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SPATIAL DISTRIBUTION OF SMALL BENTHIC INVERTEBRATES IN ROCKY UPPER INFRALITTORAL AT THE ASINARA ISLAND (NW MEDITERRANEAN): A PILOT STUDY

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COST-BENEFIT ANALYSES
DESIGN OF SAMPLING
INVERTEBRATES
MPAS
PILOT STUDY

ABSTRACT. – A pilot study was carried out in the Asinara Island National Park (NW Mediterranean) at Giordano Bay to investigate the spatial variation in abundance of small invertebrates inhabiting upper infralittoral hard bottoms covered by algae. Six experimental areas were randomly chosen within a mixed assemblage of erect algae at the bay. Samples of benthic fauna were collected by cutting through the algae to the rock surface with open-ended plastic cylinders of 40, 60 and 80 mm in diameter (small, medium and large size, respectively). The abundance of the most common taxa, for both macrofaunal and meiofaunal components, was found unaffected by the size of the sampling unit, suggesting that the distribution of organisms is spatially heterogeneous and that the average distance among aggregation of benthic assemblages is larger than the sampling unit sizes considered. Further, taxonomic resolution used is discussed as a possible cause for the results obtained. Cost-benefit analyses have determined the optimal allocation of resources to use in future sampling of small benthic invertebrates at the site.

ANALYSES AVANTAGES-COÛTS
PROGRAMME D'ÉCHANTILLONNAGE
INVERTÉBRÉS
MPAS
ÉTUDE PILOTE

RÉSUMÉ. – Une étude pilote dans le Parc National de l'Île d'Asinara (Méditerranée nord-occidentale) située dans la Baie Giordano a permis d'examiner la variation spatiale de l'abondance des petits invertébrés vivant sur les fonds rocheux couverts d'algues de l'infralittoral supérieur. Six zones expérimentales ont été sélectionnées dans un assemblage mixte d'algues dressées dans la baie. Les échantillons de faune benthique ont été prélevés en coupant les algues à la surface du rocher avec des cylindres en plastique ouverts à l'extrémité et d'un diamètre de 40, 60 et 80 mm (petite, moyenne et grande taille respectivement). Les résultats montrent que l'abondance des taxons les plus communs de la macrofaune ou de la méiofaune, n'est pas influencée par la taille de l'échantillon : ceci révèle que la distribution des organismes est hétérogène spatialement et que la distance moyenne dans l'agrégation des assemblages est plus grande que les tailles considérées des unités d'échantillonnage. De plus, la résolution taxonomique utilisée est discutée comme cause possible des résultats obtenus. L'analyse des avantages et des coûts a permis de déterminer la position optimale des ressources utilisées en vue de l'échantillonnage futur des petits invertébrés benthiques de ce site.

INTRODUCTION

Sampling procedures may vary depending on the specific hypotheses to be investigated, the complexity of habitat under investigation and spatial variability of organisms. Further, logistic and economic efforts often constrain specific sampling designs.

Evaluating spatial variability of species by means of an appropriate pilot sampling design seems to be very helpful to allocate resources efficiently (Kennelly & Underwood 1984, Andrew & Mapstone 1987, James *et al*. 1995, Benedetti-Cecchi *et al*. 1996, James & Fairweather 1996). Testing any specific hypotheses through an established proper experimental design can minimize the costs and maximize the benefits, especially in marine reserves where it is particularly necessary to reduce the interference of sampling procedures to species abundance. In fact, there is actually an increasing concern on the use of sampling techniques to be adopted in MPAs. Not destructive sampling, such as visual estimates and photographic

techniques (Fraschetti *et al*. 2003, Benedetti-Cecchi *et al*. 2003), or destructive sampling of least areas is to be preferred once reliability is assured. Knowledge of spatial variability of organisms investigated allows design of experiments and monitoring programs, testing specific hypotheses about any experimental treatment or protection effectiveness.

One way to test the precision of sampler of small invertebrates is by taking a repetitive series of samples from an area and calculating the optimal number of samples, necessary for a predetermined level of error (Christie 1976). A pilot study is generally performed to determine the optimum size of sampling units and number of both replicates and areas for experimental treatments with regard to the spatial variability of the organisms investigated (Underwood 1981, Kennelly & Underwood 1984, 1985, Benedetti-Cecchi *et al*. 1996). In fact, the choice of the size of sampling unit is fundamentally related to characteristics of the organisms being sampled, such as spatial arrangement, and this is particularly true when they are aggregated (Andrew & Mapstone 1987). Furthermore, comments on the precision and accuracy of some sampling methods suggest that no approach is generally valid and the adequacy of a given method should be evaluated in any particular situation (Benedetti-Cecchi *et al.* 1996).

In the present pilot study we describe a sampling method to determine spatial variation in the abundance of the small invertebrates inhabiting upper infralittoral hard bottoms covered by algae in the Asinara National Park (NW Mediterranean), by using several corer sizes to assess the most effective sample size. Cost-benefit and variance analyses were used to determine optimal number of replicates and areas to be sampled in any field experiment. Although there have been examples of cost-benefit analyses in the literature (e.g. Benedetti-Cecchi *et al*. 1996), these studies have focused mainly on attached organisms of relatively large size. The present pilot study provides comparable information for small macrofaunal and meiofaunal invertebrates inhabiting assemblages of turf-forming algae of rocky shores.

METHODS

This study was carried out on upper infralittoral hard bottoms covered by algae in Asinara Island MPA at Giordano Bay (Lat N 41º 05,476'; Lon E 08º 21,007'), a low-use part of the Reserve. Asinara Island was a prison island from 1885 to 1997 and, therefore, public access and construction have been forbidden for nearly a century (Villa *et al*. 2002). Although Giordano Bay has been established as a 'entry, no-take' zone, very few visitors were observed during summer.

Samples of benthic fauna were collected on 9 January 2002 between 0.2-0.4 m below MLLW by cutting through the algae to the rock surface with open-ended plastic cylinders. Three sampler size 40, 60 and 80 mm in diameter (small, medium, large) were used, corresponding to 12.56 cm^2, 28.26 cm^2 and 50.24 cm^2 in surface area scraped, respectively (e.g. Brown & Taylor 1999, Kelaher *et al.* 2003). For each sample, all the algae and benthic invertebrates were removed down to the basal crust by using a metal scraper, so including both macrofaunal and meiofaunal components. Macrofauna component is not commonly sampled using those sizes of corer and this could possibly represent a source of error. However, this is a pilot study and further experiments will investigate this aspect.

The remaining organisms on the rock that had been scraped clean and the epifauna apparently had no opportunity to escape following placement of the sampling cylinder.

At the bay, six experimental areas within a mixed assemblage of erect algae were randomly chosen. They were about 20×20 cm in size and 5 m distant apart. Two replicate areas were randomly assigned to each sampling surface and two replicate samples were collected in each area.

In the field, samples were preserved in 4% formalin in seawater and, in the laboratory, each sample was sieved in a 500-µm mesh; material filtered was sieved again in a 100-µm mesh. Organisms retained by the two meshes were attributed to macrofaunal and meiofaunal components (Coull & Bell 1979, Platt 1981, Martens & Schockaert 1986, Brown & Taylor 1999, Kelaher *et al*. 2003), identified to coarse taxonomic levels and counted under stereo microscope.

Two-way ANOVAs were performed to examine the difference in abundance of the most common taxa depending on the size of the sampling cylinder used. 'Size' was treated as fixed factor (3 levels, small, medium, and large), while 'area' was random and nested in size (2 levels). Cochran's test was used to check for the homogeneity of variances (Winer 1971). Whenever necessary data were transformed. The GMAV 5.0 software (University of Sydney) was used to perform statistics.

Cost-benefit analyses were used to determine the optimal number of replicates and areas. Standard procedures (Winer 1971, Underwood 1981) were used for calculation. The number of replicates (n) per area was determined as:

$$n = (Ca \cdot Se^2 / Cr \cdot Sa^2)^{1/2} \quad (1)$$

where Se^2 is the estimated variance among replicates and Sa^2 is the estimated variance among areas.

The optimal number of areas (a) was determined as:

$$a = Ct / nCr + Ca \quad (2)$$

where Ct (360 min) is the total time allocated to collect the samples with a specific surface in future studies, Cr is the mean cost among samples of different size (in terms of time) necessary to sort under microscope each replicate (60 min and 140 min for macrofauna and meiofauna, respectively). These mean times have been calculated beeing the cost for sorting macrofauna 30, 60 and 90 min for small, medium and large sampler size, respectively, and for meiofauna 60, 120 and 240 min. Ca (1 min) is the mean time necessary to move among experimental areas.

RESULTS AND DISCUSSION

Padina pavonica L. Lamour, *Stipocaulon scoparium* (L.) Sauv, *Polysiphonia* spp., *Cystoseira amentacea* Bory var. *stricta* Montagne, *Ceramium* spp., *Dasycladus vermicularis* (Scopoli) Krasser, *Jania rubens* (L.) Lamour were the most abundant algal species in the samples. Polychaetes, molluscs, nematodes, oligochaetes, echinoderms, gammarid amphipods, caprellid amphipods and tanaids mostly composed the macrofauna (Fig. 1). The other individuals of crustaceans found, isopods, ostracods and cumaceans have been included in 'other' group. Nematodes larger than 0.5 mm were included in this category although they are usually considered meiofaunal representatives (Martens & Schockart 1986). A total of 1298 individuals were collected in 12 samples. Polychaetes (36.5%), gammarid amphipods (25.9%) and caprellid amphipods (8.6%) were the dominant taxa.

Polychaetes, molluscs, nematodes, oligochaetes, gammarid amphipods, caprellid amphipods, isopods, tanaids, ostracods and harpacticoid copepods were the meiofauna most common taxa found in samples (Fig. 2). A total of 6139 individuals were found: harpacticoid copepods (51.7%), polychaetes (29.2%) and nematodes (9.7%) were the dominant taxa.

The abundance of each taxon was not affected by the size of sampling units. For none of the response variables, the analysis has identified a significant difference among sizes of sampling units (Table I). However, the abundance data of polychaetes found in meiofauna could not be analysed by ANOVA because of variance heterogeneity even after data transformation (Table I). For this taxon, the number of individuals seems proportionally dependent on the size of sampling unit so that the smallest sample collects about half of the organisms collected by the largest sample, consistently to different cylinder areas.

The size of sampling unit greatly affects the precision of data estimates (Andrew & Mapstone 1987). The size of the sampler is relative to the spatial aggregation of organisms studied. If the size of the sampling unit is similar to or smaller than the average distance among aggregations, variation among replicates is expected to be large (Andrew & Mapstone 1987). Conversely, estimates collected in large sampling units are expected to be less dependent on the patchiness of species. Small-scale, local heterogeneity in habitat and the distribution and abundance of organisms is well documented in marine systems in both littoral and infralittoral (Underwood & Chapman 1989, Chapman 1994a, 1994b, Chapman *et al*. 1995, Underwood & Chapman 1996, Menconi *et al*. 1999, Benedetti-Cecchi 2001, Kelaher *et al*. 2003). In fact, species distribution is dependent on the characteristics of the substrate (Gibbons 1988a, 1988b, Danovaro & Fraschetti 2002), on the macroalgae

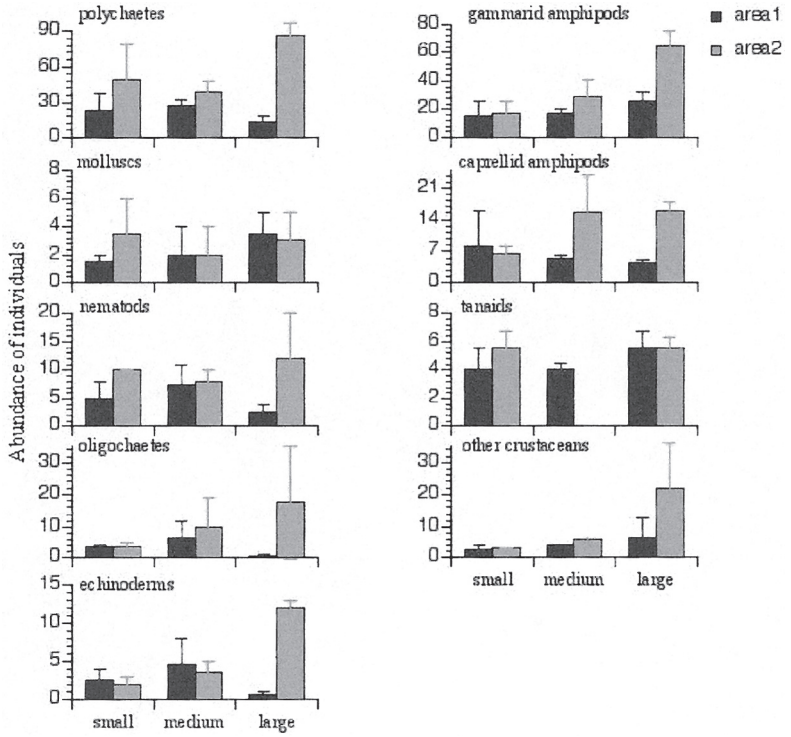

Fig. 1. – Mean abundance (±SE) of commonest macrofauna taxa sampled with samplers of different size (small, medium, large) and areas (n=2).

Table I. – Results of ANOVAs on the effects of sampler size (large, medium and small) and Area (two areas). Bold numbers indicate significance at $p<0.05$.

		Size				Area (S)				residual	
Macrofauna	df	MS	F	P	df	MS	F	P	df	MS	Cochran's test
Polychaetes	2	309,000	0,15	0,866	3	2049,660	4,77	**0,049**	6	429,660	0.652 ns
Molluscs	2	1,583	1,12	0,433	3	1,416	0,20	0,889	6	6,916	0.301 ns
Nematodes	2	0,250	0,01	0,993	3	38,500	1,26	0,368	6	30,500	0.699 ns
Oligochaetes	2	37,000	0,37	0,717	3	99,333	0,72	0,576	6	138,333	0.738 ns
Echinoderms	2	16,083	0,36	0,723	3	44,500	7,03	**0,021**	6	6,333	0.644 ns
Gammarid amphipods	2	911,083	1,63	0,332	3	560,416	3,39	0,094	6	165,250	0.266 ns
Caprellid amphipods	2	13,083	0,17	0,853	3	78,166	1,64	0,277	6	47,666	0.505 ns
Tanaids	2	14,083	3,19	0,181	3	4,416	0,71	0,582	6	6,250	0.653 ns
other*	2	1,657	1,66	0,327	3	1,000	0,83	0,523	6	1,202	Sqrt(X+1) 0.624 ns
Meiofauna											
Polychaetes											**
Molluscs	2	7,583	0,89	0,497	3	8,500	0,63	0,622	6	13,500	0.617 ns
Nematodes	2	186,333	0,13	0,887	3	1487,000	2,88	0,125	6	515,667	0.562 ns
Oligochaetes	2	3,170	0,40	0,701	3	7,899	2,14	0,196	6	3,689	Sqrt(X+1) 0.704 ns
Gammarid amphipods	2	272,583	2,25	0,253	3	121,167	7,65	**0,018**	6	15,833	0.757 ns
Caprellid amphipods	2	2,583	0,42	0,691	3	6,167	0,60	0,640	6	10,333	0.653 ns
Isopods	2	7,000	4,20	0,135	3	1,667	0,14	0,929	6	11,500	0.587 ns
Tanaids	2	2,298	1,59	0,339	3	1,447	0,99	0,457	6	1,455	Sqrt(X+1) 0.711 ns
Ostracods	2	13,583	2,23	0,255	3	6,083	0,74	0,567	6	8,250	0.363 ns
Harpacticoid copepods	2	40411,083	4,18	0,136	3	9676,167	0,61	0,633	6	15893,333	0.408 ns

*Other taxa includes isopods, ostracods and cumaceans

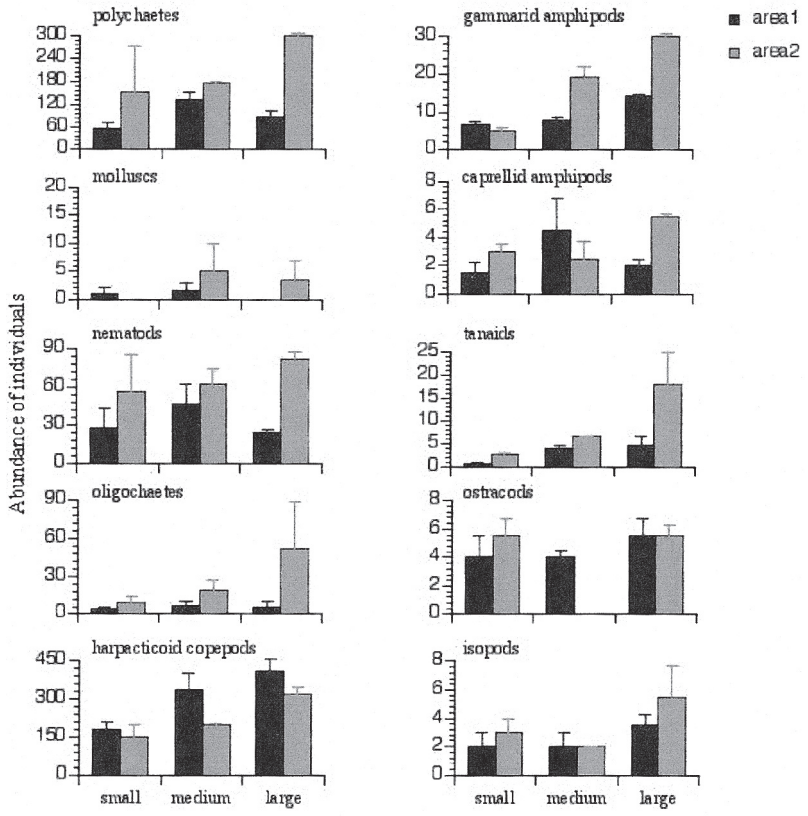

Fig. 2. – Mean abundance (±SE) of commonest meiofauna taxa sampled with samplers of different size (small, medium, large) and areas (n=2).

occurring on the substrate, that can play a major role for the recruitment of several benthic species such as polychaetes, bivalves and amphipods (Beckley 1982, Coull *et al.* 1983, Judge *et al.* 1988) and on the ethology of each species (Olafsson 1992, Hughes 1996, Blome *et al.* 1999).

In this study, the lack of a significant effect of the sampling corer size may be dependent on the heterogeneous spatial distribution of taxa, hence the average distance among aggregations is larger than the sampling unit sizes considered. If this was true, at the site, significant differences in invertebrate abundance among sampling sizes would have been likely using a sampler larger than 80 mm in diameter. However, the lack of significant differences among cores of different size might also have occurred if populations were distributed homogeneously in space. In fact, this was a pilot study and it is possible that the lack of significant effects reflected the lack of statistical power (Underwood 1997). Because this study did not test hypotheses about the spatial arrangement of organisms, it is impossible to distinguish among these alternatives.

Results of analyses obtained for cores of different size indicate that the three cylinders collect the same amount of taxa considered. This finding suggests that the smallest sampler size (40 mm in diameter) would be the most suitable for future samplings in this habitat, given the distribution of species, the minimum impact on this protected environment and the least time needed to sort each sample of this size.

Analysis of variance did not identify a significant effect of experimental areas. There was a considerable homogeneity in the distribution of taxa (Fig. 1, Table I) among areas. However, for three taxa, spatial distribution was significantly dependent on area: for polychaetes, echinoderms belonging to macrofauna and gammarid amphipods belonging to meiofauna, indeed area had a significant effect (Table I). The large spatial variation at this scale suggests the importance of using hierarchical sampling design at this site. This estimated spatial variation could be an important basis for designing conservation management at this MPA.

Results of cost-benefit analyses are summarized in table II for each taxon. The optimal number of replicate plots per area (determined from Eq. 1) was always less than two, because of the long time needed to sort the samples (Cr) rather than a high variability among areas. However, for some taxa the negative estimates of variance among areas (Sa^2) found did not allow to identify the most appropriate number of replicate cores and areas. The number of replicates was set at two to provide a

Table II. – Summary of cost-benefit analyses on macrofaunal and meiofaunal taxa.

	S_e^2	S_a^2	n	a	Sqrt V
Macrofauna					
Polychaetes	429,666	810,000	2	2	30,280
Molluscs	6,916	neg			
Nematodes	30,500	4,000	2	2	3,410
Oligochaetes	138,333	neg			
Echinoderms	6,333	19,083	2	2	4,546
Gammarid amphipods	165,250	197,583	2	2	15,456
Caprellid amphipods	47,666	15,250	2	2	5,212
Tanaids	6,250	neg			
other*	1,202	neg			
Meiofauna					
Polychaetes	5051,916	7089.499	2	2	91,392
Molluscs	13,500	neg			
Nematodes	515,666	485,666	2	2	24,791
Oligochaetes	3,689	2,105	2	2	1,740
Gammarid amphipods	15,833	52,666	2	2	7,525
Caprellid amphipods	10,333	neg			
Isopods	11,500	neg			
Tanaids	1,455	neg			
Ostracods	8,250	neg			
Harpacticoid copepods	15893,333	neg			

*Other taxa includes isopods, ostracods and cumaceans.

S_e^2, variance among replicates;

S_a^2, variance among areas;

n, optimal number of replicates;

a, optimal number of areas;

Sqrt V, estimated SE

minimum of replication in each area. From Eq. (2) and using the optimal number of replicate plots, the optimal number of replicate areas was determined. Results estimated a low optimal number always less than two because of the high relative contribution of Cr, as in Eq. 1. Although results have identified 2 replicate cores and 2 areas as the optimum sample size, in order to increase statistical power, larger samples will probably be needed to test hypotheses about spatial and temporal patterns in these assemblages.

In this paper we estimated spatial variation of invertebrates aggregated in coarse taxonomic levels, for both meiofaunal and macrofaunal components, so as they are commonly considered (Brown & Taylor 1999, Danovaro & Fraschetti 2002). Hence, abundance estimates and replication for future sampling designs suggested by the cost-benefit analyses, are likely to be appropriate for these taxonomic levels. In future monitoring program and experiments carried out at this site, following the design here drawn, the abundance of organisms with the same taxonomic resolution should be analysed, since some authors have already evidenced that results can be dependent on the taxonomic level considered (*i.e.* Somerfield & Clarke 1995, Pagola-Carte *et al*. 2002).

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ERRATA (VOLUME 54 2/3)

– La 1ere partie du tableau III de l'article de Goma *et al* qui n'avait pas été tirée, est à ajouter à la page 85 du volume 54(2/3)
The first part of Table III in the paper by Goma *et al* was not published. Please, add this first part (given hereafter) on page 85 (volume 54 2/3)

– La nouvelle figure 1-116 remplace la figure 1-116 de l'article Monnier *et al*, page 129. La placer à la page 129.
The new figure 1-116 should replace the figure on page 129 in the paper by Monnier *et al* in volume 54(2/3).

The editors apologize for this inconvenience.

Table III (First part). List of taxa identified in the studied sites. Bold: taxa which had abundance over 5% at least in one site. One asterisk: new taxa for Catalonian diatom flora (Cambra *et al.* 1991). Two asterisks: new taxa for the Iberian Peninsula (Aboal *et al.* 2003).

Achnanthes conspicua Mayer
Achnanthes exigua Grunow
Achnanthidium alteragracillima (Lange-Bertalot) Round & Bukh.**
Achnanthidium biasolettianum (Grunow) Round & Bukhtiyarova
Achnanthidium catenatum (Bílý & Marvan) Lange-Bertalot*
Achnanthidium latecephalum Kobayasi **
Achnanthidium minutissimum (Kützing) Czarnecki
Achnanthidium straubianum (Lange-Bertalot) Lange-Bertalot **
Adlafia bryophila (Petersen) Moser, Lange-Bertalot & Metzeltin
Adlafia minuscula var. *muralis* (Grunow) Lange-Bertalot
Amphipleura pellucida Kützing
Amphora inariensis Krammer
Amphora libyca Ehrenberg
Amphora montana Krasske
Amphora ovalis (Kützing) Kützing
Amphora pediculus (Kützing) Grunow
Amphora veneta Kützing
Aulacoseira dianchiensis Yang, Stoermer & Kociolek **
Aulacoseira granulata (Ehrenberg) Simonsen
Bacillaria paxillifera (Müller) Hendey
Brachysira neoexilis Lange-Bertalot
Caloneis bacillum (Grunow) Cleve
Caloneis molaris (Grunow) Krammer *
Caloneis silicula (Ehrenberg) Cleve
Cocconeis pediculus Ehrenberg
Cocconeis placentula Ehrenberg
Cocconeis placentula var. ***euglypta*** (Ehrenberg) Grunow
Cocconeis placentula var. ***lineata*** (Ehrenberg) Van Heurck
Craticula accomoda (Hustedt) Mann
Craticula ambigua (Ehrenberg) Mann
Craticula halophila (Grunow) Mann
Craticula molestiformis (Hustedt) Lange-Bertalot *
Cyclostephanos invisitatus (Hohn & Hellerman) Theriot, Stoermer & Hakansson *
Cyclotella atomus Hustedt
Cyclotella cyclopuncta Hakansson & Carter **
Cyclotella distinguenda Hustedt *
Cyclotella meduane Germain
Cyclotella meneghiniana Kützing
Cyclotella polymorpha Meyer & Hakansson **
Cyclotella radiosa (Grunow) Lemmermann
Cyclotella wuethrichiana Druart & Straub **
Cymatopleura solea (Brébisson) Smith
Cymbella amphicephala Naegeli
Cymbella delicatula Kützing
Cymbella excisa Kützing
Cymbella helvetica Kützing
Cymbella laevis Naegeli *
Cymbella lanceolata (Ehrenberg) Kirchner
Cymbella naviculiformis Auerswald
Cymbella tumida (Brébisson)Van Heurck
Cymbella turgidula Grunow *
Denticula tenuis Kützing
Diadesmis contenta (Grunow) Mann
Diatoma ehrenbergii Kützing
Diatoma mesodon (Ehrenberg) Kützing
Diatoma moniliformis Kützing *
Diatoma problematica Lange-Bertalot **
Diatoma vulgaris Bory
Diploneis elliptica (Kützing) Cleve
Diploneis oblongella (Naegeli) Cleve-Euler
Diploneis ovalis (Hilse) Cleve
Diploneis petersenii Hustedt *
Encyonema caespitosum Kützing
Encyonema lacustre (Agardh) Mills
Encyonema minutum (Hilse) Mann
Navicula cryptofallax Lange-Bertalot & Hofmann **
Navicula cryptotenella Lange-Bertalot
Navicula cryptotenelloides Lange-Bertalot **
Navicula erifuga Lange-Bertalot *
Navicula germainii Wallace **
Encyonema neogracile Krammer
Encyonema prostratum (Berkeley) Kützing
Encyonema silesiacum (Bleisch) Mann
Encyonopsis cesatii (Rabenhorst) Krammer
Encyonopsis microcephala (Grunow) Krammer
Entomoneis paludosa (Smith) Reimer
Eolimna minima (Grunow) Lange-Bertalot
Eolimna subminuscula (Manguin) Moser, Lange-Bertalot & Metzeltin
Epithemia adnata (Kützing) Brébisson
Eucocconeis laevis (Oestrup) Lange-Bertalot
Eunotia soleirolii (Kützing) Rabenhorst *
Fallacia insociabilis (Krasske) Mann
Fallacia monoculata (Hustedt) Mann
Fallacia pygmaea (Kützing) Stickle & Mann
Fallacia subhamulata (Grunow) Mann
Fistulifera saprophila (Lange-Bertalot & Bonik) Lange-Bertalot
Fragilaria arcus (Ehrenberg) Cleve
Fragilaria capucina Desmazières
Fragilaria capucina var. *amphicephala* (Kützing) Lange-Bertalot
Fragilaria capucina var. ***rumpens*** (Kützing) Lange-Bertalot
Fragilaria capucina var. *austriaca* (Grunow) Lange-Bertalot **
Fragilaria capucina var. *perminuta* (Grunow) Lange-Bertalot **
Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot
Fragilaria gracilis Oestrup *
Fragilaria nanana Lange-Bertalot *
Fragilaria parasitica (Smith) Grunow
Fragilaria tenera (Smith) Lange-Bertalot
Frustulia spicula Amossé
Gomphonema acuminatum Ehrenberg
Gomphonema affine Kützing *
Gomphonema angustum Agardh
Gomphonema clavatum Ehrenberg
Gomphonema gracile Ehrenberg
Gomphonema lateripunctatum Reichardt & Lange-Bertalot **
Gomphonema micropus Kützing *
Gomphonema minutum (Agardh) Agardh
Gomphonema olivaceum (Hornemann) Brébisson
Gomphonema parvulum (Kützing) Kützing
Gomphonema pseudoaugur Lange-Bertalot *
Gomphonema pumilum (Grunow) Reichardt & Lange-Bertalot *
Gomphonema rhombicum M. Schmidt *
Gomphonema tergestinum Fricke *
Gomphonema truncatum Ehrenberg
Gyrosigma attenuatum (Kützing) Rabenhorst
Gyrosigma nodiferum (Grunow) Reimer
Gyrosigma parkerii (Harrison) Elmore **
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski
Hippodonta hungarica (Grunow) Lange-Bertalot, Metzeltin & Witkowski
Karayevia clevei (Grunow) Round & Bukhtiyarova
Karayevia laterostrata (Hustedt) Kingston **
Kolbesia ploenensis (Hustedt) Kingston
Lemnicola hungarica (Grunow) Round & Basson
Luticola goeppertiana (Bleisch) Mann
Luticola mutica (Kützing) Mann
Luticola ventricosa (Kützing) Mann
Mastogloia smithii Thwaites
Mayamaea atomus (Kützing) Lange-Bertalot
Mayamaea atomus var. ***permitis*** (Hustedt) Lange-Bertalot
Mayamaea lacunolaciniata (Lange-Bertalot & Bonik) Lange-Bertalot **
Melosira varians Agardh
Meridion circulare (Greville) Agardh
Navicula antonii Lange-Bertalot **
Navicula capitatoradiata Germain
Navicula caterva Hohn & Hellerman *
Navicula cryptocephala Kützing
Nitzschia subacicularis Hustedt *
Nitzschia subcapitellata Hustedt *
Nitzschia supralitorea Lange-Bertalot
Nitzschia thermaloides Hustedt
Nitzschia umbonata (Ehrenberg) Lange-Bertalot

Figs 1-116. – LM. Scale bar = 10 µm. Figs 1-41: *Achnanthidium atomoides* (type material from river Ernz Blanche at Hessemillen, Luxembourg). Figs 1-14: raphe valves. Figs 15-17: girdle views. Figs 18-29: rapheless valves. Figs 30-41: two valves of the same individual. Figs 42-72: *A. atomoides* (material from river Nalón, Spain). Figs 42-49: raphe valves. Figs 50-52: rapheless valves. Figs 53-56: girdle views. Figs 57-72: two valves of the same individual. Figs 73-116: *A. atomus* (original material from type locality, Java). Figs 73-92: raphe valves. Figs 93, 94: two valves of the same individual. Figs 95-116: rapheless valves.

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