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Research Letters

Biased research generates large gaps on invertebrate biota knowledge in Brazilian freshwater ecosystems

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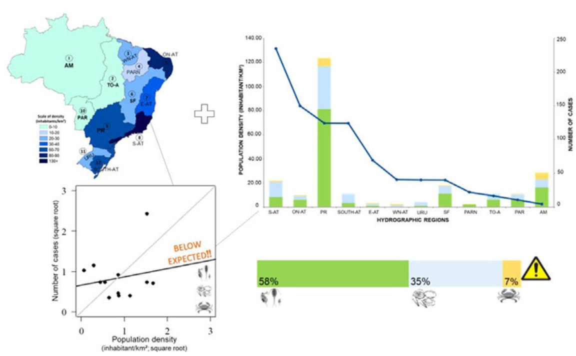
HIGHLIGHTS

- The zooplankton was the most studied taxon, followed by mollusks and crabs, and it was also dominant across the hydrographic regions.
- Cases about microplastics were reported only in three regions.
- The hydrographic region of Paraná comprised the largest number of cases for the three invertebrate groups.
- It was detected a disproportionately low increase of number of cases in relation to population density in the hydrographic regions.

GRAPHICAL ABSTRACT



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ABSTRACT

Research biases are common and constant issues in biological research, with major consequences for biodiversity conservation. Freshwaters represent one of the most threatened ecosystems worldwide, but knowledge of freshwater biota is unevenly distributed due to bias toward marine and terrestrial groups. Therefore, detecting biases and associated knowledge gaps is crucial to steer future research effort and to guide applicable conservation policies for freshwater ecosystems. In this study, we investigated the existence of biases and gaps in knowledge about the biodiversity of invertebrate fauna (zooplankton, mollusks and freshwater crabs) in the hydrographic regions of Brazil. We searched for all studies published in this country on zooplankton, mollusks and freshwater crabs, in the Web of Science database. Subsequently, the number of papers was classified by taxonomic group and hydrographic region to detect research biases. Each report within a paper between a given taxonomic group and a hydrographic region was termed as a case. We also recorded human population density for each hydrographic region. Among the taxonomic groups, zooplankton was the most studied taxon, followed by mollusks and crabs, and it was also dominant across the hydrographic regions. The hydrographic region of Paraná comprised the largest number of cases for the three invertebrate groups. We detected a disproportionately low increase of number of cases in relation to human population density in the hydrographic regions. The identification of the major gaps reported here limits our ability to draw scenarios for the conservation of hydrographic regions and their megadiverse biota in Brazil.

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Introduction

Research biases result in uneven distribution of biological surveys and subsequent flawed knowledge on identity, distribution and attributes of species, which has major consequences for conservation (Clark and May, 2002; Bini et al., 2006; Mokany and Ferrier, 2011). Taxonomic biases are persistent and pervasive issues in ecological research, limiting our knowledge of biodiversity (Clark and May, 2002; Ribeiro et al., 2016). Biogeographic biases derive in areas, countries or ecosystems neglected or poorly considered (e.g., Moerman and Estabrook, 2006; Deikumah et al., 2014; Guerra et al., 2020). Overall, research biases are particularly relevant in developing countries with limited research funding, and large countries with remote oversampled areas and uneven distribution of population and research institutions (Deikumah et al., 2014; Oliveira et al., 2016). Therefore, detecting biases and gaps is largely required to subsequently guide future research efforts, to stablish efficient conservation strategies and to provide evidence-based support for decision-making.

Freshwaters comprise one of the world's most threatened ecosystems (Jenkins, 2003; Vörösmarty et al., 2010; Carrizo et al., 2017), harboring about 10% of global biodiversity despite their low representativeness on Earth's surface (Strayer and Dudgeon, 2010). However, our knowledge of freshwater biota is unequally distributed because of the strong bias toward marine and terrestrial, sometimes charismatic, taxonomic groups in biological samplings and conservation research (Clark and May, 2002; Darwall et al., 2011; Carrizo et al., 2017). In the last decades, pollution and urban solid waste have increasingly been major and persistent causes of direct loss of biodiversity in freshwater ecosystems (Agostinho et al., 2005; Cardinale, 2011; Reid et al., 2019). Consequently, large efforts are required to improve our knowledge about the ecological role of organisms living in freshwater environments, their distribution, the consequences of pollution on them, and subsequent conservation. This is particularly important to achieve the Agenda 2030's Goals 15, that seeks to protect, restore and promote the sustainable use of freshwater ecosystems and their services (UN General Assembly, 2015), as well as the Aichi Target 6, which proposes to manage and harvest sustainably all invertebrate stocks from aquatic ecosystems for 2020 (CBD, 2010). Without a substantial change in the knowledge of freshwater ecosystems and their biodiversity, achieving the international conservation agenda and policy of the world's freshwater ecosystems remains increasingly difficult.

The need to determine knowledge gaps in freshwater systems' biota becomes more urgent in large and developing countries. In this context, Brazil represents a valuable study model for manifold reasons. First, specific laws about freshwater conservation in this country are virtually non-existent (Ferreira et al., 2014; Reid et al., 2019). Second, Brazil has the largest volume of freshwater ecosystems in the world, comprising about 8200 km^3 (12% of the world's freshwater resources: Gleick et al., 2006). Third, the vast territory of the country also includes the Amazon Basin, the world's largest river system, and some hydrographic regions (HRs) that comprise densely-populated and highly-industrialized areas (Agência Nacional das Águas (ANA, 2015). These socioeconomic particularities, together with the inaccessibility of large and remote areas, may have influenced the funding directed to scientific research in each HR over time, including research effort on knowledge, management and preservation of biodiversity, as reported for terrestrial ecosystems (Oliveira et al., 2016; Ribeiro et al., 2016; Guerra et al., 2020). Thus, temporal, geographic and taxonomic biases on biota from freshwater ecosystems and resulting shortfalls may have emerged in the country. In this regard, the occurrence of biased research and knowledge gaps on invertebrate fauna across freshwater ecosystems in Brazil may represent a threat for conservation of these human-perturbed and vulnerable environments in this megadiverse country.

In this study, we investigated the status of the research biases and the existence of knowledge gaps in the number of published papers focused on zooplankton, mollusks and crustaceans in the Brazilian HRs. Given that population, research institutions and economic resources are unevenly distributed in the country, we expect the number of cases is geographically biased, disproportionally increasing in overpopulated regions that comprise major research centers, resulting in vast, historically undersampled, hydrographic regions. Ultimately, our study may help us to guide future research and to provide useful information to better support conservation decisions in the Brazilian freshwater ecosystems.

Methods

Biological groups

The three groups of aquatic invertebrates investigated in this study (i.e. zooplankton, mollusks and crabs) play a multifold and strategic role in the aquatic trophic web. They comprise a high diversity of feeding habits, and act on energy transfer among trophic levels, nutrient cycling and in the structuring of habitats (Vaughn and Hakenkamp, 2001; Collins et al., 2006). The studies on these groups in Brazil began around the 19th century and, to date, many inventories and catalogues have revealed high richness and widespread distribution for zooplankton (625 rotifer species and 112 cladocerans) and bivalves (117 species), and high endemism indexes for crabs of the infraorder Brachyura (about 27% of species) in freshwater ecosystems (Elmoor-Loureiro, 2000; Cumberlidge et al., 2009; Garraffoni and Lourenço, 2012; Pereira et al., 2014; Pedraza et al., 2016; Zanetti et al., 2018).

Data source

We surveyed papers on 27 March 2019 in the Web of Science database. This platform was selected because it provides access to a large database comprising a high number of publications and high-quality indexed journals. To access the papers focused on freshwater biota we searched specific keywords in English and Portuguese: "zooplan?ton*" or "zooplâncton*" or "zooplan?t?nic*", "freshwater crab*" or "caranguejo* de água doce", "mollus?*" or "molusc*" or "clam*", and "Bra?il". The searches considered the title, abstract and keywords of papers between 1974 to 2019. For mollusks, we added the terms "freshwater" or "água doce" or "límnico*" to refine the search and exclude studies unrelated to the biodiversity of the group (e.g. studies of parasitology). For each particular search, we specifically excluded (using the boolean operator NOT) "marine" or "estuar*" or "coast*" or "marinho*" or "costeiro*" to exclusively seek studies from freshwater environments.

Despite the terminological precision of the survey, some studies were still excluded after an exhaustive assessment and categorization of all papers found. Thus, we excluded papers that did not include zooplankton, mollusks or freshwater crabs as study system (n = 439). We also excluded studies conducted outside Brazil, that did not indicate a precise study area (n = 71), and that used specimens obtained from zoological collections (n = 35, including databases) or experimental studies (n = 103), which precludes to know the original study area.

For the classification of Brazilian Hydrographic Regions (HRs), we considered the division according to the special edition of the "Brazilian Water Resources Report: hydrographic regions", published and available online by the National Water Agency (Agência Nacional das Águas (ANA, 2015). This document con-

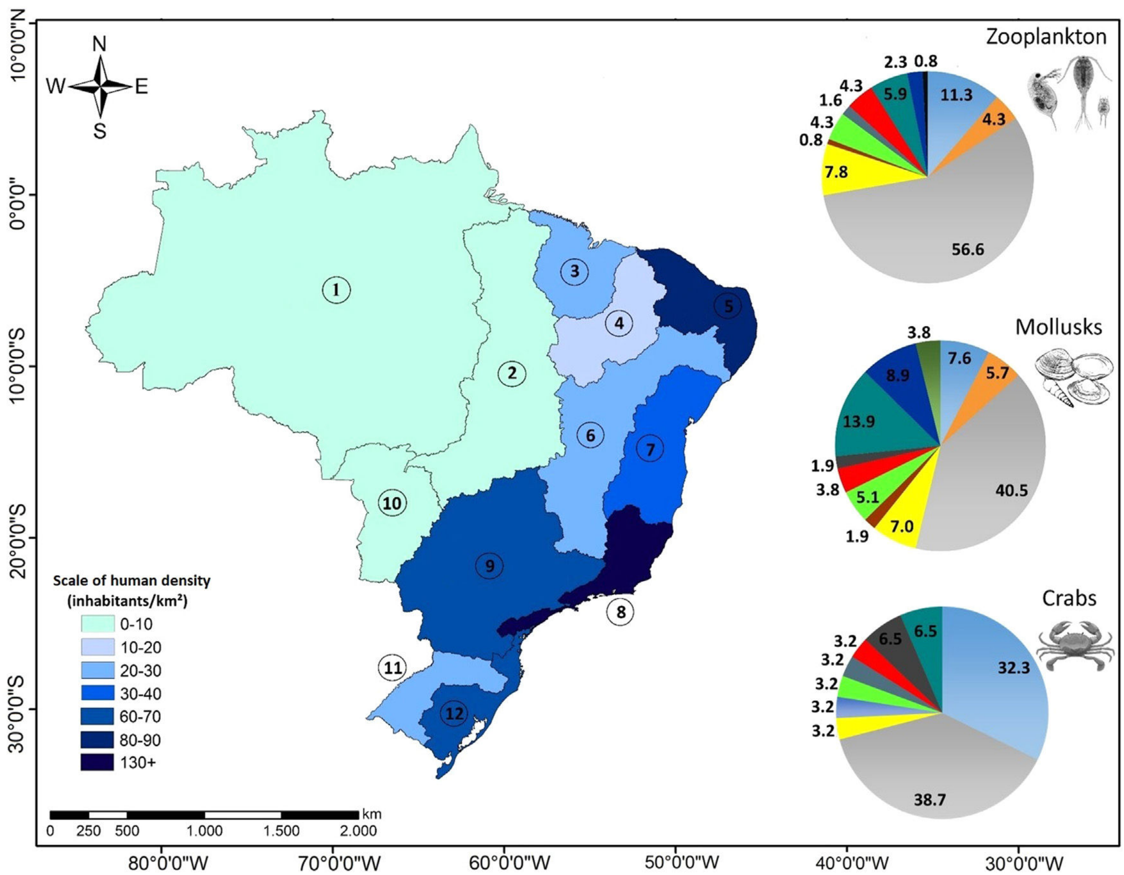

Fig. 1. Map of Brazil depicting human-population density of the 12 Hydrographic Regions (HRs). On the right side the proportion of cases studied (%) in each HR for the three invertebrate groups analyzed is shown. The numbers in the map and the color in the graphs represent each HR: **1** ■ Amazonian, **2** ■ Tocantins-Araguaia, **3** ■ Western Northeastern Atlantic, **4** ■ Parnaiba, **5** ■ Oriental Northeastern Atlantic, **6** ■ San Francisco, **7** ■ East Atlantic, **8** ■ Southeastern Atlantic, **9** ■ Paraná, **10** ■ Paraguay, **11** ■ Uruguay, **12** ■ South Atlantic.

tains information of the Brazilian freshwater ecosystems delimited into twelve HRs (Southeastern Atlantic, Oriental Northeastern Atlantic, Paraná, South Atlantic, East Atlantic, Western Northeastern Atlantic, Uruguay, San Francisco, Parnaíba, Tocantins-Araguaia, Paraguay and Amazonian; see also Fig. 1), its total area (km^2), and the number of inhabitants. These data were used to calculate human population density (inhabitant/km^2) for each HR (Table S1 in Supplementary Information; Fig. 1). Subsequently, we classified the selected studies into the corresponding Brazilian HR. Some studies, especially for mollusks and zooplankton, reported data from multiple HRs, so each report within a paper between a given taxonomic group and a HR was termed as a case, the unit of our database (Table S1).

Data analyses

To determine biases on invertebrate fauna research in the freshwater ecosystems of Brazil four linear regressions were performed: the number of study cases found on (1) zooplankton; (2) mollusks; (3) crabs; and (4) all invertebrate groups was independently regressed against the human population density of HRs. The human population density and the number of cases selected in each HR were square-root transformed to standardize the differences of the units of measure. Subsequently, we tested significant departures of the slope from 1 (i.e. the expected relationship: $\beta \pm SE = 1 \pm 0$) in the observed relationships by means of *t*-tests. When the observed slope is significantly >1 the bias is positive and when <1 the bias is negative. The analyses were conducted in software R 3.5.1 (R Core Team, 2018).

Results

We found 496, 523 and 55 papers about zooplankton, mollusks and crabs, respectively. However, the database ultimately comprised 432 papers and 445 cases focused on freshwater invertebrate groups in the Brazillian HRs following our criteria. Overall, we registered 256 cases for zooplankton (58%), 158 cases for mollusks (35%) and 31 for crabs (7%). The Southeastern Atlantic was the most densely populated HR, comprising a human population density five times higher than the Brazilian average, whereas Amazonian system, Tocantins-Araguaia and Paraguay regions were the least densely populated HRs, comprising less than ten inhabitants per Km^2 (Table S1; Fig. 1).

The Oriental Northeastern Atlantic, Paraná, Amazonian, San Francisco, Southeastern Atlantic, Tocantins-Araguaia and Paraguay HRs comprised cases for all invertebrate groups, whereas the Tocantins-Araguaia, South Atlantic and Uruguay HRs showed cases only for zooplankton and mollusks (Fig. 1). The Parnaiba HR presented cases only for zooplankton and crabs and the Western Northeastern HR for mollusks and crabs (Fig. 1). By far, the Paraná HR (the third most densely populated HR) was the most studied basin (n = 221; 49.6% of cases), followed by the Amazonian (n = 51; 11%), Southeastern Atlantic (n = 39; 9%), San Francisco (n = 32; 7%) and Tocantins-Araguaia (n = 20; 4.4%) regions. In contrast, the Parnaiba, Eastern Atlantic, Uruguay and Western Northeastern Atlantic HRs overall comprised only 24 cases (5.2%). Zooplankton and mollusks were studied in 11 Brazilian HRs and crabs in 9 (Fig. 1). Western Northeastern Atlantic did not show any study for zooplankton, Parnaiba for mollusks, and South Atlantic, Tocantins-

Araguaia, Uruguay for crabs. Approximately, 57% of cases about zooplankton and 40% about mollusks were performed in the Paraná basin, whereas 71% of cases about crabs were conducted in the Paraná (38.7%) and the Amazonian (32.3%) HRs (Fig. 1).

Overall, the distribution of research effort on invertebrate fauna in freshwater ecosystems from Brazil tended to be geographically biased. However, we found a negative bias, as the increase in number of cases was disproportionally lower than the increase of the HR population density for zooplankton (marginally significant differences, $t_{20} = 1.94$, $p = 0.06$; Fig. 2a) and mollusks (i.e. observed slope <1, $t_{20} = 2.53$, $p = 0.01$; Fig. 2b). This trend was even opposite-signed for crabs ($\beta = -0.06$), thus decreasing the number of cases with increasing HR population density ($t_{20} = 2.53$, $p = 0.02$; Fig. 2c). According to the results for taxonomic groups, we found a negative bias when considering all invertebrate groups ($t_{20} = 2.35$, $p = 0.04$; Fig. 2d).

Discussion

The results demonstrated geographic and taxonomic research biases and subsequent knowledge shortfalls on the invertebrate biota in the large Brazilian freshwater system. The zooplankton is the group with more cases and studies across the HRs of Brazil. However, contrary to our expectations, the most densely populated HRs did not show an increase in the number of cases studied. In this regard, the Paraná, the third most densely populated basin, was the most studied HR. A disproportionate relationship between the population density of HRs and the investigated groups was observed, but this ranged from positive relationships (for mollusks and considering all groups together) to negative ones (for crabs).

The Brazilian HRs with higher human population density tend to concentrate higher investments in education, research, sanitation policies and solid waste treatment (Borja, 2014). However, even unexpected, the lack of a strict relation between HR population density and number of cases and studies could be related to the division of Brazilian HRs is not political and, thus, there is a large variation across the federated states comprising each of them. Peculiarities of the HRs may influence on research bias reported here. For example, the outstanding number of studies in the Paraná HR may be related for the large and numerous artificial reservoirs (e.g. Itaipu dam, one of the most productive hydroelectric power plants in the world), and large permanent rivers and lakes formed by the Paraná river flooding area (Agência Nacional das Águas (ANA, 2015). Additionally, the Brazilian laws impose mandatories environmental impact statements and previous studies on biodiversity in any economic activity involving changes in the natural habitat, as the construction of hydroelectric power plants. These studies can generate available data for academic researchers and subsequent publications. Therefore, together with the socio-economic development in the Paraná basin during the last decades, some invertebrates have been an increasingly goal of research in this region, whereas others have accumulated a deficit in the number of publications.

Likewise, research on ecological processes, biodiversity and interactions in the Amazon region has been intensified in recent years. This region harbors one of the largest river basins in the world, with recognized importance on hydrological and climatic parameters across the Brazilian territory, also affecting several countries of South America (Val et al., 2016). Although sparsely populated, the Amazonian HR has a large territorial extension wherein areas are increasingly being used for economic development purposes. Under such circumstances, it will increasingly be necessary to join efforts and a growing investment in research and public policies that foster the sustainable development of the region.

In addition to the hydrographic and socioeconomic properties of the Brazilian HRs, the dominance of studies about the invertebrate groups examined here in some regions may be accounted for the higher number of specialist researchers working on these taxa. For example, research institutions in the Paraná region house specialists in taxonomy and biology of zooplankton. For mollusks, the higher number of studies in the Paraná, Paraguay, Southeastern Atlantic and South Atlantic HRs, especially nearly the Paraná and the Tietê rivers, is also related to the presence of large research groups. The scenario is not different for freshwater crabs. Public institutions such as Maringá State University (UEM), São Paulo University (USP) and Julio de Mesquita Filho State University (UNESP), located in the Paraná HR, and the National Institute for Amazonia Research (INPA), located in Amazonia, are evident examples of research institutions with consolidated scientific staff working on aquatic invertebrates (e.g. Troncon and Avelar, 2011; Pescinelli et al., 2014; Avelar et al., 2014; Pedraza et al., 2016; Magalhães, 2017).

One other aspect to be considered for the biases reported here is related to the biological characteristics of the groups of the HRs wherein they occur. For mollusks, for example, the Paraguay and South Atlantic HRs are two of the three richest Brazilian HRs for bivalves (Pereira et al., 2014), which has also entailed the concentration of a high number of malacologists working on these basins. Moreover, local environmental characteristics in the semiarid region (Caatinga) such as long periods of drought, high temperatures and high evaporation rates can favour the growth of invasive species populations that have adaptive strategies to protect against desiccation (Abílio et al., 2007; Paiva et al., 2018). This process may readily explain the increased number of studies about mollusks carried out in the densely populated Oriental Northeastern Atlantic HR and adjacent lowly-populated HRs that comprise the north-eastern Brazilian semiarid region, which have exclusively focused on invasive species (Azevêdo et al., 2014, 2016; Paiva et al., 2018). Additionally, the characteristics from the Caatinga ecosystem that could limit the studies for zooplankton, such as rainfall irregularities, dominance of temporary rivers and negative water balance, have been overcome, and studies have recently revealed a high biodiversity in this region (Arruda et al., 2017; Brito et al., 2017).

Freshwater crabs were the less considered species in terms of number of publications, but they occur in all Brazilian HRs (Magalhães, 2016). Despite being the largest taxonomic rank within the Brachyura infraorder, freshwater crabs have long been considered a group of low phylogenetic and biogeographical relevance when compared to marine crab species (Ng et al., 2008; Cumberlidge et al., 2009). Recently, several studies have counteracted this assessment, giving to freshwater crabs an ecological relevance among macroinvertebrate species across continental waters due to their abundance, biomass and role in the destruction of debris (Abdallah et al., 2004; Collins et al., 2006; Yeo et al., 2008; Kawai and Cumberlidge, 2016). The patterns distribution for freshwater crabs in South America have revealed that there is a large potential area for new species, especially in the vast Amazon basin, wherein large unexplored or poorly studied areas may constrain the record of new taxa (Kawai and Cumberlidge, 2016). The absence of data on freshwater crab diversity may be, thus, a taxonomic artefact that should be corrected by more recent technology on species identification.

The current scenario of knowledge described here demonstrates that the diversity of the groups of organisms in this study can still be little known, with relevant consequences for their conservation. In this context, our knowledge is still insufficient to propose the use of these organisms as biological indicators and to predict possible biodiversity losses caused by different pollutants. The need to fill this knowledge gap becomes even more urgent given the rapid human-population increase in large urban areas and, consequently, greater pollutant inflow into freshwater bodies. Among

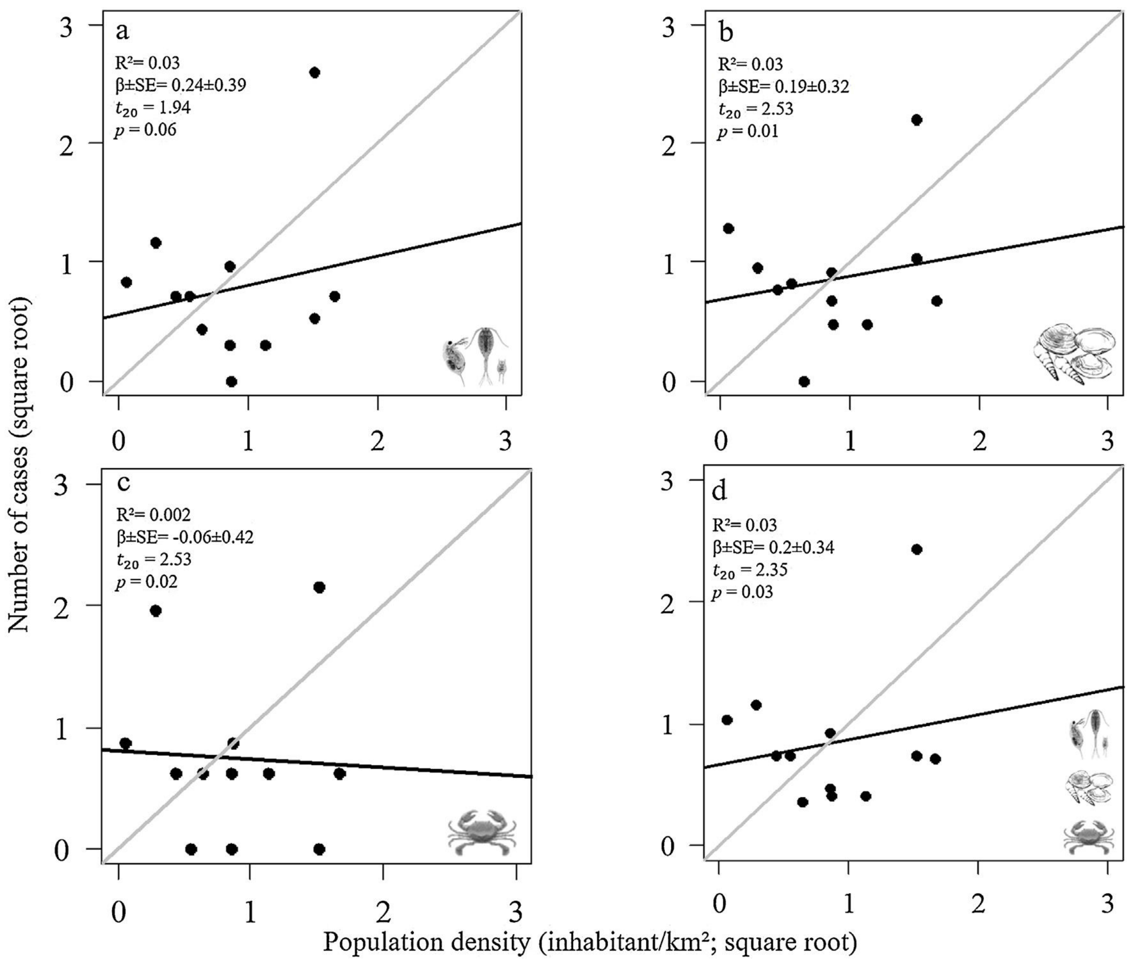

Fig. 2. Graphical representation of the expected versus observed relationship of the number of cases in relation to the human-population density in the hydrographic regions of Brazil for (a) Zooplankton, (b) Mollusks, (c) Freshwater crabs and (d) all invertebrate groups. The gray line represents the expected slope (i.e. β = 1), and the black line represents the observed slope.

potential pollutants of freshwater systems that are poorly studied in these HRs, microplastic stands out as one of the most urgent topics to be addressed. Microplastic particles represent greater hazards to aquatic organisms (e.g. Collignon et al., 2014; Reid et al., 2019), although studies about microplastic pollution and its consequences for freshwater fauna are scarce (Azevedo-Santos et al., 2019). It is expected suspended particle-eating organisms (e.g. rotifers, cladocerans and bivalves) and detritivores (e.g. crabs) to be highly susceptible to this kind of pollutant (Fendall and Sewell, 2009; Brennecke et al., 2015). In fact, microplastics have been detected in zooplankton organisms (Fendall and Sewell, 2009; Cole et al., 2013), in gills and digestive system of bivalves (von Moos et al., 2012; Farrell and Nelson, 2013) and in gills, hepatopancreas and stomach of crabs (Brennecke et al., 2015). The broad and easy distribution of microplastics in freshwater ecosystems can represent serious threats to both benthic and planktonic aquatic biota. However, the impact of microplastic in the main Brazilian HRs was only addressed for three studies (Paraná: Colabuono et al., 2009; Oriental Northeastern Atlantic: Silva-Cavalcanti et al., 2017; Amazon: Andrade et al., 2018; ongoing research is being developed in the Paraguay HR: Faria et al., 2019).

Conclusion

The uneven distribution of studies conducted across Brazilian HRs reveals that, despite their great extension and hydrographic importance, research in many HRs is strongly biased and generates relevant knowledge gaps related to the fauna of the aquatic organisms investigated here. Such negligent knowledge limits our capability to draw scenarios facing environmental change, the implementation of hydroelectric power plants and conservation of target species. Overall, this knowledge gap also limits our ability to propose public policies for HR conservation. Our study demonstrates the importance of focal studies filling the gaps in biological knowledge and suggests an increased focus on the virtually absent studies about the microplastic pollution near urban areas to understand their local impact.

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Conflict of interests

None.

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Appendix A. Supplementary data

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