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Use of Underwater Photography for in situ Monitoring of Fruit Development of *Posidonia oceanica* (L.) Delile

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Résumé : Utilisation de la photographie sous-marine pour le suivi in-situ du développement des fruits de *Posidonia océanica* (L.) Delile

« La maturation des fruits de Posidonia océanica a été suivie dans la baie de Monterosso al Mare, dans le Parc national des « Cinque Terre » (La Spezia, Italie). L'utilisation de la photographie sous-marine avec un système macro (1 :2) a permis de suivre la maturation des fruits sans récolter les inflorescences, qui étaient rares et dispersées. La longueur des fruits et le pourcentage de maturation a été analysé à partir des photographies jusqu'à l'achèvement du cycle de reproduction et la libération des fruits ».

Key words: Seagrasses, reproduction, underwater photography, Mediterranean Sea.

Abstract: The maturation of fruit of *Posidonia oceanica* was followed in the Bay of Monterosso al Mare included in the Italian 'Cinque Terre' National Park (La Spezia, Italy). Underwater photography with a macro (1:2) complex allowed maturation of the fruits to be studied without collecting the inflorescences, which were rare and sparse. Fruit length and percentage of maturation were analysed from the photographs until completion of the reproductive cycle and detachment of fruits.

INTRODUCTION

Posidonia oceanica (L.) Delile is a marine phanerogam endemic to the Mediterranean Sea that colonizes the seafloor from the low water mark to nearly 40 m depth (DREW and JUPP, 1976). Sexual reproduction of the seagrass was frequently recorded in the last two decades, when SCUBA diving became common among marine biologists. Nevertheless, few studies monitored the fruit maturation for all the reproductive cycle and, in the majority of the cases, observations were limited to noting fruit and flower density during one or two dives (THELIN and BOUDOURESQUE, 1985). Fruit development was usually followed by collecting the inflorescences (PERGENT, 1985; BALESTRI *et al.*, 1995) or periodic SCUBA observations within permanent quadrats

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(FRADÀ ORESTANO *et al.*, 1989; BUIA and MAZZELLA, 1991). These two methods are inappropriate in an area with few and sparse inflorescences, as they are destructive (the first) or do not give results when high fruit mortality occurs (the second).

In this work, underwater close-up photography was used to follow fruit development of *P. oceanica* in Liguria, (Italy, North Western Mediterranean Sea) where flowering is considered a rare event (GIRAUD, 1977; BIANCHI and PEIRANO, 1995). The non-destructive method applied in the protected area of Monterosso al Mare Bay allowed fruit maturation to be followed until completion of the reproductive cycle, despite the low flower density.

Material and methods

The study was performed on the meadow of Monterosso al Mare Bay (44° 08' 30" N, 9° 39' 00" E) included in the Italian 'Cinque Terre' National Park. The meadow covers a surface of nearly 30 ha between 5 and 20 m depth (SANDULLI et al., 1994; GÓNGORA GONZÁLES *et al.*, 1996) and is one of the largest of the eastern Liguria (BIANCHI and PEIRANO, 1995). The inflorescences were first observed between 5 and 15 m depth in winter 1992 (SANDULLI et al., 1994), when an extensive reproductive event occurred in all the Northern Mediterranean. In the following months flowering continued only in the shallow part of the meadow where *P. oceanica* covered 60 % of the seafloor with a density of 300 shoots·m^{-2} (STOPPELLI and PEIRANO, 1995).

Since December 1992 until May 1993 flowering and development of fruits were followed at 6 m depth through SCUBA diving. A Nikonos V camera was equipped with a 35 mm lens, one macro extension tube (1:2) and a Nikonos SB-103 substrobe blocked in fixed position. The complex was protected with a semi-rigid plastic net with a 0.5 cm mesh size to prevent the leaves disturbing flash photography or to drifting between the lens and the subject. The fixed frame of the macro complex and the pre-set lens aperture (f/22) assured a constant focal length and scale of reproduction and TTL (through-the-lens) camera metering system guaranteed the corrected exposure. The rigid complex allowed the diver, once an inflorescence was found among the thick canopy of leaves, to position it within the frame with one hand and push the shutter release button with the other.

During each dive a diver took photographs of the inflorescences while another diver collected data on flower density along a 50 m long transect counting the number of inflorescences encountered in a belt one metre wide.

The images were used to distinguish healthy from abortive fruits; the total length of each healthy fruit, i.e. the distance between the apex and the attachment to the spikelet (CAYE and MEINESZ, 1984), was measured on the slides under a dissecting microscope fitted with a micrometer. The accuracy of measurements was initially tested by

photographing and then collecting 4 inflorescences (with 17 fruits) during the first dive. Fruit measurements were used: a) to calculate the mean fruit length; b) to group the fruit into the four size classes of BOUDOURESQUE and THELIN (1985): flowers with green ovaries (4-6 mm), very young fruits (7-13 mm), young fruits (14-21 mm), ripe fruits (>22 mm), and to calculate the percent maturation, i.e. the percent ratio of the number of mature fruits to the total number of fruits.

Results

The difference between figures obtained from image analysis and those measured with a venier calliper on the collected fruits was not significant (P=0.756, test U of Mann-Whitney). A total of 207 inflorescences were analysed, 533 fruits were classified and 337 healthy fruits were measured (Table I). The calculated regression line of fruit length versus time

$$Lf = -0.646\ (\pm\ 0.655) + [0.115\ (\pm\ 0.005)\cdot T]$$

where Lf is length of fruit in mm and T is the time in day (starting from October the 1st), was significant (R? = 0.698, P <0.0001):

Analysis of the evolution of fruiting (Table II) showed the mean number of total fruits per inflorescence decreased from December (4.28) to May (1.59). The ratio of healthy/abortive fruit was nearly constant and equal to 1 until April when the ratio was influenced by the detachment of abortive fruits. Maturation proceeded regularly from December to May and ripe fruits appeared in March (Fig. 1). The percent maturation rate reached its maximum (98%) on May 21, a day before the first stranding of ripe fruits occurred coincident with an abrupt rise of water and air temperature (STOPPELLI and PEIRANO, 1995). Along the 200 m shore in front of the meadow 186 fruits were collected on May 22nd and 316 fruits on May 27th.

Date	N	Mean	SD
16 Dec 1992	54	8.9	3.2
27 Jan 1993	80	12.2	4.5
9 Mar 1993	59	17.7	5.4
16 Apr 1993	47	22.2	3.9
29 Apr 1993	48	24.2	2.6
21 May 1993	49	26.6	2.4
22 May 1993	186 fruits cast ashore		
27 May 1993	318 fruits cast ashore		

Table I. Fruit growth of *Posidonia oceanica* in Monterosso al Mare. Mean length of healthy fruits measured on slides on each dive (N= Number of fruits, SD= standard deviation).

Date	Density (Infl·m^{-2})	infl. (n)	flower (4-6 mm)	very young fruits (7-13 mm)	young fruits (14-21mm)	ripe fruits (>22 mm)	aborted (n)	Detached (n)	Percent maturation (%)
16 Dec 1992	5.3	25	6	38	8	0	43	0	-
27 Jan 1993	4.0	36	6	37	40	0	47	0	-
9 Mar 1993	1.1	36	0	15	29	19	30	0	30.2
16 Apr 1993	1.2	36	0	1	14	32	34	21	68.1
29 Apr 1993	0.6	37	0	0	9	39	25	18	81.2
21 May 1993	0.5	37	0	0	1	48	12	26	98

Table II. Flower density (infl·m^{-2}) of *Posidonia oceanica* and total number of fruit recorded on examined inflorescences (infl.) following the classification of BOUDOURESQUE and THELIN (1984).

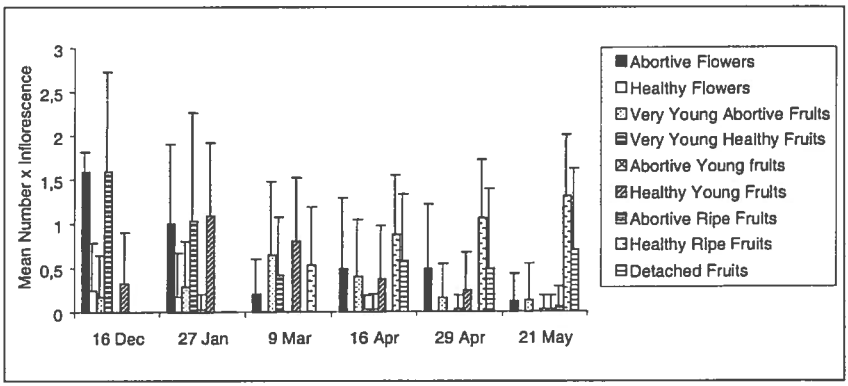

Fig. 1. Flowering evolution of *Posidonia oceanica* in Monterosso al Mare. Mean number of flowers or fruits per inflorescence are grouped into size-classes of BOUDOURESQUE and THELIN (1984). Standard deviation are evidenced with horizontal bars.

Discussion

Although underwater photography has been largely used recently to follow *in situ* growth of algae (HADDAD and ORMOND, 1994), corals (RAHAV *et al.*, 1991) and bryozoans (SGORBINI *et al.* 1996), it was not applied to phanerogams. The close-up photography proposed in this work was more appropriate, both in term of easy-of-use and quantity of data collected. The photo-camera rigid complex protected with the plastic net allowed one diver to shoot the inflorescences in the dense canopy and, in the case that the inflorescences were close to each other, allowed the shooting of two different floral stalks in one slide. The manipulation of floral stalks was reduced to the minimum. The fruits

were not touched, not interfering with the natural development or inducing early detachment.

The mean diving time for each dive was around one hour, which is acceptable at shallow sites but could be too long at higher depth. The 1:2 macro complex and the slide film assured a good definition and readability and only in some cases some pictures had to be discarded. The method assured the collection of a number of data statistically acceptable to follow flowering, calculate mean growth of fruits and estimate percent maturation rates. The close-up photography allowed fruit development to be followed in the only site of Liguria where fruits completed the maturation in 1992-1993 (BOYER *et al.*, 1995), despite the high mortality rate and the low flower density. The method was appropriate when working in protected areas or marine reserves to reduce interference with the natural dynamics of resident communities.

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