How well do ecological indicators assess environmental status? Case studies in coastal and estuarine ecosystems

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2005

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Cover designed by Hugo Andrade

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FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

Doctoral dissertation in Biology (Scientific Area of Ecology) presented to the University of Coimbra

Dissertação apresentada à Universidade de Coimbra para obtenção do grau de Doutor em Biologia (especialidade Ecologia)

To my grandparents

The things with which we concern ourselves in science appear in myriad forms, and with a multitude of attributes. For example, if we stand on the shore and look at the sea, we see the water, the waves breaking, the foam, the sloshing motion of the water, the sound, the air, the winds and the clouds, the sun and the blue sky, and light; there is sand and there are rocks of various hardness and permanence, colour and texture. There are animals and seaweed, hunger and disease, and the observer on the beach; there may be even happiness and thought. Any other spot in nature has a similar variety and influences. It is always as complicated as that, no matter where it is.

(Feynman, 1995)

CONTENTS

This thesis is based on the following papers and book chapters, which constitute or are the bases of the different chapters.

Chapter 1. Marques, J.C., Salas, F., **Patrício, J**. & Pardal, M.A. 2005. Application of ecological indicators to assess environmental quality in coastal zones and transitional waters: two case studies. *In*: Jørgensen, S.E. (ed), Handbook of Ecological Indicators for Assessment of Ecosystem Health, CRC Press LLC, 65- 102 p.

Salas, F., Marcos, C., Neto, J.M., **Patrício, J**., Pérez-Ruzafa, A. & Marques, J.C. User friendly guide for using ecological indicators in coastal and marine quality assessment. (*submitted* to *Ocean and Coastal Management*)

Chapter 2. **Patrício, J**., Ulanowicz, R.E., Pardal, M.A. & Marques, J.C. 2005. Ascendency as ecological indicator for environmental quality assessment at the ecosystem level: A case study. *Hydrobiologia* (*in press*).

Chapter 3. **Patrício, J**., Ulanowicz, R.E., Pardal, M.A. & Marques, J.C. 2004. Ascendency as an ecological indicator: a case study of estuarine pulse eutrophication. *Estuarine, Coastal and Shelf Science* 60: 23-35.

Chapter 4. **Patrício, J**., Salas, F., Pardal, M.A., Marques, J.C. & Jørgensen, S.E. Ecological indicators in a re-colonisation field experiment. Contribution to theoretical ecology. *Ecological Indicators* (*in press*)

Chapter 5. Salas, F., **Patrício, J**., Marcos, C., Pardal, M.A., Pérez-Ruzafa, A. & Marques, J.C. Are Taxonomic Distinctness measures compliant to other ecological indicators in assessing ecological status? *Marine Pollution Bulletin* (*accepted)*

ACKNOWLEDGMENTS

I would like to take this opportunity to acknowledge those people who assisted me with their advice, expertise and encouragement, without which this study would not have been undertaken and completed.

This study was financially supported by Fundação para a Ciência e a Tecnologia (Portuguese National Foundation) through the grant SFRH/BD/820/2000, and lately also supported in the scope of the research project DYNAMOD (POCTI/MGS/37431/2001).

I am truly thankful to the Institute of Marine Research (IMAR) for all the logistic support. Thanks to that I always felt at home.

I am also indebted to the Chesapeake Biological Laboratory, University of Maryland Centre of Environmental Science and to the Royal School of Pharmacy, Copenhagen, for all the facilities.

I am forever grateful to my supervisor and mentor, Professor Dr. João Carlos Marques, for his unwavering dedication and for all inputs that were always unfailingly insightful, expansive, and cheerful. No word is sufficient to express my gratitude. Thank you for all the support.

I am similarly grateful to Professor Dr. Robert Ulanowicz and Professor Dr. Sven Erik Jørgensen for their guidance, knowledge and enthusiasm. Each of them, in a special way, is an inspiration for me. Science without dreaming new paths is too poor.

Many thanks to Dr. Søren Nors Nielsen from the Royal Danish School of Pharmacy, Denmark, whose comments helped with the final stage of balancing the Mondego estuary models. Thank you for the care and friendly talks.

To Dr. Sheila Heymans, from Fisheries Centre, University of British Columbia, Vancouver, Canada, for her friendship and support during my first stay at CBL and, of course, for her expertise and helpful discussions regarding Chapter 2 content. After more than 2 years without any contact, she gently agreed to help me again.

Particular thanks to Professor Dr. Bernard Patten, who despite being in a phase of refraining from taking new responsibilities, accepted to review part of this study. I am deeply grateful for his help and encouragement. He, too, is an inspiration for me.

I would also like to acknowledge all the hours spent by Professor Dr. Miguel Pardal, helping me with field and laboratory work, and proof reading of various sections of my thesis; his time and comments were very helpful and appreciated. Even in the middle of an extremely occupied life he was able to find time to help me. Thank you very much.

Special thanks go to Dr. Fuensanta Salas for her unconditional friendship, guidance, generous scientific and emotional support. I miss our hard work mixed with endless hours of “women talks”. Muchas gracias por todo.

What can I say about Gabi? I cannot adequately thank her for all her precious help and friendship. Her bravery to finish graduation studies in Biology while having a labour intensive administrative job, her strengths together with her fragilities, her humour and her astonishing professional competency made her an example for me.

I want to extend deserving thanks to my dear colleagues and friends at IMAR who always helped me through out this journey: Lilita, Patrícia, Susana, Maria João, Matilde (I am forever grateful for your inestimable help tracking inconsistencies), Elsa, Marina, Ana Isabel Lillebø, João Neto, Ricardo (countless thanks for helping me in the tedious formatting work), Isabel, Nuno Coimbra, Sílvia, Adriana, Nelson, Aranzazu, Ricardo Leitão, Alexandra, Filipe, Rute, Helena and Irene.

To Niels and Zhang, thanks to them I always felt welcome to Copenhagen and begun to appreciate the delicious Danish pastry and the thousand different varieties of Chinese green tea.

To all my dear friends: Renata, Cândida, Catarina Moura, Hugo (my dear cover designer), Zé Pedro, Saraiva, Sandrina, Manel, João Martins, Nicas, Alexandre, La-Salete, Pileco, Joana Cortez, Biá, Marta, Ricardo, Gustavo, JC, Natacha, Hugo Cabral, Ana Viegas, Carlos, Anabela, Bilal, Heléne, Majda, Célia… without our cinema sessions, late-night dinners, tea meetings, endless talks, alternative programmes, travels, and inestimable friendship, this growing process would have been (for sure) a good deal more painful.

I am also deeply grateful to Maria do Carmo, Carlos, Ana, Eurico and Daniel for their support, true care and rewarding smiles. Thank you for making me feel part of the family.

To my family… all cousins, aunts and uncles. I am very proud to be Mateus Patrício.

I am most thankful for the unwavering encouragement and love of my parents Migú and Zé and my dear sister Inês. Thank you for believing in me and respecting my way of being.

Ultimately, I am most grateful to João. Thank you for not losing sight of the big picture and for hanging in there with me during the difficult times. As always, you have been a great comfort and friend to me, your patience I can not fathom. Thank you for helping me to connect with life, beauty and love.

SUMMARY

The main goals of this dissertation were 1) to test the behaviour of three ecological indicators with holistic characteristics, respectively i) based on network analysis (Ascendency), ii) thermodynamically oriented and often used in ecological modelling (Eco-Exergy and Specific Eco-Exergy) and iii) diversity measures that take into consideration phylogenetic links (Taxonomic Distinctness and associated measures), by the use of empirical data sets, collected in four different ecological scenarios (a gradient of eutrophication symptoms in the south arm of Mondego estuary, Portugal; different hydrodynamic regimes and impacts considering both the south and north arms of Mondego estuary; a recovery process after physical disturbance, from a field experiment carried in the Atlantic rocky shore, Papoa, Portugal, and various types of pollution in the Mar Menor coastal lagoon, Spain) and 2) to appraise the performance of these three ecological indicators in comparison with more conventional and broadly applied ones (*e.g.* Shannon-Wiener, Margalef and Pielou indices).

A brief review of the ecological indicators utilised from the benthic ecological perspective to assess the status of coastal and estuarine ecosystems was done in Chapter 1. This review was carried out aiming at describing how diverse approaches can be, and to put the selected ecological indicators in perspective in the general framework.

Chapter 2 dealt with the steps followed to develop mass balanced models of food webs in three areas along a well-documented gradient of eutrophication symptoms in the south arm of the Mondego estuary (Portugal), using the Ecopath with Ecosim software package. The sum of consumptions, exports, respiration, production, flow to detritus, TST and annual rate of net primary production was always higher in the *Zostera* meadows, followed by the strongly

eutrophic area and, finally, by the intermediate eutrophic area. The Ecopath mass balanced models successfully provided a synthesis of the current knowledge of the food web and trophic flows along the gradient of eutrophication symptoms in the Mondego estuary. This tool was particularly important to calculate the network based ecological indicator – Ascendency.

In Chapter 3, Ascendency was used as an ecological indicator. Moreover, it was tested whether the network definition of eutrophication properly encompassed changes in community structure observed along the gradient of eutrophication symptoms (Mondego estuary). Pulse eutrophication was considered as the major driving force behind a gradual shift in primary producers from a community dominated by rooted macrophytes to a community dominated by green macroalgae. The measures associated with the intermediate eutrophic area turned out not to be intermediate to those at the gradient extremes. The most likely explanation appears to be the highly unstable nature of this area. Conditions along the spatial gradient were discussed as representing various stages in the temporal evolution of the system, and analysed in the framework of the Intermediate Disturbance Hypothesis, Bifurcation, Chaos, and Catastrophe theories.

In Chapter 4, through a re-colonisation field experiment, three main questions were approached regarding the ecological indicators behaviour and the dominant growth forms during the process of recovery. Shannon-Wiener index, Margalef index, Pielou evenness, Eco-Exergy and Specific Eco-Exergy were applied to characterise the state of the community during the process. Results showed that the replacement of species over time occurred, species richness increased rather rapidly and species composition was similar in disturbed and undisturbed areas. Eco-Exergy and Specific Eco-Exergy provided useful information about the structural development of the community. Overall, the characteristics of a systems’ recovery after disturbance appear to be dependent on the spatial scale of the disturbance (openness hypothesis).

Finally, in the last chapter (Chapter 5), the robustness of Taxonomic Distinctness measures was tested in different scenarios (estuarine eutrophication, different hydrological regimes, organic and heavy metal pollution, and re-colonisation after physical disturbance), analysing, simultaneously, its correlation with other types of ecological indicators. Results showed that, in most of the case studies, only Total Taxonomic Distinctness was relatively satisfactory in discriminating between disturbed situations. Other Taxonomic Distinctness measures have not proved to be more sensitive than other ecological indicators (Shannon-Wiener, Margalef, and Eco-Exergy indices). Therefore, this approach does not seem to be particularly helpful in assessing systems' ecological status with regard to the Water Framework Directive implementation.

As a final remark, it can be said that the ecological status and development should be evaluated by combining a dynamic battery of useful and efficient indicators, which may provide complementary information.

RESUMO

Esta dissertação teve como principais objectivos 1) testar o comportamento de três indicadores ecológicos com características holísticas, respectivamente i) baseados na análise de rede (Ascendência), ii) oriundos da Termodinâmica e frequentemente utilizados em modelação ecológica (Eco-Exergia e Eco-Exergia Específica) e iii) medidas de diversidade que levam em linha de conta relações filogenéticas ("Taxonomic Distinctness" e medidas associadas), utilizando dados empíricos, recolhidos em quatro cenários ecológicos distintos (um gradiente de sintomas de eutrofização no braço sul do estuário do Mondego, Portugal; diferentes impactos e regimes hidrodinâmicos considerando, simultaneamente, o braço norte e o braço sul do estuário do Mondego; um processo de recuperação após perturbação física, através de uma experiência de campo levada a cabo na costa rochosa Atlântica, Papoa, Portugal; e vários tipos de poluição na lagoa costeira do Mar Menor, Espanha) e 2) comparar o desempenho destes três indicadores ecológicos com a performance de outros indicadores mais convencionais e largamente aplicados (*e.g.* índices de Shannon-Wiener, Margalef e Pielou).

No Capítulo 1 foi feita uma breve revisão dos indicadores ecológicos utilizados para avaliar a qualidade ecológica de ecossistemas estuarinos e costeiros, do ponto de vista da ecologia bentónica. Esta revisão foi levada a cabo com o objectivo principal de descrever quão variadas e diversas são as abordagens possíveis e contextualizar os indicadores seleccionados no panorama geral.

O Capítulo 2 lidou com as diferentes etapas necessárias para desenvolver modelos balanceados de massa das redes tróficas de três áreas ao longo de um gradiente de sintomas de eutrofização no braço sul do estuário do Mondego (Portugal), utilizando o programa "Ecopath with Ecosim". A soma

dos consumos, exportações, respirações, produções, o fluxo para os detritos, a actividade total do sistema (TST) e a taxa anual líquida de produção primária foi sempre mais elevada nas pradarias de *Zostera* sp., seguida da área fortemente eutrofizada e, finalmente, pela área intermédia. Os modelos balanceados de massa, desenvolvidos com o Ecopath, permitiram com sucesso a síntese do conhecimento actual do sistema respeitante aos fluxos energéticos e redes tróficas ao longo do gradiente de sintomas de eutrofização no estuário do Mondego. Esta ferramenta mostrou-se particularmente importante para o cálculo do indicador ecológico baseado na teoria de redes – Ascendência.

No Capítulo 3, a Ascendência foi utilizada como indicador ecológico. Foi testado se a definição de eutrofização proposta pela análise de redes detecta correctamente as alterações na estrutura das comunidades observadas ao longo do gradiente de sintomas de eutrofização (estuário do Mondego). A eutrofização por impulsos (pulse eutrophication) foi considerada como sendo a principal força motriz por trás da alteração gradual dos produtores primários de uma comunidade dominada por macrófitas para uma comunidade dominada por macroalgas verdes. As medidas associadas à área intermediamente eutrofizada não se revelaram intermédias relativamente aos extremos do gradiente. A explicação mais plausível prende-se com a natureza instável desta área. Discutiram-se as condições ao longo do gradiente espacial como sendo representantes das várias etapas da evolução temporal do sistema. Esta evolução foi analisada à luz da Hipótese dos Efeitos Intermédios e das teorias da Bifurcação, Caos e Catástrofes.

No Capítulo 4, através de uma experiência de campo de re-colonização, foram abordadas três questões referentes ao comportamento dos indicadores ecológicos e às formas de crescimento dominantes ao longo do processo de recuperação. Foram aplicados os índices de Shannon-Wiener, Margalef, Pielou, Eco-Exergia e Eco-Exergia Específica, de forma a caracterizar o estado da comunidade, ao longo do processo de re-colonização. Os resultados mostraram que ocorreu a substituição de algumas espécies ao longo do tempo, que a riqueza específica aumentou muito rapidamente e que a composição específica das áreas perturbadas e não perturbadas foi similar. A Eco-Exergia e a Eco-Exergia Específica forneceram informação útil sobre o desenvolvimento estrutural da comunidade. Em resumo, as características inerentes ao processo de recuperação de uma comunidade parecem ser dependentes da escala espacial da perturbação e da abertura do sistema ao ambiente circundante (openness hypothesis).

Finalmente, no último capítulo (Capítulo 5), foi testada a robustez da "Taxonomic Distinctness" e medidas associadas em diferentes cenários (eutrofização estuarina, regimes hidrológicos distintos, poluição por metais pesados e enriquecimento orgânico e re-colonização pós perturbação física). Simultaneamente, foi analisada a sua correlação com outros tipos de indicadores ecológicos. Os resultados demonstraram que, na maioria dos casos de estudo, apenas a "Total Taxonomic Distinctness" apresentou um comportamento relativamente satisfatório na discriminação de situações perturbadas. As outras medidas associadas não revelaram ser mais sensíveis do que outros indicadores ecológicos (Shannon-Wiener, Margalef e índices baseados na Eco-Exergia). Deste modo, esta abordagem parece não auxiliar particularmente na avaliação da qualidade ambiental dos sistemas, no âmbito da implementação da Directiva Quadro da Água (EC, 2000).

Como nota final, pode ser dito que a avaliação da qualidade ambiental dos ecossistemas deve ser feita combinando uma bateria de indicadores ecológicos eficientes e úteis que possam, através da sua acção combinada, oferecer informação complementar.

GENERAL INTRODUCTION

THE CHALLENGE THAT TRIGGERED THIS STUDY

Just after finishing my undergraduate studies I had the opportunity to be introduced to some eminent theoretical ecologists that confronted me with a new way of doing science. They showed me that scientists observe nature, search for generalisations, and provide explanations for why the world is as it is. However (and this was the novelty for me), generalisations can be of two kinds. The first are descriptive and inductive. They are derived from observations and therefore refer to observables (scientists must first describe nature as accurately as possible and then discover apparent causal relationships among observables). The second are often imaginative and form the axioms of a deductive theory. They often refer to unobservables. Contrarily to Physics, I began to recognise that Biology and Ecology have many inductive generalisations but few recognised universal laws and virtually no deductive theories (Murray, 2001) behind them. Consequently, a question arose: is it possible to develop a theoretical framework able to explain the numerous observations, rules and correlations dispersed in the ecological literature during the last few decades?

Some authors (Pickett *et al.*, 1994; Wilson, 2003; Boero *et al.*, 2004) claim that universal laws do not apply to the ecological realm, that exceptions will always be found, and that *ad hoc* explanations are not a curse to our science, but a blessing. They state that if we look at laws, we end up with Physics, but Ecology is much more than Physics (Pickett *et al.*, 1994), even if it obeys its laws, just as literature is much more than grammar, even if it obeys grammar's laws (Boero *et al.*, 2004). Even if this perspective raises several challenges, thinking that ecological predictions are possible does not imply that biologists should copy mathematicians and physicists. It means that if they were able to do it, we should also (at least) try to do it. It is undeniable that these approaches have contributed much to advance our knowledge of how ecosystems function. Moreover, these generalisation attempts counterbalance the overabundance of careful descriptions that remained sterile, each one with its limited meaning. It is patent that ecological literature from the last decades contains a large number of observations, data sets, correlations, and some rules resulting from increasing interest in sustainable development and environmental health. Yet, it must be said that such observations and rules are comparable to lonely islands in an immense ocean and only rarely do links exist between them (Patten *et al*., 2002b). Almost each new study is isolated; each latest explanation lacks coherence with a paradigm.

So, why should we care at all about theory? The answer is, because a theoretical frame provides the context wherein researchers and others can interpret and integrate empirical results. Without integration, without interpretation of raw observations within a consistent theoretical frame, and without communication to affected constituencies, there can be no organised or effective science. Only description is possible, not basic understanding that can be transmitted to the public (Patten *et al.,* 2002b).

This thesis is a collection of scientific papers focused mainly on three different types of ecological indicators, with special emphasis on the potential applicability and theoretic robustness of each of the chosen indicators. Although it would be a great pleasure for me to say that this thesis was a sound contribution for Ecosystem Theory, I suppose I still have plenty to learn before accomplishing that. Nevertheless, I strongly believe that testing ecological indicators derived from a consistent theoretical frame with real empirical data is a useful contribution to test some of the few generalisations and predictions available for Ecology.

THE ECOSYSTEM THEORY PERSPECTIVE

How did it start?

In its more than 100 years of development as a science, Ecology has progressed from early descriptions of patterns in nature to experimental studies of underlying processes in the field and in laboratory model systems (Paine, 1994). From the end of the 1950s onwards, process studies and dynamics theory for species interactions and biogeochemical cycles stimulated productive interactions of observation, modelling, experimentation, and attempts at prediction (Power *et al*., 2005). Community ecologists focused on species interactions. Their experiments often yielded surprising results that could not have been detected without manipulations. This created deep scepticism in this community about inferring process from natural, non manipulated patterns (Paine, 1994). Such experiments were necessarily limited in scope, focusing on small spatial and temporal scales and restricted subsets of “interaction webs” (Menge *et al*., 1994; Polis *et al*. 2004). When computers came along, ecosystem ecologists created large energy or material flux models in an attempt to capture ecosystem dynamics on larger (*e.g.* regional) scales. However, due to the difficulty of gathering sufficient information on complex dynamic natural ecosystems, ecologists have been more successful at explanations than predictions (Power *et al.,* 2005).

Ecosystems, hierarchical systems and ecological complexity

Ecosystem studies use widely the notions of hierarchy and complexity. They are used interchangeably in the literature, which causes much confusion. As the terms are used in relation to ecosystems throughout this thesis, first it is necessary to clarify the concepts. Therefore, according to Patten *et al*. (2002a), **complexity** is that which is difficult to understand and hard to explain (Hornby, 1995), which is generally the consequence of multiple interrelationships between systems elements. This complexity increases with the number of interacting units and their interaction intensity (Nicolis, 1986). Complex systems range across large hierarchical scales: from genomes, to cells and their organelles, organisms to populations, communities, geosystems and biogeocoenoses, ecosystems, and so on.

On the other hand, **hierarchy** (in mathematical terms) is a partially ordered set. In less austere terms, a hierarchy is a collection of parts with ordered asymmetric relationships inside a whole. That is to say, upper levels are above lower levels, and the relationship upwards is asymmetric with the relationship downwards. Once the concepts are clarified it is possible to look further.

An ecosystem could be viewed merely as a dynamic material system, with inputs and outflows in rain, winds, leaching and streams. Or it could be viewed as an active system of matter and energy deployment, as flow cycles, food webs, or even more detailed processes like control by keystone predators. Or it could be viewed as a system of specifically biological interactions, using concepts of diversity and symbiosis. What is quite fascinating is that there is an emerging consensus among ecologists and environment scientists that many of today's urgent ecological and environmental problems across spatial-temporal scales are seen as complex systems problems (Li, 2004). Ulanowicz (2004) suggests that this complexity may require an essentially different way of apprehending how nature works, and Patten *et al*. (2002a) advert that analysing complexity is a problematic issue. There exist problems of (1) description; (2) measurement; and (3) understanding (large numbers of elements, indirect causalities, nonlinear functional relationships, distributed effects, and cross-scale interactions and control issues).

There are different sources and types of complexity in Ecology. One source of complexity in nature is based on the fact that more than a single system generally occupies any locale (Salthe, 1985). While systems of the same scale might exclude each other or further each other symbiotically, systems of different scale do neither, since they cannot directly interact. They exist, not side by side, but within and around each other, and so do not directly interact, but instead mutually constrain each other, somewhat as stable constants relate to variables in an equation (Salthe, 2005). Here comes the hierarchical perspective of nature. Following Salthe (2005), nature can be modelled as a scalar hierarchy, with systems embedded in other systems and holding still others within them. They are nested, as in [ecosystem [population [organism [cell]]]] (any level could be ignored for particular descriptive purposes in a given locale). Larger more slowly changing systems regulate many smaller ones, but the aggregate effects of lower scale systems can influence larger ones too. Our world has plenty of this type of interactions. Formally, the extension of scale just keeps going endlessly. An observed or studied system is exactly in the centre of the hierarchy. Therefore, when applying Hierarchical Theory it is essential to choose the right focal level, meaning that what happens in upper levels has a very strong influence on the lower levels, but what happens in the lower levels has a much weaker influence in the upper levels. For instance, a change at the ecosystem level can have a strong influence on the population, organism or cell levels, but what happens at the single individual level does not have a similar influence on the ecosystem as a whole.

Apart from the hierarchic organisation, other sources of complexity are large numbers and high diversity of components and connections, asymmetry and strong interactions. A system may also be complex in its behaviour. Several categories of behaviour are notoriously complex, namely non-linear, chaotic and catastrophic behaviours (Patten *et al.,* 2002a). In fact, Chaos Theory has an important concept to offer to Ecology: an apparently irrelevant factor, or an apparently irrelevant change in a relevant factor, can have an important impact on the history of a complex system making it behave in a non-linear fashion (Boero, 1996). In a number of situations, drivers of change are local and apparent. For example, the transition from a macrophyte- to a macroalgae-dominated estuary at the southern arm of the Mondego River, Portugal, depends on the amount of fresh water entering the system in late winter and spring, which in turn varies according with both precipitation and river management practices in upstream agricultural lands (Martins *et al.,* 2001); or, for the same system, Leitão (2005) claims that there is a close relationship between the disappearance of *Symphodus* sp., a fish that lives in association with the macrophyte *Zostera noltii*, and the seagrass die-off due to eutrophication problems. However, other apparently irrelevant changes or behaviours may have less obvious causes and even more unpredictable consequences. A good example is the *Spartina* spp. marsh die-offs along the south-eastern USA coast that might be linked to human over-harvesting of blue crabs offshore, an inference that was enabled by the detection of hidden keystone species, a snail *(Littoraria irrorata*) and the fungi that colonise its grazing scars (for more details see Silliman & Bertness, 2002; Bertness *et al.*, 2004). Even so, the causes of marsh die-off have not yet been fully assessed. Ulanowicz (1997) suggests that living systems navigate a “window of vitality” – a middle ground between too much order and too much disorder. He shows this graphically by plotting the effective connectance per node versus topological connectance per node of 38 ecosystems based on their trophic flow networks. All 38 systems exist in a stable region between the extremes of disorder (maximally connected networks, or those at the “edge of chaos”) and order (minimally connected, mechanical or “brittle” network). According to the author, it seems as though the self-organising tendencies of ecosystems allow them to avoid chaos for the most part.

Given the above, the complexity and hierarchical structure of nature are tremendous challenges in the field of Ecology. They impact the development of theory, the conduct of field studies, and the practical application of ecological knowledge.

Holism *vs* Reductionism

As already mentioned, an ecosystem consists of so many interacting components that it is impossible ever to be able to understand how it functions by only examining these relationships. Even when it is possible to examine the parts by reduction to simple relationships, according to Allen (1988) when the parts are put together they will form a whole, which behaves differently from the sum of the parts, *i.e.,* the whole is much more than the sum of its components.

Although most Ecology textbooks start by saying that Ecology is the science of interactions and that it has emergent properties, in subsequent pages, however, ecological systems are usually split into parts, and there is no synthesis (*e.g.* ecosystem functioning is just metabolism). Reductionistic

Ecology is a contradiction that goes against the essence of Ecology but, paradoxically, is the most practised approach to Ecology. What is the other route, then? We need a holistic approach, where the entire system is considered and where it is attempted to reveal properties at the system level (Jørgensen, 2002).

Towards a consistent Ecosystem Theory: state of the art

In 1969, Ludwig von Bertalanffy, the founder of General Systems Theory, expressed the general discontent of all branches of natural and human sciences with the mechanist and reductionistic tendency, and the need for a reorientation in scientific thinking in order to deal with the complexities of organised wholes as open systems (Naveh, 2004). Apparently, 30-40 years ago, the time was not yet ripe for such a holistic theory and its realisation in scientific research. Meanwhile, the developments in computer science, a new far-from equilibrium Thermodynamics, new mathematical tools such as Catastrophe Theory, Fractal Theory, Cybernetics, Non-linear Dynamics and Network Theory, have provided efficient instruments. Moreover, thanks to Systems Theory and Hierarchy Theory, ecologists have been already aware of the interconnections and coherences within the web of life and its total environment (Naveh, 2004). Therefore, many excellent contributions to a more profound understanding of ecosystems have been launched during the last two to three decades (Jørgensen, 2002).

Knowing that the properties of an ecosystem can only be revealed by the use of a pluralistic view, it is not surprising that there have been several different ecosystem theories published in the scientific literature. Ten to fifteen years ago the presented theories seemed very inconsistent and chaotic. How could E.P. Odum's attributes (1969), H.T. Odum's Maximum Power (1983), Ulanowicz's Ascendency (1980), Patten's Environs and Indirect Effects (1992), Kay and Schneider's Maximum Exergy Degradation (1992), Jørgensen's Maximum Exergy Principle (Jørgensen & Mejer, 1977; Jørgensen 1982, 2001), and Prigogine's (1947) and Mauersberger's Minimum Entropy Dissipation (1982) be valid at the same time? According to Jørgensen (2002), new results, open discussions among the contributing scientists and mutual respect for the other possibilities have led to a formation of a pattern, where all the theories contribute to the total picture of an ecosystem development.

The first contribution to a clear pattern of the various ecosystems theories came from the network approach used by Bernard Patten. Fath & Patten (2001) used their results to determine the development of various variables used as goal functions (Exergy, Power, Entropy, etc.). Their results can be summarised as follows:

1. Increased inputs (more solar radiation is captured) mean more biomass, more exergy stored, more exergy degraded, therefore also higher entropy dissipation, more throughflow (power), increased Ascendency, but no change in the ratio indirect to direct effect or in the retention time for the energy in the system.
2. Increased cycling implies more biomass, more exergy stored, more throughflow, increased Ascendency, increased ratio of indirect to direct effects, increased retention but no change in exergy degradation.

Almost simultaneously, Jørgensen *et al*. (2000) published a paper claiming that ecosystems show three growth forms:

I. Growth of physical structure (biomass), which is able to capture more of the incoming energy in the form of solar radiation but also requires more energy for maintenance (respiration and evaporation).
II. Growth of network, which means more cycling of energy or matter.
III. Growth of information (more developed plants and animals with more genes), from r-strategists to K-strategists, which waste less energy but also usually carry more information.

Accordingly, these three growth forms may be considered an integration of E.P. Odum's attributes, which describe changes in ecosystems associated with development from the early stage to the mature stage.

Based upon five hypotheses that have been proposed to describe ecosystem growth and development (see Prigogine 1947, 1980; Mauersberger, 1983; Odum, 1983; Kay, 1984; Jørgensen & Mejer, 1977; Jørgensen, 2002; Ulanowicz, 1997), it was possible to formulate the Ecological Law of Thermodynamics, which intends to unite the above mentioned hypotheses. This hypothesis states: ecosystem development in all phases will move away from thermodynamic equilibrium and have the propensity to select the components and the organisation that yields the highest flux of useful energy through the system and the most exergy stored in the system. This also corresponds to the highest Ascendency.

The aforesaid basic hypothesis for ecosystem development was formulated in an applicable form and accepted by several systems ecologists (Jørgensen, 2002). Of course, it has to be refined and its applicability must be improved. Ecologists may do this by adding to the theoretical base but another great need is to establish the extent to which the relevant observations and experimental results appearing in the ecological literature actually conform to and support the developing rules of systems assembly and to identify where adjustments are necessary (Reynolds, 2002).

Ecological indicators

Undeniably, no area of science can be successful without a mutually supportive interaction between theory and empiricism. Having highlighted the complexity of the natural world, a major challenge for ecologists will be to provide robust inputs not only to understand, but also to solve the urgent global environmental problems. A common, integrative conceptual framework in which both theory and empiricism can engage will definitely help. Therefore, the theoretical advances should be intimately linked with their application. In order to accomplish this endeavour some tools are already available; modelling techniques and ecological indicators are two good examples.

Commonly, ecological indicators are used to supply synoptic information about the state of ecosystems. Most often, they address ecosystem structure or functioning accounting for certain aspects or components, for instance nutrient concentrations, water flows, macroinvertebrate and vertebrate diversity, plant diversity, etc.

The goal of a “new generation” of holistic ecological indices must be to address the ecological integrity at the system level. Broadening from monitoring potential environmental contaminants, based primarily on toxicological measurements, to a wider focus on environmental “health” is the challenge. True ecological indicators are inherently relational, monitoring the symmetries of exchange among components that maintain system integrity (Patten *et al*., 2002a). According to this idea, we can attempt to capture at least part of the reality developing ecological indicators that combine numerous environmental factors in a single value, and that can be useful in establishing a valuable connection between empirical research, modelling and management (Marques *et al*., 2005).

INTENDED CONTRIBUTION

Bazzaz and other ecologists (1998) argued that when they began their careers, “good science consisted of two basic activities: (1) doing first-rate research and (2) publishing it in the technical literature for the benefit of scientific colleagues”. They firmly believed that a third activity needed to be added by all scientists, namely informing the general public of the relevance and importance of Ecology. Ecologists and ecological knowledge currently play secondary roles in many of the decisions that affect the environment (Palmer *et al*., 2005). In view of the growing evidence showing that better environmental decisions result when choices are informed by dialogue among scientists, policy makers, decision makers, and the public (Parsons, 2001; Worcester, 2002), the lack of engagement of ecologists is disturbing. While ecologists need to be more proactive in conveying their knowledge, they also need to listen and respond to the needs of society. It is a reality that ecological science does not always answer the questions that matter to user groups, because user needs are not always well understood, or are not given sufficient consideration when priorities are set by the research community. As a consequence, Ecology is not always seen as relevant (Cash *et al.*, 2003), and ecologists are therefore not always included in contexts where they could make fundamental contributions. This lack of aptitude to offer solutions might be another symptom of a weak and young science, not ready yet to be taken seriously. Unquestionably, a stronger deductive theory would help to overcome the challenge.

Nonetheless, an obvious low tolerance is usually exhibited for “theory for its own sake” – for concepts that appeared too specialised, or could not be immediately transformed to serve pragmatic ends, but what is clear is that one cannot apply what one does not have. In other words, if complex hierarchical systems (*e.g.* ecosystems) related problems are inherently difficult, progress in solving them without commitment to developing the requisite science, however specialised and esoteric it may initially seem to other scientists, managers and the public, is not likely to be made (Patten *et al.*, 2002b). Complex systems thinking and approaches may provide us a new opportunity to comprehensively understand the nature of natural systems, which will allow the development of management strategies for sustainability that are adaptable and flexible, able to deal with uncertainty and surprise, and have the capacity to adapt to change (Li, 2004).

After all, if this approach could at least help all ecologists that still feel inwards the need for a more general and integrative theory able to explain their observations along with their experimental results (Marques & Jørgensen, 2002) and facilitating its application to “real-world” problems, the effort would have been worthwhile.

OBJECTIVES AND QUESTIONS EXAMINED

The main objectives of this study can be outlined as follows:

1. To test the behaviour of three ecological indicators with holistic characteristics, respectively i) based on network analysis (Ascendency),

ii) thermodynamically oriented and often used in ecological modelling (Eco-Exergy and Specific Eco-Exergy) and iii) diversity measures that take into consideration phylogenetic links (Taxonomic Distinctness and associated measures). Their performance is tested by the use of empirical data sets, collected in the following four different ecological scenarios:

   a) Gradient of eutrophication symptoms in the south arm of Mondego estuary (Portugal);
   b) Different hydrodynamic regimes and impacts considering both the south and north arms of Mondego estuary;
   c) A recovery process after physical disturbance, from a field experiment carried in the Atlantic rocky shore (Papoa, Portugal);
   d) Various types of pollution, such as organic enrichment and heavy metals contamination in the Mar Menor coastal lagoon (Spain).

2. To appraise the performance of these three ecological indicators in comparison with more conventional and broadly applied ones (*e.g.* Shannon-Wiener, Margalef and Pielou indices).

From pursuing these objectives, several main questions were approached:

1. Considering the existing framework of ecological indicators what are the particular characteristics of the three selected ones that might make them gainful when compared to others available? (Chapter 1)
2. Can these indicators perform a synthesis of our knowledge about the systems? Moreover, can the modelling tool Ecopath assist us in building up such synthesis? (Chapter 2)

3. How do different ecological indicators perform comparatively in assessing the environmental status along a gradient of eutrophication symptoms? (Chapters 3 and 5)
4. Does the network definition of eutrophication properly track changes in community structure along a known gradient of eutrophication symptoms? (Chapter 3)
5. How do different indicators elucidate the successive phases during a recovery process? (Chapters 4 and 5)
6. What does grow first during a community succession, in this case simulated through an experimental recovery process: biomass or complexity? (Chapter 4)
7. Can ecological indicators, namely the three selected, help in recognising the three growth forms proposed by Jørgensen *et al.* (2000): biomass, network and information, throughout such a recovery process? Which indicators reflect better each of the three growth forms? (Chapter 4)
8. Can the selected ecological indicators be considered effective in elucidating pollution/disturbance bearing in mind environmental management, namely the European Union Water Framework Directive implementation? (Chapter 5)

THESIS OUTLINE

This thesis presents a general introduction (Figure 1) that addresses the rationale of the work carried out, namely with regard to some of the most challenging problems and interrogations in theoretical Ecology, and identifies the study objectives and the main questions examined.

Subsequently, the thesis is organised in five chapters, written in a concise and simple format where the essential issues were emphasised over the redundant details, which are based in five papers in international scientific

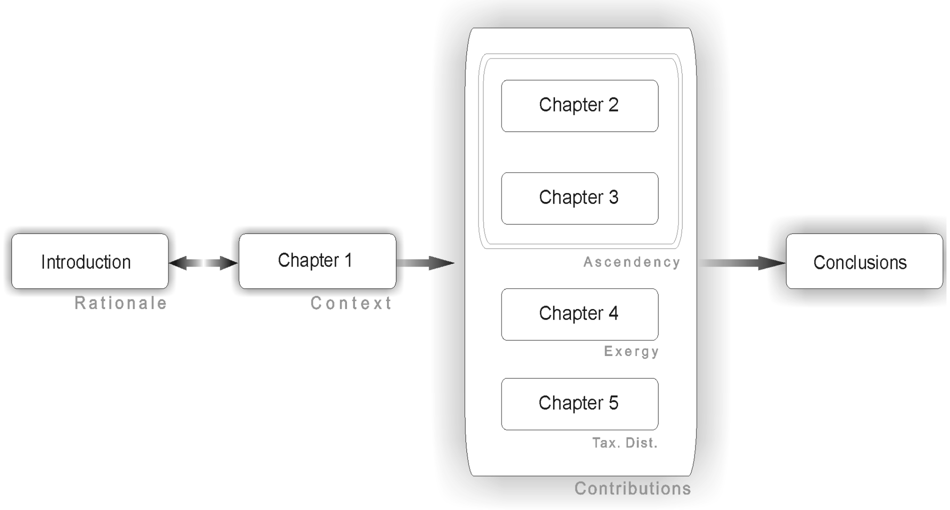


Figure 1. Thesis outline.

journals (published, in press or submitted for publication) and one book chapter (already published):

1. *The status of coastal and estuarine ecosystems assessed from the benthic ecological perspective. A brief review of the ecological indicators utilised.*
   This chapter describes what ecological indicators/indices are and their purpose. It also addresses different levels from which biodiversity can be approached, together with the concerns and challenges of using these metrics to assess an ecosystem's condition. Some criteria to evaluate and select ecological indicators are examined. Moreover, a brief review of the indices more commonly used in assessing ecological status is done in order to enlighten the context that lies beneath the selection of the three ecological indicators tested in this study (Ascendency, Exergy and Taxonomic Diversity measures). This review was carried out aiming at describing how diverse approaches can be, and to put the selected ecological indicators in perspective in the general framework.

2. *Mass balanced models of the food web in three areas along a gradient of eutrophication symptoms in the south arm of the Mondego estuary (Portugal).*

   In this chapter we describe the steps followed to develop mass balanced models with regard to three areas along the eutrophication gradient in the south arm of the Mondego estuary (Portugal), using the Ecopath with Ecosim software package. The main purpose was to build flow webs to further estimate (Chapter 3) a network based ecological indicator.

3. *Ascendency as ecological indicator: a case study on estuarine pulse eutrophication.*

   In this chapter, Ascendency is used as an ecological indicator. Moreover it is tested whether the network definition of eutrophication properly encompasses changes in community structure observed along a known gradient of eutrophication symptoms in the south arm of the Mondego estuary.

4. *Ecological indicators performance during a re-colonisation field experiment and its compliance with ecosystems' theory.*

   This chapter describes a re-colonisation field-experiment carried out in an intertidal rocky community to answer three main questions: (1) how do different ecological indicators perform in capturing the state of the system during the process of recovery (particularly, Eco-Exergy and Specific Eco-Exergy), (2) what does grow first during a community succession, biomass or complexity?, and (3) can the chosen ecological indicators help in recognising the three forms of growth: biomass, network and information, during re-colonisation?

5. *Do Taxonomic Distinctness measures accord with other ecological indicators in assessing ecological status?*

   In this chapter the robustness of Taxonomic Distinctness measures is tested, applying these measures in different scenarios (estuarine eutrophication, organic pollution, and re-colonisation after physical disturbance) and analysing, simultaneously, its compliance to other types of ecological indicators (Shannon-Wiener, Margalef, and Eco-Exergy indices). The aim is to evaluate its effectiveness as pollution biological indicator, namely in the scope of the European Union Water Framework Directive implementation.

At the end, a general discussion and conclusions Chapter examines what were the major contributions of the present study concerning the answer to our starting question: how well do ecological indicators (particularly, the three selected ones) assess environmental status?

CHAPTER 1

The status of coastal and estuarine ecosystems assessed from the benthic ecological perspective. A brief review of the ecological indicators utilised

ABSTRACT

Ecological indicators and indices are used as quantitative tools in simplifying, through discrete and rigorous methodologies, the attributes and weights of multiple parameters with the intention of providing broader indication of a resource, or the resource attributes, being assessed. These measures will only be effective if they are developed according to a hierarchical model (values, objectives, criteria and ecological indicators) that encompasses all the complex attributes that environmental systems display. In order to be useful, indicators and indices must also take into account that biodiversity has a hierarchical structure, which ranges from the ecosystem and landscape level, through the community level and down to the population and molecular level. With the purpose of choosing or developing ecological indicators or indices a number of useful criteria are listed. This brief review covers mostly those indicators more commonly used to assess the status of coastal and estuarine environments and among these the ones further concerned to benthic communities. Finally, some considerations regarding ecological metrics' real value are made.

KEYWORDS: Ecological indicators, environmental assessment, coastal systems, estuaries, diversity

ECOLOGICAL INDICATORS, INDICES AND THEIR PURPOSES

Indicator: a sign of something; a thing that shows the state or health of something else; a device that provides specific information (Hornby, 1995).

Typically, data provide information about systems' status and functioning. Although primary data are the basis for analysis, raw data are seldom meaningful to scientists or to the general public. Rather, meaning emerges through analysis and the utility of data for conveying information becomes more powerful as the data are condensed. The increasing compression of data shown in Figure 1 represents the usual progression from raw data to indicators and, finally, to indices.

Indicators are designed to provide clear signals about something of interest, to communicate information about the status of things, and, when recorded over time, can yield valuable information about changes or trends. The

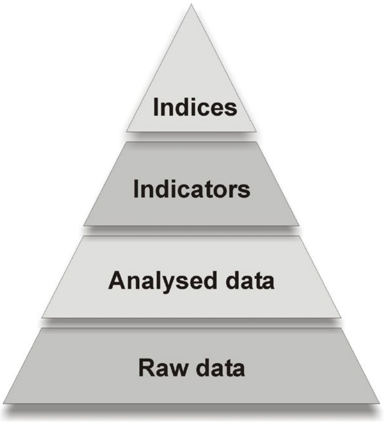


Figure 1. The relationship between raw data, analysed data, indicators and indices (adapted from Shields *et al*., 2002)

bar on a thermometer indicates if the temperature has changed or not; canaries were carried into mines to warn workers of the presence of methane and other gases that could lead to an explosion (when a bird succumbed to toxic gas, it was an indication that the miners were in imminent danger); a flashing light on a vehicle shows that it is going to change direction; blooms of green macroalgae *Enteromorpha* sp. or *Ulva* sp. in a temperate estuary indicate that eutrophication problems are developing.

Indices can be aggregates, or suites, of individual indicators. Furthermore, indices are used as quantitative tools in simplifying, through discrete and rigorous methodologies, the attributes and weights of multiple indicators with the intention of providing broader indication of a resource, or the resource attributes, being assessed (Hyatt, 2001). The values of an indicator or index over time can inform decisions about whether an intervention is desirable or necessary, which of various interventions might yield the best results, and how successful interventions have been. Indicators or indices, therefore, can and should be used to help direct research and to guide policies and environmental programs.

Although used to aid understanding and simplify communication, there is nothing simple about indices (Hyatt, 2001). Despite their complexity, why are ecological indicators needed?

Coastal and estuarine ecosystems provide many benefits to human society and as consequence are affected by many human activities. They provide fisheries and marine products, sinks for pollution, transport, leisure opportunities, etc. Notwithstanding, there is a growing recognition that the current growth of human activity cannot continue without significantly overwhelming critical ecosystems. It is widely recognised that human activities now occur on scales that impact even vary large areas and that those impacts can have cumulative effects. The Brundtland Commission (WCED, 1987) defined sustainable development as ‘development that meets the needs of the present without compromising the ability of future generations to meet their own needs. This statement addresses the concern over the extent to which ecosystems can continue to provide functions and services into the future (in terms of ecosystem trophic linkages, biodiversity, biogeochemical cycles, etc.), given the activities of human societies. Although there is an influentional body of opinion that we need coastal and estuarine management, there is much confusion about what constitutes it and how we need to proceed to achieve it (Gewin, 2003). Much has been written about general principles of ecosystem management (Christensen *et al.*, 1996) but rather less has been said about practical approaches for attempting it. Indicators and indices are needed to improve our understanding of the nature of human demands on ecosystems, and the extent to which these can be modified.

LINKING VALUES TO INDICATORS

The current spatial extent of anthropogenic impacts, combined with their increasing intensity, has endangered and, in some cases, degraded the structure and functioning of our environment (Lubchenco, 1998). We have also come to understand that the environmental issues display attributes of high uncertainty, urgency, complexity and connectivity (Shields et al., 2002). Moreover, according to Funtowicz & Ravetz (2001), nothing can be managed in a convenient isolation; issues are mutually implicated; problems extend across many scale levels of space and time; uncertainties affect data and theories alike. Therefore, ecological indicators or indices will only be effective if they are developed according to a hierarchical model (Figure 2) that encompasses all this complexity. First of all, in order to be valuable these measures have to be built upon guiding human values and objectives (goals). In order to illustrate this concept, let us use the following example. One of the objectives that society in general has clearly set for itself after the report “Our Common Future” (WCED, 1987), is to achieve a sustainable way of life, *i.e.,* the overarching goal is sustainable development. Then, criteria have to be set in order to translate the goals into systems’ characteristics and desirable systems’ states or dynamics. These criteria describe with increasing levels of specificity what should be accomplished in support of the established general goal. Finally, the

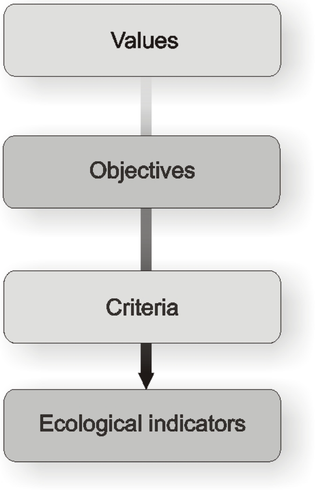


Figure 2. Hierarchical model: linking values to indicators.

measurable elements of each criterion are the indicators or indices of environmental quality, and such metrics should be used to assess the extent to which the objectives embodied in the criteria are achieved. This hierarchical approach ensures that the connections between an indicator and criteria that it refers to are clear. On the other hand, it reinforces the meaning and acceptance of these measures among other scientists and individuals working in adaptive ecosystem management (Walters & Holling, 1990), and reflects public's understanding of their values and objectives.

DIFFERENT LEVELS OF STUDY

In order to be useful, indicators and indices must also have into account that biodiversity has a hierarchical structure, which ranges from the ecosystem and landscape level, through the community level and down to the population and molecular level (Figure 3). Thus, there is a need to develop methodologies for the practicable detection of ecosystem change, as well as the evaluation of different ecological functions. It is also required a set of indicators which

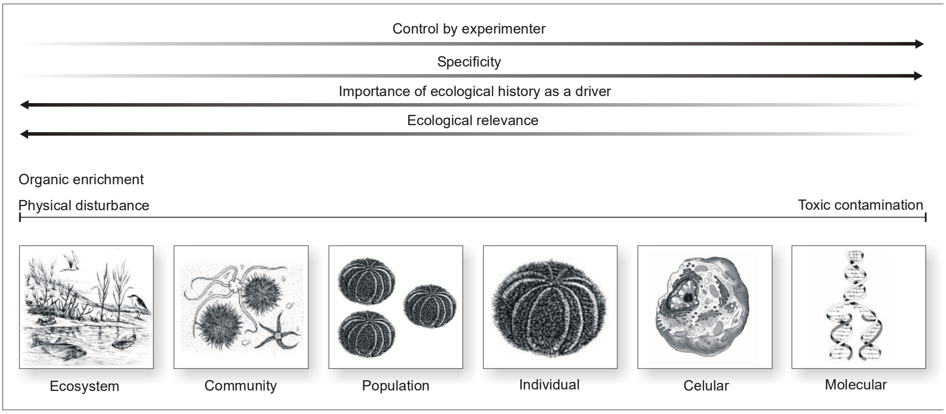


Figure 3. Comparison of different levels to study biodiversity.

facilitate the detection of change in ecosystems suffering stress and highlight possible drivers of the change process.

A hierarchical classification of ecological indicators would need to take into account existing interactions between different organisation levels since the effects of environmental stress are expressed in different ways at different levels of biological organisation and effects at one level can be expected to impact other levels, often in unpredictable ways (Turner *et al.*, 2000). On the other hand, it is widely accepted that at higher levels of biological organisation decreasing control can be exercised by the experimenter, and studies become less specific, whereas ecological history grows in importance as a driver and, even more significantly, gains in ecological relevance. Although a selected suite of measurements along this continuum of organisation levels is recommended, for the sake of our study, indicators of the higher hierarchical levels are highlighted here.

CONCERNS AND CHALLENGES

Due to the variety of environmental issues, the complexity of environmental processes, and the necessity for applied management, many types of indicators and indices have been developed for countless different purposes. They can reflect biological, chemical and physical aspects of ecological condition, and have been used to characterise status (*e.g.* Bricker *et al*., 2003; Kabuta & Laane, 2003), track or predict change (*e.g.* Marques *et al.,* 1998; Salas *et al.,* 2004), identify stressors (*e.g.* Engle & Summers, 1998), assess risk (*e.g.* Pardal *et al*., 2004), and influence management actions (*e.g.* Borja *et al.,* 2004). These metrics have been used to describe past and present conditions at a variety of geographical scales (*e.g.* Europe: Borja *et al*., 2003; Mexico: Engle *et al*, 1994; USA: Vølstad *et al.*, 2003; Egypt: Ismael & Dorgham, 2003; Asia: Rogers *et al*., 1997; South America: Muniz *et al*., 2005) and for a variety of resources. Because they are so diversified, development and selection of successful indicators has become a complex process (Kurtz *et al*., 2001).

Although few scientists deny the benefits that ecological indicators provide to research and management efforts, Dale & Beyeler (2001) pointed out three concerns that hold back their use:

1) Monitoring programs often depend on a small number of indicators and, as a consequence, fail to consider the full complexity of the ecological system;
2) Unclear or ambivalent goals and objectives can lead to a wrong choice of ecological indicator;
3) Lack of robust procedures for selecting ecological indicators makes it difficult to validate the information provided by indicators or indices.

Accordingly, these three concerns bring along three challenges:

1) Indicators should be selected from multiple levels in the ecological hierarchy in order to effectively monitor the multiple levels of complexity within an ecological system. Thus, a key challenge is to find a measure or a mix of measures which give interpretable signs and cover the spectrum of ecological variation;

2) Primary goals and objectives should be determined early in the process in order to select the measurable system characteristics that most closely relate to research or management concerns;
3) The creation and use of clear criteria and standard procedures is extremely important to allow repeatability, to avoid bias and to impose discipline upon the selection process

Each phase in an evaluation process will highlight strengths or weaknesses of an indicator in its current stage of development. Weakness may be overcome through further research and modification. Alternatively, it might be overlooked if an indicator has strengths that are particularly important to program objectives. Some users might be willing to accept a weakness in an indicator if it provides vital information. Or, the cost may be too high for the information gained. These decisions should be made on a case-by-case basis. Regardless, ecological indicators must be developed and used with the knowledge that substantial uncertainty will always exist (NRC, 2000).

CRITERIA FOR EVALUATING AND SELECTING ECOLOGICAL INDICATORS

Selection of effective indicators is a key point. In general, these measures need to capture the ecosystem complexities and yet remain as simple as possible. With the purpose of choosing or developing ecological indicators or indices, it is firstly necessary to set the selection criteria. With the purpose of helping the decision process, a number of criteria are listed in this section (see, *e.g.* Müller *et al*., 2000; Caughlan & Oakley, 2001; Dale & Beyeler, 2001; Niemeijer, 2002; Spangenberg *et al*., 2002). Hence, in order to be of practical utility, environmental indicators should be (although not all of these recommendations can always be met):

- **conceptually well founded**, based on a sound scientific foundation, widely acknowledged by the scientific community; not only derived considering pragmatic arguments, but also referring to an optimal ecosystem theoretical background (Müller *et al*., 2000)
- **relevant**, *i.e.,* they have to cover crucial aspects of environmental processes ;
- **understandable**, that is to say, clear, simple and unambiguous;
- **realisable** within the capacities of national governments, given logistics, time, technical, and other constrains;
- **indicative**, *i.e.,* an indicator must be truly representative of the phenomenon it is intended to characterise;
- **easily measured** and **easily documented**;
- **robust**, *i.e.,* directionally safe with no significant changes in case of minor changes in the methodology or improvements in the data base;
- have a **known response to disturbances, anthropogenic stresses** and **changes over time**;
- **statistically defensible** (quantifiability, reproducibility, validity, sensitivity and transparency); the statistical limitations of the indicator's performance should be documented;
- **sensitive**, *i.e.,* they have to react early and sensibly to changes in what they are monitoring, in order to permit monitoring of trends, serve as an early indicator of reduced system integrity and verify the success of policies (Spangenberg *et al*., 2002);
- **applicable in extensive geographical areas** and in the greatest possible number of communities or ecological environments and seasons, in order to have relevance to policy and management needs (Salas, 2002);
- **independent from reference conditions**; determining baseline values or a reference state is extremely problematic because it involves subjective judgment on what should be considered "normal" or "acceptable" (Niemeijer, 2002)

- **able to distinguish the components of variability** (measurement errors introduced during field and laboratory activities and natural-temporal- and spatial-variation) from a true environmental sign.

The difficult but challenging task is to derive an indicator or set of indicators that together are able to meet these criteria. In fact, despite the panoply of ecological indicators that can be found in the literature, very often they are more or less specific for a given kind of stress or applicable to a particular type of community or scales of observation, and rarely their wider validity has actually been proved conclusively. Additionally, in the process of selecting an ecological indicator or index, data requirement and data availability must be accounted for (Marques *et al.*, 2005). Unfortunately, in most of the cases, these are the leading selection factors.

ECOLOGICAL INDICATORS AND INDICES: BRIEF REVIEW

Many ecological indicators used or tested in evaluating the ecosystem status can be found in the literature, resulting from just a few distinct theoretical approaches (Salas, 2002; Marques *et al.*, 2005). A number of them focus on the presence or absence of given indicator species, while others take into account the different ecological strategies carried out by organisms, diversity, or the energy variation in the system through changes in the biomass of individuals. A last group of ecological indicators are thermodynamically oriented or based on network analysis, and look for capturing the information on the ecosystem from a more holistic perspective, as they try to see the forest through the trees and capture the total image of the ecosystem without the inclusion of details (Jørgensen *et al.*, 2005b).

This chapter covers mostly those indicators more commonly used to assess the status of coastal and estuarine environments and among these the ones further concerned to benthic communities. Other types of environmental indicators, such as physical and chemical indicators of climate change, ozone depletion, acid precipitation, or air, sediment and water quality were left out of this review, since, although they are not less important, they are not directly concerned to the present work. Table 1 summarises the type of indicators considered, each indicator algorithm or explanation, and, whenever was applicable, the indicator range of values and the ecological status classification mentioned by the authors.

1. Species as indicators

When talking about species as indicators (Table 1) two cases have to be distinguished: a) indicator species and b) bioaccumulator species.

a) Indicator species

Indicator species are defined as species which can provide information on ecological changes and give early warning signals regarding ecosystem processes in site-specific conditions, due to their sensitive reactions to them.

These species are useful because they can provide signs of impending environmental problems such as air and water pollution, soil contamination, climate change or habitat fragmentation, and they can also provide information on the integrated effect of a variety of environmental stresses and their cumulative effects on the health of an organism, population, community and/or ecosystem.

Studies on indicator species are mainly based on the understanding that particular species are extremely sensitive to different environmental stresses. These species only tolerate a narrow range of environmental conditions. Changes in the environment can affect the usual functioning of individuals, which can lead to changes in behaviour, growth, reproduction, life span, etc. This can lead to alterations of whole populations and cause shifts in species and community structures, which can affect whole ecosystems. Indicator species can also show positive alterations to environmental conditions, for example the decrease of hazardous substances or the re-stabilisation of populations. Furthermore, species emerging in the habitat because of its altered conditions, can serve as indicator species.

Several benthic groups, such as amphipods (Gómez-Gesteira & Dauvin, 2000), cumaceans (Corbera & Cardell, 1995), polychaetes (Bellan, 1967; Grassle & Grassle, 1974; Anger, 1977; Ganapati & Raman, 1976; Gray, 1976; Glemarec & Hily, 1981; Hily, 1983; Mendez-Ubach, 1988; Yan & Lu, 1989; Diaz-Castaneda & Safran,1989; Ros & Cardell, 1991; Pocklington *et al*., 1994; Mendez-Ubach, 1997; Samuelson, 2001), or molluscs (Bellan 1980; Moore *et al.*, 1987; Mora *et al.*, 1989; Broom *et al.*, 1991) have been used as indicators of stress or pollution. Usual methods for identifying pollution effects on benthic communities are based upon the species response to organic pollution and eutrophication (Pearson & Rosenberg, 1978). For example, indices such as Marine Biotic Index (AMBI) (Borja *et al*., 2000), Annelida Pollution Index (Bellan, 1980), Bellan-Santini Index (Bellan-Santini, 1980), Benthic Quality Index (BQI) (Rosenberg *et al*., 2004), Benthic Response Index (Smith *et al*., 2001), BENTIX Index (Simboura & Zenetos, 2002), Conservation Index (Moreno *et al*., 2001), Ecological Evaluation index (EEI) (Orfanidis *et al*., 2001), Indicator Species Index (Rygg, 2002) and Macrofauna Monitoring Index (Roberts *et al*., 1998) focus on the presence/absence of these species (Table 1).

There are several problems associated with the use of indicator species if their abundance is to be used in some absolute sense as a measure of the intensity of perturbation. In certain situations, many indicator species may occur naturally at relatively high densities. As a result, less subjective aspects of community structure may indicate stress effects before indicator species unequivocally suggest a pollution situation.Thus, in such situations, indicators are probably best used as confirmatory evidence or as part of a suite of other pollution assessment. Also, their appearance is not universal, and while their dominance may be used as an indicator of pollution, their absence certainly cannot be taken to indicate the absence of pollution (Warwick, 1993).

b) Bioaccumulative species

Certain taxa have the capacity of accumulating relatively large amounts of several pollutants in their tissues, without apparent noxious effects, facilitating their detection when they are in the environment at low levels, *i.e.,* pollutants levels difficult to detect through analytical techniques. These species are classified as bioaccumulators. By analysing element content from animal tissue samples at different distances from a pollution source, the type of pollution and the size of the fallout zone can be determined. The selection of a species will depend on the techniques employed and the objectives of the monitoring programme.

Macroalgae (*e.g. Fucus* sp.*, Enteromorpha* sp.), phanerogames and periphyton, which have a large free surface compared to their weight, very rapidly concentrate great quantities of various pollutants and are consequently useful indicators of local pollution levels (*e.g.* Fytianos *et al.,* 1999; Constanzo *et al.,* 2000). Other useful indicators of local pollution are zoobenthos species: 1) marine amphipods are useful indicators for several heavy metals in the sediments (*e.g.* Song & Breslin, 1998); 2) several benthic fish species are also used as indicators (*e.g.* Karr, 1981; Steckis *et al*., 1995); 3) a number of polychaete species (*e.g. Nereis diversicolor, Neanthes arenaceodentata, Glycera alba, Nephtys hombergi*) are able to accumulate toxic substances; and 4) bivalve molluscs (*e.g. Mytilus* sp.*, Cerastoderma* sp.*, Ostrea* sp.*, Donax* sp.) are effective accumulators of metals and organic micropollutants, because they filtrate large volumes of water with soluble and particulate substances, which after metabolisation, are selectively concentrated in their soft tissues or in the shell (*e.g.* Hellou & Law, 2003; Gorinstein *et al*., 2003). In addition, they are generally very resistant to several pollutants (*e.g.* organochlorines) which are dangerous for other animals living in the same environment (*e.g.* crustaceans, insects). In conclusion, all these species are especially useful as "sentinels" to evaluate the importance and spatial distribution of many pollutants. Although it is more common the simple measurement of a certain pollutant effect on those species, a few indices based on the use of bioaccumulative ability have been formulated; the Ecological Reference Index (ERI) (OSPAR/MON, 1998) and the Fish Tissue Contamination Index (USEPA, 2000) are two good examples (Table 1).

The disadvantage of using bioaccumulator species in the detection of pollutants arises from the fact that a number of biotic and abiotic variables may affect the rate at which the pollutant is accumulated, and therefore both laboratory and field tests need to be undertaken so that the effects of extraneous parameters can be identified (Marques *et a*l., 2005).

2. Indices based on diversity

Diversity is the other mostly used concept focusing on the fact that the relationship between diversity and disturbances can be seen as a decrease in the first one as stress increases (Marques *et al.*, 2005). Different diversity indices (Table 1) emphasise the species richness (this measures either simply the total number of species present or some adjust form which attempts to allow for different numbers of individuals) or evenness (this expresses how evenly the individuals are distributed among the different species) components of diversity to varying degrees. According to Magurran (1989), diversity measurements can be divided into three main categories:

1) indices that measure the number of species in a defined sampling unit (*e.g.* Margalef index);

2) models of the abundance of species, as the K-dominance curves (Lambshead *et al.*, 1983), which describe the distribution of species abundance, as of those that represent situations in which there is a high uniformity to those that characterise cases in which the abundance of the species is very unequal;

3) indices based on the proportional abundance of species that pretend to solve enrichment and uniformity in a simple expression. Such indices can be divided into those based on Statistics, Information Theory (*e.g.* Shannon-Wiener, Macrophyte Community Diversity index), count-based measures (*e.g.* Fisher's α index, Hulbert index) and equitability indices (*e.g.* Simpson, Berger-Parker, Pielou).

A new suite of univariate indices, based on taxonomic distinctness (Table 1), have been developed at the Plymouth Marine Laboratory over the last decade (Clarke & Warwick, 1998, 1999, 2001; Warwick & Clarke, 2001). In quantifying relatedness, hierarchical Linnean classifications are generally used for practical reasons. While these may not be true representations of phylogenies, they are based on cladistic principles, are the best available relationships and can be standardised between studies. These indices (Average Taxonomic Diversity, Average Taxonomic Distinctness, Total Taxonomic Distinctness, Variation in Taxonomic Distinctness) have the advantages of being independent of sample size and sampling effort, not requiring quantitative data, and being applicable across different geographic scales (Warwick & Clarke, 2001). At present, only a limited number of studies that employ taxonomic relatedness indices have been undertaken (*e.g.* Warwick & Clarke, 1998; Somerfield *et al.*, 1997; Rogers *et al.*, 1999; Somerfield & Clarke, 2003; Prior *et al.*, 2004). Therefore, further investigations are required to determine how useful these indices are as measures of biodiversity over a much greater range of taxa and environmental situations.

3. Indices based on Ecological Strategies

Some indices try to assess environmental stress effects accounting for the ecological strategies followed by different organisms (Table 1). A number is based on the different feeding strategies of the organisms (*e.g.* the Feeding Structure index proposed by Milovidova & Alyomov, 1992) and Word's (1979) Infaunal Trophic index). Others, such as the Meiobenthic Pollution Index (Losovskaya, 1983), the Mollusc Mortality index (Petrov, 1990), the Nematodes/Copepods ratio (Raffaelli & Mason, 1981), the Polychaeta/Amphipod ratio (Gómez-Gesteira & Dauvin, 2000) and the Belsher index (Belsher & Boudouresque, 1976) for algae, have as background the notion of a taxon as "functional group" - a group of not necessarily related species, the members of which exploit a common resource base in a similar fashion. Because species in a functional group share vital resources, it is expected that disturbances would affect all members of the group.

Nevertheless, the lack of knowledge of species feeding behaviour, the well known plasticity of feeding modes, the incorrectly assignment of trophic groups to species (trophic indices), or the scarce interpretation of community changes along natural gradients (*e.g.* copepods presence diminishes in depth), can all produce misunderstandings and have supported much debate on the suitability of these type of indices. Moreover, the abundance of a dominant species can be attributed to pollution when the population abundance may be caused by a natural stressed condition.

4. Indices taking into account species biomass and abundance

Other approaches account for the variation of organism's biomass as a measure of environmental disturbances. There have been numerous attempts, based on empirical findings, to categorise the response of marine benthos to varying degrees of pollution. Regarding organic enrichment, one of the most notable exercises was that of Pearson & Rosenberg (1978), whose model for successional changes in communities - summarised in SAB (Species, Abundance, Biomass) curves - has been found to be widely applicable (Table 1). The values of these statistics are known to vary in a characteristic manner along any gradient of organic enrichment.

Later, Warwick (1986) derived the abundance-biomass comparison plots (ABC) method of determining levels of disturbance (pollution-induced or otherwise) on benthic macrofaunal communities (Table 1). The ABC method involves the plotting of separate K-dominance curves for species abundance and species biomass on the same graph and making a comparison of the forms of these curves. According to the author, when the number of sites, times or replicates is large, presenting ABC plots for every sample can cumbersome and it would be convenient to reduce each plot to a single summary statistic. In order to avoid these constrains, Clarke (1990) proposed the W statistic (Table 1).

There are some cases in the literature where the ABC method has not succeeded as a measure of the pollution status of marine macrobenthic communities, because small non-polychaete species and amphipods (Beukema, 1988), that are not indicative of polluted conditions, have been dominant. Therefore, in this sort of situations, results should be interpreted with caution.

5. Integrative measures

Integrative indices (Table 1) are composed of multiple key attributes and associated metrics that are shown empirically to change in value along a gradient of human disturbance. The first successful application of the multimetric concept to biological systems (Index of Biological Integrity, IBI) occurred in freshwater systems (Karr, 1981; Karr *et al*., 1986). The most widely-used index in the USA incorporates the responses of fish or benthic invertebrates to measure biological condition (Karr, 1981; Karr *et al*., 1986). Several authors proposed other indices able to integrate a great extent of environmental information (*e.g.* physicochemical factors, diversity measures, specific richness, taxonomical composition, the trophic structure of the system). Nowadays, a huge quantity of this type of metrics is available for application in coastal areas. The following measures are only a minute part of them; the Chesapeake Bay B-IBI (Weisberg *et al.*, 1997), the Pollution Load Index (Jeffrey *et al*., 1985), the Index of Biotic Condition (Engle *et al*., 1994), the Index of Environmental Integrity (Paul, 2003), the Macrobenthic Index of Biotic Integrity (Carr & Gaston, 2002), the Pollution Coefficient (Satmasjadis, 1982) or the Trophic Index (TRIX) (Wollenweider *et al*., 1998).

Similarly, a set of specific indices of fish communities have been developed to measure the ecological status of estuarine areas: the Biotic Integrity (IBI) for Fish (McGinty & Linder, 1997), the Estuarine Ecological Index (EBI) (Deegan *et al*., 1993, 1997), the Estuarine Fish Importance Rating (Maree *et al*., 2000) and the Fish Health Index (FHI) (Cooper *et al*., 1993).

Table 1. Short review of environmental quality indicators regarding the benthic communities.

Type	Indicator	Algorithm/ Explanation	Classification
1. Species			
a) Indicator spp			
	Marine Biotic Index (AMBI) (Borja *et al.*, 2000)	$AMBI = \frac{\{(0 \times \%GI) + (1{,}5 \times \%GII) + (3 \times \%GIII) + (4{,}5 \times \%GIV) + (6 \times \%GV)\}}{100}$ I. Species very sensitive to organic enrichment and present under unpolluted conditions II. Species indifferent to enrichment, always in low densities with non-significant variations with time III. Species tolerant to excess of organic matter enrichment. IV. Second-order opportunist species, mainly small sized polychaetes V. First-order opportunist species, essentially deposit-feeders	Normal 0-1.2 Slightly polluted 1.2-3.2 Moderately polluted 3.2-5.0 Highly polluted 5.0-6.0 Very highly polluted 6.0-7.0
	Annelida Pollution Index (Bellan, 1980)	$IP = \sum \frac{pollution\ indicator\ species\ (pis)}{no - pollution\ indicator\ species\ (npis)}$ pis: *Platynereis dumerilli, Theosthema oerstedi, Cirratulus cirratus, Dodecaria concharum* npis: *Syllis gracilis, Typosyllis spp., Amphiglena mediterranea*	Pollution disturbed > 1
	Bellan-Santini Index (Bellan-Santini, 1980)	$IP = \sum \frac{pollution\ indicator\ species\ (pis)}{no - pollution\ indicator\ species\ (npis)}$ pis: *Caprella acutrifans, Podocerus variegatus* npis: *Hyale sp., Elasmopus pocillamanus, Caprella liparotensis*	Pollution disturbed > 1

<table>
<tr><td>Benthic Quality Index (BQI)
(Rosenberg <i>et al.</i>, 2004)</td><td>$$BQI = \left(\sum_{i=1}^{n} \left(\frac{Ai}{totA} \times Es500.05i \right) \right) \times {}^{10} \log\left(S + 1\right)$$
Es500.05: species tolerance value of each species found at a station; <i>A</i>: mean relative abundance of the species <i>i</i>; <i>S</i>: mean number of species</td><td>(< 20 m)
High ES ≥ 16.0
Good ES 12.0-16.0
Moderate ES 8.0-12.0
Poor ES 4.0-8.0
Bad ES < 4.0</td></tr>
<tr><td>Benthic Response Index
(Smith <i>et al.</i>, 2001)</td><td>$$Is = \frac{\sum_{i=1}^{n} pi\sqrt[3]{asi}}{\sum_{i=1}^{n} \sqrt[3]{asi}}$$
<i>Is</i> : index value for sample <i>s</i>; <i>n</i>: number of species for sample <i>s</i>; p_i: position for species <i>i</i> on the pollution gradient (pollution tolerance score); a_{si}: abundance of species <i>i</i> in sample s</td><td>Uncontaminated 0-33
Loss of biodiversity 34-43
Loss of function 44-72
Defaunation >72</td></tr>
<tr><td>BENTIX
(Simboura & Zenetos, 2002)</td><td>$$CI = \frac{L}{L + D}$$
L: the meadow of living <i>Posidonia oceanica</i>
D: the dead meadow coverage</td><td>High conservation >0.79
Low to moderate 0.56-0.79
Impacted meadow 0.33-0.56
Advanced regression < 0.33</td></tr>
<tr><td>Ecological Evaluation Index (EEI)
(Orfanidis <i>et al.</i>, 2001)</td><td colspan="2">To evaluate the ecological status of PPs (permanent-polygon) or PLs (permanent-line) the mean absolute abundance (%) of ESGs (macrophyte ecological status) I and II sampled in PPs and PLs is non-linearly correspondent to five different ESCs. The ESCs are related linearly to the EEI at PP or PL scale. The surface area of each PP or the length of each PL is multiplied by their EEI and then divided by the sum of surface areas of PPs or lengths of the PLs. The area- or length-weighted values are then summed to estimate the spatial scale weighted EEI and the equivalent ESC.
<table>
<tr><th>x coverage of ESG I (%)</th><th>x coverage of ESG II (%)</th><th>ESG</th><th>EEI at PP or PL</th><th>Spatial scale weighted EEI and equivalent ESCs</th></tr>
<tr><td>0-30</td><td>0-30</td><td>Moderate</td><td>6</td><td>≤6 to >4 = Moderate</td></tr>
<tr><td></td><td>>30-60</td><td>Low</td><td>4</td><td>≤4 to >2 = Low</td></tr>
<tr><td></td><td>>60</td><td>Bad</td><td>2</td><td>2 = Bad</td></tr>
<tr><td>>30-60</td><td>0-30</td><td>Good</td><td>8</td><td>≤8 to >6 = Good</td></tr>
<tr><td></td><td>>30-60</td><td>Moderate</td><td>6</td><td>≤6 to >4 = Moderate</td></tr>
<tr><td></td><td>>60</td><td>Low</td><td>4</td><td>≤4 to >2 = Low</td></tr>
<tr><td>>60</td><td>0-30</td><td>High</td><td>10</td><td>≤10 to >8 = High</td></tr>
<tr><td></td><td>>30-60</td><td>Good</td><td>8</td><td>≤8 to >6 = Good</td></tr>
<tr><td></td><td>>60</td><td>Moderate</td><td>6</td><td>≤6 to >4 = Moderate</td></tr>
</table></td></tr>
</table>

Table 1. (Continued)

Type	Indicator	Algorithm/ Explanation	Classification	
	Indicator Species Index (Rygg, 2002)	The ISI value of a sample is defined as the average of the sensitivity values (ES100min5) of the taxa occurring in the sample. Only presence/absence of the taxa, not their abundance, is considered. ES100: expected number of species among 100 individuals. The average of the five lowest ES100 was defined as the sensitivity value of that taxon, denoted ES100min5.	High Good Fair Poor Bad	>8.75 7.5-8.75 6.0-7.5 4.0-6.0 0-4.0
	Macrofauna Monitoring Index (Roberts *et al*., 1998)	Each of 12 indicator species is assigned a score from 1 to 10. This score reflects primarily the impact which dredge spoil dumping has on its abundance. A score of 10 indicates a species which is very intolerant to dredge spoil dumping. A score of zero indicates a species which is more common at impacted samples than oin unimpacted samples. The Index value for each sample is calculated as the average score of those scoring species present in the sample.	No impact Intermediate impact Extreme impact	>6 2-6 <2
b) Bioaccumulative spp				
	Ecological Reference Index (ERI)	$$ERI = \frac{measured\ \ concentration}{BCR}$$ BCR: value of the background/reference concentration	See OSPAR/MON, 1998	
	Fish Tissue Contamination Index (USEPA, 2000)	This Index reflects contaminants concentrations in the edible tissues of fish or shellfish species. For more details see USEPA, 2000.	Good Fair Poor	[ ] < EPA range [ ] = EPA range [ ] > EPA range
2. Diversity				
	Taxonomic Distinctness Measures	**Average Taxonomic Diversity** (Warwick & Clarke, 1995) $$\Delta = \left[\sum\sum_{i<j}\omega_{ij}x_i x_j\right]/\left[n\left(n-1\right)/2\right]$$		

	Average Taxonomic Distinctness (Warwick & Clarke, 1995) $\Delta^{*} = \left[\sum\sum_{i<j} \omega_{ij} x_i x_j\right] / \left[\sum\sum \delta_{i<j} x_i x_j\right]$ $\Delta^{+} = \left[\sum\sum_{i<j} \omega_{ij}\right] / \left[s\left(s-1\right)2\right]$ (presence/absence data)		
	Total Taxonomic Distinctness (Warwick & Clarke, 1995) $s\Delta^{+} = \left[\sum_{i} \left[\left(\sum_{i\neq j} \omega_{ij}\right) / \left(s-1\right)\right]\right]$		
	Variation in Taxonomic Distinctness (Warwick & Clarke, 2001) $\Lambda^{+} = \left[\sum\sum_{i\neq j} \left(\omega_{ij} - \bar{\omega}\right)^{2}\right] / \left[s\left(s-1\right)\right]$ x_i: abundance of the i *th* of S species observed; $n\left(=\sum_i x_i\right)$: total number of individuals in the sample; ω_{ij} : "distinctness weight" given to the path length linking species i and j in the taxonomy		
Berger-Parker Index (Berger & Parker, 1970)	$D = n_{\max} / N$ n_{max}: number of individuals of the most abundant species; N: total number of individuals	Low diversity	High values
Fisher's α Index (Fisher *et al.*, 1943)	$S = \alpha \times \ln(1 + \frac{n}{\alpha})$ *S*: number of taxa; *n*: number of individuals; *α*: Fisher's α		

Table 1. (Continued)

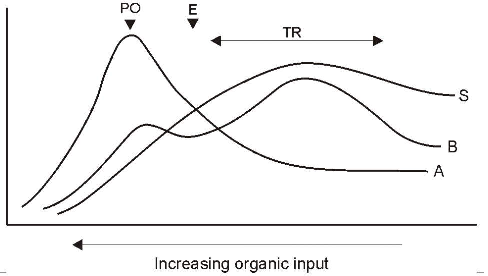
Type	Indicator	Algorithm/ Explanation	Classification	
	K-Dominance curves (Lambshead *et al*., 1983)	The K-Dominance curve is the representation of the accumulated percentage of abundance versus the logarithm of the sequence of species ordered in a decreasing order. The slope of the straight line obtained allows the valuation of the pollution grade.	High diversity	High slope
	Macrophyte Community Diversity Index (CDI) (Mitsch *et al*., 2005)	$CDI = -\sum_{i=1}^{N} (C_i \ln C_i)$ C_i :percentage cover wetland community I (0 to 1) in the wetland basin; *N*: number of wetland		
	Margalef Index (Margalef, 1969)	$D = (S-1) / Log(N)$ S : number of species found; N : total number of individuals		
	Pielou Evenness Index (Pielou, 1969)	$J' = H'/H'_{\max} = H'/\log S$ H'_{max} : maximum possible value of Shannon diversity; S : number of species found		
	Shannon-Wiener Index (Shannon & Weaever, 1963)	$H' = -\sum p_i Log_2 p_i$ p_i : proportion of abundance of species i in a community were species proportions are $p_i, p_2, p_3 ... p_n$; S : number of species found		
	Simpson Index (Simpson, 1949)	$D = \sum [n_i (n_i - 1) / N(N-1)]$ n_i: number of individuals in the species *i* ; *N*: total number of individuals	Low diversity	High values

3. Ecological strategies

a) Benthos

Feeding Structure Index (Milovidova & Alyomov, 1992)	$I = \frac{N^o\ species\ of\ \ filter - feeders}{N^o\ species\ of\ \ deposit - feeders + predators}$	At less eutrophic areas, filter feeders are 6-8 times more abundant than in highly eutrophic areas
Infaunal Trophic Index (Word, 1979)	$ITI\ = 100 - 100\big/3\left(0n_1\ + 1n_2\ + 2n_3\ + 3n_4\right)/\left(n_1\ + n_2\ + n_3\ + n_4\right)$ Macrofauna groups: (1) suspension feeders; (2) interface feeders; (3) surface deposit feeders; (4) subsurface deposit feeders n_1, n_2, n_3, n_4: number of individuals in each of the above mentioned groups	Community dominated by: Group (1) >78 Group (2) 58-77 Group (3) 25-57 Group (4) < 24
Meiobenthic Pollution Index (Losovskaya, 1983)	$MPI = \frac{\log(H+1) + \lg(P+1)}{2\lg N}$ H, P and N: number (indv/m^2) of Harpacticoida, Polychaeta and Nematoda	At higher impact nematodes replace harpacticoides and polychaetes
Mollusc Mortality Index (Petrov, 1990)	$MMI(\%)\ = \frac{Weight\ \ of\ \ shells\ of\ \ recently\ dead\ \ molluscs}{Total\ weight\ of\ \ living\ individuals\ and\ the\ shells\ of\ \ molluscs\ of\ \ the\ same\ species}$	Disturbance High values
Nematodes/Copepods Ratio (Raffaelli & Mason, 1981)	$I\ = \frac{nematodes\ \ abundance}{copepodes\ \ abundance}$	High organic pollution >100
Polychaeta/Amphipod ratio (Gómez-Gesteira & Dauvin, 2000)	$I = Log_{10}\left(\frac{Polychaetes\ abundance}{Amphipods\ abundance} + 1\right)$	Non polluted I≤1 Polluted I>1

Table 1. (Continued)

Type	Indicator	Algorithm/ Explanation	Classification
b) Algae			
	Belsher Index (Belsher & Boudouresque, 1976)	$Qualitative\ Dominance = \frac{\%species\ of\ a\ taxonomic\ group}{\Sigma\ population\ species} \times 100$ $Quantitative\ Dominance = \frac{\Sigma\ cover\ area\ by\ a\ group}{total\ cover\ area}$ The ratio between qualitative and quantitative dominance is called tension, Ψ Pollution index = $\frac{\sum \psi i}{\sum \psi j}$ *i*: groups with decreasing Ψ; *j*: groups with increasing Ψ	Polluted High values Normal Nearly null
4. Species biomass and abundance			
	SAB Curves (Pearson & Rosenberg, 1978)	The SAB curves describe the changes in the benthic community parameters of species numbers (S), total abundance (A) and total biomass (B) as the result of organic enrichment. PO: peak of opportunists E: ecotone point TR: transition zone	

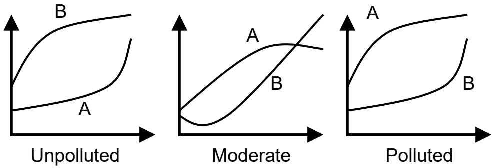
ABC Method (Warwick, 1986)	It is adapted from the K-dominance curves already mentioned, showing in one graphic the abundance k- dominance and biomass curves. The graphics are made up comparing the interval of species (in the abscise axis), decreasingly arranged and in logarithmical scale, to the accumulated dominance (in the ordinate axis).	
W-Statistic (Clarke, 1990)	$W = \sum_{i=1}^{s} (Bi - Ai) / 50(S-1)$ B_i: biomass of species *i*; A_i: abundance of species *i: S*: number of species	High status +1 Moderate status 0 Bad status -1
5. Integrative		
Chesapeake Bay B-IBI (Weisberg *et al.*, 1997)	Eleven metrics are used to calculate the Chesapeake Bay B-IBI: Shannon-Wiener species diversity index; Total species abundance; Total species biomass; % abundance of pollution-indicative taxa; % abundance of pollution-sensitive taxa; % biomass of pollution-indicative taxa; % biomass of pollution-sensitive taxa; %t abundance of carnivore and omnivores; % abundance of deep-deposit feeders; Tolerance Score; Tanypodinae to Chironomidae % abundance ratio. B-IBI is calculated by scoring each of the attributes of benthic community structure and function according to thresholds established from reference data distributions. The scores (on a 1 to 5 scale) are then averaged across attributes to calculate and index value.	Not degraded ≥ 3.0 Marginal 2.7-2.9 Degraded 2.1-2.6 Severely degraded ≤ 2.0
Pollution Load Index (Jeffrey *et al.,* 1985)	$PLI = anti\log_{10}\{1-((CP-B)/(T-B))\}$ *CP*: pollutant concentration; *B*: baseline, unpolluted; *T*: threshold, damage $Site\ PLI = (PLI_1 x PLI_2 x PLI_n)^{1/n}$, for *n* pollutants. $PLI = (PLI_1 x PLI_2 x PLI_j)^{1/j}$, for *j* sites.	

Table 1. (Continued)

Type	Indicator	Algorithm/ Explanation	Classification	
	Index of Biotic Condition (Engle *et al.*, 1994)	*Benthic index = (2.3841 x Proportion of expected diversity) + (-1.6728 x Proportion of total abundance as tubifids) + (0.6683 x Proportion of total abundance as bivalves)* The expected diversity is calculated throughout Shannon-Wiener index adjusted for salinity. $Expected\ Diversity = 0.75411 + (0.00078 \times salinity) + (0.00157 \times salinity^2) + (-0.00030 \times salinity^3)$	Degraded conditions Moderate conditions Non-degraded	< 4.1 4.1-6.1 > 6.1
	Index of Environmental Integrity (Paul, 2003)	IEI is based on the evaluation of individual measures of resources that are averaged in each level of aggregation. It is a simple sum of individual metrics that respond monotonically to environmental stress caused both by man or natural stress. This index is based on: eutrophication; sediment contamination; benthic condition.	Good Fair Poor	5 3 1
	Macroinvertebrate Index of Biotic Integrity (Carr & Gaston, 2002)	The index is composed of four metrics: Abundance of pollution sensitive organisms (ranked in a decreasing order); Abundance of pollution tolerant organisms (ranked in an increasing order); Total abundance; Species diversity. After summing each metric for each site, the scores are ranked and normalised.	Bad condition Good condition	0 1
	Pollution Coefficient (Satsmadjis, 1982)	$S' = s + t(5 + 0{,}25)$ $i_0 = \left(-0{,}0187 s'^2 + 2{,}63 s' - 4\right)\left(2{,}20 - 0{,}0166 h\right)$ $g' = i / \left(0{,}0124 i + 1{,}63\right)$ $P = g' / \left[g \left(i / i_0 \right)^{1/2} \right]$ P: pollution coefficient; *S*': sand equivalent; *s*: % sand; *t*: % silt; i_0: theoretical number of individuals; *i*: actual number of individuals; *h*: station depth (m); *g*': theoretical number of species; *g*: actual number of species	Very heavy pollution Heavy pollution Moderate pollution Slight pollution	4.0-8.0 3.0-4.0 2.0-3.0 1.5-2.0
	Trophic Index (TRIX) (Wollenweider *et al.*, 1998)	$TRIX = \frac{k}{n} \times \sum (Mi - Li)/(Ui - Li)$ In which *k*=10 (scaling the result between 0 and 10), *n*=4 (number of variables that are integrated), M_i = measured value of variable *i*, U_i = upper limit of variable $_i$, L_i = lower limit of value		

Biotic Integrity (IBI) for Fish (McGinty & Linder, 1997)	Nine metrics are used to calculate the index having in account species richness, trophic structure and abundance: Nº of species; Nº of species comprising 90% of the catch; Nº of species in the bottom trawl; Proportion of carnivores; Proportion of planktivores; Proportion of benthivores ; Nº of estuarine fish; Nº of anadromous fish; Total fish with Atlantic menhaden removed. IBI is calculated by scoring each of the attributes of benthic community structure and function according to thresholds established from the sample of unknown water quality to thresholds established by data distributions.		
Estuarine Ecological Index (EBI) (Deegan *et al*., 1993)	EBI includes the following eight metrics: Total nº of species; Dominance; Fish Abundance; Nº of nursery; Nº of estuarine spawning species; Nº of resident species; Proportion of benthic associated species; Proportion of abnormal or diseased fishes.		
Estuarine Fish Importance Rating (Maree *et al*., 2000)	$FHI = 10(J)\left[\ln(P)/\ln(P_{\max})\right]$ *J*: number of species in the system divided by the number of species in the reference community; *P*: potential species richness (number of species) of each reference community; *Pmax*: maximum potential species richness from all the reference communities.	Poor Good	0 10
Sediment Quality Triad (SQT) (Chapman *et al*., 1987)	The SQT approach is an effects-based technique that involves three components: sediment chemistry (measures of contamination), sediment toxicity testing (measures of biological effects and bioavailability) and *in situ* community parameters (benthic macroinvertebrate community structure).		
EQUATION index (Ferreira, 2000)	EQUATION is based on an aggregation of 4 different components: *vulnerability*, measuring the physical capacity of the system to react to change, *water quality*, which examines trophic status and eutrophication aspects, *sediment quality*, which looks at the sediments and benthic fauna, and *trophodynamics*, which addresses the quality and value of the top levels of the trophic web. The data requirements are reduced by the application of models and heuristic grading.	Excellent Good Fair Low Bad	5 4 3 2 1
Estuarine Trophic Status Index (NEEA/ASSETS) (Bricker *et al*., 2003)	It is a screening model that uses a pressure-state-response framework to assess eutrophication. The core methodology relies on three diagnostic tools: a heuristic index of pressure (*Overall Human Influence*), a symptoms-based evaluation of state (*Overall Eutrophic Conditions*), and an indicator of DIN, salinity, chlorophyll a, macroalgae, epiphytes, dissolved oxygen, submerged aquatic vegetation, nuisance and/ or toxic algae blooms, capacity of a system to dilute and/or flush nutrients.	High Moderate high= Good Moderate Moderate low= Poor Low= Bad	

Table 1. (Continued)

<table>
<thead>
<tr><th>Type</th><th>Indicator</th><th>Algorithm/ Explanation</th><th>Classification</th></tr>
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<tr>
<td></td>
<td>**Trophic Oxygen Status Index (TOSI)**
(Viaroli & Christian, 2003)</td>
<td>The TOSI is an extension of the Benthic Trophic Status Index of Rizzo *et al*. (1996). It provides a rapid assessment of the potential and amplitude of oxygen availability or deficiency with time. The Trophic oxygen Status Index is based on the oxygen fluxes. This index is the result of the relationship between the net maximum productivity (NP) and the dark respiration (DR). The results obtained can be expressed as graphics or as a categorical classification.</td>
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<table>
<thead>
<tr><th>Category</th><th>Condition</th><th>System qualification ($g\ O_2\ m^{-2}\ h^{-1}$)</th></tr>
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<tbody>
<tr><td>Dystrophy</td><td>$DR = NP < 0$</td><td>1–10</td></tr>
<tr><td>Total heterotrophy</td><td>$DR = NP < 0$</td><td><1</td></tr>
<tr><td>Net heterotrophy</td><td>$DR < NP \leq 0$</td><td><1</td></tr>
<tr><td>Net autotrophy</td><td>$0 < NP \leq |DR|$</td><td><1</td></tr>
<tr><td>Total autotrophy</td><td>$0 < |DR| < NP$</td><td><1</td></tr>
<tr><td>Hyperautotrophy</td><td>$0 < |DR| \ll NP$</td><td>1–10</td></tr>
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<td>**6. Thermodynamically or network oriented**</td>
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<td>**Ascendency**
(Ulanowicz, 1980)</td>
<td>$$A = \sum_{i} \sum_{j} T_{ij} \ \log\left[\frac{T_{ij} T_{..}}{T_j T_j}\right]$$
T_{ij}: Trophic exchange from taxon i to taxon j</td>
<td>Higher A: higher system growth or development</td>
</tr>
<tr>
<td></td>
<td>**Benefit/Cost Indicator (BC)**
(Palmeri, 2003)</td>
<td>$$BC = k \frac{EX}{Em} \frac{AMI}{NC}$$
EX: Exergy; Em: Emergy; AMI: Average mutual information; NC: network capacity</td>
<td></td>
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<tr>
<td></td>
<td>**Emergy**
(Odum, 1983)</td>
<td>$$Em = \sum_{i=1}^{N} Tr_i J_i$$
Tr_i : adimensional factor, expressing the quantity of solar energy units required to produce one unit of the flow J</td>
<td></td>
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</table>

Eco-Exergy (Mejer & Jørgensen, 1979)	$EX = T \times \sum \beta_i \times C_i$ *T*: absolute temperature; C_i: concentration in the ecosystem of component *i* (eg biomass of a given taxonomic group or functional group); β_i: factor able to express roughly the quantity of information embedded in the genome of the organisms	
Specific Eco-Exergy (Jørgensen, 2000)	$SpEX = Exergy \big/ Total \;\; biomass$	
Supply Demand Balance (Bendoricchio & Palmeri, 2005)	$\alpha \propto \frac{\log E}{\log B} = \log_B E$ *E*: Energy flow; *B*: biomass	Large α: high supply/demand ratio high undirected network, flow redundancies, enhanced cycling, resilience and cost of maintenance

With the Sediment Quality Triad (SQT), Chapman *et al*. (1987) suggested an integrated approach, which simultaneously investigates sediment chemistry, sediment toxicity, as well as alterations in the field, for example modifications of benthic community structure able to yield data with respect to toxic effects on selected test organisms and test systems. Additionally, Ferreira (2000) proposed the EQUATION index, based on 4 system components: vulnerability, water quality, sediment quality and trophodynamics. Bricker *et al.* (2003) developed the Estuarine Trophic Status index (NEEA/ASSETS) that uses a pressure-state-response framework to assess eutrophication. Finally, Viaroli & Christian (2003) suggested the Trophic Oxygen Status Index (TOSI) as an extension of Rizzo *et al*. (1996) Benthic Trophic Status Index.

Although very interesting, some of these indicators lack theoretical underpinnings and are still rarely used in a generalised way because they usually are site, region or ecosystem specific, which turns them dependent on the type of habitat and seasonality. On the other hand, some of them are difficult to apply as they need a large amount of data.

6. Indices thermodynamically or network analysis oriented

In the last two decades, several functions have been proposed as holistic ecological indicators (Table 1), intending a) to express emergent properties of ecosystems arising from self-organisation processes in the run of their development, and b) to act as orientors (goal functions) in models development (Marques *et al*., 2005). Such proposals resulted from a wider application of theoretical concepts, following the assumption that it is possible to develop a theoretical framework able to explain ecological observations, rules, and correlations on the basis of an accepted pattern of ecosystem theories (Jørgensen & Marques, 2001).

One of such holistic measures derives from the analysis of networks of trophic exchanges and is called the system Ascendency. Ulanowicz (1980) defines Ascendency as an index that quantifies both the level of system activity and the degree of its organisation whereby it processes material in autocatalytic fashion. Emergy was introduced by H.T. Odum (1983) as the quantity of solar energy required to obtain a given storage flow. It attempts to account for the energy required in the formation of organisms at different trophic levels. Thus, by looking into the energy flows in the ecological network, this indicator expresses the cost in solar energy of a given product. Furthermore, Mejer & Jørgensen (1979) proposed Eco-Exergy, a concept derived from Thermodynamics, which can be seen as energy with a build in measure of quality, as a measure of the ecosystem condition. Specific Eco-Exergy is defined as Eco-Exergy/biomass. Both Eco-Exergy and Specific Eco-Exergy may be used as indicators in environmental management and is advisable to use them complementary (Marques *et al*., 1997).

Another holistic measure, the Benefit/Cost indicator (Palmeri, 2003), was derived by a combination of classical thermodynamic quantities. Very recently, Bendoricchio & Palmeri (2005) developed another index, the Supply/Demand Balance indicator (SDB), on the basis of the network paradigm, and this index also considers cost and benefits of an ecosystem. According to the authors, the SDB indicator provides a single number emerging from a complex food web, it can be ecologically interpreted in terms of widely accepted allometric relations and it satisfies the mathematical properties required to be a measure of ecosystem organisation.

REALISTIC EXPECTATIONS ABOUT THE VALUE OF ECOLOGICAL METRICS

According to several authors and studies (*e.g.* Salas *et al*., 2004; Marques *et al*., 2005; Muniz *et al*., 2005; Patrício *et al*., in press) a single approach does not seem appropriate due to the complexity inherent in assessing the environmental quality of a system. Rather, ideally, this should be evaluated by combining a suite of ecological indicators, which may provide complementary information about structure, function, and composition. Nevertheless, this is not always a straightforward task. For example, it is often easier to measure structural features that can provide information about the composition or functioning of the system than to measure composition or function (Lindenmayer *et al.*, 2000) or, occasionally, measures from one scale can provide information relevant to another scale. Thus, is essential to have flexibility so as to understand the delicate interactions between the different ecosystem characteristics. This same message has intermittently been conveyed to the scientific community working on environmental quality assessment (*e.g.* Dauer *et al.*, 1993), together with an increasing concern regarding the need for a deeper understanding of ecological processes and for the development of a theoretical network able to explain observations, rules, and correlations on the basis of an accepted pattern of ecosystem theories (Jørgensen & Marques, 2001; Marques & Jørgensen, 2002). In other words, nature is too complex to be successfully described by simple ecological indicators (Marques, 2001).

Investigation and protection of ecological resources continues to change in focus and complexity. In keeping with these changes, a dynamic battery of useful and efficient indicators is essential. As Marques *et al.* (2005) left hanging; will the ecological scientific community be able to overcome the challenge?

The challenges associated with this task are formidable, but are not insuperable. Today's widely accepted economic indicators (*e.g.* gross national product, inflation) were developed over decades, not days. Developing indicators of comparable power for ecological processes will help focus attention on environmental conditions, attention that may, in turn, stimulate significant and informed management actions. These indicators must provide information in a simpler, more comprehensible form than the complex statistics usually employed on ecological issues, and the relationship between these indicators and the complex phenomena they represent must be evident (NRC, 2000).

CONCLUSIONS

Ecological indicators are used to monitor, assess, and manage natural resources. A difficulty in selecting appropriate indicators results from the complexity of ecological systems, their variability in space and time, and the great variety of human interactions with natural and modified ecosystems. Many existing measures are applicable to only limited areas, to one type of ecosystem, or to the population of one or a few species. Some indicators have been less useful than hoped because either inadequate attention was given to the values, objectives and criteria that should guide their development and use, or the measures employed were not clearly linked to underlying ecological processes. As a result, it has been difficult to interpret changes or trends. Thus, it is necessary to use a suite of indicators conceptually well founded and representative of the structure, function and composition of ecological systems (Dale & Beyeler, 2001).

As previously stated, in what concerns the more widely used ecological indicators and indices, many uncertainties persist relatively to their real efficacy in assessing ecosystems condition. Therefore, a lot of effort still has to be done, particularly when talking about holist indicators. Although their strength, from the theoretical perspective, is unquestionable, it is essential and urgent to test them with real empirical cases and compare their behaviour with further well studied indices. From this evaluation could result a better use of this class of holistic indicators. In this context, we decided, through the following chapters, to focus our attention in the applicability of the following three types of indicators: Ascendency (network based), Eco-Exergy (Thermodynamics oriented) and Taxonomic Distinctness measures (diversity measures taking into account phylogenetic relations), in different scenarios and ecosystems.

Science should help to address applications and help us to understand the most significant impacts and consequences of our decisions. Not only must we seek to discover answers to the enquiry **Why**? but we also must address the question **How do we use this information?** Ecological indicators and indices should contribute to answer these questions.

ACKNOWLEDGMENTS

This research was carried out in the scope of the research project DYNAMOD (POCTI/MGS/37431/2001) and supported by FCT (Portuguese National Board of Scientific Research) through a grant SFRH/BD/820/2000. I like to greatly thank Fuensanta Salas (Department of Ecology and Hydrology, University of Murcia, Spain) for her precious help, inspiration and valuable review of the manuscript.

CHAPTER 2

Mass balanced models of the food web in three areas along a gradient of eutrophication symptoms in the south arm of the Mondego estuary (Portugal)

ABSTRACT

Three Ecopath with Ecosim models were constructed to represent the eutrophication gradient along the south arm of the Mondego estuary (Portugal). Sampling was conducted in three areas representative of different stages along the gradient: (a) a non-eutrophic area (*Zostera noltii* meadows), (b) an intermediate eutrophic area (macrophyte absent, although residual roots can still be found in the sediment, and the occasional formation of abundant macroalgae mats) and (c) a strongly eutrophic area (macrophyte community totally absent for at least a decade and strong, regularly occurring, blooms of *Enteromorpha* spp.). Field, laboratory and literature information were used to construct the models, as well as empirical ecological knowledge gained from years of work on this system. Approximately 76 trophic groups (*e.g.* Phytoplankton and Zooplankton species), species and genera are included. These players are grouped into 43, 36 and 34 model groups for *Zostera* sp. meadows, intermediate eutrophic area and strongly eutrophic area, respectively. The groups are arranged by trophic similarity and habitat preferences; special distinction is given to macrofauna. Biomass, production, consumption, and diet are among the parameters used to describe each group. The sum of consumptions, exports, respiration, production, flow to detritus, total system throughput and annual rate of net primary production was always higher in the *Zostera* sp. meadows, followed by the strongly eutrophic area and, finally, by the intermediate eutrophic area.

KEYWORDS: Ecopath, ecological model, estuary, eutrophication, trophic structure

INTRODUCTION

Ecology can be defined as the scientific study of the relationships between organisms and their environment; and, in general, can be approached from two directions: (1) as reductionism, wherein each relationship is considered by itself and the results are assembled afterwards; and (2) as holism, whereby the system is considered in its entirety and a search is undertaken to reveal properties at the system level (Jørgensen, 2002).

Previous studies have shown that an ecosystem consists of so many interacting components that it becomes impossible ever to understand how it functions by examining the component relationships in isolation (Likens, 1985; Allen, 1988). Often, when individual components of ecosystems are studied via reductionism, the reconstructed ensemble will behave differently than the sum of the parts.

To obviate such problems, since no system can understand itself, the way it can be understood is to develop simplified models which have enough of the characteristics of the original system to resemble reality, but at the same time are simple enough to be understood (Brown, 2004). In fact, one might attempt to describe at least part of the reality of ecosystems structure by developing mass-balance models that represent a static description or a ‘snapshot’ of the trophic flows in the ecosystem (Christensen, 1994). Such snapshots can be readily compared and therefore, used to explore the evolution of a system through a series of stages or stable states (Christensen & Pauly, 1993). The study of trophic webs has a number of potential advantages, including the likely prediction of negative effects in cascade caused by anthropogenic impacts in ecosystems, and a greater understanding of ecosystem management (Cohen *et al*., 1993). Assessment of ecosystem health, conservation of living resources and biodiversity could be advanced if the consequences of trophic web modification were predictable (Arias-González *et al.,* 2004). If a trophic network is defined as a model of energy and material flow between organisms via predation processes, then the adjustment (increase or decrease) of elements from the intricate food web and the changes produced in the community structure by this process should produce a disruption in the trophic structure.

From this viewpoint, the main goal of this paper was to construct mass balanced models of the food web in three areas along an eutrophication gradient in the south arm of the Mondego estuary (Portugal), a small and well described temperate intertidal estuary (Marques *et al*., 1997; 2003; Pardal *et al*., 2000; 2004; Cardoso *et al*., 2004; Ferreira *et al*., 2004, Neto, 2004), using the “Ecopath with Ecosim” software package. This work was a first study of the Mondego estuary using a mass-balance model of trophic interactions

MATERIAL AND METHODS

Study Area

The Mondego estuary, situated along the western coast of Portugal, is bifurcated into a northern and southern arm, each exhibiting very different hydrological characteristics (Figure 1). The northern arm is deeper, while the southern arm is silted up, especially in upstream areas, which causes most of the freshwater discharge to flow through the northern arm. This siltation diverts most of the freshwater discharge into the northern arm. Consequently, the water circulation in the southern arm is dependent mainly on tidal flushing and on a relatively small input of freshwater from the Pranto River, the flow of which is controlled artificially by a sluice.

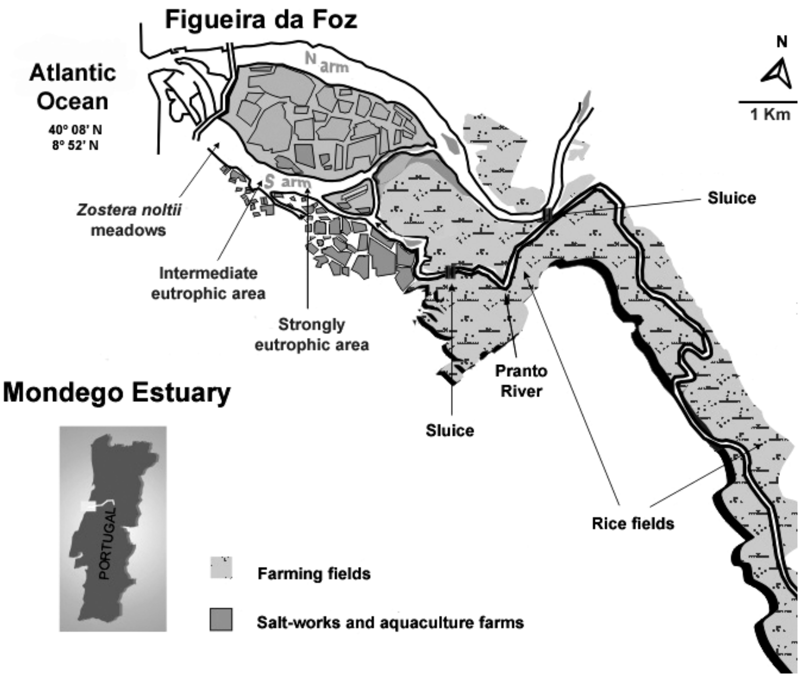


Figure 1. Mondego estuary: location of the sampling stations along a spatial gradient of eutrophication.

Macroalgal blooms of *Enteromorpha spp* have regularly been observed in the Mondego over the last twenty years (Flindt *et al*., 1997; Marques *et al*., 1997, 2003; Lillebø *et al*., 1999b; Pardal *et al*., 2000; 2004; Martins *et al*., 2001; Dolbeth *et al.*, 2003; Cardoso *et al*., 2004). Nevertheless, such macroalgal blooms may not occur in exceptionally rainy years. This is most probably due to the resulting long periods during which salinity remains below the tolerance limit of macroalgae, coupled with a limitation of phosphorous induced by a heavy nitrogen discharge from the Pranto River (Martins *et al*., 2001).

Sampling was conducted in three areas in the southern arm of the Mondego estuary that represent different stages along a spatial gradient of eutrophication (Marques *et al*., 1997; Lillebø *et al.*, 1999b; Pardal *et al*., 2000, 2004; Dolbeth *et al.*, 2003; Cardoso *et al*., 2004; Neto, 2004) (Figure 1): (a) A non-eutrophic area (*Zostera noltii* Hornem beds), (b) an intermediate eutrophic area (*Zostera noltii* absent, although residual roots can still be found in the sediment, and the occasional formation of abundant macroalgae mats) and (c) a strongly eutrophic area (macrophyte community totally absent for at least a decade and strong, regularly occurring, blooms of *Enteromorpha spp*.).

Methods

Food webs of the ecosystem in the three areas were constructed using the “Ecopath with Ecosim” software package, which assists the user in casting a balanced carbon budget for each trophic group. The core routine of Ecopath/Ecosim centres on the Ecopath program of Polovina (1984), which was extended to apply to non-steady-state systems (Christensen *et al*., 2004). It no longer assumes a steady state but instead calculates parameters on the assumption of mass balance over an arbitrary period - usually one year. Scores of applications of Ecopath with Ecosim can be found at: http://www.ecopath.org/, along with the freely distributed software and documentation. Although the formulations and basic concepts are accessible in these venues, the general approach is summarised here. When applied, Ecopath derives model parameters on the basis of two master equations. The first equation, describes how the production term for each group can be split in components (eq. 1). More specifically, it says that the net production of a functional group equals the sum of (1) the total mass (or energy) removed by predators and fisheries, (2) the net biomass accumulation of the group, (3) the net migration of the group’s biomass, and (4) the mass flowing to detritus.

$$B_i \times (P/B)_i \times EE_i - \sum_{j=1}^{n} B_j \times (Q/B)_j \times DC_{ji} - Y_i - BA_i - E_i = 0 \qquad \text{(eq. 1)}$$

where B_i and B_j are biomasses of prey (i) and predators (j), respectively; P/B_i is the production/biomass ratio, equivalent to total mortality (Z) in most circumstances (Allen, 1971); EE_i is the ecotrophic efficiency; the fraction of the total production of a group utilised in the system; Y_i is the fisheries catch per unit area and time (*i.e.,* $Y = F \text{x} B$); Q/B_j is the food consumption per unit biomass of j; DC_{ji} is the fraction of prey i in the average diet of predator j; BA_i is the biomass accumulation rate for i; and E_i is the net migration of i (emigration less immigration).

In this type of models, the energy input and output of all living groups must be balanced. The basic Ecopath eq. 1 includes only the production. When balancing a compartment in an ecosystem other flows must be considered. Energy balance is ensured within each group using eq. 2 (Christensen e*t al.*, 2004)

Consumption = production + respiration + unassimilated food (eq. 2)

The implied thermodynamic constraints of this equation underscore the power of Ecopath models as a focal point for refinement of ecosystem information. The need to reconcile energy production and demand among components of the food web narrows the possible ranges of parameter estimates for particular groups. Inclusion of a biomass accumulation factor and migration factor in the general Ecopath equation distinguishes Ecopath modelling as an 'energy continuity' approach rather than a strictly 'steady state' approach. Conservation of energy (continuity) is assumed for every identified component of the ecosystem, and the whole system. This basic constraint enables representation of changes in populations (*i.e.,* functional groups) when expressed in dynamic form.

1. Sampling program and laboratory treatment

Chlorophyll *a*, detritus, macroalgae, macrophytes and macrofauna were sampled fortnightly (February 1993 to January 1994), during low tide, at each of the three areas. All biological material was identified and separated into the lowest possible taxa (for more details concerning the technical procedures see Pardal *et al*., 2000; 2004). Between March 1996 and January 1997, monthly samples of epiphytes attached to *Zostera noltii* were separated from their substrate, dried and weighed. Zooplankton was collected monthly from sub-surface waters at each sampling site from April 1995 to April 1996, using 200 and 335 µm mesh nets (Azeiteiro *et al*., 1999). Data on fish were taken monthly from January 1991 to December 1992. The captured fish were identified and weighed (wet weight), and the dominant species in the stomach contents were analysed (Jorge *et al.*, 2002). Finally, wading birds were counted from January 1996 to January 1998 at fortnight-tide and monthly low-water to provide an accurate census across the three areas (Lopes *et al.*, 2002). Seagulls were counted monthly, from November 1993 to July 1994.

2. Compartments

Species of similar size, diets or with identical ecological niche were grouped. Different numbers of ecosystem compartments were identified in each system (Table 1): 43 in the *Zostera* sp. meadows, 36 in the intermediate eutrophic area and 34 in the strongly eutrophic area. Species that were not naturally present in one of the three areas or whose roles in the trophic network were unimportant were not considered.

3. Biomass (for data sources see Appendix A)

Chlorophyll *a* was estimated according to standard procedures (Strickland & Parsons, 1968) and values were transformed into Phytoplankton biomass using a conversion factor taken from Anderson & Williams (1998) and assuming an average depth of 0.5 m over the sampling area. Epiphytes consisted only of the material attached to the aerial part of *Zostera noltii*. Plants and macrofauna were dried at 70ºC for 72h and weighed. The ash free dry weight (AFDW) of biomass was assessed after combusting samples for 8h at 450ºC (Pardal *et al.*, 2000; 2004). The abundance of each Zooplankton taxon was estimated by multiplying the observed number of that taxon by the average AFDW of an individual belonging to it. The weights of all taxa were summed to arrive at the annual average standing stock.

Sixty-two species of fish were observed and grouped according to their ecological and trophic characteristics. The biomass corresponding to each group was determined by multiplying its wet weight by a conversion factor taken from Jørgensen *et al.* (1991).

Table 1. Input data and calculated estimates (in brackets) for the 3 areas (Z: *Zostera* sp. meadows; I: Intermediate eutrophic area; S: Strongly eutrophic area) along the eutrophication gradient. (A: macroalgae; M: macrophyte; G: gastropoda; Am: amphipoda; B: bivalvia; I: isopoda; D: decapoda; P: polychaeta; F: fish; Gu: gull; W: wader).

Groups	Biomass			P/B*			Q/B*			EE*		
	Z	I	S	Z	I	S	Z	I	S	Z	I	S
Phytoplankton (A)	0.336	0.324	0.17	185	185	185	-	-	.	0.772	(0.572)	(0.96)
Enteromorpha sp. (A)	1.800	26.975	96.784	3.4	3.4	3.4	-	-	.	0.996	(0.691)	(0.288)
Ulva sp (A)	0.373	2.504	7.658	3	3	3	-	-	.	0.957	(0.883)	(0.984)
Gracilaria sp (A)	16.081	2.054	2.322	3	3	3	-	-	.	(0.033)	(0.084)	(0.044)
Fucus sp (A)		0.084	0.203		3	3		-	.		(0.81)	(0.533)
Zostera noltii (M)	204.84			2.5			-			0.001		
Epiphytes	7.695			180			-			0.149		
Zooplankton	(0.348)	(0.278)	(0.234)	22	18	20	(73.33)	(90)	(100)	0.98	0.95	0.95
Hydrobia ulvae (G)	54.750	4.666	9.745	1.3	4.5	4.5	(6.5)	(22.5)	(22.5)	(0.209)	(0.224)	(0.092)
Gibulla umbilicallis (G)	0.072			1.76			(8.8)			(0.73)		
Littorina spp (G)	2.489	0.525		3	3		(15)	(15)		(0.082)	(0.468)	
Melita palmata (Am)	(0.109)	(0.181)	0.099	7.2	8.38	8.5	(36)	(41.88)	(42.5)	0.95	0.95	(0.973)
Ampithoe valida (Am)	(0.236)	(0.179)	0.145	5.8	4.8	4.9	(29)	(24)	(24.5)	0.95	0.95	(0.87)
Echinogammarus marinus (My)			0.002			6.3			(31.5)			(0.844)
Corophium multisetosum (Am)		0.002			10			(50)			(0.635)	
Scrobicularia plana (B)	3.260	7.762	11.347	1.8	1.8	1.8	(9)	(9)	(9)	(0.834)	(0.619)	(0.319)
Cerastoderma edule (B)	5.221	0.088	0.216	4.8	4.8	4.8	(24)	(24)	(24)	(0.305)	(0.859)	(0.956)
Modiolus barbatus (B)	0.022			2			(10)			(0.446)		
Cyathura carinata (I)	0.056	0.343	7. 268	2.03	3.17	3.17	(10.15)	(15.85)	(15.85)	(0.982)	(0.331)	(0.012)
Idotea chelipes (I)	0.040	0.027	0.02	3.8	3.8	3.8	(19)	(19)	(19)	(0.953)	(0.494)	(0.41)
Sphaeroma hookeri (I)	0.002			3.8			(19)			(0.96)		
Carcinus maenas (D)	1.09	0.58	0.419	6.4	6.4	6.4	(32)	(32)	(32)	(0.384)	(0.431)	(0.511)
Crangon crangon (D)	(0.132)	(0.317)	0.280	6	6	6	(30)	(30)	(30)	0.95	0.95	(0.964)

Table 1. (Continued)

Alkmaria romijni (P)	0.008	0.022	0.114	2.3	2.3	2.3	(11.5)	(11.5)	(11.5)	(0.443)	(0.69)	(0.804)
Capitella capitata (P)	0.006	0.062	0.038	2.4	1.6	1.6	(12)	(8)	(8)	(0.818)	(0.913)	(0.943)
Heteromastus filiformis (P)	0.610	2.2	0.192	2.4	2.2	2.2	(12)	(11)	(11)	(0.93)	(0.974)	(0.778)
Hediste diversicolor (P)	(0.866)	(0.505)	0.428	5.6	5.4	5.2	(28)	(27)	(26)	0.95	0.98	(0.981)
Diopatra neapolitana (P)	0.019			6.52			(32.6)			(0.914)		
Nephtys hombergii (P)	0.052	0.055		4.6	4.6		(23)	(23)		(0.931)	(0.346)	
Lumbrineris impatiens (P)	0.130			2.4			(12)			(0.734)		
Other macrofauna detritivores (P)	0.600	0.893	0.160	2.9	2.4	2.4	(14.5)	(12)	(129	(0.983)	(0.784)	(0.919)
Other macrofauna predators (P)	0.355	0.053	0.010	3.43	3.43	3.43	(17.15)	(17.15)	(17.16)	(0.835)	(0.822)	(0.90)
Oligochaets	0.127	0.031	0.005	2.6	2.6	2.6	(13)	(13)	(13)	(0.948)	(0.884)	(0.483)
Microalgae and detritus feeders (F)	1.685	0.894	1.1	0.51	0.49	0.51	10.5	10.5	10.5	(0.719)	(0.687)	(0.821)
Zooplankton consumers (F)	(0.335)	(0.102)	(0.063)	1.3	1	1	7.44	7.44	7.44	0.95	0.95	0.95
Endofauna consumers (F)	0.060	0.06	0.06	0.8	0.77	0.77	3.1	3.1	3.1	(0.79)	(0.617)	(0.870)
Macrofauna predators (F)	0.314	0.14	0.136	0.54	0.9	0.9	9.66	9.66	9.66	(0.347)	(0.771)	(0.789)
Trigla lucerna (F)	0.020			1.4			(7)			0.95		
Pomatoschistus (F)	(0.031)			1.7			(8.5)			0.95		
Larus ridibundus (Gu)	0.006	0.006	0.006	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Larus fuscus (Gu)	0.005	0.006	0.006	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Charadrius alexandrinus (W)	0.001	0.001	0.001	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Charadrius hiaticula (W)	0.001	0.001	0.001	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Pluvialis squatarola (W)	0.001	0.002	0.002	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Calidris alpina (W)	0.001	0.005	0.005	0.02	0.15	0.15	0.24	0.24	0.24	(0.000)	(0.000)	(0.000)
Detritus	527.31	518.76	309.8	-	-	-	-	-	-	(0.184)	(0.826)	(0.675)

* P: Production; B: Biomass; Q: Consumption; EE: Ecotrophic Efficiency

The observed density of each bird species was multiplied by the average AFDW of an individual belonging to that taxon (see Appendix A). Although combining bacteria with detritus can be problematic using the Ecopath software package, bacterial biomass was assigned to the detritus compartment, as recommended by Christensen & Pauly (1992). Finally, the amount of organic matter in the sediment was assessed to be the weight lost after combustion of dry samples for 8h at 450ºC.

4. Production, consumption and diet composition

Production refers to the increase of tissue within a compartment over a given period. Whenever possible, Production/Biomass ratios (P/B), previously calculated for local populations (*e.g.* Lillebø *et al.*, 1999b; Pardal *et al.*, 2000; Ferreira *et al.*, 2004), were used. When this was not feasible, values taken from the literature (Appendix A) were utilised. Special care was exercised to identify values coming from similar Portuguese estuarine systems.

Consumption is the intake of food by a group over a given interval of time. It was entered into Ecopath as the ratio of consumption to biomass (Q/B). Q/B values for birds and fish were taken from the literature (Appendix A). For the other heterotrophic compartments, the P/B ratios were entered into the program to estimate indirectly the Q/B ratio (Hostens & Hamerlynck, 1994).

In a trophic model, such as those constructed using the Ecopath, it is predation that links the different groups into a system. Consumption for one group becomes mortality for another, making information on predation paramount to understand the dynamics of ecosystems. Unfortunately, quantitative information on diet composition is sparse. Diet information for almost all the compartments here identified had to be obtained from the literature (*e.g.* Hughes, 1969; Costa, 1982; Pihl, 1985; Zajac, 1986; Sprung, 1994; Ansell *et al.,* 1999; Azeiteiro *et al.*, 1999; Cunha *et al.*, 2000; Pardal *et al.*, 2000, see Apendix A). Initially, all prey items of each compartment of macrofauna and fishes were listed, along with their corresponding percentages of occurrence. Each observed dietary item was then assigned to an ecologically similar species or group of species as identified in (2) above. Finally, the percentage of occurrence in the diet was assumed to be proportional to the fraction that its biomass comprised of the total biomass of the group. The diets of wading birds and gulls were obtained directly from an analysis of their droppings (Moreira, 1995; Cabral *et al*., 1999).

5. Captures

A complete network requires estimates of the rates of export from the system, including the harvests of economically important species. In the present work, the harvests of the bivalve *Scrobicularia plana* and the polychaete *Hediste diversicolor* (the only two species of economic importance on the Mondego estuary) were considered so small as to be negligible.

6. Balancing the models

For each of the three models the software has calculated the missing parameters. At first none of the models were balanced (*e.g.* negative flows to detritus, ecotrophic efficiencies higher than 1 - which indicated that the demand on them was too high to be sustainable - and some P/Q=GE values physiologically unrealistic). Given the distinct data and parameters source, this situation was predictable in advanced. An exhaustive set of guidelines for how a model should be balanced cannot be given. However, according to the methodology proposed by Christensen *et al*. (2004), the following procedures were followed.

The quality of the data used for each group in the models was variable. For some, there were empirical estimates available from samples taken from within the models areas and time frame (*e.g.* all macrofauna, macrophytes, macroalgae). For others it was necessary to use empirical data from other areas and/or time frames (*e.g.* fishes, gulls) or less specific information (*e.g.* Zooplankton, Phytoplankton). The most reliable data were macrofauna biomass and production, contrary to the majority of studies (*e.g.* Bundy *et al*., 2000; Heymans & Baird, 2000). Data proceeding from the south arm of the Mondego estuary (primary producers, macrofauna, waders and detritus biomass, as well as P/B ratios calculated from previous studies at the some location) were left unchanged. Therefore, greater confidence was placed on them. One exception was the decapods (*Crangon crangon* and *Carcinus maenas)* biomass. This parameter had to be estimated due to the fact that the original values were sub estimated owing to the sampling strategy used. This was also true for the majority of the fish groups' biomass. Regarding the Zooplankton, due to its complex tidal and seasonal dynamics, it was difficult to estimate a realistic annual average biomass. Thus, the software has calculated the missing parameter for the three models. For subsequent balancing it was necessary to re-evaluate some compartments' diet compositions (*e.g. Carcinus maenas, Crangon crangon, Hediste diversicolor, Lumbrineris impatiens* and some fish groups) since feeding habits of some organisms are highly labile and mainly depend on food sources that are available in the ecosystem. This parameter was poorly known, yet it had a large influence on the model estimates.

RESULTS AND DISCUSSION

Diagrams showing the trophic flows were constructed and are depicted in Figure 2, while a summary of all the final input data and the calculated parameters is found in Table 1.

1. Summary statistics

Table 2 summarises the ecological statistic and indices for the three estuarine networks.

The sum of consumptions, exports, respiration, production, and flow to detritus was always higher in the *Zostera* sp. meadows, followed by the strongly eutrophic area and, finally, by the intermediate eutrophic area. This is also the conclusion reached examining the trends for the total throughput (the sum of all flows: consumption, exports, respiratory flows, and flows to detritus) at each trophic level: 6817 g AFDW m^{-2} y^{-1} in *Zostera noltii* meadows, 1826 g AFDW m^{-2} y^{-1} in the strongly eutrophic area and 819 g AFDW m^{-2} y^{-1} in the intermediate eutrophic area (Table 2). All these results are, partially, explainable because the

Table 2. Summary of ecological statistic/indices for the three estuarine networks.

Statistic/indices	Area		
	Zostera sp. meadows	Intermediate eutrophic	Strongly eutrophic
Sum of all consumption (g AFDW $m^{-2}y^{-1}$)	694.91	292.14	521.33
Sum of all exports (g AFDW $m^{-2}y^{-1}$)	1707.51	34.01	169.61
Sum of all respiration (g AFDW $m^{-2}y^{-1}$)	2322.55	297.32	612.67
Sum of all flows into detritus (g AFDW $m^{-2}y^{-1}$)	2092.06	195.09	522.40
Sum of all production (g AFDW $m^{-2}y^{-1}$)	2151	223	494
Total system throughput (g AFDW $m^{-2}y^{-1}$)	6817	819	1826
Net primary production (g AFDW $m^{-2}y^{-1}$)	2014.83	165.58	391.065
Total primary production/total respiration	0.868	0.557	0.638
Total biomass/Total system throughput	0.045	0.061	0.076
Total biomass (no detritus) (g AFDW m^{-2})	304.18	49.91	139.24
Omnivory index	0.105	0.110	0.202

non-eutrophic area model has more groups than the other models. This higher number of compartments has an impact on the calculations and increases the TST and subsequently all the flows such as consumption, production, etc. Both eutrophic areas presented a similar partitioning of the total throughput, between 29 and 36% of the total was due to consumption, approximately 4-9% was exported, about 24-28% flowed into detritus, and around 34-36% was respired (Figure 3).

The major difference verified in the non-eutrophic area concerned a proportionally higher value (25%) due to exports. Why this differences in the breakdown of throughput, with so much more exports and flow to detritus in the *Zostera* sp. meadows? It is well known that macrophytes support two types of food-webs; first, an herbivorous web in which herbivorous feed directly on the standing plant or on the attached epiphytes, and second, a detritivorous web, were some species feed on plant detritus. According to Enriques *et al*. (1993), macrophytes are major producers of organic matter but little of this production enters the grazing food-chain because there is a time lag between the production and its utilisation.

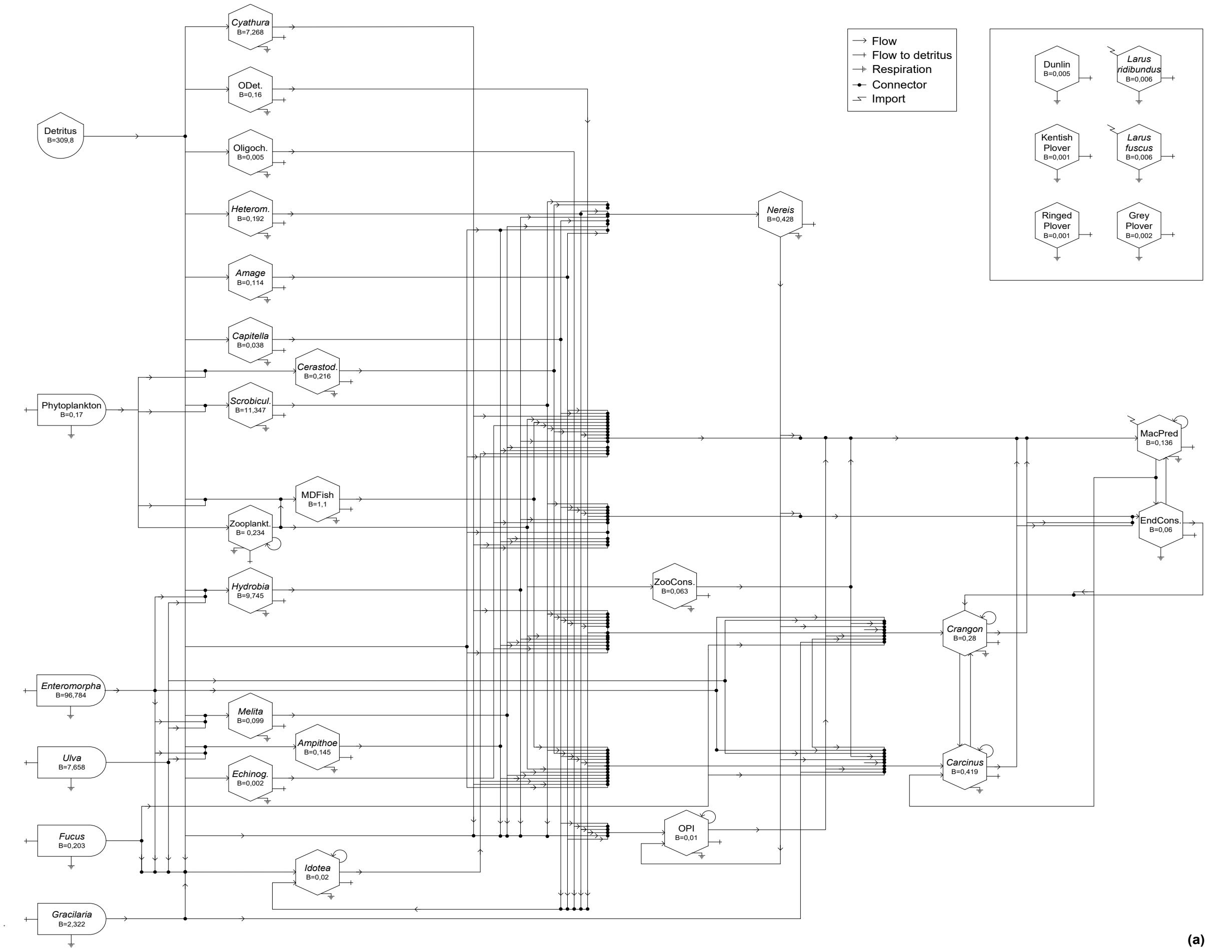


Figure 2. Energy flow diagrams: (a) strongly eutrophic area, (b) intermediate eutrophic area and (c) *Zostera noltii* meadows. Biomass is given in g AFDW m^{-2}.

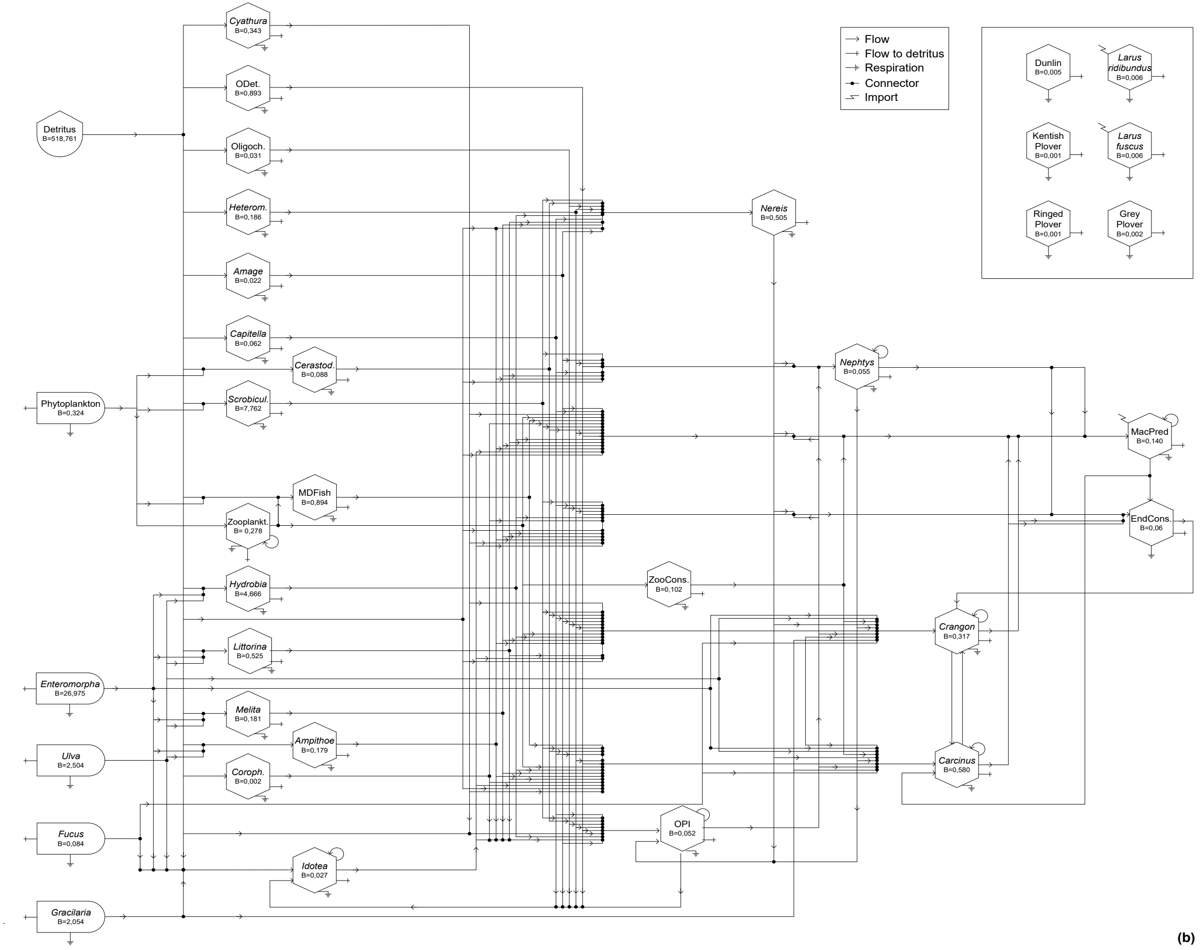


Figure 2. (Continued)

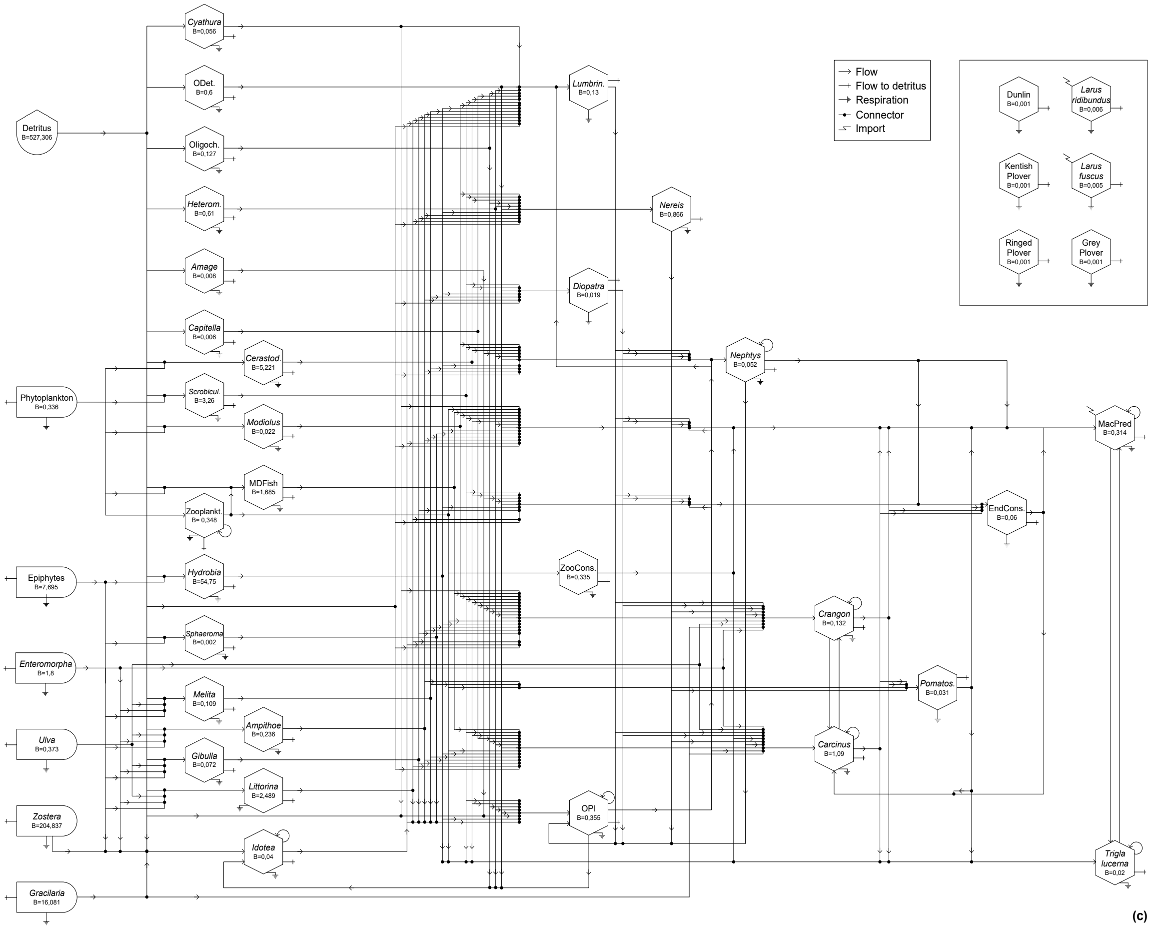


Figure 2. (Continued)

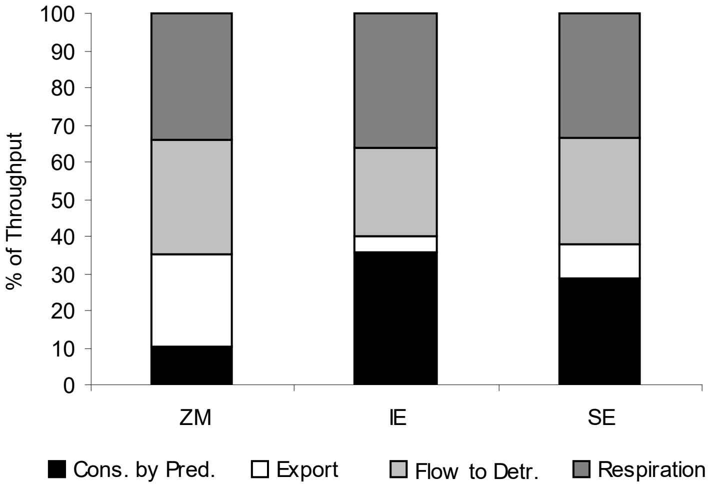


Figure 3. Partitioning of throughput among consumption by predators, exports, flow to detritus and respiration in the three estuarine areas. ZM: *Zostera* sp. meadows; IE: Intermediate eutrophic area; SE; strongly eutrophic area.

Therefore, only few animals feed directly on these plants (their production is usually used after decomposition) and a big proportion of the production decays to detritus or is washed way from the production area, being used in other systems. This fact is consistent with the results of the current study.

The annual rate of net primary production presented a similar behaviour, clearly related with the primary producers dynamic in each of the studied areas. A long-term study in the Mondego estuary has indicated that years of low precipitation have been associated with reductions in turnover rates and with increases in water column stability, salinity and light penetration (Martins *et al.*, 2001). These changes in habitat conditions favoured the initiation of macroalgal blooms, which then served to depress the previously dominant macrophyte communities (Marques *et al.*, 1997; Martins *et al.*, 2001; Dolbeth *et al.*, 2003; Cardoso *et al.*, 2004). In the intermediate and strongly eutrophic areas, primary production is largely the result of such macroalgal blooms (Marques *et al.*, 1997). As a consequence, production in these two systems appears as a strong pulse during the course of the blooms, but remains at very low levels for the rest of the year (Dolbeth *et al.*, 2003). The short duration of the abundant primary production in these areas averages over the year to a significantly lower annual rate of net primary production. Odum (1969) had suggested that less-impacted systems (*e.g. Zostera* sp. beds) should exhibit higher rates of net system production - a fact that is consistent with the results of the current study.

The system omnivory index (SOI) is the average group omnivory index weighted by the logarithm of the total food consumption. A group's omnivory index is calculated as the variance of the trophic levels of a consumer's preys (Christensen & Pauly, 1992). The SOI is a measure of how the feeding interactions are distributed between trophic levels (Vasconcellos *et al.* 1997). If a predator only has a prey on one trophic level its omnivory index will equal zero, while a large omnivory index indicates that the trophic positions of a predator's preys are variable. Heymans (2003), comparing different models of the Newfoundland, observed that the reduction of the 50 compartment models to 30 compartment models reduced the SOI in these systems. According to the author, this index is dependent on the number of compartments in the model: as more compartments would have more connections and there would be less omnivory when compartments are combined and diets consolidated. However, in this study, the *Zostera* sp. meadows despite having 43 compartments exhibited the lower value for this index. This system had the most groups, and still had the lowest SOI, fact that indicates that it is probably not an effect of different group size.

2. Transfer efficiency

According to Lindeman (1942), ecosystem components can be grouped into discrete trophic levels, and transfer efficiencies estimated. Ecopath, using the trophic aggregation routine calculates the transfer efficiencies as the fraction of total flows at each trophic level (throughput) that are either exported or transferred to another trophic level through consumption. Since Lindeman (1942), it has often been assumed that trophic transfer efficiencies vary around 10%, so that one-tenth of the energy that enters a trophic level is transferred to the next trophic level. Hence, transfer efficiencies are usually greater at the

Table 3. Trophic transfer efficiencies (%) (proportion of energy transferred from one trophic level to the next) for each trophic group for the three estuarine networks.

Trophic level	Area		
	Zostera sp. meadows	Intermediate eutrophic	Strongly eutrophic
2	6.6	8.9	3.7
3	9.6	9.0	10.0
4	4.2	5.4	5.8
5	1.8	1.6	2.2
6	1.1	1.5	0.8
7	0.2	0.8	0.0

beginning of the food web compared with higher trophic levels, because of intrinsic characteristics of organisms at different levels in the food web (Christensen & Pauly, 1993). Nevertheless, the transfer efficiencies for the three studied areas (Table 3) suggest a pattern of low herbivore transfer efficiencies (most of the production does not originate from the Phytoplankton, but from the macroalgae and macrophytes whose embodied energy is available for consumers only after decaying into detritus), higher efficiencies on trophic level 3 and lower efficiencies at the higher levels. This fact has already been reported in the literature (*e.g.* Christensen & Pauly, 1993; Baird & Ulanowicz, 1989). Based on the system and the trophic level specific transfer efficiencies, Christensen & Pauly (1993) estimated the average transfer efficiency for different systems (as geometric mean, weighted after flow). The average efficiencies in the 3 areas of the south arm of the Mondego estuary (Table 3) are within the range of those describe by the authors for temperate systems (3 to 7%).

3. Limitations and strengths of the approach

The uncertainty over the input parameters for some of the groups at the lower trophic levels (*e.g.* Zooplankton, Phytoplankton) has already been discussed above. However, there were also several areas of uncertainty for groups at higher trophic levels (*e.g.* gulls, fishes, waders). Weakness in diet data has been noted in many compartments of the three models. The present models provided estimates of the contribution by various predators or groups of predators, but it must be clear that the diet information for all these predators is inadequate for obtaining accurate estimates of their consumption of minor preys. For example, the information on predation by many fish groups came mainly from personal observations obtained at different sampling stations during a distinct time frame. These uncertainties are transmitted down the food web, since all production and losses must be balanced for each group. A high consumption at the top of the food web requires high production at all lower levels. The model is particularly sensitive to some of the groups (*e.g. Hediste diversicolor, Crangon crangon, Carcinus maenas*, Zooplankton, and Phytoplankton). The results described above indicate a system for which our information is uncertain. As a consequence, there are several possible versions of the models.

Some other limitations of the model were observed, mainly associated with the steady-state assumption: high seasonal variations occurred, special in the strongly eutrophic area with the macroalgae bloom event, and these produced major changes in trophic structure and production. This is not reflected in the present models which report average conditions, but it could have been done by constructing seasonal models for each of the three areas. Moreover, uncertainty and time delays in processes associated with ecosystem dynamics were not considered, which will constrain their direct use for management purposes. Nevertheless, lack of historical data and difficulty in measuring some ecosystem components and processes will likely always plague efforts to understand trophic structure and interactions. This is not a problem with Ecopath, but rather with aquatic ecology in general (Ludwig *et al.,* 1993).

Lastly, even with all the limitations that this approach seems to have, why are flux estimates vital to ecosystem science? Many hypotheses and concepts about ecosystem function and food web dynamics focus on the nature of flows of energy in these systems. For example, previous authors have produced a number of ecosystem attributes, and hypothesised about their relationship to the productivity, the successional state, and the level of human disturbance in an ecosystem (Odum, 1969; Ulanowicz & Kay, 1991). Some of these attributes, such as gross production, community respiration quotients, energy cycling or feedback loops require estimates of fluxes between functional groups in their calculation. Patten (1995) showed that five indices used to describe ecosystems, Ascendency, Emergy, Eco-Exergy, Indirect Effects, and Power, are related through the structure of networks and flows of energy within the networks. Thus, most of the 'descriptive statistics' for ecosystems include transfers of energy between groups, and estimating fluxes is as fundamental to ecosystem and food web ecology as estimating demographic rates is to population ecology. In addition, to simply describe ecosystems or food webs, ecologists can also use this information to test hypotheses and draw conclusions regarding management decisions. Just as food webs were summarised and analysed for their structural characteristics (Pimm, 1982; Cohen *et al*., 1993), ecologists have now begun summarising the patterns of energy and nutrient flows between functional groups in food webs and ecosystems.

CONCLUSIONS

The Ecopath models presented here provided a summary of our current knowledge of the biomass, consumption, production, food web and trophic flows in the three areas along the eutrophication gradient in the south arm of the Mondego estuary (Portugal).

The models also highlight many of the uncertainties in our knowledge of the system (diet compositions, site-specific P/B, Q/B ratios, ecological role of a number of abundant species, etc.). Unfortunately, these doubts extend over all trophic levels and many constituent groups of the models, including some parameters regarding those groups that are regularly surveyed and assessed. Nevertheless, Ecopath with Ecosim provided a useful scheme for organising the communities' trophic structures.

As a final word, it is important to note that the process of constructing models such as these is essentially open-ended. The data available for inputs are constantly being added to and revised. These models should be considered as a first step. Doubtless, the three models could be further enhanced, but these versions embody our closest approximate to the system, using the available data. Others are invited to critique the models structure, the input data and the assumptions, so that the models can be improved in the future.

ACKNOWLEDGEMENTS

This research was carried out in the scope of the research project DYNAMOD (POCTI/MGS/37431/2001) and supported by FCT (Portuguese National Board of Scientific Research) through a grant SFRH/BD/820/2000, and by the IMAR- Institute of Marine research /FLAD Grants program. I am grateful to Irene Martins, Isabel Jorge, Miguel Pardal, Ricardo Lopes, Ulisses Azeiteiro and other colleagues for making data available and helping me access and/or interpret it. I also would like to thank Søren Nielsen (Royal Scholl of Pharmacy, Copenhagen) and J.J. (Sheila) Heymans (Fisheries Centre, Vancouver, BC) for their precious help along all modelling phases and valuable review of the manuscript.

Appendix A. Models data sources.

Compartments	Parameter	Source
Phytoplankton	Biomass	Pardal, 1998
	C:Chl a ratio	Anderson & Williams, 1998
	P/B	Wolff *et al*., 2000
Enteromorpha sp.	Biomass	Pardal, 1998
Ulva sp.	P/B	Anibal, 1998
Gracilaria sp.	Biomass	Pardal, 1998
	P/B	Duarte & Ferreira, 1997
Fucus	Biomass	Pardal, 1998
	P/B	Niell *et al*, 1996
Zostera noltii	Biomass	Pardal, 1998
	P/B	Sand-Jensen, 1975 ; Pérez-Lloréns & Niell, 1993
Epiphytes	Biomass	Pardal, 1998; Martins *et al*., 1999
	P/B	Wolff *et al*., 2000
Zooplankton	Biomass, Diet	Azeiteiro *et al*., 1999
	P/B	Rosado-Salórzano & Próo, 1998
Hydrobia ulvae	Biomass	Pardal, 1998
	P/B	Lillebø *et al*, 1999b; Sola, 1996
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Hootsmans & Vermaat, 1985; López-Figuereoa & Niell, 1987; Morrisey, 1988; Philippart, 1995
Gibulla umbilicalis	Biomass	Pardal, 1998
	P/B	Baird & Milne, 1981
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Parker *et al*., 1993; Watson, 1985
Littorina sp.	Total Biomass	Pardal, 1998
L. littorea, L. saxatillis	P/B of the group	Baird & Milne, 1981
	P/Q of the group	Hostens & Hamerlynck, 1994
	Diet	Orth & Montfrans, 1984; Konan *et al*., 1992; Parker *et al*., 1993; Watson, 1985
Melita palmate	Biomass	Pardal, 1998
Ampithoe valida	P/B	Pardal, 1998
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Sprung, 1994; Alonso *et al*., 1995 ; Greze, 1968
Echinogammarus marinus	Biomass	Pardal, 1998
	P/B & Diet	Marques & Nogueira, 1991
	P/Q	Hostens & Hamerlynck, 1994

Corophium multisetosum	Biomass	Pardal, 1998
	P/B	Casabianca, 1975; Cunha *et al*., 2000
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Cunha *et al*., 2000; Smith *et al*., 1996
Scrobicularia plana	Biomass	Pardal, 1998
Cerastoderma edule	P/B	Sprung, 1994
Modiolus barbatus	P/Q	Hostens & Hamerlynck, 1994
	Diet	Hughes, 1969; Loo, 1992; Prins & Smaal, 1989
Cyathura carinata	Biomass	Pardal, 1998
	P/B and diet	Ferreira *et a*l., 2004; Pardal, 1998
	P/Q	Hostens & Hamerlynck, 1994
Idotea chelipes	Biomass	Pardal, 1998
	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Nienhuis & Groenendijk, 1986; Schaffelke *et al*., 1995
Sphaeroma hookeri	Biomass	Pardal, 1998
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Pardal, 1998
Amage adspersa	Biomass	Pardal, 1998
	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Pardal, 1998; Sprung, 1994
Capitella capitata	Biomass	Pardal, 1998
	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Tenore, 1983; Tenore & Chesney, 1985;
Heteromastus filiformis	Biomass	Pardal, 1998
	P/B, Diet	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
Hediste diversicolor	Biomassa	Pardal, 1998
	P/B	Abrantes *et al.,* 1999; Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Nielsen *et al*., 1995; Riisgård *et al*., 1996
Diopatra neapolitana	Biomass	Pardal, 1998
	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Mangum *et al*., 1968

Nephtys hombergii	Biomass	Pardal, 1998
	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Beukema, 1987; Oyenekan, 1986
Lumbrineris impatiens	Biomass	Pardal, 1998
	P/B	Venier, 1997
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Petch, 1986; Valderhaug, 1985
Other macrofauna detritivores	Total Biomass	Pardal, 1998
Aonides oxycephala, Chaetozone setosa, Lagis koreni, Polydora ligni, Pygospio elegans, Streblospio shrubsolii, Haminea hydatis, Diptera larvae	P/B of the group	Sprung, 1994
	P/Q of the group	Hostens & Hamerlynck, 1994
	Diet	Dauer *et al*., 1981; Lambeck & Valentijn, 1987; Zajac, 1986
Other macrofauna predators	Total Biomass	Pardal, 1998
Nemertini, *Glycera convoluta, M. picta, A. mucosa, P. laminosa*	P/B of the group	Sprung, 1994
	P/Q of the group	Hostens & Hamerlynck, 1994
	Diet	Commito & Ambrose, 1985; McDermott & Roe, 1985; Ockelmann & Vahl, 1970; Thiel & Reise, 1993
Oligochaeta	Total Biomass	Pardal, 1998
Tubificoides benedeni, Oligocheta sp.	P/B of the group	Sprung, 1994
	P/Q of the group	Hostens & Hamerlynck, 1994
	Diet	Giere, 1975
Carcinus maenas	Biomass	Pardal, 1998
Crangon crangon	P/B	Sprung, 1994
	P/Q	Hostens & Hamerlynck, 1994
	Diet	Ansell *et al*., 1999; Lee & Seed, 1992; Pihl, 1985
Microalgae and detritus feeders	Total Biomass	Jorge (unpublished data)
Mugil cephalus, Chelon labrosus, Liza aurata, Liza ramada, Alosa fallax, A. alosa	P/B, Q/B group	http://www.fishbase.org
	Stomach content	Correia *et al*., 1997; Oliveira & Soares, 1996
	DW/WW, C/DW	Jørgensen *et al*., 1991
Zooplankton consumers	Total Biomass	Jorge (unpublished data)
Sardina pilchardus, Syngnathidae, *Engraulis encrasicolus*	P/B, Q/B group	http://www.fishbase.org
	Stomach content	Convay *et al*., 1994; Jorge (unpublished data)
	DW/WW, C/DW	Jørgensen *et al*., 1991
Endofauna consumers	Total Biomass	Jorge (unpublished data)
Solea vulgaris, Solea senegalensis, Platichthys flesus	P/B, Q/B group	http://www.fishbase.org
	Stomach content	Costa, 1982; Gonçalves, 1990; Summers, 1980
	DW/WW, C/DW	Jørgensen *et al*., 1991

Macrofauna predators	Total Biomass	Jorge (unpublished data)
Dicentrarchus labrax, Anguilla anguilla, Gobius niger, Ciliata mustela, Sparus aurata, Diplodus sargus, Diplodus vulgaris, Mullus surmuletus, Atherina boyeri, A presbyter	P/B, Q/B group	http://www.fishbase.org
	Stomach content	Correia *et al*., 1997; Costa, 1982; Rebelo, 1993; Rosecchi, 1995
	DW/WW, C/DW	Jørgensen *et al*., 1991
Trigla lucerna	Biomass	Jorge (unpublished data)
	P/B, Q/B	http://www.fishbase.org
	Stomach content	Correia *et al*., 1997; Costa, 1982; Morte *et al*., 1997
	DW/WW, C/DW	Jørgensen *et al*., 1991
Pomatoschistus minutus	Biomass	Jorge (unpublished data)
	P/B, Q/B	http://www.fishbase.org
	Stomach content	Costa, 1982; Jorge (unpublished data)
	DW/WW, C/DW	Jørgensen *et al*., 1991
Larus ridibundus	Biomassa	Lopes (unpublished data)
Larus fuscus	P/B, Q/B	http://www.cbl.umces.edu/ãtlss
	Diet	Moreira, 1995
	Av. weight/ind.	Cramp & Simmons, 1983
	DW/WW, C/DW	Jørgensen *et al*., 1991
Charadrius alexandrinus	Biomass	Lopes (unpublished data)
Charadrius hiaticula	P/B, Q/B	http://www.cbl.umces.edu/ãtlss
Pluvialis squatarola	Diet	Lopes *et al*., 1998
Calidris alpina	Av. weight/ind.	Zwarts *et al*., 1990
	DW/WW, C/DW	Jørgensen *et al*., 1991
Detritus	Biomass	Pardal, 1998
	(O.M in the sediment)	

CHAPTER 3

Ascendency as ecological indicator: a case study on estuarine pulse eutrophication

ABSTRACT

Increasingly, management agencies are requiring that the remediation of eutrophic waters be addressed at the level of the whole ecosystem. One whole-system approach to quantify ecosystems is what has been called ecological network analysis, and Ascendency Theory, the branch of the field that deals with the quantification of whole-system status, specifically addresses the definition of eutrophication. This definition was applied to data taken over a gradient of eutrophication. Three separate areas were observed: a non-eutrophic area (with *Zostera noltii* meadows), an intermediate eutrophic area (*Zostera noltii* already absent and macroalgae abundant at times) and a strongly eutrophic area (where *Enteromorpha* spp. blooms occur with regularity). Pulse eutrophication was considered as the major driving force behind a gradual shift in primary producers from a community dominated by rooted macrophytes (*Zostera noltii*) to a community dominated by green macroalgae. The measures associated with the intermediate eutrophic region turned out *not* to be intermediate to those at the gradient extremes. The most likely explanation appears to be the highly unstable nature of this area. Conditions along the spatial gradient were discussed as representing various stages in the temporal evolution of the system, and analysed in the framework

of the Intermediate Disturbance Hypothesis, Bifurcation, Chaos, and Catastrophe theories.

KEYWORDS: Ascendency, ecological indicator, estuary, eutrophication, network analysis

INTRODUCTION

Most European and North American estuaries are affected to some degree by organic pollution and nutrient discharges, often to an extent that gives rise to eutrophication with all linked effects upon resident biota (Diaz & Rosenberg, 1995; Norkko & Bonsdorff, 1996; Flindt *et al.*, 1997; Marques *et al.*, 1997; Weaver *et al.*, 1997; Rafaelli *et al.*, 1998; Cloern, 2001). It has been observed that benthic eutrophication in estuaries and coastal lagoons can induce a shift from rooted plant communities, dominated by slow-growing species, like the eelgrass *Zostera* sp., towards free-floating (or partially free-floating), faster-growing macroalgae, like *Enteromorpha* sp. or *Ulva* sp.\(Hartog, 1994; Duarte, 1995; Borum, 1996; Marques *et al.*, 1997; Lillebø *et al.*, 1999b; Cardoso *et al.*, 2004; Pardal *et al.*, 2004).

During the past two decades, the emphasis in Ecology has shifted somewhat towards a vision of the ecosystem as a system of interactions (Fasham, 1984; Frontier & Pichod-Viale, 1995). That is, the center of interest has become less the state of the biomass of the different groups of organisms, than the status of the interactions between them, as quantified by flows of matter or energy (Niquil *et al.*, 1999). In particular, there have been attempts to define quantitatively the process of eutrophication (Cloern, 2001). Any index used in such attempts must combine the attributes of system activity level and community structure. One such measure derives from the analysis of networks of trophic exchanges and is called the system “Ascendency”. Ulanowicz (1980) defines Ascendency as an index that quantifies both the level of system activity and the degree of its organisation whereby it processes material in autocatalytic fashion. The level of activity is measured by the sum of the magnitudes of all the trophic exchanges occurring in the system, or what is called the "total system throughput" (TST) The organisation of the flow structure is captured by the average mutual information (AMI) inherent in how the flows are put together (Rutledge *et al.*, 1976.) Ascendency varies jointly as (is the product of) both of these network characteristics (see below.)

Ascendency is a rather abstract concept that reveals manifold attributes when viewed from a variety of aspects. This richness makes the measure useful in a number of practical circumstances. Ascendency was originally created to quantify the developmental status of an ecosystem. If the manager suspects that a particular disturbance has negatively impacted his/her ecosystem, Ascendency can be invoked to test that hypothesis quantitatively, provided sufficient data are available to construct networks of exchanges before and after the impact. Not only can one make before and after comparisons, but the developmental stages of disparate ecosystems can also be compared with one another (*e.g.* Ulanowicz & Wulff, 1991).

Using Ascendency, it is possible to determine quantitatively whether a system has grown or shrunk, developed or regressed. Furthermore, particular patterns of changes in the information variables can be used to identify processes that hitherto had been described only verbally (Ulanowicz, 2000). The process of eutrophication, for example, can be described in terms of network attributes, as any increase in system Ascendency (due to a nutrient enrichment) that causes a rise in TST that more than compensates for a concomitant fall in the mutual information (Ulanowicz, 1986). This particular combination of changes in variables allows one to distinguish between instances of simple enrichment and cases of undesirable eutrophication.

The aim of this study, therefore, is to test whether this network definition of eutrophication properly tracks changes in community structure along a known gradient of eutrophication in the south arm of Mondego estuary (Portugal), a well described small temperate intertidal estuary (e. g. Marques *et al.*, 1997; Cardoso *et al.*, 2004; Neto, 2004; Pardal *et al.*, 2004).

MATERIAL AND METHODS

Study area

The Mondego estuary, western coast of Portugal (Figure 1) consists of two arms, north and south, with very different hydrological characteristics. The northern arm is deeper, while the southern arm is silted up, especially in upstream areas, which causes most of the freshwater discharge to flow through the northern arm. Consequently, the water circulation in the southern arm is dependent mainly on tidal activity and on the (usually small) freshwater input of a tributary, the Pranto River, which is controlled by a sluice.

Although a large part of the southern arm intertidal area remains relatively unimpacted, consisting of sand/mud bottoms covered by *Spartina maritima*

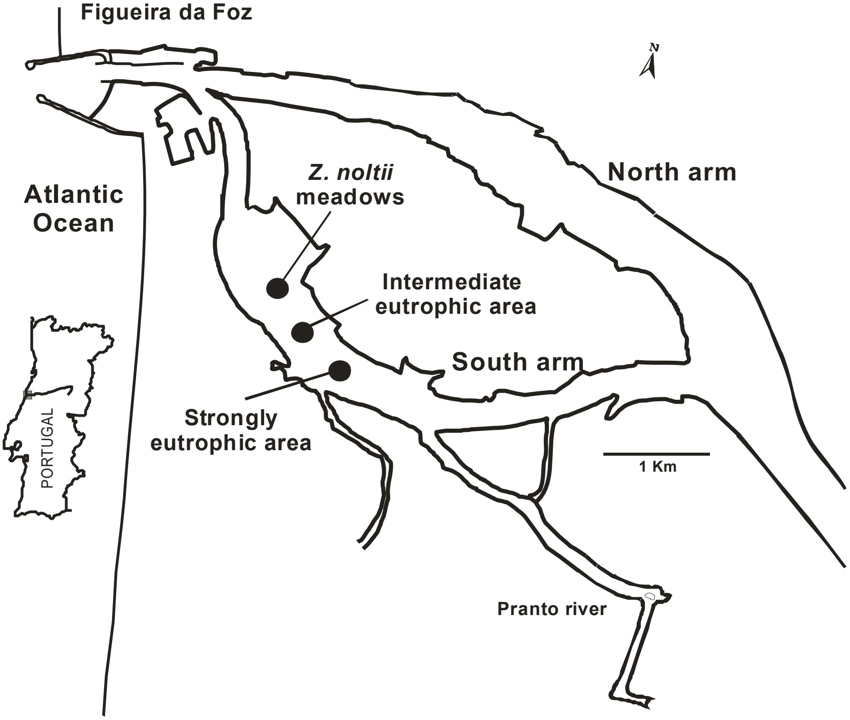


Figure 1. Mondego estuary: location of the sampling stations along a spatial gradient of eutrophication.

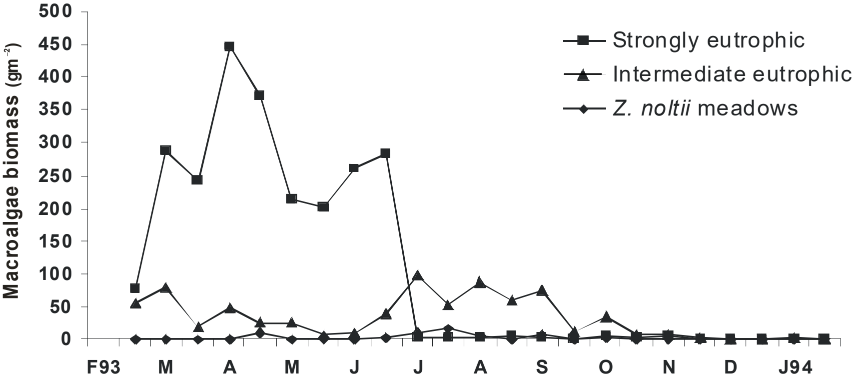


Figure 2. Interannual changes in macroalgae (*Enteromorpha* spp. and *Ulva* spp.) biomass along the eutrophication gradient.

marshes and *Zostera noltii* meadows, macroalgal blooms of *Enteromorpha* spp. have been regularly observed the last twenty years (Flindt *et al.*, 1997; Marques *et al.*, 1997; Lillebø *et al.*, 1999b; Martins *et al.*, 2001; Cardoso *et al.*, 2004; Pardal *et al.*, 2004). In general, *Enteromorpha* spp. biomass increases from early winter until July, when an algal crash usually occurs (Figure 2). These macroalgal blooms may not occur in exceptionally rainy years, due to long intervals of low salinity coupled to strong currents occasioned by discharge from the Pranto River (Martins *et al.*, 2001).

Three sampling stations representative of a spatial gradient in eutrophication were chosen along the south arm of the Mondego estuary (Marques *et al.*, 1997; Lillebø *et al.,* 1999b; Cardoso *et al.*, 2004; Pardal *et al.*, 2004) (Figure 1): A non-eutrophic area (*Zostera noltii* beds still present), an intermediate eutrophic area (*Zostera noltii* absent, although residual roots can still be found in the sediment and macroalgae is sometimes abundant) and a strongly eutrophic area (macrophyte community totally absent for a decade and *Enteromorpha* spp. blooms are regularly observed).

Methods

Estuarine food webs were constructed at the three sites, and these quantified food webs were examined using network analysis, which quantifies input-output relationships (Leontief, 1951), cycling (Finn, 1976), through-flows, storage, information-theoretic indices of whole system status (Ulanowicz, 1986), and indirect diet relationships.

Energy budgets for each of the three stations were developed using “Ecopath with Ecosim” software (www.ecopath.org), which calculates a balanced budget for each trophic group, according to the linear equations $C_i = P_i + R_i + E_i$, where C_i = consumption, P_i = production, R_i = respiration, and E_i = egestion by i (see Chapter 2 for details). Estimates of consumption, production and respiration generated by Ecopath with Ecosim were imported into NETWRK 4.2a software (Ulanowicz, 1999) to calculate annual AFDW budgets for each heterotrophic compartment during one year. The structures of trophic levels and cycling for each network were analysed and system properties were calculated using algorithms described by Ulanowicz (1986), Kay *et al.* (1989) and Monaco & Ulanowicz (1997).

i) Whole System indices

Total System Throughput, TST: The differences in system activity are gauged by the relative values of the TST. The TST is simply the sum of all transfer processes occurring in the system, including consumptions, exports, respirations, and flows into detritus. TST is a surrogate for the size of an ecosystem in the same sense that the extent of an economy may be reckoned in terms of the gross domestic product (Kay *et al*., 1989). That is $TST = \sum_{p,q} T_{pq}$ for all possible transfers T_{pq}, where *p* and *q* can represent either an arbitrary system component or the environment.

Average Mutual Information, AMI: Measures the average amount of constraint exerted upon an arbitrary quantum of currency as it is channelled from any one compartment to the next (Ulanowicz, 1997). It also measures the overall degree by which one compartment communicates unambiguously with any other (Rutledge *et al*., 1976). Ulanowicz (1986) has suggested that both the number of trophic compartments and the extent of trophic specificity (the relative lack of trophic niche overlap) are embodied in the AMI of the flow connection between compartments. A network with high mutual information is a system with many nodes (compartments) of comparable size that are connected with each other (Baird *et al*., 1991). System development then becomes any increase in the AMI, which reflects increasing internal constraint within the ecosystem. Such constraints are thought to arise as autocatalytic feedback loops, reinforcing and incrementing their own component pathways at the expense of other non-participating members. Zorach & Ulanowicz (2003) discovered that the AMI of a network is related to the logarithm of the effective number of trophic levels in the corresponding ecosystem. It is the unscaled form of the Ascendency and is written as:

$$AMI = \sum_{i,j} \frac{T_{ij}}{T_{..}} \log \left[\frac{T_{ij} T_{..}}{T_{i.} T_{.j}} \right]$$

where T_{ij} expresses the trophic exchanges from taxon *i* to taxon *j.*

Ascendency, A: This is a key property of a network of flows that quantifies both the level of system activity and the degree of organisation (constraint) with which material is being processed in autocatalytic systems such as ecosystems. The Ascendency, *A*, expressed in terms of trophic exchanges, T_{ij}, from taxon *i* to taxon *j* is calculated as:

$$A = \sum_{i} \sum_{j} T_{ij} \log \left[\frac{T_{ij} T_{..}}{T_{.j} T_{i.}} \right]$$

where a dot as a subscript indicates summation over that index.

Development Capacity, C: Functions as a mathematical upper bound on the Ascendency. Capacity is measured by the diversity of the flows (calculated using the Shannon-Wiener formula), as normalised by the TST (Ulanowicz & Norden, 1990). The capacity is so named, because it represents the scope of the system for further development. Quantitatively, it takes the form:

$$C = -\sum_{i,j} T_{ij} \log\left[\frac{T_{ij}}{T_{..}}\right]$$

Redundancy, R: This is the degree to which pathways parallel each other in a network. It can be calculated in an isolated system as the (non-negative) difference by which the system capacity exceeds the Ascendency. In terms of flows it is:

$$R = -\sum_{i,j=0}^{n} \frac{T_{ij}}{T_{..}} \log\left[\frac{T_{ij}^{2}}{T_{i.}T_{.j}}\right]$$

where *n* is the number of components in the system (for more details see Ulanowicz & Norden,1990; Ulanowicz & Wulff ,1991).

Specific Overhead of the system, **Ø/TST**: It measures the total flexibility of the system on a per-unit-flow basis. The overhead of a system is the amount by which the capacity of a non-isolated system exceeds the Ascendency. It consists mostly of redundancy, but in open systems it is also augmented by multiplicities in the external inputs and outputs. In terms of the flows it resembles the redundancy, only it also includes the transfers with the external world. In term of flows it is:

$$\Phi \Big/ TST = -\sum_{i,j=0}^{n+2} \frac{T_{ij}}{T_{..}} \log\left[\frac{T_{ij}^{2}}{T_{i.}T_{.j}}\right]$$

where the index $(n+1)$ signifies an import and $(n+2)$ an export or dissipation.

ii) Trophic analysis: Food webs that are qualitatively very different can be mapped into a standard straight-chain network topology. This standard form allows comparing corresponding trophic efficiencies between different estuaries (Baird *et al.* 1991). The trophic efficiency between any two levels is defined as the amount a given level passes on to the next one, divided by how much it received from the previous level (Ulanowicz & Wulff, 1991). The energy flow networks pertaining to the non-eutrophic and the strongly eutrophic study areas were aggregated into their canonical trophic forms (Figure 3), called the "Lindeman spine" (Ulanowicz, 1997). The connectance indices (Table 1) are estimates of the effective number of links both into and out of each compartment of a weighted network.

iii) Cycle analysis: The Cycling Index (CI) calculated by NETWRK, separates a given network into two component networks. One contains no cycles and the other consists only of cycles. The TST of the cycling network is divided by the original TST. It reveals the proportion of TST that is devoted to the recycling of carbon.

RESULTS

Table 1 and Figure 3 provide the measures that were used to characterise the trophic status of the three estuarine ecosystems.

Table 1. Network analysis ecosystem indices for the three study areas.

Information indices	Area *Zostera* sp. meadows	 Intermediate eutrophic	 Strongly eutrophic
Total System Throughput (g AFDW m^{-2} y^{-1})	10852	1154.8	2612.5
Development Capacity (g AFDW m^{-2} y^{-1}; bits)	39126	5695.2	10831
Ascendency (%)	42.3	30.4	36.7
Overhead on imports (%)	12.3	8.2	6.2
Overhead on exports (%)	1.3	1.5	2.5
Dissipative overhead (%)	17.7	22.1	19.9
Redundancy (%)	26.4	37.8	34.6
Average Mutual Information (bits)	1.53	1.50	1.52
Φ/T	2.08	3.43	2.62
Overall connectance	1.672	2.431	2.11
Intercompartmental connectance	2.406	3.573	2.63
Cycling Index	5.75E-02	0.2045	0.1946
Total number of cycles	74517	15009	9164

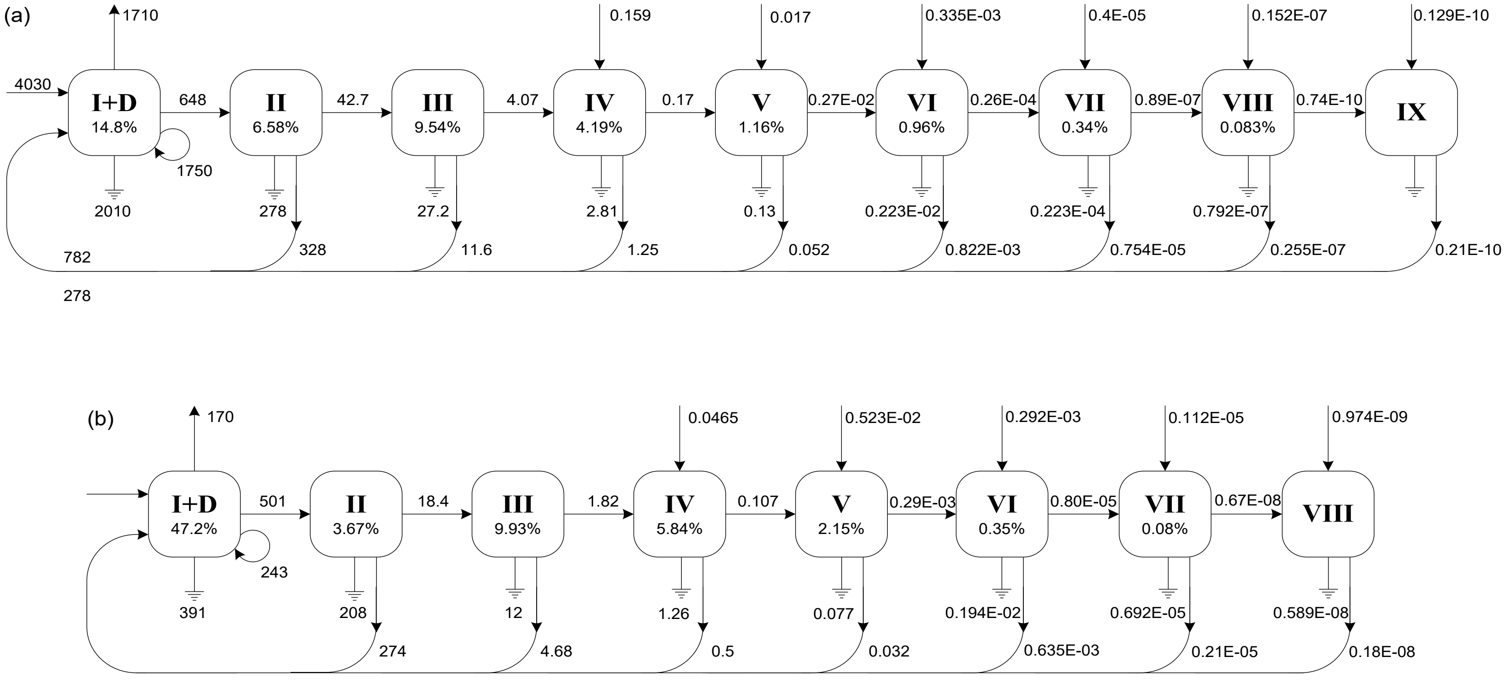


Figure 3. Linear food chains: (a) *Zostera* sp. meadows and (b) strongly eutrophic areas. Flows out of compartment boxes represent exports (g AFDW m^{-2} y^{-1}), flows arriving to compartment boxes represent outside system inputs (g AFDW m^{-2} y^{-1}) and flows out of the bottom represent respiration (g AFDW m^{-2} $year^{-1}$) . *Level* I+D corresponds to the association of autotrophs (level 1) and detritus (non-living compartment). The heterotrophic compartments are divided in *levels* II-IX (in a) or *levels* II-VIII (in b), according to their diet.

Figure 4 characterises the magnitude and structure of carbon cycling at the two endpoints of the eutrophication gradient.

As with Monaco & Ulanowicz (1997), no test of statistical significance was applied to the differences between the values of the indices pertaining to the different areas, due to the complexity of comparing information-theoretic combinations. When the results of network trophic structure and estuarine ecosystem properties were ordered according to magnitude, definitive patterns emerged, providing insights into the trophic structures, complexities and relative stresses exhibited by each community (Monaco & Ulanowicz, 1997).

Whole system indices

The current study was designed to test how the network formulation of eutrophication could be applied to the differences in trophic status among the trophic structures of three neighbouring communities along an eutrophication gradient. Although the three habitats are clearly distinct in physical appearance, network analysis revealed both differences and similarities among their trophic structures that had not been apparent at first glance.

It was possible to observe (Table 1) that the *Zostera* sp.- dominated community had the highest TST, followed (unexpectedly) by the strongly eutrophic system and finally by the intermediate eutrophic area. The development capacity was highest in the *Zostera* sp. beds and lowest in the Intermediate eutrophic area. The index differed among the three areas. Due to the logarithmic nature of this index, small differences can represent appreciable disparities in structure. The average mutual information was slightly higher in the non-eutrophic area, followed closely by the strongly eutrophic area, and was lowest in the intermediate eutrophic area.

Concerning Ascendency, it increased in order from the intermediate eutrophic to the heavily eutrophic zone to the *Zostera* sp. meadows. Regarding redundancy, the intermediate eutrophic community had the highest value, followed by the strongly eutrophic area and was least in the *Zostera* sp. beds.

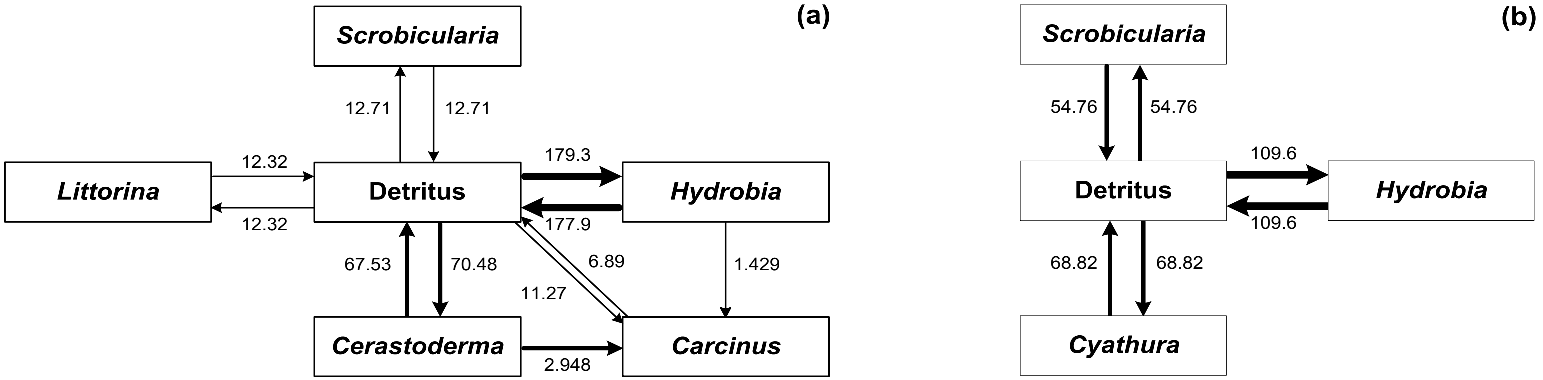


Figure 4. Cycling diagrams: (a) *Zostera* sp. meadows cycling diagram representing the compartments involved in 91% of the cycled flow (g AFDW m^{-2} y^{-1}) and (b) Strongly eutrophic area cycling diagram representing the compartments involved in 92% of the cycled flow (g AFDW m^{-2} y^{-1}).

The rankings in Ø/TST mirrored those in redundancy, which comprises the largest component of Ø/TST.

Trophic analysis

The Lindeman spine for the non-eutrophic area possesses an additional trophic level beyond those apparent in the strongly eutrophic chain (Figure 3). Although both areas exhibited their highest transfer efficiencies at the first trophic level (*Zostera* sp. beds with 14.8% and the strongly eutrophic with 47.2%), the intermediate eutrophic area was most effective in transferring material (8.9%) at the second trophic level.

In the Mondego system, connectance indices (Table 1) were relatively low, being modest in the intermediate eutrophic area, and decreasing yet further in the strongly eutrophic area and *Zostera* sp. community.

Cycle analysis

The Cycling Index percentage (Table 1) was greatest in the intermediate eutrophic area (20.45%), decreased in the strongly eutrophic zone (19.46%) and then markedly in the *Zostera* sp. - dominated system (5.75%). This would seem to indicate that the overall percentage of cycled matter increases as the degree of eutrophication rises. However, the structure of cycling changes dramatically between the pristine and eutrophic systems. The total number of cycles (Table 1) is highest in the *Zostera* sp. beds (74 517), followed by the intermediate eutrophic area (15 009), and the fewest were counted in the strongly eutrophic community (9 164). This is due to the fact that a larger number of cycles tend to be found among systems with more compartments at higher trophic levels. The preponderance of cycling occurs in both systems over cycles of length 2, and the major routes for recycle are shown in Figure 4. From this figure it is evident that less material is flowing over the more complicated web of cycles in the *Zostera* sp. community (Figure 4a), while far more medium cycles over fewer pathways occurred in the strongly eutrophic system (Figure 4b). This is a common signature of a stressed ecosystem (Ulanowicz, 1986). It

Table 2. Ecological indicators along the eutrophication gradient.

Ecological indicators		***Zostera* sp. meadows**	**Intermediate eutrophic area**	**Strongly eutrophic area**
Biodiversity	Species diversity	1.80	1.51	1.21
	Heterogeneity	0.99	1.46	1.52
Specific Eco-Exergy		85.70	150.29	165.42
Eco-Exergy		25 364	4 789	8 547
Ascendency		42.3	30.4	36.7

is also evident that the keys species, implicated in the cycle process, changed from one community to the other.

Comparison with other ecological indicators

Comparing the behaviour of the Ascendency with other ecological indicators (Table 2), showed that the heterogeneity (as computed using the Shanon-Wiener index) and the Specific Eco-Exergy (a thermodynamic measure indicating the amount of work the system can perform on a per-unit-mass basis), both increased with greater eutrophication Contrariwise, species richness, Ascendency and total Eco-Exergy declined with the degree of eutrophication.

DISCUSSION

Eutrophication as a state of an ecosystem is difficult to define quantitatively and little consensus has been reached (Christian *et al.*, 1996; Cloern, 2001). Nevertheless, there are points upon which most investigators agree. The process of eutrophication, for example, is commonly considered to be an increase in the rate of supply of organic matter to an ecosystem (Nixon, 1995). The dystrophy attendant to over enrichment is usually manifested as the loss of important species along with the system functions they help to maintain. That is, although nutrients tend to stimulate a system's growth, the organisation of the system is degraded, despite its augmented activity. Almost all studies stress both the nutrient enrichment phenomenon as well as its negative consequences for the system.

Our previous long-term study in the Mondego estuary indicated that years of low precipitation tended to be associated with reductions in turnover rates and increases in water column stability, temperature, salinity and light penetration (Martins *et al.*, 2001). These changes in habitat conditions encouraged blooms of macroalgae that gradually replaced the resident macrophytes (Marques *et al.*, 1997; Martins *et al.*, 2001). In the intermediate and strongly eutrophic areas primary production is largely the result of these macroalgal blooms (Marques *et al.*, 1997). Production appears as a strong pulse during this specific time, but remains at very low levels during the rest of the year (Figure 2). This limited temporal interval of primary production results in a markedly lower figure for the cumulative annual primary production and total system throughput (TST) in these two areas as compared with the corresponding measures in the *Zostera* sp. beds. Comparing the average mutual information values of the flow structure for the three areas, it is possible to discern a very small decrease in the measure among the three zones, suggesting that, as regards trophic structure, these areas are indeed different. The three zones appear nevertheless much more distinct to eye than illustrated by the average mutual information values.

In light of these results, the network definition of eutrophication does not appear to accord with the gradient in eutrophication in the Mondego estuarine ecosystem. Rather, it would be more accurate to describe the enrichment processes occurring in this ecosystem as pulse eutrophication. This process could be characterised as a disturbance to system Ascendency in the form of an intermittent supply of excess nutrients that, when coupled with a combination of physical factors (*e.g.* salinity, precipitation, temperature, etc), causes both a decrease in system activity and a drop in the mutual information of the flow structure. Even though a significant rise in the TST does occur during the period of the algal bloom and at that time does give rise to a strong increase of the system Ascendency (as per the network definition of press eutrophication), the longer-term annual picture suggests that the other components of the intermediate and strongly eutrophic communities were unable to accommodate the pulse in production. The overall result was a decrease in the annual value of the system TST and, as a consequence, of the annual Ascendency as well.

Regarding the results of the trophic analysis, the *Zostera* sp. community has one more trophic level than those counted in the strongly eutrophic chain, implying that this community possesses a more complex web with additional top consumers. At the same time, the *Zostera* sp. community exhibits lower transfer efficiency at the first trophic level, probably because the production of *Zostera noltii* meadows usually cannot be eaten directly, but needs first to be decomposed (Lillebø *et al.*, 1999a).

Concerning the analysis of cycled materials, the overall percentage of cycled matter, as indicated by the CI, increases as the degree of eutrophication rises. Odum (1969) suggested that mature ecosystems recycle a greater percentage of their constituent material and energy than do pioneer or disturbed communities. Hence, according to Odum, the progressive increase in the CI would suggest the maturation of the ecosystem. It has been observed, however, that disturbed systems also often exhibit greater degrees of recycling. The speculation is that such increase in cycling in disturbed systems is the homeostatic response that maintains in circulation resources which before the perturbation had been stored as biomass in the higher organisms (Ulanowicz & Wulff, 1991). This latter scenario seems consistent with the present results.

When the whole-system properties of the three areas were compared, the measures associated with the system considered to lie between the two extremes in nutrient loading did not plot intermediate to the other two. Rather, the intermediate eutrophic area exhibited the lowest Ascendency, AMI, TST and development capacity values and the highest figures for redundancy, Ø/TST and CI, so to say it appears to be the most disturbed of the three areas. Because Ascendency is scaled by the flows of material in a system, it is likely to be dominated in the *Zostera* sp. meadows and the strongly eutrophic site by the primary producers -- seagrasses and macroalgal mats, respectively. At the intermediate site there is little macroalgal material and no seagrasses, resulting in a lower value for Ascendency. A second, and not mutually exclusive, explanation is that the non-disturbed and most disturbed sites host relatively stable communities -- one dominated by seagrasses and fine sediments, and the other by macroalgal mats and coarser material. When the seagrasses are lost, however, there is a coarsening of the sediments, which makes it very difficult for seagrasses to re-invade. The reason for the depressed values found in the intermediate eutrophic area, therefore, appears to lie in its unstable nature (Marques *et al*., 2003). In fact, observations using other ecological indicators (Table 2) have indicated that the communities built around both the *Zostera* sp. and *Enteromorpha* sp. populations represent more stabilised communities at the ends of the eutrophication gradient. Regarding the intermediate eutrophic area, since it always exhibited the lowest values of total biomass and the highest species heterogeneity (as measured by the Shannon-Wiener index) (Table 2), a preliminary interpretation of the results suggested that the Intermediate Disturbance Hypothesis (IDH) (Grime, 1973; Connell, 1978) was applicable to explain the observations (Marques *et al*.1997). Nevertheless, the same data base was also utilised to test other ecological indicators thermodynamically oriented (the Eco-Exergy index and Specific Eco-Exergy), (Table 2), which allowed concluding that: a) both indicators were consistently higher in the non-eutrophic area, followed by the most eutrophic and intermediate eutrophic areas; b) their range of variation through time was smaller in the non-eutrophic area, expressing a more stable situation, while the magnitude of the variations was stronger in the other two areas, but especially in the intermediate eutrophic area (Marques *et al*., 2003). As a whole, by contrast with the other two areas, the intermediate eutrophic one clearly appeared too disturbed to allow for the establishment of a coherent community built around either mode of primary production. These results argue in favour of using a pluralistic approach to evaluate the effects of adding nutrients to ecosystems.

So, observations on the intermediate eutrophic area were not satisfactorily consistent with the IDH, which is thought to be realised in systems with a certain capacity to absorb changing environmental conditions at a larger time scale. The story could be not so simple if the system has already lost or exploited most of the possibilities to react properly, either due to natural succession or long-term stress.

Qualitative observations carried out in the Mondego estuary since the early 1980s (Marques *et al*., 1984) provide a general idea regarding how the system was at the time. Since then, the most evident feature was the increase of eutrophication symptoms and its impact on the biological communities, of which the most visible effect was the occurrence of green macroalgae blooms and a concomitant decrease of the area occupied by *Z. noltii* beds. In fact, some twenty years ago, *Z. noltii* beds covered a large part of the intertidal area, extending to the upstream section of the south arm. Moreover, we know that its disappearance first took place in the inner areas of the south arm of the estuary, what is now the most eutrophic area, and went forward to the downstream section, where it is presently restricted. In the intermediate eutrophic area, although *Z. noltii* disappeared some time ago, it is still possible to find the residuals of its rhizomes in the sediment.

The way macroalgae extended from upstream to downstream sections in the south arm of the estuary, competing with *Z. noltii*, and the way this process influenced faunal assemblages was fully described in previous works, illustrating how a shift in primary producers may determine changes at other trophic levels (Marques *et al*., 1997; Marques *et al*., 2003). Additionally, a tentative interpretation of the recent biological changes in the system was proposed (Marques *et al*., 2003), starting from a basic assumption: since *Zostera noltii* disappearance in the south arm of the estuary commenced in the inner areas and progressed downstream, spatial changes in the system may be taken as representing temporal changes. So to say, the non-eutrophic area, with *Z. noltii* beds, may represent what the system was two decades ago, the intermediate eutrophic area may represent the changing areas during the process, and the most eutrophic area may represent the most advanced stage in the observed shift of primary producers, where macroalgae has totally ousted macrophytes.

Once accepted this assumption, the recent modification in primary producers and its related food web may be seen as a dynamic shift in the ecosystem network structure, of the catastrophic type, described for instance by Scheffer *et al*. (2001). Briefly, in the prevailing conditions two decades ago, rooted macrophytes, *Z. noltii* and related epiphytic grazers dominated by *Hydrobia ulvae* were selected, and an important detrital food web was also present. As eutrophication affected an increasing area of the estuary, green macroalgae, like *Enteromorpha spp*. and *Ulva spp*. replaced the rooted macrophytes. As a consequence, the high turnover of these macroalgae, and the alternation between periods dominated by the occurrence of extensive algal mats and periods with bare bottom organically enriched sediments caused an increasing importance of the detrital pathway (Marques *et al*., 2003). Let us see how this shift can be theoretically understood.

The possible long-term development and modification, of an ecosystem exposed to various degrees of disturbances, natural as well as human caused, may be illustrated by Figure 5A. On the left part of the curve, at a relatively low level of stress corresponding to normal fluctuations in environmental conditions, we represent a normal succession towards an increase and finally stabilisation of the biomass and complexity of the system. During this period, the system will follow traditional developmental patterns (see for instance the 24 principles of E.P. Odum) such as development from r- to K-stragetists, increased cycling, importance of the network, etc. (Odum, 1971).

For a given increase in stress, literature exists that reports an additional stimulation of biomass and diversity, illustrated by a “hump” on the curve (Figure 5A), what is usually referred to as the IDH (Grime, 1973; Connell, 1978). Nevertheless, this developmental pattern is only thought to be realised in systems that possess a cache in diversity that enable them to react, buffering the disturbances at a larger time scale, which is not always the case. If a system has lost or exploited most of its overhead already, either due to natural succession or long-term stress, such capability may be limited or not exist at all. If this is the case, higher stress forces will lead to “real” macroscopic modification, which takes place through other mechanisms. First of all, continuous adaptation and fine-tuning of parameters leads to a system of highly fitted and specialised organisms, which in turn will reduce organism’s

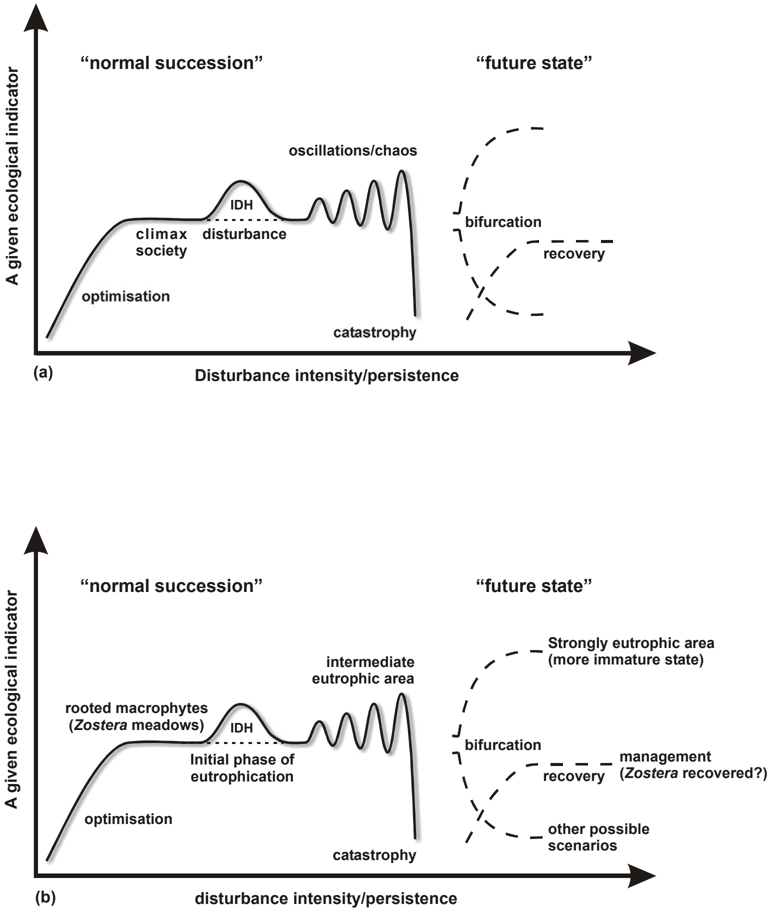


Figure 5. Ecosystem development as a function of succession and environmental stress: a) Relation between possible ways of ecosystem development and concepts from ecological theory and b) Possible interpretation of changes on going in the Mondego estuary in the frame of ecological theories.

adaptability in relation to changes in environmental conditions. As a result, it is believed that the system as a whole becomes very unstable and brittle (Jørgensen & Johnsen, 1989). This phase may be expected to correspond to large short-term fluctuations of the ecological indicators we may use to capture the state of the system (Figure 5A), which corresponds in fact to the observations by Marques *et al*. (2003). Second, adaptation of the network leads to a development where the ecosystem has decreased as much as possible its conditional entropy, or overhead in the sense of Ulanowicz (1986, 1997), *i.e.,* maximizing the utilisation of the available resources (energy) through a more and more specialised (efficient) species composition. Such specialisation also means a decrease in the possibility of the system to cope with environmental changes, which eventually leads to change through a type of Holling cycle, (Holling, 1986; Ulanowicz, 1997).

Moreover, the modification of systems is non-linear, and as time passes instability gives rise to a bifurcation to new stability points (Glansdorf & Prigogine, 1971) (Figure 5). In other words, the instability of the system will thus lead it to a break down - a **catastrophe** - with possibilities of new organisms and combinations hereof to take over and be selected because the new constellation is better able to meet the prevailing conditions. Catastrophe is here not necessarily used in its narrow mathematical sense and may also not be mathematically as such (Zeeman, 1976). Beyond the point of **bifurcation**, whatever we prefer as interpretation, several possibilities are allowed: a) a total recovery of the system to an almost identical state can take place, which would correspond to a “normal” understanding and interpretation of the Holling cycle. This of course provided that the perturbation somehow has been stopped, and sufficient biodiversity has been conserved during the stress period to allow the system to return to a quasi-original state; b) in the case these conditions are not fulfilled, the system will evolve to one or more stability points, or maybe even continuous instability. The shift to other stability points may also be viewed as if the system is leaving one Holling cycle (Holling, 1986) and entering into another.

Plausibly, several of these “states” of development can be identified in time and space along the south arm of the Mondego estuary. The following descriptions allow us to understand the present state of each studied area along the south arm and interpret the situation in accordance with a broader theoretical frame, as previously suggested by Marques *et al*. (2003):

a) The *Zostera noltii* beds may be considered as corresponding to the more or less original state of the system, identical to the climax society (Figure

5B). Meanwhile, these areas are in regression, showing that they are highly vulnerable to the present conditions;

b) The most eutrophic area has undergone a transition and, through a bifurcation, found another stability point (Figure 5B);

c) In the intermediate eutrophic area changes exceed the natural variation to a degree where the system is never able to find a new stability point, and the scenario may be described as a chaotic regime (Figure 5B), which will be followed by a catastrophic shift (Zeeman, 1976; Scheffer *et al*., 2001).

This interpretation is very much consistent to the fact that, through time, even in the short run, the highest variations of the ecological indicators estimated are found in this area (Marques *et al*., 2003). As a whole, our results and conclusions appear to point that a possible recover of the system should occur at different critical conditions than the shift to the scenario of macroalgal dominance, which is known as hysteresis (Sheffer *et al*., 2001), but at the present stage our data do not allow to elucidate this point and further work will be necessary.

Despite the little respect accorded to it by those in other fields of science, Ecology deals with some of the most complex phenomena encountered in modern science. Ecosystem analysis must encompass several disciplines in a coordinated fashion to answer specific questions concerning how large, multidimensional systems work (Livingston *et al.*, 2000; Jørgensen & Marques, 2001). Such research entails the integration of diverse studies, usually over a significant spatial area for intervals of time long enough to account for both seasonal and inter-annual variability of basic physicochemical and biological factors. Such difficulties notwithstanding, network analysis appears to provide a systematic approach to apprehend what is happening at the whole-system level. The current study on the Mondego estuarine ecosystem seems to have provided an example of how the measures coming out of network analysis can lead to an improved understanding of eutrophication process itself. Despite the considerable time and labour needed to collect all the data necessary to perform network analysis, the insights the method provided seemed to demonstrate its promise as a very useful tool for Ecosystem Theory.

ACKNOWLEDGEMENTS

The present study was carried out in the scope of the research project DYNAMOD (POCTI/MGS/37431/2001) and supported by FCT (Portuguese National Board of Scientific Research) through a grant SFRH/BD/820/2000, by the Chesapeake Biological Laboratory, University of Maryland Center of Environmental Science, through a grant from the National Science Foundation's Biocomplexity Program (Contract No. DEB-9981328), and by the IMAR-Institute of Marine Research/ FLAD Grants Programme.

CHAPTER 4

Ecological indicators performance during a re-colonisation field experiment and its compliance with ecosystems' theory

ABSTRACT

Through a re-colonisation field experiment three main questions were approached: (1) how do different ecological indicators react during the process of recovery? (2) what does grow first during a community succession, biomass or complexity? (3) can the chosen ecological indicators help in recognising the three proposed forms of growth: biomass, network and information, throughout re-colonisation?

The study was carried out in an intertidal rocky community dominated by the algae *Corallina elongata*. Experimental plots were cleared and macroalgae and fauna were removed. Multivariate analysis was performed to examine the convergence of the disturbed plots with the surrounding community during recovery. Shannon-Wiener index, Margalef index, Pielou evenness, Eco-Exergy and Specific Eco-Exergy were applied to characterise the state of the community during the process. Results show that the replacement of species over time happens both with the macroalgae and associated macrofauna community. Species richness increased rather rapidly and species composition was similar in disturbed and undisturbed areas. After 7 months, diversity was consistently higher in the community undertaking recovery. Eco-Exergy and Specific Eco-Exergy provided useful information about the structural development of the community but lacked discriminating power with regard to the informational status of the system. The observations appear to illustrate a case explainable by the Intermediate Disturbance Hypothesis (IDH). Overall, the characteristics of a systems' recovery after disturbance appear to be dependent on the spatial scale of the disturbance. If a disturbed area is small when compared to a contiguous non disturbed one, complexity (information and network) will recover prior to biomass.

KEYWORDS: Ecological indicators, rocky shore community, re-colonisation, succession, Intermediate Disturbance Hypothesis (IDH).

INTRODUCTION

The concept of succession (Odum, 1969) has been broadly applied in marine systems as the process by which species settle and are replaced on new or disturbed surfaces. In a relatively undisturbed environment or at least in an environment that is imposing only a limited degree of disturbance, the communities will follow a succession adapted to meet the long-term environmental average condition. Often, it happens that ecosystems are exposed to disturbances other than the natural ones, which adds an extra stress on the top of the natural disturbance level. Such disturbances are often related to human activities and the ecosystem response may vary drastically in both space and time (Marques *et al*., 2003). Since the 1970s, many community ecologists have focused attention on the dynamics of assemblages in marine coastal ecosystems that are recovering from different types of disturbance (*e.g.* Connell & Slatyer, 1977; Sousa, 1979; Paine & Levin, 1981; Van Tamelen, 1996; Chapman & Underwood, 1998) and there is a rich literature describing such successional changes in detail in a wide variety of coastal marine ecosystems (*e.g.* rocky shores: Kim & De Wreede, 1996; Benedetti-Cecchi & Cinelli, 1996; Dye, 1998; Williams *et al*., 2000; Hutchinson & Williams, 2003; coral reefs: Connell *et al*., 1997; Diaz-Pulido, 2002; soft sediments: Levin &

DiBacco, 1995; Rosenberg *et a*l., 2002; estuaries: Nogueira *et al.,* 2000; salt-marshes: Valiela, 1995; Levin *et al*., 1996; Craft & Sacco, 2003) from all over the world. These studies have demonstrated that succession is likely the composite result of several processes (depletion, tolerance, facilitation, inhibition, removal, allelopathy, etc) that determine if replacement takes place (Connell & Slatyer, 1977) and at what rates it is accomplished (Valiela, 1995). Probably, several of these mechanisms co-occur in most communities.

To evaluate the status of communities' ongoing recovery, a panoply of ecological indicators has been used. Nevertheless, in most cases, ecological indicators either only take into consideration some components of the ecosystem or result from non universal theoretical approaches. In general terms, a number of them are based on the presence/absence of indicator species, others take into account the different ecological strategies carried out by organisms, like diversity, or the energy variation in the system through changes in species biomass. Another group of ecological indicators is either thermodynamically oriented or based on network analysis, looking to capture the information on the ecosystem from a more holistic perspective (Patrício *et al*., 2004). In fact, Biology and Ecology are, in many ways, still lacking universal laws and predictive theory, and many ecologists feel the need for a more general and integrative theoretical network that may help to explain their observations and experimental results. Simultaneously, a broad theoretical framework needs to be in straight connection with empiricism. With that purpose in mind, it would be appealing to perceive what type of information is captured by distinct ecological measures applied to the same recovery experiment.

Over the last two decades, Ecology has changed from a largely qualitative discipline to a quantitative hypothesis-driven experimental science, and manipulative field experiments have contributed greatly to ecological theory during this period (Hawkins, 1999). Rocky shores, in particular, have proven to be good testing grounds for ideas of general ecological significance (*e.g.* Connell & Slatyer, 1977; Paine, 1994; Benedetti-Cecchi & Cinelli, 1996; Dye, 1998; Hawkins, 1999; Olobarria, 2002; Hutchinson & Williams, 2003).

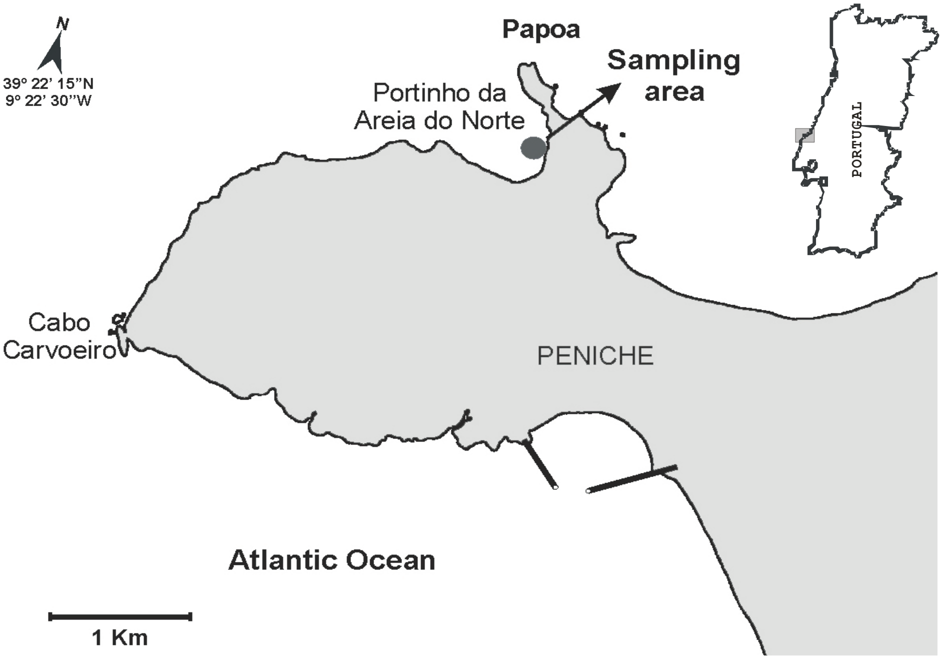


Figure 1. Map of Peniche peninsula, western coast of Portugal showing location of the study site.

The experiment carried out aimed to approach three working questions: (1) how do different ecological indicators elucidate the process of recovery? (2) what does grow first during a community succession, biomass or complexity? Regarding this second question, according to Odum (1969), the hypothesis advanced was that biomass would be the first ecosystem-attribute to recover (3) can the chosen ecological indicators help in recognising the three forms of growth proposed by Jørgensen *et al.* (2000): biomass, network and information, throughout the recovery process?

MATERIAL AND METHODS

Study Area

The experiment was carried out from February 1999 to May 2000 in a small beach called “Portinho da Areia do Norte" (39º 22’ 15’’ N, 9º 22’ 30’’ W),

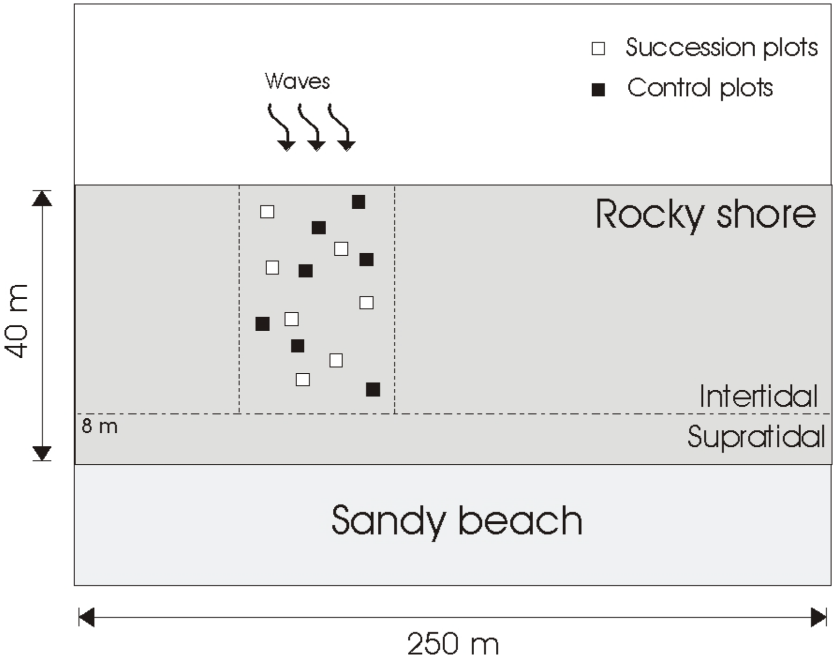


Figure 2. Schematic diagram illustrating the 25x25cm plots localization within the sampling area.

(Peniche), 70 km North from Lisbon, on the Western Coast of Portugal (Figure 1). Slender calcareous layers alternate with marls structuring a nearly horizontal and homogeneous platform, with approximately 250 m in length and 40 m in width (Figure 2). This intertidal rocky system is dominated by the turfing algae *Corallina elongata* Ellis & Solander, which forms a stiff matrix that helds some sediment. Nevertheless, encrusting coralline *Lithophillum incrustans* Philippi, other red macroalgae (*Chondria caerulescencens* (Crouan) Falkenb., *Chondrachantus compressa* Grev., *Gigartina pistillata* (Gmelin) Stackh., *Asparagopsis armata* Harv., *Jania rubens* (L.) Lamouroux, *Lomentaria articulata* (Huds.) Lyngbye, *Gastroclonium ovale* (Huds.) Kütz., *Plocamium cartilagineum* (L.) Dixon, *Callithamnion tetricum* Agardh, *Ceramium* sp., *Nitophyllum punctatum* (Stackh.) Grev., *Laurencia pinnatifida* (Gmelin) Lamouroux and *Calliblepharis jubata* (Gooden. Woodw.) Kütz., and green macroalgae (*Enteromorpha compressa* (L.) Grev., *Cladophora* sp. and *Ulva rigida* Agardh) also occur as epiphytes on the other plants or as early successional species.

Experimental design and sampling

For the present work, the broadly used concept of minimum area was applied. In February 1999, quadrats of 25 x 25 cm (625 cm^2) were randomly distributed across the study area (Figure 2).

The corners of each square plot were marked with casing nails for subsequent relocation. In this preparation phase, 27 discrete areas were created by totally removing macroalgae and the associated macrofauna with a chisel. Other plots were assigned as controls, being left undisturbed at this stage of the experiment. Subsequently, both experimental plots and control plots were sampled, during low tide, every one or two months, until May 2000. All experimental plots were replicated (3 replicates); however, we were not able to replicate the control plots at all different dates.

Samples were preserved in 4% buffered formalin in seawater and sieved through a 500 µm mesh. Later, algae and associated macrofauna were separated and identified. Both macroalgae and animals were subsequently dried at 70ºC for 72h and weighted. Small individuals were pooled to obtain measurable values. Biomass, calculated as g AFDW m^2, was assessed after combustion of samples for 8h at 450ºC.

Data analysis

Multivariate analysis were performed using the PRIMER 5 (Software package from Plymouth Marine Laboratory, UK) in order to examine for convergence of the recovering community with the surrounding area. Data (species abundance and biomass) were transformed by fourth root. Bray Curtis similarity matrix was calculated and used to generate 2-dimensional plot with the non-metric multidimensional scaling (nMDS) technique (Clarke, 1993; Clarke & Warwick, 1994). Stress values were shown for each MDS plot to indicate the goodness of representation of differences among samples.

On the other hand, the following ecological indices were applied: Shannon-Wiener index (eq. 1), Margalef index (eq. 2), Pielou evenness (eq. 3).

$$H' = -\sum p_i Log_2 p_i \qquad \text{(eq. 1)}$$

$$D = (S - 1) / Log(N) \quad \text{(eq. 2)}$$

$$J' = H' / Log(S) \quad \text{(eq. 3)}$$

where p_i is the proportion of abundance of species *i* in a community were species proportions are $p_i, p_2, p_3 \ldots p_n$, *S* is the number of species found and *N* is the total number of individuals.

Moreover, we also applied Eco-Exergy, a concept derived from Thermodynamics. Eco-Exergy (Jørgensen & Mejer, 1979) is one of the mathematical functions that have been proposed as holistic ecological indicators in the last two decades, intending a) to express emergent properties of ecosystems arising from self-organisation processes in the run of their development, and b) to act as orientors (goal functions) in models development. Such proposals resulted from a wider application of theoretical concepts, following the assumption that it is possible to develop a theoretical framework able to explain ecological observations, rules, and correlations on basis of an accepted pattern of ecosystem theories. Eco-Exergy, which has been tested in several studies, can be seen as a measure of the maximum amount of work that the system can perform when it is brought into thermodynamic equilibrium with its environment.

If Eco-Exergy is calculated only from the chemical potentials, which are extremely dominant with regard to ecosystems, the following expression is valid with good approximation (Jørgensen, 2002):

$$EX = RT \times \sum C_i \times \beta_i \quad \text{(eq. 4)}$$

where *R* is the gas constant, *T* is the absolute temperature and C_i is the concentration in the ecosystem of component *i* (*e.g.* biomass of a given taxonomic group or functional group), and β_i is a factor able to express roughly the quantity of information embedded in the biomass. β-values have previously been calculated for several organisms based upon number of coding genes (see Jørgensen, 2002). The β-values used in estimating Eco-Exergy from biomass in the present paper are provided in Table 1.

Table 1. Values for the weighting factors to estimate Eco-Exergy related to organisms biomass for different groups of organisms (*values from Jørgensen *et al*., 1995; ** values from Fonseca *et al*., 2000).

Organisms	Weighting factor (β-values)
Detritus	1**
Algae	25**
Jellyfish	30*
Sponges	30*
Annelids	50**
Insecta	70**
Crustaceans	230**
Mollusc	280**
Echinoderms	360**
Gastropods	450**
Bivalves	760**

Detritus was used as reference level, *i.e.,* β_i = 1 and Eco-Exergy in biomass of different types of organisms is expressed in detritus energy equivalents. This formulation does not correspond to the strict thermodynamic definition, but provides nevertheless an approximation of Exergy values. In this sense it was proposed to call it Eco-Exergy index (Marques *et al.,* 1997).

If the total biomass in the ecosystem remains constant, Eco-Exergy variations will rely only upon its structural complexity, and thus a Specific Eco-Exergy of the system can be defined as Eco-Exergy / total biomass (Marques *et al.,* 1997). Both Eco-Exergy and Specific Eco-Exergy have been tested as indicators in environmental assessment, being considered advisable to use them complementary (Marques *et al*., 1997, 2003; Jørgensen, 2002).

RESULTS

Variation in algal structure

The algae community in the control plots (Figure 3A) was, clearly, dominated by *Corallina elongata*, with a biomass peak in May 1999 (182 gm^{-2}) and a minimum in May 2000 (36 gm^{-2}), after a storm occurrence (April 2000) that removed a significant part of the *Corallina*'s canopy. The other red macroalgae presented a biomass oscillation between 6.7–65 gm^{-2}. The green algae presented very low values through the study period, with an exception in May 2000 (12 gm^{-2}), after the storm occurrence, indicative of a partial restart of the recolonisation process.

The succession plots were first re-colonised by the green algae *Enteromorpha compressa*, *Cladophora* sp. and *Ulva rigida*. Green algae biomass (Figure 3B) increased from the beginning of re-colonisation until May 1999 (reaching 56 gm^{-2}), when an accentuated decrease occurred. Low values (1-5.5 gm^{-2}) were registered throughout until May 2000, when another biomass peak occurred (20 gm^{-2}), after a storm event. Inversely, the red algae *Corallina elongata* evidenced a slow biomass increase until June 1999, followed by a pronounced biomass increase which took place until May 2000. *Corallina* sp. biomass peaks were observed in July 1999 (42 gm^{-2}), November 1999 (59 gm^{-2}) and March 2000 (65 gm^{-2}). The other red macroalgae showed only a slight increase throughout the study, although a pronounced biomass peak of these last ones occurred after the storm occurrence.

Variation in the macrofaunal community structure

A total of 2 637 979 individuals of 137 taxa (Table 2) were identified. In terms of abundance, the community in the control plots was dominated by different taxonomic groups according to the month considered (Figure 3C). The main taxonomic groups were Gastropoda, Oligochaeta, Polychaeta, Enoploidea and Bivalvia. Considering the biomass estimates (Figure 3E), Bivalvia and Polychaeta accounted between 72% and 92 % of the total community biomass, showing inverse temporal trends.

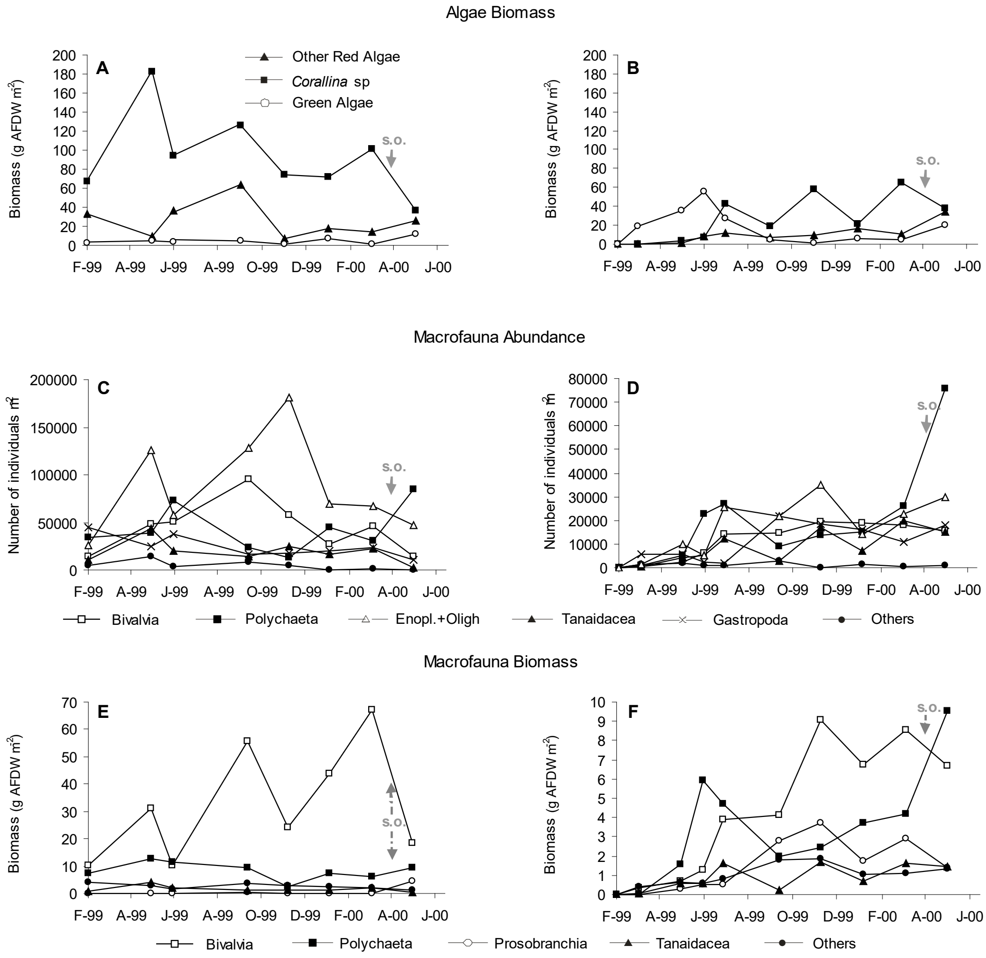


Figure 3. Changes of algae biomass, macrofauna abundance and macrofauna biomass in the control plots (A, C, E) and succession plots (B, D, F) along the studying period. s.o: storm occurrence, Enopl.+Oligh: Enoploidea+Oligochaeta.

Table 2.Taxonomic list of macrofauna species (or species groups) recorded.

- **Anthozoa**
  - Actiniaria
- **Nematoda**
  - Enoploidea
- **Diptera**
  - Orthocladinae
  - Tanytarsini
  - Rhagionidae
  - Psychodinae
- **Tanaidacea**
  - *Tanais dulonguii* (Audouin)
  - *Leptochelia savigny* (Kroyer)
- **Isopoda**
  - *Anthura gracilis* (Montagu)
  - *Paranthura costana* Bate & Westwood
  - *Limnoria lignorum* (Rathke)
  - *Cymodoce truncate* Leach
  - *Dynamene edwardsi* Lucas
  - *Dynamene magnitorata* Holdich
  - *Campecopea hirsuta* (Montagu)
  - *Ischyromene lacazei* Racovitza
  - *Idotea pelagica* (Leach)
  - *Idotea* sp
- **Amphipoda**
  - *Caprella acanthifera* Leach
  - *Caprella penantis* Leach
  - *Ampelisca rubella* A. Costa
  - *Amphilochus brunneus* Della Valle
  - *Ampithoe helleri* Karaman
  - *Lembos websteri* Bate
  - *Microdeutopus chelifer* (Bate)
  - *Apherusa jurinei* (Milne-Edwards)
  - *Dexamine spiniventris* (A. Costa)
  - *Guernea coalita* (Norman)
  - *Photis* sp
  - *Melita obtusata* (Montagu)
  - *Podocerus variegatus* Leach
  - *Stenothoe monoculoides* (Montagu)
  - *Hyale stebbingi* Chevreux
- **Decapoda**
  - *Pachygrapsus marmoratus* (Fabricius)
  - *Brachyura* sp
  - *Pirimela denticulata* (Montagu)
- **Pantopoda**
  - *Callipallene emaciata* (Dohrn)
  - *Anoplodactylus virescens* (Hodge)
- **Arachnida**
  - Arachnida sp1
  - Arachnida sp2
  - Halacaridae
- **Polyplacophora**
  - *Lepidochitona cinerea* (L.)
  - *Lepidochitona corrugata* (Reeve)
  - *Acanthochitonia crinita* (Pennant)
- **Echinodermata**
  - *Asterina gibbosa* (Pennant)
  - *Amphipholis squamata* (DelleChiaje)
  - *Paracentratus lividus* (Lamarck)
  - Holothuroidea
- **Gastropoda**
  - *Patella ulyssiponensis* Gmelin
  - *Tectura tessulata* (Müller)
  - *Tricolia pullus* (L.)
  - *Tricolia tingitana* Gofas
  - *Gibulla umbilicalis* (Da Costa)
  - *Gibulla cineraria* (L.)
  - *Calliostoma zizyphinum* (L.)
  - *Bittium simplex* (Jeffreys)
  - *Littorina neritoides* (L.)
  - *Littorina neglecta* (Bean)
  - *Skeneopsis planorbis* (Fabricius)
  - *Eatonina fulgida* (Adams)
  - *Rissoa parva* (Da Costa)
  - *Alvania semistriata* (Montagu)
  - *Setia pulcherrima* (Jeffreys)
  - *Barleeia unifasciata* (Montagu)
  - Eulimidae sp
  - *Ocinebrina edwardsii* (Payraudeau)
  - Buccininae sp
  - *Rissoela glabra* (Alder)
  - *Rissoela opalina* (Jeffreys)
  - *Rissoela globularis*
  - *Omalogyra atomus* (Philippi)
  - *Ammonicera rota* (Forbes & Hanley)
  - *Odostomia* sp
  - *Odostomia eulimoides* Hanley
  - Gstropoda sp1
  - Gastropoda sp2
  - *Runcina coronata* Quatrefages
  - *Aplysia punctata* (Cuvier)
- **Opistobranchia**
- **Bivalvia**
  - *Mytilus galloprovincialis* Lamarck
  - *Musculus costulatus* Risso
  - *Mytilaster minimus* (Poli)
  - *Modiolaria sulcata* Deshayes
  - *Hiatella arctica* (L.)
  - *Irus irus* (L.)
  - *Venerupsis* sp
  - *Turtonia minuta* (Fabricius)
  - *Parvicardium ovale* (Sowerby)
  - *Lasaea rubra* (Montagu)
  - *Cardita calyculata* (L.)
  - Bivalvia sp1
  - Bivalvia sp2
- **Oligochaeta**
- **Sipuncula**
- **Nemertina**

Table 2. (Continued)

Polychaeta
Eteone picta (Quatrefages)
Eulalia viridis (L.)
Eulalia mustela Pleijei
Perinereis cultrifera (Grube)
Platynereis dumerilii (Audouin & Edwards)
Autolytus benazzi Cognetti
Brania pusilla (Dujardin)
Pseudobrania yraidae San Martin
Sphaerosyllis taylori Perkins
Exogone naidina Oersted
Ehlersia ferrugina Langerhans
Syllis garciai (Campoy)
Syllis gracilis Grube
Syllis mediterranea (Bem- Eliahu)
Syllis truncata criptica Bem- Eliahu
Odontosyllis ctenostoma Claparède
Syllides edentatus (Westheide)
Pholoe synophthalmica (Fauvel)
Lysidice ninetta Audouin & Edwards
Lumbrineris latreilli Audouin & Edwards
Lumbrineris tetraura (Schmarda)
Protoaricia oerstedi (Claparède)
Boccardia polybranchia (Haswell)
Polydora flava Claparède
Polydora hoplura Claparède
Pseudopolydora pulchra (Carazzi)
Caulleriella spp
Cirratulus cirratus (Müller)
Cirratulus chrysoderma Claparède
Cirriformia sp
Dodecaceria concharum Oersted
Cirratulidae sp
Capitella spp
Arenicolides grubii Langerhans
Maldanidae sp
Sabellaria alveolata (L.)
Polycirrus sp
Fabricia sabella (Ehrenberg)
Sabellidae spp
Pomatoceros lamarcki (Quatrefages)
Polychaeta sp
Nematoda
Enoploidea

Bivalvia reached the highest biomass value in March 2000 and the minimum in June 1999, while Polychaeta biomass presented a peak in June 2000 and rather low values in November 1999 and March 2000. Regarding the community abundance in the succession plots (Figure 3D), during the first 3 months Gastropoda was the dominant taxonomic group, but subsequently its density decreased. In September 1999 a new peak of abundance occurred, declining afterwards until the end of the study period. Amphipoda, Diptera and Isopoda (included in the Others group) were also abundant at the very beginning of the community recovery (March 1999), but showed only residual values during the rest of the study period. Polychaeta was the prevailing group from June to July 1999 and from March 2000 to May 2000. Bivalvia, Enoploidea, Oligochaeta and Tanaidacea were also important groups in structuring the community during the whole recovery process. Regarding the biomass estimation (Figure 3F), Bivalvia was already leading during the first month of recovery. Although relatively less important in general, the Others group, specifically, Amphipoda, Diptera and Isopoda presented the highest values during this period. The other taxonomic groups aggregated in the Others

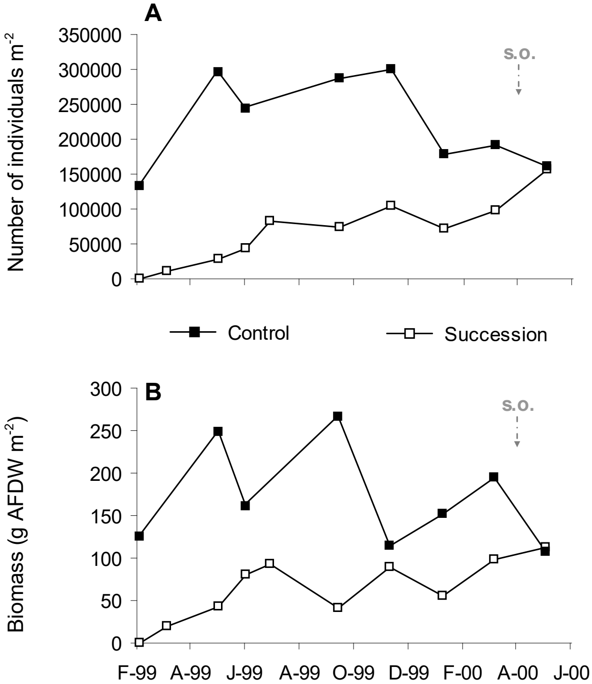


Figure 4. Comparison of temporal changes between succession plots and control plots. (A) macrofauna abundance (B) total (macrofauna+macroalgae) biomass. s.o: storm occurrence.

group, such as Decapoda, Anthozoa and Polyplacophora occurred sporadically with values below 2% of the community biomass. Polychaeta was the dominant taxonomic group from May 1999 to July 1999, being replaced by Bivalvia from September 1999 to March 2000, when Polychaeta became dominant again.

Recovery of the community as a whole

Data on macrofauna total abundance (Figure 4A) and total biomass (macrofauna and macroalgae) (Figure 4B) of both communities tend to converge by the end of the study period. Both in terms of biomass and abundances, the convergence of the recovering community, with the surrounding community is illustrated in MDS plots (Figure 5). In both cases, MDS bi-dimensional plots are associated with values of stress that fall into the categories of “good” and “excellent” representation or ordination, respectively (Clarke, 1993).

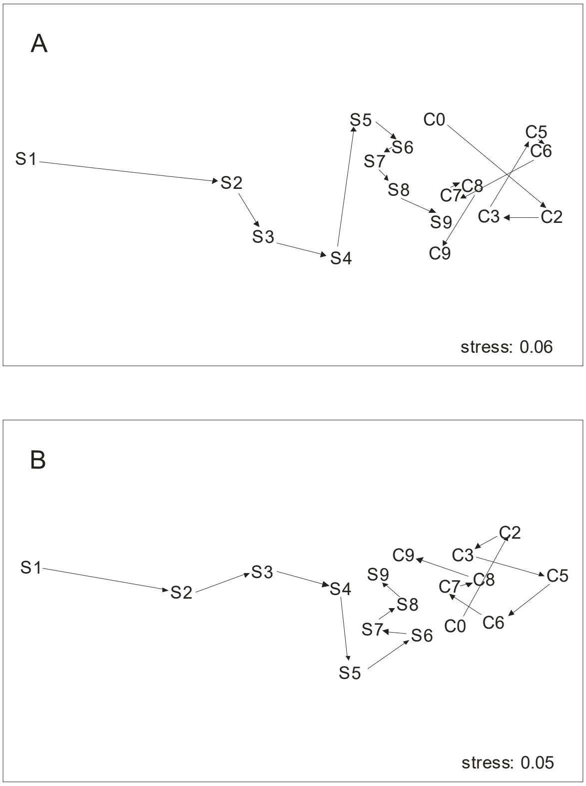


Figure 5. Two-dimensional non-metric multidimensional scaling (nMDS) ordinations comparing communities: control plots (C) and succession plots (S), regarding (A) macrofauna abundance and (B) macrofauna + macroalgae biomass data.

Ecological indicators performance

How did the different ecological indicators capture the recovery process? The variation of ecological indicators values in both communities over time is illustrated in Figure 6. In general, the indicators based in species richness and evenness presented higher values in the control plots until September 1999, but a shift is then recognisable. In fact, from September 1999 up to the end of the study period, the experimental plots representing the recovering community presented higher values. A more detailed analysis shows that the control community presented higher values of Pielou's evenness in February 1999, June 1999 and March 2000 (Figure 6B). Furthermore, the values of Margalef's index (Figure 6C) were always higher at the recovery community, except in the very beginning of the experiment, when the control assemblage, logically, exhibited higher values (7.42). Accordingly, the Shannon-Wiener index (Figure 6D) was higher at the recovering plots from September 1999. Nevertheless, these results must be examined cautiously, given that the differences between the control and the succession plots appear to be too small to be considered significant.

With regard to the Eco-Exergy index (Figure 6E), values estimated for the experimental plots gradually increased, and converged towards those observed in the control community by the end of the study period. Finally, the Specific Eco-Exergy index (Figure 6F) attained similar values both in the experimental and control communities after only 1 month of recovery, therefore expressing a more or less analogous structural complexity in both assemblages. Nevertheless, this index showed always slightly lower values in the community under recovery.

DISCUSSION

What does grow first during the community recovery succession? At least in theory, all ecological indicators accounting for the composition and abundance of biological communities might be useful in detecting the environmental situation of an ecosystem. However, since many were in practice developed to approach the characteristics of a specific ecosystem, they often lack generality. Others have been criticised or rejected due to their dependence on specific environmental parameters, or because of their unpredictable behaviour depending on the type of environmental stress. Therefore, it is not recommendable to use a single ecological indicator to assess something as complex as the recovery process of a system. Instead, different aspects must be taken into account and if possible combined.

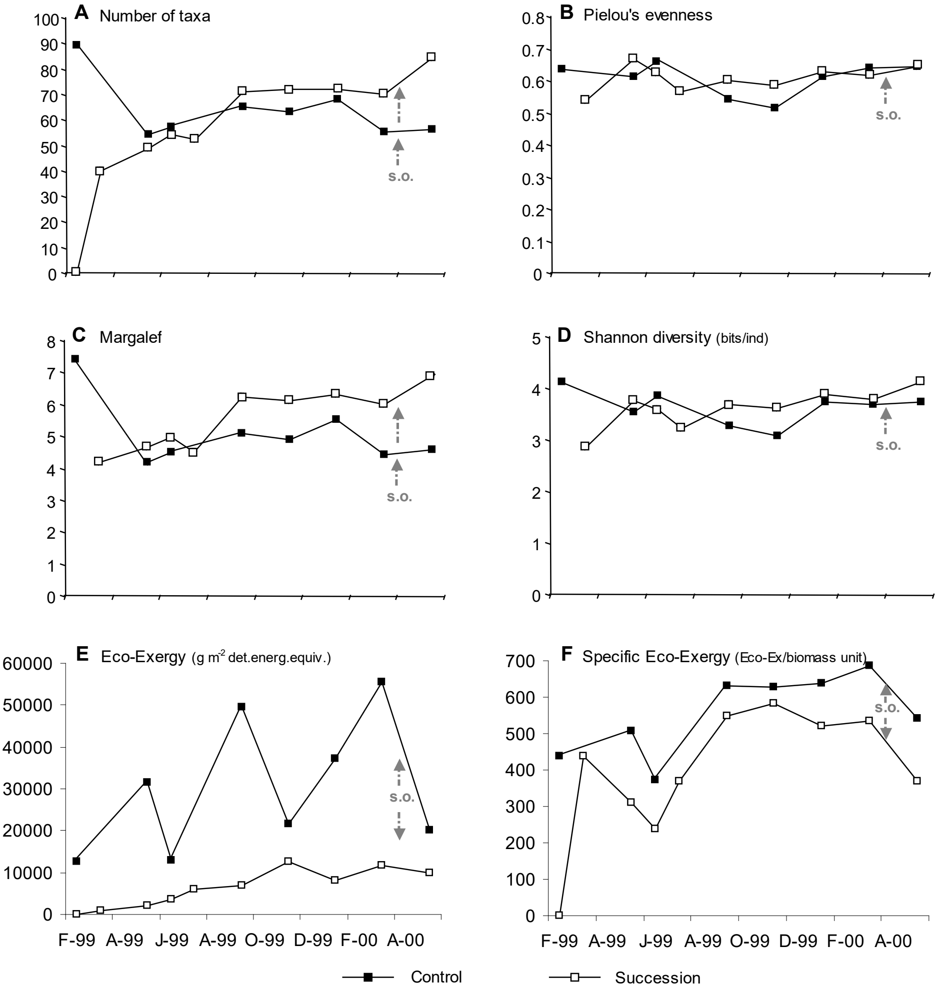


Figure 6. Variation of (A) Number of taxa (B) Pielou's Evenness (C) Margalef (D) Shannon diversity (E) Eco-Exergy and (F) Specific Eco-Exergy ecological indicators in control and succession plots, from February 1999 to May 2000. s.o: storm occurrence.

It is commonly accepted that early colonisers tend to be rapid growing, opportunistic, r-selected species, and more palatable for consumers (Valiela, 1995). This pattern was in fact clearly recognisable at the beginning of the experiment when the bare surface was firstly re-colonised by green algae. Then, these early colonisers were replaced, firstly by red macroalgae, which also occur as early successional species, and finally by the turfing algae *Corallina elongata*. This shift in algal species in the community was also followed by changes in the associated macrofauna. Some groups as Amphipoda, Isopoda, Diptera, and Gastropoda presented high abundances only in the very beginning of the recovery process, being subsequently replaced by Bivalvia and Polychaeta.

In general, it is also known that as succession proceeds, the species, in most cases tend to be larger, grow more slowly, be less productive and have more complex morphology and special requirements. As time goes on, more species accrue, and diversity increases as a result of spatial heterogeneity. In fact, the species richness not only increased throughout the recovery period, but also became consistently higher than in the control community after September 1999. Not surprisingly, the Margalef's index behaviour mirrored that of species number. Another category of diversity indexes combines the richness of species with a measure of their relative abundance, and includes the widely used Shannon-Wiener index (H'). The Shannon index was originally used in Information Theory, but it has been commonly employed to evaluate species diversity in ecological communities. Again not surprisingly, in our experiment, the Shannon-Wienner index and Pielou's evenness presented a parallel behaviour.

Two attractive ideas emerge from these observations. First, diversity increased rather rapidly. After approximately 6 months, succession plots came to resemble those of the surrounds concerning the information related to the number of species present. Additionally, species composition appeared also to be similar in both communities. This latter observation is also quite acceptable once patch size is known to exert influence on colonisation mechanisms (*e.g.* Kim & DeWreede, 1996). It has in fact been observed that after large-scale disturbances, plankton larvae are an important source of colonists, while after small disturbances, on the scale of cm or m, like in the present case, re-colonisation is often carried out by post-larvae and mobile adults from the adjacent assemblages (Levin *et al.*, 1996).

Second, after September 1999, diversity was consistently higher in the community undertaking succession. Considering the plots clearance as a small-scale but harsh disturbance event, after the first algae settlement, re-colonisation was mainly achieved by invertebrates' post-larvae and adults from the undisturbed community. Therefore, after 6-7 months, although effects of disturbance were still visible in the recovering communities, they were clearly becoming less evident.

On the other hand, our observations after September 1999 appear to illustrate in very interesting and unexpected way a case explainable by the Intermediate Disturbance Hypothesis (Grime, 1973; Connell, 1978). This hypothesis predicts that highest diversity values will be found at intermediate levels of disturbance. If the disturbance is too mild or too rare, patches will approach equilibrium and be dominated by a few species that are able to out-compete all others. If disturbance is too harsh or too frequent, then only a few species that are resistant to the disruption will persist.

In terms of biomass, the control community was, undoubtedly, dominated by Bivalvia (Mytilacea) (Figure 3E). Moreover, the other species only showed a slight recovery, after a severe storm occurrence that removed large quantities of *Corallina enlongata* (late stage primary producer) and wash way loads of associated organisms, in April 2000. Paine & Levin (1981) had already mentioned that, particularly in rocky shores, potential sources of disturbance could be herbivores, waves and wave-driven rocks.

Furthermore, growth can be interpreted as an increase in the organisation of ordered structure or information (Marques & Jørgensen, 2002), although more commonly, in practical terms, growth is expressed as the increase of measurable quantities, most often biomass and diversity. Nevertheless, Jørgensen *et al.* (2000) considered 3 forms of growth, respectively, growth-to-storage (Form I), growth-to-throughflow (Form II), and growth-to-organisation

(Form III), which when applied to our case can be considered as corresponding to biomass, network and information. Regarding these three forms of growth, Jørgensen *et al.* (2000) hypothesised that in ecological succession, energy storage in early stages is dominated by Form I growth which builds structure: the dominant mechanisms are increasing energy capture and low entropy production. In middle stages, growing interconnection of proliferating storage units (organisms) increases energy throughflow (Form II growth) and, finally, in mature phases, cycling becomes a dominant feature of the internal network, reflecting advanced organisation (Form III growth).

Jørgensen & Mejer (1979) also proposed Eco-Exergy storage, mathematically defined by both conservative (energy and matter) and non-conservative (informational) terms, as a measure of complexity, hypothesising: a) that complexity in ecosystems is associated to the presence of more complex organisms, corresponding to higher information content, and b) that ecosystems development drives them to optimise the Eco-Exergy storage levels under given environmental circumstances and with the available genetic pool. Stored Eco-Exergy expresses the distance from thermodynamic equilibrium, and reflects the size of the organised structure in terms of its content in thermodynamic information (Jørgensen, 2002).

The variation trend of the Eco-Exergy index in experimental plots appeared to reflect, essentially, changes in biomass but not in information. Rates of convergence are known to vary from shore to shore and differ from time to time (Chapman & Underwood, 1998). In our experiment, although after 7-8 months the succession plots resembled the surrounds in terms of structure (Figure 4B and Figure 5B), even after 15 months biomass had not still reached the levels of the control plots. Particularly, the algae biomass was still at a considerable lower level in the recovering community. On the other hand, taking into account Specific Eco-Exergy, or average organism complexity (an average β-value), the community at the experimental plots has rapidly recovered in terms of information. In fact, Specific Eco-Exergy, after only 1 month of experiment, showed already similar values in the succession and control plots, suggesting therefore an analogous structural complexity in both assemblages.

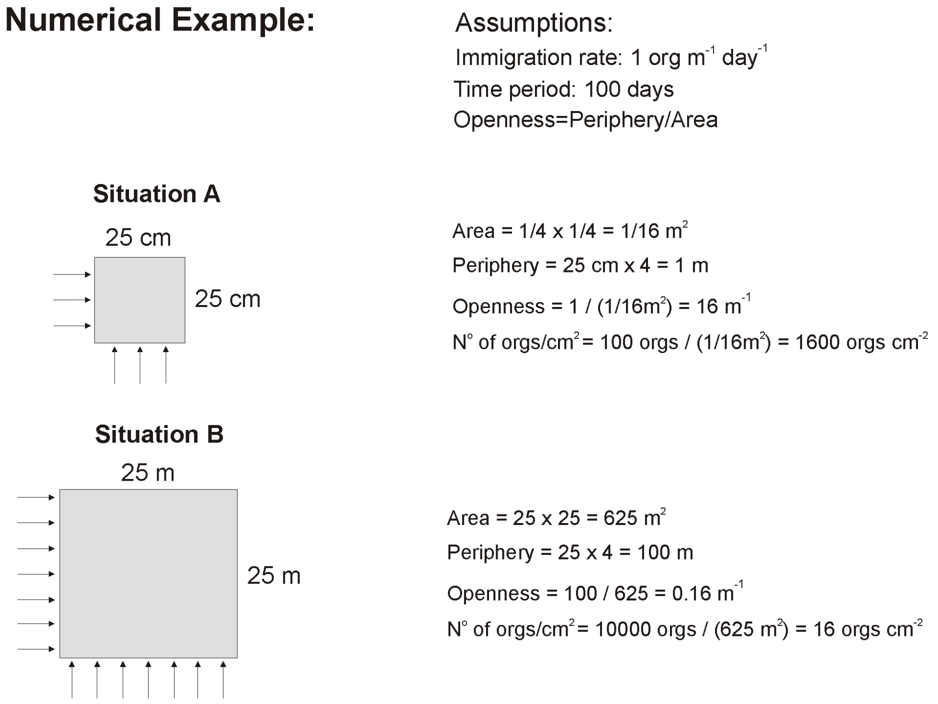
Thus, the system information appears to have recovered much faster than biomass.

A problem in applying Eco-Exergy based indices is the obvious lack of discriminating power of the weighting factors used to estimate Eco-Exergy and Specific Eco-Exergy, because organisms are considered at very high taxonomic levels. A new updated set of *β*-values, resulting from a more refined calculation methodology, will soon be available (Jørgensen *et al.*, 2005a). Nevertheless, with regard to the forthcoming weighting factors, although values are different in absolute terms, the ratio between them is similar to the ratio between the *β*-values used in this study. On the other hand, despite significant methodological progresses, in practical terms an extensive work will still be necessary to improve the discriminating power at low taxonomic levels. Therefore, the problem of estimating the *β*-values still constitutes a weak point, which will be gradually solved in the future as our knowledge about genes and their active expression increases (Fonseca *et al.*, 2000; Marques & Jørgensen, 2002). Nevertheless, despite this problem, both thermodynamic oriented indices provided useful information about the structural development of the community.

The answer to the question, what does grow first during the community succession: biomass, network or information? was to a certain extent elucidated with the help of different ecological indicators. In our experiment, contrarily to what we hypothesised based on Odum (1969), the system information (expressed by Specific Eco-Exergy) recovered rather quickly (despite still presenting lower levels in the community under recovery), closely followed by the network interactions (considering species diversity an indirect indicator of network complexity), while even by the end of the study period biomass remained lower than in the control community. Also, contrarily to what was hypothesised by Jørgensen *et al.* (2000), in our study, growth-to-organisation and growth-to-throughflow dominated the early stages of the recovery process, while growth-to-storage increased in importance as maturity approached. Nevertheless, it seems reasonable to assume that this result was related to the scale of the experiment. In fact, the cleared plots were very small in comparison with the surrounding *Corallina* sp. algal community. Due to this fact, although the re-colonisation by primary producers followed the pattern usually described in the literature, the macrofauna found at each date probably consisted not only of the species usually found in the succession, but also of other ones proceeding from the undisturbed algal cover, that carried short incursions into the small experimental plots. In such case, biomass development was probably mostly dependent on the primary producers' growth, while complexity assessment was strongly affected by these invertebrates' incursions.

Consequently, a tentative generalisation of our experimental results could be: The characteristics of a systems' recovery after disturbance appear to be dependent on the spatial scale of the disturbance according to the following pattern: a) If a disturbed area is small when compared to a contiguous non disturbed one, complexity (information and network) will recover prior to biomass; b) If a disturbed area is large in comparison to a contiguous non disturbed one, biomass will recover in first place, and complexity will gradually develop afterwards.

The abovementioned considerations may be further explored if the concept of **openness** introduced by Jørgensen (2000) is considered. The initial premise that an ecosystem must be open or at least non-isolated to be able to import the energy needed for its maintenance, is easily accepted. Furthermore, openness is here expressed as the ratio of periphery (or perimeter) to area. Figure 7 illustrates a numerical example where is clearly demonstrated that small plots compared with big cleared areas present higher values of openness. As a consequence, these small areas express higher possibility to exchange energy or matter and increased chance for immigration of organisms. In this experiment, complexity (information and network) did, indeed, recover prior to biomass, fact that is reasonable since information and network are more dependent of openness. Therefore, the higher the openness value the faster is information and network recovery. On the other hand, biomass is less dependent because the major biomass contribution is coming from slow growing species, leading this growth form to be the last one to recover. Although Debeljak (2002) examining managed and virgin forest in different development stages (*e.g.* pasture, gap, juvenile, optimum forest) has confirmed



Numerical Example:

Assumptions:
Immigration rate: 1 org m^{-1} day^{-1}
Time period: 100 days
Openness=Periphery/Area

Situation A

Area = 1/4 x 1/4 = 1/16 m^2
Periphery = 25 cm x 4 = 1 m
Openness = 1 / (1/16m^2) = 16 m^{-1}
N° of orgs/cm^2 = 100 orgs / (1/16m^2) = 1600 orgs cm^{-2}

Situation B

Area = 25 x 25 = 625 m^2
Periphery = 25 x 4 = 100 m
Openness = 100 / 625 = 0.16 m^{-1}
N° of orgs/cm^2 = 10000 orgs / (625 m^2) = 16 orgs cm^{-2}

Figure 7. Numerical example: openness calculation in situation A (25cm x 25cm plots) and situation B (25m x 25m plots). orgs: organisms.

Jørgensen *et al*. (2000) development hypothesis, the present study results stressed how openness can shape and modify the sequence of ecosystem development.

Were the chosen ecological indicators able to help in recognising the growth of biomass, network and information, throughout the recovery process? The answer to this question is obviously implicit in the discussion above: Yes, when applied in combination, almost not if used in isolation. In fact, diversity measures are obviously not suitable to capture the first form of growth (biomass), although they can provide useful hints regarding the other two (network and information). On the other hand, Eco-Exergy and Specific Eco-Exergy provided useful information about the structural development of the community but lacked discriminating power with regard to the informational status of the system.

ACKNOWLEDGMENTS

The present study was carried out in the overlapping region of the research projects DYNAMOD (POCTI/MGS/37431/2001), MEDCORE (ICA3-2001-10028), and TICOR, funded by INAG - Instituto da Água (Portugal). It was also supported by FCT (Portuguese National Board of Scientific Research) through a grant SFRH/BD/820/2000.

CHAPTER 5

Do Taxonomic Distinctness measures accord with other ecological indicators in assessing ecological status?

ABSTRACT

Assessing the ecological status, a concept implemented in the European Water Framework Directive (EC, 2000), requires the application of methods capable of distinguishing different levels of ecological quality. Somerfield *et al.* (2003) proposed Average Taxonomic Distinctness to be used as tool in this context. We tested the robustness of Taxonomic Distinctness measures applying it in different scenarios (estuarine eutrophication, organic pollution, and re-colonisation after physical disturbance), analysing simultaneously its correlation with to other types of ecological indicators. Results showed that, in most of the case studies, only Total Taxonomic Distinctness was relatively satisfactory in discriminating between disturbed situations. Other Taxonomic Distinctness measures were not proven to be more sensitive than other ecological indicators (Shannon-Wiener, Margalef, and Eco-Exergy indices). Therefore, this approach does not seem to be particularly helpful in assessing systems' ecological status with regard to the WFD implementation.

KEYWORDS: Taxonomic Distinctness measures, Diversity, Eco-Exergy, Specific Eco-Exergy, ecological status, European Water Framework Directive.

INTRODUCTION

The Water Framework Directive (EC, 2000) establishes a framework for the protection of all waters (including inland surface waters, transitional waters, coastal waters, and groundwater), aiming at achieving a good quality status for all waters by the year 2015. The concept of ecological status developed in the WFD is defined in terms of the quality of the biological community, as well as the systems' hydrological and chemical characteristics. Applying it requires methods capable of distinguishing different levels of ecological quality to classify surface water areas. Moreover, the concept of ecological status implies that in the absence of a comprehensive knowledge of all the pressures on a water body and of their combined biological effects, it will always be necessary to get direct measures with regard to the condition of the biological quality elements. This must be achieved, namely, by using biological indicators, in order to validate any biological impacts suggested by non-biological indicators. Therefore, the WFD highlights the importance of measures able to elucidate the biological effects of disturbance.

At least in theory, all ecological indicators accounting for the composition and abundance of biological communities might be useful in detecting the environmental situation of an ecosystem. Costello *et al*. (2004) surveyed the frequency of using diversity indices in the scientific literature and found that the most widely used and popular measure of diversity is species richness (*e.g.* number of species, Margalef index), immediately followed by the Shannon-Wiener and evenness indices. Although, highly applied, some authors (Warwick & Clarke, 1998; Wilkinson, 1999, Rogers *et al.* 1999; Gray, 2000) point out that these measures can be highly influenced by different sample sizes, sampling effort, habitat type or complexity, and do not show monotonic behaviour in response to environmental degradation. Finally, the Convention on Biological Diversity (UNEP, 1992) argues that biodiversity cannot be regarded as just the number of species in an area or measured by an index of their relative abundance. In the Rio declaration, biodiversity was defined as "the variability among living organisms including, for example, terrestrial, marine and other ecosystems and the ecological complexes to which they belong: this includes diversity within species, between species and diversity of ecosystems". Almost all of the components of natural systems are therefore included in this definition of biodiversity. This broad definition resulted in many different interpretations of the biodiversity concept when put into practice. According to Van der Spoel (1994), biodiversity must be considered as "the sum of taxonomic or numerical diversity, and the ecological, genetic, historical and phylogenetic diversity. In this context, Warwick & Clarke (1995) proposed that Taxonomic Distinctness measures (ecological indicators that are based on the species abundance and also on the taxonomic distances, through the classification tree, between every two pair of individuals) should incorporate more of this information than species richness measures. Although there were some attempts to use Taxonomic Distinctness measures more widely in the marine field (Somerfield *et al.,* 1997; Hall & Greenstreet, 1998; Rogers *et al*, 1999; Clarke & Warwick, 2001, Warwick & Light, 2002), these measures still need a wider testing and should be investigated, as these indices are likely to add complementary information value to existing measures of biodiversity (Costello *et al.*, 2004). In this paper we test the robustness of Taxonomic Distinctness measures applying it in different scenarios (estuarine eutrophication, organic pollution, and re-colonisation after physical disturbance) and, simultaneously, analysing its correlation with other types of ecological indicators (Shannon-Wiener, Margalef and Eco-Exergy indices). The aim was to evaluate their effectiveness as pollution/disturbance biological indicator with regard to the European Union Water Framework Directive implementation.

MATERIAL AND METHODS

Case studies and data source

Mondego estuary (Portugal)

The Mondego estuary (Figure 1A) is under severe environmental stress, and an ongoing eutrophication process has been monitored during the last decade. A detailed description of the characteristics of the system can be found in the available literature (*e.g.* Marques *et al*., 1997, 2003; Pardal *et al*., 2000, 2004; Martins *et al*., 2001; Cardoso *et al*., 2004).

Two different data sets were selected: a) subtidal communities and b) intertidal communities. The first set was provided by a study on the sub-tidal soft bottom communities, which characterised the whole system with regard to species composition and abundance, taking into account its spatial distribution in relation to the physicochemical factors of water and sediments. The infaunal benthic macrofauna was sampled using a van Veen grab (496 cm^2) during spring, in 1990, 1992, 1998 and 2000, at 14 stations (E1 to E14) (5 replicates in each station) covering the whole system (Figure 1A). The second set proceeded from a study on the intertidal benthic communities carried out from February 1993 to February 1994 in the south arm of the estuary (Figure 1A). Samples of macrophytes, macroalgae and associated macrofauna, as well as samples of water and sediments, were taken fortnightly, with a manual core (141 cm^2 to a depth of 15 cm), during low tide, at three areas, representing different conditions along a spatial gradient of eutrophication symptoms (Figure 1A): A non-eutrophic area (*Zostera noltii* beds), an intermediate eutrophic area (*Zostera noltii* absent, although residual roots can still be found in the sediment) and a strongly eutrophic area (macrophyte community totally absent with *Ulva* sp. blooms regularly observed). In both studies, samples were preserved in 4% buffered formalin and sieved through a 1 mm mesh (subtidal community) and 500 µm mesh (intertidal communities). Organisms were identified to the species level and their biomass was determined ($g.m^{-2}$ AFDW). Corresponding to each biological sample the following environmental factors were determined: salinity, pH, silica temperature, dissolved oxygen, chlorophyll *a*, ammonia, nitrates, nitrites, and phosphates in water, and organic matter content in sediments.

Mar Menor (Spain)

The Mar Menor is a coastal lagoon with an area of 135 Km^2. The lagoon is connected to the Mediterranean at some points by channels through which the water exchange takes place with the open sea (Figure 1B).

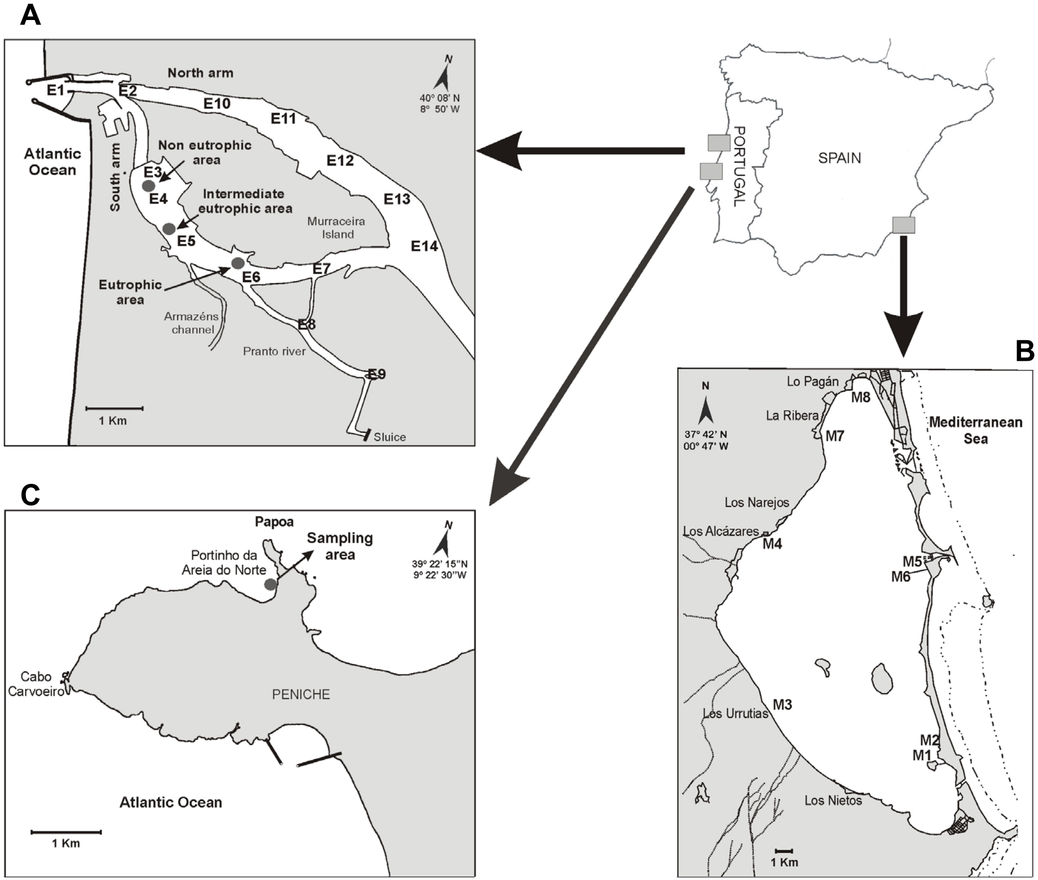


Figure 1. Case studies location. A: Mondego estuary (Portugal), B: Mar Menor (Spain) and C: Papoa (Portugal) and sample stations.

This coastal lagoon presents an environmental heterogeneity with different types of pollution sources. A detailed description of the system and of the effects of the main environmental impacts to which it is subject can be found in the available literature (*e.g.* Pérez-Ruzafa *et al*., 1987, 1989, 2000, 2005; Ros *et al*., 1987; Barcala, 1999).

Data from Pérez-Ruzafa *et al*. (1989), consisting of a complete characterization of the benthic populations in the lagoon, were used. Eight sampling stations were located on both rocky and soft bottoms along the lagoon at sites representative of the different biocenosis and main polluted areas (Figure 1B): rocky bottom stations M7 and M8 (urban direct dumping with the development of nitrophyle communities dominated by *Ulva* spp); rocky bottom stations M1 and M5 (not affected by organic enrichment); stations M2 and M6 (with high levels of organic matter in the sediment coming from the primary production of the macrophyte meadows (*Caulerpa prolifera*) that was introduced in the lagoon as a result of the dredging in one of the channels at the beginning of the 1970s, growing rapidly around the whole lagoon (Pérez-Ruzafa *et al*., 1991), leading to an increment of the organic matter in the sediment, which had important effects in the biological communities, namely a general impoverishment with regard to macrofauna) and, finally, stations M3 and M4 located in soft bottom zones with low input of organic matter (<1%).

Samples were taken seasonally (A: July, B: November, C: February, D: May), which allowed evaluating the influence of seasonal variations on the performance of different ecological indices. Divers collected biological samples from both soft bottoms and rocky areas, moving along transepts perpendicular to the coastline and also in precise spots corresponding to the eight sampling stations. Each sample corresponded to 400 cm^2. Afterwards, samples were sieved through a 500 µm mesh and preserved in 4% buffered formalin in seawater. Organisms were identified to the species level and biomass was determined ($g.m^{-2}$ AFDW). The environmental factors measured were salinity, temperature, pH, and dissolved oxygen, as well as sediment particles size, organic matter and heavy metal contents.

Papoa (Portugal)

Data used are the outcome of a re-colonisation field experiment carried out from February 1999 to May 2000 in a small beach, Papoa, on the Western Coast of Portugal (Figure 1C), where the intertidal community is dominated by the turfing algae *Corallina elongata* which forms a stiff matrix that helds some sediment (Patrício *et al.*, in press). Other red macroalgae (*e.g. Chondria* sp., *Chondrachantus* sp., *Gigartina* sp. and *Laurencia* sp.) and green macroalgae (*Cladophora* sp. and *Ulva* sp.) also occur as epiphytes or as early successional species. In February 1999, quadrates of 25 x 25 cm (625 cm^2) were randomly distributed across the study area. During the preparation phase, 27 discrete areas were created by totally removing macroalgae and the associated macrofauna with a chisel. Other plots were assigned as controls, being left undisturbed at this stage of the experiment. Subsequently, both experimental plots (3 replicates) and control plots were sampled, during low tide, every one or two months, until May 2000. Samples were preserved in 4% buffered formalin in seawater and sieved through a 500 µm mesh. Later, algae and associated macrofauna were separated, identified to the species level and biomass ($g.m^{-2}$ AFDW) assessed after combustion of samples for 8h at 450ºC.

Data analysis

Taxonomic Distinctness measurements

To estimate Taxonomic Diversity indices, a hierarchical Linnean classification was used as a proxy for cladograms representing the relatedness of individual species. For each location, a composite taxonomy was compiled and five taxonomic levels were considered (species, genus, family, order, class and phylum) and five diversity indices (Taxonomic Diversity, Δ; Taxonomic Distinctness, Δ^*; Average Taxonomic Distinctness based on presence/absence of species Δ^+; Variation in Taxonomic Distinctness Λ^+ and Total Taxonomic Distinctness, $s\Delta^+$) defined by Clarke & Warwick (1998, 2001) were then calculated from macrofauna abundances, using PRIMER 5 (Software package from Plymouth Marine Laboratory, UK):

$$\Delta = \left[\sum\sum_{i<j} \omega_{ij} x_i x_j \right] / \left[n(n-1)/2 \right] \qquad \text{(eq. 1)}$$

$$\Delta^{*} = \left[\sum\sum_{i<j} \omega_{ij} x_i x_j \right] / \left[\sum\sum_{i<j} x_i x_j \right] \qquad \text{(eq. 2)}$$

$$\Delta^{+} = \left[\sum\sum_{i<j} \omega_{ij} \right] / \left[s(s-1)/2 \right] \qquad \text{(eq. 3)}$$

$$\Lambda^{+} = \left[\sum\sum_{i \neq j} \left(\omega_{ij} - \overline{\omega} \right)^2 \right] / \left[s(s-1) \right] \qquad \text{(eq. 4)}$$

$$s\Delta^{+} = \sum_i \left[\left(\sum_{i \neq j} \omega_{ij} \right) / (s-1) \right] \qquad \text{(eq. 5)}$$

where x_i represents the abundance of the i *th* of s species observed, $n\left(= \sum_i x_i\right)$ is the total number of individuals in the sample and ω_{ij} is the "distinctness weight" given to the path length linking species i and j in the taxonomy. Taxonomic Diversity (eq. 1) can be thought of as the average path length between two randomly chosen individuals from the sample (including individuals of the same species), whereas Taxonomic Distinctness (eq. 2) is the average path length between two randomly chosen individuals, conditional on them being from different species (Rogers *et al.*, 1999). From data consisting only of presence or absence of species (*i.e.,* species list), a simpler form of Taxonomic Distinctness (eq. 3), can be thought of as the average length between any two randomly chosen species present in the sample. The degree to each certain taxa are over- or under-represented in samples is another biodiversity attribute of ecological relevance and it is reflected by the Variation in Taxonomic Distinctness (eq. 4). Finally, Total Taxonomic Distinctness (eq. 5) was proposed by Clarke & Warwick as a useful measure of total taxonomic breadth of an assemblage, as a modification of species richness, which allows for the species inter-relatedness.

Diversity measures

The Shannon-Wiener (eq. 6) and Margalef indices (eq. 7) were applied.

$$H' = -\sum p_i Log_2 p_i \quad \text{(eq. 6)}$$

$$D = (S-1)/Log(N) \quad \text{(eq. 7)}$$

where p_i is the proportion of abundance of species i in a community were species proportions are $p_i, p_2, p_3 \ldots p_n$, S is the number of species found and N is the total number of individuals.

Eco-Exergy and Specific Eco-Exergy

Eco-Exergy (eq. 8), a concept derived from Thermodynamics, was also estimated for each location. Eco-Exergy (Jørgensen & Mejer, 1979) is one of the mathematical functions that have been proposed as holistic ecological indicators in the last two decades, intending a) to express emergent properties of ecosystems arising from self-organisation processes in the run of their development, and b) to act as orientors (goal functions) in models development. Eco-Exergy, which has been tested in several studies, can be seen as a measure of the maximum amount of work that the system can perform when it is brought into thermodynamic equilibrium with its environment. If Eco-Exergy is calculated only from the chemical potentials, which are extremely dominant with regard to ecosystems, the following expression is valid with good approximation (Jørgensen, 2002):

$$Ex = RT \times \sum C_i \times \beta_i \quad \text{(eq. 8)}$$

where R is the gas constant, T is the absolute temperature and C_i is the concentration in the ecosystem of component i (*e.g.* biomass of a given taxonomic group or functional group). β_i is a factor able to express roughly the quantity of information embedded in the biomass. β-values have been previously calculated for several organisms based upon number of coding genes (see Jørgensen, 2002). Detritus was used as reference level, *i.e.,* $\beta_i = 1$ and Eco-Exergy in biomass of different types of organisms is expressed in detritus energy equivalents. This formulation does not correspond to the strict thermodynamic definition, but provides nevertheless an approximation of Exergy values. In this sense it was proposed to call it Eco-Exergy Index (Marques *et al*., 1997).

If the total biomass in the system remains constant through time, then the variation of Eco-Exergy will be a function of only the structural complexity of the biomass or, in other words, of the information embedded in the biomass, which may be called Specific Eco-Exergy (SpEx) or Eco-Exergy per unit of biomass (eq. 9). For each instant, Specific Eco-Exergy is given by:

$$SpEX = \frac{Exergy}{Total\ Biomass} \qquad \text{(eq. 9)}$$

Statistical analysis

Pearson´s correlations ($p \leq 0.05$) were estimated to evaluate the relationships between the values of the indices and environmental factors. Moreover, the values estimated for each index were submitted to a Kruskal-Wallis analysis to test their performance in detecting differences a) along spatial and temporal gradients, in the case of the Mondego estuary data set, b) between organic enriched and non organic enriched areas, in the case of Mar Menor, and c) between experimental and control plots, in the case of the Papoa beach field experiment.

RESULTS

Mondego estuary

a) Subtidal communities

Values estimated for the different indices are summarised in Table 1. It becomes clear that most of the sampling stations do not show differences when we account for the Average Taxonomic Distinctness values (Figure 2).

Table 1. Indices values in Mondego estuary (subtidal stations) in 1990, 1992, 1998 and 2000. S: Species richness; Δ: Taxonomic diversity; Δ^*:Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness (presence/absence of species); $s\Delta^+$: Total Taxonomic Distinctness; Λ^+: Variation in Taxonomic Distinctness; Eco-Ex: Eco-Exergy; Sp Eco-Ex: Specific Eco-Exergy.

	E1				E2				E3				E4				E5				E6				E7			
	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00
S	-	-	15	10	4	7	6	26	8	10	1	7	18	6	6	8	19	10	9	11	10	9	9	9	12	4	7	10
Shannon	-	-	2.85	0.87	1.56	1.74	2.45	3.45	2.56	2.94	0	2.55	3.11	2.42	1.43	2.92	1.22	2.74	2.03	2.51	0.72	1.88	1.91	1.46	1.61	1.44	1.66	2.39
Margalef	-	-	2.32	1.30	0.91	1.26	1.35	4.01	1.47	2.01	0	1.74	3.15	1.47	0.94	1.99	2.11	1.91	1.07	1.35	1.18	1.32	1.25	1.03	1.57	0.71	0.81	1.43
Δ	-	-	71.9	22.9	55.9	47.6	73.6	73.6	72.5	71.7	0	75.9	72.0	76.6	42.9	82.1	28.2	76.9	49.5	67.8	16.0	61.1	51.9	46.7	42.6	51.6	56.2	69.7
Δ^*	-	-	93.6	97.2	95.4	87.2	95.1	86.2	92.3	84.1	0	92.7	91.1	93.8	95.1	92.6	84.7	94.7	81.7	87.4	86.8	96.4	81.2	96.4	81.9	95.9	97.6	93.2
Δ^+	-	-	92.4	85.6	97.2	83.3	88.3	90.5	90.5	87.1	0	91.3	90.7	91.1	94.4	92.3	87.2	91.5	88.4	88.9	92.6	86.6	89.9	86.6	93.6	94.4	92.9	90.7
$s\Delta^+$	-	-	1386	856	388	583	442	2353	633	870	0	639	1542	547	567	738	1569	915	796	889	833	779	719	779	1030	378	650	907
Λ^+	-	-	207	390	39	476	336	296	280	579	0	255	297	217	136	208	343	205	306	309	208	476	285	445	217	154	174	266
Eco-Ex	-	-	214	3528	-	-	32	3425	-	-	6	15	-	-	7	31	-	-	33	427	-	-	15	307	-	-	311	85
Sp Eco-Ex	-	-	100	276	-	-	219	217	-	-	450	65	-	-	159	348	-	-	166	215	-	-	11	201	-	-	119	83

(cont.)

	E8				E9				E10				E11				E12				E13				E14			
	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00	90	92	98	00
S	9	8	9	9	9	3	5	5	3	5	4	10	4	8	10	4	6	0	7	2	12	4	8	3	5	3	3	7
Shannon	1.93	2.35	1.47	1.68	2.31	0.35	0.83	1.38	1.50	0.96	1.36	2.4	1.85	2.45	2.96	1.84	1.56	-	2.14	0.65	2.95	1.75	2.61	1.37	1.20	0.55	0.87	2.04
Margalef	1.25	1.18	0.98	1.15	1.37	0.38	0.72	0.8	0.77	0.72	0.89	1.53	0.95	1.38	1.99	0.9	0.99	-	1.26	0.27	1.95	0.73	1.55	0.67	0.79	0.36	0.60	1.23
Δ	47.7	72.6	40.9	47.6	62.2	10.1	24.6	42.7	63.1	25.3	47.7	44.5	59.7	69.6	74.2	67.7	39.5	-	69.0	28.5	78.7	65.3	73.6	58.9	35.4	19.3	32.6	57.1
Δ^*	84.4	95.3	85.4	78.4	85.3	99.4	96.1	91.5	93.3	81.7	95.8	59.2	100	90.1	87.5	94.1	74.1	-	95.4	100	93.7	96.3	92.6	100	88.8	99.6	100	86.2
Δ^+	90	94.1	94.6	94.1	89.8	83.3	95.0	91.7	88.9	86.7	83.3	85.2	100	90.5	86.3	97.2	76.7	-	84.4	100	88.8	94.4	89.3	100	90.0	88.9	100	89.7
$s\Delta^+$	719	752	757	752	808	250	475	367	267	433	333	852	300	724	863	389	460	-	507	200	977	378	714	300	450	267	300	628
Λ^+	304	183	140	183	313	556	114	162	247	267	278	410	0	247	374	39	289	-	425	0	304	154	322	0	233	247	0	224
Eco-Ex	-	-	72	7	-	-	3	2	-	-	21	5	-	-	3416	2	-	-	60	3	-	-	6	2	-	-	4	16
Sp Eco-Ex	-	-	180	70	-	-	146	2	-	-	123	51	-	-	230	221	-	-	59	322	-	-	202	146	-	-	222	175

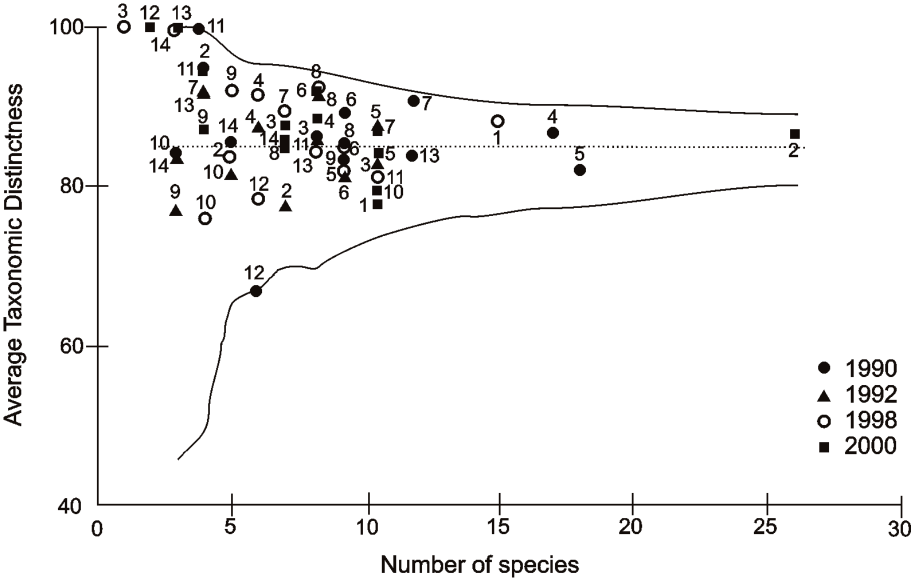


Figure 2. Confidence funnel (mean and 95% confidence interval) of the Average Taxonomic Distinctness in the Mondego estuary subtidal stations (numbers above the symbols correspond to station number) in 1990, 1992, 1998 and 2000.

In fact, even in the stations where just a few species were observed (*e.g.* E12 and E13 in 2000; E14 in 1998), the Average Taxonomic Distinctness measures present high values, and suggesting therefore high path length between species through the tree. On the contrary, the Shannon-Wiener and Margalef indices, and Total Taxonomic Distinctness (TTD) showed low values in these faunal impoverished stations, and a high correlation (r= 0.99, p<0.005) was found between the Margalef index and TTD.

None of the indices showed significant differences (p>0.05) among the various sampled years, although all of them indicate an improvement in the environmental quality status in 2000, namely in the south arm stations, which coincided with the implementation of impact mitigation practices from 1999. In spatial terms, the Margalef index and TTD were the only two indicators that clearly (p<0.05) discriminated between the north and south arms sampling stations.

Table 2. Groups obtained after the application of a Kruskal-Wallis analysis. (NEA: Non-eutrophic area; IA: Intermediate eutrophic area; EA: Eutrophic area). Total Taxonomic Distinctness ($s\Delta^+$), Margalef and Eco-Exergy indices were the ones that significantly distinguished different areas in the intertidal area of Mondego estuary ($p \leq 0.05$).

	$s\Delta^+$	Eco-Exergy	Margalef
	Average	Average	Average
Non-eutrophic area (*Zostera* sp. meadows)	2348	35048	2.3
Intermediate eutrophic area	1919	10143	2.1
Eutrophic area	1542	14893	1.6
GROUPS	1-NEA 2-IA 3-EA	1-NEA 2-IA,EA	1-NEA 2-IA, EA

b) Intertidal communities

With regard to intertidal communities along the gradient of eutrophication symptoms in the south arm of the Mondego estuary, TTD was able to discriminate between the three sampling areas, showing higher values at the *Zostera noltii* beds and lower values at the most eutrophic area (Table 2). Also, the Margalef index and Eco-Exergy index varied as theoretically expected, exhibiting higher values at the *Zostera* sp. meadows and lower values at the inner areas of the south arm, although being unable to differentiate the intermediate eutrophic area from the most heavily eutrophic one. On the contrary, the Shannon-Wiener index, the Taxonomic Diversity, the Taxonomic Distinctness and Specific Eco-Exergy indicated that environmental quality status was better at the most eutrophic area, which obviously is not in agreement with our knowledge of the system (Figure 3).

The Eco-Exergy, the TTD, and the Margalef index, all capable of discriminating the *Zostera noltii* meadows from the rest of the system, are positively and significantly correlated with the organic matter in the sediment (Table 3). Moreover, the Margalef index and Eco-Exergy are negatively correlated with the ammonium and nitrite concentration in the water column (Margalef index vs Nitrite concentration: $r=-0.25$, $p<0.05$; Eco-Exergy vs ammonium concentration: $r= -0.30$, $p<0.05$). This indicates some response to

Table 3. Pearson's correlations between values of different indices in the Mondego estuary (intertidal) and Mar Menor lagoon. (*): *p*≤0.05; (**): *p*≤0.001

Mondego estuary			
	Organic matter	Salinity	Silt (%)
Eco-Exergy	-0.62*	-	-
Total Taxonomic Distinctness	-0.76**	-	-
Margalef	-0.64*	-	-
Mar Menor lagoon			
Eco-Exergy	-0.49*	-0.60*	-0.39
Total Taxonomic Distinctness	-0.69*	+0.27	-0.69*
Margalef	-0.68*	-0.60*	-0.77*
Shannon-Wiener	-0.67*	-0.61*	-0.70*

the fact that, although indirectly, in the Mondego estuary, the benthic communities structure is negatively influenced by the concentration of nutrients in the water column, which is related with the overall eutrophication of the system (Marques *et al*., 2003).

Mar Menor

According to the hypothesis tested by Warwick & Clarke (1995) it would be expected that Taxonomic Distinctness measures should be able of elucidating pollution effects. However, only TTD, in parallel with the Margalef index, were both able to detect significant differences between organically enriched and non-enriched areas (*p*≤0.05) in soft bottom and rocky zones.

In the majority of the stations, both Average Taxonomic Distinctness (Δ^+) and Variation in Taxonomic Distinctness (Λ^+) were within the 95% confidence funnel (*p*≤0.05) (Figure 4), implying a good degree of taxonomic stability. Only station M8, characterised by the presence of nitrophyle communities, appeared out of the confidence funnel, as the few species found (7) belong only to two orders (Tanaidacea and Amphipoda), indicating a low community structural complexity.

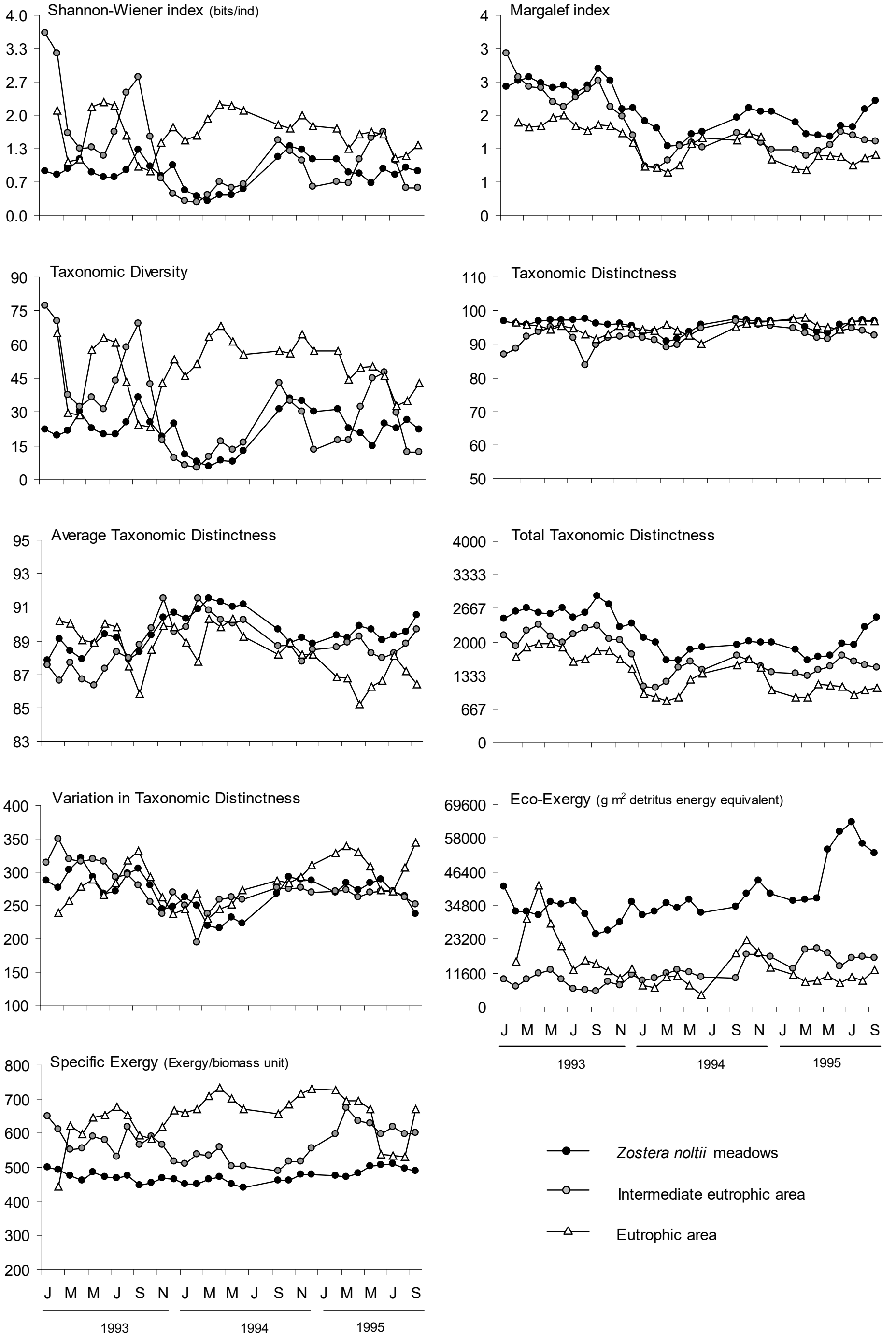


Figure 3. Temporal and spatial variation of the applied indices in the south arm of the Mondego estuary along a gradient of eutrophication.

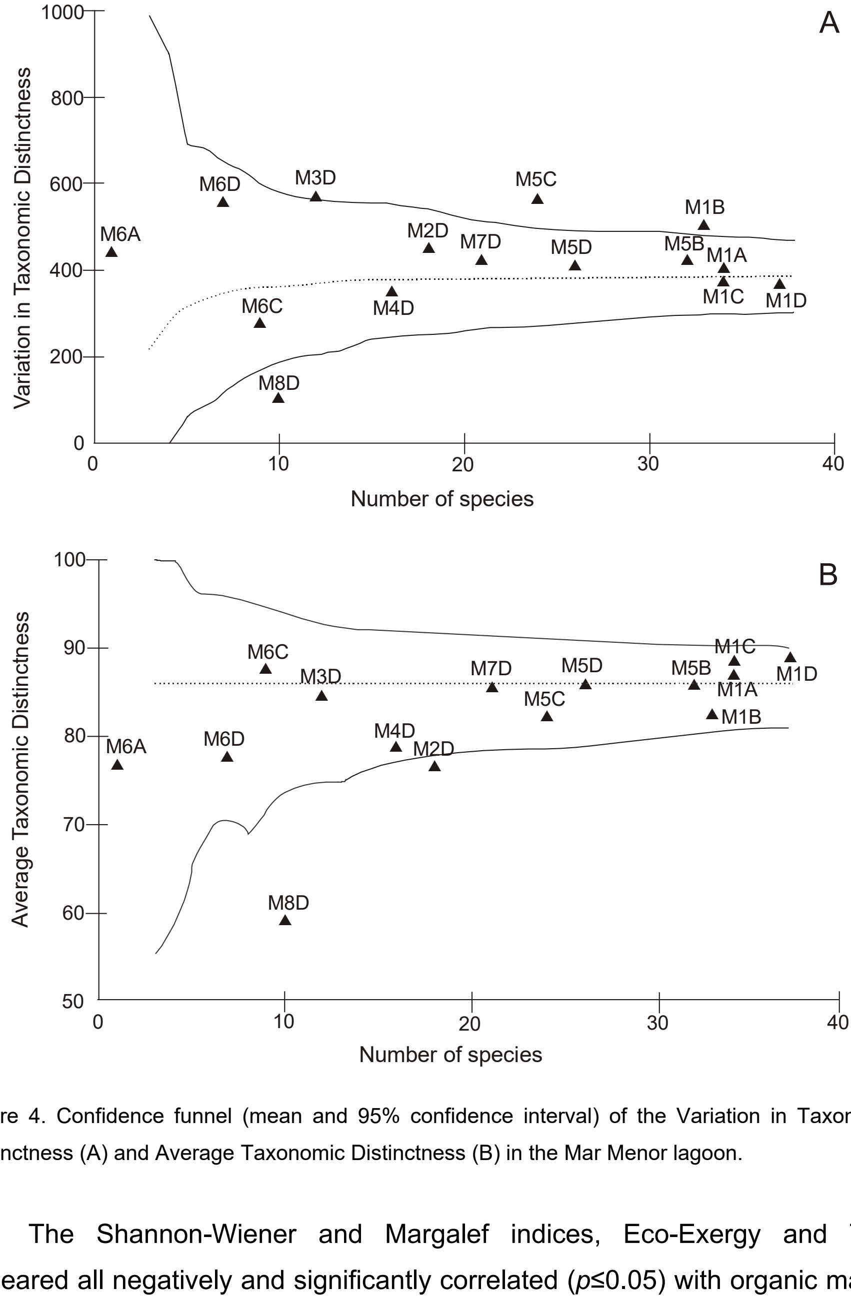


Figure 4. Confidence funnel (mean and 95% confidence interval) of the Variation in Taxonomic Distinctness (A) and Average Taxonomic Distinctness (B) in the Mar Menor lagoon.

The Shannon-Wiener and Margalef indices, Eco-Exergy and TTD appeared all negatively and significantly correlated ($p \leq 0.05$) with organic matter content in sediments and salinity. The Margalef and Shannon-Wiener indices and TTD were also negatively correlated with sediments particle size (Table 3).

Table 4. Indices values measured at different sampling stations in the Mar Menor lagoon. Δ: Taxonomic diversity; Δ^*:Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness (presence/absence of species); sΔ^+: Total Taxonomic Distinctness; Λ^+: Variation in Taxonomic Distinctness; Sp Ex: Specific Eco-Exergy.

	Shannon	Margalef	Δ	Δ^*	Δ^+	s Δ^+	Λ^+	Eco-Exergy	Sp Ex
M1A	2.24	3.72	45.0	72.42	87.25	2966.67	403.52	2885503836	149346
M1B	3.63	4.91	77.2	89.12	82.51	2722.92	532.79	183671203	155184
M1C	2.19	3.87	43.71	72.74	88.15	2996.97	386.82	546460384	76725
M1D	2.43	4.32	49.77	77.22	88.46	3273.15	370.33	192624681	70963
M2D	2.75	2.13	56.45	68.89	85.10	1021.21	514.55	15762446	603402
M3D	2.06	3.20	42.48	79.71	77.67	1398.04	418.17	211020	1592
M4D	2.71	3.20	51.78	69.85	79.03	1264.44	340.26	2523455	14250
M6A	-	0.00	0.00	0.00	0.00	0.00	0.00	285182	14990
M5B	2.46	3.87	46.34	63.63	85.25	2727.96	430.36	899957796	109861
M5C	2.55	3.26	46.10	61.49	82.19	1972.46	575.37	76867912	102457
M6C	1.44	1.78	38.33	87.56	87.96	791.67	287.21	94659	92702
M5D	1.90	3.41	49.01	95.41	86.41	2246.67	407.63	145227127	94642
M6D	1.18	1.24	25.20	70.92	76.98	538.89	621.06	1555244	109065
M7C	2.00	2.50	41.98	72.67	85.79	1801.67	421.20	3249672701	94686
M8C	2.75	1.76	47.24	58.27	59.26	592.59	105.62	301455400	70064

Moreover, Specific Eco-Exergy showed a clear positive correlation with the presence of certain heavy metals such as Pb ($r=0.89$, $p \leq 0.05$) and Zn ($r=0.71$, $p \leq 0.05$), which does not correspond to what theoretically should be expected. Station M2D, for instance, which presented the highest concentration of these two heavy metals (Pb: 3 300 ppm; Zn: 3 400 ppm), also exhibited the highest values of Specific Eco-Exergy (Table 4). Nevertheless, this can be explained if we account for the fact that, in the case of Specific Eco-Exergy, biomass fluctuations have much less influence in the values estimated than changes in the quality of the biomass, which are reflected in the β-values. Molluscs, namely bivalves, are more tolerant to heavy metals contamination, due to their ability to bio-accumulate them, and have higher β-factors than less tolerant groups like polychaetes, crustaceans and echinoderms. Therefore, despite the general macrofaunal impoverishment in areas contaminated by heavy metals, molluscs are comparatively better represented (*e.g. Venerupsis aurea* in station M2) than other groups, and it becomes immediately easy to understand why values of Specific Eco-Exergy are higher.

Papoa

In general, indicators based on species richness (Margalef and Shannon-Wiener indices) and TTD showed higher values in the control plots until September 1999 (seven months after the physical disturbance), but a shift is then recognisable. In fact, from September 1999 up to the end of the study period, the experimental plots presented higher values than the control plots (Figure 5). Furthermore, the values of Margalef index were always higher at the recovery community, except in the very beginning of the experiment, when the control assemblage, understandably exhibited higher values. Accordingly, the Shannon-Wiener index was higher at the recovering plots from September 1999 until the end of the study period. Taxonomic Diversity, Taxonomic Distinctness, Average Taxonomic Distinctness and Variation in Taxonomic Distinctness showed similar values in control and experimental plots (Figure 5), suggesting the same degree of complexity in both cases. With regard to Eco-Exergy (Figure 5), values estimated for the experimental plots increased gradually throughout the field experiment, converging towards those observed in the control plots by the end of the study period. Finally, Specific Eco-Exergy (Figure 5) showed nearly comparable values in both type of plots after only 1 month of recovery, expressing therefore a more or less identical structural complexity in both cases. Nevertheless, this index showed always slightly lower values in the community under recovery.

DISCUSSION

Among the biological quality elements for the definition of ecological status with regard to the WFD implementation are the composition and abundance of benthic invertebrate fauna.

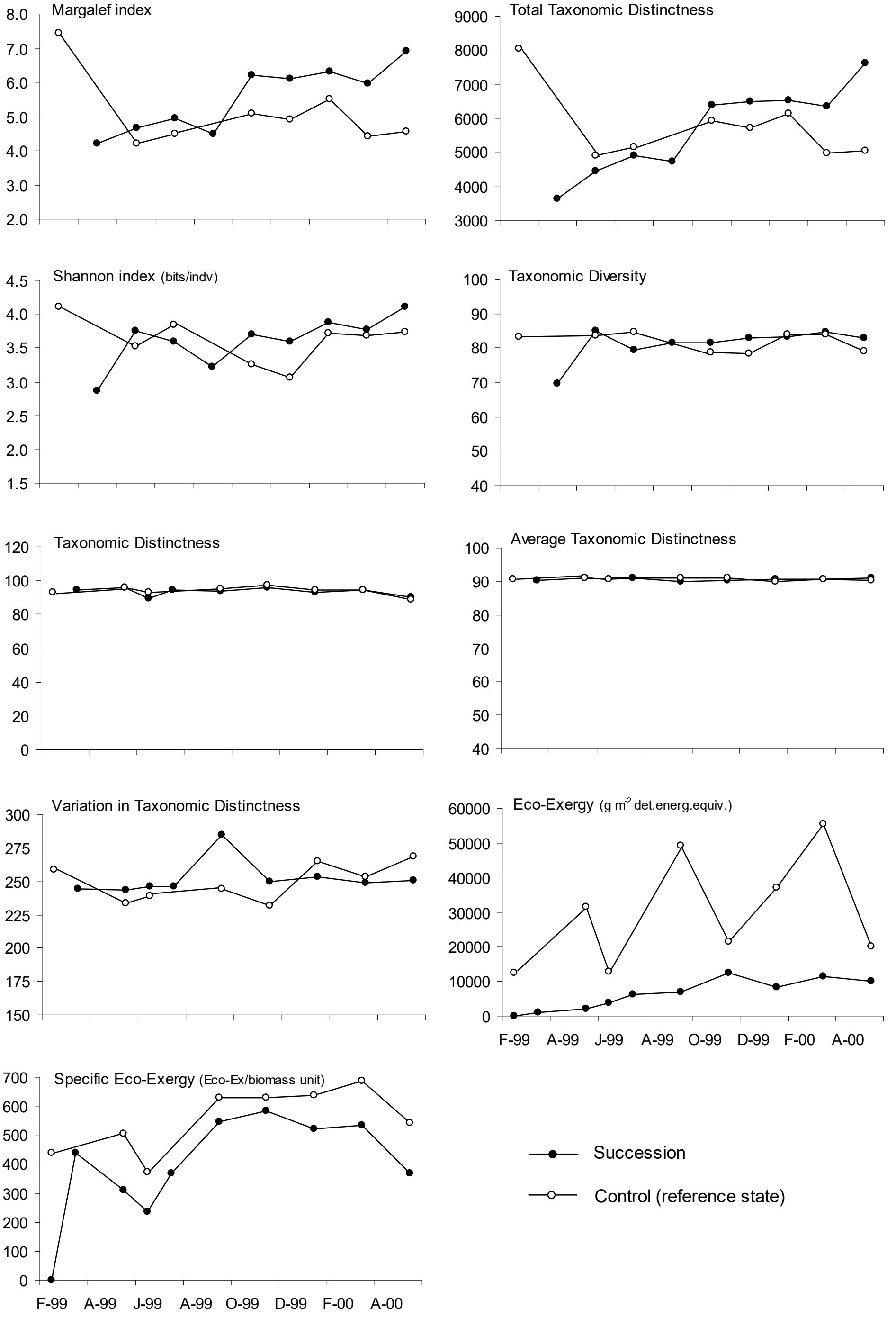


Figure 5. Variation of the applied indices in the control and succession plots in Papoa.

At least in theory, all ecological indicators accounting for those parameters might be useful in detecting the environmental quality status of an ecosystem. However, due to the fact that in practice most of them were developed to approach the characteristics of a specific ecosystem, they often lack generality. Others have been criticised or rejected due to their dependence on specific environmental parameters, or due to their unpredictable behaviour depending on the type of environmental stress. The choice of the ecological indicators set to use in a particular case is, therefore, a subtle process.

Somerfield *et al*. (2003) proposed the Average Taxonomic Distinctness to be used as a useful tool in the classification of ecological status following the European Water Framework Directive. This is due to an advantage of this index proceeding from the fact that it incorporates a master list of taxa, which reflects what could be considered to represent reference conditions. Moreover, this index incorporates a statistical framework from which to measure the distance in relation to those reference conditions.

However, in view of the results, the Taxonomic Distinctness measures have been less sensitive than other diversity measures, despite having many of the features required in order to be a good biodiversity indicator (*e.g.* independency on sample size/effort or monotonic response to environmental degradation). Therefore, the Taxonomic Distinctness measures do not appear to be very useful in establishing the ecological status proposed by the WFD. Even more, along a clear gradient of eutrophication symptoms as in the south arm of the Mondego estuary, the Taxonomic Distinctness Measures showed higher values in the most eutrophic area. Apparently, our observations do not support Warwick & Clarke (1994) statements regarding the monotonic behaviour of these indices along environmental degradation. Similarly, Somerfield *et al*. (1997) found no consistent pattern between decreasing Taxonomic Diversity of marine macrofaunal assemblages and increasing environmental impact. Hall & Greenstreet (1998), studying fish communities, found that Taxonomic Distinctness measures showed identical trends to conventional diversity indices.

Out of all Taxonomic Distinctness measures, only the TTD was able to correctly differentiate the eutrophication gradient present in the intertidal area of Mondego, apart from differentiating more organically enriched areas in the Mar Menor. Nonetheless, Warwick & Clarke (1998) consider not recommendable the use of that measure due to, in general, TTD tends to track species richness rather closely, and it is only useful for tightly controlled designs in which effort is identical for the samples being compared, or sampling is sufficiently exhaustive for the asymptote of the species-area curve to have been reached.

In the same way, Margalef index performed better, despite its simplicity compared to other indices based on the abundance of individuals, distinguishing between different eutrophication levels, (*e.g.* Mondego estuary intertidal area) and detecting organic enrichment situations as in the case of the Mar Menor lagoon. On the contrary, the Shannon-Wiener index appeared excessively influenced by the dominance of certain species (*e.g. Hydrobia ulvae* and *Cerastoderma edule* in the Mondego estuary or *Bittium* sp. in the Mar Menor) whose presence has no relation with any type of disturbance, just being favoured by abundant food resources.

It is interesting to observe how the two tested indices based on specific richness (Margalef index and Total Taxonomic Distinctness) were the most successful measures in differentiating the diverse grades of pollution, leading us to think that the increment or decrement in the number of species is one of the best disturbance indicators, and therefore, essential when it comes to differentiating ecological status. The Northeast Atlantic Geographical Intercalibration Group Benthic Expert (NEAGIG, 2004) considered that the selected metrics to be used in the WFD context need to distinguish clearly across the good/moderate boundary. Obviously, those two measurements are not able themselves alone to work out such distinction, as they will always need a previous knowledge on the number of species (reference situation) of the studied site. In that sense, few are the indices capable of establishing the different ecological status (high, good, moderate, poor and bad). That is for instance the case of AMBI (Borja *et al*., 2000, 2003; Salas *et al*., 2004) or the BENTIX (Simboura *et al*., 2002), which were not tested in this particular work.

Regarding the Eco-Exergy based indices, the results of the present study suggest that the Eco-Exergy index, as ecological indicator, captured, in fact, useful information about the state of the community. In the Mar Menor lagoon this index was able to respond to structural environmental variables such as organic matter, and salinity interval and Specific Eco-Exergy was sensitive to granulometry (the proportion by weight of particules of different sizes in granular material). However, the Eco-Exergy index did not provide explicit information about disturbed (*e.g.* polluted) scenarios. On the other hand, in the Mondego estuary, both Eco-Exergy based indicators differentiated between areas with distinct eutrophication symptoms. Efficiency differences, in both case studies, might have been due to the fact that in the Mar Menor lagoon the effects of organic pollution are, in a certain extent, diluted among other system-structuring factors, while in the south arm of the Mondego estuary eutrophication is undoubtfully the major driving force behind the ongoing changes. However, in the Mondego estuary, only the Eco-Exergy index differentiated correctly the non-eutrophic zone from the rest. Specific Eco-Exergy showed the highest values in the eutrophic area due to the dominance of the bivalve molluscs which *β*-values are higher than in other groups, as it occurred in some of the stations in the Mar Menor.

In the Papoa case study, through re-colonisation, Eco-Exergy values in experimental plots converged towards those observed in the control community. However, this trend appeared to reflect, essentially, changes in biomass, not in information (Patrício *et al.*, in press). On the other hand, taking into account Specific Eco-Exergy, or average organism complexity (an average *β*-value), the community at the experimental plots rapidly recovered in terms of information. In fact, Specific Eco-Exergy, after only 1 month of experiment, showed already resembling values in the experimental and control plots and a parallel behaviour suggesting therefore an analogous structural complexity in both assemblages. Yet, this index showed always inferior values in the community under recovery. A problem with Eco-Exergy based indices is the obvious lack of discriminating power of the weighting factors used to estimate these indices, since organisms are considered at very high taxonomic levels. Although the assessment of *β*-

values still constitutes a weak point, Jørgensen *et al*. (2005a) present an expanded list that contains 45 *β*-values, which hopefully will improve the use of these values to calculate the Eco-Exergy for assessment of ecosystem condition. The authors state that the previous found *β*-values (the ones available by the time this study was carried out) are most probably too small as they only account for the non-nonsense genes in estimating the *β*-values. On the other hand, it is stated that the application of the previous values for the assessment of ecosystem health and in the development of structurally dynamic models have been satisfactorily robust, so Jørgensen *et al.* (2005a) conclude that the previously obtained results are still valid in relative terms. Despite this difficulty, both the thermodynamic oriented indices have provided useful information about the structural development of the community, although at the present stage, the Eco-Exergy index is still not tested in a sufficient number of situations to be used as a tool for WFD application.

In conclusion, although some of the measures taken into consideration in this work have been effective in differentiating the various disturbance statuses, none of them (and specially the Taxonomic Distinctness measures) seems to be particularly helpful alone in assessing the five systems' ecological status considered in the scope of the WFD implementation.

ACKNOWLEDGMENTS

The present work was carried out in the scope of the research Project DYNAMOD (POCTI/MGS/37431/2001) and supported by FCT (Portuguese National Board of Scientific Research) through a grant SFRH/BD/820/2000. A special thanks to Fuensanta Salas for all the work carried out together.

GENERAL DISCUSSION

STUDIED ECOSYSTEMS AND CHOSEN ECOLOGICAL INDICATORS

From the vast quantity of possible ecological indicators, this study selected three types to test (see Table 1): Ascendency based on network analysis, Eco-Exergy and Specific Eco-Exergy thermodynamically oriented and often used in ecological modelling, and Taxonomic Distinctness and associated measures that take into consideration phylogenetic links.

Table 1. Ecological indicators tested in this study and their algorithms. T_{ij}: trophic exchange from taxon *i* to taxon *j;* a dot as a subscript indicates summation over that index; *R*: gas constant; T_a: absolute temperature; c_i: concentration in the ecosystem of component *i*; *β*: weighting factor; x_i: abundance of the *i th* of *s* species observed; $n\left(=\sum_i x_i\right)$: total number of individuals in the sample; ω_{ij}: “distinctness weight” given to the path length linking species *i* and *j* in the taxonomy.

Indicators	Algorithm
Ascendency	$A = \sum_i \sum_j T_{ij} \log\left[\frac{T_{ij}T_{..}}{T_{.j}T_{i.}}\right]$
Eco-Exergy	$Ex = RT_a \times \sum c_i \beta_i$
Specific Eco-Exergy	$SpEx = Eco - Exergy \big/ Total\ Biomass$
Taxonomic Diversity, Δ	$\Delta = \left[\sum \sum_{i<j} \omega_{ij} x_i x_j\right] / \left[n(n-1)/2\right]$
Taxonomic Distinctness, Δ^*	$\Delta^* = \left[\sum \sum_{i<j} \omega_{ij} x_i x_j\right] / \left[\sum \sum_{i<j} x_i x_j\right]$
Average Taxonomic Distinctness, Δ^+	$\Delta^+ = \left[\sum \sum_{i<j} \omega_{ij}\right] / \left[s(s-1)/2\right]$
Total Taxonomic Distinctness, $s\Delta^+$	$s\Delta^+ = \sum_i \left[\left(\sum_{i \neq j} \omega_{ij}\right)(s-1)\right]$
Variation in Taxonomic Distinctness, Λ^+	$\Lambda^+ = \left[\sum \sum_{i \neq j} \left(\omega_{ij} - \bar{\omega}\right)^2\right] / \left[s(s-1)\right]$

Table 2. Main characteristics of the studied ecosystems.

Ecosystem	Disturbance type	Characteristic properties
Mondego estuary (South arm)	Spatial gradient of eutrophication symptoms	Intertidal soft bottom communities. 1) *Zostera noltii* meadows 2) Intermediate eutrophic area 3) Eutrophic area (without *Z. noltii* and with macroalgal blooms)
Mondego estuary (North & South arm)	Different hydrodynamic regimes and impacts	Subtidal soft bottom communities. *North arm*: deeper, lower residence time (2 days), freshwater flows essentially through it, dredging activities and physical disturbance of the bottoms. *South arm*: upstream areas almost silted up, circulation dependent on tidal regime and small tributary input, excessive nutrient release from agricultural fields.
Papoa beach	Recovery process after physical disturbance	Intertidal rocky shore community. Control community dominated by the red algae *Corallina elongata*.
Mar Menor lagoon	Organic enrichment and heavy metals contamination	Coastal lagoon with 135 Km^2. Rocky and soft bottom communities. Environmental heterogeneity with different types of pollution sources

In order to test the behaviour of recently proposed ecological indicators using real empirical data, the present study was carried out at four different ecological scenarios representative of distinct disturbance types (Table 2): 1) the soft bottom intertidal communities in the South arm of Mondego estuary, reflecting a clear gradient of eutrophication symptoms; 2) the subtidal communities of both arms (North and South) of the Mondego estuary, exposed to different hydrodynamic regimes and impacts; 3) the intertidal rocky shore communities at Papoa beach, deliberately affected by physical disturbance, and 4) the rocky and soft bottom communities of Mar Menor coastal lagoon, submitted to diverse types of pollution (*e.g.* organic enrichment and heavy metals contamination).

MAJOR RESULTS AND THEIR RELEVANCE FOR ASSESSING ENVIRONMENTAL STATUS

Mass balanced models

Three mass balanced models were created (using Ecopath with Ecosim software) to represent different areas along the gradient of eutrophication symptoms in the south arm of the Mondego estuary. The sum of consumptions, exports, respiration, production, flow to detritus, TST and annual rate of net primary production was consistently higher at the *Zostera* sp. meadows, followed by the most strongly eutrophic area and, finally, by the intermediate eutrophic area. All these results are partially explainable due to the fact that the non-eutrophic area model has more compartments than the other models. This higher number of compartments has an impact on the calculations and increases TST and consequently all the flows. There were also differences in the breakdown of throughput in the *Zostera* sp. meadows (more exports and flow to detritus) due to the detritivorous web dominance (there was a time lag between the production and its subsequent utilization – a big proportion of the production decays to detritus and is washed way from the production area).

A higher net primary production was observed in the *Zostera* sp. meadows. This fact was probably related with the primary producers' dynamics at each of the studied areas: in the most eutrophic area, there was a strong pulse production during the course of macroalgae blooms and then very low values during the rest of the year. Moreover, the *Zostera* sp. area exhibited the lower system omnivory index, despite having the higher number of compartments, contrary to what was found by Heymans (2003). This observation needs further studies.

Regarding the transfer efficiencies for the three areas, the results suggested a pattern of low herbivore efficiencies, higher efficiencies on trophic level 3 and lower efficiencies on the higher levels. This outcome was supported by Christensen & Pauly (1993). Additionally, average efficiencies in the three areas were within the range described by the same authors for temperate systems.

In conclusion, the Ecopath models provided a summary of the current knowledge of biomass, consumption, production, food web and trophic flows along the eutrophication gradient and have highlighted many of the uncertainties regarding the studied system (diet compositions, site-specific physiological parameters, ecological role of a number of abundant species). Naturally, the process of constructing such models is open-ended and consequently these models were simply a first attempt.

Ascendency as ecological indicator

Ascendency was tested in the only case where the available data were enough to estimate it within a feasible timeframe – the Mondego estuarine intertidal communities (1993 to 1994), which was obviously a very circumscribed application. Notwithstanding data difficulties, network analysis appeared to provide a systematic approach to understand what is happening at the whole system level, which is obviously a powerful tool from the theoretical point of view.

The current study on the Mondego estuarine system seems to have provided an example of how the measures coming out of network analysis can lead to an improved understanding of the eutrophication process itself. In light of these results, Ulanowicz 1986 network definition of eutrophication did not appear to accord with the gradient of eutrophication studied. Rather, it would be more accurate to describe the enrichment processes occurring in this ecosystem as pulse eutrophication. This process could be characterised as a disturbance to the system’s Ascendency in the form of an intermittent supply of excess nutrients that, when coupled with a combination of physical factors (*e.g.* salinity, precipitation, and temperature), causes both a decrease in system activity and a drop in the mutual information of the flow structure. Even though a significant rise in the TST does occur during the period of the algal bloom and at that time does give rise to a strong increase of the system’s Ascendency (as per the network definition of press eutrophication), the longer-term annual picture nevertheless suggests that the other components of the intermediate and strongly eutrophic communities were unable to accommodate the pulse in production. The overall result was a decrease in the annual value of the system TST and, as a consequence, of the annual Ascendency as well.

When the whole-system properties in the three areas were compared, the measures associated to the intermediate eutrophic area, considered to lie between the two extremes in terms of nutrient loading did not plot intermediate. Rather, this area exhibited the lowest Ascendency, AMI, TST and Development Capacity values and the highest figures for Redundancy, System Overhead and Cycling Index, so to say it appeared to be the most disturbed of the three areas. Because Ascendency is scaled by the flows of material in a system, in the *Zostera* sp. meadows and the strongly eutrophic area it is likely to be dominated by the primary producers - seagrasses and macroalgal mats, respectively. At the intermediate eutrophic area there is little macroalgal material and no seagrasses, resulting in a lower value for Ascendency. A second, and not mutually exclusive, explanation is that the non-disturbed and most disturbed areas host relatively stable communities - one dominated by seagrasses and fine sediments, and the other by macroalgal mats and coarser material. When the seagrasses are lost, however, there is a coarsening of the sediments, which makes it very difficult for seagrasses to re-invade. The reason for the low values found in the intermediate eutrophic area appears, therefore, to lie in its unstable nature (Marques *et al*., 2003). In fact, assessments using other ecological indicators (Shannon Wiener index, Species richness and Eco-Exergy) have indicated that the communities built around both the *Zostera* sp. and *Enteromorpha* sp. populations represent more stabilised communities at both ends of the eutrophication symptoms gradient. Moreover, observations on the intermediate eutrophic area were not satisfactorily consistent with the Intermediate Disturbance Hypothesis. Rather, the recent modification in primary producers and its related food web may be seen as a dynamic shift in the ecosystem structure of the catastrophic type, described by Scheffer *et al*. (2001).

Unfortunately, there is still a major inconvenience regarding Ascendency use, namely the considerable time and labour needed to collect all the data necessary to perform network analysis, which limits its application.

Eco-Exergy and Specific Eco-Exergy performance

Regarding the Eco-Exergy based indices (Eco-Exergy and Specific Eco-Exergy), the results of the present study suggested that Eco-Exergy, as ecological indicator, captured, in fact, useful information about the state of the community. In the Mondego estuary intertidal community the Eco-Exergy index varied as theoretically expected, exhibiting higher values at the *Zostera* sp. meadows and lower values at the inner areas of the south arm. Moreover, it also enabled us to differentiate the intermediate eutrophic area from the most heavily eutrophic one. Values were, in fact, lower in the intermediate eutrophic area, and the range of variation was larger.

In contrast, in the Mar Menor lagoon, Eco-Exergy did not provide explicit information about disturbed (*e.g.* polluted) scenarios. This indicator was able to respond to structural environmental variables such as organic matter, and salinity interval. Moreover, Specific Eco-Exergy showed a clear positive correlation with the presence of certain heavy metals (Pb and Zn) which did not correspond to what was theoretically expected. Nevertheless, this observation can be explained if we account for the fact that, in the case of Specific Eco-Exergy, biomass fluctuations have much less influence in the values estimated than changes in the quality of the biomass, which are reflected in the β-values. Therefore, despite the general macrofaunal impoverishment in areas contaminated by heavy metals, bivalves that have a higher β-values (and are more tolerant to this type of contamination) were better represented than other groups (polychaetes, crustaceans or echinoderms), rising Specific Eco-Exergy values.

Efficiency differences, in both case studies, might have been due to the fact that in the Mar Menor lagoon the effects of organic pollution are, in a certain extent, diluted among other system-structuring factors, while in the south arm of the Mondego estuary eutrophication is by far the major driving force behind the ongoing changes.

In the Papoa experiment case, through re-colonisation after physical disturbance, Eco-Exergy values in experimental plots converged towards those observed in the control community. However, this trend appeared to reflect, essentially, changes in biomass, not in information. On the other hand, taking into account Specific Eco-Exergy, the community at the experimental plots rapidly recovered in terms of information. In fact, despite showing always lower values in the community under recovery, after only 1 month of experiment, the index showed already resembling values in the experimental and control plots and a parallel behaviour suggesting therefore an analogous structural complexity in both assemblages.

The other question raised by this experiment was which of the growth forms proposed by Jørgensen *et al*. (2000), would be the dominant form during the recovery? Contrarily to what was hypothesised, the system information recovered rather quickly, closely followed by the network interactions, while even by the end of the study period biomass remained lower than in the control community. Also, contrarily to what was assumed by Jørgensen *et al*. (2000), in this study, growth-to-organisation and growth-to-throughflow dominated the early stages of the recovery process. Accordingly, it was hypothesised that the property **openness** (expressed here as the ratio of periphery to area) was determinant in the sequence of development, and afterwards it was theorised that the rate of immigration (units of organisms m^{-2} d^{-1}) has to be proportional to the openness. Small plots compared with big cleared areas present higher values of openness, expressing higher possibility to exchange energy or matter and increased chance for immigration of organisms.

A problem with Eco-Exergy based indices is the lack of discriminating power of the β-values used to estimate these indices once organisms are considered at very high taxonomic levels. Although the assessment of β-values still constitutes a weak point, Jørgensen *et al.* (2005a) present an expanded list that contains 45 β-values, which will hopefully improve the use of these values to calculate Eco-Exergy for assessing ecosystem condition.

In conclusion, despite this difficulty, Thermodynamics oriented indices have provided useful information about both the ecological status and the structural development of the studied communities. Nevertheless, at the present stage, both Eco-Exergy based measures are still not sufficiently tested to be used as a reliable tool in implementing the European Water Framework Directive (WFD)

Taxonomic Diversity measures as assessment tools

Along the gradient of eutrophication symptoms as it is at present in the south arm of the Mondego estuary, the Taxonomic Diversity measures showed higher values in the most eutrophic area. Apparently, these study observations do not support Warwick & Clarke (1994) statements regarding the monotonic behaviour of these indices along environmental degradation (they should not increase with stress). Similarly, Somerfield *et al*. (1997) found no consistent pattern between decreasing Taxonomic Diversity of marine macrofaunal assemblages and increasing environmental impact, and Hall & Greenstreet (1998), studying fish communities, found that Taxonomic Distinctness measures showed identical trends to conventional diversity indices.

Out of all Taxonomic Distinctness measures, only the Total Taxonomic Distinctness (TTD) was able to correctly differentiate between areas along the eutrophication gradient in the Mondego estuary intertidal area, apart from discriminating more organically enriched areas in the Mar Menor lagoon. Nonetheless, Warwick & Clarke (1998) consider not recommendable the use of that measure due to the fact that, in general, TTD tends to track species richness rather closely, and it is only useful for tightly controlled designs in which the sampling effort is identical for the samples being compared, or sampling is sufficiently exhaustive for the asymptote of the species-area curve to have been reached.

It is interesting to observe how the two indices based on species richness (Margalef index) were the most successful indicators in differentiating between different grades of pollution, leading us to think that the increment or reduction in the number of species is one of the best disturbance indicators, and for that reason, essential when it comes to define a system's ecological status. Therefore, in view of these results, the Taxonomic Distinctness measures have not been more sensitive than other diversity measures, despite having many of the features (*e.g.* independency on sample size/effort) required in order to be a good ecological indicator. As a result, these measures do not appear to be very useful in establishing ecological status as proposed by the WFD.

SUGGESTIONS FOR FURTHER WORK

It is often the case with scientific investigations that more new questions and clues than answers are produced. This work was not an exception: it shaped new challenges and suggested a few interesting avenues that could help to improve the analysis made, namely:

1. How different the results of this study would have been if the stable isotopes methodology was available to estimate much more precisely estuarine food webs?
2. How would Ascendency, Eco-Exergy based indices and Taxonomic Diversity measures perform if longer time series have been used in the Mondego estuary intertidal communities?
3. How different or more accurate the results of the Eco-Exergy based indices would have been if more discriminating *β*-values had been available?
4. How can powerful holist indicators (such as Ascendency and Eco-Exergy) be routinely and straightforwardly applied in environmental quality assessment (namely in implementing the WFD)?

The collection of these new questions may provide potential work directions to be implemented in the future by the author or by anyone interested in these subjects.

CONCLUSION

A brief review of the more commonly used indices to assess ecological status was done in Chapter 1 in order to provide the context underlying the selection of the ecological indicators tested in this study. This was done with the purpose of describing how diverse approaches can be and to locate the selected ecological indicators in the general framework. Two of these measures (Ascendency and Eco-Exergy based indices) were chosen because they are part of the “new generation” of holistic ecological indices that aim to address the ecological integrity at the system level, therefore being useful in establishing a valuable connection between empirical research and Ecosystem Theory. Taxonomic Distinctness measures were selected due to some of their properties: 1) theoretically these measures are not influenced by different sample sizes, sampling effort, habitat type or complexity, and show monotonic behaviour in response to environmental degradation; 2) they incorporate the taxonomic diversity; 3) these metrics were still not extensively applied and 4) some scientists suggested that they could be useful in WFD implementation.

The Ecopath mass balanced models, build up in Chapter 2, successfully provided a synthesis of the current knowledge of biomass, consumption, production, food web and trophic flows along the gradient of eutrophication symptoms in the Mondego estuary. This tool was particularly important to calculate the network based ecological indicator – Ascendency. Even so, the process of constructing such models is open-ended and consequently these models were a first attempt.

Table 3 summarises the major results regarding the ecological indicators behaviour in the four selected ecological scenarios.

Regarding the **gradient of eutrophication symptoms**, the two holistic measures (Ascendency and Eco-Exergy) and Total Taxonomic Distinctness were able to differentiate the *Zostera noltii* meadows from the two eutrophic areas in the Mondego estuary case study. Contrarily, the Taxonomic Diversity, Taxonomic Distinctness and Specific Eco-Exergy index presented higher values in the eutrophic areas.

Table 3. Major results regarding ecological indicators behaviour. Δ: Taxonomic diversity; Δ^*:Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness (presence/absence of species); $s\Delta^+$: Total Taxonomic Distinctness; Λ^+: Variation in Taxonomic Distinctness.

Indicator	**Mondego intertidal** (eutrophication gradient)	**Mondego Subtidal** (hydrological regime)	**Papoa** (recovery)	**Mar Menor** (organic & heavy metal pollution)
Ascendency	Able to distinguish 3 areas with higher values in the non-eutrophic area.	Not tested	Not tested	Not tested
Eco-Exergy	Able to distinguish eutrophic and non-eutrophic areas, with higher values in the *Zostera* sp. area.	No differences detected	Reflects biomass and grows slowly	No differences detected
Specific Eco-Exergy	Reflects the presence of organisms more tolerant to organic enrichment. Higher values in the eutrophic areas.	No differences detected	Reflects information content, grows very fast but still presents lower values in the recovery community	Reflects the presence of organisms more tolerant to metal contamination. Higher values in the polluted areas.
Δ	Higher values in the eutrophic areas.	No differences detected	No differences detected	No differences detected
Δ^*	Higher values in the eutrophic areas.	No differences detected	No differences detected	No differences detected
Δ^+	No differences detected	No differences detected	No differences detected	Detects the community structural complexity
$s\Delta^+$	Able to distinguish 3 areas with higher values in the non-eutrophic area. Tracks species richness.	Able to distinguish between north and south arms, with lower values in impoverished stations	Able to distinguish the control and recovery communities' behaviour. Tracks species richness.	Able to distinguish between organically enriched and non-enriched areas in rocky and soft bottoms
Λ^+	No differences detected	No differences detected	No differences detected	Detects the community structural complexity

Additionally, Specific Eco-Exergy reflected, in this case study, the presence of organisms tolerant to organic enrichment. The other Taxonomic Distinctness measures were unable to detect differences between areas.

All the ecological indicators tested, with the exception of Total Taxonomic Distinctness, were unable to detect differences between the subtidal communities of **the two arms of the Mondego estuary exposed to different hydrological regimes and impacts** (Table 3).

Concerning the **recovery progression after physical disturbance**, the Eco-Exergy index presented a slow growth and closely reflected the evolution of biomass along the re-colonisation process (Table 3). Moreover, Specific Eco-Exergy reflected the information content, presenting similar values in both communities after only one month of recovery. Among Taxonomic Distinctness measures, only the Total Taxonomic Distinctness was able to distinguish between control communities and recovering communities' behaviour and, additionally, this index followed closely species richness.

The other two questions raised in Chapter 4 were 1) if the chosen ecological indicators could help in recognising the three growth forms proposed by Jørgensen *et al.* (2000): biomass, network and information, throughout the recovery process and 2) which would be the dominant growth forms during the recovery. In the present study, contrarily to what was hypothesised based on Odum (1969), the system information (expressed by Specific Eco-Exergy) recovered rather quickly after physical disturbance (despite still presenting lower levels in the community under recovery), closely followed by the network interactions (considering species diversity as an indirect indicator of network complexity), while even by the end of the study period biomass remained lower than in the control community. Also, contrarily to what was hypothesised by Jørgensen *et al*. (2000), in this study growth-to-organisation and growth-to-throughflow dominated the early stages of the recovery process, while growth-to-storage (captured by Eco-Exergy) increased in importance as maturity approaches. Nevertheless, it seems reasonable to assume that this result was related to the experiment scale. In fact, the cleared plots were very small in comparison with the surrounding *Corallina* sp. algal community, leading us to hypothesise the importance of **openness** (expressed here as the ratio of periphery to area) in the re-colonisation process. Small plots compared with big cleared areas present higher values regarding openness, expressing therefore higher possibility to exchange energy or matter and increased chance for immigration of organisms.

A propos of **Mar Menor case study** (Table 3), the only metric capable to distinguish between organically enriched and non-enriched areas in rocky and soft bottoms communities was Total Taxonomic Distinctness. In this coastal lagoon, Specific Eco-Exergy, revealed the presence of organisms more tolerant to metal contamination, therefore exhibiting higher values in the polluted areas.

Summarising, this study results suggested that (Table 4) Ascendency is an integrative and whole system measure able to discriminate areas along a gradient of eutrophication symptoms and very useful as a tool for Ecosystem Theory. Ascendency and Eco-Exergy, were the only two measures able to detect that the transitional communities along the gradient of eutrophication in the Mondego estuary were actually under greater stress than the end communities. However, the considerable time and labour needed to collect all the data necessary to perform the analysis made difficult the use of this metric in environmental management. Eco-Exergy is a further holistic measure able to discriminate between areas with different degrees of eutrophication. Contrarily to the previous metric, it is easy to calculate. Regrettably, the β-values essential for its calculation still lack discriminating power and confine information to the molecular level, when plenty of information exists at other levels.

Although Eco-Exergy was able to correctly track changes in biomass (*e.g.* Papoa beach recovery experiment) it needs to be tested in further cases and scenarios in order to be used *per se* as an ecological assessment tool. As for Specific Eco-Exergy, since it was able to express the “biomass quality” of the system (*e.g.* has revealed the presence of species tolerant to organic enrichment in the eutropic areas of Mondego Estuary and species tolerant to heavy metal contamination in the Mar Menor coastal lagoon), it is recommendable that its use is complementary to Eco-Exergy.

Table 4. Summary of the advantages and disadvantages of the studied ecological indicators. Δ: Taxonomic diversity; Δ^*:Taxonomic Distinctness; Δ^+: Average Taxonomic Distinctness (presence/absence of species); sΔ^+: Total Taxonomic Distinctness; Λ^+: Variation in Taxonomic Distinctness.

	Conclusion on indicators	
	Advantages	**Disadvantages**
Ascendency	• Integrative and whole system measure; • Able to discriminate areas along a gradient of eutrophication symptoms; • Useful as a tool for Ecosystem Theory.	• Considerable time and labour needed to collect all the data necessary to perform the analysis; • Difficult to use in environmental management; • Difficult to assess statistical differences between values.
Eco-Exergy	• Integrative and whole system measure; • Reasonably easy to calculate; • *β*-values are applied relatively; • Correctly tracks changes in biomass/structure; • Useful as a tool for Ecosystem Theory.	• Lack of discriminating power of the *β*-values available; • Still needs to be tested in further case studies and scenarios to be use *per se* as an ecological assessment tool.
Specific Eco-Exergy	• Reasonably easy to calculate; • Able to express the “biomass quality” of the system; • Reveals the presence of species tolerant to organic enrichment and heavy metal contamination.	• Lack of discriminating power of the *β*-values available; • It can only be used as a complementary measure of Eco-Exergy.
Δ, Δ^*, Δ^+, Λ^+	• Reasonably easy to calculate; • Able to express phylogenetic relations.	• Unable to discriminate organic enrichment, heavy metal contamination and recovery after disturbance; • Do not present monotonic behaviour along environmental degradation.
sΔ^+	• Reasonably easy to calculate; • Able to express phylogenetic relations; • Able to differentiate organically enriched areas and to discriminate areas along a gradient of eutrophication symptoms.	• Tracks rather closely species richness, therefore being dependent of sampling effort and design

Finally, although the Taxonomic Distinctness measures are easy to calculate and express phylogenetic diversity, these metrics were unable to discriminate organic enrichment and heavy metal contamination in the Mar Menor case study and to detect recovery after disturbance in the Papoa beach experiment. Besides, these measures did not present monotonic behaviour along environmental degradation, expressed in our case as an eutrophication gradient. Among the Taxonomic Distinctness measures, the exception was the Total Taxonomic Distinctness, which was capable to distinguish organically enriched areas (*e.g.* Mar Menor coastal lagoon) and to differentiate areas along a gradient of eutrophication symptoms (south arm of Mondego estuary). Nevertheless, this index tracked rather closely species richness, therefore being dependent of sampling effort and design.

As final remarks, it can be said that a single approach does not seem appropriate in assessing ecological status or development. Rather, this should be evaluated by combining a dynamic battery of useful and efficient indicators, which may provide complementary information. Nature is too complex to be successfully described by simple ecological indicators.

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Area $= 25 \times 25 =$

Periphery $= 25 \times$

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