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Research Article

Anthropogenic disturbance produces divergent effects in the community structure and composition of tropical semi-evergreen forests in the Eastern Himalaya

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Abstract

Studies documenting anthropogenic disturbance-driven changes in forest communities of the Eastern Himalaya, a global biodiversity hot spot, are largely lacking. We studied six forest sites of tropical semi-evergreen forests in Arunachal Pradesh in the Eastern Himalaya to understand the effects of varying disturbance intensities on the forest community structure and composition. Based on the magnitude of disturbance, forest sites were classified as experiencing low, moderate and high disturbance. Mean species richness (SR) of trees and shrubs decreased from low disturbance to high disturbance. Mean SR of herbs was maximum in moderately disturbed forest sites. Maximum values of the Shannon-Wiener Diversity Index (SD) were recorded for trees at sites with low disturbance, for shrubs at sites with high disturbance and for herbs in moderately disturbed forests. Pilelou Evenness Index (EI) values were maximum for trees at sites with high disturbance, while maximum EI values for shrubs and herbs were recorded in the forest sites with low disturbance. The number of tree families decreased from 18 to 13 in the forests with low and high disturbance, respectively. Moderate disturbance led to increased herb species richness and diversity, while increasing disturbance produced contrasting effects on trees. High anthropogenic disturbance led to low species richness, but high diversity amongst shrubs. Our investigations suggest that the magnitude of disturbance elicits differential responses in different physiognomic classes in the forest ecosystems and further our understanding of the effects of disturbance in tropical forest ecosystems of a global biodiversity hotspot.

Key words: biodiversity, hotspot, species diversity, species richness

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Introduction

Tropical forests form a major component of the global biodiversity hotspots, as well as the world's most endangered ecosystems (Myers et al. 2000; Mittermeier et al. 2011). As such, global biodiversity to a great degree is threatened due to the rapid degradation of tropical forests (Laurance et al. 2012). Equally well recognised is the fact that, world-over, tropical forests with the least anthropogenic impacts are potential centres of undescribed species diversity due to their greater structural complexity and richness compared with any other biome

(Giam et al. 2012). Notwithstanding their conservational significance, tropical forests face high levels of threat to their biodiversity and ecosystem services due to deforestation, agricultural expansion, urbanisation and widespread developmental projects such as dams (Wright 2005; Pandit et al. 2007, 2014, 2023). A comprehensive global assessment of the impact of disturbance on biodiversity showed that primary forests are irreplaceable in preserving tropical biodiversity (Gibson et al. 2011). When disturbed, tropical forests take a long time to recover; an average recovery time of 503 years was reported in a global assessment of tropical forests affected by disturbance (Cole et al. 2014).

The effects of disturbance on the structure and dynamics of vegetation communities have been contentious in ecological literature. Some regard disturbance as a negative influence as it destroys the climax assemblages (Clements 1936), while others regard it as a positive stimulus because it removes the dominant species and results in an increase in species diversity (Huston 1979). The proponents of the intermediate disturbance hypothesis suggest that species diversity is likely to be the highest at intermediate levels of disturbance intensity or frequency (Connell 1978; Miller et al. 2011). Yet others argue that disturbance not only reduces diversity, but also disrupts natural ecosystem services with significant effects on a forest ecosystem's vegetation, soil, water resources, wildlife and microclimate (Tilman and Lehman 2001; Pandit and Grumbine 2012; Pandit et al. 2014), even though various studies have highlighted the role of disturbance as a major factor in determining population and community structure. The causes, rates, size, pattern and trends of landscape changes in the Tropics are not extensively documented at the local and regional levels.

Arunachal Pradesh, the easternmost state of India, comprising the major part of the Eastern Himalaya global biodiversity hotspot, is regarded as one of the most sensitive areas with a high risk of deforestation (Roy and Joshi 2002; Pandit et al. 2007). The total forested area of Arunachal Pradesh is about 67,680 km^2, of which nearly 80% (53,850 km^2) exists as dense forests (Pandit et al. 2007). Of nearly 6000 species of flowering plants reported from the Arunachal Himalaya, 30–40% have been reported to be endemic to the region (Behera et al. 2002). However, in recent years, deforestation and anthropogenic pressures on land due to diverse economic interests, such as infrastructure and hydro-power development, have emerged as major drivers of land-use change and forest loss in the Eastern Himalaya (Pandit 2017). Notably, this region is poised to lose nearly one-fourth of the endemic species across various taxonomic groups by the turn of this century due to ongoing deforestation (Pandit et al. 2007) with additional species losses projected due to unprecedented dam building activities and climate change (Grumbine and Pandit 2013; Telwala et al. 2013).

Earlier studies on the effect of disturbance on the vegetation communities in the Arunachal Himalaya have been limited to the effect of disturbance only on the tree layer (Bhuyan et al. 2003), the regenerative ability and patterns of important tree species (Duchok et al. 2005) and the population structure of tree species in various forest stands (Nath et al. 2005). Little attention has been paid to understanding the effects of disturbance on the overall community structure and dynamics of these forests. This study aims to fill this gap by focusing on the effects of disturbances on all the physiognomic classes in these forests, viz. trees, shrubs and herbs. In this study, we identified and examined the effects of anthropogenic disturbances on the tropical forest stands of Arunachal

Pradesh, Eastern Himalaya in relation to their species diversity, community composition and structure. We also carried out a comparative analysis of varying disturbance intensities on different community characteristics in these tropical forests. Given the concentrated developmental activities unfolding in the region over the last decade, this empirical analysis is timely for documenting the impact of anthropogenic disturbances on the rich tropical forests of the Eastern Himalaya. The findings of this study are relevant considering the high conservation value of this global biodiversity epicentre and a general lack of community ecology studies on the tropical forest ecosystems in the Eastern Himalaya.

Materials and methods

Study area

We studied six forest sites located in Aalo Forest Division, West Siang District of Arunachal Pradesh (Fig. 1). The study area is located between 28°2°'50"N–28°40'21"N latitude and 94°21'42"E–94°42'32"E longitude with elevations ranging from 450 m to 950 m. The major forest type of the study area is tropical semi-evergreen forest, which corresponds to 2B/1S1 Sub-Himalayan light alluvial semi-evergreen forest (Champion and Seth 1968). The

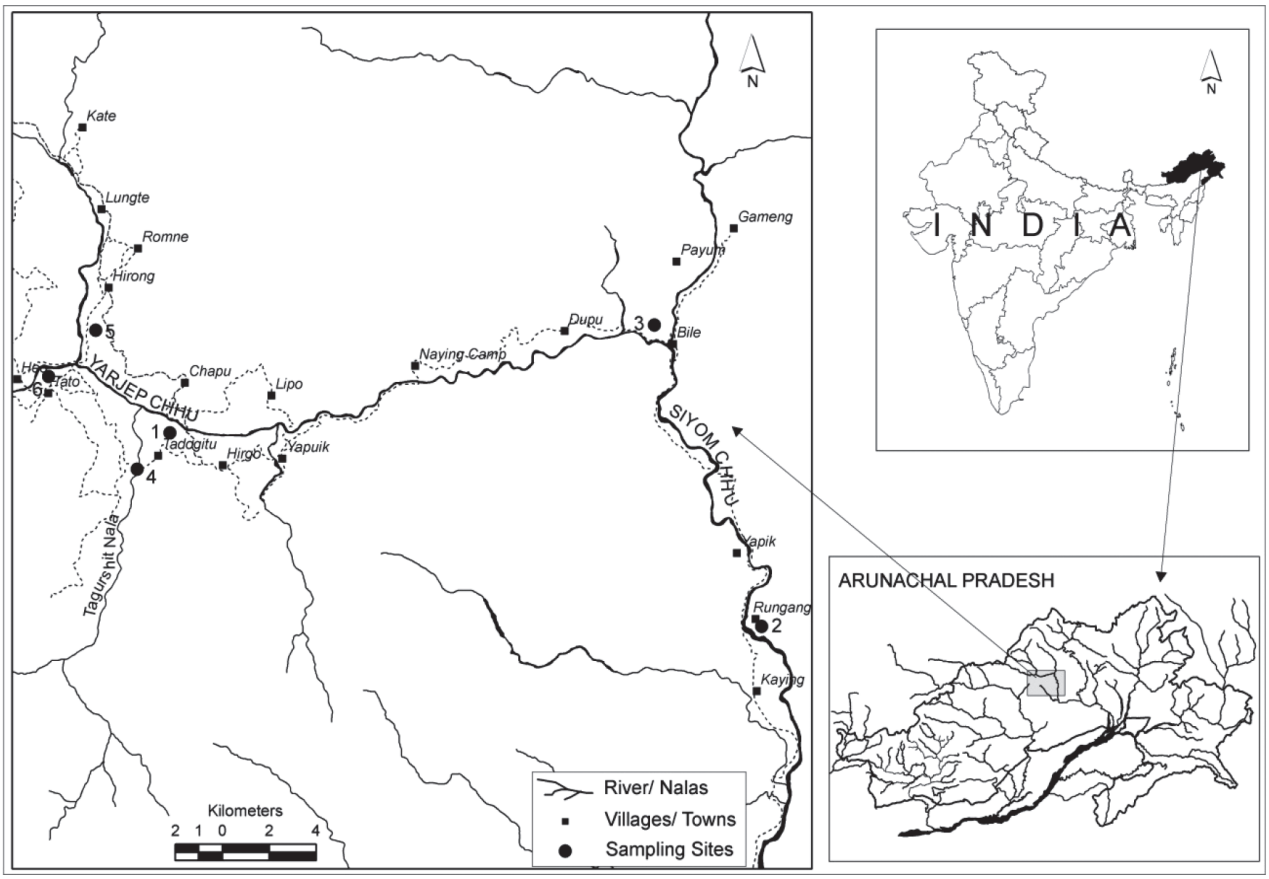

Figure 1. Map of the study area showing the locations of six sampling sites (1-Tatogito, 2-Roying, 3-Poma Basti, 4-Tagurshit, 5-BB Camp Bamboo bridge area, 6-BB Camp Basti area). Each sampling site represents a different forest site. Sampling was undertaken to assess the structure and composition of the vegetation community at each site during pre-monsoon (March-April), monsoon (July) and post-monsoon (October) seasons.

area is characterised by high monsoon rainfall with an annual average of 2300 mm, moderate temperatures ranging from 18°–22°C and high relative humidity levels between 77% and 98%. These forests, therefore, come structurally closest to the tropical rainforests of the Malayan Archipelago in Southeast Asia dominating the region east of the Bay of Bengal.

Sampling

Sampling in six forest sites was undertaken to assess the structure and composition of the vegetation community of the study area. Sampling locations were randomly chosen in each forest site in such a manner that the maximum possible representative vegetation of the respective forest site was covered. In each forest site, three physiognomic classes, i.e. trees, shrubs and herbs were surveyed, sampled and analysed using the standard nested quadrat sampling method. Each nested quadrat design contained subplots of 10 m × 10 m [for recording tree species with circumference at breast height (cbh) > 30 cm], 5 m × 5 m (for recording shrubs and saplings with 10–30 cm cbh), and 1 m × 1 m (for recording herbs with < 10 cm cbh) (Manish et al. 2017). In the context of the present study, we defined saplings as the individuals of tree species that are generally low in height (up to 5 m in height and having cbh of 10–30 cm). The nested quadrats were laid over a 1 km long line transect along the hill slope. Standard species-area curves were used to determine the number of quadrats required to cover maximum species diversity at each sampling location. To account for seasonal community fluctuations, sampling was carried out in the pre-monsoon (March-April), monsoon (July) and post-monsoon (October) seasons for three years. The data on the vegetation composition was quantitatively analysed for species richness (SR) and other phytosociological characteristics including density, abundance, frequency, dominance, importance value index (IVI), Shanon-Wiener Diversity Index (SD) and Evenness Index (EI).

Disturbance gradient

Following the methods of Sagar et al. (2003) and Sapkota et al. (2010) with minor modifications, six sampling sites were ranked according to the intensity of anthropogenic disturbance they experienced. Four different sources of disturbance were recognised, viz. road, human habitation, tree-felling/lopping and herbivory (grazing by domestic cattle) (see Table 1). The relative impact of each disturbance source was estimated as follows:

a) Road: A sampling site furthest from the road head was assumed to experience minimum disturbance and designated as having an impact equal to 1. The impact for other sites was calculated as ratios of the distance of respective sites from the road to the distance of the site with an impact of 1 (Sagar et al. 2003).
b) Human habitation: The sampling site located furthest from a village/human settlement was taken to experience an impact of 1. The ratio of the distance of other sites from their respective village/human settlements to the distance of the site with an impact of 1 was taken as the impact for other sites (Sagar et al. 2003).

c) Logging/lopping: Relative density and relative basal area of the damaged individuals were used as a measure to rank logging/lopping as impacting a site (Sagar et al. 2003; Sapkota et al. 2010). The relative density of damaged individuals was calculated as the ratio of the sum of the density of damaged individuals and the total density of individuals (damaged + standing). Likewise, the relative basal area of damaged individuals was calculated as the ratio of the sum of the total basal area of damaged individuals and the total basal area of all individuals (damaged + standing). Sampling sites with the lowest values of relative density and relative basal area of damaged individuals were assigned to experience an impact of 1. The impact on other sampling sites was determined as the ratio of the total relative basal area and relative density of a particular site to the total relative basal area and relative density of damaged individuals at the site with an impact of 1, respectively.
d) Browsing/grazing: Relative density of damaged saplings (tree individuals less than 5 m high and with 10–30 cm cbh) was calculated to estimate the impact due to herbivory (browsing/grazing) by domestic cattle. The relative density of saplings was calculated as the ratio of the sum of the density of damaged saplings to the total density of all saplings (damaged + undamaged). The sampling site with the lowest value of relative density was assigned an impact of 1. The impact on other sampling sites was determined as the ratio of the relative density of damaged saplings at a particular site to the relative density of saplings at a site with an impact of 1, respectively.

After the relative impact of each of the four disturbance sources was estimated, their individual scores were summed to yield a cumulative impact at each of the six sampling sites. This cumulative total impact was taken as a surrogate for the degree of disturbance at each site. Based on the disturbance intensity, we classified different forest sites under the following three anthropogenic disturbance classes: (i) Least disturbed forest site with a total impact of less than 10, (ii) Moderately-disturbed forest site having a total impact of less than 100 and (iii) Highly-disturbed forest site having a total impact of greater than 100. Though there may be other sources of anthropogenic disturbance in the study area like herb collection, firewood cutting and intentional fire, we focused on the relative impacts of only road construction, human habitation, logging/lopping and browsing/grazing as these were the only predominant sources of disturbance as per our field study. There are no records of any rare, endangered or threatened medicinal plant species in the study area.

Table 1. Relative impacts for each disturbance source in six tropical semi-evergreen forest sites in Arunachal Pradesh.

Disturbance source		Relative impact at forest sites					
		Tatogito	**Roying**	**Poma Basti**	**Tagurshit**	**BB Camp Bamboo bridge area**	**BBCamp Basti area**
Road		1	20	25	40	50	100
Habitation		1	4	8	1.3	80	100
Tree cutting/ lopping	Relative density	1	1.83	4	2.67	5.17	6.17
	Relative basal area	1	5	8	12	14	12
Browsing/Grazing		1	1.93	3.67	3.97	4.65	4.78
Total impact		**5**	**32.76**	**48.67**	**59.97**	**153.82**	**222.95**

Data analysis

Each of the six sites was surveyed for angiosperm taxa. The collected species were assigned to their respective plant families and the three physiognomic classes (trees, shrubs and herbs). Each site was analysed for density, abundance, frequency and dominance of constituent flora using standard procedures of vegetation sampling (Curtis and McIntosh 1950). The Importance Value Index (IVI) for each plant species was determined as the sum of relative density, relative frequency and relative dominance (Curtis and McIntosh 1950). For determining dominance, total basal area (TBA) for tree species and cover for shrub and herb layers were calculated. TBA was measured using the formula: TBA = mean basal area × density, where, mean basal area = (average circumference at breast height) $^2/4\pi$. The cover value for shrub and herb layers was measured by the formula: Cover = mean cover × density, where, mean cover = π × (average diameter of species)^2 × 0.25.

In order to characterise the community structure of the investigated sites, the following variables were analysed: (i) SR - determined as the total number of species per sampling unit (Whittaker 1975), (ii) SD - calculated following Shannon and Weiner (1963) and (iii) EI - calculated following Pielou (1969). A plant family with the largest number of species was considered the most dominant one in a site. The population structure of the studied sites was examined using the density-diameter distribution of the trees (Rao et al. 1990). Following standard literature, the trees were distributed into six girth classes with successive increments of 30 cm, i.e. 30.1–60 cm, 60.1–90 cm, 90.1–120 cm, 120.1–150 cm, 150.1–180 cm and 180.1–210 cm (Majumdar et al. 2012). Unstacked one-way analysis of variance (ANOVA) was used to test the significance of differences in mean SR and mean density for different physiognomic classes across the sampling sites. We used Minitab 16 (Minitab Inc., State College, PA, USA) software for this analysis. The total basal area of tree species was used for ordination analysis of different sites using Principal Component Analysis (PCA) to investigate if varying disturbance magnitudes exerted significant influence on tree species distribution (Sagar et al. 2003). Before PCA analysis, the values of the total basal area were log (x+1) transformed to control for skewness in the dataset. PAST version 2.13 software was used for PCA analysis.

Results

The study sites varied in nature and intensity of disturbance they experienced and a noticeable disturbance gradient between them was discerned (Table 1). Tatogito forest site was the least disturbed; Roying, Poma Basti and Tagurshit were moderately disturbed, while BB Camp Bamboo Bridge area and BB Camp Basti area were highly-disturbed forest sites (Table 1).

A total of 160 species were recorded at these sites of which 54 were trees, 30 shrubs and 76 herbs (Table 2). The emergent tree layer in almost all the forest sites mainly comprised *Macaranga denticulata* (Blume) Müll.Arg., *Alnus nepalensis* D.Don and *Sauraria punduana* Wall. The shrub layer was represented by *Melocalamus compactiflorus* (Kurz) Benth., *Boehmeria penduliflora* Wedd. ex D.G.Long, *Pinanga gracilis* Blume, *Oxyspora paniculata* (D.Don) DC. and *Alsophila spinulosa* (Wall. ex Hook.) R.M.Tryon, while the herbaceous layer mainly

Table 2. Shanon-Wiener Diversity Index and Pilelou Evenness Index for different vegetation layers in six forest sites along a disturbance gradient.

Vegetation parameters		Forest sites					
		Tatogito	Roying	Poma Basti	Tagurshit	BB Camp Bamboo bridge area	BB Camp Basti area
Shanon-Wiener Diversity Index	Trees	2.79	2.61	2.54	2.42	2.47	1.65
	Shrubs	1.75	1.51	1.34	1.62	2.04	1.73
	Herbs	1.9	2.17	2.55	2.96	2.42	2.03
Pilelou Evenness Index	Trees	0.13	0.15	0.16	0.17	0.18	0.24
	Shrubs	0.25	0.17	0.22	0.23	0.2	0.16
	Herbs	0.19	0.15	0.17	0.09	0.15	0.14

comprised *Thysanolaena latifolia* (Roxb. ex Hornem.) Honda, *Musa balbisiana* Colla and *Pilea scripta* (Buch.-Ham. ex D.Don) Wedd. Mean SR varied significantly for trees, shrubs and herbs between different sites (Fig. 2A). Mean SR for trees and shrubs decreased from the least disturbed (SR = 3.2 ± 1.03, SR = 2 ± 0.82) to highly disturbed (SR = 1.9 ± 0.57, SR = 1.5 ± 0.53) sites. However, for herbs, mean SR was the highest in moderately disturbed site (SR = 3.7 ± 1.2) (Fig. 2A**)**. Maximum SD for trees was recorded at the least disturbed sites, while the highest SD for shrubs was recorded at the highly-disturbed sites. Herbs, on the other hand, showed maximum values of SD at the moderately-disturbed forest sites (Table 2). The maximum value of EI for trees was recorded in the highly-disturbed forests in contrast to shrubs and herbs in which maximum values were recorded at the least disturbed forest sites (Table 2**)**. Similar to SR, mean density of species also showed significant variation for trees, shrubs and herbs between different sites (Fig. 2B). Maximum mean tree density (MTD) was recorded in the least disturbed forest (MTD = 3.4 ha^{-1} ± 0.5) and minimum values were recorded at the highly-disturbed sites (MTD = 2.55 ha^{-1} ± 1.2) (Fig. 2B). Maximum mean shrub density (MSD) was recorded at the highly-disturbed forest site (8.40 ha^{-1} ± 1.7) and minimum at the least disturbed forest site (6.30 ha^{-1} ± 0.8) (Fig. 2B). Maximum mean herb density (MHD) was also recorded at the highly disturbed site (23.3 ha^{-1} ± 2.7) and minimum in the least disturbed site (6.70 ha^{-1} ± 1.1) (Fig. 2B). Overall, the density-diameter distribution pattern showed a gradual decrease in the density of trees with an increase in diameter class (Fig. 3). Across the investigated sites, maximum number of trees belonged to the lower diameter classes (30.1–90 cm cbh) and minimum numbers were in the higher diameter classes (150.1–210 cm cbh). All six forest sites also showed significant variation in terms of the total basal area of tree species. The PCA ordination plot using the total basal area of tree species showed a clear separation of the six sites (Fig. 4).

Species composition showed varied dominance of different species at different forest sites. Species listed in Suppl. material 1: appendix A (10 trees, 17 shrubs and 12 herbs out of total 160 species) dominated the forest vegetation across sites with relatively higher IVI and TBA/cover. *Alnus nepalensis* D.Don, *Engelhardtia spicata* Lechen ex Blume and *Albizia odoratissima* (L.f.) Benth. emerged as the most dominant tree species in the least, moderately- and highly-disturbed forest sites, respectively. Amongst shrubs, *Boehmeria penduliflora* Wedd. ex D.G.Long was the most dominant in the least disturbed site. *Bambusa tulda* Roxb. and *Polygonum molle* D.Don were the most dominant shrub species

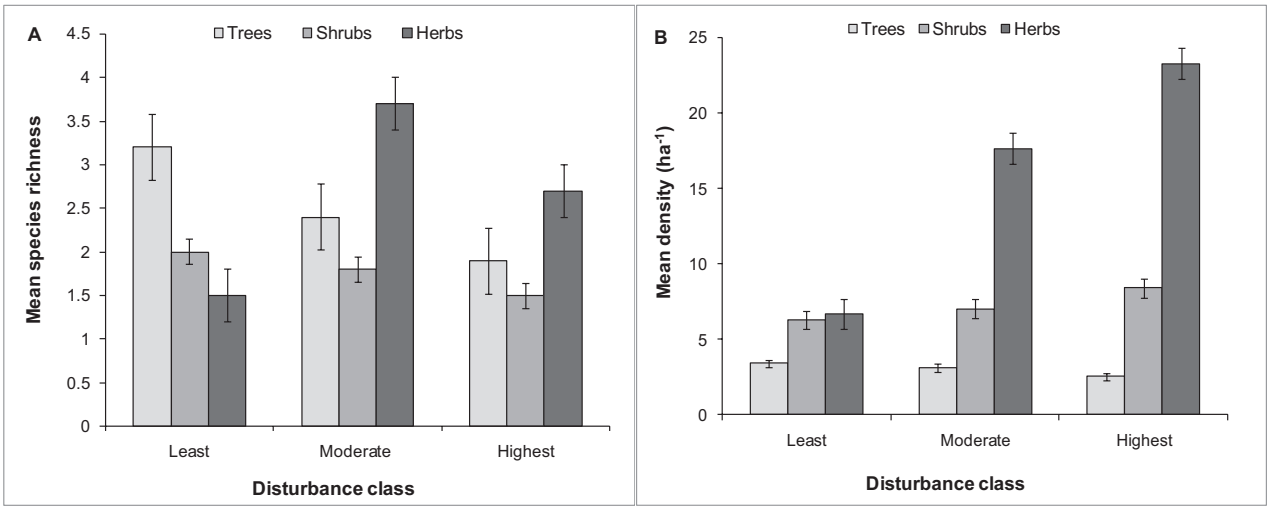

Figure 2. Effect of anthropogenic disturbance on vegetational parameters of different forest sites. (A) Variation of mean species richness with disturbance classes for trees, shrubs and herbs, (B) Mean density as related to disturbance classes for different vegetation layers. Error bars represent the standard deviation.

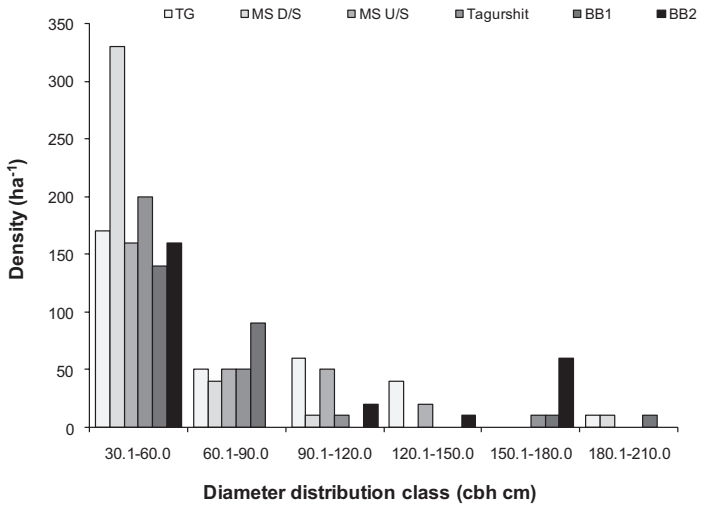

Figure 3. Density-diameter distribution of trees in different forest sites (TG - Tatogito, RO - Roying, PB – Poma Basti, TAG - Tagurshit, BB1 - BB Camp Bamboo bridge area, BB2 - BB Camp Basti area). In all the forest sites, most trees had maximum density in the lower diameter classes (30.1–90 cm cbh) and minimum density in the higher diameter classes (150.1–210 cm cbh).

in the moderately- and highly-disturbed sites, respectively. In herb strata, *Hellenia speciosa* (J.Koenig) S.R.Dutta, *Alpinia nigra* (Gaertn.) B.L.Burtt and *Ageratum conyzoides* L. were the most dominant species in the least, moderately- and highly-disturbed sites, respectively (Suppl. material 1: appendix A). The number of tree families decreased from 18 at the least disturbed site to 13 at the highly-disturbed site (Suppl. material 1: appendix B). Betulaceae and Araliaceae were the most dominant families and Moraceae was a co-dominant family in the least disturbed forest sites. Euphorbiaceae was the most dominant family and Anacardiaceae a co-dominant at the moderately-disturbed site. In

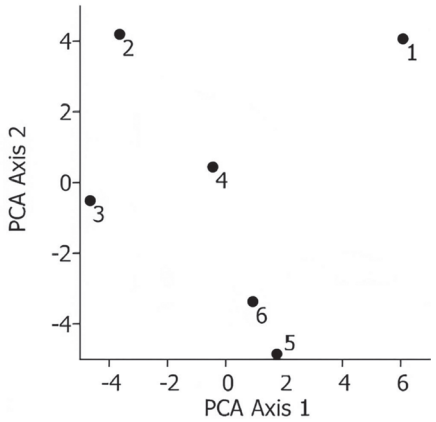

Figure 4. Principal Component Analysis (PCA) ordination plot, based on total basal area of tree species in six forest sites (1 - Tatogito, 2 - Roying, 3 - Poma Basti, 4 - Tagurshit, 5 - BB Camp Bamboo bridge area, 6 - BB Camp Basti area). A total of 51.92% variation in species composition was explained by the first two principal components (PCA axis 1 – 27.14%, PCA axis 2 – 24.78%) in the ordination plot.

the highly-disturbed forest sites, Araliaceae and Moraceae were recorded to be the most dominant families, while Betulaceae, Bignoniaceae, Anacardiaceae and Mimosaceae were the co-dominants. Amongst the recorded families, only Anacardiaceae was present in all the forest sites (Suppl. material 1: appendix B).

Discussion

Anthropogenic activities are a looming threat to the biodiversity of the Himalayan forests and have been responsible for the significant transformation of its landscape (Pandit et al. 2014). Earlier studies have shown that anthropogenic pressures on the forests of Arunachal Pradesh and adjoining hill regions of northeast India have historically been in the form of logging for traditional agriculture like *Jhum* (slash and burn) and selective felling by the Forest Department (Singh et al. 2003). The cycle of shifting cultivation in the region is 6 to 7 years. The growing human population and the increasing number of domestic cattle and livestock have necessitated more harvest of timber, fuel wood and uncontrolled grazing (Pandit 2017).

All six study sites in the present paper are directly affected due to various human activities including ongoing hydroelectric power projects in the study area. We were able to locate a total of 105 households in the study area. Scheduled tribe population accounts for more than 95% of the total population (CISMHE 2010). These villages are very poor in literacy that is attributed to low education facilities. Average literacy is 45%, being relatively high in the male population. Farming remains the main occupation of the people. The local farmers continue to practise the age-old slash-and-burn (*Jhum*) method of cultivation. The main crops grown in the region are paddy, millets and chillies. Nearly 44% of the total population constitutes the total workforce employed in agriculture (CISMHE 2010). A good number of households of Tato and Roying are employed in small-scale businesses. The level of utilisation of medicinal plants in the region is quite low. The local population use *Cautleya gracilis* (Sm.) Dandy, *Hellenia*

speciosa (J.Koenig) S.R.Dutta, *Urtica dioica* L., *Alpinia nigra* (Gaertn.) B.L.Burtt, *Piper pedicellatum* C.DC. etc., but as highlighted earlier, there are no reports of any rare, endangered or threatened medicinal plant species in the region.

We recorded significant changes in the community composition of the investigated forests with increasing disturbance levels. Moderate disturbance led to increased herb richness and diversity, while high disturbance levels had the opposite effect on trees. Shrubs showed low richness, but high diversity with increasing disturbance. These results confirm earlier findings of Bhuyan et al. (2003) in a tropical wet evergreen forest in Arunachal Pradesh and Mishra et al. (2004) in the adjoining Meghalaya forests, who reported decreased tree species diversity, density and basal area along the disturbance gradient. Our results suggest that disturbance of varying magnitudes produces differential responses by different physiognomic classes in the forest ecosystems. Higher tree species richness and diversity were observed in forests with the least disturbance. It is well known that the stability and resilience of an ecosystem depend on the magnitude of species diversity and the number of interactions between them (MacArthur 1955; Leigh 1965). Evidence, based on theoretical and empirical studies, shows that a decrease in species diversity hastens the simplification of ecological communities causing negative impacts on the ecosystem services (McCann 2000).

We observed that, with increasing disturbance, tree species richness decreased, while their Evenness increased. For shrubs and herbs, maximum values of the Evenness Index were found in forests with the least disturbance. The negative relationship between richness and Evenness has also been reported elsewhere (Symonds and Johnson 2008). Uniyal et al. (2010) also showed that the Evenness Index for tree species was highest in the most disturbed forests. In a major modelling exercise, Svensson et al. (2012) showed that Evenness increased with increasing disturbance for all levels of productivity. These findings including the present investigations suggest a negative relationship between richness and Evenness. The inverse relationship between species richness and Evenness mediated by disturbance alludes to the fact that, with increasing disturbance, abundance of dominant species is reduced. The tree basal area decreased with increasing disturbance levels and a clear gradation between disturbance and basal area was reflected by the separation of the forest sites in the PCA ordination analysis. In earlier studies, decreasing tree basal area has been directly related to the disturbance index and deteriorating forest stand productivity (Smiet 1992). The density-diameter distribution curves in the present study depicted a successive reduction in the number of trees of higher girth classes and disturbance increased thereby preventing these ecosystems from attaining a climax stage and perpetuation of seral stages. Maximum tree density in the lower diameter classes (30.1–90 cm cbh) across the forest sites suggests selective removal of individuals of higher diameter which is clear from the data on logging.

Our results suggest that disturbance impacts tree species more and arrests climax community formation by the proliferation of disturbance-tolerating shrub and exotic herbaceous species. *Ageratum conyzoides* L., an exotic invasive herb, was absent in the forests with low disturbance, but dominated the sites experiencing high disturbance. Numerous earlier studies have reported the invasion of disturbed sites by exotic invasives (Lodge 1993; Martin et al. 2009). The reasons for the proliferation of invasives in disturbed sites include reduced com-

petition from native species (Davis and Pelsor 2001), low suppression of exotic species by lack of closed cover of native species (Manish 2021), faster growth rates than native species (Burke and Grime 1996) and higher local nutrient resource levels (Levine et al. 2003). Logging creates forest gaps which becomes a critical factor for invasion. In less disturbed forest fragments, denser canopy cover leads to the presence of low light levels in the understorey. Since exotic species generally tend to be light-demanding (Fine 2002) and shade intolerant (Mack 1996), exotics are absent from undisturbed habitats and are more likely to be found in only disturbed environments where light availability is greater.

Conclusion

All five sites, except the one with the least disturbance, experienced varying intensities of biotic pressure in the form of lopping for timber and firewood and grazing by domestic animals. Loss of plant diversity and changes in community structure in terms of composition, species density and population structure have resulted from these anthropogenic disturbances in the tropical semi-evergreen forests of Arunachal Pradesh. The help of local communities can be sought in the conservation of the forests through participatory activities by the grant of title rights, delineating specific areas for browsing/grazing, fixing permits for extraction of timber wood etc. The policy-makers can do well to involve the local communities in conservation programmes and sensitise them about the ill effects of anthropogenic disturbances rather than adopting a top-down conservation approach.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Dinesh C. Nautiyal – Conceptualisation, Investigation, Methodology, Supervision; Kumar Manish – Writing original draft, review and editing, Formal analysis, Resources, Software, Validation, Visualisation

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Appendices

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Data type: pdf

Explanation note: **appendix A.** Important vegetational parameters of six forest sites. + – species present at the forest with < 10% IVI. TBA – total basal area, IVI – Importance value index. Units: density (ha^{-1}), TBA (m^2 ha^{-1}), cover (m^2 ha^{-1}). **appendix B.** The distribution of tree families and their contribution to total genera and species in six tropical semi-evergreen forest sites. Dashed entries indicate absence of respective genera/species at a sampling site.

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Research Article

The assessment of the bioaccumulation of microplastics in key fish species from the Bulgarian aquatory of the Black Sea

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Abstract

One of the main problems of the world's oceans, reported by many scientific studies, is the microplastic pollution. Within the Black Sea, one of the main sources of pollution is the same, which is caused by the diverse anthropogenic activities. The present study demonstrated detailed microplastics contamination of in five fish species important for the commercial fishing (Garfish, Mullet, Knout goby, Pontic shad and Mediterranean horse mackerel). They were collected from the Sozopol area on the Bulgarian Black Sea coast. Within each microplastic morphological group, three size classes were recognised: 100–200 µm, 25–100 µm and ≤ 25 µm. Microplastics were found in all studied tissues of the fish, but in varying proportions of pellets, fibres and fragments. Pellets were most frequently isolated, followed by irregularly-shaped fragments and fibres were the least numerous. The bulk of insulated plastics are made of polyethylene (PE) and polyethylene terephthalate (PET). Our results pointed out serious pollution with plastic particles in the Bulgarian Black Sea aquatory, which, in the future, may seriously affect the health of the fish population and also human health.

Key words: Anthropogenic pressure, bivalves, food resources, ocean, pollution, sea water

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Introduction

The pollution of the world oceans by plastic waste has been reported in many scientific papers in recent years. There is scientific evidence of plastic particles detected even in the polar regions (Corsi et al. 2021). Plastics, as water-insoluble solid polymers, are widely distributed and used in a wide range of industries due to many advantages – for example, flexibility, rigidity, temperature resistance and chemical stability (Herbort et al. 2018). It is reported that the annual share of plastic production has reached 280 million tonnes. A number of scientific papers have reported the amount of microplastic particles (< 5 mm) in seawater and sediments to be around 4.8 and 12.7 million tonnes (Kumar et al. 2021). These particles impact life in the seas and oceans. Microplastics (MPs) have recently been defined as "a synthetic solid particle or polymer matrix of industrial origin, not soluble in water, of regular or irregular shape and measuring between 1 µm and 5 mm " (Frias and Nash 2019). Plastics are categorised into three groups (macro

(> 100 mm), meso (1–10 mm) and micro (1–1000 mm). MPs can be produced as microparticles for personal care and cleaning products or occur due to the fragmentation of macroplastics in aquatic ecosystems by environmental factors, such as UV radiation and wave abrasion (Zhang et al. 2021). The main sources of microplastic particles are the plastic bags, bottles and straws that are widely used. They are not biodegradable and remain in the environment for hundreds of years (Plastics Europe 2020). For this reason, the fight against microplastic pollution is one of the main environmental priorities of the United Nations Environment Programme (UNEP 2016). Microplastic particles have been reported in the bodies of mammals, shellfish, fish, birds and decapods, particularly in the Northern Hemisphere. Entering the organisms of bivalves and fish through the food, microplastics cause disturbances and damage - from oxidative stress to behavioural disturbances. Other negative impacts, such as decline in the swimming capacity in fish, reduction of reproduction, inhibition of growth and others were reported (De S´a et al. 2015; Wang et al. 2016; Anbumani and Kakkar 2018). Once inside living organisms, microplastic particles have the ability to absorb and release toxic chemicals/organic or inorganic additives, such as bisphenol A, PCBs and DDT, which creates additional potential risks to human health. On the other hand, microbial biofilms can form on microplastic particles, which contain microbial pathogens and have an adverse effect on animals and humans (Leslie et al. 2013; Eerkes-Medrano et al. 2015; Koelmans et al. 2019). The pollution of the Black Sea, known as one of the most degraded marine ecosystems according to BSC (2007), makes this semi-enclosed sea more vulnerable to microplastic pollution. On the other hand, the estuaries of the Danube, Dnieper, Dniester and Don Rivers further pollute the Black Sea with plastic waste (BSC 2009). In their work, Lechner et al. (2014) report that 4.2 tonnes of plastic/day reach the Black Sea via the Danube River. The Black Sea is surrounded by several industrialised countries and is an important place for both small-scale and large-scale fisheries (BSC 2007). There are several studies in the region on macroscale plastics and they showed a large amount of macroplastic pollution along the Black Sea coasts (Simeonova et al. 2017; Terzi and Seyhan 2017; Oztekin et al. 2020; Terzi et al. 2020; Erùz et al. 2022). So far, more than 890 fish species have been found to ingest MPs and the abundance of MPs has been reported to be highly variable amongst different fish species (Bowen et al. 2021). In addition, out of a total of 323 fish species found to ingest MPs in different regions of the world, 262 were reported as commercial species. In the modern world, people are increasingly interested in a healthy lifestyle and healthy eating. Fish has been one of the staple foods for humans for decades. In many cases, it is recommended as a healthy food and source of very useful omega fatty acids, vitamins and minerals. On the other hand, for many people living on the Black Sea coast, fishing is the main way of livelihood. There are data on the impact that microplastic particles have on the health of marine life, but there are no data on what microplastics would do to the human body and how this would affect human health and the health of our children in the future. Popular science films have recently emerged that clearly show that people who eat seafood have circulating microplastics in their blood. There are no data on studies from the Bulgarian Black Sea water area regarding the condition and amount of microplastic particles in fish. This is the first pilot study on the composition of microplastic particles in different parts of key fish species from the Bulgarian Black Sea water area.

Materials and methods

Sampling location and duration of the study

The study was conducted at the Department of Biology, University of Shumen, Bulgaria. Probes from different anatomical structures were sampled in Garfish (*Belone belone*), Mullet (*Mugil cephalus*), Knout goby (*Mesogobius batrachocephalus*), Pontic shad (*Alosa immaculata*) and Mediterranean horse mackerel (*Trachurus mediterraneus*) caught in the region of Sozopol in February 2022.

Collection of samples

After catching, the fish was immediately transported to the laboratory at 4 °C, where it was dissected. Samples were taken from the skin, musculature, gills, intestinal tract and caviar and these probes were subjected to analysis. The probes were obtained from three specimens of each fish species, which were similar in size.

Tissue digestion and microscopic inspection

Tissues obtained from fish were minced according to Roch and Brinker (2017). To prevent contamination with microplastics, work was undertaken only with glass and metal tools. All tools used were rinsed with bidistilled water before use. Similarly, the reagents used in the analysis were tested for the presence of microplastics using the black sample method. After cutting each sample, 5 ml of 1M sodium hydroxide (NaOH) were added to it and heated to 50 °C for 15 minutes. The temperature was controlled at all times. A total of 17.5 ml of nitric acid (HNO_3) (49%) and 2.5 ml of ultrapure water were added to the NaOH-treated sample. This was followed by reheating to 50 °C for 15 minutes, with the temperature again controlled. The whole sample was heated for 15 minutes, but at the temperature up to 80 °C, which aimed to remove the remaining suspended solids from the sample. The next step was filtration, after which the samples were diluted 1:2 (v:v) with ultrapure water heated to 80 °C. Filtration was performed through a cellulose nitrate filter (Ø 47 mm, pore size 8 µm, Sartorius Stedim Biotech, Goettingen, Germany). The glassware used in the experiment was also filtered and washed. Nitrocellulose filters were dried for 24 h at 37 °C and then analysed for MPs. For microplastics identification, the filters were visually observed under a stereomicroscope SZM-D (OPTIKA Italy, 1000× magnification) coupled with Dino-Eye AM4023X eyepiece camera (Dino-Lite ANMO Electronics, Taiwan). The digital images were examined by using "DinoCapture 2.0" software and the plastic particles were quantified by size (≤ 25 µm, 25–100 µm and 100–200 ìm), based on their largest cross section and shape (pellets, fibres and fragments).

Polymer identification

To identify the polymer, we used FTIR spectroscopy according to Ibryamova et al. (2022). All results were done in triplicate.

Results

A total of three specimens from each species was analysed for the presence of microplastics. In all tables, averaged results from the three samples were plotted. The presence of microplastics was detected in 100% of the specimens. The microplastic particles ranged from 10 to 40 particles per individual (Fig. 1, Tables 1–4).

The microscopic pictures in Fig. 1 demonstrate the types of MP particles which were isolated - irregularly shaped (Fig. 1A, pellets (Fig. 1B and fibres (Figs. 1C, D) and also different colours, which are probably due of the various impurities and trace elements used by the manufacturer for the specific type of microplastic.

The data in Table 1 showed that, in the Garfish, the pellets with sizes ≤ 25 μm isolated from the gills, muscles and skin of the fish were predominant. Fibres of the same size ≤ 25 μm were isolated from the gills only. Irregularly- formed MPs were isolated in all parts of the fish, most notably from the skin and gastrointestinal tract. As in the skin, particles with larger sizes (25–100 μm) were also found (Fig. 1A). MPs with sizes of 100–200 μm were not isolated.

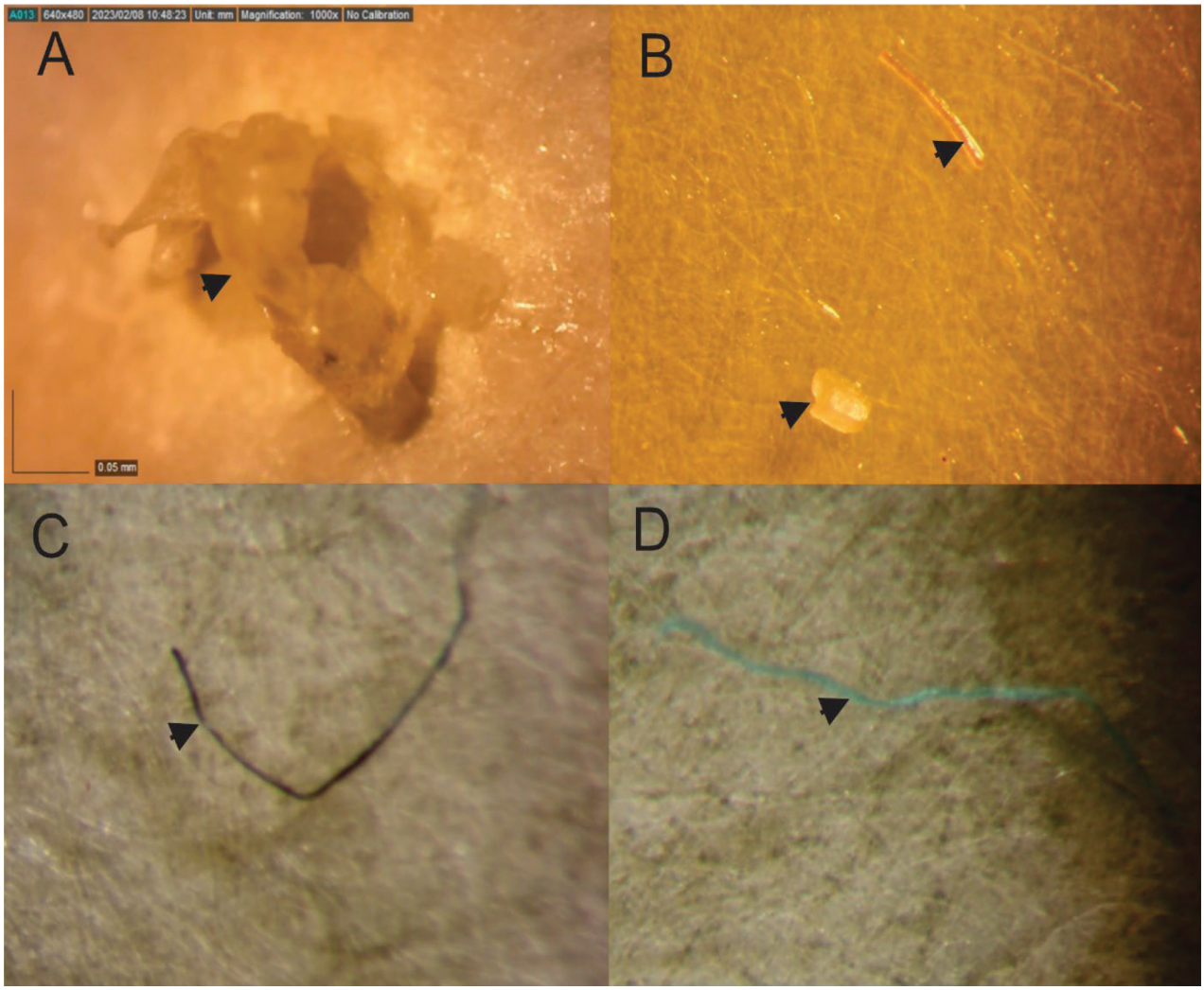

Figure 1. Stereomicroscope picture of morphological types of microplastics (arrowheads) recognised in the studied species from: A) *B. belone*; B) *M. batrachocephalus* C) *A. immaculata* and D) *T. m. ponticus*.

Table 1. Number of microplastic particles in samples from *B. belone.*

Species	*B. belone*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm
skin 1	0	0	0	0	0	0	4	2	0
skin 2	0	0	0	0	0	0	1	0	0
skin 3	0	0	0	0	0	0	4	0	0
meat 1	1	0	0	0	0	0	0	0	0
meat 2	0	0	0	0	0	0	1	0	0
meat 3	2	0	0	0	0	0	1	0	0
gills 1	10	0	0	6	0	0	0	0	0
gills 2	7	0	0	6	0	0	0	0	0
gills 3	6	0	0	9	0	0	2	0	0
gl tract 1	0	0	0	0	0	0	2	0	0
gl tract 2	0	0	0	0	0	0	3	0	0
gl tract 3	0	0	0	0	0	0	1	0	0
caviar 1	3	0	0	0	0	0	0	0	0
caviar 2	3	0	0	0	0	0	0	0	0
caviar 3	4	0	0	0	0	0	2	0	0

Table 2. Number of microplastic particles in samples from *M. cephalus.*

Species	*M. cephalus*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm
skin 1	2	0	0	0	1	0	1	0	0
skin 2	3	0	0	0	0	0	0	0	0
skin 3	0	0	0	0	0	0	0	0	0
meat 1	0	0	0	0	0	0	3	1	0
meat 2	0	0	0	0	0	0	2	1	0
meat 3	0	0	0	1	1	0	4	0	0
gills 1	3	0	0	0	0	0	1	0	0
gills 2	3	0	0	0	0	0	0	0	0
gills 3	2	0	0	2	0	0	1	0	0
gl tract 1	11	0	0	0	0	0	1	0	0
gl tract 2	8	0	0	1	1	0	1	0	0
gl tract 3	11	0	0	1	1	0	1	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

In the Mullet (Table 2), the pellets with sizes ≤ 25 μm prevailed. They were isolated mostly from in the gastrointestinal tract, gills and skin. Pellets 100–200 μm in size were detected in the muscles, gills and the gastrointestinal tract. Fibres ≤ 25 μm in size were isolated from skin, the muscles and the gastrointestinal tract. Irregularly-formed MPs were isolated from all parts of the fish, except the chaviar, but were most abundant in the muscles and the gastrointestinal tract. As in the muscles, particles with size of 25–100 μm were detected.

Table 3. Number of microplastic particles in samples from *M. batrachocephalus*.

Species	*M. batrachocephalus*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm
skin 1	2	0	0	1	0	0	2	1	0
skin 2	5	0	0	0	0	0	2	0	0
skin 3	2	0	0	0	0	0	1	0	0
meat 1	0	0	0	1	0	0	3	0	0
meat 2	0	0	0	1	0	0	1	0	0
meat 3	2	0	0	1	0	0	1	0	0
gills 1	1	0	0	0	0	0	0	0	0
gills 2	0	0	0	0	1	0	0	0	0
gills 3	2	0	0	0	0	0	2	1	0
gl tract 1	1	0	0	0	0	0	0	0	0
gl tract 2	1	0	0	0	0	0	0	0	0
gl tract 3	2	0	0	0	0	0	1	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

Table 4. Number of microplastic particles in samples from *A. immaculata*.

Species	*A. immaculata*								
Form of MPs	Pellets			Fibres			Irregular form		
	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm	≤ 25 μm	25–100 μm	100–200 μm
skin 1	1	0	0	0	3	0	0	0	0
skin 2	0	0	0	0	0	0	0	0	0
skin 3	1	0	0	0	0	0	0	0	0
meat 1	3	0	0	0	0	0	1	0	0
meat 2	3	0	0	0	0	0	1	0	0
meat 3	3	0	0	0	0	0	3	1	0
gills 1	1	0	0	7	0	0	0	0	0
gills 2	1	0	0	6	0	0	0	0	0
gills 3	0	0	0	7	1	0	0	0	0
gl tract 1	0	0	0	0	0	0	0	0	0
gl tract 2	0	0	0	0	0	0	0	0	0
gl tract 3	0	0	0	0	0	0	0	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

From *M. batrachocephalus*, pellets with sizes ≤ 25 μm were isolated from the skin, gastrointestinal tract, gills and flesh of the fish prevailed (Table 3). Fibres ≤ 25 μm in size were isolated from muscles and the skin. Fibres 100–200 μm in size were also detected in the gills. Irregularly-formed MPs were isolated in all parts of the fish except the caviar, most abundantly in the muscles and the skin. In the skin and gills, larger particles of 25–100 μm were also found (Fig. 1C).

In *A. immaculata*, the pellets with sizes ≤ 25 μm predominated and they were isolated from the skin and the gills of the fish. Fibres ≤ 25 μm in size were isolated from the gills (Fig. 1C). Fibres 25–100 μm in size were also isolated from the skin and gills. Irregularly-shaped MPs were isolated from the muscles. Particles of larger size (25–100 μm) were also found.

In *T. m. ponticus*, pellets with sizes ≤ 25 μm prevailed. They were isolated from the gastrointestinal tract, the gills, the muscles and the skin of the fish. Fibres ≤ 25 μm and 25–100 μm in size were isolated from the gastrointestinal tract (Fig. 1). In the gills and in the skin, larger particles of 25–100 μm were also found.

FTIR spectral analysis was performed to determine the nature of the isolated MPs. The obtained results are represented in Appendix 1.

From the isolated microplastic particles, the following types were identified: LDPE – low-density polyethylene used in the production of plastic cups; PA – polyamide used in the production of cords; PET – polyethylene terephthalate used in the production of bottles for soft drinks; PP – polypropylene used in the production of shampoo bottles; PC – polystyrene/polystyrene used in the production of CD cases; EPS - expanded polystyrene used in the production of packaging and PVC - plasticised polyvinyl chloride.

Some of the isolated microplastic particles were shown to be a polyamide (nylon fibres) (Appendix 1: Fig. A1B). They are specific by spectra that are characterised by strong amide I-amide II spectral bands at 1650 and 1543 cm^{-1} and polyethylene terephthalate (polyester, PET) (Appendix 1: Fig. A1C) showing C=O with bands at 1725 cm^{-1}, C–O with bands at 1250 and 1100 cm^{-1}. Two of the analysed fibres have a spectrum with a peak at 1729 cm^{-1} (Appendix 1: Figs A1E, G). This peak is consistent with the C=O stretching mode of the acrylates. The absence of a nitrile band (2237 cm^{-1}) allowed us to reject polyacrylonitrile as a component of the polymer matrix. The plain translucent fibre presents a spectrum similar to that of siloxanes, characterised by peaks at 1030–1065 cm^{-1} (Si-O-Si stretch) and 1280 cm^{-1} (Si-CH3 strain) (Appendix 1: Fig. A1C).

Discussion

This study presents a detailed assessment of MPs contamination in commercially important fish species, caught in the south of the Bulgarian Black Sea aquatory. All investigated species were contaminated with MPs. A study by (Eryaşar et al. 2022) reports on contamination with 95 MPs in three commercial fish species - *Engraulis encrasicolus*, *Merlangius merlangus* and *Mullus barbatus* from the Turkish Black Sea coast. The most contaminated with MPs was the red mullet and the MPs were mainly in the form of fibres. However, these authors did not dissect the fish into separate parts and it is not clear exactly in which part of the fish the respective microplastics were isolated.

However, they found that polyethylene and polypropylene were the most dominant type of polymers. This is in line with our results, as we also isolated mostly polyethylene. In our study, pellets were the most abundant, followed by fibres.

Neves et al. (2015) found, that, along the Portuguese coast, the number of MPs per fish was higher, compared to the study of Eryaşar et al. (2022). Compared to our results, however, these are relativelly low numbers. Additionally, a lower number of MPs was reported in the analysis of the gastrointestinal tract

in fish from the Turkish Marmara, Aegean and Mediterranean coasts (Gȕven et al. 2017; Gündoˇgdu et al. 2020), the Spanish coast (Bellas et al. 2016) and Portuguese coasts (Bessa et al. 2018).

In our study, the most MPs were reported in the gastrointestinal tract in one the species - 36 MPs particles. Differences in the number of MPs can be a consequence from the differing methodologies in the papers and from the different degrees of pollution (Neves et al. 2015; Bellas et al. 2016; Peters et al. 2017; Wang et al. 2021). In general, benthic fish species reported higher MPs content, which could be related to differences in fish feeding and behaviour and due to the sedimentation and deposition processes of the particles (Woodall et al. 2014; Jabeen et al. 2017; Wootton et al. 2021). There is evidence that bottom-dwelling fish species can ingest sediment in the process of feeding (Lusher et al. 2013), which may lead to higher levels of MPs uptake, through one of the main pathways for MPs ingestion, via the gastrointestinal tract (McGoran et. al. 2017; Wang et al. 2021).

Nevertheless, a higher percentage of MPs was reported in pelagic fish species – Garfish and Mediterranean horse mackerel (Tables 1, 5).

This type of material is commonly used to make domestic and marine sealants. The majority of isolated microplastic particles mainly contain polyethylene (PE) and polyethylene terephthalate. Polyethylene is used and is included in the composition of plastic bottles, cups, stirrers and plastic bags. This polymer is very light and floats on the surface of the sea because its density is lower than that of water. Polyethylene terephthalate, on the other hand, is denser than water and is more likely to sink and accumulate in the sea bed and benthic organisms. These polymers are widely used in fabrics, in nets, ropes and strings used for fishing - one of the main economic activities of the Black Sea. The predominant types of polymers - the PE, corresponds to the content

Table 5. Number of microplastic particles in samples from *T. m. ponticus*.

Species	***T. m. ponticus***								
Form of MPs	**Pellets**			**Fibres**			**Irregular form**		
	≤ 25 µm	**25–100 µm**	**100–200 µm**	**≤ 25 µm**	**25–100 µm**	**100–200 µm**	**≤ 25 µm**	**25–100 µm**	**100–200 µm**
skin 1	1	0	0	0	0	0	1	1	0
skin 2	0	0	0	0	0	0	1	0	0
skin 3	0	0	0	0	0	0	2	0	0
meat 1	2	0	0	0	0	0	1	0	0
meat 2	4	0	0	0	0	0	1	0	0
meat 3	2	0	0	0	0	0	1	0	0
gills 1	4	0	0	0	0	0	3	1	0
gills 2	7	0	0	0	0	0	1	1	0
gills 3	4	0	0	0	0	0	6	0	0
gl tract 1	5	0	0	6	0	0	1	0	0
gl tract 2	8	0	0	3	2	0	1	0	0
gl tract 3	7	0	0	5	0	0	3	0	0
caviar 1	0	0	0	0	0	0	0	0	0
caviar 2	0	0	0	0	0	0	0	0	0
caviar 3	0	0	0	0	0	0	0	0	0

of manufactured plastics all around Europe, as almost half of the plastics produced in Europe are reported as PE (Plastics Europe 2020). The sinking of plastics and sedimentation is related to the fact that the upper layer of the Black Sea is less dense than other seas. On the other hand, the weight of these particles increases due to the accumulation of marine plants and nutrients in them and this can affect the distribution of plastics and their sedimentation on the sea bed. In studies on the black mussel, it was reported that these polymers are dominant in the mussels (Ibryamova et al. 2022).

Regarding fish health, it has been reported that plastics < 1000 μm in size can reach the digestive tract or the gills and, in turn, can cause adverse effects, such as a weak immune response and reduced fertility (Browne et al. 2008; Prokic et al. 2019; Jaafar et al. 2021). No particles larger than 100 μm were found in our study. Considering the wide variety of types of MPs detected in the digestive tract, we assumed that the fish regularly ingest the MPs during feeding. Some researchers reported that the main mode of MPs ingestion is not by misidentifying these particles as prey, but fish passively consume MPs during the feeding process (Sun et al. 2019; Wang et al. 2021). Many nutrients are also adsorbed on the plastic particles which deceives the fish that this is food. It can be assumed that the fish do not recognise the microplastics, but identify it as a food. Bowen et al. (2021) showed that fish ingest MPs inadvertently rather than intentionally. The authors found that fish did not actively ingest microfibres and, instead, the MPs were passively absorbed by breathing. Fish have also been reported to exhibit fibre rejection behaviour by expelling them out when mixed with mucus (Bowen et al. 2021). MPs can accumulate in the predatory fish species; unfortunately, very limited research has been performed on bioaccumulation and biomagnification in food webs (Wootton et al. 2021); therefore, more studies are needed to reach this conclusion.

MPs enter seawater food chains in different pathways and threaten entire ecosystems through their ability to transport pollutants, pathogenic microorganisms and alien species. Having in mind the intensifying economic activity in the Black Sea coast and the consequent influence on the riverine water quality (Gartsiyanova et al. 2024), river mouths can be considered as a potential source of MPs. This is especially so near the Kamchia River mouth, which is the largest intra-territorial river in Bulgaria, entering directly into the Black Sea, with a cathment area above 5300 km^2 (Doychev 2023).

This cathment and the entire Black Sea coast, where agriculture is well developed (Gartsiyanova et al. 2024) is a potential source of MPs, which have the ability to absorb and release toxic chemicals of organic and inorganic origin, such as Bisphenol A (BPA), PCBs and DDT, creating an additional potential risk to human health. Humans are exposed to BPA in the environment they live in from the air we breathe, the food and drinks we consume etc. Therefore, even if BPA intake is below some accepted limits, this does not guarantee that the additive will not accumulate and cause more pronounced effects and chronic toxicity in the food chain, given the tendency to accumulate.

As a consequence of the obtained results and the amount and type of polymer found in the study and literature, the source of contamination, in our opinion, can be mainly attributed to domestic wastewater discharges coming from the washing of synthetic fabrics. However, detailed studies are needed to prove

this. In Bulgaria, wastewater is discharged directly or after purification into marine and freshwater ecosystems, as is the case in other neighbouring countries along the Black Sea coast-Our results show a wide variety of micropollutants originating from the commonly used plastic cups, stirrers, bags, soft drink bottles, fishing nets, packaging of hygiene and personal hygiene preparations and others that have systematically entered the Black Sea and are degraded into microplastic particles. The present study demonstrated the MPs contamination of five commercial fish species from the Black Sea with higher abundance of MPs in pelagic species. It is important future research to determine the toxicological side effects of plastic ingestion for fish communities in both benthic and pelagic habitats.

However, even if introducing plastics into the water system is stopped, both groups of fish will continue to be impacted, since the number of microplastics can increase due to the breakdown of larger plastics in the environment.

This study shows the need to carry out further studies and characterisation of microplastics using different types of microscopic and spectral analysis. Even though microplastics may not pose a risk to humans who consume fish, these contaminants pose a potential risk to marine food webs and endangered species. We found particles of different sizes, types and colours in different fish species. We believe that the variability of polymer species in fish can reveal/indicate the polymer species in water to some extent. Our results show that fish are important as ecological bioindicators and serve as a basis for future studies on microplastic pollution in tourist sandy beaches.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Tsveteslava V. Ignatova-Ivanova and Nikolay D. Natchev conceived and designed the study. Nikolay D. Natchev and Radoslav Ivanov obtained the samples. Teodora Koynova performed the fish dissection. Tsveteslava V. Ignatova-Ivanova and Nikolay D. Natchev supervised the data analysis and wrote the manuscript. Sevginar F. Ibryamova, Stephany Toschkova, Darina Ch. Bachvarova and Elitca Stanachkova performed the testing and contributed to data analyses and summaries. All authors have read and agreed to the published version of the manuscript.

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Appendix 1

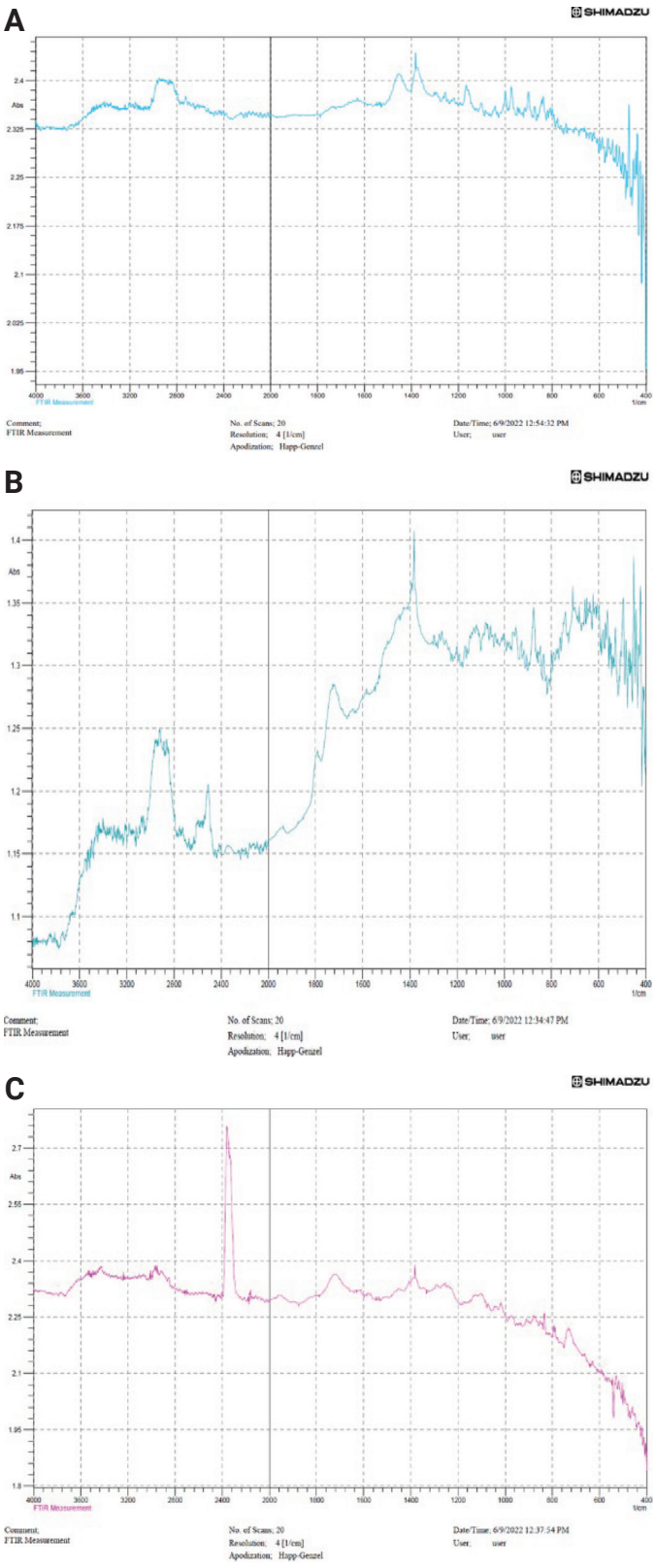

Figure A1. FTIR spectrum of the microplastic particles isolated from the fish **A** LDPE – low density polyethylene **B** PA – polyamide **C** PET – polyethylene terephthalate **D** PP – polypropylene **E** PC – polystyrene/polystyrene **F** EPS - expanded polystyrene **G** PVC – plasticised polyvinyl chloride spectrum.

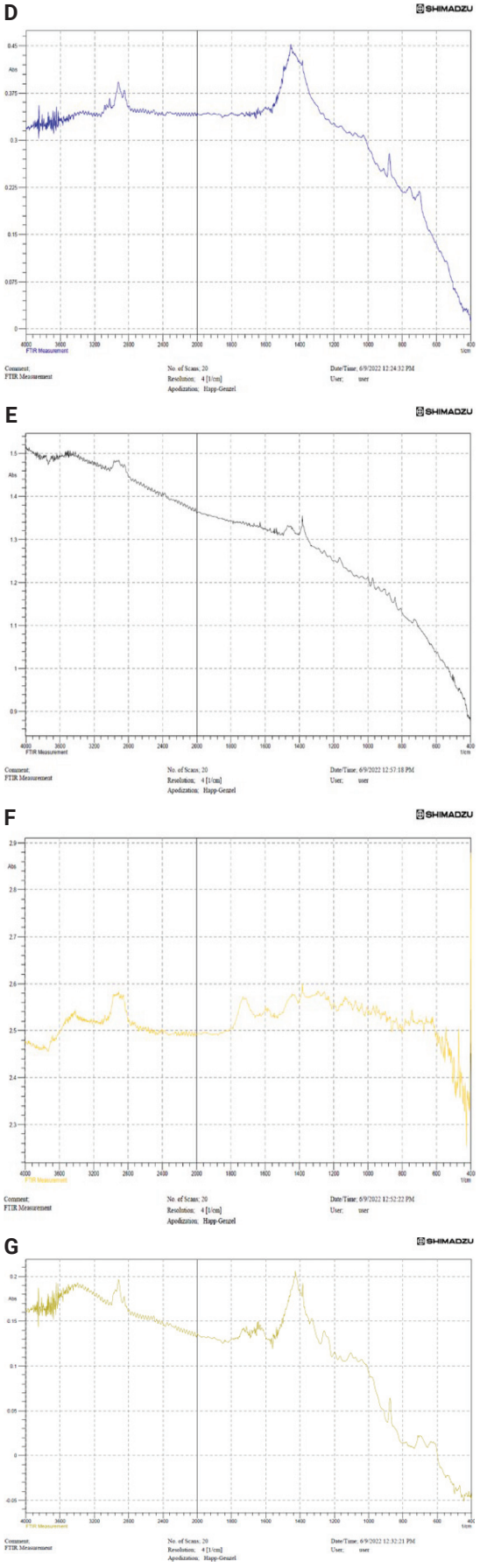

Figure A1. Continued.

BioRisk

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Review Article

Twenty years of ecosystem services research in Bulgaria: lessons learned and future directions from a geographical perspective

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Abstract

The ecosystem services (ES) concept has established itself in recent years as the predominant paradigm for framing environmental research and policy-making. The EU Biodiversity Strategy to 2020 with its task for member countries to map and assess the state of ecosystems and their services has contributed vastly to the development of the ES studies in the European countries. Bulgaria was among the countries that made substantial progress in its implementation and the contribution of the geographers was of vital importance. This paper aims to provide an overview and analysis of the ES research in Bulgaria focusing on the contributions of the geographers and the spatial aspects of the studies. The information on the ES research was performed through a literature review by collecting all available published works that address the main objectives of the study. To systematize and characterize the content of the reviewed papers, a special database with a standard nomenclature was constructed. The findings from the review allowed us to identify both achievements and research gaps in the ES studies conducted by Bulgarian geographers. This enabled us to define the main research priorities of the coming years which can trace the future directions of ES research in the country. They include the development of the spatial aspects in the methodological frameworks for mapping and assessment of ES, better use of GIS-based tools for mapping ES alongside models' integration, and improvement of the publication's quality and increase of the papers published in highly rated indexed journals.

Key words: Cultural services, literature review, mapping ES, provisioning services, regulating services

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Introduction

The ecosystem services (ES) concept has established itself in recent years as the predominant paradigm for framing environmental research and policy-making (Martin-Ortega et al. 2019). It has been introduced in scientific literature with the fundamental works of De Groot (1992), Costanza et al. (1997), and Daily (1997), and since the Millennium Ecosystem Assessment (2005) the number of publications has risen drastically. The ES concept provides an appropriate methodological basis that enables linking the state of ecosystems with human well-being which can be used as a platform to find solutions to various environmental problems (Danova 2023). The EU Biodiversity Strategy to 2020 with its

task for member countries to map and assess the state of ecosystems and their services in their national territory has contributed vastly to the development of the ES studies in the European countries. The working group on Mapping and Assessment of Ecosystems and their Services (MAES) has guided the process by establishing a methodological framework to ensure consistency across member states (Maes et al. 2013). According to the MAES barometer, which indicates each EU member state's stage of MAES implementation, Bulgaria was among the countries that made substantial progress in the implementation of Action 5 (Burkhard et al. 2018a). This is a result of significant efforts within several projects during the MAES process but it has also been determined by various research activities during the last 20 years. The spatial aspects of such works are very important and the contribution of the geographers is of vital importance.

The growing number of studies and publications both at global and national level lead to accumulation of knowledge which is stored in various types of publications and different databases. Furthermore, the ES label is used in a range of studies with widely different aims which poses problems to both researchers and policy-makers as it makes it difficult to assess the credibility of the results and reduces the compatibility of the studies (Seppelt et al. 2011). This raises the need for systematization and critical analyses of the published literature sources. The interdisciplinary of the ES concepts means also that the publications come from various disciplines and apply different methods from both natural and social sciences. The present work aims to analyze the contribution of geographers to the development of ES research in Bulgaria, especially in the field of mapping and assessment of ecosystems and their services.

Literature reviews on various aspects of ES have been conducted in many publications during the last few years. Several reviews focus on various methodological aspects such as the indicators for mapping ES (Egoh et al. 2012), and methods for mapping ES supply (Martínez-Harms and Balvanera 2012), mapping of ES values (Schägner et al. 2013), mapping of ES demand (Wolff et al. 2015), the matrix approach (Campagne et al. 2020), and the integration of ES indicators (Peng et al. 2023). ES provided by urban ecosystems has been reviewed by Haase et al. (2014), Luederitz et al. (2015), and Stoycheva and Geneletti (2023). The spatial aspects of ES research have been reviewed from the point of view of quantification and mapping of ES supply and demand (Ayanu et al. 2012), mapping of ES across scales and continents (Malinga et al. 2015), mapping ES demand (Wolff et al. 2015). Several reviews and overview studies are focused on ES research at the national level in specific countries such as Germany (Förster et al. 2019), the UK (Sing et al. 2018), the Czech Republic (Frélichová et al. 2014), Hungary (Vári et al. 2022).

Despite the undoubted achievements of ES research in Bulgaria, there is still no study that reviews the development of these fields in the country. The contribution of the researchers from different disciplines including geographers has also not been studied so far. It is necessary also to reveal the spatial aspects of the studies with a focus on the maps and mapping methods. This field of ES research has been very well developed especially after the MAES process in Europe (Burkhard and Maes 2017; Vári et al. 2024) and a study on maps and mapping methods presented in the publications from Bulgaria is much needed.

This paper aims to provide an overview and analysis of the ES research in Bulgaria focusing on the contributions of the geographers and the spatial

aspects of the studies. More specifically, we aim at: i) revealing the main stages in the ES research in Bulgaria through retrospective analysis; ii) analyzing the publications on ES and the distribution of the authorship among the geographical units in the country; iii) analyzing the distribution of ES and the methods used in the reviewed studies; iv) analyzing the spatial aspects of the ES studies focusing on the maps and the mapping methods in the reviewed studies.

General overview of the ES research in Bulgaria

The development of ES research in Bulgaria can be divided into four main stages: 1) first steps (2005–2010); 2) research stage (2010–2014); 3) MAES stage (2014–2018); post-MAES stage (after 2018). The first steps of the ES concept in Bulgaria were carried out both in academia and the public sector by two pioneering projects. The implementation of the project "Protection of Globally Significant Biological Diversity in the Landscape of the Rhodopes", a joint initiative of the United Nations Development Program (UNDP) and the Ministry of Agriculture and Food led to the first publication on ES by Zevurdakis et al. (2007). The participation of researchers from the Institute of Geography of the Bulgarian Academy of Sciences in the international project "Use of landscape sciences for environmental assessment" (2001–2006) led to the implementation of new approaches for environmental assessment including the promotion of the ES concept in 2005. As a follow-up to this work, the first research paper on the use of hydrological models for flood regulation ES assessment was published (Nedkov 2008). The first steps in the Faculty of Geology and Geography at the Sofia University "St. Kliment Ohridski" were the studies on natural capital assessment (Assenov 2009).

The second period (2010–2014) is characterized by significant development of the research methods for mapping and assessment of specific ES, as well as economic valuation approaches. The biophysical methods for mapping and assessment of flood regulation ES by application of GIS-based hydrological modeling were developed and applied in several case studies (Nedkov and Burkhard 2012; Boyanova et al. 2014). These works were further developed for other water-related ES and contributed to the concept at the international level on the development of the matrix approach (Burkhard et al. 2012) and for the blueprint of the mapping and modeling methods (Crossman et al. 2013). The economic valuation methods (especially contingent valuation) were developed and applied in several case studies mainly at the municipality level (Assenov 2010, 2012). Several studies explore the impact on ES by environmental changes in mountains (Bratanova-Doncheva et al. 2014), forest practices (Zhang et al. 2013; Grigorov et al. 2022).

The third period (2014–2018) is characterized by a significant rise in ES research which is determined mainly by the MAES process. In response to the requirements of the EU Biodiversity Strategy for member countries to map and assess the state of ecosystems and their services the Bulgarian Ministry of Environment and Waters (MOEW) initiated a national program for mapping ecosystems. It gathered researchers from various disciplines and institutions to develop methodologies for each of the nine main ecosystem types and organized projects for mapping ecosystems and their services (Bratanova-Doncheva et al. 2017). These activities led to a national information system for ecosystems

in the country, various publications, and a special issue in One Ecosystem journal (Nedkov et al. 2018). They also contributed to the development of the methodology at the European level through the systematization and development of biophysical methods (Vihervaara et al. 2019), generalization at the European level through comparative analyses (Geneletti et al. 2020), and the update of the methodological framework (Burkhard et al. 2018b).

The post-MAES period (after 2018) is characterized by further development of specific ES fields which led to important research contributions but on the other hand, the end of the MOEW program led to a decrease in interest among some of the participants in the MAES process. Therefore, the ES activities were driven back to the research groups that were active during the previous periods. The achievements of the Bulgarian geographers in the ES research were presented by significant contributions in the book (Smart Geography) dedicated to the 100th anniversary of the Bulgarian Geographical Society (Nedkov et al. 2020). The ES research is extended towards the application of new and innovative spatial approaches as well as the development of interdisciplinary research. These included the impact of climate change on the ES in Bulgaria (Bratanova-Doncheva and Gocheva 2020; Nikolova et al. 2021b), the implementation of ecosystem accounting (Nikolov et al. 2022), the implementation of the ES approach for nature-based sustainable tourism (Nikolova et al. 2021c), the nature heritage in forest areas as a source of economic, social and cultural benefits (Zhiyanski et al. 2021), the methodology for mapping of the capacity of landscapes to provide ES (Prodanova 2021), the relation between the urban and peri-urban landscapes, biodiversity and the flow of ES (Semerdzhieva and Borisova 2021) the relationship between the individual scores and final expert based assessment for natural heritage supply maps (Prodanova and Varadzhakova 2022). Another important tendency during this period is the integration of nature-based solutions into ecosystem services research.

Materials and methods

Literature review

The literature review was performed to collect all available published works that address the main objectives of the study. The overview of the ES research in Bulgaria necessitates the finding of all literature sources that contributed to the development of this research. The review was conducted in several steps: 1. Identifying the geographical institutions in Bulgaria; 2. Identifying geographers (researchers) who work in ES-related fields; 3. Searching through institutions' official web pages and authors' scientific profiles (Scopus ID, WoS researcher ID; Google Scholar profile; ResearchGate profile) for ES-related papers. However, apart from the research papers published in scientific journals, many grey literature sources reveal valuable information for the overview we were aiming at. For instance, several methodological guidelines publications play an important role in shaping ES research in the country. It is also important to reveal the full scope of the Bulgarian geographers' publication activity to analyze its strengths and weaknesses and make recommendations for future development.

The initial search resulted in a total of 145 publications. Most of them we authored or co-authored by geographers but there were also some publications

with no geographer contribution. A specific part of these publications is related to the MAES process including the methodologies for mapping and assessment of ecosystems in Bulgaria and follow-up publications concerning the mapping of ecosystems itself. As there are methodologies and mapping-related publications with geographers' participation, we decided to keep all publications from this group. All other publications without a geographer as an author or co-author were omitted from the list. The remaining publications were the subject of preliminary screening for relevance to the topic of the study. Those that do not have any kind of ecosystem services research were also omitted from the list. Thus, the final number of publications to be reviewed was reduced to 123.

To systematize and characterize the content of the reviewed papers, a special database with a standard nomenclature was constructed (Nedkov et al. 2022). A template in the form of a structured MS Excel sheet that enables easy and convenient data entry was developed. Its design follows the common structure of such templates and comprises five main parts: 1) general characteristics of the publication; 2) ecosystem services; 3) methods; 4) case study; 5) mapping. When possible, the variables in the table were entered using the binary numerical system, otherwise, inputs were made in the form of text (Nedkov et al. 2022). The first part reflects the second specific aim of the study to analyze the publications on ES and the distribution of the authorship among the geographical units in the country. It contains bibliographic data for the publication (authors, journal, year, DOI, and journal or publisher), data for the authorship (distribution between geographical entities and international collaboration), the language of the publication, the case study, the purpose, and the dimensions of the study. The second and third parts are designed to support the achievement of the third specific objective of this review to analyze the distribution of ES and the methods used in the reviewed studies. In the second part, for each publication, we count the number of ES and their distribution among the three main ES groups (provisioning, regulating, and cultural). The supply-demand side of ES research is an important aspect of any assessment therefore the template contains columns that indicate if the study concerns supply, demand, or both. In the third part, the publications were reviewed according to the three main methods (biophysical, social, and economic) used for ES assessment. The fourth and fifth parts contain data entries reflecting the fourth specific objective to analyze the spatial aspects of the ES studies with a focus on the maps and the mapping methods. The fourth part considers the case studies of the publications: presence of a case study, number of case studies, location, and scale of the case study. The fifth part is dedicated to the mapping of ES. The presence of maps in the publications is an important indicator to evaluate the geographical aspects of the studies. The use of an established ES mapping approach is an indicator of the integrity of the internationally accepted methodologies. The next five categories enable us to define the methods used to develop the ES maps. The predefined categories include matrix approach, expert score, use of statistical data, spatial proxy methods, and modeling methods. There is also an option to select the "other" option and add the name of the method that does not fit the previous options. Columns for mapping of ecosystem types and ecosystem condition are also presented in this part.

Data analyses

The analyses of ES research in the reviewed publications were performed in three main aspects: i) the character of the ES research; ii) revealing what ES is/are studied; iii) the methods applied in ES studies. The character of the ES research was analyzed on one hand the presence or absence of specific ES and research on supply/demand and on the other hand by revealing if the study is focused on supply, demand, or both. The presence of the specific ES and supply/demand is an indicator that the publication presents results of real ES assessment. The papers that do not fulfill the requirements of this indicator could be either conceptual or editorial which cover general aspects of ES research or studies on broader environmental topics that mention ES in text but do not perform real ES research. The ES in the review template were reduced to the three main groups of services (provisioning, regulating, and cultural) following the CICES classification (Haines-Young and Potschin 2018). The analyses were focused on the distribution of the ES groups in the studied publications and the number of individual ES studied in them. The methods applied in the ES studies were systematized and analyzed using the most common classification of the methods into three major groups: biophysical, social, and economic).

The spatial dimensions of the ES research in Bulgaria were analyzed in two main aspects: the case studies in the publications and the mapping of ES. The presence or absence of a case study is an important indicator of the character of the ES studies, namely the geographical element in them. The mapping is a very important aspect of ES research as maps are very useful for raising awareness about areas of ecosystem goods and services supply and demand, and to provide information about interregional ES flows (Burkhard and Maes 2017). The analyses on mapping aspects of the publication were performed at two levels: for the whole set of publications and for research papers. Metrics about the number of publications with ES maps, general ES methods used in ES mapping, specific methods used for ES mapping, and methods applied with the matrix approach were calculated.

Results

Characteristics of the publications

The literature search resulted in 123 publications in total that were authored or co-authored by Bulgarian geographers. Their list consists of various types of peer-reviewed publications and grey literature sources published between 2007 and 2023. Ten major types of papers were identified during the review process (Table 1) where research and review papers have the highest share of 40% (49 publications). One type of publication is not presented in the reviewed publications—norm documents (Category 7). Category "10", other types of publications refer to one discussion paper and one blog post. The highest number of papers were published in 2017 and 2021 (Fig. 1A). Those papers refer to two national projects that ended in the reference years—the nine national mapping projects under the MAES framework (ESMERALDA project) and the project Heritage.BG. The only year without any publications for the studies period of 2007–2023 is 2009. Out of the 123 publications 43% are indexed in Scopus or Web of Science

Table 1. Distribution of the reviewed publications by type.

Type of publication	Number of papers	%
1. Research and review papers in scientific journals	49	40
2. Other types of papers in scientific journals	11	9
3. Papers in conference proceedings	23	19
4. Books	2	1.3
5. Book chapters	17	14
6. Methodologies	11	9
7. Normative Documents	0	0
8. Publications in popular scientific magazines	2	1.3
9. Doctoral dissertations	6	5
10. Other types of publications	2	1.3

while most of them are not indexed 57% (Fig. 1C). Analysis of the accessibility of publications shows that 66% of all documents are available online with open access, 19% are with paid access, and 15% are counted as grey literature since they do not appear online (Fig. 1E). All publications are published in English, Bulgarian, or both languages (Fig. 1I). The English language is the primary publishing language with 69% of all, followed by Bulgarian language (21%). Only 10% of the publications are bilingual. The top three of the most impactful journals or publishers are the Journal of the Bulgarian Geographical Society with 16 papers, One Ecosystem with 13 published papers, and Clorind Publishing House with 11 published methodologies (Fig. 1J). Six out of ten are periodical journals, two are book series, one is conference proceedings, and another is a publishing house.

Authors profile/background

Results of the affiliation of the lead author show that most publications were authored by researchers from the National Institute of geophysics, Geodesy and Geography at the Bulgarian Academy of Sciences (NIGGG-BAS) with 41% (56) and Sofia University with 26% (35), while only 9% (12) were authored by foreign authors (Fig. 1F). Only one of the studied institutions—Shumen University, has zero authors publishing about ES. The most active lead author is Nedkov with 19 publications, followed by Assenov with 10. Five out of the ten most active authors are affiliated with the NIGGG-BAS, four with Sofia University, and only one is affiliated with a non-geographical institution (Fig. 1B). Results on the international collaboration show that only 18% (22) of the publications have foreign co-authors, and the majority of the publications 82% (101) were authored entirely by Bulgarian scholars (Fig. 1D). The geographical distribution of the foreign authors shows that the most collaborative country is Germany with 15 publications, followed by Spain with six publications (Fig. 1G). Most of the foreign co-authors are based in countries in Europe such as Finland, Italy, Sweden, Romania, and France. Authors from only two countries besides Europe appear in the publications—those are Australia with four publications and the USA with three publications. The most frequent number of authors per publication varies between one (24 publications) and three (20 publications). The highest number of authors per publication is 83 and appears for only one publication, which is out of the range (Fig. 2H).

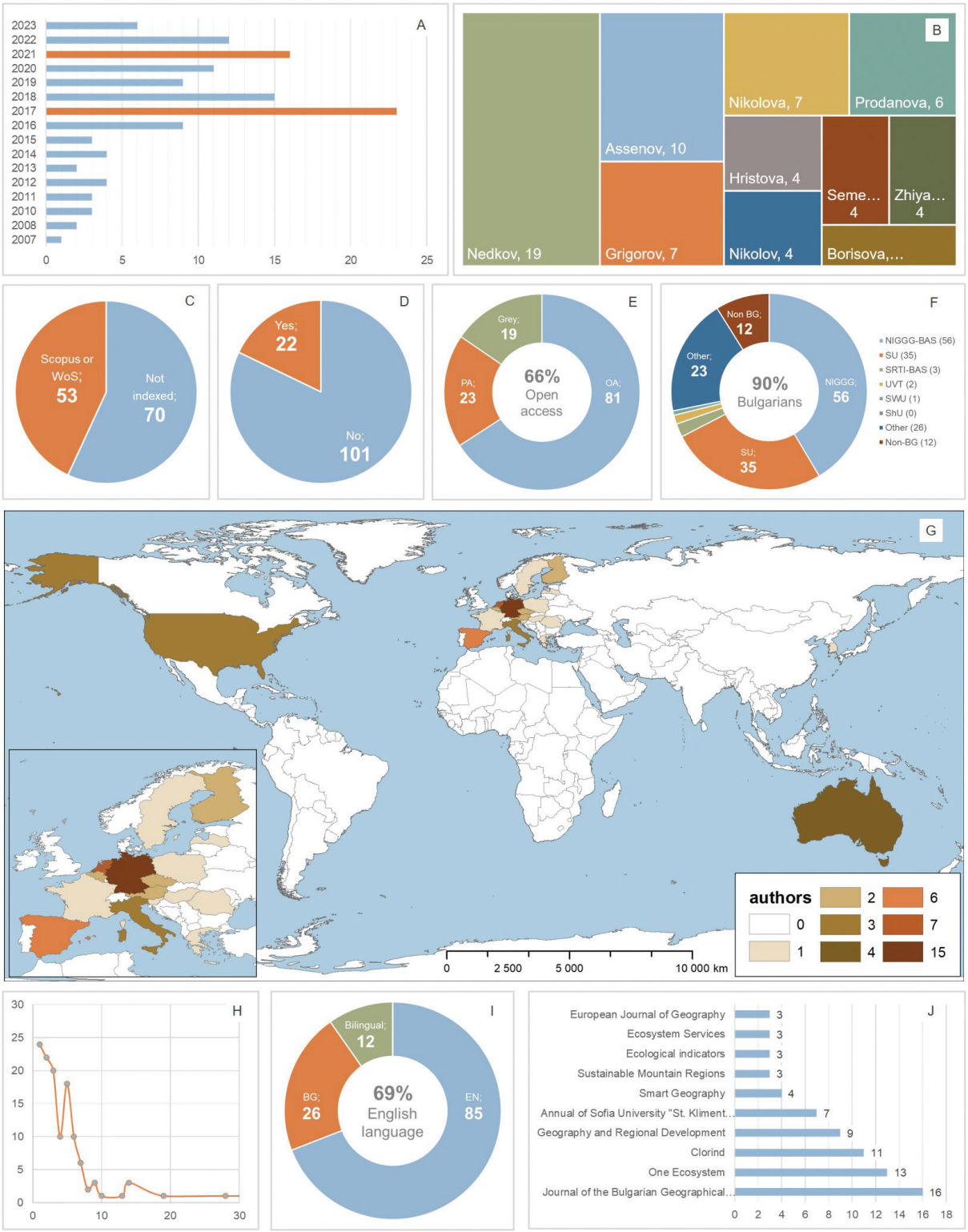

Figure 1. Characteristics of the publications and authors **A** number of publications per year **B** top 10 Bulgarian lead authors **C** number of indexed publications **D** number of publications with international collaboration **E** number of accessible publications online **F** number of publications per institution **G** global distribution of foreign co-authors **H** number of authors per publication **I** language of the publication **J** most contributing journals and publishers to the ecosystem services research in Bulgaria. Abbreviations (1F): NIGGG-BAS—National Institute of Geophysics, Geodesy and Geography - Bulgarian Academy of Sciences, SU—Sofia University "St. Kliment Ohridski", SRTI-BAS—Space Research and Technology Institute - Bulgarian Academy of Sciences, UVT—"St. Cyril and St. Methodius" University of Veliko Tarnovo, SWU—South-West University "Neofit Rilski", ShU—"Konstantin Preslavsky" University of Shumen.

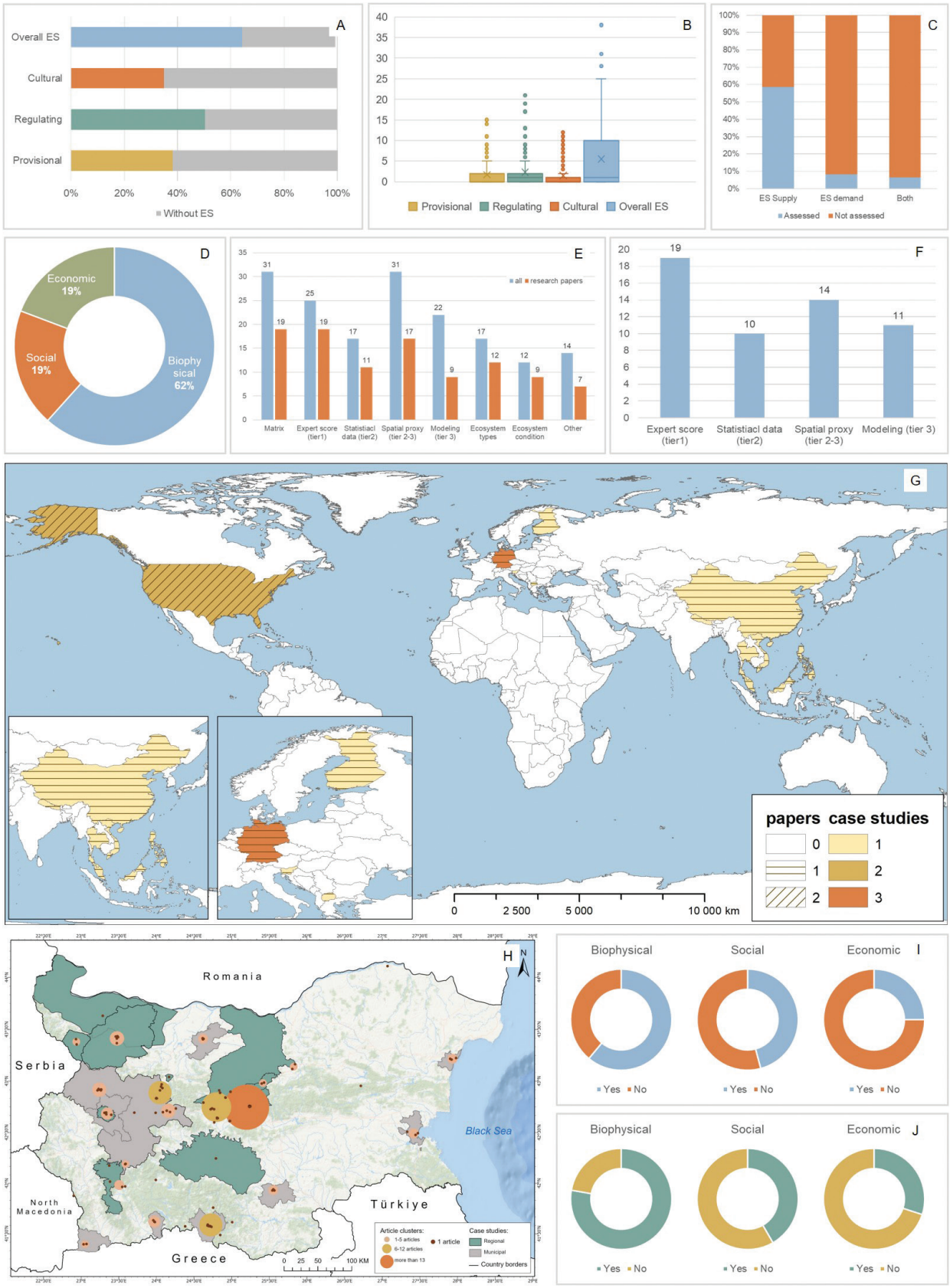

Figure 2. Characteristics of the ecosystem services research **A** assessed ES types in studied publications **B** distribution of the assessed ES **C** assessed supply-demand **D** ES assessment methods **E** mapping methods **F** matrix mapping methods **G** international case studies **H** Bulgarian case studies **I** distribution of all publications with ES maps between assessment methods **J** distribution of research papers with ES maps between assessment methods.

Ecosystem services research

The results on the character of the ES research showed that 64% (80), of the reviewed publications have assessed ES whereas the rest 36% (43) had no ES assessment. Further analyses of the latter group revealed that some of these papers are either editorial papers (five) from special issues containing ES assessment papers (such as Nikolova et al. 2021a) or conceptual papers (nine) that deal with methodological aspects of ES research. Another group contains five publications that present ES assessment without focusing on particular services. Four publications deal with ecosystem mapping (such as Hristova and Stoycheva 2021; Petkova et al. 2022) and assessment of ecosystem conditions (Uzunov et al. 2017). The largest group (21) consisted of publications that deal with broader environmental topics mentioning ES in the text but do not perform real ES research. Although these publications are evenly distributed between the four periods, their share during the first two is much larger (33% and 31%) than in the third (12%) and the fourth (17%) period. The number of studied ES (provisioning, regulating, cultural, or a bundle of ES) differs within each study.

The most studied ES group is the regulating (50% overall), followed by provisioning (38% of all papers) and cultural (35% of all papers) ones (Fig. 2A). The publications that focus only on one of the ES groups number 36 and those assessing all three ES groups number 30. The publications with two ES groups number 13. Almost all of them contain the combination provisioning-regulating, while only one contains the combination provisioning-cultural. The distribution of the number of ES in each paper varies from mostly studying one ES (mainly regulating ES) to 38 in two methodological publications that address 38 ES (Karamfilov et al. 2017; Kostov et al. 2017). The biggest difference is within regulating ES, which ranges from one to 21 ES within one paper (Fig. 2B). Research papers mainly study a bundle of ES from each ES group, varying from five up to 21 ES. The studies of the ES supply-demand side have a well-defined trend for assessing mainly ES supply (59%). The lack of research on ES demand, as well as studies assessing both supply and demand, is evidence knowledge gap (Fig. 2C). Biophysical methods are the most used ones for ES assessment (62%), while the social and economic have a similar presence in the studied papers (19% each) (Fig. 2D).

Geographical scope

The analyses showed that 86 (70%) of the publications have clearly defined and spatially outlined case studies while the rest 37 (30%) had no case study. The case studies are predominantly in Bulgaria but there are also several case studies in other countries such as Germany, USA, Finland, North Macedonia etc. (Fig. 2G). The scale of the case studies is predominantly local with 57 of the publications dealing with such cases. Regional and national level case studies number 12 and 14 respectively. There are only two multiscale studies in Bulgaria combining local–regional and local–national scales, and one international study combining all three scales. Apart from the multiscale studies, six local studies cover between two and four separate sites. Thus, the whole number of studied sites in the above-mentioned 86 publications became 109. 97 of them are in Bulgaria and the rest are located in ten other countries. The local

scale case studies in Bulgaria form several visible clusters that can be divided into three groups (Fig. 2H). The first one contains only one but the far biggest cluster located in the area around the Central Balkan National Park combining 19 cases. The second group contains three moderate size clusters combining between nine and 12 cases. The third group contains 12 clusters combining between two and five cases. The highest number (20) of local-scale case studies were municipalities. Natural objects were chosen as research objects in 18 case studies, which comprise seven mountain areas, four lakes, and six other natural objects. River basins as a spatial unit were used in 16 case studies, while urban areas (city/town) were used in 13 cases.

The publications with ES maps were 58 which comprise almost half (47%) of the reviewed sources. The figures in the research paper are slightly different with 59% of the papers presenting some kind of ES maps and 41% without ES maps. The respective distribution between the main groups of ES assessment methods showed a significant difference in the number of publications containing ES maps (Fig. 2E). The studies that use biophysical methods are predominantly with ES maps, which are presented very well in the research papers with 78% share of the publications containing ES maps (Figs 2I, 2J). The respective distribution for the publications applying social methods is almost equal but for research papers, the share of no ES maps publications is even higher which is opposite to the general trend defined for the total dataset. The ES maps in the publications applying economic methods are even lower with only one-third presenting by maps the results of their studies.

The ratio between publications with and without maps changed during the different periods. During the first period, the publications with no maps are predominant (83%). During the second period, the predominance is not so pronounced (62%). The third period is characterized by balance (52% to 48%) while during the fourth period, the share of no ES maps publications is no more predominant (47%).

The ES mapping methods applied in the studies were evenly distributed among the publications with a slight dominance of the matrix approach, expert score, and spatial proxy methods (Fig. 2F). The matrix approach and the spatial proxy methods were the most popular for the total dataset, while for the research papers, the expert score replaced the spatial proxy methods as the second best used. Modeling methods are also well represented in the total dataset but their use in the research papers rapidly declines. The studies with ecosystem types and ecosystem condition maps comprise about 25% of the mapping publications and such maps are presented predominantly in research papers.

Discussion

Lessons learned

The case studies used in ES assessment reveal three important topics for discussion. Firstly, the municipality and river basin appear as the most appropriate spatial units that ensure a clear extent and policy relevance. Both of them have clearly defined boundaries (administrative or natural) which ensure a strong rationale for the study and comparability to other studies. The river basin is the main spatial unit in water management, while the municipalities are the main ob-

jects of the local government. Therefore, both units ensure a direct relationship between research findings and decision-making. Secondly, natural objects such as mountains, lakes, and karst areas appear as quite popular case studies. However, they vary a lot in the dimensions and the definitions of their extent. Their boundaries are mostly case-specific and hardly correspond in extent to other studies. Such studies can have high research contributions but their uptake necessitates further work to be adjusted to the policy needs and decision-making. The methodological frameworks for mapping and assessment of ES give some guidance but their development at regional and local level is still in the beginning (Nedkov et al. 2021). Thirdly, the nature protection areas are rarely selected as case studies which is an obvious research gap. The recent studies on the provision of ES by the nature heritage in Rila mountain (Silvestriev et al. 2021) and the habitat maintenance ES in mountain-protected areas (Borisova et al. 2023) are positive examples but much more research in this direction is needed.

The predominance of local case studies and the limited number of multiscale studies show that the researchers prefer to focus on relatively small areas that can easily be provided by data and expertise. The efforts during the national mapping under the MAES process resulted mainly in methodological publication which applicability in the real research is still to be proved. The limited number of research papers in high-ranked journals presenting results from the national mapping (the only exception is Nedkov et al. 2017) is clear evidence of the need for further development of these methodologies. Therefore, despite the undoubtable achievements during these 20 years ES research in the country is still a relatively new field that is still to be fully come of age.

The share of publications containing specific geographical elements in the form of ES maps and spatial analyses has gradually risen from the first to the fourth period. Two main reasons are behind this tendency. The first one came from the MAES process that stimulated the mapping studies all over Europe and the Bulgarian researchers were among the most active which was proven by the results in the MAES barometer indicating the leading role of the country (Burkhard et al. 2018a). The second one is the development of the spatial aspects in ES research inspired by the activities in the Ecosystem Services Partnership (ESP) working group on mapping and modeling of ES where the Bulgarian geographers were also present (Burkhard et al. 2013; Crossman et al. 2013). However, even in the last period the share of the publications without maps remains high (47%) which is not reasonable for the geographical community. Therefore, more attention on the spatial aspect of the ES research by the Bulgarian geographers is needed.

The disproportion between the ES supply and demand studies is another important finding of our review. Although it corresponds to the general trend defined in international studies (Campagne et al. 2020) the lack of studies focused on the demand side, especially on the supply/demand balance, remains a significant research gap. In the Bulgarian case, this disproportion corresponds also to the predominance of biophysical methods at the expense of social and economic. The latter (especially the social) are more appropriate for the studies on ES demand. This is a clear message on the need for the development and application of more social methods in ES research by geographers. This need is even more pronounced taking into account the existence of the social-economic branch of geography.

Future directions

The findings from the review allow us to identify both achievements and research gaps in the ES studies conducted by Bulgarian geographers. This enables us to define the main research priorities of the coming years which can trace the future directions of ES research in the country. The main priority should be directed towards the development of the spatial aspects in the methodological frameworks for mapping and assessment of ES. This includes problems such as the identification of relevant scales for the implementation of particular indicators and the methods for their quantification, a precise statement on the spatial scale and extent of the ecosystem services assessment, a clearer and more precise definition of the spatial units used in the assessment, application of more spatially explicit indicators including those for assessment of ecosystem condition, include a sensitivity analysis to understand the effects of varying spatial resolutions, include potential trade-offs between different spatial scales and their implications on ecosystem service, etc. The achievement of such objectives will ensure both scientifically robust mapping of ES and relevance to policy and decision-making.

The next priority should be in the use of GIS-based tools for mapping ES which are developing very fast in recent years. Particularly the development of specialized ES tools such as InVEST, ESTIMAP, and ARIES ensures a variety of spatial options for a wider range of individual ES (Nedkov 2018). The tendency for models' integration in the ES research is another aspect that can be used also to incorporate more social and economic methods and further development to ES demand and supply demand studies which would contribute to overcoming this research gap. Another important priority should be the improvement of the publication quality and increase of the papers published in highly rated indexed journals. This could be achieved by focusing on internationally recognized topics, application of modern methods, and abandonment of the grey literature as a publication option.

Conclusions

The development of ES research in Bulgaria during the last 20 years has led to the accumulation of significant scientific production in the form of publications that cover almost all important aspects of the ES concept. Bulgarian geographers have the leading role in this development, especially in the mapping of ES that covers all spatial aspects of the ES research including spatial data acquisition, GIS analyses, and preparation of maps. The variety of publications reviewed in this study reveals significant achievements both in the geographical extent and the methodological robustness of the research. The case studies of the reviewed publications cover 10 countries in three different continents. Even more pronounced is the significance of international collaboration given the scope of the authors who come from 21 different countries. The studies cover a variety of ES from all three main groups of services and apply a variety of methods with a focus on the biophysical. The contributions to the mapping methods are also important with significant achievements in the development of the matrix approach, spatial proxy, and modeling methods. However, despite the undoubted achievements during these 20 years, ES research in the country is still a relatively new field, and there are still many gaps to be filled.

The perspectives of the ES research in Bulgaria, and the geographers in particular, are related to the development of several priorities. The development of the spatial aspects in the methodological frameworks for mapping and assessment of ES is necessary to ensure scientifically robust mapping of ES and relevance to policy and decision-making. Better use of GIS-based tools for mapping ES alongside models' integration is needed to ensure a variety of spatial options for a wider range of individual ES and incorporation of more social and economic methods for further development of ES demand and supply/demand balance studies. The improvement of the publication's quality and increase of the papers published in highly rated indexed journals will ensure better visibility and impact of the research.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SN, HP, VS. Data curation: HP, SN, IA, VS. Formal analysis: SN, VS, HP, YY, IA. Funding acquisition: HP, SN. Investigation: YY, HP, VS, IA. Methodology: VS, SN. Resources: HP, VS, SN. Supervision: SN. Validation: IA, HP, VS. Visualization: SN, HP. Writing - original draft: SN, VS, HP. Writing - review and editing: SN, VS, HP.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Database of the reviewed publications on ecosystem services

Authors: Stoyan Nedkov, Vanya Stoycheva, Hristina Prodanova, Ivaylo Ananiev, Yordan Yordanov

Data type: xlsx

Explanation note: Literature database on ecosystem services containing 123 records of publications reviewed in this work.

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Research Article

Possible effects of shipping routes on coral reef degradation and diversity in Karimunjawa Marine National Park, Java Sea

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Abstract

The Karimunjawa Marine National Park, situated in the Java Sea, Indonesia, is renowned for its rich biodiversity and lively coral reefs. However, amidst the backdrop of this natural beauty, concerns have been raised regarding the potential impacts of shipping activities on the health and diversity of these fragile ecosystems. The increase in maritime traffic, including commercial vessels, tourist boats, and fishing vessels, traversing through the Karimunjawa Marine National Park, raises significant environmental concerns. The movement of these vessels, especially along specific shipping routes, has the potential to disturb and damage coral reefs through various mechanisms. Hence, this study aimed to investigate the possible impacts of shipping routes on the coral abundance and diversity and the coral health in the Karimunjawa Islands, Java Sea, Indonesia.

This study categorized ship routes into West Route, East Route, and Non-Route and assessed coral health and diversity across 15 islands. Key metrics analyzed included coral disease prevalence, coral cover, diversity index, species richness, relative abundance, and evenness, using 15 × 2 m belt transects at 3 and 8 m depths with three repetitions each.

Statistical analysis revealed significant differences in coral abundance and species richness among ship-route groups, but no significant depth-related differences. These results suggest that while shipping routes affect certain aspects of coral health and diversity, other factors may be more influential in shaping coral disease prevalence and overall diversity in Karimunjawa reefs.

Key words: Biodiversity, coral reefs, degradation, Karimunjawa, shipping route

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Introduction

More than fifty thousand ships move around the world every year, lugging millions of containers (Sinay 2022). As global trade continues to expand, maritime shipping is poised for rapid growth. The primary shipping routes are expected to experience heightened congestion and a potentially unprecedented increase in traffic in the coming years (IMO 2019). As a result, all stakeholders in the maritime sector explore more effective methods for managing ships, trade routes, transit times, cargo ports, and containers (Lind et al. 2016).

In the context of environmental concerns, cruise ships are frequently observed discharging substantial amounts of sewage, food waste, and oily bilge water, containing insoluble particles, into the ocean. Byrnes and Dunn (2020) reported addressing pollution discharge from cruise ships and highlighted that the decomposition of these wastes and ocean dumping contributes to water acidification and a significant reduction in oxygen levels. This, in turn, leads to the proliferation of toxic algae blooms, posing a substantial threat to coral reefs (Lloret et al. 2021). Additionally, with the cruise industry's shift towards increasingly larger ships, tourist destinations face the challenge of adapting to the latest generation of ships. These ships require deeper and wider shipping lanes, which presents a dilemma for many tourist destinations. The location of shipping lanes close to shore, through which cruise ships pass, has significant environmental and economic impacts. Larger tract dredging can damage habitat, while ship traffic can result in sediment buildup that has the potential to cover sensitive habitats such as coral reefs and seagrass beds. Additionally, dredging costs tend to vary spatially (Sinay 2022).

Coral reefs are one of the seabed ecosystems that are very beneficial for human life because of their enchanting aesthetic beauty as a recreation area, their ecological function as a coastal barrier from waves and erosion, and because they are a fish habitat for spawning, rearing, and fishing grounds for various types of fish and other marine organisms, as well as providing active compounds for various pharmaceutical and medicinal purposes (Hoegh-Guldberg 2011; Gracia et al. 2018; Reguero et al. 2018; Zhao et al. 2019).

Shipping routes are the navigating lanes, both natural and man-made, in wide waterways used by large vessels to connect major ports and carry cargo (Pirotta et al. 2019; Sinay 2022). These routes allow efficient, safe, and economical transportation of goods while offering the shortest sailing times. Karimunjawa Marine National Park (KMNP) which is composed of 27 islands is one of seven marine national parks in Indonesia. This archipelago is located about 100 km north of Semarang, Central Java. The richness of coral and coral fish species in Karimunjawa consists of about 100 species of corals documented from more than 50 genera and approximately 250 species of fish (Edinger et al. 2000). Consequently, tourism in KMNP has increased remarkably over the last decade. Since the early 2010s, cruise ship travel has experienced nearly continuous growth, exceeding 25% annually (Sabdono 2019b). Associated with the increasing tourism boom has been an increase in the total number of cruise ships and the routes of ship traffic (Sabdono et al. 2019a). This situation in the vicinity of coral reefs is not always benevolent and corals are subject to continuous stress. The negative impacts can include coral degradation and loss of marine life.

The increasing volume of ship traffic, water pollution from ships, and increased direct physical pressure on coral reefs due to ship groundings and anchoring activities are factors that allow damage to coral reefs (Walker et al. 2019; Zhang et al. 2019; Kostianaia et al. 2020; Satya et al. 2023). Today, the study on the impact of increasing shipping routes on the existence and health of coral reefs is limited. Few previous studies reported that damage to coral reef ecosystems has long-term impacts that cause reduced marine biodiversity, decreased fish populations, and loss of habitat for marine organisms (Veron

et al. 2009; Eddy et al. 2021). Increasing human activity around KMNP due to the ever more crowded shipping traffic traversed by commercial ships, tourist ships, and local transport has become an important part of the economic life of the community and the environment in Karimunjawa. This is because the impact of increasing shipping routes on the existence and health of coral reefs is not yet fully understood. Hence in this study, the possible effects of shipping routes on coral reef degradation and diversity were investigated.

Materials and methods

Study area

The study area encompasses Karimunjawa Marine National Park, located in the Java Sea, Indonesia. The park comprises a group of 27 islands and surrounding marine areas, renowned for its diverse marine ecosystems including coral reefs, seagrass beds, and mangroves. Based on the tourism destinations and geographic locations, the ship routes were grouped into 3 categories (west, east, and non-routes). The West Route refers to regular arrival in those islands that are situated in the western part of Karimun island including Menjangan Kecil, Burung, Geleang, Cemara Besar, and Cemara Kecil islands. The East Route refers to regular arrival in those islands that are situated in the western part of Karimun island including Menjangan Besar, Gosong Saloka, Kecil, Tengah, and Sintok islands. Non-route refers to irregular arrivals of tourism in those islands including Sambangan, Seruni, Genting, Cendekia, and Menyawakan islands (Fig. 1).

Line Intercept Transects (LIT) and Belt transect

Three belt transects, with 2 × 15 meters (30 m^2) in size, were randomly established on each of the 15 islands. These transects were located at depths of 3 meters and 8 meters. The LIT *in situ* was established in the middle of the belt transect. Hence the total number of LIT and belt transects established is 90 transects. Divers identified all benthic categories and estimated the percent hard coral cover (scleractinian corals) by dividing the total length of occurrence of hard corals by the total length of the transect. Within each belt transect, all scleractinian coral colonies exceeding 5 centimeters in diameter were recorded based on their genus. Corals were further classified as either healthy or diseased (Figs 4, 5).

Health and diseased corals

Diseased colonies exhibited manifestations such as changes in color or structure, tissue loss, necrosis, and abnormal growth. Conversely, healthy colonies displayed no signs of lesions or other indications of compromised health. The prevalence of coral diseases was determined for each belt transect by dividing the number of colonies showing signs of each disease by the total number of colonies present in each transect. The other variables measured for reef degradation were % coral cover, H-index (H'), species richness (SR), and evenness (E).

Figure 1. Route ships of Karimunjawa (Note- red: east shipping route; green: west shipping route; blue: non-route).

Data analyses

Parameters observed included coral damage, underwater photography, oceanographic parameters, type and number of corals per transect, number (healthy/sick coral), and percent coral cover. To determine the prevalence and coral cover, the formula used was adopted from Raymundo et al. 2008)):

$$Percent\ Prevalence = \frac{\sum Diseased\ coral\ colonies}{\sum Total\ colonies} \times 100 \quad (1)$$

$$Percent\ Coverage = \frac{Lifeform\ coverage\ length}{\sum Total\ transect\ length} \times 100 \quad (2)$$

The Shannon-Weaver index was used to measure the H', GR, and E of coral reefs: H'= $\sum_{i=1}^{S}$ Pi *lnPi* (3); E = H'/ln*S* (4)
where: *pi* = relative abundance, S = species richness; H'= diversity index, and E-evenness.

Analyses of Variance-one way were used to explore the impact of shipping routes on disease prevalence, coral cover, and biological diversity of corals among islands by using SPSS-22 software.

Results

Impact of shipping-route traffic on the prevalence of coral diseases

The mean prevalences of coral disease in the west, east, and non-route are 7.5, 5.8, and 5.2 percent, respectively (Fig. 2). Statistically, there was no significant difference among routes in prevalence. White Syndrome (WS), White Blotch

Figure 2. Coral diseases prevalence of Karimunjawa on three shipping routes (Note: the same word characters means no significant differences)

(WB), Black Band Disease (BBD), Pigmentation Response (PR), White Plague (WP), Ulcerative White Spot (UWS), Black Disease (BD), Growth Anomaly (GA) and Skeleton Erode (SE) were detected in all routes (Fig. 3).

Impact of shipping-line activity on % coral cover

Fig. 4 demonstrated that the % coral cover in the west, east, and non-cruise lines were 68.14, 64.9, and 61.18 percent, respectively. Statistically, there was no significant difference among routes in % coral cover.

Impact of shipping-route activity on abundance and diversity

Statistical analysis showed that the impact of shipping-line activity on coral abundance and species richness is significantly different (Table 1, Fig. 6.). However, there are no significant differences in terms of diversity and evenness. Post-hoc test $LSD_{5\%}$ revealed that the coral abundance and species richness on the west route were significantly different to the east route, and no significant difference to the non-shipping route.

Depth effect on the prevalence of coral diseases, % coral cover, abundance, and diversity

The effect of shipping-route activity in response to increases in depth, specifically, whether relationships exist between depth and prevalence of coral diseases, coral cover, and diversity was presented in Table 2.

Table 1. The effect of shipping route on the Diversity, Abundance, Species Richness, and Evenness of corals.

Shipping route	Diversity (H')	Relative Abundance (RA)	Species Richness (SR)	Evenness (E)
West route	2.18 ± 0.7[a]	36.6% [a]	17.86 ± 0.65[a]	0.76 ± 0.02[a]
East route	2.02 ± 0.9[a]	26.5% [b]	14.04 ± 1.01[b]	0.78 ± 0.02[a]
Non-route	2.2- ± 0.8[a]	37.0% [a]	17.12 ± 0.61[a]	0.79 ± 0.01[a]

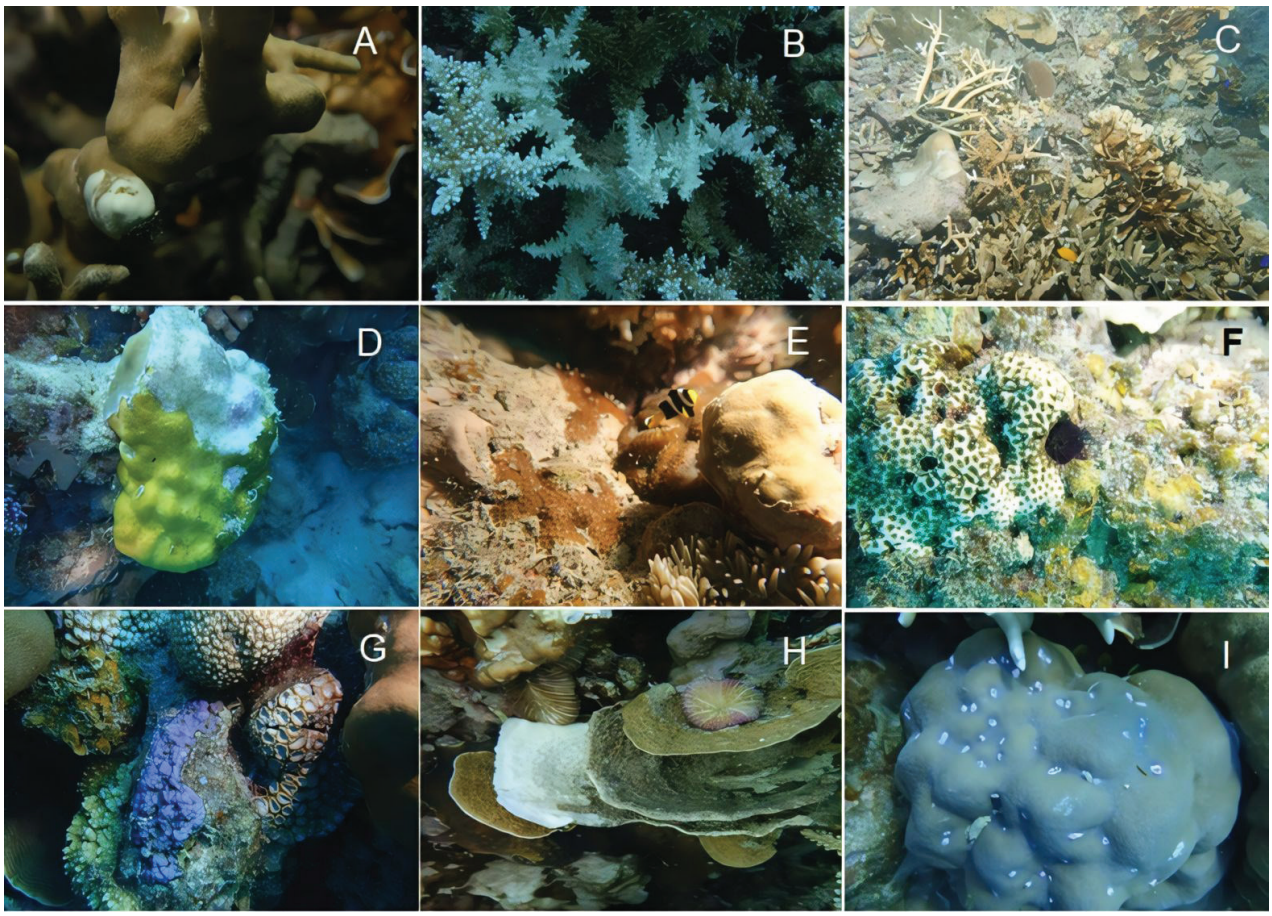

Figure 3. Coral diseases prevalence of Karimunjawa [Note: **A** White Blotch (WB) **B** White Syndrome (WS) **C** Sedimental Eroding (SE) **D** White Plague (WP) **E** Skeletal Erode (SE) **F** Bleaching Syndrome (BS) **G** Growth Anomaly (GA), **H** Black Band Disease (BBD), and **I** Pigmentation Response (PR)].

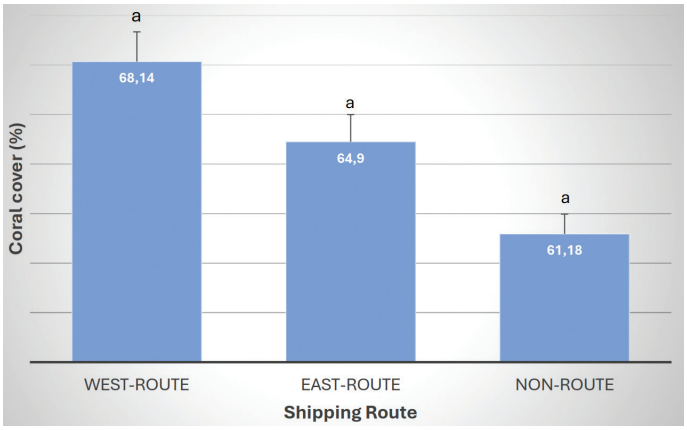

Figure 4. Percent coral cover of line-transect on shipping route.

Table 2. The effects of shipping-route activity on the prevalence of coral diseases, % coral cover, and abundance and diversity on different depths.

No.	Depth	Prevalence (%)	Coral Cover (%)	H-Index	Species Richness	Evenness
1.	3 m	6.18 ± .94 a	67.07 ± 2.42 a	2.15 ± .06 a	16.22 ± .59 a	.79 ± .01 a
2.	8 m	6.43 ± .73 a	62.85 ± 2.89 a	2.13 ± .04 a	17.07 ± .72 a	.76 ± .01 a

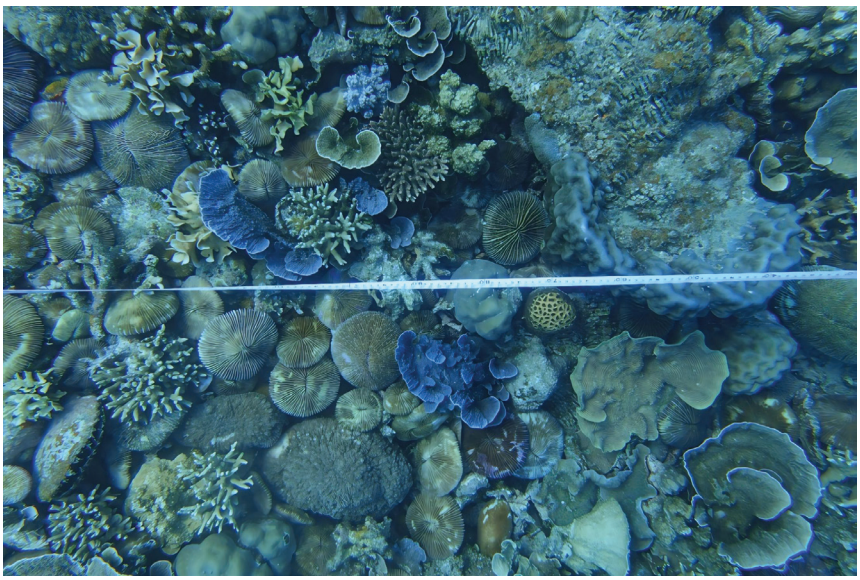

Figure 5. Line intercept transect (LIT).

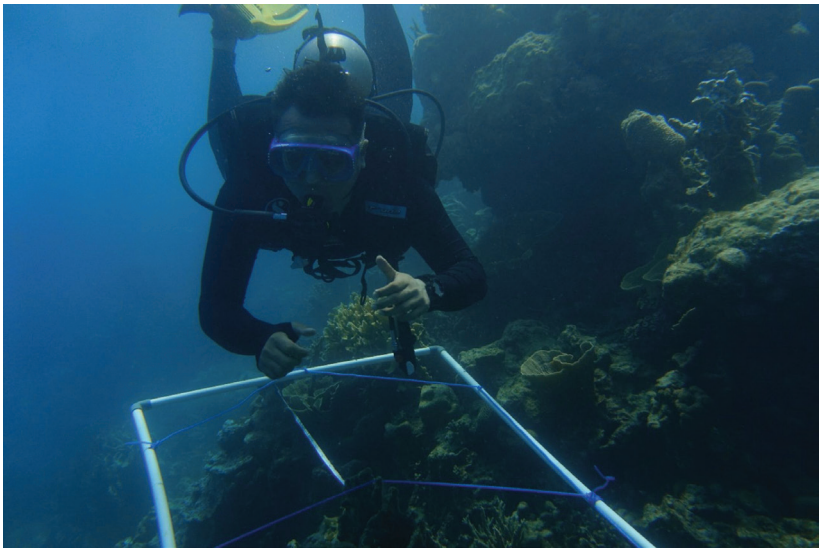

Figure 6. Impact of shipping-line activity on abundance and diversity.

Discussion

The prevalence rate of the disease is the proportion of the coral population with that disease at a point in time. It indicates how many of the corals are sick. Fig. 2 showed that statistically there was no significant difference among routes in prevalence, even though there were 9 different coral diseases detected on each shipping route. It indicates a widespread issue affecting coral reefs in these areas. The prevalence of coral disease in the west of the shipping route tends to be high and the non-cruise lines are the lowest one. Some previous studies of Karimunjawa coral diseases reported that coral disease prevalence in Menjangan Besar ranged between 10.6 and 43.61% and found BBD, BrBD, UWP, WS, YBD, PR, WP, and WBD (Nursalim et al. 2022). A swift evaluation of coral disease across three islands—Genting, Seruni, and Sambangan in the Karimunjawa revealed that human activities, specifically coastal settlement, and floating cage mariculture, were responsible for the onset of coral disease. However, there

was no notable difference in coral disease prevalence among areas frequented by the shipping route and those that were not frequented (Sabdono et al. 2019[a]; 2019[b]). Compared to other studies in different regions of Indonesia, such as the coral disease prevalence in Panjang Island (Sabdono et al. 2014), the prevalence of coral diseases in the 15 islands of Karimunjawa in this study is lower.

The discharge of pollution from cruise ships, including decaying waste and ocean dumping, leads to increased acidification of the waters and a significant reduction in oxygen levels. This, in turn, promotes the growth of harmful algae blooms, which pose a serious threat to coral reefs (EPA 2008). Even the shipping route has no significant difference in the percentage of coral cover in this study, however, shipping lines have detrimental effects on coral reef ecosystems in Southeast Florida (Walker et al. 2012). Additionally, the Great Barrier Reefs (GBR) have been changed by human activities, and live coral cover has declined overall (Sweatman et al. 2011). In this study, the west route is the most visited by tourists, vessels, boats, and ships, yet it has the highest percentage of coral cover. Based on the data available, the differences observed in coral cover between the west, east, and non-cruise lines are not significant enough to conclude that one category has significantly more or less coral cover compared to the others. However, through knowing that there were no significant differences between the pathways, environmental management can detail conservation efforts evenly across the region, without focusing too much on one pathway. It is important to continuously monitor and analyze data to support conservation policies and sustainable environmental management. Further research may be needed to understand the factors behind the differences in % coral covers in each pathway.

Table 2 showed that there were no significant differences between depths of 3 m and 8 m in prevalence of coral diseases, % coral cover, abundance, species richness, and diversity. However, the % coral cover tends to decrease with depth, likely due to reduced light. Meanwhile, reduced light exposure at greater depths can constrain the photosynthetic activities of zooxanthellae (Kahng et al. 2019; López-Londoño et al. 2024). Additionally, very low water flow may result in the formation of boundary layers around the coral surface, hindering nutrient absorption and consequently suppressing coral respiration and growth (Nelson and Altieri 2019; Hughes et al. 2020). It is important to note that each location has unique conditions, and human impacts on coral reefs are highly dependent on the local context. In addition, effective conservation and management efforts can help reduce the negative impacts of human activities on coral reefs at various depths.

Conclusion

In conclusion, the management of the KMNP area is relatively sufficient according to the currently available data, and even though the shipping lanes are quite congested, coral cover is still maintained. The most important result was that between the non-route route and the west route, there were no significant differences in coral abundance and species richness. However, it is necessary to consider again the limited data obtained and the limited research time available, so that it cannot capture long-term trends or changes in coral cover. So, it is necessary to carry out further research to find out the specific causes of differences in coral abundance and species richness in the context of shipping route activities.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

A. Sabdono, conceived and designed the study methodology, analyzed and interpreted the data, drafted the manuscript, visualization, supervision, project administration, and funding acquisition. D.P. Wijayanti, conceived and designed the study methodology, analyzed and interpreted the data, and wrote—reviewed, and edited the manuscript. M. Helmi, analyzed and interpreted the data, writing—reviewing, and editing. E.D. Satya analyzed and interpreted the data, and data curation.

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Data availability

All of the data that support the findings of this study are available in the main text. The data underpinning the analysis reported in this paper are deposited at “Data repository” at https://doi.org/10.3897/biorisk.xx.xxxxxx.

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Supplementary material 1

Coral abundance data

Authors: Agus Sabdono, Diah Permata Wijayanti, Muhammad Hilmi, Eridhani Dharma Satya

Data type: xlsx

Explanation note: Coral abundance of the West Route, East Route, and Non-Route including a total of 15 islands were recorded as raw data to calculate H indexes, evenness and relative abundance.

Link: https://doi.org/10.3897/biorisk.22.124897.suppl1

Supplementary material 2

Coral reef degradation and diversity in Karimunjawa

Authors: Agus Sabdono, Diah Permata Wijayanti, Muhammad Hilmi, Eridhani Dharma Satya

Data type: docx

Explanation note: This data is summary of data analyzed result from coral abundance and coral cover raw data.

Link: https://doi.org/10.3897/biorisk.22.124897.suppl2