Sensitivity of food webs to nitrogen pollution: a study of three transitional water ecosystems embedded in agricultural landscapes

R. Santoro[1*], F. Bentivoglio[1], P. Carlino[1], E. Calizza[1], M.L. Costantini[1], L. Rossi[1]

[1]Sapienza University of Rome, Department of Environmental Biology, via dei Sardi 70, 00185 Roma, Italy

*Corresponding author: Phone: +39-0649917802; e-mail address: rosaria. santoro@uniroma1.it

RESEARCH ARTICLE

Abstract

1 - Transitional water ecosystems constitute extremely complex and productive environments, a preferred route of migrating birds and optimal nursery locations for many fish species. In these interesting environments, descriptors of trophic relationships between species in a web have been suggested as potential integrative and sensitive endpoints for anthropogenic pressure.

2 - To better understand the effects of environmental disturbance on the resident macroinvertebrate assemblage structure, we analysed food webs in three neighbouring transitional water ecosystems located along the Thyrrenian coastal area of Central Italy (Lake Caprolace, Lake Fogliano and Lake Sabaudia), which are affected by different inputs of nitrates from agricultural, farming and urban activities.

3 - Macrozoobenthos, aquatic macrophytes and attached macroalgae, leaf detritus from sediments and suspended organic matter in the water column were sampled at four sites in each ecosystem and stable isotope analysis (C and N) was carried out in order to describe trophic pathways and the food web structure.

4 - $\delta^{15}N$ values of macroinvertebrates increased from Caprolace to Sabaudia according to increasing nitrate concentration derived from organic sources. Macrozoobenthic assemblages varied in species composition with the lowest biodiversity found in Sabaudia, the most polluted ecosystem. Also proportion of primary consumers, predators and omnivorous species varied, with cascade effects on food web topology and nutrient flow pathways.

5 - In particular, omnivorous and generalist predators were more numerous in Sabaudia, where food chains were longer and webs less compartmentalised than in Caprolace and Fogliano. In Sabaudia a lower level of web robustness to species loss was also observed.

6 - These results suggest that nitrogen pollution can be responsible of evident changes in the architecture of biodiversity between ecosystems, determining less robust trophic structures with strong implication for biodiversity management and conservation.

Keywords: transitional waters, macroinvertebrates, stable isotopes, nitrogen pollution, food webs

Introduction

Transitional waters are very complex ecosystems because of high spatial and temporal heterogeneity of environmental variables and associated biological structures (Basset et al. 2006; Chainho et al., 2007) and are among the most productive and man-impacted areas on Earth. In recent decades anthropogenic nutrient loading (in particular nitrogen) in these ecosystems has increased due to intensive land-use, such as agriculture, livestock production and urbanisation, contributing to estuarine and coastal eutrophication. Nutrient concentrations can influence also the feeding behaviour of aquatic animals (Fagan et al., 2002) with cascade effects on the decomposition rates (Menéndez et al., 2003) and matter recycling along the food web (Costantini and Rossi, 2010; Calizza et al., 2013). Thus, the description of the effect of nutrient loadings on the trophic relationships between species and biodiversity organisation represents a crucial step to understand how human activities affects ecosystem functioning and stability in transitional waters (Ponti et al., 2009; De Wit, 2007; Costantini and Rossi, 2010; Basset et al., 2013; di Lascio et al., 2013). In particular, the study of species interactions and food web structure enhances our ability to predict effects of pollution and anthropogenic pressure (Preziosi and Pastorok 2008, Clements and Rohr, 2009; Calizza et al., 2012) and the description of web topology can inform on community robustness to stressors and biodiversity loss, providing relevant information for biodiversity management and conservation (Dunne et al., 2002a; Lobry et al., 2008; O'Gorman and Emmerson, 2009).

C and N stable isotope analysis (S.I.A.) is increasingly applied to describe changes in community organisation and food web structure in aquatic ecosystems (Cabana et al., 1996; McClelland et al., 1997, McClelland and Valiela, 1998; Lake et al., 2001; McKinney et al., 2002; Cole et al., 2004), representing a powerful approach to depict changes in both nutrient pathways and interspecific interactions by starting from a trophic-functional description of ecological communities along environmental gradients (Post 2002, Layman et al. 2007, Calizza et al., 2012, 2013). $\delta^{15}N$ has been also used to distinguish between various sources of nitrogen pollution in different environments (Heaton, 1986; Costanzo et al., 2001; Schlacher et al., 2005; Hadwen and Arthington, 2007), since different sources can exhibit different $\delta^{15}N$ values. For example, high $\delta^{15}N$ signature in primary producers and animals can be associated with anthropogenic nutrient inputs (Pennock et al., 1996; Cole et al., 2004, Dailer et al., 2010, Orlandi et al., 2014). As discussed by Dailer at al. (2010) and Morrissey et al. (2013), treated sewage is relatively enriched in 15N, showing higher $\delta^{15}N$ values (from +6 ‰ to +38 ‰), due to the preferential use of 14N by the denitrifying bacteria used in wastewater treatment (Finlay and Kendall, 2007), than other nitrogen inputs such as inorganic fertilizers (ranging from -4 ‰ to +4 ‰; Owen, 1987; Dailer et al., 2010).

In the present work, we tested the hypothesis that both $\delta^{15}N$ values of macrozoobenthos and the structure of food webs varied with the levels of nitrogen inputs in three transitional water ecosystems differently affected by anthropogenic pressure.

Materials and methods

Study area and sampling activity

Study locations were Lake Caprolace, Lake Sabaudia and Lake Fogliano, which are located on the Tyrrhenian coast of central Italy (42°28'00" North - 12°51'00" East). Mean depths are 3 m, 2 m and 10 m, while surface areas are 3 km^2, 4 km^2 and 3.9 km^2 for Caprolace, Fogliano and Sabaudia,

respectively. Fogliano has two small water channels at the southern side, one connected to the sea and the other to River Rio Martino. Livestock farming activities are widespread in the surrounding land. Caprolace has two connections with the sea at the northern side while it does not receive water inputs from inlands since they are collected by a small circular independent water channel. Fogliano and Caprolace, which are located within the Circeo National Park and are relatively well-preserved, are designed as Sites of Community Importance (CSIs). Instead, Sabaudia, which is a private authority, receives nutrient inputs from the urban settlement of Sabaudia city (~20.000 inhabitants) and is intensely exploited for fishing and mussel farming. A channel at the southern side of this transitional water ecosystem represents the main connection with the sea. The increases in the rate of nutrient supply and the low water renewal, coupled with private management, have caused rapid eutrophication phenomena for decades (Brunelli, 1933; Brunelli and Cannicci, 1940; Milo, 1961; La Mura and Spezie, 1966; Milani, 2014). Based on nutrient inputs the three ecosystems can be ranked from Caprolace with low anthropogenic pressure, to Fogliano with intermediate anthropogenic pressure and, then, Sabaudia with high anthropogenic pressure.

Four sampling sites were selected within each ecosystem (Fig.1). Sampling occurred between April and May 2012. Macrozoobenthos was sampled by a bucket grab Van Venn (volume: 3.5 L) in three replicates for each sampling site and samples were transported into laboratory where specimens were sorted, counted and identified to the lowest possible taxonomic level. At each sampling site, macrophytes and algae were removed manually. In order to quantify suspended solids, 1 L of water was collected in wide-necked plastic bottles, in three replicates per sampling sites. Total suspended organic matter (hereafter, S.O.M.)

was determined by filtering water with a precombusted glass-fiber filter (yielding total suspended solids, TSS). Filters were then dried at 60 °C and stored over desiccant before stable isotopes analysis. Samples of bottom sediment were collected with 125 mL plastic jars, in three replicates per sampling sites. Coarse particulate organic matter (hereafter, detritus) in the sediments was also collected and rinsed in distilled water. All samples were stored at -80 °C and freeze dried before stable isotope analysis. Water salinity was measured at each site by an optical salinometer (Hand refractometer ATAGO), and has been expressed as Practical salinity scale (PSS). 1 L of water was collected at each sampling site in order to quantify nitrate concentration. Nitrate concentration analyses were performed by the Regional Environmental Protection Agency (A.R.P.A. provincial section of Latina) according to standard processing protocols (APAT-IRSA-CNR, 2003).

Stable isotope analysis (S.I.A.)

Living macroinvertebrates were starved for 24 h to empty guts, washed with distilled water and then conserved at -80 °C. All samples were freeze-dried and ground to a fine powder in a ball-mill (Mini-Mill Fritsch Pulverisette 23: Fritsch Instruments). 0.25 mg for animal samples and 2 mg for detritus, suspended solids, macrophyte and algal samples were placed in tin cups (in double replicate) for C and N stable isotopic analysis (SIA). Isotopic analysis was carried out using a continuous flow isotope ratio mass spectrometer (ISOPRIME 100) equipped with an elemental analyser (Elementar Vario Micro Cube). Isotopic ratios were expressed in "δ" units ($\delta^{15}N$, $\delta^{13}C$) in per mil (‰) difference from a standard [atmospheric N_2 for N; PD-Bedelemnite (PDB) carbonate for C]: δX (‰) = [(Rsample×Rstandard)/Rstandard] ×10^3 where X is ^{13}C or ^{15}N and R is the corresponding ratio ($^{13}C/^{12}C$ or $^{15}N/^{13}N$).
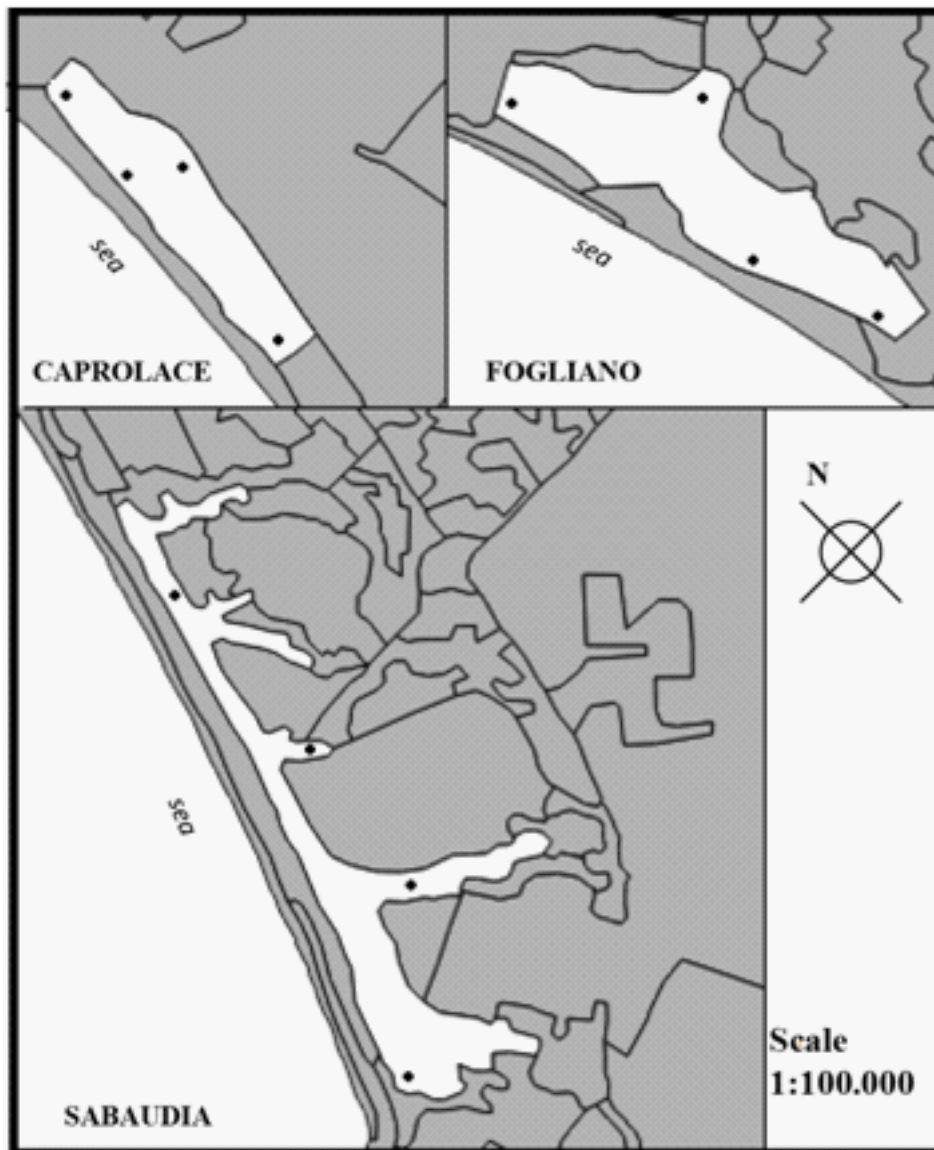
Figure 1.Sampling sites (black dots) in Caprolace, Fogliano and Sabaudia transitional water ecosystems, which are located along the Tyrrhenian coast of central Italy (42°28'00" North - 12°51'00" East). Grey areas indicate terrestrial environments.

Analysis of data

Data were tested for normal distribution and in conformity to assumption of variance homogeneity. Differences between mean values were tested by means of *one-way* ANOVA and *post-hoc* comparisons. Linear regression analysis was performed to determine the relationship between $\delta^{15}N$ values of macroinvertebrates and NO_3^- concentrations. Analysis of similarities (ANOSIM) was used to compare species assemblages between ecosystems (Fogliano, Caprolace and Sabaudia), and the contribution of each species to the observed similarity was calculated by SIMPER analysis. T-test was used to compare the Shannon index diversity among ecosystems, as described by Poole (1974) and Magurran (1988).

Rank-abundance curves were determined for each ecosystem (Fogliano, Caprolace and Sabaudia) and the differences between slopes were tested by *One-way* ANCOVA and associated test for slopes (F test for the equality of regression slopes). All statistical analyses were performed using the software R 2.15.2 and PAST 2.08. Reconstruction of the food web for each ecosystem was based on macroinvertebrate species diet as determined by SIAR, Stable Isotope analysis by software R-statistics (Parnell et al., 2008). The Bayesian Mixing Models were applied to the isotopic data (both $\delta^{15}N$ and $\delta^{13}C$) to assess the relative contributions of prey with distinct isotope signatures to predator diet. Fractionation values were obtained from literature (McCutchan, 2003). Based on mixing model outputs, food web binary matrices (0-1) were built for each sampling site within each location, with 1 indicating the occurrence of a trophic link between any given pair of taxa in each matrix. Food web 3D models were reconstructed for each sampling site within each ecosystem (food-web 3D software, Yoon et al., 2004). In order to investigate the potential effects of increasing anthropogenic pressure and nitrogen loading on the biodiversity organisation within each ecosystem (Fogliano, Caprolace and Sabaudia), the following food web parameters

were assessed at each sampling site: (i) total number of taxa (S), (ii) number of intermediate taxa (i.e. taxa both consuming and being consumed), (iii) number of top taxa (taxa consuming but not being consumed by others), (iv) the ratio between predator (taxa preying on other animal taxa) and prey taxa (taxa being preyed by other taxa), such ratio being indicative of the resource availability for predators, (v) number of linked paths (food chains) from detritus to intermediate and top consumers excluding loops, (vi) linkage density between taxa (L/S), where L is the total number of feeding links in the web, (vii) web robustness to species loss (Dunne et al., 2002a), (viii) food web compartmentalisation (Pimm, 1980), and (ix) the percentage of omnivorous taxa in each web, where an omnivorous taxon is defined as each taxon feeding on two or more different trophic levels (Pimm, 1982). Web robustness was obtained by sequentially deleting species from the most to the least connected species to the others in a web (Dunne et al., 2002a, 2002b).

Results

Water salinity was on average lower in Sabaudia than in the other two ecosystems, which did not differ from each other (Table 1; *One way* ANOVA, F=10.52, and *post hoc*

Table 1 - Means (± standard deviation) of chemical, isotopic and food web parameters in the three study ecosystems. Different letters (a, b or c) indicate a significant difference between ecosystems (*one-way* ANOVA and *post-hoc* comparison p<0.05) (no letters imply a not significant difference for a p value < 0.05).

Parameters	Caprolace	Fogliano	Sabaudia
Nitrates (µg/l)	12.25 ±2.89 a	42.37±61.31 ab	91.94±70.24 b
Salinity (PSS)	42.81 ± 1.19 a	41.85±2.27a	37.05±2.05 b
$\delta^{15}N$ of macroinvertebrates	3.62 ± 1.61 a	4.73 ± 1.55 ab	9.06 ± 0.45 b
Number of taxa	29.25 ± 5.48	27.00 ± 6.98	27.5 ± 9.11
Linkage density	1.73 ± 0.08	1.57 ± 0.29	1.95 ± 0.16
Compartmentalisation	0.27 ± 0.14	0.24 ± 0.11	0.11 ± 0.03
Web robustness	0.20 ± 0.07 a	0.13 ± 0.07 ab	0.07 ± 0.02 b
Mean chain length	1.36 ± 0.05 a	1.55 ± 0.11ab	1.83 ± 0.17b
Number of predator taxa (P)	3.50 ± 1.29 a	4.75 ± 0.95 ab	10.25 ± 5.67 b
Number of prey taxa (C)	21.75 ± 6.23 a	18.25 ± 5.12 ab	11.75 ±3.77 b
Predator-prey ratio (P/C)	0.16 ± 0.07 a	0.26 ± 0.03 ab	0.82 ± 0.19 b
Number of omnivorous taxa	0.00±0.00a	0.25 ± 0.50 b	5.25 ± 1.89 c

comparisons, all p<0.05), whereas nitrate concentration was highest in Sabaudia and lowest in Caprolace (*One way* ANOVA, F=5.43, and *post hoc* comparisons, all p<0.05). Nitrates were heterogeneously concentrated within the two most polluted ecosystems (Sabaudia and Fogliano), as indicated by the high standard deviation of nitrate values in these two systems. Mean nitrogen isotopic signature (δ^{15}N) of macroinvertebrates was highest in Sabaudia and lowest in Caprolace (*One way* ANOVA, F=18.93, and *post hoc* comparisons, all p<0.001), being positively related with nitrate inputs (d.f.=10, R^2=0.66, p<0.05). The composition of the species assemblages varied among ecosystems (see Appendix in the online supplemental material; ANOSIM, R=0.85; p<0.0005; *post hoc* pairwise comparisons,

p always <0.05). Species that mostly contributed to the observed dissimilarity were *Gammarus* sp. (14.0 %), *Corophium* sp. (11.0 %), *Paranemonia cinerea* (10.7 %) and *Idotea baltica* (9.8 %). Species diversity, assessed on the mean species abundance per ecosystem, as the Shannon-Wiener diversity index (Hs), was lower in Sabaudia than Fogliano and Caprolace (Hs: 2.33 vs 2.60 vs 2.64; pairwise Shannon-Wiener T-test, p<0.0001 in both comparisons). No difference existed between the other two ecosystems. Consistently, the slope of the rank-abundance curve describing each species assemblage was significantly higher in Sabaudia than in the other two ecosystems (Fig.2; *One-way* ANCOVA associated test for Homogeneity slopes F=9.94, p<0.0001). Food webs and the related parameters
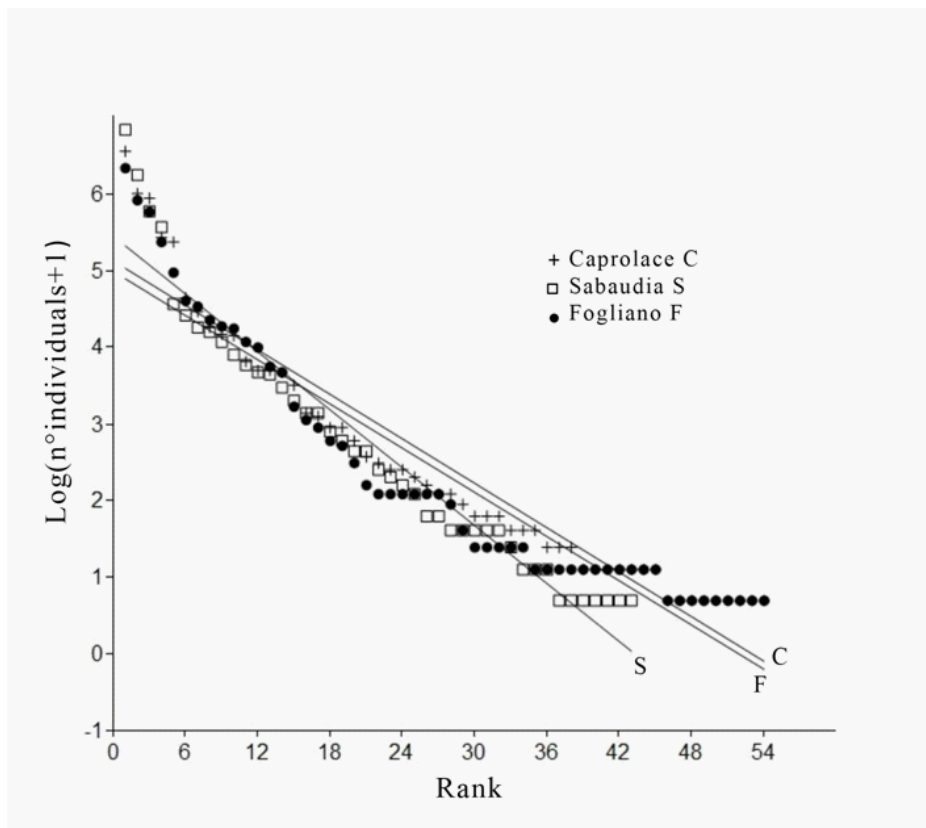

Figure 2. Rank abundance curves based on taxa abundances. Absolute value of the slopes of the rank abundance curves are -0.096 (Caprolace), -0.0096 (Fogliano) and -0.12 (Sabaudia). Taxa are ranked from the most to the least abundant within each ecosystem.

were determined at each sampling sites, but for brevity only average values are reported in Table 1 and the metawebs cumulating all taxa and feeding links in each ecosystem are showed in Fig.3.

The number of predator taxa decreased with water salinity (y=-1.10x+50.91, d.f.=10, R^2=0.64, p<0.05), whereas the number of prey taxa decreased with nitrate concentration (y=-0.063x+20.33, d.f.=10,
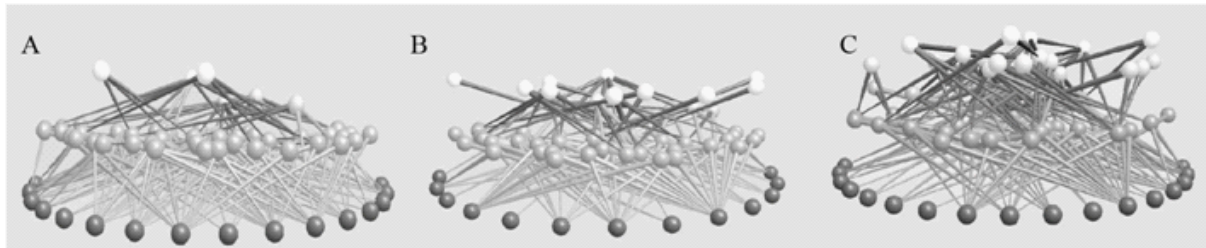

Figure 3. 3D metawebs for (A) Caprolace, (B) Fogliano and (C) Sabaudia. Metawebs integrate the occurrence of taxa and feeding links across the four sampling sites within each transitional water ecosystem, thus representing the trophic structure at the ecosystem-scale level. Black nodes: basal taxa; grey nodes: intermediate taxa; white nodes: top-taxa. Software producing 3D food web by Yoon et al., 2004. Sticks represent trophic links between nodes.

R^2=0.35, p<0.05).Globally, while the total number of taxa did not differ between ecosystems (*One way* ANOVA, n.s.), the predator-prey ratio was higher in Sabaudia than in Fogliano and Caprolace (Table 1, *One way* ANOVA, F=34.75, and post hoc comparisons, all p<0.001). From Caprolace, the less N-polluted ecosystem, to Sabaudia, the most N-polluted ecosystem, both the percentage of omnivorous taxa (i.e. taxa feeding on more than one trophic level) and the mean food chain length increased (Table 1, *One way* ANOVA, F=16.14, and post hoc comparisons, all p<0.001), whereas food web compartmentalisation and web robustness to species loss decreased (Table 1, *One way* ANOVA, F=5.08, and *post hoc* comparisons, all p<0.05).

Discussion and Conclusion

Transitional water ecosystems are complex environments, highly dependent on the surrounding terrestrial habitats (Basset et al., 2006). In our study ecosystems, consistent with increasing anthropogenic pressure, we

observed an increase in nitrate concentration affecting the water bodies, with Sabaudia presenting the highest values and Caprolace the lowest. Nitrate concentration was positively related with the nitrogen isotopic signatures of macroinvertebrates, being indicative of organic nitrate loadings (Bergfur et al., 2009; Dailer et al., 2010) and also showing that anthropogenic N loading 'propagates' from the water column to predators through autotrophs and their detritus along food chains. Therefore, isotopically distinct webs were identified in the three ecosystems, depending on the different isotopic baselines rather than the observed different species compositions. Identity and abundance of species have been shown to vary naturally in transitional waters, given the macroinvertebrate sensitivity to the different sources of abiotic heterogeneity of this type of ecosystems, such as salinity (Cognetti and Maltagliati, 2000), sediment composition and granulometry (Teske and Wooldridge, 2003; Reizopoulou and Nicolaidou, 2004), type of vegetation

(Arocena, 2007), surface area (Sabetta et al., 2007) and hydrology (Barbone and Basset, 2010). In our cases, unlike $\delta^{15}N$ values, macroinvertebrate diversity and the slopes of rank-abundance curves differed between Sabaudia and the other ecosystems but not between Fogliano and Caprolace, suggesting that diversity metrics were less sensitive than tropho-chemical parameters, such as the nitrogen isotopic value, to moderate levels of anthropogenic impact (Calizza et al., 2012; di Lascio et al., 2013). Regardless of species richness and diversity, both proportion of species between trophic levels and their feeding strategies (i.e. primary consumers, predators, omnivores) varied with nitrate concentration. The reduced number of prey and increased percentage of generalist predators in Sabaudia, the most polluted ecosystem, is consistent with the expected under disturbed conditions (O'Gorman and Emmerson, 2009; Calizza et al., 2012; Calizza et al., 2013). In the same way, the higher level of omnivory observed in Sabaudia in comparison with the less polluted ecosystem can be explained by lower prey availability for predators (Kondoh and Ninomiya, 2009; Calizza et al., 2012), which can also explain the longer mean food chain as a result of intraguild predation (i.e. predators feeding on other predators). Although a food web is an over-simplification of biological reality (Polis, 1991), if description comes from reliable techniques, it can be a useful tool to intercept important environmental anomalies affecting communities within a type of habitat (Winemiller et al., 2011; Abrantes et al., 2014). Sabaudia presented the lowest level of web compartmentalisation, due to the occurrence of the high percentage of omnivores interconnecting multiple food chains and trophic levels. Low compartmentalisation and long food chains have both profound implications for food web persistence (Post, 2002; Montoya et al., 2006;

Vander Zanden and Fetzer, 2007; Stouffer and Bascompte, 2011). In particular, since the presence of compartments slows down the propagation of a disturbance in a web, thus increasing the long-term persistence of communities (Stouffer and Bascompte, 2011), the low compartmentalisation can well explain the low level of web robustness to the loss of biodiversity observed in Sabaudia. In this perspective, changes in food web topology represent a key aspect linking anthropogenic pressure and ecosystem vulnerability to biodiversity loss (Dunne et al., 2002). Given the observed differences between the three study ecosystems, the macroinvertebrate communities in Sabaudia can be considered structurally more fragile than the other, less polluted, ecosystems. In conclusion, our results suggest that increasing anthropogenic pressure and associated high nutrient loadings can affect food web topology and dynamics in transitional waters. In particular, increasing nitrogen inputs are associated with increasing omnivory, with important implication for the trophic behaviour of predators and consequent cascade effects on food web structure and stability-related topological aspects (Dunne et al., 2002; Stuoffer and Bamcopte, 2011; Calizza et al., 2012; Calizza et al., 2013). Therefore, the inclusion of food web study in monitoring and conservation plans seems to represent a promising way to increase the comprehension of the effects of human pressure and environmental changes on the organisation, functioning and vulnerability of the communities inhabiting transitional waters.

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Appendix A - List of taxa found in Fogliano (F) Caprolace (C) and Sabaudia (S). Mean and standard deviation (st.dev.) of taxa sampled in each ecosystem. "mean" indicates the mean number of individuals on the four sites in each ecosystem. The percentage (%) of species assemblage variability across the three ecosystems, explained by each taxon, is reported in accordance with SIMPER analysis. Taxa are ranked according to their contribution to the observed species assemblage variability.

Abundance matrix	F		C		S		
Taxa	mean	st.dev.	mean	st.dev.	mean	st.dev.	%
Gammarus sp.	91.50	150.93	25.50	29.46	229.25	276.70	14.1
Corophium sp.	78.50	132.40	99.75	103.29	127.00	75.43	11.04
Paranemonia cinerea	14.25	6.34	173.00	127.21	0.75	1.50	10.77
Idotea baltica	139.00	113.91	17.25	29.88	0.00	0.00	9.67
Mytilaster sp.	0.00	0.00	94.00	177.34	64.25	67.94	8.62
Cymodoce truncata	53.00	72.97	53.00	29.18	12.00	24.00	4.91
Amphiura sp.	0.00	0.00	9.75	15.20	79.25	157.83	3.93
Gibbula sp.	1.75	3.50	56.25	19.79	0.00	0.00	3.6
Gammarus insensibilis	35.50	41.02	0.00	0.00	0.00	0.00	2.77
Cerastoderma sp.	13.25	11.79	0.25	0.50	23.50	45.68	2.54
Hydrobidae	24.50	49.00	0.50	1.00	9.50	19.00	2.03
Glyceridae	9.50	16.36	21.25	22.51	0.00	0.00	1.77
Capitellidae	19.00	38.00	0.00	0.00	5.50	9.04	1.73
Chironomidae	22.75	35.64	0.00	0.00	5.50	6.81	1.55
Abra ovata	17.00	14.00	1.75	1.26	0.00	0.00	1.26
Capitella capitata	1.75	2.06	15.50	18.77	1.75	3.50	1.17
Idotea sp.	17.50	35.00	3.75	7.50	0.00	0.00	1.15
Nereidae	3.75	5.19	5.25	3.40	20.25	16.76	1.11
Nassarius sp.	0.00	0.00	2.50	4.36	14.25	16.82	1.05
Gastropoda (alia)	0.00	0.00	0.00	0.00	16.25	16.09	1.02
Bulla striata	1.75	3.50	0.50	1.00	17.25	16.60	0.98
Erichthonius sp.	0.50	1.00	15.75	17.10	0.00	0.00	0.97
Sagartia troglodytes	6.00	10.68	0.00	0.00	10.50	16.11	0.95
Spionidae	10.25	13.05	2.00	4.00	4.25	4.19	0.77
Bittium sp.	0.00	0.00	9.50	19.00	0.00	0.00	0.77
Hippolyte sp.	0.00	0.00	11.00	13.32	0.00	0.00	0.74
Anemonia viridis	4.50	4.80	9.75	12.97	0.00	0.00	0.72
Sagartia sp.	0.00	0.00	0.00	0.00	7.75	15.50	0.67

Appendix A - Continued

Carcinus aestuarii	5.00	10.00	2.50	3.79	1.00	2.00	0.55
Hippolyte longirostris	0.00	0.00	8.00	16.00	0.00	0.00	0.5
Dexamine spinosa	1.75	3.50	4.50	7.14	0.00	0.00	0.44
Nemertea	1.00	0.82	0.50	1.00	6.50	12.34	0.44
Pseudoprotella sp.	0.00	0.00	0.00	0.00	9.25	18.50	0.38
Hippolyte inermis	0.00	0.00	5.50	6.45	0.00	0.00	0.37
Chamelea gallina	0.00	0.00	4.50	5.92	1.00	1.41	0.3
Cyclope neritea	3.50	6.35	0.75	0.96	0.00	0.00	0.29
Bouganvilia sp.	0.25	0.50	3.00	6.00	0.00	0.00	0.26
Cirratulidae	0.00	0.00	0.00	0.00	3.25	4.72	0.24
Cereus peduncolatus	2.75	5.50	0.00	0.00	0.00	0.00	0.23
Terebellidae	0.00	0.00	2.75	3.77	1.25	1.26	0.21
Lumbrinereidae	0.00	0.00	1.25	1.50	3.25	6.50	0.21
Oligochaetae	0.50	1.00	0.00	0.00	2.50	5.00	0.18
Bulla sp.	0.00	0.00	0.00	0.00	3.75	5.68	0.18
Eteone sp.	0.50	1.00	2.25	4.50	0.00	0.00	0.17
Sphaeroma sp.	0.00	0.00	0.50	1.00	2.00	2.45	0.17
Palaeomon sp.	0.00	0.00	1.25	1.50	0.50	1.00	0.15
Palaeomon elegans	1.75	1.71	0.75	1.50	0.50	0.58	0.14
Cucumaria grubei	0.00	0.00	1.75	2.06	0.00	0.00	0.13
Cerithium vulgatum	0.75	1.50	1.50	2.38	0.00	0.00	0.13
Ficopomatus enigmaticus	0.00	0.00	0.00	0.00	2.25	3.30	0.12
Aplysia sp.	0.50	1.00	1.00	2.00	0.00	0.00	0.11
Phyllodocidae	1.75	2.36	0.25	0.50	0.00	0.00	0.11
Palaeomon serratus	2.00	2.83	0.00	0.00	0.00	0.00	0.1
Conus sp.	0.00	0.00	1.00	1.41	0.00	0.00	0.08
Asterina sp.	0.00	0.00	0.50	1.00	1.00	2.00	0.08
Loripes lacteus	0.25	0.50	1.25	1.89	0.00	0.00	0.08
Sphaeroma serratum	1.50	3.00	0.00	0.00	0.00	0.00	0.08
Amphipholis squamata	0.75	1.50	0.00	0.00	0.00	0.00	0.07
Hippolyte leptocerus	0.00	0.00	1.00	2.00	0.00	0.00	0.06
Nematoda	0.00	0.00	0.75	1.50	0.25	0.50	0.06
Syllidae	0.75	1.50	0.00	0.00	0.00	0.00	0.06
Upogebia tipica	0.75	1.50	0.00	0.00	0.00	0.00	0.06
Cyathura carinata	0.00	0.00	0.00	0.00	1.00	1.15	0.06
Melita sp.	0.00	0.00	0.00	0.00	1.25	2.50	0.05
Idotea chelipes	0.25	0.50	0.50	1.00	0.00	0.00	0.05
Tapes decussatus	0.75	0.96	0.00	0.00	0.00	0.00	0.05
Zenobiana prismatica	0.50	1.00	0.25	0.50	0.00	0.00	0.05
Arenicola marina	0.50	1.00	0.00	0.00	0.00	0.00	0.04
Eulalia viridis	0.00	0.00	0.00	0.00	1.00	2.00	0.04
Harmothoe sp.	0.00	0.00	0.50	1.00	0.00	0.00	0.04

Appendix A - Continued

Abarenicola sp.	0.50	0.58	0.00	0.00	0.00	0.00	0.04
Neanthes sp.	0.50	1.00	0.00	0.00	0.00	0.00	0.04
Perinereis sp.	0.50	1.00	0.00	0.00	0.00	0.00	0.04
Hexaplex trunculus	0.00	0.00	0.25	0.50	0.00	0.00	0.03
Nainereidae	0.50	1.00	0.00	0.00	0.00	0.00	0.03
Palaeomon xiphias	0.50	0.58	0.00	0.00	0.00	0.00	0.03
Serpulidae	0.00	0.00	0.00	0.00	0.50	0.58	0.03
Balanus sp.	0.00	0.00	0.00	0.00	0.25	0.50	0.02
Flabelligeridae	0.00	0.00	0.25	0.50	0.00	0.00	0.02
Venerupis sp.	0.25	0.50	0.00	0.00	0.00	0.00	0.02
Actinia equina	0.00	0.00	0.25	0.50	0.00	0.00	0.02
Cerithium sp.	0.00	0.00	0.25	0.50	0.00	0.00	0.02
Ciona sp.	0.00	0.00	0.00	0.00	0.25	0.50	0.02
Liocarcinus sp.	0.25	0.50	0.00	0.00	0.00	0.00	0.02
Sabellidae	0.00	0.00	0.25	0.50	0.00	0.00	0.02
Seila trilineata	0.25	0.50	0.00	0.00	0.00	0.00	0.02
Spisula subtruncata	0.25	0.50	0.00	0.00	0.00	0.00	0.02
Sternaspidae	0.00	0.00	0.00	0.00	0.25	0.50	0.02
Stylochus sp.	0.00	0.00	0.25	0.50	0.00	0.00	0.02
Carcinus mediterraneus	0.25	0.50	0.00	0.00	0.00	0.00	0.01
Microdeutopus sp.	0.25	0.50	0.00	0.00	0.00	0.00	0.01
Scrobicularia sp.	0.00	0.00	0.00	0.00	0.25	0.50	0.01
Xantho sp.	0.00	0.00	0.00	0.00	0.25	0.50	0.01