

## Effect of multiple disturbances on food web vulnerability to biodiversity loss in detritus-based systems

EDOARDO CALIZZA,† M. LETIZIA COSTANTINI, AND LORETO ROSSI

*Laboratory of Trophic Ecology, Department of Environmental Biology, Sapienza University of Rome,  
Via dei Sardi 70, 00185-Rome, Italy*

**Citation:** Calizza, E., M. L. Costantini, and L. Rossi. 2015. Effect of multiple disturbances on food web vulnerability to biodiversity loss in detritus-based systems. *Ecosphere* 6(7):124. <http://dx.doi.org/10.1890/ES14-00489.1>

**Abstract.** Global biodiversity is affected by human pressure and climate change, and the present rate of biodiversity loss is probably higher than ever before. Community composition is also changing, and interspecific interactions are under severe pressure. The extinction of one species within a food web can result in further secondary extinctions, due to bottom-up effects that can be even more intense and less predictable than the direct effects of disturbance, undermining our capacity for ecosystem management and conservation. Here we investigated a metric for assessing the structural stability of food webs in the face of species loss, referred to as “Resistance”, based on two fundamental web properties: (1) the proportion of key species in the web, a “key” species being one whose deletion leads to at least one secondary extinction, and (2) the mean number of secondary extinctions observed per key species deletion. We compared web Resistance with web Robustness (Dunne et al. 2002) based on 12 detritus-based riverine food webs under four species extinction scenarios on various temporal and spatial scales. We investigated the effect of multiple disturbances (extreme flood and river basin urbanization) on community vulnerability to biodiversity loss, assessing the behavior of Robustness and Resistance under the applied species extinction scenarios and testing their dependence on web topology. We estimated the contribution of the rarest and the most dominant species, and that of the most and least connected species, to web Resistance.

Urbanization negatively affected community vulnerability to biodiversity loss. Only food web Resistance showed a significant flood effect and interaction between flood and urbanization. The most connected species contributed the most to food web resistance, whereas the rarest and the most abundant species had a similar, intermediate structural importance. Both food web Resistance and the role of selected key species varied across web description scales. Food web Resistance values were coherent across species extinction scenarios, demonstrating the suitability of the proposed approach for quantifying community vulnerability to species loss and the importance of considering food webs in monitoring and impact assessment programs. The approach is thus seen to be a promising research pathway supporting ecosystem management.

**Key words:** biodiversity loss; central Italy; flood; freshwater management; macroinvertebrates; resistance; river; robustness; urbanization.

**Received** 5 December 2014; revised 5 February 2015; accepted 12 February 2015; final version received 31 March 2015; **published** 29 July 2015. Corresponding Editor: D. P. C. Peters.

**Copyright:** © 2015 Calizza et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. <http://creativecommons.org/licenses/by/3.0/>

† **E-mail:** edoardo.calizza@uniroma1.it

## INTRODUCTION

Worldwide species extinction is now occurring at a rate probably higher than ever before, driven by numerous interconnected factors (M.E.A. 2005). The local extinction of a species within an ecological network can result in further secondary extinctions, due to indirect effects along food webs that can be even more intense and less predictable than what is expected from the direct effects of disturbance (Dunne et al. 2002, Tylianakis et al. 2008, Beckerman et al. 2010, Petchey et al. 2010). Such unpredictability in ecological response, mediated by food web structure, could seriously undermine the ability of scientists and policy makers to cope with future environmental changes, weakening ecosystem management and conservation efforts. Among others, lotic ecosystems are expected to be particularly sensitive to environmental changes, with high rates of biodiversity loss expected over the next few decades, and with extreme events expected to increase in frequency (M.E.A. 2005). Specifically, high-order rivers crossing large cities are affected by multiple stressors, given the combined effects of anthropogenic pressure and natural disturbance (e.g., floods) on river basins (Woodward and Hildrew 2002, Calizza et al. 2012, di Lascio et al. 2013). In urbanized basins, altered habitat structure and connectivity, coupled with decreased resource availability and water quality, affect habitat suitability for animal species and the potential for recolonization following disturbance (Hildrew 1996, di Lascio et al. 2013). Thus, information on the vulnerability of river communities to disturbance and to the possible consequent local extinction of species is urgently needed, in order to ensure informed management of lotic environments under global change scenarios.

To predict species extinction following disturbance is no easy task (Allesina et al. 2009). Studies of ecosystem robustness to biodiversity loss have used various species traits to simulate likely extinction scenarios, reflecting the numerous risks that threaten biodiversity in ecosystems. Both earlier and more recent studies of ecological networks' robustness have ordered primary extinctions randomly or by the degree of species connectedness (Dunne et al. 2002, Estrada 2007, Stouffer and Bascompte 2011).

Others have based extinction scenarios on ecological traits of species, such as species stress sensitivity, species rarity (i.e., local abundance) (Solan et al. 2004), the commercial importance of fish species (Coll et al. 2008), or the observed nested distribution of species through space and time, where the observed nested composition of communities reflects the sequential loss of species occurring at a given spatial or temporal scale (Zavaleta and Hulvey 2004, Srinivasan et al. 2007).

Dunne et al. (2002) and Allesina et al. (2009) observed that there exists a core, minimum set of secondary extinctions that are predictable in any case. Caused by the lack of food items for a specific consumer, they are easily deducible from a food web description. Despite representing a simplification of biodiversity organization (Polis 1991), real food webs encapsulate the complexity of ecological communities, making it possible to describe and manage such complexity. When separate food webs are reconstructed with reference to standardized protocols and assumptions, then comparison of food web structures across environmental and disturbance gradients can reliably help to quantify the effect of natural and anthropogenic stressors on biodiversity and ecosystems (Layer et al. 2010, Careddu et al. 2015). Furthermore, when samplings are replicated through space and time, food web reconstruction makes it possible to obtain time-integrated and space-integrated information on community organization, response to disturbance and vulnerability to biodiversity loss across multiple scales of observation (Ings et al. 2009, O'Gorman et al. 2009, Poisot et al. 2012).

In a recent paper, Calizza et al. (2012) addressed the combined effect of extreme flood and river basin urbanization on the structure of a detritus-based food web in a lowland stretch of the River Tiber (Rome, Italy). Despite the importance of the detritus compartment to ecosystem functioning and stability (Costantini and Rossi 2010, Rooney and McCann 2012), few descriptions of biodiversity organization in these systems exist (Tavares-Cromar and Williams 1996, Hildrew 2009, Rooney and McCann 2012) and we have only limited knowledge of the potential implications of disturbance for the dynamics of detritus-based systems. Previous studies of stream-dwelling macroinvertebrates relying on

epilithic diatoms across a pH gradient have found food webs composed of generalist taxa that are less diverse but more robust to species loss at low water pH levels (Layer et al. 2010). Similarly, increasing anthropogenic disturbance (i.e., experimental nutrient addition in sediments) in a coastal invertebrate food web has been shown to result in a less diverse and more interconnected community (O’Gorman et al. 2009), due to the loss of specialist taxa under disturbed conditions. As with aquatic systems, disturbance in terrestrial environments (i.e., habitat degradation) has been shown to result in a lower number of species and increased web connectance both in plant-herbivore and parasite-host webs (Valladares et al. 2012). Increased connectance is expected to promote food web structural Robustness to species loss (Dunne et al. 2002). On the other hand, disturbance-induced changes in community composition and organization can lead to alternative food web configurations with reduced ability to face additional stressors, depending on the identity and traits of species recovering after disturbance and the distribution of trophic interactions within the food web (Montoya and Solé 2003, Hedlund et al. 2004, Jonsson et al. 2006, O’Gorman et al. 2009).

Based on food webs previously described by Calizza et al. (2012), here we extended the analysis to quantify potential disturbance-induced changes in community vulnerability to species loss. We propose a novel food web-based metric of community vulnerability to biodiversity loss (referred to as food web Resistance, RC), and we compare the performance of the proposed index with a well-established index of food web vulnerability to species extinction, i.e., food web Robustness, proposed by Dunne et al. (2002). We hypothesized that the observed combined effect of disturbances on food web structure would be reflected in changes in food web vulnerability to species loss, with differences observed between urbanized and non-urbanized river stretches and between pre- and post-flood communities. In addition, as samplings were replicated through space and time, we investigated food web structural stability, i.e., the capacity to withstand species extinction, on different spatial and temporal scales of web description. Lastly, based on RC computation, we quantified the relative importance of the most

dominant and rarest, and the most and least connected species within each community to the overall food web Resistance, and we demonstrate that this approach could easily be extended to any desired subset of species in the web.

## MATERIALS AND METHODS

### *Study area*

We selected 12 detritus-based river food webs previously described by Calizza et al. (2012). The food webs were representative of six sampling times and two different sampling locations along the River Tiber (Appendix: Fig. A1), upstream and downstream of the city of Rome (Italy). The river flows through the city in a north-south direction. The upstream sampling location (41°57′48.902″ N, 12°30′19.432″ E, 19 m above sea level) was situated at the northern limit of the urban area. Above this location, the river receives agricultural and, to a lesser extent, industrial waste. River banks were vegetated, mainly by poplars, willows and reed beds. The downstream sampling location (41°48′59.17″ N, 12°25′15.97″ E, 7 m above sea level) was at the southern limit of the urban area, 27.5 km from the upstream location. Locally, river banks were vegetated, and river slope, depth and current velocity were similar to the upstream location. However, between the two locations the river receives the discharge of a waste water treatment plant serving the northern part of the city (average discharge: 3–3.5 m<sup>3</sup> s<sup>-1</sup>; population equivalent: 780,000), as well as the highly polluted waters of the River Aniene (Appendix: Fig. A1). In addition, this stretch of the river is affected by several direct inputs from untreated ditches and urban loadings, and the river bed is characterized by artificial banks and engineering work. The first sampling time occurred before an exceptional flood season, in July 2008, and samplings were replicated at 9, 16, 23, 31 and 71 days after the last flood event, which occurred at the end of April 2009. During the flood period, river outflow peaked at 1800 m<sup>3</sup> s<sup>-1</sup> (normal mean annual outflow is about 240 m<sup>3</sup> s<sup>-1</sup>), reaching 13.5 m above its normal level. Decreased leaf detritus and particulate organic matter inputs and increased water turbidity were observed at the downstream location after the flood season with respect to what was observed upstream,

with important implications for invertebrate community composition and organization.

### Food webs

Macroinvertebrates were sampled using litterbags containing 30 g dry weight of *Arundo donax*, the dominant macrophyte in the study area. At each sampling time and location, 10 spatially separated litterbags on each side of the river were used. In the laboratory, specimens from each litterbag were counted and identified separately. Once identified, samples were stored at  $-80^{\circ}\text{C}$  for subsequent C and N isotopic analyses. Further details of sampling locations and timing, as well as laboratory analyses, can be found in Calizza et al. (2012, and literature cited therein).

For each sampling time and location, patch-scale food webs were reconstructed based on C and N stable isotope analysis and macroinvertebrate population census data. The proportional contribution of all potential prey species to predator diet was assessed by means of isotopic mixing models (Phillips 2001). The use of litterbags that varied in terms of the quantity and palatability of colonizing microfungi allowed us to simulate discrete, spatially separated patches of a naturally amorphous resource (i.e., leaf litter) accumulating on the river bed (Rossi 1985, Costantini and Rossi 2010, Calizza et al. 2013b). Mixing model outputs were corroborated by considering the patchy distribution and the correlation between predator and prey abundance at each sampling location. The spatial distribution of specimens was compared only for those predator-prey pairs where the difference in the isotopic N signature fell within 1–4‰ (Post 2002, McCutchan et al. 2003, Fry 2006), indicative of taxa occupying different trophic positions in the food web. Similarly, as litter differed in its palatability across space and time, detritivore-detritus links were determined based on the proportion of spatially separated resource patches colonized by each taxon (Rossi 1985, Graça et al. 1993, Costantini and Rossi 1995, Costantini and Rossi 2010, Calizza et al. 2013b). Based on food web structures obtained at the patch scale, a time-integrated food web structure for each sampling location was obtained by considering together the taxa and feeding links detected across the six different sampling times. All species and links occurring in at least one

patch-scale food web were cumulated in the time-integrated food web structure. In the same way, by merging the time-integrated food webs of both the upstream and downstream locations, we obtained a time-and-space-integrated food web accounting for all taxa and trophic relationships observed in the study area during the study period (O’Gorman et al. 2009).

During samplings, 25 and 29 macroinvertebrate taxa were found at the upstream and downstream locations, respectively. Considering the minimum and maximum values from all patch-scale food webs, linkage density between taxa ( $L \div S$ ) ranged between 2.9 and 4.4 links per taxon, where L is the number of feeding links and S the number of taxa in the web; food web connectance (C), measured as  $2L \div [S(S - 1)]$ , ranged between 0.09 and 0.28; mean food chain length, as the mean number of links per food chain from detritus to top consumers excluding loops, ranged between 1.56 and 2.7; the number of prey items included in a given predaceous species’ diet ranged between 1 and 9; the number of predators feeding on a given prey species ranged between 0 and 10, and the per capita prey availability for predators, measured as the ratio of prey diversity (Shannon diversity index) to predator diversity, ranged between 0.21 and 0.88. The mean trophic generalism of predators in the web, measured as the proportion of available prey items that were actually consumed by a predator (Srinivasan et al. 2007), ranged between  $0.14 \pm 0.02$  and  $0.33 \pm 0.07$ , and the skewness of linkage distribution ranged between  $-0.38$  and  $1.90$ . Thus, relatively large differences between food web structures exist, indicating potential differences in community vulnerability to species loss between both pre- and post-flood and upstream and downstream communities (Dunne et al. 2002). Linkage density and connectance describe the complexity of the food web by accounting for differences in food web size, whereas mean chain length describes the mean number of trophic transfers from basal resources to top consumers in the web. A high number of predators feeding on a given prey reflects its vulnerability in the web and makes it a potential keystone species in the web, dominating (sensu Bodini et al. 2009) a high number of predators. In this case, a positive skewness of the linkage distribution reflects the presence in the web of

many poorly connected and few highly connected species, the latter being considered key for food web structural stability in the face of species loss. On the other hand, generalist predators feeding on a high number of prey should be considered less prone to local extinction following the loss of one or few prey species.

#### *Food web vulnerability to biodiversity loss*

In order to quantify food web vulnerability to biodiversity loss we simulated species primary extinction based on two species deletion criteria, i.e., species connectedness and species rarity, and two species deletion orders, from most to least and from least to most for each deletion criterion. This resulted in four species extinction scenarios for each food web: from most connected to least connected species (Scenario A); from rarest to most abundant species (Scenario B); from least connected to most connected species (Scenario C), and from most abundant to rarest species (Scenario D). Scenario A was expected to maximize the impact of species loss on the food web (Dunne et al. 2002), whereas scenario B was considered the most likely (Raffaelli 2004). Scenario C was expected to have the opposite effect to scenario A, whereas the comparison between scenarios B and D highlights possible differences in food web vulnerability to the loss of rare or abundant species, respectively.

The selected food webs were explicitly defined as detritus-based, the basal resource consisting of spatially separated single-species leaf litter patches differing in the amount and type of microbial colonization. Given the impossibility of comparing detritus quantity and animal density, in line with other descriptions of detritus-based systems (e.g., Mulder et al. 2013), we did not differentiate between basal species, whether leaf litter or colonizing microfungi, which are the detritivorous invertebrates' preferred food source (Rossi 1985, Costantini and Rossi 2010). Thus, we excluded the deletion of detritus as a basal node in the web, since its primary extinction would result in the inevitable loss of the entire food web (Dunne et al. 2002). Mechanisms of secondary extinction represent a bottom-up pathway for the propagation of the impact of biodiversity loss in ecosystems. Furthermore, both species rarity and trophic generalism seem to follow distinctive patterns within food webs, increasing with

trophic level. Accordingly, top predators, which would otherwise be among the first to be deleted, were not considered in primary deletion sequences, as their primary extinction could not result in any secondary extinctions and would therefore lead to an underestimate of the bottom-up effect of the primary extinction of the remaining intermediate species. Those species considered in primary deletion sequences were defined as “target species”. A species was considered secondarily extinct when all its resource items were primarily or secondarily extinct (Dunne et al. 2002, Allesina et al. 2009). We acknowledge that the extinction of a top predator could result in the exclusion of a prey via top-down cascade effects and apparent competition mechanisms (Pimm 1980). However, such mechanisms cannot be described by static food web models, making it harder to predict which competitor species would be expected to disappear following the removal of a top predator.

Based on the deletion of target species, food web Robustness (R) was computed following Dunne et al. (2002), where the Robustness value is the proportion of species that have to become primarily extinct in order to produce the overall loss of 50% of total species in the food web, considering both primary and secondary extinctions. Food web Resistance (RC) was computed with reference to (1) the proportion of target species whose primary extinction did not lead to any secondary losses (here called “buffer species”,  $\beta$ ) and (2) the mean number of secondary extinctions observed per key species deletion ( $\epsilon$ ), where a “key” species is defined as one whose primary extinction results in at least one secondary loss. Food web Resistance to biodiversity loss is quantified as the ratio of  $\beta$  to  $\epsilon$  (i.e.,  $RC = \beta \div \epsilon$ ; see Box 1 for details), which has a minimum theoretical value of 0 (poorly resistant to species loss) and a maximum theoretical value of 1 (highly resistant to species loss). Thus, food web Resistance accounts for both (1) the presence of species whose extinction would promote bottom-up effects (i.e., secondary extinctions) and (2) the magnitude of the expected bottom-up effects of each extinction event, given as the mean number of potential secondary extinctions following the loss of a given key species.

Given that RC has a maximum value of 1 and

## BOX 1

**Getting inside the index: food web Resistance and the role of target species**

Food web Resistance accounts for (1) the proportion of target species whose primary extinction does not lead to any secondary losses (here called “buffer species”,  $\beta$ ) and (2) the mean number of secondary extinctions observed per key species deletion ( $\varepsilon$ ), where a “key” species is defined as one whose primary extinction results in at least one secondary loss. Based on these food web properties, we computed RC as follows:

We deleted all target species in accordance with the selected species extinction scenario, and (1) we quantified the proportion of buffer species ( $\beta$ ) as:

$$\beta = (nT - nK) \div nT \quad (\text{A.1})$$

where  $nT$  is the total number of target species and  $nK$  is the number of key species; and (2) we calculated the mean number of secondary extinctions observed per key species deletion ( $\varepsilon$ ), as:

$$\varepsilon = N \div nK \quad (\text{A.2})$$

where  $N$  is the total number of observed secondary extinctions.

Food web Resistance (RC) was then calculated as the ratio of  $\beta$  to  $\varepsilon$ :

$$\text{RC} = \beta \div \varepsilon. \quad (\text{A.3})$$

The minimum theoretical value of  $\beta$  is 0, indicating that no buffer species are present in the food web; whereas the maximum theoretical value of  $\beta$  is 1, indicating that  $nK = 0$  (theoretically possible only when excluding the last deletion event; see below).

The minimum theoretical value of  $\varepsilon$ , by definition, is 1, as a species is defined as “key” when its deletion leads at least to one secondary extinction. The maximum theoretical value of  $\varepsilon$  is  $S$ , where  $S$  is the number of intermediate and top predator species in the web, which represent taxa potentially undergoing secondary extinction. Thus, the theoretical value of food web Resistance ranges from a minimum of 0 (a food web that is minimally resistant to species loss) to a maximum of 1 (a food web that is highly resistant to species loss).

Based on each species deletion scenario, we quantified the Specific Structural Importance (SSI) of (1) the least connected species (from scenario A), (2) the most abundant species (from scenario B), (3) the most connected species (from scenario C), and (4) the rarest species (from scenario D). SSI was calculated as:

$$\text{SSI} = \text{RC}^* - \text{RC} \quad (\text{A.4})$$

where  $\text{RC}^*$  represents the food web Resistance re-computed after excluding the last deletion event from the original species deletion sequence in each extinction scenario.  $\text{RC}^*$  can be obtained from RC by replacing  $\beta$  with  $\beta^*$  as in:

$$\beta^* = [(nT - 1) - (nK - 1)] \div (nT - 1) \quad (\text{A.5})$$

if the last deleted species in the RC computation was a key species, or

$$\beta^* = [(nT - 1) - nK] \div (nT - 1) \quad (\text{A.6})$$

if the last deleted species was a buffer species;  
and replacing  $\varepsilon$  with  $\varepsilon^*$  as in:

$$\varepsilon^* = (N - L) \div (nK - 1) \quad (\text{A.7})$$

if the last deleted species in the RC computation was a key species, or maintaining the original value of  $\varepsilon$  (Eq. A.2) if the last deleted species was a buffer species, where  $L$  is the number of observed secondary extinctions following the last species deletion in the RC computation. If no secondary extinctions were observed excluding the last deletion event (i.e. if all the secondary extinctions originally observed were dependent on the species deleted last, implying  $N = L$ ),  $\text{RC}^*$  was defined as 1.

R has a maximum value of 0.5 (Dunne et al. 2002), we calculated food web vulnerability as:  $V_{RC} = 1 - RC$  when derived from food web Resistance, and  $V_R = 1 - 2R$  when derived from food web Robustness. This made it possible to obtain comparable (i.e., on the same scale) food web vulnerability values, independently of the initial computation of R or RC, with both  $V_{RC}$  and  $V_R$  varying between 0 (minimally vulnerable food web) and 1 (highly vulnerable food web).

To test the independence of the R and RC values of each food web with respect to the applied species deletion scenarios, we tested for linear positive correlation of R or RC between the four species extinction scenarios. That is, we compared the same deletion criterion with different deletion orders (i.e., scenarios A vs. B and C vs. D), and the same deletion order with different extinction criteria (i.e., scenarios A vs. C and B vs. D). Complete independence of a given food web's R or RC from the applied species deletion scenario implies that the relative value of each food web does not vary across extinction scenarios and a positive significant correlation is expected in this case. This does not necessarily imply that each food web maintains the same absolute value of R or RC regardless of the scenario, but rather that the ranking of the food webs in terms of their R or RC values does not vary.

Considering that the maximum Robustness value is 0.5, and the maximum Resistance value is 1, for the statistical comparison of mean values we standardized Resistance as  $RC \div 2$ . In the text we refer to mean R and RC (and associated standard error) as the mean value obtained for each food web across the four extinction scenarios at each sampling time and sampling location.

Results in the text are reported as mean  $\pm$  1 SE. Means were compared by two-way ANOVA and associated Tukey's honestly significant difference post-hoc pairwise comparison. Pearson's correlation coefficient was determined to evaluate the relationships between variables where possible. When necessary, data were log-transformed in order to better normalize distributions before analysis. When the assumption of equality of variances was not respected, or log-transformation of data did not allow data normalization, the Wilcoxon matched-paired test, Kruskal-Wallis test and associated Mann-Whitney *U* test post

hoc comparison were used.

## RESULTS

For all species extinction scenarios, food web Resistance (RC) was always lower than Robustness (R) (Wilcoxon paired test:  $W$  always  $> 78$ ,  $p$  always  $< 0.003$ ) (Fig. 1). However, both the R and RC of the upstream community food webs across sampling times were higher in comparison with those of the downstream community (Table 1).

The post-flood variation pattern of RC was consistent across different species extinction scenarios, whereas that of R varied (Fig. 1). Mean R did not vary between pre- and post-flood sampling times (Table 1 and Fig. 2). In contrast, mean RC was significantly different at both sampling locations after the flood, and a combined effect of flood and river basin urbanization was detected (Table 1 and Fig. 2). Both mean R and RC varied hyperbolically with the skewness of linkage distribution between species (R:  $y = -0.07x^2 + 0.08x + 0.40$ ,  $r = 0.80$ ,  $p < 0.01$ ; RC:  $y = -0.13x^2 + 0.15x + 0.31$ ,  $r = 0.74$ ,  $p < 0.01$ ) but they were not related to food web connectance ( $p > 0.05$ ). The mean proportion of buffer species in each web ( $\beta$ ) and the mean number of secondary extinctions observed per key species deletion ( $\varepsilon$ ) were not related ( $p > 0.05$ ). Overall, neither  $\beta$  nor  $\varepsilon$  varied significantly between locations (Wilcoxon paired test,  $p > 0.05$  for both). Nevertheless, both  $\beta$  and  $\varepsilon$  varied across sampling times (Fig. 3). At the upstream location,  $\beta$  was higher after the flood than before. At the downstream location,  $\beta$  decreased temporarily, returning to its pre-flood value by the end of the monitoring period. On the other hand, 71 days after the flood  $\varepsilon$  was higher than before the flood at both the upstream and downstream locations. Both  $\beta$  and  $\varepsilon$  contributed to web RC, with  $\varepsilon$  explaining the majority of RC variability between food webs (Appendix: Table A1).

There was no correlation between food web R values across species extinction scenarios ( $p$  always  $> 0.05$ ), whereas a significant correlation was observed for RC values ( $p$  always  $< 0.01$ ) (Fig. 4). Consistent with this observation, the mean standard error across the four extinction scenarios was significantly lower for RC values than R values (paired *t* test,  $t = 2.4$ ,  $p < 0.05$ ).

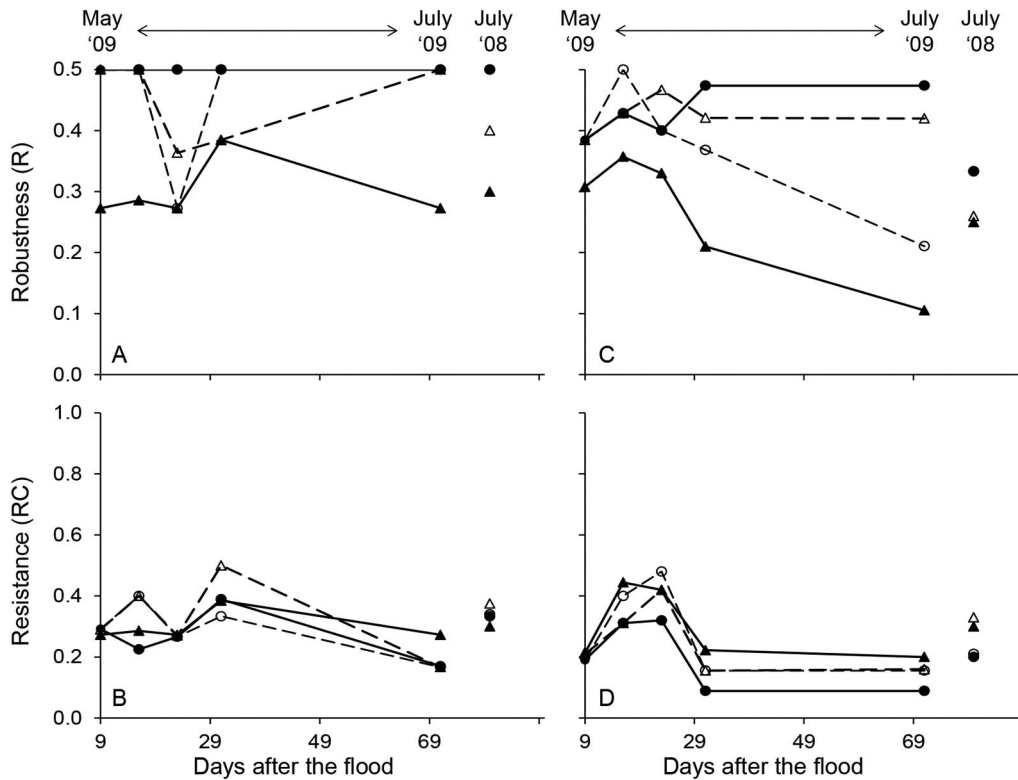


Fig. 1. River Tiber food web Robustness and Resistance before (July 2008) and after the flood season, both upstream (panels A–B) and downstream (panels C–D) of the city of Rome (Italy), in four different species extinction scenarios. Filled triangles: scenario A; empty triangles: scenario B; filled circles: scenario C; empty circles: scenario D. For details on species extinction scenarios please refer to the materials and methods section. Note that the maximum values of the y-axes are fixed according to the theoretical maximum values of Robustness (0.5) and Resistance (1.0).

Table 1. Two-way ANOVA exploring the effect of location (i.e., comparing the two sampling locations upstream and downstream of the urban area) and flood disturbance on the Robustness and Resistance of detritus-based food webs in the River Tiber (Rome, Italy). Values in boldface indicate a significant effect ( $p < 0.05$ ).

Source of variation	Effect		Error		F	p
	df	MS	df	MS		
<b>Robustness</b>						
Location	1	0.053	36	0.010	5.44	<b>0.025419</b>
Flood	5	0.007	36	0.010	0.69	0.634157
Location × Flood	5	0.010	36	0.010	1.05	0.402857
<b>Resistance</b>						
Location	1	0.060	36	0.004	14.12	<b>0.000607</b>
Flood	5	0.044	36	0.004	10.46	<b>0.000003</b>
Location × Flood	5	0.038	36	0.004	9.00	<b>0.000013</b>

Based on  $V_R$  computation, the different extinction scenarios were observed to produce substantially different rankings (Appendix: Table A2). No food web had the same ranking across

all extinction scenarios, the highest variation for a given web (9 ranks) being observed for food web “e”, corresponding to the food web reconstructed at the upstream location 31 days after the flood.



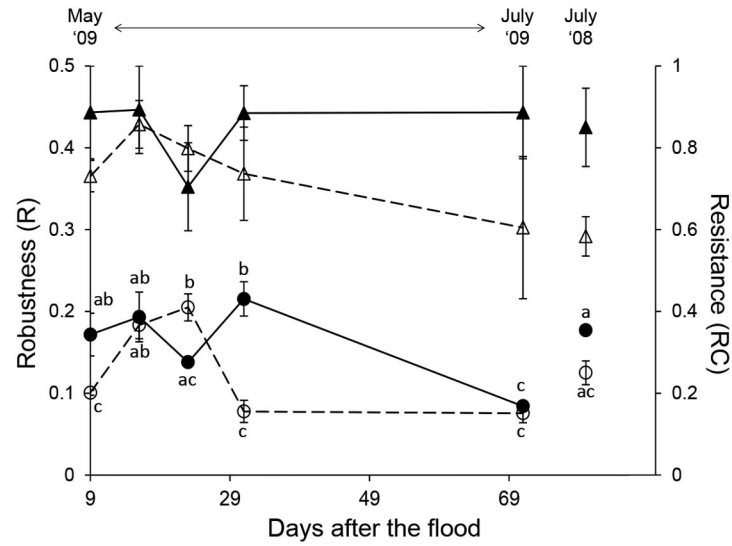


Fig. 2. Food web Robustness (triangles, left y-axis) and Resistance (circles, right y-axis) before (July 2008) and after the flood season, both upstream (filled symbols) and downstream (empty symbols) of the city of Rome (Italy) (mean  $\pm$  SE). Different letters indicate a significant difference (ANOVA and Tukey's honestly significant difference post-hoc pairwise comparison,  $p < 0.05$ ).

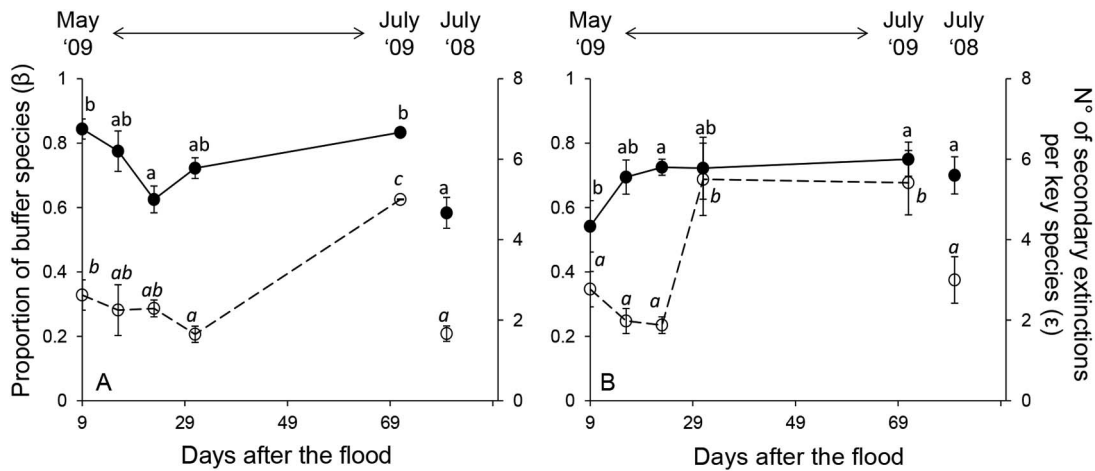


Fig. 3. Variation of the proportion of buffer species ( $\beta$ ; filled symbols, left y-axis) and the number of secondary extinctions observed per key species deletion ( $\epsilon$ ; empty symbols, right y-axis) in patch-scale food webs both upstream (panel A) and downstream (panel B) of the city of Rome (Italy) (mean  $\pm$  SE). Different letters indicate a significant difference (Kruskal Wallis and Mann-Whitney post hoc comparison,  $p < 0.05$ ). Letters in italics refer to empty symbols.

This food web was the most vulnerable (i.e., it had the highest  $V_R$  value, ranking 12th out of 12) in scenario A, but was amongst the least vulnerable food webs (ranking 3rd out of 12) in scenario B. The average food web ranking

variation across extinction scenarios was 3.8 ranks. In contrast, the mean food web ranking variation in terms of  $V_{RC}$  values was 1.4 ranks and was significantly lower (Wilcoxon paired test,  $Z = 2.7$ ,  $p < 0.01$ ). The highest variation (6

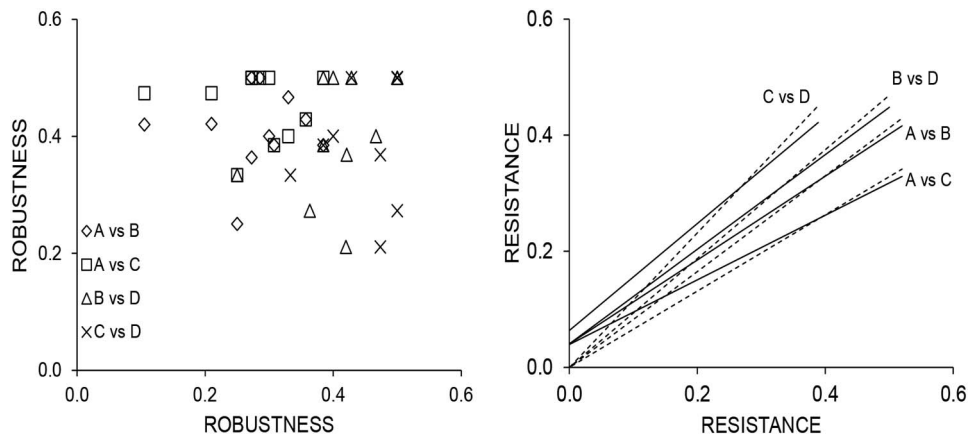


Fig. 4. Comparison of Robustness (R) and Resistance (RC) of each food web in four different species extinction scenarios (A–D). A significant linear correlation implies that food web rankings by their R or RC value do not vary between different scenarios. For food web Resistance, only the regression models (solid lines) are shown because correlations were always significant. Scenario A vs. C:  $r = 0.78$   $p$  value = 0.007; B vs. D:  $r = 0.86$   $p$  value = 0.001; A vs. B:  $r = 0.86$   $p$  value = 0.001; C vs. D:  $r = 0.77$   $p$  value = 0.009. As expected, the model intercept was never significantly different from 0 ( $t$  test for model intercept,  $p$  always > 0.05). Dotted lines represent regression models when the intercept is forced to 0. No significant correlations between different species extinction scenarios were observed for food web Robustness.

ranks) was observed for food web “c”, corresponding to the food web reconstructed at the upstream location 16 days after the flood. Nine out of 12 food webs varied by only 0, 1 or 2 ranks across extinction scenarios. No more than 2 out of 12 food webs presented an identical  $V_{RC}$  value in any extinction scenario, whereas 3 food webs in A, 7 food webs in B, 8 food webs in C and 6 food webs in D had an identical  $V_R$  value to at least one other web (Appendix: Table A2).

Based on the computation of food web RC, we were able to quantify the specific structural importance (SSI) of the last deleted species in each food web in each extinction scenario. The most connected species had the highest mean SSI ( $SSI = 0.65 \pm 0.06$ ) and the least connected had the lowest ( $SSI = 0.01 \pm 0.01$ ) (Kruskal Wallis and Mann-Whitney post hoc comparison,  $p < 0.001$ ), whereas the rarest ( $SSI = 0.22 \pm 0.09$ ) and the most abundant species ( $SSI = 0.24 \pm 0.10$ ) had intermediate SSI values that did not differ significantly ( $p > 0.10$ ). The SSI of the rarest species in each food web increased with per capita prey availability for predators ( $y = 0.49 \ln(x) + 0.82$ ,  $r = 0.75$ ,  $p < 0.01$ ). As expected, RC scaled with SSI only when the last deleted species was the most connected ( $r = -0.68$ ,  $p <$

0.05). In all other cases (i.e., considering the rarest, dominant and less connected species), SSI and RC were not related ( $p$  always > 0.05).

After the flood, Calizza et al. (2012) reported dominance by the amphipod *Echinogammarus veneris* in the macroinvertebrate community at both sampling locations. At the downstream location, *E. veneris* substituted Chironomid larvae as the dominant taxon in the pre-flood community. The specific structural importance of the dominant species (DSSI) was directly related to the mean trophic generalism of predators in the web ( $y = 3.57x - 0.60$ ,  $r = 0.71$ ,  $p < 0.01$ ). DSSI was higher after 9 days, and then lower after 23 days at both sampling locations (Appendix: Fig. A2). At the last sampling time DSSI was 0 in the downstream community, similar to its pre-flood value, while in the upstream community it presented its highest observed value.

#### Time-integrated and space-integrated food webs

Food web RC varied between different temporal and spatial scales of web description, as a consequence of different  $\beta$  and  $\varepsilon$  values in the time-integrated (T) and time-and-space-integrated (T + S) food webs with respect to patch-scale

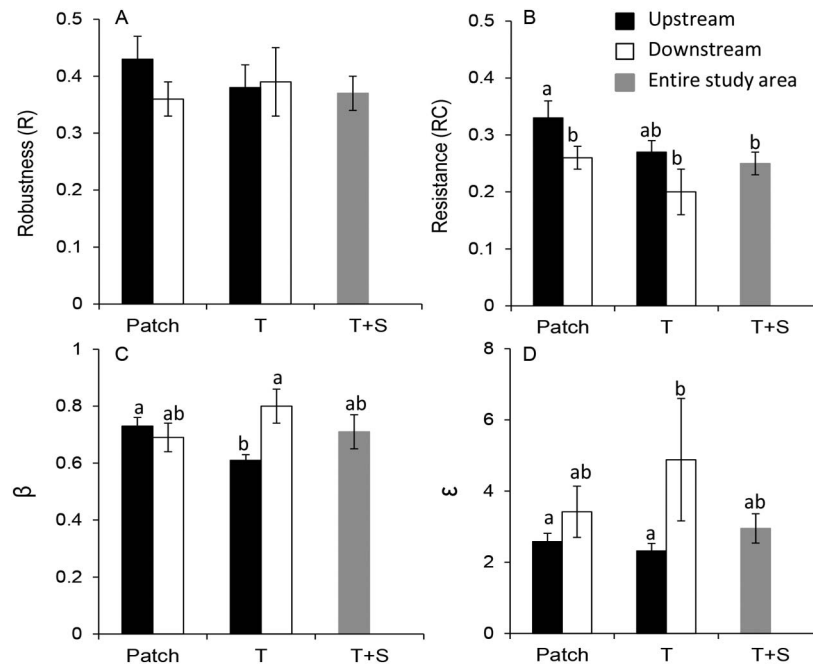


Fig. 5. Food web Robustness (R) and Resistance (RC), proportion of buffer species ( $\beta$ ) and number of secondary extinctions observed per key species deletion ( $\epsilon$ ) at different temporal and spatial scales of food web description in the River Tiber (Rome, Italy) (mean  $\pm$  SE). “Patch” refers to the 12 patch-scale food webs reconstructed at six different sampling times upstream (black bars) and downstream (white bars) of the city of Rome. “T” indicates time-integrated food webs including all taxa and feeding links occurring in at least one of the six patch-scale webs reconstructed at each sampling location. “T + S” indicates time-and-space-integrated food webs, considering the entire study area and sampling period (grey bars). The “T + S” food web includes all taxa and feeding links occurring in at least one of the two time-integrated food webs. Different letters indicate a significant difference (Kruskal Wallis and Mann-Whitney post hoc comparison,  $p < 0.05$ ).

webs (Fig. 5). At the upstream location, both R and RC were higher at the patch scale than at the T and T + S scales, although the difference was significant only for RC. At the downstream location, where both R and RC were lower at the patch scale, neither R nor RC varied significantly across web description scales. At the patch scale,  $\beta$  and  $\epsilon$  did not differ between sampling locations, whereas both  $\beta$  and  $\epsilon$  were higher at the downstream location when considering time-integrated food web structures (Fig. 5). The SSI of the most connected species, as well as that of the rarest and most abundant, was greater at patch scale than on the T and T + S scales (Fig. 6). The time-and-space-integrated food web is reported as supplemental material in the Appendix (Fig. A3).

## DISCUSSION

River ecosystems represent complex and vulnerable habitats, where both natural environmental variability and disturbance regulate ecosystem structure, functioning and stability (Wootton et al. 1994, Urban 2004, Power et al. 2008). In our study, biological interactions and environmental constraints were key to mediate the local effect of disturbance on food web structure and stability (Brown and Swan 2010, Calizza et al. 2012). Our results indicate that both natural and anthropogenic disturbance modified food web topological properties in the river invertebrate community, with such disturbance-induced changes being reflected in substantial variations in the structural stability of detritus-based food webs in the face of species loss. The consideration of two different measures of food

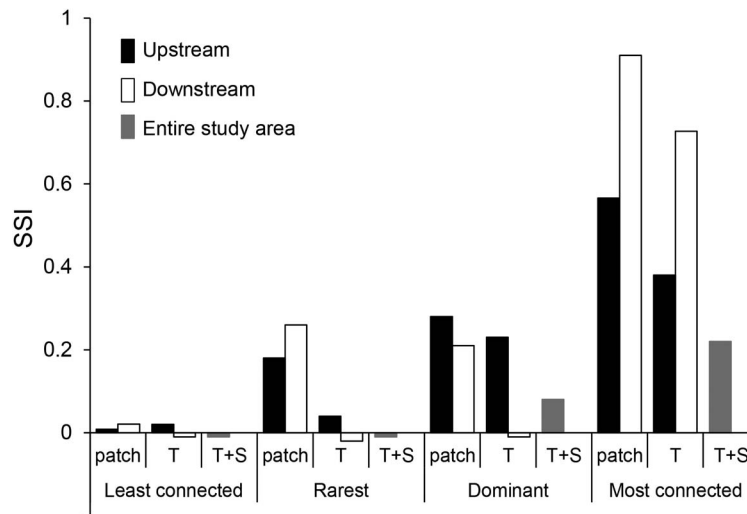


Fig. 6. Specific structural importance (SSI) of the least connected, rarest (i.e., least abundant), most connected, and dominant (i.e., most abundant) taxon at different temporal and spatial scales of food web description in the River Tiber (Rome, Italy). The dominant taxon was always the amphipod *Echinogammarus veneris*. For the calculation of SSI please refer to the materials and methods section. “Patch” refers to the 12 patch-scale food webs reconstructed at six different sampling times upstream (black bars) and downstream (white bars) of the city of Rome. “T” indicates time-integrated food webs including all the taxa and feeding links occurring in at least one of the six patch-scale webs reconstructed at each sampling location. “T + S” indicates time-and-space-integrated food webs considering the entire study area and sampling period (grey bars). The “T + S” food web includes all taxa and feeding links occurring in at least one of the two time-integrated food webs.

web stability, i.e., food web Robustness (R) and Resistance (RC), modified our perception of the effect of disturbance on web vulnerability. Both mean R and RC decreased as the river crossed the urban area, indicating that anthropogenic pressure increased invertebrate community vulnerability to species loss. On the other hand, the post-flood variation pattern of R depended on which species extinction scenario was applied, whereas RC was consistent across extinction scenarios, indicating a similar flood effect regardless of species deletion order or criterion. This highlighted a significant interacting negative effect of flood disturbance and river basin urbanisation on food web RC, consistent with the reported synergic effects of these stressors on food web topology (Calizza et al. 2012).

Food web Resistance takes account of fundamental topological properties ( $\beta$  and  $\epsilon$ ), which link food web structure and vulnerability to biodiversity loss. Thanks to the inclusion of these features, the RC of food webs tended to be lower than their R.  $\beta$  and  $\epsilon$  may have a balanced effect on RC in food webs. That is, in food webs

characterized by proportionally few key species, the mean number of secondary extinctions per key species loss might plausibly be high, indicating the presence in the web of critical nodes (i.e., species whose deletion would lead to an elevated number of secondary losses), while a high proportion of key species might be mitigated by a low mean number of secondary extinctions per key species loss. Consistent with this, both extremely high and low skewness values for linkage distribution between species were reflected in a low RC. On the other hand, neither web R nor RC were related to web connectance, as previously observed in other systems (Dunne et al. 2002). This could be ascribed to the narrower range of connectance values in our dataset with respect to food webs analysed by Dunne et al. Nevertheless, this suggests that differences in interspecific linkage distribution can produce differences in community vulnerability to biodiversity loss even between similarly connected communities (Jonsson et al. 2006).

Food web Resistance to species loss varied in

accordance with the time- and space-scales of web description, as a consequence of  $\beta$  and  $\varepsilon$  varying between observation scales. Time-integrated and time-and-space-integrated webs can be considered the result of alternative configurations that invertebrate food webs assume at the local (i.e., patch) scale, accounting for the effect of temporal and spatial dynamics of species distribution and interactions (Thompson and Townsend 2005, O’Gorman 2009, Poisot et al. 2012). This has been shown to arise from consumers switching their foraging choices between available food items through space and time, and/or the functional or physical compartmentalization of the habitat (Thompson and Townsend 2005, Ings et al. 2009, Jana and Bairagi 2014). In both cases, the existence of spatially and temporally compartmentalized configurations in food webs has been shown to promote community persistence (McCann et al. 2005, Stouffer and Bascompte 2011). On average, the invertebrate food web in the River Tiber was more resistant to species loss at the patch scale than at the time-integrated and time-and-space-integrated scales. This can be considered a stabilizing mechanism with regard to species loss in the invertebrate community. Indeed, (1) in riverine networks, the disturbance-induced extinction of a species is more likely to occur at the local than at the whole-habitat scale (Urban 2004, Brown and Swan 2010), and (2) in patchy environments, the temporary exclusion of a species from a given patch could result from natural meta-community dynamics and local environmental variability (Urban 2004, Brown and Swan 2010). This suggests that efficient meta-community dynamics and the possibility of patch recolonization after disturbance enhance the resistance of the invertebrate community to species loss at multiple spatial scales. The variation of food web RC across temporal and spatial scales at higher food web sizes remains to be tested. However, it is assumed that this approach can be applied to other kinds of ecological community and other environmental conditions regardless of habitat, disturbance or network type.

The opportunity to quantify the specific structural importance (SSI) of a given species in the description of food web RC represents a powerful tool for assessing and comparing the role of selected species in community stability in

the face of biodiversity loss. As expected, the most connected species contributed the most to food web RC (Dunne et al. 2002). Interestingly, rare and abundant species contributed similarly to food web RC, implying that rare and intermediate species could play an important role in the stability of trophic pathways in river detritus-based systems (Solan et al. 2004, Power et al. 2008, Calizza et al. 2013a), with important implications for biodiversity conservation. The structural importance of rare species decreased when the per capita prey availability for predators was low. This could be explained with reference to animal foraging optimization strategies (MacArthur and Pianka 1966, Pyke et al. 1977, Beckerman et al. 2006, Petchey et al. 2008). Indeed, at low levels of per capita prey availability and under disturbed conditions, predators are expected to generalise and feed on more abundant, and thus more frequently encountered, prey species. On the other hand, the structural importance of the dominant species (DSSI) increased during the food web recovery phase after the disturbance, and was higher in webs characterized by generalist predators. Generalist predators are expected to dominate the early phases of habitat recolonization, as was the case in the River Tiber after the flood (see Calizza et al. 2012: Fig. 3c). This may explain the observed pattern in DSSI values, suggesting that dominant species play a stabilizing role during the early phases of food web assembly or recovery.

The relative contribution of the rarest, most abundant and most connected species to food web Resistance was higher in patch-scale than in time-integrated and time-and-space-integrated webs. This indicates that the loss of selected key species would be expected to produce more pronounced bottom-up effects at the local than at the whole-habitat scale. The SSI value of a particular species represents its relative importance with respect to the other species in the web, and it is not solely dependent on the number of species feeding on it. The greater contribution to RC at patch level of selected key species may therefore be considered a direct effect of the number of intermediate species increasing with the scale of food web description, coupled with the greater opportunity for predators to vary and separate their trophic niches depending on the

turnover of potential prey taxa through space and time (Havens 1992, Thompson and Townsend 2005, Poisot et al. 2012). This suggests that the maintenance of high levels of beta-biodiversity of intermediate species pools could promote food web persistence in the face of selected key species losses from riverine invertebrate communities. We notice that the SSI computation for a single species (Box 1) can easily be extended to any given subset of species, by (1) substituting  $(nT - 1)$  with  $(nT - n_{\text{set}})$ , and  $(nK - 1)$  with  $(nK - \lambda)$  in Eq. A.5, where  $n_{\text{set}}$  is the number of species in the given subset, and  $\lambda$  is the number of key species in the given subset, and (2) substituting  $(nK - 1)$  with  $(nK - \lambda)$  and  $L$  with  $L_s$  in Eq. A.7, where  $L_s$  is the number of observed secondary extinctions resulting from the deletion of the key species in the given subset ( $\lambda$ ), assuming the contiguity of the subset of species in the deletion sequence.

#### Concluding remarks

Detritus-based food webs represent a fundamental energy input pathway in most freshwater ecosystems and consideration of the structural aspects of the stability of these systems in the face of disturbance and biodiversity loss can provide important complementary insights for freshwater ecosystem management. Although metrics of food web structural stability such as Robustness and Resistance do not directly take account of dynamic changes in population abundance or potential diet shifts of consumers through space and time, the study of food web response to species extinction can make a meaningful contribution to more traditional monitoring and conservation approaches (Dunne et al. 2002, May 2009). Mechanisms of secondary extinction in food webs, mediated by food web structure, can shed light on the relationship between biodiversity organization and vulnerability to species extinction in ecosystems, providing an effective “trophic map” with which to locate keystone species in the web. Our results indicate that the consideration of  $\beta$  and  $\varepsilon$  in food webs and the quantification of web Resistance to species loss could represent a precise measure of food web structural stability in the face of species loss, providing an effective research pathway supporting river ecosystem management and the ecological monitoring of multiple

disturbances. Indeed, the presence of a small number of highly connected key species within a community (i.e., high  $\varepsilon$ ), or the presence of a widespread risk of bottom-up effects following biodiversity loss (i.e., low  $\beta$ ), should be addressed by dedicated monitoring and conservation strategies.

In addition, when food webs are reconstructed from replicate samplings through space and time (like the time-integrated and space-integrated food webs in the River Tiber), the resulting structures can be considered descriptive of the flexible diet choices of their constituent species (O’Gorman et al. 2009, Layer et al. 2010, Poisot et al. 2012). In this study, the scale of spatial and temporal dynamics of the macroinvertebrate food webs allowed us to obtain evaluations and comparisons of community response to stressors at multiple scales of observation and at different levels of anthropogenic pressure. Macroinvertebrates are the most frequently cited group for bioindication in freshwaters, so useful comparisons with classical indicators and associated information on the environmental status of water bodies can be made, making it possible to quantify whether, how and to what degree anthropogenic pressure and climatic change are eroding the ability of aquatic systems to cope with future expected levels of biodiversity loss. On the other hand, the application of the proposed approach to other systems and more complete food webs (including other ecosystem compartments and trophic levels) would facilitate cross-habitat comparison and enable generalization regarding the relationship between (1) topology and Resistance in food webs, and (2) species’ ecological traits and their structural importance in the web, as well as the effect of disturbance on the vulnerability of natural communities to species extinction.

#### ACKNOWLEDGMENTS

We thank Mr. George Metcalf for revising the English text. We also thank an anonymous reviewer and Prof. E. Ward for their helpful comments on a previous version of the manuscript. This research was supported by Sapienza University grants 2012, PNRA CONISMA-Rossi L. 2010 and 2013, Latina Provincial Administration 2011. E. Calizza wishes to thank the Italian Ecological Society (SItE) and the “Successione Ecologica” Association, as the idea behind this paper

was originally presented at the national meeting of SItE PhD students, in Siena 2011, and further discussed with colleagues from “Successione Ecologica”.

## LITERATURE CITED

- Allesina, S., A. Bodini, and M. Pascual. 2009. Functional links and robustness in food webs. *Philosophical Transaction of the Royal Society B* 364:1701–1709.
- Beckerman, A., O. L. Petchey, and P. J. Morin. 2010. Adaptive foragers and community ecology: linking individuals to communities and ecosystems. *Functional Ecology* 24:1–6.
- Beckerman, A. P., O. L. Petchey, and P. H. Warren. 2006. Foraging biology predicts food web complexity. *Proceedings of the National Academy of Sciences USA* 103:13745–13749.
- Bodini, A., M. Bellingeri, S. Allesina, and C. Bondavalli. 2009. Using food web dominator trees to catch secondary extinctions in action. *Philosophical Transactions of the Royal Society B* 364:1725.
- Brown, B. L., and C. M. Swan. 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. *Journal of Animal Ecology* 79:571–580.
- Calizza, E., M. L. Costantini, D. Rossi, P. Carlino, and L. Rossi. 2012. Effects of disturbance on an urban river food web. *Freshwater Biology* 57:2613–2628.
- Calizza, E., M. L. Costantini, P. Carlino, F. Bentivoglio, L. Orlandi, and L. Rossi. 2013a. *Posidonia oceanica* habitat loss and changes in litter-associated biodiversity organization: a stable isotope-based preliminary study. *Estuarine Coastal and Shelf Sciences* 135:137–145.
- Calizza, E., L. Rossi, and M. L. Costantini. 2013b. Predators and resources influence phosphorus transfer along an invertebrate food web through changes in prey behaviour. *PLoS ONE* 8(6):e65186.
- Careddu, G., M. L. Costantini, E. Calizza, P. Carlino, F. Bentivoglio, L. Orlandi, and L. Rossi. 2015. Effects of terrestrial input on macrobenthic food webs of coastal sea are detected by stable isotope analysis in Gaeta Gulf. *Estuarine Coastal and Shelf Sciences* 145:158–168.
- Coll, M., H. K. Lotze, and T. N. Romanuk. 2008. Structural segregation in Mediterranean Sea food webs: testing ecological hypotheses using stochastic and mass-balance modelling. *Ecosystems* 11:939–960.
- Costantini, M. L., and L. Rossi. 1995.  $^{32}$ Phosphorus transfer in systems of fish and amphipods exploiting detritus particles. *Hydrobiologia* 302:81–87.
- Costantini, M. L., and L. Rossi. 2010. Species diversity and decomposition in laboratory aquatic systems: the role of species interactions. *Freshwater Biology* 55:2281–2295.
- di Lascio, A., L. Rossi, P. Carlino, E. Calizza, D. Rossi, and M. L. Costantini. 2013. Stable isotope variation in macroinvertebrates indicates anthropogenic disturbance along an urban stretch of the river Tiber (Rome, Italy). *Ecological Indicators* 28:107–114.
- Dunne, J. A., R. J. Williams, and N. D. Martinez. 2002. Network structure and biodiversity loss in food webs: robustness increases with connectance. *Ecology Letters* 5:558–567.
- Estrada, E. 2007. Food webs robustness to biodiversity loss: The roles of connectance, expansibility and degree distribution. *Journal of Theoretical Biology* 244:296–307.
- Fry, B. 2006. *Stable isotope ecology*. Springer, New York, New York, USA.
- Graça, M. A. S., L. Maltby, and P. Calow. 1993. Importance of fungi in the diet of *Gammarus pulex* and *Asellus aquaticus*. I. Feeding strategies. *Oecologia* 93:139–144.
- Havens, K. 1992. Scale and structure in natural food webs. *Science* 257:1107–1109.
- Hedlund, K., B. Griffiths, S. Christensen, S. Scheu, H. Setälä, T. Tschardtke, and H. Verhoef. 2004. Trophic interactions in changing landscapes: responses of soil food webs. *Basic and Applied Ecology* 5:495–503.
- Hildrew, A. G. 2009. Sustained research on stream communities: a model system and the comparative approach. *Advances in Ecological Research* 41:174–312.
- Hildrew, H. G. 1996. Whole river ecology: spatial scale and heterogeneity in the ecology of running waters. *Large Rivers* 10:25–43.
- Ings, T. C., et al. 2009. Ecological networks—beyond food webs. *Journal of Animal Ecology* 78:253–269.
- Jana, D., and N. Bairagi. 2014. Habitat complexity, dispersal and metapopulations: Macroscopic study of a predator-prey system. *Ecological Complexity* 17:131–139.
- Jonsson, T., P. Karlsson, and A. Jonsson. 2006. Food web structure affects the extinction risk of species in ecological communities. *Ecological Modelling* 199:93–106.
- Layer, K., J. O. Riede, A. G. Hildrew, and G. Woodward. 2010. Food web structure and stability in 20 streams across a wide pH gradient. *Advances in Ecological Research* 42.
- MacArthur, R. H., and E. R. Pianka. 1966. On optimal use of a patchy environment. *American Naturalist* 100:603–609.
- May, R. M. 2009. Food-web assembly and collapse: mathematical models and implications for conservation. *Philosophical transaction of the Royal Society B* 364:1643–1646.
- McCann, K. S., J. B. Rasmussen, and J. Umbahnwar. 2005. The dynamics of spatially coupled food webs. *Ecology Letters* 8:513–523.

- McCutchan, J. H., Jr., W. M. Lewis, Jr., C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. *Oikos* 102:378–390.
- Millennium Ecosystem Assessment. 2005. *Ecosystems and human well-being: synthesis*. Island Press, Washington, D.C., USA.
- Montoya, M. J., and R. V. Solé. 2003. Topological properties of food webs: from real data to community assembly models. *Oikos* 102:614–622.
- Mulder, C., et al. 2013. Connecting the green and brown worlds: allometric and stoichiometric predictability of above- and below-ground networks. *Advances in Ecological Research* 49:69–175.
- O’Gorman, E. J., J. E. Fitch, and T. P. Crowe. 2009. Multiple anthropogenic stressors and the structural properties of food webs. *Ecology* 93:441–448.
- Petchey, O. L., A. P. Beckerman, J. O. Riede, and P. H. Warren. 2008. Size, foraging, and food web structure. *Proceedings of the National Academy of Sciences USA* 105:4191–4196.
- Petchey, O. L., U. Brose, and B. C. Rall. 2010. Predicting the effects of temperature on food web connectance. *Philosophical Transaction of the Royal Society B* 365:2081–2091.
- Phillips, D. L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. *Oecologia* 127:166–170.
- Pimm, S. L. 1980. Food web design and the effect of species deletion. *Oikos* 35:139–149.
- Poisot, T., E. Canard, D. Muillot, N. Moquet, and D. Gravel. 2012. The dissimilarity of species interaction network. *Ecology Letters* 15:1353–1361.
- Polis, G. A. 1991. Complex trophic interactions in deserts: an empirical critique of food-web theory. *American Naturalist* 138:123–155.
- Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718.
- Power, M. E., M. S. Parker, and W. E. Dietrich. 2008. Seasonal reassembly of river food webs under a Mediterranean hydrologic regime: floods, droughts, and impacts of fish. *Ecological Monograph* 78:263–282.
- Pyke, G. H., H. R. Pulliam, and E. L. Charnov. 1977. Optimal foraging: a selective review of theory and tests. *Quarterly Review of Biology* 52:137–154.
- Raffaelli, D. 2004. How extinction patterns affect ecosystems. *Science* 306:1141–1142.
- Rooney, N., and K. S. McCann. 2012. Integrating food web diversity, structure and stability. *Trends in Ecology and Evolution* 27:40–46.
- Rossi, L. 1985. Interactions between invertebrates and microfungi in freshwater ecosystems. *Oikos* 44:175–184.
- Solan, M., B. J. Cardinale, A. L. Downing, K. A. M. Engelhardt, J. L. Ruesink, and D. S. Srivastava. 2004. Extinction and ecosystem function in the marine benthos. *Science* 306:1177–1180.
- Srinivasan, U. T., J. A. Dunne, J. Harte, and N. D. Martinez. 2007. Response of complex food webs to realistic extinction sequences. *Ecology* 88:67–682.
- Stouffer, D. B., and J. Bascompte. 2011. Compartmentalization increases food-web persistence. *Proceedings of the National Academy of Sciences* 108:3648–3652.
- Tavares-Cromar, A. F., and D. D. Williams. 1996. The importance of temporal resolution in food web analysis: evidence for a detritus-based stream. *Ecological Monographs* 66:91–113.
- Thompson, R. M., and C. R. Townsend. 2005. Food-web topology varies with spatial scale in a patchy environment. *Ecology* 86:1916–1925.
- Tylianakis, J. M., R. K. Didham, J. Bascompte, and D. A. Wardle. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters* 11:1351–1363.
- Urban, M. C. 2004. Disturbance heterogeneity determines freshwater metacommunity structure. *Ecology* 85:2971–2978.
- Valladares, G., L. Cagnolo, and A. Salvo. 2012. Forest fragmentation leads to food web contraction. *Oikos* 121:299–305.
- Woodward, G., and G. A. Hildrew. 2002. Food web structure in riverine landscape. *Freshwater Biology* 47:777–789.
- Wootton, J. T., R. S. Parker, and M. E. Power. 1994. Effect of disturbance on river food webs. *Science* 273:1558–1563.
- Zavaleta, E. S., and K. B. Hulvey. 2004. Realistic species losses disproportionately reduce grassland resistance to biological invaders. *Science* 306:1175–1177.



## SUPPLEMENTAL MATERIAL

## APPENDIX

Table A1. Multiple linear regression between food web Resistance (dependent variable) and (1) the proportion of buffer species in each web ( $\beta$ ), where a “buffer” species is defined as one whose deletion did not lead to any secondary losses; and (2) the mean number of secondary extinctions observed per key species deletion ( $\epsilon$ ), where a “key” species is defined as one whose deletion leads to at least one secondary loss.

Statistic	Value	Coeff.	SE	<i>t</i>	<i>p</i>	<i>R</i>
N	48					
Multiple R	0.69					
Multiple R <sup>2</sup>	0.48					
Multiple R <sup>2</sup> adj.	0.46					
<i>p</i>	3.59E-07					
Constant		0.15	0.07	2.07	0.0441	0.00
$\beta$		0.41	0.12	3.56	0.0009	0.20
$\epsilon$		-0.15	0.01	-6.48	0.0000	0.58

Table A2. Ranking of 12 patch-scale food webs in the River Tiber (Rome, Italy) across different species extinction scenarios (A-D) in terms of vulnerability to biodiversity loss, as calculated from food web Robustness (indicated as  $V_R$ ) or Resistance (indicated as  $V_{RC}$ ). Rankings range from 1 (most vulnerable) to 12 (least vulnerable). Each alphabetic letter indicates the same food web across different extinction scenarios. Webs from a to f refer to the food webs reconstructed upstream of the urban area both before (food web a) and at five different sampling times (food webs from b to f) after an exceptional flood season. Webs from g to l refer to the food webs reconstructed downstream of the urban area both before (food web g) and at five different sampling times (food webs from h to l) after the exceptional flood season.

Rank	Scenario A		Scenario B		Scenario C		Scenario D		Scenario A		Scenario B		Scenario C		Scenario D	
	Web	$V_R$	Web	$V_R$	Web	$V_R$	Web	$V_R$	Web	$V_{RC}$	Web	$V_{RC}$	Web	$V_{RC}$	Web	$V_{RC}$
1	l	0.79	g	0.48	g	0.33	l	0.58	f	0.83	k	0.84	k	0.91	k	0.84
2	k	0.58	d	0.27	h	0.23	d	0.45	l	0.80	l	0.84	l	0.91	l	0.84
3	g	0.50	e	0.23	j	0.20	g	0.33	h	0.79	f	0.83	f	0.83	f	0.83
4	b	0.45	h	0.23	i	0.14	k	0.26	k	0.78	h	0.79	h	0.81	h	0.81
5	d	0.45	a	0.20	l	0.05	h	0.23	d	0.70	d	0.73	g	0.80	g	0.79
6	f	0.45	l	0.16	k	0.05	j	0.20	g	0.70	b	0.71	c	0.78	d	0.73
7	c	0.43	k	0.16	a	0.00	a	0.00	a	0.63	i	0.69	d	0.73	b	0.71
8	a	0.40	i	0.14	b	0.00	b	0.00	j	0.58	g	0.67	b	0.71	e	0.67
9	h	0.38	j	0.07	c	0.00	c	0.00	i	0.56	a	0.63	i	0.69	a	0.66
10	j	0.34	b	0.00	d	0.00	e	0.00	b	0.50	c	0.60	j	0.68	i	0.60
11	i	0.29	c	0.00	e	0.00	f	0.00	e	0.50	j	0.58	a	0.67	c	0.60
12	e	0.23	f	0.00	f	0.00	i	0.00	c	0.48	e	0.50	e	0.61	j	0.52

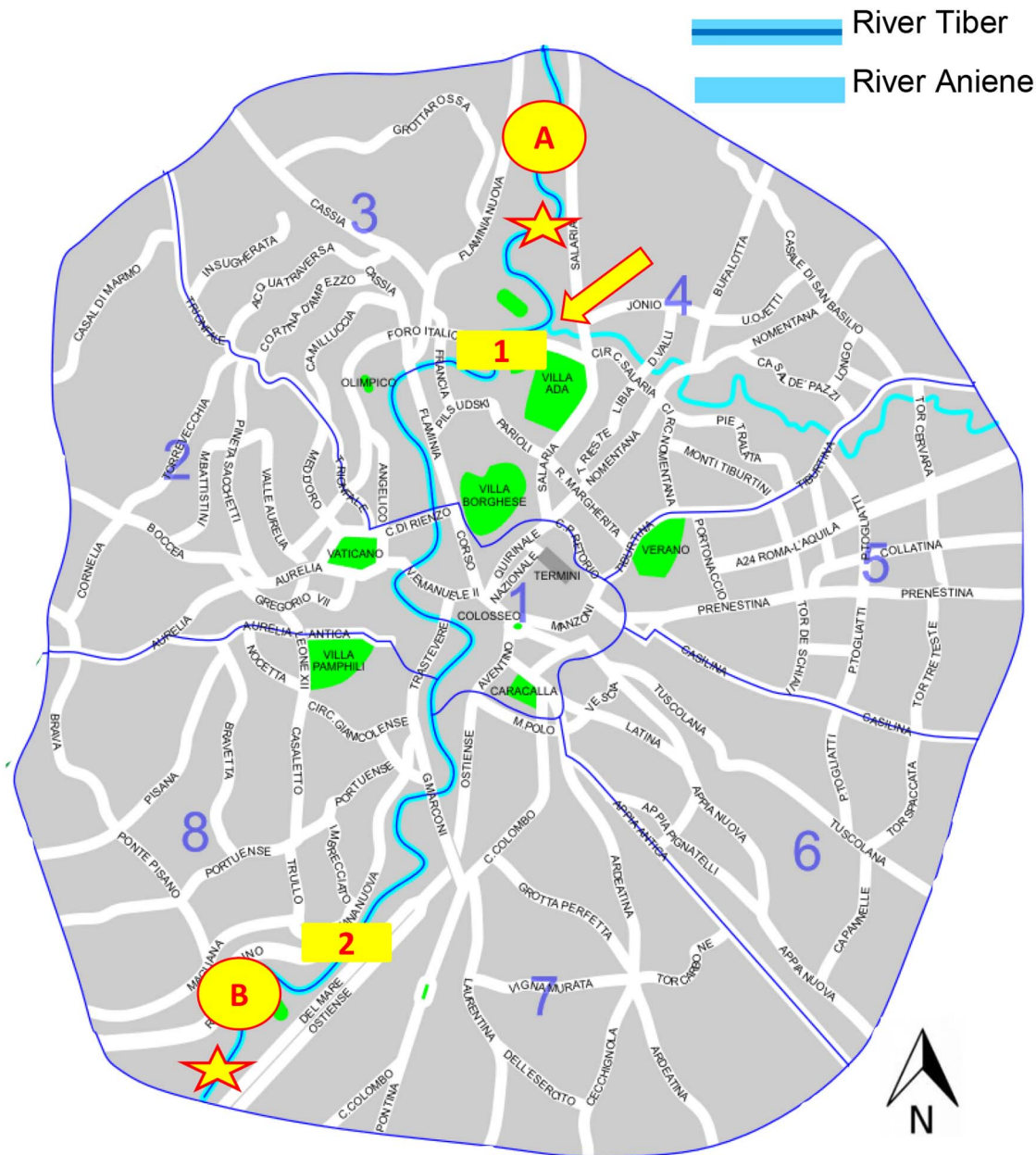


Fig. A1. Representation of the study area, the lowland stretch of the River Tiber (Rome, Italy). Samplings were conducted at one sampling location upstream (A) and one sampling location downstream (B) of the urban area of Rome. The upstream and downstream sampling locations are 27.5 km apart along the river course. The map shows the position of each sampling location, the River Aniene, characterized by highly polluted waters, and the waste water treatment plants (WWTP) serving the urban area. Stars indicate WWTP points of discharge into the River Tiber. The southern WWTP has been shown not to affect the invertebrate community upstream of the point of discharge (di Lascio et al. 2013). The arrow indicates the confluence of the River Aniene with the Tiber. Numbers 1 and 2 delimit the urban stretch where the River Tiber is characterized by artificial banks and frequent engineering works.

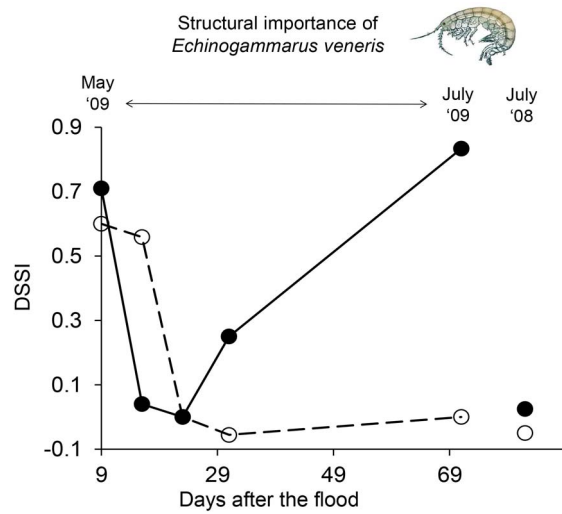


Fig. A2. Post-flood variation in the structural importance of the dominant species (DSSI) in detritus-based food webs in the River Tiber (Rome, Italy), both upstream (closed circles and solid line) and downstream (open circles and dashed line) of the urban area. *Echinogammarus veneris* was always the dominant invertebrate species, the only exception being the pre-flood sampling time at the downstream location, when Chironomid larvae were dominant.

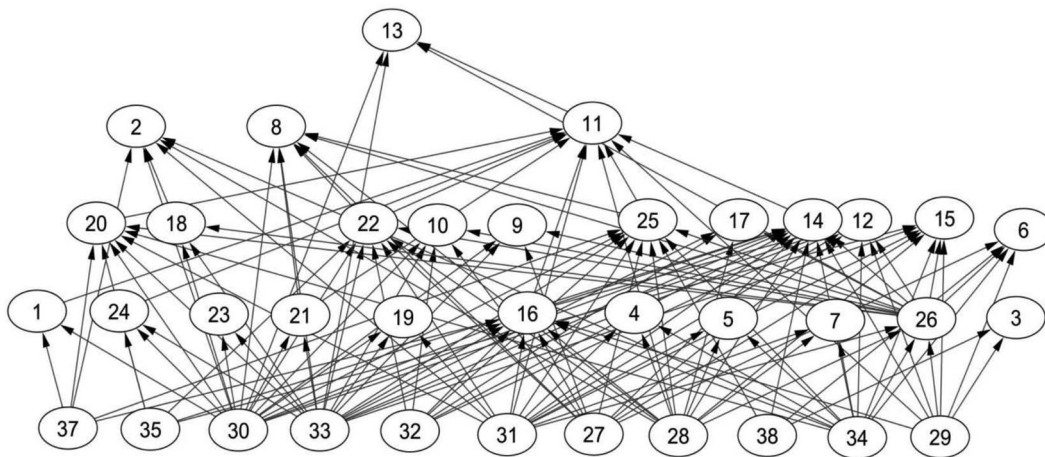


Fig. A3. Time-and-space integrated structure of the detritus-based food web in the River Tiber (Rome, Italy). The time-and-space integrated web structure was obtained by cumulating the species and feeding links detected at 6 different sampling times before (July 2008) and after (from May to July 2009) an exceptional flood season and both upstream and downstream the urban area of Rome. All species and links that occurred at least in one sampling time and location were considered in the integrated food web structure. Arrows represent feeding links pointing from a prey to its predator, and numbered circles are taxa. For each taxon, the first number in square brackets indicates its ranking position according to the degree of interconnection within the food web, i.e., from the most (rank 1) to the least (rank 38) connected, and the second number in brackets indicates its ranking position according to the relative abundance of that taxon in the community, i.e., from the most (rank 1) to the least (rank 38) abundant. Taxon 1: *Coenagrion* sp. [31–35]; 2: Odonata Zygoptera (alia) [19–14]; 3: Hydropsychidae sp. [37–9]; 4: Leptoceridae sp. [32–37]; 5: Odontoceridae sp. [33–38]; 6: Philopotamidae sp. [22–32]; 7: Polycentropodidae sp. [34–20]; 8: Hydracarina sp. [16–31]; 9: Hirudinea (alia) [29–13]; 10: *Glossiphonia* sp. [23–26]; 11: *Helobdella* sp. [10–27]; 12: *Hemiclepsis* sp. [13–36]; 13: *Dina* sp. [30–7]; 14: *Erpobdella* sp. [6–12]; 15: *Piscicola* sp. [17–11]; 16: *Ischnura* sp. [8–17]; 17: *Planaridae* (alia) [28–15]; 18: *Crenobia* sp. [26–25]; 19: *Dendrocoelum* sp. [35–28]; 20: *Dugesia* sp. [15–10]; 21: *Gonocephala* sp. [25–19]; 22: *Polycelis* sp. [12–30]; 23: *Dugesia lugubris* [36–33]; 24: *Dugesia tigrina* [27–18]; 25: Trichoptera (alia) [9–23]; 26: Diptera (larvae) [3–3]; 27: *Valvata piscinalis* [11–16]; 28: *Asellus aquaticus* [5–5]; 29: *Bithynia tentaculata* [14–6]; 30: Chironomidae (larvae) [2–2]; 31: *Echinogammarus veneris* [4–1]; 32: Ephemeroptera [18–22]; 33: Oligochaeta [1–4]; 34: *Physa fontinalis* [7–8]; 35: *Sphaerium* sp. [20–24]; 36: *Teodoxus fluviatilis* [38–24] (do not preyed by any predator); 37: *Planorbis planorbis* [21–29]; 38: *Baetis* sp. [24–21].