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Is landscape of fear of macroinvertebrate communities a major determinant of mesopredator and prey activity?

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Corresponding Author:	Raoul Manenti Universita degli Studi di Milano Milano, Milano ITALY
Corresponding Author E-Mail:	raoulmanenti@gmail.com
Order of Authors:	Raoul Manenti
	Benedetta BARZAGHI
Abstract:	Macroinvertebrate foragers play an important role on the trophic structures of freshwater environments, and multiple trophic levels occur among macroinvertebrate communities providing very interesting scenarios for testing scientific hypotheses. One of the most intriguing aspect to understand is the role played by the landscape of fear (LOF) on macrobenthos density and activity.
Additional Information:	
Question	**Response**
Suggested Reviewers:	Daniela Ghia Universita degli Studi di Pavia daniela.ghia@unipv.it Expert of invertebrates
	Ronald Sluys Naturalis Nationaal Natuurhistorisch Museum ronald.sluys@naturalis.nl Expert of behaviour and ecology of triclads
	Diego Fontaneto Istituto per lo Studio degli Ecosistemi Consiglio Nazionale delle Ricerche d.fontaneto@ise.cnr.it Studies freshwater invertebrates distribution and ecology.
Opposed Reviewers:	

Milano, 01/11/2019

Dear, Prof. Daniel Gerdeaux,

Thank you fro the quick answer.

We shortened consistently the paper to submit it as a short communication. As we are convinced that testing the landscape of fear (LOF) on freshwater macroinvertebrate communities is quite novel and very interesting we believe that a short and focused communication can be a good basis for further and more detailed studies.

We used multiple surveys and multiple season to assess if the LOF at the macroinvertebrate communities affected the density of three target invertebrate species, a detrtivor and two mesopredators.

The broad implication of the research is that the abundance of freshwater macroinvertebrates is mainly linked to some environmental than to the predation risk at the community level.

Please find enclosed the short communication entitled "Is landscape of fear of macroinvertebrate communities a major determinant of mesopredator and prey activity?", to be considered for publication in Knowledge and Management of Aquatic Ecosystems.

We confirm that:

- The enclosed work was never submitted or published and to another journal;
- its submission for publication was approved by all relevant authors and institutions
- all persons entitled to authorship have been so named
- all authors have seen and agreed to submit this version of the manuscript.

Yours sincerely,

Raoul Manenti, Benedetta Barzaghi

Title page

Is landscape of fear of macroinvertebrate communities a major determinant of mesopredator and prey activity?

Raoul MANENTI*, Benedetta BARZAGHI

Department of Environmental Science and Péolicy, univeristà degli Studi di Milano, Via Celoria, 26, 20133 Milano (Italy)

*= corresponding author: raoulmanenti@gmail.com

Running head: landscape of fear of macroinvertebrates

Abstract

Macroinvertebrate foragers play an important role on the trophic structures of freshwater environments, and multiple trophic levels occur among macroinvertebrate communities providing very interesting scenarios for testing scientific hypotheses. One of the most intriguing aspect to understand is the role played by the landscape of fear (LOF) on macrobenthos density and activity.

With this pilot study we wanted to test if LOF at the macrobenthos community levels play a role in determining the density of both prey and mesopredators.

During two consecutive years, we evaluated, with both day and night surveys, the density of two mesopredator triclad species and of one detritivor prey crustacean species comparing it to the number of respective predators occurring in the macroinvertebrate community.

LOF levels at the macroinvertebrate community did not reduce the abundance of the target taxa. One of the triclad species was instead positively related to the levels of LOF assessed for it on the basis of the available knowledge.

The broad implication of the research is that the abundance of freshwater macroinvertebrates is not mainly linked to the predation risk at the community level, suggesting that also for researches on macrobenthos LOF analyses should take in consideration the role of top predators.

Keywords: seepage, triclad, isopod, behaviour, predator.

The communities of freshwater invertebrates are regarded as fundamental indicator of the status and pollution of freshwater habitats. Several factors may determine differences in macroinvertebrate activity and distribution; in general, all the aspects under the constraints of Darwinian natural selection as: food availability, predation risk and other inter- and intra-specific interactions may concur to determine differences in macroinvertebrate species density (Elliott, 2000, 2002; Kusano and Kusano, 1991). While food availability is a well-recognised element regulating macrobenthos abundance and diel activity (Elliott, 2002; Fiser et al., 2007), in freshwater habitats less attention is paid to the predation risk that may involve different taxa. In particular, one of the most intriguing aspect to understand is the role played by landscape of fear (LOF) on macrobenthos density and activity: macroinvertebrate foragers play an important role on the trophic structures of freshwater environments, and multiple trophic levels occur among macroinvertebrate communities furnishing several opportunities to study LOF effects (Marino et al., 2016). A forager has to usefully adopt strategies to forage based on the type of risk it is likely to face (Matassa and Trussell, 2011). Generally, the activity patterns a forager must take to cope risk from habitats with high number of predators, will differ greatly from those it will take to exploit safer habitats (Melotto et al., 2019). Within predation risk both the diversity of the predator community and of the predator activity play major roles in affecting the LOF (Gaynor et al., 2019); in particular, the predator diel activity levels may strongly change the features of LOF ((Bleicher et al., 2019; Laundre, 2010) with consequent reflections on prey activity itself. However, how much LOF levels may affect macroinvertebrate species sampling and activity remains an intriguing aspect to be studied.

To assess if LOF affects the macrobenthos diel activity, we studied environments with similar aquatic top predator presence, such as day active visual predators (fish) and night active wanderer predators (the invasive crayfish *Procambarus clarkii*) and we focused only on the LOF at the macroinvertebrate community level.

First of all, we tested if LOF varied between day and night conditions; second we evaluated the relationship between LOF and density of both target mesopredator and prey invertebrate species. In particular, we tested two hypotheses:

1) Fear hypothesis; LOF affects prey and/or mesopredator density activity.

2) No fear hypothesis; The density of predator and/or prey species varies with day/night conditions notwithstanding to the levels of LOF.

In particular, during two consecutive years, we evaluated, with both day and night surveys, the density of two mesopredator triclad species and of one detritivor prey crustacean species comparing it to the predator occurring in the whole macroinvertebrate community.

We performed the study in Lombardy (NW Italy). We studied four “fontanili”; springs forming lentic habitats fed by groundwater flow. Fontanili are springs anciently managed by humans pushing tubes in the substrate to collect groundwaters and ease their flow toward the surface (Balderacchi et al., 2016). Fontanili springs are generally characterised by a large head in which the tubes occur and a straight section that allows water to flow out. We performed transects in the spring head along the outflow tubes tracing 8 transects (2 for each site). The transects were all 1 m wide, but varied in length depending on the site features (length average ± SE = 4.3 ± 0.7 m).

During winter months, from December 2017 to February 2018 and from December 2018 to February 2019 we performed for each site 12 repeated surveys both during day and during night (6 surveys during night and 6 during day). During surveys in each site we first assessed visually the occurrence and the number of the target taxa such as crustaceans of the species *Asellus aquaticus* and planarians of the species *Polycelis nigra* and *Dendrocoelum lacteum* along two transects per site.

During each survey, in each transect after 20 minutes of visual encounter numbering of the target organisms we sampled the whole macrobenthos community using a dip-net. Net samplings lasted 10 minutes in each transect and were performed by intense movement of the substrate. All the collected organisms were released in the transect of origin after having been numbered and recognised at species, genus or family level according to the guidelines for the Italian Biotic Index assessment

(Ghetti 1997). We also we assessed the occurrence of wanderer top predator species like fish. From each survey we kept at minimum 4 days of interval. During surveys we recorded also maximum illuminance of the water surface (with a PCE EM882 luxmeter) and water temperature. LOF assessment considered the taxa collected through the dip-netting of the substrate at each sampling session. LOF was calculated using the number of potential predator taxa for each target species occurring in the transects: we divided the number of occurring predators per the total number of taxonomical units collected. Predator assessment was based on the information available in the literature (Ghetti, 1997; Reynoldson and Young, 2000; Tachet, 2010).

To test if LOF was different between sites and day/night conditions, we developed a Linear Mixed Model (LMM) using the log transformed levels of LOF as dependent variables and the transect identity and the period (day/night) as fixed factors; we considered also the year of sampling as random factor. Through a Wald F test we assessed the significance of the fixed factors composing the model. We then used random-effect generalized mixed models (GLMMs) to assess the relationships between the relative abundance of the target taxa and the LOF (Barker et al., 2017). In particular, we used a negative binomial distribution to account for over dispersion as, especially for planarians we had different 0 occurrences. As a dependent variable, we considered the number of active individuals of the target taxa observed for each transect at each survey. We included the moment of observation (day/night) and the sampling method (visual/net) as covariates. We included the year of survey, the number of survey and the transect as random factors.

GLMMs and LMMs were run in R environment (R Development Core Team 2018) using a negative binomial error, using the package glmmTMB, lmerTest and car (Brooks et al., 2017).

Considering the whole samplings, *Polycelis nigra* was the more abundant species (on average (± SE) 34.9 ± 8.5 individuals per sampling). Considering night samplings only the average number of *Asellus aquaticus* observed overcame the average number of *P. nigra* (18 ± 5.2 *A. aquaticus* individuals' vs 16.9 ±5.2 *P. nigra* individuals).

Water temperature was on average (± SE) 12,17 °C ± 0.19 °C; a significant difference, assessed through ANOVA and post-hoc tukey test was observed only between two of the sites ($F= 3.2$; $P = 0.03$). Maximum illuminance in fontanili during sunny days was around 40000 lux and ranged between 0,01 and 0,1 lux during night with no significant differences between sites. Wanderer top predator taxa such as fish and the alien freshwater crayfish *Procambarus clarkii* were recorded in all the sites with at least one observation in the proximity or inside the transects during each year of monitoring. While fish were observed both during day and night, crayfish were detected mainly during night. In the transects we recorded globally 13 macroinvertebrate predator taxa at which for our crustacean target species must be added the two planarians target species. On average (± SE), considering all the predator taxa, the number of the potential predator individual for our crustacean target species was of 1.25 ± 0.34 individuals per net sampling.

LOF levels differed among sites (for both crustacean and planarians LOF levels: $F > 5.7$; $P <0.001$), but not between day/night conditions (for both crustacean and planarians LOF levels: $F < 0.22$; $P > 0.64$). *Dendrocoelum lacteum* was significantly more abundant during night (Table 1, Fig. 1). On the other hand, the abundance of *Polycelis nigra* showed a weak, unexpected and significant relationship with the LOF levels considered, being more abundant in transects with higher levels of LOF (Table 1, Fig. 1). *A. aquaticus* was more abundant when sampled with deep net (Table 1, and Fig. 2). No significant effect was played by the LOF levels considered.

The broad implication of the present research is that LOF at the macroinvertebrate community level does not seem to affect the activity of macroinvertebrate foragers. On the other side the abundance of both macroinvertebrate predators and prey is strongly related to other factors irrespective to their position on the food web and to LOF levels.

The ecological study of LOF is increasingly being recognised as central in understanding the patterns driving predator-prey interactions (Gallagher et al., 2017). LOF can determine the population density of a species, but interspecific competitive/predatory interactions in complex communities may produce various combinations of impacts (Gallagher et al., 2017; Laundre et al., 2014). In freshwaters communities, where food webs are often highly structured, the study of LOF effects can be intriguing and reveal important insights in terms of management. As examples, assessing the role of predators may increase the efficacy of restoration action in lotic environments, while understanding the role of LOF in spring habitats may reveal important insights for understanding some of the evolutionary pressures that drive groundwaters colonisation. However, our study suggests that further work is in order. From one side mesopredator taxa considered in our study may not be the most important determinants of the LOF in the system that hosts native fish and alien crayfish as top predators. Often top predators feed both on mesopredators with which they share prey (Rodriguez-Lozano et al., 2015) with likely LOF top-down control on both mesopredator and prey. Thus a finer scaled characterisation of LOF levels based on the foraging activity of top wanderer predators like fish and crayfish may reveal different patterns.

Moreover, some of our results suggest that the evaluation of LOF for planarians on the basis of the available information could not be sufficiently reliable. The assessment of LOF level has been made on the basis of the few information available for the genus *Polycelis* and its possible predator (Tachet, 2010). However some of the predator taxa included could not directly feed on *P. nigra* that it is itself considered mainly a predator of living macroinvertebrates with a minor preference for also dead invertebrates (Reynoldson and Young, 2000; Tachet, 2010). It is possible that *P. nigra* is an opportunistic mesopredator feeding on already damaged/dead invertebrates and thus favoured by other mesopredator occurrence.

A second argument of discussion originating from our results is the differential effect played by day/night conditions on the invertebrate target species. While the abundance of individuals of both crustacean and planarian pigmented species does not differ between day and night, the abundance of individuals belonging to the unpigmented *Dendrocoelum lacteum* species is slightly higher during night. Generally, nocturnal activity in both vertebrates and invertebrates is considered as an adaptive strategy to minimize risk of predation (Huhta et al., 2000; Kotler et al., 2010) and is often supported by heightened non-visual senses that allow detection of threatens in darkness conditions (Bleicher et al., 2019; Vestheim et al., 2013). In our system that assessed LOF levels of invertebrate community through substrate sampling, for both detritivor and mesopredator target species LOF levels did not vary between day and night. *D. lacteum* is an unpigmented epigean species for which some general ecological study has been performed (Herrmann, 1986; Reynoldson and Young, 2000); however, no detailed behavioural information exist; generally freshwater planarians are regarded as nocturnal (Lombardo et al., 2011), but our results indicate that between different genera and species slight differences in the diel activity may occur. As we have argued elsewhere the study of LOF in freshwater environments may be considered a promising aspect to understand evolutionary and ecological patterns shaping freshwater organisms' distribution. Our results suggest however that more studies are necessary to increase the knowledge of species composing the microbenthic community and that the potential role of top predators should be accounted at different habitat scales.

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Table and figures legends

	Variables	Estimate	z	P
Asellus aquaticus				
	Night	0.22	1.05	0.29
	LOF	0.85	0.31	0.75
	Deep netting	1.23	5.44	**< 0.001**
Dendrocoelum lacteum				
	Night	0.72	2.79	**< 0.01**
	LOF	0.09	0.05	0.95
	Deep netting	0.14	0.56	0.57
Polycelis nigra				
	Night	0.44	1.52	0.12
	LOF	7.02	1.96	**0.04**
	Deep netting	0.15	0.55	0.58

Table 1 Results of the GLMMs analysis. In bold the significant results. LOF represents the level of landscape of fear.

Figure 1. Plots and boxplots of the relationship between the number of planarians of the species *Dendrocoelum lacteum* and *Polycels nigra* and the parameters studied. A, B and C refer to *Dendocoelum lacteum*; D, E and F to *Polycelis nigra*. Fear_planarians indicates the level of landscape of fear for the planarians.

Figure 2. Plots and boxplots of the relationship between the number of crustaceans of the species *Asellus aquaticus*. Fear_crustaceans indicates the level of landscape of fear for the target taxon.

Figure 1

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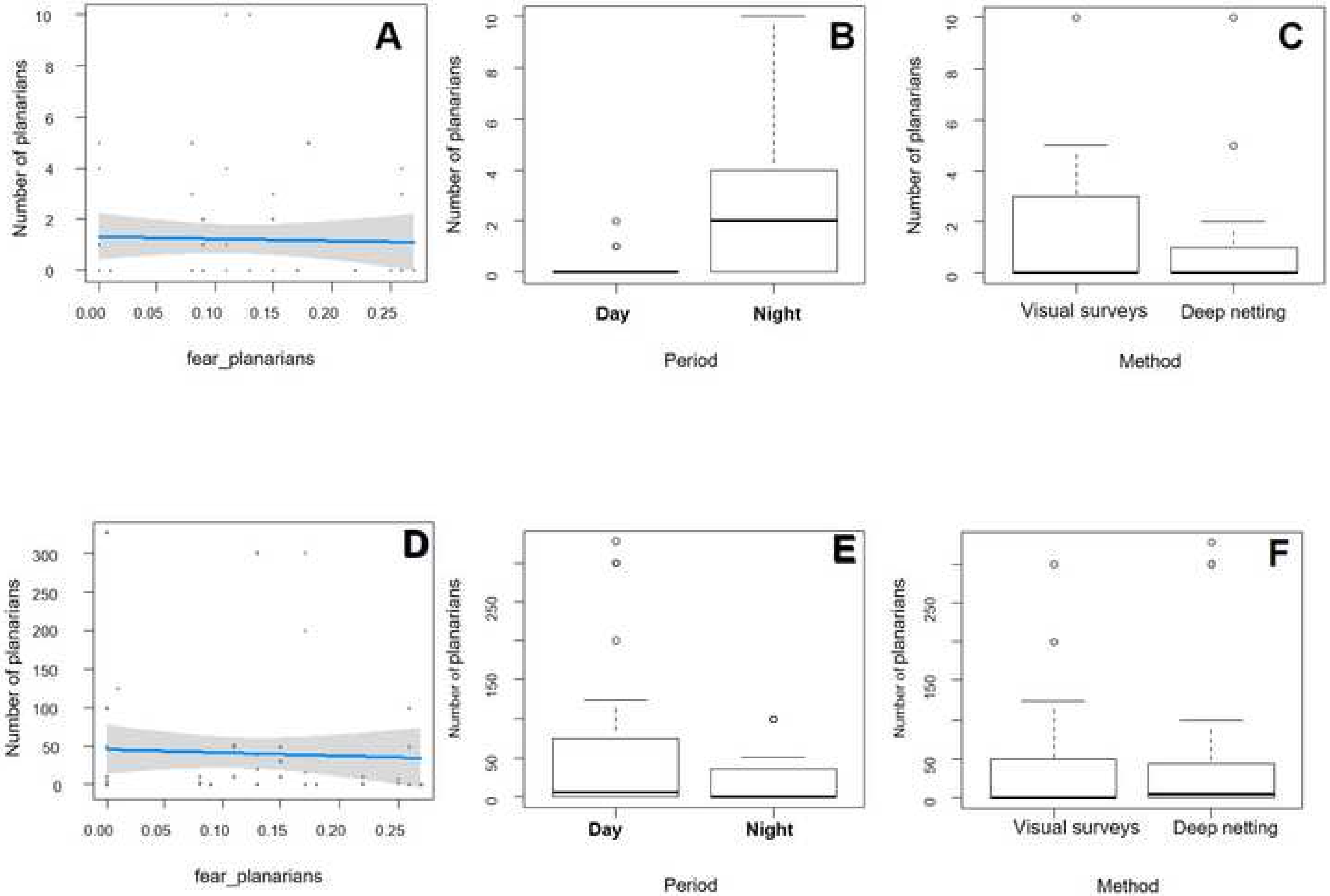

Figure 2

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