the cotyledon remained white, lengthening little (3—10 mm). The seedlings could persist for 2—3 weeks in water at 28°C, and end by decaying. Should, however, the temperature fall slightly, the plants could resume their development.

DISCUSSION

The annual, and often abundant, flowering of *C. nodosa* in our regions, produces seeds which persist, in concentrations up to 220 seeds m^{-2} in seagrass bed sediment. Under favourable laboratory conditions, seeds can germinate (up to 90%) at any time of the year. Similar large seed reserves in the sediment and the ability to germinate in situ thoughout the year are found in other marine phanerogams such as *Halodule wrightii* Aschers. and *Cymodocea rotundata* (McMillan, 1981; McMillan et al., 1982). Conditions leading to germination appear similar in *C. nodosa* and *C. rotundata* (McMillan et al., 1982).

In the laboratory, seeds of *C. nodosa* can germinate at 18°C in 6—20 days, but they germinate faster at higher temperatures. Temperatures of 28°C, or higher, however, are unfavourable to seedling development. Only temperatures between 20 and 25°C are suitable to both rapid germination and seedling development. In relation to the tropical species, *C. rotundata*, which develops in situ at 27—29°C (McMillan et al., 1982), *C. nodosa* seems to show some climatic adaptation.

Lowering of salinity is the principal factor permitting germination in *C. nodosa*. At constant salinity, seeds develop quickly with good seedling development at salinities of 15—20‰. At lower salinities, seedlings show poor growth; at salinities above 20‰ germination is slow, taking several months. Short periods (2—6 days) of marked drops in salinity followed by the salinity rising again to 27‰ are suitable for both germination and seedling development. Our experiments show that germination of *C. nodosa* depends not on the amplitude of salinity variation, but on the absolute value of salinity.

Under the climatic conditions of the French Mediterranean coasts, water temperatures are favourable to the germination of *C. nodosa* from April to October or November, depending on the year. During this period, germination can occur only when the salinity is markedly lowered. A small or moderate lowering of salinity (27‰) would have to persist for several weeks or months to lead to germination. This could happen only near the mouths of large rivers, on very shallow seagrass beds or, for deeper beds, near submarine springs. In the event of a large drop in salinity (to 10—20‰), germination might be produced in 2—6 days. This may occur after severe thunderstorms in spring or autumn.

One property of *C. nodosa* seeds is that they are able to cease their development for several days to 2 or 3 weeks, when temperatures are too low or too high, and then to resume growth if conditions become favourable once more. Although healthy seeds of *C. nodosa* are present throughout the year in the sediment of several parts of the western Mediterranean, germination of these seeds can occur only near sources of freshwater. Because *C. nodosa* seeds are not disseminated, plants arising from sexual reproduction must be limited in both time and space in the localised nursery zones.

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