Conservation and management

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■ Italian and international laws on protection

Neptune grass flower

All the species of Italian phanerogams growing in marine and/or brackish waters are considered deserving of protection as species and/or habitats, and are listed in international agreements and conventions.
Unfortunately, in the Habitats Directive, adopted by Italy with the "Regulations implementing EEC Directive 92/43 relating to the conservation of natural and semi-natural habitats, as well as the wild flora and fauna, D.P.R. 357 of 08.09.1997 (G.U. 248 of 23.10.1997)", the only marine phanerogam cited is *Posidonia oceanica*, and as a habitat (Annex I) and not as a species (Annexes II and IV). This is an enormous problem, because Directive 92/43 is the most important and cogent law for nature conservation as it is the only one with true sanctioning powers. The 1979 Berne Convention on wildlife and the natural environment in Europe was ratified by Italy in 1981 (Law 503 of 05/08/1981), but its Annexes regarding plants were only modified in 1996 (implemented by Italy in 1998) with the insertion of various species, including *Cymodocea nodosa*, *Posidonia oceanica* and *Zostera marina*. These were inserted in Annex 1, covering strictly protected plant species, for the conservation of which it is also necessary to protect the habitats in which they grow. It is the only convention, for the moment, in which *C. nodosa* is also considered as a species to be strictly protected.
A fundamental contribution towards surmounting the shortcomings of the Habitats Directives in the marine environment is provided by the Barcelona Convention (1995), which contains various protocols, including the "Protocol related to specially protected areas and the biological diversity of the Mediterranean" (SPA/BIO). This is entirely innovative and not limited to territorial

Dumping sediment into the sea clouds the water and creates problems for Neptune grass meadows waters. Each signatory country of the Barcelona Convention is asked to create specially protected areas for the conservation of habitats and species, and the creation of a SPAMI (Specially Protected Area of Mediterranean Interest) is established by the Contracting Parties, on the basis of the criteria listed in Annex 1 of the SPA/BIO Protocol. At risk or threatened species and those which require proper management are listed in Annexes 2 and 3, respectively. A classification scheme was drawn up for benthic environments according to the classical benthic binomial of Pérès and Picard and on phytosociological knowledge. Of the 161 habitats listed, 61 are classified as priorities, their protection being considered indispensable for the maintenance of Mediterranean biodiversity. The selection was made following five criteria: vulnerability, natural value, rarity, aesthetic value and economic value. Then, on the basis of the scores obtained, the habitats were divided into three categories:

P – priority (if they obtained the maximum score in at least two criteria: strict protection);

I – important (only 1 maximum score: they are considered deserving of particular attention and management);

NI – not important (those which obtained no maximum score).

Concerning habitats with phanerogams in Annex 2 of the SPA/BIO Protocol (species threatened or at risk), the marine magnoliophyte list includes: *Posidonia oceanica*, *Zostera marina*, *Nanozostera noltii* (=*Zostera noltii* and *Z. nana*).

Posidonia oceanica and *Caulerpa prolifera*

In order to strengthen protection measures and cooperation between countries, a plan of action was approved in 1999 for protecting marine plants in the Mediterranean Sea. This plan, the state of implementation of which is checked every two years by the Contracting Parties, has identified the following priority actions at national and international level:

- to ensure the conservation of the species and plant formations through improved knowledge and legal measures of protection;
- to avoid the degradation and loss of marine magnoliophyte meadows and other plant formations, as they are habitats of marine species, and to maintain them in a satisfactory state of conservation;
- to ensure the conservation of those formations which are considered as natural monuments, such as the barriers (*recifs*) of Neptune grass.

The institution of Protected Marine Areas (PMA) is in many cases an indirect measure for the protection of marine phanerogams. Indeed, many of the 26 Italian PMA (instituted in June 2007) contain magnoliophyte meadows, in particular of *P. oceanica*, within their confines, and their mapping is under way.

The criteria that contribute towards determining the insertion of an area in the SPAMI list regard the presence of rare, endemic or threatened species, ecological representativity, level of biodiversity, naturalness, peculiarities of the habitat, scientific importance, and cultural representativity.

Eunicella cavolini and *Antedon mediterranea*

The management plans of Natura 2000 sites should consider the ecological and socio-economic characteristics of each site in a comprehensive way. The aim of the Habitats Directive, and more particularly the Sites of Community Interest (SCI) management plans, is to maintain the habitats and species identified within chosen sites in a satisfactory state of conservation. The principle of integrated planning is to consider the whole set of requirements for protection and enhancement of environmental systems.

Impact evaluation. According to Article 6 of the Habitats Directive and Article 5 of Italian Law 357, which implements the Directive in Italy, any plan or project that might have significant impact on an SCI should undergo suitable impact evaluation, which takes into account the specific characteristics and conservation aims of the site in question. This is an attempt to apply the principle of prevention, to limit any degradation of habitats listed in Annex 1 or disturbance to species in Annex 2. Its purpose is also to avoid the European Commission starting up any contravention procedures.

The competent authority which evaluates environmental impact is the national one. It is worth noting that an impact valuation is not limited to interventions lying exclusively within the territories proposed as Natura 2000 sites, but also covers works that, although developed outside these areas, might in some way influence them. The evaluation should be interpreted as an instrument of prevention, which analyses the possible effects of an intervention within an ecological context, and which considers the correlations existing between the various sites and their contribution to the overall coherence of the structure and ecological function of the Natura 2000 network. According to Article 6, in the case of an evaluation of negative impact, or one lacking alternative solutions, a plan or project can be carried out in sites with non-priority habitats and species only for imperative reasons of public interest, including reasons of a social and economic nature: in this case, the Member State must adopt every compensatory measure necessary to safeguard the global coherence of the Natura 2000 network. Information on the procedure for conducting an impact evaluation correctly is given in the website of the European Commission, DG Environment at the address: http://europa.eu.int/comm/environment/eia/home.htm.

Posidonia meadow in good state of conservation

The conservation state of Neptune grass meadows plays a determinant role in the evaluation of water quality. The application of the new Waters Directive (Directive 2000/60/EC) brought innovative legislation to European water policy, in that it tackles the question of safeguarding and protection of waters, considering aquatic environments in their complexity and defining the objectives of environmental quality to be achieved through various elements of biological, hydro-morphological, chemical and physico-chemical quality. The innovation in the regulation is the fundamental role assumed by biological elements. In particular, *Posidonia oceanica* has been chosen for to evaluate the ecological status of marine coastal waters and as a representative of the phytobenthos.

Given the importance of Neptune grass meadows in the Mediterranean and the many research studies devoted to it, it is an appropriate indicator of the ecological status of coastal waters, particularly those which are above soft (incoherent) beds.

The problem is to find a global descriptive index for characterising Neptune grass which is valid for all the Mediterranean countries of the European Union. Among the various parameters proposed, the following three have been selected at European level: density of leaf bundles; percentage of plagiotrophic rhizomes, i.e., those which grow horizontally (parallel to the substrate);

leaf surface area per sheath, which integrates the set of morphological characteristics (number of leaves and their size). Other parameters may be used, the choice of which will depend on the type of control network that is organised.

Neptune grass and VEI. In the studies necessary for the valuation of environmental impact (VEI), according to Italian law and European Directive 11/1995, information is required on the vegetation and, more particularly, on the marine phanerogams of the coastal strip. It is necessary to provide a map on the scale 1:10,000, a bathymetric distribution, a floral inventory and phyto-sociological description, a report on human pressures, and an evaluation of conservation status. The protection of Neptune grass meadows is also included in Laws 426 of 09/12/98 (New Environmental Interventions) and 93 of 23/03/2001 (Environmental Regulations). Liguria was the first Italian Region to legislate specifically on marine phanerogams and in particular on *Posidonia oceanica*, with a Regional Council Resolution (D.G.R. 646 of 08/06/2001) on regulating impact evaluations of coastal management plans on the SCI. The technical regulations for evaluating the conservation status of *P. oceanica* meadows have also been approved (D.G.R. 1533, 2005). These regulations are of enormous practical use, because they provide the tools of evaluation and intervention, for both the administrators and planners of maritime works. The latter can now know, thanks also to the availability of a detailed georeferenced regional map, if their plan is compatible with the conservation of sensitive habitats (biocoenoses).

Another planning tool is the map (on the scale 1:10,000) with the official demarcation of the 26 Ligurian SCI (D.G.R. 1561, 2005), although the SCI protection of a priority habitat must be ensured independently of the identified boundaries at a given moment. The Neptune grass meadow may increase its area over time and the whole area must be protected, not just that within the boundaries reported on the map.

Butte with *Pinna nobilis*

Posidona oceanica atolls

The Habitats Directive and SCI. EEC Directive 92/43 of 21/05/1992, relating to the conservation of natural or semi-natural habitats, as well as the flora and fauna, defines the concepts of conservation, priority habitats and species and those of European interest, conservation areas, ecological corridors, and introduction and reintroduction. The constitution of a network of Special Areas of Conservation (SAC) is strategic. Designated *Natura 2000*, the network is formed by the sites in which the habitats in Annex I are found (classified according to the Corine Biotopes Project) and the species in Annex II. Each Member State is pledged to the constitution of the network of proposed sites which, once accepted by an apposite Commission, are defined Sites of Community Importance (SCI). A rather complex module is compiled for each site, describing the biotic and abiotic characteristics and providing an evaluation of the species and habitats present.

The Italian Ministry of the Environment has conducted a research project, named BioItaly, to identify and map the sites of natural value, on the basis of Annexes I (habitats) and II (species) of the 92/43 Directive and other natural criteria.

As regards the Natura 2000 Network, the Mediterranean biogeographical region is the largest of the three present in Italian territory (Mediterranean, Alpine and Continental) and involves 1337 of the 2500 SCI, the majority of which are listed in Annex B of D.M. 03/04/2000. The approximately 70 more strictly marine sites were identified mainly by the presence of Neptune grass and underwater grottoes. Unfortunately, no SCI have been created in many regions, despite the presence of extensive meadows of Neptune grass. The list of marine SCI and SAC would be much longer if the reference habitats were those of the SPA/BIO protocol and not just those of Directive 92/43. According to SPA/BIO, of the 61 habitats considered worth protecting in the Mediterranean, 60 are found in Italy; all 14 macrophytes in Annex 2 of SPA/BIO live in Italy; and, of the 37 invertebrates and 37 vertebrates (excluding freshwater species and birds) in the same Annex, 35 and 33, respectively, are also found in Italy. This country therefore has a heritage of biodiversity among the highest and most diversified in the Mediterranean, and it must be protected. One of the current problems is missing or few economic valuations of natural assets, although some attempts have been made for Neptune grass.

■ Monitoring methods

The ecological importance of the *Posidonia oceanica* ecosystem and the Italian and international protecting laws mean that its health status must be kept under control. This monitoring has three objectives:

- to maintain surveillance on an ecosystem of high heritage value, but vulnerable, in order to be able to identify any alterations quickly;
- to use this ecosystem as a biological marker of the overall quality of littoral environments;
- to evaluate the efficacy of the conservation and management policies of the coastal strip. For this purpose, it is necessary to choose sites for periodic visits, in order to follow the dynamics of seagrass meadows and their evolution over time.

Almost all the necessary data have to be collected underwater, so the researchers and technicians who conduct the seagrass meadow monitoring must be experienced scuba-divers, with specific training in scientific diving. The parameters which have to be measured must be surveyed at three different levels of ecological complexity, because ecosystem analyses involve the study of individuals, populations and communities. In the *P. oceanica* ecosystem, the individual is the plant, the population is the meadow, and the community is the set of organisms which are in association with the meadow.

Scuba-diver and cable-guided vehicle monitoring a Neptune grass meadow

Plant level. A series of biometric measurements should be conducted on *P. oceanica* plants to provide information on vegetative growth, directly usable as such, and as data useful for subsequent derived indexes.

Morphometric study may be flanked by a retrospective technique called *lepidochronology*: this is analogous to dendrochronology and permits reconstruction of the recent history of a *P. oceanica* rhizome.

- Average number of leaves per tuft. A direct count is done, distinguishing the three categories of leaves. If leaf production is continuous throughout the year, this parameter may reveal seasonal rhythms.
- Leaf length, width and surface area. These are measured in millimetres with a ruler. They are base parameters, which are also useful for calculating the derived parameters.
- Ratio between brown and green parts of the leaf. Calculated as a percentage ratio between linear measurements (in millimetres).
- Lepidochronology. At the end of their vegetative period, the leaf blades of *P. oceanica* break off, while the bases, being rich in lignin and tannins, remain attached to the rhizome for many years and are called *cataphylls*. Depending on the point of insertion along the rhizome, the thickness of the cataphylls has cyclical variations according to the period of the year in which the leaves were alive (maximum in summer-autumn, minimum in winter-spring).

Balisage and photographic survey of a lower limit of Neptune grass meadow

Meadow level. Study of the meadow involves a description of its morphology, definition of its boundaries and numerical evaluations of plant cover. The latter may be combined with the biometric measurements taken at plant level to obtain new concise indexes.

Monitoring procedures require the installation of permanent transects or quadrants in certain portions of the meadows.

Damaged Neptune grass meadow

A transect may be marked on the seabed by stakes laid out at regular intervals and linked by a tapeline, or simply be a stretch between two points located on the surface by GPS. Permanent quadrants are confined areas, usually 6x6 m (more rarely, 10x10 m), which are also marked out by stakes. A specific technique for following the evolution of boundaries is that of marking out (more frequently called by the original French term *balisage*), which yields precise photographic documentation of their position over time.

- Physiognomy of the meadow. A basic qualitative description, which is indispensable for arranging and interpreting point-source quantitative data.
- Nature and depth of boundaries. Surveyed through descriptive protocols and instrumental measurements of depth, distinguishing between indistinct, clear, or eroded boundaries, and between healthy or regressing ones.
- Marking out (*balisage*) of the boundaries. Done by planting a series of stakes along the boundary, which are then surveyed topographically and photographically at fixed intervals.
- Proportion of plagiotropic rhizomes. Evaluated as a percentage, after an adequate number of rhizomes have been counted and divided into plagiotropic and orthotropic.
- Exposure of rhizomes. Measured as the distance between the sediment level and the lower part of the rhizome, in the case of plagiotropic rhizomes; or as the distance between sediment and base of the most external leaf in the case of orthotropic rhizomes. It is an index of sedimentary deficit.
- Cover. Percentage of the portion of seabed covered (in a vertical projection) by Neptune grass, or by other phanerogams or algae (particularly species of *Caulerpa*).

- Conservation Index (CI). This represents the proportion of living *P. oceanica*, and varies from 0 (only dead matte) to 1 (only living Neptune grass).
- Substitution Index (SI). This measures the proportion of other phanerogams or *Caulerpa* species in a meadow, and ranges from 0 (no substitution, there is only Neptune grass) to 1 (no Neptune grass, only the substitute is present).
- Phase Shift Index (PSI). The abundance of dead mattes or substitute species represents a change of phase in the ecosystem of Neptune grass meadows. According to this index, which assumes values between 0 (optimal situation with only *P. oceanica*) and 1 (profoundly altered situation with only *C. racemosa*), a phase dominated by a highly invasive non-indigenous substitute is to be considered worse than one dominated by a substitute native to the Mediterranean.
- Density of leaf bundles. This represents the number of leaf bundles of *P. oceanica* per m^2.
- Relative density. Originally improperly called global density, this combines cover and density.
- Leaf Area Index (LAI). Leaf surface area per unit surface area of the seabed, calculated by multiplying the density of tufts by the average leaf area per tuft, expressed in m^2/m^2. It is a concise parameter of the health of the meadow, which can be correlated to its primary production.
- Coefficient A. The proportion of broken leaf tips to total sampled leaves.

Counting *Posidonia oceanica* sheaths

Level of the associated community. In view of the richness and complexity of the associated community, the study methods of the various components are equally varied and diversified. Many are extremely specialised and are adopted only for specific studies, not for routine monitoring of the *P. oceanica* ecosystem. The vagile invertebrates of the leaf layer are sampled with a mowing net, whereas a sucking pump is used for the vagile invertebrates associated with the rhizome layer and surface sediment. The invertebrate fauna of the matte and sediment are collected with special core samplers adapted for underwater use, or with special hoes and shovels that can penetrate and cut the matte. For all these components, traditional surface equipment like scoops and dredges are rather inefficient. Direct sampling of a given number of tufts is used for the flora and sessile fauna of the rhizomes and the boring organisms which live inside the cataphylls and include some species of polychaetes and isopods.

Blacktail (*Oblada melanura*)

For monitoring purposes, most studies relating to the associated community regard the fish fauna, leaf epiphytes, and the extent of herbivore grazing.

- Fish fauna. The traditional sampling of fish (and large vagile invertebrates) with an oyster net, a sort of small trawling net, has nowadays been almost entirely abandoned in favour of techniques of visual census. The abundance and diversity of fish, the presence of large specimens, and the numbers of juveniles may be indicators of good *P. oceanica* ecosystem conditions.
- Leaf epiphytes. Analysis of the epiphyte community can be conducted with speedy techniques of quantitative surveys of the main taxa. The Epiphytic Index is based on the cover percentage of the organisms with respect to the leaf surface area. Predefined categories of organisms are usually evaluated rather than individual species. Subsequent data processing can evaluate differing concise indexes.
- Extent of grazing by herbivores. The simplest and most rapid method for evaluating grazing intensity is the Grazing Index (percentage of leaves with the tip or edge bitten by fish, isopods or sea urchins).

Damage caused by an anchor chain

■ Causes of regression of Neptune grass meadow

The regression and destruction of marine phanerogam meadows, documented at world level, has both natural and human causes. The former, which have so far been of limited extent in Italian waters, include diseases and the action of storms. The consequences of the many human activities which have an impact on the meadows are much more serious. In many parts of the Mediterranean, the regression has accelerated since 1950, in the wake of intense urbanisation and industrialisation of the coast, especially in the north-west. In Liguria, the reduction in Neptune grass meadow has been evaluated at 10-30%, off Alicante (Spain) at 52%, and as much as 90% near the Marseilles area. As early as the 1950s, French authors had advanced the hypothesis that the reduction in Neptune grass meadows was in some way linked to their poor adaptation to the current hydrological and climatic conditions of the Mediterranean, especially in the north-western areas. This hypothesis was based on two main facts:

- the rarity of seagrass flowering and therefore seed production;
- the ages of the individuals, calculated from the thickness of the mattes, indicates that they may be thousands of years old.

However, it is difficult to believe that this substantial regression is mainly due to the above-mentioned natural phenomena, because in the past *Posidonia* has clearly been able to withstand much more serious crises, such as the Messinian crisis (when most of the Mediterranean dried up) and the various Ice Ages. It is more reasonable to suppose that man is the principal current cause of the regression. Well-documented studies demonstrate that the many human activities on the coastal belt (excessive urbanisation, mass tourism, pollution, dumping of waste, illegal fishing, uncontrolled fish farming, excessive numbers of pleasure craft, beach enrichment, etc.) threaten the survival of phanerogams, both as individuals and as a habitat. It is not easy to establish which of the two is more serious, but impact ranking, referring to the time necessary until the effect of the impact becomes reversible, shows that the destruction of the habitat is always irreversible and that the disappearance of individuals, in the best of cases, happens over long periods for annual species (such as *Ruppia cirrhosa* and *R. maritima*) or pioneer species like *C. nodosa* and *N. noltii*. In the worst of cases, it is irreversible on a human scale for climax species like *P. oceanica* or rare species like *Z. marina*.

Damage due to maritime works. The construction of maritime works can have a direct impact on Neptune grass meadows by covering and thus destroying them: i.e., the building of breakwaters, barrages or piers above the meadow, or the effects of the resulting alterations to currents and waves. Then there are indirect effects, due to harbour activities and possible pollution and discharges of various types. The building of a breakwater may sometimes confine part of a meadow inside a harbour. The possibility of its survival depends on many factors, although experience shows that, in the majority of cases, a Neptune grass meadow isolated inside a man-made structure like a harbour complex disappears within a relatively short time. However, the capacities for survival increase if the harbour is small, does not send polluting discharges from either land or boats and is well-exposed to hydrodynamics that enable good exchange of internal waters, sometimes further facilitated by jetties built on piles. A significant part of the damage caused by the construction of maritime works occurs while building work is in progress, and also depends on the techniques used.

Effects of dumping fine sediment on a Neptune grass meadow

Beach enrichment, dumping of waste. The anthropogenic effects on the coastline and, in much of Italy, the multiplication of tourist jetties and artificial enrichment of beaches, are among the major causes of environmental degradation. The latter activity is truly harmful because, on most occasions, it is carried out with materials and methods that are not ideal for repeated use on a large scale because, with the first heavy seas, the material is dispersed on the seabed. It is therefore necessary to re-enrich the beach every year or in any case at regular intervals, and the seabed is once again “muddied”, with the destruction of the Neptune grass meadow, which is the principal bulwark of protection of beaches against waves. The particle size of the seabed becomes profoundly altered and with the slightest movement of the sea, the mud rises, clouding the waters, with obvious consequences on the ecology and also on the aesthetic plane.

With the excuse of enrichment of beaches, which are being eroded in many parts of Italy, huge numbers of waste dumps have been opened every year (many fewer in recent years). A great variety of materials - from excavations for the construction of buildings, roads, railways, etc., the demolition of concrete and brick buildings, and even residues of industrial processes - have all been dumped in the sea. Even when the soft enrichment technique is used, i.e., taking relict sands from the seabed, the risks to the Neptune grass are not averted, as demonstrated by the emblematic case of the enrichment of the Maronti beach on the island of Ischia. Because of an error in choosing the site from which to extract sand, four hectares of Neptune grass meadow were destroyed by the excavator pump in less than 24 hours.

Variations in river flows. Rivers may have an impact on *P. oceanica* meadows by their inputs of freshwater (which causes a reduction in the salinity of coastal waters), nutrients (essentially nitrogen and phosphorus) and sediments. The flow of Italian rivers is subject to strong seasonal and inter-annual variations. Seasonally, there are alternating high and low waters, whereas inter-annual variations are linked to climatic cycles. These cycles are described by indexes such as the North Atlantic Oscillation index (NAO). When this index is negative, the weather in southern Europe and the Mediterranean basin is damp and rainy (and therefore outflow increases). These fluctuations are natural, and may explain why the meadows do not grow permanently in the area influenced by the outflows of the fullest spates. However, the action of man has altered natural fluctuations in river flow in various ways.
Weirs lessen peak flows during spates, at least in the initial stage; holding the water in reservoirs and using it for agriculture reduces the amount of freshwater that reaches the sea directly. Both these aspects should have a positive effect on the Neptune grass, which suffers from a fall in salinity. However, channelling of watercourses, which often accompanies river regimenting works, accentuates peak flows during spates, with clearly negative effects on the meadows. The areas of dead matte in the vicinity of river mouths have thus greatly increased in the last few decades. A recently studied example was the river Centa in Liguria. This watercourse crosses an intensively cultivated plain and has been greatly modified; the seagrass meadow which at one time stretched uninterrupted along the coastline is now just a stretch of dead matte 5 km long, corresponding to more than 200 hectares, at the river mouth and on both sides of it.

Variations in sediment flows, suspended solids and turbidity. The sedimentary regime plays a determining role in maintaining Neptune grass meadows in optimal conditions. Accumulation and erosion depend on waves and currents, which may be greatly modified by the construction of maritime works, which then act on the transport and distribution of sediments.

Structures perpendicular to the coast, especially if they are large, divert marine currents and cause strong sedimentation upstream (at the base of the breakwater) and erosion downstream. Breakwaters parallel to the coast act mainly on waves, with similar consequences.
The sedimentation rate should be compatible with the growth of Neptune grass rhizomes and that of their erosion because excessive erosion exposes the rhizomes, making them more vulnerable to breakage and uprooting. Excessive accumulations of sediments cover the growing tips of the plant. It has been observed that they die if the sedimentation rate is more than 5-7 cm/year.

Pollution, eutrophication, fish farms. Discharges of liquid effluents from urban areas, industries, or shipping may cause direct or indirect damage to marine phanerogams, modifying the chemical and physical characteristics of the water column by increasing suspended solids and the distribution of pollutants and nutrients. Nutrients also encourage the growth of epiphytes, which reduces photosynthesis of leaves by shielding the light from them and favouring their destruction by non-herbivores - in particular, fish, which eat the organisms colonising the leaves.
The reduction in light, due in particular to water turbidity, reduces the size and density of Neptune grass leaf bundles, ultimately causing their death. This is

Trawl-net otter boards plough the seabed and destroy seagrass meadows

Artificial barrier pyramid protecting a Neptune grass meadow

the principal cause of the shift of the lower limit of Neptune grass meadows towards the coast, which has been widely documented in the whole of the north-western Mediterranean. Off Latium it has moved from 35 m to 25-20 m. In Liguria, the average depth of the lower limit is now just 23 m.

Besides local effects, the general cause appears to be an increase in water turbidity and muddying of the seabed in recent decades, due to the substantial terrigenous deposits that have radically altered coastlines.

The great expansion of meadows of *C. nodosa* on the Ligurian seabed is linked to this. *C. nodosa* is generally considered as both a pioneer and a “secondary” species in the evolutionary climax stage of Neptune grass meadows. In Liguria, this species occupies around 2300 hectares, i.e., almost half the surface area covered by *P. oceanica*. The abundance of *C. nodosa* in Liguria may be related to generalised environmental degradation.

Applying a conceptual model of life strategies to marine phanerogams, *C. nodosa* is a “ruderal” species and *P. oceanica* is a “competitive” one. The species defined as ruderal gain advantage over competitive ones where there is a high level of environmental “disturbance”. Therefore, the abundance of *Cymodocea* compared with that of *Posidonia* may be a response to “disturbance” by humans.

The role of reduced light in determining the shift of the compensation level – the depth at which oxygen produced by photosynthesis just balances oxygen consumption by respiration - is fundamental. A 70% reduction in light causes the death of 90% of Neptune grass leaf bundles within three months.

Urban and industrial discharges introduce various polluting substances (heavy metals, hydrocarbons, surfactants, pesticides, etc.) into the environment, and they all have varying levels of direct and indirect effects on seagrasses, depending on their chemical characteristics. Some pollutants cause alterations in physiological activity, damage at cellular level, effects on photosynthetic pigments, reduced growth rate, etc.

As heavy metals accumulate in the plants, particularly in the rhizomes, the possibility of dating them through lepidochronology makes Neptune grass a bioindicator of enormous interest, as it is possible not only to determine metal concentrations, but also to follow their evolution in previous years. As regards mercury, it has been demonstrated that its concentration in the plants is correlated to that in sediments and that its accumulation in leaf tissues can have serious physiological consequences, leading to cell death and arresting leaf growth.

From the above, it is obviously necessary, for instance, to avoid siting a liquid manure discharge plant close to a Neptune grass meadow, or worse still, on it.

Neptune grass rhizome

Existing discharges should be extended beyond the meadow. For those being built, it is indispensable to take all possible precautions to ensure correct positioning of both pipelines (see excavations) and outflows.
Another threat to the conservation of Neptune grass meadows comes from the proliferation of fish farms, which use floating cages for rearing and/or fattening fish along Mediterranean coasts. The disastrous effects on the meadows from this type of fish farming has been well documented, so they should be completely banned - not only on the meadows, but also nearby, in relation to sea currents. This ban is amply motivated by the laws already in force on the conservation of biodiversity.
The main damage is due to the increased organic load (eutrophication) in both the water column and the sediment. As regards nutrients, it is similar to that of sewage discharge. Water eutrophication may contribute towards increasing turbidity and the numbers of epiphytes on Neptune grass leaves, with a consequent reduction in photosynthesis. The shading effect of the cages on the seabed, and thus on the Neptune grass meadows, should also be examined.

Mechanical destruction: anchoring, trawling, explosions, excavations.
There are various mechanical activities which lead to the destruction of Neptune grass meadows.
Anchoring is today one of the major causes of degradation of these meadows, due to the increased popularity of sailing and frequent visits by vessels, not only in summer, to areas of high natural interest, whether protected or not. Yet this type of damage could easily be avoided with a little common sense and greater regard for the coastal marine environment and seagrass meadows in particular.
Anchors have various kinds of effects on Neptune grass meadows:
- they break the rhizomes on which the anchor falls, or on which it is dragged along before catching;
- the anchor chain shifts on the seabed, because currents and wind move the vessel above and tear off Neptune grass leaves. The chain sometimes rotates around the anchor;
- when the anchor is raised, it breaks the rhizomes on which it was caught. Occasionally a whole block of matte may be uprooted.

French researchers have evaluated that, during the various stages of anchoring (lowering, anchored state and raising) from 16 to 34 tufts of Neptune grass are torn up per vessel. The immediate consequence of this is a reduction in the density of leaf bundles and break-up of the meadow, with the formation of holes and erosive channels.
Even more serious is damage caused by the anchoring of large merchant ships, military vessels and cruise liners, which sometimes stop in coastal areas with Neptune grass meadows. This practice should be banned, precise indications being given on nautical maps that anchoring in all coastal areas with Neptune grass, a habitat protected by the Habitats Directive and numerous other international agreements, is prohibited (see page 113).
Yachts and sailing boats are more difficult to forbid. Yachtsmen choose Neptune grass areas for the beauty of the site, but also because the anchor is easier to secure than in rocky areas (the anchor may become obstructed) or sandy-muddy ones where the anchor might not hold. To remedy the problems of uncontrolled anchoring, moorings have been laid out in many areas, which have unfortunately often been shown to be even more damaging than individual anchors. The dead weights lying on the seabed, attached to large anchoring buoys and the chains that join them may be dragged along the seabed, destroying large stretches of Neptune grass.
Illegal mechanised fishing, and trawling in particular, is very probably the main cause of the destruction of phanerogams, especially Neptune grass meadows, in many Mediterranean areas. In Latium, it has been evaluated that 40% of the reduction in surface area occupied by Neptune grass meadows is due primarily to trawling. The damage it causes may be summarised as follows:
- reduction in the surface area covered by phanerogams;
- creation of furrows and areas without Neptune grass, by uprooting leaf bundles and rhizomes;
- rising to the surface of large mounds of leaves and tufts, which then reach the shore and increase the litter on the beach;
- contribution to the reduction in biomass and density of the fish fauna, particularly that of commercial interest;
- modification of the habitat, with the formation of large areas of sand or sandy mud, with no vegetation, which attract other types of fauna, including fish.

As well as trawl nets, dredgers are also used in some areas. All forms of fishing that involve towing by motorised fishing-boats are banned within 50 m depth and three nautical miles from the coast, by both Italian law and European regulations. To impede illegal fishing, sinkers, usually made of cement (artificial

Sea urchin

barriers), have been laid in some areas with the intention of protecting the biocoenoses, and Neptune grass meadows in particular, from the effects of trawling, in order to increase biodiversity and the biomass of species of commercial interest.

The need to lay water, oil and gas pipelines, and to sink electricity and telephone cables, can have deleterious effects on seagrass meadows growing along their route. The damage is greater if it is necessary to carry out excavations, or protect pipes and cables with stones or cement in shallow areas with strong currents. Observations of electricity and telephone cables have demonstrated that simple laying, without digging, has much less impact and is therefore to be recommended wherever hydrodynamic conditions allow it. In some situations it has been possible to observe that cables laid on Neptune grass meadows, after some decades, become incorporated into the tangle of rhizomes.

It is not unusual to come across more or less circular areas with dead mattes in Neptune grass meadows. This is the result of destructive aerial bombing along the coast during the Second World War and the exploding of mines - not to mention the use of dynamite for poaching, which is still practised in some parts of the Mediterranean, including Italy. More than fifty years after the explosion, colonisation of the destroyed area is still only very partial. It would appear that the sensitivity of Neptune grass to explosions is largely due to the existence of many large intercellular spaces filled with oxygen and carbon dioxide (*aerenchyma*) in the leaves. In the event of an explosion, the aerenchyma burst.

Overgrazing. The effect of herbivores on the growth of plants plays an extremely important role in the delicate equilibrium of the food chain, which is fundamental for the stability of all ecosystems. The main consumer of *P. oceanica*, and probably of other phanerogams, is the salema (*Sarpa salpa*), the only strictly herbivorous fish in the Mediterranean. Especially towards the end of summer, divers above a Neptune grass meadow can see patches where the tufts appear to be shorter and the leaves have truncated tips and fewer epiphytes than in the surrounding areas: this is where a large shoal of salema have paused to graze. Second to salema, as grazers of Neptune grass, are sea urchins, particularly the species *Paracentrotus lividus*. Alterations of the natural mechanisms of the food chain may lead to overgrazing by these species, with the consequent risk of regression of meadows.

Competition with exotic species. Located in the warm-temperate region, at the junction between the Atlantic and the Indo-Pacific, with widespread fish farming, very intense maritime traffic, and progressive warming of its waters, since the Suez Canal opened the Mediterranean has become a “hot spot” of introduction of new species, comparable with only a few other places in the world. Today, at least 600 species (around 5% of the known flora and fauna) may be considered as new introductions, and of these at least one hundred are macrophytes, both algae and phanerogams.

A hypothesis has been attempted on biological introduction, to explain why certain habitats are invaded more than others, and why certain species, especially algal macrophytes, have shown themselves to be highly invasive (such as *Caulerpa taxifolia* and *C. racemosa* var. *cylindracea*) outside their places of origin. At the beginning of these studies, in the 1950s, a hypothesis of “biotic resistance” was formulated, according to which a habitat containing many species (with high biodiversity) is less vulnerable to invasions than a poorer one: an ecosystem rich in species is well structured, with complex and varied inter-species interactions (competition, predation, mutualism, parasitism, diseases). *P. oceanica* meadows, being well-structured ecosystems, are no exception, and invasive species often find it difficult to advance in healthy meadows, whereas they have been shown to be much more aggressive in meadows with signs of regression or weakness.

he invasion of non-indigenous chlorophytes - *Caulerpa racemosa* var. *cylindracea* and *C. taxifolia* - is considered to be the most critical for the environment. Until a few years ago, these species were recorded only in some areas of the Mediterranean, but they have now extended to all sectors, often completely covering rocky seabeds and also advancing on the sedimentary beds at the base of rocks.

Caulerpa prolifera

Transplanting Neptune grass

■ Reforestation

Recognition of the fundamental role played by *P. oceanica* meadows along Mediterranean coasts, and the obvious regression phenomena to which they have been subjected, following decades of man's activities, have encouraged attempts at reforestation aimed at restoring the previous conditions. Two of the most important drawbacks to the reforestation of *P. oceanica* are difficulty in anchoring cuttings, shoots or plantlets obtained from seed on the seabed, and also the need to use structures that must be highly resistant to wave action (if the area in question is close to the surface or very exposed) or else subjected to currents on the seabed. In Italy, the first attempts at transplanting marine phanerogams using cuttings were made off Ischia and Sicily in the mid-1970s. In conjunction with transplanting and reforestation experiments, many studies have been undertaken to obtain material, by taking cuttings from the edges of meadows, germinating beached seeds, or cultivating protoplasts (organelles of plant cells) obtained by enzymatic digestion of *P. oceanica* tissues. Eight different techniques have been proposed for reforestation, divided into two main categories:

- no anchoring: plants free of sediment, but with rhizomes covered in sediment; plants supplied with sediment and placed on the seabed; plants with sediment inserted in a hole; individual plants in biodegradable containers placed on the seabed;
- anchoring: individual plants fixed to tubes or piles; individual plants anchored by bricks; individual plants or leaf bundles fixed to wire netting; individual plants anchored to stakes embedded in the substrate.

All the systems used until now are included in one of these two categories. However, the choice of a particular technique should always be carefully evaluated according to the local situation and the aims of the reforestation. It should also not be forgotten that any attempt at restoration of regressed or degraded meadows must aim at restoring favourable conditions for the survival of Neptune grass at the site. In 1994-95, experiments on transplanting *P. oceanica* began along the coast at Livorno (Rosignano Solvay) and along the coast of Latium, north of Civitavecchia. One year after the start of the experiments, the survival rate of cuttings in the seabed off Rosignano Solvay was 89.9%, with similar values at Civitavecchia.

■ Neptune grass as a bioindicator

Marine phanerogams are essential components of the structure and ecological processes that involve coastal areas. *P. oceanica* is particularly sensitive to variations in the quality of the environment, and disappears when pollution is too high - for this reason, it is considered an excellent indicator of environmental quality. Some authors state that *P. oceanica* is demonstrating a progressive lack of adaptation to the Mediterranean environment, which is leading to natural rarefaction of the meadows, at least along the northern coasts. The low frequency and success of sexual reproduction appears to have led over time to reduced genetic variability in the populations, which may have made the species more vulnerable to changes in environmental conditions. Either an increase or a reduction in the distribution of sediment may also create serious problems for the survival of meadows, in the former case by favouring burial and consequent suffocation, and in the latter by exposing the rhizomes and therefore rendering the meadow more sensitive to erosion.

Recognition of the fact that human disturbance is critical to the process of management and conservation of resources and natural habitats is essential. The capacity to identify effects greatly depends on the techniques and procedures adopted. A critical stage of this process is a proper sampling

Red mullet among Neptune grass leaf litter


Rippled seabed with Neptune grass leaves

design. Notable progress has recently been made in developing procedures that allow the natural variability of any human effect to be distinguished. In this, it is extremely important to estimate the space-time variability of natural populations in habitats at high risk. These estimates provide the basic information necessary for thorough impact analysis, avoiding many of the problems due to a chronic lack of information relating to the situation preceding the impact.

The possibility of identifying human disturbance unequivocally is fundamental for the management and conservation of resources or natural environments. Many examples in the literature illustrate the enormous difficulties that arise in managing environmental problems when there is any ambiguity in identifying an impact. In these cases, it is extremely difficult, if not impossible, to reach an agreement between the various stakeholders involved in decision-making (private associations, public opinion, political representatives).

Natural populations are known to fluctuate widely in space and time and this variability changes according to the scale considered. A representative estimate of studied variables therefore requires analysis on more than one spatial scale.

The hypothesis that the variability in the *P. oceanica* system changes according to the scale considered may be examined with a hierarchical sampling scheme. The objective of this type of research is to quantify, for each variable, variances at different scales. Variances estimated in this way can be used to optimise future sampling schemes aimed at the study and evaluation of the conditions of seagrass meadows. In order to evidence any future alterations to the meadows and relate them to human interventions, it is also necessary to provide an estimate of expected impact. This is determined by comparing meadows currently exposed to impact (for example, those close to urban areas and marinas) with control meadows not exposed to this particular source of disturbance. Comparisons of this type allow the hypothesis of human impact to be examined directly, and at the same time provide a measurement of the expected difference between impacted condition and control condition, for use as a reference for future evaluations on the state of the meadows.

■ Posidonia oceanica meadows along the Italian coasts


Emerging *Posidonia* at Pianosa (Tuscany)

The Italian census of the priority habitat "Neptune grass meadows" shows that only 16% are protected, most being State Nature Reserves, and that more than 70% of those recorded are in unprotected areas. The need for protection, according to the census reports, regards all the meadows of *P. oceanica* in optimal and good state of conservation in all the regions considered. In Italy, 180 sites have been listed for this priority habitat, in most of the coastal regions (Liguria, Tuscany, Sardinia, Latium, Basilicata, Apulia, Friuli Venezia Giulia, Sicily, Campania and Calabria).

- Liguria: 27 different sites have been identified, 26 of which are SCI. Leaving out the many small formations, around fifty *P. oceanica* meadows are distributed in the provinces of Imperia, Savona, Genoa and La Spezia. They differ greatly in size, from a minimum of a few hectares (ha) to a maximum of around 760 ha. This comes to a total of 4800 ha of meadows, or about 10-15% of the Ligurian seabed between the coastline and a depth of 35 m. Of these 4800 ha, 3500 ha lie along the Riviera di Ponente (to the west) and the remainder in the Riviera di Levante (to the east). The Riviera di Ponente, and in particular the province of Imperia, is characterised by very extensive meadows, whereas the small and medium-sized meadows are more important in the Levante.
- Tuscany: a detailed map of *P. oceanica* meadows has been made, which provides perhaps the most complete picture of the distribution of these meadows in Italy, and demonstrates the discontinuous presence of this seagrass along the entire Tuscan coast. In the provinces of Livorno and Grosseto, 22 sites have been counted, between the continental coastal areas and the islands of the Tuscan Archipelago, covering an area of more than 28,000 ha. The largest meadows in the continental area grow on the sandbars at Meloria and Vada. *P. oceanica* is present around almost the entire coastline of the island of Elba, and is found on the seabed surrounding all the smaller islands of the Tuscan Archipelago (Gorgona, Capraia, Giglio, Formiche di Grosseto, Pianosa, Giannutri, Montecristo and Scoglio d'Africa). Pianosa and Scoglio d'Africa have vast meadows which surround their coasts without interruption;

The methods used for mapping *Posidonia oceanica* may be classified as direct or indirect, depending on whether they involve the use of scuba-divers or various electrical-acoustical optical instruments.
Direct underwater observation is an efficacious method and highly detailed maps (generally on the scale 1:5,000 or higher) can be drawn. This method is advisable for the examination of restricted areas. It is also a valid and indispensable aid in the case of large areas for calibrating other methods, such as aerial photography or echography, in the form of what some authors define as "identification dives". Direct observation is done following underwater transects marked out on the seabed with measuring tapes: for each of these, a point with known coordinates is the starting point and an established direction is then followed.
The method of direct observation by scuba-divers is certainly the most precise, in that not only is each community identified *in situ*, but even the smallest groups of tufts of *P. oceanica* can be surveyed and their location estimated within an error margin of about one metre.
The use of mini-submarines allows long stretches of the lower limit of the meadows to be surveyed and mapped with sufficient precision, verifying the type and state of the limit, which is generally at a depth of more than 30 metres. At this depth, scuba-diving encounters problems of safety that curtail diving time and therefore the area that can be studied during a dive. Direct methods also include those based on the use of optical instruments, such as telecameras mounted on R.O.V. or R.C.V. (Remote Operated or Remote Controlled Vehicles).
Indirect methods include the following: mechanical soundings; echographic soundings; ecographic recordings; conventional aerial photography; aircraft-borne tele-detection; satellite surveillance.
Mechanical soundings are the most traditional means for surveying the nature of the seabed, and corers, trawls and grab-dredgers are used. These survey methods do not require special vessels and are fast, but they provide point-source information lacking in details. They are sometimes used in mapping surveys to integrate other methods but, being destructive, are generally restricted in their applications.
Echographic soundings: echo-sounding gear is used at high or low signal frequencies. The former are those in common use which provide one-dimensional tracings of the seabed. Low-frequency echography is based on echo-sounders of the "sub-bottom profiler" type, which emit frequencies of around 2.5 Khz. They are useful in studying the deep structure of mattes, although the UNI-BOOM system has turned out to be more expedient for this purpose, as it can survey buried structures with a resolution often better than 20 cm and a penetration that may reach 200 m in optimal conditions (very soft sediment). However, these surveying methods are not very useful for mapping *P. oceanica*, because the echoes produced by the meadows are distinguishable from those of rocky outcrops only where the mattes are very tall.
Acoustic recordings are based on the use of side-scan sonar. The seabed is "illuminated" obliquely with impulses from acoustic sources, which are reflected differently according to what they strike. These modified reflected sounds return to the instrument which emitted them and their intensity is recorded. Once all the data are ordered, a sonogram is obtained, corresponding to a photograph of the seabed. The sonar equipment, mounted on a boat, is composed of the following parts: an analogical recorder for the emission of acoustic impulses and their recording; a digital recorder; an electric cable of appropriate length; a towed structure called a "fish", in which the emitting/receiving acoustic sources (transducers) are placed and which "illuminate" the seabed obliquely on either side of the fish. The sound propagates, covering two lateral flukes perpendicular to the direction of movement, with a horizontal opening of 1-2° and a vertical one of 90°.
Side-scan sonar is backed up by a positioning system connected to it, with precision of the order of one metre, constantly available to the boat personnel. Surveys by side-scan sonar make it possible to calculate the surface areas covered by vegetation and to monitor the evolution of its lower limits. Qualitative detail is also very good, as the sonograms can distinguish the meadows of *Cymodocea nodosa* (which produce dotted rasters) from those of *Posidonia oceanica* (which produce granular rasters), as well as from the other types of seabed. One of the disadvantages of this method is the impossibility, at least up to now, of gauging the density of the meadows from the sonograms and picking out other important details, although recent studies have aimed at improving instrument performance. In addition, identification dives often become necessary to check dubious recordings, which are particularly frequent in strongly degraded meadows where there are dead mattes. Side-scan sonar is also difficult to use in shallow water (10 metres or less).

Clearings and lower limit of a Neptune grass meadow, as shown on a sonogram

Posidonia meadows near Molarotto Island (Sardinia)

the extent is smaller around the other islands.

- Sardinia: *P. oceanica* meadows grow almost continuously between depths of 5 m and 30 m and in some cases up to 40 m. More than 40 sites have been counted, for a total surface area of around 27,000 ha. The ecological status of the meadows around this region appears to reflect the anthropogenic level: where this is high (e.g., north-eastern Sardinia), meadows show evident signs of degradation.

Latium: 11 sites have been counted in the provinces of Viterbo, Rome and Latina, with *P. oceanica* meadows covering a total of just under 20,000 ha. They are distributed in three precise geographical areas, with different environmental conditions and a correspondingly different state of health: one zone to the north (the most degraded), one south of the Tiber, and the third surrounding the Pontine islands.

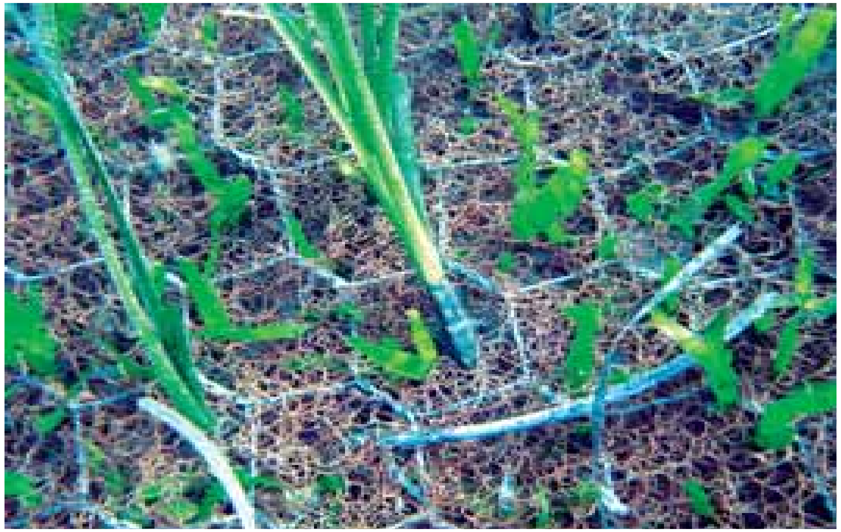
- Campania: 17 sites have been recorded in the provinces of Naples and Salerno, covering an area of about 100 ha, although it is almost certain that the surface area covered by meadows is much larger. Neptune grass is widespread along the Sorrento peninsula. The most extensive meadows are mainly found in the bays where the bathymetric gradient is less pronounced. At shallower depths, coinciding with the upper limit, signs of regression are evident - as demonstrated by many uprooted rhizomes, caused by the anchoring of pleasure craft during the summer. There are extensive meadows of *P. oceanica* in the gulf of Policastro, growing continuously on the ideal sandy bed to a depth of 30 m. At Palinuro, the presence of large “islands” of Neptune grass forming the so-called “hilly meadows” denotes a form of degradation to which the meadows are subjected in this area, perhaps because of the large numbers of boats in the area. Around the island of Ischia, *P. oceanica* forms a green belt which covers an area of 15.7 km^2, between 0.5 and 39 m in depth. The plants grow on various types of substrate, such as sand, rocks and mattes of Neptune grass. From the upper limit to a depth of 10-15 m, the meadows are generally dense, and then tend to diminish with increasing depth. The lower limits are of a different type (normal and erosive), according to hydrodynamics. Within the meadows themselves, there are more or less evident signs of erosion, such as inter-matte channels and clearings, caused by both hydrodynamics and the uprooting of rhizomes by too many anchors. In various sites, meadows of *Cymodocea nodosa* are to be found at the upper Neptune grass limit, mixed with *Nanozostera noltii* in rare cases. The meadows of *C. nodosa* which precede those of Neptune grass are also found at greater depths - to around 15 m. At Capri, *P. oceanica* grows in sparse patches all around the island; only the northern part is less colonised (due to the steepness of the seabed in that area). In the eastern sector, meadows are recorded as deep as 30 m; elsewhere, they reach a depth of 15-20 m.

Neptune grass meadow in Baia degli Infreschi (Campania)

- Basilicata: *P. oceanica* is found along most of the coastline. In particular, one of the largest continuous meadows on the Italian coasts has been recorded facing Maratea.
- Calabria: 13 sites have been counted, covering a total of about 13,000 ha. Surveys conducted in 2002-2003 demonstrated the more marked presence of meadows in a good state on the Tyrrhenian coast rather than on the Ionian.
- Sicily: extensive dense meadows of *P. oceanica* are found along the south-eastern, north-western and western coasts, corresponding to the largest carbonate and calcareous rocky outcrops of the island. In these sectors, the meadows also grow on rocky substrates, with or without a fine layer of organogenic sediment. Along the western coast of Sicily, favourable ecological conditions have allowed the settlement and growth of one of the most impressive meadows of *P. oceanica* ever found in the Mediterranean basin. The evolution of this meadow, growing extensively on deep areas of the seabed, has led to some unusual formations of Neptune grass in the area (indicated by the French terms *plateau récifale* and *récif-barrière*) and of natural ecosystems, both lagoonal (Stagnone di Marsala) and artificial (saline), of notable natural and ecological interest.

P. oceanica meadows are rare along the central-eastern Tyrrhenian and north-eastern Ionian coasts of Sicily, which are characterised by steep slopes and seasonal watercourses and where metamorphic, igneous and sedimentary (Peloritani) and volcanic (Etna) rocks dominate. On the central sector of the southern side of the island, the muddy-silty nature of the substrates and their continual remixing by the waves block the evolutionary sequence at communities of *C. nodosa* (edaphic climax), which form extensive and dense meadows, especially in the Gulf of Gela, at a depth of around 15 m. Ample meadows are found on the seabed off the Egadi islands and Lampedusa, whereas in the volcanic islands (Eolie, Ustica, Pantelleria and Linosa), *P. oceanica* finds growing conditions prevalently on rocks and volcanic sands containing calcareous debris of a biogenic nature. Overall, *P. oceanica* meadows are frequently found along the Sicilian coastal seabed but, especially close to the large urban and industrial areas, show evident signs of regression and have in places completely disappeared.

- Puglia: meadows differ widely as regards both substrate and state of health. The meadows in the gulf of Taranto, especially those facing Gallipoli, and also those off Otranto, are growing on mattes, are well-developed, and show a high density of leaf bundles. Instead, those along the Adriatic coast are more sparse and grow on rocks (Bari) or sand (Brindisi). *P. oceanica* meadows do not grow to the north of the Tremiti islands, mainly because of the nature of the seabed.
- Friuli Venezia Giulia: scattered patches of *P. oceanica* are found on solid substrates emerging from fine sands in the areas of Sant'Agata and San Gottardo, along the Grado coastline; these represent the only cases of living Neptune grass on the Italian side of the Gulf of Trieste.



Neptune grass meadow “replanted” off east coast of Sicily

■ Economic importance

The economic importance of Neptune grass meadows derives from their fundamental role in maintaining the ecological and physical equilibria of the coastal environment and the ecological services they provide - the enormous value of which we often only become aware when they have disappeared. An economic valuation should consider the benefits - direct (e.g., fishing and diving) and indirect (services rendered, i.e., protection of the coastline from erosion, oxygenation of waters) - without forgetting the value of possible future uses.

As already mentioned, Neptune grass meadows contribute to the development of tourism and seaside activities, which have an enormous economic impact in Italy, through maintaining water quality (transparency) and, more especially, stabilising beaches, which are protected against erosion (reduction of hydrodynamics, sediment traps, banks of dead leaves).

In addition, although the meadows are not places particularly sought after by scuba-divers, unless for underwater fishing, they are formidable producers of biomass and act as nurseries for species which may then move to sites preferred for diving, such as rocky environments.

Over and above the figures proposed by various authors, on a world scale, it is worth noting that marine meadows are one of the groups of ecosystems with the highest economic value. Some American authors assign a value of US $ 19,000 per hectare per year to seagrass meadows, surpassed only by estuaries (US $ 22,832) - three times that of coral reefs (US $ 6,075) and ten times that of tropical forests (US $ 2,007) - Neptune grass meadows offer a magnificent example for sustainable development, in which economic conservation and social aspects are closely correlated.

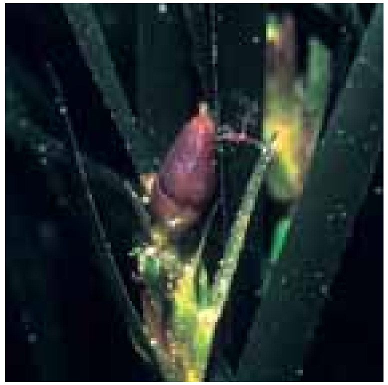
Two approaches are used in the literature to evaluate the monetary value of the services performed by an ecosystem (ecological services): identification of its direct market value (fishing), or indirect value of the ecosystem, and an energy analysis. The latter method estimates the total energy cost of all the functions of the ecosystem in question. The value of a Neptune grass meadow off a beach has been estimated, based on the cost of beach enrichment. If this is estimated at 2600 m^2/year, because every metre of Neptune grass meadow protects approximately 15 m of beach, the sum of 39,000 m^2 is obtained.

It is also worth stressing the role in CO_2 uptake that Neptune grass meadows may play in relation to CO_2 emissions of human origin and the Kyoto Protocol. Within this context, part of the meadows could be calculated for the system of credits. The monetary value of approximately 49 km^2 of the Neptune grass meadows in Liguria has been estimated at around Euros 640,000/year.

Teaching suggestions

GIUSEPPE GIACCONE

■ General description



Neptune grass fruit

● Aims: to learn the concepts of adaptation and evolution of terrestrial plants in the marine environment to form meadow landscapes in the Mediterranean.

The following topics should be covered: mechanisms of attachment to the substrate with underground stems (rhizomes); absorption mechanisms of nutrients, mainly through leaves (cuticle thinning), and transport via tracheids along the stems; photosynthesis and respiration through gaseous exchange with the water by means of simplified leaf stomata or a fine cuticle, sometimes with numerous lenticels; vegetative propagation by pieces broken off during heavy seas, or branches specialised as propagules; sexual reproduction with true flowers, fruit and seed production. Environmental factors affecting establishment and growth: light (not less than 1% of the intensity measured on the surface); temperature (10-23 °C); salinity (36-38‰); moderate currents and water movements; type of substrate (coarse organogenic sands and rocks with some sediment).

To learn the concepts of “structuring species” or “engineer species”, for which Neptune grass is particularly suited, and the “ecosystem services” provided by Neptune grass meadows. Interesting analogies may be made: for example, by comparing the Neptune grass system to a large block of flats (or a skyscraper) which, from the cellars (mattes) to the topmost floors and attics (the leaf layer), offers very diversified living spaces (refuges) for the “residents”.

● Level: secondary-school pupils 17-18 years of age; by simplifying the subjects and developing the laboratory work with field excursions to the coast, also pupils of 12-14.

Posidonia oceanica seedling

- Equipment: literature (see select bibliography); in class, pupils should prepare cards regarding the species of seagrass and principal animal species living in the meadows; documentaries should be shown and websites consulted. For field excursions, strong transparent plastic bags for plant and animal specimens collected on the shore; for more delicate specimens, transparent plastic canisters with watertight caps filled with seawater, with addition of 4% formalin for plants and denatured alcohol for animals.
For laboratory work, sheets of paper and newspapers, to prepare a herbarium with plant species and transparent plastic canisters of various sizes to preserve animal species in alcohol; all specimens must be labelled with the name of the organism, locality and collection date. Magnifying glasses are useful for field excursions; binocular microscopes (both reflected and transmitted light), a selection of tweezers and needles for optical microscopy.
- Possible collaborators: personnel of a Protected Marine Area, environmental associations active in sea-related subjects, scuba-diving clubs, with an interest in environmental education, university students or staff who are experts in marine biology and ecology. Field excursions should take place in September and October, to collect beached material and also to allow easy swimming with mask and flippers above the meadows at shallow depths. Excursions in the first weeks of May and June are optimal for collecting seeds.

Striped meadow

■ Origin, evolution and adaptation of angiosperms to the marine environment

Life originated in the ancient seas of the Earth approximately 4 billion years ago. The first organisms were bacterial cells and, around two billion years ago, some of these developed oxygen photosynthesis, i.e., the capacity to use the energy from sunlight to produce carbohydrates from carbon dioxide and water, and ultimately to release oxygen. Other bacterial cells maintained heterotrophic metabolism, and yet others drew chemical energy from the sulphur compounds released by secondary submarine volcanic phenomena. From photosynthetic bacteria, called cyanobacteria or blue-green algae, the marine algae evolved, first unicellular and then multi-cellular. Around one billion years ago, some eukaryote organisms developed sexual propagation. Due to photosynthesis, the hydrosphere and atmosphere then both became oxidising instead of reducing, and the ozone layer, capable of blocking the ultraviolet radiation, was formed. At this point, plants, together with other forms of life, first conquered the surface waters and then, approximately four hundred million years ago, dry land.
In this new environment, plants developed roots to absorb water and nutrients, and leaves equipped with stomatal openings for gaseous exchange of carbon dioxide and oxygen with the surrounding environment. Some of them were also covered with a waxy cuticle to diminish water loss, and had various pigments in the cell plastids to capture, transfer and exploit sunlight no longer filtered by seawater. Their stems transported raw materials and water from the roots to the leaves and substances processed by the leaves to the roots.
Around three hundred million years ago, terrestrial plants also acquired various systems for sexual reproduction, sometimes in different generations and individuals, but later mainly involving flowers. These plants, with evident reproductive structures in the flowers, are called phanerogams, and those which bear seeds hidden in ovaries are angiosperms.
Around one hundred million years ago, in the Cretaceous, among the angiosperms whose germinating seeds form just one germ layer, called monocotyledons, the alismatales evolved in aquatic environments, some genera of which also managed to populate marine waters from where plants had originally come. One of the Cretaceous fossils which documents these evolutionary events is a species of Neptune grass, known as *Posidonia cretacea*.
Only around 60 species of angiosperms are currently found in the marine waters of the Earth and, to judge from the few fossils, this number was never very different in the Tertiary and Quaternary eras. This signifies that it is very

difficult for higher plants to adapt entirely to living in a marine environment. Some manage to live in brackish environments, both inland and coastal, but have never penetrated stably into the sea. These plants are known as halophytes (plants of brackish environments); those which live permanently in the sea are thalassophytes.

The specific characteristics of marine plants are:

adaptation to life in an environment with salinity between 35 and 39‰ which generates an osmotic pressure higher than that of the cytoplasm;

capacity to live continually submerged, i.e., to absorb oxygen and carbonic acid dissolved in seawater for respiration and photosynthesis;

presence of an efficient system of attachment to the seabed, and a suitable leaf architecture to resist the forces of the waves and currents and to absorb nutrients;

auxiliary pigments that function as antennae to capture low light intensities and use bands of sunlight with shorter wavelengths than those of red light, and capable of resonance phenomena to transit sufficient energy to the chlorophyll molecules for photosynthesis;

hydrophilous pollination with pollen floating capacity exactly in balance with the length of the pistils and shape of the stigmas, which secrete sticky non-water-soluble substances to trap pollens and make them germinate;

capacity of seeds to disperse in water and germinate on the seabed;

Heaps of beached Neptune grass

capacity to compete with marine algae and resist excessive grazing by herbivores both by the abundant and periodic production of new leaves and by processing of chemical products, known as allelopathic substances, which render the leaves unappetising to many herbivores.

Halophila stipulacea

Five species of thalassophytes live in the Mediterranean, including the Italian coasts. The last species to arrive in 1988, from the Red Sea, is *Halophila stipulacea*, which still does not produce seeds, but only propagates vegetatively by detaching plant parts and currently produces only male flowers. The most ancient is *Posidonia oceanica*.

The name *Posidonia* was given to the genus to commemorate the Greek sea god Poseidon, so that this marine angiosperm is his plant. The specific name "*oceanica*" is the result of an error in the geographical distribution of this species: *Posidonia oceanica* does not and could not live in the Atlantic Ocean, as demonstrated by the limit of its diffusion at Gibraltar: it stops advancing where the Atlantic water maintains its typical environmental characteristics and starts to grow again where the water has typical Mediterranean characteristics. It would therefore be more correct to call it *Posidonia mediterranea*, but Linnaeus was unaware that it is endemic to the Mediterranean and, once names are attributed, right or wrong, they must be retained.

The ancient Greeks (as reported by the philosopher Aristotle, who was also a naturalist, in the fourth century B.C.) observed two natural events which occurred punctually every year: in late spring, fruit similar to acorns floated to the surface, which were thought to be produced by a "marine oak", and fibrous balls, called "marine hairballs", thought to be the excrement of tuna fish, were deposited on the beaches in late summer and autumn. The tuna migration began simultaneously with the event of the floating fruit and, the tuna returned to the Atlantic at the same time as the beaching of the hairballs. The coastal populations did not know that the tuna migrations were linked to their breeding cycle, but thought that they were attracted into the Mediterranean by the food produced by the marine oak and then, before turning back, they filled the beaches with their faeces. This explanation, reported by Aristotle, was considered valid until 1800, then naturalists discovered that Neptune grass was the source of the fruit and the hairballs the tangled fibres of its debris.

■ Marine phanerogam meadows in the Mediterranean

Of the five marine angiosperms that live in the Mediterranean, only *Posidonia oceanica* forms large submerged meadows similar to the terrestrial grasslands of subtropical regions.
Posidonia by its morphology, type of growth and the extension of its systems, is a true "engineer" or "structuring species", i.e., it constitutes the habitat for other species which it provides with living spaces, refuge and food resources (like the corals on coral reefs).
Seagrass meadows cover around 2% of the Mediterranean seabed, colonising approximately 37,000 km^2. Their extent and high density provide "services" to the environment, including: production of organic matter (biomass) and oxygen; seabed stabilisation; attenuation of wave action and coastal erosion; nurseries for many species; and obvious benefits also for the fishing industry.
The amount of carbon dioxide fixed and oxygen released by photosynthesis are comparable to those of terrestrial grasslands or forests. The geometry of seagrass meadows depends on the currents which dominate in the areas where they grow: littoral barriers on the bed of gulfs and bays; cordons in stretches of sea between islands; mosaics of patches in conditions of weak hydrodynamics, hills and atolls in environments with circular currents. The meadows filter suspended sediment and, by trapping it, gradually raise the seabed, forming mattes, until the surface is reached. A Neptune grass barrier thus defends the coast from erosion, contributes towards beach enrichment and augments coastal dunes. The development and stability of the meadows increases the animal populations of the seabed, including fish, which find food, refuge and suitable environments for breeding and raising their young. Inshore fishermen using the correct tackle can find most of their catch around the meadows.
In the various regions of Italy, Neptune grass meadows have been given several local names: Alghero, in Sardinia, takes its name from the stretch of Neptune grass (called *alga* locally, as on old nautical maps) offshore. The most common name is *tressa* in Friuli Venezia Giulia or *trezza* in Sicily - where the fishing village, the setting for Verga's "Malavoglia", is called Acitrezza, because it is sited next to a Neptune grass meadow.
The presence of meadows in the sea is manifested on beaches by heaps of leaves and rhizomes, which may reach a metre or more in height and extend for dozens of metres. These beached deposits of Neptune grass debris host important communities of detrivorous animals, fungi and bacteria, which recycle and mineralise the organic matter, fertilising the coastal strip and triggering an efficient food chain (see box on p. 152).

Heaps of beached plants

Beached leaves and rhizomes accumulating on the shore were considered a valuable resource in the past and were used for such purposes as wrapping ceramics, terracotta and glassware in a protective bundle, as bedding for livestock, and as fertiliser on farmland. Seagrass fruit were eaten in some coastal areas of North Africa, and were given, together with their leaves, as additional food for livestock.
Seagrass meadows are stable formations and resistant to climate change, but they are vulnerable to illegal trawling inshore and damage caused by the anchoring of vessels.
Furthermore, the pollution caused by the discharge of unpurified wastewaters and dumping along the coast of materials from demolished buildings, industrial wastes and plastic coverings from greenhouses all lead to the death of the meadows.

EXCURSION TO A BEACH

After heavy seas, especially in autumn and winter, Neptune grass debris containing a large number of algae and animals, both benthic and pelagic, accumulates on the beaches facing gulfs and bays where seagrass meadows grow. Choose a cloudy morning in order to find fresh organisms. As well as containers, fixing fluids, and the equipment listed above, it is worth taking a rake to dislodge the first 30-50 centimetres of the heaps, in order to uncover specimens to collect.
Assign various tasks to groups of three-five pupils, encharged with collecting the remains of angiosperms, algae, invertebrates, vertebrates, and unusual remains of natural or human origin. After about an hour, ask the groups to fix and label their specimens, and provide some information on their origin and nature. Back in the laboratory, bags and canisters should immediately be stored in a fridge.

Beached marine phenerogame

Giuseppe Giaccone

On sandy beaches or low ledges of rocky coastlines, small banks (banquettes) or piles of beached debris of sea plants accumulate, mostly of Neptune grass and other marine phanerogams. The plant residues are formed of entwined leaves, rhizomes partly covered by the bases of their leaf sheaths, balls of tangled fibres (marine hairballs) and, to a lesser extent, the animals and plants which live on and between the submerged plants. These plant residues are used by communities (facies) of detrivorous animals, which in turn are preyed upon by groups of carnivores.
In the supralittoral environments subject to sea spray, two biocoenoses are distinguished: the upper section of rapidly drying beached heaps and the slowly drying lower section. The former is found on beaches of fine sand exposed to the sun. The animal facies is characterised by the insects *Phaleria provincialis*, *Cicindela* sp., *Bledius areanarius*, *B. juvencus*, *Tridactylus variegatus*, the spider *Arctosa perita*, isopods of the genus *Porcellio* and the amphipods *Talitrus saltator* and *Orchestia stephenseni*. The latter biocoenosis is shaded from the sun inside the piles of dead Neptune grass leaves or amongst the beach pebbles. The animals found here are amphipods of the genus *Orchestia*, the isopods *Tylos sardous* and *Halophiloscia couchii*, the gastropods *Truncatella subcylindrica*, *Alexia myosotis*, *A. firmini* and *Ovatella bidentata*, centipedes, pseudoscorpions, beetles, particularly of the genus *Bledius*, various dipterans, and earwigs. Species of the genus *Orchestia* (*O. mediterranea*, *O. montagui* and *O. platensis*) are particularly abundant among the dead Neptune grass leaves. The lower section of the beached heaps of seagrass and algae lies in the mesolittoral level. The animal population which settles here is considered an impoverished facies of mesolittoral debris. Its components are essentially detrivores which feed on the plant residues of the lower section of the beached heaps and their predators. Among the amphipods found here are *Gammarus olivii* and *Allorchestes aquilinus*, among the isopods, *Sphaeroma serratum*, the decapod *Pachygrapsus marmoratus*, and the polychaete *Perinereis cultrifera*. Many of these organisms are also found in the cavities of the calcareous formations, mostly made up of algae and molluscs, in the intertidal zone.
The beached biomass is composed mainly of Neptune grass debris, and represents about 12% of the primary production of the meadows growing in the submerged environment lying off the beach. Part of this biomass is left behind by detrivores, is mineralised by bacterial flora, and transformed into nutrient salts which fertilise the submerged meadows. The mechanical removal of these beached heaps to make room for bathing facilities is prohibited by law.

Talitrus saltator

LABORATORY OBSERVATIONS

In the laboratory, ask the groups of pupils to open the containers. Many of the plants may be used to form a herbarium, with the help of a naturalist. Place the other plant samples, to be used for microscope observations, in a basin with seawater (brought back from the excursion and stored in the dark in a fridge) or in a black bag to avoid putrefaction or flowering of bacteria and unicellular algae. Prepare slides with tiny sections of the plants, to be viewed with transmitted light under the optical microscope. Animal specimens should be placed in Petri dishes with seawater and viewed under the stereomicroscope with reflected light.

Pisa sp. among Neptune grass leaves

Emphasise how important it is to observe and understand the biodiversity that characterises Neptune grass leaves and rhizomes. As regards plant architecture, show pupils how the leaves are grouped in sheaths of 5-7 leaves. The external leaves are the oldest and therefore have more epiphytes, with greater cover in the middle section and tip, because the functioning leaf produces mucous substances at the base to discourage epibionts from attaching themselves. The internal leaves are almost free of epibionts because they are more subject to mechanical stress. Pupils should also note the dark patches on adult leaves, caused by tannin, which is produced to reduce grazing by herbivores. Point out the rich variety of calcified animals and algae, which turn the leaves grey, and the networks of bryozoans and hydrozoans which attract carnivorous grazers, including some fish. On the rhizomes, note the bases of the leaf sheaths which, when they fall off, leave an alternating pattern of scars. Each centimetre in length of the orthotropic rhizomes (with vertical growth) corresponds to approximately one year of growth of the Neptune grass plant, so a rhizome ten centimetres tall is around ten years old. Plagiotropic samples (rhizomes with horizontal growth) may be found among the rhizomes, with leaf buds at the tips and adventitious roots in the sub-apical section.
One exercise might be to construct a sheath of Neptune grass with handicraft materials (cardboard, raffia, balsa-wood, etc.), on the scale 1:1 (i.e., a sheath of around 1-1.5 m in length)

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Glossary

GLOSSARIO

> Anther: the part of a male flower that contains pollen: a modified leaf that is usually borne on a stalk called filament
> Auriculate: ear-shaped
> Bionomy: a branch of ecology concerned with the distribution of benthic organisms and the factors regulating benthos on various time-scales
> Carbohydrates: groups of natural molecules composed of carbon, hydrogen and oxygen, which organisms can use either immediately for growth or store for future use
> Climax: the relatively stable stage or community attained by an available population of organisms in a given environment, often constituting the culminating development in a natural succession
> Cryptic: serving to conceal; used especially of the pattern or colouring of animals
> Cryptogamic: of, or relating to, a plant reproducing by means of spores and not producing flowers or seeds
> Dioecious: a plant with male and female flowers on separate plants
> Endolithic: growing within or between stones
> Epibiont: an organism that lives on the body surface of another
> Epiphyte: a plant that grows on another plant
> Euryhaline: species able to live in waters with a wide range of salinity
> Euryphotic: species with a wide range of light and shade tolerance
> Eurythermal: species able to live in a wide range of temperatures
> Eutrophication: the process of nutrient enrichment (usually by nitrates and phosphates) in aquatic ecosystems
> Hydrolysis: a chemical reaction whereby one compound is split by water
> Ligule: the thin spoon-shaped appendage of a leaf
> Lipase: any of a class of enzymes that accelerate the hydrolysis or synthesis of fats
> Maërl: lime-producing red seaweeds
> Matte: tiered formations made up of entwined living and dead rhizomes and roots of Posidonia oceanica, with interstices filled with sediment. They alternate with empty areas (intermattes)
> Mesoherbivore: a herbivorous animal that grazes and lives in the same environment
> Microstenotherm: communities or organisms living in environments with constantly low temperature
> Monoecious: a plant with male and female organs on the same individual
> Orthotropic rhizome: having the longer axis more or less vertical
> Pelite: a rock composed of fine particles of clay or mud
> Pericarp: the outer wall of a fruit
> Phenolic compound: a poisonous chemical compound, also known as phenic acid, present in wood tar.
> Plagiotropic rhizome: having the longer axis inclined away from the vertical
> Polyphyletic: derived (as by convergence) from more than one ancestral line
> Primary producer: an organism capable of synthesising its own food from simple inorganic substances
> Sclerite: a sclerotised (hardened) plate of an arthropod integument
> Semi-amplexicaul: partially amplexicaul; embracing the stem half way round, like a leaf
> Suspensivore: an organism feeding on suspended particles in water
> Tracheid: a long tubular cell that is peculiar to xylem, functions in conduction and support, and is characterised by tapering closed ends which are not absorbed
> Velme: a typically Venetian dialectal word, indicating a shoal in the Lagoon of Venice
> Xylem: a complex tissue in the vascular system of higher plants, consisting of tracheids (see above) and typically constituting the woody element of a plant stem

List of species

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