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Temporal trends in invasion impacts in macrophyte assemblages of the Mediterranean Sea

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Abstract: Invasive species are able to deeply alter coastal marine ecosystems ranging them among the most influential global changes of the present time. In the context of spatio-temporal variability, temporal trajectories of invasion impacts are very poorly known processes. The aim of the present study was to examine the impact of the invasive *Caulerpa racemosa* var. *cylindracea* (Chlorophyta) on benthic macrophyte assemblages in the north-western Mediterranean Sea (Marseille, France). A comparison was made between two different months (March *versus* November) and between years (2002/2003 *versus* 2006/2007). The results showed that there was significant variability between months and years in the biomass of *Caulerpa* as well as in its impact on macrophyte assemblages. In addition, *Caulerpa* outcompeted other invasive species, such as *Asparagopsis armata* and *Womersleyella setacea*, which showed strongly reduced biomass in invaded compared with non-invaded patches.

Résumé : *Evolution temporelle de l'impact des invasions dans les assemblages de macrophytes en Mer Méditerranée.* Les espèces invasives sont capables de modifier profondément les écosystèmes marins côtiers ; de ce fait elles se rangent parmi les causes majeures du changement global. Dans le contexte de la variabilité spatio-temporelle, les trajectoires temporelles des impacts provoqués par les invasions restent, à l'heure actuelle, des processus très mal connus. Le but de cette étude était d'examiner l'impact de l'espèce invasive *Caulerpa racemosa* var. *cylindracea* (Chlorophyta) sur les communautés benthiques macrophytiques en Méditerranée nord-occidentale (Marseille, France). Des comparaisons ont été réalisées entre deux différentes mois (mars *versus* novembre) et entre années (2002/2003 *versus* 2006/2007). Les résultats ont pu révéler une variabilité entre mois et années significative dans la biomasse de *Caulerpa* ainsi que dans son impact sur les communautés macrophytiques. Par ailleurs, *Caulerpa* dominait d'autres espèces invasives, telles qu'*Asparagopsis armata* et *Womersleyella setacea* qui montraient des biomasses fortement réduites dans les communautés envahies par rapport aux communautés non-envahies.

Keywords: Biological invasions ● *Caulerpa racemosa* var. *cylindracea* ● Algae ● Benthic assemblages ● Species introductions

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Introduction

Invasive species are of major concern in current ecological research due to their importance as drivers of ecosystem changes (Sala et al., 2000; Molnar et al., 2008). Understanding long-term effects of invasive species and the adaptive capacity of native assemblages is crucial when attempting to shed light on the evolution of ecosystems in a global change context. This comprehension naturally requires long-term studies. At present, a severe lack of long-term data exists, particularly concerning invasive species. Most studies dealing with invasion impacts are carried out punctually in space and time.

The introduced *Caulerpa racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman & Boudouresque (hereafter: *Caulerpa cylindracea*) (Chlorophyta) can currently be regarded as one of the most influential invasive species in Mediterranean benthic assemblages (Piazzi et al., 2005; Klein & Verlaque, 2008). The aim of the present study was to identify inter-annual variability (1) in *Caulerpa cylindracea* biomass and (2) in differences between invaded and non-invaded Mediterranean marine macrophyte assemblages.

Material and Methods

The macrophyte assemblages of dead *Posidonia oceanica* (Linnaeus) Delile matte have been studied at 17 m depth in the bay of Marseille (France) in the north-western Mediterranean Sea (43°13'44''N-5°20'41''E). Two different types of assemblages co-occur in this area: one invaded by the introduced *Caulerpa cylindracea* and the other one non-invaded. Biomass of *C. cylindracea* has been measured from November 2001 to March 2003 and from March 2005 to March 2007. Sampling of macrophytes assemblages has been conducted at two times, T1 (2002-2003) and T2 (2006-2007) and during two months, November (autumn) and March (winter). For each assemblage (Invaded and Non-Invaded), three 20 cm x 20 cm plots have been sampled by Scuba diving for each study date. Samples were stored in 4% formaldehyde seawater. The size of the quadrates was chosen to be bigger than the minimum sampling area of Mediterranean macro-algal assemblages (Dhont & Coppejans, 1977; Boudouresque & Belsher, 1979) to ensure representative sampling. The samples were analysed under a binocular microscope and under a light microscope for identification to the species level. In this way, an exhaustive species list has been drawn up for each sample (available from the author upon request). Dry weight measurements have been made by drying the sample in an oven (60°C) until constant weight.

A 3-factor analysis of variance was carried out on *C. cylindracea* biomass, macrophyte species number and cover. The factors were "Year" (2 levels, random, 2002/2003 *vs.* 2006-2007), "Month" (2 levels, fixed, November *vs.* March) and "Invasion" (2 levels, fixed, Non-invaded *vs.* Invaded). Cochran's test was used to test for homoscedasticity. If necessary, the data were log transformed to ensure homogeneity of variances. Multiple comparisons were made using the SNK test ($\alpha = 0.05$). Analyses were done with GMAV 5 for Windows (EICC, University of Sydney). A multivariate permutational analysis of variance (PERMANOVA; Anderson 2001) was done on the abundance data of the different macrophyte taxa. Data were square-root transformed prior to elaboration of the Bray-Curtis dissimilarity matrix. A multidimensional scaling (MDS) plot was constructed using the Bray-Curtis dissimilarity matrix after square root transformation of the abundance data. PRIMER-E v6 package software (Primer-E, Plymouth) was used for the MDS plot.

Results and Discussion

Overall 143 macrophyte taxa have been identified. Besides *Caulerpa cylindracea*, four other introduced taxa have been detected, *Acrothamnion preissii* (Sonder) E.M. Wollaston, *Antithamnion amphigeneum* A.J.K. Millar, *Asparagopsis armata* Harvey and its tetrasporophyte "*Falkenbergia rufolanosa*" and *Womersleyella setacea* (Hollenberg) R.E. Norris.

Caulerpa cylindracea *biomass*

Biomass of *Caulerpa cylindracea* was significantly higher in the Invaded than in the Non-invaded assemblage except for March 2003 where the species almost completely disappeared (Mean biomass [g dry weight m^{-2}] ± SE: Nov 2001[1]: 33.81 ± 5.29; Mar 2002[1]: 5.35 ± 1.65; Nov 2002[1]: 64.79 ± 5.35; Mar 2003[1]: 0.88 ± 0.66; Mar 2005[2]: 31.85 ± 5.55; Nov 2006[2]: 56.06 ± 3.70; Mar 2007[2]: 15.97 ± 3.86) (Table 1).

Highest biomasses were recorded in November 2002. The winter regression of *C. cylindracea* observed in March 2003 could not be confirmed in March 2005 and 2007 where *C. cylindracea* was abundant throughout the cold period. On one hand, the quasi-complete regression observed in winter 2002/2003 may have been due to unfavourable environmental conditions (violent storms, low water temperatures). On the other hand, the perenniality of the populations in subsequent years may illustrate an increased adaptation of *C. cylindracea* to the conditions of the north-western Mediterranean Sea. In fact, the perennial character of the *C. cylindracea* populations increases with decreasing latitude in Italy.

[1] Data from Ruitton et al. 2005

[2] Data from Klein & Verlaque 2011

Table 1. Results from the analysis of variance on *Caulerpa racemosa* biomass. Significant results are in bold.

Tableau 1. Résultats de l'analyse de variance sur la biomasse de *Caulerpa racemosa*. En gras : résultats significatifs.

Source	df	Mean square	F	p
Year, Y	1	57.8306	2.00	0.1760
Month, M	1	4794.7334	166.18	**< 0.001**
Invasion, I	1	6368.7384	220.73	**< 0.001**
Y x M	1	189.3098	6.56	**0.0209**
Y x I	1	5.4626	0.19	0.6693
M x I	1	3849.4001	133.41	**< 0.001**
Y x M x I	1	373.5126	12.95	**0.0024**
Residual	16	28.8534		
Total	23			

Multiple comparisons (SNK test, at α = 0.05) are as follows:
2002-2003 (March): Non-invaded assemblage = Invaded assemblage; 2002-2003 (November): Non-invaded assemblage < Invaded assemblage; 2006-2007: Non-invaded assemblage < Invaded assemblage.
Non-invaded assemblage: 2002-2003 = 2006-2007; Invaded assemblage (March): 2002-2003 < 2006-2007; Invaded assemblage (November): 2002-2003 > 2006-2007.
Non-invaded assemblage: March = November; Invaded assemblage: March < November.

Macrophyte assemblages

The mean number of macrophyte species per plot was always significantly higher in the Non-invaded than in the Invaded assemblage (Fig. 1 & Table 2). In the Non-invaded assemblage, the number of species remained relatively stable over time, whereas significant inter-annual variability (2002-2003 *vs.* 2006-2007) was revealed in the Invaded assemblage especially in March. Seasonal variations were more pronounced in 2006-2007 than in 2002-2003. Mean macrophyte cover (excluding *Caulerpa cylindracea*) was significantly higher in the Non-invaded than in the Invaded assemblage (Table 3, ANOVA, p = 0.0017). In March 2007 a relatively large number of macrophyte species has been recorded in comparison with November 2002 and 2006. These observed differences between months could be explained by the fact that *C. cylindracea* populations are colonised by large amounts of epiphytes during the cold winter months. In March 2003, the complete disappearance of *C. cylindracea* and its epiphytes has led to a very low number of species.

The cover of other introduced macrophytes excluding *C. cylindracea* was smaller in the Invaded than in the Non-invaded assemblage (Fig. 2 & Table 4, ANOVA, p < 0.001) and was constituted mainly by the two species *Asparagopsis armata* and *Womersleyella setacea*. Thus, it seems that *C. cylindracea* is capable of outcompeting the other two invasive species which are present in the north-western Mediterranean Sea. This finding has been confirmed by another study in Italy (Piazzi & Cinelli, 2003).

Table 2. Results from the analysis of variance on the number of macrophyte species. Significant results are in bold.

Tableau 2. Résultats de l'analyse de variance sur le nombre d'espèces de macrophytes. En gras : résultats significatifs.

Source	df	Mean square	F	p
Year, Y	1	693.3750	31.22	**< 0.001**
Month, M	1	12.0417	0.54	0.4722
Invasion, I	1	1890.3750	85.12	**< 0.001**
Y x M	1	176.0417	7.93	**0.0124**
Y x I	1	187.0417	8.42	**0.0104**
M x I	1	45.3750	2.04	0.1721
Y x M x I	1	0.0417	0.00	0.9660
Residual	16	22.2083		
Total	23			

Multiple comparisons (SNK test, at α = 0.05) are as follows:
In 2002-2003 and 2006-2007: Non-invaded assemblage > Invaded assemblage;
Non-invaded assemblage: 2002-2003 = 2006-2007; Invaded assemblage: 2002-2003 < 2006-2007;
In 2002-2003: March = November; in 2006-2007: March < November;
In March: 2002-2003 < 2006-2007; in November: 2002-2003 = 2006-2007.

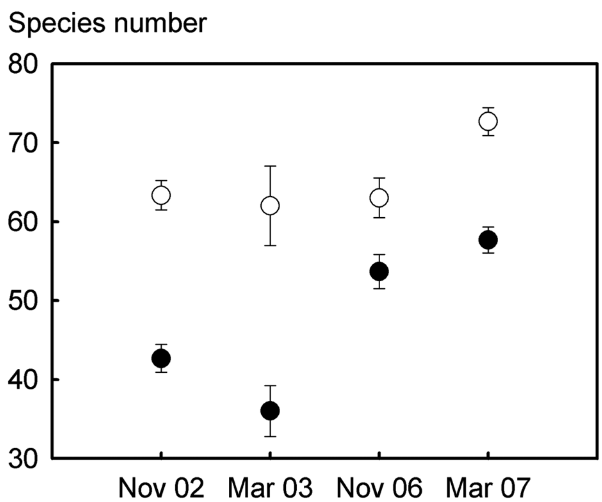

Figure 1. Number of macrophyte species per plot (mean ± SE) in November 2002, March 2003, November 2006 and March 2007. ○ Non-invaded, ● Invaded.

Figure 1. Nombre d'espèces de macrophytes par quadrat (moyenne ± SE) en novembre 2002, mars 2003, novembre 2006 et mars 2007. ○ Non-envahie, ● Envahie.

Results from multivariate analysis (PERMANOVA) showed significant differences in macrophyte species composition and assemblage structure between the Non-Invaded and the Invaded assemblage. Furthermore there were significant differences between years and months (except for the Invaded assemblage in November between the years 2002-2003 and 2006-2007). The MDS plot showed a clear separation between the samples of the two

Table 3. Results from the analysis of variance on macrophyte cover. Significant results are in bold.

Tableau 3. Résultats de l'analyse de variance sur le recouvrement des macrophytes. En gras : résultats significatifs.

Source	df	Mean square	F	p
Year, Y	1	3617.1971	1.19	0.2910
Month, M	1	2244.2136	0.74	0.4024
Invasion, I	1	43173.3803	14.23	**0.0017**
Y x M	1	4623.7056	1.52	0.2348
Y x I	1	5155.6291	1.70	0.2108
M x I	1	10148.4163	3.35	0.0861
Y x M x I	1	3509.9691	1.16	0.2980
Residual	16	3033.3756		
Total	23			

Multiple comparisons (SNK test, at α = 0.05) are as follows: Non-invaded assemblage > Invaded assemblage.

Table 4. Results from the analysis of variance on introduced macrophyte cover. Significant results are in bold.

Tableau 4. Résultats de l'analyse de variance sur le recouvrement des macrophytes introduits. En gras : résultats significatifs.

Source	df	Mean square	F	p
Year, Y	1	3.6635	4.82	**0.0433**
Month, M	1	0.8559	1.13	0.3045
Invasion, I	1	69.9339	91.94	**< 0.001**
Y x M	1	8.1478	10.71	**0.0048**
Y x I	1	0.7433	0.98	0.3376
M x I	1	0.3494	0.46	0.5076
Y x M x I	1	2.8918	3.80	0.0690
Residual	16	0.7607		
Total	23			

Multiple comparisons (SNK test, at α = 0.05) are as follows:
Non-invaded assemblage > Invaded assemblage.
In 2002-2003: March = November; in 2006-2007: March < November
In November: 2002-2003 = 2006-2007; in March: 2002-2003 > 2006-2007

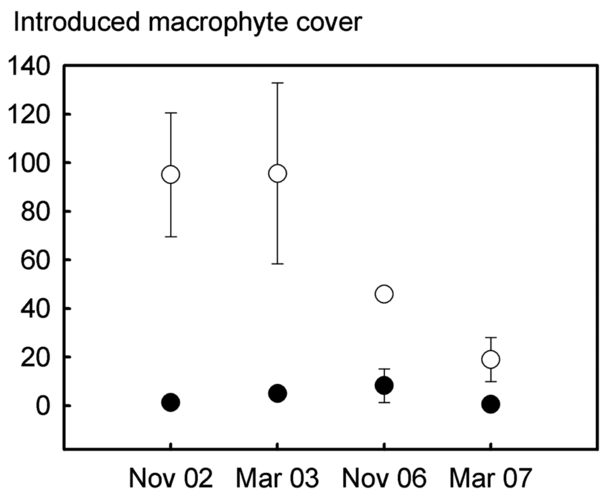

Figure 2. Cover of introduced macrophytes (cm^2 $plot^{-1}$) excluding *Caulerpa cylindracea* in November 2002, March 2003, November 2006 and March 2007. ○ Non-invaded, ● Invaded.

Figure 2. Recouvrement des macrophytes introduits (cm^2 $plot^{-1}$) (C. cylindracea exclue) en novembre 2002, mars 2003, novembre 2006 et mars 2007. ○ Non-envahie, ● Envahie.

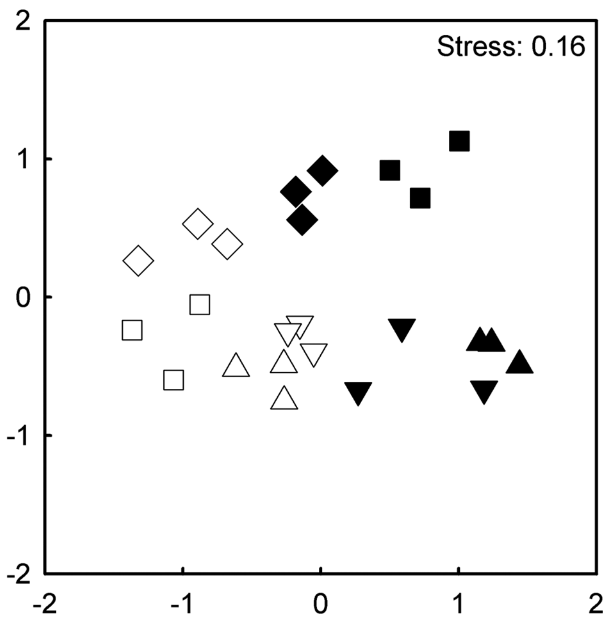

Figure 3. MDS plot for the samples from November 2002 (triangle pointing upwards), March 2003 (square), November 2006 (triangle pointing downwards) and March 2007 (diamond).

Open symbols: Non-invaded; full symbols: Invaded.

Figure 3. Plot de MDS représentant les échantillons de novembre 2002 (triangle vers le haut), mars 2003 (carré), novembre 2006 (triangle vers le bas) et mars 2007 (losange).

assemblages (Non-invaded *vs.* Invaded) and within each assemblage between months and years (Fig. 3). The samples from March 2003 were most distant indicating highest dissimilarities between the two assemblages. This was confirmed by the PERMANOVA results.

Conclusions

Long-term studies on macrophyte assemblages are scarce, just like long-term studies on the impact of invasive species on native assemblages. Large-scale temporal variations in biological systems may occur due to (1) natural variations inherent to the dynamics of the assemblage, (2) natural variations in environmental variables, or (3) human induced disturbances. Through the interplay of various factors, complex population and assemblage dynamics arise over different temporal and spatial scales. It is now essential to initiate long-term studies on invasion impacts and community dynamics to further our comprehension of the evolution of coastal assemblages.

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References

Anderson M.J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*, **26**: 32-46.

Boudouresque C.F. & Belsher T. 1979. Le peuplement algal du port de Port-Vendres: Recherches sur l'aire minimale qualitative. *Cahiers de Biologie Marine*, **20**: 259-269.

Dhont F. & Coppejans E. 1977. Résultats d'une étude d'aire minima des peuplements algaux photophiles sur substrat rocheux à Port-Cros et à Banyuls (France). *CIESM report*, **24**: 141-142.

Klein J. & Verlaque M. 2008. The *Caulerpa racemosa* invasion: a critical review. *Marine Pollution Bulletin*, **56**: 205-225.

Klein J.C. & Verlaque M. 2011. Experimental removal of the invasive *Caulerpa racemosa* triggers partial assemblage recovery. *Journal of the Marine Biological Association of the United Kingdom*, **91**: 117-125.

Molnar J.L., Gamboa R.L., Revenga C. & Spalding M.D. 2008. Assessing the global threat of invasive species to marine biodiversity. *Frontiers in Ecology and the Environment*, **6**: 485-492.

Piazzi L. & Cinelli F. 2003. Evaluation of benthic macroalgal invasion in a harbour area of the western Mediterranean Sea. *European Journal of Phycology*, **38**: 223-231.

Piazzi L., Meinesz A., Verlaque M., Akçali B., Antolić B., Argyrou M., Balata D., Ballesteros E., Calvo S., Cinelli F., Cirik S., Cossu A., D'Archino F., Djellouli A.S., Javel F., Lanfranco E., Mifsud C., Pala D., Panayotidis P., Peirano A., Pergent G., Petrocelli A., Ruitton S., Žuljević A. & Ceccherelli G. 2005. Invasion of *Caulerpa racemosa* var *cylindracea* (Caulerpales, Chlorophyta) in the Mediterranean Sea: an assessment of the spread. *Cryptogamie Algologie*, **26**: 189-202.

Ruitton S., Verlaque M. & Boudouresque C.F. 2005. Seasonal changes of the introduced *Caulerpa racemosa* var. *cylindracea* (Caulerpales, Chlorophyta) at the northwest limit of its Mediterranean distribution. *Aquatic Botany*, **82**: 55-70.

Sala O.E., Chapin F.S. III, Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. &Wall D.H. 2000. Global biodiversity scenarios for the year 2100. *Science*, **287**: 1770-1774.