

Universidade Federal do Rio Grande – FURG

Instituto de Oceanografia

Programa de Pós-Graduação em Oceanologia

***Aurelia coerulea* (Cnidaria, Scyphozoa)**
como organismo modelo: revisão
sistemática e avaliação dos efeitos de
partículas de tintas anti-incrustantes em
diferentes vias de exposição

Laís Olivera das Neves

Dissertação apresentada ao
Programa de Pós-Graduação
em Oceanologia, como parte
dos requisitos para a
obtenção do Título de
Mestre.

Orientador: *Profª. Dra. Grasiela Lopes Leães Pinho*
Universidade Federal do Rio Grande (FURG), Brasil.

Coorientador: *Prof. Dr. Renato Mitsuo Nagata*
Universidade Federal do Rio Grande (FURG), Brasil.

Rio Grande, RS, Brasil

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por

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Às quatorze horas do dia primeiro de junho do ano dois mil e vinte e seis, de forma Virtual: <https://conferenciaweb.rnp.br/sala/grasiela-lobes-leaes-pinho-grasiela> e Presencial, Sala: 5201- Pav. 5 – FURG-Carreiros, reuniu-se a Comissão Examinadora da Dissertação de **MESTRADO** intitulada: “EFEITOS DE PARTÍCULAS DE TINTAS ANTI-INCRUSTANTES EM *AURELIA COERULEA* (CNIDARIA, SCYPHOZOA)”, da Acad. **Laís Olivera das Neves**. A Comissão Examinadora foi composta pelos seguintes membros: Profa. Dra. Grasiela Lopes Leães Pinho – Orientadora – (PPGO-IO/FURG); Prof. Dr. Renato Mitsuo Nagata – Co-orientador - (IO/FURG); Profa. Dra. Barbara Estevão Clasen – (UERGS); Prof. Dr. Adalto Bianchini – (FURG) e Profa. Dra. Eunice da Costa Machado – (PPGO-IO/FURG). Dando início à reunião, a Coordenadora do PPGO, Profa. Dra. Grasiela Lopes Leães Pinho, e orientadora da aluna, agradeceu a presença de todos e fez a apresentação da Comissão Examinadora. Logo após esclareceu que a candidata teria um tempo de 45 a 60 min para explanação do tema, e cada membro da Comissão Examinadora, um tempo máximo de 30 min para perguntas. A seguir, passou à palavra a candidata que apresentou o tema e respondeu às perguntas formuladas. Após ampla explanação, a Comissão Examinadora reuniu-se em reservado para discussão do conceito a ser atribuído a candidata. Foi estabelecido que as sugestões de todos os membros da Comissão Examinadora, que seguem em pareceres em anexo, foram aceitas pelo Orientadora/Candidata para incorporação na versão final da Dissertação. Finalmente, a Comissão Examinadora considerou a candidata APROVADA, por unanimidade. Nada mais havendo a tratar, foi lavrada a presente ATA, por mim, Clabrisnei Moura de Melo – Secretário PPGO, que após lida e aprovada, será assinada pela Comissão Examinadora, pela Candidata e pela Coordenadora do Programa de Pós-Graduação em Oceanologia.

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Prof. Dr. Renato Mitsuo Nagata

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Prof. Dr. Adalto Bianchini

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Lista de Acrônimos e Abreviações

A

ANOVA – Análise de Variância

APPs (*Antifouling Paint Particles*)

– Partículas de tintas anti-incrustantes

C

CE₅₀ (**EC₅₀**) – Concentração de Efeito para 50% dos organismos

CL₅₀ (**LC₅₀**) – Concentração Letal para 50% dos organismos

CONECO – Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática

Cr – Cromo

Cu – Cobre

Cu⁺ / **Cu²⁺** – Íons de cobre (monovalente / divalente)

D

DCOIT – 4,5-dicloro-2-n-octil-4-isotiazolin-3-ona

Diuron – Herbicida utilizado como biocida

Dietborne – Exposição via alimentação (transferência trófica)

G

GF AAS – Espectrometria de Absorção Atômica com Forno de Grafite (*Graphite Furnace Atomic Absorption Spectrometry*)

GLM – Modelo Linear Generalizado (*Generalized Linear Model*)

I

Irgarol – Biocida orgânico (Irgarol 1051)

L

Labzoo – Laboratório de Zooplâncton

M

Mn – Manganês

N

Ni – Níquel

P

Pb – Chumbo

PTAs – Partículas de Tintas Anti-incrustantes

S

Sn – Estanho

T

TBT – Tributilestanho

TCMTB – 2(tiocianometiltio)benzotiazol

W

Waterborne – Exposição via água

Z

Zn – Zinco

Resumo

As partículas de tintas anti-incrustantes (PTAs) são amplamente utilizadas para prevenir a bioincrustação em estruturas submersas, porém representam uma fonte relevante de contaminantes no ambiente marinho devido à liberação de biocidas. Apesar de sua ocorrência em sedimentos e na coluna d'água, ainda são escassos os estudos que avaliam seus efeitos ecotoxicológicos considerando múltiplas vias de exposição. Nesse contexto, o presente estudo teve como objetivo investigar a toxicidade de PTAs em diferentes estágios do ciclo de vida da água-viva *Aurelia coerulea*, comparando exposições via água (*waterborne*) e via alimentação (*dietborne*), além de integrar uma análise da literatura sobre o uso do gênero como organismo modelo em bioensaios. A análise da literatura revelou alta variabilidade metodológica entre estudos com *Aurelia*, especialmente em parâmetros como temperatura, fotoperíodo, regime alimentar, tempo de exposição e seleção dos estágios de vida, fatores que influenciam diretamente as respostas dos organismos e dificultam a comparabilidade entre os estudos. Ensaios laboratoriais foram conduzidos com pólipos e éfiras de *A. coerulea*, expostos diretamente às PTAs ou alimentados com náuplios de *Artemia franciscana* previamente contaminados, simulando a exposição trófica. Adicionalmente, ensaios com *A. franciscana* foram realizados para determinar concentrações capazes de induzir efeitos subletais nos náuplios sem comprometer totalmente sua sobrevivência. Nos pólipos, foram avaliadas a desintegração e a contração dos tentáculos; nas éfiras, a imobilidade e a frequência de pulsação; e, em *A. franciscana*, a imobilidade e a alteração natatória. Os resultados demonstraram que a toxicidade das PTAs varia em função da formulação da tinta, da via de exposição e do estágio do ciclo de vida, sendo a exposição via água mais tóxica em comparação à via alimentar, embora efeitos também tenham sido observados na exposição via dieta. De maneira geral, as éfiras demonstraram maior sensibilidade em relação aos pólipos. A quantificação de cobre nos lixiviados evidenciou a liberação de contaminantes para o meio aquoso, contribuindo para os efeitos observados. Assim, a integração de múltiplas vias de exposição e estágios de vida em um mesmo delineamento experimental, como realizado neste trabalho, contribui para uma

avaliação mais abrangente dos efeitos ecotoxicológicos das PTAs. Os resultados mostram que a toxicidade de PTAs não é consistente entre vias de exposição e estágios do ciclo de vida, e que a variabilidade metodológica observada na literatura influencia a interpretação desses efeitos, indicando que abordagens baseadas em um único modelo experimental podem levar a interpretações incompletas do risco ecotoxicológico.

Palavras-chave: Avaliação de risco ambiental; Bioensaios; Ciclo de vida; Cifozoários; Contaminação marinha.

Abstract

Antifouling paint particles (APPs) are widely used to prevent biofouling on submerged structures; however, they represent a relevant source of contaminants in the marine environment due to the release of biocides. Despite their occurrence in sediments and the water column, studies evaluating their ecotoxicological effects considering multiple exposure routes remain scarce. In this context, the present study aimed to investigate the toxicity of APPs across different life stages of the jellyfish *Aurelia coerulea*, comparing waterborne and dietborne exposure, while also integrating a literature analysis on the use of this genus as a model organism in bioassays. The literature analysis revealed high methodological variability among studies using *Aurelia*, particularly regarding parameters such as temperature, photoperiod, feeding regime, exposure time, and life-stage selection, which directly influence organism responses and limit the comparability of results. Laboratory experiments were conducted using polyps and ephyrae of *A. coerulea* and nauplii of *Artemia franciscana*, assessing both lethal and sublethal responses, including disintegration, immobilization, tentacle contraction, pulsation frequency, and swimming alteration. The results demonstrated that APP toxicity varies according to paint formulation, exposure route, and life stage, with waterborne exposure being more toxic than dietborne exposure, although effects were also observed under dietborne conditions. In general, ephyrae showed higher sensitivity compared to polyps. Copper quantification in leachates revealed the release of contaminants into the aqueous medium, contributing to the observed effects. Thus, the integration of multiple exposure routes and life stages within a single experimental design, as performed in this study, provides a more comprehensive assessment of the ecotoxicological effects of APPs. The results show that APP toxicity is not consistent across exposure routes and life stages, and that methodological variability reported in the literature influences the interpretation of these effects, indicating that approaches based on a single experimental model may lead to incomplete assessments of ecotoxicological risk.

Keywords: Environmental risk assessment; Bioassays; Life cycle; Scyphozoa; Marine contamination.

Prefácio

A presente dissertação está estruturada em forma de capítulos, conforme as normas propostas pelo Programa de Pós-Graduação em Oceanologia da Universidade Federal do Rio Grande. Os artigos científicos publicados e submetidos encontram-se apresentados em sua forma completa, de acordo com as normas exigidas por cada periódico. O primeiro artigo (Capítulo IV), intitulado “Approaches to the methodological standards in the use of *Aurelia* Lamarck, 1816 (Cnidaria, Scyphozoa) as a model organism in bioassays and experimental applications”, foi publicado no periódico internacional *Marine Environmental Research* e teve como objetivo compilar e avaliar criticamente a literatura sobre o uso do gênero *Aurelia* como organismo modelo, com foco na variabilidade metodológica entre estudos e suas implicações para a comparabilidade e reprodutibilidade dos resultados. O segundo artigo (Capítulo IV), intitulado “The route of exposure determines the toxicity of antifouling paint particles (APPs) in

different life stages of *Aurelia coerulea* Lamarck, 1816 (Cnidaria, Scyphozoa)”, foi submetido no periódico internacional *Environmental Toxicology* e avaliou experimentalmente a toxicidade de partículas de tintas anti-incrustantes considerando diferentes vias de exposição, bem como diferentes estágios do ciclo de vida do organismo, contribuindo para a compreensão dos efeitos ecotoxicológicos desses contaminantes em organismos gelatinosos.

Capítulo I: Introdução

As partículas de tintas anti-incrustantes (PTAs) derivam de revestimentos aplicados em cascos de embarcações e outras estruturas submersas para evitar a colonização por organismos incrustantes (Torres & De-la-Torre, 2021). Essas tintas geralmente contêm biocidas que são liberados gradualmente para o ambiente aquático com o objetivo de impedir a fixação desses organismos (Watermann et al., 2005; Torres & De-la-Torre, 2021). Nos sistemas anti-incrustantes com tecnologia de auto-polimento, os biocidas são liberados da matriz da tinta de maneira controlada, embora não estejam necessariamente ligados quimicamente (Omae, 2003). Essas tintas permitem um desempenho previsível ao garantir uma liberação constante dos biocidas por períodos prolongados (Omae, 2003). No entanto, com o tempo, a eficácia dessas tintas diminui devido à lixiviação dos biocidas para o ambiente aquático, o que leva à formação de novas incrustações (Almeida et al., 2007). Isso requer a docagem da embarcação para remover o sistema anti-incrustante antigo e aplicar uma

nova camada de tinta, resultando no aporte de PTAs aos ecossistemas marinhos (Almeida et al., 2007).

As PTAs possuem grande heterogeneidade química em suas camadas (Singh & Turner, 2009). Mesmo após o uso em embarcações, podem ser encontradas altas concentrações de metais, especialmente Cu e Zn, além de co-biocidas, nas camadas antigas (Soroldoni et al., 2018). Estudos de caracterização química demonstram que o Cu é frequentemente o elemento dominante nas partículas, podendo ocorrer em concentrações superiores a $200.000 \mu\text{g g}^{-1}$, atuando como principal biocida em formulações anti-incrustantes modernas (Soroldoni et al., 2017). O Cu é comumente combinado com co-biocidas orgânicos para potencializar a eficiência das tintas, e o descarte inadequado desses resíduos contribui para o enriquecimento desses compostos no ambiente (Soroldoni et al., 2018). Em áreas próximas a estaleiros e marinas, altas concentrações de biocidas foram encontradas em sedimentos superficiais (Rees et al. 2014, Soroldoni et al. 2018). A ausência de controle e legislação abrangendo a geração e o descarte das PTAs no Brasil, reforçam a importância de estudos avaliando seus efeitos ecotoxicológicos em organismos aquáticos (Soroldoni et al., 2018). Embora acordos internacionais, como a International Convention on the Control of Harmful Anti-fouling Systems on Ships, tenham restringido compostos altamente tóxicos como o tributilestanho (TBT), muitos co-biocidas e metais utilizados nas formulações atuais, ainda são liberados no ambiente. Partículas de tinta apresentam elevadas concentrações de aditivos potencialmente tóxicos, com liberação de íons como $\text{Cu}^+/\text{Cu}^{2+}$, Pb^{2+} e outros compostos a partir da matriz da tinta (Turner, 2021).

As tintas anti-incrustantes e seus componentes ativos são reconhecidos como poluentes ambientais há décadas (Kyei et al., 2020). No entanto, o conhecimento sobre a ecotoxicidade das PTAs ainda é escasso (Torres & De-la-Torre, 2021). Tolhurst et al. (2007) testaram, isoladamente, o efeito da ressuspensão de sedimentos contaminados com PTAs à base de Irgarol 1051 na macroalga *Ulva intestinalis* e constataram um efeito negativo em seu crescimento. Soroldoni et al. (2020) avaliaram a toxicidade de sedimentos contaminados com PTAs e elutriato das partículas, aos crustáceos bentônicos *Monokalliapseudes schubarti* e *Hyalella azteca*. O resultado foi de alta sensibilidade dos organismos, com CL50 em 10 dias de 0,45% e 0,16% de partículas no sedimento, respectivamente (Soroldoni et al., 2020).

Entretanto, a maioria dos estudos que avaliaram a toxicidade das PTAs, foram feitas exclusivamente sobre a ressuspensão de sedimentos ou elutriato (e.g. Zaaboub et al. 2015, Othman et al. 2017, Soroldoni et al. 2020). Essa abordagem decorre do fato de que esses resíduos tendem a se acumular em sedimentos de áreas portuárias, estaleiros e marinas após atividades de manutenção e raspagem de cascos (Turner, 2010; Rees et al., 2014). No entanto, as PTAs não permanecem restritas aos sedimentos dos ambientes aquáticos. Processos hidrodinâmicos podem ressuspender essas partículas, mantendo parte do material na coluna d'água (Turner, 2010), onde compostos presentes na matriz da tinta continuam sendo liberados para o meio aquoso (Torres & De-la-Torre, 2021). Nesse contexto, evidências recentes demonstram que fragmentos de tinta estão amplamente distribuídos na coluna d'água e podem representar uma fração significativa do microdetrito marinho. Por exemplo, Lacerda et al. (2019) registraram PTAs em todas as amostras superficiais analisadas na Antártica,

com abundâncias até 30 vezes superiores às de plásticos convencionais, enquanto Turner et al. (2022) observaram que a maior parte dos microplásticos não fibrosos no Atlântico Norte correspondia a fragmentos de tinta, muitos deles associados a formulações anti-incrustantes ricas em metais.

Apesar das evidências de que as PTAs podem permanecer em suspensão e atuar como fontes móveis de contaminantes na coluna d'água, ainda são escassos os estudos que investigam os efeitos ecotoxicológicos das PTAs considerando diferentes rotas de exposição, particularmente o contato direto com a água contaminada (*waterborne*) e a transferência trófica por meio da ingestão de presas contaminadas (*dietborne*), mecanismo já descrito para outros contaminantes particulados e metais associados a sedimentos (Wang & Fisher, 1999). Dessa forma, surge uma lacuna sobre os riscos associados às PTAs em ambientes costeiros, onde organismos podem ser expostos simultaneamente por múltiplas vias.

Aurelia coerulea é uma água-viva (Cnidaria, Scyphozoa) com ciclo de vida metagênico que inclui dois estágios: o pólipo, fase sésil e bentônica, e a medusa, fase planctônica e vágil (Figura 1) (Arai, 1997). Os pólipos produzem inúmeras éfiras, as medusas juvenis, através do processo de estrobilização, que representa um tipo de reprodução assexuada (Arai, 1997). A presença de fases bentônicas e planctônicas expõe esses organismos a contaminantes presentes tanto no sedimento quanto na coluna d'água (Arai, 1997; Lucas et al., 2012). Além disso, diferenças fisiológicas entre pólipos e éfiras podem influenciar a resposta a agentes estressantes (Olguin-Jacobson et al., 2020; Nagata et al., 2024; Morales et al., 2025). Espécies de *Aurelia* apresentam ampla distribuição em ambientes costeiros e estuarinos, colonizando tanto substratos naturais,

como rochas e conchas, quanto estruturas artificiais, como embarcações e estruturas de concreto. Em áreas costeiras e estuarinas antropizadas, medusas de *Aurelia* spp. frequentemente formam *blooms* intensos que podem gerar efeitos ecológicos importantes e prejuízos a atividades econômicas costeiras (Goldstein & Steiner 2020; Morales et al., 2025).

Por essas características, espécies do gênero *Aurelia* têm sido amplamente utilizadas como organismos modelo em estudos experimentais. Além da alternância entre fases bentônicas e planctônicas (Figura 1), esses organismos apresentam facilidade de manutenção em condições laboratoriais e respostas rápidas e mensuráveis a alterações químicas e ambientais (Neves et al., 2026). Os diferentes estágios do ciclo de vida oferecem oportunidades experimentais complementares, permitindo a avaliação de múltiplas respostas, como crescimento, reprodução assexuada, comportamento natatório e alterações morfológicas sob estresse. Além disso, *Aurelia* apresenta ampla distribuição geográfica, baixa sazonalidade e já possui informações genéticas disponíveis para algumas espécies, o que favorece a reprodutibilidade e a comparabilidade entre estudos (Neves et al., 2026).

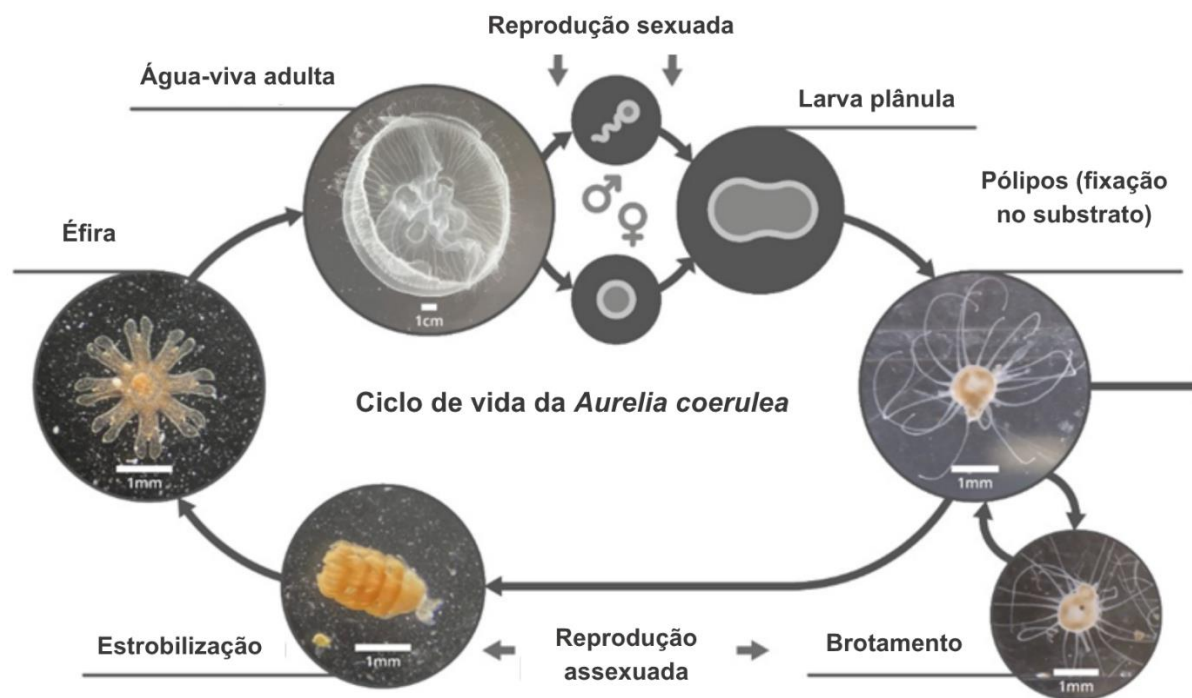


Figura I- 1: Ciclo de vida da *Aurelia coerulea* (Adaptado de Morales et al., 2025).

Náuplios de artêmia são usualmente utilizados como alimento em ensaios ecotoxicológicos com *Aurelia* spp., tanto em estudos que avaliam efeitos letais quanto subletais (e.g., Brunelli et al., 2024; Wang et al., 2025; Park et al., 2025). Além disso, *Artemia* também é reconhecida como um organismo modelo em ensaios toxicológicos devido à facilidade de obtenção e manutenção na forma de cistos, bem como à rápida eclosão desses (Nunes et al., 2006; Libralato et al., 2016). Esses organismos têm capacidade de assimilar e acumular contaminantes, sendo empregados em estudos que investigam rotas de exposição e transferência trófica em cadeias alimentares aquáticas (Mathews & Fisher, 2008; Tangaa et al., 2016). Dessa forma, o uso de *Artemia* permite avaliar experimentalmente a transferência trófica de contaminantes para organismos predadores.

Apesar do crescente aumento de estudos com *Aurelia*, eles apresentam uma grande variação metodológica. Diferenças em temperatura, salinidade,

alimentação, estágio de vida e condições de exposição afetam diretamente os resultados obtidos. Essas variações influenciam parâmetros como sobrevivência, morfogênese, frequência de pulsação e respostas fisiológicas (Jordano et al., 2018; 2022; von Montfort et al., 2023; Schiariti et al., 2015; Wang et al., 2023; Olguín-Jacobson et al., 2021; Chen et al., 2025). Trabalhos que avaliam contaminantes também utilizam diferentes concentrações, tempos de exposição e critérios de análise, o que dificulta a comparação entre estudos (Ringwood et al., 2025; Park et al., 2025; Goldstein & Javidpour, 2023). Essas diferenças podem alterar a sensibilidade dos organismos e interferir na interpretação dos resultados (Lopes et al., 2021).

Diante dessas lacunas, este estudo inclui uma revisão da literatura sobre o uso de *Aurelia* em ensaios ecotoxicológicos, com foco nas condições experimentais empregadas, nos estágios de vida avaliados e nos tipos de contaminantes testados. O trabalho também avalia os efeitos das PTAs considerando duas vias de exposição, via água e via dieta, em pólipos e éfiras de *A. coerulea*. Em conjunto, os dois manuscritos permitem situar os resultados experimentais dentro do que já foi descrito para o gênero e identificar diferenças associadas ao delineamento dos estudos.

Capítulo II: Objetivos

Geral:

Investigar a ecotoxicidade de partículas de duas tintas anti-incrustantes (PTAs) e seus efeitos no cifozoário *Aurelia coerulea*.

Específicos:

- I. Realizar uma revisão sistemática com análise cientométrica sobre o uso de *Aurelia* como organismo modelo em bioensaios;
- II. Verificar a toxicidade aguda (respostas letais) e crônica (respostas subletais) das PTAs em pólipos e éfiras de *A. coerulea* e náuplios de *Artemia franciscana*;
- III. Avaliar diferentes vias de exposição das PTAs em pólipos e éfiras de *A. coerulea*, comparando a toxicidade via água e via dieta (artêmias pré-contaminadas com PTAs).

Capítulo III: Material e métodos

3.1. Obtenção e isolamento dos organismos-teste

Os pólipos de *Aurelia coerulea* (Figura 2) foram obtidos a partir de uma cultura mantida pelo Laboratório de Zooplâncton (Labzoo), do Instituto de Oceanografia da Universidade Federal do Rio Grande (IO-FURG). No laboratório Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática (CONECO) do IO-FURG, a cultura foi replicada e mantida em béqueres de 300 mL com água marinha artificial de salinidade 35 (VeroSal Corais), a 20 °C, sob fotoperíodo de 12:12, alimentados uma vez por semana com náuplios recém-eclodidos de *Artemia franciscana*. Para a obtenção de éfiras (Figura 2), pólipos foram induzidos à estrobilização por indução térmica a 15 °C, sem alimentação e sob fotoperíodo de 12:12 (Morales et al., 2025). As éfiras recém-liberadas foram coletadas e utilizadas nos experimentos. Cistos de *A. franciscana* foram obtidos em loja de aquarismo e eclodidos em béqueres de 1 L contendo água marinha

artificial de salinidade 35, a 25 °C, sob iluminação constante de lâmpada fluorescente de 25 W e aeração contínua (Figura 2).



Figura 1- 2: A: Éfira de *Aurelia coerulea*; B: Pólipo de *A. coerulea*; C: Náuplio de *Artemia franciscana*.

Antes do início dos ensaios, todos os pólipos foram alimentados *ad libitum* com náuplios de *A. franciscana*, de modo a padronizar as condições nutricionais dos organismos.

3.2. Soluções-teste

Os ensaios com as partículas de tintas anti-incrustantes (PTAs) foram realizados com pólipos, éfiras e artêmias com o objetivo de estimar a toxicidade desses materiais (Tabela 1). As PTAs foram coletadas manualmente em 2014, durante a manutenção de embarcações em dois estaleiros no município de Rio Grande, RS, por meio da raspagem das tintas anti-incrustantes Supermarine AF Ionex (Renner®) e Micron Premium (International®), conforme descrito em Soroldoni et al. (2018). As amostras foram peneiradas (entre 180 µm e 1 mm) e mantidas sob refrigeração (4 °C) (Figura 3). Ambas as amostras foram previamente caracterizadas quimicamente quanto às concentrações de Cr, Cu, Mn, Ni, Pb, Sn, Zn, Diuron, Irgarol, TCMTB e DCOIT (Soroldoni et al., 2018). O tratamento controle consistiu em água marinha artificial com salinidade 35, preparada nas

mesmas condições utilizadas para a preparação das soluções contendo PTAs, porém sem adição de partículas.



Figura I- 3: Armazenamento das partículas de tintas anti-incrustantes Renner (partículas azuis) e International® (partículas vermelhas).

As concentrações testadas são ambientalmente realistas para áreas impactadas por atividades de manutenção de embarcações, onde PTAs podem atingir concentrações de até 44.300 mg g^{-1} no sedimento e representar uma fração significativa do material particulado no ambiente (De-la-Torre et al., 2023; Torres & De-la-Torre, 2021). Além disso, as concentrações foram selecionadas de modo a provocar efeitos tóxicos sem causar mortalidade total dos organismos, permitindo a avaliação de respostas comportamentais.

Tabela I- 1: Tratamentos e respostas analisadas dos organismos-teste durante os ensaios com partículas de tintas anti-incrustantes.

Tratamentos	Pólipos	Éfiras	Artêmias
PTAs Renner® (mg L ⁻¹) exposição via água	0, 1, 2, 3, 4	0, 0.25, 0.5, 1, 2	0, 0.5, 1, 2, 4
PTAs Renner® (mg L ⁻¹) exposição via dieta	0, 1, 2, 3, 4, 12	0, 0.5, 1, 2, 4, 10	-
PTAs International® (mg L ⁻¹) exposição via água	0, 0.5, 1, 2, 4	0, 0.25, 0.5, 1, 2	0, 0.5, 1, 2, 4
PTAs International® (mg L ⁻¹) exposição via dieta	0, 0.5, 2, 4, 14	0, 0.5, 1, 2, 4, 10	-
Respostas agudas	Desintegração	Imobilidade	Imobilidade
Respostas crônicas	Contração dos tentáculos	Frequência de pulsação	Alteração natatória
Períodos de observação (h)	1, 6, 24, 48, 72 e 96	24 e 48	24

3.3. Solução de exposição com as partículas de tintas anti-incrustantes

As partículas foram pesadas em balança de alta precisão (Mettler Toledo XPR504S/A) (Figura 4), considerando cada pesagem como uma repetição independente, e ajustadas para um volume final de 50 mL de água marinha artificial (salinidade 35). As partículas de tintas anti-incrustantes (PTAs) foram transferidas individualmente para tubos tipo Falcon de 50 mL, sem mistura entre marcas. Os tubos foram mantidos horizontalmente em mesa agitadora orbital (Marconi Ltda.) a 110 rpm, no escuro, durante 7 dias, para permitir a liberação de compostos presentes na matriz da tinta para o meio aquoso (Figura 5) (adaptado de Soroldoni et al., 2018). A incubação no escuro foi adotada para

evitar a degradação fotoquímica de compostos orgânicos presentes nas formulações das tintas. Após esse período, os próprios tubos Falcon contendo a solução de exposição foram utilizados nos ensaios, sendo os organismos adicionados diretamente ao meio. Dessa forma, os testes foram realizados na presença simultânea do lixiviado em água salina e das PTAs em suspensão.



Figura I- 4: Pesagem das partículas de tintas anti-incrustantes na balança de alta precisão.



Figura I- 5: Agitação dos lixiviados em mesa agitadora orbital.

Durante os ensaios, os tubos permaneceram horizontalmente em agitador orbital (110 rpm) com o objetivo de manter os organismos em contato contínuo com as PTAs, além de mantê-las em suspensão ao longo da coluna d'água e evitar sua sedimentação. A agitação também favorece a liberação contínua de compostos presentes nas partículas para o meio aquoso.

3.4. Ensaio de toxicidade

Todos os ensaios foram realizados em tubos tipo Falcon de 50 mL, e as observações das respostas biológicas foram feitas em microscópio estereoscópio (Olympus SZX9) nos períodos previamente definidos (Figura 6).

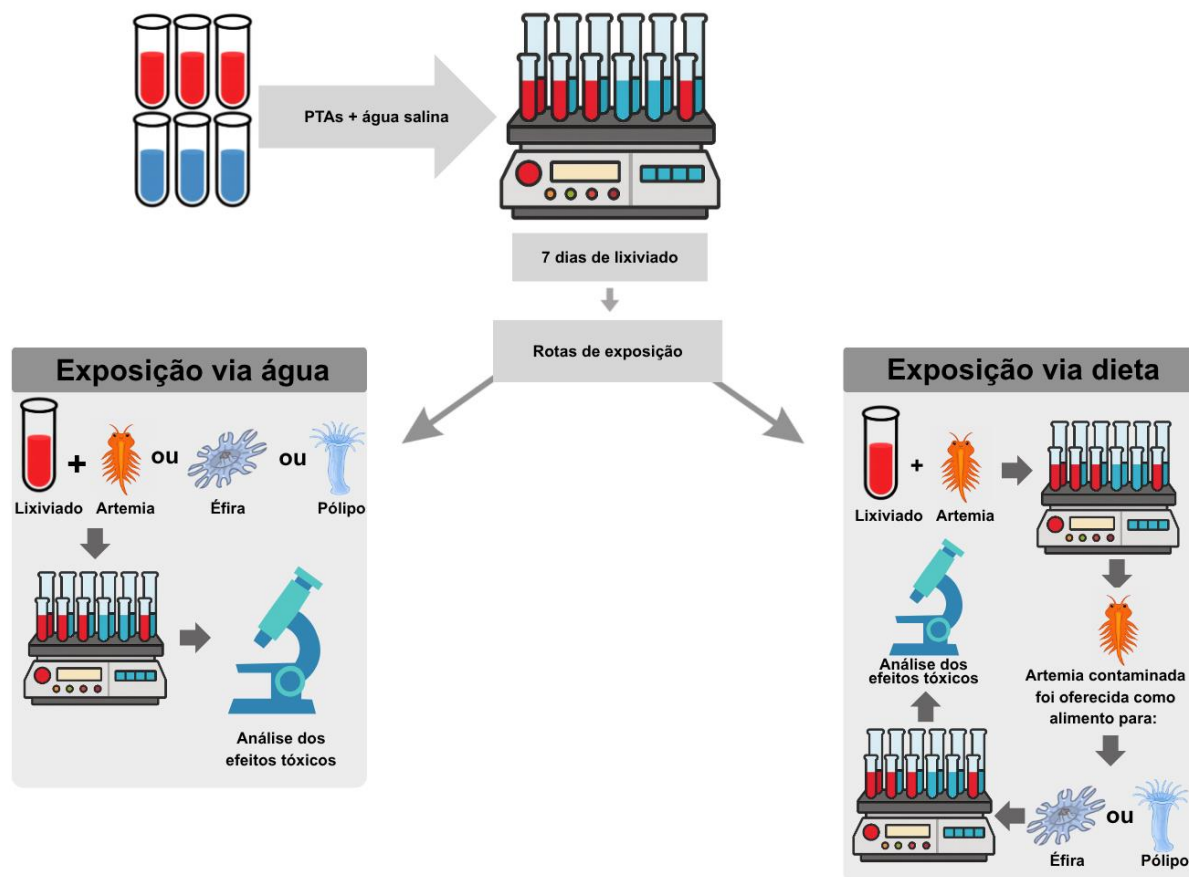


Figura I- 6: Execução dos testes de toxicidade via água e via dieta. Tubos vermelhos indicam mistura com tinta Renner®, tubos azuis, com International®. Todos os ensaios foram realizados com cinco repetições por tratamento.

3.4.1. Pólipos

Os testes foram realizados conforme metodologia de Morales et al. (2025), com pólipos sem idade definida, em incubadora a 20 °C e fotoperíodo de 12:12. Para cada ensaio, foram utilizadas 5 repetições contendo 5 pólipos cada, totalizando 25 pólipos por tratamento. As respostas crônicas incluíram a contração dos tentáculos, considerada quando todos os tentáculos se encontravam contraídos, ainda que ligeiramente curvados. A resposta aguda foi determinada pela desintegração da estrutura corporal dos pólipos.

3.4.2. Éfiras

Os testes foram realizados com éfiras de 0 a 5 dias de vida, em incubadora a 20 °C e fotoperíodo de 12:12, conforme Morales et al. (2025). Para cada ensaio, foram utilizadas 5 repetições contendo 5 éfiras cada, totalizando 25 éfiras por tratamento. A resposta crônica foi quantificada pela frequência de pulsação (pulsos por minuto). Para cada éfira, foram realizadas três medições de frequência de pulsação com duração de 10 segundos cada. A resposta aguda consistiu na imobilidade dos organismos, determinada pela ausência de movimento durante 10 segundos, mesmo após estímulo com pipeta Pasteur (Morales et al., 2025).

3.4.3. Artêmias

Os testes com *Artemia franciscana* tiveram como objetivo estabelecer uma faixa de concentração na qual o efeito das PTAs afetasse os náuplios sem comprometer totalmente sua sobrevivência, permitindo posterior avaliação da transferência da toxicidade para *Aurelia coerulea*. Os ensaios foram realizados a 25 °C no escuro, com organismos de 72 horas de idade (adaptado de Libralato et al. 2007). Para cada ensaio, foram utilizadas 5 repetições contendo 15 artêmias cada. Após 24 horas de exposição, foram avaliadas a alteração natatória, como resposta crônica, e a imobilidade, como resposta aguda.

3.4.4. Ensaio de ingestão de presas contaminadas

Para estimar efeitos crônicos e agudos da toxicidade por exposição via dieta, pólipos e éfiras de *Aurelia coerulea* foram alimentados com náuplios de *Artemia franciscana* previamente expostos às PTAs durante 24 horas. Os ensaios foram conduzidos em água marinha artificial sem PTAs, garantindo que a exposição

ocorresse exclusivamente pela ingestão dos náuplios contaminados. Durante os ensaios de exposição via dieta, foram oferecidos 10 náuplios de artêmia contaminados para cada pólip ou éfira. A alimentação ocorreu uma única vez no início do experimento, e os períodos de observação foram contabilizados a partir desse momento. Assim, no caso das éfiras, por exemplo, os organismos foram alimentados com os náuplios contaminados e as observações ocorreram 24 e 48 horas após essa alimentação.

A ingestão das presas foi confirmada visualmente em microscópio estereoscópio por meio da observação da captura dos náuplios e pela presença de coloração alaranjada na cavidade gastrovascular dos organismos, característica da ingestão de náuplios de *Artemia* spp. por organismos gelatinosos transparentes. As respostas agudas e crônicas avaliadas foram as mesmas descritas para os ensaios de exposição via água.

3.5. Quantificação de cobre nos lixiviados

As concentrações de cobre foram determinadas nos lixiviados utilizados nos ensaios toxicológicos, com o objetivo de quantificar a fração dissolvida liberada pelas partículas de tintas anti-incrustantes (PTAs). Foram preparadas soluções das tintas Renner® e International® nas concentrações de 0,5, 1, 2, 4 e 10 mg L⁻¹, além do controle, seguindo o mesmo procedimento descrito na seção 2.3, com lixiviação por 7 dias em água marinha artificial. Após esse período, as alíquotas dos lixiviados foram previamente filtradas (0,45 µm) e dessalinizadas segundo o método de Nadella et al. (2009), que consistiu na adição de óxido de lantânio e carbonato de sódio, seguida de aquecimento (80 °C, 30 min), centrifugação (~3000 g, 15 min) e dissolução do precipitado em ácido nítrico. A

determinação do cobre foi realizada em espectrômetro de absorção atômica com forno de grafite (GF AAS; ContrAA 700), com limite mínimo de quantificação de $0,05 \mu\text{g L}^{-1}$, conforme Fonseca et al. (2021). A curva de calibração foi definida a partir de solução padrão multi-elementar certificada (Sigma-Aldrich), com concentração máxima de $100 \mu\text{g L}^{-1}$. A exatidão analítica foi avaliada por meio de ensaios de recuperação, que apresentaram valores superiores a 90%.

3.6. Análise estatística

Para as respostas agudas, as CL_{50} (concentração letal para 50% dos organismos) foram estimadas para 96 horas de exposição em pólipos e 24 horas em *Artemia franciscana*, enquanto a CE_{50} (concentração de efeito para 50% dos organismos) foi estimada para éfiras após 48 horas de exposição, utilizando análise Probit (Finney, 1952). A frequência de pulsação das éfiras foi tratada como uma variável resposta contínua. Para esse endpoint, as diferenças entre concentrações foram avaliadas separadamente para cada tempo de exposição. Os pressupostos de normalidade dos resíduos e homogeneidade de variâncias entre tratamentos foram avaliados pelos testes de Shapiro-Wilk e Levene, respectivamente. Quando esses pressupostos foram atendidos, foi aplicada uma análise de variância de uma via (ANOVA). Para os demais endpoints, foram utilizados modelos lineares generalizados (GLMs) com distribuição binomial, uma vez que as respostas analisadas correspondiam a desfechos binários ou proporções. Quando foi detectado efeito significativo da concentração, comparações múltiplas entre concentrações, dentro de cada período de observação, foram realizadas por meio de testes pós-hoc com ajuste de Tukey. As análises estatísticas foram realizadas utilizando o software GraphPad Prism 8.4 para Windows (San Diego, EUA).

Capítulo IV: Artigos científicos

Para a obtenção do título de Mestre pelo Programa de Pós-Graduação em Oceanologia é requerido que o discente realize a submissão de pelo menos um artigo científico como primeiro autor em periódico com corpo indexado. Desse modo, os resultados da pesquisa desenvolvida durante o período de mestrado e a discussão dos resultados serão apresentados em forma de artigos neste Capítulo. O primeiro manuscrito, de autoria de Laís Olivera das Neves, Mikael Luiz Morales Pereira, Sanye Soroldoni, Renato Mitsuo Nagata e Grasiela Lopes Leães Pinho, é intitulado “Approaches to the methodological standards in the use of *Aurelia* Lamarck, 1816 (Cnidaria, Scyphozoa) as a model

organism in bioassays and experimental applications” e foi publicado no periódico *Marine Environmental Research*. O segundo manuscrito, de autoria de Laís Olivera das Neves, Mikael Luiz Morales Pereira, Sanye Soroldoni, Renato Mitsuo Nagata, Camila de Martinez Gaspar Martins e Grasiela Lopes Leães Pinho, é intitulado “The route of exposure determines the toxicity of antifouling paint particles (APPs) in different life stages of *Aurelia coerulea* Lamarck, 1816 (Cnidaria, Scyphozoa)” e está em revisão no periódico *Environmental Toxicology*.



Approaches to the methodological standards in the use of *Aurelia* Lamarck (1816) (Cnidaria, Scyphozoa) as a model organism in bioassays and experimental applications

Laís Olivera das Neves^{a,*}, Mikael Luiz Pereira Morales^{a,b}, Sanye Soroldoni^a, Renato Mitsuo Nagata^c, Grasiela Lopes Leães Pinho^a

^a Programa de Pós-graduação Em Oceanologia (PPGO), Instituto de Oceanografia (IO), Universidade Federal Do Rio Grande (FURG), Rio Grande, Rio Grande Do Sul, Brasil. Av. Itália, Km 8, Carreiros, CEP, Rio Grande, Rio Grande do Sul, 96203-900, Brazil

^b Programa Institucional de Pós-Doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PIPD-CAPES/PPGO), Brazil

^c Programa de Pós-Graduação Em Oceanografia Biológica, Instituto de Oceanografia da Universidade Federal Do Rio Grande (FURG), Av. Itália, Km 8, Carreiros, CEP, Rio Grande, Rio Grande do Sul, 96203-900, Brazil

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ABSTRACT

The use of the jellyfish *Aurelia* has expanded considerably across marine research, including in ecotoxicology, physiology, biotechnology, and environmental assessment. Despite this growing interest, methodological approaches vary widely, which can limit the comparability and reproducibility of results. In this review, we compiled and evaluated 46 peer-reviewed articles published between 2015 and 2025 to identify patterns in how *Aurelia* has been used as a model organism in bioassays and experimental studies. Our synthesis revealed a high degree of methodological heterogeneity, particularly with respect to temperature, photoperiod, feeding regime, life-stage selection, exposure duration, and endpoints. The studies were geographically concentrated in China and Italy, with limited representation from other regions, including tropical and developing countries. Polyps and ephyrae were the life stages most frequently used in ecotoxicological assays, whereas adult medusae were mainly employed in biochemical and biotechnological applications. Overall, our findings highlight the urgent need for clearer reporting practices and standardized experimental approaches to improve reproducibility and support the reliable use of *Aurelia* as a model organism in marine research.

1. Introduction

Species of the genus *Aurelia* (Cnidaria, Scyphozoa) are among the most common, widely distributed, and well-studied scyphozoans in the world's oceans (Albert, 2011; Jarms and Morandini, 2023). Like most scyphozoans, *Aurelia* has a complex life cycle, alternating between sexual reproduction in the medusa stage and asexual reproduction in the polyp stage (Brekman et al., 2015). After embryogenesis, the planula larva settles and metamorphoses into a sessile polyp capable of reproducing by asexual propagation (Brekman et al., 2015; Lucas et al., 2012). The transition to the medusa stage occurs through strobilation, during which the polyp produces segmented discs that detach as ephyrae, young medusae that develop into adults (Brekman et al., 2015). The remaining polyp can regenerate and restart the cycle under favorable conditions (Brekman et al., 2015).

Several studies have demonstrated the ease of maintaining *Aurelia* under laboratory conditions, as well as its rapid and measurable responses to chemical and environmental changes (Echols et al., 2015; Costa et al., 2020; Roveta et al., 2022; Morales et al., 2025). The different stages of its life cycle also provide complementary experimental opportunities: the benthic polyp stage is suitable for evaluating chronic exposure, fouling-related impacts, and asexual reproduction, whereas the planktonic and motile ephyrae allow for the investigation of swimming behavior, morphogenesis, growth, and phenotypic plasticity under chemical or environmental stress (Echols et al., 2015; Costa et al., 2020). In addition, biochemical components of the organism, such as mucins and mucus-associated proteins, have been explored for biotechnological applications, including nanoparticle capture and the investigation of molecules with bioactive potential (Geum and Yeo, 2022; Ladewig et al., 2023). Owing to this diversity of responses and

* Corresponding author.

E-mail address: laisdneves00@gmail.com (L.O. das Neves).

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applications, as well as the possibility of use in both its planktonic and benthic stages, *Aurelia* has become widely employed across different fields of marine research.

The selection of a model organism is based on criteria such as sensitivity to different chemical agents, prior knowledge of its biology (reproduction, feeding, physiology, and behavior), low seasonality, broad geographic distribution and genetic stability (Domingues et al., 2006). More broadly, model organism selection requires understanding why a particular taxon is appropriate for addressing specific research questions. To assess environmental risks, test for contaminants, or propose solutions, the organism must reflect relevant ecological traits, exhibit measurable responses and possess biological requirements that enable its reliable maintenance under laboratory conditions.

In this context, *Aurelia* stands out because of its suitable size and well-defined life cycle stages, which facilitate its maintenance in the laboratory (Giussani et al., 2016). In addition, reproduction can be readily stimulated, resulting in the formation of polyps within a few days or weeks. Moreover, it is a widely cultivated species in laboratories around the world, which favors comparisons and the replication of results (Lucas et al., 2012). More recently, important advances in the taxonomy of the genus have been achieved with the delimitation of several species based on genetic characteristics, which will allow for greater replicability in various laboratory studies (Lawley et al., 2021). Finally, genomic information is available for some *Aurelia* species, enabling studies on gene expression and molecular responses (Gold et al., 2019).

Despite the growing number of studies on moon jellyfish, there is considerable methodological variation among investigations, including differences in factors such as temperature, salinity, photoperiod, polyp density, ephyrae size and age, feeding regime and strobilation conditions. These aspects directly influence endpoints such as morphogenesis (Jordano, 2018, 2023), the pulsation rate (von Montfort et al., 2023), reproduction rates (Schiariti et al., 2015), survival (Wang et al., 2023), metabolism (Olguín-Jacobson et al., 2021) and oxidative stress (Chen et al., 2025). Beyond environmental and husbandry conditions, in studies involving exposure to substances such as metals, microplastics and nanoparticles, researchers employ distinct concentrations, exposure times, feeding regimes and evaluation criteria (Ringwood et al., 2025; Park et al., 2025; Goldstein and Javidpour, 2023), which limit the scope of comparisons. Given this variability, it is essential to compile and organize existing methodologies to identify patterns, critical differences, and knowledge gaps, thus reinforcing the need for standardization. Standardization is particularly important because methodological differences can alter organismal sensitivity and measured endpoints, compromising comparisons and the interpretation of results among studies (Lopes et al., 2021). With respect to *Aurelia*, the standardization of experimental conditions is even more necessary because changes in factors such as diet and water temperature modify reproductive and morphogenesis patterns, highlighting the phenotypic plasticity of the organism (Jordano, 2018; Nawroth et al., 2010; Chiaverano and Graham, 2017). Moreover, standardized procedures promote the generation of more consistent and comparable datasets, strengthening the reliability of conclusions (Monserrat et al., 2007).

Although *Aurelia* has been used as a model organism for several decades, earlier studies were primarily focused on descriptive biology, life cycle characterization, and ecological observations. In contrast, more recent studies have increasingly incorporated ecotoxicological approaches, molecular tools, and emerging contaminants. This shift reflects broader changes in marine research and justifies a focused evaluation of recent methodological developments. The aim of this study is to critically evaluate existing research that employs jellyfish of the genus *Aurelia* as a model organism to identify methodological patterns and approaches. To achieve this goal, a systematic review was conducted that included a literature search, study selection, data extraction and analysis, interpretation of results and preparation of the final report. We also incorporated a scientometric approach to evaluate

trends and patterns in this research field and to identify knowledge gaps and provide recommendations for future studies.

2. Methodology

The literature search was conducted on the Web of Science and Scopus databases, following the criteria established by the PRISMA protocol (Moher et al., 2009). The search strategy was designed to maximize sensitivity by retrieving a broad range of records across databases, including those not explicitly indexed in titles or abstracts. The review involved the following stages: literature search, study selection, data extraction and analysis, interpretation of results and preparation of the final report. The search involved the following combination of keywords: (“toxicology” OR “ecotoxicology”) AND (“cnidarian” OR “*aurelia*”) AND (“tests” OR “biomarkers” OR “assays” OR “bioassays” OR “acute” OR “chronic”).

During the search, we considered articles published between 2015 and July 30, 2025. This temporal restriction was applied to focus on recent methodological approaches and current experimental practices in studies using *Aurelia* as a model organism, particularly those involving emerging contaminants and advances in ecotoxicological and molecular tools. We acknowledge that earlier studies have contributed substantially to the biological and ecological understanding of *Aurelia*; however, these studies often differ in scope and methodological detail compared to more recent work. Therefore, restricting the analysis to the last decade allowed for a more consistent comparison of experimental designs and reporting practices. This choice may have excluded some relevant earlier studies, and this limitation is acknowledged. To broaden the scope of the review, additional relevant studies cited in previous reviews were also considered.

Initially, 2353 articles were identified in Scopus and 54 in Web of Science, reflecting differences in database coverage and indexing criteria. The abstracts were then screened to assess their relevance to the objective of this study. Literature reviews, theses, dissertations, conference papers, gray literature, studies not published in English, extended abstracts, and duplicate records were excluded. After this screening process was complete, 69 articles remained, and these were subjected to full-text reading. Only studies that employed *Aurelia* jellyfish, regardless of the life stage in the assays, were subsequently retained, resulting in a final dataset of 46 scientific articles (Fig. 1).

Data extraction included information related to both the systematic review and the scientometric analysis. For the systematic assessment, we collected data on the *Aurelia* species used, life stage, type of toxicological response (acute or chronic), type of substance tested (micro/nano-plastics, metals, organic compounds, natural products, physicochemical factors, or absence of substances), exposure duration/test duration, application of pre-test feeding, origin of the organisms, temperature and photoperiod of the assays. In the scientometric review, we considered the country of origin of the study, year of publication, and first author to identify geographic patterns in study distribution.

All the data extracted from the selected articles, including tables and supplementary material, were organized in spreadsheets for subsequent analysis. The results are presented in graphs and maps produced using R Studio 4.5.1 (R Core Team and R, 2025) with the packages ggplot2 (Wickham, 2016), dplyr (Wickham et al., 2023a), readr (Wickham et al., 2023b), forcats (Wickham, 2023), sf (Pebesma, 2018), rnatuarearth (South, 2017a) and rnatuarearthdata (South, 2017b).

3. Results and discussion

3.1. Geographical distribution of the studies

The survey of authors (Fig. 2A) and countries (Fig. 2B) with the greatest number of publications indicates a greater concentration of studies in China (n = 16) and Italy (n = 9). This pattern is expected since both *A. aurita* in the Mediterranean/Italy (Brunelli et al., 2024) and

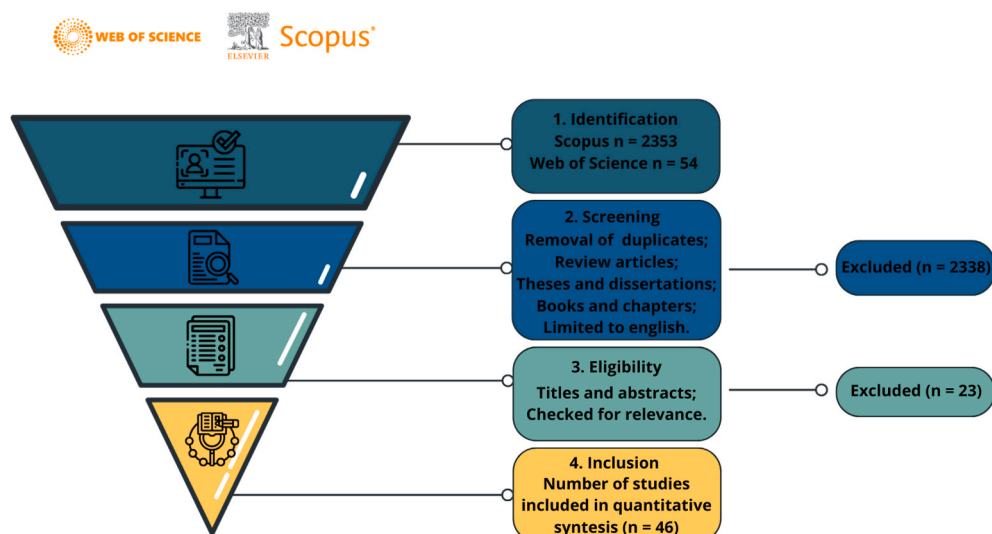


Fig. 1. Flowchart of the review of studies employing *Aurelia* as a model organism. The search was conducted up to July 2025.

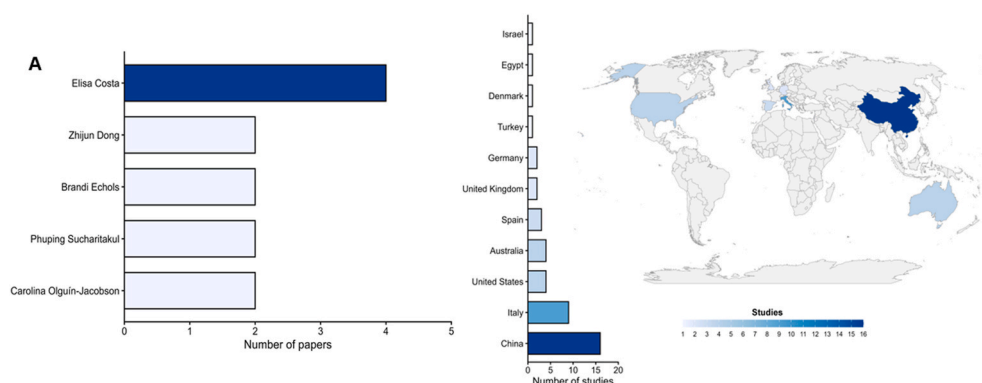


Fig. 2. Geographical and authorship distribution of the articles. A - Ranking of the five most active authors in studies on *Aurelia* worldwide from 2015 to 2025. B - Overview of the number of studies on *Aurelia* worldwide from 2015 to 2025.

A. coerulea in the China Sea (Dong et al., 2019) occur in coastal areas with strong anthropogenic influence and are subject to frequent bloom events. In China, research has shown that the polyp populations of *A. coerulea* in Jiaozhou Bay play a central role in bloom formation because of their high tolerance to variation in environmental conditions and their high rate of asexual reproduction (Zang et al., 2023). Similarly, studies in the Mediterranean have demonstrated that *A. coerulea* can be readily collected in situ, highlighting the ease of obtaining specimens for experimental studies (Stabili et al., 2021).

In China, studies have shown that copper exposure can inhibit the asexual reproduction of polyps, indicating the direct effects of contaminants on the life cycle of this species and possible implications for bloom recurrence (Liu et al., 2024). Other studies reported behavioral and morphological changes in *A. aurita* ephyrae exposed to harmful microalgae (Yang et al., 2024). In Italy, research has shown, for example, that exposure to nanoplastics alters pulsation behavior in ephyrae, demonstrating the sensitivity of the species to emerging contaminants and its potential as an environmental bioindicator (Costa et al., 2023). In addition, biochemical analyses conducted in the Mediterranean have revealed antioxidant and antimicrobial activity in extracts from *A. aurita*, reflecting increasing interest in its physiology and ecotoxicological responses (Stabili et al., 2021).

While China and Italy account for much of the scientific output on *Aurelia*, several countries, including developing nations and tropical regions such as Brazil, show limited representation in the dataset

analyzed. This pattern may reflect not only geographical gaps, but also differences in research focus, laboratory infrastructure, access to cultures, and the temporal restrictions applied in this review. In addition, studies conducted in these regions may not always meet the specific inclusion criteria of this systematic analysis. As a result, much of the available knowledge remains concentrated in a few research centers, which may lead to regional biases and constrain a broader understanding of the biological responses of the genus. This geographic asymmetry may reflect limitations in laboratory infrastructure, access to cultures, funding, or technical capacity, as well as the lack of widely adopted protocols that facilitate the use of the organism in different institutional contexts. The scarcity of studies in other regions therefore represents a relevant knowledge gap and highlights opportunities to expand the use of *Aurelia* into new environmental and socioeconomic contexts.

Finally, the consolidation of scientific infrastructure and the long-standing tradition of research on gelatinous organisms help explain why Italy and China stand out with respect to scientific publications on *Aurelia*. In China, coastal laboratories equipped for culturing and experimentation have enabled significant advances in toxicological and physiological studies (Liu et al., 2024; Wang et al., 2025). In Italy, the role of institutions such as the universities of Naples and Lecce has also supported research on the genus (Stabili et al., 2021; Zuccarotto et al., 2025). In addition to these universities, the work of Dr. Elisa Costa stands out; she is the most cited first author in studies on *Aurelia* between

2015 and 2025 ($n = 4$) (Fig. 2A). She is affiliated with the Institute for the Study of Anthropogenic Impacts and Sustainability in the Marine Environment (CNR-IAS), which is part of the Italian National Research Council (CNR) in Rome. The CNR-IAS leads national initiatives for monitoring and assessing anthropogenic impacts, which includes the “Difendiamo il mare” campaign (Garaventa et al., 2021), in partnership with Greenpeace, providing infrastructure and collaborative networks that support research on the moon jellyfish, from laboratory culture to ecotoxicological assays.

3.2. Temporal trends in publications

The increase in the number of studies employing *Aurelia* as a model in laboratory assays (Fig. 3) reflects shifts in research priorities over the past decade. First, global attention to emerging pollutants such as microplastics has increased substantially after 2015, prompting researchers to seek alternative marine invertebrates with high sensitivity and reliable laboratory maintenance, a role for which *Aurelia* has proven particularly suitable (Costa et al., 2020; Di Giannantonio et al., 2022). Second, interest in the biotechnological potential of jellyfish-derived compounds has also expanded (Geum and Yeo, 2022; Stabili et al., 2021; Li et al., 2022). Combined with its ease of cultivation and well-defined life cycle (Giussani et al., 2016), these factors have contributed to the expanded use of *Aurelia* across toxicological and biochemical studies. Between 2015 and 2025, there is a clear upward trend in scientific output involving *Aurelia* (Fig. 3). It is important to note that the number of studies in 2025 may be underestimated, as the dataset analyzed in this review includes only studies published up to July of that year.

3.3. Experimentally relevant traits of the organisms

3.3.1. Species used

The predominance of studies using *A. aurita* ($n = 24$) over *A. coerulea* ($n = 14$) (Fig. 4A) reflects the complex taxonomic history of the genus, in which all species were previously classified under the name *A. aurita* until molecular analyses revealed that this species actually represented a combination of cryptic species (Dawson and Jacobs, 2001). This revision and other studies (Gómez Daglio and Dawson, 2017) provided evidence of more than 16 possible species in *Aurelia*, which is supported by molecular genetics and morphological analyses. More recently, the number of species has increased to 28 (Lawley et al., 2021). However, species identification, especially in polyp, ephyra, and juvenile jellyfish, which are most often used in bioassays, is only possible through molecular analysis for most species. As a result, the number of studies attributed to *Aurelia aurita* may be overestimated, especially in laboratories that have

maintained colonies for several decades without molecular revalidation of their lineages.

The formal recognition of *A. coerulea* as a valid species (Scorrano et al., 2017) likely contributed to the correction of this nomenclature in more recent studies, particularly those conducted in Asia and the Mediterranean. The term *Aurelia* sp., in turn, reflects caution, indicating that identification was based solely on morphological traits, without genetic confirmation, a practice that is still common in ecotoxicological, ecological and physiological research. However, given the high cryptic diversity within the genus, this lack of taxonomic resolution may introduce variability in experimental outcomes, as different species can exhibit distinct physiological and ecological responses. Therefore, genetic identification of laboratory cultures prior to experimental assays is essential to improve reproducibility and comparability across studies, as demonstrated by (Morales et al., 2025).

3.3.2. Life stages

The distribution of *Aurelia* life stages employed across assays was relatively balanced, with similar numbers of studies conducted with polyps ($n = 13$), ephyrae ($n = 13$), and medusae ($n = 11$) (Fig. 4B). This balance indicates that no single life stage dominates literature; different stages are used for different purposes in experiments. While polyps and ephyrae are mainly employed in ecotoxicological assays because of their ease of cultivation and sensitivity to contaminants (Chen et al., 2025; Wu et al., 2025), adult medusae appear more frequently in studies focused on biochemical or biotechnological characterization because of their size and greater biomass, which facilitates the extraction of mucins, extracts, or bioactive metabolites (Stabili et al., 2021; Li et al., 2022; Ben-David et al., 2023).

In addition, most studies employ only one life stage per assay ($n = 37$), whereas combinations of stages (e.g., polyps and ephyrae or polyps and medusae) are less common ($n = 7$). This pattern is likely related to methodological and logistical constraints, since each life stage of *Aurelia* requires distinct conditions for cultivation, feeding, and monitoring, which complicates the implementation of simultaneous and standardized tests. However, because *Aurelia* has a metagenic life cycle, alterations in early stages may affect subsequent phases of development. Therefore, studies integrating multiple life stages can provide a more comprehensive understanding of organismal responses, particularly in ecotoxicological contexts (Morales et al., 2025).

3.3.3. Acquisition of organisms

Analysis of the sources of organisms (Fig. 4C) revealed that most studies employed jellyfish obtained from laboratory cultures ($n = 23$), followed by organisms collected from the environment ($n = 15$) and those acquired from private sources ($n = 9$), such as aquaria. This

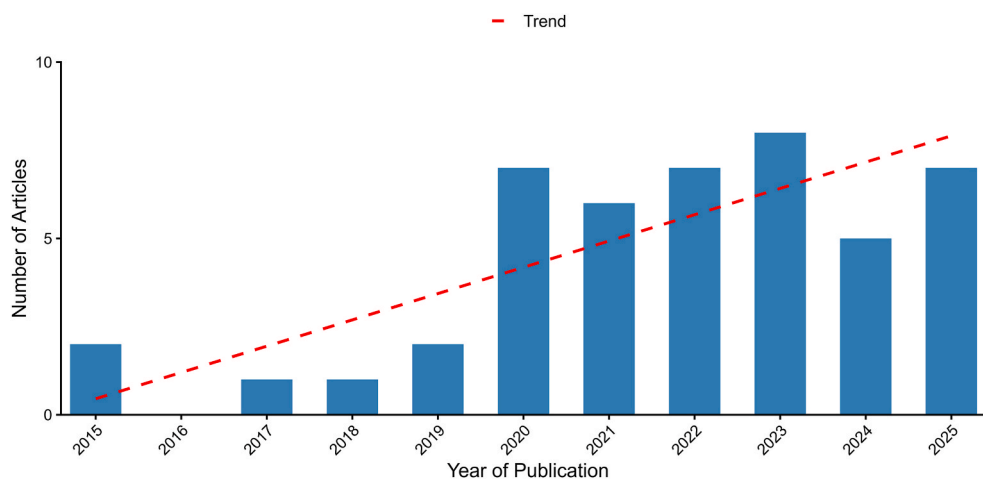


Fig. 3. Temporal trend of studies on ecotoxicology employing *Aurelia* spp. worldwide from 2015 to July 2025.

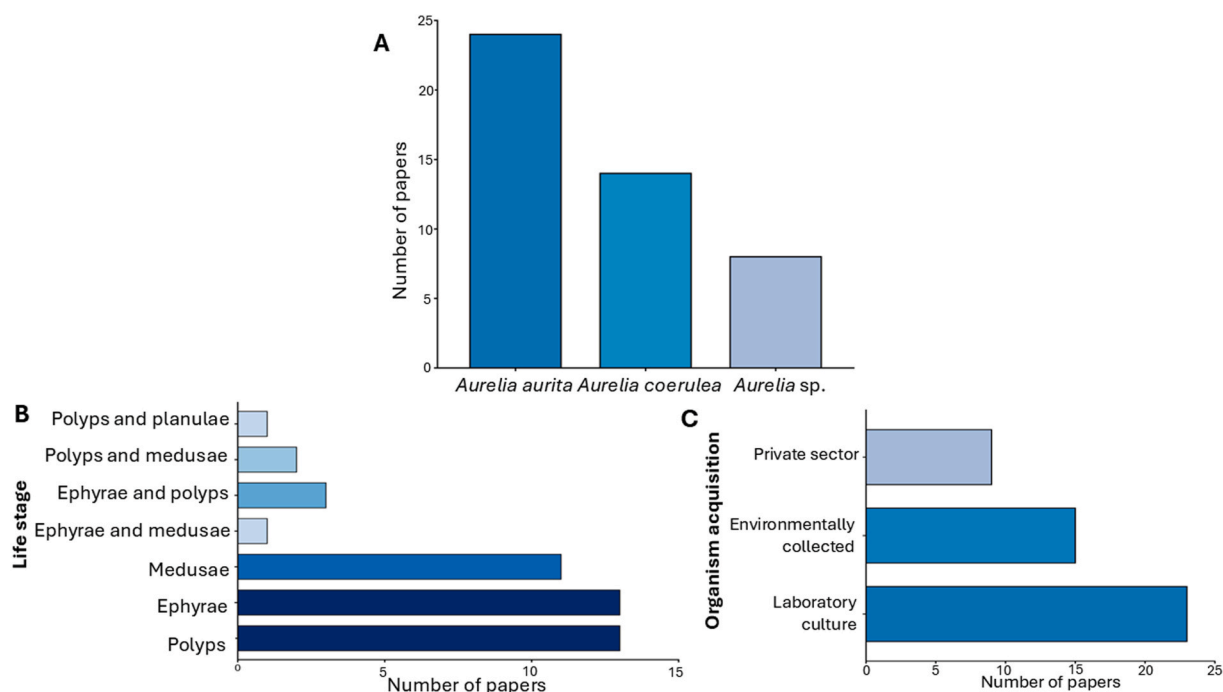


Fig. 4. Experimental parameters of studies on *Aurelia* worldwide from 2015 to 2025. A - Species used. B - Life stages used. C - Origin of the organisms.

pattern likely reflects the widespread use of *Aurelia* in laboratory-based research. Environmental collection, while less frequent, remains feasible because of the natural abundance of the species in coastal areas, particularly during bloom events (Zang et al., 2023). Acquisition from the private sector is typically associated with research laboratories that do not maintain their own cultures. In this context, the predominance of laboratory cultures may indicate a global tendency to prioritize replicability and experimental control, reinforcing the importance of standardized methods to ensure reproducibility and comparability of results

(Open Science Collaboration, 2015; Baker, 2016).

3.4. Experimental conditions

3.4.1. Temperature

With respect to the temperatures used in the studies (Fig. 5A), most assays ($n = 25$) were conducted at moderate temperatures (20–25 °C). This range matches the temperatures commonly used for culturing polyps and ephyrae (Ballesteros et al., 2022) and the conditions under

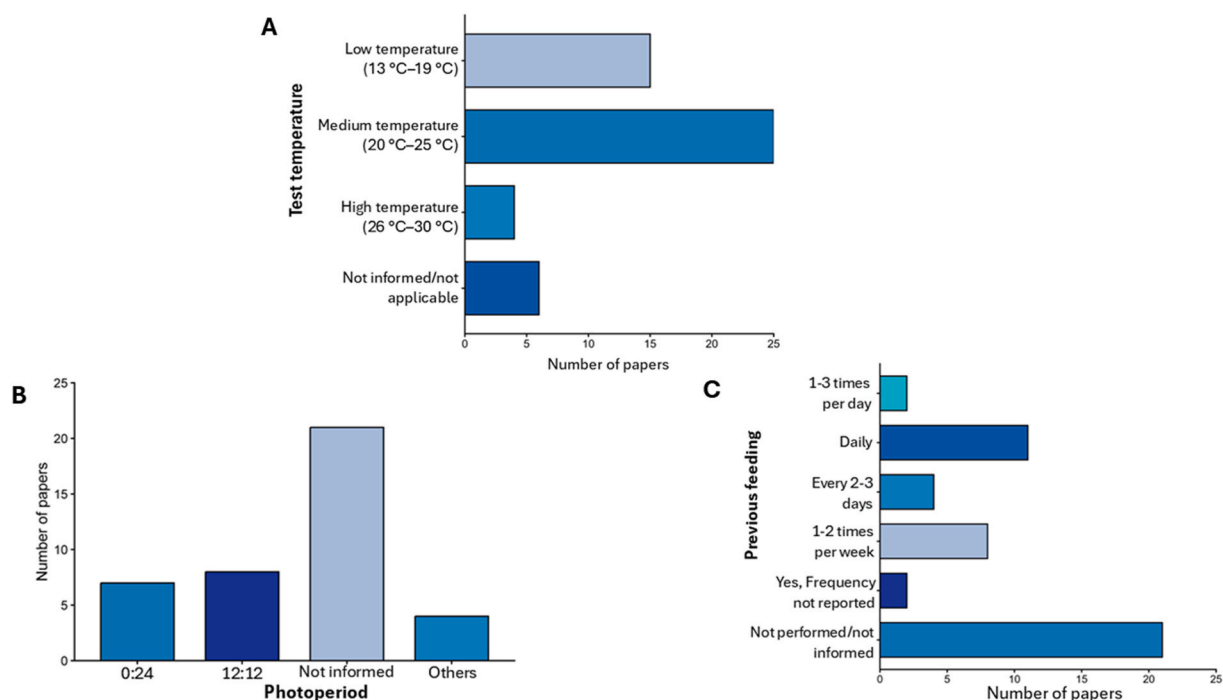


Fig. 5. Experimental conditions of studies on *Aurelia* from 2015 to 2025. A - Temperatures adopted in the assays. B - Photoperiods used in the assays. C - Pretest feeding conditions.

which ephyrae production is not inhibited (Holst et al., 2012). Low temperatures (13–19 °C) were used in 15 studies and were generally associated with toxicity tests involving microplastics and metals (Ringwood et al., 2025; Goldstein and Javidpour, 2023; Brunelli et al., 2024; Wang et al., 2025; Devereux et al., 2021; Romero-Kutzner et al., 2022). In most cases, these temperatures are reported as standardized experimental conditions for culture and testing.

Even so, this temperature range is frequently used in tests with polyps and ephyrae, which suggests that its use follows a methodological convention replicated in the literature, likely related to keeping the organisms under stable conditions during experimentation. As with many poikilothermic organisms, lower temperatures induce behavioral changes; in the case of the ephyrae and medusae of *Aurelia*, this means a lower pulsation frequency, slower growth rates, and changes in morphogenesis patterns (Jordano, 2018; Nawroth et al., 2010; Fu et al., 2020). On the other hand, higher temperatures can intensify metal toxicity by increasing metabolic activity and ionic accumulation in the tissues of aquatic invertebrates (Sokolova and Lannig, 2008; Haque et al., 2020). Similarly, recent reviews on microplastics highlight that synergistic interactions between temperature and emerging contaminants may enhance physiological and oxidative responses (Klasios et al., 2024). Therefore, one possible explanation for the use of this temperature range is the intention to minimize confounding effects and isolate the impact of contaminants, since warming may amplify the response and confound the interpretation of results.

Only four studies (Fig. 5A) employed high temperatures (26–30 °C). In one study, elevated temperatures were used to simulate future climate scenarios (Pinnow et al., 2023), and the response of *A. aurita* to gradual increases in temperature and salinity was evaluated. The other three studies focused primarily on derived products and tested the toxicity and applications of mucin and tentacle extracts from moon jellyfish (Geum and Yeo, 2022; Stabili et al., 2021; Li et al., 2022). A similar pattern was observed in studies in which temperature was either not reported or not applicable (n = 5) (Fig. 5A), which included experiments focused on biochemical, molecular, or technological characterization rather than direct exposure of organisms (Zuccarotto et al., 2025; Liu et al., 2018; Killi et al., 2020; Bleve et al., 2021; Khalil et al., 2022). These studies analyzed antioxidant compounds, structural proteins, or natural venoms, as well as industrial applications such as food processing and bioprospecting, which explains the absence of temperature control, since they did not involve live organisms in testing.

In these cases, the higher temperatures reflect the laboratory conditions required for biochemical or biotechnological assays (such as antioxidant activity, hemolysis, or protein stability tests) rather than an attempt to evaluate thermal tolerance in the medusa. Thus, both the “high temperature” and “not applicable” categories involve studies focused on the functional properties and applications of *Aurelia* products rather than on ecotoxicological investigations involving live organisms.

3.4.2. Photoperiod

In most studies (n = 21), the photoperiod (light:dark cycle, L:D) was not reported (Fig. 5B), which hinders reproducibility and limits comparisons. The “other” category (n = 4) includes photoperiods of 14:10 h, 16:08 h and 16:24 h (L:D) (Roveta et al., 2022; Park et al., 2025; Yang et al., 2024; Ha et al., 2020), likely reflecting methodological adjustments according to the experimental goals. Eight studies employed a 12:12 h cycle, whereas in seven, the organisms were kept under constant darkness (0:24 L:D), mainly in assays involving microplastics and nanoplastics (Costa et al., 2023; Wu et al., 2025; Gambardella et al., 2024).

Although the literature recognizes that light availability can influence physiological processes in cnidarians, such as cnidocyte discharge and behaviors mediated by visual stimuli (Picciani et al., 2021), there is no consensus on a standardized photoperiod for ecotoxicological assays with *Aurelia*. Experimental studies, however, have shown that light can modulate strobilation. Under continuous darkness, the polyps of

A. aurita display significantly higher strobilation rates, reaching nearly 95%, compared with less than 30% under constant light (Ishii and Shioi, 2003). Liu et al. (2008) also reported that illumination, although secondary to temperature, can accelerate the onset of strobilation when combined with favorable thermal conditions. Thus, darkness tends to favor strobilation, whereas controlled photoperiods (such as 12:12 h) maintain behavioral and physiological stability, making the organisms more suitable for prolonged experiments or those involving behavioral endpoints.

3.4.3. Feeding

In most studies, pre-test feeding was either not performed or not reported (n = 21) (Fig. 5C). The lack of this information compromises the interpretation and reproducibility of the results, since many endpoints used in *Aurelia*, such as pulsation frequency, behavior, and growth, depend on the physiological status of the organisms and are sensitive to environmental stressors (Costa et al., 2020; Yang et al., 2024; Pinnow et al., 2023). Thus, when the nutritional condition prior to an assay is unknown, it becomes difficult to distinguish whether the observed changes reflect contaminant exposure or the initial condition of the animals, such as starvation and underfeeding. Notably, in the vast majority of studies in which organisms were fed, they were given *Artemia* nauplii (Park et al., 2025; Wang et al., 2025).

In the second most common group, the organisms were fed daily (n = 11). One possible explanation is the need to maintain stable body conditions prior to exposure to stressors. Studies have shown that increased food availability (i.e., ad libitum) is associated with increased pulsation rates and growth in the early stages of *Aurelia*, as well as accelerated morphological development (Holst et al., 2012). In recent assays, higher prey availability also resulted in greater biomass gain and filtration activity in medusae (Goldstein and Javidpour, 2023). On the other hand, starvation may delay the growth of jellyfish, cause shrinkage (Jordano, 2023), interrupt spawning (Hamner and Jenssen, 1974), and cause malformation (Widmer, 2015). The rapid response of jellyfish to a lack of food results from the absence of usual storage tissues (wax esters) and low lipid content (Nagata et al., 2024), which results in physiological responses that may be considered in experimental design.

The stages of polyp and ephyra are less sensitive to the lack of daily feeding, as they can delay death for months without food. The point of no return for ~50% of *Aurelia* spp. ephyrae was between 33 and 58 days, reaching 100 days without feeding (Fu et al., 2014). However, it is still unclear when the physiological effects of fasting begin in these phases. Feeding frequencies of 1–2 times per week (n = 8) or every 2–3 days (n = 4) may reflect simpler maintenance routines used in laboratory cultures, especially considering that many of these studies focused on polyps (Wang et al., 2025; Wu et al., 2025; Chen et al., 2024), in which strobilation can occur even under moderate feeding regimes (Holst et al., 2012).

A small subset of studies reported feeding the organisms 1–3 times per day (n = 2), and these studies did not focus on polyps but on the planktonic stage. In Ballesteros et al. (2022), frequent feeding with *Artemia* nauplii was used to maximize growth and survival in a system designed for early-stage biomass production. In Sucharitakul et al. (2020), regular feeding functioned as a behavioral stimulus to ensure that ephyrae maintained capture activity, allowing for the accurate assessment of microplastic ingestion and egestion. In two studies, feeding was mentioned, but the frequency was not specified (Zuccarotto et al., 2025; Devereux et al., 2021). Taken together, pre-test feeding conditions appear to be poorly standardized and inconsistent among studies, despite their direct influence on physiological responses. Therefore, feeding protocols should be clearly standardized and reported, including food type, quantity, and frequency, to ensure reproducibility and allow comparisons across studies. For example, the amount of food offered to experimental organisms can be quantitatively standardized through dilution techniques or direct counting of *Artemia* nauplii with the aid of micropipettes (Jordano et al., 2022).

3.4.4. Tested substances

Most studies involving *Aurelia* have focused on micro- and nano-plastics (n = 16) (Fig. 6A), likely because these are emerging pollutants of major environmental concern that are capable of affecting behavior, reproduction and metabolism across different developmental stages of the moon jellyfish (Costa et al., 2020; Di Giannantonio et al., 2022; Wu et al., 2025). Metals such as copper and cadmium were also frequently tested (n = 11) (Fig. 6A). These elements are common contaminants in coastal environments and have well-documented ecotoxicological effects on *Aurelia*, including the inhibition of asexual reproduction in polyps and physiological alterations in ephyrae (Wang et al., 2023; Dong et al., 2019; Liu et al., 2024). In a considerable number of studies (n = 10), external substances were not used; instead, the biological and bioactive properties of *Aurelia* itself were investigated, such as its antioxidant and antimicrobial activity and mucus properties, suggesting that interest in biotechnological applications of the moon jellyfish has been increasing (Stabili et al., 2021; Li et al., 2022; Bleve et al., 2021).

In some studies, organic compounds (n = 6), such as pesticides and saponins, were tested (Olguín-Jacobson et al., 2021; Dong et al., 2017), and physiological and behavioral responses were evaluated, whereas in others, natural products (n = 2) and physicochemical factors (n = 1) were tested (Yang et al., 2024; Pinnow et al., 2023; Dong et al., 2017). In other words, *Aurelia* stands out as an organism that enables multiple approaches, functioning both as a model organism for ecotoxicological studies and as a source of biotechnological compounds.

3.4.5. Observed responses

With respect to endpoints (Fig. 6B), the most abundant group comprises studies that simultaneously assessed behavioral and lethal effects (n = 15), indicating a preference for assays that capture both sensitive responses and more extreme toxicological outcomes within a single experiment. The second most common group corresponds to studies classified as “not applicable” (n = 12), in which alive organisms belonging to *Aurelia* were not exposed to stressors. In these cases, the species was used as a source of biomaterials or bioactive compounds or as a molecular model, with goals distinct from those of experimental ecotoxicology. Examples include studies employing medusa extracts for

nanomaterial removal or antimicrobial testing (Park et al., 2025; Shim et al., 2025), molecular analyses of ovothiol biosynthesis (Zuccarotto et al., 2025), and biochemical investigations of lysosomal antioxidant activity in *Aurelia* extracts (Stabili et al., 2021).

Studies that assessed only behavioral responses were less common (n = 8) and were associated with continuous endpoints such as growth, budding, and strobilation (Goldstein and Javidpour, 2023; Brunelli et al., 2024). These parameters have been monitored in polyps because they allow for physiological and reproductive responses to be tracked over time under laboratory conditions, particularly under environmental change, as discussed by Holst (Holst et al., 2012). Studies that simultaneously evaluated behavioral and cellular responses (n = 6) and those exclusively focused on cellular endpoints (n = 3) have shown that exposure to stressors can trigger measurable sublethal alterations, such as increased reactive oxygen species production, lipid peroxidation (MDA), and the modulation of antioxidant enzymes (Wang et al., 2023; Liu et al., 2024). In some cases, these responses are accompanied by histological damage, including vacuolization and injury to the ectoderm (Chen et al., 2025), suggesting structural impairment even in the absence of mortality.

Only one study exclusively assessed acute responses, indicating that this type of design is uncommon. Similarly, only a single study simultaneously integrated acute, chronic, and cellular biomarker responses, probably because multilevel methodologies are still rarely applied in *Aurelia* because of logistical and/or laboratory constraints. However, recent evidence has shown that behavioral, cellular, and histological responses do not always occur in parallel and may even diverge within the same experiment, highlighting the need to integrate multiple levels of analysis to fully elucidate toxic effects (Olguín-Jacobson et al., 2021; Chen et al., 2025; Pinnow et al., 2023). Furthermore, biochemical and physiological responses can be modulated by different factors and may not immediately reflect effects at higher levels of biological organization, emphasizing the importance of integrating multiple levels of biological organization in toxicity assessments (Monserrat et al., 2007).

3.4.6. Exposure duration

The exposure duration in the assays (Fig. 6C) was balanced across

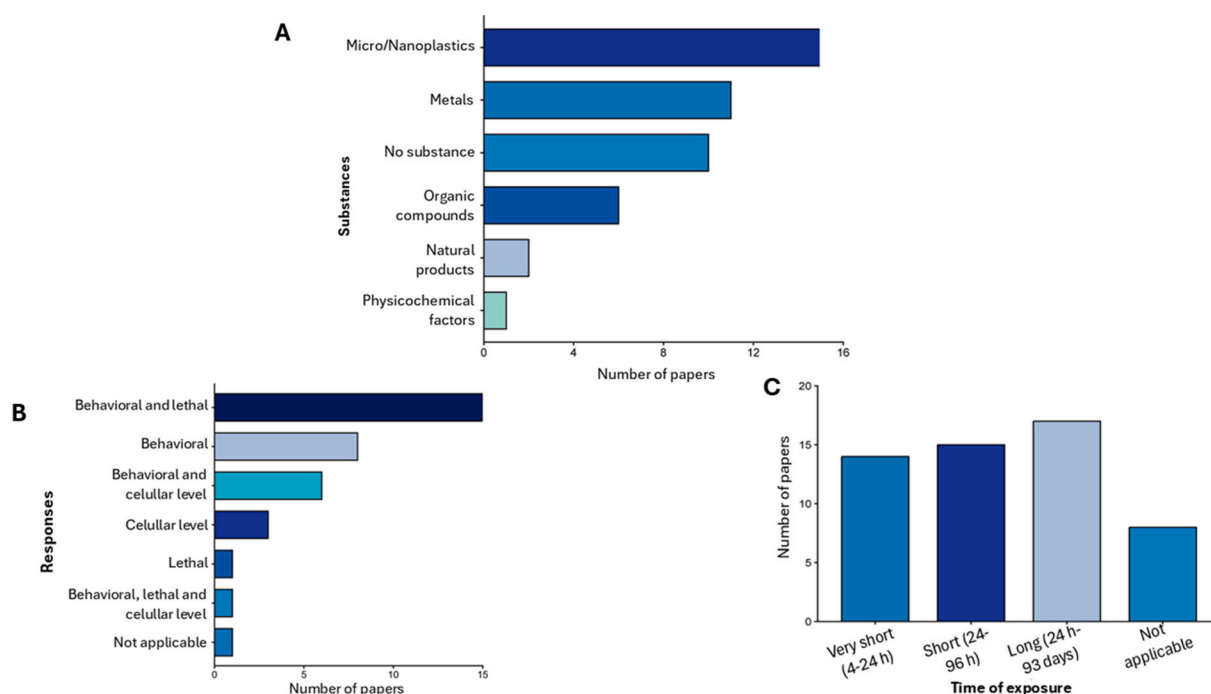


Fig. 6. Experimental conditions in studies on *Aurelia* from 2015 to 2025. A - Substances tested in the assays. B - Types of responses observed. C - Duration of the assays.

different categories: very short (4–24 h), short (24–96 h), long (96 h–93 days), and not applicable. Very short assays ($n = 14$) were focused on acute toxicity and immediate responses such as pulsation, immobilization, or early mortality, especially in ephyrae exposed to rapid-acting contaminants such as pesticides, surfactants, or microplastics (Costa et al., 2020; Gambardella et al., 2024; Dong et al., 2017).

In the short-duration category ($n = 15$), experiments were predominantly aimed at observing both behavioral effects (pulsation, swimming, and immobilization) and physiological alterations, such as enzymatic variation and oxidative indicators (Echols et al., 2015; Costa et al., 2015, 2023). Differences among studies indicate that the 24–96 h period can reveal early sublethal effects as well as rapid lethal responses, depending on the life stage and the toxicity of the compound.

Long-duration assays ($n = 17$), mostly chronic in nature, were focused on cellular-level responses and were conducted with polyps, which allowed for the continuous observation of sublethal effects such as enzymatic, oxidative, and reproductive alterations (Chen et al., 2024, 2025; Wang et al., 2025; Wu et al., 2025; Romero-Kutzner et al., 2022). In these studies, periods of 7–28 days, or even several months, allowed for the investigation of cumulative effects, including impacts on asexual reproduction via budding or strobilation.

The “not applicable” group ($n = 8$) included studies that did not expose live organisms to environmental stressors but used *Aurelia*-derived products in bioprospecting or biotechnological contexts (Geum and Yeo, 2022; Stabili et al., 2021; Zuccarotto et al., 2025; Li et al., 2022; Ballesteros et al., 2022). In these cases, experimental duration was not associated with biological exposure time but rather with the period of chemical incubation or analytical assays. Notably, some studies appeared in more than one temporal category because multiple tests were performed using *Aurelia* in different life stages. For example, Shim et al. (2025) and Wu et al. (2025) combined rapid contaminant-removal assays with prolonged exposure in polyps.

3.5. Guidelines for protocol standardization and future research

Although the use of *Aurelia* has increased in recent years, there are still no clear guidelines for cultivation practices, experimental design, or endpoint selection. This contrasts with other well-established aquatic model organisms widely used in ecotoxicology, such as *Danio rerio*, *Daphnia magna*, and *Acartia tonsa*, for which protocol standardization has been progressively achieved through decades of coordinated experimental research and comparative methodological assessments (Ringwood et al., 2025; Lopes et al., 2021; Monserrat et al., 2007). For *Aurelia*, a key priority is the development of life-stage-specific protocols that account for distinct physiological requirements, coupled with comparative studies across laboratories to identify which experimental variables most strongly drive variation in biological responses.

To improve reproducibility and comparability across studies, we propose that future experimental designs using *Aurelia* should incorporate a set of minimum standardized conditions. These include: (i) clear taxonomic identification of the species used, preferably supported by molecular tools; (ii) explicit reporting of life stage, including developmental criteria (e.g., size or age); (iii) detailed description of environmental conditions, particularly temperature and photoperiod (light:dark cycle); (iv) standardized feeding protocols, including food type, quantity, and frequency; and (v) clear definition of exposure conditions, including duration, concentration, and pre-test procedures. The data gathered in this review highlight key aspects of this advancement. Based on the patterns identified in this review, environmental conditions should be explicitly defined and controlled, particularly temperature and photoperiod, as these factors strongly influence metabolism, pulsation, and strobilation in *Aurelia* (Jordano, 2018; Holst et al., 2012). Most ecotoxicological assays have been conducted at moderate temperatures (20–25 °C), suggesting that this range is suitable for ensuring physiological stability while minimizing confounding stress effects. Feeding and culturing routines should also be clearly described, with

Artemia nauplii being the most used food source and feeding frequency should be defined according to the life stage under investigation.

Furthermore, accurate taxonomic identification of the species and a clear definition of the evaluated endpoints are essential, as these factors represent major sources of variation among studies. At present, the lack of specific guidelines limits comparisons between studies involving *Aurelia* and may result in methodological variation that outweighs biological effects. Thus, the development and validation of standardized, life-stage-specific protocols are critical steps for increasing reproducibility and supporting the future inclusion of *Aurelia* in regulatory and applied ecotoxicology frameworks.

4. Conclusion

This review critically evaluated the use of *Aurelia* as a model organism in experimental studies, identifying key methodological patterns and sources of variability across the analyzed literature. The studies analyzed reveal an increasing number of publications over the past decade and a wide diversity of tested contaminants, endpoints, and experimental durations, demonstrating the versatility of the organism. However, this diversity is accompanied by substantial methodological heterogeneity, particularly in environmental conditions, feeding regimes, life-stage selection, and endpoint definition. On the other hand, there is uneven geographical distribution, with research concentrated mainly in China and Italy and with few studies conducted in other regions, including developing countries. This trend reflects not only the growing scientific interest in the moon jellyfish but also highlights gaps that may limit broader understanding of the species.

Despite this heterogeneity, the literature indicates that *Aurelia* is a promising model organism for both investigating the ecological effects of pollutants and for biotechnological applications. Its use is likely to expand as future studies adopt more standardized and transparent experimental designs, enabling more robust comparisons and strengthening its role as a model organism in marine ecotoxicology and applied research.

CRediT authorship contribution statement

Laís Olivera das Neves: Investigation, Methodology, Validation, Writing – original draft. **Mikael Luiz Pereira Morales:** Writing – review & editing. **Sanye Soroldoni:** Writing – review & editing. **Renato Mitsuo Nagata:** Writing – review & editing. **Grasiela Lopes Leães Pinho:** Writing – review & editing.

Declaration of competing interest

The authors declare no conflicts of interest.

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Data availability

Data will be made available on request.

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The route of exposure determines the toxicity of antifouling paint particles (APPs) in different life stages of *Aurelia coerulea* Lamarck, 1816 (Cnidaria, Scyphozoa)

Laís Olivera das Neves^{a*}; Mikael Luiz Pereira Morales^{a,b}; Sanye Soroldoni^a;
Renato Mitsuo Nagata^c; Camila de Martinez Gaspar Martins^d; Grasiela Lopes
Leães Pinho^a;

^a Programa de Pós-graduação em Oceanologia (PPGO), Instituto de Oceanografia (IO), Universidade Federal do Rio Grande (FURG), Rio Grande, Rio Grande do Sul, Brasil. Av. Itália, Km 8, Carreiros, CEP: 96203-900 - Rio Grande, Rio Grande do Sul, Brasil.

^b Programa Institucional de Pós-Doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PIPD-CAPES/PPGO).

^c Programa de Pós-Graduação em Oceanografia Biológica, Instituto de Oceanografia da Universidade Federal do Rio Grande (FURG). Av. Itália, Km 8, Carreiros, CEP: 96203-900 - Rio Grande, Rio Grande do Sul, Brasil.

^d Programa de Pós-Graduação em Ciências Fisiológicas, Instituto de Ciências Biológicas (FURG). Av. Itália, Km 8, Carreiros, CEP: 96203-900 - Rio Grande, Rio Grande do Sul, Brasil.

***Corresponding author:** laisdneves00@gmail.com

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HIGHLIGHTS

- Exposure route modulates the toxicity of antifouling paint particles (APPs) in *Aurelia coerulea*
- Waterborne exposure induced greater toxicity than dietborne exposure
- International® paint showed higher toxicity than Renner® paint
- Responses to APPs differed between the polyp and ephyra life stages
- Prey-mediated exposure at the highest APP concentration induces both lethal and sublethal effects in jellyfish

ABSTRACT

Antifouling paint particles (APPs) are an emerging source of biocides in coastal environments; however, the influence of different exposure routes on their toxicity remains poorly understood. In this study, we assessed how waterborne and dietborne exposure influence the toxicity of APPs derived from two

commercial antifouling paints at different ontogenetic stages of the jellyfish *Aurelia coerulea*. Dietborne exposure was simulated through the ingestion of *Artemia franciscana* previously exposed to the particles. Under waterborne exposure, high toxicity was observed in both *Artemia franciscana* and *A. coerulea*, with 100% mortality in *A. franciscana* at a concentration of 4 mg L⁻¹ for International® paint. Total polyp mortality over the exposure period, and reduced pulsation frequency accompanied by immobility were found in ephyrae. Under dietborne exposure effects occurred primarily at the highest concentrations, with no mortality in polyps exposed to APPs from Renner® paint and mortality observed only at 14 mg L⁻¹ for International® paint. Differences between formulations were evident. For International® paint, 100% mortality was recorded in *A. franciscana* at 4 mg L⁻¹, whilst for Renner® paint, mortality at the same concentration was 68.2%. Furthermore, lethal and sublethal responses in *A. coerulea* were recorded at lower concentrations for International® paint compared to Renner®. Distinct toxicological responses were observed between the polyp and ephyra stages, indicating stage-specific susceptibility to APP exposure. The results demonstrate that exposure route influences APP toxicity and that the ingestion of contaminated prey represents an effective pathway for contaminant transfer, indicating the potential of these particles to propagate through marine food webs.

1. INTRODUCTION

Antifouling paint particles (APPs) are derived from coatings applied to vessel hulls and other submerged structures to prevent colonisation by fouling organisms (Torres & De-la-Torre, 2021). These paints generally contain biocides that are gradually released into the aquatic environment with the aim of preventing the settlement of such organisms (Watermann et al., 2005; Torres & De-la-Torre, 2021). In self-polishing antifouling systems, biocides are released from the paint matrix in a controlled manner, although they are not necessarily chemically bound within it (Omae, 2003). The gradual hydrolysis or dissolution of the paint surface in seawater continuously exposes fresh layers containing biocides, ensuring a predictable and sustained release of these compounds over extended periods (Omae, 2003). However, over time, the efficacy of these paints diminishes as biocides leach into the aquatic environment, leading to the renewed formation of fouling (Almeida et al., 2007). This necessitates the drydocking of

the vessel to remove the old antifouling system and apply a fresh coat of paint, resulting in the introduction of APPs into marine ecosystems (Almeida et al., 2007).

APPs exhibit considerable chemical heterogeneity across their layers (Singh & Turner, 2009). Even following use on vessels, high concentrations of metals, as well as co-biocides, can be found in older layers (Soroldoni et al., 2018). Chemical characterisation studies demonstrate that Cu is frequently the dominant element in these particles, occurring at concentrations exceeding 200,000 $\mu\text{g g}^{-1}$, and acting as the principal biocide in modern antifouling formulations (Soroldoni et al., 2017). Cu is commonly combined with organic co-biocides to enhance paint efficacy, and the improper disposal of such waste contributes to the enrichment of these compounds in the environment (Soroldoni et al., 2018). In areas adjacent to shipyards and marinas, high concentrations of biocides have been recorded in surface sediments (Rees et al., 2014; Soroldoni et al., 2018). The absence of comprehensive regulation and legislation governing the generation and disposal of APPs in Brazil further underscores the importance of studies evaluating their ecotoxicological effects on aquatic organisms (Soroldoni et al., 2018). Although international agreements, such as the International Convention on the Control of Harmful Anti-fouling Systems on Ships, adopted in 2001 and fully enforced in 2008, have restricted highly toxic compounds such as tributyltin (TBT), many co-biocides and metals used in current formulations continue to be released into the environment.

Paint particles contain high concentrations of potentially toxic additives, with ions such as $\text{Cu}^+/\text{Cu}^{2+}$ and Pb^{2+} released from the paint matrix (Turner, 2021), reinforcing their recognition as environmental pollutants for decades (Kyei et al., 2020). However, knowledge of the ecotoxicity of APPs remains limited (Torres & De-la-Torre, 2021). Tolhurst et al. (2007) investigated, in isolation, the effect of resuspension of sediments contaminated with Irgarol 1051-based APPs on the macroalga *Ulva intestinalis* and reported a negative effect on its growth. Soroldoni et al. (2020) evaluated the toxicity of APP-contaminated sediments and particle elutriate to the benthic crustaceans *Monokalliapseudes schubarti* and *Hyaella azteca*. The organisms exhibited high sensitivity, with 10-day LC_{50} values of 0.45% and 0.16% particles in sediment, respectively (Soroldoni et al., 2020).

However, most studies evaluating the toxicity of APPs have focused exclusively on resuspended sediments or elutriate (e.g., Zaaboub et al., 2015; Othman et al., 2017; Soroldoni et al., 2020). This approach stems from the fact that such waste tends to accumulate in sediments in port areas, shipyards, and marinas following hull maintenance and scraping activities (Turner, 2010; Rees et al., 2014). Nevertheless, APPs are not confined to the sediments of aquatic environments. Hydrodynamic processes can resuspend part of these particles to the water column (Turner, 2010), where compounds present in the paint matrix continue to be released (Torres & De-la-Torre, 2021). In this context, there is evidence demonstrating that paint fragments are widely distributed throughout the water column and may represent a significant fraction of marine microdebris. For instance, Lacerda et al. (2019) recorded APPs in all sea surface samples analysed in Antarctica, at abundances up to 30 times greater than those of conventional plastics, whilst Turner et al. (2022) observed that the majority of non-fibrous microplastics in the North Atlantic corresponded to paint flakes, many of which were associated with metal-rich antifouling formulations.

Despite evidence that APPs may remain in suspension and act as mobile sources of contaminants within the water column, studies investigating the ecotoxicological effects of APPs across different exposure routes remain scarce, particularly those considering direct contact with contaminated water (waterborne exposure) and trophic transfer through the ingestion of contaminated prey (dietborne exposure), a mechanism already described for other particulate contaminants and sediment-associated metals (Wang & Fisher, 1999). Thus, a knowledge gap persists regarding how different exposure routes influence the toxicity of APPs in coastal environments.

The jellyfish *Aurelia coerulea* (Cnidaria, Scyphozoa) was the biological model chosen for this work. In fact, species of the genus *Aurelia* have been used as model organisms in experimental marine ecotoxicology studies evaluating different life cycle stages (e.g., Devereux et al., 2021; Geum & Yeo, 2022; Li et al., 2022; Goldstein & Javidpour, 2023). *Aurelia* has a metagenic life cycle comprising two stages: the polyp, a sessile and benthic phase, and the medusa, a planktonic and vagile phase (Arai, 1997). Polyps produce numerous ephyrae (the juvenile medusae) through the process of strobilation, which represents a form of asexual reproduction (Arai, 1997). The presence of both benthic and planktonic

phases exposes these organisms to contaminants present in both the sediment and the water column (Arai, 1997; Lucas et al., 2012). Furthermore, physiological differences between polyps and ephyrae may influence their responses to stressors (Olguin-Jacobson et al., 2020; Nagata et al., 2024; Morales et al., 2025). Moreover, *Aurelia* species are widely distributed in coastal and estuarine environments, colonising both natural substrata, such as rocks and shells, and artificial structures, such as vessel hulls and concrete structures, where they frequently form blooms in anthropogenically impacted areas (Morales et al., 2025). Given these characteristics, this species is particularly suitable as an experimental model organism, as it is frequently exposed to contaminants associated with anthropogenic activities, including suspended particles and materials accumulated in sediments.

The other biological model used for this work was *Artemia* nauplii which is commonly used as prey in ecotoxicological assays with *Aurelia* sp., both in studies evaluating lethal and sublethal effects (e.g., Brunelli et al., 2024; Wang et al., 2025; Park et al., 2025). Furthermore, *Artemia* is recognised as a model organism in toxicological assays owing to the ease of culture and the rapid hatching of its cysts (Nunes et al., 2006; Libralato et al., 2016). These organisms can assimilate and accumulate contaminants, and have been employed in studies investigating exposure routes and trophic transfer in aquatic food chains (Mathews & Fisher, 2008; Tangaa et al., 2016). As such, the use of *Artemia* allows the experimental evaluation of the trophic transfer of contaminants to predatory organisms.

In this context, the present study aims to evaluate the toxicity of APPs at different ontogenetic stages (ephyra and polyp) of *Aurelia coerulea*, comparing the effects of waterborne and dietborne exposure. Additionally, differences in responses between polyps and ephyrae were investigated with the aim of understanding how exposure route and ontogenetic stage influence the ecotoxicological response to APPs. By integrating different exposure routes and life cycle stages within a single model organism, this study contributes to broadening the understanding of the risks associated with APPs in coastal environments, an aspect that remains largely unexplored in the assessment of the impacts of antifouling systems on marine biota.

2. METHODOLOGY

2.1. Collection and maintenance of test organisms

Polyps of *Aurelia coerulea* were obtained from a culture maintained by the Zooplankton Laboratory (Labzoo) of the Instituto de Oceanografia (IO) at the Universidade Federal do Rio Grande (FURG). At the CONECO laboratory (Laboratório de Microcontaminantes Orgânicos e Ecotoxicologia Aquática, IO-FURG), the culture was replicated and maintained in 300 mL beakers containing artificial seawater at a salinity of 35 (VeroSal Corais) at 20 °C, under a 12:12 h light:dark photoperiod, and fed once weekly with freshly hatched *Artemia franciscana* nauplii. To obtain ephyrae, polyps were induced to strobilation by thermal induction at 15 °C, without feeding and under a 12:12 h light:dark photoperiod for a week (Morales et al., 2025). Newly released ephyrae were collected and used in the experiments. Cysts of *A. franciscana* were obtained from an aquarium supply store and hatched in 1 L beakers containing artificial seawater at a salinity of 35, at 25 °C, under constant illumination from a 25 W fluorescent lamp and continuous aeration (adapted from Libralato et al., 2007). Prior to the commencement of the assays, all polyps were fed *ad libitum* with *A. franciscana* nauplii 24 h before the experiments to standardise their nutritional condition.

2.2. Test solutions

Assays with antifouling paint particles (APPs) were conducted using polyps, ephyrae, and *Artemia* to estimate the toxicity of these materials (Table 1). The APPs were collected manually in 2014 during vessel maintenance at two shipyards in the municipality of Rio Grande, southern Brazil, by scraping the antifouling paints Supermarine AF Ionex (Renner®) and Micron Premium (International®), as described in Soroldoni et al. (2018). The samples were sieved (between 180 µm and 1 mm) and stored under refrigeration at 4 °C. Both samples were previously characterised chemically with respect to the concentrations of Cr, Cu, Mn, Ni, Pb, Sn, Zn, Diuron, Irgarol, TCMTB, and DCOIT (Soroldoni et al., 2018). The control treatment consisted of artificial seawater at a salinity of 35, prepared under the same conditions used for the preparation of solutions containing APPs, but without the addition of particles.

The concentrations tested are environmentally realistic for areas impacted by vessel maintenance activities, where APPs may reach concentrations of up to 44,300 mg g⁻¹ in sediment and represent a significant fraction of particulate matter in the environment (De-la-Torre et al., 2023; Torres & De-la-Torre, 2021). Furthermore, the concentrations were selected to elicit toxic effects without causing total mortality of the organisms, thereby allowing the assessment of sublethal endpoints such as pulsation frequency, immobility, and tentacle contraction.

Table 1. Treatments and endpoints assessed for test organisms during assays with antifouling paint particles (APPs).

Treatments	Polyps	Ephyrae	<i>Artemia</i>
APPs Renner® (mg L ⁻¹) waterborne exposure	0, 1, 2, 3, 4	0, 0.25, 0.5, 1, 2	0, 0.5, 1, 2, 4
APPs Renner® (mg L ⁻¹) dietborne exposure	0, 1, 2, 3, 4, 12	0, 0.5, 1, 2, 4, 10	-
APPs International® (mg L ⁻¹) waterborne exposure	0, 0.5, 1, 2, 4	0, 0.25, 0.5, 1, 2	0, 0.5, 1, 2, 4
APPs International® (mg L ⁻¹) dietborne exposure	0, 0.5, 2, 4, 14	0, 0.5, 1, 2, 4, 10	-
Acute responses	Desintegration	Immobility	Immobility
Chronic responses	Tentacle contraction	Pulsation frequency	Swimming impairment
Observation time (h)	1, 6, 24, 48, 72 and 96	24 and 48	24

2.3. Exposure solution with antifouling paint particles

The particles were weighed using a high-precision balance (Mettler Toledo XPR504S/A), with each weighing treated as an independent replicate, and adjusted to a final volume of 50 mL of artificial seawater (salinity 35). The antifouling paint particles (APPs) were transferred individually into 50 mL

Falcon tubes, without mixing between brands. The tubes were maintained horizontally on an orbital shaker (Marconi Ltda.) at 110 rpm, in the dark, for 7 days, to allow the release of compounds present in the paint matrix into the aqueous medium (adapted from Soroldoni et al., 2018). Incubation in the dark was adopted to prevent the photochemical degradation of organic compounds present in the paint formulations. Following this period, the Falcon tubes containing the exposure solution were used directly in the assays, with organisms added directly to the medium. The tests were therefore conducted in the simultaneous presence of the leachate in saline water and the APPs in suspension.

During the assays, the tubes were maintained horizontally on an orbital shaker (110 rpm) with the aim of keeping the organisms in continuous contact with the APPs, maintaining the particles in suspension throughout the water column, and preventing their sedimentation. Agitation also promotes the continuous release of compounds from the particles into the aqueous medium.

2.4. Toxicity assays

All assays were conducted in 50 mL Falcon tubes, and observations of endpoints were made using a stereoscopic microscope (Olympus SZX9) at the previously defined time points (Figure 1).

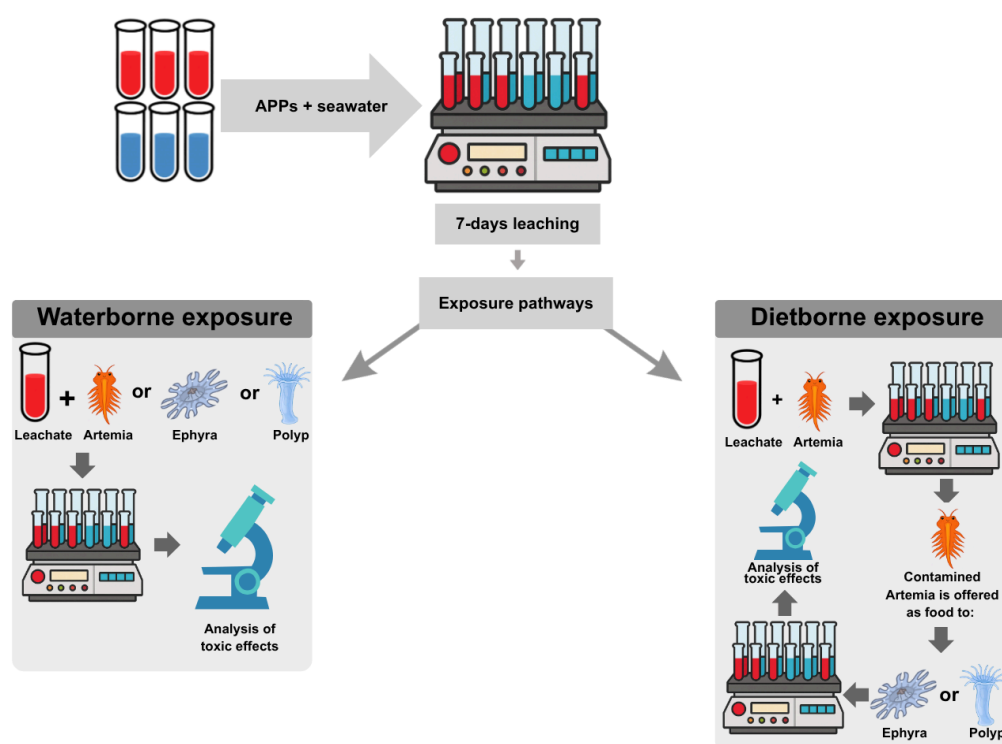


Figure 1. Waterborne and dietborne toxicity tests. Red tubes indicate mixtures with Renner® paint; blue tubes indicate mixtures with International® paint. All assays were performed with five replicates per treatment.

2.4.1. *Artemia*

Tests with *Artemia franciscana* aimed to establish a concentration range at which the effects of APPs would affect the nauplii without completely compromising their survival, thereby allowing subsequent evaluation of toxicity transfer to *Aurelia coerulea*. The assays were conducted at 25 °C in the dark, using organisms aged 72 h (adapted from Libralato et al., 2007). For each assay, five replicates of 15 *Artemia* each were used, totalling 75 individuals per treatment. After 24 h of exposure, swimming impairment was assessed as the sublethal response, and immobility as the acute response.

2.4.2. *Polyps*

Tests were conducted following the methodology of Morales et al. (2025), using polyps of undefined age, in an incubator at 20 °C under a 12:12 h light:dark photoperiod. For each assay, five replicates of five polyps each were used, totalling 25 polyps per treatment. Sublethal responses included tentacle contraction, defined as the condition in which all tentacles were contracted, even if only slightly curved. The lethality was determined by disintegration of the polyp body structure.

2.4.3. *Ephyrae*

Tests were conducted with ephyrae aged 0 to 5 days, in an incubator at 20 °C under a 12:12 h light:dark photoperiod, following Morales et al. (2025). For each assay, five replicates with five ephyrae each were used, totalling 25 ephyrae per treatment. The sublethal response was quantified by pulsation frequency (pulses per minute). For each ephyra, three measurements of pulsation frequency were taken, each of 10 seconds duration. The acute response consisted of immobility of the organisms, determined by the absence of movement for 10 seconds, even following stimulation with a Pasteur pipette (Morales et al., 2025).

2.4.4. *Contaminated prey ingestion assays*

To estimate the chronic and acute effects of dietborne exposure toxicity, polyps and ephyrae of *Aurelia coerulea* were fed *Artemia franciscana* nauplii

previously exposed to APPs for 24 h. The assays were conducted in artificial seawater without PTAs or leachate, ensuring that exposure occurred exclusively via dietary intake of contaminated prey. During the dietborne exposure assays, 10 contaminated *Artemia* nauplii were offered to each polyp or ephyra. Feeding occurred once at the start of the experiment, and observation periods were counted from that moment. Thus, in the case of ephyrae, for example, organisms were fed the contaminated nauplii and observations were made 24 and 48 h after feeding.

Prey ingestion was confirmed visually under a stereoscopic microscope through observation of nauplii capture and, at subsequent time points, by the presence of orange colouration within the organisms, characteristic of the ingestion of *Artemia* spp. nauplii by gelatinous organisms. The acute and sublethal endpoints assessed were the same as those described for the direct exposure assays.

2.5. Quantification of copper in leachates

Copper concentrations were determined in the leachates used in the toxicological assays, with the aim of quantifying the dissolved fraction released by the APPs. Solutions of Renner® and International® paints were prepared at concentrations of 0.5, 1, 2, 4, and 10 mg L⁻¹, in addition to the control, following the same procedure described in Section 2.3, with leaching for 7 days in artificial seawater.

Following this period, 10 mL aliquots of the leachates were filtered (0.45 µm), acidified with 1% nitric acid and desalinated according to the method of Nadella et al. (2009), which consisted of the addition of lanthanum oxide and sodium carbonate, followed by heating (80 °C, 30 min), centrifugation (~3,000 g, 15 min), and dissolution of the precipitate in nitric acid (Suprapur Merck®). Copper determination was performed using a graphite furnace atomic absorption spectrometer (HR-CS GF AAS, Analytik Jena, Jena, Germany), at the Institute of Biological Sciences (ICB), Federal University of Rio Grande (FURG), Brazil, with a minimum limit of quantification of 0.05 µg L⁻¹ Cu. The calibration curve was established using a multi-element standard solution (Sigma-Aldrich), with a maximum concentration of 5.0 µg L⁻¹. Samples were read in triplicate. All

procedures employed for sample preparation and analysis were performed in the absence of the sample (procedural blanks). Instrument calibration was performed using certified analytical standards (Sigma-Aldrich), and method performance was verified through recovery tests, which consistently yielded recoveries above 90%.

2.6. Statistical analysis

For acute responses, the LC₅₀ (lethal concentration causing mortality in 50% of organisms) was estimated after 96 h of exposure for polyps and 24 h for *Artemia franciscana*, while the EC₅₀ (effective concentration causing effects in 50% of organisms) was estimated for ephyrae after 48 h of exposure using Probit analysis (Finney, 1952), with corresponding 95% confidence intervals (95% CI). Ephyrae pulsation frequency was treated as a continuous response variable. For this endpoint, differences among concentrations were evaluated separately for each exposure period. The assumptions of residual normality and homogeneity of variances among treatments were assessed using the Shapiro–Wilk and Levene tests, respectively. When these assumptions were met, a one-way analysis of variance (ANOVA) was performed. For the remaining endpoints, generalized linear models (GLMs) with a binomial error distribution were applied, as the responses corresponded to binary outcomes or proportions. When a significant effect of concentration was detected, multiple comparisons among concentrations within each observation period were performed using Tukey-adjusted post hoc tests. Statistical analyses were conducted using GraphPad Prism 8.4 for Windows (San Diego, CA, USA).

3. RESULTS

3.1. Toxicity in *Artemia franciscana*

Swimming impairment in *Artemia franciscana* nauplii varied according to the paint tested (Figures 2A and 2C). For Renner® (Figure 2A), a low frequency of impairment was observed in the control and an intermediate response at 0.5 mg L⁻¹. At concentrations of 1, 2, and 4 mg L⁻¹, a marked increase in swimming impairment was observed, with no differences amongst these concentrations, indicating a response threshold from 1 mg L⁻¹ onwards. Compared to the control, a significant increase in swimming impairment was recorded at concentrations of

1 mg L⁻¹ (p = 0.0381), 2 mg L⁻¹ (p = 0.0038), and 4 mg L⁻¹ (p = 0.0257). For International® (Figure 2C), swimming impairment increased at concentrations of 2 and 4 mg L⁻¹, which did not differ from one another but showed significantly higher values than the control (p = 0.0323 and p = 0.0487, respectively).

Mortality also followed a distinct pattern between paints (Figures 2B and 2D). For Renner® (Figure 2B), a progressive increase in mortality was observed, with a statistically significant difference only at a concentration of 4 mg L⁻¹ (p = 0.0466), with an estimated LC₅₀ of 0.67 mg L⁻¹ (CI: 0.21–2.13 mg L⁻¹). For International® (Figure 2D), the highest concentration resulted in total mortality (100%), with an estimated LC₅₀ of 0.73 mg L⁻¹ (CI: 0.19–2.78 mg L⁻¹), indicating greater toxicity relative to Renner®.

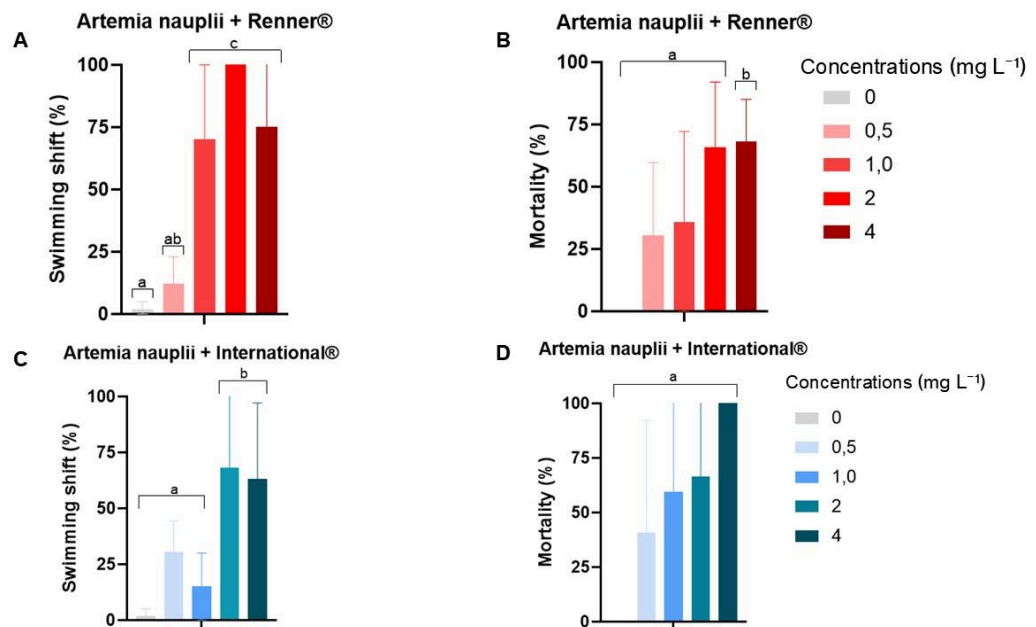


Figure 2. Toxicological responses of *Artemia franciscana* nauplii exposed to antifouling paint particles (APPs) at different concentrations. A and B – APPs from Renner® paint. C and D – APPs from International® paint. A and C – Swimming impairment (%). B and D – Mortality (%). Data are presented as mean ± standard deviation (SD). Different letters indicate statistically significant differences between concentrations.

3.2. Toxicity in *Aurelia coerulea*

3.2.1. Polyps

3.2.1.1. Tentacle contraction

Tentacle contraction in polyps of *Aurelia coerulea* followed a similar pattern between paints under waterborne exposure (Figures 3A and 3C). For both antifouling paint particles, a rapid increase in the proportion of polyps with contracted tentacles was observed during the first h of exposure. For Renner® (Figure 3A), differences relative to the control were recorded as early as 1 h at concentrations of 2, 3, and 4 mg L⁻¹ ($p < 0.0001$), a pattern that was repeated at 6 h and 24 h ($p < 0.0001$). From 48 h onwards, all concentrations differed from the control ($p < 0.0001$), and exposed polyps reached 100% contraction, maintaining this value through to 96 h. For International® (Figure 3C), all concentrations differed from the control from 1 h through to 96 h ($p < 0.0001$), with 100% contraction in all exposed treatments throughout the entire experimental period.

Under dietborne exposure, the pattern differed between paints (Figures 3B and 3D). For Renner® (Figure 3B), tentacle contraction occurred during the first 48 h, followed by a reduction at the 72 h and 96 h assessments, with no significant differences amongst concentrations at any of the time points evaluated. For International® (Figure 3D), the initial contraction was followed by an increase at the highest concentration of 14 mg L⁻¹ over time, remaining elevated from 24 h until the end of the assay.

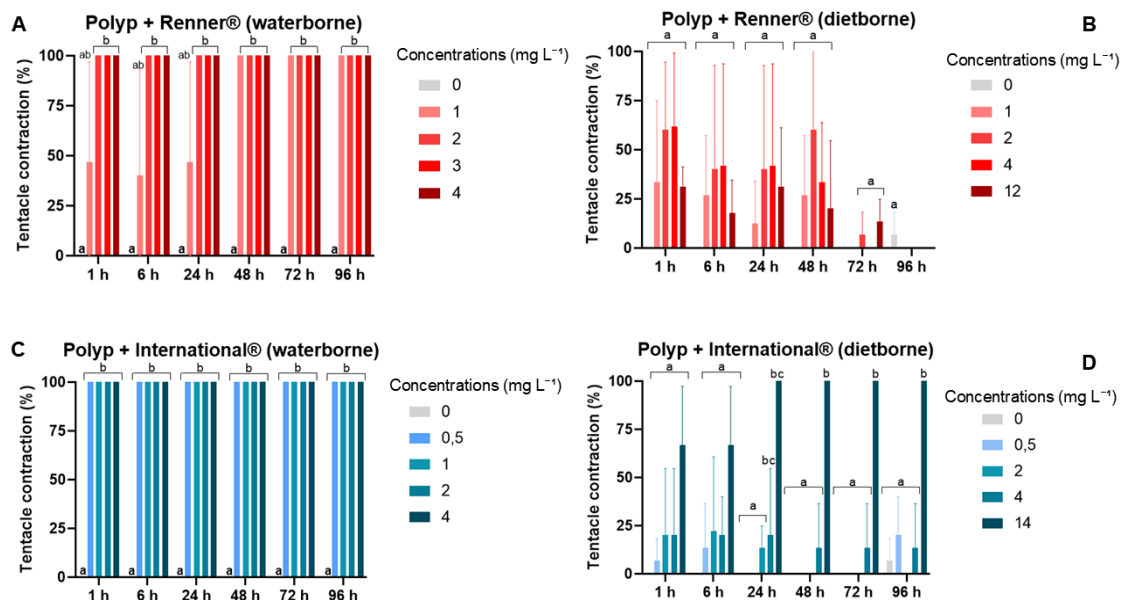


Figure 3. Tentacle contraction (%) in polyps of *Aurelia coerulea* exposed to antifouling paint particles (APPs) at different concentrations. A and B – APPs from Renner® paint. C and D – APPs from International® paint. A and C – Waterborne exposure. B and D – Dietborne exposure. Data are presented as

mean $\pm$ standard deviation (SD). Different letters indicate statistically significant differences between concentrations.

3.2.1.2. Polyp mortality

Polyp mortality differed between exposure routes (Figure 4). Under waterborne exposure to Renner® antifouling paint particles (APPs) (Figure 4A), mortality increased over time at all concentrations. At 1 h, concentrations of 3 and 4 mg L⁻¹ differed from the control ($p < 0.0001$). At 6 h, differences relative to the control were recorded at 2 mg L⁻¹ ($p = 0.0004$), 3 mg L⁻¹ ($p < 0.0001$), and 4 mg L⁻¹ ($p < 0.0001$). At 24 h, concentrations of 2, 3, and 4 mg L⁻¹ also differed from the control ($p < 0.0001$). At 48 h, significant differences were observed at 2, 3, and 4 mg L⁻¹ ($p < 0.0001$). From 72 h onwards, all concentrations differed from the control (1 mg L⁻¹: $p < 0.0001$; 2, 3, and 4 mg L⁻¹: $p < 0.0001$), reaching 100% mortality from that point onwards. A similar pattern was observed for International® (Figure 4C), although with no effect during the first hours. At 6 h, concentrations of 1 mg L⁻¹ ($p = 0.0021$) and 2 mg L⁻¹ ($p = 0.0029$) differed from the control. At 24 h, a difference was observed only at 1 mg L⁻¹ ($p = 0.0021$). At 48 h, significant differences were observed at 1 mg L⁻¹ ($p = 0.0247$), 2 mg L⁻¹ ($p < 0.0001$), and 4 mg L⁻¹ ($p < 0.0001$). At 72 h and 96 h, all concentrations differed from the control, with $p = 0.0335$ and $p = 0.0301$ for 0.5 mg L⁻¹, respectively, and $p < 0.0001$ for 1, 2, and 4 mg L⁻¹ at both time points. The estimated LC₅₀ for polyps under waterborne exposure was 0.38 mg L⁻¹ (CI: 0.20–0.70 mg L⁻¹) for Renner® and 0.31 mg L⁻¹ (CI: 0.23–0.42 mg L⁻¹) for International®.

Under dietborne exposure, the pattern differed between APPs (Figures 4B and 4D). For Renner® (Figure 4B), no mortality was recorded throughout the experiment. For International® (Figure 4D), mortality occurred only at the highest concentration. At 24 h, the 14 mg L⁻¹ treatment differed from the control, from 0.5 mg L⁻¹, and from 2 mg L⁻¹ ($p = 0.0004$ in all cases), as well as from 4 mg L⁻¹ ($p = 0.0074$). At 48 h, 72 h, and 96 h, the concentration of 14 mg L⁻¹ differed from the control, from 0.5 mg L⁻¹, and from 2 mg L⁻¹ ($p < 0.0001$ in all cases), as well as from 4 mg L⁻¹ ($p < 0.0001$). For this exposure route, the LC₅₀ was estimated at 6.27 mg L⁻¹ (CI: 4.39–8.96 mg L⁻¹) for International®, whilst

for Renner® it was not possible to calculate an LC₅₀ owing to the low mortality observed.

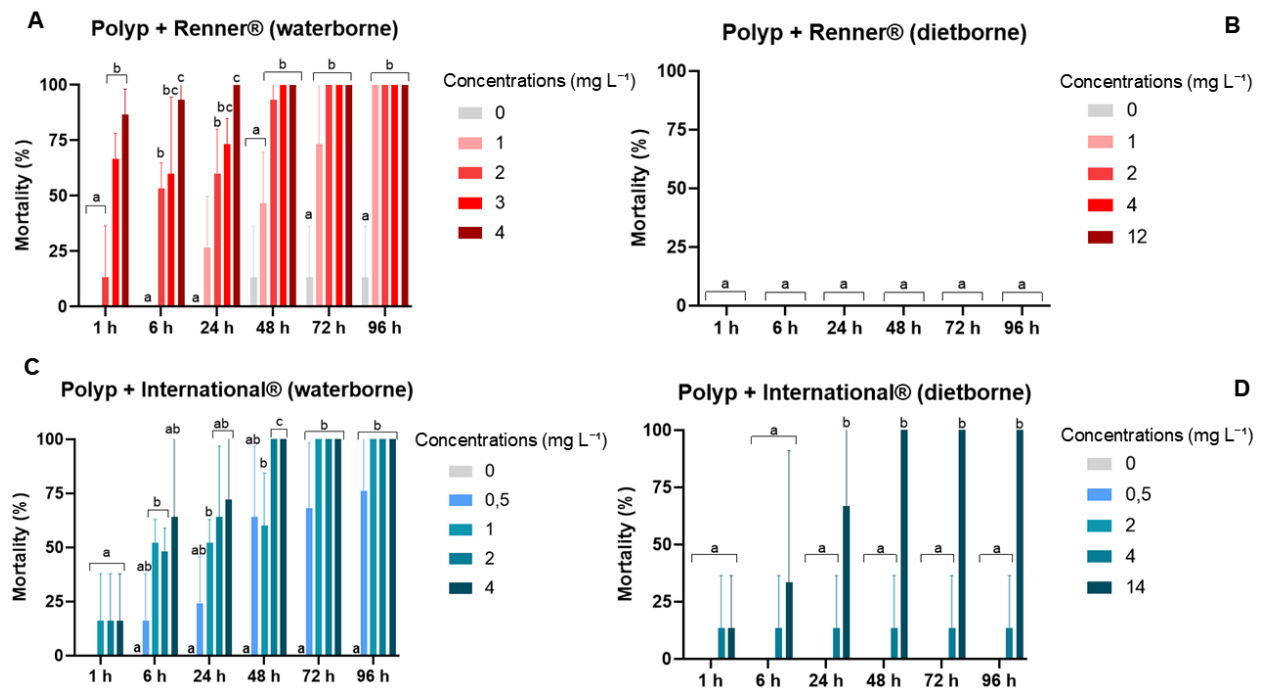


Figure 4. Mortality (%) of polyps of *Aurelia coerulea* exposed to antifouling paint particles (APPs) at different concentrations. A and B – APPs from Renner® paint. C and D – APPs from International® paint. A and C – Waterborne exposure. B and D – Dietborne exposure. Data are presented as mean $\pm$ standard deviation (SD). Different letters indicate statistically significant differences between concentrations.

3.2.2. Ephyrae

3.2.2.1. Pulsation frequency

Pulsation frequency in ephyrae varied according to exposure route and paint tested (Figure 5). Under waterborne exposure to Renner® antifouling paint particles (APPs) (Figure 5A), a marked reduction in pulses per minute was observed after 24 h, with differences relative to the control at all concentrations ($p < 0.0001$), followed by a further decrease at 48 h, with the significant difference between the control and treatments maintained ($p < 0.0001$). For International® (Figure 5C), the reduction occurred progressively with increasing concentration. At 24 h, all concentrations differed from the control ($p < 0.0001$), and additional differences were observed between the lowest concentration (0.25 mg L⁻¹) and concentrations of 1 and 2 mg L⁻¹ ($p = 0.0067$ and $p = 0.0002$,

respectively). At 48 h, the control continued to differ from all concentrations ($p < 0.0001$), whilst 0.25 mg L^{-1} also differed from the remaining concentrations ($p < 0.0001$).

Under dietborne exposure (Figures 5B and 5D), the response was less pronounced. For Renner®, reductions in pulsation frequency were observed primarily at the higher concentrations, particularly at 2 and 10 mg L^{-1} , which differed from the control at 24 h ($p < 0.0001$) and 48 h ($p < 0.0001$). For International®, the majority of treatments did not differ from the control, and a significant effect was recorded primarily at the highest concentration. At 24 h and 48 h, 10 mg L^{-1} differed from the control ($p < 0.0001$) and from the remaining concentrations ($p \leq 0.0243$).

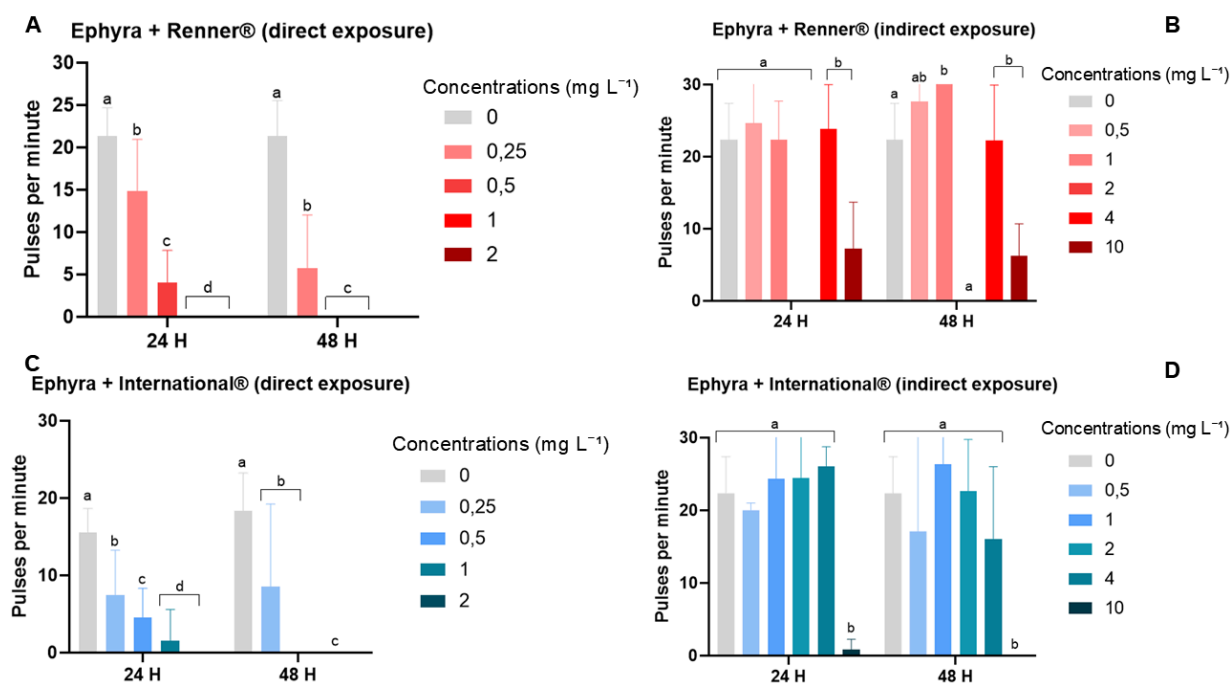


Figure 5. Pulses per minute of ephyrae of *Aurelia coerulea* exposed to antifouling paint particles (APPs) at different concentrations. A and B – APPs from Renner® paint. C and D – APPs from International® paint. A and C – Waterborne exposure. B and D – Dietborne exposure. Data are presented as mean $\pm$ standard deviation (SD). Different letters indicate statistically significant differences between concentrations.

3.2.2.2. Immobility

Under waterborne exposure to Renner® antifouling paint particles (APPs) (Figure 6A), concentrations of 1 and 2 mg L^{-1} resulted in 100% immobility as early as 24 h, differing from the control ($p = 0.0006$), and this pattern was

maintained at 48 h ($p = 0.0006$). For International® (Figure 6C), the effect was more pronounced. At 24 h, concentrations of 0.5, 1, and 2 mg L⁻¹ differed from the control ($p = 0.0400$, $p < 0.0001$, and $p < 0.0001$, respectively), and at 48 h, 100% immobility was recorded at these three concentrations, all of which continued to differ from the control ($p < 0.0001$). The estimated LC₅₀ for ephyrae under waterborne exposure was 0.38 mg L⁻¹ (CI: 0.20–0.70 mg L⁻¹) for Renner® and 0.23 mg L⁻¹ (CI: 0.14–0.38 mg L⁻¹) for International®.

Under dietborne exposure (Figures 6B and 6D), the pattern differed. For Renner®, no immobility was recorded at 24 h, and at 48 h the response occurred only at the highest concentration, with no significant difference relative to the control. For International®, the effect was restricted at the highest concentration. At 24 h, 10 mg L⁻¹ differed from the control and from all other treatments ($p = 0.0013$), and at 48 h this difference was maintained ($p < 0.0001$). For this exposure route, the LC₅₀ was estimated at 0.16 mg L⁻¹ (CI: 0.05–0.48 mg L⁻¹) for International®, whilst for Renner® it was not possible to calculate an LC₅₀ owing to the low immobility observed. Overall, ephyrae responded to APP exposure at lower concentrations and over shorter exposure periods than polyps under waterborne exposure. However, comparisons between life stages should be interpreted with caution, as different biological endpoints were evaluated for each stage and physiological differences may influence their responses to chemical stressors.

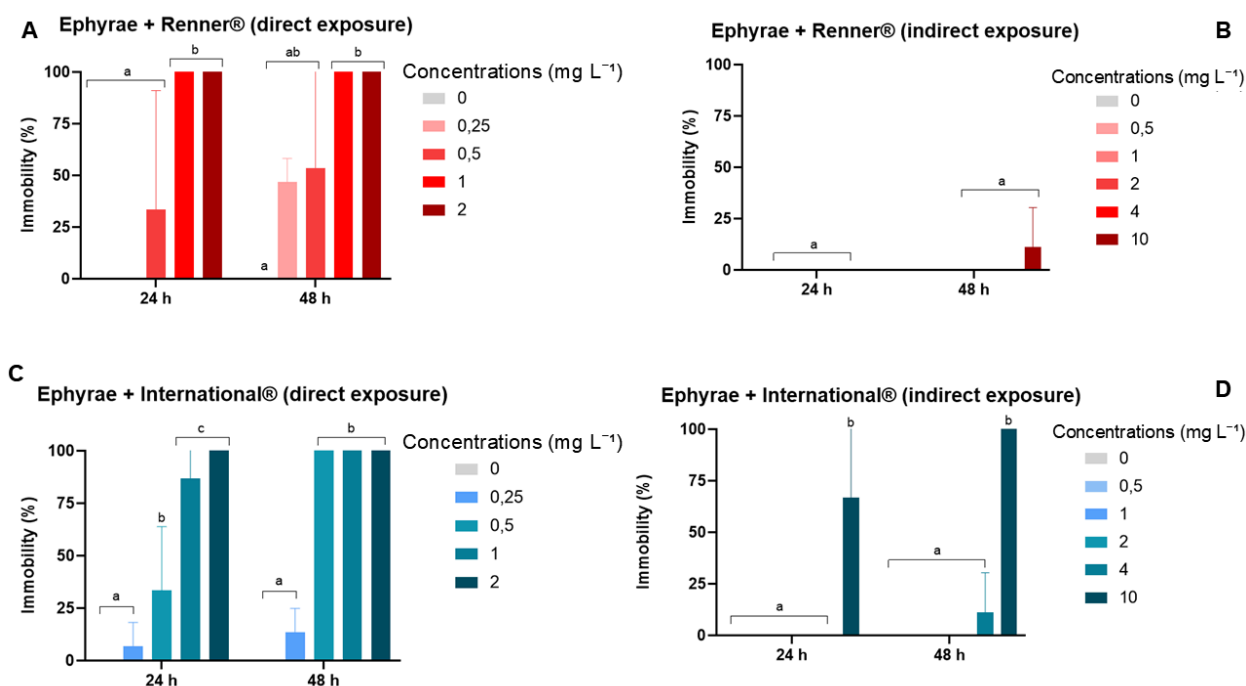


Figure 6. Immobility of ephyrae of *Aurelia coerulea* exposed to antifouling paint particles (APPs) at different concentrations. A and B – APPs from Renner® paint. C and D – APPs from International® paint. A and C – Waterborne exposure. B and D – Dietborne exposure. Data are presented as mean $\pm$ standard deviation (SD). Different letters indicate statistically significant differences between concentrations.

3.3. Copper concentration in leachates

Copper (Cu) concentrations in the leachates increased with increasing antifouling paint particle (APP) concentrations for both paints (Table 1), demonstrating that both formulations release this metal into the aqueous medium. Differences between formulations were observed, with higher values recorded for International® paint at all concentrations tested.

At the lowest concentration (0.5 mg L⁻¹), International® yielded approximately four times more Cu in the leachate compared to Renner®. This pattern was maintained at higher concentrations, reaching maximum values of 202.6 $\mu\text{g L}^{-1}$ for International® and 35.31 $\mu\text{g L}^{-1}$ for Renner® at the highest concentration tested (10 mg L⁻¹).

Table 2. Copper concentration ($\mu\text{g L}^{-1}$) in leachates of APPs from Renner® and International® paints at the different concentrations tested.

Treatment	Cu ($\mu\text{g L}^{-1}$)	SD
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Control	< LOD	-
Renner® 0,5 mg L ⁻¹	2.38	0.12
Renner® 1 mg L ⁻¹	2.87	0.12
Renner® 2 mg L ⁻¹	5.74	0.09
Renner® 4 mg L ⁻¹	26.34	0.10
Renner® 10 mg L ⁻¹	35.31	0.22
International® 0,5 mg L ⁻¹	10.77	0.05
International® 1 mg L ⁻¹	33.49	0.21
International® 2 mg L ⁻¹	45.24	0.15
International® 4 mg L ⁻¹	88.24	0.41
International® 10 mg L ⁻¹	202.60	1.03

4. DISCUSSION

4.1. Differences between antifouling paint particle (APP) formulations

Overall, antifouling paint particles (APPs) from International® paint exhibited greater toxicity compared to those from Renner®. This difference would be due to the chemical composition of the formulations, as International® paint particles contain higher concentrations of copper (Cu) ($234,247 \pm 268 \text{ mg kg}^{-1}$) and the co-biocide DCOIT ($134 \pm 30 \text{ ng g}^{-1}$) relative to those of Renner® paint ($178,471 \pm 6,448 \text{ mg kg}^{-1}$ Cu and $88.6 \pm 7.5 \text{ ng g}^{-1}$ DCOIT, respectively) (Soroldoni et al., 2018). In the present study, quantification of Cu in the leachates demonstrated that both formulations release the metal in a dose-dependent manner, albeit at higher levels for International® paint, reaching values exceeding $200 \text{ } \mu\text{g L}^{-1}$, whilst Renner® paint yielded values of approximately $35 \text{ } \mu\text{g L}^{-1}$. This indicates that differences between formulations are not restricted to particle composition but also extend to the dissolved fraction released into the medium, which likely contributed to the more pronounced effects observed, particularly under waterborne exposure, where APPs and leachate were present simultaneously.

However, the toxicity observed cannot be attributed exclusively to copper, as other metals and co-biocides previously identified in these APPs may also

have contributed to the biological responses. Furthermore, the bioavailability and toxicity of copper in aquatic environments are strongly influenced by environmental variables such as pH, salinity, dissolved organic matter (DOM), and suspended particulate matter. Because the assays were conducted in artificial seawater, which does not contain natural DOM, copper may have remained predominantly in more bioavailable dissolved forms than would be expected under natural environmental conditions. Therefore, the toxicity observed in the present study should be interpreted considering these experimental conditions.

Given that antifouling paints contain a complex mixture of metals and organic and organometallic biocides, Cu was adopted as an indicator of chemical exposure owing to its predominance in the formulations and its well-documented toxicity to marine organisms (Dafforn et al., 2011; Torres & De-la-Torre, 2021). As the principal active compound in these systems, Cu can interfere with fundamental physiological processes, including cellular respiration, electron transport, and enzymatic activity (Nadella et al., 2009; Leal et al., 2016). At elevated concentrations, it may also induce oxidative stress and cellular damage, affecting the integrity of membranes, proteins, and DNA (Rehman et al., 2019), in addition to compromising essential metabolic processes and organismal growth (Leal et al., 2016). Review studies indicate that the environmental toxicity of these paints varies according to the type and concentration of biocides present in the formulations (Dafforn et al., 2011; Torres & De-la-Torre, 2021).

4.2. Exposure route as a modulator of toxicity

Waterborne exposure caused greater sensitivity than dietborne exposure, likely owing to differences in exposure levels between the two routes. Contaminants present in the water can be incorporated directly by aquatic organisms from the dissolved phase through exposed body surfaces (Luoma & Rainbow, 2008).

In cnidarians, exchanges with the surrounding medium occur across the entire body surface, as these organisms lack an exoskeleton or specialised organs for absorption (Arai, 1997). However, these exchanges are not restricted to passive diffusion. The presence of motile cilia generates continuous water flows,

promoting mixing of the boundary layer and facilitating exchanges with the medium (Jordano et al., 2024). Experimental studies demonstrate that this movement enhances solute transport by reducing boundary layer thickness and maintaining concentration gradients at the organism–environment interface (Shapiro et al., 2014). In species of the genus *Aurelia*, the thin body wall, combined with intense ciliary activity, renders this interface particularly efficient for chemical exchange (Arai, 1997; Lucas et al., 2012).

Under dietborne exposure, contaminant incorporation depends on additional physiological steps. In this case, compounds must first be assimilated by the prey and subsequently released during the digestive process of the predator before being absorbed across the gastrovascular epithelium. For this reason, the amount incorporated by the consumer will depend on the concentration of contaminants present in the prey, the efficiency of digestive assimilation, and the retention of the compound within the tissues of the consuming organism (Wang & Fisher, 1999; Mathews & Fisher, 2008).

4.3. Ontogenetic responses to Antifouling Paint Particles exposure

The results of the assays revealed distinct toxicological responses between ephyrae and polyps, which likely reflect physiological and functional differences between life cycle stages. Polyps are sessile organisms with a relatively simple body structure, whereas ephyrae continuously perform rhythmic pulsations of the umbrella for locomotion and maintenance in the water column (Arai, 1997). Consequently, physiological or neuromuscular disturbances caused by stressors may be more readily detected through changes in pulsation frequency and mobility in ephyrae (Di Giannantonio et al., 2022; Costa et al., 2023; Mercado et al., 2023; Gambardella et al., 2024; Yang et al., 2024; Morales et al., 2025).

Physiological differences between ontogenetic stages may also influence contaminant uptake, detoxification, and overall responses to environmental stressors. Early developmental stages often possess less developed enzymatic systems and different capacities for contaminant metabolism and excretion (Newman, 2015). Previous studies with *Aurelia* have likewise reported stage-specific responses to environmental stressors and contaminants (Dong et

al., 2017; Olguin-Jacobson et al., 2021; Morales et al., 2025), reinforcing the importance of considering multiple life stages in ecotoxicological assessments.

Although direct comparisons of sensitivity between polyps and ephyrae should be interpreted with caution, since different biological endpoints were evaluated in each stage, the present results demonstrate that APP exposure affects both life stages through distinct response pathways. In ephyrae, effects were primarily detected through reductions in pulsation frequency and increased immobility, whereas in polyps the main responses involved tentacle contraction and body disintegration.

Nonetheless, both polyps and ephyrae demonstrated susceptibility to APP exposure, which is consistent with the chemical composition of the particles tested, in which copper is the predominant metal. In scyphozoans, Cu may accumulate in tissues at levels exceeding those observed for other metals owing to the capacity of these organisms to regulate intracellular copper concentrations (Rainbow, 2002; Templeman & Kingsford, 2015). Even when a proportion of this metal does not remain metabolically available, its presence may induce oxidative stress and cellular damage associated with the production of reactive oxygen species (Morales et al., 2025).

The responses observed in *A. coerulea* are consistent with those reported for other marine organisms exposed to APPs and their associated biocides (Dafforn et al., 2011; Torres & De-la-Torre, 2021). Previous studies have demonstrated high toxicity of APP-contaminated sediments in benthic crustaceans (Soroldoni et al., 2020), as well as lethal and sublethal effects in planktonic organisms exposed to copper and co-biocides commonly used in antifouling formulations (Nadella et al., 2009; Leal et al., 2016). Together, these findings support the use of *A. coerulea* as a relevant model organism for ecotoxicological studies involving antifouling contaminants.

4.4. Ecological implications and trophic transfer

Dietborne exposure suggests that contaminants released from antifouling paint particles (APPs) can be transferred to predators through the ingestion of previously exposed prey. *Artemia franciscana* exposed to APP leachates likely

accumulated or adsorbed dissolved contaminants, acting as a vector through which these substances were made available to *Aurelia coerulea* during feeding. The observed effects indicate that these contaminants remain bioavailable after transfer via prey. This resulted in both lethal and sublethal responses, including mortality in ephyrae and polyps at higher concentrations, as well as tentacle contraction in polyps. Although these responses were generally less pronounced than those observed under waterborne exposure, their occurrence indicates that dietborne exposure constitutes a relevant pathway of contamination. Even at lower apparent toxicity, this route may contribute to sustained exposure and biological effects across trophic interactions.

In pelagic systems, where plankton represents a key interface between contamination in the water column and higher trophic levels (Chouvelon et al., 2019), the contamination of gelatinous zooplankton may be of particular concern. As planktonic organism, *A. coerulea* contribute to trophic dynamics and may facilitate the entry and redistribution of contaminants within pelagic food webs. Jellyfish are suspensivorous predators that filter large volumes of water and can reflect regional sources of pollutants in the composition of their tissues (Nagata et al., 2024). Given that they can occur in high abundances and are consumed by a wide range of predators, including fish, seabirds, and sea turtles (Hays et al., 2018), their contamination may represent a pathway for the transfer of APP-derived substances to higher trophic levels. In regions where the abundance of these organisms is increasing, exposure to APPs may increase the availability of contaminants within the food web.

It is important to note that the present study evaluated trophic exposure through prey previously exposed to APP leachates, rather than through the direct ingestion of particles. Therefore, the observed effects are likely associated with the transfer of dissolved contaminants, and may differ from scenarios in which organisms are exposed to intact particles or complex environmental mixtures. Experimental evidence demonstrates that dietary transfer can occur across multiple trophic levels (Mathews & Fisher, 2008), and that contamination of basal prey may enhance consumer exposure and prolong the persistence of contaminants within ecosystems, even in the absence of biomagnification (Saidon et al., 2024). In natural systems, however, multiple exposure pathways

may occur simultaneously, including direct contact with contaminated water, ingestion of particles, and dietary intake. As such, the responses observed here likely represent only part of the overall toxicological impact of antifouling paint particles in marine environments.

4.5. Limitations and future perspectives

The assays were conducted under controlled laboratory conditions, which allowed the effects of the antifouling paint particles to be isolated, but do not fully reproduce the complexity of coastal environments, where factors such as hydrodynamics, physicochemical variability, and co-exposure to other contaminants may influence the behaviour and availability of these particles. Furthermore, dietborne exposure was evaluated using a single trophic step and a single feeding event with previously contaminated *Artemia franciscana* nauplii, representing a simplified scenario compared to natural conditions, where exposure may be continuous and involve multiple prey types. In addition, the endpoints assessed were sufficient to detect relevant lethal and sublethal effects, but do not allow the underlying biochemical mechanisms or internal contaminant uptake to be fully understood. Future studies should therefore focus on quantifying contaminant uptake in exposed organisms and evaluating repeated dietary exposure, in order to better link exposure routes to internal dose and to more realistically represent trophic exposure dynamics.

CONCLUSION

The results demonstrated that exposure route decisively modulates the toxicity of antifouling paint particles (APPs) in *Aurelia coerulea*, with more pronounced effects under waterborne exposure than under dietborne exposure. Of the formulations tested, APPs from International® paint exhibited greater toxicity than those from Renner® paint, which is consistent with their chemical composition, characterised by higher concentrations of metals and co-biocides. Furthermore, ephyrae proved more sensitive than polyps, indicating that ontogenetic differences influence the response to these contaminants, and that assessments across different life stages should be considered in ecotoxicological assays. Although less pronounced, the toxicity observed under dietborne exposure demonstrates that the ingestion of contaminated prey may also

contribute to the exposure of marine organisms to these waste materials. Taken together, the results highlight the importance of considering multiple exposure routes and different life cycle stages in the assessment of ecological risks associated with APPs in coastal environments. Moreover, they contribute to addressing a knowledge gap regarding the ecological impacts of these particles, particularly in port areas and marinas, where they may be continuously released into the environment.

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Capítulo V: Síntese da Discussão

No presente estudo, a toxicidade de partículas de tintas anti-incrustantes (PTAs) variou conforme a formulação da tinta, a via de exposição e o estágio do ciclo de vida de *Aurelia coerulea*. A maior toxicidade observada na exposição via água indica que a fração dissolvida liberada pelas partículas foi influente nos efeitos registrados. Nesse caso, a disponibilidade direta dos contaminantes no meio aquoso favorece o contato contínuo com os organismos. Em cifozoários, essas trocas ocorrem ao longo de toda a superfície corporal, uma vez que esses organismos não possuem exoesqueleto ou estruturas especializadas de proteção e absorção (Arai, 1997). Além disso, a superfície corporal é recoberta por cílios móveis que geram fluxos contínuos de água ao redor do corpo, promovendo a mistura da camada limite e aumentando as taxas de troca com o meio (Jordano et al., 2024). Estudos experimentais demonstram que esse movimento intensifica o transporte de solutos ao reduzir a espessura

da camada limite e manter gradientes de concentração na interface organismo–ambiente (Shapiro et al., 2014).

Na exposição via dieta, embora tenha sido confirmada a ingestão de presas contaminadas, os efeitos foram menores. Esse padrão sugere que a transferência trófica não depende apenas da presença do contaminante na presa, mas também de processos como digestão e assimilação, que podem limitar a quantidade efetivamente incorporada (Wang & Fisher, 1999; Mathews & Fisher, 2008). A variabilidade na retenção dos compostos ao longo do tempo também pode influenciar a expressão dos efeitos, especialmente em exposições de curta duração.

As diferenças entre pólipos e éfiras indicaram variação na sensibilidade ao longo do ciclo de vida, com maior resposta nas éfiras. Esse resultado pode estar relacionado à maior atividade metabólica desse estágio, associada às pulsações contínuas utilizadas para locomoção e manutenção na coluna d'água (Arai, 1997; Lucas et al., 2012). Em comparação aos pólipos, as éfiras mantêm contato com um maior volume de água ao longo do tempo, o que pode aumentar a exposição a contaminantes dissolvidos e partículas em suspensão.

A presença de cobre nos lixiviados confirma que as partículas atuam como fonte de contaminantes para o meio aquoso. Considerando que o cobre é utilizado como biocida nessas formulações, sua liberação contribui para a toxicidade observada. Ainda assim, os efeitos registrados não podem ser atribuídos a um único composto, já que as PTAs contêm uma mistura de metais e biocidas orgânicos, cuja interação pode influenciar as respostas biológicas.

A comparação com a literatura mostra que estudos com *Aurelia* utilizam condições experimentais bastante distintas, incluindo diferenças em temperatura, alimentação, duração dos ensaios e critérios de análise. Essas variações interferem diretamente nos resultados e dificultam a identificação de padrões consistentes entre estudos. Nesse sentido, parte das diferenças observadas não está necessariamente relacionada ao contaminante testado, mas às condições em que os ensaios foram conduzidos.

Capítulo VI: Conclusões

Este trabalho reuniu evidências experimentais e análise da literatura para avaliar a toxicidade de partículas de tintas anti-incrustantes (PTAs) em *Aurelia coerulea* sob diferentes condições de exposição. A abordagem adotada permitiu examinar, de forma conjunta, fatores que normalmente são investigados separadamente, incluindo via de exposição, estágio do ciclo de vida e características das formulações. Os resultados demonstraram que PTAs apresentam elevada toxicidade, induzindo efeitos letais e subletais, mais intensos sob exposição direta à água.

Os resultados obtidos ajudam a explicar a interação entre contaminantes associados a partículas e organismos gelatinosos, ao mesmo tempo em que evidenciam limitações na comparabilidade de estudos decorrentes da variabilidade metodológica descrita na literatura. Nesse contexto, o uso de *Aurelia* como organismo modelo se mostra relevante, principalmente quando consideradas as condições experimentais que influenciam suas respostas.

Capítulo VII: Perspectivas futuras

Os ensaios foram conduzidos em condições laboratoriais controladas, o que permite isolar os efeitos das partículas, mas não representa a complexidade dos ambientes naturais. Estudos futuros podem incorporar condições ambientais variáveis, exposições prolongadas e cadeias tróficas mais complexas nos delineamentos experimentais. A quantificação de contaminantes nos organismos expostos também pode contribuir para o entendimento dos processos de absorção e bioacumulação, assim como a aplicação de biomarcadores pode esclarecer os mecanismos envolvidos nas respostas observadas.

Além disso, a literatura evidencia a necessidade de maior padronização metodológica em estudos com *Aurelia*, incluindo a definição de condições experimentais, protocolos de alimentação, estágios de vida e identificação taxonômica, a fim de melhorar a comparabilidade e a reprodutibilidade dos resultados. Com base na variabilidade observada entre os estudos revisados,

recomenda-se que futuras pesquisas relatem e, sempre que possível, padronizem parâmetros como temperatura, fotoperíodo, salinidade, frequência e tipo de alimentação, duração da exposição e estágio de vida utilizado nos ensaios. A adoção de protocolos mais consistentes também facilitaria comparações entre estudos e contribuiria para o estabelecimento de *Aurelia* como organismo modelo na ecotoxicologia marinha.

Capítulo VIII: Referências Bibliográficas

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