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## 4. Morpho-functional study and transplantation essays of *habitat forming species*

### Research unit

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### Introduction

The colonisation of macrophytobenthos on artificial reefs has often been considered to be marginal when compared to that of organisms economically more important (Falace & Bressan, 2000; Miller & Falace, 2000), yet both are interrelated. Moreover biodiversity does not only vary depending on the physical nature of the environment, but the biotic community itself may influence the physical features of the habitat and, therefore, the distribution and the abundance of species (Ray *et al.*, 1997).

In particular, at a small spatial scale of observation ( $10^{-2}$ ,  $10^{-3}$ m), the development of canopy-forming algae can promote spatial heterogeneity of coastal rocky bottoms, leading to an increase of additional habitats and providing 3-dimensional complexity (Hicks, 1980; Dean & Connell, 1987; Gibbons, 1988; Jenkins *et al.*, 1999; Benedetti-Cecchi *et al.*, 2001; Chemello & Milazzo, 2002).

Canopy algae (referred as keystone species *sensu* Paine, 1969 or ecological engineering species *sensu* Jones *et al.*, 1994) play a fundamental role in affecting the understory assemblages, by modifying physical factors such as light (Reed & Foster, 1984; Kennelly, 1989; Figueiredo *et al.*, 2000; Melville & Connell, 2001) desiccation (Menge, 1978; McCook & Chapman, 1991), hydrodynamism (Hawkins, 1983; Duggins *et al.*, 1990; Jenkins *et al.*, 1999) and sedimentation rate (Hawkins, 1983; Jenkins *et al.* 1999; Bulleri *et al.*, 2002; Airoldi, 2003; Piani *et al.*, 2004). Furthermore, a good relationship exists between algal structural complexity and epibiotic diversity/abundance (Hicks, 1980; Edgar, 1983; Dean & Connell, 1987b; Gibbons, 1988; Russo, 1997; Aarnio & Mattila, 2000; Hernández-Carmona *et al.*, 2000; Chemello & Milazzo, 2002). These species may affect the density and efficiency of predators, providing shade and shelter for diversified assemblages of animals and plants, and modulate the structure of the biotic community, modifying rates of recruitment and post-settlement mortality of epibionts (Hicks, 1980; Edgar,

1983; Dean & Connell, 1987; Menge & Sutherland, 1987; Gibbons, 1988; Belegratis & Bitis, 1989; Jones *et al.*, 1994; Russo, 1997; Aarnio & Mattila, 2000; Hernández-Carmona *et al.*, 2000; Bulleri *et al.*, 2002; Chemello & Milazzo, 2002; Piraino *et al.*, 2002).

In the Mediterranean Sea the genus *Cystoseira* (Fucales, Phaeophyta), characterised by a large perennial dendroid thallus, are widely distributed in shallow and sublittoral waters (Boudouresque, 1971; Ribera *et al.*, 1992; Russo, 1997; Bulleri *et al.*, 2002). The complex morphology of stipes and fronds of members of the *Cystoseira* group, offers rich and diverse substrata for the attachment of benthic fauna (Russo, 1997). At the same time, this habitat complexity also permits an epiphytic stratification similar to that found in vascular plants (Rull & Gomez Garreta, 1989; Otero-Schmitt & Pérez-Cirera, 1996; Belegratis *et al.*, 1999).

The importance of macroalgae in processes leading to the evolution of biotic communities has led to a search for technical-engineering solutions aimed at optimizing the algal cover on artificial reefs (Falace & Bressan, 1997; 2000a; 2002; Miller & Falace, 2000) and on natural substrata for mitigation/restoration purposes. For instance, algal colonisation may be induced by means of the inoculation of spores or transplantation of juvenile or adult thalli in reproductive phase (Carter *et al.*, 1985a; Yoshida *et al.*, 2004; Heise & Bortone, 1999; Dean & Jung, 2001; Hernández-Carmona *et al.*, 2000). Such experiences have been accomplished in USA and Japan on artificial reefs using both spore cultures together with kelp transplantation and the employment of plastic algae (McPeak, 1977; Dayton *et al.*, 1984; Carter *et al.*, 1985b; Rice *et al.*, 1989; Dean & Deysher, 1994; Patton *et al.*, 1994; Vasquez & McPeak, 1994; Hernández-Carmona *et al.*, 2000; Deysher *et al.*, 2002; Godoy & Coutinho, 2002; Holbrook *et al.*, 2002; Terawaki *et al.*, 2003).

In the Mediterranean Sea only few studies have been conducted on macroalgae transplantation (Gros & Knoepffler-Peguy, 1978; Susini *et al.*, 2004) and most of them have been carried out for aquaculture purposes (Falace & Bressan, 1997).

## **Objectives**

The study deals with two species, *Cystoseira barbata* (Stackhouse) C. Agardh and *Cystoseira compressa* (Esper) Gerloff & Nizamuddin, characterized by different life-history strategies which, for their wider tolerance towards environmental stress, could contribute to the recolonisation and restoration of damaged habitat.

During the present research the following aspects were considered:

Phenological analyses, to determine the variability of diacritic characters and to evaluate the growth and reproduction of *C. barbata* and *C. compressa*;

Morphogenesis of *C. barbata* by means of sporeling cultures to investigate the feasibility for the transplant of juveniles.

Transplantation of adult thalli of *C. barbata* and *C. compressa*, with innovative techniques to increase the vegetal colonization and to improve habitat enhancement procedures as a restoration tool. These procedures applied to artificial reefs may increase the primary productivity and, thus, the secondary productions.

Structural complexity evaluation of *habitat-forming species*, to characterize the habitat former architecture. The analysis conducted on the algae complexity may also find a useful application in the design of artificial plants that mimic these morphologies (artificial algae) for projects aimed at saving and restoring the environment.

## **Materials and methods**

### Sampling area

Sampling was carried out at two locations (Fig. 4.1) with different hydrodynamic and bottom sediment characteristics:

Izola (lat. 45°32'41.3", long. 13°40'39.2") on exposed rocky low-shores;

Muggia (lat. 45°35'58", long. 13°46'19") in a sheltered shallow sandy zone.

Samples were taken monthly for one year (April '02 - March '03), apart from June, August and December, due to international bureaucratic impediments.

### Phenology

To describe the phenology of *C. compressa* and *C. barbata*, during each sampling, 3 thalli of each species were taken randomly and fixed in a 4% formalin seawater solution. For every thallus the morphology was described and the following measures were carried out:

#### *C. compressa*:

- the overall height from the basal disc to the apex of the frond;
- the length of 10, randomly chosen, I branches;
- the diameter of 10 I branches, at 1cm from the ramification from the cauloid and at half the length of the branch;
- the length and diameter in the middle of the axis of 5, randomly selected, II branches.

#### *C. barbata*:

- the overall height from the basal disc to the apex of the frond;
- the length of 10, randomly chosen, I branches;
- the length of 5, randomly selected, II branches.
- the length of 5, randomly selected, adventitious branches.

For both species the reproductive phenology was analysed highlighting the presence of fertile conceptacles.

### Sporeling cultures

On the basis of previously phenological studies conducted, I branches from 10 fertile thalli were collected in May '04 at Izola and Muggia from the natural population, refrigerated in sea water and taken to the laboratory. In order to obtain monospecific cultures the mature receptacles were separated from the primary branches, accurately washed with sterile filtered sea water (FSW) and cleaned from sediment and epibionts. They were then placed in sterilised Petri dishes using slides as a support for the zygotes. These slides were kept well above the bottom of the dishes by means of glass supports in order to avoid the formation of anoxic and stagnant zones (Fig. 4.2a - 4.2b). The dishes were then placed in culture chambers at 16-17° C with a photoperiod of L:D 12:12 (120mmol fot m<sup>-2</sup> s<sup>-1</sup>) (Fig. 4.3). The Von Stosch (1963) medium culture diluted to 50% with FSW was replaced every three days and enriched with GeO<sub>2</sub> (2mg l<sup>-1</sup>) to inhibit diatom contamination (Lewin, 1966). Samples were observed daily during the first stages of development and segmentation of the zygote, and weekly in the following period.

### Transplantation

The investigation was carried out at Izola, using adult thalli of *C. barbata* and *C. compressa*. During the first part of the investigation different methodologies were tested in order to choose the most suitable for the morphology of the two species. Thalli were removed from parent populations, marked with a numbered tag, and fixed with several types of non-toxic adhesives and resins onto artificial units (made up of four bricks hooked up to a plastic box) (Fig. 4.4). The success of the *Cystoseira* transplants was defined as thalli survival rate.

Following the results of preliminary surveys the transplantation techniques chosen were: 1) polymat-box units: the base of *C. barbata* was covered in polyurethane foam before placing it securely into the holes of the bricks; 2) hooking mat-box units: the *C. compressa* holdfasts were attached using arched hooks, previously anchored on cubes of cork, fitted into the holes of the bricks.

In March '02, 24 reef units for each species were arranged at Izola into which 288 thalli were transplanted. The length of the plant from the holdfast to the apex of each transplanted thallus was measured before placing it into the transplantation unit. The modules were randomly interspersed among the respective parent population at a depth ranging from 2 to 4m.

From April '02 to March '03 the growth and survival rates of the sporophytes were monitored by collecting randomly three thalli from the natural and artificial populations of both species. International bureaucratic impediments did not allow us to sample in August and December '02. Samples were fixed in a 4% formalin seawater solution while loose, naturally occurring, thalli were counted and the

position recorded. Morphometric and thalli surface area measures were recorded in order to compare the natural and artificial populations.

#### Analysis of the complexity

To quantify the substratum geometry some architectural characteristics were measured as structural (total area, perimeter) and spatial (intercepting area, volume and interstitial area) attributes. To calculate the intercepting area of a 3-dimensional substratum of irregular shape we used an original method deploying a square-based glass prism, on the base of which a framework was placed; this permitted rotation of the thallus by 45° each time with respect to the longitudinal axis (Fig. 4.5). The shape of the thalli was then analysed in 3-dimensions, from 4 non-specular perspective angles. A reticulum, with a mesh dimension of 1cm, was placed on two sides of the prism and the percentage cover in each grid was estimated. The data from the 4 readings of each sample were averaged. The matrices obtained were elaborated using the software Surfer. Thus, the 3-dimensional complexity of the sample and the intercepting area was summarized in a plane-image. These images were further elaborated, grouping the areas of equal “probability of presence” in 5 classes of cover: 1 (d 20%); 2 (21 - 40%); 3 (41- 60%); 4 (61 - 80%); 5 (e 81%). The percentage of each class in each image was calculated using Adobe Photoshop 6.0 software.

The *areas* and the *perimeters* of the collected thalli were measured by scanning images of each specimen. The procedure utilized was different for the two species: *C. compressa* was laid directly onto the scanner glass (Fig. 4.6) while *C. barbata*, which show a thick epiphytes cover, was analysed inside the prism. For each sample two images were scanned rotating the thallus 90° inside the prism. The images were acquired at 600 dpi, on a grey scale to avoid possible interference due to the water column located between the scanner sensor and the object. The digital images were elaborated by means of Adobe Photoshop 6.0.

The *volume* of each thallus was measured by placing the alga in a graduated cylinder containing water, and determining the volume of water displaced. The volume provided information on the size of a body and an indirect appraisal of the primary production of the investigated populations using a non-destructive methodology. For *C. barbata* this measure was conducted firstly by analysing together the basiphyte-epiphyte system and then the single components, after careful removal of any visible epiphytes.

Using the collected data, the following bio-structural indices of the thalli were analysed, each being considered as useful for the evaluation of potential microhabitats available for the colonisation of epibionts and substrate microtopography:

-*Fragmentation Index* (CP/A). This index was proposed by Austin (1984) to compare figures that do not present a regular geometrical shape. The correction of the ratio Perimeter/Area ( $CP/A = 0.282 \text{perimeter}/\sqrt{\text{area}}$ ) enables the comparison of the irregular surfaces. This index was applied to both species.

Only for *C. barbata* other two indices have been proposed:

-*Interstitial Area Index* (cm<sup>2</sup>) (IA), that is related to the amount of free space within the fronds. It was calculated as:  $IA = \text{total area}/\text{sum of the interstitial areas}$ .

-*Surface Complexity Index* (SC) refers to the degree of roughness or irregularity of the surface of a structure. It was calculated, from the digital images, as:  $SC = \text{Perimeter}/\text{Tangent Rectangle Perimeter}$ .

## Results

The main results of the research may be summarized as it follows:

### *C. compressa*

*C. compressa* is a caespitose plant attached to the substratum by a small disc. From November to January the analysed samples have a “rosette-shaped” form, characterized by flattened I branches, smooth borders and rounded apex. The II branches are short, distichous and alternate. In January the ramification appears denser near the apex and sparse in the basal portion of the thallus; the I and II branches are still flattened. In February-March the examined thalli have a transitory shape, with the coexistence on the same plant of 2 different typologies of I branches: - short and flattened like in the winter form; - lengthened and thin towards the upper 3/4 of the axis. Near the apex of the fronds, the II branches are thin and thick, ramified, and disposed on more layers, whereas at the base they are alternate-distichous.

In April and May the thallus changes its bearing, becoming erect and densely ramified, especially in the apical region. The II branches become longer, thinner and ramified. In July some thalli present roundish I branches with a bare section in the basal portion above which the II branches are less densely ramified. At the end of summer-beginning of autumn the thallus regain the winter “rosette-shaped” bearing; the change occurs with the fall of older branches and the birth of new ones with a flattened portion of relatively growing importance, and alternated distichous II branches.

The average monthly length of the thalli analysed ranged from a minimum 54mm in November to a maximum 448,3mm in May. The I branches (Tab. 4.1 - Fig. 4.7) were more developed in the April-July period (average length: 209,0mm) with a maximum in May (324,3mm) and a minimum in October (24,9mm). The

morphological variations highlighted were characterized also by changes in the measurements of the I branches diameter. In winter, when the photoperiod length is shorter, the thallus seem to present a mechanomorphic adaptation that determines the presence of broad flattened branches which offer a larger surface for the radiant flux. The diameter of the I branches at 1cm from the ramification from the cauloid varied between a minimum of 1,9mm in July and September to a maximum of 2,7mm in November and March (Tab. 4.1 - Fig. 4.8). A similar pattern may be observed for the diameter values at half the length of the I branch: the minimum (1,5mm) was reached between April and July, when the branches are cylindrical, while the maximum (2,7mm) was observed between October and November, when the thallus gains the winter *habitus*. The length/diameter ratio of the II branches followed the same trend recorded for I branches (Fig. 4.9).

The *C. compressa* thalli of the studied population were fertile in the period May-July (Tab. 4.1 - Tav. 4.1).

As far the *intercepting area* was concerned (Fig. 4.10) in the natural population the spatial density value was over 61% (classes 4 and 5), with the exception of November and January when the thallus gained the “rosette” shape. During this phase the density values did not exceed 40% and were located at the base of the caespitose cauloid, where the overlapping of the branches is higher. At the beginning of spring, following the development along the central-basal area of the thallus of I and II branches, spatial density value over 60% was recorded (class 4). In April and May when the frond is characterized by a high structural complexity and vegetative development, the *intercepting area* exceeds 80% (class 5).

The areas average monthly values of *C. compressa* are reported in Tab. 4.1.

To evaluate the transplantation efficiency mean values of algae areas, belonging to the natural and artificial population, were compared. All individuals transplanted at Izola immediately reduced in size relative to controls (April D= 366,5cm<sup>2</sup>; May D= 770,6cm<sup>2</sup>). ANOVA performed on *C. compressa area* showed significant differences between natural and transplanted thalli ( $F_{1, 20}=24,98$ ;  $p < 0,001$ ). The survival rate of the thalli of *C. compressa* transplanted on modules was 25%.

The volumes average monthly values of *C. compressa* are reported in Tab. 4.1 and Fig. 4.11. For both populations the highest values were recorded in April (natural: 41,6cm<sup>3</sup>; artificial: 11,6cm<sup>3</sup>) and May (natural: 62,3cm<sup>3</sup>; artificial: 12,4cm<sup>3</sup>); the lowest were recorded in January (natural: 0,9cm<sup>3</sup>; artificial: 4,9 cm<sup>3</sup>). The volume, used as an indirect estimation of the biomass, confirmed that the primary production was higher in the natural population compared to the artificial one.

***C. barbata***

The thallus of *C. barbata* is characterized by a perennial, monoaxial cylindrical cauloid with a smooth and prominent apex, small aerocysts and receptacles terminating in a mucron (Tav. 4.2). The I and II branches, densely ramified, reached their maximum development in spring; the adventitious ones, at the base of the cauloid, are generally shorter. The receptacles along the II branches are simple or cylindrical, generally pedicellated and could sometimes overlap one or two aerocysts (Tav. 4.2).

From July the apices of analysed thalli were less prominent, the I branches reduced in length and presented a great number of short and thin II branches. The aerocysts in the apical region of the II branches appeared isolated. At the end of summer the I branches further reduced in length and the II ones were filiform and sterile; in September-October the older branches fell and the I branches progressively became sterile with short, lanceolate or cylindrical receptacles. At the beginning of winter the I branches were more developed, with aerocysts isolated or arranged in chain and numerous cylindrical and pedicellate receptacles. The oldest I branches were scarcely ramified and presented reduce-sized lanceolate receptacles.

The phenology described for the samples of the Gulf of Trieste partially coincide with those reported by Marzocchi *et al.* (2003) for the Venice lagoon; particularly the samples studied in the Gulf are less developed, probably due to the different hydrodynamic conditions.

The thallus height, which in this species is mainly due to the cauloid length, ranged from a minimum of 13,2cm in September to a maximum of 40,5cm in April, with an annual average value of 23,1cm (Tab. 4.2 - Fig. 4.12). The I branches were more developed in April (average length: 13,3cm), while the minimum were recorded in October (1,7cm) (Fig. 4.13).

The II and adventitious branches presented a similar seasonal, with a maximum in March-April (II branches: 5,5cm, adventitious: 6,4cm). Starting from July, before the vegetative resting period (September-November), it is possible to highlight a gradual decrease in their length. The minimum values were recorded in September-October (II branches: 0,5cm, adventitious: 1,1cm) (Fig. 4.13).

During the vegetative resting period the spatial density values, reached values of cover ranging between 20-60% (class of cover 2 and 3) in the central basal area of the thallus, due to the perennial cauloid. In March (Fig. 4.14), with the branches growing, the recorded cover density values were over 80% (class 4 and 5). In April and May, because of the expansion of the frond, the areas with the highest spatial density (covering 60-100%) were found in the central part of the frond. The classes with a lower cover, where the branching was less dense, occupied a wider area. The



3-dimensional structures of all the samples are summarized in Fig. 4.15. The larger class of cover (class 5) was found in March-April in both populations, and in May in the transplanted thalli, when the species reached its maximum architectural complexity.

The *C. barbata* areas are reported in Tab. 4.2. The highest average values were measured in April (artificial: 391,3cm<sup>2</sup>; natural: 443,4cm<sup>2</sup>), May (artificial: 436,8cm<sup>2</sup>) and March (natural: 553,3cm<sup>2</sup>); the minimum average values were recorded in September (artificial: 45,6cm<sup>2</sup>; natural: 43,5cm<sup>2</sup>).

The *C. barbata* CP/A are reported in Tab. 4.3: the highest monthly average values were associated with the samples with an irregular thallus; the lowest values were reached when the shape was more compact.

In view of the fact that the values of the bio-structural indices did not vary seasonally, but depended exclusively on the structure of the frond and on the morphological variability of different individuals, only a few cases are given below as examples:

- Interstitial Area Index (IA): to explain the range of this index two characteristic examples of the process of evolution of the frond were chosen and compared (Fig. 4.16). In the case of the thallus showed in Fig. 4.16a, characterised by a developed frond with several spaces between fronds, IA= 11. On the contrary, the thallus in Fig. 4.16b, characterised by the absence of the frond, therefore not revealing any holes, IA = 113.

- Surface Complexity Index provides the degree of roughness or irregularity of thallus outline (Fig. 4.17). In September (Fig. 4.17b), in thalli characterised by the absence of the frond and by the presence of the ramified perennial cauloid, the surface complexity value was 0.13, while in April (Fig. 4.16a), when the thallus had the typical dendroid branched frond arising from the cauloid, it reached 0,50.

The monthly averages volumes of the basiphyte (Tab. 4.2 - Fig. 4.18) were higher in April (34,1cm<sup>3</sup>) and May (39,9cm<sup>3</sup>) in the transplanted population, and in April (38,3cm<sup>3</sup>) and March (62,7cm<sup>3</sup>) in the natural one. As far as epiphytes were concerned, the highest average values were recorded for both populations in the spring period with a peak in March in the natural population (79,6cm<sup>3</sup>). The contributions to the basiphyte-epiphyte system of the epiphytes varied between 40 and 70%. In February, for example, the average volume of epiphytes (32,4cm<sup>3</sup>) removed from the thalli of the natural population was almost double that of the basiphyte (14,3cm<sup>3</sup>).

As far as the potential available area for the colonization of epibionts is concerned, the annual average value of the surface of *C. barbata* was 194cm<sup>2</sup> and of *C. compressa* was 162,6cm<sup>2</sup>. Six thalli were fixed on every transplantation unit, which

mean that, theoretically, the annual average increase of the available area for the colonization of epibionts is of 1164cm<sup>2</sup>/module for *C. barbata* and 975,7cm<sup>2</sup>/module for *C. compressa*. Since the annual average volume of the epiphyte on each thallus of *C. barbata* was 23,28cm<sup>3</sup> the annual average theoretical estimation of epiphytes for each unit with 6 thalli of *C. barbata* is 139.68cm<sup>3</sup>. The highest theoretical increment is 398,38cm<sup>3</sup>, when the thallus reach its greatest development.

The survival rate of *C. barbata* transplanted thalli with the polymat-box technique is ca. 80%. The sporophytes of both populations showed a seasonal development and no significant differences were highlighted between the thalli belonging to the two populations (ANOVA:  $F_{1,36} = 0,78$ ,  $p = 0,38$ ).

With regard to the *C. barbata* sporeling culture, after about 24h the oospheres were fecundated with the formation of zygotes. The adhesion to the substrata was observed after 5-6h after fecundation. After 4-5 days, the formation of embryos was complete and after 1 month they took on an erect carriage. During the second month of culture the young plants were completely erect and they gained the maximum development (200-250µm). After four months of observation the plants were completely developed, gaining a height of 10-25mm and showing all the adult structures, but they remained floating in the culture medium (Tav. 4.3).

### Conclusions

In the Gulf of Trieste *C. barbata* and *C. compressa* showed a significant morphological plasticity due to the seasonal development of the frond, which is very changeable in size and appearance in relation to the principal environmental parameters.

The spatial and structural attributes analysed, as well as the definition of the architectural complexity of *Cystoseira*, permitted the evaluation of the area available for the attachment and shelter of epibionts together with the number and type of potential habitats. The representation of 3-dimensional plant architecture provides replicated and standardisable measures of algal structure, in order to develop a broader understanding of the role of canopy species in the creation and maintenance of habitat diversity. Furthermore, the indices proposed may enable the evaluation of degrees of complexity and a classification of habitat-former species complexity in comparative studies.

After one year of survey, the transplanting methods applied seemed to be suitable for *C. barbata*, while they were not successfully adapted for *C. compressa*.

The poor success obtained for *C. compressa* is most likely due to the cauloid structure of this species. In fact, *C. barbata* thalli are characterised by a monopodial

development, while *C. compressa* thalli have a sympodial development with a cauloid more or less densely ramified. Transplantation time plays a pivotal role in determining the ability of species to colonize the artificial substratum with new individuals, and thus it must be taken into account when artificial seaweed beds are designed and planned. The best period for the biogeographical area under examination takes place in spring when thalli are fertile. In fact, the artificial modules were re-colonised by juvenile plants of *C. barbata* after some months.

Transplantation units, with reproductive thalli arranged on new artificial reefs, could reduce the time of colonization and facilitate the achievement of canopy maturity.

Furthermore, the transplantation of juveniles may represent a good alternative to the traditional destructive methodologies, which require the removal of adult individuals or part of them from the natural site.