



**PROCEEDINGS OF THE 4<sup>th</sup> MEDITERRANEAN  
SYMPOSIUM ON MARINE VEGETATION**  
Yasmine-Hammamet, Tunisia 2-4 December 2010

**ACTES DU 4<sup>ème</sup> SYMPOSIUM MÉDITERRANÉEN  
SUR LA VÉGÉTATION MARINE**  
Yasmine-Hammamet, Tunisie 2-4 Décembre 2010

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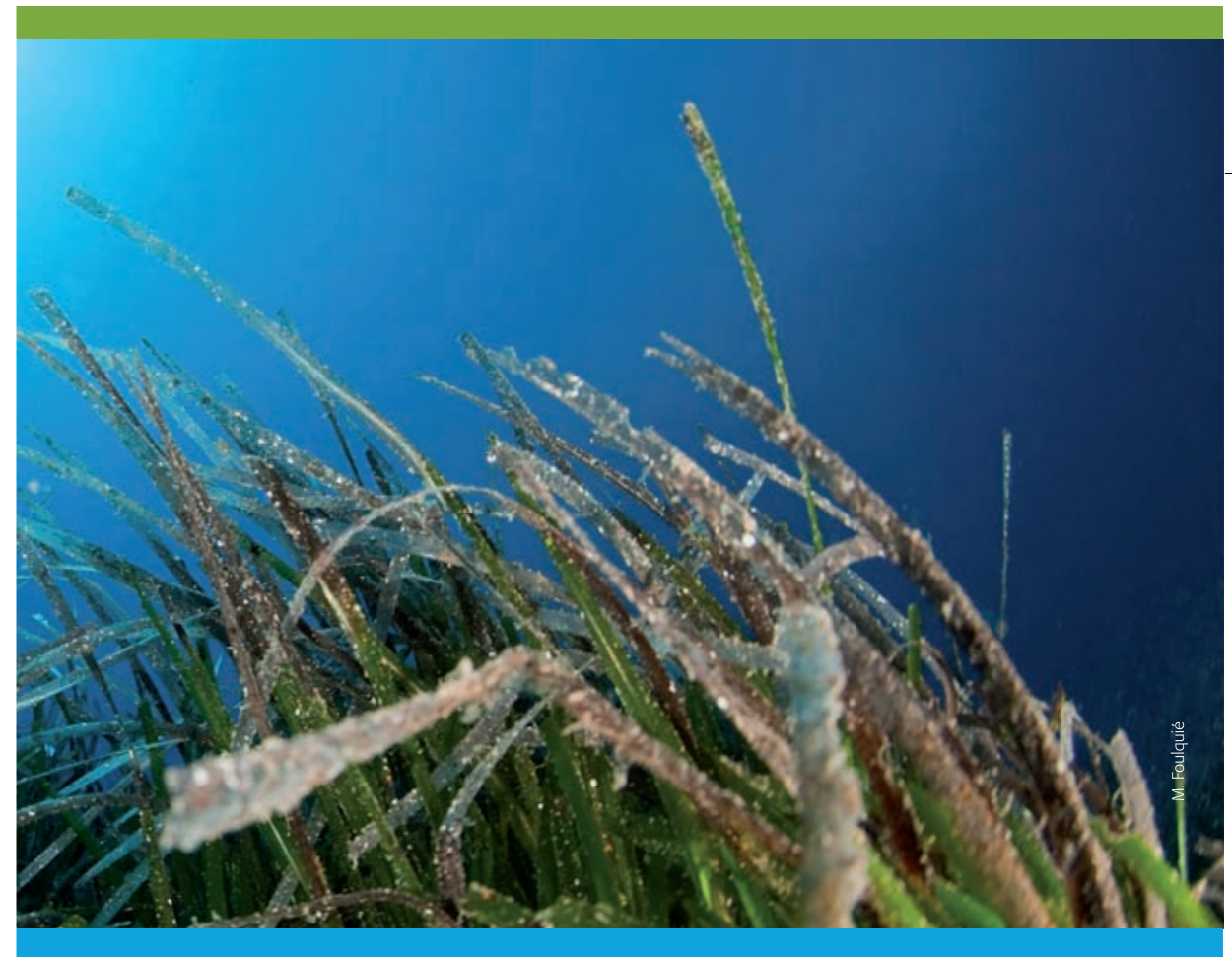
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Tunis, December 2010

United Nations Environment Programme  
Mediterranean Action Plan  
Regional Activity Centre for Specially Protected Areas

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Citation: UNEP - MAP - RAC/SPA, 2010. Proceedings of the Fourth Mediterranean Symposium on Marine Vegetation (Yasmine-Hammamet, 2-4 December 2010), EL ASMI S., LANGAR H., BELGACEM W. edits., RAC/SPA publ., Tunis: 251 p.

PNUE - PAM - CAR/ASP, 2010. Actes du Quatrième Symposium Méditerranéen sur la Végétation Marine (Yasmine-Hammamet, 2-4 Décembre 2010), EL ASMI S., LANGAR H., BELGACEM W. édits., CAR/ASP publ., Tunis: 251 p.

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## AVANT-PROPOS

Suite à une recommandation du Plan d'action pour la conservation de la végétation marine en mer Méditerranée (adopté par les Parties contractantes à la Convention de Barcelone, en 1999) et dans le but du développement des connaissances, une série de symposiums scientifiques, dédiée à la végétation marine méditerranéenne, a été initiée en 2000. Cette initiative vise essentiellement à faire le point sur les données scientifiques disponibles et à promouvoir la coopération entre les spécialistes méditerranéens.

Le premier symposium méditerranéen sur la végétation marine (Ajaccio, octobre 2000) a réuni 43 participants de 15 pays méditerranéens et s'est traduit par 32 communications orales et posters. Lors des tables rondes des sujets d'importance, tels que la taxonomie dans la région et l'utilisation de la végétation marine comme outil de gestion de la zone côtière, ont été abordés. Les recommandations ont permis de promouvoir, dans le cadre du Plan d'Action pour la Méditerranée, une Initiative méditerranéenne sur la Taxonomie, s'inspirant de la démarche développée, dans le cadre de la Convention sur la Diversité Biologique au plan mondial.

Le deuxième symposium (Athènes, décembre 2003), organisé en collaboration avec le Centre Grec pour la Recherche Marine, a regroupé 67 participants issus de 15 pays méditerranéens. 40 communications orales et posters ont été présentées à cette occasion. Les groupes de travail qui se sont réunis ont mis l'accent sur la nécessité de standardiser les techniques de cartographie et de développer des outils pour la taxonomie de la végétation marine, à l'échelon méditerranéen.

Le troisième symposium (Marseille, mars 2007) a, quant à lui, réuni 121 participants de 16 pays méditerranéens et autres et a été organisé en collaboration avec la Région Provence-Alpes-Côte d'Azur et l'Association Seagrass 2000. 60 communications orales et posters ont été présentées lors de ce symposium, qui a en outre débattu des méthodes de cartographie et de surveillance selon leurs différents objectifs, et de la végétation marine en tant qu'outil d'évaluation environnementale. Nombre de discussions ont porté sur le niveau de standardisation, et deux principales propositions en sont ressorties: la définition de protocoles précis (comme cela existe pour d'autres conventions internationales) et la proposition de procédures standardisées mais aussi adaptables aux besoins de chacun.

Le quatrième symposium programmé à Yasmine-Hammamet (Tunisie) du 2 au 4 décembre 2010, a vu l'inscription de plus de 110 participants en provenance de 16 pays méditerranéens. Ce ne sont pas moins de 72 communications orales et posters qui devraient y être présentées.

Cette quatrième édition sera aussi l'occasion d'aborder des sujets d'actualités tels que les invasions biologiques, le réchauffement global, et leurs impacts sur les peuplements végétaux de Méditerranée; et de renforcer les liens entre les institutions scientifiques.

Le Directeur du CAR/ASP  
Abderrahmen GANNOUN



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## 4<sup>th</sup> Mediterranean Symposium on Marine Vegetation

Yasmine-Hammamet, 2-4 December 2010

Alhambra-Thalasso Hotel

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# PROGRAMME

### Thursday 2 December 2010

- 8:00-9:30** Participants welcome and registration
- 9:30-10:30** **Opening of the Symposium:**
- Welcome speech by RAC/SPA Director
  - Speech of UNEP/MAP Officer in Charge and Deputy Coordinator
  - Opening speech by His Excellency the Minister of Environment and Sustainable Development
- 10:30-10:50** **Presentation of the Symposium programme and objectives** by RAC/SPA
- 10:50-11:00** **Action Plan for the conservation of marine vegetation in the Mediterranean Sea: Assessment of ten years of activity** by Ms. Souha EL ASMI (Programme Officer at RAC/SPA)
- 11:00-11:30** *Coffee break*
- Session 1: Mediterranean Marine Vegetation: population, biology, ecology and dynamics**  
Chair: Mr Dr. Marc VERLAQUE, Rapporteur: Mr Dr. Aslam Sami DJELLOULI
- 11:30-11:50** "Flora and vegetation of the Italian transitional water systems: an Italian editorial initiative" by Ester CECERE, PETROCELLI A , SFRISO A
- 11:50-12:10** "*Nouvelles connaissances sur les herbiers tigrés de Méditerranée*" by Gérard PERGENT, CALVO S , CANCEMI G , DJELLOULI A , DUPUY DE LA GRANDRIVE R , LANGAR H , PERGENT-MARTINI C , TOMASELLO A
- 12:10-12:30** "Invasion susceptibility of Mediterranean rocky benthic assemblages to red alga *Lophocladia lallemandii* (Montagne) F. Schmitz" by Emma CEBRIAN, BALLESTEROS E
- 12:30-12:50** "Light and temperature requirements for survival and growth of Mediterranean *Womersleyella setacea*" by Conxepció RODRÍGUEZ-PRIETO, JOHER S , CEBRIAN E
- 12:50-13:10** Discussion
- 13:10-14:30** *Lunch*
- Session 1: Mediterranean Marine Vegetation: population, biology, ecology and dynamics**  
(continued) Chair: Ms Dr. Conxepció RODRÍGUEZ-PRIETO, Rapporteur: Mr Robert TURK
- 14:30-14:50** "Biometry and leaf lepidochronology of the seagrass *Posidonia oceanica* in the Croatian Adriatic" by Tatjana BAKRAN-PETRICIOLI, Stewart T. SCHULTZ
- 14:50-15:10** "*Variations des paramètres biométriques de la bryopsidophycées Caulerpa racemosa var. Cylindracea en interaction avec la magnoliophyte marine Posidonia oceanica*" by Yosr MEZGUI, ZERZERI A , DJELLOULI A S

- 15:10-15:30 "Seasonal pattern of biomass and growth of *Cymodocea nodosa* in the Ghar El Melh lagoon in response to lagoon condition" by Yassine-Ramzi SGHAIER, ZAKHAMA-SRAIEB R , CHARFI F
- 15:30-15:50 "*Dynamique spatio-temporelle de la faune carcinologique associée à l'herbier de Posidonie dans la région de cap Zebib (Nord Est de la Tunisie)*" by Walid BELGACEM, LANGAR H , BEN ISMAIL D , BEN HASSINE O K
- 15:50-16:10 "Algal-dominated seascapes from the continental shelf off Mallorca and Menorca (Balearic Islands, Western Mediterranean)" by Sergi JOHER, BALLESTEROS E , CEBRIAN E, SÁNCHEZ N , RODRÍGUEZ-PRieto C
- 16:10-16:30 Discussion
- 16:30-16:50 *Coffee break*
- 16:50-18:15 **Poster Session**
- 18:30-19:30 *Side event*
- Photo exhibition of seagrass meadows around the world by Mr Mathieu FOULQUIE

## Friday 3 December 2010

### **Session 2: Mapping and monitoring of Mediterranean marine vegetation**

Chair: Mr Prof. Gérard PERGENT, Rapporteur: Mr Dr. Habib LANGAR

- 8:30-8:50 "Mapping *Posidonia oceanica* meadows using Quickbird and World View images west of Gulf of Alexandroupolis, Evros and Rodopi Prefecture, Greece" by Maria Venetia APOSTOLOPOULOU, PARCHARIDIS I , PAVLOPOULOS K , DEHAIRS F
- 8:50-9:10 "*La microcartographie par télémétrie acoustique, une nouvelle méthode de suivi des herbiers de posidonie - Application sur 9 sites sur les côtes françaises*" by Pierre DESCAMP, HOLON F , BOISSERY P , BALLESTA L , GUILBERT A , GUILLOT M , DETER J
- 9:10-9:30 "*Les relations entre les herbiers de posidonies, leur substrat et l'hydrodynamisme*" by Philippe CLABAUT, PERGENT-MARTINI C , PERGENT G , AUGRIS C , PASQUALINI V , CHAMLEY H
- 9:30-9:50 "Multi-sensors data fusion method devoted to sea bottom vegetation mapping and monitoring" by Claire NOEL, VIALA C , COQUET M , ZERR B , BLOUET S , DUPUY DE LA GRANDRIVE R
- 9:50-10:10 Discussion
- 10:10-10:40 *Coffee break*

### **Session 2: Mapping and monitoring of Mediterranean marine vegetation**

(continued)

Chair: Mr Prof. Enrique BALLESTEROS, Rapporteur: Ms Dr. Zohra EL ASMI-DJELLOULI

- 10:40-11:00 "New descriptors to evaluate conservation of *Posidonia oceanica* meadows" by Luciana MIGLIORE
- 11:00-11:20 "*Posidonia oceanica* meadows as a priority habitat for the sustainable management of the Greek coastal environment" by Panayotis PANAYOTIDIS, DRAKOPOULOU P
- 11:20-11:40 "*Progression de Caulerpa racemosa (Caulerpales, Chlorophyta) dans la région d'Alger*" by Halima SERIDI, KABRANE K
- 11:40-12:00 "*Suivi de l'expansion de Caulerpa taxifolia le long du littoral tunisien: situation au 30 avril 2009*" by Habib LANGAR, BELGACEM W , DJELLOULI A S , PERGENT G
- 12:00-12:20 Discussion

12:20-13:00 *Side event*

**Presentation of the Guidelines on the Standardisation of mapping and monitoring methods of Mediterranean marine magnoliophyta** by Ms Dr. Christine PERGENT- MARTINI

Discussion

13:00-14:30 *Lunch*

**Table Ronde: Biological invasions and global warming**

Chair: Ms Dr. Christine PERGENT-MARTINI, Rapporteur: Ms Dr. Cristina BUIA

14:30-15:00 Keynote conference: **Biological invasions and sea warming: Is the Mediterranean marine vegetation heading for disaster?** by Charles-François BOUDOURESQUE and Marc VERLAQUE

15:00-16:00 Debate

16:00-16:30 *Coffee break*

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14:30-16:30 *Side event*

Awareness raising workshop on marine vegetation and particularly seagrass meadows, addressed to a group of children from the region, led by Ms Rym ZAKHAMA-SRAIB

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16:30-18:00 **Poster Session**

Election of the Best Poster

Jury : Mr Dr. Panayotis PANAYOTIDIS, Mr Prof. Gérard PERGENT, Ms Dr. Christine PERGENT-MARTINI, Mr Prof. Rachid SEMROUD and Mr Dr. Marc VERLAQUE, Secretaries: Mr Dr. Habib LANGAR and Mr Walid BELGACEM

20:00 *Social dinner*

Prize-giving for the Best Poster

Saturday 4 December 2010

**Session 3: Mediterranean vegetal assemblages: Impact, disturbance and management**

Chair: Mr Dr. Panayotis PANAYOTIDIS, Rapporteur: Mr Dr. Leonardo TUNESI

8:30-8:50 "Evaluation of the contamination of the Mediterranean Sea based on the accumulation of trace-metals by *Posidonia oceanica*" by Maÿlis SALIVAS-DECAUX, BONACORSI M , PERGENT G , Christine PERGENT-MARTINI

8:50-9:10 "An integrated approach to study the effects of chronic water acidification" by Lucia PORZIO, ARENA C , PATTI F P , LORENTI M , Maria Cristina BUIA

9:10-9:30 "Recovery trajectories of temperate reefs benthic communities in the Ligurian Sea under global change scenarios" by Valentina ASNAGHI, CHIANTORE M , THRUSH S , MANGIALAJO L , HEWITT J , ATTANEO-VIETTI R

9:30-9:50 Discussion

9:50-10:20 *Coffee break*

<b>Session 3: (continued)</b>	<b>Mediterranean vegetal assemblages: Impact, disturbance and management</b> Chair: Ms Prof. Luciana MIGLIORE, Rapporteur: Mr Dr. Alain JEUDY DE GRISSAC
10:20-10:40	"Heavy Metal Monitoring in Marine Seaweeds from the Southeastern Mediterranean Sea off the Egyptian Coast, 2006 -2009" by Maha A. M. ABDALLAH
10:40-11:00	" <i>Posidonia oceanica</i> (L.) Delile, a useful tool for the biomonitoring of chemical contamination along the Mediterranean coast: a multiple trace element study" by Jonathan RICHIR, GOBERT S , SARTORETTO S , BIONDO R , BOUQUEGNEAU J M , LUY N
11:00-11:20	"Trace metal pollution increases beta-diversity in <i>Cystoseira</i> spp. epiphytic assemblages <i>Posidonia oceanica</i> (Linnaeus.) Delile meadows identified as SAC (Special Areas of Conservation) of International Importance in the Maltese territorial" by Marta SALES, CEBRIAN E , BALLESTEROS E
11:20-11:40	" <i>Impact des actions anthropiques sur le phytobenthos de la baie de Monastir</i> " by Jeanne ZAOUALI, BEN CHARRADA R
11:40-12:00	Discussion
12:00-13:20	<i>Side events</i>
12:00-12:40	<b>Impacts of climate change on seagrass meadows</b> by Mr Prof. Gérard PERGENT
12:40-13:20	<b>Blue Carbon</b> by a representative of UNEP
13:20-13:50	<b>Closure of the Symposium:</b> <ul style="list-style-type: none"><li>• Conclusions-Recommendations</li><li>• Date and place of the 5<sup>th</sup> Mediterranean Symposium on Marine Vegetation</li><li>• Closure speech</li></ul>
13:50	<i>Lunch</i>





## 4<sup>ème</sup> Symposium Méditerranéen sur la Végétation Marine

Yasmine-Hammamet, 2-4 décembre 2010

Hôtel Alhambra-Thalasso

# PROGRAMME

### Jeudi 2 décembre 2010

- 8h00-9h30** Accueil et inscription des participants
- 9h30-10h30** **Ouverture du symposium:**
- Mot de bienvenue par le Directeur du CAR/ASP
  - Allocution de l'Administratrice Chargée et Coordinatrice Adjointe du PNUE/PAM
  - Discours d'ouverture par Son Excellence Monsieur le Ministre de l'Environnement et du Développement Durable
- 10h30-10h50** **Présentation du programme et des objectifs du symposium** par le CAR/ASP
- 10h50-11h00** **Plan d'action pour la conservation de la végétation marine en mer Méditerranée : bilan de dix ans d'activités** par Mme Souha EL ASMI (Chargée de Programmes au CAR/ASP)
- 11h00-11h30** *Pause café*
- Session 1: Végétation marine de Méditerranée: population, biologie, écologie et dynamique**  
Président: M. Dr. Marc VERLAQUE, Rapporteur: M. Dr. Aslam Sami DJELLOULI
- 11h30-11h50** "*Flora and Vegetation of the Italian Transitional Water Systems: an Italian editorial initiative*" par Ester CECERE, PETROCELLI A., SFRISO A
- 11h50-12h10** "*Nouvelles connaissances sur les herbiers tigrés de Méditerranée*" par Gérard PERGENT, CALVO S., CANCEMI G., DJELLOULI A., DUPUY DE LA GRANDRIVE R., LANGAR H., PERGENT-MARTINI C., TOMASELLO A
- 12h10-12h30** "*Invasion susceptibility of Mediterranean rocky benthic assemblages to red alga Lophocladia lallemandii (Montagne) F. Schmitz*" par Emma CEBRIAN, BALLESTEROS E
- 12h30-12h50** "*Light and temperature requirements for survival and growth of Mediterranean Womersleyella setacea*" par Conxepció RODRÍGUEZ-PRIETO, JOHER S., CEBRIAN E
- 12h50-13h10** Discussion
- 13h10-14h30** *Déjeuner*
- Session 1: Végétation marine de Méditerranée: population, biologie, écologie et dynamique (suite)**  
Président: Mme Dr. Conxepció RODRÍGUEZ-PRIETO, Rapporteur: M. Robert TURK
- 14h30-14h50** "*Biometry and leaf lepidochronology of the seagrass Posidonia oceanica in the Croatian Adriatic*" par Tatjana BAKRAN-PETRICIOLI, Stewart T. SCHULTZ

- 14h50-15h10 "Variations des paramètres biométriques de la bryopsidophycées *Caulerpa racemosa* var. *Cylindracea* en interaction avec la magnoliophyte marine *Posidonia oceanica*" par Yosr MEZGUI, ZERZERI A , DJELLOULI A S
- 15h10-15h30 "*Seasonal pattern of biomass and growth of Cymodocea nodosa in the Ghar El Melh lagoon in response to lagoon condition*" par Yassine-Ramzi SGHAIER, ZAKHAMA-SRAIEB R , CHARFI F
- 15h30-15h50 "Dynamique spatio-temporelle de la faune carcinologique associée à l'herbier de Posidonie dans la région de cap Zebib (Nord Est de la Tunisie)" par Walid BELGACEM, LANGAR H , BEN ISMAIL D , BEN HASSINE O K
- 15h50-16h10 "*Algal-dominated seascapes from the continental shelf off Mallorca and Menorca (Balearic Islands, Western Mediterranean)*" par Sergi JOHER, BALLESTEROS E , CEBRIAN E, SÁNCHEZ N , RODRÍGUEZ-PRIETO C
- 16h10-16h30 Discussion
- 16h30-16h50 *Pause café*
- 16h50-18h15 **Session Poster**
- 18h30-19h30 *Evènement parallèle*  
Exposition de photos d'herbiers marins à travers le monde par M. Mathieu FOULQUIE

## Vendredi 3 décembre 2010

### **Session 2: Cartographie et surveillance de la végétation marine de Méditerranée**

Président: M. Prof. Gérard PERGENT, Rapporteur: M. Dr. Habib LANGAR

- 8h30-8h50 "*Mapping Posidonia oceanica meadows using Quickbird and World View images west of Gulf of Alexandroupolis, Evros and Rodopi Prefecture, Greece*" par Maria Venetia APOSTOLOPOULOU, PARCHARIDIS I , PAVLOPOULOS K , DEHAIRS F
- 8h50-9h10 "La microcartographie par télémétrie acoustique, une nouvelle méthode de suivi des herbiers de posidonie - Application sur 9 sites sur les côtes françaises" par Pierre DESCAMP, HOLON F , BOISSERY P , BALLESTA L , GUILBERT A , GUILLOT M , DETER J
- 9h10-9h30 "Les relations entre les herbiers de posidonies, leur substrat et l'hydrodynamisme" par Philippe CLABAUT, PERGENT-MARTINI C , PERGENT G , AUGRIS C , PASQUALINI V , CHAMLEY H
- 9h30-9h50 "*Multi-sensors data fusion method devoted to sea bottom vegetation mapping and monitoring*" par Claire NOEL, VIALA C , COQUET M , ZERR B , BLOUET S , DUPUY DE LA GRANDRIVE R
- 9h50-10h10 Discussion
- 10h10-10h40 *Pause café*

### **Session 2: Cartographie et surveillance de la végétation marine de Méditerranée**

(suite)

Président: M. Prof. Enrique BALLESTEROS, Rapporteur: Mme Dr. Zohra EL ASMI-DJELLOULI

- 10h40-11h00 "*New descriptors to evaluate conservation of Posidonia oceanica meadows*" par Luciana MIGLIORE
- 11h00-11h20 "*Posidonia oceanica meadows as a priority habitat for the sustainable management of the Greek coastal environment*" par Panayotis PANAYOTIDIS, DRAKOPOULOU P
- 11h20-11h40 "Progression de *Caulerpa racemosa* (Caulerpales, Chlorophyta) dans la région d'Alger" par Halima SERIDI, KABRANE K
- 11h40-12h00 "Suivi de l'expansion de *Caulerpa taxifolia* le long du littoral tunisien: situation au 30 avril 2009" par Habib LANGAR, BELGACEM W , DJELLOULI A S , PERGENT G
- 12h00-12h20 Discussion

12h20-13h00 *Evènement parallèle*

Présentation des Lignes Directrices sur la Standardisation des méthodes de cartographie et de surveillance des magnoliophytes marines en Méditerranée par Mme Dr. Christine PERGENT-MARTINI

Discussion

13h00-14h30 *Déjeuner*

### Table Ronde: Invasions biologiques et réchauffement global

Président: Mme Dr. Christine PERGENT-MARTINI, Rapporteur: Mme Dr. Cristina BUIA

14h30-15h00 Conférence introductive: Invasions biologiques et réchauffement des mers: La végétation marine de Méditerranée va-elle droit au désastre? par Charles-François BOUDOURESQUE et Marc VERLAQUE

15h00-16h00 Débat

16h00-16h30 *Pause café*

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14h30-16h30 *Evènement parallèle*

Atelier de sensibilisation à la végétation marine et en particulier aux herbiers de phanérogames destiné à l'attention d'un groupe d'enfants de la région, animé par Mme Rym ZAKHAMA-SRAIB

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16h30-18h00 **Session Poster**

Election du Meilleur Poster

Jury: M. Dr. Panayotis PANAYOTIDIS, M. Prof. Gérard PERGENT, Mme Dr. Christine PERGENT-MARTINI, M. Prof. Rachid SEMROUD et M. Dr. Marc VERLAQUE, Secrétaires: M. Dr. Habib LANGAR et M. Walid BELGACEM

20h00 *Dîner de gala*

Remise du Prix du Meilleur Poster

## Samedi 4 décembre 2010

### Session 3: Formations végétales de Méditerranée: Impact, nuisances et gestion

Président: M. Dr. Panayotis PANAYOTIDIS, Rapporteur: M. Dr. Leonardo TUNESI

8h30-8h50 "Evaluation of the contamination of the Mediterranean Sea based on the accumulation of trace-metals by *Posidonia oceanica*" par Maylis SALIVAS-DECAUX, BONACORSI M., PERGENT G., Christine PERGENT-MARTINI

8h50-9h10 "An integrated approach to study the effects of chronic water acidification" par Lucia PORZIO, ARENA C., PATTI F. P., LORENTI M., Maria Cristina BUIA

9h10-9h30 "Recovery trajectories of temperate reefs benthic communities in the Ligurian Sea under global change scenarios" par Valentina ASNAGHI, CHIANTORE M., THRUSH S., MANGIALAJO L., HEWITT J., ATTANEO-VIETTI R.

9h30-9h50 Discussion

9h50-10h20 *Pause café*

<b>Session 3:</b> (suite)	<b>Impact et nuisances menaçant les formations végétales de Méditerranée</b> Président: Mme Prof. Luciana MIGLIORE, Rapporteur: M. Dr. Alain JEUDY DE GRISSAC
10h20-10h40	" <i>Heavy Metal Monitoring in Marine Seaweeds from the Southeastern Mediterranean Sea off the Egyptian Coast, 2006 -2009</i> " par Maha A. M. ABDALLAH
10h40-11h00	" <i>Posidonia oceanica (L.) Delile, a useful tool for the biomonitoring of chemical contamination along the Mediterranean coast: a multiple trace element study</i> " par Jonathan RICHIR, GOBERT S , SARTORETTO S , BIONDO R , BOUQUEGNEAU J M , LUY N
11h00-11h20	" <i>Trace metal pollution increases beta-diversity in Cystoseira spp. epiphytic assemblages Posidonia oceanica (Linnaeus.) Delile meadows identified as SAC (Special Areas of Conservation) of International Importance in the Maltese territorial</i> " par Marta SALES, CEBRIAN E , BALLESTEROS E
11h20-11h40	" <i>Impact des actions anthropiques sur le phytobenthos de la baie de Monastir</i> " par Jeanne ZAOUALI, BEN CHARRADA R
11h40-12h00	Discussion
12h00-13h20	<i>Evènements parallèles</i>
12h00-12h40	Impacts des changements climatiques sur les herbiers marins par M. Prof. Gérard PERGENT
12h40-13h20	<i>Blue Carbon</i> par un représentant du PNUE
13h20-13h50	<b>Clôture du symposium:</b> <ul style="list-style-type: none"><li>• Conclusions-Recommandations</li><li>• Date et lieu du 5<sup>ème</sup> Symposium Méditerranéen sur la Végétation Marine</li><li>• Allocution de clôture</li></ul>
13h50	<i>Déjeuner</i>

# **ORAL COMMUNICATIONS**

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# **COMMUNICATIONS ORALES**



**Maha A.M. ABDALLAH**

National Institute of Oceanography and Fisheries (NIOF), Qait Bey, Alexandria, Egypt  
e-mail: [mahaahmed001@yahoo.com](mailto:mahaahmed001@yahoo.com)

## **HEAVY METAL MONITORING IN MARINE SEAWEEDS FROM THE SOUTHEASTERN MEDITERRANEAN SEA OFF THE EGYPTIAN COAST, 2006 -2009**

### **Abstract**

*During the period 2006 - 2009, marine seaweeds samples were collected at different stations of the semicircular bay (Abu Qir Bay) at the Egyptian coast of the Mediterranean Sea in order to establish the concentration of selected heavy metals. Certain seaweed species were chosen among the green (Ulva lactuca, Ulva fasciata, Enteromorpha compressa, Enteromorpha linza), brown (Padina pavonica) and red (Laurencia pinnatifida, Jania rubens, Hypnea musciformis, Gracilaria spp., Pterocladia capillacea, Corralina spp.) algae species. Heavy metals analyzed were Cd, Pb, Cr, Cu, Zn, Co and Mn. The results showed that, Abu Qir Bay coast is facing heavy metal pollution and almost most of metal levels in certain species have gradually decreased during past years. The concentrations of the heavy metals in certain seaweed species are compared with previous studies in other areas. At the same time, Cd, Co, Cr, Cu, Zn, Mn and Pb levels in the present study are lower than in Red Sea seaweeds for some species and Cd, Co, Cr, Pb are higher than that in Black Sea algae. According to these findings the heavy metal pollution slightly decreased in Abu Qir Bay coast of the Mediterranean Sea during the years investigated.*

**Key-words:** Marine seaweeds, Mediterranean Sea, Metals, Egypt

### **Introduction**

Contaminants such as metals are introduced into the Mediterranean Sea through rivers or direct discharge of industrial wastes and agricultural and municipal usage. Moreover, the metal levels in the Egyptian coast of the Mediterranean Sea have increased due to oil pollution, airborne contaminants, discharge of mixed wastes from huge drains and the industrial activities in the hot spots. Heavy metals in aquatic environment can remain in solution or in suspension and precipitate on the bottom or be taken up by organisms. On the other hand, marine seaweeds can be used as monitors to give information on concentrations of heavy metal or changes in metal availabilities in the surrounding environment. Some papers have been published concerning heavy metal levels observed in macroalgae (Abdallah *et al.*, 2005; Abdallah 2007; Güven *et al.*, 1998; Topcuo?lu *et al.*, 2003), at the Mediterranean and Black Sea Coast.

In previous studies, heavy metal concentrations were determined in seaweed samples after collected from Egyptian Coast of the Mediterranean and Red Sea. In that study it was found that the highest metal levels in different seaweeds were: Mn, Zn, Co and Cu (Abdallah & Abdallah, 2007) in the other study in Red Sea the highest metal levels in different seaweeds were: Zn, Pb, Mn and Cu (Abdallah *et al.*, 2005).

The aim of the present study was to find the concentration of Cd, Co, Cr, Ni, Zn, Fe, Mn, Pb and Cu in seaweed species collected from Abu Qir Bay from 2006 to 2009. In

addition, to compare the present results with the similar studies that has been carried out in the region.

### Material and Methods

Samples of seaweeds collected from Abu Qir Bay were *Ulva lactuca*, *Ulva fasciata*, *Enteromorpha compressa*, *Enteromorpha linza* (green algae); *Padina pavonia* (brown algae); *Pterocladia capillacea*, *Laurencia pinnatifida*, *Jania rubens*, *Hypnea musciformis*, *Gracilaria spp.*, *Corralina spp.* (red algae). About 200 g fresh weight were harvested during spring, summer and autumn seasons and chose thalli as similar stage of each species from 2006 to 2009. The samples were washed in seawater at the sampling station and transferred to the laboratory under refrigerated conditions. In laboratory, they were rinsed in seawater to remove sand and other contaminants. Lastly, samples were rinsed in distilled water. They were dried in air then at 85 °C (to constant weight), homogenized and kept away from metallic materials to avoid contamination. One gram from each algal sample was dissolved in conc. HNO<sub>3</sub> (5 ml) and heated on 70–80°C. Small amount of HClO<sub>4</sub> and HNO<sub>3</sub> mixture (1:2) was added very slowly and heated to 120°C. After near dryness, the solution was diluted to 100 ml with 2% HNO<sub>3</sub> in a volumetric flask. The metal concentrations were determined by an Atomic Absorption Spectrophotometer (AAS, Varian Techtron-Model 1250). The concentration values were expressed as mean of three-subsamples, replicates. Blank samples were used inside each batch. The accuracy of the analysis was verified by analyzing the IAEA's certified reference materials

### Results and discussion

The concentration values of heavy metal in algae are summarized in Table 1. The levels of the metals in algae varied widely depending on sampling date, and algae species. The highest accumulation of different metals in the algae species were: Cd, Pb, Cr in *Jania rubens*, Cu and Mn in *Enteromorpha compressa*, Zn and Co in *Ulva lactuca*. In addition high concentrations of Cd and Cr were measured in *Gracilaria spp.* In general, for species that are repeated twice or more over the study period, Cd, Pb, Cu, Zn, Mn and Co concentrations in *Ulva lactuca*, *Enteromorpha compressa*, *Ulva fasciata*, *Jania rubens* were decreased from 2006 to 2009. On the other hand, in case of *Hypnea musciformis*, the levels of Cu, Zn and Co were increased while the levels of Cd, Pb, Cr and Mn have not changed during the study period from 2007 to 2009.

Among the tested algae species, the green species (*U. lactuca* and *Enteromorpha compressa*) were two of the most abundant at the Egyptian marine environment for sampling throughout the year. The concentrations of heavy metals in *U. lactuca* are comparable to those reported for same algae species collected from the Egyptian coast of Red Sea in 2002 (Abdallah *et al.*, 2005). These results are given by the bar diagram in Figure 1. The Pb, Co and Mn in 2006, Cu and Zn in 2007, Cd in 2008, Cr in 2009 were reached peak levels in *Ulva lactuca*. The annual high concentration values in *Enteromorpha compressa* found as follows: Cr and Mn in 2006, Co, Cd, Pb and Cu in 2007, Co in 2008 and Zn in 2009.

It is well known that the heavy metals are introduced via rivers or direct discharge of industrial wastes into the Mediterranean Sea coast. In addition, levels of heavy metals in the Mediterranean Sea are increased by oil pollution and airborne contaminants. Moreover, especially western part of Nile Delta Coast (Abu Qir Bay), has been polluted by industrial wastes from 22 different factories including food processing and canning,



paper industry, fertilizer industry and textile manufacturing (Khairy *et al.*, 2009). On the other hand, the high concentrations of heavy metals in the algae are gradually decreased after 2008. This event may be occurred after preventive measures were enforced by the Egyptian Environmental affairs Agency to prevent discharging industrial wastes without treatment and ballast waters from ships in the study area.

Meanwhile, Co levels in tested all algae species in 2009 were found to be below lower limit detections, except *P. pavonia*. This result showed that the data are limited in space and time and further sampling would be needed to follow the seasonal and annual changes.

Direct Comparison with other published works are fraught with problems, including those associated with sample handling and processing (Gledhill *et al.*, 1998).

**Tab. 1: Heavy metal concentrations ( $\mu\text{g/g}$  dry wt) in algae sample collected from Abu Qir Bay from 2006 – 2009.**

Sample	Cd	Pb	Cr	Cu	Zn	Mn	Co
<b>2006</b>							
<i>U. lactuca</i>	0 78±0 9	6 65±3 5	3 44±1 1	5 87±3 2	57 4±5 1	92 1±21 2	17 3±1 6
<i>U. fasciata</i>	2 42±1 6	3 53±2 7	5 28±2 9	7 18±4 9	50 9±19 0	97 15±5 7	9 13±4 8
<i>E. compressa</i>	1 11±0 8	11 17±2 3	8 92±1 9	7 02±1 1	44 1±10 6	100 7±10	12 9±7 3
<i>L. pinnatifida</i>	1 41±0 8	3 55±2 1	2 18±0 98	2 03±1 1	17 32±3 1	1 22 78±3 2	0 1±0 02
<b>2007</b>							
<i>U. lactuca</i>	0 92±0 6	5 7±1 3	6 51±2 2	8 61±4 5	97 5±45 5	11 38±3 2	14 3±7 5
<i>E. compressa</i>	1 29±0 6	15 9±1 0	4 74±3 1	12 6±4 8	28 4±6 1	19 6±58 6	10 9±1 9
<i>J. rubens</i>	3 39±0 6	24 3±6 3	12 54±2 2	3 81±1 5	2 37±1 3	18 81±3 2	7 91±1 5
<i>H. musciformis</i>	1 07±0 8	4 26±1 7	2 15±3 8	1 77±0 4	11 78±4 3	34 34±5 4	3 4±0 46
<i>Gracilaria spp.</i>	3 96±1 4	18 0±9 4	11 4±4 4	4 71±1 9	5 34±4 0	17 82±1 8	3 41±1 1
<b>2008</b>							
<i>U. lactuca</i>	1 6±0 8	5 68±0 4	3 15±1 3	3 17±0 5	13 93±2 5	19 1±1 6	3 6±1 9
<i>E. compressa</i>	0 96±0 5	6 92±1 9	5 52±4 6	3 16±0 8	58 36±8 7	41 2±16 6	9 56±1 9
<i>J. rubens</i>	1 05±0 3	10 3±5 9	4 01±1 7	2 04±0 8	13 46±1 2	32 32±8 5	2 95±1 5
<i>P. capillacea</i>	1 20±0 1	6 67±1 7	2 55±1 2	3 25±1 0	24 85±3 9	43 14±5 3	7 69±3 8
<b>2009</b>							
<i>U. lactuca</i>	0 18±0 11	4 50±2 3	2 6±1 8	2 0±1 78	10 0±6 2	8 07±5 8	ND
<i>E. compressa</i>	0 76±0 21	5 64±2 6	3 13±0 95	3 38±2 1	21 6±11 9	14 8±5 8	ND
<i>H. musciformis</i>	1 02±0 5	3 84±1 3	2 06±1 1	2 2±0 98	16 85±6 5	32 2±11 8	ND
<i>P. pavonia</i>	0 79±0 3	2 63±1 2	3 46±1 5	2 7±0 20	35 1±10 7	64 4±25 4	5 41±1 3
<i>Corralina spp.</i>	0 23±0 1	15 1±3 8	3 89±1 6	2 3±0 95	12 54±4 9	17 48±6 4	ND

*Ulva* and *Enteromorpha* are two of the most widely used seaweed biomonitors of trace metals in various parts of the world; it is possible to obtain and estimate of the expected range of concentrations within these seaweed species sites varying in metals contamination. In previous studies, the heavy metal levels were investigated in *Ulva lactuca* and *Pterocladia capillacea* collected from the Turkish Coast of the Black Sea during the period of 1998–2000 (Topcuo?lu *et al.*, 2003). Our results show that Cd, Co, Cr and Pb concentrations are higher than the Black Sea algae, but Mn and Zn concentrations are within the same range of the Black Sea algae. In addition, Cd and Pb in *Ulva lactuca* and *Enteromorpha compressa* in our study are higher than that recorded

for the same species in Aegean Sea (Sawidis *et al.*, 2001), on the other hand Cd, Cr, Cu, Pb and Zn are extremely lower than that recorded by Abdallah *et al.* (2005) in Suez Gulf (Red Sea) for *Ulva lactuca*, *Enteromorpha compressa* and *Padina Pavonia*.

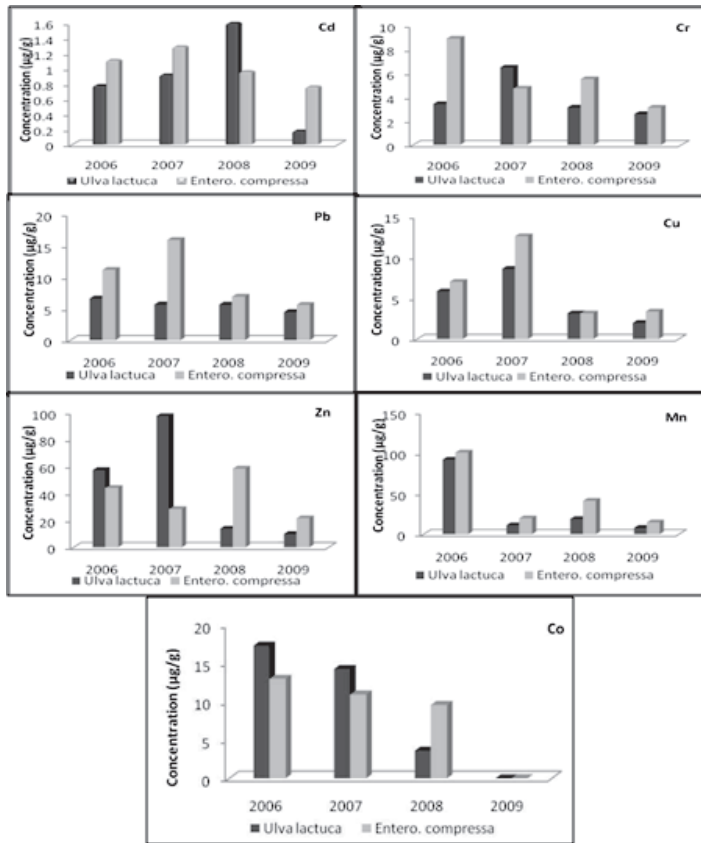


Fig. 1. Mean concentration of heavy metals in *U. lactuca* and *E. compressa* during the period of 2006 - 2009.

### Conclusion

Heavy metals concentrations when measured in macroalgae species can give a picture of the quality of our surrounding environment. Heavy metal levels in algae species are dependent both on environmental parameters (salinity, temperature, pH, light, oxygen, nutrient concentrations) and the structural differences among the algae species (Favero *et al.*, 1996). It is not possible to compare the heavy metal concentrations in macroalgae reported from other marine environment with present results due to wide variations of the environmental parameters and systematic position of the algae and also due to the multiplicity of different types of algae in the environments. A comparison of the present results with data reported for similar algae species from other Mediterranean and Egyptian marine environment, suggest that the heavy metal concentrations are higher in the Egyptian Coast Mediterranean Sea algae than the Turkish Coast of the Black Sea

algae especially Cd, Co, Cr and Pb. On the other hand, the heavy metal concentrations in the Egyptian Coast Red Sea algae are higher than that in the Egyptian coast Mediterranean Sea algae, could be due to the contamination of the Suez area as a result of shipping and industrial activities.

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## **MAPPING *POSIDONIA OCEANICA* MEADOWS USING QUICKBIRD AND WORLDVIEW IMAGES: WESTERN PART OF ALEXANDROUPOLIS GULF (NE GREECE)**

### **Abstract**

*Since March 2007 the construction of an oil pipeline is under deliberation by the governments of Russia, Greece and Bulgaria. The aim of this development is to transport Russian oil through the Black Sea, Bulgaria, Greece and the Aegean Sea to the worldwide market. A possible result of this construction will be annoyance and decline of marine flora. *Posidonia oceanica* is the dominant endemic seagrass in the Mediterranean Sea and its meadows are considered as one of the most important and productive ecosystems in coastal waters. On the other hand, increasing human pressure as a result of industrial (including oil spills) and domestic waste disposal in the coastal ecosystem is a threat to benthic communities. In this research, sensing approaches have been used to map seagrass extent before the construction of the pipeline, using multispectral data from Aster (2007), Quickbird (2007) and WorldView-2 (2010) imagery for bottom classification in a shallow coastal area west of Gulf of Alexandroupolis (Evros and Rodopi Prefecture, Greece). After applying a supervised classification, pixels are automatically classified in four classes: sand, unvegetated area, dense *P.oceanica* and *Cymodocea nodosa* beds. The change detection between different dates is examined. The method is evaluated using ground truth data.*

**Key-words:** *Posidonia oceanica*, Remote Sensing, Classification, Quickbird, Worldview

### **Introduction**

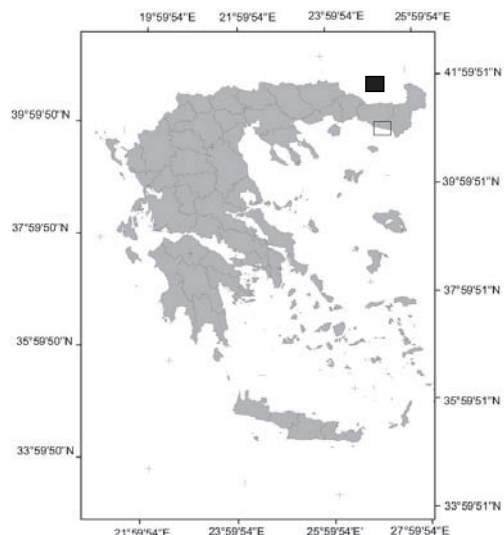
*Posidonia oceanica* is the dominant endemic seagrass in the Mediterranean Sea and its meadows are considered as one of the most important and productive ecosystems in coastal waters, covering the seabed up to depths of about 40m. In addition, these seagrass meadows host a variety of other primary producers (i.e. planktonic, epiphytic and benthic algae), support a rich community of secondary producers and also have the ability to stabilize coastal area, limiting erosion. On the other hand, increasing human pressure via industrial (including oil spills), domestic waste disposal in the coastal ecosystem as well as anthropogenic modification of the coastal zone for constructions, sand winning and aquaculture activities, is a threat to benthic communities. For instance, increased organic matter input from agricultural activities and domestic waste disposal has been identified to be a key factor responsible for the decline of *P. oceanica* in the Mediterranean Sea (Hemminga and Duarte, 2000). The extent of seagrass habitat is a general indicator of coastal ecosystem health. Accordingly, mapping of marine flora is critical (Peneva E. *et al.*, 2008). A variety of techniques have been applied in mapping seagrass distribution and coverage. Seagrass meadows exhibit differences in colour, tone and texture that can be detected via remotely sensed imagery. Aerial photography and remote sensing have been used since long to map seagrass ecosystems. Among them, satellite imagery such Landsat, SPOT, Quickbird, IKONOS (Fornes *et al.*, 2006; Vela *et al.*, 2008) and Worldview are widely used to map seagrasses.

The most common classification techniques are the supervised and unsupervised methods. These methods were proven to be highly effective for mapping dense canopy seagrasses and delineate their presence over hundreds of square kilometers (Holmes *et al.*, 2007). Factors for choosing the appropriate tool depend on the study area, the lower limit of the seagrass communities (maximal depth), the degree of precision required and the cost-effectiveness in relation to time spent (Fornes *et al.*, 2006). Under favourable measurement conditions (i.e., clear water), *P. oceanica* seagrass meadows have been mapped at depths from 0 to 20 m with 73% to 96% accuracy (Pasqualini *et al.*, 2004)

The present paper provides a comparison of different satellite images for mapping *Posidonia oceanica* meadows in the Northeast Aegean Sea (Thracian coastal Sea) in the framework of a wider research dealing with the effects of crude oil derivatives on *P. oceanica* meadows. The incentive for this research was the agreement signed on March 15<sup>th</sup> 2007 between the governments of Russia, Greece and Bulgaria concerning the construction of an oil pipeline from Bourgas (Bulgaria) to Alexandroupolis port. However, in June 2010 it was announced that Bulgaria most likely will not proceed with the project, due environmental and supply concerns. Nevertheless meadows, which are considered habitats with priority for conservation under the *EU Habitats Directive 92/43/CEE of 21/05/1992* (posterior adaptation at *97/62 CE of 27/11/1997*), remain an important issue for the Northeast Aegean Sea area. Especially the present day extent of these meadows and the reasons for their documented decline need to be investigated.

### Materials and methods

The study area was west of the Gulf of Alexandroupolis, the border between the Evros and Rodopi prefectures (Fig. 1). The coastline has a length of more than 50 km. The water depth over the continental shelf of the Alexandroupolis Gulf is less than 35m. Superficial sediments are mostly fine-grained and are zonally distributed, mainly in SE–NW direction, almost parallel to the shoreline (Kanellopoulos *et al.*, 2008). *P. oceanica* are found in Mediterranean in the depth of 0-50m (Pasqualini *et al.*, 2004). However, in the current research Gulf is found to have extended meadows between 5 and 15m depth.



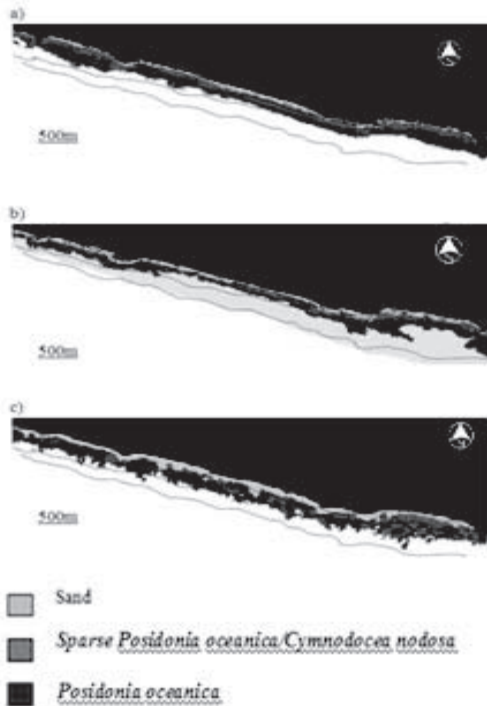
**Fig. 1: Study site of Greece**

Four different satellite images were analysed in the laboratory of the Department of Geography, Harokopio University of Athens. Cloud free images were acquired on September 9, 2007 for Aster image (panchromatic, 15m spatial resolution) and at October 10, 2008 for Ikonos image (multispectral, spatial resolution 2m and spectral resolution in the visible blue 0.45-0.52µm, green 0.52-0.60 µm, red 0.63-0.69 µm and in the near-infrared channel 0.76-0.90 µm) covering the gulf of Alexandroupolis. Quickbird satellites images obtained on November 12, 2007 with spatial resolution

0.6m for panchromatic band (spectral range 0.45–0.90  $\mu\text{m}$ ) as well as multi-spectral bands (spectral ranges: 0.45–0.52, 0.52–0.60, 0.63–0.69, 0.79–0.90  $\mu\text{m}$ ). Worldview -2 images were acquired May 9, 2010 with spatial resolution 0.5m for panchromatic and 2m for multispectral images (spectral range of coastal blue detector 0.40 – 0.45  $\mu\text{m}$ ). Data were geometrically and radiometrically preprocessed (Greek Reference System, GGRS'87) in GeoTIF format and then saved as a 16-bit standard imagery product with digital numbers. A test common area with subsets of  $\sim 23\text{km}^2$  for each image was selected for their comparison. The majority analysis filter (3x3) was applied to the results in order to change spurious pixels within a large single class to that class. To discriminate between those pixels corresponding to the sea versus those that are land, a mask on land was applied. Afterwards, we differentiated between dense and sparse *P. oceanica* meadows, sandy and unvegetated bottoms, using the supervised classification. In this phase the Maximum Likelihood Classification (MLC) technique was adopted. After first classification, the Ikonos image was rejected due to waves effect and corresponding errors. The accuracy assessment process done by equalized random method in ERDAS, with 60 points per class per image. The degree of agreement is presenting with a contingency matrix (Tab. 1).

Subsequently, 25 defined points were imported for the classes; dense, sparse *P. oceanica* meadows and sand which were obtained using a GPS (Global Positioning System Magellan. SporTrak Pro Marine, UTM; WGS 84) on July 2009 (Tab. 2). These data confirmed after diving and enabled the identification of the communities and types of seabed.

Change detection was used to identify significant differences in imagery acquired at different times. There is a wide range of techniques used for land cover/land use change detection. Three different composite image detection options were applied for the images Quickbird and Worldview-2 using the limits  $\pm 25\%$  of values (tab. 3): a) Quickbird image was standardised multiplied by the gain value of original image Worldview ( $\times 0.5$ ). The histogram of Worldview-2 was matched to that of the standardized Quickbird image. Change detection was applied; b) Creation of 2 NDVI Images from the original input data files for each date and overlay; c) The classified



**Fig. 2: Classification: a) Worldview-2 (pixel 2m); b) Quickbird (pixel size 0.6m); c) Aster 0.6397 (pixel 15m)**

Quickbird image was reclassified into four classes (adding the Unvegetated class to that of Deep water) in order to have same number of categories to the classified Comparison of the two images was followed and given through matrix (Tab. 4).

### Results

For the Worldview-2, Quickbird and Aster images the equalized random method yielded overall accuracies between 68 percent and 87 percent (Tab. 1). The highest overall accuracy of the main benthic assemblages and bottom types in Northeast Aegean Sea observed in the Quickbird test-area image (86,67%). Worldview-2 image was comparable to that of the Aster image classification (68.33% and 75%, respectively) and these were both higher than IKONOS (35%). Accuracies for classes of interest (e.g., dense *P. oceanica*, sparse *P. oceanica*/*Cymnodocea nodosa* and sand) could be mapped in most cases with an accuracy exceeding 80 percent, excepting the Ikonos image. Apart from Quickbird and Ikonos images, unvegetated bottom area next to *P. oceanica* meadows couldn't be identified. However, in this study the Ikonos image is not considered due to low accuracy and error in k-statistics.

**Tab. 1: Accuracy of mapping habitats done by equalized random method in ERDAS (%)**

<b>WORLDVIEW-2</b>	Sand	Sparse <i>P.oceanica</i> / <i>Cymn.nodosa</i>	<i>P.oceanica</i>	Deep water	Unvegetated bottom area	Overall accuracy
Sand	100	0	0	6		
Sparse <i>P.oceanica</i> / <i>Cymn. nodosa</i>	0	80	0	12		
<i>P.oceanica</i>	0	13	82	0		
Deep water	0	7	7	65		68 33
K statistics	0 5957					
<b>Quickbird</b>						
Sand	100	0	0	8	0	
Sparse <i>P.oceanica</i> / <i>Cymn. nodosa</i>	0	100	0	0	15	
<i>P.ceanica</i>	0	7	90	8	7	
Deep water	0	0	10	73	0	
Unvegetated bottom area	0	0	0	0	80	86 67
K statistics	0 8347					
<b>ASTER</b>						
Sand	100	0	0	7		
Sparse <i>P.oceanica</i> / <i>Cymn. nodosa</i>	0	91	18	0		
<i>P.oceanica</i>	0	9	71	7		
Deep water	0	0	12	73		75
K statistics	0 6791					
<b>IKONOS</b>						
Sand	100	0	20	9	17	
Sparse <i>P.ceanica</i> / <i>Cymn. nodosa</i>	0	67	10	0	17	
<i>P.oceanica</i>	0	33	50	9	17	
Deep water	0	0	10	64	17	
Unvegetated bottom area	0	0	10	18	33	35
K statistics	0 2896					

Inspection of Table 2 reveals that the mean overall accuracy using point based field data is lower. However, *P. oceanica* meadows were mapped very accurately with the Worldview-2 (100% user accuracy), whereas Sparse *Posidonia oceanica* / *Cymnodocea nodosa* meadows had low accuracy with the Quickbird and Aster test-area image. A classified image is considered to be accurate and close to ground data, when the value of overall accuracy is high and the Kappa Coefficient above 0.5 (Fornes *et al.*, 2006).



Consequently, the classifications of both Worldview-2 and Quickbird multispectral images are considered to be accurate and adequate to continue for the change detection process. The different change detection approaches yielded quite different results. However, as is shown in Table 3, the area measured in hectares which has changed or didn't change is similar between the two dates for the original and classified images. The vegetation produced images demonstrate a composite image in which all hectares seem to be changed. The summary matrix (Tab. 4) of the change detection between classified Quickbird (2007) vs Worldview-2 (2010) (% of zone) demonstrate that sand is changed especially to the area of deep water which was not easy to classify.

High percent of sand change detection in the other categories between the two images may not reflect the actual situation. Posidonia oceanica and Sparse P.oceanica/C. nodosa seem not to be widely changed with the exception the parts that are mixed with sand.

**Tab. 2 : Accuracy check statistics**

	Producers Accuracy	Users Accuracy	Overall Accuracy
<b>Worldview-2</b>			
Sand	37 50%	100 00%	
Sparse <i>P.oceanica</i> / <i>C. nodosa</i>	85 71%	85 71%	
<i>P.oceanica</i>	90 00%	100 00%	<b>72.00 %</b>
Overall Ks	0 6212		
<b>Quickbird</b>			
Sand	92 31%	92 31%	
Sparse <i>P.oceanica</i> / <i>C. nodosa</i>	75 00%	60 00%	
<i>P.oceanica</i>	62 50%	83 33%	<b>75.00%</b>
Overall ks	0 5338		
<b>Aster</b>			
Sand	100 00%	58 33%	
Sparse <i>P.oceanica</i> / <i>Cymn. nodosa</i>	37 50%	75 00%	
<i>P.oceanica</i>	70 00%	87 50%	<b>68.00%</b>
Overall ks	0 6397		

**Tab. 3: Change detection Quickbird vs Worldview-2 in hectares**

	Original images	NDVI images	Classified images
Decreased	1127	1530	879
Some Decrease	0	0	0
Unchanged	402	0	379
Some Increase	949	857	255
Increased	95	187	1060
<b>Total change</b>	<b>2.171</b>	<b>2.574</b>	<b>2 191</b>
<b>Unchanged</b>	<b>402</b>	<b>0</b>	<b>379</b>

**Tab. 4: The summary matrix between classified Quickbird vs Worldview-2 (% of zone)**

	Unclassified	Sand	Sparse <i>P.oceanica</i> / <i>Cymn.nodosa</i>	<i>P.oceanica</i>	Deep water
Unclassified	0,00	0,00	0,00	0,00	0,00
Sand	0,63	0,06	0,58	0,23	0,96
Sparse <i>P.oceanica</i> / <i>Cymn.nodosa</i>	0,00	0,01	0,30	0,04	0,01
<i>P.oceanica</i>	0,01	0,09	0,09	0,30	0,01
Deep water	0,36	0,84	0,03	0,43	0,01

### Discussion and conclusions

In general remote sensing data have been proven to be useful for monitoring and mapping submerged vegetation.

Besides the fact that satellite data for mapping seagrasses, present limitations due to a relatively coarse spatial resolutions (Peneva *et al.*, 2008), small seagrass patches at increased depths are more difficult to be identified, because the proportion of reflectance reaching the remote sensing device that could be attributed to the seagrass



canopy, will diminish with depth (Ferwerda J. *et al.*, 2007). Thus, in most images the situation at depths exceeding 20m couldn't be evaluated with exemption of the Quickbird image in which we were able to map the unvegetated area up to 25m. Even if the spatial structure of *P. oceanica* can be masked better by larger pixel size images (Pasqualini *et al.*, 2004,) classification results for Aster image classification (15m) presented lower overall accuracy relative to ground truth. This paper compares common methods of change detection based on multi-temporal, NDVI and classified images, using ERDAS software, and analyzing their results referring to the area of change. Investigating the change detection of the original images (first case) the sun elevation/sun azimuth is crucial and must be considered. However, in this study due to lack of digital elevation model (DEM) of the area, sun elevation/sun azimuth correction of the images could not be applied. Normalized Difference Vegetation Index (NDVI), which is developed for estimating vegetation cover from the reflective bands of satellite data, is the appropriate way to measure changes over time. On the other hand, factors such as time of the year when images were captured, ratios of vegetation density/diversity, and healthy-green/not healthy yellowish seagrasses need to be assessed. Thus, the method of change detection after classification was the simplest approach.

In this work, the Quickbird and Worldview images were identified as more appropriate than Ikonos and Aster images for a successful identification of shallow marine environments. Even if the overall accuracies obtained with the Quickbird and Worldview images for benthic categories and bottom types were satisfactory, still more work has to be done using alternative techniques, such as acoustic sampling. Finally, the use of remote sensing appears valuable for the preservation and management of the coastal area of the Thracian Sea.

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## **RECOVERY TRAJECTORIES OF TEMPERATE REEF BENTHIC COMMUNITIES IN THE LIGURIAN SEA UNDER GLOBAL CHANGE SCENARIOS**

### **Abstract**

*Global changes, such as climate changes (increase of temperature and atmospheric CO<sub>2</sub>, variations in the carbon cycle, more frequent extreme climatic events) and human impacts acting at a local scale but having consequences at a global level (coastal urbanization, overfishing), may result in severe phenomena causing troubles to marine ecosystem. Rocky reefs, sitting at the interface between land and sea, are affected by both terrestrial and marine impacts and threatened by multiple stressors. Temperate reef communities are very sensitive to human-induced changes and their structure and dynamics should be studied in order to predict the effect of global and local scale human-induced phenomena. In particular, macroalgae belonging to the order Fucales and Corallinales play a major role in the dynamics of temperate reefs in the presence of multiple impacts. Disturbance-recovery experiments have been proposed as an assay of resilience in ecological systems. In this study we experimentally disturbed 1m<sup>2</sup> plots at 5 sites (5 replicate plots at each site) along the Ligurian coast (North-western Mediterranean). We assessed recovery within our plots over about one year to identify variations in priority effects and growth of dominant taxa. Different recovery trajectories were observed linked to the colonization and growth of furoid and coralline algae. These taxa are already considered to represent alternative stable states, and seem to be differently affected by human pressure. Consequently, the different recovery of communities alternatively dominated by the two taxa provides evidence of cumulating global and local human induced impacts: the shift from Fucales to Corallinales dominated communities, with the loss of canopy forming species, may lead to a general decrease in habitat complexity and biodiversity.*

**Key-words:** global changes, multiple impacts, resilience, coastal ecosystem, macroalgae

### **Introduction**

Human induced global changes, such as climate change (*e.g.* the rising of greenhouse gas concentrations and atmospheric CO<sub>2</sub>), coastal urbanization and overfishing continue to increase with growing concern about their potential consequences on marine ecosystem extending from local to global effects (Solomon *et al.*, 2007). In this scenario of changing oceans, threatened by the increase of temperature, atmospheric and ocean CO<sub>2</sub>, variations in the carbon cycle, rise in sea-water level and increased frequency of extreme climatic events (IPPC, 2001), it is important to understand the consequences of these environmental changes on biodiversity loss and change in coastal ecosystem functioning (Kleypas *et al.*, 2006).

Coastal ecosystems are highly threatened, affected by human impacts coming both from marine and terrestrial activities (Halpern *et al.*, 2008); temperate reef communities are very sensitive to human-induced changes and accordingly they are used for ecological status assessment under the European Water Framework Directive 2000/60/EC (see Asnaghi *et al.*, 2009 and Mangialajo *et al.*, 2007 for an application along the Ligurian coast). Macroalgal communities are dominant along the North-western Mediterranean

and species belonging to the orders Fucales and Corallinales represent the most common and abundant algal groups. Communities dominated alternatively by the two taxa are known as alternative stable states, because of their different sensitivity to human pressure, particularly overfishing (which may cause a shift to communities dominated by encrusting corallinales, see Sala *et al.*, 1998) and coastal urbanization (which may cause a regression of Fucales macroalgae in favour of coralline turf, see Mangialajo *et al.*, 2008).

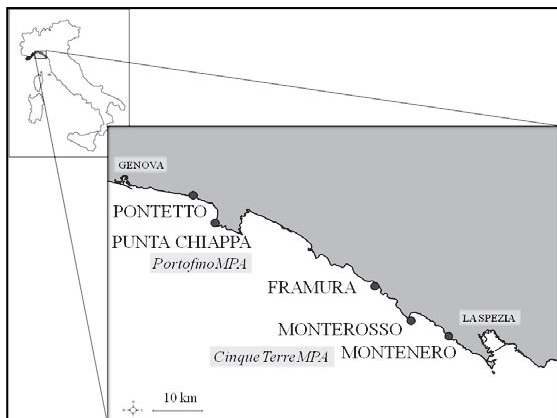
In the present study, we assessed temperate reef communities resilience after a generic perturbation (in relation to the foreseen future scenarios of global change), by setting up a disturbance-recovery experiment along the eastern Ligurian rocky coast (North-Western Mediterranean). Five sampling sites, differing in terms of environmental variables (*e.g.* wave exposure, rock type) and MPA protection status, were chosen and regularly sampled over about one year. The different sites show different recovery trajectories, mainly driven by macroalgae of the Fucales and Corallinales orders.

### Material and methods

Sampling sites are distributed along the eastern side of the Ligurian coast (NW- Mediterranean; Fig.1): Pontetto (PON, near Genoa), Punta Chiappa (POR, in the C zone of Portofino MPA), Framura (FRA), Monterosso (MES) and Montenero (MON), both in the A zone of the Cinque Terre MPA.

In each site, the biotic component in 5 plots (1m X 1m), located in the upper infralittoral rocky shore, was mechanically removed with both high pressure water and sand blasting (160 bar).

Within each plot, in order to evaluate the percent cover of the species we sampled 5 visual quadrats (20cm X 20cm) located in the middle of the plot (to reduce edge effects), following the method by Dethier *et al.* (1993). Sampling has been repeated over one year (t1: July 2009; t2: September 2009; t3: December 2009; t4: April 2010), in order to describe the recovery dynamics of the resident community. We considered pre-impact conditions to be represented by previous surveys carried out in spring-summer 2008. The data were analyzed in order to highlight recovery trajectories and possible interactions between taxa that typically characterize superficial macroalgal communities.



**Fig. 1: Sampling sites along the eastern Ligurian coast**

## Results

Data from the disturbance-recovery experiment were analyzed both in terms of the number of taxa and percent cover of the different taxa.

In terms of the number of taxa, some sites, *e.g.*, Pontetto (PON) and Monterosso (MES), after 1 year (time 4, April 2010) seem to be close to a complete recovery, while others are still far from it. The patterns highlighted by the species accumulation curves displayed in Figure 2 do not show any positive effect of the protection regime in speeding up the recovery (*e.g.* POR and MON seem very far from reaching the complete recovery).

The first sampling time after the disturbance is characterized by presence of very few taxa with all the sites looking homogeneous, and dominated by filamentous algae. The following sampling times show an increasing complexity in community structure, although the different sites behave differently (in Table 1 the average values of percent coverage of dominant taxa are reported). The MDS analysis on taxa percent cover displays very different recovery trajectories for the different sites (Fig. 3) with the PON and FRA sites most different from the pre-disturbance condition. Preliminary analyses suggest that the different trajectories are mainly driven by Fucales or, alternatively, Corallinales algae. Both these taxa are relatively large with architectures that need more time to reach a complete recovery in terms of percent cover. This, in turns, affects the rate at which these taxa can influence recovery dynamics in the experimental plots.

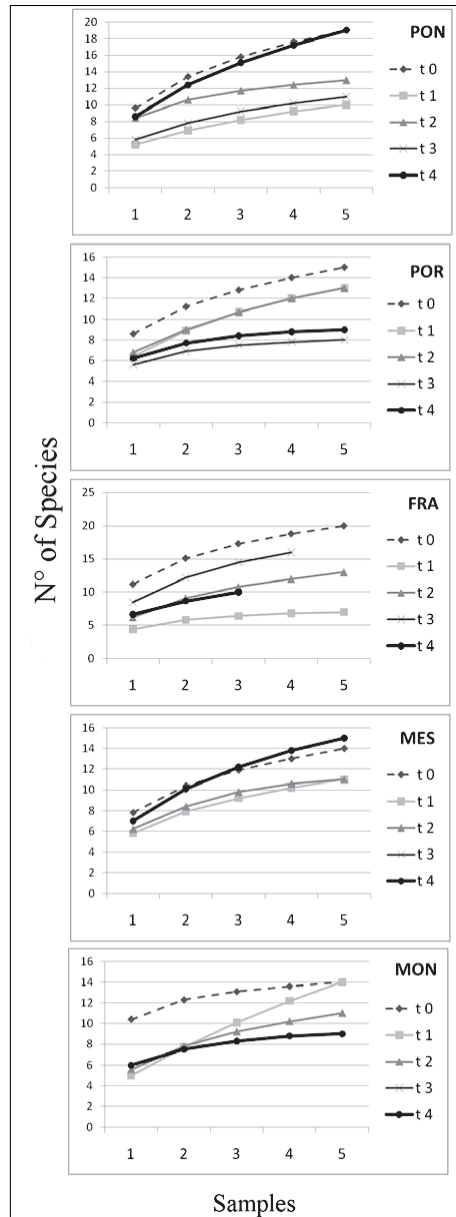
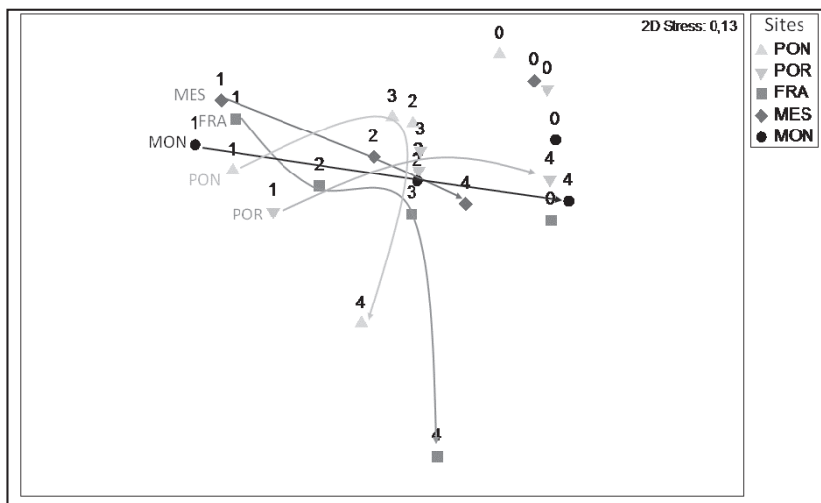


Fig. 2: Species accumulation curves for each sampling time and site (each line correspond to a sampling time; t0: broken line ; t4: thick black line)

**Tab. 1: Average values of % cover of dominant taxa in the five sampling sites, in the different sampling times**

Sites	Time	<i>Cystoseira compressa</i>	<i>Corallina elongata</i>	<i>Jania rubens</i>	Encrusting Corallinales	Turf*	Filamentous algae	<i>Laurencia complex</i>
PON	0	1,2	28	37,8	4,6	14,2	0,6	4,6
	1	0	0	3,4	24,8	0	64,8	0
	2	2,2	0,4	32	26,6	7,6	7,6	11
	3	0,2	2,8	45,2	34,2	0	0,6	8,8
	4	43,6	3,4	1,6	24,2	0	2,2	0,8
POR	0	0,4	23,8	26,6	1,6	24,4	2	8,2
	1	0	1	1	42,4	0	33,8	4
	2	0	3,8	16,6	27,2	2	1,2	30,4
	3	0	5,2	36	19,2	0	2	30,8
	4	0	6,4	0	6,8	52	3,4	11,8
FRA	0	5,4	2,6	5,8	5,8	49,8	2,4	5,4
	1	0	0	4,6	15,4	0	53,8	0
	2	0	3,4	2,2	43,4	0	17,4	12,4
	3	0,5	1,75	5,25	22,75	5	16,5	35,75
	4	37,33	2,67	0	6	0	0	0,33
MES	0	0	38,2	13,8	3,6	36,2	2,4	2,6
	1	0	9,2	0,6	6,2	0	78,2	0,6
	2	0	3,2	20,8	25,2	0	16,6	28,8
	4	0,4	12,6	0	11,6	21,8	6,2	38
	0	3,4	28	11,6	6,6	47,6	1	5
MON	1	0	1,4	3,8	10	0	80,6	0,2
	2	2,6	3,2	22,6	26,4	0	2	36
	4	1,2	5	0	3,8	47,4	0,2	36,8

\*Turf: generally made up by small Corallinales, Ceramiales, Sphacelariales and other filamentous algae



**Fig. 3: MDS analysis on percent cover data, displaying recovery trajectories (from time 1 to time 4). Numbers refer to sampling times (see MM section). POR, MON and MES show almost linear trajectories towards the pristine condition (time 0), although MES is still quite far from it. PON and FRA, instead, show a trajectory pointing really far from time 0 condition.**

## Discussion and conclusions

Along the Ligurian rocky shores, canopy forming species, belonging to the order Fucales (e.g. *Cystoseira* spp.), characterize stretches of coast least affected by coastal urbanization and water pollution. The most sensitive species, *Cystoseira amentacea* var. *stricta*, which is dominant along western part of the region and in the south-side of the Portofino promontory, is historically absent in the eastern part of the region, probably due to basin dynamics (Asnaghi *et al.*, 2009), where, instead, the more tolerant *C. compressa* is the dominant furoid species. Species belonging to the order Corallinales (e.g. *Corallina* spp.) tend to dominate in more impacted areas (Mangialajo *et al.*, 2008). Accordingly, the relative abundance of these two taxa (Fucales and Corallinales) is used in the ecological status assessment under the European Water Framework Directive (Asnaghi *et al.*, 2009; Mangialajo *et al.*, 2007).

Our results show, independently of the community structure previous to the clearing, a spatially homogeneous response of macroalgal taxa to disturbance in the early stages of recovery (time 1), when only the less complex filamentous algae have colonized the bare substrates. Subsequently, at sampling time 2 (nearly 3 month after disturbance) and 3 (nearly 6 month after disturbance) coralline algae, in particular encrusting Corallinales and *Jania* sp., together with the *Laurencia* complex (Ceramiales order) become dominant at all the sites. At Portofino (time 0), *Corallina* sp., *Jania* sp. and turf were all more or less equally dominant, while on the last sampling time, nearly one year after the experimental disturbance, we found mainly turf, suggesting that filamentous algae cover the corallines, forming an complex matrix. Note that in our samples we defined algal turf as a complex matrix of macroalgae, mainly composed by Corallinales species (e.g. *Corallina* sp., *Jania* sp.), Ceramiales (e.g. *Laurencia* sp., *Chondria* sp.) and other filamentous algae (Cladophorales, Sphacelariales, etc.). At Monterosso and Montenero similar trends were observed: at time 0 *Corallina* sp. and algal turf were dominant, while one year after the disturbance (time 4) we recorded the presence of *Laurencia* sp., which together with algal turf dominated the community. This trend suggests that *Corallina* sp., which has a complex structure, needs more time to recover, leaving space for these fleshy algae to establish.

Pontetto and Framura are the two sites that show the most drastic change in the recovery trajectory, being at time 4 quite still far from the community composition observed at time 0. In these sites, plots reached a higher complexity compared to the undisturbed condition, showing a high percent cover of canopy-forming species (*Cystoseira compressa*) compared to Corallinales (*Corallina* sp. and *Jania* sp.) in Pontetto, and turf forming species in Framura. For both sites this implies that the removal of the present community led to the establishment of a *Cystoseira* dominated community, although present with the most tolerant species (*C. compressa*), whose presence seems to be precluded by competition with coralline and filamentous turfs.

Recovery dynamics of coastal community after a disturbance event are controlled by a combination of physical and ecological features, operating at different spatial scales (Thrush *et al.*, 2008) and we expected that different sites could recover in different ways, both in relation to large-scale variability in anthropogenic pressure and to local scale factors. Recovery dynamics in our sampling sites are generally similar and lead to the reestablishment of the previous community, probably mirroring that stressors there have not changed in the last decade. In Pontetto and Framura, instead, a change in the trajectory is shown: the resident community is mostly auto maintained by competitive mechanisms that, if removed (such as in the plots), could lead to a different community.

We then may point out that something has changed there in terms of ecological status comparing the present day to the past, or maybe that competitive interactions get stronger later on in the succession and maybe that in one more year we go back to the time 0 condition.

### Acknowledgments

We thank Danilo Pecorino and Eleonora Costa for their help in the field. We also thank the managers of the Portofino and Cinque Terre Marine Protected Areas for permission to work in their reserves, and the MPAs staff (particularly Claudio Valerani - Cinque Terre and Valentina Cappanera - Portofino) for the boat support. This research was supported by a Marie Curie International Incoming Fellowship to SFT (FP7-PEOPLE-2007-4-2-IIF-ENV221065).

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## **BIOMETRY AND LEAF LEPIDOCRONOLOGY OF THE SEAGRASS *POSIDONIA OCEANICA* IN THE CROATIAN ADRIATIC**

### **Abstract**

*The endemic Mediterranean marine phanerogam Posidonia oceanica (L.) Delile forms dense beds characteristic of the photic infralittoral zone. As elsewhere in the Mediterranean, they are considered to be the most important shallow benthic habitat in the Adriatic Sea. This complex habitat/biocoenosis, with high primary production and high biodiversity, is important for the oxygenation of the seawater and stabilization of the sediment. However, in the Croatian part of the Adriatic the biocoenosis is poorly known. The aim of this research was to compare biometry and leaf lepidochronology of seven Posidonia meadows in the Middle and South Adriatic studied from 2002 to 2006 (on Islands Dugi otok, Krapanj, Vis and in the Lastovo Archipelago). In well-developed meadows, 20-50 Posidonia shoots per meadow were taken along the depth gradient. The upper and the lower bathymetric extension of the meadows and the number of shoots along the depth/light gradient (in 10 quadrats 40 x 40 cm per each depth) were determined in situ. Variables measured included shoot density, leaf area index, biomass, and estimated annual primary production. Meadow density and the effect of depth on estimated primary production were compared among the researched meadows as well as with the published data for Posidonia beds in the Mediterranean. Generally, meadows under the influence of open Adriatic clear waters showed the highest primary production: in Lojisce cove (Dugi otok) and Rukavac cove (Vis). Estimated primary production in our studied Posidonia meadows was similar to the estimated primary production of other meadows in the Mediterranean Sea. However, meadows in enclosed bays (Cuska Dumboka, Sascica) showed remarkably lower primary production, probably due to natural eutrophication and lower water clarity.*

**Key-words:** Adriatic Sea, *Posidonia oceanica*, lepidochronology, leaf biometry

### **Introduction**

Meadows of the marine phanerogam *Posidonia oceanica* (L.) Delile are considered to be one of the most important habitats in the Adriatic Sea because of their complexity, high primary production, and biodiversity. They also oxygenate seawater and stabilize the sediment. Since they are still poorly known in the Croatian part of the Adriatic, we undertook a series of studies from 2002 to 2006 in order to characterise seven *Posidonia* meadows in the Middle and South Adriatic using the standard and widely accepted descriptors of shoot density, meadow bathymetric extension, leaf biometry, and estimated primary production (Pergent-Martini *et al.*, 2005).

### **Materials and methods**

In well-developed meadows on Islands Dugi otok, Krapanj, Vis and in Lastovo Archipelago (Fig. 1), 20-50 *Posidonia* shoots per meadow were taken along the depth gradient. The upper and the lower bathymetric extension of the meadows and the number of shoots along the depth/light gradient (in 10 quadrats 40 x 40 cm per each



depth) were determined *in situ*. Biometry and lepidochronology were performed in the laboratory following the methods of Pergent-Martini & Pergent (1994). Leaf area index, biomass, and estimated annual primary production were calculated.



**Fig. 1:** Position of the studied *Posidonia* beds; 1 – three on Dugi otok Island: in Sascica, Cuska Dumboka and Lojisce coves; 2 – two around Krapanj Island (one along SW side of the Island and the other on the N side of Krapanj, near Brodarica); 3 - one in Rukavac cove, Vis Island; 4 – one on the SE side of Saprun Island, Lastovo Archipelago.

## Results

The results are shown in the Tables 1 and 2.

**Tab. 1:** Basic descriptors noted *in situ* on seven *Posidonia* meadows in the Middle and South Adriatic: meadow bathymetric extension and shoot density per depth.

Station	Bathymetric extension	Depth of sampling	Shoot density/m <sup>2</sup>
Sascica (Dugi otok)	7-25 m	7 m	274±52
		20 m	122±29
Cuska Dumboka (Dugi otok)	2-25 m	6 m	315±81
		19 m	155±94
		24 m	107±88
Lojisce (Dugi otok)	1-32 m	3 m	882±106
		5 m	426±48
		15 m	313±37
		20 m	165±18
		32 m	125±25
Krapanj	1-4 m	1 m	1282±28
Brodarica	2-4 m	2m	390±45
Rukavac (Vis)	5-34 m	5 m	385±19
		15 m	252±27
		20 m	163±14
		32 m	55±5
Saprun Island (Lastovo archipelago)	1-36 m	5 m	462±72
		15 m	264±13
		20 m	211±16
		32 m	37±5

**Tab. 2: Descriptors of seven *Posidonia* meadows in the Middle and South Adriatic: leaf area index and estimated primary production, calculated on the basis of leaf biometry and lepidochronology combined with meadow density (Tab. 1).**

Station	Depth of sampling	Leaf area index, LAI, m <sup>2</sup> /m <sup>2</sup> leaf area per sea bottom area	Estimated average annual leaf primary production	
			g of dry weight per shoot	g of dry weight per m <sup>2</sup> of bottom area
Sascica (Dugi otok)	7 m	17.7	0,58 - 0,84	156 - 231
	20 m	6.2	0,42 - 0,68	51 - 83
Cuska Dumboka (Dugi otok)	6 m	18.2	0,44 - 0,58	138 - 184
	19 m	13.6	0,72 - 0,95	112 - 147
Lojisce (Dugi otok)	5 m	58.2	1,34 - 1,42	506 - 607
	15 m	50.7	1,57 - 1,83	491 - 573
	20 m	24.9	2,13 - 2,24	342 - 369
	32 m	20.1	1,89 - 2,16	237 - 270
Krapanj	1 m	77.9	1,75 - 1,95	2244 - 2500
Brodarica	2 m	20.2	1,02 - 1,28	398 - 500
Rukavac (Vis)	5 m	22.2	1,72 - 2,49	663 - 958
	15 m	16.1	0,77 - 2,70	195 - 681
	20 m	6.9	0,78 - 1,55	127 - 253
	32 m	1.9	0,44 - 1,19	24 - 62
Saplun Island (Lastovo archipelago)	5 m	17.0	0,47 - 0,62	216 - 320
	15 m	15.7	0,73 - 0,91	192 - 240
	20 m	21.5	0,52 - 0,91	110 - 192
	32 m	3.6	0,59 - 0,98	22 - 36

### Discussion and conclusions

Due to the light gradient in each of the meadows studied, considerable differences along the depth were noted in shoot density, leaf area index and estimated annual primary production. Estimated primary production in the studied *Posidonia* meadows is similar to the estimated primary production of other meadows in the Mediterranean Sea (e.g. Guidetti, 2000; Pergent-Martini & Pergent, 1994;). Generally, our study meadows under the influence of open Adriatic clear waters showed the highest primary production: in Lojisce cove (Dugi otok) and Rukavac cove (Vis). However, meadows in enclosed bays (Cuska Dumboka, Sascica) showed remarkably lower primary production, probably due to natural eutrophication and lower water clarity.

Our results indicate the importance of maintaining high water quality in the ecological health of *Posidonia* beds in the eastern Adriatic, and the need for regular monitoring of water quality. Informed management requires comprehensive and regular surveys of ecological condition of *Posidonia* throughout the eastern Adriatic Sea.

### Acknowledgments

TBP would like to thank her students: Iva Kovacic, Julijana Ivanisevic, Damir Rubcic, Buga Berkovic, Andrea Obarcanin and Jasna Vuljan for technical assistance during field work and for the laboratory work. The field-work was partly supported by BIUS – the Biology Students Association, Zagreb.

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## **SPATIO-TEMPORAL DISTRIBUTION OF CRUSTACEANS FAUNA ASSOCIATED WITH *POSIDONIA* BED IN CAP ZEBIB (NORTH-EASTERN TUNISIA)**

### **Abstract**

*Crustacean communities associated with Posidonia oceanica bed were investigated in Cap Zebib (North-Eastern Tunisia). Samples were monthly collected (from May 2007 to May 2008) in two stations located at 3 (S1) and 12 m depths (S2). The shoot density and Leaf Area Index varied with depth and were correlated with temperature and dissolved Oxygen. A total of 53 species represented by 3227 individuals were collected. The species richness was found to be generally higher at the shallow station. Shannon-Wiener and Pielou indices varied with depth and seasons. Multivariate analyses showed a pronounced temporal variation of collected populations' structure. Moreover, it was shown by ANOSIM that the population structure is influenced by "depth" and "time" factors ( $r=0.368$ ;  $p<0.01$  and  $r=0.292$ ;  $p<0.01$  respectively). Otherwise, the influence of "season" factor on the composition of crustacean assemblage was found to be significant for each of the two stations (ANOSIM:  $r=0.732$ ;  $p<0.01$  for S1;  $r = 0.579$ ;  $p<0.01$  for S2). Species richness was correlated with Leaf Area Index ( $r=-0.482$ ;  $p<0.05$ ), dissolved oxygen ( $r=0.447$ ;  $p<0.05$ ) and temperature ( $r=-0.638$ ;  $p<0.01$ ). As for  $H'$ , this index was found to be correlated with depth ( $r=-0.644$ ;  $p<0.01$ ), shoot density ( $r=0.644$ ;  $p<0.01$ ) and dissolved oxygen ( $r=0.427$ ;  $p<0.05$ ).*

**Key-words:** *Posidonia oceanica*; Plant features; Crustaceans; Tunisia.

### **Introduction**

The seagrass meadows formed by the endemic species *Posidonia oceanica* (L.) Delile are widely distributed along the Mediterranean Sea (Procaccini *et al.*, 2003) and exert a very important ecological role in the coastal system. The plant canopy provides a hydrodynamic buffering of water movements and enhances bottom stability and sedimentation of fine material (Gambi *et al.*, 1989).

Along the Tunisian coastline, few studies were done on crustaceans associated to *Posidonia oceanica* meadows, and most of them were restricted to a particular taxon e.g. amphipods (Zakhama-Sraeib *et al.*, 2006). The latter study was carried out in the Eastern and Southern coasts of Tunisia, but no studies were done about the crustacean fauna of *Posidonia oceanica* along the Northern Tunisian coastline. In the present study, we focused on the characterization of crustacean fauna associated to a *Posidonia oceanica* meadow in Cap Zebib, a northern Tunisian locality. The aims of this study are i) to investigate the composition of crustacean populations inhabiting the leaf (canopy) and rhizome layers of the *Posidonia oceanica* bed in this locality, ii) to test its depth and temporal variability, iii) to check whether some relationships exist between the faunal biodiversity and the meadow features, in particular shoot density and Leaf Area Index,

and iv) to check the influence of environmental factors on population's composition and plant features variability.

### Materials and methods

Cap Zebib is located in the north-east of Tunisia and hosts a developed *Posidonia oceanica* meadow covering large surfaces and a depth up to 30m (Ben Mustapha & Hattour, 1992).

In order to study the spatial variability of *P. oceanica* features and crustacean communities, two stations located at different depths, were sampled: **S1** (37°15'38,97''N; 10°04'28,78''E) at 3m depth corresponding to the upper limit of *Posidonia* meadow and **S2** (37°15'45,92''N; 10°04'47,31''E) at 12m depth representing a homogeneous meadow structure.

At each station, abiotic factors, *Posidonia* shoots and fauna samples were monthly estimated and collected by SCUBA diving. Samples were done from May 2007 to May 2008. Meadow density was estimated using a 35x35 cm quadrat. A total of twenty-one shoot density measurements were performed within each station; the density was expressed in number of shoots m<sup>2</sup> (Pergent & Pergent-Martini, 1991).

Samples of fauna were performed using a 25cm diameter cylindrical core, according to the method described by Borg (2002). Three corer replicates were monthly collected within each station. For each sample (replicate), abundance N, number of species S, Shannon-Wiener's diversity (H'; log<sub>2</sub>) and Pielou index were calculated. In order to investigate the similarities/dissimilarities between assemblages, Bray-Curtis similarity coefficient was employed. The Cluster analysis, (based on group average linkage), two-way nested ANOSIM and similarity percentage analysis (SIMPER) were also applied. To test the relationships between abiotic factors, plant and faunal features, Spearman correlation was applied. Statistical and multivariate analyses were performed by means of the PRIMERE 6 package.

### Results

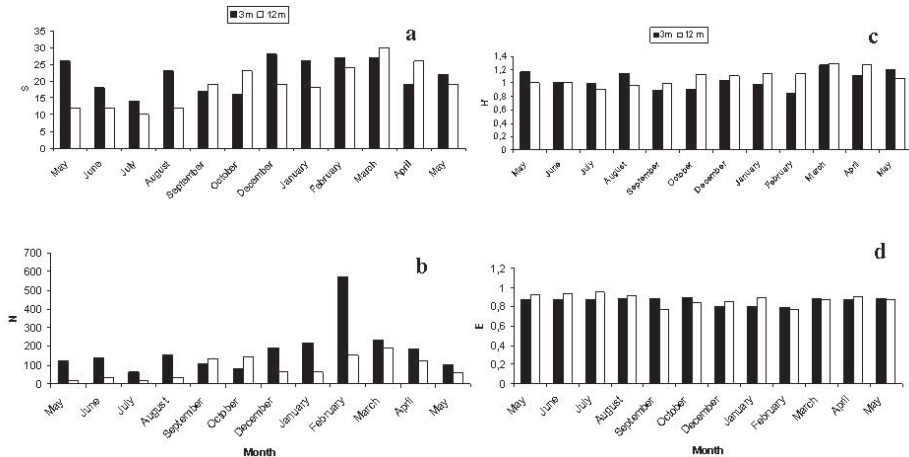
During the sampling period, 480 orthotropic rhizomes of *P. oceanica* were collected and analysed. *Posidonia* shoot density was found to vary with depth, showing clear difference among the shallow and deep localities (Spearman test,  $r=-1.000$ ;  $p<0.001$ ). With respect to the classification adopted by Pergent (2007), the density was bad at 3m depth ( $377\pm 2.82$  shoots m<sup>2</sup>); while at 12m, the number of shoots m<sup>2</sup> corresponded to a mediocre density ( $292\pm 2.72$  shoots m<sup>2</sup>). The highest Leaf Area Index (LAI) was noted in May 2007 at 3m depth ( $14.5\text{m}^2/\text{m}^2$ ) and the lowest in October 2007 at 12 m depth ( $4.5\text{m}^2/\text{m}^2$ ). The results of Spearman test showed a correlation as well between temperature and Leaf Area Index ( $r = 0.489$ ;  $p<0.05$ ) as between dissolved oxygen and Leaf Area Index ( $r=0.435$ ;  $p<0.05$ ). No significant correlations were obtained with the other factors (pH and salinity).

A total of 3227 individuals belonging to 53 species were collected. The most dominant crustacean species were *Maera ineaquipes*, *Elasmopus brasiliensis*, *Leptocheirus pilosus*, *Athanes nitesens*, *Pisidia longicornis* and *Cymodoce truncata* which were collected during all the sampling period. In contrast, some crustaceans were represented by only one or two individuals e.g. *Pisa tetradon*, *Pirimila denticulata*, *Unciolella lunata*, *Cymadusa hirsute*, *Lambos sp* and *Talitrus saltator*.

The species richness (S) varied according to depth and season. In fact, during summer and winter, the values of this index were higher at 3m depth. While, in autumn and spring, S2 showed a high species richness than S1 (Fig. 1a).

For the number of crustacean individuals, except of September and October, it was always more important at S1 than S2 (Fig. 1b). The maximum of individuals was observed during February at 3m depth (573 individuals).

The H' and Pielou values varied according to depth and season factors (Fig. 1c and d).



**Fig. 1: Number of species (a), Individuals (b), Shannon-Wiener values (c) and Pielou values (d) within crustacean assemblages at two depth ranges**

The hierarchical clustering analysis (using group-average linking) showed a clear separation of the samples collected in the two stations (Fig. 2) demonstrating that the structure of this community seems to be primarily influenced at spatial and temporal scales.

Moreover, it was shown by ANOSIM that the population structure is influenced by “depth” and “time” factors ( $r=0.368$ ;  $p<0.01$  and  $r=0.292$ ;  $p<0.01$  respectively). Otherwise, the influence of “season” factor on the composition of crustacean assemblage was found to be significant for each of the two stations (ANOSIM:  $r=0.732$ ;  $p<0.01$  for S1;  $r=0.579$ ;  $p<0.01$  for S2). SIMPER analysis, done based on crustaceans’ data, showed 69.1% of average dissimilarity between the stations (*i.e.* at the two depths). The species *Elasmopus brasiliensis*, *Leptocheirus pilosus* and *Athanes nitesens* most contributed to the dissimilarity at 3m depth. Whereas, at 12m depth, high contributions of dissimilarity were due to the species *Cymodoce truncata*, *Pisidia longicornis* and *Ampelisca rubella*. Regarding the relationship between the composition of crustaceans’ community and the features of the seagrass *P. oceanica*, Spearman test showed different correlations between plant features, environmental factors and the structure of crustaceans’ assemblage. In fact, species richness was correlated with Leaf Area Index ( $r=-0.482$ ;  $p<0.05$ ), dissolved oxygen ( $r=0.447$ ;  $p<0.05$ ) and temperature ( $r=-0.638$ ;  $p<0.01$ ). As for H', this index was found to be correlated with depth ( $r=-0.644$ ;  $p<0.01$ ), shoot density ( $r=0.644$ ;  $p<0.01$ ) and dissolved oxygen ( $r=0.427$ ;  $p<0.05$ ).

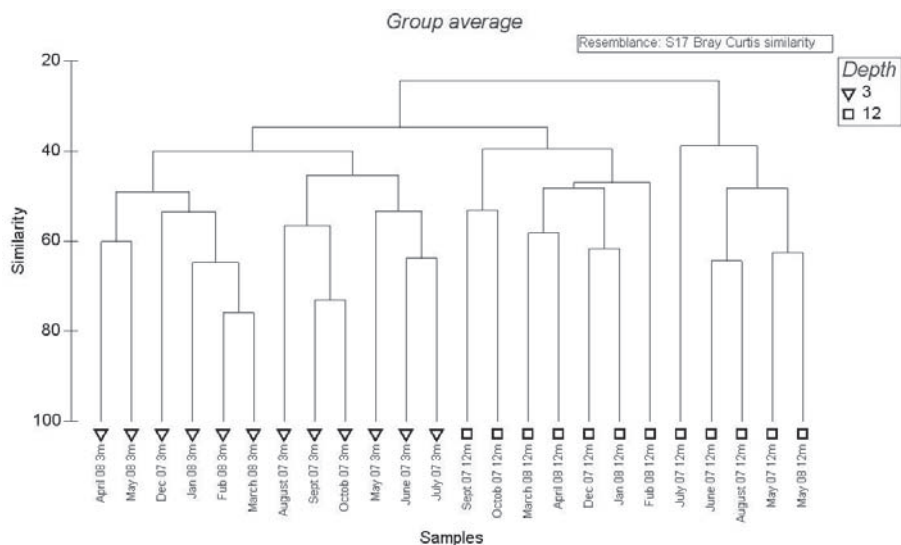


Fig. 2: Cluster plot of the 24 quantitative samples, indicated as depths.

## Discussion

The present study demonstrated that the structure of *Posidonia oceanica* meadow parameters in Cap Zebib is influenced by depth and environmental factors. The shoot density was low at the two stations; and hence this parameter was negatively correlated with the latter factors. The low shoot density can be explained by the important water movements characterizing the study area and that may induce an important loss of shoots. Such observation was noted in the sampling area. As to the mat structure, it was found more compact and tangled in the shallow area, where the highest shoot density was recorded. In addition, the decrease of shoot density with depth could be related to the decrease of solar illumination (with depth) which constitutes an important and essential factor for the development of *Posidonia oceanica*. Otherwise, the Leaf Area Index was found to be correlated with temperature and dissolved oxygen. In fact, the increase of temperature favors the photosynthetic activity of the plant and induces an important increase of the production in the leaf stratum.

Crustaceans were mainly represented by amphipods and decapods. Moreover, the highest abundance of crustaceans was found in February and seems to be related to the dominance of three amphipod species: *Maera inaequipes*, *Ampelisca rubella* and *Leptocheirus pilosus*. In fact, during this month, *Maera inaequipes* was represented by more than 300 individuals and characterized the shallow station. The latter results are different from the findings of Anabella *et al.* (2006) who noted that the highest abundance of crustaceans, in the Ligurian Sea, was observed in August.

It is worth mentioning that the species *Leptocheirus pilosus*, which is one of the most dominant amphipod species collected herein is a brackish/freshwater species which was previously recorded in Tunis northern lagoon (Zaouali & Baeten, 1984) and northern of Tunisia (Bizerta) (Chevreux, 1911). This is the first record, from a marine context, of the latter species in Cap Zebib, along the northern Tunisian coast. Thus, it seems that *Leptocheirus pilosus* migrated from the lagoon system (Tunis north lagoon) and has

adapted to sea conditions. Otherwise, the species can be induced by freshwater during rain periods. Moreover, the species *Elasmopus rapax* recorded during the present study was noted for the first time in association with Tunisian *P. oceanica* meadows. This is also the case of the species *Lysianassa longicornis* which was reported for the first time in Tunisia. This species was not noted in the studies about benthic fauna along the Tunisian coastline. As for decapods, the species *Athanas nitesens* and *Pisidia longicornis* were the most dominant and most contributed to the dissimilarity between the two localities, and hence between the two depth ranges. The dominance of the latter species was previously mentioned in Spain (Garcia-Raso, 1990). The distribution of the other decapods species varied with depth and their abundance changed with seasons. Such variability can be explained by the migration of species within the depth range of the study, as a consequence of seasonal and hence environmental changes. The Isopod group was represented by 5 species of which *Cymodocea truncata* was the most dominant. This species was almost absent in the shallow station, but was the most abundant at 12m depth. It seems that *Cymodocea truncata* prefers to occur in the deep stand and that the conditions at this level are favourable for its development.

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## **INVASION SUSCEPTIBILITY OF MEDITERRANEAN ROCKY BENTHIC ASSEMBLAGES TO RED ALGA *LOPHOCLADIA LALLEMANDII* (MONTAGNE) F. SCHMITZ.**

### **Abstract**

*Lophocladia lallemandii* (Montagne) F. Schmitz is a filamentous marine red alga with an indopacific distribution which is currently invading several areas of the Mediterranean Sea. *L. lallemandii* is known to thrive in a large variety of habitats and depths but there is no assessment on which are the features of the habitats where it becomes more aggressive. Main drivers determining establishment and invasion success of invasive species are believed to be both the abiotic and biotic factors of the invaded habitat. Although it is known that *L. lallemandii* is able to grow in different algal assemblages between 0 and 60 m depth, there are no studies on the susceptibility of the habitats to be invaded according to their species richness. Here, we explore the relationship between *L. lallemandii* invasion and its hosting native habitat species richness in order to elucidate if most diverse habitats are more susceptible to be invaded or, in contrast, biodiversity prevents *L. lallemandii* invasion. Our correlational study has demonstrated that the “invasibility” of a new area in function of the native species richness (NSR) is scale-dependent, being negative when considering small areas and positive at a broad scale.

**Key-words:** invasive algae, species richness, spatial scales, Mediterranean

### **Introduction**

*Lophocladia lallemandii* (Montagne) F. Schmitz is a red filamentous marine alga, which produces disc-like holdfasts along the thallus that allow formation of a mat of red filaments intermingled themselves or with other algae. Several authors report the high invasive potential of *L. lallemandii*, which is able to cover most kind of substrates and give to the benthic seascape a homogeneous appearance (Ballesteros *et al.*, 2007; Boudouresque & Verlaque, 2002; Cebrian & Ballesteros, 2007; 2010; Patzner, 1998). The great ability of *L. lallemandii* to colonize and thrive in new habitats has been unequivocally demonstrated through its high reproductive potential (Cebrian & Ballesteros, 2010) as well as its ability to escape from native predators (Cebrian *et al.*, 2010; Tomas *et al.* submitted). However other factors contributing to *L. lallemandii* invasion can come from particular features of the hosting habitats, such as native species richness (NSR). Yet a large body of evidences from terrestrial and marine habitats indicates that relationships between NSR and susceptibility to invasion are scale dependent: relationships are universally positive at large scales, but this relationship decays to non-significant or negative as spatial grain or extent decreases (Shea & Chesson, 2002). Here we aim to determine the susceptibility of Mediterranean rocky habitats to be invaded by *L. lallemandii* according to their species richness considering both small and large scales.

## Material and methods

The present study was carried out in the Marine Reserve of Els Freus d'Eivissa i Formentera (hereafter Els Freus MPA) and the National Park of Cabrera (hereafter Cabrera NP) (Balearic Islands) (Fig. 1). *Lophocladia lallemandii* invaded both sites during 2001 and 2003 but *L. lallemandii* was already widespread to nearly all habitats between 5 and 45 m depth (Cebrian & Ballesteros, 2007; 2010) when sampling was performed (October 2003 and 2007).

In order to assess the relationship between *L. lallemandii* abundance and the NSR of the habitats at small and large scales, two different approaches were conducted. At the large-scale study, a total of 30 sites of 25 m<sup>2</sup> belonging to photophilic and sciaphilic communities were sampled. The NSR was estimated by an expert diver who recorded all the species observed within the site area during five minutes. The abundance of *L. lallemandii* was assessed by means of 25 cm x 25 cm quadrats, divided into 25 subquadrats of 5 cm x 5 cm (Cebrian & Ballesteros, 2004; Sala & Ballesteros, 1997). At each site twenty quadrats (total area of 1,25 m<sup>2</sup>) were randomly positioned and the number of subquadrats in which *L. lallemandii* appeared was recorded as unit of measure. *L. lallemandii* abundance was the mean abundance found within the 20 random quadrats sampled.

To assess the relationship between *L. lallemandii* abundance and NSR at a small scale we related the abundance of *L. lallemandii* of each quadrat of 25 cm x 25 cm with the number of native species present at the same quadrat. A total of 15 random quadrats were sampled at 2 studied depths (10 and 20 m depth).

Relationships between *L. lallemandii* abundance and the NSR and their significance were studied by means of simple regression analyses performed using a STATISTICA 8.0 Package.

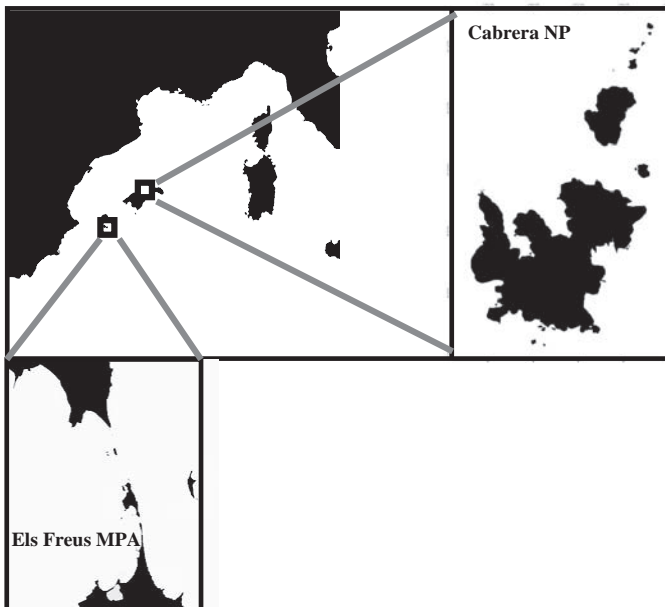
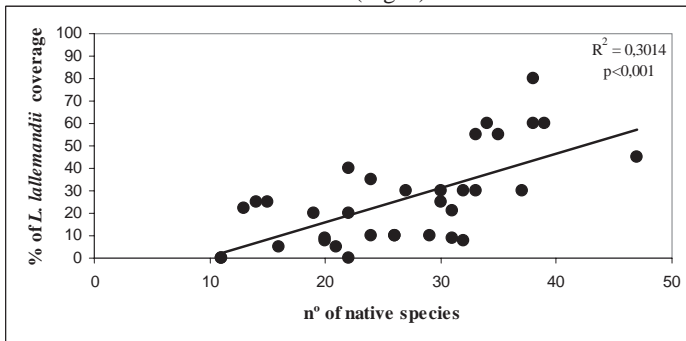


Fig. 1: Localization of the study area.

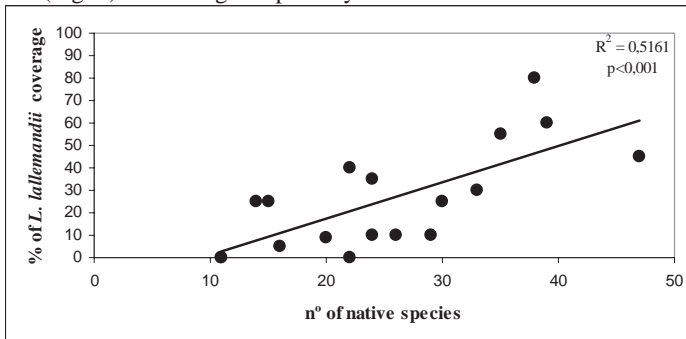
## Results

At a large scale a significant positive relationship was found between the % of coverage of *Lophocladia lallemandii* and the NSR (Fig. 2).

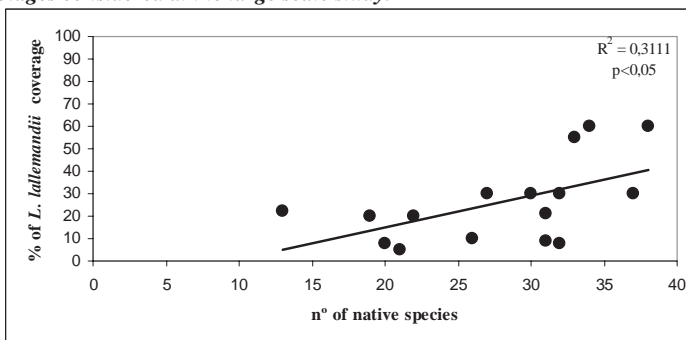


**Fig. 2:** Relationship between the % of *L. lallemandii* coverage and native species richness (NSR) for all sites considered at the large scale study.

Similarly, the relationship between the % of coverage of *L. lallemandii* and the NSR remained also positive and significant when considering the photophilic (Fig. 3) and sciaphilic (Fig. 4) assemblages separately.



**Fig. 3:** Relationship between % of *L. lallemandii* coverage and NSR found in the photophilic assemblages considered at the large scale study.



**Fig. 4:** Relationship between % of *L. lallemandii* coverage and NSR found in the sciaphilic environments considered at the large scale study.

In contrast, the relationship between *L. lallemandii* abundance and NSR at small scale showed opposite results. When considering overall quadrats at the two depths considered (10 and 20 m) a negative and significant regression was found (Fig. 5). Similarly, the relationship between *L. lallemandii* and NSR in quadrats at shallow (Fig. 6) and deep water assemblages (Fig. 7) was negative.

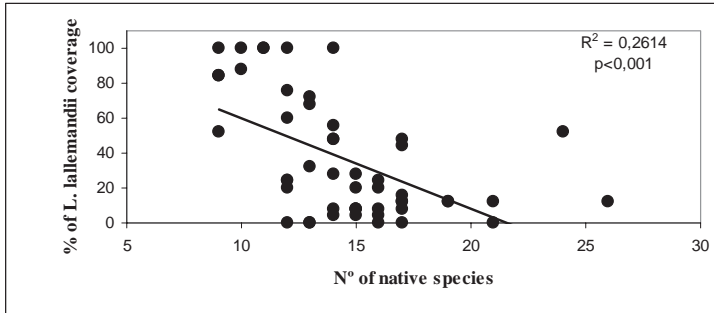


Fig. 5: Relationship between % of *L. lallemandii* coverage and NSR at the small scale study.

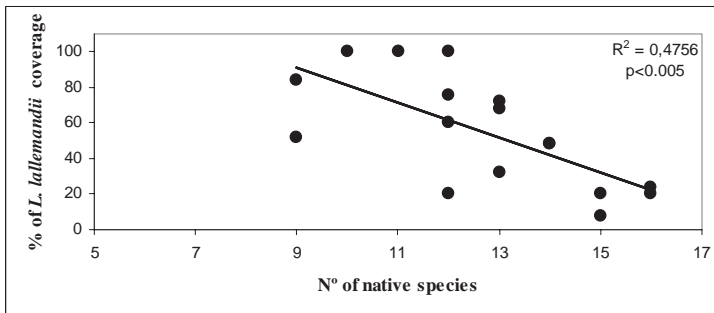


Fig. 6: Relationship between % of *L. lallemandii* coverage and NSR at the small scale study at 10 m depth.

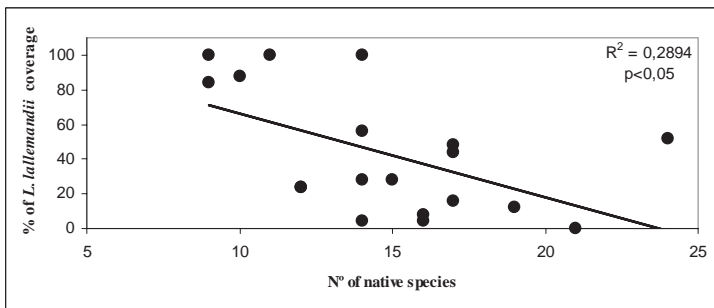


Fig 7: Relationship between % of *L. lallemandii* coverage and NSR at the small scale study at 20 m depth.

## Conclusion

Relationships between invasibility and NSR was always significantly positive in the large scale approach. So, environments with a high biodiversity seem to be more susceptible to *L. lallemandii* invasion than the less diverse ones. In contrast the study approach at a small scale revealed negative significant regressions between % of *L. lallemandii* invasion and NSR. These findings are in agreement with reasonings by Shea & Chesson (2002) who reconciliated the contrasting results usually found in several studies depending on the spatial scale considered. At large-scales NSR and invasion success could be positively correlated because the response of both native and exotic species to heterogeneity in abiotic factors at such scales overwhelms the positive effects of diversity on the invasion resistance that prevails at smaller scales (Levine & d'Antonio, 1999; Shea & Chesson, 2002). In contrast, the negative relationships found at small scale between *L. lallemandii* coverage and NSR are in agreement with the experimental study by Stachowicz *et al.* (2002) where decreasing native diversity of a subtidal marine invertebrate community increased the survival and final percent cover of invaders. The most commonly cited mechanism explaining these negative correlations is that as species accumulate, competition intensifies and fewer resources remain available for new colonists (Case 1990; Elton 1958). Here, the negative correlation found for the small scale study could reflect the consequences of the competition that inherently operates between *L. lallemandii* and the native species at smaller scale. However with this correlational study we are not able to know whether *L. lallemandii* is a driver or a passenger of NSR, and experimental studies are necessary to decipher it. However, due to findings by Ballesteros *et al.* (2007) where shoots of seagrass *Posidonia oceanica* were killed by dense mats of *L. lallemandii*, we hypothesize that when *L. lallemandii* is very abundant it can outcompete native species and decreases NSR by space occupation, light depletion and other changes in micro-environmental conditions. Thus, and awaiting further experimental work, we conclude that the contrasting relationships between invasion success of *L. lallemandii* and NSR is mainly due to competition processes at a small scale and high heterogeneity and niche opportunities at a large scale that prevent total space occupation by the invader.

## Acknowledgements

Financial support was provided by Fundación Biodiversidad. EC was funded by a post-doctoral fellowship Juan de la Cierva of the Spanish MINCIN and an European Reintegration grant (ERG-2009-248252). We would like to thank managers and staff of the Els Freus MPA and Cabrera NP for sampling permissions.

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## **FLORA AND VEGETATION OF THE ITALIAN TRANSITIONAL WATER SYSTEMS: AN ITALIAN EDITORIAL INITIATIVE**

### **Abstract**

*An editorial initiative concerning the macrophytobenthos of the Italian TWSs is here presented. From the analysis of 16 TWSs in 8 Italian regions came out that only few of them are well known from a phytobenthic point of view, in particular the Lagoon of Venice, the Mar Piccolo of Taranto, the Stagnone of Marsala and the Orbetello lagoon. A total of 447 species was assessed, among them 244 Rhodophyta, 77 Ochrophyta, 113 Chlorophyta, 3 Xanthophyceae, 1 Chrysophyta and 9 Spermatophyta. 22 non-indigenous species (NIS) belonging to Rhodophyta (14), Ochrophyta (5) and Chlorophyta (3) were recorded, mostly in the Lagoon of Venice and the Mar Piccolo of Taranto. Moreover, several peculiarities of the species in TWSs emerged in comparison with the open sea. In the absence of similar information about the Mediterranean Sea, we propose a similar initiative concerning all the Mediterranean eco-region.*

**Key-words:** Italy, Macroalgae, Mediterranean Sea, Phanerogams, Transitional Water Systems

### **Introduction**

Transitional Water Systems (TWSs) consist of different kinds of environments which, according to McLusky & Elliot (2007), include estuaries, fjords, lagoons, enclosed bays, ponds, river mouths and lakes, sharing several common features, such as high spatio-temporal variability of the physico-chemical variables, shallowness, high productivity mainly due to angiosperms and macroalgae rather than phytoplankton (Zaldívar *et al.*, 2008). From an ecological point of view, they are very important ecosystems, representing hot spots of biodiversity and natural laboratories for studies of long term ecology; in addition, they are “nursery” areas for the juveniles of several fish and mollusc species (Courrat *et al.*, 2009), and house a rich variety of both non-migratory and migratory birds. Nevertheless, just for their geo-morphological features, they are strongly affected by anthropogenic pressures, mainly resulting from urban, aquacultural, agricultural and industrial activities. Nowadays, one of the most evident effects of this negative action on TWSs is the “biological pollution” (Elliot, 2003; Olenin *et al.*, 2007). In these environments, macroalgae and phanerogams play a fundamental role: as primary producers, but also as oxygen consumers in the case of decomposition of big unattached biomasses of annual macroalgae; as biofilters in both the removal of dissolved nutrients (i.e. nitrogen and phosphorus) (Cahill *et al.*, 2010) and the adsorption of heavy metals (Gadd, 2009); as refuge and pabulum for several fish and benthic invertebrates (Roberts *et al.*, 2008; Liuzzi & Lopez Gappa, 2010); as efficient obstacles for erosion and resuspension of superficial sediments (Cabaço *et al.*, 2010). In the application of the European Water Framework Directive (WFD, 2000/60 EC), macroalgae and phanerogams represent effective biological indicators for the assessment of the ecological status in TWSs (Orfanidis *et al.*, 2001; Panayotidis *et al.*, 2004; Sfriso

*et al.*, 2009). Therefore, in these environments, the knowledge of the vegetation is important also for the definition of environmental quality indices.

In Italy, there are a lot of TWSs scattered along the coasts, many of which have been widely studied from several points of view, with the exception of macrophytobenthos, notwithstanding its evident importance; so that, in many cases, neither the dominant species were known. Therefore, within the Italian Association on Coastal Lagoon Ecology “LaguNet”, in February 2006, an editorial initiative was undertaken for the preparation of a monography on the state of knowledge about the macrophytobenthos in the Italian TWSs, in order to collect, compare and gather together the available and scattered information concerning this important biotic component in these environments. Finally, in July 2009, with the contribution of the Venetian consortium CORILA, the monography was published as a comprehensive and up-to-date framework about this subject (Cecere *et al.*, 2009a).

On the basis of the data present in each chapter, some speculative and general considerations were elaborated highlighting the importance of macrophytobenthos in TWSs and, consequently, the need of its knowledge.

### **Materials and methods**

The first step taken for the accomplishment of the editorial initiative was to contact all the researchers who worked on the phytobenthos of Italian TWSs, both directly and on the net through the Association LaguNet. Once they declared their willingness to take part in the initiative dealing with the TWS on which most of their studies are focused, a scheme was sent to each author, or group of authors, as a guide for the draft of the chapter in their competence, in order to gather information as much homogeneous as possible. The asked information, to be acquired from the available literature (i.e. scientific publications and grey literature) and from the personal experience, were both general about the environment (e.g. highly/scarcely studied, subject of extant research, morphology, main chemical-physical features, presence and kind of anthropogenic impacts, presence of anoxic crises, and so on) and specific about the vegetation. This last consisted in: a floristic list of the present species, organized in a table and noting for each species name and authorities, list of publications in which it was reported, reproductive phenology (i.e. male/female gametophyte, tetrasporophyte, specialized propagules production), settlement status (i.e. attached, unattached, epiphyte), its behaviour if non-indigenous (i.e. invasive/non-invasive); a possible list of disappeared species; a possible list of taxa *inquirenda*, *excludenda*, *nuda*. Moreover, where available, authors were also asked for the description of the vegetation considering the kind of communities, their distribution, biomass of dominant species, spatial-temporal variation, net and gross production, relations with the environmental variables and polluting substances.

The manuscripts were successively revised by the Editors as referees and from the group of Catania University for the species nomenclature, and then adequately formatted and printed.

### **Results**

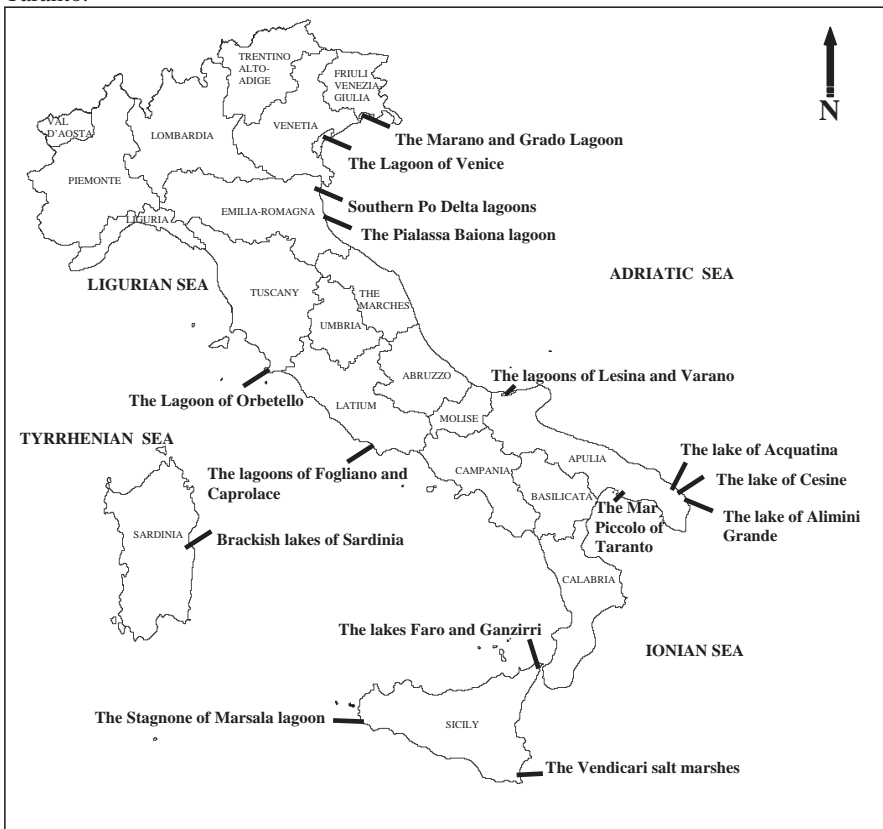
The adhesion to the project was really enthusiastic and, at the end, 16 chapters were drawn about as many TWSs in 8 Italian regions (i.e. Friuli-Venezia Giulia, Venetia, Emilia-Romagna, Tuscany, Sardinia, Latium, Apulia, Sicily) out of the 15 Italian sea side regions (Fig. 1); however, completely unknown remain TWSs from some regions,



among which Campania. The highest number of examined TWSs are from the Tyrrhenian Sea (n=35), since only in Sardinia 30 brackish lakes were considered, those from the Adriatic Sea (n=8) follow and lastly those from the Ionian Sea (n=3).

Among them, the widest information about macrophytobenthos came out from the Lagoon of Venice (in Venetia) and the Mar Piccolo of Taranto (in Apulia), followed by the Stagnone of Marsala (in Sicily) and the Orbetello lagoon (in Tuscany), while for the most part of the remaining TWSs, floristic lists reported scarce data, often very old and fragmented. Therefore, for such environments information on the reproductive phenology and the settlement status were often lacking.

A total of 447 species were catalogued: 244 Rhodophyta, 77 Ochrophyta, 113 Chlorophyta, 3 Xanthophyceae, 1 Chrysophyta and 9 Spermatophyta. 22 non-indigenous species (NIS) belonging to Rhodophyta (14), Ochrophyta (5) and Chlorophyta (3) were recorded, mostly in the Lagoon of Venice and the Mar Piccolo of Taranto.



**Fig. 1: Map of the Italian Transitional Water Systems (in bold) examined within the monography of Cecere et al. (2009).**

## Discussion and Conclusions

Surely, one of the reasons for the limited number of environments discussed in the monography, and for the different size of available information, is the little number of researchers on macrophytobenthos working in the Italian both public (i.e. University, Public Research Centers) and private organizations, which becomes scarcer considering TWSs. Currently, this is in contrast with a well-known Italian tradition regarding both angiosperm studies and phycological studies dating back to almost two centuries ago (Andreoli, 2006; Mazzella *et al.*, 1987). In addition, unfortunately, vegetation has always been neglected in the integrated studies of both coastal and TWS macrobenthos, and often in scientific meetings the term “macrobenthos” is still used referring only to zoobenthos.

All the same, the study of phytobenthos in TWSs allowed the knowledge of some peculiarities of the vegetation in these environments, which differentiates from that in open waters. For example, the Stagnone of Marsala is the only Italian TWS where the seagrass *Posidonia oceanica* (L.) Delile is surely present and forms particular structures unique in the Mediterranean Sea (Calvo *et al.*, 2009). Many species of seaweeds present in the Mar Piccolo of Taranto showed particular strategies of vegetative reproduction, never described for species from open waters (Cecere & Petrocelli, 2009), as well as the peculiarity of producing ball-like forms (Cecere *et al.*, 2009b; Petrocelli *et al.*, 2009b), which are morphologically very different from the conspecific attached thalli, making their identification as belonging to the same species sometimes very difficult. The presence of NIS, a new threaten for the world biodiversity, is particularly reported in TWSs. Indeed, these environments are preferable seats of aquaculture activities which are one of the main cause of the phenomenon also known as “biopollution”. The Lagoon of Venice, (Sfriso *et al.*, 2009) and the Mar Piccolo of Taranto (Cecere & Petrocelli, 2009; Gravili *et al.*, 2010), both with 12 NIS, appear as the principal hot spots for NIS introduction in the Italian seas, surely because subject to an intense traffic of imported molluscs (Petrocelli & Cecere, 2010). But, NIS were reported also in other Italian TWSs (Falace *et al.*, 2009; Petrocelli *et al.*, 2009a; Serio *et al.*, 2009).

As far as known, there was no similar initiative anywhere in the Mediterranean countries, only some systems of general information on transitional waters are present, such as the Greek ElNet (Faulwetter *et al.*, 2008), a lagoon observation network, or the MedOBIS, a online database which collects information about the distribution of some groups of zoobenthos from the Eastern Mediterranean and the Black Sea (Arvanitidis *et al.*, 2006; <http://www.medobis.org>). On this basis, any kind of initiatives, either on paper or on the net, would be desirable for a wider knowledge of vegetation in TWSs in all the Mediterranean eco-region. In this respect, we would like to suggest the drafting of a similar monography, likely under the auspices of RAC/SPA, considering all the TWSs along the Mediterranean coast, to widen their knowledge and better their management and conservation.

## Acknowledgments

The effort of all the authors, the economic contribution of “CORILA” and the support of the Italian Association “LaguNet” for the publication of the monography is greatly acknowledged.

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## **LES RELATIONS ENTRE LES HERBIERS DE POSIDONIES, LEUR SUBSTRAT ET L'HYDRODYNAMISME**

### **Résumé**

*La structure des herbiers à *Posidonia oceanica* a fait l'objet de plusieurs descriptions qui ont donné lieu à une typologie précise. En revanche, les facteurs responsables de l'origine de ces structures et de leurs variations temporelles ne sont que rarement identifiés.*

*L'ensemble des informations recueillies autour de la Corse lors de campagnes de cartographie à l'aide d'un sonar à balayage latéral a permis de mettre en évidence une grande diversité des fonds à herbiers. De façon générale, le faible niveau d'anthropisation du littoral insulaire permet de relier ces résultats à des phénomènes essentiellement naturels.*

*Si la lumière conditionne de manière déterminante la profondeur de la limite inférieure des herbiers, l'hydrodynamisme et le type de substrat peuvent également jouer localement un rôle important. Ainsi à l'Ouest du Cap Corse, l'extension des limites inférieures paraît dépendre directement de l'existence d'un substrat rocheux, et non pas seulement de la quantité de lumière disponible.*

*L'existence de nombreux herbiers ondoyants va de pair avec une direction générale et une longueur d'onde qui paraissent dépendre des paramètres de la houle. Un tel contrôle hydrodynamique est confirmé par la présence de mégarides façonnant les fonds sableux situés à proximité des herbiers, présentant la même orientation et des longueurs d'ondes voisines. Cette dépendance combinée de la houle, montrée par le faciès ondoyant des herbiers de posidonies corses et par le faciès des mégarides sableuses, traduit des convergences singulières quant aux mécanismes hydrodynamiques agissant localement.*

*Enfin les images acoustiques relevées pour les herbiers continus et pour les herbiers morcelés correspondent à une série régulière de faciès, comportant des stades intermédiaires bien différenciés. Les variations morphologiques montrées par ces stades semblent résulter de phases progressives de morcellement, résultant également pour l'essentiel des variations de paramètres de la houle.*

**Mots clés :** *Posidonia oceanica*, substrat, hydrodynamisme, sonar à balayage latéral, cartographie.

### **Contexte de l'étude**

La position bathymétrique des limites inférieures des herbiers à *Posidonia oceanica* est essentiellement conditionnée par la quantité de lumière disponible (Meinesz et Laurent, 1978). Toutefois, la présence de perturbations naturelles ou anthropiques, de nature à engendrer une augmentation de la turbidité des eaux, peut entraîner un recul de la position bathymétrique de cette limite (Boudouresque *et al.*, 2006).

En Corse, du fait de la transparence élevée des eaux, l'herbier se développe jusqu'à des profondeurs importantes (en moyenne -32 m) (Pasqualini *et al.*, 2000). La quantité de lumière apparaît donc comme le facteur limitant principal. Toutefois, l'analyse conjointe des informations sur la nature et la morphologie des substrats et des herbiers, les figures sédimentaires et les données bathymétriques au voisinage de la limite

inférieure de l'herbier, conduit à identifier, dans la tranche bathymétrique où la lumière disponible permet la présence de l'herbier, plusieurs autres facteurs limitant son implantation. Certains de ces facteurs semblent également responsables du façonnement de l'herbier, et d'un abaissement local de sa limite supérieure.

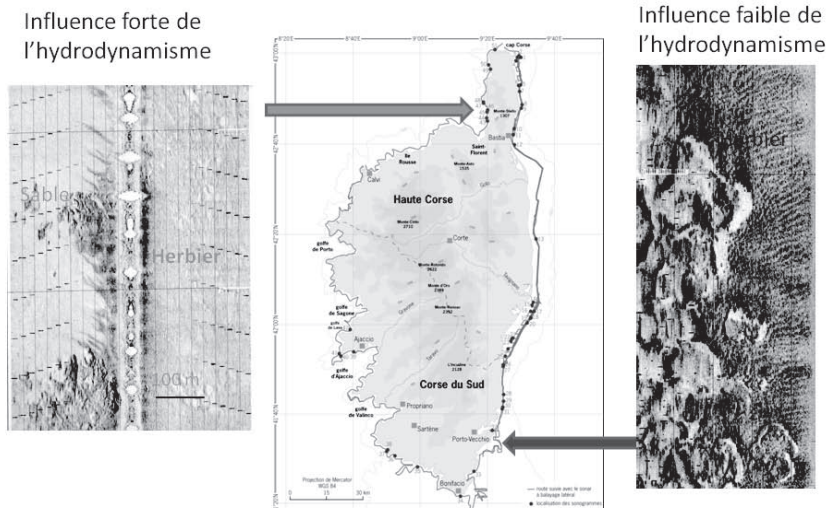
### Matériel et méthodes

La démarche consiste à aborder l'analyse de la répartition de l'herbier en fonction des caractéristiques des substrats, durs (rocheux) ou meubles (sableux) d'une part, et en fonction de l'intensité de l'hydrodynamisme d'autre part, notamment par le biais de figures sédimentaires associées (Clabaut *et al.*, 2007, Clabaut, 2010).

Cette démarche, originale, n'est que très peu abordée dans la bibliographie ; elle s'inscrit en complément de travaux, tels que ceux de Jeudy de Grissac et Boudouresque, (1985), qui traitent de l'influence de l'herbier sur la stabilité du littoral, mais concernent essentiellement les herbiers peu profonds. Elle n'est possible qu'à la faveur d'un gros volume de données disponibles comme le long du littoral de la Corse, où 1 200 km de profils ont été réalisés avec un sonar à balayage latéral (Pasqualini *et al.*, 2000).

### Résultats

Sur un substrat rocheux, l'extension de l'herbier en limite inférieure peut être limitée à la fois par la limite rocheuse et par l'intensité de l'hydrodynamisme. Ainsi à l'Ouest du cap corse, la limite inférieure de l'herbier coïncide avec celle de la roche (Fig. 1). Au-delà de celle-ci, le substrat est meuble, remanié par la houle, et dépourvu d'herbier. C'est l'extension de la roche, dans un milieu à hydrodynamisme intense, qui est à l'origine de la profondeur de la limite inférieure de l'herbier et qui détermine le caractère irrégulier de celle-ci, comprise entre 28 et 39 m de profondeur.



**Fig. 1 : Position de la limite inférieure de l'herbier vis-à-vis du substrat rocheux à l'Ouest du Cap Corse et au Nord de Porto-Vecchio.**

A l'inverse, au Sud de Porto-Vecchio, l'herbier ne se limite pas au substrat rocheux. Il est implanté à la fois sur la roche, près de la côte, et sur les fonds meubles, plus au large



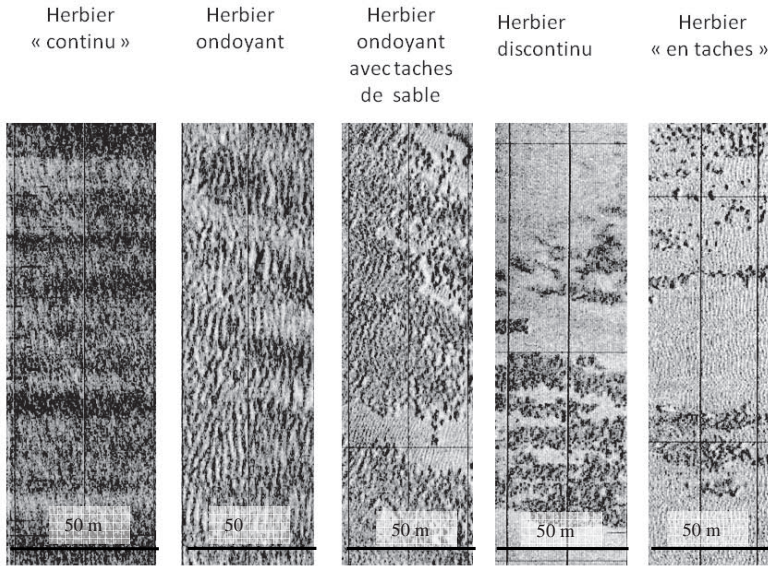
(Fig. 1). Au contact entre les fonds rocheux et sableux, l'herbier présente un faciès ondoyant, qui peut traduire un hydrodynamisme modéré. Le facteur limitant de son extension est alors la quantité de lumière disponible (transparence de l'eau).

Ces deux configurations opposées de l'herbier vis à vis de son substrat rocheux illustrent bien l'influence variable de l'hydrodynamisme. Ils indiquent également les différents paramètres limitant l'extension de l'herbier (quantité de lumière, extension et morphologie du substrat rocheux, intensité de l'hydrodynamisme) et de les hiérarchiser pour chaque secteur côtier.

La limitation de la profondeur de la limite inférieure de l'herbier par l'hydrodynamisme est par ailleurs évoquée pour le site des Lavezzi, tout proche, dans le cadre de la mise en œuvre du RSP Corse (Pergent *et al.*, 2008).

La morphologie des herbiers implantés sur substrat sableux est très variée, notamment au niveau de leur morcellement. En simplifiant, on peut discerner différents « stades » (Fig 2) :

1 – l'herbier continu, à faciès acoustique homogène et à fort recouvrement apparent. Toutefois, des intermattes de taille insuffisante (inférieures au mètre) pour être détectées par le sonar peuvent être présentes (Ifremer *et al.*, 2007).



Superficie de chaque extrait de sonogramme 1 ha.

**Fig. 2 : Faciès acoustiques d'herbiers montrant le morcellement progressif de l'herbier sur substrat sableux.**

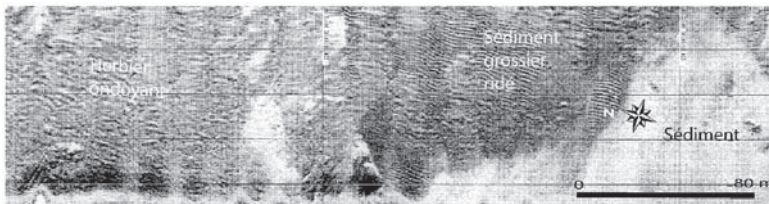
2 – l'herbier à faciès ondoyant décrit d'après des observations en plongée, est « caractérisé par des bandes d'herbier larges (jusqu'à une dizaine de mètres), parallèles entre-elles et anastomosées, en très léger relief, séparées par des bandes de sable recouvertes éventuellement de matte morte, également parallèles entre-elles » (Boudouresque *et al.*, 2006). Sur les sonogrammes, cet herbier ondoyant présente un aspect de rides assez régulières, proche de celui des rides de houle, de longueur d'onde métrique (Fig.3).

3 – l'herbier interrompu par des taches de matte ou de sable (intermatte), de taille et de fréquence variables. La nature sableuse du sédiment des intermatte est ici indiquée par la présence de mégarides de houle.

4 – l'herbier plus ou moins morcelé et discontinu, qui ne subsiste ici que sous forme de taches de taille et de forme variables.

5 – l'herbier en taches isolées, localement réduit à des taches de taille métrique, dissociées les unes des autres.

Au sein de l'herbier ondoyant, l'alternance de bandes d'herbier et de sable laisse supposer une certaine mobilité du sédiment, plus importante que dans l'herbier à faciès continu. De plus, l'orientation des « ondulations de l'herbier » est identique à celle des mégarides de houle, qui façonnent les sédiments grossiers, à proximité immédiate de celui-ci. Comme les mégarides observées dans les sables grossiers, dans des environnements de haute énergie, cette orientation des ondulations des herbiers ondoyants et des rides façonnant les sables correspond à échelle régionale à l'orientation des houles dominantes, de secteur Ouest.



*Fig.3: Disposition de l'herbier ondoyant et des sables ridés, au Nord de Punta Bianca (Ouest du Cap Corse), vers 26 m de profondeur.*

Les rides de houle et les bandes d'herbier présentent ici une orientation identique, et une « longueur d'onde » comparable, de l'ordre de 5 mètres. Ces divers éléments laissent penser que la houle est à l'origine de ce faciès « herbier ondoyant » ; Si tel était le cas, ce type d'herbier, serait, comme les mégarides de houles façonnant un fond meuble, un indicateur des directions de courant sur le fond.

Ceci rejoint les observations de Clairefond et Jeudy de Grissac (1979), qui en se basant sur de nombreuses observations en plongée dans les passes de l'île de Port-Cros, avaient identifié deux types d'herbier ondoyant, simple ou entrecroisé, liés respectivement à un ou deux régimes de houles préférentiels.

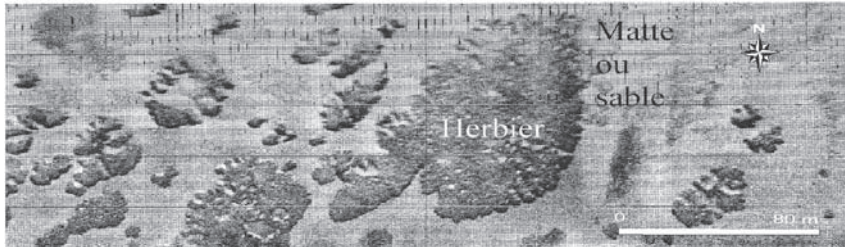
Ce faciès « ondoyant » est sans doute plus répandu que ne l'indique la bibliographie ; on l'a observé en différents sites côtiers de PACA, notamment en Baie de Cassis, aux abords du Cap Canaille, dans la Passe des Grottes, entre les îles du Levant et de Port-Cros, au Nord de Porquerolles, au Nord de la baie de La Ciotat, et entre l'Île Verte et l'anse du Mugel. En Corse, outre le site de Canari, cité précédemment, il est assez développé, notamment autour du Cap Corse, dans le golfe de Saint-Florent, et dans le golfe de Porto-Vecchio.

#### **Discussion et conclusions**

En particulier en Corse, où les impacts anthropiques sont faibles, où on dispose d'une grande quantité de données, et où l'action des houles peut façonner des fonds meubles jusqu'à une profondeur de 50 m, on peut s'interroger sur l'influence de l'hydrodynamisme sur la morphologie de l'herbier et sur son morcellement. Il serait tentant de corrélérer le morcellement de l'herbier à l'intensité de l'hydrodynamisme, les



deux paramètres croissant dans le même sens. Mais l'herbier « en taches isolées » par exemple peut avoir d'autres origines que l'action de la houle. C'est notamment un faciès de transition, au débouché des rivières, entre l'herbier « continu » et les zones dépourvues d'herbier (Fig.4). Comme le souligne C.F. Boudouresque (2006), « toutes les intermattes n'ont pas une origine anthropique » ; elles ne sont sans doute pas toutes liées à l'hydrodynamisme.



**Fig.4 : Morphologie d'herbier au large d'une embouchure de fleuve sur la façade orientale de la Corse.**

D'autres formes peuvent être liées à l'hydrodynamisme, comme les limites inférieures d'herbier érosives, soulignées par un tombant de matte, ou les tombants de matte, au sein même de l'herbier

La disposition de l'herbier vis à vis du substrat à proximité de sa limite inférieure peut refléter l'influence de l'hydrodynamisme. Une influence faible à modérée de l'hydrodynamisme, mais surtout moindre que celle de la transparence de l'eau, se traduirait :

- soit par un herbier qui se développe sur substrat rocheux et/ou sableux, jusqu'à une profondeur déterminée par la transparence de l'eau, alors que les sédiments ne sont pas différenciés et dépourvus de figures sédimentaires,

- soit par un herbier qui se développe sur un substrat rocheux, qui « déborde » au-delà de la limite de la roche sur le fond sableux, puis devient ondoyant et morcelé, Une influence forte de l'hydrodynamisme se traduirait quant à elle par un herbier limité au substrat rocheux ; dont la limite inférieure coïncide avec la bordure inférieure de la roche, avec une profondeur variable, au-delà de laquelle le fond sableux est façonné de mégarides. C'est le cas également lorsque l'herbier sur sable se termine par un tombant (limite inférieure érosive).

Au-delà du morcellement de l'herbier, ces sonogrammes, par le biais des figures sédimentaires, fournissent une information sur la mobilité du sable dans l'herbier. On peut penser qu'en fonction de l'intensité de la houle, du recouvrement de l'herbier et des caractéristiques du sédiment (taille, teneur en particules fines...), la mobilité du sédiment peut varier notablement. Par exemple, dans un herbier à faciès ondoyant, le sédiment semble conserver une certaine mobilité au sein de l'herbier. Toutefois, peu d'enregistrements hydrodynamiques semblent avoir été réalisés dans l'herbier.

La mise en évidence du lien entre limite inférieure d'herbier et substrat rocheux, dans des environnements à hydrodynamisme intense pourrait conduire à proposer une « limite inférieure d'herbier liée au substrat rocheux, contrôlée par l'hydrodynamisme ». Ce type de limite ne semble pas spécifique à la Corse ; d'autres exemples sont observés en Tunisie (Nord du Golfe de Tunis) et en Algérie (Parc National d'El Kalaa) (R. Semroud, *Comm. Pers.*).

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## **LA MICRO CARTOGRAPHIE PAR TELEMETRIE ACOUSTIQUE, UNE NOUVELLE METHODE DE SUIVI DES HERBIERS DE POSIDONIE. APPLICATION SUR 9 SITES DES CÔTES MEDITERRANNEENNES FRANCAISES.**

### **Résumé**

*Nous démontrons l'intérêt de la télémétrie acoustique (Descamp et al., 2005), pour l'obtention de cartographies sous marines de précision permettant le suivi des limites d'herbiers. Après une première phase de test de précision de mesure et de comparaison aux données constructeurs, neuf microcartographies ont été réalisées sur des herbiers de Posidonia oceanica (F. Posidoniaceae) localisés entre -7 m et -32 m de profondeur le long des côtes méditerranéennes françaises.*

*Nos résultats montrent une bonne efficacité de la méthode grâce au grand nombre de points relevés en un temps relativement court (quelques heures par site pour enregistrer environ 900 points espacés d'environ 40 cm, précision unitaire hors points remarquables inférieure à 20cm, précision des points remarquables : 3 cm à 30m de la balise). La variété des herbiers microcartographiés (en tâche, à limite franche ou éparse, etc.) a permis de tester et de valider l'adaptabilité de la télémétrie acoustique aux différents types de limites. Ces cartographies seront répliquables dans le futur et constituent d'ores et déjà un état de référence.*

**Mots clés:** Outil de cartographie, *Posidonia oceanica*, Dynamique d'herbiers sous marins, Suivi spatio-temporel, Approche coût/efficacité

### **Introduction**

Que ce soit dans un cadre de gestion de la biodiversité (Natura 2000), d'aménagement du littoral (étude d'impact) ou de suivi de la qualité des masses d'eau (Directive Cadre Eau), le suivi d'herbiers sous marins nécessite des moyens de surveillance efficaces. La méthode habituellement utilisée (méthode RSP) est basée sur l'utilisation de balises permanentes immergées en limite d'herbier. Cette méthode pose des difficultés méthodologiques liées au nombre restreint et à la pérennité des balises sur le fond rendant difficile les suivis long terme, l'étude des herbiers morcelés à limites éparse et/ou à évolution rapide (Descamp et al., 2009) (Boudouresque et al., 2006). Une autre méthode appelée télémétrie acoustique utilise un système de positionnement acoustique sous marin. Le système se compose d'un pointeur tenu par un plongeur et permettant de signaler précisément les contours de l'herbier et d'une base détectant les ultrasons émis par le pointeur (Descamp et al., 2005). Le but de cette étude est de tester la télémétrie acoustique en tant que technique « non -invasive » (aucune balise risquant d'empêcher la plante de croître) pour la cartographie de précision et le suivi temporel d'herbiers de *P. oceanica* de types variés sur les côtes méditerranéennes françaises.

## Matériel et méthodes

Les microcartographies ont été réalisées avec un AQUA-METRE D100 (PLSM) autocalibrant. Excepté pour les tests de précision, les limites d'herbiers ont été dessinées à l'aide de points enregistrés tous les 40 cm avec la fonction moyenne intelligente sur trois points activée (Il faut trois points proches avant calcul de la position moyenne et enregistrement de la donnée). La télémétrie acoustique se base sur deux points de référence (balises ou points remarquables naturels) situés à moins de 100 m de la base. Le géoréférencement de ces points à l'aide d'un GPS sous-marin USBL tracking system (Tritech Micron Nav, Tritech International Limited, United Kingdom) permet l'intégration des résultats à un système d'information géographique (SIG). Les points sont ensuite reliés pour dessiner les contours et limites d'herbiers, calculer des surfaces et leur évolution. La précision de la méthode a été évaluée par l'enregistrement d'un point fixe trois fois de suite à différentes distances de la base (10, 20, 30, 40 and 50 m) et avec la fonction moyenne intelligente (i) inactivée, (ii) activée sur deux points et (iii) activée sur trois points. Toutes les plongées ont été réalisées à l'aide de recycleurs à circuit fermé INSPIRATION VISION (Ambiant Pressure Diving Ltd., United Kingdom) permettant des plongées sans bulle et plus longues (Bahuet *et al.*, 2007).

Les limites inférieures d'herbiers de posidonie ont été étudiées sur neuf sites variés de la côte méditerranéenne française (Tab. 1). Ces sites choisis en coopération avec l'Agence de l'Eau étaient tous situés en eau de bonne qualité. Ils ont été cartographiés entre avril et août 2008 (sauf Palavas les flots en août 2006). L'évolution temporelle d'un dernier site (suivi en août 2006, Juillet 2008 et juin 2010 sur la réserve marine de Cerbère-Banyuls) a été étudiée. La comparaison des cartes obtenues en termes de progression, régression et stabilité a été réalisée à l'aide d'ArcGIS 9.3 (ESRI, France). Plusieurs de ces sites étant préalablement suivis par la méthode RSP, les balises ont été pointées lorsqu'elles étaient encore visibles.

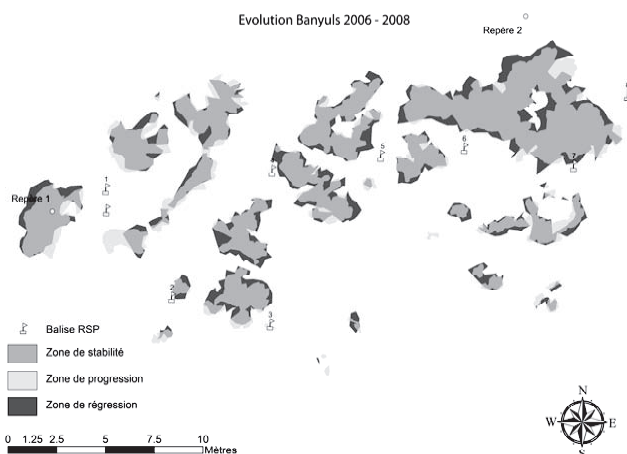
## Résultats

Les tests de précision montrent une variation de localisation de 1,02 à 12,04 cm pour un pointeur situé à 60 m de la base. Globalement, aucune différence significative n'a été observée entre les variations moyennes observées à des distances inférieures à 50 m (10 à 50 m, Kruskal Wallis test :  $\chi^2 = 6,964$ ,  $P = 0,138$ ) et quelque soit la fonction utilisée (Kruskal Wallis test :  $\chi^2 = 3,750$ ,  $P = 0,153$ ). Toutes distances confondues, la plus faible variation (4,22 cm) a été notée avec la fonction moyenne intelligente sur trois points.

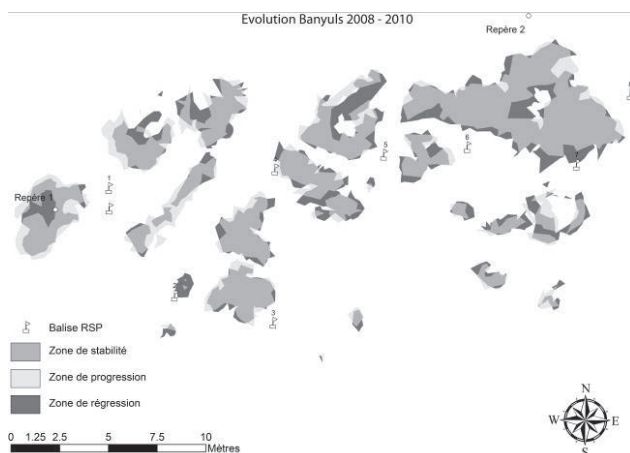
Quelque soient leurs morphologies ou leurs types de limite, un maximum d'une journée de travail (5h à trois personnes) par site a suffi pour cartographier les neuf herbiers sur la base de 137 à 1243 points. Le suivi temporel de l'herbier de Cerbère-Banyuls (Fig. 1, 2 et 3) montre une limite inférieure de type régressif stable à -19,6 m. Entre 2006 et 2010, l'herbier montre une surface totale plutôt stable à légèrement progressive (86 m<sup>2</sup> en 2006 et 91 m<sup>2</sup> en 2010). Ceci est le résultat d'une évolution globale positive (+10 m<sup>2</sup>) entre 2006 et 2008 suivie d'une évolution globale négative (-5 m<sup>2</sup>) entre 2008 et 2010. Des surfaces de colonisation similaires ont été observées durant les périodes 2006-2008 (23 m<sup>2</sup>) et 2008-2010 (19 m<sup>2</sup>) quand une disparition plus importante de l'herbier a par contre été observée durant cette dernière période (24 m<sup>2</sup> > 13 m<sup>2</sup>) et plus particulièrement au niveau de sa partie la plus profonde.

**Tab.1: Coordonnées géographiques et caractéristiques des herbiers de *posidonie* cartographiés durant l'étude. Les types d'herbiers et de limites sont classés d'après Meinez et Laurent (1978) et Pergent et al. (2008).**

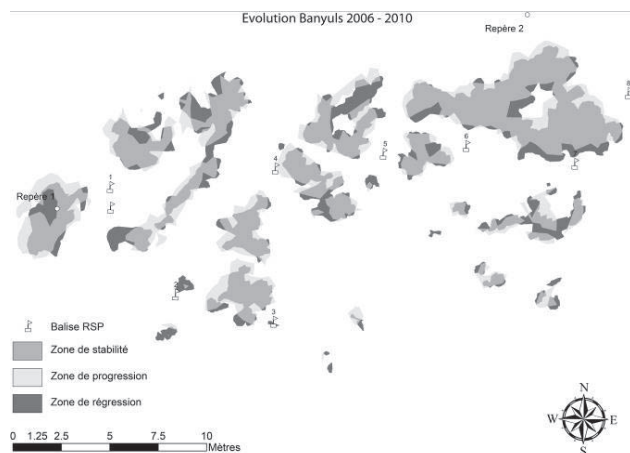
Site	Date	Coordonnées	Type d'herbier	Type de limite	Limite inférieure
1. Site Natura 2000 d'Antibes	Juin 2008	43 32'44.7"N 07 06'21.8"E	continu	régressive	28.7 m
2. Site Natura 2000 d'Esterel	Juin 2008	43 26'53.6"N 06 55'42.4"E	continu	régressive	27.5 m
3. par national de Port Cros	Juin 2008	43 00'496"N 06 21'426"E	continu	franche, faible recouvrement	31.2 m
4. Site Natura 2000 de La Ciotat	Juin 2008	43 10'254"N 05 39'470"E	continu	éparse	32.1 m
5. Marseille	Avril 2008	43 18'43"N 05 21'17"E	en tâche	franche, fort recouvrement	20.3 m
6. Parc marin « côte bleue »	Mai 2008	43 19'11"N 05 09'37"E	continu	régressive et éparse	30.2 m
7. Site Natura 2000 de Palavas-les-flots	Août 2006	43 32'11"N 04 01'48"E	en tâche	régressive	10 m
8. Site Natura 2000 du Cap d'Agde	Juillet 2008	43 16'22"N 03 28'04"E	en tâche	franche, fort recouvrement	7 m
9. Reserve marine de Cerbère/Banyuls sur mer	Août 2006 Juillet 2008 Juin 2010	42 27'45"N 03 09'43"E	en tâche	régressive	19.6 m



**Fig. 1: Evolution de l'herbier de *Posidonia oceanica* cartographié par télémétrie acoustique dans la réserve marine de Cerbère/Banyuls-sur-mer durant la période 2006-2008. Progression en gris clair, régression en noir, stabilité en gris foncé. Les repères posés durant l'étude ainsi que les balises RSP sont reportées sur les cartes.**



**Fig. 2:** Evolution de l'herbier de *Posidonia oceanica* cartographié par télémétrie acoustique dans la réserve marine de Cerbère/Banyuls-sur-mer durant la période 2008-2010. Progression en gris clair, régression en noir, stabilité en gris foncé. Les repères posés durant l'étude ainsi que les balises RSP sont reportées sur les cartes.



**Fig. 3:** Evolution de l'herbier de *Posidonia oceanica* cartographié par télémétrie acoustique dans la réserve marine de Cerbère/Banyuls-sur-mer durant la période 2006-2010. Progression en gris clair, régression en noir, stabilité en gris foncé. Les repères posés durant l'étude ainsi que les balises RSP sont reportées sur les cartes.

## Discussion

La méthode de cartographie par télémétrie acoustique montre une bonne précision dans les conditions où nous l'avons utilisée (< 10 cm pour un pointeur à moins de 60 m de la base). Nos résultats montrent une bonne efficacité de la méthode grâce au grand nombre de points relevés en un temps relativement court (quelques heures par site pour enregistrer environ 900 points espacés d'environ 40 cm). Contrairement à la méthode RSP, tous les faisceaux d'une limite régressive peuvent être précisément suivis. De plus, la rapidité de mise en œuvre sur le terrain rend la méthode relativement peu coûteuse (pointage rapide, peu de travail de balisage) pour un gain scientifique énorme (données quantitatives, précision). La variété des herbiers microcartographiés (en tâche, à limite franche ou éparse, etc. avec 137 à 1243 points) a permis de tester et de valider l'adaptabilité de la télémétrie acoustique aux différents types de limites. Le suivi temporel sur quatre ans faisait suite à un suivi RSP de onze ans (1989-2000) qui montrait une régression de 11% (Descamp *et al.*, 2007). Nos résultats sont en faveur d'un herbier stable voire en légère progression malgré l'alternance entre période de progression et régression. Toutefois, nos résultats montrent également comment l'herbier peut rapidement se trouver érodé ; la régression plus importante (et la disparition de la plupart des balises RSP) observée entre 2008 et 2010 est à mettre en relation avec deux tempêtes exceptionnelles (Décembre 2008 et Klaus en Janvier 2009, données de l'observatoire océanologique de Banyuls sur mer <http://observation.obs-banyuls.fr/>).

## Conclusion

Le suivi des herbiers de posidonie par télémétrie acoustique produit des microcartographies précises, répliquables et aisées d'interprétation. Associées à des mesures de recouvrement, biométrie, densité et déchaussement des rhizomes (données non montrées ici), elles permettent un suivi complet de l'herbier, facilement répliquable dans le futur et constituant d'ores et déjà un état de référence. Ceci explique sans doute que les gestionnaires en fassent une utilisation de plus en plus répandue puisque 22 balisages sont d'ores et déjà en place sur le littoral français et dix autres prévus à court terme.

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## **ALGAL-DOMINATED SEASCAPES FROM THE CONTINENTAL SHELF OFF MALLORCA AND MENORCA (BALEARIC ISLANDS, WESTERN MEDITERRANEAN)**

### **Abstract**

*Current knowledge of deep-water algal Mediterranean seascapes is scarce due to the difficulty of acquiring samples at depths below -50 m. In fact, most available information involves only floristic data, and information about the species composition and structure of the different seascapes is lacking. Therefore, the aim of the present study was to describe the different algal seascapes of the deep Mediterranean continental shelf from Mallorca and Menorca (Balearic Islands, Western Mediterranean) according to their species composition and abundance. A total of 31 samples were obtained in June 2007 and June 2008 by means of bottom trawl surveys between -50 and -100 m depth. Nine different seascapes were identified using nMDS and SIMPROF. Four of these seascapes were situated between -50 and -70 m depth, and were dominated by the erect red algae *Phyllophora crispa* and *Osmundaria volubilis*; they differed each other by the relative abundance of accompanying species. The rest of identified seascapes developed at higher depths (-71 to -100 m); three of them were also dominated by *P. crispa* and *O. volubilis*, one was mainly composed by crustose corallines and was almost devoid of erect algae, and the last one was dominated by the Mediterranean endemic kelp *Laminaria rodriguezii*. These results show that the continental shelf off Mallorca and Menorca is composed of patches of different algal-dominated assemblages.*

**Key words:** Algae, seascape, deep-water, Western Mediterranean.

### **Introduction**

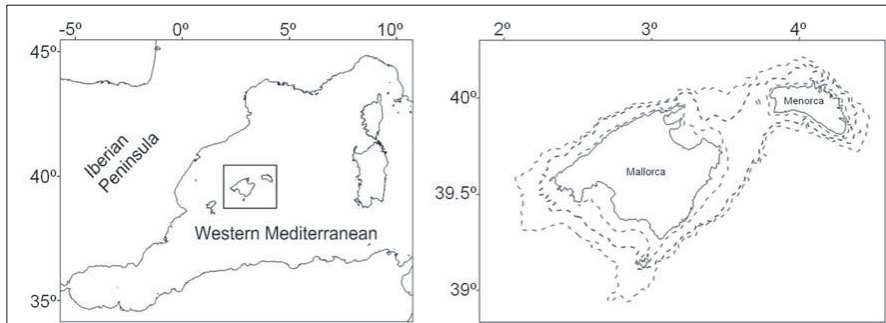
Deep-water algal-dominated seascapes, growing below -50 m, are poorly known due to the difficulty of sampling (Ballesteros, 1990; Spalding *et al.*, 2003), although some extensive studies at community level have been previously performed using SCUBA diving (e.g. Ballesteros, 1988; 1990; 1994; Giaccone, 1967). Contrastingly, studies related with seascapes are scarce and mainly based in floristic data (Pérès & Picard, 1964; Spalding *et al.*, 2003).

Scientific SCUBA diving is limited in deep waters due to the risk associated to nitrogen narcosis and long decompression times. Instead, bottom trawl methods allow sampling in much deeper waters although their use has to be restricted to the seascape description and can not be used at community level since sampling area is usually too big.

The main goal of this study was to use bottom trawl samples to describe the main coastal detritic bottom assemblages representative of the deep-water continental-shelf seascapes from Mallorca and Menorca (Balearic Islands, Western Mediterranean).

## Materials and methods

The present study was located in the continental shelf off Mallorca and Menorca (Fig. 1). Due to their bathymetry and distance to the continent, these two islands can be considered as a geographical region isolated both from the Iberian Peninsula and from the shelf of the other Balearic Islands, Eivissa and Formentera (Massutí & Reñones, 2005).



**Fig. 1:** Maps of the studied area off Mallorca and Menorca (Balearic Islands, Western Mediterranean). At right, the -50, -100 and -200 m isobaths are displayed.

Thirty one samples were collected during the MEDITS\_ES05 bottom trawl surveys in June 2007 and June 2008, on board of the R/V Cornide de Saavedra, at depths ranging from -50 to -100 m. Samples were preserved in 4% formalin with sea water and once at the laboratory, they were sorted and identified at the lowest taxonomic level. Abundance was measured as algal surface in cm<sup>2</sup> according to Ballesteros (1992a).

Similarity matrix was constructed with untransformed data and Bray-Curtis similarity index. Non-parametric multidimensional scaling (nMDS) was performed to visualize patterns of samples similarities (Kruskal & Wish, 1978). A hierarchical group average agglomerative clustering method accompanied by SIMPROF test (Clarke *et al.*, 2008) was used to explore potential grouping structures among samples. Finally, similarity of percentages routine (SIMPER) was run in order to quantify the contribution of each species to the similarity/dissimilarity between the different groups identified by the SIMPROF test. All the analyses were performed with the PRIMER v.6 software package (Clarke & Warwick, 2001).

## Results

A total of 142 taxa were identified, 121 of them to species level. Rhodophycophyta was the best represented group, with 113 identified taxa, followed by Heterokontophycophyta with 21 taxa and Chlorophycophyta with 8 taxa. The most abundant species were *Laminaria rodriguezii*, *Phyllophora crispera*, *Osmundaria volubilis* and *Halopteris filicina*.

The nMDS on species data showed patterns of resemblances in community structure among the samples. The SIMPROF analysis indicated nine groups of samples (Fig. 2), and according to the SIMPER test, *P. crispera*, *O. volubilis*, *H. filicina*, *L. rodriguezii* and *Spongites fruticulosus* were the species with the highest contribution to the similarity/dissimilarity between the different groups.

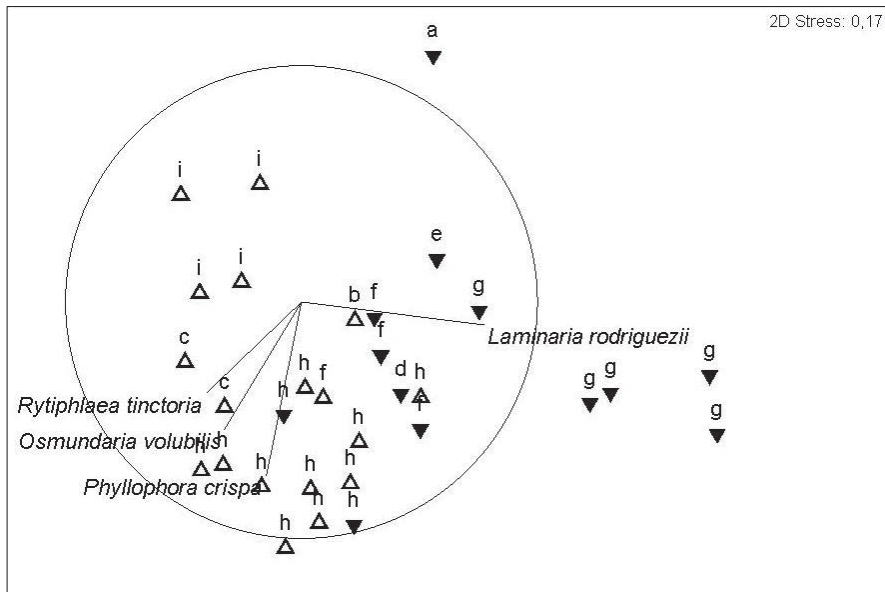


Fig. 2: nMDS ordination. White triangles indicate depths between -50 and -70 m and black triangles, depths between -71 and -100 m. Letters indicate SIMPROF significant groups. Species with a Pearson correlation > 0.5 are represented as overlaid vectors (full line circle,  $r=1$ ).

At shallower depths (-50 to -70 m), four different groups of samples were found (groups b, c, h and i). All these groups corresponded to a seascape dominated by the erect red algae *P. crispa* and *O. volubilis* and differed each other by the relative abundance of accompanying species. Group h was mainly characterized by *P. crispa*, *O. volubilis* and *Cryptonemia tuniformis*. Group b was differentiated mainly by the presence of the invasive species *Acrothamnion preissii*, and also by *L. rodriguezii*. Group c differed by the presence of *Dictyota dichotoma* and *Rytiphlaea tinctoria*. And finally, *Peyssonnelia inamoena*, *Codium bursa*, *C. tuniformis*, *S. fruticosus*, *A. preissii* and *H. filicina* distinguished group i.

At deeper waters (-71 to -100 m), five groups of samples were distinguished (groups a, d, e, f and g). Group a corresponded to a maërl seascape dominated by *S. fruticosus* and with few erect algae. Groups e and g were characterized by the deep-water kelp *L. rodriguezii*. The rest of the groups corresponded to the same seascape found at shallower depths, dominated by *P. crispa* and *O. volubilis*, and differing on the abundance of other accompanying species: group d was distinguished by *Flabellia petiolata*, *L. rodriguezii*, *Aeodes* sp. and *S. fruticosus*; and group f, by *F. petiolata* and *H. filicina*.

### Discussion and conclusions

All the groups found at shallow depths (b, c, h, i) and two of the ones found at deep waters (groups d, f) corresponded to the facies of *O. volubilis* described by Pérès & Picard (1964) or to the shallow communities dominated by *O. volubilis* described by Serio & Pizzuto (1990; 1992) and Ballesteros (1992b). The presence of other species at

local scale, such as *D. dichotoma*, *F. petiolata* or *Peyssonnelia* spp., explained the differences observed within the six different groups included in this seascape. Some of these variations have been previously described in other works (e.g. *Peyssonnelia* beds; Ballesteros, 1994; Pérès & Picard, 1964).

The seascape dominated by *S. fruticulosus* (group a) corresponded to the facies of maërl (Pérès & Picard, 1964) previously studied by other authors (e. g. Jacquotte, 1962; Augier & Boudouresque, 1978; Ballesteros, 1988), and presented a bathymetric distribution similar to that of other Mediterranean maërl beds (Ballesteros, 1988; Ballesteros *et al.*, 1993; Bordehore *et al.*, 2000). These assemblages were characterized by crustose corallines accounting for more than 90% of the abundance of the samples, and had few erect species. Similarly, Ballesteros (1988) observed that Corallinales and *Peyssonnelia* spp. were the most abundant taxa in the maërl beds of Tossa de Mar (NE Spain), and that in those bottoms erect species were not relevant.

Finally, the groups g and e were characterized by the kelp *L. rodriguezii*. This species was accompanied by *H. filicina* in low abundances in samples from the first group while in group e higher values were found. *Laminaria rodriguezii* bottoms are poorly known (Feldmann, 1934; Giaccone, 1967); however, as it was highlighted by these authors, *L. rodriguezii* can be found in zones with unidirectional currents and low water temperatures. Moreover, probably its upper bathymetric limit is -60 m while its lower limit depends on light conditions (UNEP/UICN/GIS Posidonie, 1990).

This work provides a first approach in the description of detritic coastal seascapes developing at depths ranging from -50 to -100 m in the Balearic Islands. Maërl beds, kelp forests with *Laminaria rodriguezii* and *Phyllophora crispa-Osmundaria volubilis* beds are here distinguished as the main seascapes. *P. crispa* and *O. volubilis* beds include some assemblages already described in the Western Mediterranean Sea such as those dominated by *Peyssonnelia* spp. At this moment we are not able to provide any explanation for the high variability observed in our samples, not even to suggest which are the environmental factors accounting for much of this observed variability. Further studies will be necessary for a better understanding of the processes shaping these deep-water assemblages and seascapes in the Mallorca-Menorca shelf and even in the whole Mediterranean Sea.

### Acknowledgments

This study was supported by the EVADEMED and INDEMARES projects, financed by the Spanish Institute of Oceanography (IEO) and the European Union (EU). EC was funded by a Juan de la Cierva postdoctoral fellow (MICINN). We acknowledge Instituto Español de Oceanografía (IEO) for the organization and provision of all facilities needed for the sampling surveys. We also thank to all the crew of the R/V Cornide de Saavedra, all the participants who took part in the MEDITS surveys, and to Natàlia Comalada and Núria Orra who help in laboratory tasks.

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## **SUIVI DE L'EXPANSION DE *CAULERPA TAXIFOLIA* LE LONG DU LITTORAL TUNISIEN : SITUATION AU 30 AVRIL 2009**

### **Résumé**

*Découverte pour la première fois sur la rive sud de la Méditerranée en Tunisie en 2000, Caulerpa taxifolia (Caulerpaceae, Bryopsidales) n'est signalée à ce jour que dans deux zones du littoral tunisien : la rade de Sousse (centre-est) et la région du Cap Bon (nord-est). La mise à jour en 2009 de la carte de répartition de cette espèce, dressée initialement en 2003, a révélé que les surfaces concernées par la présence de C. taxifolia sont maintenant évaluées à 50.8 km<sup>2</sup> dans la rade de Sousse et à 39.7 km<sup>2</sup> au Cap Bon. Le linéaire de côte est respectivement de 16 km et de 28 km. Dans la rade de Sousse l'extension est évaluée à 226 % en termes de superficie concernée et à 230 % en termes de linéaire côtier, tandis qu'au Cap Bon, l'extension n'est que de 76 % en termes de surface, et de 95 % en termes de linéaire côtier. La présence d'herbiers à Posidonia oceanica aux limites des zones concernées serait un facteur limitant de la progression naturelle de C. taxifolia.*

**Mots clés :** *Caulerpa taxifolia*, cartographie, distribution, Tunisie

### **Introduction**

Observée en Tunisie pour la première fois en 2000 dans la rade de Sousse (centre-est de Tunisie), la Chlorobionte *Caulerpa taxifolia* (Vahl) C. Agardh (Langar *et al.*, 2000) fait, depuis, l'objet d'une surveillance de la part de l'Institut National des Sciences et Technologies de la Mer de Tunisie (INSTM). Cette surveillance consiste à répertorier, localiser, cartographier et décrire les nouvelles colonies puis suivre leurs évolutions au fil du temps.

L'objectif de ce papier est de dresser un état des lieux de l'expansion de *Caulerpa taxifolia* le long du littoral tunisien arrêté au 30 avril 2009.

### **Matériel et méthodes**

#### **Collecte des données biogéographiques**

Conformément au protocole du réseau de surveillance des caulerpes mis en place en Tunisie depuis l'année 2000 (Langar *et al.*, 2007), il n'est considéré comme zone concernée par la présence de *Caulerpa taxifolia* que (i) les zones signalées auprès de l'organisme officiel chargé du suivi de l'expansion des caulerpes le long des côtes tunisiennes, *i.e.* l'INSTM, (ii) les zones citées dans des publications scientifiques nationales ou internationales après la vérification des signalisations sur le terrain, et (iii) les zones découvertes par le groupe de travail chargé de la surveillance des Caulerpes en Tunisie.

### **Cartographie des zones concernées**

La cartographie de *C. taxifolia* a été réalisée courant les mois de Mars et Avril 2009, suite à des prospections sous marines et à des prospections de surface, par observation des fonds marins à l'aide d'un sseau de Calfat à partir d'une embarcation. Les prospections sous marines ont eu lieu dans les zones où la profondeur dépasse les 2 mètres.

Les positions géographiques des colonies de Caulerpe les plus périphériques des zones concernées délimitées par Langar *et al.* en 2003 (Langar *et al.*, 2006) ont été les points de départ des prospections menées lors de la présente étude. Les prospections ont été réalisées selon des transects perpendiculaires à la côte et des trajectoires parallèles au rivage. A partir d'une ancienne position périphérique de Caulerpe, un plongeur confirmé est tracté perpendiculairement à la côte soit vers le large, si le point de départ est une limite inférieure de l'ancienne zone concernée, soit vers la côte si le point de départ est une limite supérieure. Quand le point de départ est une limite d'expansion latérale, le plongeur est tracté parallèlement à la côte du côté opposé à l'ancienne zone concernée. Le long du parcours du plongeur tracté, toute colonie de *C. taxifolia* est marquée par un signal rattaché à un flotteur visible de la surface. En surface, les positions géographiques des flotteurs signalant les colonies les plus périphériques sont acquises à l'aide d'un GPS type Garmin 12<sup>TM</sup> et reportées sur une carte afin de délimiter les nouvelles zones concernées. Quand la profondeur devient inférieure à deux mètres, la prospection de surface se substitue à la prospection sous marine. La prospection le long d'une trajectoire donnée est arrêtée 100 mètres après la dernière colonie de *Caulerpa* trouvée le long de cette trajectoire.

### **Résultats et discussion**

A ce jour, seules les régions du Cap Bon (nord-est de la Tunisie) et de Sousse (centre-est de la Tunisie), déjà signalées depuis 2001 (Langar *et al.*, 2006) sont concernées par la présence de *C. taxifolia*. Il est cependant à souligner que de nombreuses signalisations relatives à *Caulerpa racemosa* ou à des colonies de *C. taxifolia* déjà répertoriées nous sont parvenues de la part de pêcheurs, de plaisanciers et de diverses autorités portuaires. Ceci témoigne de la bonne prise de conscience, du moins, par les usagers de la mer (professionnels et plaisanciers) du phénomène de l'introduction des Caulerpes envahissantes.

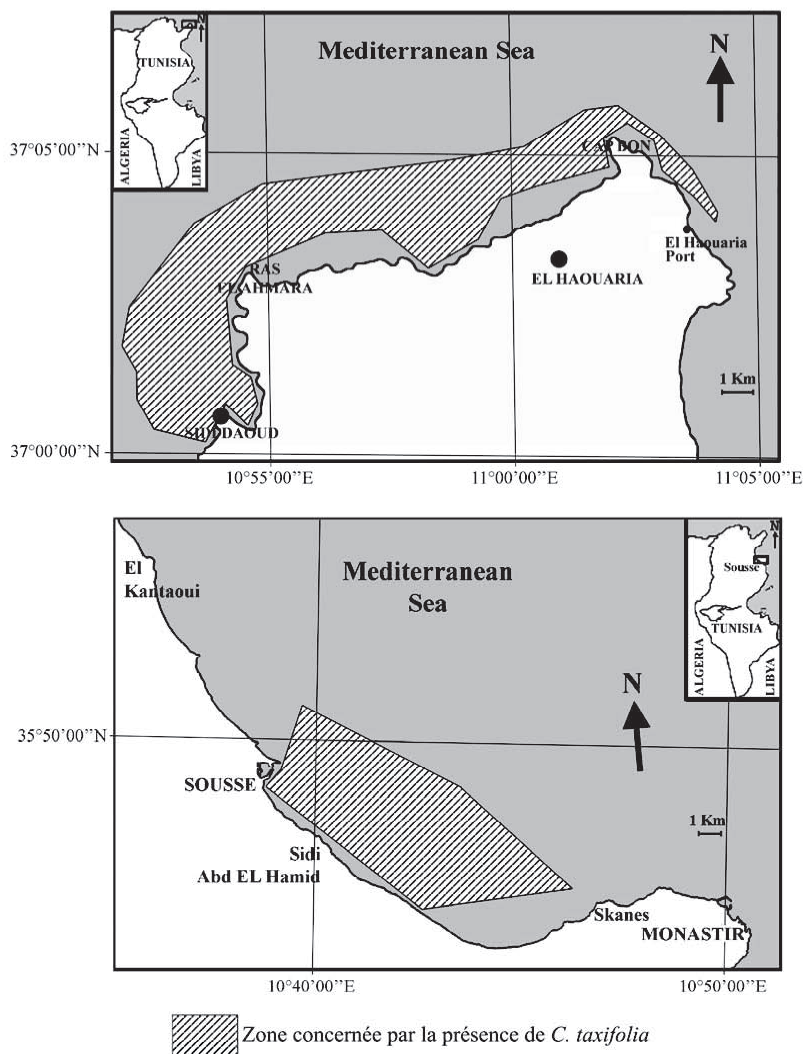
La distribution mise à jour de *C. taxifolia* dans la rade de Sousse et au Cap Bon est présentée dans la figure 1. Dans les deux régions, la superficie concernée a nettement augmenté depuis 2003, passant de 15,6 km<sup>2</sup> à 50, 8 km<sup>2</sup> dans la rade de Sousse et de 22,5 km<sup>2</sup> à 39,7 km<sup>2</sup> au Cap Bon soit une augmentation respective de la surface de 226% et de 76%.

La limite profonde de la répartition de *C. taxifolia* est demeurée inchangée, située à 50 m au Cap Bon et à 20 m dans la rade de Sousse. Par contre, la limite superficielle a nettement diminuée de profondeur devenant par endroit, et dans les deux zones, située à moins de 1 mètre de profondeur, reflétant une progression de l'algue vers la côte. Cette progression vers la côte a été probablement facilitée par le transport de proche en proche, par les courants et les vagues, de boutures arrachées soit par des opérations d'ancrages, soit par des opérations de pêches ou suite à des tempêtes. A cette progression vers la côte, s'est rajoutée une progression latérale de part et d'autre des zones atteintes, les côtés situés sous les courants progressant plus vite, *i.e.*, Sud pour la zone de Sousse, étendant la zone concernée à presque la totalité de la baie de Sousse, et



Sud-Ouest pour la zone du Cap Bon, étendant la zone concernée jusqu'au sud du port de Sidi Daoud.

Dans la région du Cap Bon, les quatre stations décrites par Langar *et al.*, (2006) se sont rejointes pour ne constituer à présent qu'une unique station au sens de de Vaugelas *et al.*, (1999). Les colonies de *Caulerpa taxifolia* apparues dans les zones qui séparaient les anciennes stations seraient le résultat d'une dissémination anthropique liée à l'intense activité de pêche au filet dans la région.



**Fig. 1: Localisation de *Caulerpa taxifolia* dans les régions du Cap Bon (carte du haut) et de Sousse (carte du bas).**



En termes de linéaire côtier concerné, l'extension enregistrée dans la région de Sousse a été de l'ordre de 11,5 km soit un accroissement de 230 % depuis l'année 2003. L'algue ayant progressé dans presque la totalité de la baie de Sousse, le linéaire côtier mesuré par projection sur la côte de la nouvelle zone concernée s'est retrouvé exagérément accru. Cet accroissement bien que relativement important ne devrait donc pas être interprété par une expansion de *C. taxifolia* dans une grande zone géographique. Au Cap Bon, l'extension du linéaire côtier concernée d'environ 13,8 km (soit un accroissement de 95 % depuis l'année 2003), bien que peu importante par rapport à celle enregistrée à Sousse, se traduit par une expansion de la zone concernée sur une zone géographique relativement étendue dans la région.

La vigueur et l'importante vitesse de croissance de *Caulerpa taxifolia* vérifiées dans les conditions de la rade de Sousse (Langar *et al.*, 2006), et les températures saisonnières de l'eau de mer dans les zones concernées en Tunisie (12 à 25°C) favorables à la croissance de l'espèce, laissaient présager une expansion naturelle de l'algue, à moyen terme, beaucoup plus importante que celle observée actuellement. L'expansion de *Caulerpa taxifolia* semble donc avoir été ralentie. Au Cap Bon, l'algue aurait atteint son aire d'expansion extrême en profondeur (50 m) et ne semble guère pouvoir continuer à progresser, probablement en raison d'une mauvaise pénétration de la lumière dans la colonne d'eau. Dans sa progression latérale, *C. taxifolia* semble aussi avoir été freinée. Elle le serait en partie, à la présence d'herbiers à *Posidonia oceanica*, qui selon Ben Mustapha et Hattour (1992) sont :

- en bon état, de type 2 et 3 vers l'ouest de Sidi Daoud et s'étendant jusqu'à des profondeurs de 20 m, ce qui correspond aux limites ouest/sud-ouest de la zone concernée par la présence de *C. taxifolia* dans la région du Cap Bon,
- en très bon état, de type 3, atteignant des profondeurs de 25 m sur la côte est du Cap Bon et sur les fond de 28 à 35 m à El Haouaria, ce qui correspond respectivement aux limites est et sud-est de la zone concernée du Cap Bon.

Dans la zone de Sousse, malgré la présence d'un herbier qualifié par Ben Mustapha et Hattour (1992) d'herbier en bon état de type 2 et 3, presque la totalité de la baie de Sousse - de la jetée-abri du port de Sousse au port de plaisance de Skanès (Fig. 1, carte du haut) et de 0 à l'isobathe -20 m - a été envahie par *Caulerpa taxifolia*. Cet envahissement ne peut en aucun cas être expliqué par la seule progression naturelle de l'algue ; il serait favorisé par les activités de pêche artisanale, le secteur envahi étant une pêcherie fréquentée par les artisans pêcheurs de Sousse. Au nord-est de ce secteur, très peu fréquenté par les pêcheurs, et au-delà de l'isobathe -20 m, *Caulerpa taxifolia* a très peu progressé naturellement probablement en raison de la présence d'un herbier à *Posidonia oceanica* jugé en bon état par Ben Mustapha et Hattour (1992). L'hypothèse du ralentissement de la progression de *C. taxifolia* par les Herbiers de posidonies pourrait être conforté par Pergent *et al.*, (2008) qui rapportent qu'en cas de confrontation, bien que dotée d'un potentiel de croissance élevé, *C. taxifolia* par ses frondes plus courtes que les feuilles de posidonies serait incapable de rivaliser avec *Posidonia oceanica* pour la ressource lumineuse indispensable au développement des organismes photosynthétiques.

Ainsi, à la fin du mois d'Avril 2009, la Tunisie se retrouve avec une superficie concernée globale d'environ 90,5 km<sup>2</sup> devant un linéaire côtier total de 44 km. Cette superficie est répartie entre deux stations classées niveau III sur l'échelle de colonisation de de Vaugelas *et al.*, (1999) rendant sa cartographie avec précision et l'estimation des superficies réellement couvertes par l'algue impossibles.

## Conclusion

La présente situation montre clairement que depuis son introduction, *Caulerpa taxifolia* n'a pas cessé de progresser. Une grande part de sa progression semble être liée à des activités anthropiques, essentiellement en relation avec la pêche artisanale, et ce malgré l'effort de sensibilisation aux modes de disséminations anthropiques de l'algue. La présence d'herbiers à *Posidonia oceanica* en bon état serait un facteur limitant de la progression naturelle de *C. taxifolia*. Le suivi de cette progression et l'étude des facteurs la limitant devraient être poursuivis et approfondis.

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## **VARIATIONS DES PARAMETRES BIOMETRIQUES DE LA BRYOPSIDOPHYCEAE CAULERPA RACEMOSA VAR. CYLINDRACEA EN INTERACTION AVEC LA MAGNOLIOPHYTE MARINE POSIDONIA OCEANICA**

### **Abstract**

*In the region of Cape Zebib (North East of Tunisia), we studied the biometric parameters of the introduced species *Caulerpa racemosa* var. *cylindracea* in interaction with the native species *Posidonia oceanica*. In order To identify the different parameters, a seasonal sampling was conducted between April 2009 and April 2010. It appears that the biometrics *C. racemosa* var. *cylindracea* shows a significant variation in function of the season and in interaction with *P. oceanica*. Whatever, the station (with, or without interaction), *C. racemosa* presents a maximum development in autumn (October), and a minimum marked by zero values in winter (February). On the other hand, the maximum biomass, density and height of the fronds are recorded when the interaction is zero: fresh weight 639.9g / m<sup>2</sup>, 4512.5 fronds / m and 2.9 cm, while, we note that, only 391.3g fresh material / m<sup>2</sup>, 3112.5 fronds / m and 1.8 cm in height during an interaction. In the studied site, the development of *C. racemosa* var. *cylindracea* is lower in the herbarium of *P. oceanica* than outside.*

### **Résumé**

*Dans la région de Cap Zebib (Nord Est de la Tunisie), nous avons étudié les paramètres biométriques de l'espèce introduite *Caulerpa racemosa* var. *cylindracea* en interaction avec l'espèce indigène *Posidonia oceanica*. Afin d'identifier les différents paramètres, un échantillonnage saisonnier a été effectué entre avril 2009 et avril 2010. Il apparaît que la biométrie de *C. racemosa* var. *cylindracea* présente des variations significatives en fonction de la saison et en interaction avec *P. oceanica*. Quelle que soit la station (avec ou sans interaction), *C. racemosa* présente un développement maximum en automne (octobre) et un minimum marqué par des valeurs nulles en hiver (février). D'autre part, les maxima de biomasse, de densité et d'hauteur des frondes sont enregistrés lorsque l'interaction est nulle à savoir, 639.9g de matière fraîche/m<sup>2</sup>, 4512.5 frondes/m<sup>2</sup> et 2.9 cm de hauteur, tandis qu'on note uniquement 391.3g de matière fraîche/m<sup>2</sup>, 3112.5 frondes/m<sup>2</sup> et 1.8 cm de hauteur lors d'une interaction. Dans le site étudié, le développement de *C. racemosa* var. *cylindracea* est plus faible dans l'herbier de *P. oceanica* qu'en dehors.*

**Key-words:** *C. racemosa* var. *cylindracea*; biometric parameters; interaction; *P. oceanica*.

### **Introduction**

*Caulerpa racemosa* (Forsskål) J. Agardh var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque a été observée pour la première fois au large des côtes de la Lybie (Nizamuddin, 1991) et depuis, elle ne cesse de s'étendre progressivement à la quasi-totalité de la Méditerranée (Piazzi et al., 2005). Elle est considérée comme envahissante par de nombreux auteurs (Piazzi et al. 2005; Verlaque et al. 2003 et 2004 ;). Elle présente toutefois, un cycle saisonnier marqué, avec une période de croissance maximale en automne et une nette régression en hiver (Mezgui et al., 2007; Piazzi & Cinelli, 1999). D'autre part, la quasi-totalité des études menées concernant la biomasse

et les caractéristiques morphométriques, montrent une grande variabilité en fonction de la profondeur et de la nature du substrat. Les fonds détritiques côtiers (Klein & Verlaque, 2009) et la matte morte de *Posidonia oceanica* (L.) Delile (Mezgui et al., 2010; Piazzini et al., 1997 ; Tolay et al., 2001) semblent constituer des biotopes très favorables à l'extension de *C. racemosa*. Dans la perspective de contribuer à la connaissance du développement de la variété invasive de *C. racemosa* en interaction avec la Magnoliophyte marine *Posidonia oceanica*, nous avons effectué une étude biométrique comparative de deux populations de *C. racemosa* var. *cylindracea*, une se développant à l'intérieur d'un herbier de *Posidonia oceanica* et l'autre à l'extérieur.

### Matériel et Méthodes

Cette étude a été réalisée dans la région de Cap Zebib (Nord Est de la Tunisie) où les populations de *C. racemosa* var. *cylindracea* se développent à la fois à l'intérieur (C+P) et à l'extérieur (C-P) de l'herbier de *Posidonia oceanica* (Fig. 1.). Ces deux stations sont situées à des profondeurs comprises entre 0.3 et 1 m. Des prélèvements mensuels ont été effectués entre avril 2009 et avril 2010. Les récoltes sont réalisées à l'intérieur d'un cadre métallique de 400 cm<sup>2</sup> de surface. Au laboratoire, les thalles sont préalablement soigneusement séparés des débris organiques et des autres végétaux présents dans les relevés. Nous avons par la suite procédé aux mesures des différents paramètres biométriques (biomasse, nombre et hauteur des frondes).

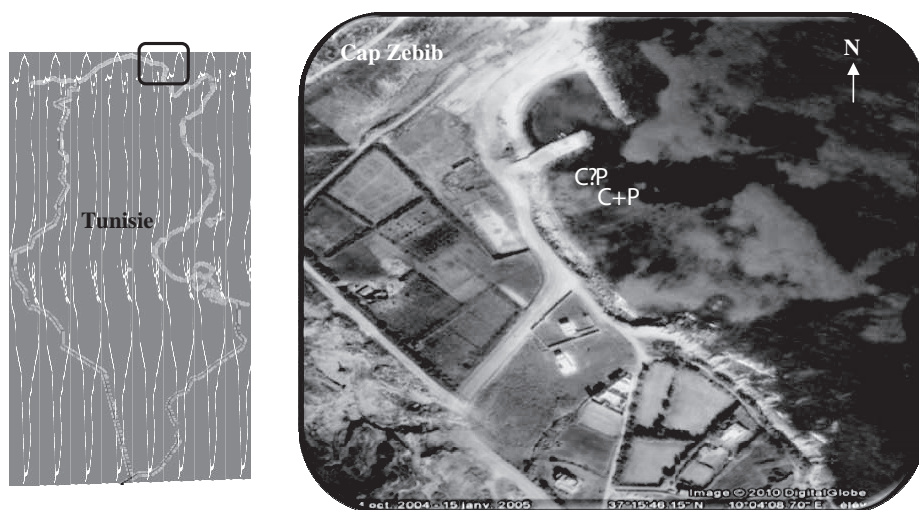


Fig. 1 : Localisation des stations de prélèvement

### Résultats

Les estimations mensuelles de la biomasse de *C. racemosa* avec ou sans interaction révèlent une période de disparition macroscopique totale des thalles de l'espèce en hiver

(février) et d'une période de croissance des thalles qui atteint un maximum en automne (octobre), soit une valeur de  $639.9 \pm 58.3 \text{ g/m}^2$  du poids frais pour (C-P) et  $391.3 \pm 71.2 \text{ g/m}^2$  du poids frais pour (C+P) (Fig. 2).

On note aussi que le nombre total moyen de frondes par  $\text{m}^2$  (Fig. 3) atteint un maximum au mois d'octobre de  $4512.5 \pm 526 \text{ frondes/m}^2$  pour (C-P) et de  $3112.5 \pm 408.2 \text{ frondes/m}^2$  pour (C+P).

La hauteur moyenne des frondes (Fig. 4) présente un maximum de  $2.9 \pm 0.5 \text{ cm}$  pour (C-P) au mois de juin et de  $1.8 \pm 0.3 \text{ cm}$  pour (C+P) au mois d'avril.

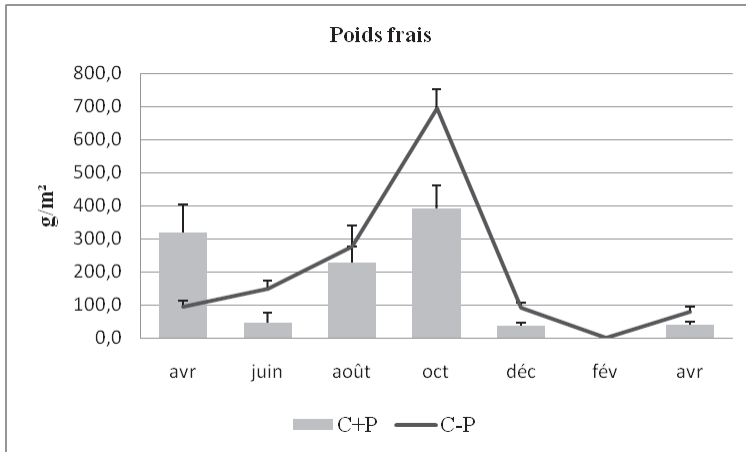


Fig. 2 : variations mensuelles du poids frais de *C. racemosa var. cylindracea*

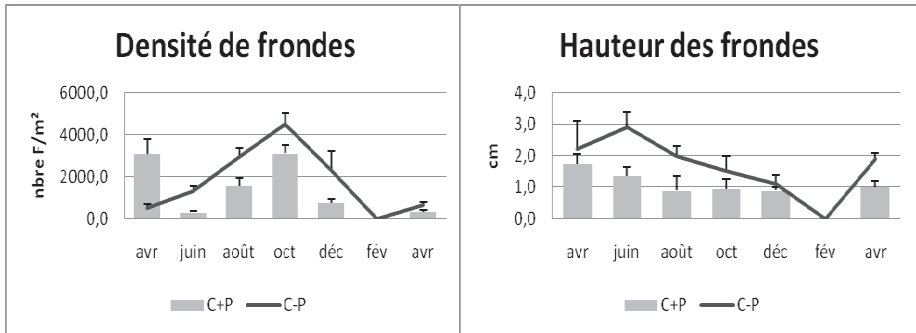
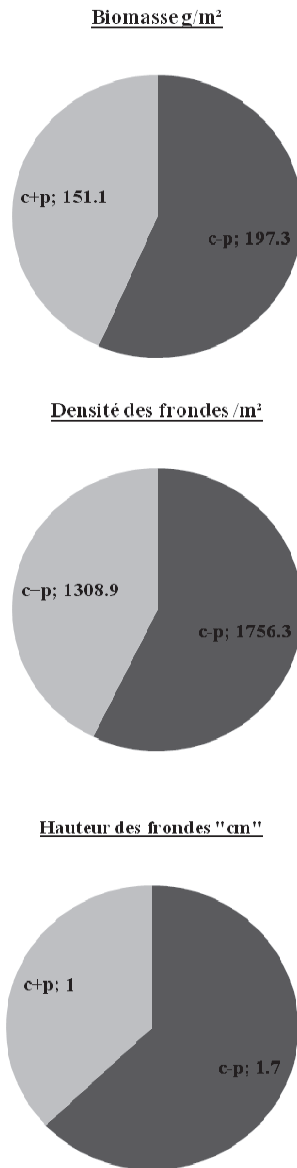


Fig. 3 : Variations mensuelles de la densité des frondes de *C. racemosa var. cylindracea*

Fig. 4 : Variations mensuelles de la hauteur des frondes de *C. racemosa var. cylindracea*

On note ainsi, que les biomasses annuelles cumulées ainsi que la densité et la hauteur des frondes de *C. racemosa var. cylindracea* récoltée dans l'herbier montrent des valeurs supérieures par rapport à ceux des caulerpes récoltées à l'extérieur de l'herbier (Fig. 5).



*Fig. 5 : Variations annuelles des paramètres biométriques de C. racemosa var. cylindracea*

## Discussion et Conclusion

La comparaison des paramètres biométriques des deux populations de *C. racemosa* var. *cylindracea* avec ou sans interaction avec l'herbier de *P. oceanica*, permet de révéler les points suivants :

- Dans les deux cas, *C. racemosa* présente un cycle saisonnier marqué, avec une période de croissance maximale en automne et une période de disparition macroscopique totale en hiver ;
- Dans l'herbier de *P. oceanica*, les valeurs des paramètres biométriques de *C. racemosa* sont plus faibles, contrairement aux observations de Dumay (2002) qui note une augmentation de la hauteur des frondes dans un herbier à 2 m de profondeur.

Ces variations de réponse de *C. racemosa* peuvent être liées à de nombreux paramètres comme une profondeur très faible en Tunisie (0.3-1 m), des différences d'hydrodynamisme, des caractéristiques différentes de l'herbier.

La grande variabilité de *C. racemosa* var. *cylindracea* observée en fonction de la profondeur, de la nature du substrat et de l'interaction avec *Posidonia oceanica* mérite un suivi régulier et approfondi.

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THIS PAPER IS IN MEMORY OF THE LATE PROF. EUGENIO FRESI

## **NEW DESCRIPTORS TO EVALUATE THE CONSERVATION STATUS OF *POSIDONIA OCEANICA* MEADOW**

### **Abstract**

*Human activities and natural events are responsible for a widespread regression of the Posidonia oceanica (Magnoliophyta, Liliopsida) meadows. Early warning indicators are necessary to implement coastal management procedures to quickly guide managers in determining recovery actions. We recently proposed an innovative "diagnostic" tool to monitor the plant conservation status: the quantification of the phenols content, as a putative stress indicator, coupled to the 2-D electrophoresis analysis of proteins from rhizomes.*

*The increased concentration of total phenols in Posidonia rhizome represents a generic response to different kinds of environmental stress and phenols determination is an easy and rapid assay for the screening of the meadows health state. The pattern of protein expression, the proteome, is the entire set of proteins expressed in a given moment. It is a dynamic entity varying in response to endogenous or exogenous stimuli, such as changing environmental conditions.*

*In this work, by analysing 2005-2010 samples from S. Marinella (Roma, Italy) meadow, we demonstrate that the decreasing plant density has been physiologically marked by the increase of total phenols content. Peculiar 2D-electrophoresis protein maps are associated to the different phenols content, i.e. protein spots were differentially expressed at high or low phenols content. These data superimpose to an increased anthropic pressure in the area where a new harbor is under construction not so far (Civitavecchia, since 2005) and agricultural practices are continuously intensifying. Hence, the combined approach, based on phenols content determination and 2-D electrophoresis protein pattern analysis, is confirmed as a promising tool to monitor the health state of Posidonia meadows.*

**Key-words:** *Posidonia oceanica*, descriptors, 2-D electrophoresis analysis, total phenols, proteome

### **Introduction**

*Posidonia oceanica* (L.) Delile is the dominant endemic seagrass in the Mediterranean Sea, where it forms highly productive meadows playing a crucial role in coastal ecosystems dynamics (Boudouresque *et al.*, 2006). It is regarded as a keystone species, as it is listed in the Habitat Directive 92/43/EEC. According to the Water Framework Directive (WFD 2000/60/EC) and Marine Strategy Framework Directive (MSFD

2008/56/EC), *Posidonia oceanica* is recognized as a good biological indicator of marine environmental quality (Montefalcone, 2009).

Throughout most of the Mediterranean Sea, natural processes and human activities are responsible for a widespread meadows regression (Boudouresque *et al.*, 2006). Hence, the identification of early warning “diagnostic” tools to monitor meadows conservation status is a critical issue. In recent years, a new tool was proposed: quantification of phenolic compounds and 2-D electrophoresis protein analysis (Migliore *et al.*, 2007; Rotini *et al.*, 2010).

In *Posidonia oceanica* phenolic compounds have been quantified as a biochemical marker of environmental stress (total phenols determination is an easy and rapid assay), as in terrestrial plants (Dixon and Paiva, 1995). Phenolic compounds are a class of secondary metabolites widely distributed in all organs of both terrestrial (Bate-Smith, 1968; Karolewski and Giertych, 2000) and aquatic plants (Mc Clure, 1970; Pip, 1992; Zapata and McMillan, 1979). They have several structural and physiological roles, including plants defense.

In *Posidonia* higher phenols concentrations were found in leaves exposed to different environmental pressures: competition with the invasive seaweed *Caulerpa taxifolia* (Cuny *et al.*, 1995; Dumay *et al.*, 2004), contamination by heavy metals (Cannac *et al.*, 2006), proximity to intensive fish cages (Ferrat *et al.*, 2003). Nevertheless, in *Posidonia* leaves, which are temporary structures, total phenols concentration varies with depth and season (Dumay *et al.*, 2004; Fresi *et al.*, 2004). On the contrary, in rhizomes the levels of synthesis and accumulation of phenolic compounds are more stable (Fresi *et al.*, 2004; Migliore *et al.*, 2007). They represent a generic response to different kinds of environmental stress and can be utilized for the screening of the meadows health state.

The protein expression pattern is a dynamic entity varying from cell to cell; it is modulated by external and internal signaling and reflects changes in the physiological state. The proteomic approach, based on the simultaneous separation of hundreds of proteins in the same two dimensional electrophoretic gel (2-DE), represents a powerful tool to monitor the “health state” of ecosystems, allowing the comparative analysis of quantitative/qualitative changes in protein expression patterns in organisms living under different physiological and environmental conditions (Lopez, 2007; Nunn and Timpermann, 2007).

Proteomics have been applied to many different organisms (microbes, plants, animals) to obtain information on the protein profile changes as a response to different environmental conditions (Hu *et al.*, 2003; Lopez, 2007; Rossignol *et al.*, 2006;).

In the present study the combined approach phenols quantifications/2-DE has been challenged on samples collected from the S. Marinella meadow between 2005 and 2010. In this time period there has been an increased anthropic pressure in the area: a new harbor is under construction not so far (Civitavecchia, since 2005) and the agricultural activities are continuously intensifying.

## **Materials and methods**

### Study Site and Sampling

The study was conducted on the *Posidonia oceanica* meadow of Santa Marinella (Rome, Italy), a Site of Community Importance (according to Habitat Directive 92/43/EEC). This meadow spans from Capo Linaro to Santa Severa, for 13.5 km coastline covering a surface of 1,800 ha. It skirts coastline for the entire stretch, the lower limit is at -20 m depth. The great majority of the meadows lies on hard bottom.

The meadow can be considered a “pure bed” according to Pergent *et al.* (1995) classification, characterized by the presence of dead matte. Sampling was carried out in late spring from 2005 to 2010 by SCUBA diving in 20 stations (central area of the meadow), on a relatively homogeneous topography (from 7.5 to 13.5 m depth). In each station, at least 3 orthotropic rhizomes were collected for phenols determination and 2-D electrophoresis analysis. Data were analyzed by non parametric Mann-Whitney test.

#### Total phenols

Total phenols determinations were carried out in duplicate on 3 different rhizomes for sampling site, but in 2009 (2 rhizomes for sampling site), according to Migliore *et al.* (2007). The same experimental protocol was used for plants sampled in a reference meadow Talamone, (Grosseto, Italy; 30 sampling sites at comparable depth) in 2005. Phenolic compounds were extracted according to Legrand (1977), modified for *Posidonia*, on 100 mg (fresh weight) of basal, intermediate and apical rhizome sections. Quantification was performed by spectrophotometry [ $\lambda=724$  nm, chlorogenic acid (Sigma) as standard] using the Booker and Miller (1998) method. The amount of proteins, known to interfere with Folin-Ciocalteu reagent (Sigma), was determined by the Bradford assay.

#### 2-D electrophoresis protein analysis

About 250 mg of basal section from each rhizome (3 rhizomes for each selected sampling site) were used for protein extraction, carried out according to Migliore *et al.* (2007). The two electrophoretic runs were carried out according to Rotini *et al.* (2010). After electrophoresis, resolved proteins were visualized by acidic silver staining. Each protein sample was subjected to at least 2 parallel runs of isoelectrofocusing and second dimension electrophoretic separation to assess proteomic pattern reproducibility. Silver-stained gels were acquired as Tiff format images then processed by PD Quest 2-D Analysis Software (Biorad, version 8.0.1). The protein spots were characterized for isoelectric point (pI) and relative molecular weight (MW); for the attribution of isoelectric points and relative molecular masses, a mixture of protein with known identities (pI markers from SERVA Electrophoresis-Heidelberg) was used as internal standard.

### **Results and discussion**

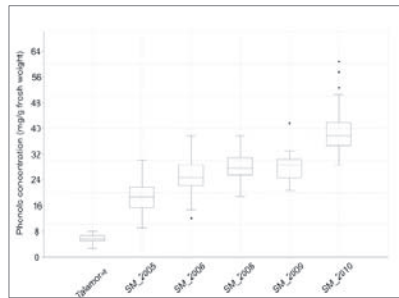
#### Total phenols

Total phenols content values in rhizomes are presented as box-plots (Fig. 1). The results indicated that the mean rhizome content progressively increased with time, from 18.7 mg/g in 2005 (n=65; SE 0.5), to 25.2 mg/g in 2006 (n=60; SE 0.7), to 26.2 mg/g (n=60; SE 0.6) in 2008, to 27.7 mg/g (n=40; SE 0.7) in 2009, up to a final 38.9 mg/g (n=60; SE 0.8) in 2010. Differences between subsequent years were always statistically significant ( $p<0.01$ ), except for the 2008-2009 comparison.

In *S. Marinella* plants both total phenols values and data dispersion around median are significantly higher than in plants from the Talamone (Grosseto, Italy) reference site meadow, which is considered well preserved according to phenological and lepidochronological analysis (Fresi *et al.*, 2004).

Phenols concentrations increase with decreasing conservation status of the meadow, in particular an inverse correlation with meadow density was found. In particular, the maximum density values (the highest among all sampling stations per year) clearly decreased with the increase of the mean of total phenols concentrations (linear

correlation -0.99,  $p < 0.01$ ). Hence, phenols concentration superimposes to the meadow regression and gives an early warning. As already mentioned, it is worthy of note that during the survey period an increasing anthropic pressure occurred, *i.e.* the construction of a new harbour in the vicinity (Civitavecchia, Rome) and a progressive intensification of agricultural practices.



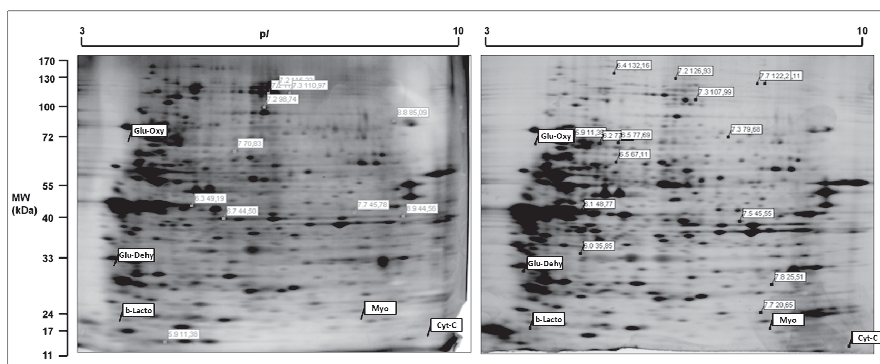
**Fig. 1:** Total phenols content (as mg/g fresh weight, f.w.) in *Posidonia* rhizomes collected in *S. Marinella* meadow (Rome, Italy) from 2005 to 2010 (SM\_05-SM\_10) and in a reference meadow, Talamone, (Grosseto, Italy) in 2005.

#### 2-D electrophoresis protein analysis

Maps of *Posidonia oceanica* rhizomes from 2006, 2008 and 2009 samples were obtained by 2-D electrophoresis. Analyses were performed on rhizomes characterized by the lowest or the highest phenols content. Maps from both low and high phenols showed a comparable number and/or arrangement of spots.

As shown in Figure 2, differences between the two phenols conditions are relative to specific spot protein constellations: some spots are exclusively expressed in low phenols rhizomes and others are exclusively expressed in high phenols ones. These differences, probably due to variations in metabolic and physiological pathways in response to stress, might give specific indications either on stressed conditions (high phenols) or good conservation status (low phenols). No significant changes in the overall high phenol plants expression pattern were found, whereas the percentage of protein spots characterizing the low phenols plants decreased with time. The incidence of high phenols-specific spots, on the contrary, remained quite constant in the same time interval. These observations suggest that adaptation to stress conditions represent an early phenomenon in *Posidonia* which results in medium/high phenols content and the rapid expression of a certain number of proteins. Less stressed conditions are associated to intermediate phenols content and to a gradually achieved loss of characteristic protein spots/function.

In conclusion, the analysis of samples from *S. Marinella* meadow showed that the increasing disturbed condition of the meadow during 2005-2010 period has been physiologically marked by an overall increase of the total phenols content and is associated to typical 2D-electrophoresis protein maps.



**Fig. 2:** Representative 2-DE maps from plants characterized by high (left) or low (right) total phenols content. High and low phenols-specific protein spots are highlighted and labeled, pI and molecular weight values are indicated. Protein markers (in alphabetic order) are: Cyt-C (Cytochrome C); Glucose-1-Dehydronase (Glu-Dehy); Glucose oxidase (Glu-Oxi); ?-Lactoglobuline (b-Lacto); Myoglobine (Myo). Molecular weight and pI ranges are reported.

The increase of total phenols concentration in plants can be considered a valuable early warning marker of environmental pressure. The correspondence between phenols content and specific protein patterns confirms our previous observations and open interesting perspectives to the validation of the combined tool, phenols content/2-DE protein pattern to evaluate the ‘health’ of *Posidonia oceanica* meadows. In the long run, this analysis may lead to the identification of specific biomarkers.

### Acknowledgments

The authors are grateful to E. Fresi, M. Scardi e L. Valiante for samples collection in all these years and for their insightful comments during all the phases of the work. This research was supported by CNR/MiUR funds to AG; ECON grant by commitment of ENEL Produzione S.p.A. (n. 3000043814/2004) and RSU grant from University of Tor Vergata to LM.

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## **MULTI-SENSORS DATA FUSION METHOD DEVOTED TO SEA BOTTOM VEGETATION MAPPING AND MONITORING**

### **Abstract**

*This paper first presents multi-sensors mini-oceanographic survey unit devoted to sea bottom mapping and monitoring developed by SEMANTIC TS, which is an acoustic oceanography research and development company. Then it deals with research tasks conducted by SEMANTIC TS in collaboration with GESMA and ENSIETA, to develop a mapping method for underwater seabed. First stage is to develop methods for characterizing vegetation and sediment on the seabed using the acoustic response from a conventional single beam echo sounder. These new methods are then operated simultaneously with multi-beams sonar producing micro-relief information and side scan sonar providing gray scale levels associated with bottom reflectivity. Then fusion of these data is processed. We show efficiency of these multi-sensors survey unit and multi-sensors data fusion concepts to get very precise seabed vegetation mapping allowing monitoring, in a way optimizing truth control (video and diving investigations). Examples are given from mapping made with ADENA manager on 2000 Natural Site « Posidonies du Cap d'Agde » and in Thau lagoon. The Cap d'Agde site presents the particularity of having discontinuous seagrass forming a patched meadow with frequent marine turbidity conditions that makes visual observations and aerial mapping difficult.*

**Key-words:** Marine vegetation, Mapping, Monitoring, Multi-sensors data fusion, Acoustic classification

### **Introduction**

Research tasks presented here referred to a global project relative to development of an operational bottom mapping method devoted to underwater vegetations, from the development of a multi-sensors survey unit to data fusion of collected data. This project comes out observation of similar mapping needs in civil and military environments, because vegetation may hide bottom mines and can significantly affect the performance of acoustic sensors used for sea-mines detection.

### **Materials and methods**

Semantic TS has begun the project by setting up a coastal survey vessel devoted to environmental submarine surveys and maps. This survey unit is a small size semi-rigid boat (6.5 m) with removable cabin, allowing moving easily on a road-trailer, boat and all its equipments and instrumentation systems (Fig. 1). Mini-survey unit characteristics are:

Professional category; Useful load: 1000 kg; Draught: 30 cm ; Length: 6.4 m; Motor: 115 CV; 220 V in board; Available power 24/24: direct: 500W / spike: 800 W.

This mini-survey unit is a French professional registered boat, driven by STCW professional pilots (captains 200). Objectives of this boat are to improve mapping of coastal submarine, harbors, lakes, rivers (zero state area studies and their time



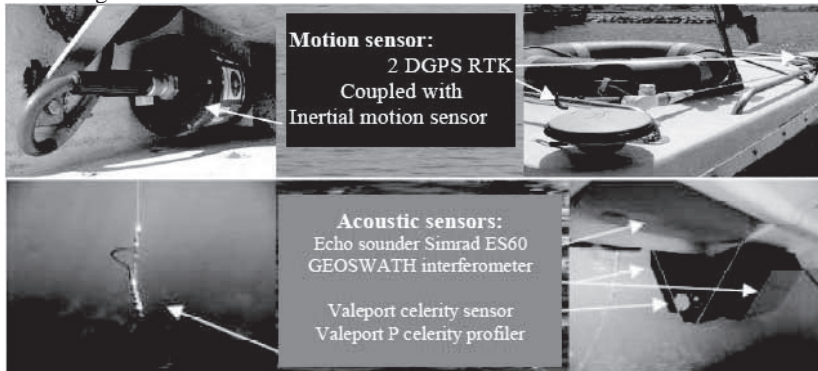
evolution), submarine bottom survey for structures establishment, monitoring of submarine vegetation boundaries or sediment movements. Small size is useful to move precisely in narrow sea areas or to strictly follow predefined survey lines, which is a necessary condition for monitoring purposes.



**Fig. 1: Survey unit SEMANTIC : semi-rigid with removable cabin.**

This small size survey unit presents high level of technology, both for platform positioning systems and for acoustic sensors. Boat is able to produced energy to process simultaneously all the instrumentation in 24h/24h. High speed internet aboard is process by 3G to pass on D-GPS RTK corrections, from land reference D-GPS station, in real time.

Instrumentation systems are simultaneously deployed on the mini-survey vessel as shown on Figure 2.

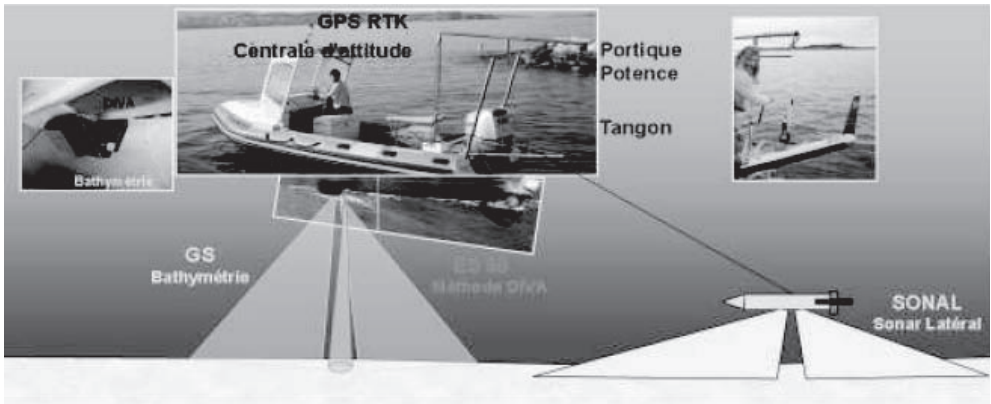


**Fig. 2: Instrumentation systems. D-GPS differential RTK Leica GX1230 centimetric system - Motion sensor Coda Octopus FS185+**

- Interferometric system Geoswath+ NG      Multibeam bathymetry
- Interferometric side scan sonar Geoswath+ NG      Precisely geo-located mosaic picture at 250 kHz
- Towed side scan sonar Klein 3900 / 5000      High resolution acoustic imagery
  - Frequency 455 kHz / 900 kHz
  - Resolution 20 cm at 75 meters
  - Range max: 150 meters
- Echo sounding Simrad ES60 high precision (scientific)      Bathymetry/biocenoses/sedimentology

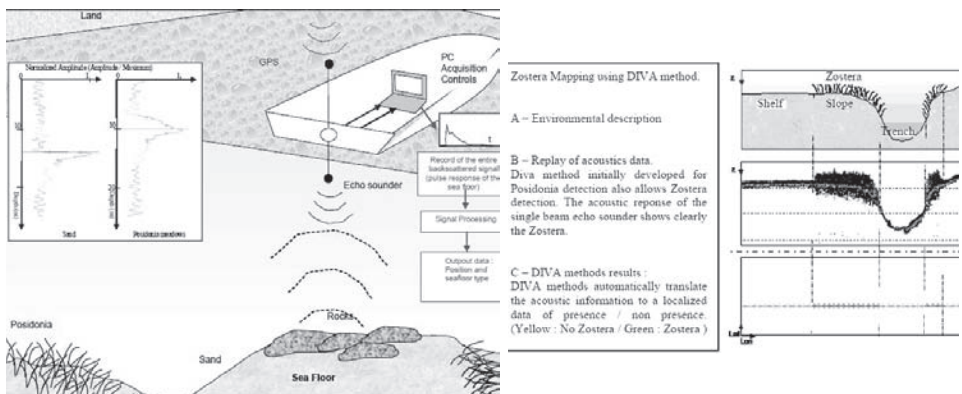






**Fig. 5: Principle of multi-sensors acquisition.**

Principle of DIVA method (Detection & Investigation Vertical Acoustic), devoted to vegetation, is resumed on Figure 6. The shape of acoustic bottom impulse response from a scientific echo sounder is recorded simultaneously with centimetric GPS position. As sand and vegetation have different acoustic signature shapes, we have developed a signal processing algorithm based on discriminant analysis and energy level of the bottom reflected impulse response (Viala *et al.*, 2007, Noel *et al.*, 2005, Noel *et al.*, 2006). Box in Figure 6 shows samples of sand and Zostera acoustic signature.



**Fig. 6: Left : Principle of DIVA method. In box : Representatives echoes.- Right : Illustration of DIVA approach in Zostera mapping.**

The DIVA method initially developed to Posidonia characterization is also efficient for Zostera localization and cartography. But as there is only one vertical acoustic echo, it's a low coverage method. The multibeam bathymetry of the survey area is a real advantage in addition to DIVA results, because it gives help, to circle bathymetric stage (between -2 m and -3.5 m), where zostera may be present. Simultaneous measurement

using DIVA method should then increase the performance of multi sensors fusion method.

We can also recommend to implement 3 to 5 DIVA sensors on a pole, perpendicularly to the ship course.

The DIVA method, developed by SEMANTIC TS, leads to various data, accurate and very well localized, in complement with those recorded from a towed video system which can be considered as “field truth”. It also allows a rapid mapping of the vegetation in coastal plain; it can cover about 100 km line of survey per day and can help to georeferenced surface picture. The main inconvenience is the difficulty to find discriminant parameter when rock and vegetation are both present in the survey area. It can be noticed that DIVA method also gives excellent results on kelp mapping (VIALA *et al.*, 2009). In addition, considering its high accuracy in data positioning (less than 1 meter), DIVA method can easily be used in time evolution growing surveys of various species of coastal marine vegetation.

Furthermore, due to its rapid capacity of deployment and data collecting, the DIVA method can be considered as a low cost mapping method when used on its own. The effective surface covered is small but can easily detect and define boundaries of coastal marine vegetation by multiplying the survey lines. It also allows localized intervention in case of doubt. By this way, the DIVA method can be a substitute for more accurate but slower method (such as diving or towed video system) or less accurate but faster method (such as towed side scan sonar which are not so well georeferenced due to underwater side scan imprecised location). (NOEL *et al.*, 2010).

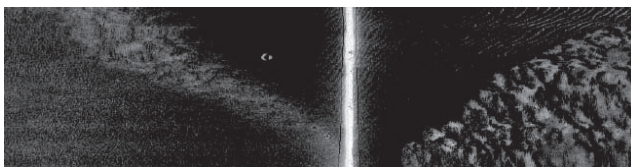
Moreover, we are now working to extend DIVA method into sediment classification (CLASS Method in progress), by the mean of hardness/roughness characterization, based on Chivers and al works (Chivers *et al.*, 1990).

So as to produce precise 3D sea bottom mapping, we have developed on a second stage **a method based on the fusion of data provided by acoustic systems** (Noel *et al.*, 2008):

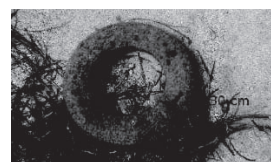
- Geoswath bathymetric system
- Side scan sonar systems (Geoswath or Klein)
- Echo sounder

Methods are operated simultaneously, and data fusion is realized by combining, after signal processing, the data collection obtained:

- 3D bathymetric data
- Side scan sonar imagery in grey levels, producing information about reflectivity, and so about bottom nature. New generation of side scan sonars are able to deliver very high resolution picture as shown on Fig 7. & Fig. 8.
- Micro-roughness derived from precise bathymetry
- DIVA method information about presence and absence of vegetation.
- CLASS method (in progress) information about bottom roughness and hardness

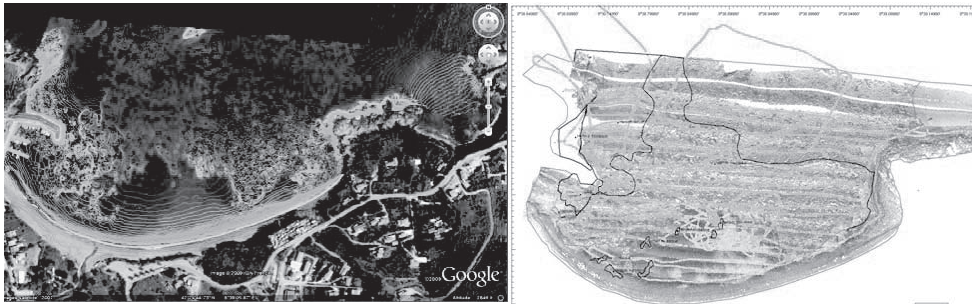


**Fig. 7: Example of high resolution acoustic picture by Klein 3900.**  
**Left : Tire on ripple sand (photo on Fig.8). Right : Posidonia meadow**

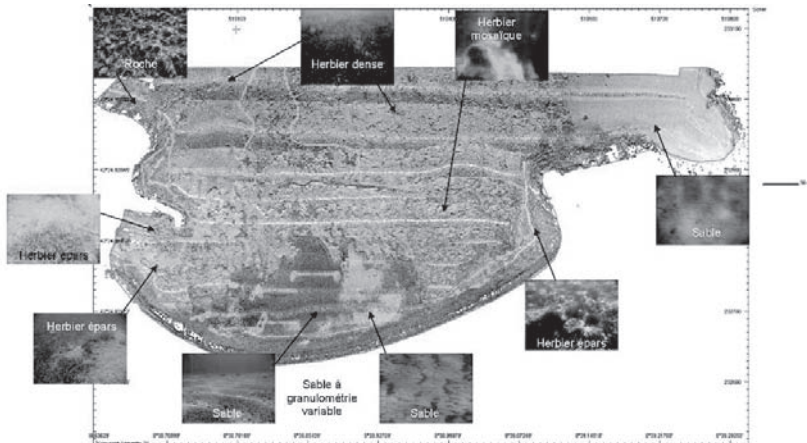


**Fig. 8 : Submarine picture of Fig.7, tire.**

These methods have been successfully applied in Corsica and French Riviera (see. Fig. 9 &10). It has also been especially tried with ADENA team, on the complex case of Natural 2000 site of Cap d'Agde, showing excellent mapping results in a turbid water area and typically patched meadow. Figure 11 illustrates efficiency of boundary determination using DIVA method in case of dense posidonia meadows and sand. Right part of Figure 11 shows 3D echography of the bottom, which can be considered as a



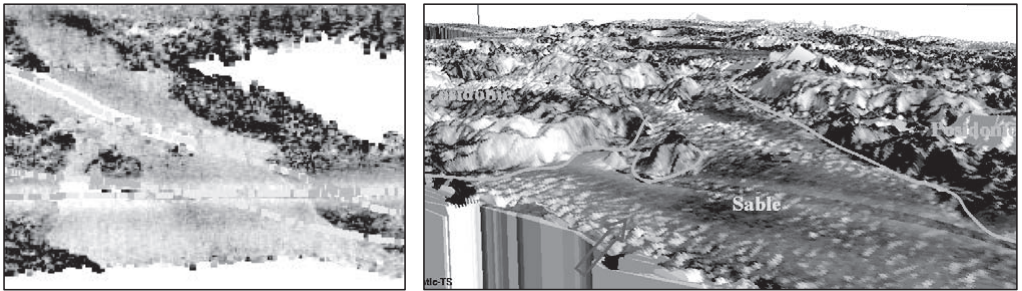
**Fig. 9:** Left: Micro-roughness results derived from precise bathymetry method results in posidonia/sand area may help to sonar mosaic interpretation - Right: Illustration of DIVA method results in Mediterranean area (in green : posidonia, in yellow: sand) superposed to gray scale side scan sonar mosaic from Geoswath system.



**Fig. 10:** Illustration of multi-sensors data fusion method results in posidonia case, allowing meadows and sand areas contouring.

Such precise and innovative 3D scan pictures of vegetation could only be obtained with very high accuracy at each step of data acquisition and processing. For this we had to develop a specific GIS software inside which, we have incorporated processing algorithms.





**Fig. 11: Natura 2000 Site « Posidonies du Cap d'Agde »** Left : view from sky : Results of DIVA method in Mediterranean area (in green : posidonia, in yellow: sand) superposed to gray scale side scan sonar mosaic from Geoswath system. Right : 3D scan picture of posidonia meadow: micro relief (from Geoswath bathymetric system) fused with DIVA results and side scan sonar mosaic.

### Discussion and/or conclusions

Data fusion concept is innovative and powerful. It allows producing like in medical applications, very accurate 3D scan pictures of seabed derived from different sources (side-scan, multi-beams, echo sounder) and information (aerial pictures, classification methods results, divers/video observations ...). Power of data fusion concept remains on the quality of the data and on their complementarities. In this context such a mini-survey unit, able to operate and synchronize several complementary high resolution acoustic sensors simultaneously, and to precisely process motion and geo-positioning, appears as a very efficient tool in the crucial data collection first step of the data fusion process.

### Acknowledgments

The authors thank the D4S/MRIS from DGA which supports financially this work under contract N° 2008.34.0029 as well as PACA Region, Agence de l'Eau RMC for help to initialize the multi-sensors acquisition project in 2005. They also thank E. Bauer, S Marchetti, S. Hernandez from Semantic TS for their collaboration in this project.

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## **POSIDONIA MEADOWS AS A PRIORITY HABITAT FOR THE SUSTAINABLE MANAGEMENT OF THE GREEK COASTAL ENVIRONMENT**

### **Abstract**

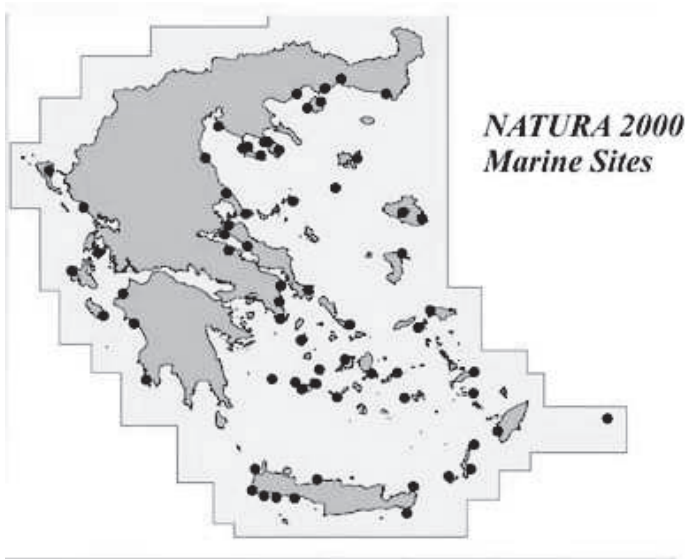
*Posidonia meadows are listed as a “priority habitat type” in Annex I of the European Habitats Directive (92/43/EEC). The practical meaning of this is that every EU member state has the legal obligation of to place under specific sustainable management measures at least 60% of the total surface of this habitat by the end of 2012. Although to date 28000 ha of Posidonia meadows in Greece are protected through the Natura-2000 network, the total coverage of this habitat is estimated to be more than 100000 ha, which makes conservation of 60% of it expensive and unrealistic within the required time frame. The present paper proposes a methodology for the estimation of the total surface covered by Posidonia meadows and a practical re-designation of the Natura-2000 sites, in such way that the Directive requirements are met in a realistic socioeconomic and temporal context.*

**Key-words:** Habitats Directive, Natura-2000 sites, Posidonia meadows

### **Introduction**

During the last decades of the 20<sup>th</sup> century, a number of legal texts aiming to protect Posidonia meadows came into force in France, Spain and Italy, due to the dramatic loss of this habitat in the north western Mediterranean coastline. At European level, the Habitats Directive (92/43/EEC) defines Posidonia meadows as “priority habitat type” and all EU member states have the legal obligation to place under specific sustainable management at least 60% of the total surface of this habitat, through the designation of “Natura-2000” sites, until 2012 at the latest. From 1996 to 1999 for the implementation of the Directive, the competent Greek authorities have selected and presented to the European Commission a map of about 300 “Natura-2000” sites. Almost 250 of these sites have a marine front. However, from 1999 to 2001, marine habitats have been mapped only in 67 of those (Fig. 1). The choice of the sites to be mapped was mainly based on the existing information about the presence of important Posidonia meadows. The result of the 1999-2001 mapping effort was a total of 28000 ha of Posidonia meadows, on about 2000 Km of coastline, actually under specific protection status. Nevertheless, the requirements of the Directive are not fluffed, as the 2001-2006 progress report (foreseen in Article 17 of the Directive), claims that Posidonia meadows are very common in Greece, present in many locations. Theoretically, these meadows will also have to be sustainably managed, in order to fulfil the legal obligations of Greece towards the EU. However, this now seems as a rather lost cause, since the Greek coastline measures more than 15000 Km and is meaningless to put 60% of the coastline under specific sustainable management.

The aim of this paper is to propose a review of the Greek Natura-2000 sites, based on expert judgment, which would ensure the protection of Posidonia meadows and the achievement of the requirements of the Directive, within the specified time limits.



*Fig. 1: The Greek marine Natura-2000 sites mapped during 1999-2001*

### **Materials and methods**

For the needs of the 2001-2006 progress report of the Habitats Directive a pan-European grid of pixels 10X10 Km was created. Every member state had to put on this standard grid all the habitats of the Annex I of the Directive. Given the great diversity of the Greek coastal and marine environment, the adverse topography of the bottom and with the nature of areas where *Posidonia* can be found, the 10X10 km pixel seems as rather impractical for the case of Greece. In order to adapt the methodology to the Greek coastal environment, we have subdivided every pixel of the 10x10 km grid into 100 pixels of 1x1 km. An assessment of the geo-morphological characteristics of each pixel as well as typical characteristics of *Posidonia* enabled the selection of those pixels (seafloor surfaces) that are most likely to host meadows. For the Greek Seas, only the pixels found between 0 and 50 m depth were taken into consideration, as the deeper limit of *Posidonia* lies usually at 40-45 m depth. The pixels were then distinguished on expert judgment in 4 groups and a conventional *Posidonia* meadow coverage was given to each group as follows:

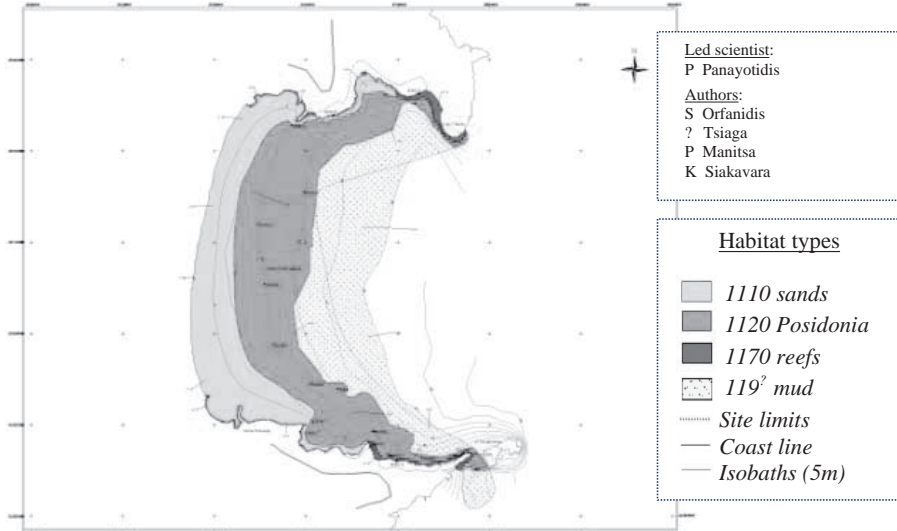
- Group A, “high density meadow”, where the estimated coverage of the seafloor is >30% and the proposed conventional mean coverage value is 35%
- Group B, “moderate density meadow”, where the estimated coverage of the seafloor is 10-30% and proposed conventional mean coverage value is 15%
- Group C, “low density”, where the estimated coverage of the seafloor is < 10% and the proposed conventional mean coverage value is 5%
- Group D, “absent” with conventional mean coverage value 1%

The choice of the percentages was based on the experience gathered from the marine Natura-2000 sites which are already mapped.

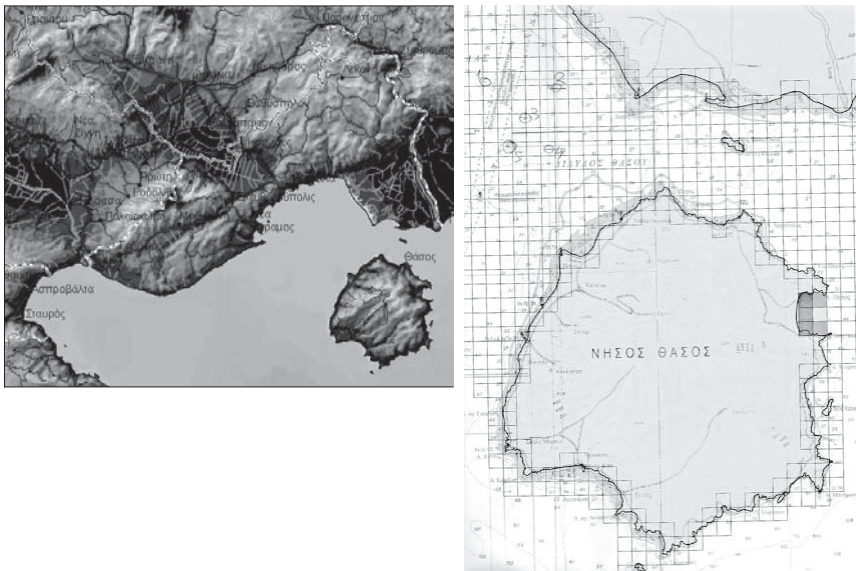
### **Results**

The proposed methodology was applied in the Natura site GR 1150008 “Ormos Potamias” in Thassos Island (North Aegean). The distribution of *Posidonia* meadow in this site (Fig. 2) is 171 ha, according to the map produced by S. Orfanidis and his

collaborators (Ministry of Environment, 2001). Using the pan-european grid of 1KmX 1Km in the same site (Fig. 3) we observe 3 pixels of “high density”, 2 pixels of “moderate density” and 1 pixel of “low density”. The estimated coverage of Posidonia meadow in the site GR 1150008 is 170 ha according to the numbers given in Table 1.



**Fig. 2: Distribution of the marine habitat types in the Natura site GR1150008. (Source: Greek Ministry of Environmentt, 2001)**



**Fig. 3: Thassos Island in the North Aegean Sea (left) and the application of the 1Km X 1Km grid of pixels**



**Tab. 1: Estimations of *Posidonia meadow* coverage in the site GR 1150008**

Type of <i>Posidonia</i> meadow	Conventional coverage (%)	Proposed mean coverage (%)	Number of pixels	Estimated coverage (ha)
High density	> 30	35	3	105
Moderate density	10-30	15	2	30
Low density	< 10	5	1	5
				Total 170

The proposed methodology will be applied in the whole coastline of Greece. The first results of this application gave as total number about 10.000 of the pixels where *Posidonia* meadows are eventually able to grow, representing an area of 1.000.000 ha. Table 2 presents the distribution of the pixels in the 4 groups of density.

**Tab. 2: Estimations of the seafloor surface covered by *Posidonia meadows***

Number of pixels	coverage %	Estimated surface (ha)	Conventional mean value (ha)
Group D = 4.000	absent (conventional 1)	0 to 4.000	4.000
Group C = 3.000	< 10 (conventional 5)	15.000	15.000
Group B = 2.000	10 to 30 (conventional 15)	20.000 to 60.000	30.000
Group A = 1.000	> 30 (conventional 35)	35.000	35.000
Total		70.000 to 114.000	84.000

According to the results presented on Table 2, the estimated total surface of *Posidonia* meadows in the Greek coastal environment could exceed 100000 ha. If we consider as more probable value the conventional value of each group, the estimated *Posidonia* meadows surface on the Greek coasts is 84.000 ha.

## Conclusion

During the last three decades of the 20<sup>th</sup> century many scientists have argued on the importance of *Posidonia* meadows to the Mediterranean coastal ecosystem. The legal status of “priority” habitat type designated to *Posidonia* meadows by Directive 92/43/EEC was a big success of the European marine scientific community. Nevertheless, in the different Mediterranean sub-ecoregions (Western, Central, and Eastern) the abundance and the distribution of *Posidonia* meadows are different but the legislation remains the same. A question rises: how do we decide upon which areas should be placed under specific sustainable management measures?

In the case of Greece, with more than 15000 km of coastline, the habitat mapping using traditional methodology seems unrealistic. Although 28000 ha of *Posidonia* meadows in Greece are protected through the “Natura-2000” network, the total coverage of this habitat is believed to exceed 100000 ha, which makes its conservation truly impractical within the time frame set by the EU.

In our point of view, managing 60% of the Greek coastline has a huge socioeconomic impact and small conservation effectiveness. It becomes clear that the boundaries of the Greek Natura-2000 network have to be reconsidered in order to include larger areas of *Posidonia* meadows. Two actions are proposed:

- extend the limits of all marine Natura-2000 sites down to the deeper limit of *Posidonia* meadows and

- include a marine part in more than 100 existing terrestrial Natura-2000 sites, which now stop at the coastline.

Exceptionally, some new sites could be considered for inclusion in the Natura-2000 network.

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## **NOUVELLES CONNAISSANCES SUR LES HERBIERS TIGRES DE MEDITERRANEE**

### **Résumé**

*Les herbiers à *Posidonia oceanica* présentent plusieurs types morpho-structuraux généralement conditionnés par les conditions de milieu (profondeur, hydrodynamisme, substrat, pente, turbidité, sédimentation). Du fait de leur rareté, deux structures particulières, les récifs barrière et les herbiers tigrés, sont considérées depuis une vingtaine d'années comme des paysages marins menacés. Initialement décrits dans le golfe de Gabès, à proximité des îles Kerkennah, les herbiers tigrés se présentent sous forme « de rubans assez étroits qui se développent entre 0.5 et 3 m de profondeur sur matte ». La mise en place de nouveaux programmes d'études sur les herbiers à *Posidonia oceanica* et l'optimisation de l'imagerie satellitaire (accessibilité, résolution, coût) ont permis d'acquérir de nombreuses données dans plusieurs secteurs de Méditerranée et d'actualiser notre connaissance de ces structures.*

*Il apparaît ainsi que les herbiers tigrés sont mieux représentés au niveau de la rive Sud du bassin (Golfe de Gabès, littoral de Zarzis en Tunisie, lagune de Farwa et littoral à l'Est de Tripoli en Libye) et dans des îles méditerranéennes (Marsala-Stagnone en Sicile et Porto-Vecchio en Corse). D'autre part la présence d'herbiers tigrés sur roche a été observée pour la première fois en Libye sur un platier rocheux à l'Est de Tripoli.*

*Compte tenu de leur rareté et des pressions qu'elles subissent, il apparaît urgent de classer ces structures comme « monuments naturels » et mettre en œuvre une politique efficace pour assurer leur conservation*

**Mots clés:** Magnoliophytes, *Posidonia oceanica*, structure,

### **Introduction**

Les herbiers à *Posidonia oceanica* présentent plusieurs types morpho-structuraux généralement conditionnés par les conditions de milieu (profondeur, hydrodynamisme, substrat, pente, turbidité, sédimentation). Ces différents types ont fait l'objet de description standardisées (Boudouresque *et al.*, 2006). Du fait de leur rareté, deux structures particulières, les récifs barrière et les herbiers tigrés, sont identifiées dans le « Livre Rouge « Gérard Vuignier » des végétaux, peuplements et paysages marins menacés de Méditerranée » comme des paysages marins menacés (PNUE, 1990). Initialement décrits dans le golfe de Gabès, à proximité des îles Kerkennah (Blanpied *et al.*, 1979), les herbiers tigrés se présentent sous forme « de rubans assez étroits qui se développent entre 0.5 et 3 m de profondeur sur matte ». La mise en place de nouveaux programmes d'études sur les herbiers à *Posidonia oceanica* et l'optimisation de l'imagerie satellitaire (accessibilité, résolution, coût) ont permis d'acquérir de

nombreuses données dans plusieurs secteurs de Méditerranée et d'actualiser notre connaissance de ces structures.

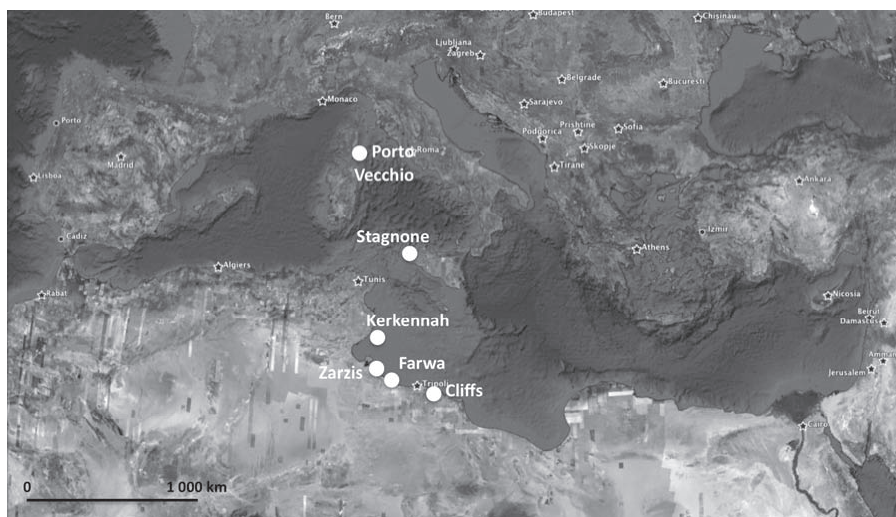
### Matériel et méthodes

Du fait de leur localisation très superficielle la présence d'herbiers tigrés a été recherchée à travers l'utilisation d'images satellitaires ponctuelles (Spot 5, Ikonos, Quickbird et Geoyes), les synthèses Google Earth et des photographies aériennes (Institut Géographique National pour le littoral français).

Des missions terrain ont permis de vérifier la présence effective de ces structures, de préciser leur extension et de les décrire de façon plus détaillée (substrat, profondeur, espèces associées et dynamique).

### Résultats et discussion

Il apparaît ainsi que les herbiers tigrés sont mieux représentés au niveau de la rive Sud du bassin (Tunisie, Libye) et dans des îles méditerranéennes (Sicile, Corse). Ces herbiers se développent aussi bien en milieu lagunaire (lagunes de Farwa et de Stagnone) qu'en mer ouverte. D'autre part la présence d'herbiers tigrés sur roche a été observée pour la première fois en Libye sur un platier rocheux à l'Est de Tripoli (Fig. 1).



*Fig.1 : Localisation des principaux herbiers tigrés identifiés le long du littoral méditerranéen.*

Si la largeur des bandes d'herbier est assez homogène (2-3 m : Calvo *et al.*, 2003), l'espacement entre ces bandes est assez variable ; il peut être ainsi relativement important autour des îles Kerkennah ou en Libye (jusqu'à 30 m, Fig. 2a) ou très réduit en Corse (moins d'1 m, Fig. 2b).

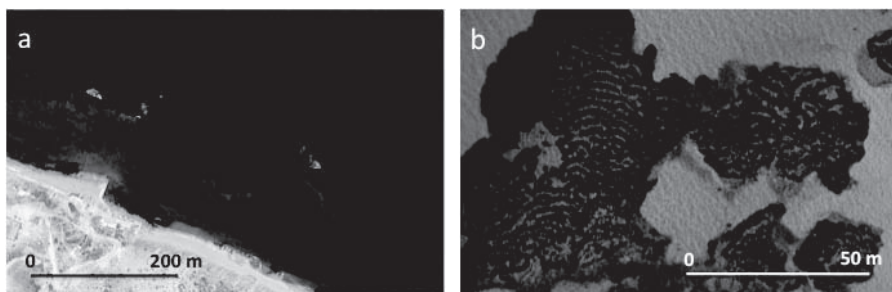


Fig. 2: Herbiers tigrés à l'Est de Tripoli (a) et dans le Golfe de Porto-Vecchio (b).

Ces herbiers se développent généralement dans des sites où l'action de l'homme est réduite; l'anthropisation et les pressions environnementales (température, salinité) peuvent générer une régression significative de ces structures (Calvo *et al.*, 2003, 2009 ; Tomasello *et al.*, 2009). Des atolls de *Posidonia oceanica* sont souvent associés aux herbiers tigrés, (Pergent *et al.*, 2007). En effet, leur localisation bathymétrique, les facteurs à l'origine de leur mise en place et leur dynamique sont assez proches (Fig. 3).

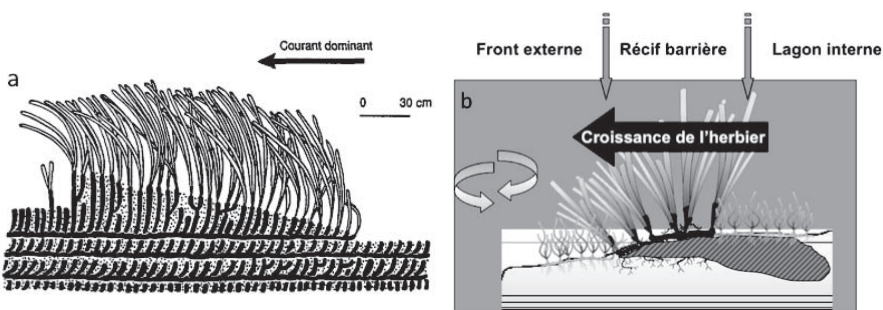


Fig. 3 : Dynamique des herbiers tigrés (a - PNUE 1990) et des atolls (b - Pergent inédit).

La rareté de ces structures et les pressions qu'elles subissent, du fait de leur localisation, menacent fortement leur pérennité à moyen terme. Il apparaît urgent de les classer comme « monuments naturels », au même titre que les récifs barrières, et mettre en œuvre une politique efficace pour assurer leur conservation.

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## **AN INTEGRATED APPROACH TO STUDY THE EFFECTS OF CHRONIC WATER ACIDIFICATION**

### **Abstract**

*Recent researches, performed in a naturally acidified site (Castello Aragonese d'Ischia - Gulf of Naples, Italy) where volcanic carbon dioxide vents cause long-term changes in seawater carbonate chemistry, lowering the pH from 8.17 down to 6.57, reveal winners and losers within the benthic community. In the same site, we chose to address the impact of ocean acidification on the algal community with an integrated approach by means of ecological, physiological and molecular tools.*

*Qualitative and quantitative changes in algal composition have been detected. Results showed a less structured community at low pH, characterized by few dominant species and the lack of calcareous taxa.*

*Due to their different tolerance to pH variations, three target species (Sargassum vulgare, Dictyota dichotoma and Jania rubens) have been selected to carry out transplant experiments in order to detect short term stress signals. Variations in fluorometry-derived parameters of the photosynthetic performance of these species were detected in situ at different pH conditions by means of a Diving-PAM.*

*In order to understand if the chronic lowering of pH of this site has promoted any local adaptations (morphological and/or genetic), the Dictyota complex species have been selected.*

*Results indicate that our integrated approach is the key factor leading to understand how these changes can drive deteriorations in the structure and function of algal assemblages under the effects of water acidification.*

**Key-words:** Water acidification; macroalgal communities; PAM; molecular taxonomy.

### **Introduction**

In coastal benthic environment, highly exposed to anthropic impact, macroalgal communities play important structural and functional roles; for long time they have been used to study coastal system response to pollution (Belsler & Boudouresque, 1976; Cormaci & Furnari, 1999; Levine, 1984; Pergent, 1991; Pinedo *et al.*, 2007; Sales & Ballesteros, 2009; Thibaut *et al.*, 2005) and, recently, to evaluate ecological status in marine coastal system (Ballesteros *et al.*, 2007; Panayotidis *et al.*, 1999).

During last years more and more attention has been addressed to climate change and, in particular, to ocean acidification. In fact a decrease of 0.5 in pH has been estimated by 2100 because of continuous CO<sub>2</sub> emission in the atmosphere (IPCC, 2007).

Volcanic underwater CO<sub>2</sub> vents offer a unique opportunity to study *in situ* water acidification effects and simulate future scenarios of shallow marine coastal systems. In this work, the effects of marine acidification has been evaluated on shallow algal assemblages in a naturally acidified site (Castello Aragonese d'Ischia - Gulf of Naples, Italy) where volcanic carbon dioxide vents cause long-term changes in seawater



carbonate chemistry, lowering the pH from 8.17 down to 6.57 (Hall-Spencer *et al.*, 2008; Martin *et al.*, 2008).

An integrative approach has been chosen by means taxonomic, physiological and molecular tools, aimed to detect changes in community structure and functioning at different temporal scales.

## Materials and methods

### Study area

The study area is Castello Aragonese in Ischia. It is a rocky volcanic dome linked to the Island through a bridge (Fig. 1). Seawater close to the “Castello” is characterized by shallow underwater CO<sub>2</sub> emissions from the rocky bottom at ambient temperature without any thermal toxic compounds such as sulphur. Emissions are present both on the Northern and on the Southern side of the Castello: on the Northern coastal the area interested by the pH gradient is 200 m long and the pH values range from 7 to 8.1; on the Southern side the area is 300 m long, and pH varies from 6.5 to 8.1 (Fig. 1) (Hall-Spencer *et al.*, 2008).

Each side has been divided into three sectors according to their pH mean value; on the North side, N1, N2 and N3 are characterized by 8.14, 7.87 and 7.09 pH mean values, respectively, while, S1, S2 and S3 on the South side, have 8.14, 7.83 and 6.57, correspondingly. N1 and S1 have been considered as control. In each sector 3 zones (5m long) were chosen and 3 replicates were randomly taken for each zone by scraping off all macroalgae from a 20 x 20 cm<sup>2</sup> surface. Samples were collected in autumn 2007. Species present in each sample square have been identified and their percentage cover has been estimated according to Ballesteros (1986).

In the present paper results referred only to the south side, being the others already in progress.

### Taxonomic approach

For each sector, Shannon-Weaver diversity index has been calculated and differences have been estimated through ANOVA and *post hoc* Tuckey's test (GraphPad Prism package). To assess structure and differences in community composition, a n-MDS analysis has been performed on species percentage cover using Primer-E software (Clarke & Warwick, 2001). K-Dominance curves of coverage data were obtained using PRIMER statistical package (version 5.0) in order to compare diversity patterns between different sectors.

### Ecophysiological approach

The maximum photochemical efficiency ( $F_v/F_m$  ratio) has been measured *in situ*, as stress indicator on three selected species, by means of a diving-PAM (Walz-Germania): *Dictyota dichotoma* var. *intricata* (C. Agardh) Greville, present all along the pH gradient, *Jania rubens* (Linnaeus) J.V. Lamouroux, dominant at normal pH (8.1) and

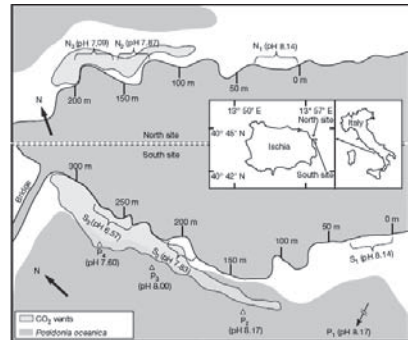


Fig. 1: Study site (from Hall Spencer *et al.*, 2008)



*Sargassum vulgare* C. Agardh, only present at the lowest pH conditions. After PAM measures on wild populations, we transplanted ten replicates of the three species at pH conditions different from their native one. After a three weeks acclimation period, fluorescence measures have been repeated both on transplanted thalli and on wild populations. Analysis of variance has been performed to test any differences after treatments.

### Molecular approach

In order to understand if the chronic lowering of pH of this site has promoted any local adaptations and to study the species plasticity, the *Dictyota* complex has been selected due to its spread occurrence in the study area. Five thalli of *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *Dictyota dichotoma* var. *intricata* and *Dictyota* sp. J.V. Lamouroux, selected on morphological characters, were sampled at S1 and S3. Genomic DNA has been extracted using a modified CTAB method (Doyle and Doyle, 1987). Two molecular markers useful to detect species variability, PsbA (Plastid encoded photosystem II reaction center protein D1) and RbcL (RuBisCO large subunit) have been amplified with specific primers and then sequenced.

## Results

### Taxonomic approach

A decrease of algal diversity was found along the pH gradient of the Southern side assessed by significant differences in the Shannon-Wiener's index ( $H'_{S1}=2.29 \pm 0.07$ ;  $H'_{S2}=1.88 \pm 0.07$  and  $H'_{S3}=1.18 \pm 0.05$ , respectively) ( $F=25.45$ ;  $p < 0.001$ ).

This different species richness affected the structure of the communities (Fig. 2 and 3). An evident separation is clear between S3 (the lowest pH condition), S2 and S1 (medium and normal pH conditions, respectively) (Fig. 2). In figure 3, where species are ranked in a logarithmic scale and their cumulative percentage dominance is represented, K-Dominances curves display different length and shapes. The lowest algal community complexity is evident in S3 where few species were dominant with high cover values, whereas in S2 and S1 the algal communities (characterized by a sigmoidal pattern), are characterized by a more homogeneous relative abundance.

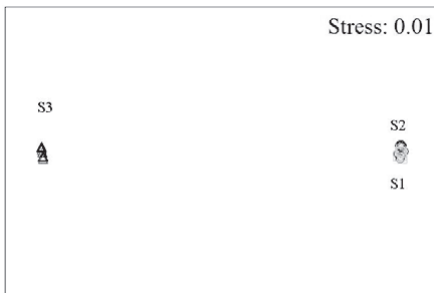


Fig. 2: nMDS for S1, S2 and S3

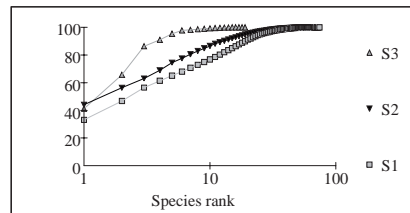
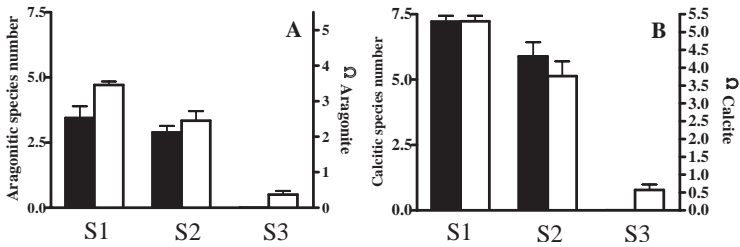


Fig. 3: K-dominance plot

The change in species composition is largely due to the lack of calcareous species with the increase in water acidity. In fact, the number of the aragonitic species (Fig. 4A), such as *Peyssonnelia* sp. Decaisne, *Padina pavonica* (Linnaeus) Thivy and *Halimeda tuna* (J. Ellis & Solander) J.V. Lamouroux, dropped to zero in the lowest pH site,

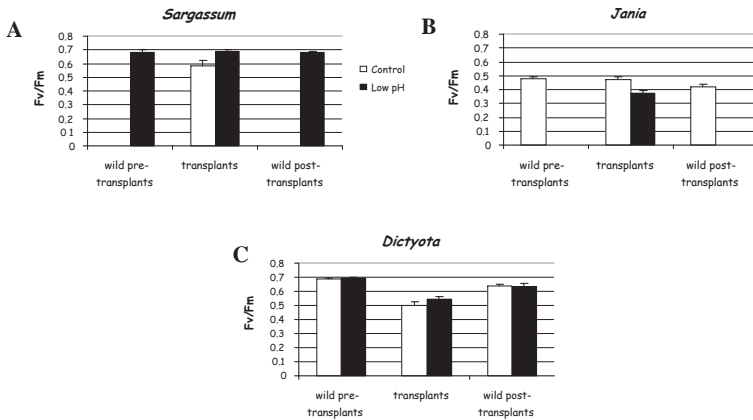
according to their omega ( $\omega$ ) values, lower than 1. The same pattern was observed in calcitic species, such as *Jania rubens*, *Amphiroa rigida* J.V. Lamouroux, *Lithophyllum sp.* Philippi (Fig. 4B). Differences among sectors S1, S2, S3 for both calcitic and aragonitic species number were highly significant according to ANOVA results ( $F=130.5$  and  $F=38.65$  respectively;  $p < 0.0001$  for both).



**Fig.4:** Bar plot representing aragonitic (A) and calcitic species number (B) (white bars) compared with omega aragonite (A) and calcite (B) (black bars) in each sector S1 (control), S2 (intermediate pH) and S3 (lowest pH).

*Ecophysiological approach*

Figure 5 shows  $F_v/F_m$  mean values in wild populations before the transplant, in transplanted thalli and in wild populations after the transplants. *S. vulgare* showed similar  $F_v/F_m$  values at low pH conditions, both in wild populations before and after transplanting ( $0.68 \pm 0.02$ ,  $0.68 \pm 0.01$ ), and in transplanted thalli ( $0.69 \pm 0.02$ ). Values were significantly lower ( $F=4.773$   $p < 0.05$ ) when this species was transplanted at normal pH ( $0.58 \pm 0.04$ ) (Fig. 5A).



**Fig.5:**  $F_v/F_m$  mean values  $\pm$  SE for *S. vulgare* (A), *J. rubens* (B) and *D. dichotoma var. intricata* (C) in natural and transplanted thalli at low pH (black bars) and in control site (white bars)

*Jania rubens* showed a similar but opposite pattern: no differences between wild populations ( $0.48 \pm 0.02$  and  $0.42 \pm 0.02$ ) and in transplanted thalli ( $0.47 \pm 0.02$ ) at normal pH but  $F_v/F_m$  value were lower when thalli were transplanted in S2 ( $0.37 \pm 0.02$ ) ( $F=6.54$ ,  $p < 0.01$ ) (Fig. 5B). No significant difference were detected for *D. dichotoma* in wild population at low S3= $0.69 \pm 0.03$ ,  $0.64 \pm 0.02$  and normal pH S1= $0.70 \pm 0.01$ ,  $0.63 \pm 0.02$ ). On the opposite, a stress was evident in transplanted thalli, regardless the pH values (S3= $0.50 \pm 0.03$ ; S1= $0.54 \pm 0.03$ ) ( $F=13.15$ ,  $p < 0.05$ ) (Fig. 5C).

#### *Molecular approach*

The analysis with *psbA* and *RbcL* yielded respectively a fragment of 900bp and of 700bp.

With both markers there were, as expected, no dissimilarity among the two sites.

Two main haplotypes were detected among the individuals of *D. dichotoma* var. *intricata* with the analysis of *RbcL* fragment. The comparison with the two concatenated genes and the sequences of *D. dichotoma* annotated in genbank revealed no differences with the three species collected.

#### **Discussion and conclusions**

Our data confirm an evident impact of water acidification on shallow macroalgal assemblages, both at community and photosynthetic levels. A strong reduction in species richness and community complexity is the first relevant result. Differences in species pH tolerance are evident, not only as it concerns calcareous species. In fact, significant physiological stress responses have been recorded for those species that, only confined to normal pH (calcareous algae) or low pH (*S. vulgare*), were transplanted in non native conditions.

*D. dichotoma* var. *intricata* results physiologically tolerant to pH variations (S1 and S3); in fact, no significant differences in  $F_v/F_m$  have been measured. However, the lower toughness of this species compared to the other target species could be responsible of the stress signals recorded after transplants experiments.

Results support the relevance of integrated approach in detecting degradation in the structure and function of vegetated communities impacted by climatic changes. Recently, interest has been concentrated on the high morphological plasticity of *Dictyota* complex (Tronholm *et al.*, in press).

The preliminary results from the molecular analysis showed that there is no association among the morphotypes detected and the gene variability of the *D. dichotoma* complex. Further studies are in progress to select hotspot genes involved in the local adaptive response.

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## **POSIDONIA OCEANICA (L.) DELILE, A USEFUL TOOL FOR THE BIOMONITORING OF CHEMICAL CONTAMINATION ALONG THE MEDITERRANEAN COAST: A MULTIPLE TRACE ELEMENT STUDY**

### **Abstract**

*The concentrations of 19 trace elements (TE): Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi as well as Cr, Fe, Ni, Cu, Zn, Cd and Pb, were analyzed by DRC ICP-MS in Posidonia oceanica (L.) Delile leaves from the Mediterranean French coast. The first 12 TE have little been studied nowadays. Except for Al, Cr, Fe, Cu and Ag, TE were preferentially accumulated in photosynthetic part of leaves. Moreover, trace element concentrations of the third intermediate leaf are representative of the integral shoot, and could be used alone in biomonitoring. Environmental background concentrations of the 12 little studied TE were determined, and spatial variations were related to anthropic activities. Compared to previous publications, concentrations of the 7 other TE classically investigated present a diminution or a stabilization, reflecting the change of anthropogenic inflows. In conclusion, P. oceanica is a sensitive bioindicator for chemical contamination, even for the twelve little studied TE.*

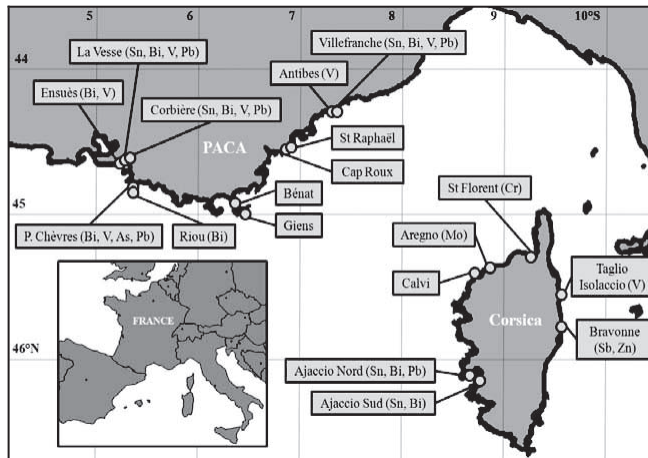
**Key-words:** Trace elements; Seagrass; *Posidonia oceanica*; Mediterranean; Pollution.

### **Introduction**

*Posidonia oceanica* (L) Delile, the endemic seagrass of the Mediterranean, is sensitive to human disturbances, notably pollution by chemicals (Boudouresque, et al., 2006). Many metals, metalloids and non-metals are regarded as serious pollutants due to their toxicity, persistence and tendency to concentrate in organisms (Ikem & Egiebor, 2005). Concentrations of some trace elements (e.g. Pb, Hg, Cd, etc) were largely studied in *P. oceanica* tissues (Luy, et al., submitted). For example, *P. oceanica* leaves give indications of trace element (TE) concentrations in seawater with accuracy (Pergent-Martini, et al., 2005), while sheaths and rhizomes memorize their temporal trends (Ancora, et al., 2004). Romero et al., (2007) also suggested that the third intermediate leaf was representative of the integral shoot. However, human activities modify environmental TE concentrations (Zhou, et al., 2008); quantification of other previously little studied potential pollutants (e.g. Sb, Bi, Mo, etc) is henceforth now relevant. The objectives of this study were: (i) To measure concentrations of 12 TE little or never studied in *P. oceanica* (Be, Al, V, Mn, Co, As, Se, Mo, Ag, Sn, Sb and Bi), to examine their tissue speciation and spatial variation, and to evaluate the potential use of *P. oceanica* as bioindicator. (ii) To investigate tissue speciation, spatial and temporal variations of 7 TE classically studied (Cd, Cu, Cr, Pb, Zn, Fe and Ni). (iii) To evaluate the ability to use *P. oceanica* third intermediate leaf instead of the integral shoot for the biomonitoring of these 19 TE.

### **Materials & Methods**

In April 2007, 15 shoots of *P. oceanica* were collected at  $15 \pm 1$  m depth in 18 sites located along the French Mediterranean coasts (Fig. 1).



**Fig. 1:** Localization of the study area and repartition of sampling sites along the Provence-Alpes-Côte d'Azur (PACA) and Corsican coasts; for each site, trace elements presented in high amounts are listed.

*Posidonia oceanica* shoots were dissected according to the biometric method proposed by Giraud (1979). Epiphytes were scraped from leaves. Each shoot was sorted and noted as follows: third intermediate leaves (3IL), other intermediate leaves (OIL), blades of adult leaves (BAL) and sheaths of adult leaves (SAL). Sorted tissues were lyophilized, weighed, pooled, cryogenic ground and mineralized (closed microwave digestion labstation). Trace element concentrations were determined by DRC-ICP-MS. Analytical accuracy was checked by analyzing Certified Reference Materials: BCR 60, BCR 62, GBW 07603 and V463. Detection decision ( $L_C$ ), detection limit ( $L_D$ ) and quantification limit ( $L_Q$ ) were calculated for each TE.

## Results & Discussion

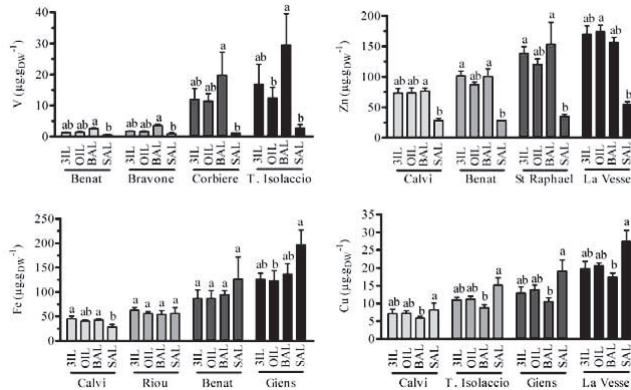
### 1. Tissue speciation

Tissue speciation of V, Zn, Cr and Cu are illustrated below in 4 contrasted sites (Fig. 2). Profiles of the other TE were similar to 1 of these 4 characteristic graphs.

Most TE were preferentially concentrated in photosynthetic tissues (*i.e.* 3IL, OIL and BAL) rather than in non-photosynthetic tissue (SAL): V, Mn, Co, Ni, Zn, As, Se, Mo, Cd, Sb, Pb and Bi. Furthermore, Mn, Co, Ni, As, Mo, Sb, Pb, Bi and particularly V were present in higher concentrations in BAL (*e.g.* V in Fig. 2), contrary to Zn, Se and Cd. These TE presented similar concentrations in all 3 photosynthetic tissues (*e.g.* Zn in Fig. 2). In the particular case of Be and Sn, no clear tissue speciation could be found. Al, Fe, Ag and Cr concentrations were higher in SAL only in sites which presented the highest concentrations (*e.g.* Fe in Fig. 2). Concerning Cu, concentrations in SAL were systematically higher than in other tissues, but differences were quite small (Fig. 2). This findings were in agreement with many previous studies: Campanella *et al.*, (2001) for Cr, Zn, Cd and Pb, but not for Cu; Lafabrie *et al.* (2008) for Cr, Co, Ni, Cd and Pb; Conti *et al.* (2010) for Cr, Cu, Zn, Cd and Pb.

A preferential assimilation from the water column to photosynthetic tissues can be assumed for V, Mn, Co, Ni, Zn, Mo, Cd, Sb, Pb and Bi, which show higher concentrations, as suggested by Lafabrie *et al.* (2008) for Co, Ni, Cd, Hg and Pb. At least 2 hypotheses can be used to explain upper values in BAL: (i) A longer exposure to TE loaded in the ambient habitat (Warnau, *et al.*, 1996); (ii) A dilution effect due to the

higher growth rate of intermediate leaves. These hypotheses can only play a major role for TE characterized by low kinetics of accumulation and little regulation. The different behaviour of TE preferentially accumulated in SAL suggests different uptake and distribution routes. For Cu, the systematic but quite limited upper level in SAL could be explained by an increase in metabolic activity during growth, when Cu is needed (Conti, *et al.*, 2010). Moreover, this work supports the hypothesis of Romero *et al.*, (2007), as the TE concentrations of the 3IL are representative of the integral shoot.



**Fig. 2: Tissue speciation of V, Zn, Fe and Cu in 4 of the 18 sites; concentrations are expressed as mean  $\pm$  standard deviation ( $\mu\text{g.g DW}^{-1}$ ); letters represent significant differences between tissues.**

## 2. Spatial (temporal) variation of TE concentrations

Based on average concentrations found in the shoots from the 18 sites (Tab. 1), TE levels decreased in the order: Zn, Fe, Al > Mn > Ni > Cu > V > Co, Cd, Pb, Mo, As > Ag > Cr, Se, Sb > Sn > Bi, Be. This confirms the sequence commonly observed in *P. oceanica* (e.g. Campanella, *et al.*, 2001, Conti, *et al.*, 2010). Some TE presented an important spatial variation of their concentrations in *P. oceanica* (higher than a factor 10 for Mo, Zn, V, Bi, Sn and As), others a moderate (from a factor 4 to 10 for Al, Cr, Fe, Pb and Sb) and many ones presented just a small (lower than a factor 4 for Cd, Cu, Ag, Be, Co, Ni, Mn and Se) spatial variation (Tab. 1).

Be, Se and Sn presented values close to  $L_D$  (0.007, 0.25 and 0.025  $\mu\text{g.gDW}^{-1}$  respectively); Bi levels were closed to  $L_Q$  (0.0048  $\mu\text{g.gDW}^{-1}$ ). Be and Se concentrations, quite similar per site, reflected the background level of the northwestern (NW) Mediterranean Sea. A significant spatial variation was observed for Sn and Bi, the upper values being found in Marseille Bay (Ensuès, La Vesce and Corbière), Plateau des Chèvres, Riou, Villefranche and Ajaccio. Since they are principally used in advanced industry, it is not surprising to encounter them in industrialized areas and harbours.

V is a tracer of hydrocarbon pollutants (Amiard, *et al.*, 2003). The presence of 4 oil refineries in the department of Bouches-du-Rhône, an old oil-exporting harbour at Antibes and the petroleum depot of Lucciana can explain the high V concentrations in Marseille Bay, Antibes and Taglio Isolaccio. V concentrations varied from 1.6  $\mu\text{g.gDW}^{-1}$  at Riou and Aregno, to 22.3  $\mu\text{g.gDW}^{-1}$  at Antibes. The highest concentrations determined by Amiard *et al.*, (2003) in 3 mollusc species (from 0.57 to 1.42  $\mu\text{g.gDW}^{-1}$ ) along the south coast of Brittany after the sinking of the tanker *Erika* are in the lower range of the concentrations determined in this study. It confirms the use of *P. oceanica* as a good biological indicator of hydrocarbon pollution.



**Tab. 1: Minimum (Min.), maximum (Max.) and average (Av.) TE concentrations in *P. oceanica* shoots from the 18 sites ( $\mu\text{g.g}_{\text{DW}}^{-1}$ ).**

	Be	Al	V	Cr	Mn	Fe	Co	Ni	Cu	
<b>Min.</b>	0.004	20	1.6	0.16	42	41	1.6	20	6.8	
<b>Max.</b>	0.011	151	22.3	0.98	95	180	4.5	48	22.9	
<b>Av.</b>	0.008	97	8.2	0.34	62	104	2.6	31	13.3	
	Zn	As	Se	Mo	Ag	Cd	Sn	Sb	Pb	Bi
	65	0.89	0.18	1.2	0.52	1.08	0.017	0.16	1.1	0.004
	1273	9.44	0.31	27.1	1.63	4.21	0.115	0.70	4.8	0.049
	163	2.24	0.24	3.7	0.92	2.46	0.048	0.22	2.3	0.013

Mn presented just a small spatial variation ( $62 \pm 15 \mu\text{g.g}_{\text{DW}}^{-1}$ ). Furthermore, our SAL concentrations ( $14 \pm 3 \mu\text{g.g}_{\text{DW}}^{-1}$ ) were in the lower range of the values determined by Ancora *et al.*, (2004) in *P. oceanica* scales in the Gulf of Naples (up to  $200 \mu\text{g.g}_{\text{DW}}^{-1}$ ). Mo is a micronutrient of significance for citrus nutrition (Srivastava & Shyam, 2007). The high Mo concentrations measured at Aregno ( $23.1 \mu\text{g.g}_{\text{DW}}^{-1}$ ) might be due to this specific agricultural activity. However, the 17 other sites presented concentrations ( $1.2 - 5.2 \mu\text{g.g}_{\text{DW}}^{-1}$ ) lower than those determined by Augier *et al.*, (1991) in *P. oceanica* leaves ( $5.2 \pm 0.6 \mu\text{g.g}_{\text{DW}}^{-1}$ ) at the uncontaminated site of La Palu (Port Cros Island, France). So, average concentrations of these 2 TE reflect the background level of the NW Mediterranean Sea.

Ag contamination results from urban or industrial diffuse sources. RNO (2006), using wide mussel, determined the Ag contamination along the French Mediterranean coast as homogeneous and limited compared to the Atlantic coast. Moreover, lowest values of this study ( $0.52 \mu\text{g.g}_{\text{DW}}^{-1}$ ) were found in Corsica. They corroborate the low values measured by Lopez y Royo *et al.*, (2009) and reflect the Ag background level.

Sb, Zn and As had relatively small spatial variation, with the exception of Bravone (Sb and Zn) and Plateau des Chèvres (As). Average concentrations of Zn ( $163 \mu\text{g.g}_{\text{DW}}^{-1}$ ) and As ( $2.24 \mu\text{g.g}_{\text{DW}}^{-1}$ ) corresponded to unpolluted sites (Fourqurean, *et al.*, 2007). The high Sb concentrations only detected at Bravone could be attributed to the As/Sb deposit at Matra. However, if the high Sb concentrations were associated with high Zn concentrations, it is not the case with As, as previously shown in mussels by Andral *et al.*, (2004). The high As concentrations detected at Plateau des Chèvres could be attributed to the industrial history of the south of Marseille (Andral, *et al.*, 2004), which suffers from a chronic contamination of As and Pb (Lassalle, 2007).

Al and Fe presented similar profiles. This is consistent with Barabasz *et al.*, (2002) observation: one effect of Al on plants is the stimulation of Fe absorption by roots. Our Fe average concentration ( $104 \mu\text{g.g}_{\text{DW}}^{-1}$ ) corroborates the low values determined by Fourqurean *et al.*, (2007) on the Illes Balears. Fe and Al spatial variations would result from a natural heterogeneity rather than an anthropogenic disturbance.

The high Cr levels determined at Saint Florent ( $0.98 \mu\text{g.g}_{\text{DW}}^{-1}$ ) corroborates similar high values measured at west of Cape Corse ( $1.07 \mu\text{g.g}_{\text{DW}}^{-1}$ ) by Lafabrie *et al.*, (2008). These values have been linked to waste from the disused Canari asbestos mine. Contrary to Lafabrie *et al.*, (2008), the high Cr levels at Saint Florent were not associated with high levels of Co or Ni. Furthermore, Co, Ni and Cd spatial distributions were similar, and their measured concentration ranges were low and should reflect a diffuse contamination. Moreover, when compared to Lafabrie *et al.*, (2008), a slight decrease in Cd level in Corsica was likely a result of the application of European rules (CE, 2009).

Our Cu average concentration ( $13.3 \mu\text{g.g}_{\text{DW}}^{-1}$ ) is similar to the NW Mediterranean background level determined by Campanella *et al.*, (2001). All sites sampled can thus



be considered as having low levels of Cu contamination. Since Pb interdiction of use as an anti-knock additive, contamination levels are in decline, as determined by Ancora (2004) between 1989 and 1999 in the Gulf of Naples. However, its significant environmental persistence in the sediment explains the relatively higher end values determined in the highest industrialized sites (Marseille Bay, Plateau des Chèvres, Villefranche and Ajaccio).

### Conclusion

Tissue speciation allows us to construct hypotheses concerning the uptake and distribution routes of these 19 TE. Such hypotheses have to be confirmed by the analysis of different parts of the same leaf and both above and below-ground tissues, and by experimental contaminations. Furthermore, future analyses of most TE in *P. oceanica* could be realized only on the single 3IL. The NW Mediterranean natural levels of most of the 12 little studied TE measured in *P. oceanica* could be determined, and their spatial variation and their contamination sources (punctual, diffuse and/or chronic) could be explained. *P. oceanica* might be used *de facto* as a sentinel species for their monitoring. The temporal variations of the other 7 widely studied TE present a diminution, or at least a stabilization of their concentrations, probably due to their reduced anthropogenic inflows. These observations suggest that *P. oceanica* is a sensitive bioindicator for the monitoring of the 19 TE studied.

### Acknowledgements

Funding was provided by Agence de l'eau, FRS-FNRS (FRFC 2.4.502.08) and French Community of Belgium (ARC Race 05/10-333). Authors are also grateful to IFREMER and STARESO for sampling of biological material.

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## **LIGHT AND TEMPERATURE REQUIREMENTS FOR SURVIVAL AND GROWTH OF MEDITERRANEAN WOMERSLEYELLA SETACEA**

### **Abstract**

The tropical red alga *Womersleyella setacea* (Rhodomelaceae, Rhodophyta) is considered to be invasive in the Mediterranean Sea since its introduction in late's 80s. To determine if its ability to adapt to different light and temperature conditions can explain its colonization success through the Mediterranean, laboratory experiments were set up in order to assess its short (1 month), and long term (8 months) light and temperature requirements for survival and growth. Results showed that survival was both temperature and light dependent, and the higher survival was observed at 10°C combined with 5-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , at 12°C combined with 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , and at 16°C combined with 50-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Besides, at short term, *W. setacea* was able to survive and grow in a large range of environmental conditions, but at higher temperatures (18-26°C) it displayed a positive growth rate only at 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Lower photosynthetic photon flux densities (5  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) showed the minimal survival and growth at all the temperatures assayed. According with these requirements, *W. setacea* is able to develop in the shallow infralittoral Mediterranean zone, where temperatures rise in summer, only at 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , whereas it can hold on deep infralittoral and shallow circalittoral bottoms when light intensities are 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . The development of massive turfs of *W. setacea* in the upper circalittoral is probably related with its high and hold growth rate at photosynthetic photon flux densities of 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  combined with temperatures ranging from 12 to 16°C.

**Key-words:** Culture, growth, physiological requirements, survival, *Womersleyella setacea*

### **Introduction**

The red alga *Womersleyella setacea* (Hollenberg) R.E. Norris is one of the eight macroalgae considered at present to be invasive in the Mediterranean Sea (Boudouresque & Verlaque, 2002). It forms thick persistent turfs that completely covers substrata, and investigations have revealed that it cause substantial negative effects on native communities (Piazzi & Cinelli, 2001; Piazzi *et al.*, 2002; Serio *et al.*, 2006), becoming the dominant species of these communities and modifying benthic assemblages (Airoldi, 2000; Airoldi *et al.*, 1995; Antoniadou & Chintiroglou, 2007; Piazzi & Balata, 2009), and/or taking the place of keystone species (Cebrian *et al.*, *submitted*).

There is a very limited knowledge on the intrinsic ecological and biological *W. setacea* traits which may support its rapid and widespread invasion and its bathymetrical distribution, although see Rindi *et al.*, (1999) for mid-term (3-3.5 months) requirements. The main goal of this work was to determine the short (1 month) and long-term (8 months) physiological requirements of light and temperature for survival and growth of Mediterranean population of *W. setacea*, owing to relate results obtained under laboratory conditions with its colonization success.

## Materials and Methods

Specimens of *W. setacea* were collected by SCUBA at L'Imbuttu, Scandola, Corsica (France), the 16<sup>th</sup> October 2009, on coralligenous assemblages situated at depths of 30 m, where it formed a dense turf between 27 and 35 m depth. Some vegetative tips of around 1.5 cm<sup>2</sup> were excised and cultured in 250 ml vessels in incubators (Radiber, Barcelona, Spain) equipped with 30 W cool white fluorescent bulbs. Culture medium was a quarter-strength modified Von Stosch enriched seawater medium (Guiry & Cunningham, 1984), and was changed weekly in order to avoid depletion of nutrients, using medium previously preheated to the experimental temperature.

To examine the temperature and photosynthetic photon flux densities (PPFD) requirements for survival and growth of the specimens, 4 replicates were grown in a variety of temperatures (10°C, 12°C, 16°C, 18°C, 24°C and 26°C) and PPFD (5, 20, 50 and 75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ). Monitoring was made weekly by means of a photograph taken with a Canon EOS 350D (Canon, Tokyo, Japan). Survival of the cultured thalli was determined as the time between the collecting data and the death of the specimen, and was followed for up to 8 months. The thallus surface (in mm<sup>2</sup>) and the surface growth rate were determined at the initial time of the culture and every 2 months. Measures of thallus surface were made from the periodic photographs using the program ImageJ (National Institutes of Health, USA). The surface growth rate (in month<sup>-1</sup>) was determined as  $\text{Growth rate} = (\text{area gained between } t_i \text{ and } t_{(t+1)}) / (\text{area at } t_i) / \text{time between } t_i \text{ and } t_{(t+1)}$ . Representative specimens of material used for cultures are deposited in the Herbarium of the University of Girona (HGI-A).

Survival, surface and surface growth rate data were analyzed by a two-way ANOVA where temperature (6 levels) and PPFD (4 levels) were fixed orthogonal factors, and for each combination of factors there were 4 replicated measurements.

## Results

### Survival

At short-term *W. setacea* was able to survive in all the combinations of temperature and PPFD assayed (Fig. 1). At long-term, survival was both temperature ( $p < 0.001$ ) and PPFD ( $p < 0.001$ ) dependent and there was a significant interaction between both factors ( $p < 0.001$ ). So, survival was maximum at 10°C combined with 5-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , at 12°C combined with 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , and at 16°C combined with 50-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Fig. 1). Among the specimens growing in optimal conditions, those looking healthier at the end of the cultures were those cultured at 10°C and 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , at 12°C and 50-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , and at 16°C and 50  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Fig. 1). On another hand, *W. setacea* was able to survive close to 8 months at 18-24°C and 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , but survival was very low at 24°C combined with PPFD of 5, 50 or 75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Fig. 1, 2). Finally, at 26°C it survived less than 3 months (Fig. 1) and it was severely damaged at the end of the first month in culture.

### Growth rate

In most cases growth rate was maximal during the first 2 months of culture and strongly decreased afterwards (Fig. 2). This strong decrease was partially due to the fact that at the beginning of cultures thalli increased meanly in surface, but afterwards specimens became massive and grew also in volume. At short-term, growth was maximal at 16°C combined with 50-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , but at long term specimens growing at 16°C and 75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  died, whereas the growth was slow but maintained at 50

$\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Fig. 1, 2). Consequently, after 8 months of culture, the maximal surface was obtained at 16°C combined with 50  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  (Fig. 3). At higher temperatures (18, 24 and 26°C) maximum growth was observed at 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , followed by 50-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . In all the assayed temperatures the lower growth was observed at 5  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  and growth was lower at colder temperatures. Thallus surface after 8 months of culture was both temperature ( $p < 0.001$ ) and PPFD ( $p < 0.001$ ) depending and there was a significant interaction between these factors ( $p < 0.001$ ), whereas growth rate was only PPFD depending ( $p = 0.049$ ).

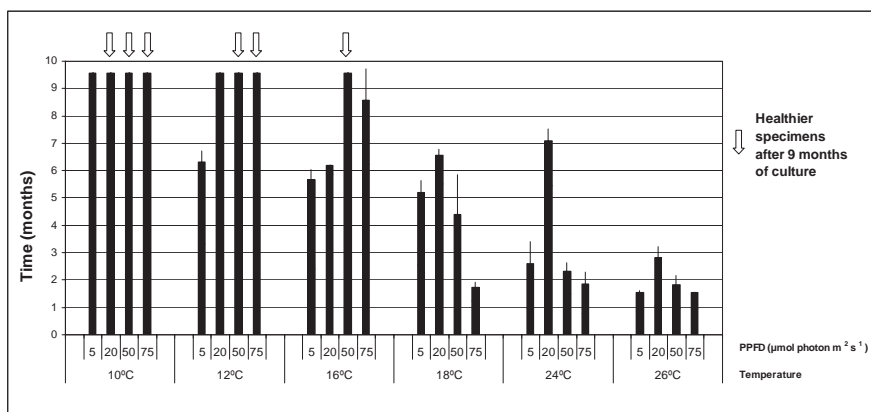


Fig. 1: Survival of thalli of *W. setacea* as a function of temperature and PPFD. Statistical deviation of the total survival for each culture condition is also shown ( $n = 4$ ).

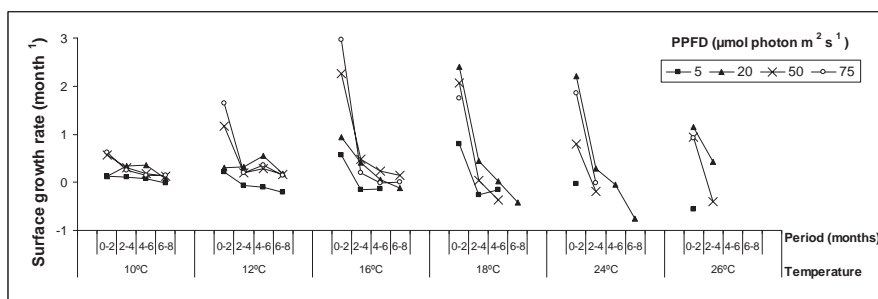


Fig. 2: Thallus surface growth rate of *W. setacea* as a function of temperature and PPFD.

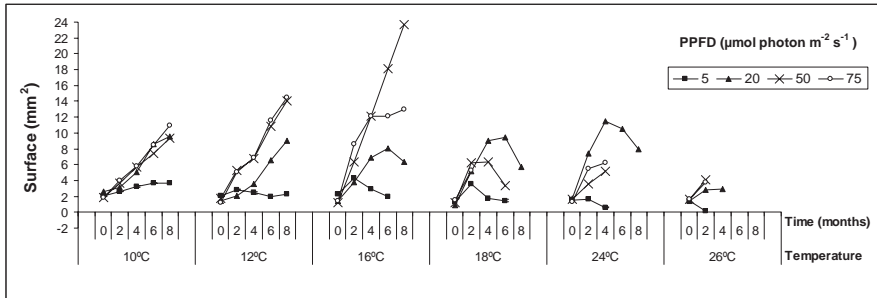


Fig. 3: Thallus surface of *W. setacea* as a function of temperature and PPFD.

## Discussion

*Womersleyella setacea* is distributed all around the Mediterranean (see Guiry & Guiry 2010). Our study indicates that light and temperature are obviously critical factors determining the ability of *W. setacea* to spread across the Mediterranean, but are also decisive to explain its present bathymetric distribution.

Our results showed that *W. setacea* is able to survive hot summer periods (up to 24°C) of the upper infralittoral Mediterranean zone but only at 20  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Optimal temperatures are quite in agreement with those found by Rindi *et al.*, (1999), which showed 15°C to be the optimal temperature for the development of *W. setacea* at 30  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Furthermore, *W. setacea* is considered to be a sciaphilic species (Rindi *et al.*, 1999) and it usually grows in dim places (authors pers. observ.). Its light requirements confirm its sciaphilic behaviour, thus presenting a maximal survival and growth at PPFD compressed between 20 and 75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  depending on water temperature. Low survival and growth was observed at lower PPFD (5  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) and this could explain why at deep circalittoral zones, where light is 0.05-3% of the water surface irradiance (Ballesteros, 1992), it does not form dense turfs (Rindi *et al.*, 1999). A decrease of growth at 3  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  was also observed by Rindi *et al.* (1999). Finally, the development of massive turfs of *W. setacea* in the upper circalittoral is probably related with its high growth rate at PPFD of 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  combined with temperatures ranging from 12 to 16°C and to the possibility of presenting a high growth during at least 4 months at 18-24°C and 20-75  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Growth of *W. setacea* is higher than those recorded for other Mediterranean perennial or pseudoperennial species growing on coralligenous bottoms (unpublished data of the first author).

## Acknowledgments

We like to thank E. Ballesteros, A. Colom, C. Gifre, B. Hereu, L. Navarro, A. Papió and B. Weitzman for several and important technical assistances during this study. This work was supported by an EU Reintegration grant (ERG-2009-248252) and a grant from the Spanish Ministry of Science and Technology (CGL2004-05556-C02-01).

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## **TRACE METAL POLLUTION INCREASES BETA-DIVERSITY IN CYSTOSEIRA SPP. EPIPHYTIC ASSEMBLAGES**

### **Abstract**

*Changes in beta-diversity of epiphytic assemblages thriving on *Cystoseira* specimens (Fucales, Heterokontophyta) in relation to trace metal pollution were evaluated. Replicated individuals belonging to three different *Cystoseira* species were subjected to different pollution treatments (control, slightly polluted and highly polluted). Beta diversity of *Cystoseira* epiphytes (measured as percentage of epiphyte species change between *Cystoseira* individuals) increased significantly in the specimens subjected to the highly polluted treatment, coinciding with increasing trace metal concentrations in environment and algae. Our results suggest that an increase in beta diversity of *Cystoseira* epiphytic assemblages at a small scale could be used as an early indicator of pollution stress.*

**Keywords:** *Cystoseira*, epiphyte assemblages, pollution, trace metals, beta-diversity

### **Introduction**

High levels of pollution have generally negative effects on biodiversity (Lotze *et al.*, 2006). In the Mediterranean Sea, macroalgae of the genus *Cystoseira* Agardh, the primary foundation species in sublittoral rocky bottoms (Giaccone, 1973), have suffered a decline during the last decades which has been mainly attributed to increased pollution (Arévalo *et al.*, 2007; Munda & Veber, 1996). Specimens of the genus *Cystoseira* generally support considerable epiphytic assemblages (Belegratis *et al.*, 1999). Epiphytes display shorter life cycles than *Cystoseira*, being thus more dynamic; therefore, changes in epiphytic assemblages may indicate pollution stress before *Cystoseira* starts showing any stress symptoms. The aim of this study is to evaluate the effect of trace metal pollution on beta-diversity of *Cystoseira* epiphytes.

### **Materials and methods**

Between 16 and 20 specimens of *Cystoseira barbata*, *C. crinita* and *C. spinosa* v. *tenuior* were subjected to four different experimental treatments: Control (labelled but untouched specimens, C), transplanted specimens at the Slightly Polluted (SP) and the Highly Polluted areas (HP), and a transplant control at the control area (TC). The polluted areas are situated in a bay with strong human influence (urban and industrial activities). HP area is located 1 km away from the source area, while SP area is located 3 km away from it. The area chosen as Control is an area morphologically identical to the polluted area but with very low human influence. Pollution levels at the different areas and effects of pollution on *Cystoseira* specimens are reported in Sales *et al.*, (submitted).

The experiment lasted 9 months, and then *Cystoseira* specimens were collected and transported to the laboratory. The sessile epiphytes were identified and relative

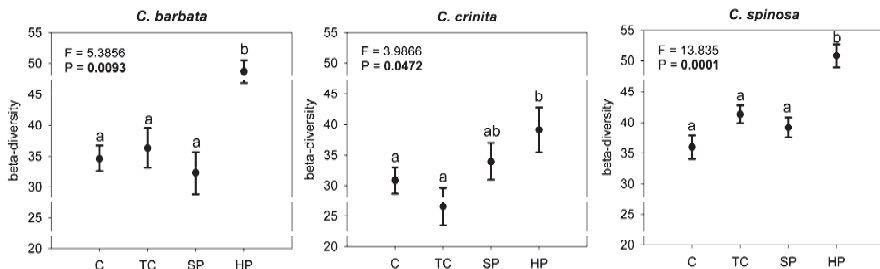


abundances of species were measured as horizontal coverage in cm<sup>2</sup> (Ballesteros 1986). Pieces of *Cystoseira* tissue were cut and stored for trace metal analysis. Concentrations of Pb, Cu, Cr, Zn and V were determined in sediments and algae following the methods explained in Cebrian *et al.*, (2007).

For the data analysis, differences in beta-diversity between experimental treatments were evaluated for each species using PERMDISP routine (available in PRIMER 6 + PERMANOVA). PERMDISP is a routine for evaluating the degree of dispersion of a group of samples based on a distance matrix. When used on a Jaccard distance matrix (presence/absence) is directly interpretable as a test for similarity in beta diversity among groups (Anderson 2006). dbRDA analyses (McArdle & Anderson 2001) were applied to study and visualize the relationship between the composition of the epiphytic assemblage and the trace metal concentrations.

## Results

Environmental concentrations of trace metals were significantly higher at the HP area than at the SP area, and lower at the C area than at the SP area (ANOVA,  $p < 0.05$ ; Sales *et al.*, submitted). The epiphytic assemblages of the species *C. barbata* and *C. spinosa* v. *tenuior* showed significantly higher values of beta diversity in HP treatment than in the rest of treatments. In the case of *C. crinita*, beta-diversity of epiphytes was significantly higher for the HP treatment than for C and TC treatments only (Fig. 1).



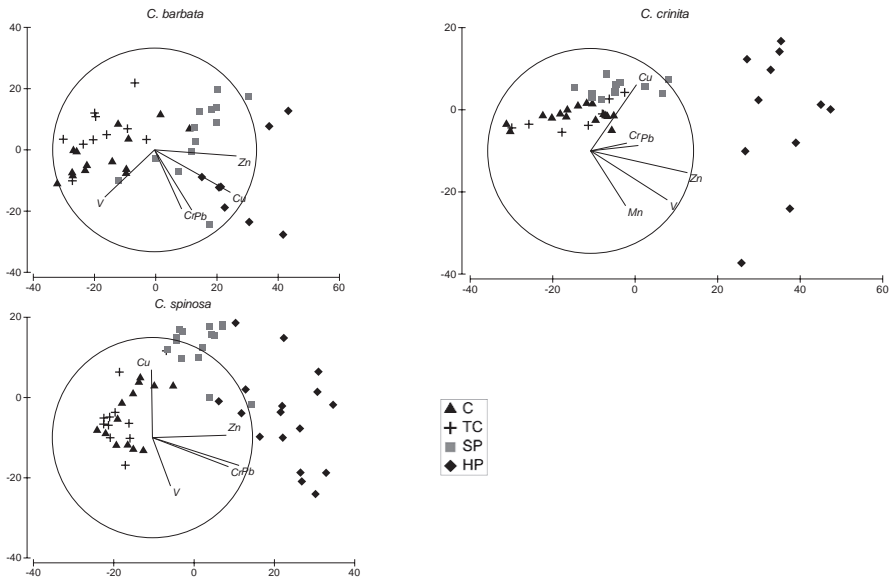
**Fig. 1:** Mean ( $\pm 1SE$ ) epiphytes beta-diversity for each species and treatment showing results of PERMDISP analyses.

In the dbRDA plots (Fig. 2), an increase in the multivariate dispersion of the samples can be appreciated for the treatment HP, coinciding with higher concentrations of trace metals in algae.

## Conclusion

An increase in beta-diversity at small scale has been found in epiphytic assemblages growing over three *Cystoseira* species coupled to an increase in trace metal pollution. In contrast, Piazzini and Balata (2008) found a decrease in beta diversity in impacted assemblages (invaded by *Caulerpa racemosa* v. *cylindracea*). However, the scale at which they studied the phenomenon was much greater than our scale (they considered assemblages at different depths and we considered different plants at the same area). On the other hand, Warwick and Clarke (1993) noted that, in a variety of environmental

impact studies, the variability among samples collected from impacted areas was much greater than that from control sites. Although results from this study have to be considered as preliminary, our findings agree with those by Warwick & Clarke (1993), thus reinforcing the idea that the increase of beta diversity at small scale could be used as an indicator of stress.



**Fig. 2:** dbRDA plots showing relationship between epiphyte community composition and trace metal concentrations.

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## **EVALUATION OF THE CONTAMINATION OF THE MEDITERRANEAN SEA BASED ON THE ACCUMULATION OF TRACE-METALS BY *POSIDONIA OCEANICA***

### **Abstract**

*The ability of *Posidonia oceanica* to concentrate contaminants has been used to evaluate trace-metal contamination in 84 sites, around the entire Mediterranean basin. Based on these measurements, evaluation scales have been established for seven metals (As, Ag, Cd, Cu, Hg, Ni and Pb), based on the calculation of the mean value of each quintile as class limit, allows the ranking of all sites and their classification in five classes between “very low” until “very high” contamination.*

*The application of these preliminary scales to bibliographic data demonstrated a good accuracy with the previous classification of these sites as “non contaminated” or “contaminated” sites, and allowed to propose a first map of contamination for different trace-metals at the sub regional level (e.g. Western Mediterranean basin, Adriatic basin). These maps will be very useful in the framework of the monitoring of the reduction of trace-metal contaminations (e.g. Horizon 2020 goals).*

**Key-words:** Mediterranean Sea, seagrass, *Posidonia oceanica*, trace metal contamination, evaluation scale.

### **Introduction**

The Mediterranean basin occupies the 7<sup>th</sup> place out of the 35 in the list of the most threatened seas (PNUE, 1996) and represents, one of the regions of the world with the most serious environmental problems (UNEP, 2005). Since few years, a number of programmes have been setting up, at international level, aiming at the improvement of the environmental quality (EU Water Framework Directive, EU Marine Strategy, Horizon 2020, etc.). Since the last decades, evaluation of trace metal in coastal environment have been realized using bioindicators, like mussels placed in artificial stations (“caging”) in coastal water (Andral *et al.*, 2004). This reliable method offers the opportunity to compare the contamination between sites, everywhere, but requires time and money (Pergent-Martini *et al.*, 2005). As many seagrass, *Posidonia oceanica* (L.) Delile is highly sensitive to environmental changes and according to its wide distribution in the Mediterranean basin, its longevity, its permanent presence whatever the season, this species is particularly suitable for environmental studies (Augier, 1985). The ability of *Posidonia oceanica* to concentrate trace metals has been clearly established since several years (Augier *et al.*, 1977; Maserti *et al.*, 1988) and recent researches have proved the efficiency (Lafabrie *et al.*, 2007) of this species as trace-metal accumulator.

Therefore, the aim of this study is, using *Posidonia oceanica*, to evaluate the metal contamination of coastal water in several sites of the Mediterranean basin in order to propose quality scales, for seven metals, that can be useful in the framework of the

implementation of the WFD and the monitoring of coastal contamination (e.g. Horizon 2020 initiatives).

### Material and Methods

*Posidonia oceanica* leaves were collected at  $10 \pm 5$  m depth by SCUBA diving, between April and July 2006, 2007 and 2008 in 84 sites, located in 13 countries (Fig 1). The season has been respected each year to avoid possible seasonal variations of metal concentrations. Only the two external leaves of *Posidonia oceanica* (Adult 1 and Adult 2) were sampled, that avoids the mortality of the plant. The blades of the leaves were cleaned (epiphytes scraped off), rinsed (ultrapure water) and either lyophilised (Heto<sup>®</sup> FD4-85 freeze dryer, HetoHolten A/S) or dried at 30°C to constant weight, before they were manually reduced to powder.

Metals analysed were silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), mercury (Hg), nickel (Ni) and lead (Pb). All these metals are considered as priority contaminants under the Barcelona Convention (UNEP, 2006). Mercury concentrations (Hg) were determined according to the method described in Ferrat *et al.*, (2003). Mineralized samples were analysed with a cold vapour atomic absorption spectrometer (CV-AAS – Perkin Elmer<sup>®</sup>). Other trace metals analyses were run by atomic absorption spectrometry with quality assurance procedures at the Laboratory of Rouen / ETSA (France). The accuracy of the method was evaluated by calibration vs. an international standard (CRM 60: *Lagarosiphon major*, Community Bureau of Reference – BCR, Brussels).

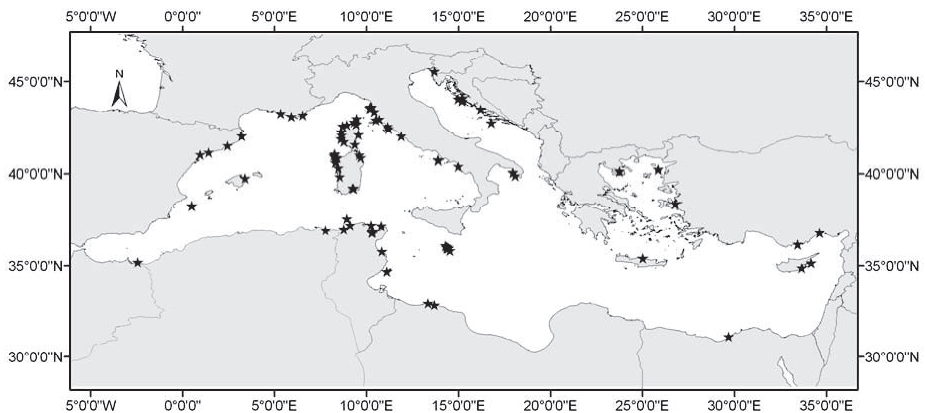


Fig. 1: Mediterranean sampling sites

### Results and discussions

Among the four metals (Cd, Hg, Ni and Pb) analyzed in the 84 sites, Porto (Corsica) presents the maximum value of Cd ( $4.43 \pm 0.31 \mu\text{g.g}^{-1}$  dry wt.); Urla (Turkey) the maximum value of Hg ( $0.204 \pm 0.004 \mu\text{g.g}^{-1}$  dry wt.); Canari (Corsica) the maximum value of Ni ( $123.00 \pm 4.62 \mu\text{g.g}^{-1}$  dry wt.) and Rades (Tunisia) the maximum value of Pb ( $16.83 \pm 0.78 \mu\text{g.g}^{-1}$  dry wt.). On the other hand, this last site presents the minimum value of Cd ( $0.23 \pm 0.01 \mu\text{g.g}^{-1}$  dry wt.) and Ni ( $1.50 \pm 0.10 \mu\text{g.g}^{-1}$  dry wt.). From a general point of view, sites located in Ionian Sea are significantly less contaminated in Ni than those situated in Tyrrhenian Sea, Algero-provencal Basin and Adriatic Sea (Kruskal-Wallis,  $p < 0.1$ ). The minimum value of Hg ( $0.027 \pm 0.007 \mu\text{g.g}^{-1}$  dry wt.) is

recorded in Kalogria (Greece) and the minimum value of Pb ( $0.60 \pm 0.15 \mu\text{g.g}^{-1}$  dry wt.) is present in Macinaggio (Corsica).

Three others metals (Ag, As and Cu) are only analyzed in 76 sites (the sites of Livorno and Porto Torres are excluded). Ile Rousse (Corsica) presents the maximum value of Ag ( $1.55 \pm 0.20 \mu\text{g.g}^{-1}$  dry wt.) but many sites present value of Ag under the detection level. Overall, sites located in Aegean Sea and in Levant Sea are significantly more contaminated in Ag than those in Tyrrhenian Sea and in Algero-provencal (Kruskal-Wallis,  $p < 0.05$ ) Basin. For As the maximum value ( $5.30 \pm 0.35 \mu\text{g.g}^{-1}$  dry wt.) is found in Brbinjsica (Croatia) and the minimum ( $0.13 \pm 0.03 \mu\text{g.g}^{-1}$  dry wt.) in St Paul's Bay (Malta). The maximum value of Cu ( $27.70 \pm 2.06 \mu\text{g.g}^{-1}$  dry wt.) is recorded in Olbia (Sardegna) and the minimum ( $9.07 \pm 0.43 \mu\text{g.g}^{-1}$  dry wt.) in Monastir (Tunisia). In general, sites situated in Ionian Sea are significantly less contaminated in Cu than those situated in Tyrrhenian Sea (Kruskal-Wallis,  $p < 0.05$ ).

The 84 sites present different levels of metal contamination, ranging from pristine sites to highly impacted sites and a gradient of contamination for some metals can be established between the East Basin and the Western Basin. Tyrrhenian Sea and Algero-provencal basin are significantly more contaminated in Ni than Ionian Sea, and significantly more contaminated in Ag than Levant Sea and Aegean Sea. Another gradient could be pointed out: stations situated in the North of Western Mediterranean Basin present significantly higher concentrations in Ag, As, Cd, Hg, Ni and Pb (Kruskal-Wallis;  $p < 0.1$ ) than stations located in the South of this basin. These two main observations could be linked with the urbanization, more important in the western Mediterranean basin, especially in North (UNEP, 2005).

Therefore, the range of level of impact and metal contamination available allow proposing "quality scales" based on quintile. According to Sanchiz *et al.*, (2001) the values below the detection limit are considered as half the value of the detection limit, then the mean value of each quintile is calculated and considered as class limit (Tab. 1).

**Tab. 1: Preliminary scales based on quintile method (values in  $\mu\text{g.g}^{-1}$  dw)**

	Very low contamination level	Low contamination level	Moderate contamination level	High contamination level	Very high contamination level
Ag	< 0.08	0.08 - 0.22	0.23 - 0.36	0.37 - 0.45	> 0.45
As	< 0.37	0.37 - 0.47	0.48 - 0.66	0.67 - 1.16	> 1.16
Cd	< 1.68	1.68 - 2.01	2.02 - 2.44	2.45 - 2.84	> 2.84
Cu	< 5.47	5.47 - 6.93	6.94 - 8.91	8.92 - 13.03	> 13.03
Hg	< 0.051	0.051 - 0.064	0.065 - 0.075	0.076 - 0.088	> 0.088
Ni	< 14.59	14.49 - 19.51	19.52 - 22.05	22.05 - 27.74	> 27.74
Pb	< 1.17	1.17 - 1.43	1.43 - 1.80	1.81 - 3.23	> 3.23

On the basis of this preliminary quality scales (Tab. 2) it is then possible to evaluate the level of metal contamination of studied sites. Thus, for Ag, 14 sites are considered as having a high level of quality (Very low contamination level), 16 a good level (Low contamination level), 15 a moderate level, 14 a poor level (High contamination level) and 17 a bad level (Very high contamination level).

In order to estimate the coherence of this preliminary scale, it has been applied on existing bibliographic data and a comparison of results were realised (Tab. 2). Only less than 10 % of the studied sites show a different classification (comparison between previous evaluation and present classification). So, the use of such scales appears

promising in the framework of contamination monitoring all around Mediterranean Sea, even if it would need to be implemented by the inclusion of others Mediterranean sites, with different levels of human pressure.

**Tab. 2: Metal concentrations in *P. oceanica* ( $\mu\text{g}\cdot\text{g}^{-1}$  dry wt) from different Mediterranean sites**

Metal	Station	Country	Reference study	Value ( $\mu\text{g}\cdot\text{g}^{-1}$ dw)	Level of contamination in reference study	Level of contamination with the present scale
Ag	Var	France	Grauby <i>et al.</i> , 1991	1.90	No	Significant
As	Var	France	Grauby <i>et al.</i> , 1991	3.00	Contaminated	Significant
Cd	Carry-le Rouet	France	Augier <i>et al.</i> , 1993	1.15	No	No
	East coast	Spain	Sanchiz <i>et al.</i> , 1999	3.70	Contaminated	Significant
	Favigna Island	Italy	Campanella <i>et al.</i> , 2001	2.22	No	No
	Canari	France	Lafabrie <i>et al.</i> , 2007	5.38	Contaminated	Significant
Cu	Carry-le Rouet	France	Augier <i>et al.</i> , 1993	56.96	Contaminated	Significant
	Calvi	France	Warnau <i>et al.</i> , 1995	10.20	Contaminated	Significant
	Ischia	Italy	Warnau <i>et al.</i> , 1995	16.20	Contaminated	Significant
	Marseille	France	Warnau <i>et al.</i> , 1995	12.10	Contaminated	Significant
	Favigna Island	Italy	Campanella <i>et al.</i> , 2001	11.60	No	Significant
Hg	Calvi	France	Pergent & Pergent-Martini, 1999	0.01	No	No
	Marseilles	France	Pergent & Pergent-Martini, 1999	0.18	Contaminated	Significant
	East coast	Spain	Sanchiz <i>et al.</i> , 1999	0.11	Contaminated	Significant
	Livorno	Italy	Lafabrie <i>et al.</i> , 2007	0.13	Contaminated	Significant
Ni	Carry-le Rouet	France	Augier <i>et al.</i> , 1993	13.99	No	No
	Canari	France	Lafabrie <i>et al.</i> , 2007	60.30	Contaminated	Significant
	Calvi	France	Warnau <i>et al.</i> , 1995	5.96	Contaminated	Significant
	Ischia	Italy	Warnau <i>et al.</i> , 1995	8.35	Contaminated	Significant
Pb	Marseille	France	Warnau <i>et al.</i> , 1995	7.76	Contaminated	Significant
	Carry-le Rouet	France	Augier <i>et al.</i> , 1993	<1.00	No	No
	East coast	Spain	Sanchiz <i>et al.</i> , 1999	19.00	Contaminated	Significant
	Favigna Island	Italy	Campanella <i>et al.</i> , 2001	0.91	No	No
	Porto-Torres	Italy	Lafabrie <i>et al.</i> , 2009	2.70	Contaminated	Significant

In addition, the interest of this approach resides in the standardization of the sampling protocol, that can be applied to all Posidonia meadows (even in Marine Protected Areas or when the species is legally protected), and in the fact that the analyses have been carried out in the same laboratory (Laboratoire Municipal de Rouen, NF EN ISO/CEI 17025) with the use of reference samples. This allows to propose for the first time a global distribution of trace metal at the sub regional level, based on a unique bio-indicator, sampled and analyzed by the same way.

### Acknowledgements

This work was supported by a grant from the “Collectivité Territoriale de Corse” and the collaboration of several Mediterranean researchers (Dr Akçali, Dr Apostolaki, Dr Bakran-Petricioli, Dr Belbacha, Dr Borg, Dr Buia, Dr Celebi, Dr Fernandez-Torquenada, Dr Hadjichristoforou, Dr Langar, Dr Lipej, Dr Llagostera, Dr Marcou, Dr Mavric, Dr Panzalis, Dr Pérez, Dr Romero, Dr Salgado, Dr Semroud, Dr Skoufas, Dr Turk, Dr Weitzmann).

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## **PROGRESSION DE *CAULERPA RACEMOSA* (CAULERPALES, CHLOROPHYTA) SUR LE LITTORAL ALGERIEN**

### **Résumé**

*Apparue en 2007 dans quelques localités de la région d'Alger, la prolifération de cette algue commence à être perceptible. En effet Caulerpa racemosa colonise de plus en plus des surfaces relativement importantes du médiolittoral, et de l'infralittoral, sa répartition verticale s'étale depuis la surface jusqu'à environ -26m de profondeur.*

*L'étude du peuplement algal photophile montre des perturbations de l'aspect floristique. L'algue prolifère sur différents substrats : des substrats durs aux substrats meubles et même vaseux, puisqu'elle a été récoltée à proximité d'un débouché d'émissaire d'égout.*

*La prolifération de Caulerpa racemosa se traduit par un appauvrissement des algues photophiles (richesse spécifique faible dans les relevés). L'algue est souvent associée à Corallina elongata J.Ellis et Solander, espèce indicatrice de perturbation du milieu par apport d'eaux usées riches en matières organiques.*

**Key-words:** *Caulerpa racemosa*, progression, disturbance, population, Alger

### **Introduction**

En Algérie, longtemps la seule espèce du genre *Caulerpa* existante a été *C. prolifera* (Forsskal) J. V. Lamouroux, (Hamel, 1930). Depuis les signalisations de *Caulerpa taxifolia* (M.Vahl) C. Agardh et de *C. racemosa* var. *cylindracea* (Sonder) Verlaque, Huisman et Boudouresque dans le Nord de la Méditerranée, le suivi de leur expansion est devenu une question d'actualité en particulier pour *C. racemosa* en Algérie.

*Caulerpa racemosa* a été observée pour la première fois en Algérie en 2005 sans pour autant être abondante. C'est essentiellement depuis 2007 que *C. racemosa* est devenue fréquente (Ould Ahmed et Meneisz, 2007). Elle est signalée de plusieurs localités du centre et de l'ouest algérien ce qui montre sa rapide progression.

Les différents recensements sont de simples signalisations, n'ayant pas fait l'objet de travaux à l'exception de Ould Ahmed et Meneisz, (2007) et Seridi (2007) qui mentionnent respectivement sa présence dans les localités de Sidi Fredj (Est d'Alger) et de Bordj El Kiffan (Ouest d'Alger).

D'autres signalisations ont été faites toujours dans les régions centre et est à différentes profondeurs allant de la surface (+ 0,20 m) à plus de 20 m, de profondeur. Ces signalisations ont été faites pour la plupart par des amateurs et n'ont donc pas fait l'objet ni de publications ni de suivi. *Caulerpa racemosa* a été récoltée sur différents substrats, vaseux à rocheux et dans l'herbier de posidonie.

Ces observations soulèvent le problème des espèces invasives et leurs conséquences sur les communautés autochtones non protégées et non suivies.

La situation actuelle de la distribution de *Caulerpa racemosa* en Algérie n'est que partielle car l'expansion de l'algue sur l'ensemble du littoral algérien n'est pas connue.

## Matériel et Méthodes

Quelques opérations de sensibilisation ont été réalisées auprès des usagers de la mer mais aucune n'a fait l'objet d'un suivi.

*C. racemosa* a été découverte au cours du suivi phytosociologique des peuplements de macrophytes de différentes localités de la région d'Alger. Les prélèvements ont été fixés dans de l'eau de mer formolée à 5%. D'autres récoltes ont été effectuées par des plongeurs amateurs sur la côte ouest de l'Algérie. Ces dernières n'ont pas fait l'objet d'étude descriptive mais constituent une source d'information sur la répartition et l'extension de l'algue en Algérie.

Très récemment une approche cartographique succincte a été réalisée (Lamouti, 2010).

## Résultat et Discussion

L'ensemble des observations réalisées sur de *Caulerpa racemosa* en Algérie a permis de délimiter les zones colonisées notamment au centre et à l'ouest de l'Algérie, par contre peu d'information sont disponibles pour la région Est.

La surface exacte recouverte par l'algue n'a pas été évaluée mais celle-ci peut être importante aux vues des signalisations. Les prairies de *C. racemosa* se distribuent de +0,20m à plus de 20m de profondeur. Des surfaces importantes ont été observées dans quelques stations ayant fait l'objet de suivi pour d'autres études. En générale ces stations présentaient des conditions idéales pour son introduction et sa prolifération : ce sont des stations touristiques, parfois on note la présence de port de pêche, ou de plaisance, d'autre stations sont caractérisées par une activité commerciale, une urbanisation importante, la proximité d'égout (Kabrane, Observation personnelle).

L'implantation et le développement de l'algue sont certainement dus à l'arrachage et au transport par les différents moyens (filets, plaisance, etc.).

En raison de l'absence de campagne de sensibilisation et de signalisation le nombre exact de stations colonisées et la surface occupée par l'algue reste indéterminés.

Dans la région centre, d'Ouest en Est, l'algue a été trouvé à :

Sidi Fredj, Rais Hamidou, Bainem, Port d'Alger, Bordj El Kiffan, Tamentfoust.

Dans la région ouest, les signalisations faites par les usagers de la mer, concernent : Béni Saf et Mostaganem (Fig. 1).

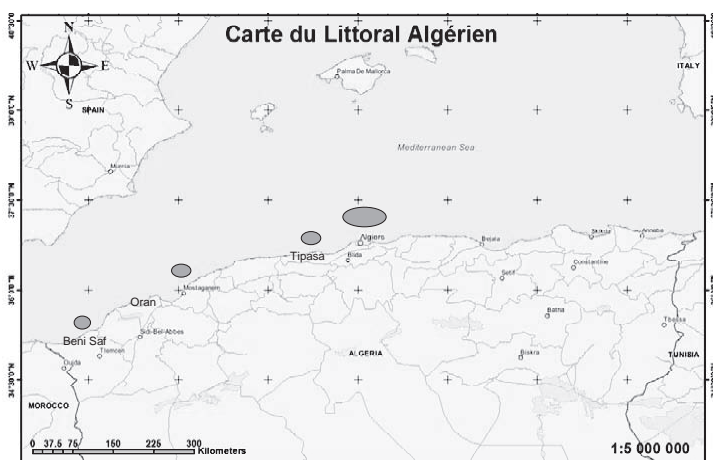


Fig. 1: Répartition de *Caulerpa racemosa* var. *cylindracea* sur le littoral algérien

Tous les spécimens récoltés présentaient le même aspect avec des stolons ramifiés irrégulièrement et enchevêtrés, fixés aux substrats par des rhizoïdes incolores (Fig. 2).



*Fig. 2: Surface occupée par Caulerpa racemosa ( Forskaal)J.Agardth*

Signalée pour la première fois en 2005, l'algue montre une potentialité de croissance importante qui semble être favorisée par des températures de l'eau modérément élevées (environ 20°C), des eaux chargées en matières organiques (stations d'épuration insuffisantes).

Ces conditions laissent présager une dynamique de croissance de l'algue importante.

La progression rapide et constante de *C. racemosa* justifie un suivi méthodique sur les zones envahies ; cela implique l'instauration d'un réseau d'observation afin de préciser les exigences écologiques de l'espèce.

Jusqu'à présent, l'absence de suivi qualitatif et quantitatif des zones colonisées empêche une analyse détaillée de la dynamique de l'expansion de *C. racemosa* en Algérie. Les observations montrent que l'herbier de posidonie n'est pas épargné. Au cours du suivi saisonnier (analyse en cours, Kabrane,) un impact similaire à celui décrit dans la littérature a été constaté à savoir une régression des autres espèces algales. *C. racemosa* est souvent associée à *Corallina elongata* J.Ellis et Solander, espèce indicatrice de perturbation du milieu (apport d'eaux usées riches en matière organiques). L'expansion rapide *C. racemosa* constitue une menace sérieuse pour la biodiversité marine de la côte algérienne ; sachant que celle-ci est assez bien conservée particulièrement dans les secteurs situés loin des agglomérations.

### **Conclusion**

Si *Caulerpa taxifolia* n'a pas été pour le moment trouvée sur la côte algérienne, *Caulerpa racemosa* s'est répandue en Algérie en très peu d'année (2005-2010). Elle est très présente dans les localités colonisées où elle recouvre des substrats très variés, de la vase à la roche.

L'algue est probablement bien distribuée sur le littoral algérien malgré le peu d'informations émanant de la région de la région Est.

L'expansion de *C. racemosa* et son impact sur les biocénoses restent inquiétants car peu de sensibilisation et de campagne sont préconisés pour freiner sa progression et limiter sa dissémination à partir des zones déjà atteintes.

Il conviendrait donc d'informer au mieux les usagers de la mer de la propagation de l'algue et sur ses conséquences au niveau de l'écosystème marin.

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## **A SEASONAL PATTERN OF BIOMASS AND GROWTH OF CYMODOCEA NODOSA IN THE GHAR EL MELH LAGOON IN RESPONSE TO LAGOON CONDITION**

### **Abstract**

*The primary production and the biomass cycle of Cymodocea nodosa located in the Ghar El Melh lagoon (N-E of Tunisia) were calculated monthly from November 2006 to October 2007. Two sampling stations were selected in order to assess the response of the seagrass to lagoon condition; the first station was close to the communication channel with the sea and the second inside the lagoon at the limit of C. nodosa distribution. Leaf growth was estimated using Zieman method and rhizome production was evaluated by marking rhizome terminals.*

*The growth of C. nodosa show a clear unimodal cycle in the tow stations reaching maximum leaf development in summer. Shoot density decreased from 915 to 500 shoot m<sup>-2</sup> from the communication channel toward the lagoon. C. nodosa meadow inside the lagoon exhibited several morphological variables (shoot density, leaf length, total biomass etc.) significantly low from those near to the communication channel. Whereas, the leaf plastochrone interval (22 days) and rhizome production (2.5 m apex<sup>-1</sup> day<sup>-1</sup>) was positively correlated to lagoon conditions. The annual leaf production decrease significantly inside the lagoon, from 519 to 54 g dry wt.m<sup>-2</sup> per year. The seasonal changes of water temperature and salinity, especially in the lagoon condition, were considered to play an important role in the seasonal growth cycle of C. nodosa.*

**Key-words:** Primary production, biomass, *Cymodocea nodosa*, lagoon condition.

### **Introduction**

*Cymodocea nodosa* (Ucria) Ascherson is seagrass species common in the Mediterranean Sea, the North-Atlantic coast of Africa, South-Atlantic coast of Europe and colonizing also coastal areas of the Canary Islands (Den Hartog, 1970). *C. nodosa* shows a high environmental plasticity: (i) colonises from the intertidal (Vermaat *et al.*, 1993) to depths of 33–35 m (Canals & Ballesteros, 1997); (ii) lives on a wide range of substrata, from coarse sand to muddy sediments (Peduzzi & Vukovic, 1990), and (iii) forms widespread meadows in shallow, sheltered area such as lagoons (Agostini *et al.*, 2003), bays (Pérez & Romero, 1994) or harbor areas (Reyes *et al.*, 1995).

The aims of this research were: (1) to assess the growth and the vitality of *C. nodosa* meadow (density, phenology, biomass and primary production during the annual cycle 2006–2007), (2) to define its temporal variability (3) to analyze the influence of lagoon condition (water temperature and salinity) on seagrass morphology and production.

## Materials and methods

The studied *C. nodosa* meadow was located at the Ghar El Melh lagoon, on the north part of the Gulf of Tunis, between Ras Tarf (Cape Farina), and the estuary of Mejerda wadi (Fig. 1). This lagoon is 34 km<sup>2</sup>. Its maximum depth is approximately 3.8 m, with an average of 0.8 m, and the bottom is relatively flat and muddy. The connection with the Mediterranean Sea is ensured by one small, 10-70 m wide channel. In Ghar El Melh, *Ruppia cirrhosa* and the species belonging to the genus *Cladophora* are the most dominant whereas *Cymodocea nodosa* is only located in a small zone of approximately of 7.8 km<sup>2</sup> exposed to the marine influence (Shili *et al.*, 2002).

Two sampling stations were selected in order to assess the response of the seagrass to lagoon condition; the first station was close to the communication channel with the sea and the second inside the lagoon at the limit of *C. nodosa* distribution. The two stations were at a depth of 0.5 m (Fig. 1).

Water temperature and salinity were measured using a salinometer (WTW Cond 315i). Sampling was performed from November 2006 to October 2007 mainly monthly except in summer season (2 samplings per month). To estimate the dynamic pattern of the meadow, the shoot density of *C. nodosa* was measured in plots of 20x20cm (10 replicates at each sampling event); the plant biomass (above and belowground compartments) was furthermore estimated using a metal cylinder of 15 cm diameter. In the laboratory, shoots, rhizomes and roots were separated and dried at 60°C to a constant weight. The length of leaves was also measured.

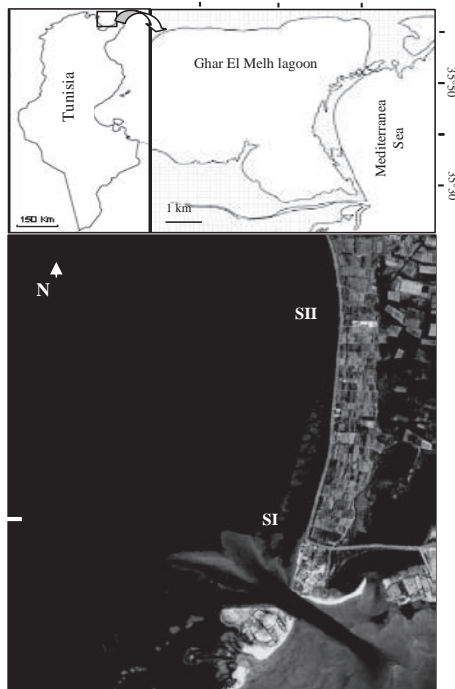


Fig. 1: Map of Ghar El Melh Lagoon. Localisation of sampling sites (SI and SII).



**Leaf and rhizome production** - Twenty shoots of *C. nodosa* were marked monthly 1 cm above the sheath of the oldest leaf according to Zieman (1974) method to estimate the leaf production; in the laboratory, the newly formed tissue was measured by the shift of the marking hole along each leaf. The leaf growth elongation (mm shoot<sup>-1</sup> d<sup>-1</sup>) was determined. The number of new leaves produced per year and the leaf Plastochrone Interval (PI) (Short & Duarte, 2001) were also calculated. Every three months 20 plagiotropic rhizomes were tagged (with a plastic string before the last rhizome node) and collected three months later. Their length increase (mm day<sup>-1</sup>) was measured to estimate the seasonal rhizome growth.

## Results

Water temperature displayed a clear annual pattern, with the lowest values (14°C) in February, and the highest one in September (31°C) for the station I and in August (34°C) for the station II. Salinity generally ranged from 37 to 39 PSU for the station I and to 45 PSU the station II with an important increase in summer (Fig. 2).

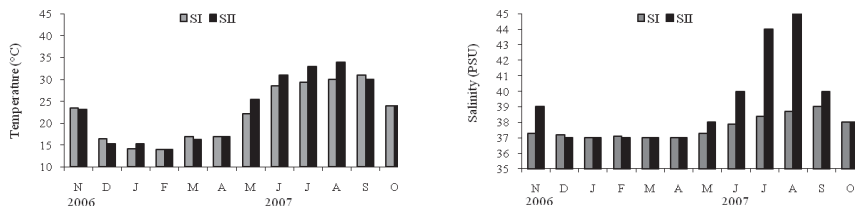


Fig. 2: Water temperature (A) and salinity (B) at the studied stations from November 2006 to October 2007.

Shoot density showed a clear seasonal pattern ( $P < 0.001$ ). The mean annual density of the *C. nodosa* shoots was  $915 \pm 166$  shoots  $m^{-2}$  for the station I and decrease to  $500 \pm 65$  shoots  $m^{-2}$  inside the lagoon (Fig. 3).

Concerning leaf length, both SI and SII showed a regular seasonal fluctuation ( $P < 0.001$ ), with a maximum values reached in July of the SI and in June for the SII (Fig. 4). Leaf length was significantly ( $P < 0.001$ ) higher at SI than SII.

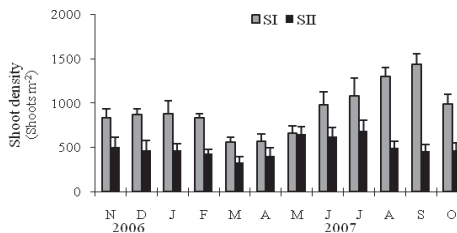


Fig. 3: Annual pattern of the *C. nodosa* shoot density within the two considered stations in the Ghar El Melh lagoon.

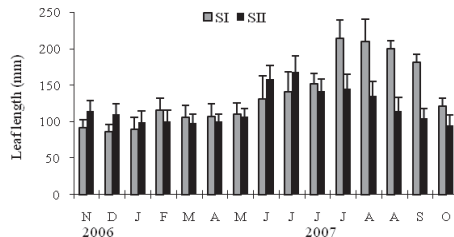
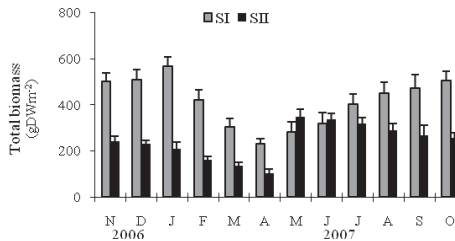


Fig. 4: Annual trend of length of the differentiated leaves (without sheath) in the two considered stations.

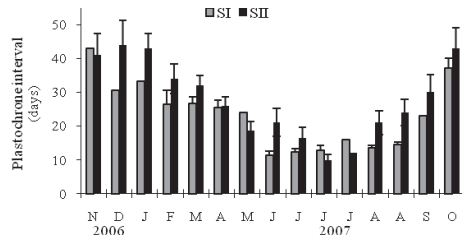
Concerning biomass, the SI and SII showed opposite trends (Fig. 5). In April, the total biomass reached its lowest value at the station II. Whereas the SI exhibited the highest

ones in May. The average total biomass varied respectively between  $241.6 \pm 34$  g dry wt.  $m^{-2}$  and the SI and  $413.8 \pm 46$  g dry wt.  $m^{-2}$  at the SI.

Leaf plastochrone intervals (PI) varied seasonally ( $P < 0.001$ ) with an annual average of about 23 days at the SI and 28 days at the SII (Fig. 6).



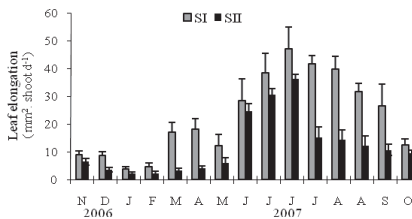
**Fig. 5 :** Annual variation of total, above- and below-ground biomass in the two considered stations.



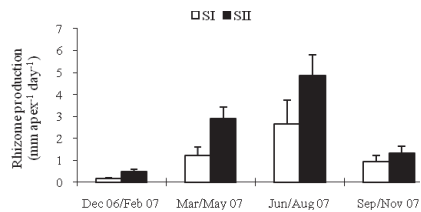
**Fig. 6 :** Annual pattern of plastochrone interval in the two considered stations.

The daily leaf elongation followed a clearly unimodal seasonal diagram (Fig. 7), increased between May to July. Moreover, an important decrease was observed in the second part of July at SII.

The lowest rates of the daily rhizome production were recorded in winter and the highest in summer for the both stations. Their mean values were respectively  $1.2 \pm 1$  mm d<sup>-1</sup> at S1, and  $2.4 \pm 1.9$  mm d<sup>-1</sup> at SII (Fig. 8).



**Fig. 7:** Annual pattern of daily leaf growth in the two considered stations.



**Fig. 8:** Annual pattern of daily rhizome production in the two considered stations.

The Annual leaf production was approximately ten times higher at SI ( $518.6$  g. DW  $m^{-2}$  year<sup>-1</sup>) than at SII ( $54$  g. DW  $m^{-2}$  year<sup>-1</sup>)

### Discussion and conclusions

The shoot density observed showed a unimodal annual within one order of magnitude from winter and spring to summer and autumn. The shoot densities were a bit lower than those found in other localities ( $2000$  shoot  $m^{-2}$  - Pérez, 1989;  $1925$  shoot  $m^{-2}$  - Cancemi *et al.*, 2002;  $2302$  shoots  $m^{-2}$  - Rismondo *et al.*, 1997;  $1520$  shoots  $m^{-2}$  - Agostini *et al.* 2003), with the exception of plants growing in banc d'arguin ( $576$  shoots  $m^{-2}$  - Van Lent *et al.*, 1991).



The biomass cycle of *C. nodosa* showed a seasonal model identical to that observed for the same seagrass species in marine Mediterranean and Atlantic environments (Agostini *et al.*, 2003; Cancemi *et al.*, 2002; Reyes *et al.*, 1995; Terrados & Rós, 1992). The total biomass was lower than those recorded in Ebro Delta (631 g dry wt. m<sup>-2</sup> by Pérez, 1989) and in the lagoon of Venice (1700 g dry wt. m<sup>-2</sup> by Rismondo *et al.*, 1997).

The annual leaf production was similar to many Mediterranean sites: 388-467 g. DW m<sup>-2</sup> year<sup>-1</sup> (Pérez 1989); 427 g. DW m<sup>-2</sup> year<sup>-1</sup> (Terrados & Ros, 1992); 412 g. DW m<sup>-2</sup> year<sup>-1</sup> (Cancemi *et al.*, 2002).

Seasonal variations in seagrass characteristics have been attributed to seasonal changes in irradiance and in water temperature (Duarte, 1989). *C. nodosa* productivity, biomass and shoot size usually increase with increasing water temperature and light availability during spring and summer and decrease during autumn and winter (Agostini *et al.*, 2003; Cancemi *et al.*, 2002; Cunha & Duarte, 2007; Reyes *et al.*, 1995; Terrados & Rós, 1992).

Our results show also that the vegetative development of *C. nodosa* inside the lagoon at the limit of their distribution is different from that observed close to the communication channel with the sea. The values of the studied morphological variables were significantly low from those recorded in the station near to the communication channel. The reduction of seagrass vegetative development could be explained by the high level of salinity recorded in summer. In fact, according to Pagès *et al.* (2010), salinity should never exceed 44 psu inside the meadows, to prevent deterioration of *C. nodosa* plant preceding the deterioration of *C. nodosa* habitat.

### Acknowledgements

This study was largely financed by the WADI project (ICA3-CT2002-10003) as well as by the ARUB (Support Research of University Base).

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## **IMPACT DES ACTIONS ANTHROPIQUES SUR LE PHYTOBENTHOS DE LA BAIE DE MONASTIR.**

### **Résumé**

*Cette évaluation a été faite à partir de l'établissement de 6 profils bio écologiques concernant la zone de la baie comprise entre la ville de Monastir à l'ouest et les îles Kuriat au nord est et la ville de Teboulba au sud est. Les observations effectuées sur la macro flore benthique ont montré que l'état environnemental de la zone étudiée a, dans son ensemble, une qualité globale médiocre avec une très forte déstructuration des écosystèmes de la partie littorale comprise entre la partie sud de la lagune de Khniss et la zone de Teboulba. La caractérisation des principaux indicateurs de stress et des réponses des écosystèmes a permis l'élaboration de cartes phyto écologiques mettant en évidence la bipartition des biotopes de la baie : « lagon versus herbier » : à savoir une zone « para lagunaire » côtière fortement déstructurée par l'impact direct des actions anthropiques aussi bien urbaines qu'agricoles ou industrielles et, plus au large, la zone de l'herbier de posidonie où les facteurs majeurs de déstructuration environnementale sont à mettre en relation avec les activités liées à la pêche ou/ et à la plaisance.*

**Key-words :** *Posidonia*, phytobenthos, anthropic impact, Monastir.

### **Introduction**

L'étude d'impact de l'ensemble des activités anthropiques sur les écosystèmes littoraux et marins de la baie de Monastir, faite sous les auspices du Ministère de l'environnement tunisien, a eu pour but de faire le point sur la situation écologique de cette baie afin de programmer, à l'échelle régionale, un ensemble d'actions de réhabilitation des zones les plus dégradées. Cette étude a comporté plusieurs volets, mais nous ne retiendrons dans ce qui suit que la seule partie purement bionomique.

Les études de bionomie benthique concernant la baie de Monastir ont, depuis le début de notre siècle (le 21<sup>e</sup>), été nombreuses. Parmi les travaux botaniques les plus importants, nous citerons ceux concernant les herbiers de Posidonies de la baie menés par l'équipe qui s'est intéressée aux impacts des actions anthropiques (el Asmi-Djellouli *Z et al.*, 2001) et à la relation entre l'hydrodynamisme et la typologie de l'herbier (el Asmi - Djellouli *Z et al.*, 2003). Ces travaux ont été suivis en 2004 par une étude morphologique et phénologique très complète (Sghaier *et al.*, 2006) au niveau des isobathes 2, 5 et 10 qui a mis en évidence le mauvais état de l'herbier vivant à 5 m. Enfin le projet MedPosidonia, sous l'égide du PNUE –RAC/SPA (2009), s'est, entre autres, attaché à l'étude de la frange haute des herbiers de posidonies de la baie de Monastir entre les villes de Lemta et de Teboulba.

### **Matériel et méthodes**

Les prélèvements d'échantillons ont été réalisés au cours de la dernière quinzaine du mois de mars 2009 dans 31 stations réparties selon 6 radiales perpendiculaires au trait de côte à l'ouest et avoisinant les hauts fonds entre le continent et l'archipel des Kuriat à

l'est (Fig.1). L'ensemble des données qualitatives et quantitatives obtenues à partir des observations faites en plongée et des échantillons récoltés ont été examinés selon un angle écosystémique (partie concernant le seul macro phytobenthos). Dans le cadre de cette étude d'impact l'accent a été mis sur les points forts et faibles de l'état environnemental au niveau de chaque radiale et de chaque station. Dans ce qui suit, l'étude biocénotique est faite en partant du large (zone nord) vers le fond de la baie (zone sud) pour les radiales, et, pour chaque radiale, en partant du continent (zone ouest) vers le « large » zone est.

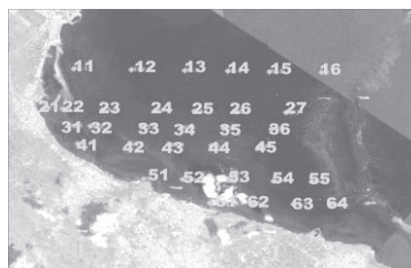


Fig. 1 Baie de Monastir - emplacements des stations.

## Résultats

**La radiale 1** correspond à la partie nord de la baie et comporte 5 stations (st 11 à 16). *Posidonia oceanica* est présente entre 3,5 et 13 m dans toutes les stations. On l'y rencontre sous des formes très diverses : feuilles courtes et arquées dans la zone des 3 m de profondeur, sous forme de litière dans la cuvette centrale (13 m –station 14-), sous forme de prairie mixte à *Cymodocea nodosa* et *Caulerpa prolifera* à la station 15, association que l'on peut assimiler à un faciès de dégradation de l'herbier. Dans presque tous les cas, les rhizomes sont assez fortement dénudés et leur croissance se fait horizontalement. Deux blocs écologiques peuvent être distingués tout au long de cette radiale. Un bloc occidental (stations 11 à 13) où le milieu est peu perturbé. Un bloc oriental où l'on enregistre, au contraire, une légère déstructuration environnementale avec l'apparition d'éléments du coralligène. Cette détérioration peut, en grande partie, être liée à des actions de pêche illicites (labourage des fonds par des engins prohibés) ou/et à des ancrages intempestifs (navigation de plaisance liée à la présence voisine de nombreuses marinas).

**La radiale 2** comprend 7 stations (21 à 27) allant de 0,5 m (st ouest proche de l'embouchure de l'«oued» de Khniss) à 11 m de profondeur (zone centrale). Les espèces bio indicatrices d'eutrophisation, *Ulva rigida*, *Chaetomorpha linum*, constituent une énorme biomasse dans les stations les plus côtières (stations 21 et 22) où elles se surimposent à une prairie mixte à *Zostera noltii* et *Caulerpa prolifera*. Dans la zone côtière sont aussi présentes les deux espèces invasives exotiques *Valonia aegagropila* et *Caulerpa racemosa* var *cylindrea* (station 21). *Posidonia oceanica* est, quant à elle, présente à partir de l'isobathe des 4 m dans toutes les stations prospectées (stations 23 à 27). Tout au long de cette radiale, trois blocs écologiques peuvent être distingués. : un bloc occidental (stations 21 et 22) où le milieu peu profond est très fortement perturbé par les impacts liés à la proximité d'une côte très largement anthropisée et à de nombreux déversements « sauvages » d'eaux usées ménagères (voire industrielles); un bloc central occupé par un herbier en bonne condition (st 23 et 24) mettant en évidence

une atténuation importante des impacts liés aux activités côtières ; un bloc oriental (st 25 à 27) avec la présence d'un herbier très fragmenté de type « inter mattes érosives » (Boudouresque *et al.*, 2006). Les fonds, dans cette zone relativement éloignée de la côte, subissent, en effet, le contre coup des activités halieutiques.

**La radiale 3** comprend 7 stations (30 à 36) allant des profondeurs de 1 m (st 30, la plus à l'ouest) à 7m (st 35 zone est). On rencontre *Posidonia oceanica* dans toutes les stations dont la profondeur est supérieure à 1,5 m (stations 32 à 36). Les espèces bio indicatrices d'eutrophisation constituent une énorme biomasse dans les stations les plus côtières (stations 30 à 32 « zone du lagon ») où elles se surimposent à une prairie mixte de *Cymodocea nodosa* et *Caulerpa prolifera*, où, par ailleurs, prolifèrent *Caulerpa racemosa* et *Valonia aegagropila*. Les observations *in situ* ont montré un herbier fragmenté en massifs de taille assez réduite, séparés par de larges plages sableuses parsemées de feuilles de posidonies déchetées. Sur cette radiale, deux blocs écologiques ont été identifiés : un bloc occidental (stations 30 à 32) où le milieu très peu profond (lagon) occupé par les algues nitrophiles et les espèces exotiques invasives est très fortement déstabilisé par les impacts liés à la proximité d'une côte très largement anthropisée et un bloc central (st 33 à 36) où l'eutrophisation est atténuée mais où l'on trouve de nombreux témoignages de la présence d'un herbier « en pain de sucre » entouré de plages nues ou secondairement occupées par des algues fixées sur les mattes mortes.

**La radiale 4** comprend 5 stations (41 à 45) allant des profondeurs de 1,2 m (st 42, à l'ouest) à 7m (st 43 zone centrale). Dans cette zone proche du fond de la baie, les espèces bioindicatrices d'eutrophisation qui se surimposent à une prairie mixte à *Caulerpa prolifera*, cymodocées et /ou posidonies, sont partout présentes (stations 41 à 45). *Posidonia oceanica* est présente à partir de la station 42 sous forme d'un « récif barrière », puis, dans toutes les autres stations, sous forme d'un herbier clairsemé aux feuilles largement épiphytées. Trois blocs écologiques peuvent être distingués tout au long de cette radiale : un bloc occidental (stations 41 et 42) où le milieu très peu profond (lagon et frange externe du « récif barrière ») est très fortement déstructuré ; un bloc central (st 43) légèrement plus profond où l'eutrophisation a un impact limité et qui est occupé par un herbier en assez bonnes conditions ; un bloc oriental (st 44 et 45) où l'impact de l'eutrophisation, à nouveau sensible, se traduit par une détérioration plus ou moins importante de l'herbier.

**La radiale 5** comprend 5 stations (51 à 55) allant des profondeurs de 1 m (st 51 et 52 stations, à l'ouest, proches de celles explorées par les chercheurs du projet MedPosidonia) à 7m (st 53 et 54). Les algues polluo résistantes sont présentes dans les stations ouest (stations 51 à 53). Celle dont la biomasse est la plus forte est la chlorophycée *Chaetomorpha linum*. Les rhodophycées sont représentées par *Gigartina acicularis* (st 51 et 52) et *Aspargopsis* sp (très abondante à la st 51). Nous ajoutons à cette liste l'espèce invasive exotique *Caulerpa racemosa* (st 52 et 53). *Posidonia oceanica* est présente à l'est, entre 3,5 et 5 m (st 53 à 55). Dans leur ensemble les plants, partiellement déchaussés, ont une croissance majoritairement horizontale (plagiotope). Deux blocs écologiques peuvent être distingués : un bloc occidental (stations 51 et 52) où le milieu très peu profond (lagon), très largement eutrophisé, peut être considéré comme totalement déstructuré ; un bloc oriental (st 53 à 55) occupé par les posidonies où l'impact de l'eutrophisation est assez largement atténué.

**La radiale 6** correspond au fond de la baie ; elle comprend 4 stations (61 à 64) avec des profondeurs allant de 1,8 m (st 61 à l'est) à 4,5 m (st 64 à l'ouest). Les algues

nitrophiles sont abondantes dans les stations ouest (stations 61 et 62). Celle dont la biomasse est la plus forte reste la chlorophycée *Chaetomorpha linum*. Nous devons ajouter à cette liste la présence de *Valonia aegagropila* (st 61). L'herbier à *Posidonia oceanica* est présent entre 3 et 4,5 m (st 62 à 64 sous une forme mixte (présence conjointe d'une prairie de Cymodocées). Dans leur ensemble les feuilles des posidonies sont très longues (100 cm et plus), assez fortement épiphytées. Les rhizomes sont, le plus souvent, déchaussés. Deux blocs écologiques peuvent être distingués : un bloc occidental (st 61 et 62) où le milieu est très largement eutrophisé ; un bloc oriental (st 63 et 64) occupé par un herbier mixte où l'impact de l'eutrophisation est assez largement atténuée.

## PRINCIPALES CARACTERISTIQUES DES BIOCENOSSES PHYTALES

### La biodiversité

Au total 30 espèces macrobenthiques ont été recensées. Selon les radiales, le taux de biodiversité est très peu différent : il oscille entre 11 et 16 espèces. En ce qui concerne la répartition des espèces selon leur signification écologique et notamment celle des espèces indicatrices d'eutrophisation, il y a peu de divergences entre les radiales avec un taux de présence des espèces nitrophiles allant de 30 et 35% de l'ensemble floristique (Fig.2), à l'exclusion de la radiale 1 qui est celle, à la fois, la plus « au large » et donc celle de plus grande profondeur.

### Les espèces les plus fréquentes

Dans la baie de Monastir, parmi les espèces dont la fréquence est supérieure à 25% (Fig.3), *Posidonia oceanica* avec une fréquence de 70% arrive en premier lieu. Lui succèdent *Halimeda tuna* (40%) et, avec une fréquence très légèrement plus faible (38%), *Ulva rigida*. Il faut, toutefois, souligner que si *Posidonia oceanica* obtient le rang 1, ceci nous permettant de conclure à la persistance d'un herbier sur la plus grande partie de la baie, il ne faut pas perdre de vue que les stations étudiées ont une profondeur qui ne dépasse pas 14 m : profondeur à laquelle nous avons observé la présence de larges dépôts de feuilles de posidonie en voie de rouissage (stations 14 et 15).

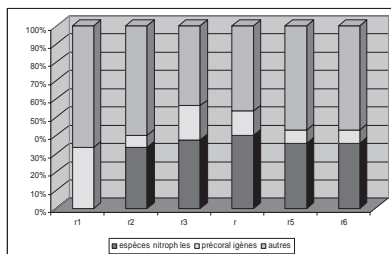


Fig. 2: baie de Monastir - phytobenthos - répartition (radiales 1 à 6) des différentes catégories écologiques - En rouge : espèces nitrophiles, en jaune : espèces précoraligènes, en vert : les autres

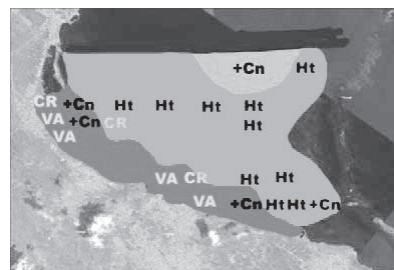
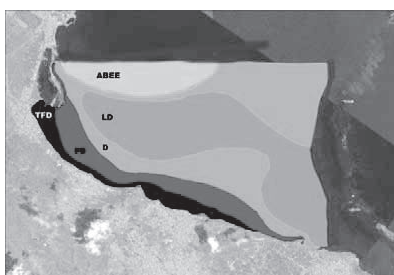


Fig. 3: la baie de Monastir - carte phytosociologique schématique ; En vert : l'herbier de posidonies. En kaki : les « fonds rouis ». En rouge: les algues nitrophiles. En jaune: les espèces exotiques invasives. Cr: *Caulerpa racemosa*, Va : *Valonia aegagropila*, Cn: *Cymodocea nodosa*, Ht: *Halimeda tuna*.

### Phyto écologie - les bio faciès

La carte phyto sociologique (Fig. 4) met nettement en évidence 2 faciès antinomiques : un faciès littoral « lagonaire », très largement eutrophisé et déstructuré (zone rouge) et un faciès « du large », dominé par la présence des posidonies, où l'impact de l'eutrophisation côtière est atténué.

Entre 0 et 2 m – le « lagon »- Dans cette zone plus ou moins transformée en une longue suite de dépotoirs, l'impact des facteurs anthropiques est très important, avec notamment une baisse des salinités liée à la présence de nombreux points d'écoulement d'eaux usées ; une augmentation nette des éléments nutritifs en provenance des exsudats des décharges d'ordures éparpillées tout au long du littoral ; la formation d'une épaisse couche sédimentaire réductrice avec diffusion d'hydrogène sulfuré. Ceci se traduit du point de vue bionomique par une sélection des espèces les plus opportunistes ; une dominance des espèces thio nitrophiles euryvalentes ; une compétition jouant en faveur des espèces invasives tropicales. Autrefois occupée par une prairie de zostères, et, notamment, par des cymodocées, cette zone est aujourd'hui, envahie par des ulves et des chaetomorphes relayées, en de nombreux points par les espèces exotiques invasives *Caulerpa racemosa* et *Valonia aegagropila*. Cette zone déstabilisée, où la production primaire benthique est énorme, suit toute la côte où elle occupe une bande d'une largeur moyenne d'environ 200 m. Cette bande, toutefois, s'amenuise en allant vers l'est. La déstructuration de cet écosystème par hyper eutrophisation se traduit le cas échéant (saison estivale 2006) par l'apparition « d'eaux rouges ».



**Fig. 4: carte phyto écologique de la baie de Monastir -mars 2009-**  
TFD= très forte dégradation environnementale, FD= déstructuration forte, D= déstructuration, LD= légère déstructuration, ABEE= assez bon état environnemental

Entre 2 et 13 m – la biocénose de l'herbier de posidonies- Dans cette zone, l'impact direct des nuisances liées à l'urbanisation l'agriculture ou /et l'industrialisation est notablement réduit, en particulier du fait du rôle de « lagunarisation » (épuration naturelle et décantation des boues) joué par le lagon. Les nuisances restent cependant fortes avec une prise de relais par les activités de pêche (chalutage ou utilisation du « kiss », les ancrages intempestifs et répétés des bateaux de plaisance venus des marinas voisines, les dégazages sauvages d'une importante flotte de bateaux côtiers). En conséquence, si cette partie de la baie reste occupée par un herbier de posidonies, nous avons vu qu'il ne se présentait pas comme un tapis continu, loin de là. La présence entre les massifs ou même les touffes de posidonies, de nombreuses « plages inter mattes » recouvertes de sédiments détritiques coquilliers plus ou moins grossiers, est le signe d'un déséquilibre environnemental qui se traduit aussi par la persistance de la



présence de *Caulerpa racemosa* ; l'importance du nombre de rhizomes déchaussés ; une densité de feuilles par pied faible (entre 102 et 456 par exemple contre 984 à Madhia dans un herbier dense, très peu perturbé) ; une biomasse par pied de 0,3 gr à Monastir contre 0,8 à Madhia (Sghaier, 2006), en d'autres termes, par la présence d'un herbier globalement de qualité médiocre.

### Conclusion

La carte phyto écologique dressée à partir des observations faites en mars 2009 nous montre que l'état environnemental de la baie n'est pas le même dans toutes ses parties avec, au niveau côtier, une forte déstructuration environnementale (bandes grenat et rouge), et, plus au large, une qualité environnementale majoritairement médiocre (secteurs rose et orange). La détérioration environnementale de la zone des lagons est à imputer exclusivement aux nuisances d'origine « continentale » alors que celle des herbiers de la baie est plus complexe avec, à la fois, le contre coup de la très mauvaise qualité des eaux côtières, bien que cette mauvaise qualité soit légèrement minimisée par l'« effet lagunage » et celui des impacts d'origine mécanique, liées aux pressions halieutiques (chalutages et autres) et plaisancières (ancrages trop fréquents) et d'origine physico chimique, notamment avec la formation de mini nappes d'hydrocarbure (dégazages ou tout simplement suintements des diesels de la flottille de pêche). Par ailleurs, nous devons noter que si, du point de vue éco morphologique, la bipartition des biotopes de la baie est évidente : « lagon versus herbier », ces « compartiments », en fait, ne sont pas étanches. On constate en effet la présence d'enclaves de « caractère positif », au niveau de la zone des « lagons » nous citerons, entre autres, la présence d'espèces rares relativement strictes quant à leurs exigences telles les cystoseires ou les poissons syngnathidés, et, vice versa, de « caractère négatif », dans la zone de l'herbier, avec la présence d'espèces nitrophiles telles les ulves que l'on trouve tout au long de la radiale 4 et avec la relative abondance d'espèces suspensivores et notamment des vers serpulidae présents sur la plupart des organismes benthiques. Il faut, enfin, ne pas perdre de vue que le « mitage » actuel de l'herbier, s'il ne fait pas l'objet de drastiques et urgentes mesures de conservation, risque de s'étendre de façon rapidement exponentielle.

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# POSTERS

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## **PRESENCE OF RHODOLITH ASSOCIATIONS IN THE ITALIAN SEAS: CURRENT STATE OF KNOWLEDGE**

### **Abstract**

*This contribution synthesises the results of an analysis on the scientific publications available on rhodolith associations in the Italian seas, an activity conducted in support of the rules established by the EC Council regulation n.1967/2006 concerning the usage of towed gear over mäerl beds in the Mediterranean Sea. 131 scientific documents reporting the species responsible for rhodolith association development were screened for the Italian seas. Publications clearly describing the presence of these associations amount to 34, and only 10 contain cartographies. The collected information is the baseline for the planning of future research activities.*

**Key-words:** Rhodophycean calcareous algae; bibliographic review; geographic distribution.

**Introduction** - The EC Council Regulation No.1967/2006 concerning management measures for the sustainable exploitation of fishery resources in the Mediterranean Sea, recognises the importance of protecting rhodolith beds through the prohibition to fish with trawl nets, dredges, shore seines or similar nets and the implementation of research and mapping activities on such habitats. The present contribution focuses on the results of a preliminary investigation concerning rhodolith beds in Italian waters. The activity was financed by the MiPAAF (Italian Ministry of the Agricultural, Alimentary and Forest Politics). This activity involved, amongst other things, a review of all the available scientific publications concerning rhodolith associations in the Italian seas. The species considered, are those which are characteristic of the rhodolith associations. Such concretions are generally found in free-living form on the soft bottoms of the Infralittoral and Circalittoral zones, in the presence of currents and dim light.

**Materials and methods** - The bibliographic search focused on the characterising species (Tab. 1) and the main typologies of associations (Tab. 2) (Bressan & Babbini, 2003). The search was performed using the following databases / bibliographic collections: ASFA, CASPUR service, AIOL (Italian Society of Oceanography and Limnology), SIBM (Italian Society of Marine Biology, years 1995-2004), RAC/SPA proceedings on the Mediterranean Symposia on marine vegetation. Grey literature was identified through internet research engines. The resulting list was then integrated with one produced by RAC/SPA on coralligenous and rhodolith beds (Agnesi *et al.*, 2009). The documents were analysed so as to identify the location of each study area (a single publication could contain more than one site or species), and to collect information on the range of depth of each species and of each association. The information was then sorted according to the Mediterranean sub-regions proposed by the EU Directive 2008/56/EEC (western Mediterranean, Adriatic, Ionian and Central Mediterranean).

### **Results**

131 scientific documents (Tab. 1), published from 1837 to 2010, were found concerning characteristic species. The publications describing the presence of the associations (n=34) were published from 1973 to 2009; and only 10 contain cartographies (Tab. 2). The western Mediterranean appears the most largely studied sub-region. Few studies describe the associations in the Ionian and Adriatic sub-regions.

**Tab. 1: List of the considered species, number of publications describing their presence in the Italian waters per Mediterranean sub-region and overall depth range reported.**

Species	Mediterranean Sea sub-regions <i>Sensu</i> EU Directive 2008/56/EEC			Depth Range(m)
	Western N. of publ.	Adriatic N. of publ.	Ionian & Central N. of publ.	
<i>Lithothamnion corallioides</i>	24	8	6	27-150
<i>Phymatolithon calcareum</i>	28	11	7	15-150
<i>Lithothamnion minervae</i>	8	3	1	19-98
<i>Neogoniolithon brassica-florida</i>	45	10	15	0-137
<i>Spongites fruticulosus</i>	32	11	14	0-150
<i>Mesophyllum lichenoides</i>	33	6	21	0-137
<i>Lithophyllum racemosus</i>	27	12	13	10-98
<i>Lithothamnion valens</i>	12	6	10	27-98
<i>Peyssonnelia rosa.-marina</i>	13	1	2	4-120
Non identified rhodolith species	15	1	3	

**Tab. 2: List of the considered rhodolith associations, number of studies in the Italian waters per Mediterranean sub-region, number of available maps and reported range of depth.**

Association name	Mediterranean Sea sub-regions - <i>sensu</i> EU Directive 2008/56/EEC								
	Western			Adriatic			Ionian & Central		
	N. of publ.	N. of maps	Depth (m)	N. of publ.	N. of maps	Depth (m)	N. of publ.	N. of maps	Depth (m)
<i>Facies of Maerl</i>	23	9	27-137	4	1	20-100	4	1	50-100
<i>Facies of Prálines</i>	9	4	27-98				1		
Boxwork rhodolithes	4	1	27-137						
<i>Facies of free Peyssonneliaceae</i>	5	1	20-100	1			1		

## Discussion and conclusions

Though rhodolith species have been described in Italian seas during the last two centuries, the occurrence of the various associations seems to have been documented only in the last decades. Higher investigative effort also seems to have been applied to describing the maerl facies and this may be linked to the greater ease with which this association is more easily recognizable to the naked eye. A geographic skew seems to be present with most studies occurring in the western Mediterranean and this may be due to the larger spatial extension of the circalittoral zone in this subarea. There is a paucity of available cartographies on rhodolith beds which is likely due to the low interest paid in the past to this topic but also to the need of availability of modern oceanographic instrumentation capable of producing maps of such habitats. Considering the conservation implications defined in the actions set out by the EC legal framework for the marine environment (EC Council Reg. 1967/2006, EU Dir. 92/43/EEC, EU Dir. 2008/56/EEC) as well as by the UNEP-MAP Action Plan on marine vegetation, it is clear that wide-scale mapping initiatives are imperative for the conservation success of these associations in the Mediterranean.

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## **DISTRIBUTION OF DEEP-SEA LAMINARIANS AROUND THREE SPANISH MARINE PROTECTED AREAS**

### **Abstract**

*Most marine protected areas (MPAs) have been declared marine extensions of protected terrestrial ecosystems. Therefore, these protected sites are around or beside coastal or island marine reserves, natural parks, etc. That means that most of the protected seabeds are normally shallow coastal areas usually no deeper than the infralittoral zone. Deep-sea laminarians extend down to circalittoral seabeds that are not always included inside protected areas. We are presenting data on deep-sea laminarians' distribution in places surrounding three marine protected areas in Spain (Columbretes Islands Marine Reserve, Alboran Island Marine Reserve and Cabrera National Park) that lie outside the protected zone because they are on deeper floors. Therefore, they remain unprotected.*

**Key-words:** *Laminaria rodriguezii*, *Phyllariopsis*, deep-sea, Mediterranean Sea, vulnerable marine vegetation.

### **Introduction**

Laminarian species like *Laminaria rodriguezii* Bornet, *Phyllariopsis purpurascens* (C. Agardh) Henry & South and *Phyllariopsis brevipes* (C. Agardh) Henry & South, can reach deep beds: more than -25 m for *P. purpurascens* (Flores-Moya, 1997), and even deeper for *P. brevipes* (Ballesteros, 1984; González-García & Conde, 1993; Henry, 1987). *L. rodriguezii* distribution has been normally accepted down to -50/-120 m. (Giaconne, 1967), although new publications extend the species' range to -200 m deep (Boero *et al.*, 2008). *L. rodriguezii* and *P. purpurascens* are species considered as threatened species in the Mediterranean Sea (PNUE, 1990). *Laminaria rodriguezii* is a species also protected by 1976 Barcelona and 1979 Bern Conventions. In presenting this information, we are attempting to show that current MPA's do not take into account a deeper distribution of vulnerable marine vegetation, and we are promoting the enlargement of those areas to include a wider variety of habitats and algal species.

### **Materials and methods**

Through the use of a remotely operated vehicle (ROV), deepwater zones inside and outside of three Spanish MPAs were inspected: Columbretes Islands and Alborán Island Marine Reserves, where the transects did not follow a sample-taking pattern, and Cabrera Archipelago National Park, where a grid of transects was pre-designed, thus covering an area of approximately 8,000 Ha. The transects were made between depths of -20 and -200 m, traversing distances between 300 and 3,000 m in length at speeds between 0.2 and 0.4 knots and maintaining a field of vision between 1.5 and 2 m wide.

### **Results**

In total, 42 transects were made, covering a distance of 96,550 m and covering an area of 168,350 m<sup>2</sup>: 7 transects, 7,200 m traveled and 12,600 m<sup>2</sup> documented in the Columbretes area; 14 transects, 19,000 m traveled and 33,250 m<sup>2</sup> in the Alboran area and 21 transects, 70,350 m traveled and 122,500 m<sup>2</sup> documented in the Cabrera area. The species encountered in the Columbretes and Cabrera areas were chiefly *Phyllariopsis* spp. (-60/-70 m) and *L. rodriguezii* (-50/-75 m). In the case of Alboran, the species *Phyllariopsis* sp., *L. ochroleuca* Bachelot de la Pylaie and *Saccorhiza*

*polyschides* (Lightfoot) Batters were found at depths greater than -50/-60 m as had already been previously observed by Templado *et al.* (2006). Both on the Columbretes and in the Cabrera areas, laminarians were found outside the seabeds of the protected areas, whereas on Alboran, they were found in and outside the protected area, given the laminarians' greater expanse in this zone and how quickly the nearby seabeds drop off. *Phyllariopsis* spp. usually appears more spread out. Some major concentrations can reach deep seabeds (-60/-70m) off Alboran Island and to the west of the Columbretes area. *L. rodriguezii*, is specially abundant to the east of Cabrera, around -65 m, forming major colonies on the detritic sandy seabeds especially around large coralligenous concretions. *L. ochroleuca* and *S. polyschides* forests, in turn, are known on the Alborán Island seabeds, and they extend beyond the protected area in all directions.

### Discussion and/or conclusions

As exhaustive samplings were not performed, it is very possible that the distribution and abundance of these species are greater both inside and outside these MPAs. However, the intention behind these samplings was not to make complete bionomic cartographies, but to learn about the benthic communities that extend beyond the protected areas. Since most MPAs have been declared around islands or coastal areas, the seabeds involved rarely go beyond the upper circalittoral zone, although there may be zones that even surpass -100 m. The lack of MPAs that accommodate more ample bathymetric ranges or, even, on the high seas, makes many deepwater laminarian and other algal communities remain largely outside the protected areas, in spite of their ecological importance, and being species included in several different catalogs and marine conservation conventions.

### Acknowledgments

Oceana thanks the General Secretariat of the Sea, the guards and people in charge of running the protected areas, Fundación Biodiversidad and the Government of the Balearic Islands.

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## **AN OTHER THREAT ON *POSIDONIA OCEANICA* BEDS: MUCILAGE EFFECT IN GÖKÇEADA ISLAND, NORTH AEGEAN SEA, TURKEY**

### **Abstract**

*In this study, conducted seasonally between spring 2009 and summer 2010, we presented overall algological data and basic environmental data in order to evaluate the extensive mucilage formation impact on Posidonia beds in the coasts of Gökçeada Island. During the mucilage formation we recorded very high phytoplankton abundance and micro-epiphytes on Posidonia leaves.*

**Key-words:** *Posidonia oceanica*, epiphytic algae, mucilage, Gökçeada, North Aegean

### **Introduction**

*Posidonia oceanica* (L.) Delile is a seagrass endemic to the Mediterranean Sea and Mediterranean coastal systems dominated by seagrasses are locals of very high primary production that have been regarded as net autotrophic systems. However, the *Posidonia* ecosystem is threatened by environmental stress due to climate change and anthropogenic factors. The massive presence of mucilaginous organic matter, resulting from planktonic and benthic algal blooms, has become more frequent in many coastal waters around Europe in recent years (Aktan *et al.*, 2008; Giani *et al.*, 2005; Lorenti *et al.*, 2005), as a result of climatic fluctuations or increased eutrophication (Bianchi and Morri, 2000). Mucilage heavily affects marine ecosystems. Seagrass are among the benthic systems that is most affected by the deposition of mucilaginous aggregates.

During the winter and spring periods of 2010, the huge mucilage formation was observed surface and whole water column in North Aegean Sea and the coast of Gökçeada Island too. In this study, epiphytic flora on *Posidonia oceanica* together with environmental data presented during the mucilage formation and the results were compared with the period when no aggregates appeared.

### **Material and methods**

In this study, conducted seasonally between spring 2009 and spring 2010 in Gökçeada, we presented overall algological data (phytoplankton in water column, micro and macro epiphytes on *Posidonia oceanica* leaves) and basic environmental data in order to evaluate the extensive mucilage formation impact on *Posidonia* beds in the coasts of Gökçeada Island.

### **Results and discussion**

In terms of epiphytic algae, a total of 36 macroalgae and 47 microalgae belongs to six divisions were recorded on the *Posidonia* leaves. Diatoms and rhodophytes were the most important groups in species number, but significant seasonal changes were found in density. Their species number was reached their high values during the summer

period, while it has minimum values in winter. *Prorocentrum lima* belongs to Dinophyta and filamentous species from cyanobacteria during winter period were also recorded as dominant in density. In the late winter period of 2010, we recorded a huge mucilage formation, in the surface, water column and bottom in Gökçeada coasts. During these phenomena, phytoplankton density reached high numbers. Diatoms and dinoflagellates were dominant in phytoplankton. A shift was observed in the composition of micro-epiphytes on *Posidonia* leaves. Filamentous cyanobacteria density reached high numbers in micro-epiphytic flora while the macro algal species numbers in their lowest values. Mucilage accumulation on the bottom may be affected in this low number of macro-epiflora species, in addition to decreasing water temperatures. During this observation neither hypoxia nor anoxia were recorded but the large quantity of mucilage aggregates had accumulated on the sediments and *Posidonia* leaves. The high N/P ratio is also characteristic in water column before, during and after mucilage.

The chlorophyll a concentrations of the water column and the epi-flora on *Posidonia* leaves ( $1.56 \mu\text{g Chl-a g}^{-1}$  dry weight per leaves) were relatively high during mucilage formation. However, the highest chl-a concentrations of the epi-flora on *Posidonia* leaves were recorded in the autumn period due to the maximum growth of *Dictyota linearis* and the summer period due to the maximum growth of *Lyngbia* sp (Tab. 1).

Our primarily results show that if the mucilage phenomenon and their extensive accumulation continuous to increase in this frequency and duration, and to spread around the coastal areas of the Mediterranean Sea, vitality of *Posidonia oceanica* beds and their ecosystems may result with a dramatic disruption.

**Tab.1. Physico-chemical and biologic characteristics in surface (S) and bottom (B) water of lower limits of *Posidonia oceanica* beds. (\*indicated mucilage period).**

	Spring'09		Summer'09		Autumn'09		Winter'10 *		Spring'10 *	
	S	B	S	B	S	B	S	B	S	B
Temperature (°C)	20.5	16.9	25.6	24.6	19.6	20.8	12.6	14.7	16.3	16.6
Dissolved oxygen (mg l <sup>-1</sup> )	7.6	9.5	8.9	8.4	14.0	10.3	18.0	14.4	14.0	15.0
pH	8.2	8.2	8.2	7.9	8.0	8.7	8.2	8.2	8.2	8.2
Salinity (‰)	34	38	31	39	27	34	33	36	31	33
DIN ( $\mu\text{g l}^{-1}$ )	1.2	1.4	7.7	9.3	5.0	7.0	10.0	10.3	11.6	7.3
PO <sub>4</sub> ( $\mu\text{g l}^{-1}$ )	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	0.1
SiO <sub>2</sub> (mg l <sup>-1</sup> )	0.014	0.017	0.022	0.023	0.058	0.088	0.063	0.038	0.012	0.01
Suspended solids (mg l <sup>-1</sup> )	24.8	34.1	15.2	16.0	29.7	35.6	20.0	20.9	24.3	35.0
Chlorophyll -a ( $\mu\text{g l}^{-1}$ )	0.03	0.02	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.04
Phytop density (x10 <sup>3</sup> cells l <sup>-1</sup> )	365	131	9	3	31	10	168	23	346	543
Secchi depth (m)	10		12		18		11		10	

### Acknowledgements

The present study is a part of a project funded by TÜBİTAK project number 109Y014.

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## **INFLUENCE OF SUBSTRATE TYPE AND ALGAL COVER ON SEEDLINGS RECRUITMENT AND GROWTH PERFORMANCES OF THE MEDITERRANEAN SEAGRASS *POSIDONIA OCEANICA***

### **Abstract**

*In seagrass systems the period between seed germination and seedling establishment constitutes one of the most vulnerable phases of the whole life cycle. This study analyses the influence of substrate nature and algal canopy composition on seedling settlement and recruitment of the seagrass *Posidonia oceanica*. Results point out that substrate stability strongly influences these processes: after two years seedlings persisted only on rocky substrates with an average density of  $1.85 \pm 0.37$  seedlings  $m^{-2}$ . In-depth knowledge of factors affecting seagrass early life history phases is required to better understand colonisation and recovery potentials of species also with regard to improving management and restoration strategies.*

### **Introduction**

The seagrass *Posidonia oceanica* presents highly variable flowering and fruit production, with synchronous massive events occurring every 8-10 years (Diaz-Almela *et al.*, 2006). Nevertheless seedlings recruitment in the field has seldom been observed, and the successful initiation of a new patch from a seedling is considered rare (Diaz-Almela *et al.*, 2008). Owing to the rarity of direct observation of this kind of events, which factors are major drivers of seedlings establishment is poorly understood. In summer 2004 we observed a high number of *P. oceanica* seedlings on a rocky coast and we followed survival and growth over two years. We compare seedling persistence and growth between habitats that differ in substrate type (rock, sand, gravel) and algal cover (*Halopteris* spp. - *Dilophus* spp. assemblage vs *Cystoseira* spp. assemblage).

### **Materials and methods**

The study was carried out along the northern coast of Favignana, in the Egadi archipelago (37°56'N-12° 22'E), north-west coast of Sicily (Italy). Two environmentally similar sites were chosen. Seedlings density was recorded on five distinct habitats found in each site: sand (Sand), gravel (Gravel), rock covered by *Cystoseira* spp. 1m deep (Cysto 1m), and 3m deep (Cysto 3m), rock covered by algal turf composed mainly by *Halopteris* spp. and *Dilophus* spp. (Halo). Were not specified sampling depth was 3m. Density counts were carried out annually, always in July.

Seedlings for morphological analysis were collected at random in three replicates on rock covered by *Cystoseira* spp. (Cy) and rock covered by turf (Ha) in July 2004, 2005 and 2006. Analysis of Variance was performed with appropriate designs.

### **Results**

Few months seedlings density significantly differed between habitats ranging from  $0.13 \pm 0.08$  on gravel to  $16 \pm 1.73$  seedlings/ $m^2$  on rock covered by *Halopteris* spp. and *Dilophus* spp. (Fig.1). After two years seedlings persisted only on consolidated, rocky substrates with an average density of  $1.85 \pm 0.37$  seedlings/ $m^2$  (Fig.1). Morphological

variables show better growth performances on rock covered by *Halopteris* spp. and *Dilophus* spp. respect to rock covered by *Cystoseira* spp. (Tab.1).

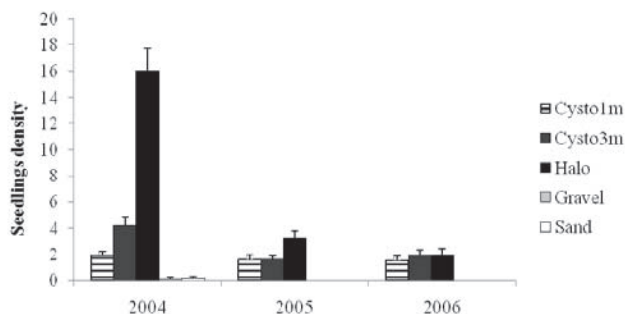


Fig.1: Value of seedling density from 2004 to 2006 on five habitats identified. Bars equal SE.

Tab.1 Student-Newman-Keuls test performed on seedling morphological variables in response to different habitats, over two years. Results are showed only for variables for which significant interaction between Time and Habitat were detected.

Variable	Effect	Interpretation				
		Within habitat			Within time	
		2004	2005	2006	Cy	Ha
Leaf number	YearXHabitat	Cy=Ha	Cy<Ha	Cy=Ha	2004>2005=2006	2004>2005>2006
Max leaf lenght	YearXHabitat	Cy=Ha	Cy=Ha	Cy<Ha	2004<2005<2006	2004<2005=2006
Max leaf width	YearXHabitat	Cy=Ha	Cy<Ha	Cy<Ha	2004=2005=2006	2004<2005<2006
Rhizome lenght	YearXHabitat	?	Cv=Ha	Cv<Ha	2005<2006	2005<2006

### Discussion and conclusion

Substrate stability and complexity are relevant factors for seedlings settlement and recruitment in the study area. Consolidated substrates offer a stable setting for seedlings anchorage respect to sand and gravel, furthermore algal coverage can increase topographic complexity at the seedlings scale, favouring seedlings retention and recruitment probability. We can hypothesize that the structure and composition of algal canopy influences seedlings growth due to stronger competitive interactions for light and/or space on rock covered by *Cystoseira* spp. or to higher level of nutrients availability on turf. Moreover differences in local physical conditions between habitats (as hydrodynamics), can contribute to explain pattern observed.

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## **ETUDE DE L'EFFET DE L'ALCOOL ISOPROPYLIQUE SUR LE RENDEMENT ET LA QUALITE DE L'AGAR-AGAR DE *GRACILARIA VERRUCOSA* (HUDSON) PAPENFUSS**

### **Abstract**

*This study performed on Gracilaria verrucosa (Rhodophyta, Gracilariales, Gracilariaceae) collected in Bizerte Lake, involves the study of the effect of isopropyl alcohol on the yield and the quality of agar. The parameters studied are: the agar-yield, the gel strength, the gelling point and the melting point. The results showed that the optimum agar -yield (18.75%) was obtained with a concentration of 0.5% isopropyl alcohol. The maximum (322.5g/cm<sup>2</sup>) and the minimum (105 g / cm<sup>2</sup>) of the gel strength were obtained, respectively, with 1% and 7%. The gelling point varied between 29 ° C and 39.5 ° C, while the melting point ranged between 70.5 ° C and 86.8 ° C.*

**Key-words:** *Gracilaria verrucosa*, agar, isopropyl alcohol, agar-yield and quality.

### **Introduction**

Utilisées depuis des millénaires par les populations littorales pour leurs hautes valeurs nutritives, les algues constituent aujourd'hui un enjeu majeur de développement économique. En Tunisie, l'algue rouge *Gracilaria* a fait l'objet de plusieurs travaux dont l'étude de ses potentialités d'exploitation (Ksouri et Ben Saïd, 1998), de la teneur et de la qualité de son agar-agar (Ben Saïd et Ksouri, 1999). Par ailleurs, des essais de culture de cette algue ont été réalisées dans la lac de Bizerte par bouturage (Ksouri *et al.*, 2008). Ce travail entre dans le cadre des travaux de recherche menés à l'INSTM en vue d'apporter des améliorations sur la technique d'extraction de l'agar-agar de *Gracilaria verrucosa* afin d'augmenter aussi bien le rendement que la qualité de l'extrait obtenu en étudiant l'effet de la concentration de l'alcool isopropylique.

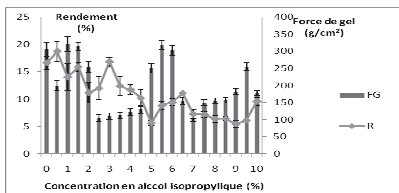
### **Matériel et méthodes :**

Le matériel biologique utilisé est la rhodophycée *Gracilaria verrucosa* (Hudson) Papenfuss. Elle a été récoltée dans le lac de Bizerte en octobre 2009. Après leur séchage à l'air libre puis à l'étuve à 60°C, différents échantillons de 15 g ont été immergés pendant 2 h dans 500 ml d'alcool isopropylique, utilisé pour chaque échantillon à une concentration différente, puis rincés à l'eau de robinet pendant 15 mn. Les autres étapes d'extraction et d'analyse de la qualité de l'agar-agar sont décrites dans la méthode de Ben Saïd *et al.*, (1999). Pour chaque concentration, les échantillons d'algues ont été traités en quatre réplicats.

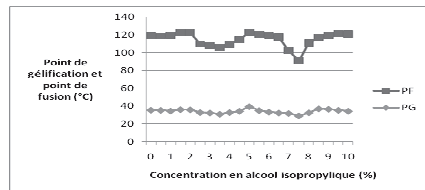
### **Résultats et discussion**

Les résultats obtenus montrent que le rendement d'extraction a varié selon la concentration en alcool isopropylique (Fig.1). Les valeurs maximales, soient respectivement 18,75% et 16,81% du poids sec de l'algue ont été obtenues pour des concentrations de 0,5% et 3%. Ces valeurs sont supérieures au témoin (16,62 %), non traité à l'alcool et aux résultats trouvés précédemment par Ben Saïd et Ksouri (1999), soit (14,51%). Yenigül (1993) trouve un rendement d'extraction à partir de *G. verrucosa*

de Turquie qui varie entre 24 et 43 %. Concernant la force de gel, les résultats obtenus montrent que les valeurs ont dépassé 300g/cm<sup>2</sup> et ce, à différentes concentrations en alcool, soient 1% ; 1,5% ; 5,5% et 6%. Le maximum a été enregistré avec une concentration de 1 %, soit 322,5 g/cm<sup>2</sup>. Cette valeur est supérieure au témoin (305 g/cm<sup>2</sup>) et à la celle trouvée par Ben Saïd et Ksouri (1999), pendant la même période de l'année, soit 235 g/cm<sup>2</sup>. Les valeurs maximales de la force de gel enregistrées par Marinho-Soriano et Bourret (2003) sont de 828 g/cm<sup>2</sup> et 168 g/cm<sup>2</sup>, respectivement pour *Gracilaria gracilis* et *G.bursa-pastoris*, récoltées en été dans l'étang de Thau (France). Concernant le point de gélification, la valeur la plus élevée (39,5°C) a été enregistrée à 5%, tandis que la valeur minimale (29 °C) a été obtenue à 7,5 % (Fig.2). Le point de fusion a atteint ses valeurs maximales (entre 86 et 87 °C) à 1,5% ; 2% ; 9,5% et 10%. Toutes les valeurs maximales obtenues sont supérieures aux témoins. Les résultats obtenus avec les concentrations de 9,5% et 5%, concordent avec ceux décrit par Ben Saïd et Ksouri (1999). Marinho-Soriano et Bourret (2003) trouvent des valeurs maximales de point de gélification de 48 °C et 46 °C, pour *Gracilaria gracilis* et *G.bursa-pastoris*, enregistrées en automne.



**Fig.1: Variation du rendement d'extraction et de la force de gel en fonction de la concentration en alcool isopropylique**



**Fig.2: Variation du point de gélification et du point de fusion en fonction de la concentration en alcool isopropylique**

## Conclusion

Tous les résultats obtenus nous permettent de dire que la méthode préconisée dans ce travail montre un effet positif de l'alcool isopropylique sur le rendement et la qualité de l'agar-agar extrait de *G.verrucosa* du lac de Bizerte.

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## **INFLUENCE OF HYDROTHERMAL VENTS ON PHYTOBENTHIC COMMUNITIES IN THE AEOLIAN ISLANDS (TYRRHENIAN SEA): PRELIMINARY RESULTS**

### **Abstract**

*The species composition and structure of photophilic algal community and macroalgal epiphytic assemblages on leaves of *Posidonia oceanica*, as well as the density and leaf biometry of the seagrass, were studied in the shallow hydrothermal vents of the Aeolian Islands (Tyrrhenian Sea). The samples of the algal community were collected in three sites within a hydrothermally active area, whereas *P. oceanica* shoots were sampled in two patched meadows exposed to different levels of gas emission. The effects of hydrothermal activity on communities were detectable only in areas very close to vents where bacterial mats were observed and algal cover and number of species were reduced. The lacking of Corallinaceae and the abundance of species with warm-water affinity may be related to a homogeneous influence of vent activity throughout the area. *P. oceanica* meadows showed an "abnormal density", whereas mean shoot surface of meadow close to vents was 60% lower than meadows far from vents.*

**Key-words:** benthic marine algae, epiphytes, shallow hydrothermal vents, *Posidonia oceanica*, Tyrrhenian Sea.

### **Introduction**

Marine CO<sub>2</sub> and H<sub>2</sub>S vents occur in the Mediterranean, especially around Italy and Greece. Floristic studies were carried out in vent areas of Aegean Sea (Sartoni & De Biasi, 1999) and the effect of vents on phytobenthic communities was showed in the Tyrrhenian Sea (Acunto *et al.*, 1996; Giaccone, 1969; Hall-Spencer *et al.*, 2008; Vizzini *et al.*, 2010). The present study aims to contribute, as far as possible, the knowledge about the photophilic macroalgal community and *Posidonia oceanica* meadows (density, leaf biometry and leaves epiphytic assemblages) of Aeolian Islands hydrothermal vents, and to evaluate the effects of acidification on both communities.

### **Materials and methods**

The study was carried out in early September 2009 in Bottaro, a small islet of the Aeolian archipelago. The area is affected by a high level of hydrothermal activity with emission of CO<sub>2</sub> and H<sub>2</sub>S. Two sampling areas were established at depths ranging from 6 to 12 m. In the first one, three rocky sampling sites were chosen randomly within the hydrothermal area and two samples of 400 cm<sup>2</sup> were collected in each site; two additional samples were collected close to vents. In the second one, in two patched *P. oceanica* meadows exposed to different levels of gas emission, shoots density was measured in a 0.4 m square. For each site 20 orthotropic *P. oceanica* shoots were randomly sampled and leaf surface area per shoot was calculated. Moreover, for each shoot the internal face of the oldest part of the two external leaves were examined to analyze epiphytic assemblages (Alcoverro *et al.*, 2004). All biological samples were collected by SCUBA diving and fixed in 4% formalin seawater.

### **Results**

The photophilic algal community was scarcely developed, showing a low number of

taxa (43 taxa in total; 21 taxa per inventory in average) and a low mean cover (199.6 cm<sup>2</sup>). It was mainly dominated by *Cystoseira sauvageauana*, *Anadyomene stellata*, *Dictyota mediterranea* and *Derbesia tenuissima*. The community was characterized by quiet photophilous infralittoral species (23.3%), with thermophilous and thionitrophile species more quantitatively abundant. Qualitatively, cosmopolitan and tropical species were the best represented in the community (39.5% and 20.9%, respectively). Vents were surrounded by a white flocculent bacterial mat dominated mainly by sulphur bacteria and the cyanobacterium *Calothrix scopulorum*. Epiphyte assemblages of *P. oceanica* leaves were also scarcely developed (27 epiphyte taxa in total; 8.1 taxa for shoot). The mean cover was very low (less than 0.5 cm<sup>2</sup>). The best represented species were *Ascocyclus orbicularis*, *Phaeophyla dendroides*, *Sphacelaria cirrosa*, *Blastophysa rhyzopus*, *Ulvella lens* and *Ceramium diaphanum*. The leaves were mainly colonized by species of large ecological repartition (22.2%) and typical epiphytic species of *Posidonia* leaves (18.5%). Cosmopolitan species (55.6%) were the most representative. It was noteworthy the lacking Corallinaceae on both communities. Mean density values of *P. oceanica* were 218 and 178 shoot/m<sup>2</sup> and leaf surface area were 148 and 241 cm<sup>2</sup>, close to and far from the hydrothermal vents respectively.

### Discussion

The results of this preliminary study showed a low algal cover and the scarcity of species both on rocky and *P. oceanica* leaves, with no differences between the epiphytic assemblages at vent and non-vent meadows. This could be related to a homogeneous influence of vent activity throughout the area and/or to a seasonal sampling. On rocky close to vents we observed small scale effects, with the lack of macroalgae for a short distance from which fluid escaped. The absence of coralline algae may be due to low pH values that cause the carbonate dissolution. The two *P. oceanica* meadows showed an “abnormal density” (Pergent *et al.*, 1995), whereas mean shoot surface of meadow close to vents was 60% lower than meadows far from vents.

### Acknowledgments

We thank Drs. M. Hernández, A. Gómez and M.A. Ribera for the identification of algae.

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## **PREMIERS RESULTATS SUR LA CULTURE DES SPORES DE *PADINA PAVONICA* (LINNAEUS) THIVY LAMOUREUX**

### **Abstract**

*A preliminary study on the culture of spores of the brown alga Padina pavonica (Dictyotales, Dictyotaceae) has been carried out on the laboratory. Four irradiance levels and three photoperiods were tested. Also, red, green, blue and yellow lights were experimented. The results showed that the highest growth was obtained under 2200 Lux with a photoperiod of 12h light: 12 h dark. Besides, spores grew similarly under blue and yellow lights. In spite of being preliminarily, these results open the horizon to cultivate P. pavonica on an important scale in order to maintain natural resources, in the one hand, and to use this alga in industry especially in pharmaceutical and cosmetic ones, in the other hand.*

**Key-words:** *Padina pavonica* ; culture ; spores ; light; growth

### **Introduction**

L'intérêt porté aux végétaux marins et en particulier aux macroalgues en vue de leur valorisation ne cesse de croître de par le monde. Parmi les algues recherchées figurent la phéophycée *Padina pavonica*, en raison de sa capacité de donner des molécules ayant un effet bioactif (Ktari *et al.*, 1999). Les algues appartenant au genre *Padina* sont actuellement récoltées des champs naturels et leur culture n'est pas encore maîtrisée comme celle des gracilaires. En Tunisie, l'intérêt à *Padina* a commencé il y a quelques années (Ben Saïd *et al.*, 2002). Ce travail entre dans le cadre de la valorisation des algues de Tunisie et constitue une première tentative de culture de *P. pavonica* à partir des spores en vue d'acquérir certaines connaissances sur les conditions optimales de leur croissance en milieu contrôlé.

### **Matériel et méthodes**

Le matériel biologique utilisé dans ce travail est la phéophycée *Padina pavonica*. Elle a été récoltée à Cap Zebib (au nord de la Tunisie) en juillet 2008 à une profondeur entre 1 et 2 m. Les thalles ramenés au laboratoire ont été bien rincés à l'eau de mer stérilisée par autoclavage afin d'éliminer le sable et les épiphytes. Par la suite, ils ont été coupés en petits fragments après avoir observé les spores présents sur les stries concentriques et remplis de spores. Dans chaque boîte de Pétri, cinq fragments de *Padina* ont été placés. Le milieu de culture utilisé est SWM3 modifié (Chen *et al.*, 1983). Quatre intensités lumineuses (600, 1400, 2200 et 3400 Lux) et trois photopériodes différentes (8/16, 12/12 et 16/8) ont été testées. De même, quatre radiations lumineuses ont été expérimentées (rouge, bleue, verte et jaune). Le test t au seuil de 95 % a été utilisé pour la comparaison des moyennes de croissance observées au terme de l'expérience.

### **Résultats**

Les résultats obtenus en lumière blanche sont représentés dans la Fig. 1 (a, b et c). Ils montrent l'évolution de la taille des spores de *Padina* au cours du temps. Ainsi, avec une photopériode de 8/16, c'est l'intensité lumineuse de 1400 Lux qui favorise le mieux

la croissance des spores qui passent d'une longueur de 50  $\mu\text{m}$  à environ 111  $\mu\text{m}$ . Sous la photopériode 12/12, la taille maximale (134  $\mu\text{m}$ ) a été enregistrée sous l'intensité de 2200 Lux. Lorsque la durée d'éclairage s'allonge (16/8), la croissance des spores est affectée, quelle que soit l'intensité lumineuse. En effet, la taille maximale a été enregistrée entre les moyennes sont significatives sauf entre les deux photopériodes de 8/16 et 12/12, respectivement à 600 Lux et 1400 Lux. La variation de la croissance des spores sous différentes radiations lumineuses est représentée sur la Fig. 1(d). Les résultats montrent que la taille augmente généralement d'une façon progressive d'une semaine à l'autre. La croissance est comparable entre les radiations bleue et jaune au bout de quatre semaines, mais au bout de deux mois environ, la taille des spores cultivées sous la radiation bleue devient plus nette. A la fin de l'expérimentation, les différences observées entre les moyennes sont significatives sauf entre les radiations verte et jaune.

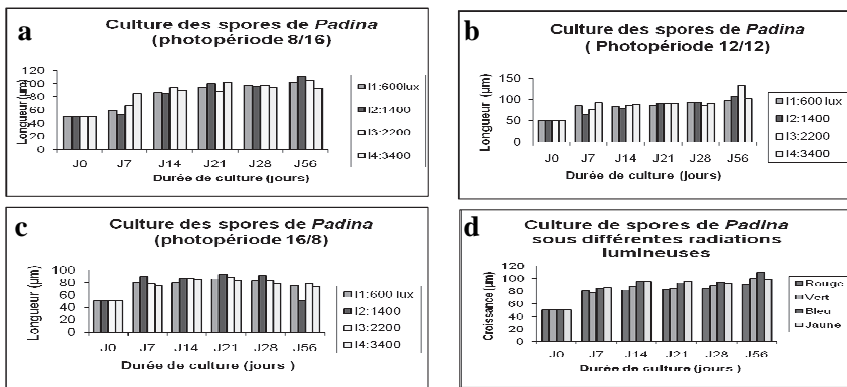


Fig. 1 : Variation de la croissance des spores de *Padina pavonica* en lumière blanche (a, b et c) et colorée (d)

### Discussion et conclusion

Les résultats obtenus montrent que les spores de *P. pavonica* se développent d'une façon plus ou moins importante sous l'influence de l'intensité lumineuse et la qualité de la lumière, à l'instar d'autres espèces voisines telle que *P. boergesenii* (Ganesan *et al.*, 1999), vivant en Inde. Nos résultats sont encore préliminaires, d'autres recherches permettraient de cerner les conditions optimales de culture des spores et de petites plantules jusqu'à l'obtention de plantes adultes.

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## **LONG-TERM DYNAMICS OF *POSIDONIA OCEANICA* MEADOWS IN THE MURCIA REGION (SE SPAIN)**

### **Abstract**

*Declining Posidonia oceanica meadows are usually associated with environmental impacts caused by the diverse human activities concentrated in the coastal zone, but global declines have also been suggested even in pristine areas. Scientific monitoring networks have been established in many Mediterranean regions during the last few decades to assess the long-term dynamics of P. oceanica meadow structure and to make inferences about the magnitude, sign and nature of such declines. In the present contribution, we provide preliminary data on the time trends of meadow structure descriptors measured in P. oceanica meadows in the Murcia Region (SE Spain). Since 2004 (through to 2009), shoot density and meadow cover have been measured once a year at 14 sampling stations, most of them located at sites where human pressure is almost absent or diffuse. Shoot density was measured using two different methods: random 400 cm<sup>2</sup> quadrats and fixed 1,600 cm<sup>2</sup> quadrats. Net interannual changes in this seagrass descriptor were consistent in the two methods. In nine of the meadows, seagrass descriptors (mainly shoot density) showed a positive time trend, indicating a progressive meadow dynamic; in another three meadows no net time trends were observed, indicating a stable meadow dynamic; in two meadows only, the time trends of one or both descriptors were negative, indicating a declining meadow dynamic. One of these two meadows was the only one in which there was localized human impact (diving frequentation and anchoring), but in the other meadow, the nature of the decline remains unclear since there is no evidence of human impact. Therefore, at least for the time period and scale considered, the symptoms of global decline were not detected in P. oceanica meadows in the Murcia Region.*

**Key-words:** long-term monitoring, Mediterranean, *Posidonia oceanica*, seagrass.

### **Introduction**

It has been suggested that *Posidonia oceanica* meadows in the Mediterranean Sea are undergoing an overall decline, although the evidence available is scarce and contradictory (Boudouresque et al., 2009; Gonzalez-Correa et al., 2007; Marba & Duarte, 2010). Long-term seagrass monitoring networks have been set up around the world and in many Mediterranean countries to test this hypothesis. The *Posidonia oceanica* meadow monitoring network of the Murcia Region (SE Spain) is a volunteer-based project which aims to determine long-term trends in the meadow structure. This paper outlines the results obtained in the period of 2004-2009.

### **Material and methods**

Once a year since 2004, shoot density (n=12 shoot counts in random 400 cm<sup>2</sup> quadrats) and the percentage of meadow cover (n=60 visual estimations in 1,600 cm<sup>2</sup> quadrats) were measured at 14 permanent stations around the Murcia coast (S1-S14). Time trends for the annual means of both descriptors were analysed using ANOVA and regression analysis. In addition, highly precise repeated counts of shoot numbers were performed in six fixed 1,600 cm<sup>2</sup> quadrats installed in each station in 2007. For each fixed quadrat, the annual net shoot change was estimated based on the difference between two

successive annual counts. Significant trends with negative signs indicated meadows with declining dynamics. The opposite pattern indicated progressive dynamics, and a lack of time trends meant a stable meadow structure.

## Results

Total net changes in meadow descriptors between 2004 and 2009 are shown in Figure 1. Nine of the meadows monitored showed a progressive status, mainly due to a significant 30-100% net increase in shoot density during the overall study period. Of the five remaining stations, three had a stable meadow structure and only two showed regressive meadow dynamics. In one of these two cases (S4), the meadow disappeared from a surface area of several tens of m<sup>2</sup> due to mechanical erosion caused by diving and anchoring. In the other regressive meadow (S11), the meadow cover in 2009 was 70% less than it was in 2004, although no changes in shoot density were observed. This suggests the influence of some erosive factor affecting the size of the meadow patches, but not its internal structure. However, since no human impact was present in this station (located within a marine reserve) the nature of this decline could not be determined. Annual net shoot changes estimated from the fixed quadrats were highly correlated with those of shoot density measured in random quadrats ( $r=0.75$ ,  $p < 0.001$ ), indicating that this classic descriptor gave reliable estimates of real interannual variations in the shoot population.

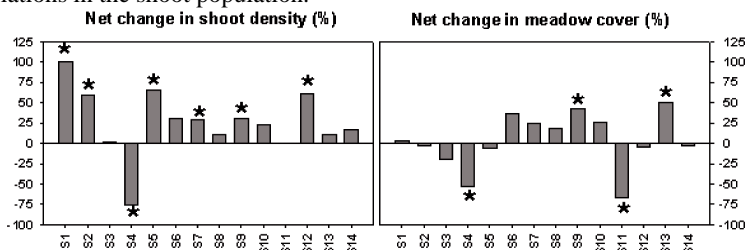


Fig. 1: Net changes of meadow descriptors between 2004 and 2009 in the sampling stations (S1-S14). \*indicates significant differences between initial and final mean values ( $p < 0.05$ ).

## Conclusion

The results obtained in this study do not support the hypothesis that the decline of *P. oceanica* meadows is a widespread phenomenon in the coast of the Murcia Region. Declines in the meadow were found to be local processes, although not always clearly linked to human disturbances.

## Acknowledgments

Servicio de Pesca y Acuicultura de la Consejería de Agricultura y Agua de la Comunidad Autónoma de Murcia.

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## **ASSESSMENT OF THE LONG-TERM DYNAMIC OF *POSIDONIA OCEANICA* MEADOWS IN SITES INVADED BY THE GREEN ALGA *CAULERPA RACEMOSA*.**

### **Abstract**

*Dense *P. oceanica* meadows seems to be the most resistant benthic assemblage to the invasion of the green alga *C. racemosa* var. *cylindracea*, although the hypothesis that the alga can outcompete the seagrass in the long-term has been stressed by some authors. To evaluate this hypothesis, algal biomass, meadow structure (% cover) and the dynamics of the seagrass shoot population are being measured since 2007 in the meadow edges of three invaded sites off the coast of Murcia (SE Spain) with contrasting depths (S = 10 m; I = 20 m; D = 26 m). Another three non-invaded sites at similar depths were used as controls. Preliminary results obtained in the period 2007–2009 are presented. Inside the seagrass leaf canopy, the mean algal biomass was rather variable (0.9–16.3 g DW m<sup>-2</sup>), but very low compared to that measured in substrates outside the leaf canopy (8 to 20-fold higher). No significant differences in meadow cover and the net shoot population dynamics were found between invaded and non-invaded sites. The annual net shoot balance was always positive (S = +30–40% and I = +15–20%) or almost in equilibrium (D ≈ 0%) at invaded sites. Our results show a stable gradient of standing biomass of *C. racemosa* between both sides of the meadow edge, and no signs of shoot decline were detected in the meadow margin. At least for the studied period, our results indicate a high resistance of the *P. oceanica* meadow to be invaded by *C. racemosa*, although longer time periods should be required to obtain more conclusive data about the long-term interaction between the alga and the seagrass.*

**Key-words:** *Caulerpa racemosa*, *Posidonia oceanica*, long-term interaction, Mediterranean.

### **Introduction**

The introduced green alga *C. racemosa* has invaded a wide variety of benthic habitats in the Mediterranean Sea, although some biocenoses have proved to be more resistant to colonisation by this alga than others (Klein & Verlaque, 2008). In most of the invaded sites, seagrass *P. oceanica* meadows are usually the least colonised community, although our knowledge of the long-term interactions between these species is still very limited. The hypothesis is that *C. racemosa* will penetrate and out-compete *P. oceanica* meadows in the long term, but no empirical or experimental evidence supports such a hypothesis as yet.

### **Material and methods**

This study was carried out in three highly-colonised sites of the Murcia Region (SE Spain) which have been invaded by *C. racemosa* since 2005: Grosa Island (S = shallow, 12 m), Tiñoso Cape (I = intermediate depth, 20 m) and Calblanque (D = deep, 26 m). Algal biomass (400 cm<sup>2</sup> quadrats; g DW m<sup>-2</sup>; n=20), seagrass meadow structure (percentage of meadow cover; n=40) and net shoot population change (1,600 cm<sup>2</sup> fixed quadrats; as percentage relative to initial values; n=6) were measured once a year throughout the period of 2007 to 2009 in the margin of the *P. oceanica* meadow

adjacent to the substrates heavily colonised by *C. racemosa*. All measurements were performed at the end of summer and algal biomass was measured both outside and inside the seagrass leaf canopy.

## Results

Algal biomass measured inside the *P. oceanica* meadow showed significant interannual variations in sites S (0.86-4.9 g DW m<sup>-2</sup>) and I (6.3-16.3 g DW m<sup>-2</sup>) with maximum mean values in 2008 (Fig. 1A). In the deepest site (D) *C. racemosa* biomass was within the range observed in S, but the annual mean values were more stable through time. In any case, these values were considerably lower than the algal biomass measured outside the seagrass leaf canopy, which were about 8 to 20 times higher. The interannual variation in meadow cover was not significant in any of the studied sites (Fig. 1B). For the annual periods of 2007-08 and 2008-09, the mean values of the annual net shoot change (Fig. 1C) were clearly positive in the *P. oceanica* meadow of sites S (13-15%) and I (26-30%) and close to zero in site D (-0.46 and -2.3%). These values indicate that the *P. oceanica* meadow showed progressive dynamics in sites S and I and was a nearly balanced population in site D.

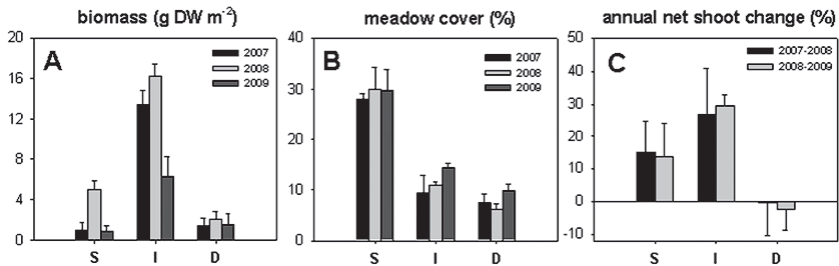


Fig. 1. Variation in mean (SE) values of *C. racemosa* biomass outside the *P. oceanica* leaf canopy (A), percentage of *P. oceanica* meadow cover (B) and annual net balance of *P. oceanica* shoots (C) between 2007 and 2009 at the studied sites.

## Conclusion

Our results indicate the presence of a marked and stable gradient of *C. racemosa* biomass at the edge of the *P. oceanica* meadow, with the very low values observed inside the leaf canopy reflecting the existence of some kind of limiting factor that prevented further penetration of this alga from its densely colonized substrates outside the leaf canopy. Furthermore, the spatial-temporal variations in meadow structure and net shoot population dynamics did not show any relationships with those of the algal biomass. Thus, at least for the studied period, our results are consistent with the high resistance to algal invasion postulated for this seagrass species, although longer time periods should be studied to confirm these results.

## Acknowledgments.

Servicio de Pesca y Acuicultura de la Consejería de Agricultura y Agua de la Comunidad Autónoma de Murcia

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## **PROSPECTION DES POPULATIONS DE *CYTOSEIRA BARBATA* ET DES PEUPELEMENTS DES MICROALGUES BENTHIQUES EPIPHYTES DANS LE SITE DE KRATTEN (KERKENNAH)**

### **Résumé**

*En méditerranée, les cystoseires appartiennent au genre le plus représentatif des algues brunes. Dans le site de Kerkennah en particulier, l'espèce Cystoseira barbata recouvre un espace important du site de Kratten et constitue un indicateur biologique de l'équilibre de l'habitat et de la qualité de l'écosystème. Ainsi, nous nous sommes intéressés à l'étude de l'écologie de cette espèce et nous avons essayé d'estimer son abondance dans ce site par la méthode du quadrat. Pour cela, nous avons suivie les biomasses sèches de cette espèce. Les résultats obtenus montrent que les biomasses les plus importantes sont enregistrées au printemps en particulier au mois d'avril avec un poids sec de 229,23g /m<sup>2</sup>. Cependant nous avons remarqué l'absence de cette algue pendant les 4 mois : juillet, Aout, septembre et le mois d'octobre. L'analyse mensuelle des prélèvements d'eau et d'algues a mis en évidence la structure particulière de la communauté associée. Cette structure se caractérise par l'abondance et la dominance de quelques espèces caractéristiques du mode calme. En particulier nous avons effectué une étude qualitative et mensuelle des dinoflagellés et diatomées associés à cette espèce dans le site de Kratten (Kerkennah) qui représentait la majorité des phytoplanctons présents dans cette zone. Au cours de ce suivi, nous avons constaté tout d'abord la dominance des diatomées avec une distribution tout au long de l'année pour la majorité. La présence des dinoflagellés a été notée au printemps en particulier en Mai, période d'abondance de Cystoseira barbata.*

**Mots clés** : variation mensuelle, *Cystoseira barbata*, abondance, épiphyte, phytoplancton

### **Introduction**

Les cystoseires sont des algues brunes (macrophytes phycophycées) qui sont pour la grande majorité d'entre elles endémique de la méditerranée. Elles abritent une faune et une flore diversifiées notamment au niveau d'espèces phytoplanctoniques. Dans un axe de recherche que nous développons sur cette thématique, nous nous sommes intéressés à l'étude de corrélation entre l'abondance de l'espèce *Cystoseira barbata* dans son milieu naturel Kratten (Kerkennah - Tunisie) et des populations phytoplanctoniques associées.

### **Matériel et méthodes**

La collecte des algues est effectuée à marée basse entre 40 et 50 cm de profondeur par la méthode du quadrat dans la zone de Kratten à Kerkennah (N34°45'720'' E11°15'201'') de Novembre 2008 à Octobre 2009. Les cystoseires sont nettoyées

étuvées à (40°C) et enfin broyées et pesées. Pour la détermination du phytoplancton nous avons utilisé la méthode d'Uthermol (1958), dont le principe repose sur la décantation de l'échantillon préalablement fixé au lugol dans des cuves de numération.

## Résultats et discussion

### 1/ Variation mensuelle des biomasses algales

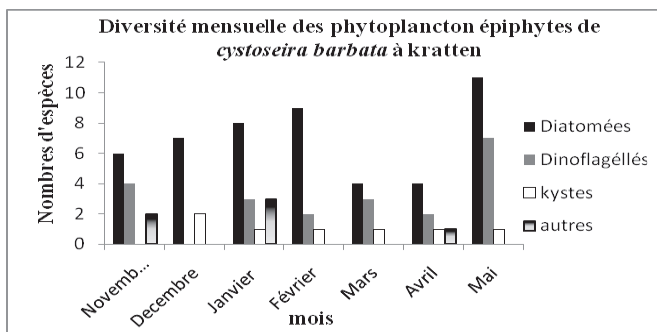
Les variations quantitatives des biomasses sèches semblent en relation avec les saisons. En effet, nous avons noté une abondance durant la période printanière surtout au mois d'avril et une absence quasi-totale durant la période estivale.

**Tab.1: variation mensuelle de la biomasse de *Cystoseira barbata* (Kerkennah).**

	Nov-08	Déc-08	Janv-09	Févr-09	Mars-09	Avr-09	Mai-09	Juin-09	Juill-09	Aout-09	Sep-09	Oct-09
poids sec g/m <sup>2</sup>	175	98,33	26,66	104	127,70	229,23	135,91	-	-	-	-	-

### 2/ Suivi de la diversité phytoplanctonique

L'étude de la composition en phytoplancton associé à la population de *Cystoseira barbata* nous a permis de noter la grande diversité des espèces de dinoflagellés Prorocentrales (6/19). Une dominance des diatomées et des dinoflagellés a été observée en particulier au mois de Mai. Cependant, nous avons remarqué une faible proportion de ces microalgues épiphytes au mois d'avril période d'abondance maximale des *Cystoseires*. Des espèces classées toxiques comme *Coolia monotis* et *Alexandrium sp.* ont été aussi répertoriées hors cette période de maximum croissance.



**Fig.1 : Diversité mensuelle des phytoplanctons épiphytes de *Cystoseira barbata* à Kratten**

## Conclusion

Le mois d'Avril constitue la période d'abondance de l'algue *Cystoseira barbata* à Kerkennah. Cette période correspond cependant à la plus faible diversité en espèces de diatomées et dinoflagellés et même en espèces toxiques associées aux *Cystoseires*. Nous pouvons supposer donc que ces algues secrètent pendant cette période de croissance maximale des substances pour lutter contre l'épiphytisme.

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## **ETUDE DU POTENTIEL ANTI-MICROFOULING DE DEUX CODIACEAE : CODIUM FRAGILE ET CODIUM BURSA**

### **Abstract**

*Les peintures antisalissure utilisées récemment sont à base de tributylétain (TBT) et/ou de sulfate de cuivre très toxiques en milieu marin. Dans le but de développer des produits antifouling moins toxiques que ceux utilisés actuellement, nous avons choisi de tester le potentiel antisalissure d'extraits organiques préparés à partir de 2 espèces de Codium : C. fragile et C. bursa récoltées sur la côte nord tunisienne. En effet ces deux espèces d'algues vertes appartenant à la famille des Codiaceae sont rarement 'foulées'.*

*Les extraits bruts réalisés au CH<sub>2</sub>Cl<sub>2</sub> ont été testés pour leurs éventuelles activités antibactériennes (vis-à-vis de 8 espèces de bactéries foulantes), et algicides (vis-à-vis de 2 diatomées). C. fragile est l'espèce qui a montré les activités les plus significatives aussi bien antibactérienne qu'algicide. Une comparaison entre l'activité de deux variétés de cette espèce : C. fragile var. tomentosoides et C. fragile var. atlanticum a été réalisée. Les résultats obtenus permettent de considérer cette algue comme intéressante pour l'extraction de principes actifs antifouling.*

**Key-words:** Fouling, seaweed, *Codium*, antibacterial, algicidal.

### **Introduction**

La colonisation des surfaces artificielles immergées est une réaction immédiate qui se traduit dès les premières heures d'immersion, par la formation d'un film primaire, composé essentiellement de macromolécules organiques, puis de micro-organismes, enfin de macro-organismes. Cette colonisation indésirable est considérée comme salissure biologique ou encore "biofouling". Les peintures antifouling largement utilisées pour lutter contre les effets néfastes des biosalissures sont souvent toxiques vis-à-vis de l'environnement et peuvent avoir des conséquences désastreuses vis-à-vis de la faune et de la flore. D'où le besoin urgent d'identifier de nouvelles molécules naturelles biocides pouvant se substituer aux substances déjà utilisées. C'est dans ce cadre que s'inscrit cette étude sur les propriétés antifouling de deux *Codiaceae*.

### **Matériel et méthodes**

#### Matériel biologique

*Codium fragile* et *Codium bursa* ont été collectées en Juin 2006 dans la Baie de Carthage (36°50'47.66''N et 10°19'41.62''E) à une profondeur inférieure à 2m. Les échantillons d'algues sont nettoyés pour enlever le maximum d'épiphytes puis séchés pendant quelques jours, à température ambiante et à l'abri de la lumière dans un endroit sec. Les extraits bruts ont été effectués à l'aide du dichlorométhane par macérations successives et évaporation au rotaévaporateur.

#### Test antifouling

- L'activité antibactérienne a été testée sur 8 bactéries foulantes (B1-B8) isolées à partir d'un dispositif expérimental immergé dans la baie de Carthage (Chérif *et al.*, 2010). L'activité antimicrobienne est évaluée par la méthode des disques par mesure du diamètre de la zone d'inhibition en mm. Chaque test a été réalisé en duplicate.

- Deux diatomées ont été utilisées pour l'évaluation de l'activité algicide : *Navicula* sp. (M1) et *Chaetoceros calcitrans* (M2). Les tests ont été réalisés selon la méthode décrite par Guenther (2007) avec quelques modifications. Les extraits bruts sont testés à une concentration de 50 µg/cm<sup>2</sup>. Le TBT (10 ppm) a été utilisé comme témoin positif. Tous les tests ont été effectués en triplicate.

### Résultats

Le tableau 1 résume les résultats des tests antibactériens des extraits algaux au CH<sub>2</sub>Cl<sub>2</sub>, sur les 8 souches utilisées, ainsi que les résultats du test d'inhibition des diatomées. *C. fragile* montre une activité moyenne vis-à-vis de 4 souches bactériennes, tandis qu'aucune activité antibactérienne n'est remarquée pour l'extrait de *C. bursa*. Concernant l'activité algicide, c'est également *C. fragile* qui a montré l'activité la plus importante avec une inhibition supérieure à 50% de *Chaetoceros calcitrans*.

**Tab. 1 : Résultats des tests antibactériens et algicides.**

	Activité antibactérienne**								Activité algicide*	
	B1	B2	B3	B4	B5	B6	B7	B8	M1	M2
<i>C. fragile</i>	-	-	7	7	7	-	-	8	+	++
<i>C. bursa</i>	-	-	-	-	-	-	-	-	-	+

\* - : 0-25%, + : 25-50%, ++ : 50-75% ; \*\* - : pas d'activité

Une comparaison entre l'activité de deux variétés de cette espèce : *C. fragile* var. *tomentosoïde* et *C. fragile* var. *atlanticum* a été réalisée ; les résultats obtenus ne montrent aucune variabilité entre les deux variétés.

### Discussion et conclusion

Les résultats obtenus dans le présent travail sont en accord avec ceux obtenus par Chiheb *et al.* (2009) qui ont étudié l'activité antibactérienne de ces deux espèces récoltées sur les côtes méditerranéennes du Maroc et ont obtenu une activité positive pour *C. fragile* et négative pour *C. bursa*. Une faible activité algicide a été rapportée pour l'espèce *C. fragile* collectée sur les côtes japonaises (Alamsjah *et al.*, 2005).

La macroalgue *Codium fragile* étudiée dans le présent travail a montré une activité algicide intéressante, ainsi qu'une activité antibactérienne non négligeable et de ce fait pourrait être considérée comme intéressante pour l'extraction de principes actifs antifouling.

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## **RSP OU TELEMETRIE ACOUSTIQUE? COMPARAISON DES DEUX METHODES POUR LE SUIVI SPATIO-TEMPOREL D'HERBIERS DE POSIDONIES**

### **Résumé**

*Sensibles aux perturbations anthropiques, les posidonies sont utilisées pour suivre la qualité de l'eau. La méthode habituellement utilisée (méthode RSP) pour leur surveillance, est basée sur l'utilisation de balises permanentes immergées en limite d'herbier. Elle pose des difficultés méthodologiques liées au nombre restreint et à la pérennité des balises sur le fond. Affranchie de ces contraintes, la télémétrie acoustique, permet d'obtenir des cartographies sous marines de précision permettant le suivi des limites d'herbiers. Ces deux méthodes ont été utilisées en parallèle en 2008 et 2010 sur trois stations du site des Trois Caps (France, 83). Les résultats montrent les nombreux avantages de la télémétrie acoustique pour le suivi de la dynamique d'herbiers sous marins : facilité de mise en œuvre, finesse de l'échelle d'étude, mesures quantitatives d'évolution, suivi précis d'espèces remarquables.*

**Mots clés :** Cartographie ; Dynamique d'herbiers ; Posidonie

### **Introduction**

De part sa compétence d'observatoire marin, le SIVOM (Syndicat Intercommunal à Vocations Multiples) du littoral des Maures (France), a établi un programme d'actions prévoyant un suivi de la qualité du milieu marin sur sa zone d'intervention. *Posidonia oceanica* (L.) Delile est une espèce bioindicatrice couramment utilisée pour le suivi de la qualité de l'eau.

### **Materiel et méthodes**

L'évolution d'un herbier de Posidonie a été suivie sur deux ans au niveau de trois stations : une limite supérieure (-9m), une limite inférieure (-32 m) et un carré permanent de 36 m<sup>2</sup> (-9 m). Deux méthodes ont été utilisées en 2008 et 2010 et sont ici comparées :

a) la méthode RSP (immersion de dix à onze balises permanentes en limite d'herbier par Bonhomme *et al.*, 2000). Une série de photographies est réalisée le long des balisages comme preuves visuelles de l'évolution de l'herbier.

b) la cartographie par télémétrie acoustique (Descamp *et al.*, 2005 et Descamp *et al.*, 2010). C'est un système autocalibrant de positionnement local subaquatique basé sur un principe d'interférométrie acoustique 3D (USBL). Il se compose d'une base fixe et d'un pointeur mobile avec lequel un plongeur pointe (tous les 40 cm) les contours de l'herbier comme tout points remarquables (*Pinna nobilis*, balises RSP).

## Résultats

Sur la base des photographies, la limite supérieure semble relativement stable entre 2008 et 2010 sur l'ensemble des balises sauf au niveau des balises 1, 2 et 7 qui présentent une régression forte (30 cm) et les balises 5 et 8 qui présentent une régression plus faible (10 cm). La cartographie par télémétrie montre une progression globale de 48 m<sup>2</sup> en deux ans (64 m<sup>2</sup> de substrat nouvellement colonisé quand 16m<sup>2</sup> d'herbier ont disparu). Excepté au niveau des balises 2 à 4, l'herbier est plutôt stable avec des tâches de colonisation situées en amont des balises 8 à 11 qui sont en régression. La régression de ces petites tâches pourrait préfigurer une future atteinte de l'herbier au niveau de sa limite balisée en deçà.

En limite inférieure, la distance entre les premières balises (posées en 2000) avec la limite de l'herbier ne permet plus aux photos de donner une estimation sur l'évolution de cette limite. Il semble cependant que la régression observée les précédentes années continue excepté au niveau des balises 4 et 11 où elle est stable et à proximité de la balise 10 qui présente deux nouveaux faisceaux. La télémétrie acoustique montre qu'entre 2008 et 2010, 41 m<sup>2</sup> d'herbier ont disparu quand 8 m<sup>2</sup> sont apparus (régression globale de 33 m<sup>2</sup>). La régression est particulièrement visible en arrière des balises 1 à 5 et à proximité des balises 10 et 11. La légère progression a lieu entre les balises 6 et 7 et à proximité de la balise 9. Quelques faisceaux isolés persistent principalement entre les balises 4 et 5 au niveau de l'ancienne limite de l'herbier.

Le suivi du carré permanent montre un herbier en progression. Aucune différence n'a été observée entre les deux méthodes de microcartographie pour cette station.

## Conclusions

Cette étude souligne l'intérêt de la télémétrie pour le suivi des limites d'herbier à partir de 3 m de profondeur, sans contraintes liées à une progression ou régression trop forte pouvant nécessiter un re-balisage et donc un surcoût. Cette méthode permet l'obtention d'une cartographie précise et de données quantitatives d'évolution de l'herbier en un temps relativement court (500 à 1000 points espacés de 40 cm en une journée). Enfin, elle permet le repérage d'espèces remarquables telles que les nacres. Malgré tous ces avantages, la télémétrie acoustique ne dispense naturellement en rien des mesures de déchaussement, recouvrement et densité foliaire afin de suivre la vitalité générale de l'herbier.

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## **VARIATION SPATIALE DE LA PETITE FAUNE VAGILE DES HERBIERS DANS LA LAGUNE NORD DE TUNIS**

### **Résumé**

*La diversité spécifique n'a cessé d'augmenter dans la lagune Nord de Tunis depuis son assainissement, particulièrement celle de la faune benthique associée aux herbiers à Magnoliophytes. Cependant, malgré qu'on soit à plus de deux décennies de l'achèvement des travaux de restauration de la lagune Nord de Tunis, on ne dispose encore que de peu de données sur la distribution de la petite faune vagile des herbiers et son rapport avec les conditions du milieu. Notre étude, menée sur la petite faune vagile des herbiers, a permis de révéler la présence de 94 espèces (sans considération de l'ichtyofaune) qui se répartissent différemment de part et d'autre de la digue de séparation. La diversité la plus importante a été relevée dans la partie nord où prédominent les Magnoliophytes *Cymodocea nodosa*, *Nanozostera noltii* et *Ruppia cirrhosa*, et la plus faible diversité dans la partie sud où prédominent la Chlorobionte *Chaetomorpha linum* et où les variabilités des conditions du milieu sont relativement plus importantes.*

### **Abstract**

*Specific diversity did not cease increasing in the Northern lagoon of Tunis since its cleansing, particularly that of the benthic fauna associated with the Magnoliophyta beds. However, although we are more than two decades after the completion of restoration work of the Northern lagoon of Tunis, we have few data on the distribution of the small vagile fauna of the seagrass beds and its relationship with the lagoon environmental conditions. Our study, undertaken on small vagile fauna of the beds, made it possible to reveal the presence of 94 species (without consideration of ichthyofauna) which are distributed differently on both sides of dividing dyke. The most important diversity was recorded in the northern part where Magnoliophyta *Cymodocea nodosa*, *Nanozostera noltii* and *Ruppia cirrhosa* are prevalent and lowest diversity in the southern part where the Chlorobionta *Chaetomorpha linum* prevails and where variations of the environmental conditions are relatively more important.*

**Key-words:** *Zoobenthos, Phytobenthos, North lagoon of Tunis, Magnoliophyta, Restoration.*

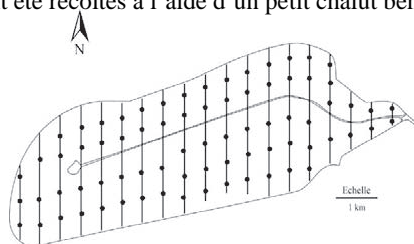
### **Introduction**

La lagune Nord de Tunis est située au fond du golfe de Tunis. Peu profonde, elle est longue de 10 km et large de 3 km (Ben Charrada, 1997). Avant les travaux de restauration il y'a eu une forte pollution aboutissant à l'eutrophisation du milieu et l'élimination de plusieurs espèces caractéristiques du milieu au profit d'autres espèces indicatrices de la pollution, mais après l'assainissement du milieu, il y'a eu une nette amélioration de la qualité des eaux lagunaires ainsi que la diversité spécifique (Ben Maïz, 1997 ; Shili, 2008).

### **Matériel et méthodes**

Les campagnes de prospection et d'échantillonnage de la faune benthique effectuées à bord d'une embarcation côtière ont intéressé 75 stations réparties dans toute la lagune

de part et d'autre de la digue de séparation et suivant 19 transects (Fig. 1). Les échantillons ont été récoltés à l'aide d'un petit chalut benthique d'un maillage de 1 mm.



**Fig. 1 : Répartition des stations d'échantillonnage de la petite faune vagile des herbiers de la lagune Nord de Tunis.**

### Résultats et discussion

Le sens de variation de la diversité spécifique de la faune est semblable à celui de la qualité des eaux lagunaires. En effet, la zone nord s'avère la plus diversifiée. Il s'agit d'une zone de transition où l'influence marine est importante et les variables physico-chimiques ne sont pas encore bien changées (442 µg/l pour l'azote total et 14 µg/l pour le phosphore total). Dans cette zone, on note la présence de certaines espèces caractéristiques telles que : *Carcinus aestuarii* Nardo, 1847, *Palaemon adpersus* Rathke 1837, *Cucumaria* sp. et *Amphipholis squamata* Delle Chiaje, 1828. La diversité spécifique diminue nettement en s'éloignant de la zone Nord-Est. Dans la zone ouest, il n'y a pas de fluctuations significatives des variables physico-chimiques mais une nette diminution de la diversité de la faune a été observée (Tab. 1). Pour la zone sud (zone de sortie des eaux lagunaires), l'atténuation de la vitesse de circulation des eaux, la nature du fond non dragué et la faible profondeur expliquent l'état d'eutrophisation marqué surtout par l'abondance de *Ruditapes decussatus* (Linnée, 1758) ainsi que par la faible diversité spécifique. La diversité la plus importante a été relevée dans la partie nord où prédominent les Magnoliophytes *Cymodocea nodosa*, *Nanozostera noltii* et *Ruppia cirrhosa* et la plus faible diversité dans la partie sud où prédominent la Chlorobionte *Chaetomorpha linum*.

**Tab. 1: Variation des paramètres physico-chimiques et de la diversité de la faune benthique dans la lagune Nord de Tunis (printemps, 2009). \*Espèces benthiques sans considération de l'Ichtyofaune.**

Paramètres physico-chimiques /Secteur	Nord	Ouest	Sud
Salinité (psu)	35.5	35.5	35.7
O <sub>2</sub> (%)	86.3	91	84.9
pH	8.3	8.4	8.4
Chlorophylle <i>a</i> (mg/l)	1.6	0.9	1.6
MES (mg/l)	15	14	17
N/P	42	42.5	60.3
Nombre d'espèces*	62	32	28

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## **INFLUENCE OF SEASONAL VARIABILITY ON SEAGRASS INDICATORS DEVELOPMENT: STUDIES IN CADIZ BAY (SOUTH-WEST IBERIAN PENINSULA)**

### **Abstract**

*The recognition of seasonal fluctuation of indices based on seagrasses as biological quality elements constitutes a topic of major importance in the assessment of ecological status, because avoids misclassification problems by discarding those changes due to natural variability in the seagrass ecosystem. This study was conducted within the Cadiz Bay and the measured variables were percentage of cover, shoot density, rhizome burial, epiphyte biomass and leaf length. The results showed significant differences among seasons for those variables and suggested that summer is the more appropriate season to carry out monitoring studies, as the maximum growth capacity of the meadows was reached during this period. A further question that needs to be addressed is to test if the seasonal variability on the parameters may cause a seasonal variation on indicators based on seagrasses.*

**Key-words:** seagrass indicators, Water Framework Directive, *Cymodocea nodosa*, *Zostera noltii*, Cadiz Bay.

### **Introduction**

The use of biological indicators for management and conservation purposes has increased during the last years due to different environment protection directives, such as the Water Framework Directive (WFD). This directive requires European Union Member States to achieve a good quality status of transitional and coastal waters by the year 2015 (European Commission 2000). According to the WFD the ecological status should be quantified based on Biological Quality Elements (BQE), being angiosperms one of these BQE selected for coastal and transitional waters.

A main issue on establishing ecological status is to discriminate between the ecosystem changes caused by natural variability or by anthropogenic pressures, in order to avoid misclassification problems (Common Implementation Strategy, 2003). For that reason, the main objective of this study was to determine how natural seasonal variability could modify the current values of different indices based on seagrasses as BQE.

### **Materials and methods**

The sampling was conducted seasonally during 2010 in two different sites within the Cadiz Bay. Two seagrass species were sampled: *Zostera noltii* Hornem. and *Cymodocea nodosa* (Ucria) Ascherson For this study, the variables were chosen at different levels of organization. Population variables such as shoot density and percentage of seagrass cover were measured in the field, whereas individual morphology, and community variables (e.g., mean leaf length and epiphyte biomass) were studied in the laboratory.

Statistical analysis (ANOVA, t-test) were used for significance of the seasonal variability on the above-mentioned parameters, selecting finally those with less fluctuations.

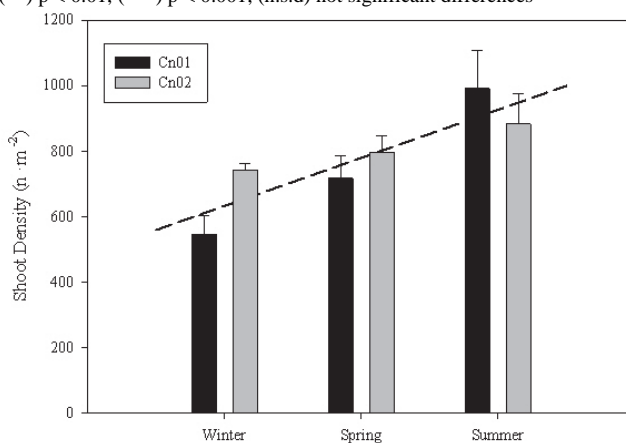
## Results

The ANOVA results (**Tab. 1**) showed that significant differences occurred not only between sites, but also among seasons for the measured parameters. Moreover, a general increasing trend from winter to summer was found in some of the variables (**Fig. 1**).

**Tab. 1: Significance of ANOVA test for the effects of site and season on seagrass variables.**

Variables	Site	Season	Site <sup>^</sup> Season
Cover percentage	**	***	*
Shoot density	n.s.d.	**	n.s.d.
Rhizome burial	***	***	***
Epiphyte biomass	n.s.d.	***	n.s.d.
Mean leaf length	n.s.d.	***	n.s.d.

(\*) < 0.05 (\*\*) p < 0.01, (\*\*\*) p < 0.001, (n.s.d) not significant differences



**Figure 1: Seasonal variation of shoot density of *C. nodosa* between two different sites (bars represent mean + SE).**

## Discussion and conclusions

The results suggested that summer is the more suitable season to carry out monitoring studies, due to the maximum growth capacity of the meadows reached during this period. Also it is important to remark that some parameters (percentage of cover and rhizome burial) seem to be more variable, while others (shoot density, epiphyte biomass and leaf length) remain more constant between different sites. This could indicate that, for instance, shoot density is a more useful variable in indices based on seagrasses as BQE than percentage of cover due to its higher stability.

## Acknowledgments

This work was supported by the Interreg project ECOLAGUNES of the SUDOE European region (SOE1/P2/F153), and the national project Imachydro (CTM2008-00012) from the Spanish Ministry of Education and Science.

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## **COMPARATIVE EFFECTS OF INCREASED SALINITY ON WATER RELATIONS AND ION CONCENTRATION IN THE MEDITERRANEAN SEAGRASSES *CYMODOCEA NODOSA* AND *POSIDONIA OCEANICA***

### **Abstract**

*The aim of this study was to analyse the effects of increased salinity on ion accumulation and osmotic adjustment in the Mediterranean seagrasses *Cymodocea nodosa* and *Posidonia oceanica*. To this end, plant fragments were exposed for 7 days to 37 psu (control treatment), 41.5, 46, 50.5, 55 and 59.5 psu. Water relations were determined by measuring water ( $\psi_w$ ) and osmotic ( $\psi_s$ ) potentials in leaf discs and concentrations of  $Cl^-$ ,  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$  and  $SO_4^{2-}$  were also analysed in leaf tissues. As expected for plants undergoing osmotic adjustment processes,  $\psi_w$  and  $\psi_s$  became more negative as salinity increased in both species, although they were always more negative in *C. nodosa* than in *P. oceanica*. In the more extreme hypersaline treatments *C. nodosa* leaves showed increased  $Cl^-$ ,  $Na^+$  and  $SO_4^{2-}$  concentrations (53%, 15% and 60%, respectively) with respect to the control plants. In contrast, these concentrations in *P. oceanica* leaves decreased (47%, 10% and 42%, respectively), suggesting the disruption of  $Cl^-$  passive influx due to cation exclusion, or the transport of ions to other parts of the plant. In both species,  $K^+$  concentrations increased to a maximum in the 50.5 psu treatment and then decreased, probably due to competition with  $Na^+$  at the membrane transporter level. In *C. nodosa*, the  $Na^+:K^+$  and  $Na^+:Ca^{2+}$  molar ratios were lower than in *P. oceanica*, suggesting a higher capacity of the former to maintain normal cytosolic enzyme activities under hypersaline conditions. The results indicate different physiological strategies in the two seagrasses for tolerating increases in salinity. *Cymodocea nodosa* seems to be able to osmoregulate through ion accumulation, while *P. oceanica* seems to avoid ion accumulation and it is more likely to osmoregulate through compatible solutes.*

**Key-words:** water relations, ions, hypersaline stress, *Posidonia oceanica*, *Cymodocea nodosa*.

### **Introduction**

When plant cells are exposed to hypersaline stress conditions, steady-state kinetics of ion transport (mainly  $Na^+$ ,  $Cl^-$ ,  $K^+$  and  $Ca^{2+}$ ) are affected. Ion homeostasis acts as the first rapid osmoregulatory response required to maintain a positive water balance in plant cell tissues in order to avoid a severe reduction in turgescence. Nevertheless, our knowledge of these relationships in seagrasses is still very limited (Touchette, 2007). The aim of this study was to analyse the effects of increased salinity on ion accumulation and osmotic adjustment in two Mediterranean seagrass species: *Cymodocea nodosa* and *Posidonia oceanica*.

### **Materials and methods**

Plant fragments of both species were placed in a series of 100 l aquaria and exposed to the following salinity treatments for 7 days: 37 psu (ambient salinity, control treatment), 41.5, 46, 50.5, 55 and 59.5 psu. Water relations were determined by measuring water ( $\psi_w$ ) and osmotic ( $\psi_s$ ) potentials in leaf discs using vapour pressure osmometry and

expressed in pressure units (Mpa). Leaf concentrations of  $\text{Cl}^-$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{SO}_4^{2-}$  were determined by ICP and ionic chromatography.

## Results

In both species, the mean values of  $\psi_w$  and  $\psi_p$  became more negative as the salinity increased, as can be expected for plants undergoing osmotic adjustment processes. However, *C. nodosa* showed more negative  $\psi_w$  and  $\psi_p$  values than *P. oceanica* across all saline treatments (Fig.1). In *C. nodosa* leaves,  $\text{Cl}^-$ ,  $\text{Na}^+$  and  $\text{SO}_4^{2-}$  concentrations increased by 53%, 15% and 60%, respectively, in the more extreme hypersaline treatments compared with the plants in ambient salinity conditions. Meanwhile, in *P. oceanica*, these values decreased by 47%, 10% and 42%, respectively, suggesting that in *P. oceanica* the passive influx of  $\text{Cl}^-$  may be disrupted due to cation exclusion or a transport mechanism to other parts of the plant. In both species,  $\text{K}^+$  concentrations increased to a maximum in the 50.5 psu treatment and then decreased, probably due to competition with  $\text{Na}^+$  at the membrane transporter level. In *C. nodosa*,  $\text{Na}^+:\text{K}^+$  and  $\text{Na}^+:\text{Ca}^{2+}$  molar ratios were lower than in *P. oceanica*, suggesting a higher capacity of the former to maintain the activities of cytosolic enzymes under hypersaline conditions.

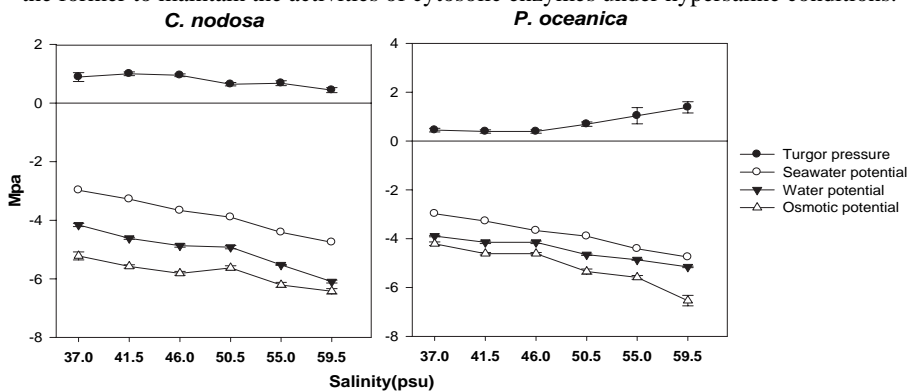


Fig. 1: Water relations in *P. oceanica* and *C. nodosa* leaf tissues. Mean  $\pm$  SE.

## Conclusion

The results obtained clearly show significant differences in the physiological strategies adopted by the two species for tolerating an increase in salinity. *Cymodocea nodosa* is able to osmoregulate through ion accumulation and maintains high levels of  $\text{K}^+$  and  $\text{Ca}^{2+}$  compared to  $\text{Na}^+$ . *Posidonia oceanica* seems to avoid ion accumulation in leaf tissues, probably because this seagrass species is less tolerant to ion toxicity, and osmotic adjustment is probably achieved through compatible solutes. These results are consistent with their respective ecological strategies, since *P. oceanica* is adapted to open water environments with constant salinities and *C. nodosa* is a more eurybiontic species.

## Acknowledgments

Ministerio de Medioambiente y Medio Rural y Marino (OSMOGRASS Project Ref. 021/SGTB/2007/1.3) and the Ministerio de Ciencia e Innovación (OSMOGRASS II project Ref. CTM2009-08413MAR).

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## **SEAGRASS BEDS AND THE ROLE OF CANOPY ARCHITECTURE ON THE ASSOCIATED BENTHIC COMMUNITY**

### **Abstract**

*This work aims to decrypt under natural conditions how the interactions between seagrass canopy properties and flow characteristics alter the abundance and species richness of benthic organisms. Faunal abundance and species richness were quantified during one year in two seagrass species with contrasting canopy properties (*Zostera noltii* and *Cymodocea nodosa*) and in three locations with different hydrodynamic properties in the surroundings of Cádiz Bay. Additional samples from surrounding bare areas were also collected in each sampling event. Species richness, abundance and plant characteristics were determined. As a general outcome, our results showed that independently of the hydrodynamic conditions, season and/or sediment granulometry the highest species richness and abundance were usually found in seagrass beds when compared to bare sediment. Furthermore, higher values of abundance were found in *Z. noltii* meadows in comparison with *C. nodosa* ones. In addition, when multiple correlations between species richness (or abundance) and canopy properties were conducted, organisms abundance was positively correlated with shoot density but, interestingly, different slopes were recorded for both seagrass species. In conclusion, seagrass beds increased the abundance and richness of macrobenthic organisms in coastal areas when compared to unvegetated areas, while canopy properties also seem to play an important role modulating the final outcome.*

**Key-words:** diversity, species richness, seagrasses, ecosystem engineers, hydrodynamics

### **Introduction**

Seagrasses provide important ecosystem functions and services. One of the primary services is the maintenance of high biodiversity levels in coastal areas. A combination of different factors has been usually stated as a potential explanation for such outcome, such as the increase of habitat complexity, the reduction of foraging efficiency of the predators and the amelioration of hydrodynamic conditions. Most of these factors are directly related with the role played by aboveground seagrass structures as ecosystem engineers. Therefore, we will test if canopy properties (shoots density, leaf length, patch configuration...) will interact with hydrodynamics altering local microenvironmental conditions and particle fluxes (e.g. edible particles for organisms) within the canopy, affecting to seagrass associated fauna. The objective of this study will be to evaluate how the interactions between canopy properties and flow characteristics alter the abundance and species richness of benthic organisms under natural conditions.

### **Materials and methods**

Three locations with different hydrodynamic properties and sediment types were sampled in the surroundings of Cádiz Bay (Bajo de la Cabezuela (Bajo C.), Cádiz and

Santibañez (Santb)) in a seasonal basis (spring, summer, autumn, winter). Every sampling time, four 16x16x25cm samples were taken at low tide in two seagrass beds with contrasting canopy properties (*Zostera noltii* and *Cymodocea nodosa*) on each location. Additional four samples from surrounding bare areas were also collected. The samples were cleaned in running seawater with a mesh (1mm) to remove the sediment and transported in a fridge to the lab, where species richness, abundance, and plant characteristics were determined.

### Results and Discussion

Significant differences were found for organism abundance between seagrass habitats and bare sediment in each location (Fig. 1). The highest abundance was usually found in *Z. noltii* meadows while bare sediment showed the lowest ones. Significant differences were also found for species richness (Fig. 2), recording more species in vegetated areas. The shoot abundance and the organisms abundance showed a highly direct correlation for both type of canopies, although interestingly, followed different slopes (Fig. 3). Therefore, seagrass beds increased the abundance and species richness when compared to unvegetated areas, independently of hydrodynamic conditions or sediment type (data not shown). This result found in *C. nodosa* and *Z. noltii* meadows highlight the importance of canopy properties on the animal diversity levels.

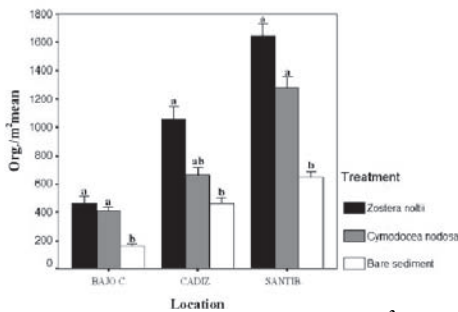


Fig. 1: Organisms abundance ( $org.m^{-2}$ ) per treatment in each location. Letters show significant differences in each location on Tukey test ( $p < 0.05$ ).

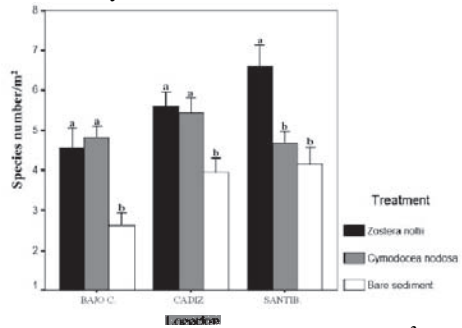


Fig. 2: Species abundance ( $species.m^{-2}$ ) in each location. Letters show significant differences among each location on Tukey test ( $p < 0.05$ ).

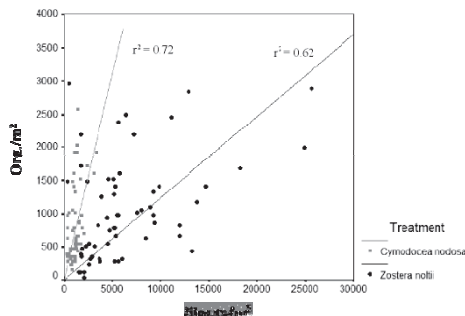


Fig. 3: Correlation between shoot density ( $shoots.m^{-2}$ ) and organisms abundance ( $org.m^{-2}$ ) in both seagrass canopies.

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## **SMALL SCALE SPATIAL VARIABILITY IN THE PHENOLIC CONTENT OF *ZOSTERA* SPP. FROM SALSSES-LEUCATE LAGOON**

### **Abstract**

*The presence and abundance of phenolic compounds of Zostera spp. from Salses-Leucate lagoon (Languedoc Roussillon, France) were studied. Seven sites were sampled on August 2009, and analyzed for both the identity and quantity of phenolics present using NMR and HPLC. The concentrations found are greater in Zostera noltii Hornem (mean value 20 mg.g<sup>-1</sup>) than in Z. marina var. angustifolia Hornem (mean value 6 mg.g<sup>-1</sup> dw). The proportions of the phenolics varied substantially with the major proportion comprising flavonoids (56-99%), whereas zosteric acid and caffeic acid each represent 0-5%. Important discrepancies appeared between the 7 sampling sites, especially in the rosmarinic acid content which varies in the range 0-56%. The results show the negative effect of epiphytic load on the phenolic concentration.*

**Key-words:** Salses-Leucate lagoon, *Zostera noltii* Hornem, *Z. marina* var. *angustifolia* Hornem, phenolic content, variability

### **Introduction**

Mediterranean lagoons are highly productive areas representing more than 50% of the coastline in Languedoc-Roussillon (South of France). Many of them suffer from eutrophication and concomitant deterioration of water quality. Seagrass meadows are susceptible to coastal environmental impacts and can serve as early indicators of system-wide degradation. It is of interest to develop and validate chemical biomarkers for monitoring conservation status and ecotoxicological impact in *Zostera* meadows. In this context, phenolic compounds constitute good candidates, which may provide insights into water quality. They play several important functions in plants, and they are common in marine ecosystems. Documenting the presence of those compounds in alive tissues, and how they vary in abundance becomes crucial to understand how human activities influence marine communities. To do this we analyzed the abundance of specific phenolic compounds (rosmarinic (RA), caffeic (CAF), zosteric acids (ZA) and flavonoids (F)) of *Zostera* spp. from different sites located across the Salses Leucate lagoon (Achamlale *et al.*, 2009a, b).

### **Materials and Methods**

Samples were collected at Salses-Leucate lagoon (42.8°N 3.0°E, France) on August 29th 2009 at 7 stations (S1-S7) providing a large gradient of physico-chemical and environmental parameters. After collection, the seagrass material was sorted and divided into all green leaves (A) without visible epiphytes and leaves still loaded with epiphytes and macroalgae (B). This led to 11 samples of *Z. noltii* and 5 samples of *Z. marina* var. *angustifolia* (Tab. 1). Methanolic extracts were prepared from the plant material, and analyzed for both the identity and quantity of phenolics present using NMR and HPLC. (Tab. 1, data are expressed in mg (gdw<sup>-1</sup>) of dry matter of *Zostera*).

## Results and Discussion

The phenolic profile was largely dominated by flavonoids whatever the species, but the concentration mean values are greater in *Z. noltii* (20 mg.g<sup>-1</sup>) than in *Z. marina* var. *angustifolia* (6 mg.g<sup>-1</sup> dw). The proportions remain constant with *Z. marina* var. *angustifolia*, for which only traces of RA, ZA and CAFF were found, whereas important variations of the amount of RA were observed with specimen (A) of *Z. noltii*: 42, 31 and 39% respectively at S4, S6 and S7, but only 9% at S2 and S3. Concentrations in specimen A are always higher than in B, showing the negative effect of macroalgae and epiphytes, which were particularly abundant at S3 and S6. Interestingly, the lowest values were found for the specimen B from these two stations. The highest values were found at S7, in the northern part of the lagoon, which is less eutrophicated than S5 and S6 (Ifremer, 2010). The lowest content with *Z. marina* var. *angustifolia* was found at S1, an artificial channel affected by pollution and physical disturbance of the sediment due to the passage of boats. Understanding the specific functional role of each of these compounds is now necessary. However, this first approach highlights the potential of polyphenols as indicators of the coastal lagoon environmental quality.

**Tab. 1: Spatial variation of the phenolic content of *Zostera* spp.**

Species	Sampling station	Extract codes	Phenolic proportions (% of the total)				Total mg.g <sup>-1</sup> dw	
			F	RA	ZA	CAFF		
<i>Z. noltii</i>	Pointe de la Coudalère (S2)	2-A-Zn	88	9	1	1	19.84	
		2-B-Zn	93	4	1	2	15.35	
	Les petites dosses (S3)	3-A-Zn	89	9	1	1	21.98	
		3-B-Zn	94	3	2	1	8.742	
	Chenal du portel (S4)	4-A- Zn	56	42	1	1	27.83	
	St Laurent de la Salanque (S5)	5-A-Zn	81	15	3	1	25.54	
		5-B- Zn	88	7	3	1	16.45	
	Anse de la Roquette (S6)	6-A- Zn	66	31	2	1	24.86	
		6-B- Zn	79	15	5	1	2.76	
	Iles des Sidrières (S7)	7-A- Zn	59	39	1	1	41.57	
		7-A- Zn	77	20	1	2	16.20	
	<i>Z. marina</i> var <i>angustifolia</i>	Port Barcares (S1)	1-A- Za	98	1	1	0	6.02
			1-B-Za	99	0	1	0	2.42
		Les petites dosses (S3)	3-A-Za	92	3	1	3	9.17
3-B-Za			94	2	1	4	6.80	
St Laurent de la Salanque (S5)		5-A-Za	97	1	1	1	5.92	

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## **PHENOLIC CONTENT OF *CYMODOCEA NODOSA* BEACH WASTE**

### **Abstract**

*Detrital leaves of the seagrass Cymodocea nodosa (Ucria) Ascherson (Cymodoceaceae) were screened for their phenolic content. For the first time, the major polyphenols in the leaves were unambiguously identified as chicoric acid (CA) and caftaric acid (CAF). They were quantified in the crude extracts using high-performance liquid chromatography. The concentrations found respectively ranged from 0.24-2.9 mg g<sup>-1</sup> dw for CAF and 8.13-30.13 mg.g<sup>-1</sup> dw for CA. This is the first report of CAF and CA in C. nodosa. Considering the demonstrated therapeutic applications of CA, its high value-added on the nutraceutical market, and its rare occurrence in the plant kingdom, the high content found in C. nodosa detrital leaves makes this abundant renewable raw material of interest for pharmaceutical and food industry.*

**Key-words:** chicoric acid; caftaric acid; *Cymodocea nodosa*; detrital leaves; quantitative HPLC.

### **Introduction**

*Cymodocea nodosa* (Ucria) Ascherson (Cymodoceaceae family, common name: lesser Neptune grass) is widely distributed along the Mediterranean coasts, the North Atlantic coast of Africa and the Canary Islands, reaching its southern limit of distribution in Senegal. Under the influence of physical factors such as winds, currents, waves and tides, sizeable volumes of seagrass material periodically wash ashore on the coast lines. Local managers are under a lot of public pressure to remove this wrack, whenever they accumulate on beaches and shorelines used for recreational purposes. In most cases, the collected biomass is disposed of in waste disposal sites. Compared to algae, seagrasses remain very little exploited as raw material for the production of bioactive compounds despite they offer tremendous opportunities to find new commercially valuable phytochemicals (Achamlale *et al.*, 2009a,b; Nuissier *et al.*, 2008, 2010). Little is known of the phenolic acid content of *C. nodosa*, despite they are very good candidate for valorization. Considering the economic potential of phenolic acids within the pharmaceutical, cosmetic and food industries, it appears of interest to investigate the phenolic content of *C. nodosa*.

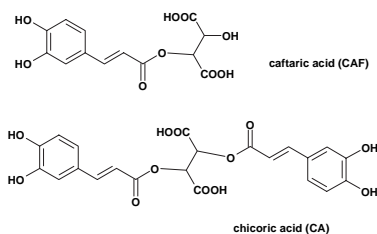
### **Materials and Methods**

Detritus of *C. nodosa* were collected from piles in the intertidal zone at San Agustin, Gran Canaria, (Canary Islands, 27°46 N, 15°32 W), at Sahline Sebkh beach (35°46 N, 10°43 E, Tunisia) and at Delta del Ebro (40°43N, 0°52 E, Spain). After collection, the samples were quickly washed in freshwater to remove sand and salt, then air-dried at room temperature. All the samples were separated as leaves and rhizomes.

Dried finely crushed leaves (10 g) were sequentially extracted with MeOH (120 mL, 24 h, rt) then aqueous methanol (80:20; 60 mL, 24 h, rt). The two extracts were evaporated to dryness or freeze-dried and analysed separately using NMR and quantitative HPLC.

## Results and discussion

HPLC-DAD was used for both qualitative and quantitative analyses of the extract composition. The identification of products was performed by matching both their HPLC Rt and 220-400 nm spectra with those of 43 standards belonging to 7 classes of phenolics, ie phenols, benzoic acids, benzaldehydes, acetophenones, cinnamic acids, cinnamaldehydes, flavonoids. All the extracts show a phenolic profile dominated by two phenolics, unambiguously identified as caftaric (CAF) and chicoric acid (CA) on the basis of UV, LC/MS and NMR spectra, and comparison with standards (Fig. 1). The concentrations found respectively ranged from 0.24-2.9 mg.g<sup>-1</sup> dw for CAF and 8.13-30.13 mg.g<sup>-1</sup> dw for CA. To the best of our knowledge, it is the first time CA and CAF were found in *C. nodosa*.



**Fig. 1: Formulae of caftaric and chicoric acid**

The large amount of CA is of special interest due to its rareness in the plant kingdom and its large spectrum of biological properties, including anti-HIV (Bailly & Cotelle, 2005; Charvat *et al.*, 2006). Commercial chicoric acid-based herbal medicines are produced from *Echinacea purpurea* roots (19.27 mg.g<sup>-1</sup> dw) (Pellati *et al.*, 2004). Our results show that *C. nodosa* detritus could compete with this traditional raw materials. Considering the demonstrated therapeutic applications of CA, and the success of chicoric acid-based herbal medicine on the nutraceutical market, detrital leaves of *C. nodosa* constitute a promising new raw material for the production of health benefits products and bring a solution to an environmental problem.

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## **POSIMED: PROJECT FOR THE INTEGRATION OF THE SPANISH VOLUNTEER ENVIRONMENTAL REGIONAL NETWORKS FOCUSED IN MONITORING *POSIDONIA OCEANICA***

### **Abstract**

*POSIMED is a project designed for coordinating the volunteer environmental networks created in the Spanish Mediterranean regions for monitoring seagrass Posidonia oceanica. There are active networks in Catalonia, Balearic Islands, Region of Valencia, Murcia, Andalusia and the City of Melilla. The stations sampled were 113 in 2009 and 276 volunteer divers participated in the activities. The integration of regional networks implies the first national network for monitoring underwater ecosystem.*

*The implementation of unique methodology has not been possible because regional networks applies different sampling techniques, even objectives and concepts are not the same (type of sampling, sample area, scale of sampling). The methodologies were contrasted in field work on the islands of Tabarca (Alicante) and Formentera (Balearic Islands), where exist the major seagrass of Posidonia oceanica on the Spanish coast. Although the regional results are dissimilar, the parameters of density are comparable when the relationship between area sampled and accumulated error is similar.*

*POSIMED continues through its website (<http://www.posimed.org/>) and annual meetings with the aim of informing and exchanging data on the conservation status of Posidonia oceanica.*

**Key-words:** *Posidonia oceanica*, Volunteer environmental networks, monitoring programmes, Spain.

### **Introduction**

The main objective of POSIMED is the national integration of volunteer environmental networks that have been created in the Spanish Mediterranean regions for monitoring seagrass *Posidonia oceanica*. The national network for monitoring the *P. oceanica* seeks to achieve the following objectives: i) Integrate the regional networks created in Spain. ii) Coordinate the methodologies used for sampling the *P. oceanica*. iii) Distribute results iv) Promote social participation and volunteer activity.

The national network involves the monitoring networks created in Catalonia, Balearic Islands, Region of Valencia, Murcia, Andalusia and City of Melilla.

### **Materials and methods**

The methodology for monitoring *P. oceanica* is similar to others used for meadows based on the calculation of density and coverage (Giraud, 1977; Romero, 1985; Sánchez Lizaso, 1993), although each network adapts the methods and techniques to the peculiarities of each area (IEL, 2009). Before diving volunteers receive a training course, and dives for collecting data always are supervised for a technician responsible for the program. The volunteer environmental networks provide all the material and

means for the realization of these dives and the results are available on the website of the program: <http://www.posimed.org/>.

### **Results**

POSIMED has involved all the regions where *P. oceanica* is distributed, a total of 113 stations and 279 volunteer participants in 2009. The volunteers programmes have been attended from 400 to 600 people every year, and around 50% of divers develop the environmental skills in field work. The networks also have distributed 10,000 leaflets on the importance of *Posidonia oceanica* in the Mediterranean Sea.

The implementation of unique methodology has not been possible because regional networks applies different sampling techniques, even objectives and concepts are not the same. However the parameters of density are comparable if standard error is not high.

### **Conclusions**

POSIMED has coordinated the regional networks that carry out monitoring activities on the Spanish coast. The project also has encouraged these volunteer activities in Andalusia and Melilla, enhancing the role of volunteers and the access to environmental information obtained.

### **Acknowledgments**

To the “Fundación Biodiversidad” (Minist. de Medio Ambiente, y Medio Rural y Marino), and the Dir. General de Pesca de la Generalitat de Catalunya; Dip. Prov. of Alicante; Dir. General de Pesca del Govern de les Illes Balears; Serv. de Pesca y Acuicultura de la Comunidad Autónoma de Murcia; Cons. de Medio Ambiente de la Junta de Andalucía; Sec. General de Mar (Minist. de Medio Ambiente, y Medio Rural), and to all volunteers who participate every year.

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## **POPULATIONS OF SEAGRASSES *ZOSTERA MARINA*, *Z. NOLTII*, AND *CYMODOCEA NODOSA* OCCUPY PREDICTABLE PHYSICO-CHEMICAL REGIONS AND HARBOR CHARACTERISTIC ANIMAL COMMUNITIES WITHIN AND AMONG CENTRAL CROATIAN ADRIATIC EMBAYMENTS**

### **Abstract**

*Although seagrass meadows are known for their value as animal habitat, little information exists on the relative habitat value of individual species. We investigated populations of co-occurring seagrasses *Zostera marina*, *Z. noltii*, and *Cymodocea nodosa* and their associated macro-fauna within their physical context in seven distinct embayments in the central Croatian Adriatic Sea. Total seagrass cover varied significantly among embayments, was negatively affected by slope of the sea bottom, and positively affected by marina developments. Variation in coverage of individual seagrass species within and between bays significantly correlated with depth, wave exposure, and salinity. At three meters depth, faunal abundance was higher in monocultures of *C. nodosa* (four times) or *Z. noltii* (seven times) than in beds of *Z. marina* (pure or invading *Z. noltii*). Likewise, the effective number of taxa (diversity) was higher in *Z. noltii* and *C. nodosa* than in *Z. marina*. Several taxa were exclusively found either in *C. nodosa* or *Z. noltii* meadows. Our results are consistent with the hypothesis that physical processes control seagrass cover and distribution, and that this species-specific cover and distribution has significant and predictable effects on the associated animal assemblage.*

**Key-words:** seagrass, *Zostera*, *Cymodocea*, Adriatic, faunal community, physico-chemical

### **Introduction**

Physical processes can control total seagrass cover at various spatial scales as well as the spatial distribution of individual species, either directly based on species' intrinsic tolerances (Krause-Jensen *et al.*, 2003) or indirectly by influencing competition among co-occurring species (Kruschel *et al.*, 2009). Although seagrass meadows are generally known for their value as animal habitat (Vizzini, 2009), little information exists on the relative habitat value of individual species.

### **Materials and methods**

In seven shallow water locations in the central Croatian Adriatic (Zadar region, spring 2008) benthic habitats including macroalgae/rock, bare sediments, and seagrass (*Zostera noltii*, *Z. marina* and *Cymodocea nodosa*) were continuously video recorded along -3m isobath and 0 - 6 m transects. Videos had overlaid satellite time, depth, and horizontal DGPS coordinates with submeter accuracy (Schultz, 2008). A diver, holding the camera, recorded all macrofauna (>1 cm) within 1 m on each side of the camera's path and the time of observation. Habitat selection was quantified as the abundance ratio R of fauna in/out a video segment scaled by the ratio of available habitat in/out of a video segment.  $R > 1$  implies a higher probability of occupation of a random segment within the habitat by a taxon than of a random segment outside of that habitat. This constitutes

positive habitat selection (Schultz *et al.*, 2009). Salinity was measured with a refractometer. Bottom slope was calculated from the shortest distance between 0 and 6 m. Depth-dependent wave exposure ( $REI_d$ ) was calculated using the formulas given in Krause-Jensen *et al.* (2003). Fetch distances were measured as the distance between a sampling point and the nearest shoreline in each of eight compass directions (N, NW, W, SW, S, SE, E, NE) using Google Earth 5.0 (2009).

## Results

Total seagrass cover varied significantly among embayments ( $p = 0.003$ ), was negatively affected by slope of the sea bottom ( $p = 6e-07$ ) and positively affected by marina developments ( $p = 0.0006$ , 50% of the variation). *C. nodosa* occurred at the greatest depths (to 6 meters), and its cover was positively correlated with wave exposure ( $p = 0.0002$ ) and salinity ( $p = 0.01$ , 26% of the variation). *Z. noltii* occurred at the shallowest depths (to < 1 m), and in mixed meadows of *Z. noltii* and *Z. marina*. *Zostera* cover was negatively correlated with wave exposure ( $p = 0.001$ ) and salinity ( $p = 0.02$ ) explaining a total 47% of the variation. At three meters depth, the mean megafaunal abundance was 0.22 observations per transect meter in pure *Z. noltii*, 0.12 in pure *C. nodosa*, 0.029 in pure *Z. marina*, and 0.032 in mixed *Zostera* beds. Faunal diversity was 8 in pure and invaded *Z. noltii*, 7 in pure *C. nodosa* and lowest in pure *Z. marina* (4). Of the 50 faunal taxa observed at 3 m, 44 favored at least one of the available habitats. For 22 taxa seagrass was at least one of the favored habitats ( $R > 1$ ). Five taxa were exclusively associated with *C. nodosa* communities and four with *Z. noltii* communities, while no taxon was solely favoring pure *Z. marina*.

## Conclusions

Our results are consistent with the hypothesis that physical processes control seagrass cover and distribution within and between embayments, and that this species-specific cover and distribution has significant and predictable effects on the associated animal community. Individual seagrass species do not have equivalent effects on animal habitat and use, and within our study context faunal diversity and abundance were substantially greater in *Cymodocea nodosa* and *Zostera noltii* than in *Z. marina*.

## Acknowledgments

We thank the University of Zadar and the Croatian Ministry of Science and Education for providing funding for this project under a grant to SS for scientific project 3207.

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## **POUR UNE COLLECTE RATIONNEELE DE L'ALGUE BRUNE *PADINA PAVONICA (L.) THIVY* : EVALUATION DE SES POTENTIALITES NATURELLES A CAP ZEBIB (NORD DE LA TUNISIE)**

### **Résumé**

*A Cap Zebib (Nord-Est de la Tunisie), l'algue brune Padina pavonica se rencontre dans la frange côtière comprise entre 0 et 10 m le long de 1600 m de linéaire de côte et couvre une surface de 132 ha. La biomasse naturelle disponible est évaluée à 112,5 tonnes de matière fraîche, soit 25 tonnes de matière sèche (22,2%). Etant donné que la majeure partie de la biomasse, 106 tonnes représentant 94% de la biomasse totale, provient de la zone comprise entre 0 et 6 m de profondeur, zone d'exploitation optimale, le rendement peut être considéré de 1,3 t/ha.*

**Mots clés :** *Padina pavonica*. Algue brune. Cap Zebib. Surface. Biomasse.

### **Introduction**

L'algue brune *Padina pavonica* a désormais un intérêt économique puisque ses extraits contiennent une molécule active permettant l'amélioration de la fixation du calcium par les tissus biologiques et ayant des applications pharmaceutiques et alimentaires.

En Tunisie, elle se rencontre pratiquement sur tout le littoral (Ktari & Langar, 2004), mais se présente à l'état naturel sous forme de peuplements dispersés, quelques fois denses. Le présent travail, effectué dans le cadre d'une convention entre l'INSTM et la société « Algues Marines de Tunisie » en complément des résultats obtenus par Ben Said *et al.*, (2002), vise la connaissance de l'état du stock naturel de cette espèce dans un site atelier, Cap Zebib (Nord-Est de la Tunisie), en vue d'assurer son exploitation rationnelle par la collecte.

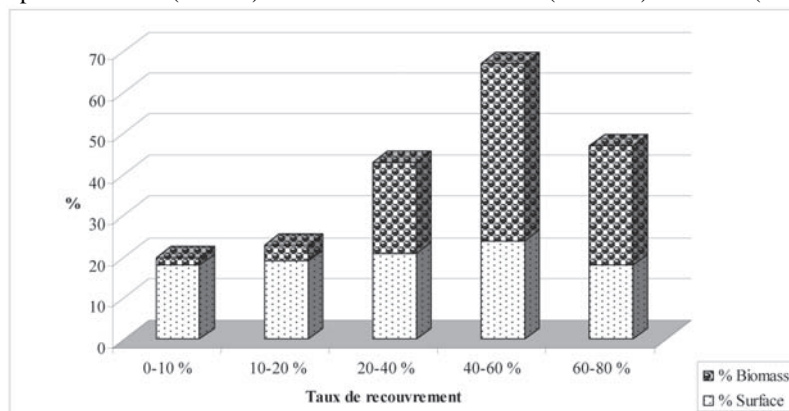
### **Matériel et méthodes**

La cartographie et les prélèvements pour la biomasse (juillet-août 2006) ont été effectuées le long de 13 transects parallèles distants de 100 m entre la côte et l'isobathe 10 m. Les observations ont été faites soit à partir de la surface lorsque le fond est nettement visible, soit en plongée. Les limites de recouvrements de *Padina* ont été repérées par GPS.

### **Résultats et discussion**

A Cap Zebib, *Padina pavonica* occupe une superficie totale de 132 ha et génère une biomasse évaluée à 112,5 t de matière humide, soit 25 t de matière sèche; la teneur en matière sèche de *Padina* au cours de la période d'étude étant de 22,2%. Les peuplements moyens, situés entre 1,5 à 4 m et ayant un taux de recouvrement de 40 à 60

%, sont majoritaires aussi bien en surface (24 %) qu'en biomasse (Fig. 1). Les peuplements denses (60 à 80 %) et clairsemés (20 à 40 %) contribuent d'une façon notable dans la biomasse, à hauteur respectivement de 29 et 22 %; la part des peuplements rares (0-10 %) et de ceux en tâches isolées (10-20 %) est faible (2 et 4 %).



**Fig. 1 : Contribution des différents types de recouvrement de *Padina pavonica* au niveau des surfaces d'extension et des biomasses.**

Nos résultats relatifs à la biomasse par unité de surface (152-300 g/m<sup>2</sup>) sont comparables à ceux mentionnés par d'autres auteurs (Turna *et al.*, 2002 ; Neto, 2000 ; Ganeson *et al.*, 2000).

A Cap Zebib, le rendement global de *Padina* est légèrement inférieur à 1 t de matière humide par hectare (0,851 t/ha). Etant donné que l'espèce est exploitable entre 0 et 6 m de profondeur sur une superficie de 83 ha, on peut considérer que le rendement moyen dans cette zone (1,3 t/ha) est le rendement de référence.

Par ailleurs, les rendements naturels de la majorité des espèces algales étant faibles, l'algoculture permettrait d'approcher des rendements bien plus importants, compatibles avec une exploitation industrielle.

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## **TUNALGOBASE: A LITERATURE BASED DATABASE ON TUNISIAN SEAWEEDS BIODIVERSITY AND POTENTIAL BIOTECHNOLOGICAL APPLICATIONS**

### **Abstract:**

*The macroalgal biodiversity of Tunisian coasts, with more than 400 species reported, represent an important potential for biotechnological uses. TunAlgoBase is a database aiming to list macroalgae found on Tunisian coasts and store at the same place different information on species such as taxonomy, biology, ecology, geographical distribution and uses. In addition to the basic taxonomic data (name, synonymy, classification), morphological description, distribution records and pictures, the database contains natural compounds information that can be extracted from seaweeds including chemical structures, formulae, molecular weight, biological activity if occurs,... All of these items can be searched for, either individually or in various combinations. TunAlgoBase has a relational database design build with MsAccess Software. Structural model and different user interfaces are presented.*

**Key-words:** Macroalgae, data scheme, relational database, biodiversity, natural products.

### **Introduction:**

During the last decade, with the increase of data sets, the interest to represent taxonomic data and related information in numerical databases has increased. In marine community the concept of Ocean Biodiversity Informatics (OBI) has been developed and concerns the use of computer technologies to manage marine biodiversity information, including data capture, storage, search, retrieval, visualisation, mapping, modelling analysis and publications (Costello and Vanden Berghe, 2006). Several databases have been established to represent biodiversity in general (ITIS ([www.itis.gov](http://www.itis.gov)), Species 2000 ([www.sp2000.org](http://www.sp2000.org)),...) and in particular to commercially important groups (Fishbase ([www.fishbase.org](http://www.fishbase.org)), Cephbase ([www.cephbase.utmb.edu](http://www.cephbase.utmb.edu)), ..).

TunAlgoBase has been initiated in 2004 (Ktari and Langar, 2004) aiming to establish a computerized database storing in the same structure different information on Tunisian seaweeds making these data, once available on the internet, easily retrieved and used.

This paper summarise the current state of TunAlgoBase data structures, available datasets and some development perspectives.

### **Material and methods**

*Principles used in database construction: overview*

In TunAlgoBase all data are stored in a relational database built on Microsoft Access software. All information is stored only once to avoid redundancy and data sets (literature, taxonomy, geography,...) are linked to each other via unique serial numbers. An overview of the database structure is displayed in Figure 1. In addition, each taxa is related to seaweed natural compounds information (when available). This data set has been structured following Lei and Zhou (2002) and contains information on the compounds origins, detailed chemical structures, isolation techniques and biological

properties. The user interface makes use of JSP web technologies (Java Server Pages) combining all the separate datasets in a friendly user format.

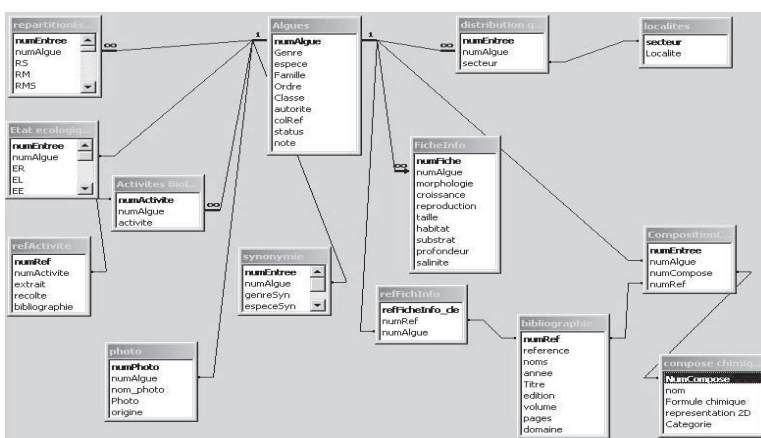


Fig. 1: Relational database diagram of the TunAlgoBase System

## Results

TunAlgoBase currently includes data on about 412 Latin names of macroalgae and 700 synonyms, 1700 distributional records, 200 biological descriptions, 120 bibliographic references, 150 images and 75 natural compounds descriptions.

Currently accepted names and synonyms referring to Algaebase ([www.algaebase.org](http://www.algaebase.org)) are about 80% completed. The most serious lack of coverage, in the database, is biological descriptions due to scarce and non available documentation. Natural products data are still in reduced amount but constitute a large task that is in continual enhancement considering the important quantity of natural substances isolated from seaweed.

## Discussion and Conclusion

As described, TunAlgoBase constitute an important amount of data set on Tunisian seaweed that can be queried at different levels (simple or combined queries). Marine natural products information opens new perspectives for seaweeds use. The generic structure of the database enables adding possible future data types (genetic information, aquaculture strategies,...). In the beginning TunAlgoBase has been available at INSTM local network, now being improved it is planned to be accessible on the internet by the end of 2010.

## Acknowledgements

Authors want to thank Miss Cheima Mdallel, technician, for the important work in data capture.

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## **ALLELOPATHIC EFFECTS OF *ZOSTERA* SPP. ON THE GROWTH AND PHOTOSYNTHETIC ACTIVITY OF THE TOXIC DINOFLAGELLATE *ALEXANDRIUM CATENELLA***

### **Abstract**

*The ability of Zostera spp. to produce allelochemicals have been tested in laboratory using bioassays. Aqueous or methanol extracts from leaves of Zostera marina and Z. noltii were assayed to determine their allelopathic effects on Alexandrium catenella, a dinoflagellate blooming worldwide and responsible of paralytic shellfish intoxications. Both growth and photosynthetic activity of the microalgae were significantly inhibited by all the extracts even at low concentrations. Alexandrium cells showed severe deformities and lysis in acute cases. A long-term inhibition was observed without the need of continuous addition of extracts, suggesting that the allelochemicals produced by Zostera are stable in the medium. These results support the hypothesis that Zostera spp. have potent allelopathic activity against Alexandrium.*

**Key-words:** *Alexandrium*, allelopathy, *Zostera*, growth, photosynthesis

### **Introduction**

Aquatic macrophytes have long been suspected of suppressing phytoplankton growth through the production and excretion of chemical substances, and there is now strong evidences that allelopathic interferences exist in freshwater ecosystems (Gross *et al.*, 2007). Water-soluble extracts of the marine macrophyte *Z. marina* have been shown to inhibit the growth of micro-algae and marine bacteria and control microbial growth and amphipod grazing (Harrison & Chan, 1980). *Z. marina* and *Z. noltii* are important seagrasses occurring along European and north African coasts. They produce bioactive phenolics among which zosteric acid, rosmarinic acid and flavonoids (Achamlale *et al.*, and ref. therein, 2009a,b). *Alexandrium catenella* is a widespread PSP toxin-producing dinoflagellate species. Since 1998, recurrent *A. catenella* blooms have been observed in the Thau lagoon (French Mediterranean coast), leading to the closing of shellfish farms (Collos *et al.*, 2007), but never in Arcachon lagoon (French Atlantic coast). Analyses of REPHY and REBENT monitoring network database showed a low occurrence of *Alexandrium* blooms in the vicinity of extensive *Zostera* beds. This led us to investigate the non-nutrient relationship between the *Zostera species* and *A. catenella*.

### **Materials and Methods**

Methanolic and aqueous extracts were prepared with *Zostera leaves* from the Bay of Arcachon and the Thau lagoon. They were analyzed for both the identity and quantity of phenolics present, and tested on the growth of *A. catenella* in 6-well sterile plates (72h). The nutritive medium was brought entirely at T0 time. *A. catenella* was added to each well (inoculation concentration of 800-1000 cells ml<sup>-1</sup>) and the growth was monitored by direct microscopic counts of cells. The effect of the crude extracts on the rate of photosynthesis in *A. catenella* was determined by sensitive fluorimetric measurement of chlorophyll fluorescence of photosystem II (PS II) using Phyto-Pam.

## Results and discussion

Extracts obtained from detrital and fresh leaves of *Z. marina* and *Z. noltii* strongly inhibited the growth of *A. catenella* at very low concentration whatever the extracts (Tab. 1). *Alexandrium* cells showed severe morphological anomalies when exposed to the extracts. Significant reduction of their photosynthetic activity was systematically observed after 4h of exposure, which became more marked as time went on. Allelochemicals are known to inhibit growth, PSII and cellular mortality (Hagmann & Jüttner, 1996; Kearns & Hunter, 2000). The significant amounts of phenolics contained in the extracts could be responsible of the inhibition effects observed. This is the first report of the allelopathic potential of *Zostera* species against a dinoflagellate. Additional experiments will be dedicated to test the effect of continuous release of allelochemicals from the fresh seagrass tissues on the growth and the physiological activity of *A. catenella*. Considering the deleterious impacts of HAB on public health and economic resources, the allelochemicals from *Zostera* detritus may be considered as potential candidates to mitigate the effects of *Alexandrium* blooms on aquaculture.

**Tab. 1: Inhibitory effects of *Zostera* spp. on the growth of *A. catenella* : IC50 values**

Species	Plant material	Extraction Method	Extract code*	IC50 (mg/L)
<i>Z. noltii</i>	detrital leaves	Water, rt	Zn A-D-W	199.7
		MeOH reflux	Zn A-D-M	79.8
	Fresh leaves	MeOH reflux	Zn A-F-M	36.1
		MeOH reflux	Zn Th-F-M	39.6
	detrital leaves	MeOH reflux then purification	Zn A-D-MS	60
			Zn A-D-MRA	16.8
<i>Z. marina</i>	detrital leaves	Water, rt	Zm A-D-W	239.5
		MeOH reflux	Zm A-D-M	92.2
	Fresh leaves	MeOH reflux	Zm A-F-M	45
		MeOH reflux	Zm Th-F-M	36

## Acknowledgments

This work was supported by the SUDOE program (ECO-LAGUNES project).

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## USE OF *POSIDONIA OCEANICA* AS BIOINTEGRATOR OF POLYCYCLIC AROMATIC HYDROCARBONS

### Abstract

*Terrestrial plants are used in standard process to evaluate environmental contamination. In coastal environment seagrasses, presenting a worldwide distribution could play a similar role. The aim of this study is to characterize the accumulation of an organic pollutant: Polycyclic Aromatic Hydrocarbons (PAHs) by Posidonia oceanica.*

*Results show that, (i) PAHs concentrations are higher in blades than in sheaths, (ii) foliar tissues report seasonal variations of PAHs, with maximum values in winter and minimum in summer, linked to run-off and foliar growth rate, (iii) impacted sites exhibit higher concentration than pristine and (iv) Posidonia oceanica exhibit a similar trend than Mytilus galloprovincialis in relation with the vicinity of contamination source. Thus, this preliminary study points out the potential use of Posidonia oceanica as indicator of aquatic PAH contamination.*

**Key-words:** PAHs; seagrass; contamination, Mediterranean

### Introduction

Among the organic pollutants, Polycyclic Aromatic Hydrocarbons (PAHs) are known to be persistent and some carcinogenic and/or mutagenic. They are listed as priority substances of the European Union Water Framework Directive (WFD) and as priority pollutant by several international organizations (e.g. US Environmental Protection Agency, Barcelona Convention). In marine environment, the main organisms investigated as PAH indicators are molluscs (Baumard *et al.*, 1999). Nevertheless, seagrasses are increasingly used as indicators of chemical contamination due to their worldwide distribution in coastal environments and their bioaccumulation properties (Lafabrie *et al.*, 2007). Following the strategy carried out for terrestrial plants, the aim of this study is to characterize the accumulation of PAHs by the seagrass *Posidonia oceanica* and more precisely the concentration according to the different organs, the season, the distance to potential anthropogenic sources and to compare these results to those measured in an other bioindicator (*Mytilus galloprovincialis*).

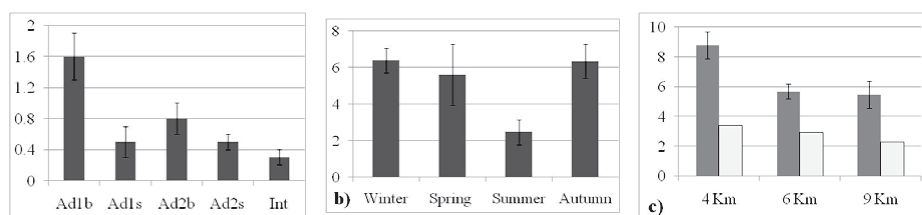
### Materials and methods

*P. oceanica* foliar shoots were collected closed to Toulon harbor (France). In order to determine the impact of studied organ, it was divided in blade (b) and sheets (s) of the two oldest adults (Ad1 and Ad2) and intermediate leaves (Int). In this site, Ad1b was also study according to season. Impact of the distance to the potential source (Livorno harbour) was analysed on foliar shoots. Samples were lyophilized and reduced to powder. PAHs were extracted by ASE and analysed by HPLC-Fluorescence.

### Results

Between the four organs of *P. oceanica* considered, PAHs were preferentially accumulated in blades and more especially in blade of the oldest leaves (Fig. 1a), while PAHs present minimum values in summer and a maximum in winter/autumn (Fig. 1b). Maximum values were recorded in the vicinity of contamination sources (harbour,

industrial complex) and concentrations present a negative correlation with distance (Fig. 1c).



**Fig. 1:** a) Anthracene in the different part of the foliar shoot, Ad : Adult leaves; Int : Intermediate leaves; b : blade; s : sheath); b) Seasonal evolution of Acenaphthene; c) Pyrene evolution according to distance to Livorno harbor in *Posidonia oceanica* (grey) and *Mytilus galloprovincialis* (white).

## Discussion

Season evolution of PAHs concentration in *P. oceanica* leaves are in agreement with mussels (Baumard *et al.*, 1999) and are related to leaves surface, inputs and run-off variations.

The values recorded according to the distance to a potential contamination source (Livorno harbour) exhibit higher concentration in the vicinity of the source and a negative correlation to the distance in *Posidonia oceanica* and in *Mytilus galloprovincialis*. In the same way higher values of PAHs (Medium and High Molecular weight) recorded in Toulon (industrial harbour) in comparison with Calvi (pristine site) confirm a previous study using mussels (Andral *et al.*, 2004).

On a general point of view, the number of PAHs detected and the values recorded appeared in same order, even higher, that for *Mytilus galloprovincialis* considered as a validate biomonitor of PAHs contamination (Andral *et al.*, 2004; Baumard *et al.*, 1999; Stella *et al.*, 2002).

## Acknowledgments

This study was supported by a grant from the "Collectivité Territoriale de Corse" and the INTERREG IIIA/MONIQUE and GdR Programs (University of Corsica/Ifremer).

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## **CARACTERISATION ET SURVEILLANCE DE L'HERBIER A *POSIDONIA OCEANICA* DE L'ÎLOT DE BOUNETTAH (REGHAÏA, ALGERIE)**

### **Résumé :**

*A partir de juin 2006, l'APPL (Agence pour la Protection et la Promotion du Littoral de la wilaya d'Alger), a lancé, les travaux de mise en place d'un réseau de surveillance des herbiers à Posidonia oceanica (magnoliophyte), des habitats remarquables et des espèces invasives dans la wilaya d'Alger. Après plusieurs prospections, le site de l'îlot de Bounettah a été choisi pour l'amorce des travaux de surveillance. Afin de décrire, évaluer et surveiller l'état de santé de l'herbier, plusieurs plongées de reconnaissance y ont été effectuées. En 2007 un balisage de la limite inférieure y a été installé et les travaux suivants réalisés : Cartographie de la limite le long du balisage, mesure des paramètres de vitalité (recouvrement et densité) détermination préliminaire de la faune et la flore qui lui sont associées. Un premier suivi vient d'être réalisé en août 2010. Les résultats indiquent une limite stable, un taux de recouvrement élevé, une baisse de la densité et l'apparition de Caulerpa racemosa (Chlorophyte).*

**Mots clés :** *Posidonia oceanica*, balisage, vitalité, faune et flore.

### **Introduction :**

En 2007 Dans le cadre du projet AMIS-SMAPIII et en collaboration avec le CIRSA (Centre Interdépartemental de Recherche pour les Sciences de l'Environnement), l'APPL (Agence pour la Protection et la Promotion du Littoral de la wilaya d'Alger) installe un balisage au niveau de la limite inférieure de l'herbier à *Posidonia oceanica* (Linnaeus) Delile de l'îlot de Bounettah à Reghaia. Depuis la fin du projet, l'agence a organisé annuellement des missions pour l'entretien des balises et un premier suivi vient d'être réalisé (Lamouti, 2010). Nous présenterons dans ce travail les résultats déjà obtenus et les perspectives de l'APPL.

### **Matériel et méthodes :**

Îlot de Bounettah, se trouve dans la baie de Zemmouri, à l'Est d'Alger et se situe à environ 1 km du rivage de la plage de Reghaia. Il se présente sous forme d'un ensemble d'affleurements rocheux dont la partie principale s'étend sur environ 600m de long et est orientée sur un axe Nord-Est/Sud-Ouest. Nous avons choisie d'installer le balisage, matérialisé par 15 balises le long de 75m entre 12,3 et 13 m de profondeur même si quelques touffes isolées peuvent atteindre 18 m de profondeur, car cette portion de limite inférieure est continue et présente un sédiment facilitant l'enfouissement des balises et piquets. Entre juillet et août 2007 les travaux suivants ont été réalisés : Cartographie de cette portion de la limite inférieure, mesure des paramètres de vitalité (recouvrement et densité) et détermination préliminaire de la faune et la flore qui lui sont associées. Depuis les balises ont été annuellement entretenues et un suivi vient d'être réalisé (août 2010). Lors de cette dernière mission en plus des paramètres mesurés lors de la première, le déchaussement des rhizomes a été estimé et des prélèvements ont été réalisés en vue de prochaines analyses phénologique et

lepidochronologique. Pour chaque paramètre nous avons calculé la moyenne des mesures, l'écart type et le coefficient de variation (CV). Pour l'interprétation des résultats de densités et de recouvrements nous avons utilisé la grille d'évaluation établie selon le protocole validé par le programme MedPosidonia du CAR /ASP (PNUE-PAM-CAR/ASP, 2009) et pour le déchaussement celle du Réseau de Surveillance Posidonie (Charbonnelle *et al.*, 2000)

### Résultats :

Les fonds sont très accidentés avec plusieurs affleurements rocheux et l'herbier est installé sur fond meuble et substrat dur. La pente est relativement importante avec une valeur moyenne de 7 à 8°.

La moyenne des densités, mesurées en aout 2007, est de 379 (CV=38,11%) faisceaux/m<sup>2</sup> ; elle indique que l'herbier présentait une densité « normale », trois ans plus tard les valeurs sont plus faibles avec une moyenne de 291 faisceaux/m<sup>2</sup> (CV=36,4%) indiquant une densité « médiocre ». Le taux de recouvrement, traduit une « très bonne » vitalité, avec un taux moyen de 84% (CV=26,5%) en 2007 et 87% (CV=22,6%) en 2010. Le déchaussement est faible avec une moyenne de 1,4cm (CV=196,5%).

Parmi les espèces rencontrées le long du balisage, depuis 2007, nous avons noté la présence d'au moins trois *Pinna nobilis* (Linnaeus) et de nombreux organismes filtreurs. L'élément nouveau est l'observation, en novembre 2009, d'une première colonie de l'espèce introduite à caractère invasif *Caulerpa racemosa var. cylindracea* (Sonder) Verlaque, Huisman et Boudouresque, en 2010 plusieurs colonies sont présentes le long de la limite.

### Conclusion

L'excellent taux de recouvrement, le bon équilibre sédimentaire et la stabilité de la limite, indiquent un bon état général de l'herbier, cependant la baisse de la densité pourrait traduire une vulnérabilité. La progression de *Caulerpa racemosa* devrait être surveillée surtout que le succès de l'invasion de cette espèce est lié à la baisse de la densité de *Posidonia oceanica* et à la présence de sable (Ceccherelli *et al.*, 2000).

Les futures études phénologiques et lepidochronologique, ainsi que le prochain suivi envisagé par l'APPL devraient apporter plus d'informations.

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**SYSTEME D'INFORMATION GEOGRAPHIQUE DE LA  
VEGETATION MARINE ET COTIERE EN ALGERIE**

**Résumé**

*La stratégie nationale de protection et de conservation des ressources naturelles initiée par le Ministère de l'Aménagement du Territoire et de l'Environnement réserve une part importante au volet amélioration des connaissances et renforcement des capacités d'expertises dans le domaine de la biodiversité en général et de la végétation marine et côtière en particulier.*

*L'étude réalisée par Grimes et al. (2004) et complétée par Grimes (2006), a mis en évidence la présence d'un cortège conséquent d'espèces "à statut" et pour certaines d'entre elles "symbolique" de la méditerranée. Il y a lieu de citer à cet effet quelques espèces végétales telles que Cystoceira amentacea, Laminaria, Goniolithon byssoides, Corallina elongata et Lithophyllum lichenoides. De même, des herbiers splendides tapissent les fonds côtiers notamment les prairies à Posidonia oceanica.*

*Un système d'information géographique (SIG) relatif à la végétation marine en Algérie a été initié et financé par le Ministère de l'Aménagement du Territoire et de l'Environnement (MATE).*

**Mots clés:** système d'information géographique, cartographie, végétation marine, base de données

**Introduction**

Le système d'information géographique est une démarche de gestion territoriale et sectorielle qui repose sur la manipulation, la mémorisation, l'affichage et la production des informations.

L'objectif attendu du SIG de la végétation marine est en effet, celui de maîtriser la dynamique des espèces et des habitats et les transformations qui résultent de leur usage ainsi que des pressions qui sont exercées sur les espèces et leurs milieux.

**Matériel et méthodes**

La construction du SIG et de la base de données associée a requis des investigations très poussées avec une analyse et une investigation systématiques d'une série de documents, de cartes et d'enquêtes de terrain:

- Ouvrages, Thèse d'état, Thèses de Magister, Articles scientifiques, Atlas, Rapports techniques, Documents (PNUD, PNUE, PAM, CAR ASP, Banque Mondiale, MedWet Coast, MedMPA), Rapports techniques (du MATE, de la DGF, des Parcs nationaux côtiers (Gouraya, El Kala)), Rapports de campagnes d'évaluation des ressources halieutiques, Enquêtes sur le terrain (clubs de plongés, plongeurs amateurs, plongeurs professionnels).
- Les cartes de base utilisées sont soit : des cartes topographiques, cartes marines, et dans certains cas des images satellites ou des photos aériennes ;
- Une gamme de logiciels telle que : Arcview 9.0 (SIG bureautique), Erdas 8.5 (SIG raster), SGBD (Access) et Excel (tableur).

Le SIG est organisé en jeux de classes d'entités (groupe de couches homogènes) où chaque groupe de couches est composé de plusieurs classes d'entités (couche), chaque couche est caractérisée par des attributs, un primitif cartographique et une échelle d'apparition.

La cartographie de la répartition des espèces a été réalisée selon des échelles individualisées et cela en fonction de plusieurs paramètres :

- l'étendue géographique naturelle de la distribution de l'espèce ;
- les secteurs ayant fait l'objet d'investigations terrain et d'études ;
- l'information disponible au moment de la construction du SIG.

### **Résultats et discussion**

L'efficacité du SIG est tributaire de la conception méthodologique, il a été procédé à une réflexion sur la nature des informations et leur traitement par rapport aux objectifs qui sont ceux de :

- Constituer une base de données sur des thématiques liées à la cartographie des milieux marins et l'organiser sous forme de couches et groupes de couches connectés à des tables attributaires ;
- Requête et croiser les informations et les couches cartographiques pour exécuter des analyses multicritères ;
- Préparer des plans d'intervention et de protection des espèces marines en cas de risques majeurs (pollution) ;
- Programmer des aménagements divers en fonction de l'état des lieux visualisé sur le SIG.

En outre cette base dynamique de données a permis de :

- Mettre à la disposition de la communauté scientifique un instrument d'orientation pour les investigations scientifiques.
- La mise à la disposition des décideurs et donc des gestionnaires et des planificateurs un instrument d'aide à la prise de décision concernant la mise en place et la planification des aires marines et côtières protégées, les études d'impacts pour les installations industrielles et les aménagements en zone côtière.

### **Conclusion**

Pour la gestion du SIG de la végétation marine et de sa base de données, il a été cependant recommandé d'élargir le cercle des pourvoyeurs des données relatives à la diversité biologique marine et côtière et de les diversifier.

Car il est évident que la cartographie présentée n'est pas exhaustive et ne reflète pas la distribution réelle mais plutôt le niveau de connaissance actuel.

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## **PRESENTATION D'UN SYSTEME INNOVANT DE RESTAURATION D'HERBIER**

### **Résumé**

*Pour améliorer l'efficacité d'une opération de restauration d'herbier marin dans le cadre de renforcement de populations de phanérogames, nous avons considéré qu'il était pertinent de :*

*1- Faciliter le développement rhizomique des boutures*

*2- Réduire l'hydrodynamisme au droit de la transplantation*

*Aussi les opérations de restauration s'accompagnent :*

*1-Dans un premier temps de bouturage dans une « galette » d'engrais racinaire bio*

*2- Dans un second temps*

*a) D'immersion d'une structure anti-affouillement apparentée à un « herbier artificiel », si possible sur un ouvrage à protéger (ex: câble sous-marin)*

*b) Cette structure réduit localement le courant marin et se comble en partie, produisant un dôme de sable dense sur lequel les galettes végétales précitées sont fixées*

**Mots-clés :** Restauration, développement rhizomique, hydrodynamisme, anti-affouillement

### **Introduction**

En matière de restauration de phanérogames de nombreux essais de renforcement de populations locales ont été expérimentés en Méditerranée. Il apparaît qu'une part des échecs soit imputable à la faible tenue des boutures dans leur nouveau milieu. Nous avons donc concentré nos efforts sur l'amélioration de la croissance des racines lignifiées durant les premiers stades de la transplantation ainsi que sur la protection physique des rhizomes implantés.

### **Matériel et méthodes**

Sur un site où l'espèce est présente et pour laquelle la cause d'altération du milieu a disparu : immersion de boutures sur les structures décrites. Comparaison de transplantations (répliquats) avec celles effectuées directement dans le sédiment, par ailleurs comparatif avec des sites témoins d'herbier existant. Suivi de paramètres (nombre de faisceaux...).

### Résultats attendus

L'herbier artificiel est composé d'une natte de 25m<sup>2</sup>/u et de « feuilles » de polypropylène de 1,20m de haut, une fois immergé, déroulé, fixé, l'ensemble est comblé par sédimentation accélérée à hauteur de 1,05 m en 30 jours (Fig. 1)

Croissance accrue des racines lignifiées des rhizomes plagiotropes et amélioration du taux de survie (Fig. 2) (ex : Pour *Posidonia oceanica* obtenir un taux pour des boutures plagiotropes >76% de survie en moyenne, pour les boutures orthotropes > 60% de survie).

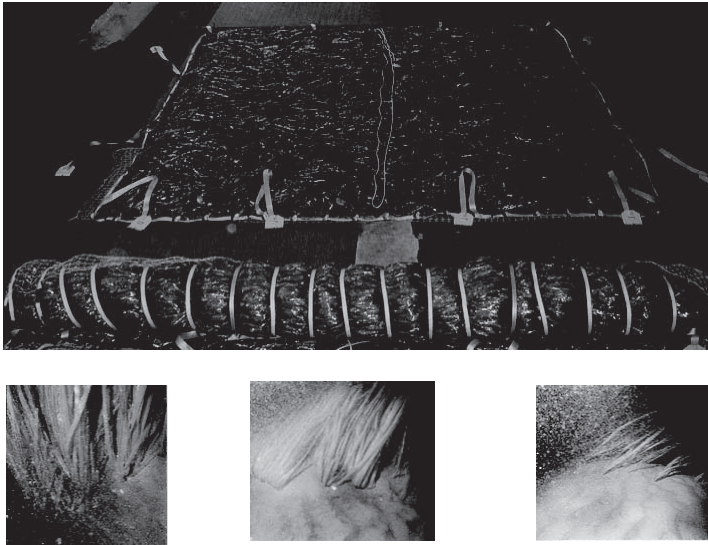


Fig.1: Photographie du comblement de l' « herbier artificiel », respectivement au 1<sup>er</sup>, 10<sup>e</sup> et 30<sup>e</sup> jour (hauteur apparente des « feuilles » ramenée à 15 cm).

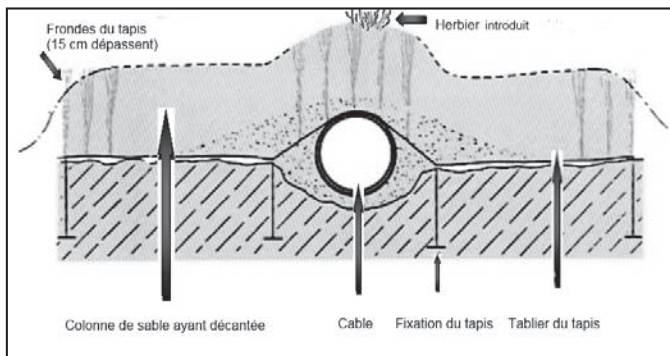


Fig.2 . Schéma coupe du système innovant

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## **CONTRASTING TWO METHODS FOR COVERAGE ESTIMATIONS IN *POSIDONIA OCEANICA* (L) DELILE: LINE INTERCEPT AND GRID**

### **Abstract**

*Considering the environmental and socio-economic value of Mediterranean seagrass beds, different aspects such as phenology, physiology or associated fauna have been studied. In the case of *Posidonia oceanica*, one of the main parameters to be measured is the coverage (proportion of seabed covered in relation to other substrata within the seagrass bed). A comparative analysis between two methods of measurement of coverage has been carried out in *Posidonia oceanica* meadows from the MPA “Paraje Natural de Acantilados de Maro-Cerro Gordo” (Málaga-Granada, southern Spain), in 3 different sampling sites and at 3 different depths (7, 9 and 11 m) within each site. Coverage measurements (n=3 by site and depth) were carried out using two methods: (a) “Line intercept” (Li) that consists of horizontal and linear measurements of plant intercepts along a transect, and (b) “Grid”(G) consisting of measurements of areas, with a transparent grid plate and diving at a constant distance from the sediment. Coverage values ranged between 1.3 to 49.3%, with a general decrease of values with depth. This trend was found similar using both methods, but in most cases Li method resulted in larger values than those obtained with Grid method. Nevertheless, these differences were not significant (U Mann-Whitney;  $Z = -0.235$ ;  $p > 0.05$ ). Difference between methods were related to mean coverage and to depth, but these values were not significant (for depth in all cases  $Z > -0.323$ ;  $p > 0.05$ ; for low vs high coverage  $Z = -0.177$ ;  $p > 0.05$ ). The use of these methods shows a similar trend, but the Li method has a lower range of values than the Grid method. The latter seems to be better than Li method for measurements of large areas with less sampling effort.*

**Key-words:** Seagrass; Coverage; Line intercept; Grid; Alboran Sea.

### **Introduction**

The environmental importance of seagrass beds is well known, mainly for those of *Posidonia oceanica* in the Mediterranean Sea that are used as a biological indicator of the quality of coastal environments as well as for evaluating the efficiency of management policies (Bonhomme *et al.*, 2010). The monitoring, therefore, is necessary for assessment of their health status/condition. Among all the monitored seagrass parameters, coverage estimation is probably the most relevant to the configuration of the meadow (continuous, fragmented), and its trend over time (progressive, regressive or stable) in successive controls. Thus, choosing the most suitable method for measuring the coverage becomes important, particularly if we take into account that the depth, may affect these measurements. For all the above reasons, it is desirable to contrast different methods for coverage measurements and to identify the most reliable one with the lowest sampling effort.

## Materials and methods

Two sampling methods, Line intercept (Li) and Grid (G), were compared in a *Posidonia oceanica* bed (7-11 m depth) located within the MPA of Acantilados de Maro- Cerro Gordo (southern Spain). The study was carried out in three sampling sites at three different depths (7, 9 and 11 m). Li method consist in horizontal, linear measurements of meadows intercepts along a 20 m transect, while G method considers a manageable area of 4 m<sup>2</sup> for quantifying the coverage, with a transparent grid plate and diving at a constant distance from the sediment (~3 m).

## Results

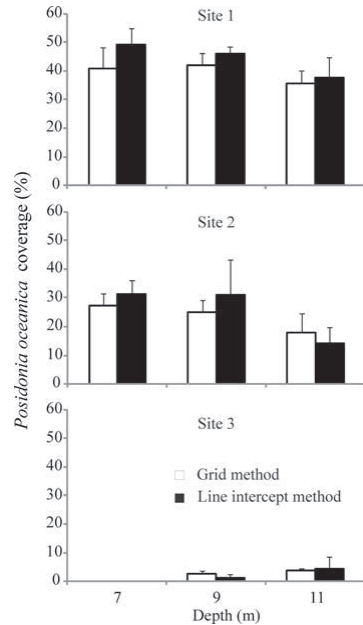
The coverage values ranged from 1.3 to 49.3%, with a general decrease with depth (Fig. 1). This trend was similar with both methods, but in many samples Li method resulted in larger values than those obtained with G. Nevertheless, these differences were not significant (U Mann-Whitney;  $Z = -0.235$ ;  $p > 0.05$ ). Differences between methods were related to mean coverage and to depth, but these values were also not significant (for depth in all cases  $Z > -0.323$ ;  $p > 0.05$ ; for low versus high coverage  $Z = -0.177$ ;  $p > 0.05$ ). The use of these methods shows a similar trend, but the Li method has a higher variability of values than the G method. Differences between methods were minimal at low coverage values that generally occur in deep meadows (9-11 m).

## Discussion and conclusions

Both methods are valid for estimating coverage at different depths and resulted in very similar values as found in studies for measuring coverage in terrestrial plants (Hanley, 1978). Percentage differences between values were never higher than 10%, being minimal at low coverage values. This could be due to the low difference of maximum possible values obtained with each method at low coverage beds, when compared to intermediate ones. We consider, however, as the best choice the Grid method because it requires a lower sampling effort.

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**Fig. 1:** Coverage of *Posidonia oceanica* obtained with two different methods at three depths in southern Spain (Alboran Sea).

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## **RESISTANCE OF *POSIDONIA OCEANICA* MEADOWS TO THE INVASION OF THE INTRODUCED *CAULERPA RACEMOSA*: THE ROLE OF LIGHT**

### **Abstract**

We tested the hypothesis that light availability within undisturbed *P. oceanica* meadows is a critical factor that limits the invasion by the introduced alga *C. racemosa*. To this end, we studied light climates and *C. racemosa* photosynthesis and morphology outside and inside the leaf canopies of three *P. oceanica* meadows located at different depths under winter and summer conditions. The results indicated that light experiences a severe reduction inside the seagrass canopies and in response, *C. racemosa* significantly reduces the saturation and compensation points for photosynthesis. Even with the showed photoacclimative adjustments, the algae penetrating the meadows in summer photosynthesised at saturation rates for less than 4 hours (28–36% of the time estimated outside the meadows). In winter, *C. racemosa* is not able to achieve maximum photosynthetic rates during the day beneath the seagrass canopy, and as a consequence, the metabolic carbon balance was negative, unlike the positive balances obtained in summer. Positive carbon balances found in summer inside the seagrass canopy are likely due to the notable reduction in respiration rates (up to 89%), which suggests a significant slowdown of the metabolism (and growth) of the algae penetrating the meadows. These results may explain the very low algal biomass found within the meadows, which was 77 to 99% less than that measured just outside. In conclusion, our results provide evidence that light reduction within *P. oceanica* meadows plays a key role in the high resistance to invasion of *C. racemosa* reported for undisturbed meadows.

**Key-words:** *Posidonia oceanica*, *Caulerpa racemosa*, photoacclimation, resistance to invasion.

### **Introduction**

The invasive alga *Caulerpa racemosa* var. *cylindracea* has spread throughout the Mediterranean Sea, colonising a wide variety of native substrate communities at depths of up to 60 m (Klein & Verlaque, 2008). The distribution of the invasive alga overlaps with that of the endemic Mediterranean seagrass *Posidonia oceanica*. However, little is currently known about the interaction between these species. Existing evidence shows that undisturbed dense meadows are more resistant to colonisation by the alga than other heavily-invaded benthic communities. These differences in susceptibility to invasion seem to be associated with the structural complexity of the different communities, but very little is known about the specific factors involved. In this study, we test the hypothesis that light availability within *P. oceanica* meadows is a critical factor that limits the invasion of this important Mediterranean habitat.

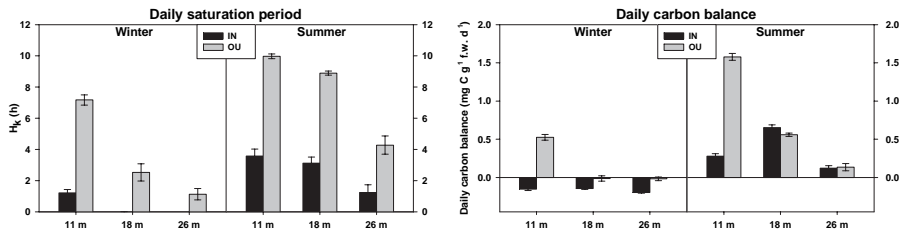
### **Materials and methods**

We studied the light climates (daily irradiance curves) and *C. racemosa* photosynthesis (P vs. E curves) and morphology (frond height and biomass) both outside (OUT) and inside (IN) the leaf canopies of three *P. oceanica* meadows located at different depths (11 m, 18 m and 26 m) and in different seasons (winter and summer). Using the

estimated photosynthetic parameters and continuous light recordings, we estimated the mean daily compensation ( $H_c$ ) and saturation ( $H_k$ ) periods and the daily carbon balances of *C. racemosa* fronds.

## Results

Our results indicate that less than 5% of subsurface irradiance reaches the basal parts of the three studied meadows in both seasons. In response to such a severe reduction in light, *C. racemosa* showed a significant increase in frond length and in chlorophyll content in order to increase light absorption efficiency. In addition, *C. racemosa* significantly reduced the saturation ( $I_k$ ) and compensation ( $I_c$ ) points for photosynthesis, lengthening  $H_k$  and  $H_c$ . Even with these photoacclimation adjustments, the algae growing inside the leaf canopy photosynthesised at saturation rates for a period less than  $H_k=4$  hours per day in the summer (Fig. 1), which represented only 28-36% of the mean  $H_k$  value obtained for the algae growing outside the leaf canopy in this season. In winter, this alga is not able to achieve maximum photosynthetic rates during the day at all ( $H_k=0$  h; Fig. 1). Consequently, during the winter season the metabolic carbon balance of *C. racemosa* penetrating the meadows was negative, unlike the positive balances obtained in summer (Fig. 1). These positive carbon balances are possible primarily because of a notable reduction in respiration rates (up to 89%), which suggest a significant slowing down of metabolism (and growth) of the algae growing inside the leaf canopies of the meadows studied. These results may explain the very low algal biomass found within the leaf canopy, which was 77% to 99% lower than that measured in the densely colonised stands just outside the seagrass leaf canopy.



**Fig 1: Mean ( $\pm$ SE) daily saturation period ( $H_k$ ) and daily carbon balance of *C. racemosa* populations growing inside (IN) and outside (OU) the three studied meadows located at different depths (11 m, 18 m and 26 m) in summer and winter.**

## Conclusions

Our results provide substantial evidence that the resistance of undisturbed *P. oceanica* meadows to *C. racemosa* invasion could be largely attributed to the extreme light reduction that occurs within the seagrass leaf canopy. Thus, despite the high photoacclimation capacity of *C. racemosa*, the low light levels measured inside dense seagrass *P. oceanica* meadows are limiting for the development of the invasive alga.

## Acknowledgments

Servicio de Pesca y Acuicultura de la Consejería de Agricultura y Agua de la Comunidad Autónoma de Murcia.

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## CRUSTACEAN DECAPOD ASSEMBLAGES ASSOCIATED WITH *POSIDONIA OCEANICA* FRAGMENTED MEADOWS IN WESTERN ALBORAN SEA

### Abstract

The crustacean decapod assemblages associated with *Posidonia oceanica* meadows were studied in two stations, located in a protected area in Southern Spain (Málaga, Alboran Sea), close to the Strait of Gibraltar. This area represents the westernmost geographical limit for this seagrass, so it was interesting to compare their faunistic assemblages with those of other similar beds from the Mediterranean Sea. From July 2007 to June 2008 a total of 34 species were captured. The most dominant species were those associated with the rhizome stratum, such as *Pisidia longimana* (Dominance ~18%), *Pilumnus hirtellus* (~15%), *Athanas nitescens* (~14%), *Calcinus tubularis* and *Cestopagurus timidus* (~7); although differences in the dominance values between both zones were found. In general, the dominant species are similar to those mentioned in other Mediterranean meadows of *Posidonia* but with differences in abundance and with the presence of some unusual species. The assemblage displays seasonal changes ( $R_{ANOSIM}=0.304$ ;  $p=0.001$ ), with maxima abundances in autumn (in Punta de Calaburras) or summer-autumn (in Calahonda). Species richness was also maximal in autumn in both zones. Diversity ( $H'$ ) displayed also a similar trend with maximal values (~2 bits) in autumn. These trends are related to some biometric features of the seagrass as well as the depth. At this biogeographical distribution limit, with strong Atlantic influence, *P. oceanica* are structured in patches of unequal sizes, but it still preserves similar components of its associated decapod fauna.

**Key-words:** *Posidonia oceanica*, community, decapoda, Alboran Sea.

### Introduction

In the Mediterranean there are four seagrasses able to form extensive beds: *Posidonia oceanica*, *Cymodocea nodosa*, *Zostera marina* and *Zostera noltii*. Among these, *Posidonia oceanica* is the most abundant and forms the most extensive meadows. This seagrass presents two well defined strata, the leaves and the rhizomes, that support different biotic assemblages (Kikuchi, 1980) and high biodiversity. The aim of this study is to analyse the composition and temporal variations of the decapod assemblage in the westernmost meadow of *P. oceanica*, as well as their relationships with the life cycle and biometric features of the plant and with some environmental variables.

### Material and methods

The studied area is located in Málaga, southern Spain, in a marine Site of Community Importance (SCI) known as "Calahonda" (code ES6170030), included in the Natura 2000 Network. Two sampling stations were selected, Calahonda (36°29'21''N-04°41'55''W) and P. de Calaburras (36°30'23'' N - 04°38'41''W).

Samples were taken seasonally, from July 2007 to May 2008, at 2-3 m depth. Samples were collected with a manual airlift pump in quadrats of 50x50 cm (5 replicates) and all decapods were identified and counted. This same quadrat was used for estimating shoot density (shoot/m<sup>2</sup>). Within each quadrat, ten randomly-selected shoots were analysed in situ for counting number of leaves per shoot and measuring length and width of leaves. Statistical and PRIMER softwares were used to analyse the relationship and structure of the community.

## Results

*P. oceanica* displays top values of shoot density in autumn (1055.20±574.0 shoot / m<sup>2</sup>) for P. de Calaburras and in winter (906.50±25.03 shoot / m<sup>2</sup>) for Calahonda. In P. de Calaburras, the number and width of leaves show maximum values in winter (6.12 ±0.10 leaves per shoot / 1.028±0.01cm respectively) and the shoot height in spring (39.38±1.12cm). In Calahonda, the number of leaves is maximum in winter (6.14±0.10), while the maximal shoot height and width are in spring (38.72 ±1.18cm / 1.028±0.01cm). A total of 1178 individuals were captured belonging to 34 species. The top dominant species were *P. longimana* (Dominance = 18.76%), *P. hirtellus* (15.45%), *A. nitescens* (14.18%), *C. tubularis* (7.56%) and *C. timidus* (7.47%). The assemblages (data of both areas) displays slight seasonal differences ( $R_{ANOSIM}=0.304$ ;  $p=0.001$ ) with maximum values of diversity index ( $H'$ ) in autumn, in both zones (2.20±0.02 / 2.11±0.15), and of evenness index ( $J'$ ) in winter and spring (0.93±0.02 / 0.92±0.04) for P de Calaburras and Calahonda respectively. The abundance shows a negative correlation with the number of leaves ( $R_{Spearman}= -0.348$ ;  $p=0.035$ ), while richness is positively correlated with the shoot density ( $R_{Spearman}=0.365$ ;  $p=0.026$ ).

## Discussion

The studied meadow is fragmented but it still presents a high species richness. In *P. oceanica* meadows of Almería the decapod assemblage was dominated by two hermit crabs, *C. tubularis* and *C. timidus* (García Raso, 1990), similar results were found in Malta where *C. timidus* and *A. nistencens* were the top dominant species (Borg & Schembri 2000). While in the SCI of Calahonda, *P. longimana*, *P. hirtellus* and *A. nitescens* are the most dominant species, probably due to differences in the development rhizome stratum, the shallower depth of the meadows and the proximity of adjacent habitats. The species richness and diversity values are related to some aspect of the plant phenology, although less marked than in other smaller seagrass such as *C. nodosa* (personal observations). This is possibly due to the more complex and stable structure of the *P. oceanica* habitat.

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## **LA COMPOSITION CHIMIQUE DES ALGUES, UNE PASSERELLE VERS DES APPLICATIONS BIOTECHNOLOGIQUES. LE CAS DE L'ALGUE ROUGE *GRACILARIA GRACILIS* (STACKHOUSE) TEENTOFT ET AL.**

### **Résumé**

*L'algue rouge Gracilaria gracilis a été cultivée en mode suspendu dans la lagune de Bizerte (Tunisie) durant le printemps 2009. Au cours de cette période d'étude, la croissance de l'algue, ainsi que ses teneurs en protéines, carbohydrates et pigments ont été étudiées dans différents sites et profondeurs de la lagune de Bizerte afin d'évaluer l'effet du mode de culture, du site et de la profondeur sur les rendements. L'algue contient des teneurs élevées en protéines (20-30%) et en R-phycoérythrine (3 à 6,5 mg/g matière fraîche) et une faible teneur de carbohydrates (30%-35%). Les teneurs en protéines et R-phycoérythrine corroborent le potentiel d'utilisation de cette algue, pour l'extraction de molécules à haute valeur ajoutée et le développement d'activités innovantes.*

**Mots clés :** *Gracilaria gracilis*, Lagune de Bizerte, Protéines, R-phycoérythrine, Sucres totaux

### **Introduction**

L'algue rouge *Gracilaria gracilis* a désormais un intérêt économique dans le monde, elle est utilisée essentiellement pour la production de l'agar. De même elle contient des teneurs élevées en protéines et en pigments essentiellement la R-phycoérythrine, molécule active ayant plusieurs applications pharmaceutiques et alimentaires. Le présent travail, vise la connaissance de la composition chimique de cette espèce dans la lagune de Bizerte (Nord de la Tunisie) en vue de développement d'activités innovantes.

### **Matériel et méthodes**

#### **a- Sites d'études**

Coordonnées géographiques des deux stations de culture A1 (37°13.00'), C1 (37°09.500' ; 9°50.000), A0 (37° 13.391' ; 9°49.490) et C0 (37° 09.00' ; 9°51.000).

#### **b- Méthodes de dosage**

- 1 - Protéines (Bradford, 1976).
- 2 - Sucres totaux (Dubois et al., 1956).
- 3 - R-phycoérythrine (Abdeladhim, 2005).

### **Résultats et discussion**

Les résultats relatifs aux teneurs en protéines, sucres totaux et R-phycoerythrine sont mentionnés dans la tableau1. Les concentrations en protéines et carbohydrates des thalles de *Gracilaria verrucosa*, collectés dans les deux stations, sont proches de celles enregistrées dans une partie polluée de la lagune de Venise en Italie et signalées par

Sfriso *et al.*, (1994) qui indiquent des valeurs de protéines comprises entre 26 et 30 % et des valeurs de carbohydrates ne dépassant pas les 35 %. Cependant, nos valeurs sont différentes de celles d'un milieu lagunaire peu pollué (Sfriso *et al.*, 1994). Ces auteurs indiquent des valeurs de protéine comprises entre 11 et 22% et des teneurs en carbohydrates atteignant les 60 %. La variabilité entre les résultats pourrait être due à la teneur en ammonium du milieu..

La relation entre la teneur en R-phycoérythrine (RPE) et la profondeur n'est pas bien établie. L'algue rouge *Gracilaria verrucosa* montre une stratégie photobiologique qui permet à ces thalles de se développer dans des conditions de faible lumière, tout en augmentant le nombre de pigments (Gomez *et al.*, 2005). L'absence de corrélation entre la teneur en R-phycoérythrine de *Gracilaria verrucosa* et la profondeur de l'eau de la lagune pourrait être attribuée aux nombreux autres facteurs qui influencent la concentration de ce pigment tout au long de la colonne d'eau (température, salinité, nutriments et régime du courant d'eau).

**Tab. 1 : Teneur en protéines (mg/g), teneur en R-phycoérythrine (RPE ; mg/g) et teneur en sucres totaux (%) dans différentes stations et profondeurs de la lagune de Bizerte**

Stations	A					C				
Profondeurs(m)	0	1	2	3	4	0	1	2	3	4
Protéines (mg/g)	296.27	216.39	251.95	214.06	260.57	302.13	232.55	209.52	284.288	266.024
R-phycoérythrine (mg/g)	0.92	0.858	0.90	0.50	0.515	0.47	0.413	0.392	0.790	0.615
Sucres totaux (%)	314.03	326.79	336.11	318.40	321.96	349.50	326.18	316.86	325.714	327.447

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## **ASSESSMENT OF THE ECOLOGICAL STATUS OF THE GALITE ISLAND COASTAL WATERS (NORTHERN OF TUNISIA) USING THE "CARLIT" METHOD**

### **Abstract**

*A first application attempt of an ecological quality index based on the cartography of the littoral and upper-sublittoral rocky-shore communities dominated by macroalgae (CARLIT : Ballesteros et al. 2007) was carried out to assess an initial ecological status in the Galite island coastal waters (Northern of Tunisia) in May 2009. The CARLIT method was selected to evaluate the water quality of l'Escueil de Pasques bay coasts which represents the gate of the marine coastal protected area of the Galite island (and the main anchoring zone). Two major ecological status of l'Escueil de Pasques bay water bodies were identified: a very good ecological status in the western area, characterized by the presence of *Cystoseira belt* (*Cystoseira stricta*, *Cystoseira spp.*) almost along all the rocky shore, and a moderate ecological status in the eastern area of the bay where *Cystoseira belt* appears only at level of the la Garde headland. The presence of decimetric rocky blocks in the eastern area is linked to the instability of the rocky shore caused by the intensive and continuous wave erosion which is not favorable to the development of *Cystoseira belts*.*

**Key-words:** Galite Island, Ecological status, Index, Macroalgae, Cartography.

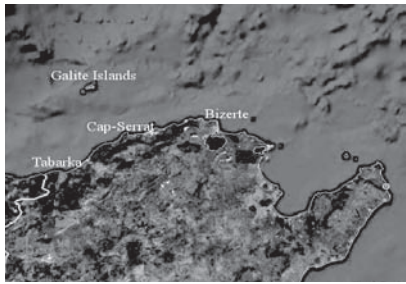
### **Introduction**

To assess the ecological status of marine coastal waters, macrophytes such as seagrasses and macroalgae are considered as basic quality elements for the evaluation of ecological status, according to the Water Framework Directive 2000/60/CE. Two indices are currently applied to the mediterranean rocky shore macroalgae dominated community. One of them, the CARLIT index (Ballesteros et al. 2007), is based on the cartography of the littoral and upper-sublittoral rocky-shore communities. The aim of this study was to assess an initial ecological status in the Galite island coastal waters (Northern of Tunisia) using the CARLIT index, as a first application attempt, to evaluate the water quality of l'Escueil de Pasques bay coasts which represents the gate of the marine coastal protected area of the Galite island and the main anchoring zone.

### **Materials and methods**

The Galite island (752 ha), a marine coastal protected area, is the main island of the Galite archipelago Islands. It is a rocky volcanic origin islands located 38 km northwest of Cape Serrat, the closest point of the Tunisian mainland coast, from which they are separated by the Galite Channel, almost 80 km NNE of the city of Tabarka, and 150 km south of Cape Spartivento in the south of Sardinia (Fig. 1).

The study was carried out in May 2009 along the rocky shore of the l'Escueil de Pasques bay (Fig. 2). First of all, the littoral and upper-sublittoral communities were identified by snorkeling observations in 27 sectors, each of 50m (scale of 1:5000) and then the ecological status of the coast was evaluated using the CARLIT method according to Ballesteros *et al.*, (2007).



**Fig. 1:** Map of the location of the archipelago of the Galite Islands

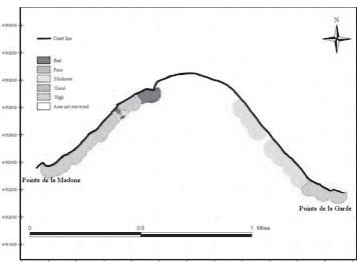
**Fig. 2:** Location of the l'Escueil de Pasques bay

**Results and discussion**

This assessment represents the first attempt of an ecological evaluation for the Tunisian marine coastal protected area of the Galite Island based on the application of the CARLIT index.

The ecological status of water bodies inside the l'Escueil de Pasques bay is shown in Fig. 3.

Two major ecological status were identified: a very good ecological status (blue color) in the western area, characterized by the presence of *Cystoseira* belt (*Cystoseira stricta*, *Cystoseira* spp.) almost along all the rocky shore, as its noticed on the first Campaign of submarine prospecting of the archipelago of Galite Islands on 2001 (APAL, 2001); and a moderate ecological status in the eastern area of the bay where *Cystoseira* belt appears only at level of Pointe de la Garde headland. The presence of decimetric rocky blocks in the eastern area is linked to the instability of the rocky shore caused by the intensive and continuous wave erosion which is not favorable to the development of *Cystoseira* belts (Thibaut *et al.*, 2005).



**Fig. 3:** Representation of the ecological status of l'Escueil de Pasques bay coastal waters

**Acknowledgments**

The authors are grateful to Rami ben Aloui for technical support and Faouzi Fersi for his support during the fieldwork.

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## **VISION 1.0, EXPERIMENTAL SOFTWARE TO CALCULATE SEAGRASS COVERAGE PERCENTAGE.**

### **Abstract**

*The use of underwater photographic images and videos obtained by remote operated vehicles (ROV) has become very important for marine and coastal monitoring. There are several open source softwares that make possible the analysis of such images and videos and provide a quick, statistically valid and efficient calculation of coverage*

**Key-words:** Seagrass coverage percentage, experimental software, connectivity and fragmentation degree, MPA Capo Rizzuto (Calabria, Italy).

### **Introduction**

Morphology and extension of the *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson meadows, depend on various factors like seaboard conformation, seabed, interaction with plant growth, main currents and sedimentation (natural factors) but also by no natural factors like industrial and seaport drain, fishing, climatic change, etc. If a change happens for one or all of this environmental parameters, these marine vegetation systems can regress: several examples of human – induced or natural regression have been registered in the Mediterranean Sea. This preliminary research has been realized in order to verify the applicability of photo frame-analysis for the estimation of the degree of connectivity and fragmentation of *Posidonia oceanica* and *Cymodocea nodosa* meadows and its relationship with substrata. This study has been realized in the MPA of Capo Rizzuto (Calabria, Italy). These areas extend for 14721 hectares.

### **Materials and Methods**

The photo frame-analysis has been conducted by using the software Vision 1.0 (Rende *et al.*, 2009a). Photographic sampling has been realized by placing a metric spacer on both *Posidonia oceanica* and *Cymodocea nodosa* meadows. The analysis has been realized on 10 random photo frames: 5 frames on a “normal” meadow called site A (control), and 5 frames on a “disturbed” meadow (Pergent *et al.*, 1995) that we indicate as site B (worst). The frame-analysis consisted in an esteem of the seagrass coverage percentage vs substrate (Fig. 1) (Rende *et al.*, 2009b).

### **Results**

The photo frame-analysis done on *Posidonia oceanica* meadows has underscored, for site A (control), a coverage of 84,8% (STD:  $\pm 7,7$ ), whereas for site B (worst) a coverage of 59,36% (STD:  $\pm 12,9$ ) (Tab. 1). Site B (worst) is in fact more exposed to sea storm coming from the SE 135° sector that buries with sand the *Posidonia oceanica* shoot with a consequent damage of the meadow. For what concerns fragmentation, site

A (control) resulted “Mixed” (coverage 60-90%, with a low level of modification), while site B (worst) resulted “Fragmented” (coverage 10-60%), with a high level of modification. The photo frame-analysis done on *Cymodocea nodosa* meadows has underscored, for site A (control), a coverage of 98,1% (STD:  $\pm$  2,4) (Tab. 1), and for site B (worst) a coverage percentage of 87,4% (STD:  $\pm$  9,8) (Tab. 1). For what concerns fragmentation, site A (control) resulted “Intact” (coverage >90%) with a low modification level, while site B (worst) resulted “Mixed” (coverage 60-90%) with a low modification level.

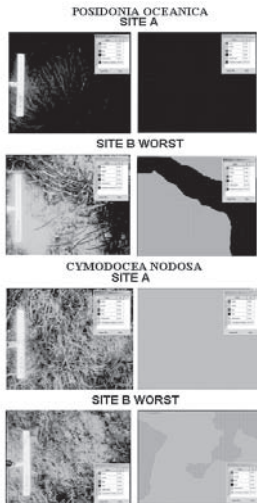


Fig. 1: Lay-out of the photo frame-analysis “software Vision 1.0”

Tab. 1: Coverage percentage estimated with software Vision 1.0

	% <i>Posidonia oceanica</i>	
	Site A (control)	Site B (worst)
Photo-frame 1	100	37,6
Photo-frame 2	80,4	57,9
Photo-frame 3	78,9	57,9
Photo-frame 4	82,4	76,6
Photo-frame 5	82,3	66,8
<b>MEAN</b>	<b>84,8</b>	<b>59,36</b>
<b>ST.DEV</b>	<b>7,7</b>	<b>12,9</b>
	% <i>Cymodocea nodosa</i>	
	Site A (control)	Site B (worst)
Photo-frame 1	100	89,9
Photo-frame 2	100	70,1
Photo-frame 3	94,7	85,6
Photo-frame 4	95,8	91,5
Photo-frame 5	100	100
<b>MEAN</b>	<b>98,1</b>	<b>87,42</b>
<b>ST.DEV</b>	<b>2,4</b>	<b>9,8</b>

## Conclusions

The preliminary experimentation has defined the fragmentation degree of seagrass meadows; seemingly the adopted sampling design looks very useful both for connectivity and fragmentation study, and for defining the types of habitat (Fahring, 2003). The next step in future will be to apply the proposed methodology on a larger scale using geo-referenced ROV systems and analyzing the video track with the software Vision 1.0.

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## **CLASSICAL AND EXPERIMENTAL APPROACHES TO THE CONSERVATION OF *POSIDONIA OCEANICA* AT S. MARINELLA (ROME, ITALY).**

### **Abstract**

*The Posidonia oceanica (Magnoliophyta, Liliopsida) meadow at S. Marinella (Rome, Italy) is endangered as recorded in 2004. It has been characterized by both classical and two newer methods: phenol content and RAPD markers. By the former the meadow was classified in poor conditions, with low density and high heterogeneity in leaf biometry, LAI values, "A" coefficient and primary production. Results obtained by the newer methods (total phenols and RAPD) correspond to the classical one. In fact the plants clearly shown high total phenols content typically scattered in response to high stress and a very low genetic polymorphism with high similarity among the plants. Total phenols content and RAPD analyses clearly identified the endangered situation of the meadow. These results demonstrated the value of the new methods which link seagrass biodiversity to their eco-physiology evaluating the health of the ecosystem. They are repeatable, cheap and easy to use; hence, both the approaches are promising tools to monitor the meadows health state and the environmental quality.*

**Key-words:** *Posidonia oceanica*, RAPD genetic diversity, total phenols, Central Tyrrhenian Sea

### **Introduction**

*P. oceanica* (L.) Delile meadows show widespread regression and the identification of early warning "diagnostic" tools is a critical issue. Recently two tools were applied to monitor seagrass meadows: phenolic compounds (Migliore *et al.*, 2007) and RAPD genetic markers (Micheli *et al.*, 2005). In rhizomes the levels of synthesis and accumulation of phenolic compounds represent a generic response to different environmental stress, hence they could be utilized to monitor the meadow health state.

Random Amplified Polymorphic DNA (RAPD) technique has been used to assess genetic diversity of seagrasses (Alberto *et al.*, 2001; Procaccini *et al.*, 1996; Waycott, 1998) highlighting a decreased genetic diversity in *P. oceanica* along an anthropogenic disturbance gradient (Micheli *et al.*, 2005).

With the aim to assess the conservation status of the *P. oceanica* at S. Marinella (Rome, Italy), we investigated the meadow using the total phenols and RAPD markers combined with the structural descriptors.

### **Materials and methods**

*P. oceanica* meadow of S. Marinella (Rome, Italy) is characterized by patchy distribution and a regressive limit (Pergent *et al.*, 1995). Sampling was carried out in spring 2004 by SCUBA divers at 30 stations on *matte* (from 7.5 to 13.5 m depth). In each station, 15 shoots of orthotropic rhizomes were collected for lepidochronological and phenological analyses, 5 for RAPD analysis and 3 orthotropic rhizomes for phenols determination. Shoot density was evaluated by counting the shoots by 40 x 40 cm standard quadrates (shoots/m<sup>2</sup>) whilst leaf biometry was studied according to Giraud (1977). "A" coefficient (% leaf with lost apex), Leaf Area Index (LAI, cm<sup>2</sup>/shoot) and lepidochronological analysis (according to Pergent *et al.*, 1995) for primary production of rhizomes (mg dry weight/shoot/year) were also investigated. Total phenols were determined according to Migliore *et al.* (2007); genetic analysis according to Micheli *et al.* (2005).



## Results and Discussion

Phenological and lepidochronological analyses classified the bed as a disturbed one, with low density and high heterogeneity in leaves biometry, LAI, “A” coefficient and primary production.

The total phenols content and RAPD analysis agreed with the classical descriptors. Mean phenols content in plants were quite high (18.7 mg/g f.w.) and scattered (from 8.8 to 30.2 mg/g), typical of a high stress response. The RAPD analyses showed a low genetic variability among the samples: we found a low polymorphism and a very high similarity of specimens at a local scale (mean similarity 0.87) much higher than those found in the Mediterranean basin (0.81). As a general rule, both total phenols content and RAPD analyses clearly identified the endangered situation of the meadow.

S. Marinella meadow could be defined as a “disturbed bed” undergoing regression, with high spatial heterogeneity, low productivity and under high mechanical or grazing pressure.

The endangered situation of the meadow is also clearly identified by both total phenols content and RAPD analyses. In fact, total phenols are very high, as happens in stressed conditions, much higher than in the reference site. Genetic polymorphism (RAPDs) showed a low genetic diversity in the population and a very high similarity of specimens at a local scale.

These results regarding the meadow at S. Marinella highlight interest in both two new tools, linking seagrass biodiversity (RAPDs) and their eco-physiology (phenols) to the health of ecosystem. Furthermore, they are repeatable, cheap and easy to perform; hence, both approaches are promising tools to monitor the health state of *P. oceanica* meadows (Micheli *et al.*, 2010; Rotini *et al.*, 2010).

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**HERBIVORY AND ECOLOGY OF THE GASTROPOD  
SMARAGDIA VIRIDIS ON MEDITERRANEAN SEAGRASSES,  
WITH SPECIAL EMPHASIS ON ZOSTERA MARINA AND  
CYMODOCEA NODOSA**

**Abstract**

*The neritid Smaragdia viridis is the only known marine mollusc that feeds on seagrasses in the European coasts, and is therefore strongly associated with different species, especially Cymodocea nodosa and Zostera marina. In this study we document radular marks and faecal content of this gastropod when feeding on 3 Mediterranean seagrasses, including both pioneer (C. nodosa, Z. marina) and climax species (Posidonia oceanica) from southern Spain. We compared seasonal dynamics, shell and radular morphology, growth and feeding in populations associated with C. nodosa and Z. marina in order to contrast trends resulting from a different type of substrate and food source. In beds of both seagrass species in southern Spain, stable populations of this gastropod occur at similar densities (70-80 indiv 222m<sup>-2</sup>) and growth rates (K values 0.9-1.2). Individuals collected on C. nodosa are narrower than those collected on Z. marina and their radulae also displayed differences. In both seagrasses, a pre-ingestive selection for young epidermal tissues occurs and higher ingestion rates were obtained in C. nodosa than in Z. marina. If both seagrasses are present, most individuals ingested preferentially Z. marina rather than C. nodosa, probably due to the lower digestibility of the epidermal tissues in the latter. The feeding of Smaragdia viridis on Posidonia oceanica was observed for first time and in spite of the strong lignification of the cell walls, the neritid displayed empty epidermal cells in faeces. As in other parts of the world, seagrass beds, especially those of Z. marina, are suffering a strong regression in southern Spain and therefore the populations of this gastropod. Nevertheless, its ability to ingest different seagrass species, and develop stable populations in those, may represent an advantage against local extinction.*

**Key-words:** *Smaragdia viridis*; Temporal Dynamics; Growth; Selection; Alboran Sea

**Introduction**

Seagrass beds are important habitats for several species world-wide but they have suffered a strong decline. In seagrass beds, most animal species do not feed on the seagrass itself, and because of that they are not strictly dependent on the seagrass plant. Molluscs feeding on seagrass tissues represent an alternative pathway for the mobilisation of carbon from seagrasses to higher trophic levels. Most of these molluscs are small size gastropods (< 1-2 cm). The use of different seagrass species as habitat and food source may result in differences of the feeding biology or growth of the grazer but these topics have never been investigated for any seagrass feeding invertebrate. The emerald neritid *Smaragdia viridis* (Linnaeus, 1758) is a small gastropod generally associated with *Cymodocea nodosa* and *Zostera marina* beds (Rueda & Salas, 2007), but also with *Posidonia oceanica*. Feeding and growth of *S. viridis* display differences that may promote the persistence of *S. viridis* populations in these seagrass species.

## Materials and methods

Seasonal abundances of *S. viridis* were studied in *C. nodosa* and *Z. marina* beds by collecting monthly samples with an Agassiz trawl (sampled area 222 m<sup>2</sup>). Shells of individuals from each seagrass and sample were measured (shell height and width). Growth was also calculated using the software Fysat II. Feeding on *Posidonia oceanica*, *C. nodosa* and *Z. marina* was characterized in laboratory conditions, using trays containing one individual of *S. viridis* and one shoot of each seagrass species. These experiments were two times replicated for each seagrass and with several individuals of *S. viridis*. After 24 h, faeces were collected and radular marks located, photographed and measured. Absorption of seagrass cells was estimated as percentage of cells without cytoplasm over 10 fecal pellets. Ingestion was only estimated for *C. nodosa* and *Z. marina* by measuring the area of all radular marks produced in 24 h. Preingestive selection on these two seagrasses was studied by counting radular marks in different parts of the shoot (sheath, central or lateral leaves).

## Results

Stable populations of *S. viridis* occur at similar densities (70-80 indiv 222m<sup>-2</sup>) and similar growth rates (K values 0.9-1.2) in *C. nodosa* and *Z. marina* beds from southern Spain. Individuals from *C. nodosa* have, however, narrower shells than those from *Z. marina*, and radular marginal teeth proportionally longer and extending further over lateral than the individuals from *Z. marina*. A pre-ingestive selection for young epidermal tissues occurs in both seagrasses, with higher ingestion rates in *C. nodosa* than in *Z. marina*. Absorption of tissues seems lower in *C. nodosa* than in *Z. marina* or *P. oceanica*. If *C. nodosa* and *Z. marina* are present, most individuals ingested preferentially the latter rather than the former, probably due to a better digestibility of the tissues. Feeding on *P. oceanica* was observed for first time and in spite of the strong lignification of the cell walls, the neritid displayed empty epidermal cells in faeces.

## Discussion

Phenotypic variation (shell and radulae) in *S. viridis* may be ecotypes related to the inhabited microhabitat or substrate and to the environmental characteristics, as observed in other seagrass gastropods. This neritid feeds mainly on the epidermal tissues of basal areas, which generally represent the youngest parts of the shoot with lower lignification than the apical parts and with the lowest concentrations of epiphytes (Rueda *et al.*, 2009). This is similar to other seagrass feeding gastropods such as *Naccula parva*, *Tectura depicta* or *Elysia cerca* but it is different to that observed in the small periphyton grazers, such as *Jujubinus striatus*. Seagrass beds are suffering a strong decline in southern Spain in the last years, particularly the beds of *Z. marina*, but the ability of this neritid to ingest efficiently different seagrass species may represent an advantage against its local extinction.

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## **LOSS OF *ZOSTERA MARINA* IN SOUTHERN SPAIN AND EFFECTS ON ITS ASSOCIATED MOLLUSCAN ASSEMBLAGE**

### **Abstract**

*Eelgrass beds of southern Spain have recently experienced a strong decline to the point of becoming extinct in some coastal areas. Changes in the sediment, the eelgrass density and the molluscan assemblage were studied in an extinct bed of *Zostera marina* (12-14 m depth) in the MPA of Paraje Natural de Acantilados de Maro- Cerro Gordo (Málaga-Granada, southern Spain). Sampling was carried out in 2004 (with *Z. marina*), 2007 (just after the decline), 2008 and 2009 (without *Z. marina*). Shoot density of *Z. marina* declined from 404 shoots m<sup>-2</sup> in 2004 to zero in 2007-2009. Organic matter in the sediment increased after eelgrass loss (3.04 %) and decreased in 2008 and 2009 (2.5 %). Mud percentage displayed a similar pattern with maximum values (17.2%) in 2007. Species richness declined from 32 spp. in 2004 to 18 spp. in 2009. The periphyton grazers *Jujubinus striatus* and *Rissoa membranacea*, the seagrass feeder *Smaragdia viridis* and the egg mass feeder *Mitrella minor* were the first species that disappeared, as well as the infaunal bivalve *Solemya togata*. The abundance of molluscs displayed fluctuant values, with minimum values just after eelgrass loss in 2007 (1528 indiv m<sup>-2</sup>). Evenness and diversity of Shannon-Wiener displayed a fluctuant decrease after eelgrass loss. Changes in the composition and structure of the molluscan assemblage and of the infaunal bivalves were more acute just after the eelgrass decline than in posterior years (2008 and 2009).*

**Key-words:** Eelgrass, gastropod, bivalve, Alboran Sea, interannual dynamics

### **Introduction**

In the last five years, eelgrass beds of southern Spain (from Málaga to Almería) have experienced a strong decline to the point of becoming extinct in most coastal areas where they used to form extensive beds (Rueda *et al.*, 2009). This decline has been mostly attributed to illegal trawling and probably to secondary effects of this anthropogenic impact on the stressed eelgrass beds (pathogens, changes in water column and sediment). This represents an important ecological loss involving the seagrass species itself but also the habitat that supported a rich and highly diverse benthic community. The immediate effects of eelgrass loss have been studied in other parts of the world, including a general decrease of the faunistic and floristic biodiversity among others, but information on the long-term changes of the fauna in the unvegetated bottoms after eelgrass loss is very scarce. In this study we analyzed the molluscan fauna when eelgrass beds were present and the interannual changes in the newly formed unvegetated bottom.

### **Materials and methods**

Changes in the sediment, the eelgrass and the molluscan assemblage were analyzed in an extinct bed of *Zostera marina* (12-14 m depth) located within the MPA of Paraje Natural de Acantilados de Maro- Cerro Gordo (Málaga-Granada, southern Spain). Sampling was carried out at the end of summer/beginning of autumn in 2004 (with *Z. marina*) and in 2007 (just after the decline), 2008 and 2009 (without *Z. marina*). In each

occasion we studied (1) the shoot density (5 quadrats of 50x50 cm) and shoot attributes, (2) grain size distribution and organic matter content of the sediment (5 replicates) and (3) the molluscan fauna in 5 quadrats of 25 x 25 cm (one sample collected in each 50x50 cm quadrat down to a sediment depth of 5 cm) that retained in a sieve of 0.5 mm.

## Results

Shoot density of *Z. marina* was  $404 \pm 20.1$  shoots  $m^{-2}$  in summer 2004 and declined to  $220.8 \pm 51.8$  shoots  $m^{-2}$  in winter 2005 and to zero in summer 2007 with no presence of eelgrass in 2008 and 2009. Remains of seeds and rhizomes were still present in the unvegetated bottoms in 2009. Organic matter in the sediment reached a peak just after eelgrass loss ( $3.04 \pm 0.54$  %) with a posterior decrease and similar values in 2008 and 2009 (values around 2.5 %). Mud percentage increased just after eelgrass loss (17.2% in 2007), displaying fluctuations since then (5.4 % in 2008 and 11.3% in 2009). Species richness declined from 32 spp. in 2004 (with eelgrass) to 18 spp. in 2009. The first species that disappeared were the periphyton grazers *Jujubinus striatus* and *Rissoa membranacea*, the seagrass feeder *Smaragdia viridis* and the egg mass feeder *Mitrella minor*, as well as the infaunal bivalve *Solemya togata*. Molluscs abundance displayed fluctuant values, with high values in 2004 (2512 indiv  $m^{-2}$ ), declining after eelgrass loss in 2007 (1528 indiv  $m^{-2}$ ) and a reestablishment of the values in 2008 (2938 indiv  $m^{-2}$ ) and 2009 (2475 indiv  $m^{-2}$ ). This is mainly linked to the recruitment of soft bottom bivalve species such as *Tellina distorta* and *Chamelea gallina*. Evenness and diversity of Shannon-Wiener displayed a fluctuant decrease after eelgrass loss, from 0.8 to 0.7 and from 4.1 to 3.2, respectively. Changes on the composition/structure of the molluscan assemblage were more acute just after the eelgrass decline than in posterior years (2008 and 2009). A similar pattern was observed in multivariate analyses when only considering the infaunal bivalve assemblage data.

## Discussion

Loss of *Z. marina* has not only resulted in a reduction of the habitat complexity but also on the available food sources, affecting in first place to species feeding on the plant itself or on organisms that occurred on the eelgrass shoots. This loss also affected the sediment characteristics, increasing the organic matter just after eelgrass loss due to the decomposition of remains of plants and epibionts. This increase of organic matter could influence the decrease of the abundance of infaunal species in 2007. Deposit feeding bivalves have displayed fluctuant densities whereas filter feeding bivalves have increased their densities representing the dominant feeding guild. Changes in the last years were less acute suggesting stabilization of the sediment characteristics as well as of the molluscan fauna associated with these soft bottoms that once were covered by eelgrass.

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## **MEASURING THE EFFECTS OF HYPERSALINE STRESS ON SEAGRASS (*POSIDONIA OCEANICA*) PHOTOSYNTHESIS USING FLUORESCENCE IMAGING.**

### **Abstract**

*In this work we analysed the effect of hypersaline stress on the heterogeneity of the photosynthetic activity along leaves of the Mediterranean marine angiosperm *Posidonia oceanica* by means of fluorescence imaging techniques. Plants from two population of contrasting light regimes (high and low light) were maintained in a controlled laboratory mesocosm system and exposed hypersaline condition (i.e. 43 psu) during two months. Hypersalinity caused substantial reductions in the maximum quantum yield (Fv/Fm), efficiency of PSII photochemistry (?<sub>PSII</sub>) and relative electron transport rate (rETR), which can be indicative of both accumulated damage on PSII and alterations of the photosynthetic carbon metabolism. These responses were concomitant with a decrease of the yield of basal fluorescence (F<sub>i</sub>) and the increment of non-photochemical quenching (qN), which indicated the induction of photoprotective mechanisms based in the thermal dissipation of excess excitation light energy. The strength of the reported effects of hypersalinity on photochemistry varied depending upon the light regime to which the plant is acclimated and the tissue age (basal vs. apical leaf portions).*

**Key-words:** *Posidonia oceanica*, imaging PAM fluorescence, hypersalinity, photosynthesis.

### **Introduction**

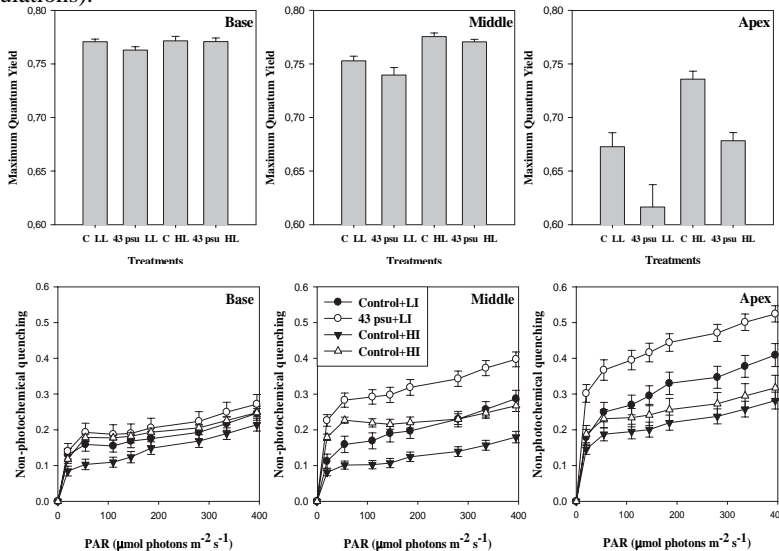
The Mediterranean seagrass *Posidonia oceanica* has been shown to be very sensitive to small increments in the ambient salinity caused by brine discharges from seawater desalination plants (Ruíz *et al.*, 2009). However, our understanding of the salt tolerance physiology in these plants is still very limited. Imaging techniques of Chlorophyll *a* fluorescence are emerging and they are promising tool for the study of physiological stress situations since they allow real-time and two-dimensional analysis of photosynthetic changes across an entire leaf surface (Ralph *et al.* 2005). The aim of the present study was to evaluate the responses of *P. oceanica* photochemistry to hypersaline stress by means of Imaging-PAM (Waltz) fluorescence techniques.

### **Material and methods**

The experiment was carried out in a controlled laboratory mesocosm system using plants from two populations adapted to contrasting light regimes: a) shallow (-5 m; high light, HL: 500  $\mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ ) and b) deep (-17 m; low light, LL: 200  $\mu\text{mol}$  quanta  $\text{m}^{-2} \text{s}^{-1}$ ). Acclimated plants from each population were exposed to hypersaline conditions (43 psu) for 2 months and compared with plants maintained at ambient salinity (37 psu). Fluorescence measurements were taken on whole *P. oceanica* leaves at the end of the experimental period using a Imaging-PAM Fluorometer (Waltz, Germany). Patterns of variation of fluorescence parameters obtained in dark-adapted (F<sub>o</sub>, F<sub>m</sub>, F<sub>v</sub>/F<sub>m</sub>) and light-adapted (F<sub>i</sub>, F<sub>m</sub>' , ? PSII, rETR and qN) leaves exposed to each salinity treatment were characterized for different leaf age portions (i.e. basal, middle and apical) of each studied populations.

## Results

After two months, the hypersaline treatment caused an overall significant reduction in the PSII photochemistry ( $F_v/F_m$ ,  $\Phi_{PSII}$ , rETR) and a significant increase in qN (Fig. 1), being these effects more pronounced in the apical and middle parts of the leaf (oldest tissues) than in the basal ones (youngest tissues). These response patterns were also more evident for rETR and qN in LL plants, suggesting that photosynthesis of mature tissues of low-light adapted plants (e.g. those from deep populations) is more sensitive to hypersaline stress than those of high-light adapted plants (e.g. those from shallow populations).



**Fig. 1:** Mean values  $\pm$  SE ( $n=10$ ) of  $F_v:F_m$  ratios (upper panel) and qN (lower panel) in different leaf portions (basal, middle, apical) obtained from rapid light curves of leaves from each salinity-light treatment combination.

## Conclusion

The results obtained in this work indicate that i) the photochemistry of *P. oceanica* leaves was significantly affected by increased salinity due to both accumulated damage in PSII and alterations of photosynthetic carbon metabolism and that ii) the oldest leaf tissues of low-light adapted plants were the most sensitive to the experimental hypersaline stress applied in this study. Our results illustrate that fluorescence imaging techniques are useful and effective tools for assessing the photosynthetic responses of *P. oceanica* leaves to hypersalinity stress.

## Acknowledgments

Ministerio de Medioambiente y Medio Rural y Marino (OSMOGRASS Project Ref. 021/SGTB/2007/1.3) and the Ministerio de Ciencia e Innovación (OSMOGRASS II project Ref. CTM2009-08413MAR).

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## **EFFECT OF HYPERSALINE STRESS ON PHOTOSYNTHESIS, GROWTH AND SURVIVAL OF THE MEDITERRANEAN SEAGRASSES *POSIDONIA OCEANICA* AND *CYMODOCEA NODOSA*.**

### **Abstract**

We examined the effect of salinity increase in the photosynthesis, leaf growth and shoot survival of the Mediterranean seagrass species *Posidonia oceanica* and *Cymodocea nodosa*. To this end both species were maintained in a laboratory mesocosm system for 45 days under ambient (i.e. 37 psu, control) and hypersaline (39, 41 and 43 psu) conditions. Changes in pigment content and the maximum photochemical efficiency of PSII ( $F_v/F_m$ ) indicated that the photosynthetic apparatus was not substantially affected by increased salinity. In both species photosynthetic rates declined at salinities immediately higher than the ambient values, with *P. oceanica* clearly being more sensitive than *C. nodosa*. Increased salinity stimulated increased respiration rates in both species (by up to 98% higher than control values) and it was the major cause of the lowered net photosynthesis, although gross photosynthesis was also affected (especially in *P. oceanica*). The photosynthesis:dark respiration ratio indicated substantial leaf carbon imbalance in *P. oceanica* (49–56% lower than in control plants). Accordingly, leaf growth rates and shoot survival were not affected in *C. nodosa*, but declined in *P. oceanica*, supporting previous hypothesis that the former seagrass species is more tolerant to salinity increments than the latter. These results are discussed in relation to the ecology of both seagrass species.

**Key-words:** *Posidonia oceanica*, *Cymodocea nodosa*, salinity, photosynthesis, Mediterranean.

### **Introduction**

The Mediterranean species *P. oceanica* inhabits marine environments with constant salinities; previous studies have shown that it is highly sensitive to increases in salinity (Fernández-Torquemada & Sánchez-Lizaso, 2005; Ruiz *et al.*, 2009). Since *C. nodosa* is able to colonise a wider range of coastal environments with contrasting salinity regimes it has been postulated that this seagrass is more tolerant to hypersalinity than *P. oceanica*. In this study, we assessed this hypothesis by comparing variations in photosynthesis, growth and survival of both seagrass species in response to increased salinity.

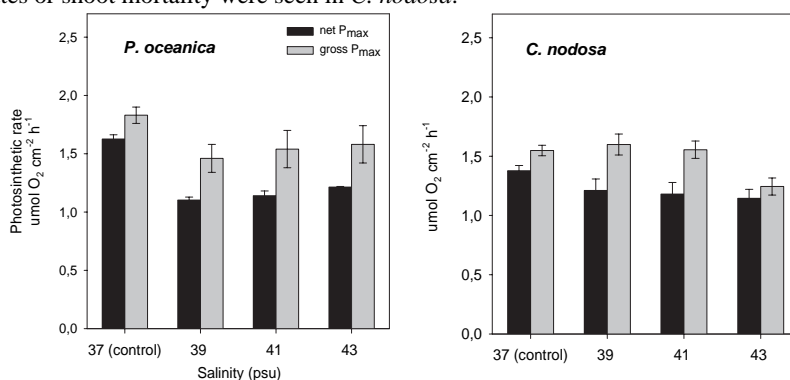
### **Materials and methods**

Large rhizome fragments were maintained in a laboratory mesocosm system for 45 days under the following salinity conditions: 37 psu (ambient seawater, control), 39, 41 and 43 psu. Pigment content (Chla, Chlb, total carotenoids, Chlb:a and carotenoids:Chla), light absorption, photosynthetic characteristics (P-E curves and fluorescence measurements), shoot features, leaf growth rates and shoot survival were determined at the end of the experimental period. The photosynthesis:respiration ratio ( $P:R_d$ ) was calculated and used as a proxy of the daily leaf metabolic carbon balance.

### **Results**

In hypersaline conditions the net and gross photosynthetic rates of *P. oceanica* were 25-

33% and 13-29% lower than in control plants, respectively. In *C. nodosa*, net photosynthesis also decreased in all hypersaline treatments but only by 12-17%, while gross photosynthesis was only affected in the most severe hypersaline treatment (Fig. 1). Dark respiration rates substantially increased by up to 98% in both species at salinities higher than ambient levels, except for *C. nodosa* at 43 psu. Treatment effects on the maximum photochemical efficiency of PSII ( $F_v/F_m$ ), pigment content, light absorption and photosynthetic efficiency (?) were generally small and/or not significant, indicating that hypersaline stress did not alter the functionality of the photosynthetic apparatus. The reduction in net photosynthesis was mainly explained by changes in respiration rates, but the decrease in gross photosynthesis suggests that hyperosmotic stress could also interfere with photosynthetic carbon metabolism. Alterations in photosynthesis resulted in a substantial impairment of the leaf carbon balance in *P. oceanica* and a subsequent decline in leaf growth rates and shoot numbers. No changes in leaf growth rates or shoot mortality were seen in *C. nodosa*.



**Fig. 1:** Mean net and gross photosynthesis rates at saturating irradiances ( $P_{max}$ ) obtained for both seagrass species in each salinity treatment. Vertical bars are standard errors.

## Conclusion

The results of this study provide experimental evidence that the photosynthesis of *P. oceanica* is affected by increasing salinity to a greater degree than of *C. nodosa*. This supports the hypothesis that *C. nodosa* is physiologically more tolerant to hypersaline conditions than *P. oceanica*, which in turn is consistent with their respective ecological strategies.

## Acknowledgments

Ministerio de Medioambiente y Medio Rural y Marino (OSMOGRASS Project Ref. 021/SGTB/2007/1.3) and the Ministerio de Ciencia e Innovación (OSMOGRASS II project Ref. CTM2009-08413MAR).

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## CHANGES IN WATER RELATIONS AND OSMOLYTE CONCENTRATIONS IN THE MEDITERRANEAN SEAGRASSES *POSIDONIA OCEANICA* AND *CYMODOCEA NODOSA* IN RESPONSE TO HYPERSALINITY STRESS.

### Abstract

The effects of hypersalinity on water relations and osmolyte concentrations were investigated in the Mediterranean marine angiosperms *Posidonia oceanica* and *Cymodocea nodosa* to determine their specific tolerances to hypersaline stress. The experiment was carried out under laboratory mesocosm conditions and the plants were exposed to increasing salinities (39, 41 and 43 psu) for 45 days. Leaf tissue osmolality and concentrations of non-structural carbohydrates (soluble sugars and starch) and free amino acids (FAAs) were determined in leaf tissues, together with leaf growth rates and shoot survival. In response to increasing salinity the water potential decreased in both species whereas the osmotic potential only responded in *P. oceanica* leaves, which was a signal of osmoregulatory activity. Turgor pressure was significantly reduced (61%) in *C. nodosa*, whereas in *P. oceanica* reductions of 34% were only found in the 41 and 43 psu treatments. In *P. oceanica* leaves soluble sugars and some FAAs (Pro, Gly) increased significantly as salinity increased. No clear response pattern was found for soluble sugars in *C. nodosa* whereas FAA concentrations decreased as salinity increased. Leaf growth rate and shoot survival declined as salinity increased but only in *P. oceanica*. These results clearly indicate that both seagrass species developed different physiological strategies to respond to hypersaline stress which supports the hypothesis that *P. oceanica* is less tolerant to salinity increments than *C. nodosa*, which in turn is in agreement with their respective ecological strategies.

**Key-words:** osmolyte, hyperosmotic stress, Mediterranean, seagrass.

### Introduction

Hypersaline effluents from desalination plants can adversely affect Mediterranean seagrass meadows formed by *Posidonia oceanica* and *Cymodocea nodosa*. The physiological mechanisms of seagrasses for tolerating sustained salinity increments are species specific (Tyerman, 1989). Both species differ in their structural, physiological and ecological attributes, with *C. nodosa* being considered as a more eurybiontic species than *P. oceanica*. In the present work, we tested this hypothesis by analysing the specific responses of water relations and osmolyte concentrations (carbohydrates and free amino acids) in both species to simulated hypersaline stress.

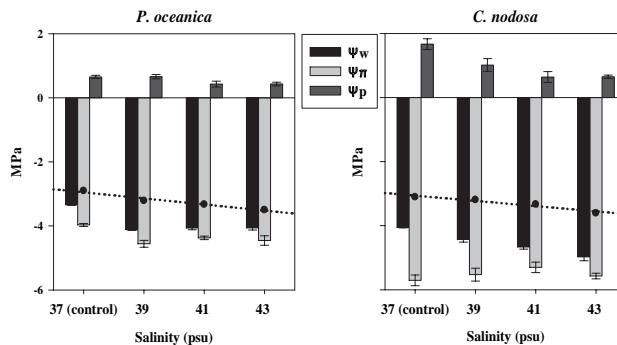
### Material and methods

Large rhizome fragments were maintained in a laboratory mesocosm system at ambient salinity (37 psu; control) before being exposed to increased salinity treatments (39, 41 and 43 psu) for 45 days. At the end of the experimental period the following measurements were performed: i) leaf tissue osmolality to determine water ( $\psi_w$ ) and osmotic ( $\psi_\pi$ ) potentials (turgor pressure,  $\psi_p$ , as the difference between  $\psi_w$  and  $\psi_\pi$ ), ii) non-structural carbohydrates (soluble sugars and starch) and free amino acids (FAAs) and iii) growth rates and shoot survival.

### Results

The  $\psi_w$  of *P. oceanica* was significantly reduced by 21-23% in all hypersaline treatments relative to the control plants (Fig. 1). In *C. nodosa* leaves this variable was also reduced but it followed a negative linear relationship with external salinity. The  $\psi_\pi$

of *P. oceanica* leaves showed similar mean values in hypersaline treatments but they were significantly lower (10-15%) than the control mean values. In contrast, the  $\psi_s$  of *C. nodosa* leaves were quite similar between all experimental treatments. The  $\psi_p$  in *P. oceanica* leaves significantly decreased by 34% in the 41 and 43 psu treatments relative to the control and 39 psu treatments. Meanwhile, *C. nodosa* leaves experienced a more marked and significant decrease of  $\psi_p$  in all hypersaline treatments (40-61%) relative to the control mean values. Soluble sugar concentrations in *P. oceanica* leaves showed a close positive linear relationship with seawater salinity and reached mean values up to 34% higher than the control in the most severe hypersaline treatment of 43 psu. Soluble sugar concentrations in *C. nodosa* leaves significantly increased by 23-41% in the 39 and 41 psu treatments. In *P. oceanica*, only the amino acids Pro and Gly increased in all hypersaline treatments while some others (Trp, Asn and Asp) increased only in the 43 psu treatment. *Cymodocea nodosa* showed a general decline in the concentration of FAAs. Leaf growth rates and shoot numbers declined in *P. oceanica*, but no changes in these variables were found for *C. nodosa*.



**Fig. 1:** Water relations in leaves of *P. oceanica* and *C. nodosa*. The dotted line represents the osmolality of external seawater transformed to pressure units. Values are means  $\pm$  SE.

### Conclusion

The analysis of water relations and osmolytes highlighted different strategies adopted by *P. oceanica* and *C. nodosa* under hypersaline stress. *Posidonia oceanica* reduced leaf  $\psi_w$  by means of osmoregulatory processes as suggested by the decrease in  $\psi_s$  and the accumulation of soluble sugars and some FAAs. *Cymodocea nodosa* did not show osmoregulatory activity and more negative  $\psi_w$  values were reached through a significant decrease in  $\psi_p$ , which suggests that cell wall hardening processes (Tyerman, 1989) could be involved in the strategy of this seagrass species for coping with hypersaline conditions. The high metabolic cost of osmoregulatory mechanisms could account for the decrease in growth and survival rates in *P. oceanica*. Meanwhile, *C. nodosa* seems to be better adapted to respond to increasing salinity, which is in agreement with the ecology of this seagrass species, present in a wide variety of coastal environments with contrasting salinity regimes.

### Acknowledgments

Ministerio de Medioambiente y Medio Rural y Marino (OSMOGRASS Project Ref. 021/SGTB/2007/1.3) and the Ministerio de Ciencia e Innovación (OSMOGRASS II project Ref. CTM2009-08413MAR).

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## **MOLECULAR CHARACTERIZATION OF THE GENUS *CERAMIUM* ROTH (CERAMIACEAE, RHODOPHYTA) IN THE TRANSITIONAL ENVIRONMENTS OF THE VENICE LAGOON (ITALY)**

### **Abstract**

*The cosmopolitan genus Ceramium Roth (Ceramiaceae, Rhodophyta) is one of the largest and most systematically complex Rhodophyta group. Taxonomical problems are mainly tied to the unusually highly variable morphology observed inside the genus. In spite of this, Ceramium species inventory in many areas is based only on morphological observations and several classification problems are still opened. One of these areas is the Venice Lagoon, where we have recently sampled several Ceramium specimens. Through accurate morphological observations and phylogenetic analyses, based on the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase gene (rbcL) and the RuBisCo intergenic spacer (rbcL-rbcS spacer) as molecular markers, we identify three distinct Ceramium taxonomic entities, two of which never reported before in this environment according to the available checklists based only on morphology. The validity of the three entities as recognizable species is also discussed.*

**Key-words:** *Ceramium*, taxonomy, *rbcL*, *rbcL-rbcS* spacer, Venice Lagoon

### **Introduction**

The cosmopolitan genus *Ceramium* Roth (Ceramiaceae), with about 190 described species, is one of the largest Rhodophyta groups. This taxon is characterized by cylindrical or slightly compressed thalli, whose axial cells can be incompletely to completely covered by cortical cells, with alternate to pseudo-dichotomous branching, and straight to inrolled apices (Maggs & Hommersand, 1993). The nomenclature and the taxonomy of this genus are currently in a state of chaos. Taxonomic problems are, indeed, tied to a high degree of variation in the morphological characters classically used in species recognition. In particular, recent studies have questioned the validity of morphological features in order to discriminate different *Ceramium* species and, as the inventory of *Ceramium* species in some areas has been only based on morphological observations, more and more authors have started using molecular markers to accomplish this scope (de Barros-Barreto *et al.*, 2006; Maggs *et al.*, 2002; Skage *et al.*, 2005).

Here we combine morphological with phylogenetic analyses (*rbcL* gene and *rbcL-rbcS* spacer sequences) to identify different *Ceramium* samples collected from the transitional waters of the Venice Lagoon, where up to now the presence and biodiversity of this genus have been only based on morphological observations.

### **Materials and methods**

*Ceramium* specimens were collected between May and June 2010 along the Alberoni breakwater in the Venice Lagoon (Italy). Stereo microscope and light microscope observations of 4% formalin/seawater preserved samples or their sections were carried out. Amplifications of the plastid *rbcL* gene and *rbcL-rbcS* intergenic spacer were performed as described by Freshwater & Rueness (1994) and Freshwater *et al.* (1994). Phylogenetic analyses were carried out according to the maximum likelihood method

with the PHYML 2.4.4 program, applying the GTR+I+G evolutionary model. Non parametric bootstrap re-sampling was performed to test the robustness of the tree topologies (1000 replicates).

## Results

The collected Venice specimens clustered in three distinct clades in the *rbcL-rbcS* intergenic spacer tree. In particular, the partially corticated samples 1, 7, 10, 11 grouped with *C. tenuicorne*, *C. siliquosum*, *C. pallidum* and two *Ceramium* sp. specimens. The fully corticated samples 4, 5, 6 clustered with *C. botryocarpum* and *C. secundatum*. The spiny samples 2, 3, 8, 9 formed a clade that resulted sister taxon to *C. ciliatum*.

In the phylogenetic reconstruction based on the *rbcL* gene, samples 1, 7, 10, 11 clustered again together and with *C. pallidum*. Sample 2 resulted sister taxon to the above described clade. Samples 4 and 6 clustered together and grouped again with *C. botryocarpum* and *C. secundatum*. Unfortunately, for the moment, no *rbcL* sequences were obtained for samples 3, 5, 8, and 9.

## Discussion and conclusions

Our morphological and molecular analyses suggest that the partially corticated samples (1, 7, 10, 11) could be identified with *C. strictum sensu* Harvey (1849). Currently a valid name has not been determined for this species that, at the moment, is in synonymy with *C. tenuicorne* (Kützinger) Waern, but our phylogenetic reconstructions indicate that it could be a distinct species.

The fully corticated specimens (4, 5, 6) were morphologically identified as *C. nodulosum* (Lightfoot) Ducluzeau, 1806. The lacking of other *C. nodulosum* sequences in the public databases and the clustering of samples 4, 5, 6 with other corticated species in both the phylogenetic trees enforce their identification with *C. nodulosum* and also the taxonomic validity of this species, which is still questioned.

Finally, the morphology of the spiny samples 2, 3, 8, 9 lead to their identification as *C. ciliatum* var. *robustum* (J. Agardh) Mazoyer, 1938, but the phylogenetic results suggest that it could be a distinct species instead a variety of *C. ciliatum*.

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## **DONNEES SUR LA PROLIFERATION DE LA RHODOBIONTE ASPARAGOPSIS TAXIFORMIS (DELILE) TREVISAN DE SAINT- LEON SUR LES COTES NORD DE TUNISIE**

### **Résumé**

*En Tunisie, le genre *Asparagopsis* (Bonnemaisoniaceae, Florideophyceae, Rhodobionta) est resté longtemps uniquement représenté par *A. armata* Harvey. Depuis 2004, la souche invasive d'*A. taxiformis*, qui a été introduite en Méditerranée occidentale et dans l'Adriatique, se répand le long des côtes nord de Tunisie. L'espèce se développe dans l'infralittoral, sur différents substrats, meubles ou durs, et sur d'autres macrophytes, dans des sites ombragés à ensoleillés. Son abondance, dans les stations étudiées, a fortement augmenté depuis 2007.*

### **Abstract**

*For a long time, in Tunisia, the genus *Asparagopsis* (Bonnemaisoniaceae, Florideophyceae, Rhodobionta) was only represented by *A. armata* Harvey. Since 2004, the invasive strain of *A. taxiformis*, which was introduced into the western Mediterranean and the Adriatic Sea, is spreading along the northern coasts of Tunisia. The species is thriving in the sublittoral, on soft and hard substrates, and on other macrophytes, in low to high irradiance habitats. Its abundance in the study sites conspicuously increased since 2007.*

**Key-words:** *Asparagopsis taxiformis*, invasive species, distribution, biomass.

### **Introduction**

*Asparagopsis taxiformis* et *A. armata* sont considérés comme étant introduits en Méditerranée (Boudouresque et Verlaque, 2002). Sous le nom d' *A. taxiformis*, deux taxons cryptiques coexistent : un taxon peu invasif, décrit en 1813 en Egypte (Alexandrie), et qui reste à ce jour confiné au bassin oriental, et un taxon invasif qui a été récemment introduit en Méditerranée occidentale et dans l'Adriatique. Le premier taxon aurait une origine atlantique alors que le second a une origine indo-pacifique (Andreakis *et al.*, 2004, 2007 ; Ní Chualáin *et al.*, 2004). Sur les côtes tunisiennes, comme dans d'autres pays méditerranéens, le taxon invasif est fréquent dans l'infralittoral (Sartoretto *et al.*, 2008). En Tunisie, l'espèce présente sa croissance maximale au printemps. Ce travail s'est intéressé particulièrement à sa répartition le long des côtes nord de Tunisie et à son abondance dans la région de Bizerte.

### **Matériel et méthodes**

Les campagnes de prospection et d'échantillonnage ont été réalisées dans plusieurs stations réparties le long de la côte nord de Tunisie. L'évolution de la biomasse et du recouvrement d'*A. taxiformis* a été étudiée au printemps, de 2007 à 2009, dans la station de Rimel (Bizerte), à 1,5 m de profondeur. Les prélèvements ont été réalisés à l'aide d'un quadrat de ¼ de m<sup>2</sup> (3 répliqués).

## Résultats et discussion

*Asparagopsis taxiformis* est en constante progression le long des côtes du nord de la Tunisie. En 2009, l'espèce était présente sur divers substrats, durs et meubles, et sur d'autres macrophytes, au Cap Négro, La Galite, Bizerte, Rafraf, Ghar El Melh, Gammarth, Korbous et Sidi Daoud. Le suivi pluriannuel du recouvrement et de la biomasse d'*A. taxiformis* confirme son comportement invasif sur les côtes nord de la Tunisie (Tab. 1).

**Tab.1 : Recouvrement et biomasse moyenne (écart-type) du gamétophyte d'*Asparagopsis taxiformis* au printemps à Bizerte (Tunisie septentrionale). MS = masse sèche.**

Position géographique	Année	Recouvrement (%)	Biomasse (g MS/m <sup>2</sup> )
Station Rimel	2007	5-10	2,68 (0,95)
Longitude : 37°15'18''	2008	10-20	10,78 (4,73)
Latitude : 9°55'35''	2009	20-50	28,16 (8,84)

La prospection réalisée le long des côtes nord de la Tunisie nous a permis de dresser la liste des espèces associées à *A. taxiformis* :

**Chlorobionta** : *Bryopsis duplex* De Notaris, *Caulerpa prolifera* (Forsskål) J.V. Lamouroux, *C. racemosa* (Forsskål) J. Agardh, *C. racemosa* var. *cylindracea* (Sonder) Verlaque et al., *Cladophora prolifera* (Roth) Kützing, *Codium fragile* (Suringar) Hariot, *Codium vermilara* (Oliv) Delle Chiaje, *Halimeda tuna* (J. Ellis et Solander) J.V. Lamouroux, *Ulva linza* Linnaeus, *U. rigida* C. Agardh.

**Chromobionta** (Phaeophyceae) : *Colpomenia sinuosa* (Roth) Derbès et Solier, *Cystoseira brachycarpa* J. Agardh, *C. crinita* Duby, *C. mediterranea* Sauvageau, *Dictyopteria polypodioides* (A.P. De Candolle) J.V. Lamouroux, *Dictyota dichotoma* (Hudson) J.V. Lamouroux, *D. dichotoma* var. *intricata* (C. Agardh) Greville, *Padina pavonica* (Linnaeus) Thivy, *Sargassum vulgare* C. Agardh.

**Rhodobionta** : *Asparagopsis armata* Harvey, *Centroceras* sp., *Hypnea musciformis* (Wulfen) J.V. Lamouroux, *Jania rubens* (Linnaeus) J.V. Lamouroux, *Laurencia obtusa* (Hudson) J.V. Lamouroux, *Peyssonnelia squamaria* (S.G. Gmelin) Decaisne, *Sphaerococcus coronopifolius* Stackhouse.

**Magnoliophyta** : *Cymodocea nodosa* (Ucria) Ascherson, *Posidonia oceanica* (Linnaeus) Delile.

Il est nécessaire de continuer le suivi de la prolifération d'*A. taxiformis* en Tunisie.

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## **STRUCTURE AND DYNAMICS OF THE COMMUNITY OF *LITHOPHYLLUM STICTAEFORME* (J.E. ARESCHOUG) HAUCK IN THE CATALONIAN COASTS. PRELIMINARY RESULTS.**

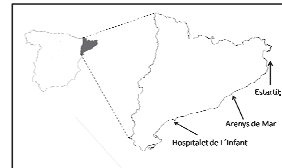
### **Abstract**

We present the preliminary results of a seasonal study of the *Lithophyllum stictaeforme* community in the Catalan coast. Samples were collected in three sites along the coast. The community was characterized by an encrusting stratum dominated by *Mesophyllum alternans*, *Lithophyllum stictaeforme* and *Neogoniolithon mamillosum* over which a big number of non calcified species develops. The mean number of taxa per sample was 35 (160 taxa recorded in total) and the mean cover 320.1 cm<sup>2</sup> (an 80%). The taxonomic group with higher coverage is the order Corallinales, whereas the order Ceramiales is the most abundant qualitatively. The best represented ecological groups are *Sciaphilous Infralittoral* and *Circalittoral (SIC)* and *Quiet Sciaphilous (SQ)* both in coverage and species number. In general, the community shows little seasonal variation, being the non calcified species the ones that show a high variability along the year.

**Key-words:** marine algae, benthic communities, coralligenous, *Lithophyllum stictaeforme*.

### **Introduction**

The *Lithophyllum stictaeforme* community is one of the most important components of the coralligenous in the Mediterranean. It's a very heterogenic community that shows its optimum growth at depths higher than 35 meters (Ballesteros, 1992). Although the coralligenous in general has been object of several studies there are very few studies concerning the *L. stictaeforme* community (Ballesteros, 1992; Silva *et al.*, 2008), undoubtedly due to the difficulty in the sampling procedure. In this paper, we provide information on a seasonal study of the *L. stictaeforme* community in the Catalan coast.



**Fig. 1: Study area.**

### **Materials and methods**

The study was carried out in three localities of the Catalan coast (Fig. 1): L'Estartit (Girona), Arenys de Mar (Barcelona) and Hospitalet de l'Infant (Tarragona). In each locality, two samples were collected in every season. Samples were taken by scraping off all organisms from a 20 x 20 cm<sup>2</sup> surface using a hammer and chisel. They are collected by SCUBA diving and preserved in 4% formalin seawater. The species were quantified as coverage in cm<sup>2</sup> (Ballesteros, 1992) and other parameters were considered following Boudouresque (1971). In the results, coverage and species number refer to the sampling area (400 cm<sup>2</sup>).

### **Results**

A total of 160 taxa (22 Chlorophyta, 29 Phaeophyta and 109 Rhodophyta) were identified in the community. The mean number of taxa per sample was 35 and the mean coverage was 320.1 cm<sup>2</sup> (80%). The community was characterized by an encrusting

stratum of calcareous algae, mainly dominated by *Mesophyllum alternans*, *Lithophyllum stictaeforme* and *Neogoniolithon mamillosum*, over which grows several species scarcely or not at all calcified. It is worth noting among them *ErythroGLOSSUM sandrianum*, *Aphanocladia stichidiosa*, *Rhodymenia ardissoni* and different species of *Peyssonnelia*. Qualitatively, the encrusting stratum is not significant (3 species (8 %) in average) but represents the major part of the community coverage (256.7 cm<sup>2</sup> (80.2%) in average). In contrast, the stratum consisting of non calcified species is qualitatively very important in the community (32 taxa (92%) in average) but relatively insignificant in terms of coverage (63.3 cm<sup>2</sup> (19.8%) in average) (Fig. 2). The taxonomic group with higher coverage is the order Corallinales (242.4 cm<sup>2</sup> (75.7%) in average), whereas the order Ceramiales is the most abundant qualitatively (14 taxa (40.6%) in average). The best represented ecological groups are Sciaphilous Infralittoral and Circalittoral (SIC) and Quiet Sciaphilous (SQ), both in coverage [145.9 cm<sup>2</sup> (45.6%) and 72.1 cm<sup>2</sup> (22.5%) respectively] and species number [4 (11.3%) and 7 (19.1%) species respectively]. The mean diversity (Shannon index) of the community is low (1.8). In winter the community appears scarcely developed, showing the lowest values of species number (28 species). In spring the number of species increases reaching their maximum in summer (44 species). In contrast, the coverage remains more or less constant along the year, varying between 312.5 cm<sup>2</sup> (78.1%) and 325.7 cm<sup>2</sup> (81.4%). Ceramiales (qualitatively) and Corallinales (quantitatively) are the taxonomical groups dominant in the community along the year. Likewise, SQ (qualitatively) and SIC (quantitatively) are the best represented ecological groups along the year (Tab. 1).

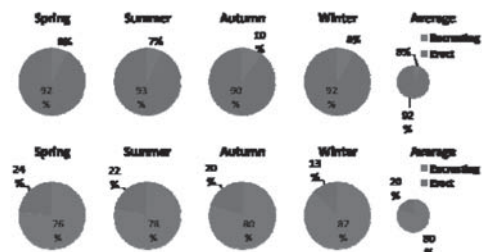


Fig. 2: Qualitative (lower) and Quantitative (upper) proportions of encrusting and erect algae.

Tab. 1: Resume table of the community parameters.

	Spring	Summer	Autumn	Winter	Average
Average Sp. Num.	39,47	44,75	35,4	28	35,255
Total Sp. Num.	93	107	94	54	86,5
Coverage (cm2)	325,7/393	320,7/75	312,4/4	325,45	320,053/208
Diversity	1,7	2,05	1,99	1,3	1,76
Tax. Group					
Corallinales (Sp. num./cov.)	2,3/238,5	2,5/240,1	2,8/238,1	1,7/252,7	2,3/242,38
Ceramiales (Sp. num./cov.)	13,3/38,5	15,3/38,9	15,2/35,5	15,3/41,01	14,3/37,6
Eco. Group					
SIC (Sp. num./cov.)	4,3/138,5	3,5/95,5	4/148,8	4,3/180,9	4,04/145,9
SQ (Sp. num./cov.)	6,7/54,3	7,8/63,5	7,6/59,5	3/73,9	6,5/72,1

### Discussion and conclusions

In general, the community of *L. stictaeforme* studied shows a low coverage and little seasonal variation. However, in winter both the number of species and diversity reaches their lowest values. This is mainly due to the non calcified species, which shows their minimum in this period and provides a great relative importance to the encrusting stratum. Our results agree with that of Ballesteros (1992) although the coverage is lower (80.2 % in comparison with 122.5 % according to this author).

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**TEMPORAL DYNAMICS IN TWO SEAGRASS SPECIES  
(*POSIDONIA OCEANICA* AND *CYMODOCEA NODOSA*) LOCATED  
CLOSE TO THE STRAIT OF GIBRALTAR  
(WESTERN ALBORAN SEA)**

**Abstract**

The seasonal variation in biometric features of two shallow (1-3 m depth) *Posidonia oceanica* (Linnaeus) Delile and two *Cymodocea nodosa* (Ucria) Ascherson beds was studied seasonally from July 2007 to March 2010 in the western coast of Málaga (southern Spain, Alboran Sea). This area is remarkable for being one of the westernmost points in the distributional range of *P. oceanica*. Both seagrass species displayed a seasonal trend throughout the years, like in other parts of the Mediterranean at similar depths. For *P. oceanica*, mean shoot height, leaf width and number of leaves per shoot varied seasonally in both areas with significant seasonal differences ( $p < 0.05$ ) and maximum values in autumn for leaf width, in winter for leaf number and in spring-winter for shoot height. Shoots with inflorescences were observed in March and November 2009, being the first time reported for this part of the Mediterranean. Some of the studied biometric features (i.e. shoot length ~39 cm) displayed lower values than those previously reported for other areas, whereas others were within the same range (i.e. shoot density ~1000 shoots  $m^{-2}$ ). For *C. nodosa*, mean values of most features were maximal in spring-summer (2700-4500 shoots  $m^{-2}$ ; 15-28 cm shoot length; ~3 leaves per shoot in Calahonda and Punta de Calaburras respectively), displaying significant changes according to season ( $p < 0.05$ ). Beds of both seagrasses are located in a highly built-up coastal zone and deserve urgent environmental conservation. This is particular important for *P. oceanica* because they represent some of the westernmost beds, displaying similar seasonal trends than other mediterranean beds including flowering events.

**Key-words:** *Posidonia oceanica*, *Cymodocea nodosa*, phenology, flowering, Alboran Sea

**Introduction**

*Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Anderson are the most important and widespread seagrass species along the coasts of the Mediterranean Sea. The growth dynamics and phenology of these two species have been widely studied throughout the basin in the last 30 years. However, there is a lack of data about the phenology of both seagrass species in areas close to the Strait of Gibraltar, where shallow fragmented and sparse meadows of both species are found, and where *Posidonia oceanica* reaches its westernmost distributional range.

**Materials and methods**

Sampling was done in two different sites: Punta de Calaburras (PC) (36° 30,4'N - 04° 38,3'W) and Calahonda (C) (36° 29,2'N - 04° 42,04'W), ca. 100 km to the Strait of Gibraltar. Phenological and flowering data were seasonally recorded from July 2007 to March 2010 in patches located at 2-3 m depth in both sites (5 quadrates of 50 x 50 cm in each site and season, 10 shoots in each quadrate for shoot morphology). The area of patches was measured and the density of shoots counted in quadrats of 0.25  $m^2$ . Number

of leaves per shoot, shoot height (from the base to the leaf tip of the longest leaf of the shoot) and leaf width (at the mid-point between the base and the leaf tip) were measured *in situ* in 10 randomly selected shoots within each quadrat. Statistical analyses (one-way ANOVA and Kruskal-Wallis test) were carried out for testing seasonal differences in *P. oceanica* and *C. nodosa* patches.

## Results

Mean shoot density, shoot height and number of leaves per shoot of *Posidonia oceanica* varied seasonally in both areas with no significant differences between sites ( $p>0.05$ ). Mean shoot density reached higher values in C ( $1055.2\pm 120.7$  shoots  $m^{-2}$  in autumn) than in PC ( $951.2\pm 73.1$  in summer). Maximum mean shoot height and leaf number were observed in spring ( $39.4\pm 7.9$  cm in C;  $38.7\pm 8.4$  cm in PC), and in winter ( $6.3\pm 1.06$  leaves shoot<sup>-1</sup> in C;  $6.1\pm 0.8$  in PC) respectively. Mean leaf width displayed maxima values in autumn ( $1.04\pm 0.07$  cm in C;  $1.06\pm 0.07$  in PC). Shoots with inflorescences were only observed in March 2009 in PC, and in November 2009 in both areas, with a density of inflorescences up to  $69.0 \pm 15.3$  inflorescences  $m^{-2}$  in C. *Cymodocea nodosa* displayed a shoot density with a seasonal trend, with higher mean values in PC (up to  $4592\pm 464.8$  shoots  $m^{-2}$  in summer) than in C ( $2731.2\pm 311$  in autumn). Shoot height displayed a seasonal pattern with a peak in summer ( $15.1\pm 0.7$  cm in C;  $28.4\pm 0.9$  in PC). The same trend was observed for the mean leaf number and leaf width in both areas, reaching the peak in spring-summer ( $3.04\pm 0.08$  leaves per shoot and  $2.42\pm 0.07$  mm of leaf width respectively in C;  $3.08\pm 0.07$  leaves per shoot and  $3.07\pm 0.03$  mm of leaf width in PC). No inflorescences were observed during the study.

## Discussion

The *Posidonia* meadows studied in the western Alboran Sea displayed seasonal patterns similar to those observed in other parts of the Mediterranean, but with lower values in some biometric features (Buia *et al.*, 1992). This could be probably due to the special oceanographic characteristics of this area, with cool waters (Atlantic and deep Mediterranean). The flowering events described here are the westernmost ones ever recorded for *Posidonia* (Urria *et al.*, submitted). Phenological parameters of *Cymodocea* followed a seasonal trend, reaching most of the highest values in spring-summer, as found in other parts of the Mediterranean (Cancemi *et al.*, 2002). Mean shoot density and leaf number was generally higher and lower than in other Atlantic and Mediterranean areas respectively, but mean shoot height and leaf width displayed similar trends. Conservational efforts should be made to preserve these seagrass meadows due to potential risks (i.e. illegal trawling, coastal constructions).

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## **ALIEN SEAWEED INTRODUCTION IN THE MEDITERRANEAN SEA: *HYPNEA FLEXICAULIS* (GIGARTINALES, RHODOPHYTA) FIRST FINDING BASED ON A MOLECULAR APPROACH**

### **Abstract**

*Hypnea flexicaulis* Yamagishi and Masuda (Gigartinales, Rhodophyta) is an economically important macroalga described for the first time in 2000 in some localities of Japan and collected also in Korea, Taiwan and Philippines. The species of the genus *Hypnea* are often difficult to discriminate on morphological grounds alone due to their high morphological variability among individuals of the same species. Here we report the first finding of the alien macroalga *Hypnea flexicaulis* in the Mediterranean Sea (Lagoon of Venice, Italy), identified through molecular analyses using the plastid ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*) and the mitochondrial protein-coding cytochrome *c* oxidase subunit I (*cox1*) genes. It is highly probable that this species has been introduced from Indo-Pacific populations, in particular the Korean one, via ship traffic or shellfish transfers, as also suggested by the phylogenetic analyses.

**Key-words:** alien macroalgae, *Hypnea flexicaulis*, Mediterranean Sea, *cox1*, *rbcL*.

### **Introduction**

Progress in molecular systematics has led to the use of DNA barcoding as a way to identify macroalgal species without clear morphological diagnostic criteria (Robba *et al.*, 2006). In particular, the mitochondrial *cox1* gene has recently proven to be a sensitive marker for revealing population structure and the hidden diversity of red algal species (Saunders, 2005).

The genus *Hypnea* Lamouroux, an economically important seaweed taxon including about 54 species, is abundant in intertidal and subtidal zones of tropical and warm temperate waters (Masuda *et al.*, 1997). Species discrimination in *Hypnea* is complicated by the high degree of morphological plasticity influenced by environmental factors (Masuda *et al.*, 1997). *Hypnea flexicaulis* was described for the first time by Yamagishi & Masuda (2000) from samples collected in Japan. The same species was also identified in Korea, Taiwan and Philippines (Geraldino *et al.*, 2006). Here we report the first finding of *H. flexicaulis* in the Mediterranean Sea based on the use of DNA barcoding combined with morphological observations.

### **Materials and methods**

Samples were collected in July 2009 in some localities of the Venice Lagoon and morphological observations were carried out by a stereo zoom microscope and a light microscope. For amplification and sequencing reactions of each gene we used specific primer pairs described by Freshwater & Rueness (1994), for *rbcL*, and by Geraldino *et al.*, (2006), for *cox1*. Identity of new sequences was checked by using the BLAST program (Altschul *et al.*, 1990). The phylogenetic analyses were performed according to the maximum likelihood (ML) method with the PHYML 2.4.4 program by applying the GTR+I+G evolutionary model. Non parametric bootstrap re-sampling was performed to test the robustness of the tree topology (1000 replicates).

## Results

Our samples matched the morphological description of *Hypnea flexicaulis* reported by Yamagishi & Masuda (2000), which is characterized by flexuous percurrent main axes and antler-like branches with wide branching angles showing abrupt abaxial bending. The partial plastid *rbcL* obtained sequences (741 bp) showed 100% identity with the sequences of 18 *H. flexicaulis* specimens collected by Geraldino *et al.*, (2006) in five different locations in Korea. There was only one base pair difference between our Mediterranean specimens and those from Taiwan, Philippines (Geraldino *et al.*, 2006) and Japan (Yamagishi & Masuda, 2000). The *cox1* obtained sequences (1470 bp) showed a percentage of similarity of 99% with those of 20 *H. flexicaulis* specimens recovered from Korea and Taiwan (Geraldino *et al.*, 2006). The sequence of the specimen from Philippines (GenBank EF136591), instead, differed from the Mediterranean samples in 33 bp (similarity percentage of 97%). The phylogenetic reconstruction based on *rbcL* + *cox1* multiple alignment and obtained from ML analysis showed that all specimens of *H. flexicaulis* from Venice, Korea, Philippines and Taiwan were included in a monophyletic group supported by a bootstrap value of 100%.

## Discussion and conclusions

The molecular results combined with the morphological features strongly suggest that our samples are identifiable as *H. flexicaulis*, a species never recorded before in the Mediterranean Sea. We can also hypothesize that our specimens have been introduced from the Korean populations. This is supported by the intraspecific divergence between the *cox1* gene sequences of our Mediterranean samples and that of the Philippines isolate and by the 100% identity of their *rbcL* sequences with those obtained from the Korean samples. Moreover the phylogenetic tree shows that the Venice isolates of *H. flexicaulis* match with all Korean specimens forming a separate clade.

The employment of molecular analyses could represent an easier and rapid way to identify alien algal species threatening the biodiversity of the Mediterranean Sea flora, so that appropriate precautions can be taken before they become invasive.

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## **TAXONOMIC DATA ON DIATOMS OF THE COASTAL LAGOON OF KARAVASTA**

### **Abstract**

*The present paper focuses on diatom flora of the Karavasta lagoon. A total of about 215 taxa were recorded in planktonic and epiphytic samples taken in different stations and habitats of the lagoon. Pennate diatoms were dominant to those centric in all samples. Most abundant species were: Cyclotella choctawhatcheeana, C. ocellata, Melosira nummuloides, Achnanthes amoena, A. brevipes, Amphora copulata, A. pediculus, Cocconeis scutellum, Fragilaria capucina var. vaucheriae, Navicula tripunctata, Neosynedra provincialis, Nitzschia closterium, Planothidium delicatulum, Striatella unipunctata, Thalassiosira weissflogii etc.*

**Key-words:** Diatom, Karavasta lagoon

### **Introduction**

Albania features large wetland areas along the Adriatic coast which have a very substantial economic potential for tourism, as breeding and refuge habitats for fish, and as valuable aquaculture sites. The most important coastal wetlands are Karavasta, Narta, Lezha, and Patoku on the Adriatic, and Butrinti on the Ionian coast. The lagoons extend along the coastline and are separated from the sea by rather narrow sandy spits, which continuously change in size and shape. They are generally characterized by brackish waters, being connected with the sea through one or more channels. Recently studies on ecology and taxonomy of brackish water diatoms have been intensified and supported by integrated environmental programs, in order to enhance knowledge on this group with a lot of importance as contributors in primary production of the lagoons.

### **Materials and methods**

The material was collected during spring and autumn 2008 at eight stations of the lagoon. The sampling mode was performed by using a planktonic net (mesh size 25 µm) or collecting periphyton. Samples were stored immediately in formaldehyde 4%. The cleaning of diatom frustules was done using the acid method (Krammer & Lange-Bertalot, 1986-2001). Permanent slides were mounted in Naphrax (index 1.71). Diatom analyze was performed using a NIKON ECLIPSE 600 light microscope with a NIKON camera system and an x100/1.25 oil-immersion objective. Diatom identifications were based on the works by (Krammer & Lange-Bertalot, 1986-2001; Witkowski, 1994; Witkowski *et al.*, 2000).

### **Results**

Microscopic observations revealed probably 215 species of diatoms, of which 20 taxa belong to centric diatoms and 195 to pennate diatoms. The genus *Cyclotella* (7 taxa), *Thalassiosira* (4) and *Stephanodiscus* (2) dominated among centric diatoms; while

amongst pennates most dominant were the genus *Nitzschia* (35), *Navicula* (33), *Fragilaria* (10), *Amphora* (9), *Mastogloia* (9), *Cocconeis* (8), *Cymbella* (7) and *Surirella* (6). The number of taxa per station varied from 25 in station 2 to 53 in station 5 and 7. It is significant the fact that pennate diatoms were dominant in all stations. The most abundant taxa were *Cyclotella choctawhatcheana*, *C. ocellata*, *Melosira nummuloides*, *Achnanthes amoena*, *A. brevipes*, *A. longipes*, *Amphora copulata*, *A. pediculus*, *Cocconeis placentula*, *C. scutellum*, *Fragilaria capucina* var. *vaucheriae*, *Navicula gregaria*, *N. tripunctata*, *N. trivialis*, *N. viridula*, *Neosynedra provincialis*, *Nitzschia amphibia*, *N. closterium*, *N. frustulum*, *Planothidium delicatulum*, *Striatella unipunctata*, *Thalassiosira weissflogii*.

### Conclusions

Most of the diatom species found in the sampling stations was widely distributed and characteristic mainly for brackish and marine waters. Only about 30 species belong to fresh water diatoms. We can also confirm the presence, in the Albanian Adriatic waters, of the centric diatom *Cyclotella choctawhatcheana*, recently found for the first time in the Croatian Adriatic waters (Buric *et al.*, 2007).

### Acknowledgements

The authors are grateful to Prof. A. Witkowski, Department of Paleocyanology, Institute of Marine Sciences, Szczecin, Poland for using his laboratory facilities and the assistance during a research visit, supported financially by Project TEMPUS CD\_JEP-17099-2002.

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## **PHENOLOGY AND BIOMASS OF THE SEAGRASS *CYMODOCEA NODOSA* (UCRIA) ASCHERSON IN THE GULF OF GABES**

### **Abstract**

*Cymodocea nodosa* (Ucria) Ascherson is one of the most common seagrass in the Tunisian coasts. However, studies on this seagrass are inexistent except of very scarce data.

Fieldwork is conducted in July 2009 in nine sites distributed along the Gulf of Gabes in shallow water (not exceed 0.5m of depth). Three sites in Kerkennah islands, two in Jerba Island, one site in Boughrara lagoon and two in Bibane lagoon are selected. Shoot density and recovery of *C. nodosa* meadows, number of blade per shoot, Leaf Area Index, and biomass are studied in each site.

**Key-words:** *Cymodocea nodosa*, Gulf of Gabes, phenology, biomass.

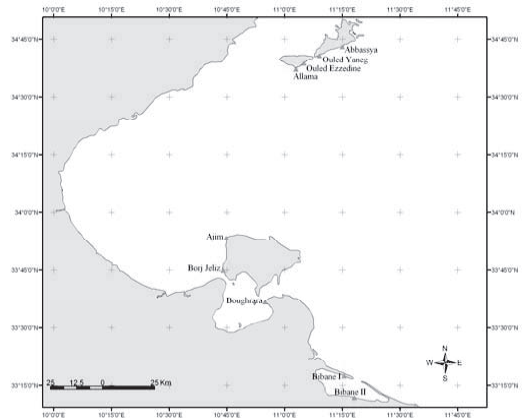
### **Introduction**

*Cymodocea nodosa* (Ucria) Ascherson is one of the most abundant seagrass in shallow water on the Gulf of Gabes in Tunisia (Ben Mustapha *et al.*, 1999); however studies on this seagrass are inexistent in this area. The aim of this study is to provide data about phenology and biomass of this seagrass in the Gulf of Gabes.

### **Materials and methods**

Sampling was carried out in July 2009 and concerned a total of nine localities distributed along the Gulf of Gabes coast (Fig. 1). In order to avoid time and depth-related effects, in each localities sampling was done in each of tem at a depth of 0.50 m. In each selected locality, shallow *C. nodosa* meadows covered the bottom beginning at 0.2m depth.

Shoot density was estimated *in situ* by counting the number of shoots present in a 20cm x 20 cm quadrat with ten replicates. Concurrently, twenty *C. nodosa* shoots were randomly collected. In the laboratory, the leaves of each shoot were removed and their length and the width measured. The Leaf Area Index, corresponding to the leaf surface area of *C. nodosa* for 1m<sup>2</sup> of surface, was also calculated. The biomass of *Cymodocea* leaves was estimated by weighing it after being dried in an oven at 70°C for 48h.



**Fig. 1: Sampling sites**

### **Results**

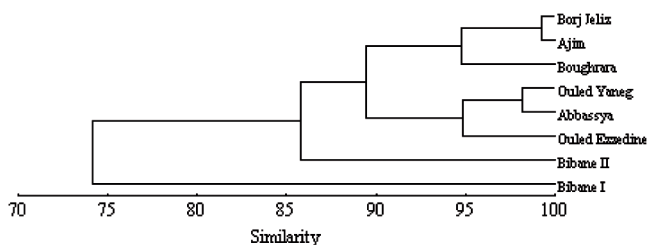
Table 1 summarizes different meadows features during this study. The highest value of shoot density has been observed at Ajim (814.40 shoots/m<sup>2</sup>), while Bibane II and Ouled

Ezzedine showed the lowest values with 204.80 and 256 shoots/m<sup>2</sup> respectively. The shoot densities were lower than those found in other localities (2000 shoot m<sup>-2</sup>, Pérez, 1989; 1925 shoot m<sup>-2</sup>, Cancemi *et al.* 2002; 2302 shoots m<sup>-2</sup>, Rismondo *et al.* 1997; 1520 shoots m<sup>-2</sup>, Agostini *et al.* 2003).

The Cluster Analysis in Fig. 2 based on the mean values, suggests that the *C. nodosa* meadows could be grouped into two categories: the first category is composed by three groups: Group I containing the three sites of Kerkennah island (Abbassya, Ouled Yaneg and Ouled Ezzedine), Group II containing the two sites of Jerba (Ajim and Borj Jeliz) and Boughrara site. The Group III is composed by Bibane II. The second category contains the meadows of Bibane I only.

**Tab. 1: Different meadows features in the Gulf of Gabes**

	Mean shoot density/m <sup>2</sup>	Mean blade length (cm)	Mean blade number/shoot	LAI (m <sup>2</sup> /m <sup>2</sup> )	Mean blade biomass (g/m <sup>2</sup> )
<b>Abbassya</b>	723.20±129.19	10.60±0.35	4.86±0.15	1.27	32.87
<b>Ouled Yaneg</b>	635.20±124.65	12.76±0.90	5.20±0.23	1.77	37.46
<b>Ouled Ezzedine</b>	256.00±218.06	12.24±0.82	5.45±0.20	0.75	18.00
<b>Ajim</b>	814.40±72.79	44.16±5.71	2.61±0.40	4.13	123.60
<b>Borj Jeliz</b>	657.60±57.19	39.58±2.91	4.20±0.39	1.18	98.00
<b>Boughrara</b>	776.00±97.30	23.07±7.92	5.00±0.55	4.50	169.17
<b>Bibane I</b>	307.20±52.64	43.00±0.24	4.69±0.24	6.02	154.96
<b>Bibane II</b>	204.80±50.95	37.94±0.28	4.64±0.28	1.37	28.98



**Fig. 2: Cluster analysis of *C. nodosa* of Gulf of Gabes**

### Conclusions

The data obtained reflected the general condition of the studied meadows, defining similar values for meadows with similar extension. The parameters considered in the period of major development of the plant, hence, resulted useful in the analysis and comparison of the health conditions of these important coastal ecosystems.

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## **PHENOLOGY AND GROWTH DYNAMICS OF THE SEAGRASS *CYMODOCEA NODOSA* IN BEKALTA (EAST OF TUNISIA)**

### **Abstract**

*The seasonal changes in the structure and growth dynamics of a Cymodocea nodosa meadow off the Bekalta (Monastir Bay, Tunisia) were studied from November 2006 to November 2007. Seagrass phenological parameters were monthly measured. Leaf and rhizome growth was estimated using marking methods. A clear seasonality was shown with maximum values of phenological parameters recorded in summer and minimum ones in winter. Shoot density ranged from 492.5±81.02 shoots m<sup>2</sup> in fall to 930±93.76 m<sup>2</sup> in summer. The leaf Plastochrone Interval varied from 11.89±1.29 days in summer to 62.22±0.20 in winter.*

**Key-words:** *Cymodocea nodosa*, dynamic, primary production, Bekalta, Tunisia.

### **Introduction**

Although *Cymodocea nodosa* (Ucria) Ascherson is widely distributed throughout the Mediterranean (Den Hartog, 1970), studies on the vegetative development of this seagrass are scarce particularly in the south side of the Mediterranean Sea. The aim of this paper was to assess the growth and the vitality of *C. nodosa* meadow (density, phenology, biomass and primary production) during the annual cycle 2006–2007.

### **Materials and methods**

Bekalta is located on the eastern coast of Tunisia with the following geographical coordinates: 35° 37' N and 11° 02' W. Sampling was performed from November 2006 to October 2007 mainly monthly except in summer season (2 samplings per month). Shoot density was measured in plots of 20x20 cm (10 replicates at each sampling event); the plant biomass was furthermore estimated using a metal cylinder of 15 cm diameter. In the laboratory, shoots, rhizomes and roots were separated and dried at 60°C to a constant weight. The length of leaves was also measured. Twenty shoots of *C. nodosa* were marked monthly 1 cm above the sheath of the oldest leaf according to Zieman, (1974) method to estimate its leaf production; in the laboratory, the newly formed tissue was measured by the shift of the marking hole along each leaf. The daily leaf elongation and the leaf Plastochrone Interval (PI) were calculated.

### **Results and discussions**

Shoot density showed a clear seasonal pattern ( $P < 0.001$ ), it's ranged from 492.5±81.02 shoots m<sup>2</sup> in fall to 930±93.76 m<sup>2</sup> in summer with maximum in summer (Fig. 1). The shoot densities were similar to other localities in Tunisia at the same depth (Sghaier *et al.*, 2008).

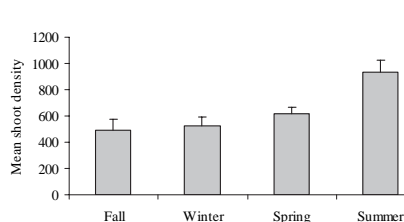
Table 1 summarizes different meadows features during this study. Growth of *C. nodosa* is continuous throughout the year with a unimodal cycle.

Concerning the biomass, leaves and roots showed opposite trends (Fig. 2). The maximum total biomass is reached in winter, whereas the minimum is observed in Fall. This model is already observed for the same seagrass species in marine Mediterranean

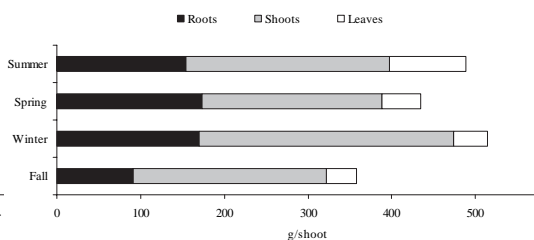
and Atlantic environments (Agostini *et al.*, 2003; Cancemi *et al.*, 2002; Reyes *et al.*, 1995; Terrados and Rós 1992).

**Tab. 1: Features of *C. nodosa* meadows in Bekalta**

	Fall	Winter	Spring	Summer
<b>Mean blade number/shoot</b>	5.10±0.24	4.25±0.27	3.80±0.44	4.55±0.33
<b>LAI (m<sup>2</sup>/m<sup>2</sup>)</b>	1.28±0.21	0.24±0.03	0.44±0.04	2.96±0.29
<b>Plastrone interval (day)</b>	13.79±3.38	62.22±0.00	45.00±0.00	11.89±1.29
<b>Daily leaf elongation (mm/shoot)</b>	11.46±1.35	0.53±0.28	2.88±0.79	9.57±1.42



**Fig. 1: Annual variation of *C. nodosa* mean density shoot in Bekalta**



**Fig. 2: Annual variation of *C. nodosa* biomass (roots, shoots and leaves)**

The primary production and biomass cycle of *C. nodosa* seems to be related to water temperature regimes.

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## **MACROPHYTOBENTHOS DES REGIONS DE BIZERTE, CAP-ZEBIB ET RAF-RAF (TUNISIE, MEDITERRANEE)**

### **Résumé**

Un total de 80 espèces, ont été identifiées : 47 Rhodophyta, 16 Ochrophyta, 15 Chlorophyta et 2 Magnoliophyta. Sur l'ensemble des espèces inventoriées, les Rhodophyta constituent le groupe le mieux représenté avec 59% de l'ensemble des espèces recensées. Une des espèces inventoriées est une nouvelle signalisation pour les côtes tunisiennes : *Acrothamnion preissii* (Sonder) E.M.Wollaston (Rhodophyta, Florideophyceae, Ceramiales, Ceramiaceae). Par ailleurs, le rapport R/P (Rhodophyceae/Phaeophyceae), calculé pour l'ensemble des sites étudiés est de 2.9 et reflète un caractère d'affinité tempérée, correspondant au bassin occidental. De plus, le regroupement des espèces inventoriées en fonction de leurs affinités biogéographiques, nous a permis de constater que l'élément Atlantique est dominant.

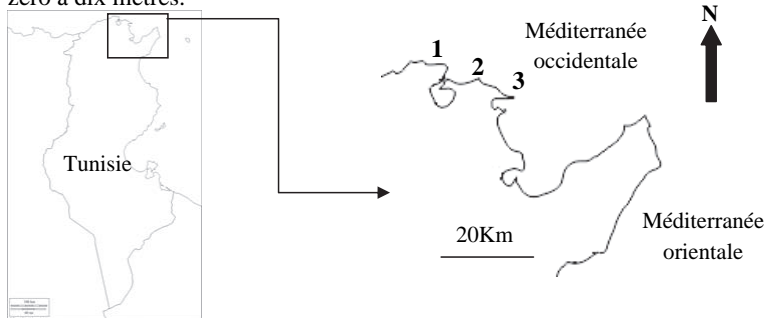
**Key-words:** Macrophytobenthos, biogeography, *Acrothamnion preissii*, Tunisia.

### **Introduction**

À cheval entre les deux bassins occidental et oriental de la Méditerranée, le littoral tunisien est largement ouvert à des influences variées de part sa position et la morphologie de ses côtes. Dans l'objectif de connaître la diversité de la macroflore benthique des côtes tunisiennes, nous avons entrepris l'étude des caractéristiques systématiques et écologiques du macrophytobenthos d'une partie du littoral tunisien.

### **Matériel et méthodes**

L'étude porte sur un ensemble de trois sites, répartis le long du littoral Nord et Nord-est des côtes tunisiennes : Bizerte, Cap-Zebib et Raf-Raf (Fig. 1), situés entre les parallèles 37°17'17'' et 37°16'57'' Nord et les méridiens 10°13'57.9'' et 10°14'10.5'' Est. Le secteur d'étude est généralement soumis à l'influence des courants atlantiques, il offre une côte présentant une succession de cap et de nombreuses anses. Du fait de cette morphologie particulière, les biocénoses de cette région sont très nombreuses. Les prospections ont été réalisées selon un plan d'échantillonnage mensuel, durant une période s'étalant de juillet 2005 à juillet 2006 (inclus) et à une profondeur variant de zéro à dix mètres.



**Fig. 1: Répartition des sites d'étude : 1 = Bizerte, 2 = Cap-Zebib et 3= Raf-Raf**

## Résultats

L'analyse de l'ensemble des prélèvements nous a permis d'identifier au total 80 espèces comprenant 47 Rhodophyta (59%), 16 Ochrophyta (20%), 15 Chlorophyta (19%) et 2 Magnoliophyta. Les Ceramiales constituent l'ordre dominant pour les Rhodophyta avec 59.6% (Tab. 1). Pour les Ochrophyta, les Dictyotales constituent l'ordre le mieux représenté avec 37.5% (Tab.1). Enfin, pour les Chlorophyta, les ordres dominants sont les Bryopsidales et les Halimedales avec 26.7% pour chaque ordre (Tab.1). Par ailleurs le rapport R/P (Rhodophyceae/Phaeophyceae), calculé pour l'ensemble des stations est de 2.9.

## Discussion et Conclusion

Le nombre d'espèces inventoriées dans ce travail (80 espèces) représente 19.32% de la flore marine connue à ce jour sur le littoral tunisien et reporté par Ben Maïz (1987). Une des espèces inventoriées est nouvelle pour la Tunisie, il s'agit d'une espèce introduite d'origine Indo-pacifique : *Acrothamnion preissii* (Sonder) E.M.Wollaston (Rhodophyta, Florideophyceae, Ceramiales, Ceramiaceae). Le rapport R/P qui est de 2.9, est identique à celui enregistré à Banyuls sur mer (Méditerranée nord-occidentale) (Boudouresque *et al.*, 1984), mais il est légèrement inférieure à 3.1, rapport enregistré pour le reste du littoral tunisien (Ben Maïz, 1995). De plus, le regroupement des espèces inventoriées en fonction de leurs affinités biogéographiques (Cormaci *et al.*, 1982), nous a permis de constater que l'élément Atlantique est dominant avec 45% suivi de l'élément Cosmopolite avec 34% puis de l'élément Méditerranéen avec 11%. Les espèces à affinité tropicale représentent, quant à elles, 10% et sont formées par 6% pour l'élément indo-pacifique et 4% pour l'élément pantropical. Enfin, d'après l'ensemble de nos observations, il apparaît que, même si la composition floristique de la région étudiée est un mélange hétérogène, constitué par plusieurs espèces d'affinités diverses, la macroflore benthique de la zone d'étude reste largement à caractère tempéré. Ceci pourrait-être expliqué par le fait que, appartenant au bassin occidental de la méditerranée, notre zone d'étude est soumise aux influences des eaux froides d'origine atlantique.

**Tab. 1: Dominance des différents ordres des (a) Rhodophyta, (b) Chlorophyta et (c) Ochrophyta**

<b>a</b> Ceramiales	59.6%	<b>b</b> Bryopsidales	26.7%	<b>c</b> Dictyotales	37.5%
Corallinales	12.8%	Cladophorales	13.3%	Ectocarpales	12.5%
Erythropeltidales	6.4%	Dasycladales	13.3%	Fucales	18.75%
Gigartinales	12.8%	Halimedales	26.7%	Scytosiphonales	12.5%
Nemaliales	4.2%	Syphonocladales	6.7%	Sphacelariales	18.75%
Rhodymeniales	4.2%	Ulvales	13.3%		

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