

UNIVERSIDADE CATÓLICA DOM BOSCO  
PROGRAMA DE PÓS-GRADUAÇÃO *STRICTO SENSU* EM  
CIÊNCIAS AMBIENTAIS E SUSTENTABILIDADE  
AGROPECUÁRIA

Avaliação Da Presença De Microplásticos Em Trato  
Gastrointestinal e Pulmão De Gambás (*Didelphis albiventris*)  
Residentes Em Fragmentos Florestais De Campo Grande/ MS

Autora: Fernanda Mayara Gauto de Melo  
Orientadora: Dra. Gisele Braziliano de Andrade  
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Co-orientadora: Dra. Nayara Yoshie Sano

Campo Grande  
Mato Grosso do Sul  
Fevereiro – 2025

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**Aprovado em 11 de fevereiro de 2025.**

A presente defesa foi realizada na Sala D-107, Bloco L, setor Biosáude da Universidade Católica Dom Bosco.

Eu, Gisele Braziliano de Andrade, como presidente da banca, assinei a folha de aprovação com o consentimento de todos os membros.



Prof. Dr. Gisele Braziliano de Andrade – PPGAGRO

Prof. Dr. Nayara Yoshie Sano - PPGAGRO

Prof. Dr. Priscila Sabioni Cavalheri - UCDB

Prof. Dr. Wesley Arruda Gimenes Nantes – PPGAGRO

Profa. Dra. Magyda Arabia Araji Dahroug - UCDB

*“Mas nos gloriamos até das tribulações. Pois sabemos que a tribulação produz a paciência, a paciência prova a fidelidade e a fidelidade, comprovada, produz a esperança. E a esperança não engana.”*

*Romanos 5*

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## LISTA DE ABREVIATURAS E SIGLAS

|        |                                                       |
|--------|-------------------------------------------------------|
| MPs    | Microplásticos                                        |
| PUR    | Poliuretano,                                          |
| PE     | Polietileno                                           |
| PP     | Polipropileno                                         |
| PVC    | Policloreto de vinila                                 |
| PET    | Tereftalato de polietileno                            |
| PS     | Poliestireno                                          |
| EPS    | Poliestireno expandido                                |
| PA     | Poliamidas                                            |
| PC     | Policarbonato                                         |
| TPE    | Elastómeros termoplásticos                            |
| CEUA   | Comitê de Ética no Uso de Animais                     |
| SISBIO | Sistema de Autorização e Informação em Biodiversidade |
| CC     | Condição Corpórea                                     |
| GLM    | Generalized Linear Models                             |
| ABREMA | Associação Brasileira de Resíduos e Meio Ambiente     |
| RSU    | Resíduos Sólidos Urbano                               |
| POPs   | Poluentes Orgânicos Resistentes                       |

## RESUMO

Entre as diversas espécies que compõem a fauna dos fragmentos florestais de Campo Grande, MS, o gambá-de-orelha-branca (*Didelphis albiventris*) é um dos principais representantes. Coleta de dados no entorno dessas áreas revelou diversas atividades com potencial poluidor e de contaminação. Microplásticos (MPs) são poluentes que, quando presentes no ambiente, podem provocar efeitos adversos nos seres vivos em diferentes níveis. Este trabalho teve como objetivo investigar a presença de microplásticos em gambás-de-orelha-branca e avaliar seus efeitos teciduais. As coletas foram realizadas de abril a dezembro de 2023, resultando na captura de 22 animais em 6 regiões florestais na região urbana de Campo Grande, MS. Foram coletados dados sobre os animais como o peso, o estado reprodutivo, o sexo e o comprimento cabeça-corpo. Estômago, íleo e pulmão foram coletados durante as necropsias para as análises laboratoriais de digestão alcalina com hidróxido de potássio (KOH) das amostras, e histopatologia. As amostras digeridas passaram por filtragem à vácuo em papel filtro de fibra de vidro e levados à estufa para secagem. Os filtros secos foram avaliados em microscópio ótico. Foi detectado um total de 270 microplásticos nos 22 animais examinados neste estudo. O íleo foi o órgão que apresentou maior quantidade de MPs (n = 141), seguido de estômago (n = 86) e pulmão (n = 43). As fibras foram o tipo de MP mais encontrado, com 185 peças, seguido de fragmentos, espuma e filme. A azul (n = 140) foi a coloração mais predominante, acompanhada de transparente (n = 43), preto (n = 38), âmbar (n = 20), vermelho (n = 16), laranja (n = 5), roxo (n = 2), cinza (n = 2), branco (n = 1), verde (n = 1), colorido (n = 1) e marrom (n = 1). Em relação à constituição química, os mais encontrados foram: polietileno tereftalato (PET), a crilonitrila butadieno estireno (ABS) e o policloreto de vinila (PVC). Embora não tenha sido observado efeitos de MPs nos tecidos avaliados e na condição corpórea dos gambás-de-orelha-branca, conclui-se que devido ao fato de todos os animais apresentarem MPs, sugerimos que esses animais podem atuar como sentinelas da contaminação ambiental.

Palavras-chave: Bioindicador, condição corpórea, contaminante, histopatologia, polietileno.

## ABSTRACT

Among the various species that make up the fauna of the forest fragments of Campo Grande, MS, the white-eared opossum (*Didelphis albiventris*) is one of the main representatives. Data collection in the surroundings of these areas revealed several activities with potential for pollution and contamination. Microplastics (MPs) are pollutants that, when present in the environment, can cause adverse effects on living beings at different levels. This study aimed to investigate the presence of microplastics in white-eared opossums and evaluate their tissue effects. Collections were carried out from April to December 2023, resulting in the capture of 22 animals in 6 forest regions in the urban region of Campo Grande, MS. Data on the animals such as weight, reproductive status, sex, and head-body length were collected. Stomach, ileum and lung were collected during necropsies for laboratory analysis of alkaline digestion with potassium hydroxide (KOH) of the samples, and histopathology. The digested samples were vacuum filtered through glass fiber filter paper and taken to the oven to dry. The dried filters were evaluated under an optical microscope. A total of 270 microplastics were detected in the 22 animals examined in this study. The ileum was the organ that presented the highest amount of MPs (n = 141), followed by stomach (n = 86) and lung (n = 43). Fibers were the most common type of MP, with 185 pieces, followed by fragments, foam and film. Blue (n = 140) was the most predominant coloration, followed by transparent (n = 43), black (n = 38), amber (n = 20), red (n = 16), orange (n = 5), purple (n = 2), gray (n = 2), white (n = 1), green (n = 1), colored (n = 1) and brown (n = 1). Regarding the chemical composition, the most found were: polyethylene terephthalate (PET), acrylonitrile butadiene styrene (ABS) and polyvinyl chloride (PVC). Although no effects of MPs were observed in the evaluated tissues and in the body condition of the white-eared opossums, it is concluded that due to the fact that all animals presented MPs, we suggest that these animals can act as sentinels of environmental contamination.

Keywords: Bioindicator, body condition, contaminant, histopathology, polyethylene.

## 1. INTRODUÇÃO

A elevada produção e consumo de plásticos a nível mundial, associada à sua eliminação inadequada e lenta degradação, resulta na presença onipresente e duradoura de microplásticos (MPs) no meio ambiente (Barnes *et al.*, 2009; Kubowicz; Booth, 2017; PLASTICSEUROPE, 2022). Os MPs são vetores de poluentes (Hartmann *et al.*, 2017) e de microrganismos patogênicos presentes no ambiente (Foulon *et al.*, 2016), bem como seus próprios aditivos químicos, que são adicionados durante a fabricação (Hahladakis *et al.*, 2018).

De acordo com Talbot e Chang (2022), a abundância global de MPs em ambientes aquáticos tende a ser diretamente afetada pela cobertura urbana do solo que detém atualmente 80% dos resíduos plásticos, alta densidade populacional e proximidade de estação de tratamento de águas residuais. Do mesmo modo, grandes bacias hidrográficas, ricas em população humana, transportam a maioria da carga total de detritos plásticos fluviais (Schmidt *et al.*, 2017).

Apesar de amplamente abordado na atualidade, a presença de partículas plásticas em escalas milimétricas já havia sido relatada em redes de plânctons em águas costeiras na região da Nova Inglaterra, Estados Unidos (Carpenter *et al.*, 1972). Essas micropartículas podem ser classificadas em primárias e secundárias, de acordo com sua origem (Caixeta; Morais, 2022). Os MPs primários são aqueles produzidos propositalmente até 5mm e são lançados no ambiente neste formato (Fendall; Sewell, 2009; Browne *et al.*, 2011). Por sua vez, os secundários são aqueles que resultam da fragmentação de resíduos maiores (Barnes *et al.*, 2009).

A presença destes contaminantes foi descrita em água doce, água do mar, solo, sedimento, ar e até na biota, de um modo geral (Wang *et al.*, 2021; Nunes *et al.*, 2023; Ding *et al.*, 2021; Uddin *et al.*, 2021; Syafei *et al.*, 2019; Prata *et al.*, 2021). Estudos relatam a presença de MPs nos sistemas digestivos e brânquias de peixes (Neves *et al.*, 2015; Da Silva *et al.*, 2020), mexilhões (Reguera *et al.*, 2019), aves (Carlin *et al.*, 2020) e mamíferos marinhos (Nelms *et al.*, 2019). Em seres humanos foram

encontrados em pulmão (Amato-Lourenço *et al.*, 2021), fígado (Hovartits *et al.*, 2022) e placenta (Ragusa *et al.*, 2021) sugerindo translocação (Ma *et al.*, 2021).

Seus efeitos adversos podem resultar em dano tecidual devido à abrasão na mucosa do sistema digestório com o detrito plástico, causando interrupção da absorção alimentar (Prata *et al.*, 2021), respostas inflamatórias, alterações no metabolismo (Lwanga *et al.*, 2016; Song *et al.*, 2019), deficiência reprodutiva, insuficiência nos níveis de energia e interação com outros contaminantes (Prata *et al.*, 2021).

Embora diversos estudos tenham sido realizados (Kiliç *et al.*, 2022; Nessi *et al.*, 2022; Li *et al.*, 2023), ainda existem muitas incertezas em relação ao real impacto da poluição por micropartículas plásticas na vida dos seres humanos e animais que vivem em áreas antropizadas e os efeitos patológicos dos mesmos. A grande maioria dos estudos vêm sendo realizados em pequena escala, em condições de laboratório e com curto tempo de exposição (Avio *et al.*, 2015; Oliveira *et al.*, 2013). Porém, para se entender, de fato, os efeitos dos MPs são necessárias avaliações também em condições reais da ocorrência desses poluentes nos tecidos dos mamíferos terrestres que habitam fragmentos florestais em centros urbanos.

## 2. OBJETIVOS

### 2.1 OBJETIVO GERAL

Investigar a presença de microplásticos em gambás (*Didelphis albiventris*) que habitam fragmentos florestais do município de Campo Grande – MS.

### 2.2 OBJETIVOS ESPECÍFICOS

- Identificar a presença e quantificar os microplásticos em conteúdo gástrico, mucosa de estômago e íleo e pulmão de gambás (*Didelphis albiventris*).
- Tipificar os microplásticos encontrados nos gambás-de-orelha-branca; de acordo com as características morfológicas (cor e formato) e composição química
- Verificar se a presença de microplásticos no estômago, íleo e pulmão dos gambás MPs afeta estes tecidos e a condição corpórea.

### 3. REVISÃO BIBLIOGRÁFICA

#### 3.1 Plásticos e microplásticos

Os plásticos já fazem parte da vida cotidiana de bilhões de pessoas em todo o mundo, devido à sua versatilidade, transparência, durabilidade, peso reduzido e baixo custo, tornou-se indispensável para a humanidade (PLASTICSEUROPE, 2018). A partir do final da década de 1950, consolidada pela cultura dos descartáveis, a produção global de plásticos aumentou de forma exponencial, atingindo cerca de 390,7 milhões de toneladas em 2021 (PLASTICSEUROPE, 2022). Levantamentos recentes apontam que entre a década de 50 e o ano de 2015, foram gerados 6,3 bilhões de toneladas de resíduos plásticos e até 2050, está previsto que aproximadamente 12 bilhões de toneladas de resíduos plásticos serão lançados no ambiente caso a produção persista em um ritmo crescente (Tourinho *et al.*, 2019; Geyer *et al.*, 2017).

Os plásticos são materiais que possuem como elemento fundamental ligações moleculares orgânicas, obtidas por síntese ou pela transformação de produtos naturais, sendo possível produzir diversos polímeros a partir de uma mesma matéria-prima, alterando o processo de produção ou realizando diferentes misturas, cujas principais fontes de matéria-prima são o petróleo e o gás natural (Michaeli *et al.*, 1995).

Os polímeros plásticos podem ser classificados quanto ao seu comportamento mecânico em duas categorias: termofixos e termoplásticos (ABIPLAST, 2018). Os plásticos termofixos são uma família de plásticos que passam por alterações químicas quando aquecidos. Depois de aquecidos e formados, estes plásticos não podem ser reformados ou refundidos. Podem ser citados como exemplos, a resina de fenol-formaldeído (baquelite), utilizada em tomadas e na inserção de amostras metalográficas, epóxi (araldite), Poliuretano (PUR), entre outros, apresentando características que favorecem a reciclagem química ou energética (Schelte; Hendrickx; Severengiz, 2023).

Já os termoplásticos podem ser derretidos e endurecidos quando arrefecidos, características que favorecem a reciclagem mecânica (Figura 1), sendo encontrados em diversos materiais que utilizamos no dia-a-dia (Schelte; Hendrickx; Severengiz, 2023). Exemplos de termoplásticos são: polietileno (PE), polipropileno (PP), Policloreto de vinilo (PVC), Tereftalato de polietileno (PET), Poliestireno (PS), Polietileno de alta densidade (PEAD), Polietileno de baixa densidade (PEBD), Policarbonato (PC), Poliamida (PA), polimetilmetacrilato (PMMA), Acrilonitrila butadieno estireno (ABS), Acrilonitrila Estireno Acrilato (ASA), Copolímero Estireno-Acrilonitrilo (SAN) e Elastômeros Termoplásticos (TPE) (PLASTICSEUROPE, 2018).

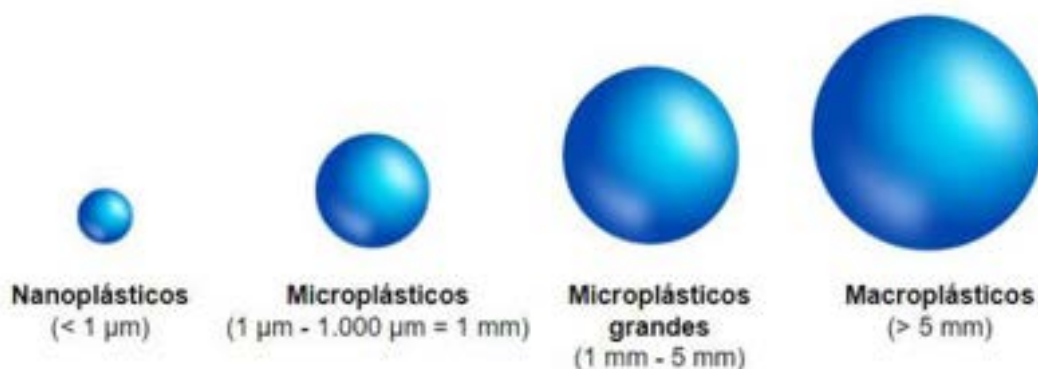
|                                                                                     |                                                                                                                                          |                                                                                                                         |
|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
|    | <b>PET</b><br>Tereftalato de polietileno<br>            | Fibras de poliéster, filmes, embalagens de alimentos, garrafas de bebidas.                                              |
|  | <b>PEAD</b><br>Polietileno de alta densidade<br>      | Garrafas de plástico, recipientes para produtos de limpeza, canos para gás e água potável, utensílios domésticos.       |
|  | <b>PVC</b><br>Policloreto de vinila<br>               | Botas, cortinas de chuveiro, caixilhos de janelas, canos, revestimentos de piso, cabos elétricos, imitação de couro.    |
|  | <b>PEBD</b><br>Polietileno de baixa densidade<br>     | Sacos de plástico, filme plástico, sacos de lixo, tubos, revestimentos de caixa de leite.                               |
|  | <b>PP</b><br>Polipropileno<br>                        | Embalagem de alimentos, 4 estojos para DVD, acabamento interno de carros, para-choque, cadeirinha de criança.           |
|  | <b>PS</b><br>Poliestireno<br>                         | Embalagem de alimentos, material de embalagem, isolamento.                                                              |
|  | <b>Outros</b><br>PC, PA, PMMA, PUR, ABS, ASA, SAN<br> | Malas, CDs e DVDs, roupas, cordas, paraquedas, cerdas para escovas de dente, brinquedos, caixas de aparelhos elétricos. |

**Figura 1.** Tipos de materiais plásticos, suas aplicações na indústria e classificação de reciclagem.

Dados do Panorama de Resíduos Sólidos no Brasil da Associação Brasileira de Resíduos e Meio Ambiente (ABREMA) apontam para o registro de cerca de 77 milhões de toneladas de Resíduos Sólidos Urbanos (RSU) gerados no país em 2022 (ABREMA, 2023), sendo que 16,8% desses RSU são constituídos por plásticos (Ministério do Meio Ambiente, 2022). Os tipos de plásticos comumente encontrados em RSU são o PVC, PET, PEAD, PEBD, PP e o PS. O PEAD vem sendo um dos plásticos mais utilizados no mercado nacional devido ao aumento da sua utilização em embalagens de descarte rápido, atrás apenas do PET (Cruz; Zanin, 1999).

Para obter algumas propriedades funcionais, são adicionadas substâncias tóxicas aos plásticos, como ftalatos, ácido bórico, bisfenol A, hidroxitolueno butilado, cádmio, composto de chumbo, amidas de ácidos graxos, diaminodifenilmetano e compostos de arsênio (Sánchez, 2020). Contudo, essas características são também uma das causas relacionadas aos problemas com resíduos plásticos, já que esses aditivos resultam em sua fragmentação e degradação (Andrady, 2011; Cole *et al.*, 2011). Os processos de degradação dos plásticos reduzem rigorosamente a massa molecular média dos polímeros (Cózar *et al.*, 2014), resultam no aumento da molhabilidade e da cristalização, bem como a formação de fissuras, gerando os fragmentos de MP (Waldman; Rilling, 2020). Esses processos podem ser físico-químicos, biológicos e principalmente mecânicos, devido à erosão por ação dos ventos, ondas e radiação solar (fotólise) (Moore, 2008).

Os microplásticos são partículas em escalas milimétricas, variando sua dimensão de 25  $\mu\text{m}$  a 5 mm (Galgani *et al.*, 2013; Gigault *et al.*, 2018), definição usada pela maioria dos autores. Recentemente foi publicada a norma “Plastics – Environmental aspects – State of Knowledge and Methodologies”, que define “microplásticos” como qualquer partícula plástica insolúvel em água e de dimensões entre 1 $\mu\text{m}$  e 1000 $\mu\text{m}$ , “microplásticos grandes” como partículas de 1mm a 5 mm, e “macroplásticos” como partículas ou objetos plásticos insolúveis com dimensões acima de 5 mm (ISO/TR 21960:2020), como demonstra a Figura 2.



**Figura 2.** Classificação de tamanho de plásticos segundo a norma (ISO/TR 21960:2020).  
Fonte: Pinhatti, 2022.

Os MPs apresentam morfologias variadas, mais comumente reportadas na literatura são os pellets, fragmentos e fibras, e em menor frequência os filmes, filamentos, esponjas, espumas e microesferas (Frias; Nash, 2019). As fibras foram descritas como um dos MPs mais frequentes no oceano (Cole *et al.*, 2011), representando os principais constituintes de roupas sintéticas e redes de pesca. Considerando a mesma área de amostragem, as fibras foram apontadas como os MPs mais comuns em peixes (Neves *et al.*, 2015; Bessa *et al.*, 2018). No entanto, esse padrão distinto pode estar relacionado a diversas fontes de poluição e ilustra a importância do monitoramento e da coleta de dados ambientais, como amostras de água, para correlacionar os dados com a disponibilidade de MP. No estudo de Bessa *et al.* (2018) a maioria dos MPS encontrados na água eram fibras (62%) e assim eram a maioria das formas de MPS encontradas em organismos.

A cor, por sua vez, é útil para identificar fontes potenciais de resíduos plásticos e pode ser dividida em sete categorias de acordo com suas cores cromáticas: marrom, vermelho, laranja, amarelo, verde, azul e roxo. As colorações preto, branco e cinza são cores acromáticas. (Zhao *et al.*, 2022). Cada coloração possui diferentes comprimentos de onda, influenciando sua absorbância solar e transmitância UV, afetando o fotoenvelhecimento do plástico (Martí *et al.*, 2020). Geralmente, pigmentos amarelos e vermelhos possuem comprimentos de ondas mais longos e podem absorver luz de comprimentos de onda curta com maior energia, enquanto os pigmentos azuis absorvem comprimentos de onda longos com menor energia o que faz com que a energia transmitida ao plástico azul é maior que a transmitida aos

plásticos amarelos e vermelhos, tornando o fotoenvelhecimento mais intenso (Zhao *et al.*, 2022).



**Figura 3.** Exemplos das classes morfológicas de microplásticos mais reportadas na literatura.

Fonte: Moraes (2024)

Os MPs podem ser classificados de acordo com sua origem em primários e secundários (Caixeta; Morais, 2022). Os MPs primários são aqueles produzidos propositalmente até 5mm e são lançados no ambiente neste formato, como: cosméticos com glitter, esfoliantes, sabonetes e cremes dentais, pellets e abrasivos industriais (Fendall; Sewell, 2009; Browne *et al.*, 2011). Por sua vez, os MPs secundários são aqueles que resultam da fragmentação de resíduos maiores (Barnes *et al.*, 2009).

### 3.2 Emissão e distribuição de microplásticos no ambiente

O primeiro estudo que revelou resíduos plásticos foi realizado por Carpenter *et al.* (1972), no qual foi identificada a presença de MPs nas águas costeiras do Sul da Nova Inglaterra. Atualmente, os MPs vêm sendo amplamente descritos em oceanos, rios, lagos, reservatórios, estuários, regiões polares, estações de tratamento de esgoto e água potável (Kasavan *et al.*, 2021; Wang *et al.*, 2022). Foram encontrados

MPs em organismos de fossas profundas do oceano, como as fossas Mariana (Jamieson *et al.*, 2019), na neve do Monte Everest (Napper *et al.*, 2020) e no ar das cidades com maiores números de população na China (Liu *et al.*, 2019). Allen *et al.* (2019) encontraram partículas de MPs em regiões montanhosas situadas entre a Espanha e a França, que devido à distância de grandes cidades e centros industriais, são consideradas intocadas, retratando, portanto, a facilidade que essas micropartículas são transportadas por longas distâncias através da ação do vento.

Muitas práticas e atividades usam e lançam resíduos plásticos no ambiente, tornando-se principais fontes de MPs distribuídos em diferentes compartimentos ambientais (Montagner *et al.*, 2021). Dentre essas atividades destacam-se o descarte de resíduos plásticos em lixões e aterros (He *et al.*, 2019), o descarte inadequado de resíduos sólidos no ambiente, o desgaste de pneus de borracha (Boucher; Billard, 2019). Adiciona-se também a utilização de produtos de higiene pessoal e cosméticos compostos por microesferas poliméricas (UNEP, 2015), a lavagem de roupas sintéticas com desprendimento de fibras poliméricas (Cesa *et al.*, 2017), o aporte de microfibras e microesferas no esgoto e entrada na Estação de Tratamento de Esgoto (Kalčíkova *et al.*, 2017) e o uso de filmes plásticos na agricultura (Steinmetz *et al.*, 2016).

Em ambientes aquáticos, a forma e a densidade dos MPs são fatores de grande importância para sua distribuição ao longo da coluna de água (Melkebeke *et al.*, 2020). Aditivos e ar quando incorporados à partícula, promovem um aumento ou diminuição em sua densidade, tornando-o mais propenso ou não à decantação. Outro fenômeno que também pode promover o aumento da densidade da partícula é a bioincrustação, causada pela formação de biofilme e colonização de micro-organismos, facilitando seu acesso em camadas mais profundas (Wagner; Lambert, 2018). No oceano, a neve marinha é um processo de sedimentação de partículas com materiais provenientes da decomposição de células, além de ser fonte de alimento para o fundo do mar, também é uma via de transporte de MPs. Argila, fitoplâncton e outros detritos orgânicos aderem-se aos MPs podendo deslocá-los em diferentes profundidades ou até atingir o sedimento. (Cauwenberghe, 2013).

As pesquisas sobre MPs são bem mais abundantes em ambientes aquáticos (Horton *et al.*, 2017; Mendoza; Karapanagioti; Álvarez, 2018; Hidalgo-Ruz *et al.*, 2012; Anderson *et al.*, 2017; Free *et al.*, 2014 ; Su *et al.*, 2016), enquanto que em ambientes

terrestres os estudos ainda são escassos. Após Riling (2012) ter apontado o problema da poluição dos MPs no solo e nos ecossistemas terrestres, novas pesquisas vêm alertando sobre os impactos potenciais dos MPs no solo (Liu *et al.*, 2014; Rochman *et al.*, 2015) e seus possíveis efeitos adversos aos seres vivos (Nizzetto *et al.*, 2016). Estudos indicam que a presença de MPs constitui um risco para a saúde do solo, impactando as características físicas e químicas, as funções vitais de animais, insetos e microrganismos, induzindo mudanças nas atividades enzimáticas e influenciando o crescimento de plantas (De Souza Machado *et al.*, 2019; Rillig; Leifheitt; Lehmann, 2021).

A distribuição espacial de MPs no solo é bastante desigual, exibindo variações regionais. Por exemplo, campos agrícolas do Chile apresentaram maior quantidade de MPs/kg (Corradini *et al.*, 2019) de solo do que campos agrícolas no leste da Alemanha (Piehl *et al.*, 2018). As concentrações de MPs no solo também podem variar de acordo com o local, podendo atingir altas concentrações em solos industriais (Fuller; Gautam, 2016), quando comparadas com áreas de várzea, que apresentam concentrações mais baixas (Scheurer; Bigalke, 2018). Geralmente, a concentração mais elevada de MPs se dá em estuários, litoral, áreas agrícolas, áreas industriais e áreas urbanas (Gao *et al.*, 2021; Zhang *et al.*, 2022).

### 3.3 Potenciais Riscos dos Microplásticos

Microplásticos são poluentes que podem provocar efeitos adversos nos organismos vivos, em diferentes níveis, quando presentes no ambiente. A sua toxicidade depende de uma série de fatores, como o polímero constituinte, tamanho e formato da partícula e presença de aditivos na matriz polimérica (Pompêo *et al.*, 2022).

Uma classe importante de compostos químicos e com grande relevância são os aditivos, sendo capazes de modificar as características originais dos plásticos, sendo adicionados na matriz polimérica no momento da produção (Da Costa *et al.*, 2023). Dentre os aditivos mais utilizados estão os retardantes de chama, plastificantes, estabilizadores de calor, modificadores de impacto, enchimentos, corantes, antioxidantes, lubrificantes e estabilizadores de luz (Geyer *et al.*, 2017). Como resultado da degradação, os aditivos que antes estavam ligados à estrutura química

do polímero passam a ser liberados no ambiente ou diretamente para biota com mais facilidade (La Nasa *et al.*, 2021).

Além dos aditivos, outros compostos tóxicos podem ser carregados por MPs, dentre os quais organoclorados (PCBs e pesticidas), hidrocarbonetos policíclicos aromáticos (PHAs) (Hirai *et al.*, 2011; Rochman *et al.*, 2013; Rochman *et al.*, 2014; Tanaka *et al.*, 2015; Turner; Holmes, 2015), poluentes orgânicos persistentes (POPs), metais (Rochman *et al.*, 2019), fármacos (Santos *et al.*, 2021a) e drogas ilícitas (Qu *et al.*, 2020). Esses compostos tóxicos presentes no ambiente entram em contato com os MPs por meio da adsorção ou da absorção (Rochman *et al.*, 2019). A adsorção ocorre quando o poluente se fixa na superfície da partícula, enquanto a absorção é caracterizada pela entrada do poluente por entre as cadeias poliméricas (Lambert; Wagner, 2017). Ambas as formas resultam no mesmo fim, onde os MPs se comportam como vetores químicos, transportando e, eventualmente, liberando os compostos novamente no ambiente (Bakir *et al.*, 2016).

### 3.4 Exposição da biota aos microplásticos - efeitos nos organismos

O habitat e o nível de exposição são fatores importantes para a contaminação da biota, pois peixes que vivem em zonas pelágicas tendem a consumir quantidades maiores de MPs em comparação com peixes de regiões oceânicas (Hantoro *et al.*, 2019). Portanto, peixes que vivem em locais com maior concentração de partículas na água, em geral, ingerem mais partículas. Concentrações maiores que 1 µg/L são capazes de interferir na aptidão reprodutiva de nematoides, enquanto no solo, concentrações de MPs maiores que 1g/kg reduzem o crescimento e sobrevivência das minhocas (Ji *et al.*, 2021).

A ingestão de MPs pode acontecer direta ou indiretamente através do consumo de organismos contaminados (Cole *et al.*, 2013). Há evidências da transferência trófica dos MPs no ambiente aquático, mostrando que a ingestão indireta através de algas foi maior que a ingestão direta pela água, demonstrada por Hasegawa e Nakaoka (2021) onde o peixe bentônico *Myoxocephalus brandti* ingeriu de 3 a 11 vezes mais micropartículas de PE por transferência trófica ao se alimentar de sua presa *Neomysis* spp.

Em ambientes marinhos, os MPs podem se acumular nos animais filtradores, como os moluscos, as ascídias e o zooplânctons (Ribeiro *et al.*, 2021). Em mexilhões e ostras, relatou-se concentrações de MPs de cerca de 37 e 48 partículas plásticas por 100 gramas de tecido mole respectivamente (Van Cauwenberghe; Janssen; Colin, 2014). Entretanto, a acumulação de MPs em região de branquispinhas depende das diferentes morfologias dos aparelhos respiratórios dos peixes (Da Silva *et al.*, 2020).

Estudos realizados em roedores mostraram que os MPs são capazes de se translocar da cavidade intestinal para o sistema circulatório, com potencial de se alojarem em diversos órgãos, acumulando-se nos tecidos e nas células. (Browne *et al.*, 2008). Em *Rattus norvegicus*, foram observadas nanopartículas de PS em placenta e fígado fetal através de microscopia hiperespectral aprimorada, sugerindo translocação do tecido pulmonar materno para o feto após exposição às nanopartículas no final da gestação (Fournier *et al.*, 2020).

A exposição aos MPs pode ocorrer através de diversas vias, como ingestão, inalação, contato pela pele, captação pelas brânquias, transferência transplacentária e transferência trófica (Scherer *et al.*, 2017; Lu *et al.*, 2021; Aristizabal *et al.*, 2024; Zhang *et al.*, 2021; Ragusa *et al.*, 2021; Au *et al.*, 2017.) Para várias espécies de mamíferos, a ingestão é vista como a principal via de exposição (Meaza *et al.*, 2021), já em moluscos bivalves, as brânquias são o primeiro local de captação de MPs (Franzellitti *et al.*, 2019). Cada caminho de exposição traz consigo riscos específicos e possíveis consequências na saúde dos mamíferos (Rahman *et al.*, 2021).

A ingestão de MPs pode causar danos tanto por abrasão, como por acúmulos de partículas no sistema gastrointestinal dos peixes (Nadal *et al.*, 2016; Cannon *et al.*, 2016). Posteriormente, através de exame histopatológico, Hamed *et al.* (2021) avaliou os tecidos do peixe *Danio rerio* expostos a diferentes cargas de MPs, confirmando que a exposição à partícula pode causar danos a diferentes órgãos e ter impactos negativos sobre a vitalidade e à vida dos peixes, como por exemplo toxicidade comportamental (Araujo; Malafaia, 2020), dilatação dos vasos sanguíneos, infiltração, congestão, degeneração hidrópica, hipertrofia e hiperplasia no fígado (Araujo; Gomes; Malafaia, 2020), modificação na homeostase (Rainieri *et al.*, 2018), danos a mucosa intestinal, elevação da permeabilidade, inflamação e interrupção do metabolismo (Qiao *et al.*, 2019).

O contato com MPs pode causar impactos fisiológicos, já que pesquisas indicam que os MPs têm a capacidade de provocar estresse oxidativo (Deng *et al.*, 2018) e inflamação em tecidos de mamíferos, resultando em danos celulares e prejuízos na função dos órgãos (Deng *et al.*, 2017). A presença de MPs no intestino pode causar perturbação à microbiota intestinal, que tem um desempenho direto na função imunológica e na manutenção da saúde geral (Deng *et al.*, 2020), podendo aumentar a susceptibilidade a infecções e doenças, impactando o sucesso reprodutivo e a sobrevivência (Hirt; Body-Malapel, 2020).

Modelos experimentais realizados em camundongos demonstraram alterações patológicas no intestino, como redução na secreção do muco (Lu *et al.*, 2018), inflamação intestinal (Li *et al.*, 2020), e disfunção da barreira intestinal (Jin *et al.*, 2019). As alterações patológicas hepáticas incluem inflamação (Lu *et al.*, 2018) e alteração no perfil lipídico (Luo *et al.*, 2019). Outros problemas metabólicos identificados incluem distúrbio no metabolismo energético (Deng *et al.*, 2017) e dos ácidos biliares (Jin *et al.*, 2019). Em mamíferos domésticos, foram detectados MPs em leite, carne e sangue de vacas e porcos, e na ração fornecida a eles (Van Der Veen *et al.*, 2022). Nos animais de companhia os achados foram em tecido de pulmão, rim, íleo, fígado e coágulos de sangue, destacando a possibilidade de internalização e distribuição de MPs em tecidos internos (Prata *et al.*, 2022), podendo resultar em dano físico de órgãos, devido a abrasão com o detrito plástico, desencadeando deste modo, respostas inflamatórias, insuficiência e redução nos níveis de energia (Lwanga *et al.*, 2016; Song *et al.*, 2019).

Um estudo com *Nephrops norvegicus* tratados experimentalmente com MPs demonstrou a redução da massa corporal média de 0,0189% destes animais, por dia, quando comparados aos animais controles (Welden; Cowie, 2016). Esses achados corroboram com relatos prévios de alimentação reduzida em *Carcinus maenas* alimentadas com MPs (Watts *et al.*, 2015), *Arenicola marina* alimentada com microesferas (Besseling *et al.*, 2013) e *Daphnia magna* alimentada com nanopoliestireno, supondo que a diminuição na frequência alimentar seja um sinal de falsa sensação de saciedade, já que as agregações plásticas ocupam uma quantidade crescente no estômago (Besseling *et al.*, 2014).

### 3.5 Métodos de diagnóstico

A amostragem para detecção de MPs é um desafio, pois estão dispersos de forma não homogênea na amostra (Primpke *et al.*, 2017; Primpke *et al.*, 2020). Cada matriz tem suas particularidades quanto a amostragem e o preparo, sendo que esse último pode ser por filtração e/ou peneiração, separação por densidade, e digestão (remoção da matéria orgânica) (Montagner *et al.*, 2021). Para digestão de matéria orgânica é necessária a utilização de métodos que não danifiquem as propriedades químicas e estruturais dos polímeros. Os mais comuns são aqueles que fazem uso de soluções ácidas ou alcalinas, digestão por oxidantes e digestão enzimática (Montagner *et al.*, 2021).

Para identificação e quantificação dos MPs diversas técnicas podem ser utilizadas baseando-se na composição química e nos grupos funcionais específicos (BOLAN *et al.*, 2020). A caracterização por tamanho, cor e formato pode ser alcançada com auxílio de uma lupa manual, ou por equipamentos mais aprimorados, como o uso de um microscópio óptico, estereoscópio, microscópio eletrônico de varredura, microscópio de fluorescência, microscópio de Raman/FTIR, e ionização e dessorção a laser assistida por matriz (Song *et al.*, 2015, Huppertsberg; Knepper, 2018, Mukotaka; Kataoka; Nihei, 2021, Siegel *et al.*, 2021).

Evitar a contaminação cruzada é uma etapa que deve estar presente em todo o processo, independentemente do método analítico utilizado. A utilização de amostra controle (“branco”) garante a precisão e confiabilidade dos resultados (Lima *et al.*, 2022). Os principais meios de contaminação remetem ao local de manuseio das amostras e ao analista, devido a isso é necessário evitar o uso de materiais plásticos sempre que possível durante a análise de MPs, e é extremamente indicado o uso de utensílios de vidro ou metal, bem como a boa higienização deles (Dehaut *et al.*, 2019).

As alterações teciduais microscópicas identificadas e medidas por meio do exame histopatológico são uma ótima opção para avaliar o nível de saúde dos animais (De Oliveira, *et al.* 2016). O exame histopatológico é considerado um indicador sensível e fundamental na definição de alterações celulares que possam ocorrer nos órgãos-alvo (Hamed *et al.*, 2021). A histopatologia vem sendo utilizada como biomarcador em diversos estudos para avaliação de alterações teciduais causada por MPs (Haredi *et al.*, 2020; Mobin; Kanai; Yoshikoshi, 2000; Hamed *et al.*, 2021),

possibilitando a identificação de lesões específicas e diferenciação de alterações agudas e crônicas (Ameur, *et al.* 2012).

### 3.6 Mamíferos Silvestres em fragmentos florestais urbanos

Um fragmento florestal corresponde a uma unidade de floresta natural contínua interrompida por barreiras naturais ou antrópicas (Firmino *et al.*, 2016). Dentre as ações antrópicas, a urbanização vem alterando significativamente o meio ambiente, gerando interferências da população sobre os recursos naturais (Moura-Fujimoto, 2000). Algumas cidades possuem remanescentes de florestas nativas resistentes à intensa urbanização, mas devido à sua localização em ambientes antrópicos, esses remanescentes florestais acabam sofrendo diversos impactos, como: acúmulos de resíduos, poluição hídrica e efeitos da poluição atmosférica externa (Melo *et al.*, 2011).

O município de Campo Grande, no Mato Grosso do Sul, abriga diversos remanescentes florestais em seu território urbano, alguns deles protegidos pelo código florestal, lei 12.651 de 25 de maio de 2012, por estarem próximos a nascentes (Brasil, 2012). Porém, um estudo de coleta de dados amostrais no entorno de uma área de preservação permanente (APP) revelou diversas atividades com potencial poluidor e de contaminação (Macedo *et al.*, 2015).

Esses fragmentos florestais são um importante refúgio da vida silvestre, abrigando inúmeras espécies de animais (Torres *et al.*, 2024; Nantes *et al.*, 2019), podendo ser dividida em duas categorias: as espécies residentes, que podem ser observadas durante todo o ano, se alimentam e procriam na região; e as espécies sazonais, que se deslocam para a área conforme a disponibilidade de alimento (IMASUL, 2022).

Uma pesquisa conduzida em um remanescente florestal da cidade de Campo Grande revelou 18 espécies de mamíferos silvestres de médio e grande porte, dentre eles anta, tamanduá bandeira, tamanduá mirim, macaco prego, macaco bugio, gambá, lobinho, capivara, irara, cateto, queixada, veado campeiro, jaguatirica, dentre outros (Santos *et al.*, 2013). Dentre as diversas espécies relatadas, o gambá-de-orelha-branca (*Didelphis albiventris*) é um dos principais representantes, podendo

habitar áreas mais conservadas como também as menos conservadas (IMASUL, 2011; Nantes *et al.*, 2021).

O *Didelphis albiventris* possui hábitos noturnos (Silva, 1984) e se desloca principalmente pelo solo, no entanto, o hábitat arbóreo tende a ser explorado também (Fonseca, 2003). Seu hábito alimentar é de natureza onívora, sendo composto por frutos, pequenos vertebrados, ovos, insetos e outros invertebrados. (Marinho Filho *et al.*, 1998). Considerado generalista, é uma das espécies de mamíferos silvestres mais frequentes no Brasil Central (Fonseca, 2003).

Esses animais se adaptaram bem aos pequenos fragmentos de florestas localizados em zonas urbanas, apresentando altas taxas de migração durante a estação das chuvas e uma rotação completa ou quase completa da população em um período de um ano (Cáceres, 2000). Considerados seminômades, percorrem trechos bastante longos em busca de alimentos (Ceballos *et al.*, 2006), podendo até mesmo procurar ativamente alojamentos humanos para obter acesso a alimentos (Parera *et al.*, 2002).

Devido à sua capacidade de explorar os recursos gerados pela presença humana e à sua resistência às mudanças climáticas, torna-se possível a frequente observação desse marsupial em ambientes antrópicos rurais e urbanos (Silva, 1984; Olifiers *et al.*, 2005; Cruz-Salazar *et al.*, 2014). Neste sentido, podem atuar como espécies sentinelas, pois são capazes de refletir perturbações causadas ao meio ambiente, servindo como indicadores de conservação do ecossistema, ajudando também a esclarecer aspectos ecológicos e sanitários que vêm ocorrendo em uma determinada região (Deem *et al.*, 2001; Aguirre *et al.*, 2002).

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INVESTIGAÇÃO DE MICROPLÁSTICOS EM TRATO GASTROINTESTINAL E  
PULMÃO DE GAMBÁS-DE-ORELHA-BRANCA (*Didelphis albiventris*) RESIDENTES  
EM FRAGMENTOS FLORESTAIS DE CAMPO GRANDE/MS

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## RESUMO

Este trabalho teve como objetivo investigar a presença de microplásticos em gambás-de-orelha-branca e avaliar seus efeitos nos tecidos avaliados e na condição corporal destes animais. As coletas foram realizadas de abril a dezembro de 2023, resultando na captura de 22 animais em 6 regiões florestais na região urbana de Campo Grande, MS. Foram coletados dados sobre os animais como o peso, o estado reprodutivo, o sexo e o comprimento cabeça-corpo. Estômago, íleo e pulmão foram coletados durante as necropsias para as análises laboratoriais de digestão alcalina com KOH das amostras, e análise histopatológica. As amostras digeridas passaram por filtragem à vácuo em papel filtro e levados à estufa para secagem. Os filtros secos foram avaliados em microscópio ótico. Foi detectado um total de 270 microplásticos nos 22 animais examinados neste estudo. O íleo foi o órgão que apresentou maior quantidade de MPs ( $n = 141$ ), as fibras foram o tipo de MP mais encontrado ( $n=185$ ) e o azul foi a coloração predominante ( $n = 140$ ). Em relação à constituição química, os MPs mais encontrados foram: Policloreto de vinilo (PVC), Tereftalato de polietileno (PET), Acrilonitrila butadieno estireno (ABS). Embora todos os gambás tenham apresentado microplásticos nos tecidos, não se observou efeitos na condição corporal e nem danos teciduais nestes animais. Porém, a presença desse contaminante sugere que estes animais podem funcionar como sentinelas da contaminação ambiental.

Keywords Bioindicator, body condition, contaminant, histopathology, polyethylene.

## Introdução

Urbanização e meio ambiente têm uma relação direta, de forma que o crescente nível de urbanização vem afetando significativamente o meio ambiente, resultando em impactos degradadores com efeitos sinérgicos e persistentes (Jatoba, 2011). A piora dos índices de poluição gerada pela urbanização vem gerando preocupações devido ao seu potencial efeito adverso sobre os fragmentos florestais (Melo *et al.*, 2011).

Os microplásticos (MPs) são partículas com tamanho  $<5\text{mm}$ , que agem como contaminantes antropogênicos onipresentes e persistentes, encontrados em ambientes urbanos (Dris *et al.*, 2018). Estas substâncias têm capacidade de causar diversas alterações patológicas em espécies residentes em áreas antropizadas devido à abrasão dos tecidos com o detrito plástico, desencadeando deste modo, respostas inflamatórias, insuficiência e redução nos níveis de energia (Lwanga *et al.*, 2016; Song *et al.*, 2019). Em animais experimentais, como *Nephrops*

*norvegicus*, foi constatada a redução da massa corporal média dos indivíduos alimentados com plástico, indicando que a retenção de MPs resulta em menores taxas de crescimento (Welden & Cowie, 2016).

Em mamíferos, a exposição a MPs pode ocorrer através de diversas vias, como ingestão, inalação, contato pela pele, transferência transplacentária e transferência trófica. Para várias espécies de mamíferos, a ingestão é descrita como a principal via de exposição (Meaza *et al.*, 2021). Cada caminho de exposição traz consigo riscos específicos e possíveis consequências na saúde dos animais (Rahman *et al.*, 2021).

O reduzido tamanho desses plásticos resulta em uma maior disponibilidade para os níveis tróficos inferiores (Lehtiniemi *et al.*, 2018). Por sua semelhança de tamanho e aparência com o plâncton, são frequentemente confundidos com a sua presa e o risco de ingestão cresce (Wagner & Lambert, 2018). Por outro lado, a cor das partículas pode afetar a ingestão, pois os predadores podem preferencialmente ingerir MPs com cores que lembram sua presa, bem como, algumas espécies tendem a ser atraídas por cores específicas (Wagner & Lambert, 2018; Wright, Thompson & Galloway, 2013).

Diversas técnicas têm sido utilizadas na identificação dos MPs baseando-se na composição química e grupos funcionais específicos (Bolan *et al.*, 2020) são realizadas com o auxílio de equipamentos mais aprimorados como estereoscópio, microscópio eletrônico de varredura, microscópio de fluorescência, microscópio de Raman/FTIR e ionização e dessorção a laser assistida por matriz (Song *et al.*, 2015; Huppertsberg & Knepper, 2018; Mukotaka *et al.*, 2021, Siegel *et al.*, 2021). Ainda, destaca-se que a aplicação de biomarcadores como os exames histopatológicos é cada vez mais usada em estudos toxicológicos experimentais de MPs devido aos seus primeiros sinais de estresse ambiental para os organismos (Prata *et al.*, 2022).

O gambá-de-orelha-branca (*Didelphis albiventris*) é o pequeno mamífero mais comum nos fragmentos florestais do município de Campo Grande- MS (Nantes *et al.*, 2021). Por ser considerado generalista e de hábito alimentar onívoro (Fonseca, 2003), eles possuem a capacidade de aproveitar os recursos disponibilizados pela presença humana e, por compartilhar um ambiente de vida comum, tornam-se sentinelas da exposição humana (Noireau *et al.*, 2009). Coleta de dados no entorno de fragmentos florestais de Campo Grande, MS revelou diversas atividades com potencial poluidor e de contaminação (Macedo, 2015)

Neste contexto, o presente estudo tem como objetivo avaliar a presença de MPs no trato gastrointestinal e pulmão de gambás-de-orelha-branca residentes em fragmentos florestais, e sua influência nestes tecidos e na condição corpórea, fornecendo conhecimentos sobre a

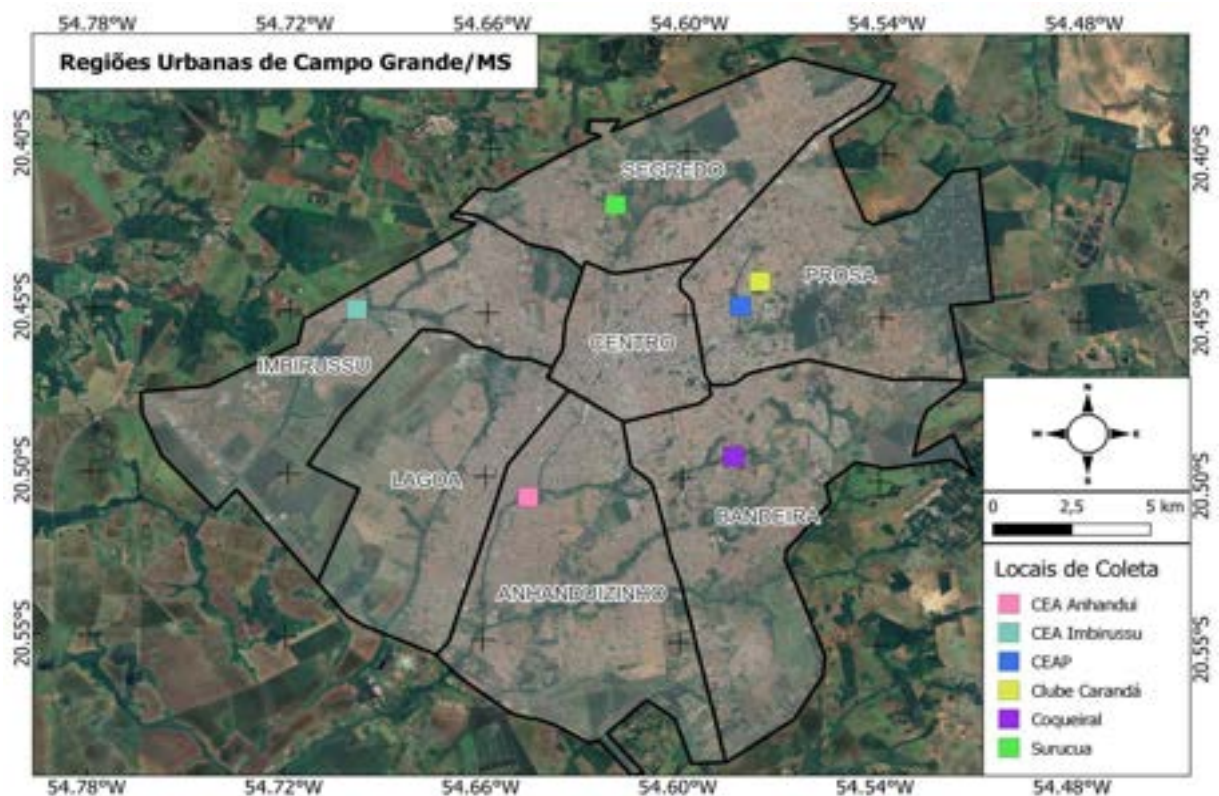
presença MPs em uma espécie silvestre terrestre abundante em áreas antropizadas da cidade de Campo Grande, MS.

## Material e métodos

### *Área de estudo*

A coleta de animais foi realizada em seis fragmentos florestais dentro do perímetro urbano do município de Campo Grande, Mato Grosso do Sul, em áreas determinadas a partir do grau de urbanização de seu entorno. As áreas amostradas foram: Reserva do Surucuá, CEAP, Clube Carandá, Chácara Coqueiral, CEA Imbirussu e CEA Anhanduí (Fig. 1).

Os procedimentos realizados durante essa pesquisa estão de acordo com o Sistema de Autorização e Informação em Biodiversidade (SISBIO) protocolo nº 70946-5 e nº89586-1. A manipulação e processamento das amostras seguem em concordância com o proposto pelo Comitê de Ética e Utilização de Animais (CEUA) da Universidade Católica Dom Bosco, protocolo nº. 013/2020.



**Fig. 1** Mapa da área urbana de Campo Grande, MS. Destacada em cinza está a região central de Campo Grande e cada quadrado colorido é referente aos fragmentos florestais de coleta de gambás-de-orelha-branca.

### *Coleta de Dados*

Vinte e dois gambás-de-orelha-branca (*Didelphis albiventris*) foram capturados pelo grupo de pesquisa INSANA HUNA/UCDB no período de abril a dezembro de 2023, com campanhas semanais em cada ponto. As armadilhas permaneceram por cinco noites seguidas em cada fragmento. Foram utilizadas para a captura armadilhas do tipo Tomahawk® (45 x 16 x 16 cm) instaladas no solo, dispostas a cerca de 10 metros de distância entre elas e em locais pré-avaliados quanto à movimentação dos animais. As armadilhas permaneceram abertas durante a noite e foram checadas diariamente pela manhã e iscadas durante o mesmo período. As iscas utilizadas consistiram em uma mistura de sardinha, fubá, paçoca e banana, dispostas em porções no interior das armadilhas. Após a captura, os animais foram transferidos dentro das armadilhas ao Laboratório de Patologia Animal do Hospital Veterinário Dom Bosco (HOVET) da Universidade Católica Dom Bosco – UCDB.

### *Necropsia e coleta de amostras biológicas*

No laboratório, a sedação dos animais foi realizada com Cetamina (20mg/kg) e Xilazina (2mg/kg) por via intramuscular. Em seguida, realizou-se a coleta de dados individuais e biométricos do animal, como comprimento cabeça-corpo, comprimento corpo-cauda, peso, sexo, estado reprodutivo, estimativa de faixa etária a partir da dentição e escore corporal. A eutanásia foi realizada com a utilização do Cloreto de Potássio (75-150 mg/kg) por via intravenosa, de acordo com o protocolo para eutanásia disponibilizado pela UNIFESP (2019). A necropsia ocorreu logo em seguida à eutanásia, seguindo a técnica de exame externo do animal, para relatar qualquer lesão, sinal ou processo patológico, na cobertura de pelos, cavidade oral, nasal, olhos, orelha, ânus, sistema reprodutor externo. A técnica empregada para retirada dos órgãos foi a de Gohn, em que os sistemas e órgãos foram retirados de forma ordenada, em monoblocos separados para a inspeção das vísceras. Durante a necropsia foram coletados pulmão, íleo e o estômago. Para coleta do estômago, foram realizadas duas amarrações, uma na região do cárdia e outra em região de piloro, preservando o conteúdo gástrico. Os materiais destinados ao exame de microplásticos foram pesados em fragmentos que variaram de 0,13g a 7,49g de pulmão, 0,37g a 5,76g de íleo e 0,5g a 12,5g de estômago, armazenados em papel alumínio e congelados em freezer em temperatura de -20° para serem processados. Fragmentos dos mesmos órgãos medindo de um a dois centímetros de espessura,

comprimento e largura, foram acondicionados em frascos de vidro com tampa larga, contendo formalina a 10%, para exame histopatológico.

### *Processamento das amostras*

Amostras de estômago, íleo e pulmão foram retiradas do freezer e descongeladas à temperatura ambiente. Para a avaliação de microplástico foi realizada a digestão de matrizes biológicas utilizando solução de hidróxido de potássio (KOH) 10%, que se mostrou eficiente na digestão de amostras sem danificar o microplástico (Kühn *et al.*, 2017). As amostras foram lavadas em água milli-Q para a retirada de resíduos, pesadas em balança semi analítica Marte Ad500 510G x 0,001G com capela e colocadas em *beckers* de vidro, juntamente com a solução de hidróxido de potássio pré-preparada a 10% (diluição de 1g de KOH para 10 ml de água milli-Q, mantidos em frasco de vidro opaco), na proporção de três vezes a massa biológica (3:1) (Dehaut *et al.*, 2016). Os *beckers* foram vedados com papel alumínio e deixados à temperatura ambiente. O conteúdo foi mantido em temperatura ambiente e agitado duas vezes ao dia para ajudar na dissolução da matéria orgânica até a completa digestão poder ser observada.

A filtragem da solução digerida foi realizada com um aparelho de filtração a vácuo, utilizando microfiltro fibra de vidro GF-1 47mm Macherey Nagel (porosidade 0.7µm). Água milli-Q foi adicionada para melhorar o desempenho da filtração e retirada completa do material digerido do *beckers*. O microfiltro foi disposto em placas de petri de vidro individualmente e colocados para secar em estufa a 70°C por 1 hora. Os filtros foram observados utilizando-se o deslocamento em zigue-zague, com auxílio de um microscópio óptico composto de luz Carl Zeiss Microscopy GmbH (MOC) modelo Axio Scope A1, e capturadas com o auxílio do software Zen.

As imagens dos tecidos foram fotografadas por uma câmera colorida Axiocam 503 acoplada ao MOC. Os materiais encontrados foram contados e classificados de acordo com suas características de tipo e sua coloração seguindo o protocolo usado por Clere *et al.* (2022). As fibras foram medidas utilizando a distância de comprimento e largura, enquanto que para os fragmentos, foi realizada a medida da área. Após a análise dos filtros, os mesmos foram lavados com água milli-Q dentro de *beckers* fazendo um pool de todas as amostras de pulmão, íleo e estômago separadamente. Os *beckers* contendo o conteúdo decorrente da lavagem dos filtros foram colocados em estufa a 70°C até completa evaporação do líquido, sobrando apenas a parte

sólida que foi enviada para o laboratório de Química da Universidade Federal do Mato Grosso do Sul – UFMS para a análise espectroscópica.

#### *Espectroscopia no infravermelho por transformada de Fourier*

Os dados espectrais na região de infravermelho médio foram obtidos utilizando-se um espectrômetro FT/IR – Perkin Elmer modelo Frontier, com transformada de Fourier. As análises foram adquiridas na janela espectral de 500 até 4000  $\text{cm}^{-1}$ . As amostras foram preparadas por dispersão em pastilhas de KBr. Os espectros obtidos foram comparados com os da biblioteca de referência criada por Shimadzu (2020). Todos os filtros contendo microplásticos foram enviados em amostra única para cada órgão. Apenas os espectros que tiveram uma correspondência de 70% ou mais com o banco de dados padrão foram considerados confiáveis e registrados como um microplástico verificado (Zhao et al., 2018).

Todo processamento das amostras foi realizado tomando os cuidados para limitar a contaminação. As bancadas e materiais utilizados na preparação da digestão foram lavados com detergente e enxaguados com água milli-Q, e higienizados com etanol 70% antes da utilização. Para cada grupo de tecidos foram utilizados 2 filtros controles, sendo o primeiro para contaminação do ar e ambiente, e o segundo foi submetido à filtragem com a solução de KOH a 10 % e água milli-Q para garantir a pureza das soluções.

#### *Exame histopatológico*

Para o exame histopatológico foram coletados fragmentos de pulmão, íleo e porção inicial do estômago, cárdia. Os tecidos foram clivados em fragmentos de 3mm a 5mm, acomodados em cassetes e encaminhados ao Centro Técnico Histopatológico de Curitiba para o preparo e coloração das lâminas com Hematoxilina e Eosina (HE). As lâminas histopatológicas foram analisadas no laboratório do bloco Biossaúde da Universidade Católica Dom Bosco utilizando microscópio óptico composto de luz Carl Zeiss Microscopy GmbH (MOC) modelo Axio Scope A1, e capturadas com o auxílio do software Zen. As imagens dos tecidos foram fotografadas por uma câmera colorida AxioCam 503 acoplada ao MOC.

### *Análise estatística*

Foi avaliado um conjunto de modelos para explorar os efeitos do tamanho dos MPs, encontrados nos órgãos avaliados individualmente (íleo, estômago e pulmão) e em conjunto, na condição corporal dos gambas coletados. A condição corporal foi calculada utilizando os resíduos padronizados de uma regressão linear simples entre peso corporal e comprimento cabeça-corpo, calculados separadamente para machos e fêmeas. Neste estudo, a condição corporal foi usada como parâmetro de avaliação de saúde. Além disso, foi criado um modelo nulo sem variável explicativa. Utilizamos modelos lineares generalizados (GLM) comparados em uma abordagem baseada no critério de informação de Akaike corrigido para pequenas amostras (AICc) (Akaike 1974), considerando como plausíveis todos os modelos com  $\Delta AICc \leq 2$  (Burnham e Anderson 2004), utilizando o pacote ‘AICcmodavg’ versão 2.3–1 (Mazerolle, 2020) no R 3.5.0 (Equipe de Desenvolvimento do R, 2018).

### **Resultados e Discussão**

Foi detectada a presença de um total de 270 MPs no material filtrado obtido da digestão do trato gastrointestinal e do pulmão dos 22 animais capturados nas seis áreas de coleta da região urbana do Município de Campo Grande-MS. Observamos nas áreas Surucuá e Clube Carandá, que são áreas não preservadas, o total de 6 animais coletados, sendo coletados 4 e 2 animais de cada área respectivamente. O CEAP, CEA Anhandui, CEA Imbirussu e Coqueiral, que são áreas de proteção ambiental, foram amostrados 16 animais no total, sendo 2, 6, 3 e 5 de cada área, respectivamente. O Clube Carandá foi a área que apresentou maior média em relação à quantidade de MPs/animal, que foi de 21,5. A segunda maior média de MPs foi da área CEA Anhanduí que obteve uma média de 13,1 MPs/animal, seguido de 11,5 do Surucuá, 10,8 do Coqueiral, 10,6 do CEA Imbirussu e 8 MPs/animal na área CEAP (Tab. 1).

**Tab. 1** Locais onde foram capturados os gambás-de orelha branca e quantidade e média de microplásticos encontrados nesses animais por área.

| <i>Local</i>         | <i>Nº de animais</i> | <i>Quantidade de MPs</i> | <i>Média</i> |
|----------------------|----------------------|--------------------------|--------------|
| <i>Surucuá</i>       | 4                    | 32                       | 11,5         |
| <i>Clube Carandá</i> | 2                    | 43                       | 21,5         |
| <i>CEA Polonês</i>   | 2                    | 16                       | 8            |
| <i>CEA Anhandui</i>  | 6                    | 79                       | 13,16        |
| <i>CEA Imbirussu</i> | 3                    | 32                       | 10,66        |
| <i>Coqueiral</i>     | 5                    | 54                       | 10,8         |
| <b><i>TOTAL</i></b>  | <b>22</b>            | <b>270</b>               |              |

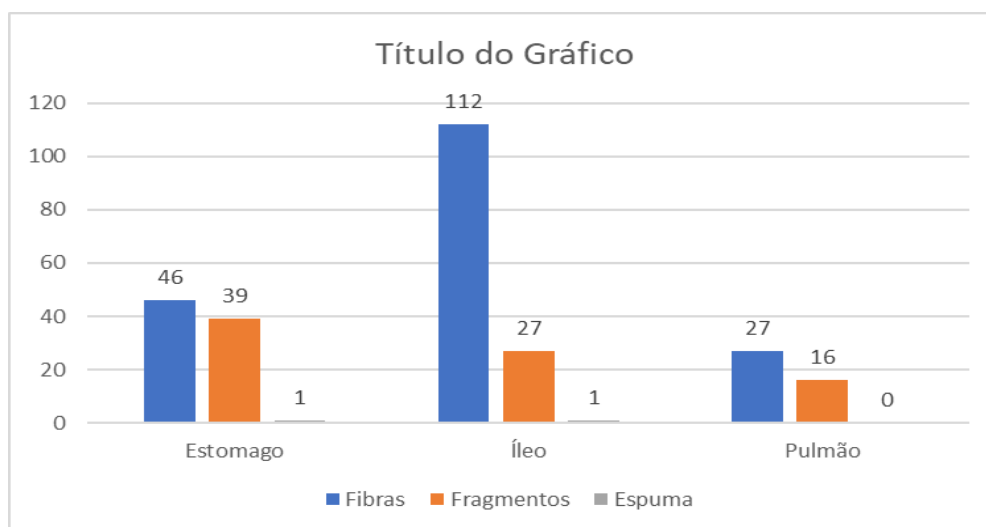
No presente estudo, todos os gambás-de-orelha-branca apresentaram MPs no trato gastrointestinal e pulmão, resultado que contribui para o aumento do número de estudos sobre estes contaminantes em mamíferos terrestres, uma vez que a maioria dos relatos se refere a animais marinhos, como bivalves (Li *et al.*, 2015), foca (Perez-Venegas *et al.*, 2018), peixes comerciais (Neves *et al.*, 2015), peixes pelágicos e demersais (Neves *et al.*, 2013), anêmona-do-mar (Morais *et al.*, 2020), arraias (Pegado *et al.*, 2021) e camarão (Hossain *et al.*, 2020). Em aves já foi detectado em trato gastrointestinal (Carlin *et al.*, 2020). Em animais terrestres, vem sendo relatado em ovelha (Beriot *et al.*, 2021), cães e gatos (Zhang, Wang e Kannan, 2019), frango (Lwanga *et al.*, 2017) e minhoca (Lwanga *et al.*, 2018).

Nosso estudo revela que os gambás-de-orelha-branca são passíveis de contaminação por MPs, sendo este resultado esperado pois são considerados a espécie mais comum em áreas com maior impacto antrópico (Cerqueira, 1985). Ainda, tem sido reportada a utilização de detritos plásticos do consumo humano como sacolas, em ninhos de *Didelphis albiventris* e como refúgio de *Thylamys sp.*, outro marsupial sul-americano (Bletter e Mitchell, 2021).

O Íleo foi o órgão que apresentou maior quantidade de MPs com 141 peças registradas (52%), seguido pelo estômago com 86 peças (32%) e pulmão com 43 peças (16%). Dos MPs presentes no íleo 112 peças eram fibras, que apareceram em número superior também no estômago (46) e no pulmão (27). O estômago foi o órgão que apresentou maior número de fragmentos, seguido do íleo com 27 fragmentos e pulmão com 16 fragmentos (Fig. 2). A maior

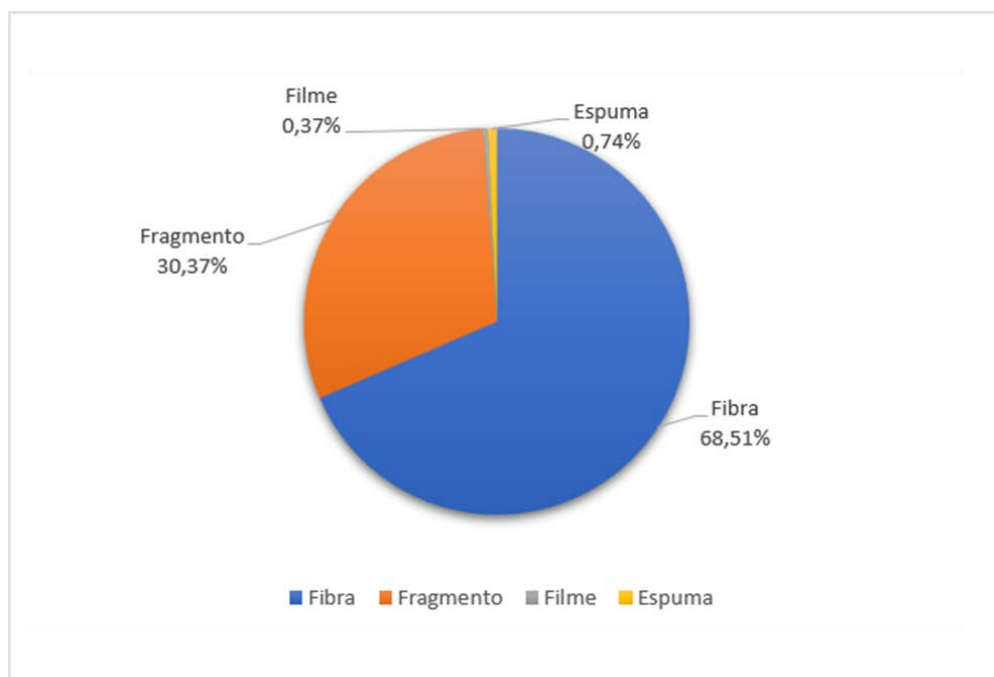
concentração de MPs no trato gastrointestinal nos gambás-de-orelha-branca do nosso estudo, pode indicar que a via de contaminação por ingestão foi a mais comum. De fato, para várias espécies de mamíferos, a ingestão é vista como a principal via de exposição (Meaza *et al.*, 2021), sendo os MPs mais detectados em trato digestório, especialmente no íleo devido à presença das células microdobradas (M) da placa de Peyer, localizada no íleo (Smith *et al.*, 1995; Arumugasaamy *et al.*, 2019).

Em relação característica alimentar onívora dos gambás, a presença de MPs nos gambás-de-orelha-branca do nosso estudo corrobora com os achados de Mizraji *et al.* (2017) e Ortíz *et al.* (2021), que demonstraram uma maior quantidade de partículas de MPs em peixes com dietas onívoras, que são mais amplas e menos seletivas, em comparação com peixes com dietas herbívoras e carnívoras.



**Fig. 2** Quantidades de microplásticos no estômago, íleo e pulmão em relação à sua morfologia

As fibras foram o tipo de MPs mais encontrado, com 185 peças (68,51%), seguido de fragmentos que totalizou 82 peças (30,37%), espuma (0,74%) e filme (0,37%) (Fig. 3). Esfera não foi observada.



**Fig. 3** Porcentagem das diferentes formas de microplásticos encontradas em estômago, íleo e pulmão de gambás-de-orelha-branca

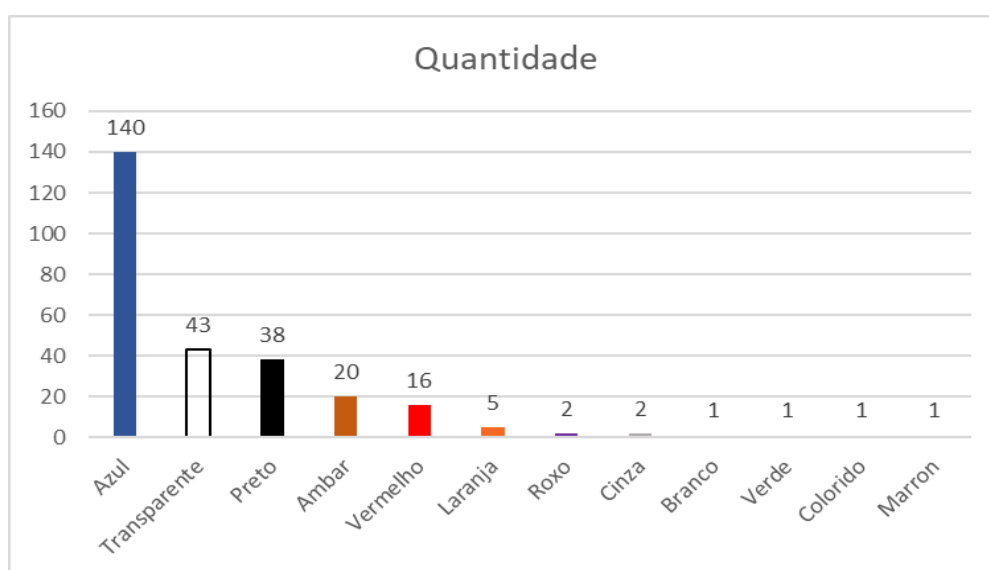
A fibra também foi a morfologia de maior prevalência em um estudo com peixes pelágicos, sendo relatada a ocorrência de fibras em 81% a 87% (Lopes *et al.*, 2023). Outros estudos como o de Bahrani *et al.* (2024) identificaram prevalência de fibras de MPs em trato intestinal de vaca e ovelha, trato intestinal de patos (Susanti *et al.*, 2021), assim como em trato gastrointestinal de aves terrestres na Flórida, EUA (Carlin *et al.*, 2020) e Xangai, China (Zhao *et al.*, 2016). As fibras também apareceram em maior quantidade no tecido pulmonar, sendo semelhantes aos achados encontrados em tecido pulmonar de porcos (Li *et al.*, 2023) e tecido pulmonar humano de pacientes com câncer de pulmão (Pauly *et al.*, 1998).

As fibras encontradas em maior quantidade podem estar relacionadas ao ambiente, já que são um dos tipos mais comuns observados tanto em ambiente marinho (Browne *et al.*, 2011) como no solo (Sing *et al.*, 2020; Zhou *et al.*, 2020). Os estudos de Pozo *et al.* (2019) e Zheng *et al.* (2019) demonstraram que fibras de MPs detectadas na biota de peixes coincidem com fibras detectadas em amostras de água de seus habitats. Qu *et al.*, (2018) reportam que existe uma correlação quantitativa entre fibras encontradas em mexilhões e o local onde vivem, representando 80% do total de MPs na água de 23 locais e em mexilhões de 18 locais.

Outra hipótese para a maior presença de fibras de MPs é que elas possuem um grande potencial de bioacumulação, quando comparado às outras formas. Estudo realizado em

mexilhão *Mytilus galloprovincialis* demonstrou um acúmulo maior de fibras de acordo com o período de exposição, se mantendo no organismo por maior tempo do que fragmento e esferas (Park *et al.*, 2024). No pulmão, por exemplo, as fibras podem se alinhar em paralelo ao eixo do fluxo respiratório, podendo penetrar na periferia do pulmão e nos alvéolos (Pauly *et al.*, 1998), e quando penetram profundamente no pulmão, as partículas ficam retidas (Timbrell, 1982). Uma pesquisa realizada com zebra-fish demonstrou que o acúmulo de fibras no intestino era maior quando comparado a fragmentos e esferas, sendo elas responsáveis por uma toxicidade intestinal mais grave do que as outras formas, como necrose mais séria das células epiteliais e diminuição notável a cobertura de células caliciformes e o volume de muco (Qiau *et al.*, 2019).

Em relação às colorações, os MPs azuis dominaram as amostras, totalizando 52% das peças, aparecendo em maior número em pulmão e íleo. Os MPs transparentes foram o segundo polímero encontrado em maior quantidade, com uma porcentagem de 16%, seguido de preto 14%, âmbar 7,4%, vermelho 6%, laranja 1,85%, roxo e cinza com 0,74%. Verde, branco, marrom e colorido foram as cores que menos apareceram, sendo 0,37% para cada cor, representado pela Fig. 4.

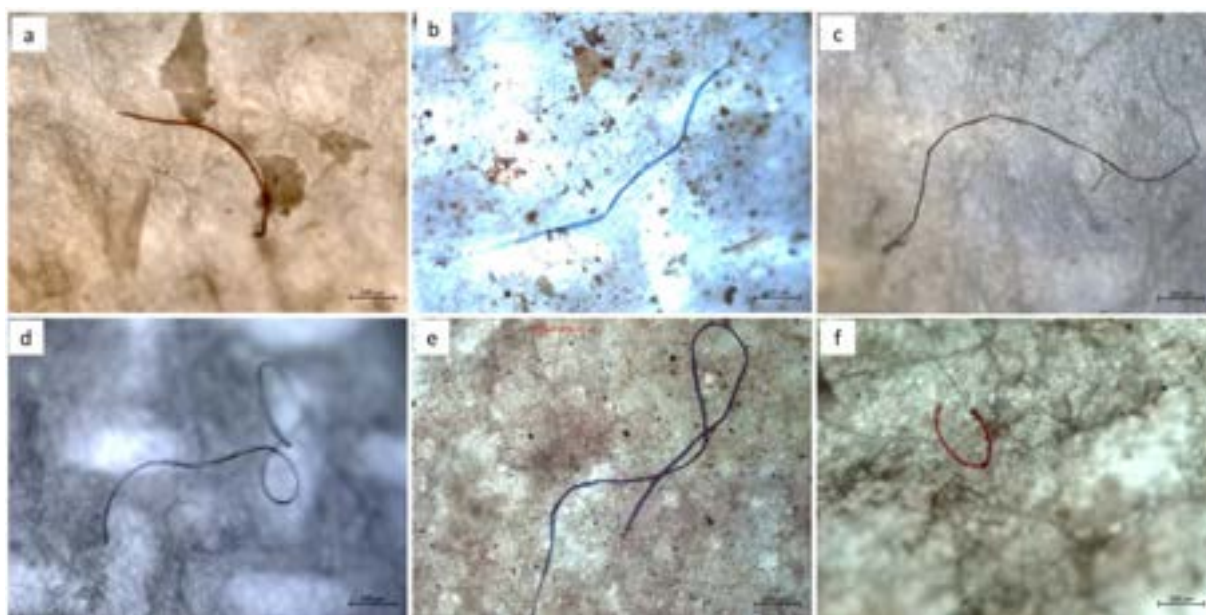


**Fig. 4** Quantidade de microplásticos encontrados em trato gastrointestinal e pulmão de gambás-de-orelha-branca, de acordo com a coloração.

As fibras de MPs foram encontradas em variadas cores, tais como azul, preto, vermelho e transparente. As fibras mais encontradas foram de coloração azul com 128 peças, seguida do transparente com 37 peças, preto com 23 peças e vermelho com 16 peças. Na Fig. 5, observa-se as diferentes cores de fibras encontradas no pulmão, como no íleo e no estômago. Avio e

colaboradores (2015) identificaram em peixes e invertebrados do mar que 47% das fibras plásticas eram de coloração azul, sendo a maioria, seguidas das cores transparente (30%) e preta (11%), sendo essa coloração semelhante à obtida no presente trabalho. Das fibras obtidas no estudo de Dhimmer (2017) com peixes da costa portuguesa, 37% também eram de coloração azul.

A observação de maiores quantidades de fibras azuis, encontradas nos gambás-de-orelha-branca do nosso estudo, também reportado na literatura, é explicada pelo fato dos plásticos azuis tenderem a se deteriorar mais rapidamente ao sol, por não conseguirem absorver adequadamente a luz UV, aumentando a taxa de fragmentação e, consequentemente, a quantidade de MPs azuis encontrados no ambiente, especialmente nos menores tamanhos (Martí *et al.*, 2020). Devido a isso, as cores devem ser levadas em conta nos estudos de plásticos, pois possuem um papel interativo na fragmentação e formação de MPs (Zhao *et al.*, 2022).

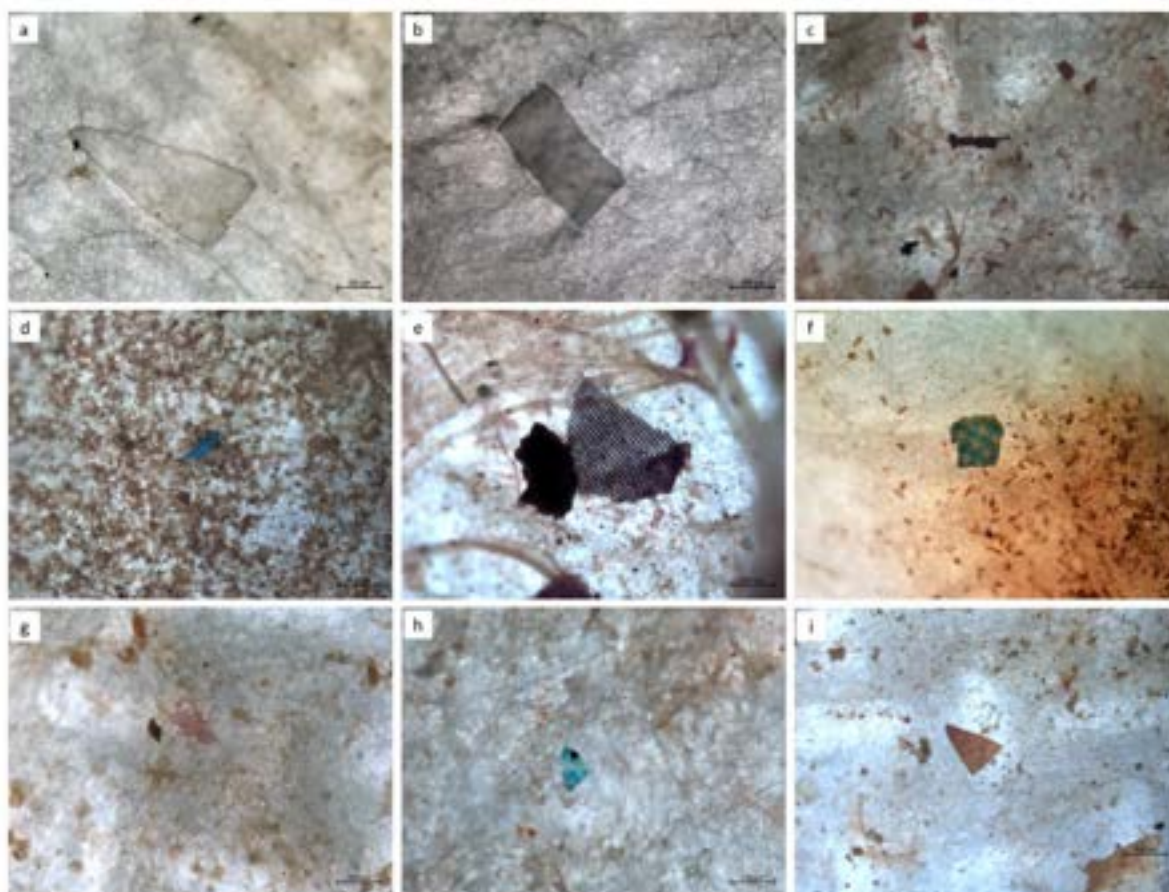


**Fig. 5** Fotomicrografia de trato gastrointestinal e pulmão de gambás-de-orelha-branca mostrando diferentes colorações das fibras de microplásticos. Estômago: fibra vermelha (a). Fígado: fibra azul (b); fibra preta (c); fibra transparente (d). Pulmão: fibra azul-royal (e); fibra vermelha (f).

Os fragmentos de MPs foram o segundo tipo morfológico mais encontrado, aparecendo em variados formatos. As cores encontradas foram azul, preto, âmbar, roxo, colorido, transparente e cinza, conforme Fig. 6. Os fragmentos âmbar foram os que apareceram mais vezes com 20 peças, enquanto que os pretos totalizaram 15 peças, azuis 12 peças, transparentes

6 peças, roxo e cinza 2 peças cada, e colorido 1 peça. Os fragmentos, assim como as fibras, também foram encontrados em pulmão, íleo e estômago.

A detecção de MPs de coloração âmbar em tratogastrointestinal de animais, é muito pouco reportada, apenas um estudo realizado na Colômbia encontrou pellets de coloração âmbar em uma praia turística em Cartagena (Acosta-Coley; Olivero-Verbel, 2015). A presença da coloração âmbar pode estar relacionada com o fotoenvelhecimento dos plásticos, que durante a exposição solar prolongada altera gradualmente a sua cor (descoloração) podendo mudar para amarelo ou âmbar (Martí *et al.*, 2020).



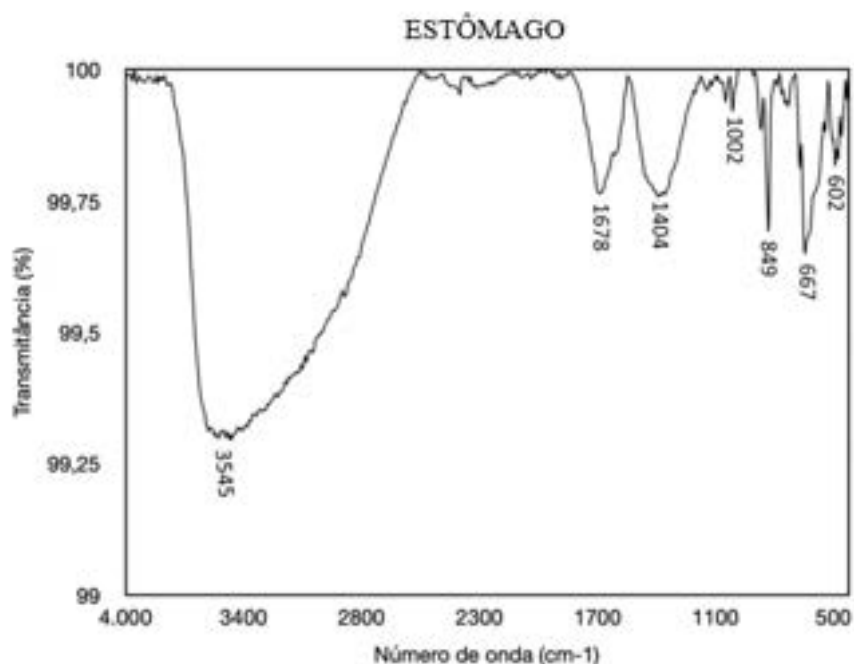
**Fig. 6** Fotomicrografia de trato gastrointestinal e pulmão de gambás-de-orelha-branca mostrando os diferentes formatos e colorações dos fragmentos de microplásticos. Estômago: fragmento transparente (a); fragmento cinza (b). Íleo: fragmento preto (c); fragmento azul (d); fragmento preto (e); fragmento azul com bolinhas amarelas (f). Pulmão: fragmento roxo (g); fragmento azul (h); fragmento âmbar (i).

Apesar de ter sido detectado MPs nos tecidos avaliados dos gambás-de-orelha-branca do presente estudo, não observamos influência na condição corporal (CC) dos indivíduos

(Material Suplementar 1), através das análises estatísticas. Provavelmente, a quantidade de MPs no trato gastrointestinal dos animais do estudo era muito pequena que pudesse causar bloqueio intestinal ou sentimento de saciedade, como De Vries *et al.*, 2020 descrevem em peixes de grande tamanho, como o bacalhau e escamudo, hipotetizando que, especialmente em indivíduos grandes, os MPs não são retidos em grande extensão e, se assim for, a CC provavelmente não é afetada. Diferentemente do que ocorre em peixes de pequeno tamanho (Critchell & Hoogemboon, 2024).

A análise em espectrômetro no infravermelho por transformada de Fourier, da amostra de estômago (Fig. 7) revelou bandas que permitem identificar a provável composição do material. A banda em  $1678\text{ cm}^{-1}$  sugere a presença de grupos C=O (carbonila), característica de polímeros contendo grupos éster, cetonas ou como resultado de degradação oxidativa, sendo especialmente comum em PVC que sofreu exposição ambiental, levando à formação de carbonilas. Adicionalmente, a banda em  $1404\text{ cm}^{-1}$  foi atribuída à deformação angular de C-H em grupos metilênicos ( $\text{CH}_2$ ), um traço comum em polímeros alifáticos como o PVC.

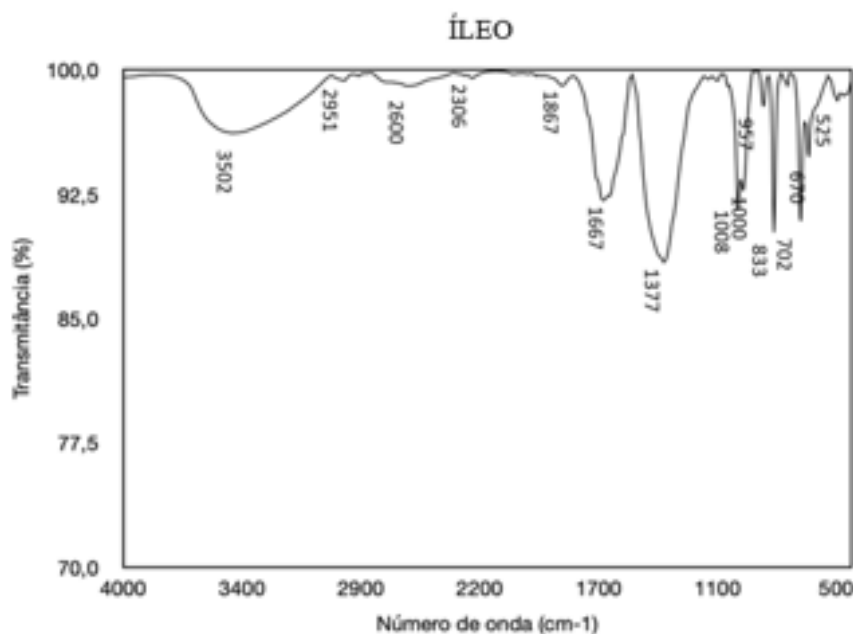
A banda em  $1002\text{ cm}^{-1}$  representa vibrações de C-O ou C-C, frequentemente observadas em materiais com grupos éster ou anéis aromáticos. A banda em  $849\text{ cm}^{-1}$  é indicativa de vibrações fora do plano em anéis aromáticos, que também podem estar presentes no PVC. Finalmente, as bandas em  $667\text{ cm}^{-1}$  e  $602\text{ cm}^{-1}$  são evidências diretas da presença de ligações C-Cl (cloro-carbono), que constituem uma assinatura espectral inequívoca do PVC. Com base nessas informações, conclui-se que a amostra analisada consiste predominantemente em PVC degradado.



**Fig. 7** Análise espectroscópica no infravermelho de microplásticos presentes em estômago de gambá-de-orelha-branca, mostrando a possível composição dos microplásticos de acordo com os comprimentos de onda e transmitância.

A análise do espectro FTIR da amostra de íleo (Fig. 8) revelou andas características que indicam a presença de PVC, como a observada em  $702\text{ cm}^{-1}$ , atribuída à vibração de ligação C-Cl, uma assinatura clara desse polímero, e a banda em  $833\text{ cm}^{-1}$ , relacionada a deformações fora do plano, também típica do PVC. A presença de PET é sugerida por várias bandas. A banda em  $1667\text{ cm}^{-1}$  pode ser atribuída ao estiramento do grupo C=O nos ésteres do PET. Bandas em  $1008$  e  $1000\text{ cm}^{-1}$  estão relacionadas às vibrações de C-O ou C-C, típicas de estruturas ésteres presentes no PET. A banda em  $833\text{ cm}^{-1}$ , que também indica deformações em anéis aromáticos, é característica do PET.

Bandas adicionais podem estar associadas à degradação ou à presença de misturas. A banda em  $1867\text{ cm}^{-1}$  sugere a formação de carbonilas, um indicativo de degradação em ambos os polímeros. A banda em  $2306\text{ cm}^{-1}$  pode estar relacionada à adsorção de dióxido de carbono, indicando exposição ambiental. Já a banda em  $1377\text{ cm}^{-1}$  representa a deformação de C-H, que é comum tanto no PVC quanto no PET. Com base nessa análise, conclui-se que a amostra contém uma mistura de PVC e PET, ambos degradados devido à exposição ambiental.



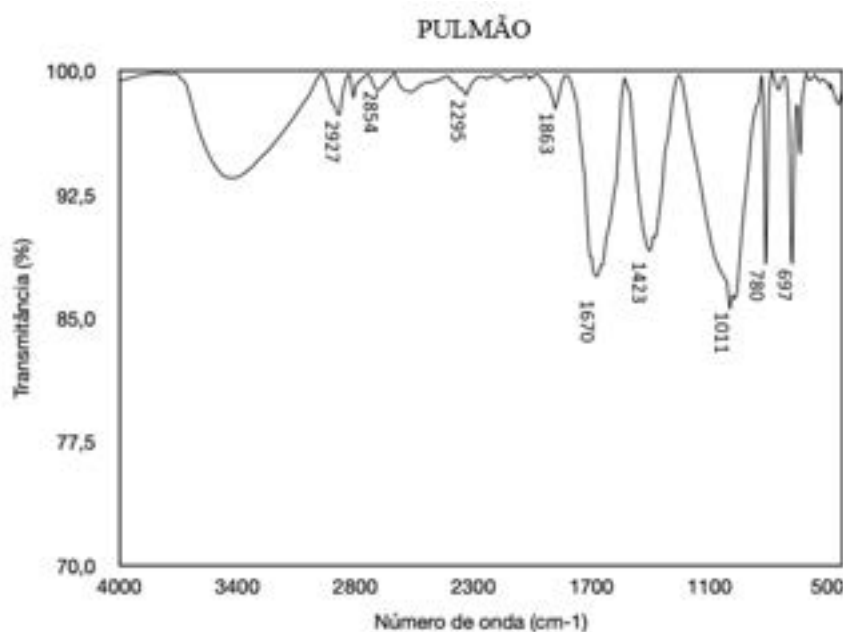
**Fig. 8** Análise espectroscópica no infravermelho de microplásticos presentes em óleo de gambá-de-orelha-branca, mostrando a possível composição dos microplásticos de acordo com os comprimentos de onda e transmitância.

A análise espectroscópica realizada da amostra de pulmão (Fig. 9) indica que as bandas em 2927 e 2854  $\text{cm}^{-1}$  foram atribuídas às vibrações de estiramento de C-H em grupos metilênicos ( $\text{CH}_2$ ) e metila ( $\text{CH}_3$ ), características de polímeros alifáticos como o PVC (Policloreto de Vinila) e outros plásticos. A banda em 2295  $\text{cm}^{-1}$ , por sua vez, é atribuída ao estiramento do grupo nitrila ( $\text{C}\equiv\text{N}$ ), uma assinatura típica de polímeros como o ABS (Acrilonitrila-butadieno-estireno). A banda em 1670  $\text{cm}^{-1}$  sugere a presença de estiramentos de  $\text{C}=\text{O}$ , que são comumente encontrados em poliésteres como o PET (Politereftalato de Etileno).

Além disso, a banda em 1423  $\text{cm}^{-1}$  foi identificada como devida à deformação angular de C-H ou estiramento de  $\text{C}=\text{C}$ , característica de polímeros como PVC ou outros materiais aromáticos. A banda em 1011  $\text{cm}^{-1}$  indica vibrações de C-O ou deformações em anéis aromáticos, que podem ser atribuídas tanto ao PET quanto ao ABS. Já as bandas em 780 e 697  $\text{cm}^{-1}$  fornecem informações adicionais relevantes: a banda em 697  $\text{cm}^{-1}$  é uma confirmação de deformações fora do plano de C-Cl, indicando a presença de PVC, enquanto a banda em 780  $\text{cm}^{-1}$  pode ser associada a deformações aromáticas, sugerindo PET ou ABS.

A partir dessas informações, conclui-se que a amostra contém uma mistura de diferentes microplásticos. O PVC é o principal componente, conforme evidenciado pela banda forte em 697  $\text{cm}^{-1}$ , juntamente com outras bandas associadas ao mesmo polímero. A presença de PET é

sustentada pelas bandas em 1670 e 1011  $\text{cm}^{-1}$ , que indicam a presença de grupos éster e anéis aromáticos. Finalmente, a banda em 2295  $\text{cm}^{-1}$  confirma a existência de grupos nitrila, característicos de ABS. Portanto, a amostra analisada é composta predominantemente por PVC, com indícios adicionais de PET e ABS.



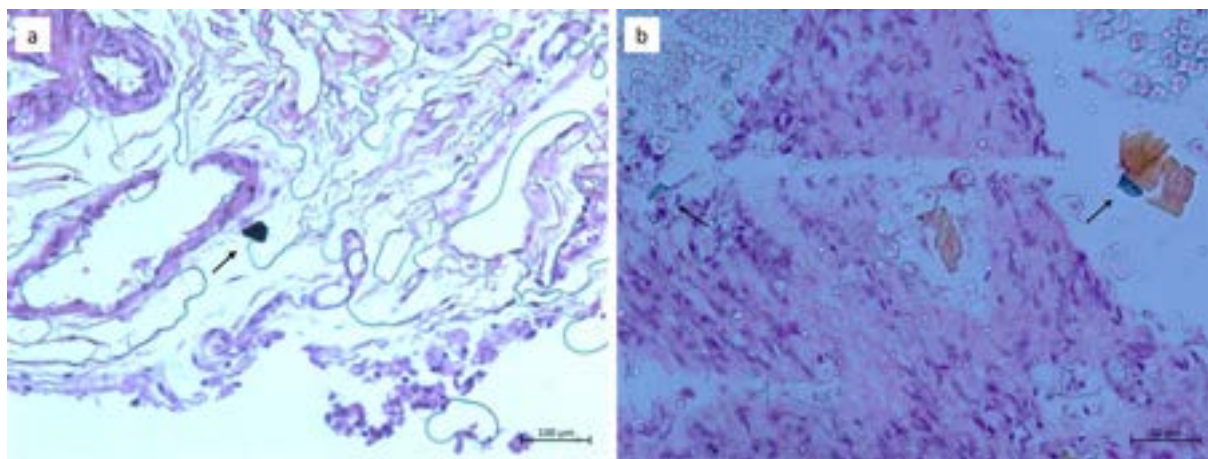
**Fig. 9** Análise espectroscópica no infravermelho de microplásticos presentes em pulmão de gambá-de-orelha-branca, mostrando a possível composição dos microplásticos de acordo com os comprimentos de onda e transmitância.

No total, três tipos de polímeros foram identificados. Eles são polietileno tereftalato (PET), Acrilonitrila Butadieno Estireno (ABS) e policloreto de vinila (PVC), ambos plásticos que se encontram dentro da classificação de termoplásticos, (PLASTICEUROPE, 2018). Observamos que todas as amostras tinham a presença de PVC. Íleo e pulmão apresentaram também a presença de PET e o pulmão foi o órgão onde foram identificados os três tipos de polímeros.

A presença do PVC e do PET no trato gastrointestinal e pulmão dos gambás-de-orelha-branca pode ser atribuído à presença grande quantidade desses polímeros no ambiente (Akdogan & Guven, 2019), pois estão entre os MPs mais encontrados tanto no solo (Yang et al., 2022) quanto no ar (Yao et al., 2022). Os animais podem ingerir PVC e PET de sacolas plásticas, garrafas, cordas e itens comumente usados em embalagens (Wang, Zhao e Xing, 2021). Estudo de Panpong e Duengkae (2024) revelaram que PET foi um dos polímeros predominantes encontrados em vertebrados terrestres e girinos no oeste da Tailândia.

Já o ABS, que foi encontrado apenas em amostra de pulmão, tem sido pouco reportada a presença desse polímero no ambiente. Entretanto, em uma pesquisa realizada em um pântano utilizado para tratamento de transbordamento de esgoto, utilizaram diferentes índices para classificação de risco ecológicos associados aos MPs, que demonstrou que os perigos ambientais representados pelo ABS superaram consideravelmente os ligados a outros tipos de polímeros (Sarti *et al.*, 2024), sendo de grande importância novas pesquisas para avaliar a presença desse polímero.

A observação das lâminas histopatológicas revelou a presença de poucos MPs em apenas 2 fragmentos, localizados no interstício do intestino e na luz do estômago, em meio ao conteúdo, sem reação inflamatória nos órgãos (Fig. 10). O parênquima pulmonar não apresentou MPs e nem alterações.



**Fig. 10** Fotomicrografia mostrando a presença de MPs em trato gastrointestinal de gambá-de-orelha-branca. (a) intestino apresentando fragmento de microplásticos de coloração preta ao lado da glândula intestinal (seta); (b) fragmento de microplásticos de coloração de HE.

Um estudo realizado na Noruega investigou MPs em tecidos de peixes, aves marinhas, mamíferos terrestres e marinhos de uma área costeira altamente poluída por plásticos, não identificando presença de MPs na histopatologia, mesmo em tecidos positivos para MP pela análise química, nenhuma reação tecidual foi encontrada (Haave *et al.*, 2021).

A maioria dos estudos realizados contendo avaliação histopatológica de efeitos dos MPs são experimentais, realizados em laboratório (Rodriguez-Seijo *et al.*, 2017; Hamed *et al.*, 2021; Ali *et al.*, 2023), com indução do dano (Hoseini *et al.*, 2022; Sayed *et al.*, 2023; Saleh *et al.*, 2025). Esses estudos de exposição de MPs por indução usam concentrações muito altas de MPs comerciais que não foram considerados ambientalmente relevantes (Allen *et al.*, 2022; Bour *et*

*al.*, 2018; Sun *et al.*, 2021). Desta forma, são necessários estudos experimentais em mamíferos terrestres com concentrações de MPs normalmente encontradas na natureza para compreender seus efeitos como biomarcadores histopatológicos em organismos.

## **Conclusão**

Todos os indivíduos de gambás-de-orelha-branca avaliados neste estudo apresentaram MPs em pelo menos um dos três órgãos avaliados, sugerindo que estes animais podem funcionar como sentinelas da contaminação ambiental. Apesar de não ter sido observado efeito dos MPs nos tecidos avaliados e na condição corpórea dos animais, nossos resultados contribuem para a detecção destes contaminantes em tecidos de mamíferos terrestres, e demonstra a necessidade de mais pesquisas sobre os riscos ecológicos associados à poluição por PVC, PET, ABS e seus efeitos na saúde dos animais.

## **Aprovação ética**

A pesquisa teve aceite do Comitê de Ética no Uso de Animais da Universidade Católica Dom Bosco (nº. 013/2020) e do Sistema de Autorização e Informação em Biodiversidade (70946-5 e nº89586-1). Os autores não têm interesses financeiros ou não financeiros relevantes a declarar.

## **Declaração de Conflitos de Interesses**

O(s) autor(es) declarou(aram) não haver potenciais conflitos de interesse com relação à pesquisa, autoria e/ou publicação deste artigo.

## **Disponibilidade dos dados**

Não aplicável.

## **Responsabilidades éticas dos autores**

Todos os autores leram, compreenderam e cumpriram, conforme aplicável, a declaração sobre "Responsabilidades éticas dos autores", conforme encontrado nas Instruções para autores”

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## APÊNDICE

### Material suplementar

Tabela 1. Conjunto de modelos criados para explorar os efeitos da área do microplásticos, encontrados nos órgãos avaliados individualmente (íleo, estômago e pulmão) e total, na condição corporal dos gambas coletados. K=Número de parâmetros; AICc=Critério de Informação Delta de Akaike; AICcWt=Peso de Akaike; Cum.Wt=Peso de Akaike Cumulativo.

| <b>Modelos</b>                                                   | <b>K</b> | <b>AICc</b> | <b><math>\Delta</math>AICc</b> | <b>AICcWt</b> | <b>Cum.Wt</b> |
|------------------------------------------------------------------|----------|-------------|--------------------------------|---------------|---------------|
| Modelo 5 (Condição Corporal ~ 1)                                 | 2        | 290.66      | 0                              | 0.48          | 0.48          |
| Modelo 2 (Condição Corporal ~ área de microplástico no pulmão)   | 3        | 293.23      | 2.57                           | 0.13          | 0.61          |
| Modelo 3 (Condição Corporal ~ área de microplástico no íleo)     | 3        | 293.26      | 2.6                            | 0.13          | 0.74          |
| Modelo 1(Condição Corporal ~ área de microplástico total)        | 3        | 293.29      | 2.63                           | 0.13          | 0.87          |
| Modelo 4 (Condição Corporal ~ área de microplástico no estômago) | 3        | 293.33      | 2.67                           | 0.13          | 1             |



# Submission guidelines

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## **Instructions for Authors**

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### **Types of Papers**

The journal welcomes a variety of article types.

**Original research papers** are original manuscripts that contain new findings in research consistent with the Journal's aims and scope.

**Review articles** do not contain new information, but rather summarize emerging trends or recent developments. These papers critically evaluate and summarize existing data and fields of research.

**Short Communications** can include new research, technologies, reviews, case studies, and practices as it relates to environmental monitoring and assessment. The Editorial Board will attempt to streamline the review process for this type of manuscript. Short Communications should contain about 3000 words of text, a maximum of two tables and/or figures, and no more than 20 references.

**Letter to the Editor** addressing previously published articles may be edited for clarity or length and may be subject to peer review at the Editors' discretion. The letters will be sent to the corresponding author of the paper on which comments are submitted, and the authors of that paper will be given an opportunity to respond to the comments if they wish. Both the original comment and any responses are then published at the same time.

## **Paper Length and Page Charges**

Papers must be concise and well written. While there are no specific word limits, the average article contains approximately 9,000 words. Longer papers may be

considered if the information justifies the length.

There are no page charges to publish in this journal under the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription).

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## Manuscript Submission

### Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

### Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) for both the print and online format and to include evidence that such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to originate from the authors.

### Online Submission

Please follow the hyperlink “Submit manuscript” and upload all of your manuscript files following the instructions given on the screen.

## Source Files

Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

## Submitting Declarations

Please note that [Author Contribution information](#) and [Competing Interest information](#) must be provided at submission via the submission interface. Only the information submitted via the interface will be used in the final published version. Please make sure that if you are an editorial board member and also a listed author that you also declare this information in the Competing Interest section of the interface.

Please see the relevant sections in the submission guidelines for further information on these statements as well as possible other mandatory statements.

## Language

We appreciate any efforts that you make to ensure that the language is corrected before submission. This will greatly improve the legibility of your paper if English is not your first language.

## **When submitting your manuscript**

The author should suggest three to five potential reviewers who are qualified to judge the work objectively, providing full names, institutions, and current e-mail addresses.

When submitting your paper, please include your field of interest/expertise in the cover letter. Authors are expected to at least review two papers for the journal when invited.

Please choose the appropriate article type (e.g. Original Research Article, Review, etc.) while submitting your paper to the journal. Please do not submit your paper to a Topical Collection if you have not been invited to do so by a guest editor.

## **Manuscript Structure**

### **Original Research Articles**

#### **Abstract**

The Abstract should contain 150 to 250 words. It should clearly explain the novelty of the findings versus current knowledge. The Abstract should not contain any undefined abbreviations or unspecified references.

#### **Keywords**

This should include 4 to 6 keywords that can be used for indexing purposes.

## Text

The text of original research articles should be divided into the following sections:

### Introduction

The Introduction should state the purpose of the investigation and identify clearly the gap of knowledge that will be filled in this study.

### Materials and methods

The Materials and Methods section should provide enough information to permit repetition of the experimental work. It should include clear descriptions and explanations of sampling procedures, experimental design, essential sample characteristics and descriptive statistics, hypothesis tested, exact references to literature describing the tests used in the manuscript, number of data involved in statistical tests, etc.

### Results and Discussion

The Results section should describe the outcome of the study. Data should be presented as concisely as possible – if appropriate in the form of tables or figures, although very large tables should be avoided. The Discussion should be an interpretation of the results and their significance with reference to work by other authors.

## Conclusions

This section should highlight the major, firm discoveries, and state what the added value of the main finding is, without literature references.

## References

The journal uses APA reference style. See References for more details.

## Appendices

If there is more than one appendix, they should be numbered consecutively. Equations in appendices should be designated differently from those in the main body of the paper, e.g. (A1), (A2) etc. In each appendix equations should be numbered separately.

## Springer Author Academy

Beginning authors or authors who have not submitted to international journals before may find the Springer tutorials on the 'Springer Author Academy' homepage of assistance when writing their articles. Click 'Springer Author Academy' tab in the sidebar or go to

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## Title Page

Please make sure your title page contains the following information.

### Title

The title should be concise and informative.

### Author information

The name(s) of the author(s)

The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country

A clear indication and an active e-mail address of the corresponding author

If available, the 16-digit [ORCID](#) of the author(s)

If address information is provided with the affiliation(s) it will also be published.

For authors that are (temporarily) unaffiliated we will only capture their city and country of residence, not their e-mail address unless specifically requested.

Large Language Models (LLMs), such as [ChatGPT](#), do not currently satisfy our [authorship criteria](#). Notably an attribution of authorship carries with it accountability for the work, which cannot be effectively applied to LLMs. Use of an LLM should be properly documented in the Methods section (and if a Methods section is not

available, in a suitable alternative part) of the manuscript. The use of an LLM (or other AI-tool) for "AI assisted copy editing" purposes does not need to be declared. In this context, we define the term "AI assisted copy editing" as AI-assisted improvements to human-generated texts for readability and style, and to ensure that the texts are free of errors in grammar, spelling, punctuation and tone. These AI-assisted improvements may include wording and formatting changes to the texts, but do not include generative editorial work and autonomous content creation. In all cases, there must be human accountability for the final version of the text and agreement from the authors that the edits reflect their original work.

## **Abstract**

Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

*For life science journals only (when applicable)*

Trial registration number and date of registration for prospectively registered trials

Trial registration number and date of registration, followed by “retrospectively registered”, for retrospectively registered trials

## **Keywords**

Please provide 4 to 6 keywords which can be used for indexing purposes.

## **Statements and Declarations**

The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

**Competing Interests:** Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to “Competing Interests and Funding” below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

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## Text

### Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using [Springer Nature's LaTeX template](#).

## Headings

Please use no more than three levels of displayed headings.

## Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

## Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other

statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

## **Acknowledgments**

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

## **Additional request Text**

- All manuscript files should be formatted to contain line numbers.
- Quotations of more than 40 words should be set off clearly, either by indenting the left-hand margin or by using a smaller typeface. Use double quotation marks for direct quotations and single quotation marks for quotations within quotations and for words or phrases used in a special sense.

## **Additional instructions Headings**

Level one headers: typed in bold, lowercase except for first letter of first word, left justified, followed by one blank line

Level two headers: typed in normal font, lowercase except for first letter of first word, left justified, followed by one blank line

Do NOT number headings and subheadings.

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## References

### Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson, 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott, 1991; Barakat et al., 1995; Kelso & Smith, 1998; Medvec et al., 1999).

Authors are encouraged to follow official APA version 7 guidelines on the number of authors included in reference list entries (i.e., include all authors up to 20; for larger groups, give the first 19 names followed by an ellipsis and the final author's name). However, if authors shorten the author group by using et al., this will be retained.

### Reference list

The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal names and book titles should be *italicized*.

If available, please always include DOIs as full DOI links in your reference list (e.g. “<https://doi.org/abc>”).

Journal article Grady, J. S., Her, M., Moreno, G., Perez, C., & Yelinek, J. (2019). Emotions in storybooks: A comparison of storybooks that represent ethnic and racial groups in the United States. *Psychology of Popular Media Culture*, 8(3), 207–217. <https://doi.org/10.1037/ppm0000185>

Article by DOI Hong, I., Knox, S., Pryor, L., Mroz, T. M., Graham, J., Shields, M. F., & Reistetter, T. A. (2020). Is referral to home health rehabilitation following inpatient rehabilitation facility associated with 90-day hospital readmission for adult patients with stroke? *American Journal of Physical Medicine & Rehabilitation*. Advance online publication. <https://doi.org/10.1097/PHM.0000000000001435>

Book Sapolsky, R. M. (2017). *Behave: The biology of humans at our best and worst*. Penguin Books.

Book chapter Dillard, J. P. (2020). Currents in the study of persuasion. In M. B. Oliver, A. A. Raney, & J. Bryant (Eds.), *Media effects: Advances in theory and research* (4th ed., pp. 115–129). Routledge.

Online document Fagan, J. (2019, March 25). *Nursing clinical brain*. OER Commons. Retrieved January 7, 2020, from <https://www.oercommons.org/authoring/53029-nursing-clinical-brain/view>

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## Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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## Artwork and Illustrations Guidelines

### Electronic Figure Submission

Supply all figures electronically.

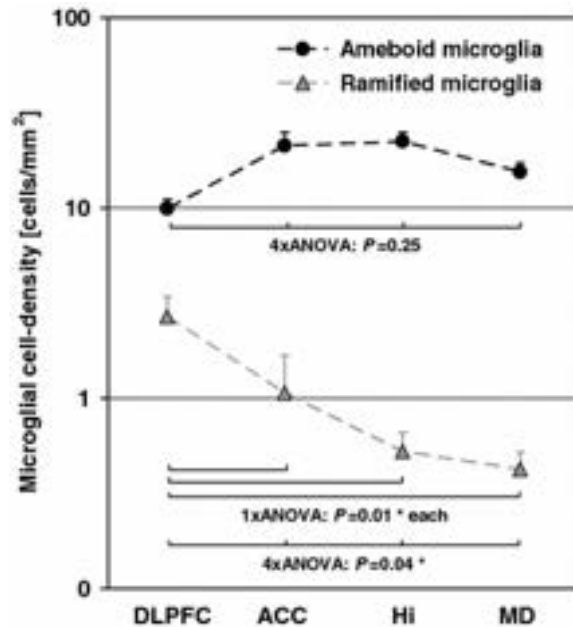
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

## Line Art



Definition: Black and white graphic with no shading.

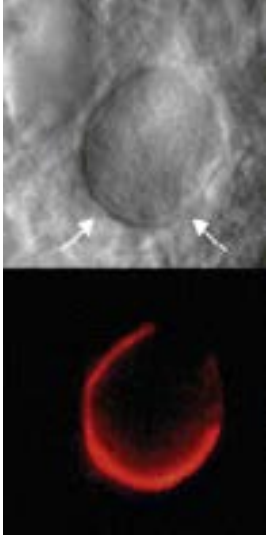
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

## Halftone Art

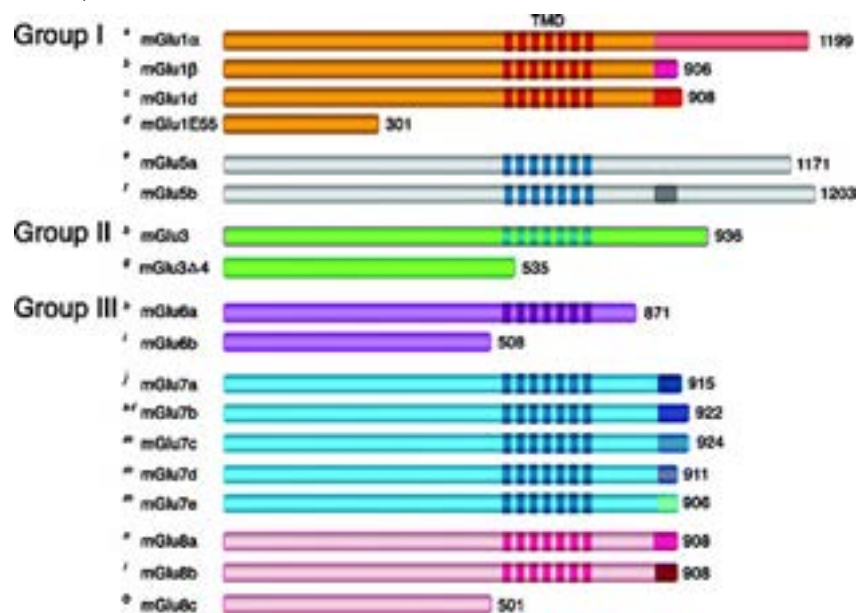


Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

## Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

## Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

## Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

## Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

## Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

## **Figure Placement and Size**

Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.

When preparing your figures, size figures to fit in the column width.

For large-sized journals the figures should be 84 mm (for double-column text areas), or 174 mm (for single-column text areas) wide and not higher than 234 mm.

For small-sized journals, the figures should be 119 mm wide and not higher than 195 mm.

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## Accessibility

In order to give people of all abilities and disabilities access to the content of your figures, please make sure that

- All figures have descriptive captions (blind users could then use a text-to-speech software or a text-to-Braille hardware)

- Patterns are used instead of or in addition to colors for conveying information (colorblind users would then be able to distinguish the visual elements)

- Any figure lettering has a contrast ratio of at least 4.5:1

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## Supplementary Information (SI)

Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

Before submitting research datasets as Supplementary Information, authors should read the journal's Research data policy. We encourage research data to be archived in data repositories wherever possible.

## Submission

Supply all supplementary material in standard file formats.

Please include in each file the following information: article title, journal name, author names; affiliation and e-mail address of the corresponding author.

To accommodate user downloads, please keep in mind that larger-sized files may require very long download times and that some users may experience other problems during downloading.

High resolution (streamable quality) videos can be submitted up to a maximum of 25GB; low resolution videos should not be larger than 5GB.

## Audio, Video, and Animations

Aspect ratio: 16:9 or 4:3

Maximum file size: 25 GB for high resolution files; 5 GB for low resolution files

Minimum video duration: 1 sec

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## Text and Presentations

Submit your material in PDF format; .doc or .ppt files are not suitable for long-term viability.

A collection of figures may also be combined in a PDF file.

## Spreadsheets

Spreadsheets should be submitted as .csv or .xlsx files (MS Excel).

## Specialized Formats

Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

## Collecting Multiple Files

It is possible to collect multiple files in a .zip or .gz file.

## Numbering

If supplying any supplementary material, the text must make specific mention of the material as a citation, similar to that of figures and tables.

Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.

Name the files consecutively, e.g. “ESM\_3.mpg”, “ESM\_4.pdf”.

## Captions

For each supplementary material, please supply a concise caption describing the content of the file.

## Processing of supplementary files

Supplementary Information (SI) will be published as received from the author without any conversion, editing, or reformatting.

## Accessibility

In order to give people of all abilities and disabilities access to the content of your supplementary files, please make sure that

The manuscript contains a descriptive caption for each supplementary material

Video files do not contain anything that flashes more than three times per second (so that users prone to seizures caused by such effects are not put at risk)

## Generative AI Images

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内容が適切に組み立てられ、質の高い英語で書かれた論文を投稿すれば、編集者や査読者が論文を理解し、公正に評価するための最善の機会となります。多くの研究者は、個別のサポートを受けることで、研究結果を可能な限り最高の形で発表できと思っています。

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## Korean (한국어)

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After online publication, further changes can only be made in the form of an Erratum, which will be hyperlinked to the article.

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The article will be published online after receipt of the corrected proofs. This is the official first publication citable with the DOI. After release of the printed version, the paper can also be cited by issue and page numbers.

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If it is not possible to share research data publicly, for instance when individual privacy could be compromised, this statement should describe how data can be accessed and any conditions for reuse. Participant consent should be obtained and documented prior to data collection. See our [guidance on sensitive data](#) for more information.

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AND

2) drafting the article or revising it critically for important intellectual content;

AND

3) final approval of the version to be submitted for publication.

All of these conditions should be met by all authors.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section.

All authors must agree on the sequence of authors listed before submitting the article.

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If the article is accepted for publication, after acceptance, no changes in authorship, the order of authors, or designation of the corresponding author will be permitted.

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## Statement of Editorial and Publication Policies

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Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are

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Research involving Human Participants and/or Animals

Informed consent

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## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

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No funding was received to assist with the preparation of this manuscript.

No funding was received for conducting this study.

No funds, grants, or other support was received.

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**Non-financial interests:** none.

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The authors have no competing interests to declare that are relevant to the content of this article.

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## **Research involving human participants, their data or biological material**

### **Ethics approval**

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the [1964 Declaration of Helsinki](#) and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the

1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

## **Retrospective ethics approval**

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Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

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individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

## Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the [NCBI database](#) for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

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The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

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## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

- All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).
- This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).
- Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.
- The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

- Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.
- This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our

study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

- This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

- This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.
- The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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## Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

- Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.
- Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

### **Consent and already available data and/or biologic material**

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

### **Data protection, confidentiality and privacy**

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered “informed”. However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

## **Consent to Participate**

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

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Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

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## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for "**Consent to participate**":

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

### Sample statements for “Consent to publish”:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

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Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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## Peer Review Guidelines for Authors and Reviewers

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# Submission guidelines

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## Instructions for Authors

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### Types of Papers

The journal welcomes a variety of article types.

**Original research papers** are original manuscripts that contain new findings in research consistent with the Journal's aims and scope.

**Review articles** do not contain new information, but rather summarize emerging trends or recent developments. These papers critically evaluate and summarize existing data and fields of research.

**Short Communications** can include new research, technologies, reviews, case studies, and practices as it relates to environmental monitoring and assessment. The Editorial Board will attempt to streamline the review process for this type of manuscript. Short Communications should contain about 3000 words of text, a maximum of two tables and/or figures, and no more than 20 references.

**Letter to the Editor** addressing previously published articles may be edited for clarity or length and may be subject to peer review at the Editors' discretion. The letters will be sent to the corresponding author of the paper on which comments are submitted, and the authors of that paper will be given an opportunity to respond to the comments if they wish. Both the original comment and any responses are then published at the same time.

## **Paper Length and Page Charges**

Papers must be concise and well written. While there are no specific word limits, the average article contains approximately 9,000 words. Longer papers may be

considered if the information justifies the length.

There are no page charges to publish in this journal under the normal publication process (whereby an article is submitted to the journal and access to that article is granted to customers who have purchased a subscription).

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## Manuscript Submission

### Manuscript Submission

Submission of a manuscript implies: that the work described has not been published before; that it is not under consideration for publication anywhere else; that its publication has been approved by all co-authors, if any, as well as by the responsible authorities – tacitly or explicitly – at the institute where the work has been carried out. The publisher will not be held legally responsible should there be any claims for compensation.

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Please follow the hyperlink “Submit manuscript” and upload all of your manuscript files following the instructions given on the screen.

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Please ensure you provide all relevant editable source files at every submission and revision. Failing to submit a complete set of editable source files will result in your article not being considered for review. For your manuscript text please always submit in common word processing formats such as .docx or LaTeX.

## Submitting Declarations

Please note that [Author Contribution information](#) and [Competing Interest information](#) must be provided at submission via the submission interface. Only the information submitted via the interface will be used in the final published version. Please make sure that if you are an editorial board member and also a listed author that you also declare this information in the Competing Interest section of the interface.

Please see the relevant sections in the submission guidelines for further information on these statements as well as possible other mandatory statements.

## Language

We appreciate any efforts that you make to ensure that the language is corrected before submission. This will greatly improve the legibility of your paper if English is not your first language.

## **When submitting your manuscript**

The author should suggest three to five potential reviewers who are qualified to judge the work objectively, providing full names, institutions, and current e-mail addresses.

When submitting your paper, please include your field of interest/expertise in the cover letter. Authors are expected to at least review two papers for the journal when invited.

Please choose the appropriate article type (e.g. Original Research Article, Review, etc.) while submitting your paper to the journal. Please do not submit your paper to a Topical Collection if you have not been invited to do so by a guest editor.

## **Manuscript Structure**

### **Original Research Articles**

#### **Abstract**

The Abstract should contain 150 to 250 words. It should clearly explain the novelty of the findings versus current knowledge. The Abstract should not contain any undefined abbreviations or unspecified references.

#### **Keywords**

This should include 4 to 6 keywords that can be used for indexing purposes.

## Text

The text of original research articles should be divided into the following sections:

### Introduction

The Introduction should state the purpose of the investigation and identify clearly the gap of knowledge that will be filled in this study.

### Materials and methods

The Materials and Methods section should provide enough information to permit repetition of the experimental work. It should include clear descriptions and explanations of sampling procedures, experimental design, essential sample characteristics and descriptive statistics, hypothesis tested, exact references to literature describing the tests used in the manuscript, number of data involved in statistical tests, etc.

### Results and Discussion

The Results section should describe the outcome of the study. Data should be presented as concisely as possible – if appropriate in the form of tables or figures, although very large tables should be avoided. The Discussion should be an interpretation of the results and their significance with reference to work by other authors.

## Conclusions

This section should highlight the major, firm discoveries, and state what the added value of the main finding is, without literature references.

## References

The journal uses APA reference style. See References for more details.

## Appendices

If there is more than one appendix, they should be numbered consecutively. Equations in appendices should be designated differently from those in the main body of the paper, e.g. (A1), (A2) etc. In each appendix equations should be numbered separately.

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## Title Page

Please make sure your title page contains the following information.

### Title

The title should be concise and informative.

### Author information

The name(s) of the author(s)

The affiliation(s) of the author(s), i.e. institution, (department), city, (state), country

A clear indication and an active e-mail address of the corresponding author

If available, the 16-digit [ORCID](#) of the author(s)

If address information is provided with the affiliation(s) it will also be published.

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Please provide an abstract of 150 to 250 words. The abstract should not contain any undefined abbreviations or unspecified references.

*For life science journals only (when applicable)*

Trial registration number and date of registration for prospectively registered trials

Trial registration number and date of registration, followed by “retrospectively registered”, for retrospectively registered trials

## Keywords

Please provide 4 to 6 keywords which can be used for indexing purposes.

## Statements and Declarations

The following statements should be included under the heading "Statements and Declarations" for inclusion in the published paper. Please note that submissions that do not include relevant declarations will be returned as incomplete.

**Competing Interests:** Authors are required to disclose financial or non-financial interests that are directly or indirectly related to the work submitted for publication. Please refer to “Competing Interests and Funding” below for more information on how to complete this section.

Please see the relevant sections in the submission guidelines for further information as well as various examples of wording. Please revise/customize the sample statements according to your own needs.

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## Text

### Text Formatting

Manuscripts should be submitted in Word.

Use a normal, plain font (e.g., 10-point Times Roman) for text.

Use italics for emphasis.

Use the automatic page numbering function to number the pages.

Do not use field functions.

Use tab stops or other commands for indents, not the space bar.

Use the table function, not spreadsheets, to make tables.

Use the equation editor or MathType for equations.

Save your file in docx format (Word 2007 or higher) or doc format (older Word versions).

Manuscripts with mathematical content can also be submitted in LaTeX. We recommend using [Springer Nature's LaTeX template](#).

## Headings

Please use no more than three levels of displayed headings.

## Abbreviations

Abbreviations should be defined at first mention and used consistently thereafter.

## Footnotes

Footnotes can be used to give additional information, which may include the citation of a reference included in the reference list. They should not consist solely of a reference citation, and they should never include the bibliographic details of a reference. They should also not contain any figures or tables.

Footnotes to the text are numbered consecutively; those to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other

statistical data). Footnotes to the title or the authors of the article are not given reference symbols.

Always use footnotes instead of endnotes.

## **Acknowledgments**

Acknowledgments of people, grants, funds, etc. should be placed in a separate section on the title page. The names of funding organizations should be written in full.

## **Additional request Text**

- All manuscript files should be formatted to contain line numbers.
- Quotations of more than 40 words should be set off clearly, either by indenting the left-hand margin or by using a smaller typeface. Use double quotation marks for direct quotations and single quotation marks for quotations within quotations and for words or phrases used in a special sense.

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Level one headers: typed in bold, lowercase except for first letter of first word, left justified, followed by one blank line

Level two headers: typed in normal font, lowercase except for first letter of first word, left justified, followed by one blank line

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## References

### Citation

Cite references in the text by name and year in parentheses. Some examples:

Negotiation research spans many disciplines (Thompson, 1990).

This result was later contradicted by Becker and Seligman (1996).

This effect has been widely studied (Abbott, 1991; Barakat et al., 1995; Kelso & Smith, 1998; Medvec et al., 1999).

Authors are encouraged to follow official APA version 7 guidelines on the number of authors included in reference list entries (i.e., include all authors up to 20; for larger groups, give the first 19 names followed by an ellipsis and the final author's name). However, if authors shorten the author group by using et al., this will be retained.

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The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

Reference list entries should be alphabetized by the last names of the first author of each work.

Journal names and book titles should be *italicized*.

If available, please always include DOIs as full DOI links in your reference list (e.g. “<https://doi.org/abc>”).

Journal article Grady, J. S., Her, M., Moreno, G., Perez, C., & Yelinek, J. (2019). Emotions in storybooks: A comparison of storybooks that represent ethnic and racial groups in the United States. *Psychology of Popular Media Culture*, 8(3), 207–217. <https://doi.org/10.1037/ppm0000185>

Article by DOI Hong, I., Knox, S., Pryor, L., Mroz, T. M., Graham, J., Shields, M. F., & Reistetter, T. A. (2020). Is referral to home health rehabilitation following inpatient rehabilitation facility associated with 90-day hospital readmission for adult patients with stroke? *American Journal of Physical Medicine & Rehabilitation*. Advance online publication. <https://doi.org/10.1097/PHM.0000000000001435>

Book Sapolsky, R. M. (2017). *Behave: The biology of humans at our best and worst*. Penguin Books.

Book chapter Dillard, J. P. (2020). Currents in the study of persuasion. In M. B. Oliver, A. A. Raney, & J. Bryant (Eds.), *Media effects: Advances in theory and research* (4th ed., pp. 115–129). Routledge.

Online document Fagan, J. (2019, March 25). *Nursing clinical brain*. OER Commons. Retrieved January 7, 2020, from <https://www.oercommons.org/authoring/53029-nursing-clinical-brain/view>

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## Tables

All tables are to be numbered using Arabic numerals.

Tables should always be cited in text in consecutive numerical order.

For each table, please supply a table caption (title) explaining the components of the table.

Identify any previously published material by giving the original source in the form of a reference at the end of the table caption.

Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

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## Artwork and Illustrations Guidelines

### Electronic Figure Submission

Supply all figures electronically.

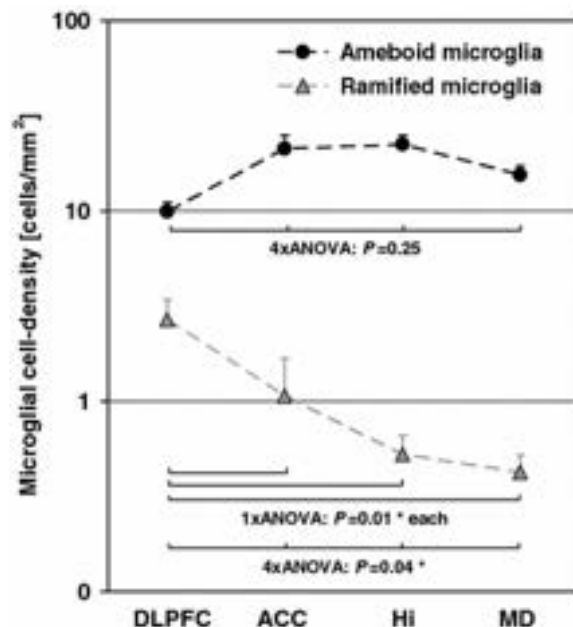
Indicate what graphics program was used to create the artwork.

For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.

Vector graphics containing fonts must have the fonts embedded in the files.

Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

## Line Art



Definition: Black and white graphic with no shading.

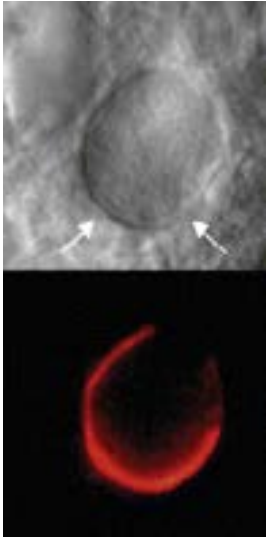
Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.

All lines should be at least 0.1 mm (0.3 pt) wide.

Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.

Vector graphics containing fonts must have the fonts embedded in the files.

## Halftone Art

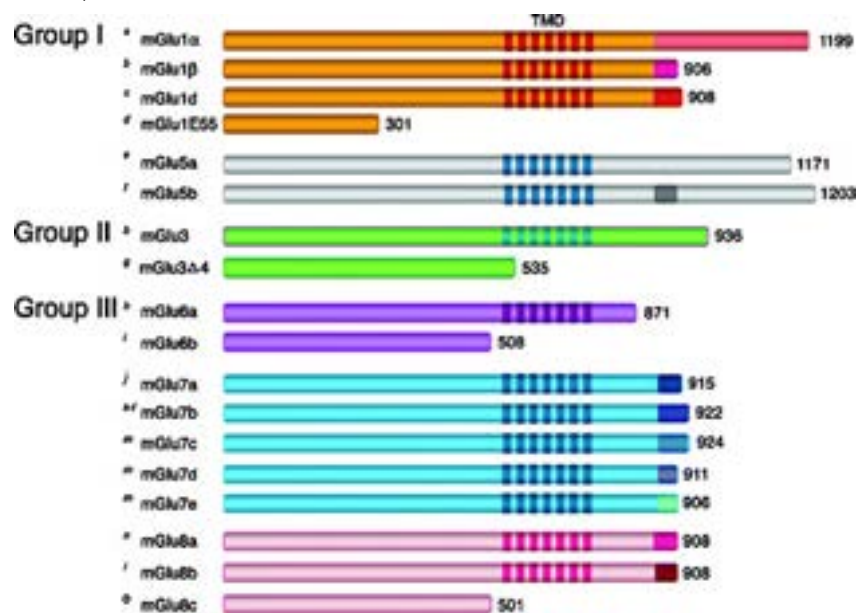


Definition: Photographs, drawings, or paintings with fine shading, etc.

If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.

Halftones should have a minimum resolution of 300 dpi.

## Combination Art



Definition: a combination of halftone and line art, e.g., halftones containing line drawing, extensive lettering, color diagrams, etc.

Combination artwork should have a minimum resolution of 600 dpi.

## Color Art

Color art is free of charge for online publication.

If black and white will be shown in the print version, make sure that the main information will still be visible. Many colors are not distinguishable from one another when converted to black and white. A simple way to check this is to make a xerographic copy to see if the necessary distinctions between the different colors are still apparent.

If the figures will be printed in black and white, do not refer to color in the captions.

Color illustrations should be submitted as RGB (8 bits per channel).

## Figure Lettering

To add lettering, it is best to use Helvetica or Arial (sans serif fonts).

Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).

Variance of type size within an illustration should be minimal, e.g., do not use 8-pt type on an axis and 20-pt type for the axis label.

Avoid effects such as shading, outline letters, etc.

Do not include titles or captions within your illustrations.

## Figure Numbering

All figures are to be numbered using Arabic numerals.

Figures should always be cited in text in consecutive numerical order.

Figure parts should be denoted by lowercase letters (a, b, c, etc.).

If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices [Supplementary Information (SI)] should, however, be numbered separately.

## Figure Captions

Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.

No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.

Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.

Identify previously published material by giving the original source in the form of a reference citation at the end of the figure caption.

## **Figure Placement and Size**

Figures should be submitted within the body of the text. Only if the file size of the manuscript causes problems in uploading it, the large figures should be submitted separately from the text.

When preparing your figures, size figures to fit in the column width.

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- Any figure lettering has a contrast ratio of at least 4.5:1

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Springer accepts electronic multimedia files (animations, movies, audio, etc.) and other supplementary files to be published online along with an article or a book chapter. This feature can add dimension to the author's article, as certain information cannot be printed or is more convenient in electronic form.

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Supply all supplementary material in standard file formats.

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Name the files consecutively, e.g. “ESM\_3.mpg”, “ESM\_4.pdf”.

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## Accessibility

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If the journal that you are submitting to uses double-anonymous peer review and you are providing reviewers with access to your data (for example via a repository link, supplementary information or data on request), it is strongly suggested that the authorship in the data is also anonymised. There are [data repositories that can assist with this](#) and/or will create a link to mask the authorship of your data.

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AND

2) drafting the article or revising it critically for important intellectual content;

AND

3) final approval of the version to be submitted for publication.

All of these conditions should be met by all authors.

Acquisition of funding, collection of data, or general supervision of the research group alone does not constitute authorship.

All contributors who do not meet the criteria for authorship should be listed in an acknowledgments section.

All authors must agree on the sequence of authors listed before submitting the article.

All authors must agree to designate one author as the corresponding author for the submission. It is the responsibility of the corresponding author to dialogue with the co-authors during the peer-reviewing and proofing stages and to also act on their behalf.

If the article is accepted for publication, after acceptance, no changes in authorship, the order of authors, or designation of the corresponding author will be permitted.

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## Statement of Editorial and Publication Policies

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Authors should refrain from misrepresenting research results which could damage the trust in the journal, the professionalism of scientific authorship, and ultimately the entire scientific endeavour. Maintaining integrity of the research and its presentation is helped by following the rules of good scientific practice, which include\*:

The manuscript should not be submitted to more than one journal for simultaneous consideration.

The submitted work should be original and should not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. (Please provide transparency on the re-use of material to avoid the concerns about text-recycling ('self-plagiarism')).

A single study should not be split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (i.e. 'salami-slicing/publishing').

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Results should be presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation (including image based manipulation). Authors should adhere to discipline-specific rules for acquiring, selecting and processing data.

No data, text, or theories by others are presented as if they were the author's own ('plagiarism'). Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks (to indicate words taken from another source) are

used for verbatim copying of material, and permissions secured for material that is copyrighted.

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Authors should make sure they have permissions for the use of software, questionnaires/(web) surveys and scales in their studies (if appropriate).

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Authors are strongly advised to ensure the author group, the Corresponding Author, and the order of authors are all correct at submission. Adding and/or

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Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results presented. This could be in the form of raw data, samples, records, etc. Sensitive information in the form of confidential or proprietary data is excluded.

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“All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors”.

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To ensure objectivity and transparency in research and to ensure that accepted principles of ethical and professional conduct have been followed, authors should include information regarding sources of funding, potential conflicts of interest (financial or non-financial), informed consent if the research involved human participants, and a statement on welfare of animals if the research involved animals.

Authors should include the following statements (if applicable) in a separate section entitled “Compliance with Ethical Standards” when submitting a paper:

Disclosure of potential conflicts of interest

Research involving Human Participants and/or Animals

Informed consent

Please note that standards could vary slightly per journal dependent on their peer review policies (i.e. single or double blind peer review) as well as per journal subject discipline. Before submitting your article check the instructions following this section carefully.

The corresponding author should be prepared to collect documentation of compliance with ethical standards and send if requested during peer review or after publication.

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Please note that, in addition to the above requirements, funding information (given that funding is a potential competing interest (as mentioned above)) needs to be disclosed upon submission of the manuscript in the peer review system. This information will automatically be added to the Record of CrossMark, however it is **not added** to the manuscript itself. Under 'summary of requirements' (see below) funding information should be included in the '**Declarations**' section.

## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Funding' and/or 'Competing interests'. Other declarations include Ethics approval, Consent, Data, Material and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

When all authors have the same (or no) conflicts and/or funding it is sufficient to use one blanket statement.

#### **Examples of statements to be used when funding has been received:**

Partial financial support was received from [...]

The research leading to these results received funding from [...] under Grant Agreement No[...].

This study was funded by [...]

This work was supported by [...] (Grant numbers [...] and [...])

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The authors did not receive support from any organization for the submitted work.

No funding was received to assist with the preparation of this manuscript.

No funding was received for conducting this study.

No funds, grants, or other support was received.

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**Non-financial interests:** Author A is on the board of directors of Y and receives no compensation as member of the board of directors.

**Financial interests:** Author A received a speaking fee from Y for Z. Author B receives a salary from association X. X where s/he is the Executive Director.

**Non-financial interests:** none.

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The authors have no relevant financial or non-financial interests to disclose.

The authors have no competing interests to declare that are relevant to the content of this article.

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## **Research involving human participants, their data or biological material**

### **Ethics approval**

When reporting a study that involved human participants, their data or biological material, authors should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee) and certify that the study was performed in accordance with the ethical standards as laid down in the [1964 Declaration of Helsinki](#) and its later amendments or comparable ethical standards. If doubt exists whether the research was conducted in accordance with the

1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that an independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study. If a study was granted exemption from requiring ethics approval, this should also be detailed in the manuscript (including the reasons for the exemption).

## **Retrospective ethics approval**

If a study has not been granted ethics committee approval prior to commencing, retrospective ethics approval usually cannot be obtained and it may not be possible to consider the manuscript for peer review. The decision on whether to proceed to peer review in such cases is at the Editor's discretion.

## **Ethics approval for retrospective studies**

Although retrospective studies are conducted on already available data or biological material (for which formal consent may not be needed or is difficult to obtain) ethics approval may be required dependent on the law and the national ethical guidelines of a country. Authors should check with their institution to make sure they are complying with the specific requirements of their country.

## **Ethics approval for case studies**

Case reports require ethics approval. Most institutions will have specific policies on this subject. Authors should check with their institution to make sure they are complying with the specific requirements of their institution and seek ethics approval where needed. Authors should be aware to secure informed consent from the

individual (or parent or guardian if the participant is a minor or incapable) See also section on **Informed Consent**.

## Cell lines

If human cells are used, authors must declare in the manuscript: what cell lines were used by describing the source of the cell line, including when and from where it was obtained, whether the cell line has recently been authenticated and by what method. If cells were bought from a life science company the following need to be given in the manuscript: name of company (that provided the cells), cell type, number of cell line, and batch of cells.

It is recommended that authors check the [NCBI database](#) for misidentification and contamination of human cell lines. This step will alert authors to possible problems with the cell line and may save considerable time and effort.

Further information is available from the [International Cell Line Authentication Committee](#) (ICLAC).

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## Research Resource Identifiers (RRID)

Research Resource Identifiers (RRID) are persistent unique identifiers (effectively similar to a DOI) for research resources. This journal encourages authors to adopt

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**Cell Line:** RST307 cell line RRID:CVCL\_C321

**Antibody:** Luciferase antibody DSHB Cat# LUC-3, RRID:AB\_2722109

**Plasmid:** mRuby3 plasmid RRID:Addgene\_104005

**Software:** ImageJ Version 1.2.4 RRID:SCR\_003070

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## Clinical Trial Registration

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maintain, promote or modify health, functioning or health conditions” and a health-related outcome is generally defined as a change in the health of a person or population as a result of an intervention.

To ensure the integrity of the reporting of patient-centered trials, authors must register prospective clinical trials (phase II to IV trials) in suitable publicly available repositories. For example [www.clinicaltrials.gov](http://www.clinicaltrials.gov) or any of the primary registries that participate in the [WHO International Clinical Trials Registry Platform](#).

The trial registration number (TRN) and date of registration should be included as the last line of the manuscript abstract.

For clinical trials that have not been registered prospectively, authors are encouraged to register retrospectively to ensure the complete publication of all results. The trial registration number (TRN), date of registration and the words 'retrospectively registered' should be included as the last line of the manuscript abstract.

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Diagnostic/prognostic studies ([STARD](#)) and ([TRIPOD](#))

Case reports ([CARE](#))

Clinical practice guidelines ([AGREE](#)) and ([RIGHT](#))

Qualitative research ([SRQR](#)) and ([COREQ](#))

Animal pre-clinical studies ([ARRIVE](#))

Quality improvement studies ([SQUIRE](#))

Economic evaluations ([CHEERS](#))

## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Ethics approval'.

Examples of statements to be used when ethics approval has been obtained:

- All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of the Medical University of A (No. ...).
- This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University B (Date.../No. ...).
- Approval was obtained from the ethics committee of University C. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.
- The questionnaire and methodology for this study was approved by the Human Research Ethics committee of the University of D (Ethics approval number: ...).

Examples of statements to be used for a retrospective study:

- Ethical approval was waived by the local Ethics Committee of University A in view of the retrospective nature of the study and all the procedures being performed were part of the routine care.
- This research study was conducted retrospectively from data obtained for clinical purposes. We consulted extensively with the IRB of XYZ who determined that our

study did not need ethical approval. An IRB official waiver of ethical approval was granted from the IRB of XYZ.

- This retrospective chart review study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The Human Investigation Committee (IRB) of University B approved this study.

Examples of statements to be used when no ethical approval is required/exemption granted:

- This is an observational study. The XYZ Research Ethics Committee has confirmed that no ethical approval is required.
- The data reproduced from Article X utilized human tissue that was procured via our Biobank AB, which provides de-identified samples. This study was reviewed and deemed exempt by our XYZ Institutional Review Board. The BioBank protocols are in accordance with the ethical standards of our institution and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Authors are responsible for correctness of the statements provided in the manuscript. See also Authorship Principles. The Editor-in-Chief reserves the right to reject submissions that do not meet the guidelines described in this section.

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## Informed consent

All individuals have individual rights that are not to be infringed. Individual participants in studies have, for example, the right to decide what happens to the (identifiable) personal data gathered, to what they have said during a study or an interview, as well as to any photograph that was taken. This is especially true concerning images of vulnerable people (e.g. minors, patients, refugees, etc) or the use of images in sensitive contexts. In many instances authors will need to secure written consent before including images.

Identifying details (names, dates of birth, identity numbers, biometrical characteristics (such as facial features, fingerprint, writing style, voice pattern, DNA or other distinguishing characteristic) and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scholarly purposes and the participant (or parent/guardian if the participant is a minor or incapable or legal representative) gave written informed consent for publication. Complete anonymity is difficult to achieve in some cases. Detailed descriptions of individual participants, whether of their whole bodies or of body sections, may lead to disclosure of their identity. Under certain circumstances consent is not required as long as information is anonymized and the submission does not include images that may identify the person.

Informed consent for publication should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort meaning.

Exceptions where it is not necessary to obtain consent:

- Images such as x rays, laparoscopic images, ultrasound images, brain scans, pathology slides unless there is a concern about identifying information in which case, authors should ensure that consent is obtained.
- Reuse of images: If images are being reused from prior publications, the Publisher will assume that the prior publication obtained the relevant information regarding consent. Authors should provide the appropriate attribution for republished images.

### **Consent and already available data and/or biologic material**

Regardless of whether material is collected from living or dead patients, they (family or guardian if the deceased has not made a pre-mortem decision) must have given prior written consent. The aspect of confidentiality as well as any wishes from the deceased should be respected.

### **Data protection, confidentiality and privacy**

When biological material is donated for or data is generated as part of a research project authors should ensure, as part of the informed consent procedure, that the participants are made aware what kind of (personal) data will be processed, how it will be used and for what purpose. In case of data acquired via a biobank/biorepository, it is possible they apply a broad consent which allows research participants to consent to a broad range of uses of their data and samples which is regarded by research ethics committees as specific enough to be considered “informed”. However, authors should always check the specific biobank/biorepository policies or any other type of data provider policies (in case of non-bio research) to be sure that this is the case.

## **Consent to Participate**

For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript. In the case of articles describing human transplantation studies, authors must include a statement declaring that no organs/tissues were obtained from prisoners and must also name the institution(s)/clinic(s)/department(s) via which organs/tissues were obtained. For manuscripts reporting studies involving vulnerable groups where there is the potential for coercion or where consent may not have been fully informed, extra care will be taken by the editor and may be referred to the Springer Nature Research Integrity Group.

## **Consent to Publish**

Individuals may consent to participate in a study, but object to having their data published in a journal article. Authors should make sure to also seek consent from individuals to publish their data prior to submitting their paper to a journal. This is in particular applicable to case studies. A consent to publish form can be found

[here. \(Download docx, 36 kB\)](#)

## Summary of requirements

The above should be summarized in a statement and placed in a 'Declarations' section before the reference list under a heading of 'Consent to participate' and/or 'Consent to publish'. Other declarations include Funding, Competing interests, Ethics approval, Consent, Data and/or Code availability and Authors' contribution statements.

Please see the various examples of wording below and revise/customize the sample statements according to your own needs.

Sample statements for "**Consent to participate**":

Informed consent was obtained from all individual participants included in the study.

Informed consent was obtained from legal guardians.

Written informed consent was obtained from the parents.

Verbal informed consent was obtained prior to the interview.

### Sample statements for “Consent to publish”:

The authors affirm that human research participants provided informed consent for publication of the images in Figure(s) 1a, 1b and 1c.

The participant has consented to the submission of the case report to the journal.

Patients signed informed consent regarding publishing their data and photographs.

Sample statements if identifying information about participants is available in the article:

Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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