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Nematode/copepod ratio and nematode and copepod abundances as bioindicators of pollution: a meta-analysis

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Abstract. Meiofauna has been considered a suitable group for monitoring pollution effects. Based on different pollution tolerance, a nematode/copepod ratio was proposed as an easy tool for monitoring the effect of anthropogenic activities. Although the validity of this tool has been subject to debate due to controversial results, it is still widely used. To establish a general pattern in the response of the ratio and nematode and copepod abundances to the effects of organic enrichment, oil pollution and metal enrichment in the marine environment, we conducted a global-scale meta-analysis. The database consisted of 715 pairs of data obtained from 46 studies published during the last 39 years. We could not find a general trend in the response of nematode and copepod abundances to these pollutants. Regarding the ratio, the only significant difference we found is under the effect of oil pollution. However, this difference appears to be an artifact due to publication bias. The information gathered in this study suggests that the ratio and mean abundances are not reliable tools for monitoring purposes.

[Keywords: global-scale analysis, heavy metal, organic matter, oil pollution, marine meiofauna, monitoring]

Resumen. Índice nematodo/copépodo y las abundancias de nematodos y copépodos como bioindicadores de contaminación: un meta-análisis. La meiofauna ha sido considerada un grupo adecuado para monitorear los efectos de la contaminación. En base a diferencias en la tolerancia a la contaminación, se propuso el índice nematodo/copépodo como una herramienta sencilla para monitorear el efecto de las actividades antropogénicas. Aunque la validez de esta herramienta ha estado sujeta a discusión debido a resultados controversiales, todavía se la usa ampliamente. Para determinar si existe un patrón general en la respuesta del índice y de las abundancias de nematodos y copépodos a los efectos del enriquecimiento orgánico, la contaminación por petróleo y la presencia de metales pesados en el ambiente marino, realizamos un meta-análisis a escala global. La base de datos consistió en 715 pares de datos obtenidos a partir de 46 estudios publicados durante los últimos 39 años. No pudimos encontrar un patrón general en la respuesta de la abundancia de nematodos y copépodos. En cuanto al índice, la única diferencia significativa que encontramos fue bajo el efecto de la contaminación por petróleo. Sin embargo, esta diferencia parece ser un artefacto debido a un sesgo de publicación. La información obtenida en este estudio sugiere que tanto el índice como las abundancias medias no son buenas herramientas para el monitoreo ambiental.

[Palabras clave: análisis a escala global, metales pesados, materia orgánica, contaminación por petróleo, meiofauna marina, monitoreo]

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Introduction

There is overwhelming evidence that human activities are driving rapid changes in the environment (Hoegh-Gulberg and Bruno 2010); thus, there is a crucial need for targeted natural and social science research that builds our understanding of the consequences of these changes for life on Earth and human societies (Rudd 2014). In this context, the consequences of human actions need to be understood to mitigate their impacts on ecosystems (Zeppilli et al. 2015). Usually, an ecosystem can be considered healthy when patterns of species abundance and interactions between these species are relatively unaltered by contaminants (O´Brien and Keough 2014). Marine ecosystems are very important to society since they provide great benefits, especially in coastal areas, but, at the same time, are affected by multiple stressors, inter alia, pipeline input of industrial or domestic wastes, harbour dredgings, oil spills and shipping (Polese et al. 2018; Rees and Eleftheriou 1989). Persistent contaminants are absorbed by sediments in the aquatic environment, where they remain over long periods and can affect organisms living therein (Chapman 1990).

The benthos is considered a suitable ecological compartment for monitoring the effects of pollution (Salas et al. 2006). The presence of these organisms is ubiquitous in the marine environment; they play an important role in the food chain since they represent the link between the organic detritus and the higher trophic levels. Besides, as they live and feed in the sediment, they are expected to be susceptible to pollutants present in it (Elarbaoui et al. 2015). Within benthic assemblages, the meiofauna have been increasingly used as an indicator of the health of marine ecosystems due to their wide distribution, high number and abundance of species, high sensitivity of some taxa to pollutants, rapid life cycle and low-cost sampling and handling (Balsamo et al. 2010).

Most of the studies concerning meiofauna have considered the most representative components from the numerical point of view, which are nematodes and harpacticoids copepods (Sandulli 1986). Nematodes are usually the most abundant taxon, comprising 60-90% of the total fauna, while copepods are typically second and represent 10-40% (Coull 1999). Based on the different pollution tolerance of these two meiobenthic taxa, Raffaelli and Mason (1981) proposed the nematode/copepod ratio (N:C) as a fast and easy tool for monitoring the effect of organic pollution in intertidal environments. This ratio relates the abundances of nematodes and copepods, and is expected to be higher in the polluted area due to nematodes are more resistant to environmental stress than copepods. In addition, the N:C ratio considers the taxonomic sufficiency principle proposed by Ellis (1985), which is an attractive feature considering the difficulty of taxonomic identification of both nematodes and copepods (Rubal et al. 2009). After its publication, the ratio began to be used associated with different kinds of pollutants and environments (intertidal, shallow waters and deep sea). But, at the same time, the validity of this tool was the subject of discussion due to controversial results. Opposite responses of both meiobenthic taxa to an environmental stressor have been reported (e.g., Ansari and Ingole 2002; Boucher 1985; Danovaro et al. 1995; Gee et al. 1985; Lambshead 1984; Lee et al. 2001; Veiga et al. 2010; Vidaković 1983).

Despite the criticism received, N:C ratio and nematode and copepod abundances have been widely used as a tool to assess the effects of many anthropogenic activities in both field and laboratory studies, probably because it is quite easy to calculate it even when taxonomic expertise is lacking. Most of the time, they are reported together with other ecological parameters such as diversity indices and community structure (e.g., Alves et al. 2013; Baguley et al. 2015; Carman et al. 2000; Elarbaoui et al. 2015; Frontalini et al. 2011; Huang et al. 2005). Nevertheless, quite often, these are the only data provided in pollution studies (e.g., Bertocci et al. 2019; Bohórquez et al. 2013; Dal Zotto et al. 2016; Gao and Liu 2018; Kim et al. 2014; Montagna et al. 2013; Morad et al. 2017; Pereira et al. 2017; Riera et al. 2013; Sun et al. 2014).

The response of these meiobenthic taxa to different forms of pollution can range from positive to negative or be even neutral; so, a general pattern is difficult to determine. To analyse this pattern in the marine ecosystem, we conducted a global-scale meta-analysis to evaluate the effect of anthropogenic pollutants on the most important components of the meiofauna (nematodes and copepods), and test the validity of the nematode/copepod ratio. This study aimed to quantitatively synthesize existing information on the N:C ratio and nematode and copepod abundances to elucidate if they can be used as bioindicators of organic enrichment, oil pollution and metal enrichment. At the same time, we performed a bibliography review to evaluate how the use of the N:C ratio and abundances of nematodes and copepods in pollution studies has changed over the years.

Materials and Methods

Data selection

This study was reported according to the guideline of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al. 2021). To evaluate the response of the two major meiobenthic taxa to different forms of pollution in marine environments, we performed a meta-analysis using data collected from Google Scholar and Scopus databases. Our search covered a period of 39 years (from 1981 to 2019) and included the following keywords: 'nematode', 'copepod´, 'meiofauna´, 'monitoring´, 'bioindicator´. We considered the studies that met the following requirements: 1) abundance of nematodes, abundance of copepods or the nematode/copepod ratio, and the amount of the pollutant were measured at the same site; 2) statistical information (i.e., mean values, standard deviation values and number of replicates) provided in graphs, text or tables. We only considered field studies and so we excluded data from laboratory experiments. Data available in graphs were digitized using GetData Graph Digitizer 2.26.0.20. When standard deviation (SD) was not reported it was calculated as follows:

$$SD = SE * \sqrt{n}$$

where SE is the standard error and n the number of replicates. We considered three kinds of pollution: organic enrichment, oil and heavy metals. In our analysis, for each article and kind of pollutant, we considered as control the site with the least amount of pollutant, and pollution of other sites (treatments) was compared to it.

Data analysis

The meta-analysis was carried out using R Software (version 3.6.0; R Development Core Team 2019), Metafor package (Viechtbauer 2010). For each data pair, we calculated the 'pollutant magnitude' (PM) as follows:

$$PM = LT / LC$$

where LT is the pollutant load of treatment (site with more amount of pollutant) and LC the pollutant load of control (site with less amount of pollutant) (for a similar approach see O'Brien and Keough 2014). It was done for each kind of pollutant (organic enrichment, oil, and heavy metals) independently. To obtain the effect size for each data pair we compared the response variable mean from treatment vs control; we selected Hedges' d (escalc function) as a measure of the effect size, which is an estimate of the standardized mean differences that is not biased by small sample sizes (Hedges and Olkin 1985).

We ran three weighted multilevel random-effects models using the rma.mv function in Metafor. The models considered each category of pollutants as moderator and 'papers' (publication from which data were obtained) as a random factor. We removed the intercept in the models to view estimates for each level, as opposed to setting one as a reference level (for a similar approach see Kroeger et al. 2021). Each model evaluated the effect of different kinds of pollutants on nematode abundance (Model 1), copepod abundance (Model 2) and nematode/copepod ratio (Model 3). When the model was significant to any of the categories considered, we explored the possibility of publication bias using the Rosenthal´s fail-safe N analysis in Metafor, which is the number of unreported studies averaging a null result that would have to exist before the overall results could reasonably be ascribed to sampling bias (Rosenthal 1979), and funnel plots, which allow one to visually assess whether studies with small effect sizes are missing from the distribution of all published effect sizes. Heterogeneity was quantified by three types of information: Q statistics, I^2 statistics and Tau-squared.

Results

The use of the N:C ratio and abundances in pollution studies over the years

Out of 658 studies examined, 172 dealt with pollution impact and its effects on meiofauna. Through an analysis from 1981 to 2019, the use of the N:C ratio decreased over the years. In the '80s, ~40% of the pollution studies based on meiofauna reported the ratio; in the '90s and 2000s, the percentage decreased to 35% and 26%, respectively; in the last decade, ~14% of these studies reported the ratio (Figure 1). Although most of these works reported the N:

C ratio as one of many parameters considered, nearly 48% of them only used the ratio (or sometimes N:C ratio + the abundance of nematodes and copepods) to evaluate the impact of pollution.

The abundance of meiobenthic taxa is a widely used parameter in pollution studies. As with the ratio, the abundance usually is one of many parameters reported in pollution studies; however, 19% of the studies were only

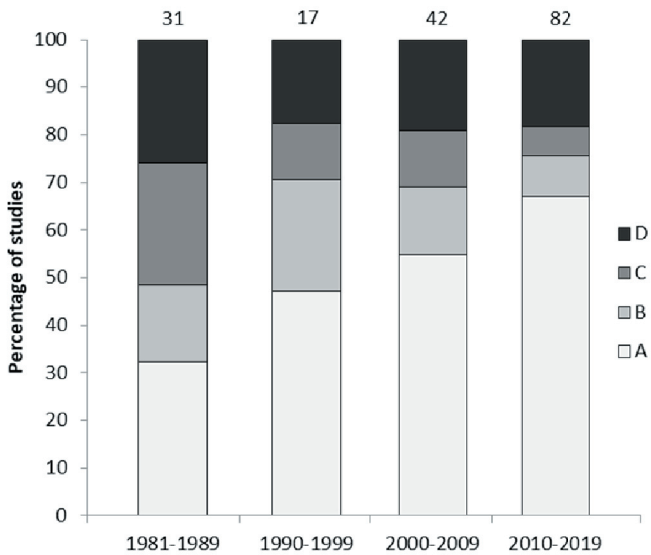

Figure 1. Percentage of pollution studies focused on meiobenthic taxa in the last four decades. Category A represents papers that do not report the N:C ratio; category B represents papers that report the ratio together with other ecological parameters; category C represents papers that report the ratio alone or with nematode or copepod abundances; category D represents papers that report only nematode or copepod abundances. The number of studies considered in each decade is indicated on the top of the column.

Figura 1. Porcentaje de estudios de contaminación que consideran taxones meiobentónicos en las últimas cuatro décadas. A: trabajos que no reportan el índice N:C; B: trabajos que reportan el índice junto con otros parámetros ecológicos; C: trabajos que reportan solamente el índice o el índice junto con las abundancias de nematodos o copépodos; D: trabajos que reportan solo las abundancias de nematodos y copépodos. Sobre cada columna se indica el número de estudios considerados en cada década.

Table 1. Results of Model 1 (M1). Data: nematodes abundance. SE standard error, ci.lb (ub) confidence interval, lower boundary (upper boundary). M1: moderator=pollutant; random=paper. QM(df=3)=1.895; P-value=0.594. QE(df=354)=1602.154; P-value <0.0001; I^2=92.725; Tau-squared=5.064.

Tabla 1. Resultados del modelo 1 (M1). Datos: abundancia de nematodos. SE error estándar, ci.lb (ub) intervalos de confianza, límite inferior (límite superior). M1: moderator=pollutant; random=paper. QM(df=3)=1.895; P-value=0.594. QE(df=354)=1602.154; P-value <0.0001; I^2=92.725; Tau-squared=5.064.

	Effect size	SE	Z-value	P-value	ci.lb	ci.ub
Oil	-1.168	1.103	-1.058	0.290	-3.330	0.995
Heavy metals	-0.782	1.363	-0.573	0.566	-3.453	1.890
Organic enrichment	0.566	0.848	0.668	0.504	-1.095	2.228

Table 2. Results of Model 2 (M2). Data: copepods abundance. SE: standard error, ci.lb (ub) confidence interval, lower boundary (upper boundary). M2: moderator=pollutant; random=paper. QM(df=3)=4.250; P-value=0.236. QE(df=176)=563.458; P-value <0.001; I^2=55.908; Tau-squared=0.396.

Tabla 2. Resultados del modelo 2 (M2). Datos: abundancia de copépodos. SE: error estándar, ci.lb (ub) intervalos de confianza, límite inferior (límite superior). M2: moderator=pollutant; random=paper. QM(df=3)=4.250; P-value=0.236. QE(df=176)=563.458; P-value <0.001; I^2=55.908; Tau-squared=0.396.

	Effect size	SE	Z-value	P-value	ci.lb	ci.ub
Oil	-0.803	0.470	-1.710	0.087	-1.724	0.118
Heavy metals	-0.215	0.414	-0.518	0.604	-1.027	0.598
Organic enrichment	0.381	0.362	1.053	0.292	-0.328	1.090

Table 3. Results of Model 3 (M3). Data: nematode/copepod ratio. The category 'Oil simulated' includes the same data that 'Oil' plus the simulated data pair. SE: standard error, ci.lb (ub) confidence interval, lower boundary (upper boundary). M3: moderator=pollutant; random=paper. QM(df=3)=4.999; P-value=0.172. QE(df=176)=888.411; P-value <0.001; I^2=72.747; Tau-squared=0.716.

Tabla 3. Resultados del modelo 3 (M3). Datos: índice nematodo/copépodo. La categoría 'Oil simulated' incluye los mismos datos que 'Oil' más el par de datos simulado. SE: error estándar, ci.lb (ub) intervalos de confianza, límite inferior (límite superior). M3: moderator=pollutant; random=paper. QM(df=3)=4.999; P-value=0.172. QE(df=176)=888.411; P-value <0.001; I^2=72.747; Tau-squared=0.716.

	Effect size	SE	Z-value	P-value	ci.lb	ci.ub
Oil	1.712	0.799	2.143	0.032	0.147	3.278
Oil simulated	0.773	0.800	0.967	0.344	-0.794	2.341
Heavy metals	0.516	0.490	1.052	0.293	-0.445	1.477
Organic enrichment	-0.300	0.518	-0.578	0.563	-1.315	0.715

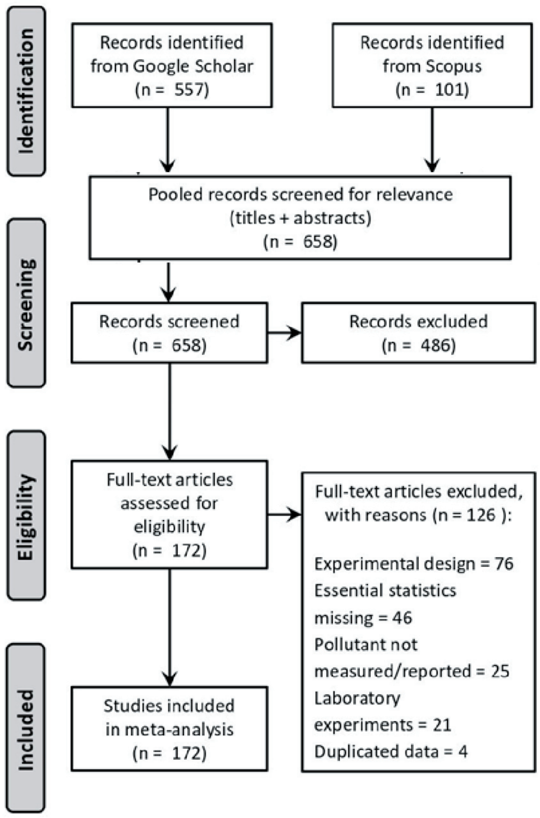

Figure 2. Diagram showing the flow of information from data search to the final data set.

Figura 2. Diagrama que muestra el flujo de información desde la búsqueda hasta el conjunto final de datos.

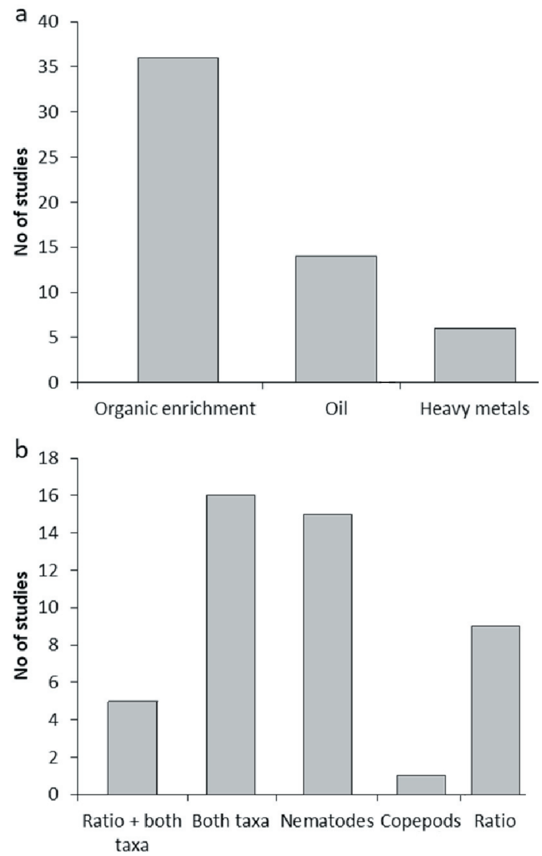

Figure 3. Number of studies examined during the analysis. Studies are characterized by (a) the kind of pollution that is analysed in the study; (b) taxa reported by the study.

Figura 3. Número de estudios examinados. Los estudios están caracterizados por (a) el tipo de contaminación analizada; (b) los taxones reportados.

based on abundances and this percentage has not changed much over time (Figure 1). If we consider the papers that based their assumptions on the use of N:C ratio or abundances, without considering other parameters, they represent 31% of the total of pollution studies based on meiofauna.

The data set

Out of 658 studies examined, a total of 46 papers fulfilled the criteria for inclusion in the analysis (Figure 2 summarizes the reasons for exclusion according to Nakagawa et al. 2017). The final database consisted of 715 data pairs obtained from 46 studies published during the last 39 years (Supplementary Material-Table SM1). The vast majority of research (64.29%) reported pollution by organic enrichment (Figure 3a); research about oil and heavy metal pollution was less frequent (25% and 10.71% respectively) (Figure 3a). Most of the studies reported information about both nematodes and copepods (34.78%) or nematodes only (32.61%); ~11% of the papers reported both abundances and the N:C ratio together; studies based just on copepod abundance were extremely rare (2.17%) and ~20% of the studies reported the N:C ratio as the only parameter (Figure 3b).

About the meta-analysis

Model 1, which evaluated the effect on nematode abundance, did not show a significant difference for any kind of pollutant between control and treatment sites (Table 1, Figure 4a). Model 2, which evaluated the effect on copepod abundance, did not show a significant difference for any kind of pollutant between control and treatment (Table 2, Figure 4b). Model 3 did not show a significant difference for heavy metal pollution and organic enrichment, but it showed a significant difference between control and treatment sites for oil pollution (P=0.032) (Table 3, Figure 4c). Nevertheless, the fail-safe N for this model

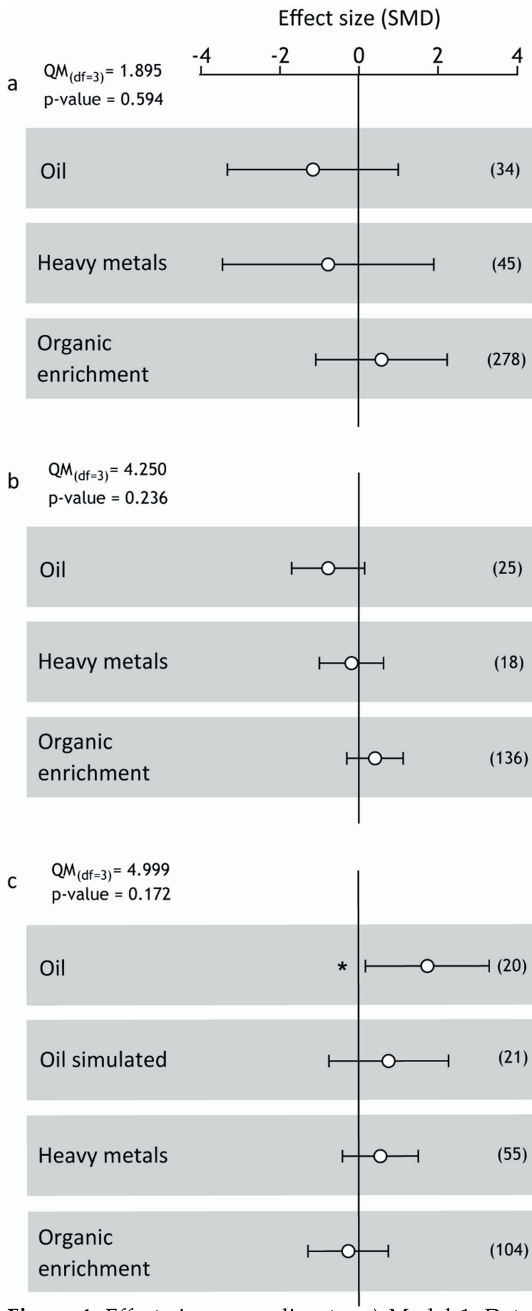

Figure 4. Effect sizes according to: a) Model 1. Data: nematode abundance. b) Model 2. Data: copepod abundance. c) Model 3. Data: nematode/copepod ratio. The category 'Oil simulated' includes the simulated data pair. Horizontal bars for each point denote the 95% confidence intervals for effect sizes. The number in brackets represents the amount of data used to calculate the effect size for each category.

Figura 4. Tamaños del efecto del: a) Modelo 1. Datos: abundancia de nematodos. b) Modelo 2. Datos: abundancia de copépodos. c) Modelo 3. Datos: índice nematodo/copépodo. La categoría 'Oil simulated' incluye el par de datos simulado. Las barras horizontales de cada punto representa el intervalo de confianza del 95% para los tamaños del efecto. El número entre paréntesis representa la cantidad de datos usados para calcular el tamaño del efecto para cada categoría.

was 0 and the funnel plot showed a tendency to publish positive differences that could be influencing the results (Figure 5). Given these and for demonstratives purposes, we added one simulated data to the dataset (Supplementary Material-Table SM1, value in bold) with a negative difference (opposite to that shown by the funnel plot). To generate the simulated data, we chose one of the data pairs that reported the index and exchanged the values of the index between the two sites, the one considered as the control was considered as the treatment and vice versa. This new model did not show a significant difference for oil pollution (Table 3).

Discussion

The information gathered using the meta-analytic approach suggests that the N:C ratio and the mean abundances of nematodes and copepods are not reliable indicators of organic enrichment, oil pollution or metal enrichment. We tested the responses of both taxa to the three different kinds of pollution, and we did not find a common trend in these responses. It suggests that abundance data is not a useful parameter to determine which sites are the most polluted, and, therefore, should not be used as a pollution indicator. When we tested the response of the ratio, we found a significant difference between the control and treatment sites when we considered oil pollution; nevertheless, the fail-safe N and the funnel plot suggested a publication bias with a tendency to publish positive results. In these views, we decided to test again the model with the addition of a simulated data pair with an opposite result, and we failed to find the significant difference anymore. This situation suggests the significant difference at first found with oil pollution was just an artifact.

There has been a great deal of discussion regarding the use of the N:C ratio in assessing pollution impact on meiofaunal assemblage. After all the criticism, the use of the ratio decreased over the years, but it is still widely used. Around 14% of the pollution works based on meiofaunal organisms in the last decade reported the ratio, and almost half of them reported it as the only parameter measured. The same situation occurs with the use of abundances since a non-negligible percentage of pollution studies based their assumption on the effect of the pollutant on taxa abundances only. Even when the use of the N:C ratio has reduced after the critics, it

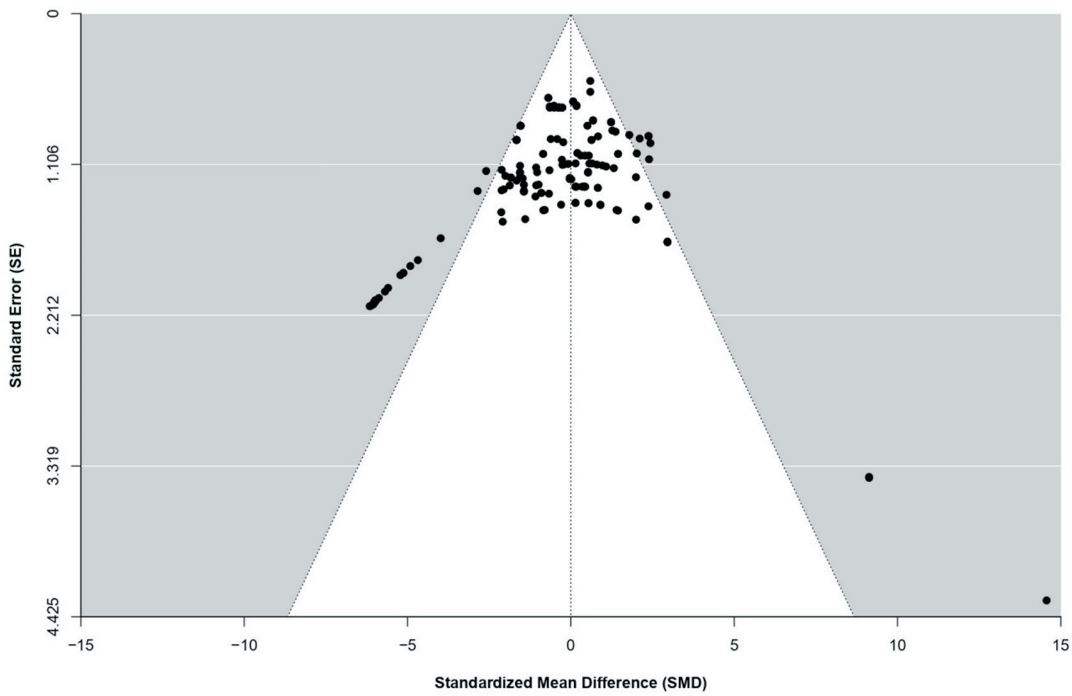

Figure 5. Funnel plots for Model 3 (without the simulated data pair). Data: nematode/copepod ratio. Moderator=pollutant, random=paper.

Figura 5. *Funnel plots* del Modelo 3 (sin el par de datos simulado). Datos: índice de nematodo/copépodo. Moderator=contaminante, random=trabajo.

seems that the use of abundances as the only parameter analysed has not changed over the years.

While nematode and copepod abundances do not seem to be reliable tools for biomonitoring purposes, this could change if the abundance of species is included in the analysis. It is estimated that in 100 cm^3 of marine sediments there are about 20 species of nematodes, although in deep-sea sediments this number could be higher, while copepods usually represents the second most important group in these samples (Giere 2009). Even if the total abundance of nematodes and copepods could not change due to pollution, the abundance of sensitive species may decrease, while the abundance of tolerant species increases. Carman et al. 2000 found that even when diesel contamination may have minimal effect on copepod abundance, it reduces the copepod species diversity. Similarly, other studies found species-sensitivity differences with nematodes (Ansari et al. 2016; Austen and McEvoy 1997).

Nematode and copepod populations may react in different ways according to a variety of environmental parameters, and pollution is just one of them (Lambshead 1984). This could be the reason why it is hard to find a generalized response to the three kinds of pollutants considered in this study. In light of our results, nematode/copepod ratio or nematode and copepod abundances by their side are inadequate tools for biomonitoring purposes. We know there is evidence suggesting meiofauna is a good indicator of anthropogenic activities (Zeppilli et al. 2015), and we are not suggesting the opposite. We want just to draw attention on the uncritical use of simplistic tools. Additional taxonomic resolution and diversity indices are being widely used to assess pollution impacts and these parameters could shed light on more subtle impacts that are not observed by investigating major taxa.

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