BENTHIC RECOVERY DURING OPEN SEA FISH FARMING ABATEMENT IN WESTERN MEDITERRANEAN, SPAIN

Running title: BENTHIC RECOVERY DURING FISH FARMING ABATEMENT

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ABSTRACT

Fish farming is an important source of organic matter input in coastal waters, which contributes to eutrophication. In this study, the macrofaunal benthic community was studied after the cessation of fish farming with the aim of improving our understanding of benthic succession and sediment recovery in a marine ecosystem. The results showed that the best environmental variables for assessing organic pollution were AVS and redox potential. Succession and recovery was best explained by a macrofaunal analysis based on community composition as well as for trophic groups. The patterns of recovery differed between each impacted station. For this reason, succession could not be accurately predicted due to the unique environmental parameters and the singular community functional structure of each location. The Azti Marine Benthic Index (AMBI) proved its validity for assessing pollution but did not distinguish, between successional stages.

Keywords: Benthic, succession, feeding guilds, trophic groups, organic enrichment, aquaculture.

INTRODUCTION

During recent decades, fish farming in the open sea has undergone almost exponential growth (FAO, 2004). Fish-farms produce a large quantity of wastes (Gowen & Bradbury, 1987), which results in the accumulation of organic matter on bottom sediments, causing severe modification of the physical and chemical characteristics of the benthic environment (Diaz & Rosenberg, 1995; Karakassis, Tsapakis, Hatziyanni, Papadopoulou & Plaiti, 2000).

Many studies have focused on processes related to the environmental impact produced by aquaculture, using macrofaunal analysis and measuring a great number of environmental variables (Karakassis et al., 2000; Pawar, Matsuda & Fujisaki, 2002). But very few studies have focused on the benthic recovery after fish farming cessation.

In previous studies of benthic recovery after fish farming cessation (Karakassis, Hatziyanni, Tsapakis & Plaiti, 1999; Brooks, Stierns, Mahnken & Blackburn, 2003; Pereira, Black, McLusky & Nickell, 2004) the recovery rates observed in the different experiments differed to a large extent. In Greece, (Karakassis et al., 1999), total benthic recovery had not been achieved after 23 months, while in British Columbia, Brooks et al. (2003) reported complete biological remediation after 6 months. At a Scottish sea loch, Pereira et al. (2004) found that sampled stations were highly to moderately disturbed after 15 months. In all these experiments recovery was considered to have been achieved when benthic fauna assemblages were similar to those of control stations.

The study was carried out at a fish farm located in the Mediterranean Sea on the SE coast of Spain. At the time of the study, fish culture had been practised for more than a decade, with a mean fish biomass of between 30 and 60 tonnes per year. From January 2001 to March 2003 the installation was progressively dismantled and fish were transferred to another farm located 3 km NE. The singularity and interest of this study is based on two facts: (1) fish culture abatement involved different groups of cages at different times, which enabled us to study, the way succession occurs before, during and after organic pollution abatement in different locations within a single site over 2 years; (2) for a period of two months, in the summer of 2002, production increased enormously as extra fish cages were deployed. This fact produced substantial disturbance in the surroundings, including the sampled stations, each of which was in a different stage of succession at the time of the disturbance. The aim of the study was to monitor the three different groups of fish cages of the same fish-farm, which were in different stages of succession.

MATERIALS AND METHODS

Location and sampling

The study area was located at Hornillo Cove, Águilas, SE Spain (Western Mediterranean) (Fig. 1). The cove has an area of approximately 700,000 m^2 with an average depth of 21 m and a maximum depth of 37 m.

Four stations were sampled. Replicates were taken over an area of 10 m from the anchoring point. Three stations (N, S, and P) corresponded to each of the different fish cage groups (Fig. 1). The reference station, F, was chosen outside the cave due to its biotic and physico-chemical resemblance with the other stations. The depth for each station was N: 14 m, S: 18 m, P: 15 m and F: 20 m.

The fish farm had produced guilthead sea bream (*Sparus aurata*) and sea-bass (*Dicentrarchus labrax*) since 1989. During the last year of full production (2000) the cultured fish biomass was around 12, 12 and 6 tonnes and feeding rate was 13, 12 and 2 metric tons of food per month for stations N, S and P, respectively. During abatement, the cultured fish biomass and feeding rate fluctuated (Fig. 2). The cages were removed in January 2001, July 2001 and March 2003, (N, S and P stations, respectively) and moved to the new area leased for fish farming.

Sampling was carried out in October 2001, January 2002, May 2002, October 2002, April 2003, July 2003 and November 2003. In the first survey, only stations N, S and P were sampled, while all four stations were sampled in subsequent surveys. Four replicate samples were taken from each station at every sampling time. All the samples were collected by scuba divers.

Physico-chemical analysis

Physico-chemical sediment parameters such as redox potential, ammonia, acid-volatile sulfides (AVS), organic matter, carbon-nitrogen ratio (C:N) and grain size were measured. For the granulometric analysis, sediment samples were first dried at 60 ºC and then sieved through a series of sieves on a mechanical sieve shaker (Buchanan, 1984). Redox potential was measured in sediment cores of 6 cm diameter and 25 cm length, which were immediately frozen. In the laboratory, cores were thawed, sliced into 2 cm sections and redox potential was measured with an Orion ORP 91-80 electrode. To measure ammonia, interstitial water was extracted from the top 2-3 cm of the sediment. The ammonia content was measured with an Orion 95-12 ammonia electrode. The sediment samples used for measuring sulfides were stored in plastic bags without air bubbles to prevent oxidation and were frozen until analysis. The sulfide content was extracted and measured following Allen's protocol (Allen, Fu & Deng, 1993). The organic matter content was measured by weight difference, heating dry sediment at 450ºC for 5 hours. The atomic C/N ratio was measured by Elemental Analyzer C, N mod. EA1108 by a Carlo Erba Instrument. Total organic carbon was also measured using the same equipment after a treatment with 2 N HCl and then drying at 105ºC.

A two-way factorial analysis of variance (ANOVA) was used for testing significant differences between stations and times for all the physico-chemical sediment parameters. ANOVA was performed after checking for normality with Kolmogorov Smirnov's test and homogeneity of variances with Levene's test. To achieve normality, values were $\log_{10}(x+1)$ transformed.

Biological analysis

Macrofaunal samples were taken using a hand grab (400 cm^2). Samples were washed through a 0.5 mm sieve. The remaining sediment was fixed in a 4% formalin buffered solution, separated into major faunal groups and stored in a 70 % alcohol solution for later identification. Determination of benthic groups was made to the lowest possible taxonomic level.

Using macrofaunal data, abundance and species richness were obtained. These parameters were used to calculate the faunal descriptive Shannon-Wiener index for measuring diversity.

The Azti Marine Biotic Index (AMBI) was calculated using the September 2004 species list. This index assesses environmental benthic quality by quantifying the occurrence of macrofaunal species. The species are divided into five groups according to their sensitivity to an increasing stress, obtaining a marine biotic index (BI). Because of the limitations of using such an index with discrete values, the continuous index, Biotic Coefficient (BC) was obtained (Borja, Franco & Pérez, 2000). AMBI was applied to see how well it assessed organic enrichment and to compare it with the other tools used.

Taxa that contributed < 4% to the total abundance were removed from the dataset and a Bray-Curtis similarity matrix (Bray & Curtis, 1957) was calculated after transformation of log(x+1). Non-parametric multidimensional scaling (MDS) ordination analysis (Clarke & Warwick, 1994) was performed to represent the similarity between the samples (MDS-A). The BioEnv routine was used to find which environmental parameters best explained the MDS pattern, based on the macrofaunal data. Percentage of organic matter, percentage of silt and clay (fines), ammonia, AVS, redox potential (measured between 0 and 2 cm at the sediment surface) and the C/N ratio were used as environmental variables. BIOENV was performed using Spearman’s rank correlation.

The SIMPER routine was applied to know which species are the most important in terms of their contribution to the similarity or dissimilarity and to identify indicator species in different stages of succession. Analysis was made with a cut-off of 90 %. Multivariate analyses were obtained with the statistical package Primer version 5.

Functionality was compared between the different stations and periods of time by grouping species according to their feeding guilds following Pearson's (2001) recommendations without considering absorbers due to our lack of knowledge of species with such a feeding habit. The trophic groups included: predators, surface deposit feeders, sub-surface deposit feeders, suspension feeders and grazers. Taxa were associated to their feeding guild according to bibliography as well as our own previous knowledge. Feeding guild abundance was plotted against time, as well as by MDS (MDS-F). The MDS was also based on a Bray-Curtis similarity matrix with a transformation of log(x+1).

RESULTS

Physico-chemical parameters

Organic matter did not show any marked trend during the studied period. The silt and clay percentage remained stable for all the stations, except N, where it decreased (Table

1). Ammonia values showed similar levels in stations N, S and F but where higher in P most of the time (Fig. 3). AVS values remained low in the reference station throughout the experiment compared with the impacted stations, where the values clearly fell, although at different rates (Fig. 4).

The redox potential depth was fairly stable at the reference station (F) and only towards the end of the surveyed period did it slightly decrease. In all the other stations, to a greater or lesser extent, redox potential showed a rising trend. At the last survey time all the redox values for the top 2 cm were positive or only slightly negative. Station N showed the lowest redox values (Fig. 5).

The atomic C/N ratio at station P presented the lowest values while the reference station showed the highest values for the most of the surveyed time. Organically enriched stations (N, S and P) showed an initial rise and then a fall to the initial levels (Table 1).

ANOVA pointed to significant temporal and spatial differences ($p<0.001$) for the physico-chemical sediment parameters analysed (Table 2). These results showed that the surveyed sediment parameters varied greatly in time as in space due to changes in organic input.

Biological analysis

A total of 17637 individuals corresponding to 184 taxa were collected. The organically enriched stations showed similar behaviour as far as the Shannon-Wiener is concerned, decreasing at first and ending with a marked increase, with values similar to the reference station (Fig. 6). The values recorded at the reference station remained fairly constant. The Shannon-Wiener index for stations N and S clearly decreased in the October 2002 survey.

According to the AMBI pollution classification (Table 3), the reference (F) and station N were unpolluted, except in October 2002. Station S was slightly polluted except in January 2002 and November 2003. The last two surveys classified station P as unpolluted.

Feeding guild abundance fluctuated in N, S and P, especially as regards the surface deposit feeder group (Fig. 7). Stations N and S showed the highest variation in the October 2002 survey, while at station P the most important peaks of surface deposit feeder abundance were observed in May 2002 and April 2003. At stations N, S and P the ratio between the different feeding guilds underwent substantial variations. At reference station (F), this ratio was more stable, only presenting a slight peak (corresponding to the predator group) in April and July 2003.

MDS based on species abundance (MDS-A) as well as MDS based on feeding guild abundance (MDS-F) indicated a clear trend for station N, followed by station S, to approximate the reference station. Station P showed a much lower degree of similarity with the rest of the stations, approximating the reference station much more slowly and fluctuating greatly in position (Fig. 8a, 8b). First surveys of station S (SA, SB and SC) share considerable similarities with mid-time surveys of station P (PB, PC, PD and PE). Station P during July and November 2003 surveys showed very low similarity to other stations. The difference was mainly due to the lack of some functional groups at station P during most of the surveys and at station S during the first surveys.

According to BioEnv, the variable that best explained the community pattern was the AVS with a correlation of 0.169, followed by ammonia with a correlation of 0.163 and C/N with a correlation of 0.111. The highest correlation was 0.303 and was obtained using these three environmental variables together.

SIMPER was applied using the individual abundance matrix, comparing every station and the different surveyed times. The analysis showed that polychaete families, Capitellidae, Spionidae and Nereidae (in this order), were the most representative taxa of the impacted stations when fish-farming was in operation. The taxa which best represented the reference station and impacted stations after abatement were the order Gammaridea, *Apseudes latreillei* (Tanaidacea) and the polychaete family Onuphidae. The polychaete family Sabellidae also seemed to be useful as an indicator of non-polluted areas, although to a lesser extent.

DISCUSSION

This work is the first study in the Western Mediterranean related with benthic recovery after open sea fish-farm abatement. Its relevance is based on the comparison of species abundance, trophic groups and a benthic index (AMBI) to assess benthic recovery.

As regards the physico-chemical parameters, AVS was the most suitable chemical parameter for assessing and monitoring eutrophication in fish farming, as Pawar et al. (2002) and Vita, Marin, Jiménez-Brinquis, Cesar, Marín-Guirao & Borredat (2004) also found. Redox potential was the second best variable, although in the BioEnv analysis, ammonia and C/N seemed to be more robust variables. Because this type of analysis does permit evaluation of the whole redox profile, the redox potential can on some occasions lead to misleading conclusions. The first abandoned station (N) did not seem to have recovered because the redox potential on the surface was still strongly negative, perhaps due to the existence of dead mats of the seagrass *Posidonia oceanica*, which would favour the accretion of limes and clays. However, dead mats may also act as a substrate and encourage benthic faunal establishment. BioEnv as well as ANOVA showed that the measured environmental parameters differed significantly in time and space, and presented low correlations with the macrofauna. Clearly, environmental parameters on their own could not explain the succession and recovery trends, although some parameters did help to throw light on the status of the benthic ecosystem.

The benthic index (AMBI) has been shown to be a useful tool for assessing organic enrichment, since it provides a quite accurate picture of the real situation.

The potential recovery of an area after organic enrichment depends on the abiotic and biotic factors that determine the benthic community and on the hypoxia and anoxia tolerance of the species involved (Diaz & Rosenberg, 1995). Such recovery seems to follow defined patterns (Pearson & Rosenberg, 1978; Diaz-Castañeda, Frontier & Arenas, 1993). Going against the classical paradigm of succession as a continuum, Karakassis et al. (1999) introduced a different perspective for the recovery process. These authors considered that recovery has the same "final point" as Pearson & Rosenberg (1978) proposed, but with a highly fluctuating rate that produces forward and backward movements, due to stochastic secondary perturbances in the ecosystem.

In our experiment, succession did not follow a predetermined temporal pattern according to trophic structure and MDS analysis. There was a considerable spatial variation, although each station seemed to tend to the same successional end. This observation coincides with that of Rosenberg, Agrenius, Hellman, Nilsson & Norling (2002) who found that the pioneering and mature benthic successional stages were predictable but not the intermediate stages.

Less stable habitats usually recover more quickly than stable habitats (Dernie, Kaiser & Warwick, 2003), and so this kind of environment studied was expected to make a rapid recovery. Since the impact of open sea fish farms is restricted to a relatively small perimeter around the cages, benthic recovery is made possible by the recolonization of nearby non-affected areas.

The recovery rate and the way in which it takes place can only be understood by taking into consideration the great physico-chemical variability in environmental parameters (Dernie et al., 2003). Furthermore, basic characteristics of the water area such as topography, hydrodynamic conditions, water turbidity presence or absence of a sharp temperature stratification and water exchange patterns should also be considered (Kraufvelin, Sinisalo, Leppäkoski, Mattila & Bonsdorff, 2001). These parameters give to each location an almost unique configuration that makes it difficult to compare with others. However, we propose that not only environmental variability influences the time of recovery, but that trophic structure (feeding guilds) may also play a major role in recovery processes.

MDS analysis and the Shannon-Wiener index pointed to a clear pattern of recovery in the impacted stations. Both analyses suggested that station N and S had fully recovered by the last survey, while station P had not. Even so, no indicators of heavy organic pollution, such as Capitellidae, Spionidae or Nereidae families, were found in the sampled community during the last survey. This fact leads us think that station P followed a way of succession that differed considerably from that followed by the other impacted stations.

Our results suggest that AMBI is appropriate for assessing organic pollution levels but not for evaluating community structure status.

MDS analyses based on abundance and on feeding guilds were performed because species diversity does not always necessarily reflect functional diversity. Ecosystem processes are affected by the functional characteristics of organisms involved, rather than by taxonomic identity (Loreau, Naeem & Inchausti et al., 2002). The number of functionally different roles represented in an ecosystem may be a stronger determinant of ecosystem processes than the total number of species, *per se* (Tilman, Knops, Wedin, Reich, Ritchie & Siemann, 1997; Hector, Schmid, Beierkuhnlein, Caldeira, Diemer, Dimitrakopoulos et al., 1999). The fact that both MDS-A and MDS-F showed a similar ordination of successional changes indicated that there were differences in species composition and abundance but also in trophic structure (feeding guilds). Functional groups provide a perspective of the ecological processes and the status of the ecosystem. MDS analysis based on functional groups, such as feeding guilds, could be used as a complementary tool for assessing organic enrichment impact.

The analysis based on feeding guilds through time provided useful information concerning organic matter perturbation. Fish farm impacted stations showed an unbalanced feeding guild ratio, where the surface deposit feeders were the prevailing group. However, the reference station remained with a relatively constant feeding guild ratio throughout the experiment. Feeding guild ratio stability seems to be a logical expectation for non-disturbed ecosystems with considerable diversity and species redundancy. This fact coincides with the view of Andrew & Hughes (2005), who found that feeding guild ratios between insects living in the same tree species was constant in pristine environments, even at different latitudes.

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TABLE LEGENDS

Table 1. Organic matter, % silt / clay (i.e. <63 µm) and C:N ratio data (mean ± SD, n=4). For location of sampling stations F, N, S and P, see Fig. 1.

Table 2. Two-way factorial ANOVA without replication on differences in physico-chemical sediment parameters among the effects, stations (df=3) and times (df=6).

Table 3. AMBI biotic coefficient (BC), biotic index (BI) and pollution classification. For location of sampling stations F, N, S and P, see Fig. 1.

Table 1

Date	Station	% Organic matter	% silt / clay	C:N
Oct 01	N	4.3 ± 1.29	14.5 ± 6.19	9.1 ± 0
	S	2.6 ± 0.38	5.5 ± 1.85	9.6 ± 0
	P	2.8 ± 1.16	3.4 ± 0.95	6.3 ± 0.53
Jan 02	F	1.9 ± 0.29	1.2 ± 0.15	19.4 ± 9.16
	N	8.3 ± 2.8	11.0 ± 2.18	12.4 ± 1.67
	S	4.3 ± 2.38	3.6 ± 0.57	24.0 ± 19.55
	P	6.4 ± 3.49	3.6 ± 0.58	10.4 ± 2.40
May 02	F	1.8 ± 0.13	1.5 ± 0.22	13.3 ± 3.45
	N	14.0 ± 1.98	14.7 ± 3.63	16.2 ± 5.06
	S	6.5 ± 3.07	5.5 ± 1.04	17.1 ± 4.48
	P	3.9 ± 0.31	4.5 ± 0.70	11.1 ± 2.48
Oct 02	F	1.6 ± 0.11	1.0 ± 0.48	13.9 ± 3.67
	N	5.3 ± 1.56	13.6 ± 6.10	8.6 ± 1.62
	S	3.4 ± 0.85	3.3 ± 0.82	7.5 ± 1.77
	P	2.1 ± 0.35	2.8 ± 0.53	13.0 ± 1.82
Apr 03	F	4.1 ± 0.33	2.9 ± 0.73	6.3 ± 1.23
	N	3.8 ± 0.50	5.5 ± 0.77	6.6 ± 1.31
	S	3.7 ± 1.02	3.7 ± 0.80	14.4 ± 5.87
	P	1.6 ± 0.56	1.5 ± 0.53	6.2 ± 1.70
Jul 03	F	3.9 ± 0.34	3.8 ± 0.97	7.8 ± 1.24
	N	4.0 ± 0.53	5.9 ± 2.49	6.3 ± 1.29
	S	3.5 ± 0.34	2.0 ± 0.22	4.2 ± 0.23
	P	3.2 ± 1.07	1.9 ± 0.53	3.7 ± 0.77
Nov 03	F	4.1 ± 0.47	4.0 ± 1.07	9.0 ± 0.67
	N	3.6 ± 0.42	3.8 ± 0.75	7.8 ± 1.42
	S	4.2 ± 0.25	4.4 ± 0.91	6.0 ± 0.44
	P	2.9 ± 0.21	1.6 ± 0.30	8.4 ± 4.80

Table 2

Variable	Source of variability	F	p
AVS	Station	107.748	<0.001
	Time	9.975	<0.001
Ammonia	Station	21.886	<0.001
	Time	34.165	<0.001
Redox (-2 cm)	Station	49.436	<0.001
	Time	16.757	<0.001
% organic matter	Station	51.944	<0.001
	Time	13.230	<0.001
% silt / clay	Station	176.353	<0.001
	Time	10.376	<0.001
C:N	Station	12.645	<0.001
	Time	28.564	<0.001

Table 3

Date	Station	BC	BI	Pollution Classification
Oct 01	N	0.694	1	Unpolluted
	S	1.882	2	Slightly polluted
	P	4.274	3	Moderately polluted
Jan 02	F	0.363	1	Unpolluted
	N	0.717	1	Unpolluted
	S	3.971	3	Moderately polluted
	P	5.582	6	Heavily polluted
May 02	F	1.087	1	Unpolluted
	N	1.057	1	Unpolluted
	S	3.156	2	Slightly polluted
	P	5.666	6	Heavily polluted
Oct 02	F	1.367	2	Slightly polluted
	N	2.436	2	Slightly polluted
	S	2.815	2	Slightly polluted
	P	5.323	5	Heavily polluted
Apr 03	F	1.106	1	Unpolluted
	N	1.015	1	Unpolluted
	S	2.305	2	Slightly polluted
	P	4.235	3	Moderately polluted
Jul 03	F	1.107	1	Unpolluted
	N	1.063	1	Unpolluted
	S	1.458	2	Slightly polluted
	P	0.138	0	Unpolluted
Nov 03	F	0.730	1	Unpolluted
	N	0.476	1	Unpolluted
	S	1.018	1	Unpolluted
	P	0.469	1	Unpolluted

FIGURE LEGENDS

Figure 1. Location of Hornillo Bay and sampling sites: N (37º 24’ 34.5” N, 1º 33’ 26.6” W), S (37º 24’ 30.2” N, 1º 33’ 28.9” W), P (37º 24’ 32.3” N, 1º 33’ 33.1” W) and F, (37º 24’ 33.3” N, 1º 32’ 35.9” W)

Figure 2. Feeding rate of the different fish cage groups from December 2000 until April 2003. Data of station P from January 2002 until April 2003 was estimated according to the number of fish cultured due to the inexistence of feeding rate data for this period of time

Figure 3. Temporal changes in ammonia concentration in the interstitial water extracted from the top 2-3 cm of sediment (mean ± SE, n=4)

Figure 4. Temporal fluctuations in acid volatile sulphides (AVS) in the top 2-3 cm of sediment (mean ± SE, n=4)

Figure 5. Temporal variation in REDOX profiles in the top 10 cm of sediment (mean, n=4)

Figure 6. Changes in Shannon-Wiener diversity index (mean ± SE, n=4)

Figure 7. Time variation of feeding guilds abundance for each station (mean, n=4)

Figure 8. a) MDS based on species at the different stations in different surveys. The MDS results have been grouped according to the cluster results, each assemblage showing approximately 60% or greater similarity. b) MDS based on feeding guilds of the different stations in different surveys. The MDS results have been grouped according to the cluster results, each assemblage showing approximately 75 % or greater similarity. The first letter of each point corresponds to the station (F, N, S and P) and the second one corresponds to the time. A: October 2001, B: January 2002, C: May 2002, D: October 2002, E: April 2003, F: July 2003, G: November 2003

Figure1

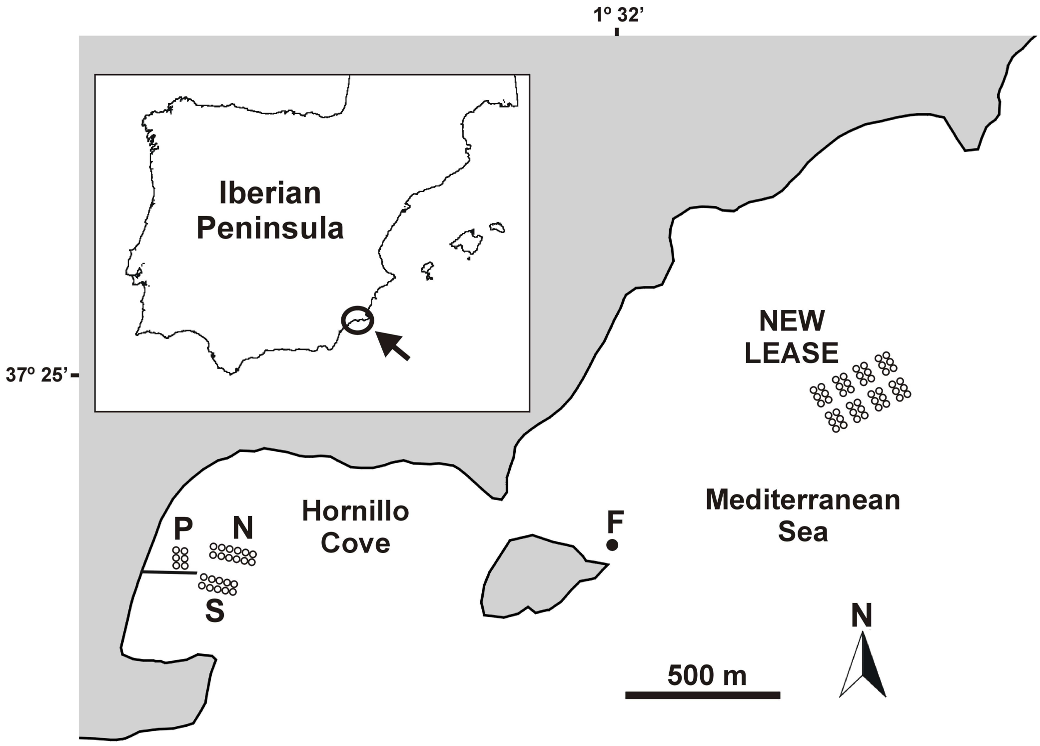


Figure 2

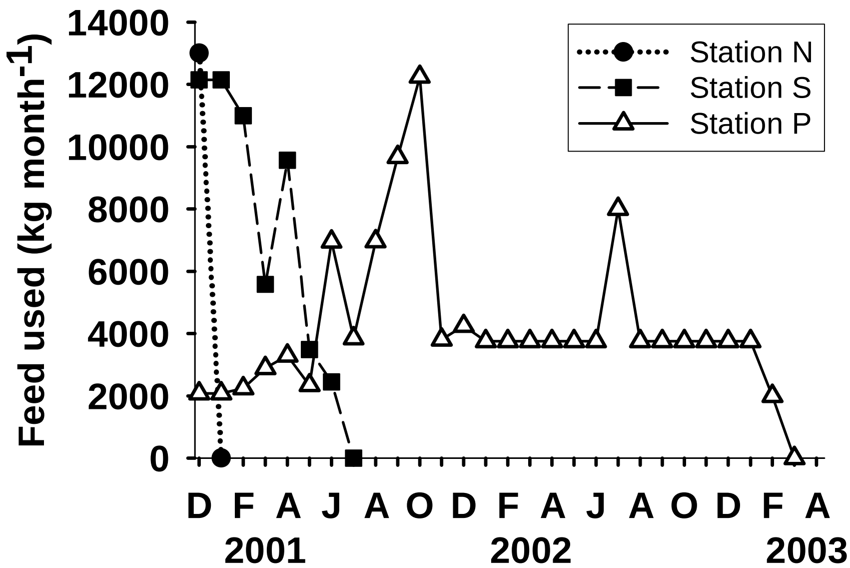


Figure 3

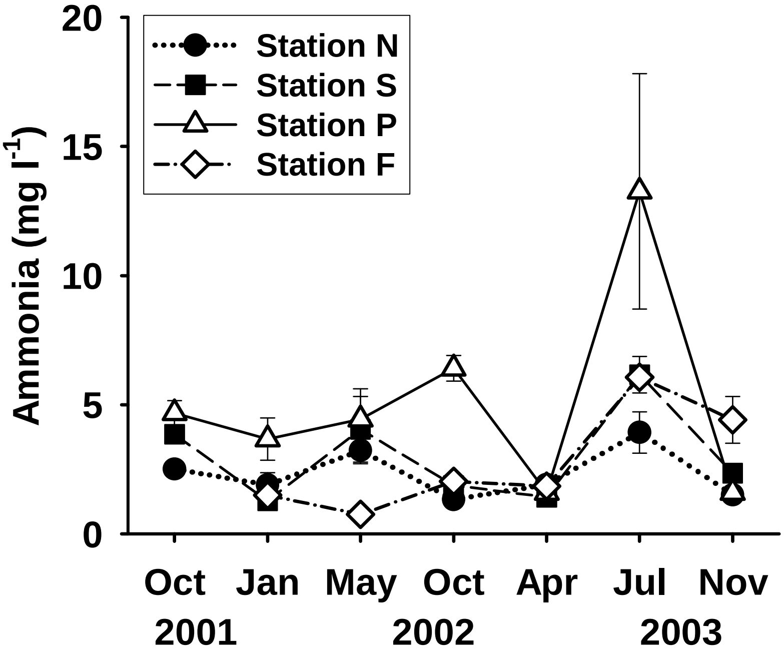


Figure 4

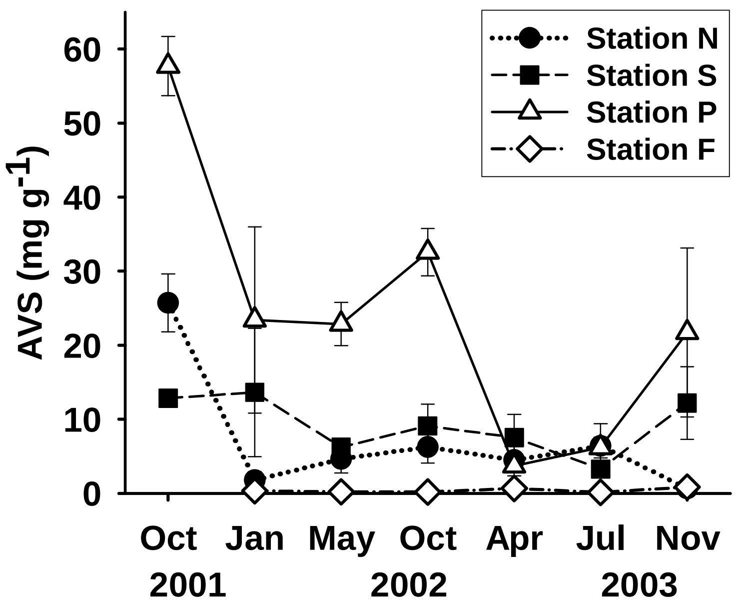
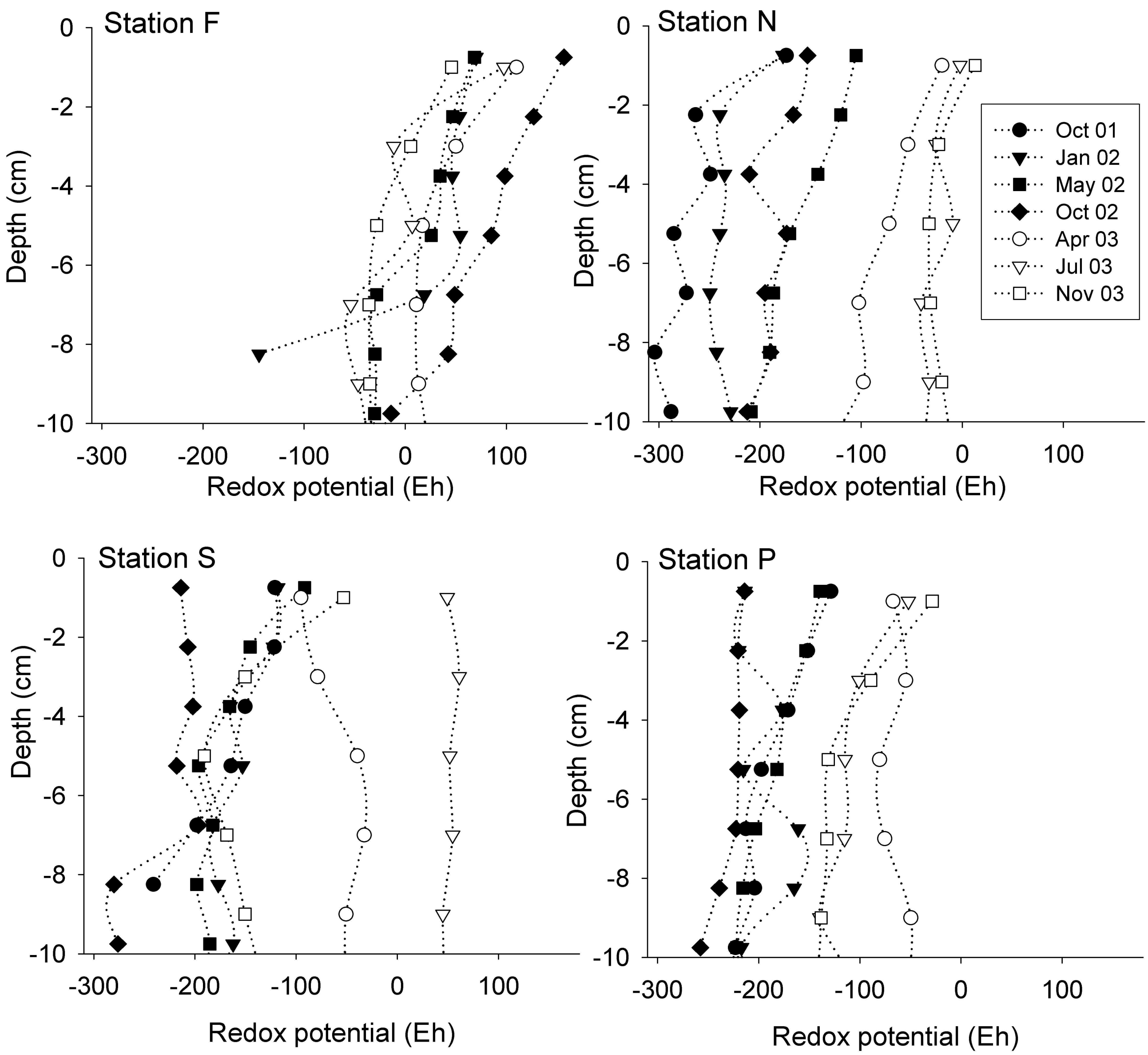
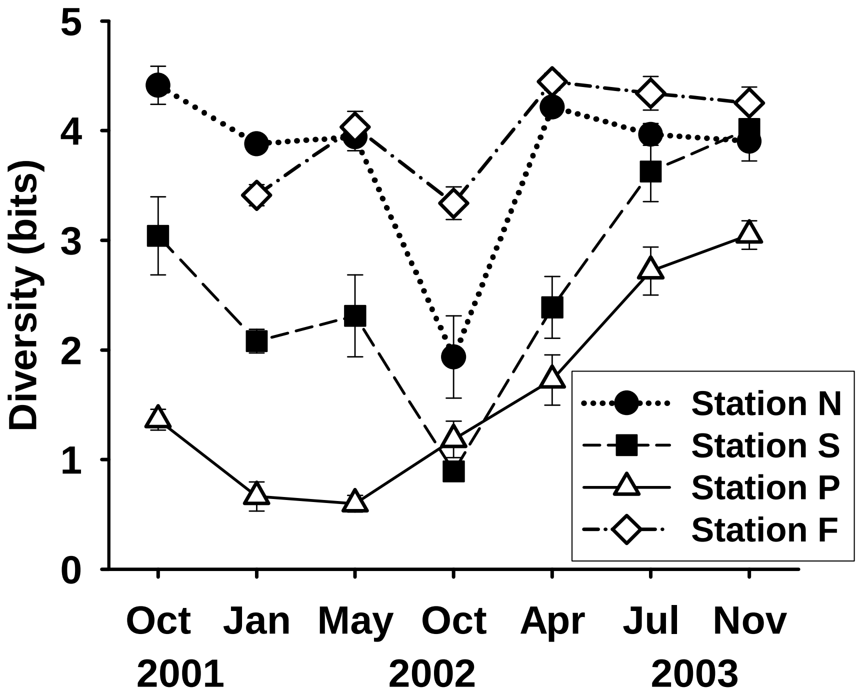


Figure 5



Station F
Station N
Station S
Station P
Depth (cm)
Redox potential (Eh)
0
-2
-4
-6
-8
-10
-300
-200
-100
0
100
Oct 01
Jan 02
May 02
Oct 02
Apr 03
Jul 03
Nov 03

Figure 6



Diversity (bits)
5
4
3
2
1
0
Station N
Station S
Station P
Station F
Oct
Jan
May
Oct
Apr
Jul
Nov
2001
2002
2003

Figure 7

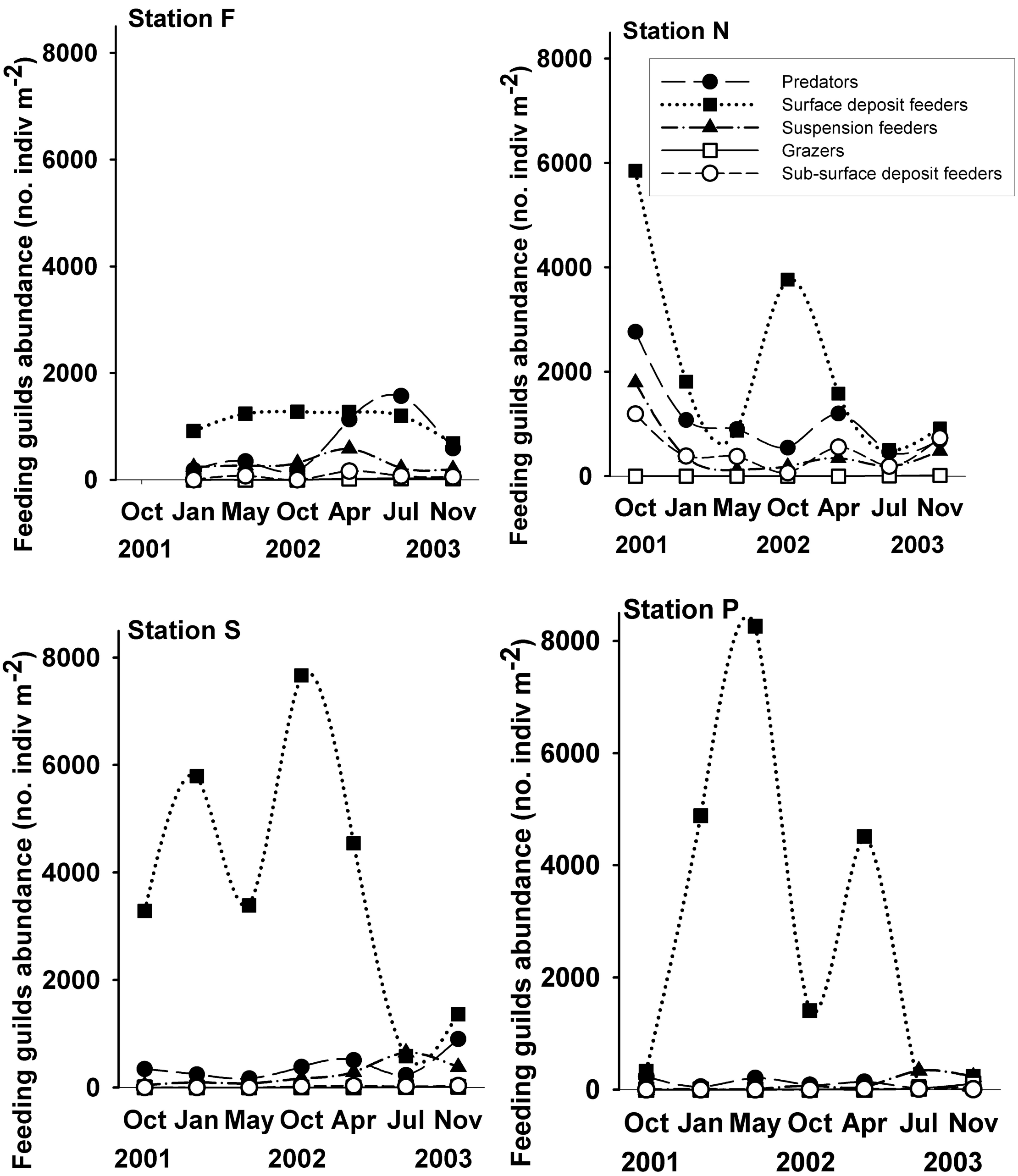
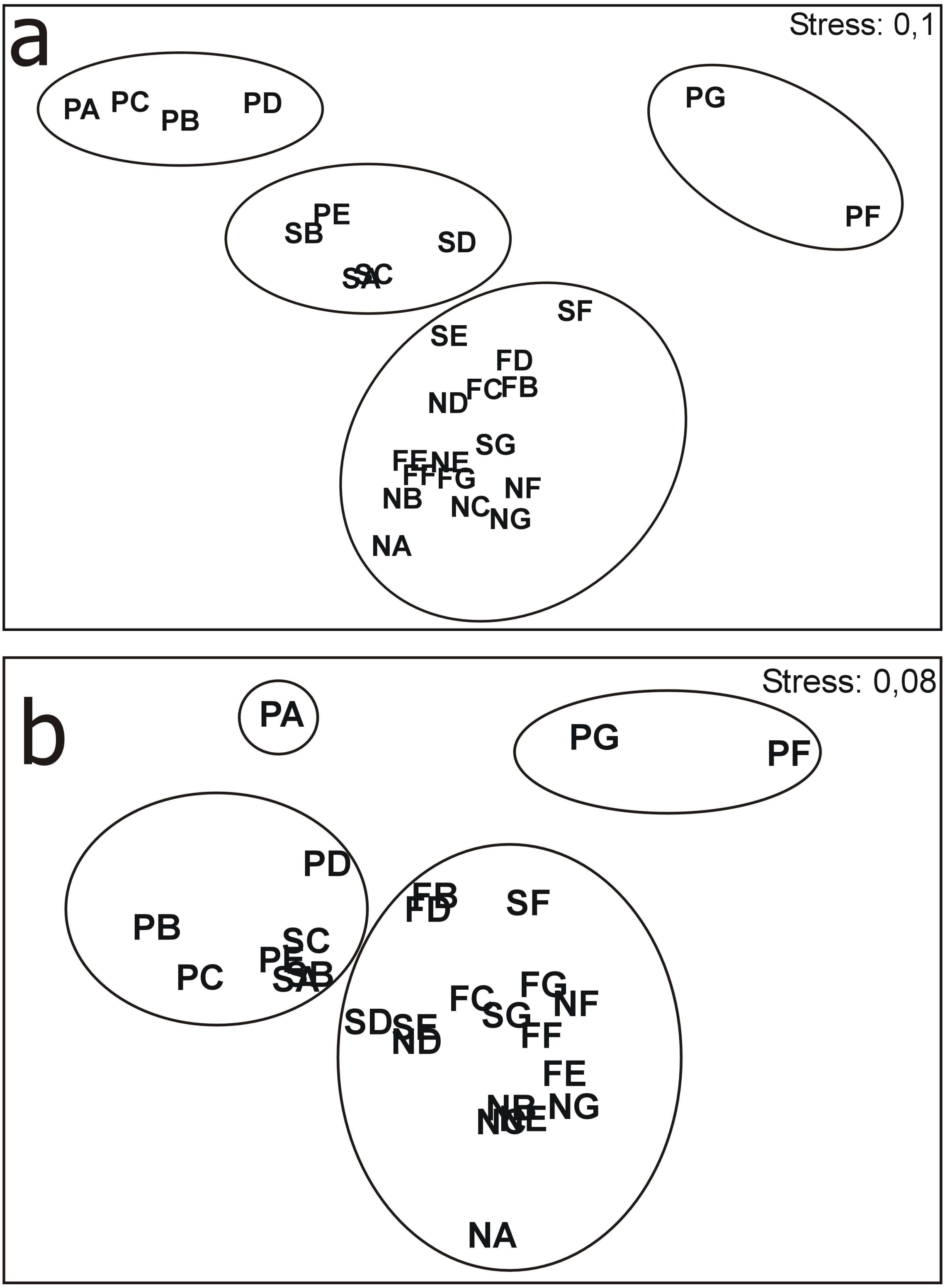

Station F
Station N
Station S
Station P
Feeding guilds abundance (no. indiv m-2)
0
2000
4000
6000
8000
Oct Jan May Oct Apr Jul Nov
2001 2002 2003
Predators
Surface deposit feeders
Suspension feeders
Grazers
Sub-surface deposit feeders

Figure 8


a
Stress: 0,1
PA
PC
PB
PD
PG
PE
SB
SD
SA
PF
SF
SE
FD
FC
FB
ND
SG
NB
NF
NC
NG
NA
b
Stress: 0,08
PA
PG
PF
PD
PB
SC
PC
SF
FC
FG
SG
NF
SD
FF
FE
NG
NA