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Global Importance of Local Experience

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APPLIED RESEARCH IN THE MONDEGO ESTUARY – EXPLORING THE USE OF DNA CONTENT OF ORGANISMS TO ESTIMATE ECOLOGICAL EXERGY

Abstract

The Mondego estuary is an extensively studied (eco)system. Studies developed in the last years provided researchers with a large compilation of data covering both the physical-chemical qualities of the system and the biology of the autochthonous fauna and flora. This database and the 'accessibility' to the estuary incite the use of this system as a 'field laboratory', allowing the study under 'natural' conditions and improved hypothesis validation. Concerning the evaluation of ecosystems state of development, we have studied the use of nuclear DNA contents of organisms (C-values) as a more practical approach to the ecological estimation of exergy (Jørgensen et al. 1995), according to the proposal by Marques et al. (1997). Methodologies for the estimation of C-values from organisms are presented. We exemplify the use of this methodology with estuarine organisms to obtain weighing factors (b) that may estimate the exergy content per unit of biomass. The applicability of this methodology in the determination of exergy estimates from organisms (biomass) is discussed, both in theoretical and practical aspects.

Introduction

The Mondego estuary has been extensively studied in the last two decades, providing a wide embracing comprehension of this system. Researchers have assessed estuarine physical and chemical characteristics and several studies were devoted to biological processes and ecological relations concerning the local estuarine fauna and flora (Flindt et al. 1999, Lillebø et al. 1999, Lopes et al. 2000, Pardal et al. 2000, Martins

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et al. 2001, Duarte et al. 2001). Present work is being developed to complement the acquired knowledge about this ecosystem, but providing insights into new questions, also (Marques et al. 1997). From this continuous process of research, has came out an conceptual framework allowing to work in this estuary as if it was a "field laboratory", where studies can be developed and theoretical hypothesis tested under 'natural' conditions. These working conditions permit to develop 'laboratorial' (field) work that, although under a reductionist practice, foresees the development of methodologies with broad application. In this scope, we have been developing a methodology to obtain estimates for the exergy associated to a (eco)system from its qualities, in view of characterising system's state of development.

Ecological exergy is a concept derived from thermodynamics as a function expressing the built-in measure of quality for energy, and a potential ecological indicator of ecosystem state of development (Jørgensen and Mejer 1977, 1979, 1981, Jørgensen 1992a, Jørgensen et al. 1995). Exergy is interpreted as an estimate of the maximum capacity of energy to perform useful work as the system proceeds to equilibrium with its surroundings (Brzustowski and Golem 1978, Ahern 1980. Quoted from: Schneider and Kay 1995). The ecological exergy does not correspond exactly to the thermomechanical availability/exergy functions - the work potential of a system at a certain state relatively to the state of equilibrium with the environment (dead state). Instead, it is an operative system interpretation reflecting the quality corresponding to a certain quantity of energy, and can be understood as a measure of contrast or distance from the system to the thermodynamic equilibrium (Schneider and Kay 1994, Jørgensen and Nielsen 1998a,b).

As ecosystems evolve in response to external changes (forcing functions), alterations occur as structural qualitative changes and at the energy flows through the system. As result alterations in the energy and matter conversion processes are verified (Bendoricchio and Jørgensen 1997, Marques et al. 1997, Zhou et al. 1996). Ecosystems are highly complex dynamic systems that may be seen as self-organising 'Prigoginean' systems (Prigogine 1980). In this context, several ecoenergetic considerations may be drawn, regarding its development: (a) ecosystems use high quality energy as 'fuel' in its metabolic processes to convert matter and energy (Schrödinger 1944); (b) the energy flows through the system are used to maintain its functioning and to build up new structures; while (c) systems deviate from thermodynamic equilibrium, (d) low quality energy is returned by the system, and (e) entropy in the surroundings increase (Wall 1996, Jørgensen and Nielsen 1998a). Therefore, as ecosystems develop it may be considered that (see: Bass 1998): (a) its living components are selected according to the pressure imposed by evolutionary competitions ('newdarwinean' processes) and energetic imperatives, (b) it's moved away from thermodynamic equilibrium by the work done on it. Hence, its departure from the thermodynamic equilibrium can be associated to its state of development, in terms of 'potential-work'. Furthermore, energetic assessments of certain ecosystems pointed out an increasing energy 'degradation' with more mature or less perturbed ecosystems, while a decreased ability to dissipate incoming energy of stressed ecosystems (Schneider and Kay 1995). Thus, ecosystems are expected to evolve towards a state of 'optimal exergy configuration'

(Jørgensen 1992b,c) and to improve its ability to withdraw the exergy content of energy (Schneider and Kay 1994a,b). Therefore, it has been suggested that changes of exergy may express alterations in ecosystems structure or functioning, and may be applied in studies as a suitable system-oriented indicator of ecosystem states of development and health (Jørgensen and Mejer 1981, Jørgensen 1988, 1992a,b,c, Jørgensen et al. 1995, Nielsen 1990, 1994, 1995, Fuliu 1997, Marques et al. 1997, 1998, Müller 1997, Jørgensen and Nielsen 1998a,b, Patten 1998).

With reasonable approximations exergy can be computed as (Jørgensen et al. 1998a):

$$\frac{Ex}{R \cdot T} = \sum \beta_i \cdot c_i \qquad \text{(Equation 1)}$$

where ci is the biomass concentration of the i-th species and ßi is a parameter weighing the relative amount of exergy per unit of biomass, expressing the 'quantity of information' embedded in the biomass. This approximate calculation of exergy is processed in terms of a global sum over all components of an ecosystem, where for each term it is considered the relative concentration of the corresponding component and its 'distance' from a common reference state - detritus or organic dead matter. Since detritus is assumed as a reference level ($\beta = 1$) the different weighing factors are determined in terms of the probability, for each component, of producing organic matter (detritus) and the probability of 'selecting' its 'genetic information', regarding the ß parameter as a discriminator of the organisational level of organisms to that reference level (see: Jørgensen et al. 1995). These ('probabilistic') considerations take into account organisms gene number in order to determine the different conversion factors (ß). Since these data is not available for most species, for many cases rough estimates will result from grossly approximate figures for the number of genes. Therefore, Marques et al. (1997) suggested the use of nuclear DNA content of organisms (C-values) in the determination of the ß parameters, in view of a more efficient approach for the estimation of ecological exergy from organism biomass.

In this work we report C-values for several organisms from different groups, and present putative values of ß calculated from these data. Moreover, the use of nuclei DNA contents of organisms as an approach to estimate ecological exergy is discussed in theoretical and operative aspects, regarding its eventual practice in ecological 'exergetic ' studies.

Methodologies and results

The expression used to obtain estimates of ecological exergy:

Jørgensen et al. (1995) developed probabilistic calculations for the approximate estimation of ecological exergy, for each component (Pi), in terms of the probability (P1) of producing organic matter (detritus) and the probability of 'selecting' the 'genetic

information', for that component, assuming detritus as a common reference state. For detritus ($i = 1$), calculations were developed considering the thermodynamic expression for the chemical potential (Jørgensen et al. 1995), such as:

$$c_i^{eq} = c_1 \cdot e^{-(\mu_1 - \mu_1^{eq})/RT} \quad \text{and} \quad P_1 \approx (c_1/c_i^{eq}) \cdot e^{-(\mu_1 - \mu_1^{eq})/RT}$$

On the other hand, for the living components ($i > 1$) the probabilities were obtained from the number of possible permutations of 20 amino-acids relatively to a genome of g genes – note that it's assumed that organisms use an universal code of 20 amino-acids:

$$P_i = P_1 \cdot P_{i,a} \quad \text{and} \quad P_{i,a} = 20^{-700 \cdot g}, \quad i > 1$$

where g represents the number of genes of an organism and 700 is assumed as the mean value of amino-acids encoded per gene. Subsequently, by means of thermodynamic formulations Jørgensen et al. (1995) developed a function that permits the estimation of an ecological 'index' of exergy as:

$$Ex/R \cdot T = (\mu_1 - \mu_1^{eq}) \cdot \sum_{i=1}^{N} c_i / R \cdot T - \sum_{i=2}^{N} c_i \ln P_{i,a}$$

where R is the gas constant, T the absolute temperature ci the concentration in the ecosystem of the i-th component and 700·g stands for an average value for the number of encoded amino-acids in the genome of species i.

Determination of nuclei DNA content of organisms:

The nuclei DNA content of organisms (i.e. the quantity of DNA per cell nucleus) is a characteristic value for each species, referred to as C-value or 2C-value, regarding to the haploid or diploid genome, respectively. The determination of C-values is easily achieved following available laboratory methodologies such as flow cytometry (FCM) (Shapiro 1995, Fonseca 1999). Concerning the estimation of this quantity for a new sample, quantification is performed by reference to nuclei internal standards (usually chicken red blood cell; CRBC 2C = 2.33 pg), which permits to calibrate the data in terms of absolute DNA units (e.g. pg or base-pairs) (Rayburn 1993). Table 1 reports values obtained for the nuclei DNA content of several organisms of different groups, following FCM methodologies, assuming that: C-value ≅ (2C-value)/2

Organisms	DNA content (pg) C-value
Annelida	
Polychaeta	
Spionida	
Spionidae	
Prionospio malmgreni	0.55
Capitellidae	
Notomastus latericeus	1.32
Phyllodocida	
Phyllodocidae	
Nereiphylla paretti	2.7
Hesionidae	
Ophiodromus obscurus	1.6
Ophiodromus culveri	0.35
Kefersteinia sp.	0.22
Hesiospina sp.	0.53
Nereididae	
Platynereis dumerilii	1.0
Laeonereis culveri	0.8
Nereis succinea	2.2
Nereis diversicolor	2.3
Neanthes caudata	2.25
Nephtydae	
Nephtys incisa	7.2
Nephtys sp.	2.2
Glyceridae	
Glycera americana	3.5
Glycera lapidum	1.46
Dinophilida	
Dinophilidae	
Dinophilus gyrociliatus	0.07
Eunicida	
Onuphidae	
Onuphis eremita oculata	1.7
Onuphis sp.	2.0
Diopatra cuprea cuprea	2.0
Americonuphis magna	1.2
Lumbrineridae	
Lumbrineris tenuis	2.4
Ninoe nignpes	5.3
Terebellida	
Pectinariidae	
Pectinaria gouldii	1.3
Sabellida	
Sabellidae	
Amphiglena mediterranea	0.39
Branchiomma luctuosum	1.2
Branchiomma crispum	1.3
Myxicola infundibulum	1.6
Sabella spallanzanii	0.65

Echinodermata	
Asteroidea	
Forcipulata	
Asteriidae	
Marthasterias glacialis	0.6
Echinoidea	
Diadematoida	
Arbaciidae	
Arbacia lixula	0.6
Mollusca	
Gastropoda	
Archeogastropoda	
Pattelidae	
Pattela sp.	0.8
Trochidae	
Gibbula umbilicallis	1.1
Mesogastropoda	
Hidrobiidae	
Hydrobia ulvae	0.68
Littorinidae	
Littorina littorea	1.1
Bivalvia	
Veneroida	
Cardiidae	
Cerastoderma edule	1.36
Scrobiculariidae	
Scrobicularia plana	1.6
Veneridae	
Ruditapes decussata	1.81
Venerupis pullastra	1.78
Maetridae	
Spisula solidissima	1.16
Mytiloida	
Mytilidae	
Mytilus galloprovincialis	6.92
Ostreida	
Ostreidae	
Ostrea edulis	1.16
Pterioida	
Pectinidae	
Pecten maximus	1.42
Chlamys opercularis	1.16
Arthropoda	
Crustacea	
Maxillopoda	
Thoracica	
Pollicipedidae	
Pollicipes pollicipes	0.35

Amphipoda	
Gammaridae	
Echinogammarus marinus	4.9
Isopoda	
Cyathura carinata	1.4
Spharomatidae	
Sphaeroma hookeri	3.1
Decapoda	
Crangonidae	
Crangon crangon	4.2
Portunidae	
Carcinus maena	4.5
Insecta	
Diptera	
Chironumus sp.	0.35
Pisces	
Salmo gairdneri indeus	2.5
Oncorhynchus mykiss	2.49
Cyprinus carpio	1.94
Lampreia planeri	1.73

Approximate figures are given. Sources: Cavalier-Smith 1978, Li and Graur 1991, Gambi et al. 1997, Fonseca 1999. (1 pg = 0.98×10^9 bp). CRBC ($2C = 2.33$ pg).

Use of nuclei DNA content of organisms in the determination of b parameters:

To use C-values in the determination of the β_i parameter, regarding to the C-value paradox (Cavalier-Smith 1978), it is necessary to work with the minimum genome size (lowest C-value) for each group of organisms (e.g. taxonomic or functional) instead of the C-value for each specie in the group (Fonseca 1999). Subsequently, as a topmost limit the DNA data (minimum genome sizes) may be considered as an approximate (although rough) of the overall 'coding capacity' of the genome and used in the evaluation of b parameters, accordingly to both Jørgensen et al. (1995) and Marques et al. (1997) proposals. This implies that these (C-)values are used replacing the corresponding term for 'genome dimension' (700 g) of Jørgensen et al. (1995) proposal (Fonseca 1999).

As an example, the estimation of b for the biomass of the annelid can be worked as follows:

find, in Table 2, the lowest C-value for the group Annelids:

☞   0.07 pg

☞

convert to base-pairs (bp) of nucleotides using the relation: 1 pg = 0.98×10^9 bp

☞ $C^* = 6.86 \times 10^7$ bp

☞

Since only one polynucleotide chain is considered ('single strand DNA'), we have:

☞ $C^{**} = (C^* / 2) = 3.43 \times 10^7$ nucleotides

☞

Following the work hypothesis, the obtained number of nucleotides is considered as a topmost limit for the 'maximum coding capacity'. Since the DNA code is (indirectly) transcribed into an amino-acid code, the number of nucleotides is converted to total nucleotides triplets (thus, the topmost limit for the number of triplets):

☞ $C^{***} = (C^{**} / 3) = (3.43 \times 10^7) / 3 = 1.14 \times 10^7$ triplets

☞

This result (C^{***}) is used replacing "700 g" in the estimation of the expression 'In P_L' from Jørgensen et al. (1995) proposal:

☞ In P_u = In 20 $^{-C^{***}}$ = 3.42×10^7

Subsequently, this value is used to obtain estimates from the expression for Ex/RT derived by Jørgensen et al. (1995).

Following the calculations according to the proposal of Jørgensen et al. (1995), it is assumed an average molecular weight for detritus of 100 000, and the free energy released per g of organic matter is ca. 18.5 kJ/mol. Taking T = 300 K and R ≈ 8.3 J / mol K, it is possible to estimate the corresponding 'index' of ecological exergy expressed in terms of 'g of detritus exergy equivalents', accordingly to the proposal of Jørgensen et al. (1995). This means (operatively) that the obtained value is divided by 7.43×10^5, assumed as the contribution of detritus in terms of g/L:

$$Ex / RT \cong \ldots C_{Annelids} \times (3.42 \times 10^7) + \ldots + (C_{Detritus} + \ldots + C_{Annelids} + \ldots) \times (7.43 \times 10^5)$$
(exergy 'equivalents', g/L)

$$Ex / RT \cong \ldots C_{Annelids} \times (50) + \ldots + C_{Detritus}$$
(g of detritus exergy 'equivalents', g Detritus/L)

Therefore, the contribution to the ecological exergy 'index' from an organism of the considered group (Annelids) can be calculated as:

$$Ex_{Annelids} / RT \cong \ldots C_{Annelids} \times (50)$$
thus, $\beta_{Annelids} \cong 50$

where $C_{Annelids}$ represents the biomass of the organism (i. e., its concentration in the ecosystem in g/L) and $\beta_{Annelids}$ is the corresponding weighing factor for the group Annelids.

Table 2 lists the lowest values for the haploid DNA content in several groups of organisms, and estimates for ß, obtained according to Jørgensen et al. (1995) and based in C-values.

Table 2. Typical values for biological parameters (number of genes and cell types) and for the weighing factor (β^*) to estimate exergy from organisms biomass according to Jørgensen et al. (1995), for different groups of organisms. It is presented, also, the lowest C-values in these groups of organisms and the corresponding weighing factors (β^{**}) accordingly to the methodologies presented in this work.

Organisms	No. genesa	No. cell Typesa	β^* C-value	Lowest	β^{**}
Detritus	0	----	1[ab]	----	1
Bacteria	600	1-2	3[ab]	0.0017[c]	2
Algae	850	6-8	3-4[ab]	0.04[c]	25
Fungi	3000	6-7	10[ab]	0.005[c]	3
Annelids	10500; 100000[b]	60	35[a]; 287[b]	0.07[d]	50
Arthropods	----	----	----	0.1[c]	70
Insects	10000-15000	----	30-46[a]; 144[b]	0.1[c]	70
Crustaceans	----	----	144[b]	0.35[e]	230
Molluscs	----	----	287[b]	0.68[e]	450
Gastropods	----	----	----	0.68[e]	450
Bivalves	----	----	----	1.16[e]	760
Echinoderms	----	----	144[b]	0.54[e]	360
Chordates	----	----	----	0.20[e]	130
Fish	100000-120000	70	287-370[ab]	0.39[e]	260
Amphibians	120000	----	344[b]; 370[a]	1.2[c]	800
Reptiles	130000	----	344[b]; 400[a]	1.5[c]	1000
Birds	120000	----	344[a]; 390[b]	1.7[e]	1100
Mammals	140000	100	402[b]; 430[a]	3.0[e]	2000
H. sapiens	~ 30000[f]	----	----	2.0[e]	1300

[a] values provided in Jørgensen et al. 1995, 1998.
[b] figures presented by Marques et al. 1997; c figures representing the lowest values in the group according to Cavalier-Smith 1978; d values from Gambi et al. 1997; e Levin, 1994. f Lander et al, 2001

Discussion

The ecological exergy is an operative interpretation of the thermomechanical availability functions (i. e., the work potential of a system at a certain state relatively to the state of equilibrium with the environment - dead state), proportional to the available energy invested by ecosystem in building up its 'structure' (information and mass). Several methodologies to estimate ecological exergy have been developed on the basis of thermodynamic principles (Jørgensen and Mejer 1979, 1981, Shieh and Fan 1982, Jørgensen et al. 1995, Zhou et al. 1996, Marques et al. 1997, 1998), stressing the relevance of these principles in the assessment of ecosystems state of development. Ecosystem structure and energy-matter balance will evolve to a state of optimal thermodynamic balance, although conditioned by the prevailing environmental parameters (Marques et al. 1998). Therefore, ecosystems are expected to evolve optimising the storage of the available energy (Jørgensen 1992b,c, Marques et al. 1998) and increasing its dissipation to maintain the acquired levels of biomass and (higher) complexity, during development (Schneider and Kay 1994a,b, 1995).

According to Jørgensen et al. (1995) the estimation of the contribution of ecological exergy from organisms (biomass) is based upon corresponding weighing factors (b) for different organisms. These authors suggested to estimate the b parameter using the number of encoded amino-acids (700·g), assuming different number of genes (g), for each organism, and that each gene codes for an average number of 700 amino-acids. These b parameters envisage weighing the contribution to the ecological exergy of the 'genetic information', but the data for the number of genes required for its determination (see Table 2), at the present, are very unreliable.

On the other hand, Marques et al. (1997) proposes the estimation of the total amount of DNA per cell nucleus (C-value) assuming this value as an approximate "estimate" for the "information content" of the genome, accounting for organisms structural 'complexity'. Table 1 illustrates two important features regarding the nature of these data: (a) C-values may vary widely in closely related species (MacGregor 1982, Gold et al. 1992, Gambi et al. 1997), and (b) some organisms may exhibit larger C-values than mammals although less morphologically "complex". Thus, for higher eukaryotes, it is evident the lack of correlation between structural "complexity" and total DNA content, at the species level (Cavalier-Smith 1978, 1985), reinforcing the concept of the "C-value paradox" (see: Futuyma 1998). This feature is essentially consequence of the repetitive (noncoding) DNA sequences in eukaryotic genome, in some organisms accounting for more than 50% of the total genome (John and Miklos 1988). Therefore, instead of calculating weighing factors, ßi, for each species from the corresponding C-values, it is preferable to use the lowest (known) C-value for different groups of organisms (Table 2).

Following this procedure the biomass of organisms from different groups is "weighed" (in terms of exergy content) according to the assumed level of complexity for each group. This way, the C-value for each group is considered as an estimate (although rough) of the overall coding capacity of the genome, and it should be understood as a topmost limit value for the "information content" of genomes from the considered group. The total DNA may affect biological events, from cell size and division, to ecological effects. Higher C-values are frequently associated with species having slower development (Bennet 1982, Rees et al. 1982, Sessions and Larson 1987), and closely related organisms may reach similar dimensions with different number of cells (MacGregor 1982). Nevertheless, although the suggested values relate to biological parameters (C-values), which were selected during the evolution processes, regarding what is focused, it should be used with great attention to the implications of the 'C-value paradox'.

Different figures for the ß parameter were estimated from the Jørgensen et al. (1995) and Marques et al. (1997) proposals (Table 2). Clearly, the estimation of the "information content" of genomes of organisms may be largely biased, from both the assumed number of genes (Jørgensen et al. 1995) and the total nuclear DNA contents (Marques et al. 1997), as discussed. However, before any definite conclusions regarding the merit of these approaches, comparative studies are required, along with other ecological indicators (e.g. diversity indices, ascendancy, emergy) aiming to assess the efficiency in capturing any additional information regarding ecosystems health and

integrity. As well, it is important to stress that the nonrepetitive DNA fraction of genome relates better to the complexity of organisms (see: Levin 1994). Thus, the development of methodologies to estimate the nonrepetitive DNA content of genome, such as the technique of reassociation kinetics, should be attempt in view to replace the FCM estimation of total nuclear DNA.
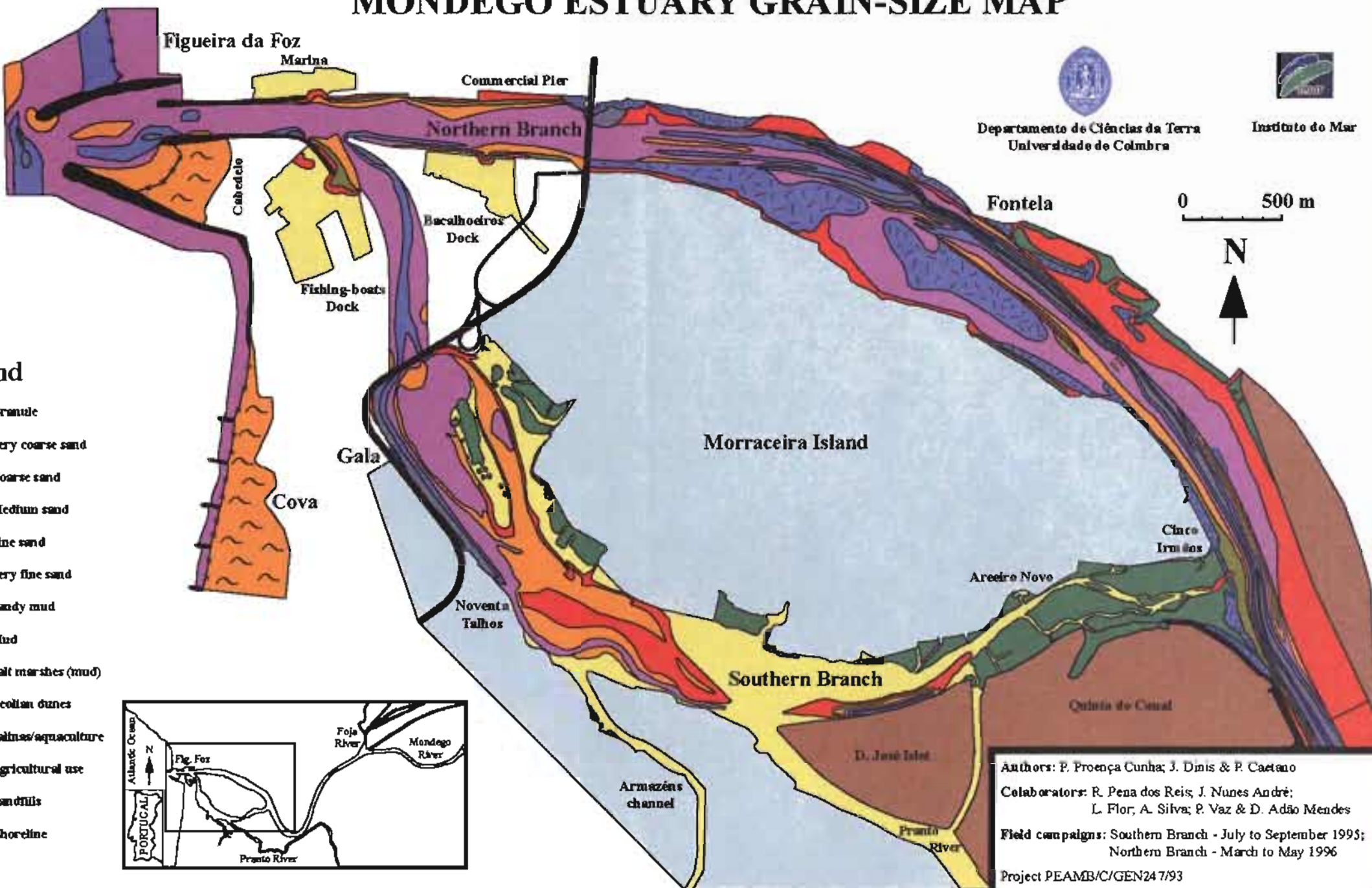
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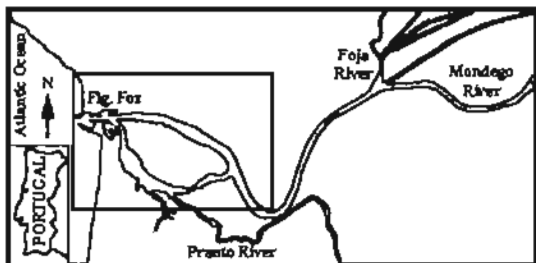
MONDEGO ESTUARY GRAIN-SIZE MAP

Atlantic Ocean

Figueira da Foz
Marina
Commercial Pier
Northern Branch
Cabedelo
Bacalhoeiros Dock
Fishing-boats Dock
Fontela

Departamento de Ciências da Terra
Universidade de Coimbra

Instituto do Mar

0   500 m

N

Gala
Cova

Morraceira Island

Cinco Irmãos

Areeiro Novo

Noventa Talhos

Southern Branch

Quinta do Canal

Legend

Sediments

- Granule
- Very coarse sand
- Coarse sand
- Medium sand
- Fine sand
- Very fine sand
- Sandy mud
- Mud
- Salt marshes (mud)
- Aeolian dunes
- Salinas/aquaculture
- Agricultural use
- Landfills
- Shoreline

Atlantic Ocean
N
Fig. Foz
Foja River
Mondego River
PORTUGAL
Pranto River

D. José Islet

Armazéns channel

Pranto River

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