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Title and subtitle: Freshwater ecosystems under natural and anthropogenic stressors: the impact of ultraviolet radiation and nanoplastics on plankton and the ecosystem

Abstract:
Since life emerged on Earth, organisms have had to handle threats and stressors of different kinds. In freshwater ecosystems, one of these ancient stressors is the solar ultraviolet (UV) radiation. Life has been continuously exposed to it, and various behavioural and morphological adaptations have evolved to cope with the stressor. On the other hand, human activities have introduced novel stressors to the Earth system, for example, different pollutants. Plastic pollution is a novel stressor in the history of Earth, and a complex, global, socio-environmental issue. Nanoplastics, the smallest size fraction (< 1 μm) formed when plastic material breaks down, negatively affect a variety of freshwater organisms. However, a comprehensive understanding of the effects of nanoplastics on natural freshwater ecosystems is still needed.

The aim of this thesis is to address how these two different stressors affect freshwater plankton, including effects at other levels of biological organization. To do this, I performed both laboratory and mesocosm scale experiments to assess the effect of UV radiation and nanoplastics, exploring, for example, organism growth, morphology, behaviour, and fitness, but also population abundances, community composition, and ecosystem processes.

Solar UV radiation is a temporally variable abiotic factor. It fluctuates during the year, but also daily, and over short time scales with the position of the sun and rapidly occurring variations in cloudiness. Despite its variable nature, most studies on organisms' responses to UV radiation have assessed the effects of a constant exposure. In **Paper I**, I experimentally investigated individual survival, reproduction, and behaviour of the zooplankter *Daphnia magna* when exposed to constant or fluctuating UV radiation. I found that *D. magna* has the potential to adopt alternative behavioural strategies to deal with either constant exposure or repeatedly fluctuating UV radiation, and that the response to the fluctuating environment implies a fitness cost.

Nanoplastics, compared to larger-sized plastics, have different transport pathways, interact differently with organisms, and require the use of specific analytical techniques for their characterization and quantification. Currently, there is a need to assess the transport, fate, uptake, and effects of nanoplastics in natural ecosystems. I investigated all these aspects in freshwater wetland mesocosms and laboratory experiments, using model plastic nanoparticles. The results of these studies showed that nanoplastics can be retained by freshwater wetland mesocosms, being mostly accumulated in sediments of the aquatic compartment, but also in organisms as *D. magna*, *Asellus aquaticus* and macrophytes (**Paper II**). Moreover, nanoplastics negatively affect the abundance of freshwater key organisms, as *D. magna*, and change the community composition of phytoplankton, favouring cyanobacteria over diatoms (**Paper III**). Further, these plastic nanoparticles interact differently with two different phytoplankton species (**Paper IV**) causing differential effects on growth and group formation.

Collectively, this thesis analysed different organism responses to both natural and anthropogenic stressors. Despite long-standing adaptations, organisms still face costs associated with responding to natural stressors as UV radiation, and these differential costs might fuel population differentiation and local adaptation. The impacts that humans are causing on natural ecosystems through plastic pollution also press natural populations and communities. Further, these pollutants can cause shifts that might be irreversible and harmful to the whole freshwater ecosystem, and to us humans. New regulations but also major social changes are needed to really change the current trend of increasing plastic pollution.

Key words: plastic pollution, ultraviolet radiation, zooplankton, phytoplankton, mesocosm, wetland

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Para Guille y Guidaí
Gracias por tanta luz

La naturaleza no es muda

El mundo pinta naturalezas muertas, sucumben los bosques naturales, se derriten los polos, el aire se hace irrespirable y el agua intomable, se plastifican las flores y la comida, y el cielo y la tierra se vuelven locos de remate.

Y mientras todo esto ocurre, un país latinoamericano, Ecuador, ha elaborado una nueva Constitución. Y en esa Constitución se abre la posibilidad de reconocer, por primera vez en la historia universal, los derechos de la naturaleza.

La naturaleza tiene mucho que decir, y ya va siendo hora de que nosotros, sus hijos, no sigamos haciéndonos los sordos. Y quizás hasta Dios escuche la llamada que suena desde este país andino, y agregue el undécimo mandamiento que se le había olvidado en las instrucciones que nos dio desde el monte Sinaí:
«*Amarás a la naturaleza, de la que formas parte.*»

Eduardo Galeano

Montevideo, Uruguay (2008)

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Abstract

Since life emerged on Earth, organisms have had to handle threats and stressors of different kinds. In freshwater ecosystems, one of these ancient stressors is the solar ultraviolet (UV) radiation. Life has been continuously exposed to it, and various behavioural and morphological adaptations have evolved to cope with the stressor. On the other hand, human activities have introduced novel stressors to the Earth system, for example, different pollutants. Plastic pollution is a novel stressor in the history of Earth, and a complex, global, socio-environmental issue. Nanoplastics, the smallest size fraction (< 1 μm) formed when plastic material breaks down, negatively affect a variety of freshwater organisms. However, a comprehensive understanding of the effects of nanoplastics on natural freshwater ecosystems is still needed.

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Nanoplastics, compared to larger-sized plastics, have different transport pathways, interact differently with organisms, and require the use of specific analytical techniques for their characterization and quantification. Currently, there is a need to assess the transport, fate, uptake, and effects of nanoplastics in natural ecosystems. I investigated all these aspects in freshwater wetland mesocosms and laboratory experiments, using model plastic nanoparticles. The results of these studies showed that nanoplastics can be retained by freshwater wetland mesocosms, being mostly accumulated in sediments of the aquatic compartment, but also in organisms as *D. magna*, *Asellus aquaticus* and macrophytes (**Paper II**). Moreover, nanoplastics negatively affect the abundance of freshwater key organisms, as *D. magna*, and change the community composition of phytoplankton, favouring cyanobacteria over diatoms (**Paper III**). Further, these plastic nanoparticles interact differently with

two different phytoplankton species (**Paper IV**) causing differential effects on growth and group formation.

Collectively, this thesis analysed different organism responses to both natural and anthropogenic stressors. Despite long-standing adaptations, organisms still face costs associated with responding to natural stressors as UV radiation, and these differential costs might fuel population differentiation and local adaptation. The impacts that humans are causing on natural ecosystems through plastic pollution also press natural populations and communities. Further, these pollutants can cause shifts that might be irreversible and harmful to the whole freshwater ecosystem, and to us humans. New regulations but also major social changes are needed to really change the current trend of increasing plastic pollution.

Resumen

Desde los inicios de la vida en la Tierra, los organismos han tenido que hacer frente a diversos estresores y amenazas. En los ecosistemas de agua dulce, uno de estos estresores es la radiación solar ultravioleta (UV). Los organismos vivos han estado continuamente expuestos a esta radiación, y desde entonces, diversas adaptaciones morfológicas y comportamentales han evolucionado, permitiendo afrontar este estresor. Por otro lado, las actividades humanas han introducido nuevos estresores en los ecosistemas, como ser, distintos contaminantes. La contaminación por plásticos es un estresor nuevo en la historia de la vida en la Tierra y un creciente problema socioambiental global. Los nanoplásticos, la fracción de tamaño más pequeño (< 1 μm) que se forma cuando el material plástico se fragmenta, afectan negativamente a diversos organismos de agua dulce. Sin embargo, el conocimiento respecto a los efectos de los nanoplásticos en los ecosistemas naturales es aún escaso.

El objetivo de esta tesis es evaluar cómo afectan estos dos estresores al plancton de agua dulce, incluidos los efectos a niveles superiores de organización biológica. Para ello, realicé experimentos a escala de laboratorio y de mesocosmos para evaluar el efecto de la radiación UV y de los nanoplásticos. Analicé, por ejemplo, el crecimiento, la morfología, el comportamiento y el fitness de los organismos, pero también el tamaño poblacional, la composición de las comunidades y procesos ecosistémicos.

La radiación solar UV es un factor que varía temporalmente, a lo largo del año, diariamente, e inclusive durante el día, con la posición del sol y las variaciones en nubosidad que ocurren rápidamente. A pesar de su naturaleza variable, la mayoría de los estudios sobre las respuestas de los organismos a la radiación UV han evaluado los efectos de una exposición constante. En el **Artículo I**, investigué experimentalmente la supervivencia, la reproducción y el comportamiento del zooplancton *Daphnia magna* cuando se expone a radiación UV presentada de manera constante o fluctuante. Observé que *D. magna* tiene la capacidad de adoptar estrategias comportamentales alternativas para hacer frente a una exposición constante a radiación UV o a una que fluctúa repetidamente. Determiné, además, que la respuesta al ambiente fluctuante implica un costo para el fitness del organismo.

Los nanoplásticos, en comparación con los plásticos de mayor tamaño, son transportados de forma diferente, interactúan de manera distinta con los organismos y requieren del uso de técnicas analíticas específicas para su caracterización y cuantificación. En la actualidad, existe la necesidad de evaluar el transporte, la distribución, la absorción y los efectos de los nanoplásticos en los ecosistemas naturales. Durante la tesis, investigué estos aspectos en mesocosmos de humedales de agua dulce y en experimentos de laboratorio, utilizando nanopartículas de

plástico como modelo. Los resultados de estos estudios muestran que gran parte de los nanoplásticos pueden ser retenidos por los humedales de agua dulce, acumulándose mayoritariamente en los sedimentos del compartimento acuático, pero también en organismos como *D. magna*, *Asellus aquaticus* y macrófitas (**Artículo II**). Asimismo, observé que los nanoplásticos afectan negativamente a la abundancia de organismos clave, como *D. magna*, y cambian la composición de la comunidad fitoplanctónica, favoreciendo a las cianobacterias frente a las diatomeas (**Artículo III**). Además, estas partículas interactúan de forma diferente con dos especies de fitoplancton, causando efectos diferenciales en el crecimiento y la formación de grupos (**Artículo IV**).

En conjunto, esta tesis analizó diferentes respuestas de los organismos a estresores naturales y antropogénicos. A pesar de las adaptaciones evolutivas, los organismos siguen afrontando costos asociados a la respuesta a estresores naturales como la radiación UV, y estos costos diferenciales podrían contribuir a la diferenciación de las poblaciones y la adaptación local. Los impactos que el ser humano está causando en los ecosistemas naturales a través de la contaminación por plásticos también presionan a las poblaciones y comunidades naturales. Además, estos contaminantes pueden provocar cambios que podrían ser irreversibles y perjudiciales para todo el ecosistema, incluyendo los humanos. Se necesitan nuevas regulaciones basadas en el conocimiento científico, así como también grandes transformaciones sociales, para cambiar la tendencia actual de aumento de la contaminación por plásticos a nivel mundial.

Popular science summary

Since life emerged on Earth, organisms have had to handle threats and stressors of different kinds. In freshwater ecosystems, one of these ancient, and very harmful stressors is the solar ultraviolet (UV) radiation. The first forms of life had to cope with high levels of UV radiation, until an oxygenated atmosphere was formed and reduced the levels of UV radiation that reached our planet. Therefore, life has been continuously exposed to this stressor, and various adaptations have evolved that allowed organisms to cope with it. On the other hand, human activities have introduced novel stressors to the Earth system, for example, different pollutants. Plastics are pollutants with widespread global distribution, and novel stressors in the history of Earth. When plastic material breaks down, smaller plastics called micro- and nanoplastics are formed. Nanoplastics, the smallest size fraction (as small as most virus, or even smaller!), harm a variety of freshwater organisms, but a comprehensive understanding of the effects of nanoplastics on natural freshwater ecosystems is still lacking.

The aim of this thesis is to address how UV radiation and nanoplastics affect freshwater organisms and their ecosystem. To explore this, I performed laboratory experiments which included small aquatic crustaceans, known as zooplankton, or micro algae and cyanobacteria, called phytoplankton. I also worked with wetland mesocosms, which are closer to real natural conditions. A mesocosm is a small ecosystem, set in an enclosure, where it is possible to manipulate some variables, and which can be replicated. This allowed me to explore how nanoplastics are distributed in the freshwater wetlands and the organisms that inhabit it, how a direct toxic effect on one organism may have indirect effects on others, and to test if nanoplastics affect organic matter decomposition.

Solar UV radiation fluctuates during the year, but also daily, and over short time scales with the position of the sun and rapidly occurring variations in cloudiness. Despite its variable nature, most studies on organisms' responses to UV radiation have assessed the effects of a constant exposure instead of fluctuating. In this thesis, I experimentally investigated how the survival, reproduction, and behaviour of the zooplankton species *Daphnia magna* is affected when exposed to constant or fluctuating UV radiation. I found that this species changes its behaviour depending on if it is exposed to a constant exposure or a repeatedly fluctuating UV radiation. I also found that the organisms exposed to the fluctuating UV radiation reproduced less, which indicates a cost to the organism due to the response to the fluctuating environment. This shows that the repeated vertical movements that *D. magna* performs daily imply a cost, something that has been debated.

All plastic items polluting the environment will inevitably break down into smaller pieces, and most will pass through the nanometre size scale before being completely degraded. Compared to the larger original plastic, these smaller plastic particles have a much higher surface in relation to their volume. This is the reason why nanoplastics can interact differently with the environment. Further, because of their tiny size, they can enter cells and cause harm to cellular processes. Therefore, it is very important to also investigate these smallest versions of plastic pollution and not only micro-sized plastic particles as previously often focused on. However, nanoplastics are too small to detect for most analytical methods and are therefore difficult to study. Therefore, we still know very little about their effects on organisms and ecosystems, and how they move and accumulate.

I navigated the challenge of detecting these tiny particles by using artificial plastic particles that had a metal (gold) core in their centre, which is possible to detect with certain analytical methods. When I added these artificial nanoplastics to the freshwater wetland mesocosms, I found that most of the nanoplastics were retained in the wetlands. The nanoplastics mostly accumulated in the sediments of the aquatic compartment, but also in organisms such as the zooplankton *D. magna*, a benthic invertebrate called *Asellus aquaticus*, and aquatic plants. Later, in a similar study, I added artificial nanoplastics to the wetland mesocosms but this time they were made of full plastic. We knew these tiny plastics were harmful for *D. magna* in laboratory tests, and now I wanted to investigate what happened when these particles were added to a complex ecosystem, more like the natural environment. I found that *D. magna* went extinct when the wetland mesocosms were exposed to nanoplastic concentrations above 2 mg of plastic per litre. *D. magna*, feeds by filtering water and trapping the phytoplankton that is floating in the water with its filtering apparatus. The extinction of *D. magna* likely favoured the abundance of cyanobacteria, that did not have efficient predators. On the other hand, other phytoplankton species were negatively affected by the nanoplastics, because they reduced their abundances when exposed to increasing concentrations of nanoplastics. This made me wonder why the nanoplastics affected the different members of the phytoplankton community differently. I found, in a laboratory experiment, that nanoplastics interact differently with two phytoplankton species: where one had nanoplastic particles sticking to its cells while the other didn't. In addition, nanoplastics caused differential effects on phytoplankton growth and group formation, where fewer groups of cells were formed in the first, while nanoplastics induced cells to team up in groups in the other. These changes in group formation will likely impact where these species are in the aquatic system (either the bottom or suspended in the water column), and which species of zooplankton can eat them.

Collectively, this thesis analysed the responses of different organisms to both natural and human-introduced stressors. Despite long-standing adaptations, organisms still face costs associated with their response to natural stressors, as UV radiation. The impacts that humans are causing on natural ecosystems through plastic pollution also press organisms' natural populations. Further, these pollutants can cause shifts on the ecosystem that might be irreversible and harmful to the whole freshwater ecosystem, and to us humans. Therefore, the current trend of increasing plastic pollution is problematic, and new regulations but also major social changes are needed to change this path.

Resumen de divulgación científica

Desde que surgió la vida en la Tierra, los organismos han tenido que hacer frente a amenazas y estresores de diversa índole. En los ecosistemas de agua dulce, uno de estos antiguos estresores es la radiación solar ultravioleta (UV). Las primeras formas de vida en la Tierra tuvieron que hacer frente a altos niveles de radiación UV, que son muy dañinos, hasta que se formó una atmósfera oxigenada que redujo los niveles de radiación UV que llegaban a nuestro planeta, y la hizo habitable para otras formas de vida. Los organismos vivos han estado continuamente expuestos a este factor estresante, y desde ese entonces diversas adaptaciones para enfrentar la radiación UV han aparecido a lo largo de la evolución. Por otro lado, las actividades humanas han introducido nuevos estresores en los ecosistemas, por ejemplo, diversos contaminantes. Para la historia de la vida en la Tierra, la contaminación por plásticos es un estresor muy nuevo, presente en la naturaleza desde hace aproximadamente 70 anos. Actualmente, la contaminación por plásticos es un problema socioambiental global. Cuando el material plástico se fragmenta, se forman plásticos más pequeños denominados microplásticos y nanoplásticos. Los nanoplásticos, la fracción de tamaño más pequeño (tan pequeño como la mayoría de los virus, ¡o incluso más diminutos!), son nocivos para diversos organismos de agua dulce. Sin embargo, aún carecemos de un conocimiento integrado acerca de los efectos de los nanoplásticos en los ecosistemas de agua dulce.

El objetivo de esta tesis es evaluar cómo afectan la radiación UV y los nanoplásticos a los organismos de agua dulce y su ecosistema. Para explorarlo, realicé experimentos de laboratorio que incluían pequeños crustáceos acuáticos, conocidos como zooplancton, o microalgas y cianobacterias, conocidas como fitoplancton. También trabajé con mesocosmos de humedales, que se parecen más a los ecosistemas naturales reales. Un mesocosmos es un pequeño ecosistema que está en un recinto, donde podemos manipular algunas variables y puede ser replicado. Esto me permitió explorar cómo se podrían distribuir los nanoplásticos en los humedales de agua dulce y los organismos que los habitan, cómo un efecto tóxico que impacta directamente sobre un organismo puede tener efectos indirectos sobre otros, y si los nanoplásticos afectan a la descomposición de la materia orgánica.

La radiación solar UV fluctúa a lo largo del año, pero también diariamente, y en escalas temporales cortas con la posición del sol y las rápidas variaciones en la presencia de nubes. A pesar de su naturaleza variable, la mayoría de los estudios sobre las respuestas de los organismos a la radiación UV han evaluado los efectos de una exposición constante en lugar de fluctuante. En esta tesis, investigué experimentalmente cómo se ve afectada la supervivencia, la reproducción y el comportamiento de la especie de zooplancton *Daphnia magna*, cuando es expuesta a radiación UV de forma constante o fluctuante. Observé que esta especie cambia su comportamiento dependiendo de si está expuesta a radiación UV presentada de forma constante, o que fluctúa repetidamente. También encontré que los organismos expuestos a la radiación UV fluctuante, se reproducían menos, lo que indica un costo para el organismo, ocasionado por la respuesta al ambiente con mayor variabilidad. Además, esto evidencia que los repetidos movimientos verticales que *D. magna* realiza a diario conllevan un costo, algo que ha sido debatido.

Todos los plásticos que contaminan el medio ambiente se descompondrán inevitablemente en trozos más pequeños, y la mayoría pasará por la escala de tamaño nanométrico antes de degradarse por completo. En comparación con el plástico original de mayor tamaño, estas diminutas partículas de plástico tienen una superficie mayor con relación a su volumen. Esta es la razón por la que los nanoplásticos pueden tener interacciones químicas diferentes con el medio ambiente. Además, debido a su diminuto tamaño, podrían entrar en las células y causar daños en los procesos celulares. Por lo tanto, es muy importante investigar también estas diminutas partículas de plástico y no sólo las partículas de plástico de tamaño micrométrico, como se ha hecho hasta ahora. Sin embargo, los nanoplásticos son demasiado pequeños para ser detectados por la mayoría de los métodos analíticos que existen hasta el momento, por lo que son difíciles de estudiar. Por esto, aún sabemos muy poco sobre los efectos de los nanoplásticos en los organismos y los ecosistemas, y sobre cómo se mueven y acumulan en el ambiente.

Para poder detectar las diminutas partículas de plástico en el ambiente, utilicé partículas artificiales de plástico que tienen un núcleo metálico (de oro) en el centro, que puede detectarse con determinados métodos analíticos. Cuando añadí estos nanoplásticos artificiales a los mesocosmos de humedales, encontré que la mayor parte de los nanoplásticos quedaban retenidos en los humedales. Los nanoplásticos se acumularon sobre todo en los sedimentos del compartimento acuático, pero también en organismos como el zooplancton *D. magna*, un invertebrado bentónico llamado *Asellus aquaticus* y en las plantas acuáticas. Luego, en un estudio similar, añadí nanoplásticos artificiales a los mesocosmos de humedales, pero esta vez las partículas eran por completo de plástico. Sabíamos que estos plásticos diminutos eran perjudiciales para *D. magna* en pruebas de laboratorio, y ahora quería investigar qué ocurría cuando estas partículas se añadían a un ecosistema parecido a los reales. Descubrí que *D. magna* se extinguía cuando los mesocosmos de humedales eran expuestos a concentraciones de nanoplásticos superiores a 2 mg de plástico por litro. Este organismo, *D. magna*, se alimenta filtrando el agua y atrapando con su aparato filtrador el fitoplancton que flota en ella. La extinción de *D. magna* probablemente favoreció la abundancia de cianobacterias, que no tenían depredadores eficaces. Por otra parte, otras especies de fitoplancton se vieron afectadas negativamente por los nanoplásticos, ya que redujeron su abundancia cuando se expusieron a concentraciones crecientes de nanoplásticos. Esto me hizo preguntarme por qué los nanoplásticos afectaban de forma diferente a los distintos miembros de la comunidad fitoplanctónica. En un experimento de laboratorio, observé que los nanoplásticos interactuaban de forma diferente con dos especies de fitoplancton: en una de ellas las partículas de nanoplásticos se adherían a sus células, mientras que en la otra no. Además, los nanoplásticos causaron efectos diferentes en el crecimiento del fitoplancton y la formación de grupos: mientras que en la primera se formaban menos grupos de células, en la otra los nanoplásticos inducían a las células a agruparse. Estos cambios en la formación de grupos probablemente repercutirán en dónde se encuentran estas especies en el sistema acuático (en el fondo o suspendidas en el agua), y en las especies de zooplancton que pueden alimentarse de ellas.

En conjunto, esta tesis analizó las respuestas de distintos organismos a estresores naturales e introducidos por el hombre. Cuando son enfrentados a estresores naturales, como la radiación UV, los organismos aún enfrentan costos asociados a la respuesta ante el estresor, a pesar del surgimiento de diversas adaptaciones a lo largo de la historia evolutiva. Los impactos que el ser humano está causando en los ecosistemas naturales debido a la contaminación por plásticos también presionan negativamente a las poblaciones naturales de organismos. A su vez, la contaminación por plástico puede provocar cambios en el ecosistema que podrían ser irreversibles y perjudiciales para todo el ecosistema de agua dulce, y para nosotros, los humanos. La tendencia actual de aumento de la contaminación por plásticos es problemática, y se necesitan nuevas normativas, pero también cambios sociales profundos, para cambiar este rumbo.

Populärvetenskaplig sammanfattning

Sedan liv uppstod på jorden har organismer varit tvungna att hantera stressfaktorer av olika slag. En skadlig och naturligt förekommande stressfaktor är solens ultravioletta (UV) strålning. De första livsformerna var tvungna att klara av höga nivåer av UV-strålning, fram till dess att en syresatt atmosfär bildades och minskade strålningsnivåerna som nådde jordens yta. Därför har livet på jorden kontinuerligt utsatts för denna stressfaktor, och olika anpassningar för att hantera det har utvecklats. Utöver dessa naturliga stressfaktorer har mänskliga aktiviteter introducerat nya, till exempel olika föroreningar. Plast är föroreningar med utbredd global spridning och en ny stressfaktor på jorden. När plastmaterial bryts ner bildas mindre partiklar som kallas mikro- och nanoplaster. Nanoplast, är den minsta storleksfraktionen (så små som de flesta virus eller ännu mindre!) och är skadligt för en mängd olika sötvattensorganismer. Trots omfattande forskning saknar vi fortfarande en heltäckande förståelse för nanoplasters effekter på naturliga sötvattensekosystem.

Syftet med denna avhandling är att studera hur UV-strålning och nanoplast påverkar sötvattensorganismer och deras ekosystem. För att undersöka detta utförde jag laboratorieexperiment som inkluderade djurplankton, små vattenlevande kräftdjur, eller växtplankton, mikroalger och cyanobakterier. Jag arbetade också med våtmarksmesokosmer. En mesokosm är ett litet ekosystem, placerat i en inhägnad, där det är möjligt att manipulera vissa variabler och som kan replikeras. Detta gjorde det möjligt för mig att undersöka till exempel var nanoplaster fördelar sig i sötvattensvåtmarker och de organismer som lever i dem. Jag undersökte också hur effekter på en organism indirekt kan påverka andra organismer, och tittade på hur nanoplaster påverkar ekosystemprocesser såsom nedbrytningen av organiskt material.

Solens UV-strålning varierar under året, men också dagligen, på grund av solens position och snabba växlingar i molnighet. Trots dess varierande natur har de flesta studier av organismers reaktioner på UV-strålning undersökt effekterna av en konstant exponering. I denna avhandling har jag experimentellt undersökt hur överlevnaden, reproduktionen och beteendet hos en djurplanktonart, *Daphnia magna*, påverkas när den utsätts för konstant eller fluktuerande UV-strålning. Jag upptäckte att denna art ändrar sitt beteende beroende på om den utsätts för en konstant exponering eller upprepad fluktuerande UV-strålning. Jag visade också att fluktuerande UV-strålning utgjorde en kostnad för organismerna som ledde till att de reproducerade sig mindre.

Alla plastföremål som förorenar miljön kommer oundvikligen att brytas ner i mindre bitar, och de flesta når till sist storlekar i nanometerskalan innan de slutligen bryts ned helt. Jämfört med den större originalplasten har dessa mindre plastpartiklar en mycket större yta i förhållande till sin volym – från en liten mängd material får vi en stor yta som kan interagerar på olika sätt med miljön. På grund av att partiklarna är så små kan de dessutom komma in i cellerna och orsaka skada på cellulära processer. Därför är det mycket viktigt att undersöka denna minsta fraktion av plastföroreningar och inte bara mikrostora plastpartiklar som det tidigare ofta fokuserats på. Nanoplaster är dock svåra att studera eftersom de är för små för att upptäcka med de flesta analysmetoder och vi vet därför fortfarande väldigt lite om deras effekter på organismer och ekosystem, och hur de rör sig och ackumuleras. Jag kringgick detta problem genom att använda konstgjorda plastpartiklar som hade en metallkärna av guld. Guldkärnan gör det möjligt att upptäcka partiklarna genom att leta efter guld med etablerade analysmetoder för grundämnesanalys. När jag tillsatte dessa konstgjorda nanoplaster till våtmarksmesokosmer, fann jag att merparten av nanoplasten stannade i våtmarkerna. Nanoplasten ansamlades mestadels i sedimenten, men också i vattenväxter, samt i organismer som *D. magna* och den bottenlevande sötvattensgråsuggan *Asellus aquaticus*.

I laboratorietester har det tidigare visats att konstgjorda modellpartiklar av nanoplast (gjorda av endast plast) är skadliga för *D. magna*, och nu ville jag undersöka vad som hände när dessa partiklar tillsätts i ett komplext ekosystem, som mer liknar den naturliga miljön. Mina resultat visade att *D. magna* minskade i antal och försvann helt när våtmarkerna exponerades för nanoplastkoncentrationer över 2 mg plast/L. Denna organism livnär sig på att filtrera vatten och fånga växtplankton som flyter i vattnet. Utdöendet av *D. magna* gynnade sannolikt tillväxten av cyanobakterier, som inte hade effektiva betare. Andra växtplanktonarter minskade istället i förekomst när de exponerades för ökande koncentrationer av nanoplast. Detta fick mig att undra varför nanoplasterna påverkade de olika arterna av växtplanktonsamhället olika. Jag upptäckte, i ett laboratorieexperiment, att nanoplast interagerar på olika sätt med två växtplanktonarter: den ena hade nanoplastpartiklar som fastnade på celler medan den andra inte hade det. Dessutom orsakade nanoplast olika effekter på deras tillväxt och gruppbildning. I det första fallet bildades färre grupper av celler medan nanoplast inducerade celler att slå sig samman i grupper hos den andra arten.

Sammanfattningsvis undersökte jag i denna avhandling olika organismers reaktioner på både naturliga och mänskliga introducerade stressfaktorer. Trots långvariga anpassningar står organismer fortfarande inför kostnader förknippade med deras anpassningar och respons till naturliga stressfaktorer, som UV-strålning. De effekter som människor orsakar på naturliga ekosystem genom plastföroreningar påverkar också organismernas naturliga populationer och kan orsaka förändringar som kan vara irreversibla och skadliga för hela sötvattensekosystemet och för oss människor. Därför är ökande plastföroreningar problematisk, och nya regleringar och stora sociala förändringar behövs för att ändra denna negativa trend.

List of papers

This doctoral thesis is based on the following publications and a manuscript. These are referred in the text by their Roman numerals.

Paper I

Stábile F.*, Brönmark C., Hansson L.A. & Lee M. (2021) Fitness cost from fluctuating ultraviolet radiation in *Daphnia magna*. Biology Letters, 17, 8, 20210261. https://doi.org/10.1098/rsbl.2021.0261.

Paper II

Stábile F.*, Ekvall M.T., Gallego-Urrea J.A., Nwachukwu T., W.G. Soorasena C.U., Rivas-Comerlati P.I. & Hansson L.A. (2024) Fate and biological uptake of polystyrene nanoparticles in freshwater wetland ecosystems. Environmental Science: Nano, 11, 3475–3486. https://doi.org/10.1039/D3EN00628J

Paper III

Ekvall M.T, **Stábile F.** & Hansson L.A*. (2024) Nanoplastics rewire freshwater food webs. Communications Earth & Environment, 5, 486. https://doi.org/10.1038/s43247-024-01646-7.

Paper IV

Stábile F.*, Ekvall M.T., Sjöstedt J. & Hammer E.C. Unity is strength: contrasting responses in group formation between the green algae *Tetradesmus obliquus* and the cyanobacteria *Dolichospermum flos-aquae* when exposed to nanoplastics. *Manuscript.*

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Author contributions

My contributions to each paper, and those of the other authors, are listed following the standard “Contributor Role Taxonomy” - CRediT (NISO CRediT Working Group, 2022).

Paper I

Conceptualization: **F.S.**, C.B., L.A.H. & M.L. Formal analysis: **F.S.** & M.L. Funding acquisition: **F.S.**, M.L. & L.A.H. Investigation: **F.S.** & M.L. Methodology: **F.S.** & M.L. Project administration: **F.S.**, M.L. & L.A.H. Supervision: L.A.H. Visualization: **F.S.** & M.L. Writing – original draft: **F.S.** with contributions from M.L. & L.A.H. Writing – review & editing: **F.S.**, C.B., L.A.H. & M.L.

Paper II

Conceptualization: L.A.H., **F.S.** & M.T.E. Data curation: **F.S.** Formal analysis: **F.S.** Funding acquisition: L.A.H. & J.A.G.U. Investigation: **F.S.**, L.A.H., J.A.G.U., T.N., W.G.C.U.S., P.I.R.C. & M.T.E. Methodology: **F.S.**, L.A.H., J.A.G.U. & M.T.E. Project administration: **F.S.**, L.A.H. & M.T.E. Supervision: L.A.H. & M.T.E. Visualization: **F.S.** Writing – original draft: **F.S.** Writing – review & editing: **F.S.**, M.T.E, J.A.G.U., T.N., W.G.C.U.S., P.I.R.C. & L.A.H.

Paper III

Conceptualization: L.A.H, M.T.E. & **F.S.** Formal analysis: M.T.E., **F.S.** & L.A.H. Funding acquisition: L.A.H. Investigation: **F.S.**, M.T.E & L.A.H. Methodology: M.T.E., **F.S.** & L.A.H. Project administration: M.T.E. & L.A.H. Supervision: M.T.E. & L.A.H. Visualization: M.T.E. & L.A.H. Writing – original draft: M.T.E. & L.A.H. Writing – review & editing: M.T.E., **F.S.** & L.A.H.

Paper IV

Conceptualization: **F.S.** with contributions from M.T.E., J.S. & E.C.H. Data curation: **F.S.** Formal analysis: **F.S.** Funding acquisition: **F.S.** supervised by L.A.H. and M.T.E. Investigation: **F.S.** Methodology: **F.S.** Project administration: **F.S.** Supervision: **F.S.**, M.T.E., J.S. & E.C.H. Visualization: **F.S.** Writing – original draft: **F.S.** Writing – review & editing: **F.S.**, M.T.E., J.S. & E.C.H.

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Publications not contained in this thesis

Odnevall I., Brookman-Amissah M., **Stábile F.**, Ekvall M.T., Herting G., Bermeo Vargas M., Messing M.E., Sturve J., Hansson L.A., Isaxon C. & Rissler J. (2023) Characterization and Toxic Potency of Airborne Particles Formed upon Waste from Electrical and Electronic Equipment Waste Recycling: A Case Study. ACS Environmental Au, 3, 6, 370–382. https://doi.org/10.1021/acsenvironau.3c00034.

Cornwallis C. K., Svensson-Coelho M., Lindh M., Li Q., **Stábile F.**, Hansson L.A. & Rengefors K. (2023) Single cell adaptations shape evolutionary transitions to multicellularity in green algae. Nature Ecology and Evolution, 7, 889–902. https://doi.org/10.1038/s41559-023-02044-6.

Abbreviations

Au	Gold
DLS	Dynamic light scattering
ICP-MS	Inductively coupled plasma mass spectrometry
NP	Nanoplastic
PE	Polyethylene
PET	Polyethylene terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinylchloride
PURs	Thermoplastics, thermosets, and polyurethanes
SiO_2	Silicon dioxide, also known as silica
TEM	Transmission electron microscopy
UV	Ultraviolet

Introduction

Since life emerged on Earth, organisms have had to handle threats and stressors of different kinds (Box 1). In freshwater ecosystems, such ancient stressors are, for example, solar ultraviolet (UV) radiation, changes in temperature or droughts. Conditions on Earth have changed during geological time due to natural phenomena. For example, variations in Earth´s orbit through time have changed the incoming solar radiation on Earth surface, causing glacial–interglacial cycles which have occurred during the past 2.6 million years (Berger and Loutre, 1991; Lisiecki and Raymo, 2005). Evolutionary processes, like biological variation and natural selection, have led to the emergence of adaptations, which allowed the species to cope with threats and environmental changes, and persist, otherwise perish.

Contrary to natural environmental changes, which have happened during long periods of time, humans have changed the conditions on Earth in an accelerating way (Steffen et al., 2015a). From the mid- 20th century, there is clear evidence for fundamental shifts in the state and functioning of the Earth system driven by human activities; changes which are far beyond the range of natural variability (Steffen et al., 2015a, 2011). For this reason, a new geological period, the Anthropocene has been proposed (Lewis and Maslin, 2015). During the Anthropocene, human activities have introduced novel stressors by altering Earth´s major biogeochemical cycles, the composition of the atmosphere, and by the introduction of different pollutants (Lewis and Maslin, 2015; Steffen et al., 2011).

During the last decades one of these novel stressors, plastic pollution, has received considerable attention by the scientific community, policy makers, the media, and the society in general. This might be explained by the visibility of the problem (Bank and Hansson, 2022), coupled with the growing number of studies reporting presence of plastics in diverse organisms, including humans (Gregory, 2009; Ragusa et al., 2021; Rochman et al., 2016), in air (Chen et al., 2020), tap water (Vega-Herrera et al., 2022) and remote areas (González-Pleiter et al., 2020; Jamieson et al., 2019; Materić et al., 2022a; Ter Halle et al., 2017). Nanoplastics, the smaller size fraction (< 1 µm) formed when plastic material breaks down, negatively affect a variety of freshwater organisms (Besseling et al., 2014; Bucci et al., 2020; Castro-Castellon et al., 2022; Chae and An, 2017; Kukkola et al., 2021; Larue et al., 2021), although a comprehensive understanding of their effects on freshwater ecosystems is still needed.

Box 1

In this thesis, *threat* is understood as the possibility of death, injure, or damage to an organism. In nature, it could be, for example, the possibility of being predated.

A *stressor* refers to an external factor that causes a negative physical effect on the organism. It could be, for example, a change in temperature or other environmental conditions, ultraviolet radiation, or the presence of a pollutant.

The two terms are related, as one can mean the other. For this reason, how these terms are used during the thesis is exemplified here, although this may not match how it was written in **Paper I**.

Aim of the thesis

The overall aim of this thesis is to address how two different stressors affect freshwater plankton and their ecosystem. Specifically, I explored ultraviolet radiation, a natural ancient stressor, and nanoplastics, a novel anthropogenic stressor. Furthermore, I explored how organism responses to these stressors can impact higher levels of biological organization, for example, the community structure or ecosystem processes.

Freshwater ecosystems and human use

It is commonly said that our planet is blue, since 71% of its surface is covered by water. Most of this water is deposited in the oceans (97.61 %), followed by polar ice and glaciers (2.08%). Besides ground water (ca. 0.3%), soil moisture (0.005%), and atmospheric water vapour (0.0009%), the inland waters from rivers, and freshwater and saline lakes, together represent 0.02 % of the total water in the biosphere (Vallentyne, 1972). Despite this low percentage, these waters are essential for sustaining the life in them and life on land. Besides water supply for drinking water and other uses, freshwaters also offer a variety of ecosystem services to humans, for instance, as food sources, for recreational uses, for transportation, for sustaining biodiversity, for aesthetics, among others (Wetzel, 2001).

The history of humans is linked to water. As other animals, we need water for drinking, but we have expanded its uses for other domestic needs as well, and since ancient times, for agriculture, and later in history, for industry. All these uses of water, together with the exponential growth of humans, have enormously increased the pressure on freshwater ecosystems. In this context, the concept of *demotechnic growth*, coined by John R. Vallentyne, is relevant. It refers to "the joint action of increasing numbers of humans and increasing per capita rates of resource consumption and waste production" - textual from (Hurlbert, 2012). This concept includes both the direct increase in production and consumption per capita and the technology that has promoted the growth of population and urbanization. Both processes increase the pressure for the use of water resources, and at the same time pollutes them (Wetzel, 2001). Inland surface waters reflect their surrounding landscape and the activities developed there, and depending on land use, different types of pollutants eventually reach these ecosystems. Different pollutants from agriculture, industries and urban areas affect and compromise water quality globally, such as nutrients, biocides, metals, particulates, plastics, and pharmaceuticals (Meybeck, 2003; Moss, 2008).

Acting in conjunction with pollution, climate change is directly and indirectly affecting freshwater ecosystems. The increase in the global average surface air temperature has changed the distribution of rainfall, increased the frequency of extreme weather events and raised the sea level (Kernan et al., 2010; Pörtner et al., 2022), which all directly affect freshwater ecosystems. But climate change also causes multiple other direct and indirect effects on all biomes on Earth. The Earth system is tightly interconnected, and models have shown amplifying interactions between nutrient pollution, climate change, and land-use change, especially threatening freshwater ecosystems integrity (Lade et al., 2019). This context makes freshwater ecosystems highly vulnerable, risking their natural state, their resilience capacity and the fundamental services they provide (Kernan et al., 2010; Pörtner et al., 2022). The problem is clear. Renewable freshwater resources are unevenly distributed in time and space (Oki and Kanae, 2006), the demand for high-quality freshwater is increasing with human population, and these environmental changes and pollution are already threating its accessibility in many places (De Wit and Stankiewicz, 2006; Steffen et al., 2015b).

Plankton and the trophic web

A key component of aquatic and freshwater ecosystems is the plankton community. Plankton is a diverse group of organisms from very different taxonomic groups, like bacteria, protozoa, algae, and animals. This diverse group is traditionally subdivided, depending on if they are autotrophs or heterotrophs. Phytoplankton is a group of autotrophic plankton organisms that can produce organic matter and oxygen from inorganic compounds, like CO_2, and an external source of energy, sunlight, through the process of photosynthesis. The other group, zooplankton, refers to plankton organisms that are consumers (heterotrophs) (Wetzel, 2024). When zooplankton organisms are eaten by fish and insect larvae, this links the energy produced by phytoplankton with organisms from higher trophic levels, like piscivorous fish or birds. Intimately linked with the plankton community, are the microorganisms (i.e., bacteria, viruses, and protist). The feeding interactions between those auto- and heterotroph microorganisms, the dissolved organic carbon, plankton organisms and their exudates are known as the "microbial loop" (Porter, 1996).

The food web theory is a central subject in ecology, which conceptualizes trophic interactions as fluxes of energy and matter (Polis and Winemiller, 1996). In brief, two energy channels can be distinguished in different food webs (Rooney et al., 2008). In lake ecosystems, for instance, the phytoplankton-zooplankton-fish energy channel is referred as "the pelagic way". The other, referred as the "littoral or benthic way", includes primary producers from the littoral and benthic areas (i.e., periphyton and macrophytes), the macroinvertebrates that consume those, and fish that feed on them (Rooney et al., 2008). Both trophic paths are tied by larger fish that feed on organisms from both energy channels, connecting them (Vander Zanden and Vadeboncoeur, 2002). Thus, the food web theory links foraging behaviours of individuals to biogeochemical cycling (Rooney et al., 2008), connecting different levels of biological organisation and the inorganic and organic world. Beside this, food web theory is a useful framework to understand community stability and responses to perturbations (Rooney and McCann, 2012).

One possible example of how food web theory allows the understanding of perturbations is the process of eutrophication, i.e., nutrient pollution. This process causes excessive growth of phytoplankton, which increases it biomass as a response to increased nutrient inputs (Carpenter et al., 1998). If the high nutrient levels persist in time, the increased phytoplankton biomass, so-called harmful algal (or cyanobacterial) blooms, can change the conditions of the whole ecosystem. In this situation, the zooplankton community is not able to control the phytoplankton excess through feeding. The excess of phytoplankton increases water turbidity, causes changes in pH, and dissolved oxygen depletion in the benthos, negatively affecting benthic primary producers and consumers. Consequently, the biodiversity decreases and the trophic chain is simplified, affecting therefore the whole ecosystem function (Dodds, 2007; Huisman et al., 2018; Schindler, 2006; Smith, 2003; Smith et al., 1999).

Pollutants can be propagated through the food web. When pollutants accumulate in organisms, named bioaccumulation, the incorporation rate of the pollutant is higher than the excretion rate. Besides this, pollutants can be magnified through the food web, known as biomagnification, when the concentration of the pollutant increases with the trophic position. Pollutants with a high tendency of biomagnification are often organic, lipophilic, and highly persistent, as some pesticides and persistent polychlorinated biphenyls (Walker et al., 2005).

Ultraviolet radiation: an ancient stressor that has shaped life on earth

Life on Earth emerged about 3800 million years ago and has coped with stressors ever since the beginning. Solar UV radiation, for example, has reached Earth long before the first forms of life appeared, and the first organisms had to cope with high levels of UV radiation until an oxygenated atmosphere was formed. Life has been continuously exposed to UV radiation, and various adaptations to cope with the stressor have evolved. Therefore, UV radiation has shaped life throughout the evolutionary time (Hessen, 2008).

In the atmosphere, more specifically in the stratosphere, the ozone layer strongly absorbs ultraviolet-B (UV-B) radiation from the sun, protecting life on Earth from harmful levels of UV radiation. Human emissions of chlorofluorocarbons, halons, and other ozone-depleting substances to the atmosphere have reduced stratospheric ozone over the globe, increasing incident UV-B radiation on Earth above naturally occurring amounts (Fahey and Hegglin, 2011; Rowland, 2006). This radiation is harmful to life, including humans and a diversity of terrestrial, marine and freshwater organisms (Bancroft et al., 2007; Fahey and Hegglin, 2011). Fortunately, international efforts, like the Montreal protocol and its amendments (UN environment programme, n.d.), have been successful in protecting the ozone layer and ozone is no longer declining, although the slow recovery to pre-damage values will take decades (Rowland, 2006).

Although the ozone layer absorbs most of the solar UV radiation, the intensities that reach the Earth´s surface are enough to negatively affect a range of different organisms. Increased mortality rates and reduced reproduction of several zooplankton species have been documented in response to UV radiation (Hansson and Hylander, 2009a; Williamson et al., 1994), besides the induction of avoidance behaviours . Furthermore, UV radiation can negatively affect primary producers, inhibiting phytoplankton photosynthesis, but can also favour the abundance of toxic cyanobacteria strains (Hansson et al., 2016; Hansson and Hylander, 2009b; Rhode et al., 2001; Xu et al., 2019).

Adaptations to the ultraviolet radiation

Depending on their own scale of perception, organisms experience environmental changes, threats but also opportunities for feeding and reproduction. Individuals from the same species can respond differently to the same threat. When the individual variation affects the reproductive success (i.e., fitness), and when these differences in fitness are consistent through time, and at least part of the variation can be inherited, natural selection occurs (Futuyma, 2005). From a biological point of view, threats and stressors are selective pressures that drive population change and evolution (Steinberg, 2012).

Adaptations at the organismic level have impacts at higher levels of biological organization, for example, in community composition and ecosystems processes. Freshwater zooplankton have different morphological and behavioural adaptations to cope with UV radiation. Solar radiation is a spatially variable factor, which is rapidly attenuated through water (Morris et al., 1995). Some species have morphological adaptations, such as photo-protective compounds (detailed by Hansson and Hylander (2009a)), that allow the species to stay at the surface, in UV exposed waters during the day (Hansson, 2000, 2004; Rautio and Korhola, 2002). Other species migrate to deep waters during day to get refuge, both from UV radiation and visual predators, causing a process known as diel vertical migration (Hansson et al., 2007; Hansson and Hylander, 2009a; Leech and Williamson, 2001; Rhode et al., 2001). These different morphological and behavioural adaptations relate with the habitat those species use, and how they feed, affecting the spatial and temporal distribution of organisms in the ecosystem (Hansson et al., 2016; Hylander and Hansson, 2010), and structuring the community.

Solar UV radiation is also a temporally variable abiotic factor, it fluctuates during the year, and over short time scales with the position of the sun and rapidly occurring variations in cloudiness (see for example: Cabrera et al. (1995), and Hernández et al. (2012)). Despite its variable nature, most studies on the effects of UV radiation have studied the effects of a constant exposure to UV radiation (Connelly et al., 2009; Fernández et al., 2018; Grad et al., 2001; Hansson et al., 2016; Leech and Williamson, 2000). On the other hand, studies which included natural variations in UV radiation in the experimental design, did not evaluate how those affect organism responses (Hylander and Hansson, 2010; Leech and Williamson, 2001; Williamson et al., 1994). In particular, the freshwater zooplankter *Daphnia magna* has been shown to exhibit a repeatedly and strong negative phototaxis in response to UV radiation (Hansson et al., 2016; Storz and Paul, 1998).

When individuals are exposed to a threat, an optimal adaptive response is the one which fits the intensity and duration of the threat. However, responding to a threat, even to an ancient one as UV radiation, might imply a cost, as missed opportunities for feeding and reproduction. Moreover, the individual condition, such as its physiological state, age, or health, also affect the final outcome of the response (Gaynor et al., 2019). Beyond the individual, variable environments and fluctuating conditions, as the natural fluctuations of UV radiation, could promote different individual responses in zooplankton, and might set the ground for different life strategies and plastic responses to arise (Franch-Gras et al., 2017; Sommer, 2020).

The specific aim of this section of the thesis is to explore how a natural and ancient stressor as UV radiation, and its fluctuations, affect the behaviour and fitness of *Daphnia magna*.

Plastic pollution: a novel and ubiquitous material

Contrary to the ancient stressor of UV radiation, the wide presence of anthropogenic pollutants in nature, mainly associated with steadily increases in the use of pesticides, fertilizers and other substances, have a recent history starting around the second half of 20th century (Pimentel, 1996; Steffen et al., 2015a). For the case of plastics, although their widespread use started in the 50´s, the increasing reports raising concerns about their ubiquity and possible adverse effects did not start until the late 90´s (Fig. 1). It has been estimated that, from 1950 to 2015, 60% of all plastics ever produced, approximately 4900 million metric tons, were discarded and are accumulating in landfills or in the natural environment (Geyer et al., 2017). Nowadays plastics can be found in all ecosystems in the world, even remote ones (Jamieson et al., 2019; Materić et al., 2022b, 2022a; Ter Halle et al., 2017), and plastic pollution (Box 2) has become a global environmental problem (Eriksen et al., 2023; Stubbins et al., 2021).

Plastic material moves between the different Earth compartments, land, water and the atmosphere (Stubbins et al., 2021), mainly dispersed through water and air. It can enter freshwater ecosystems through runoff and atmospheric deposition (Brahney et al., 2020; Kallenbach et al., 2022; Xia et al., 2020), and from point sources, such as landfills and wastewater treatment plants (Hale et al., 2022). Another significant source of plastic pollution is from the domestic washing of synthetic textiles (Cai et al., 2020; Carney Almroth et al., 2018). Freshwater ecosystems are part of the plastic cycle. Besides transporting plastic to the ocean as through streams and rivers (Lebreton et al., 2017), freshwaters can transform plastics as they get weathered and break down in smaller pieces. Moreover, these ecosystems can also be sinks of plastic pollution (Kallenbach et al., 2022; Windsor et al., 2019). Recently, it was found that plastic concentrations in surface waters of lakes can be higher than those reported in the subtropical oceanic gyres, and larger lakes and lakes in highly populated areas seem to be the most vulnerable to plastic pollution (Nava et al., 2023).

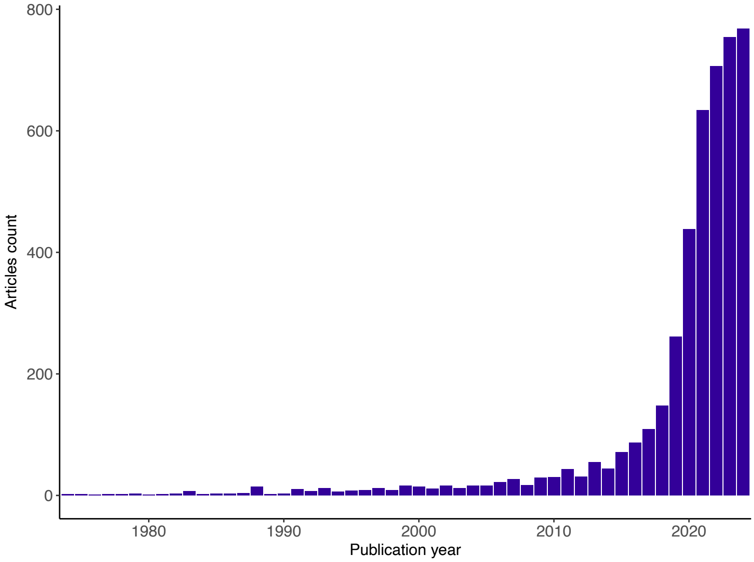

Figure 1. Number of articles in plastic pollution related topics published between 1974 and 2024. Research articles published in international journals (excluding reviews, editorials and other types) that included the word “plastic*” in the title and “pollution” or “contamination” in the topic. Articles published in 2025 or before 1974 (older than 50 years) were excluded. A total of 4504 articles were found. Data extracted from Web of Science database, accessed on December 17th, 2024.

Box 2

In this thesis, the term « plastic pollution » follows the definition proposed by Villarubia- Gómez et al.: « *plastics pollution refers to all ways that plastics cause harm to the environment and society throughout the impact pathway* » (Villarrubia-Gómez et al., 2024).

This term, as defined by the authors, incorporates the complexity of the topic, including the different polymers and chemical substances that comprise plastics, the different size classes of plastics, and their impacts.

The complexity of plastic pollution

Plastics are persistent synthetic polymers, and from a geological perspective, anthropogenic carbon-based “geomaterials with chemistries not previously seen in Earth history” (Stubbins et al., 2021). The term plastic spans diverse materials and there is still no consensus on how to define it (Hartmann et al., 2019). Conventional plastics are derived from fossil hydrocarbons and include, for example, different non-fiber forms of plastic (i.e., resins or pellets) as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), polyethylene terephthalate (PET), and thermoplastics, thermosets and polyurethanes (PURs), but also fibers of polyester, polyamide and acrylic (Geyer et al., 2017). Plastic materials usually contain additives, which are chemical substances that are added during the production of plastic, that enhance the polymer properties. Some examples of additives are plasticizers, flame retardants, flow modifiers and stabilizers (Hahladakis et al., 2018).

Another diverse aspect of plastic materials is their broad size range. The different size categories proposed for plastics are: nano- (1 to < 1000 nm), micro- (1 to < 1000 μm), meso- (1 to <10 mm), macro- (1 cm to <1 m), and megaplastics (1 m and larger), considering the largest dimension of the particle (Hartmann et al., 2019; Stubbins et al., 2021) (Fig. 2). Plastics are modified by fragmentation and degradation processes which break plastics down to smaller fragments and chemically transform them (Nicholson, 2017). Those processes are driven by environmental factors such as light (both visible and UV), heat, moisture, mechanical forces and biological activity, that change the physicochemical characteristics of the original material (Andrady et al., 2022; Masry et al., 2021). As a result, smaller plastic particles called secondary particles are formed (Mattsson et al., 2018). Furthermore, plastic particles can be intentionally manufactured at the micro- and nano-scale, known as primary particles, and added to different products, such as cosmetics (Hosseinkhani et al., 2015), shampoos (Günay et al., 2017), laundry detergents and softeners (Murphy, 2015). When these particles are not retained by the wastewater treatment plants, they reach the aquatic environment through the sewage systems (Hale et al., 2022; Mattsson et al., 2018). In addition to size, plastics found in nature can vary in other characteristics, as their shape and other physicochemical properties (Hartmann et al., 2019).

Plastics are considered emerging pollutants. These diverse materials harm organisms through entanglement, ingestion, food dilution, gastrointestinal blockage and internal abrasion (Bucci et al., 2020; Gregory, 2009; Rochman et al., 2016), but also due to chemical effects from the leaches of toxic additives or adsorbed pollutants (Thompson et al., 2024). Furthermore, although plastic impacts on the Earth biogeochemistry are still under-researched (Villarrubia-Gómez et al., 2024) some studies indicate that plastics could alter the cycle of carbon (Ziervogel et al., 2024) and nitrogen (Seeley et al., 2020; Yang et al., 2020; Zeng et al., 2023). Despite the ubiquity of plastic pollution, and possible due to its intrinsic complexity, our understanding regarding plastics effects in nature is still limited.

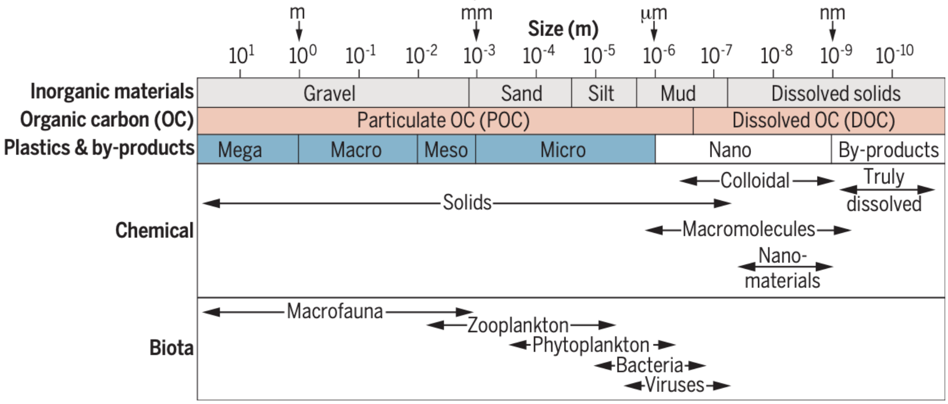

Figure 2. The size range of plastics in perspective with the environment.
The scheme shows the size range for plastics, classified as mega-, macro-, meso-, micro- and nanoplastics. Plastic leaches, as non-plastic by-products, are also included. The size classification for plastics is shown in comparison with other geomaterials, such as inorganic materials and the size range for organic carbon. This is presented on a chemical context, including different chemical classes and their behaviour, and commonly applied size distributions for biota. Reproduced from Stubbins et al. (2021). Reprinted with permission from AAAS.

Nanoplastics

To date, there is no consensus regarding the definition for nanoplastics. In this thesis I followed the criteria proposed by Hartmann et al. 2019, which define nanoplastics as solid, insoluble, synthetic and semi-synthetic polymers with a size between 1 to < 1000 nm in its largest dimension.

Our understanding of nanoplastics behaviour and toxicity has increased considerably during the last years, thanks to a rising number of laboratory and field studies. Most of the toxicity studies have been performed in simplified experimental designs using specific target organisms, whereas studies assessing nanoplastics transport, fate, and toxicity in complex systems are less but growing. Despite the fact that quantification of nanoplastics in complex environmental matrices is still analytically challenging (Mitrano et al., 2021), the presence and chemical composition of nanoplastic in environmental water samples has been successfully determined in some studies (Materić et al., 2022b; Ter Halle et al., 2017).

Compared to microplastics, plastics in the nano-scale have different transport pathways, interact differently with organisms, and require the use of specific analytical techniques for their characterization and quantification (Gigault et al., 2021; Mitrano et al., 2021). Regarding transport, when nanoplastics are in suspension, Brownian motion dominates the movement over sedimentation and buoyancy (Gigault et al., 2021). Regarding how they interact, nanoplastics exhibit strong surface reactivity per given mass due to their large surface to volume ratio

(Oberdörster et al., 2005). This results in a higher relative importance of surface interactions compared to physical interactions. In addition, the size of nanoplastics is comparable to the size of environmental macromolecules (Fig. 2), which makes it possible the passive biological uptake and transport across biological membranes (Gigault et al., 2021).

Nanoplastics are toxic to several organisms, including diverse freshwater organisms, such as phyto- and zooplankton (Besseling et al., 2014; Kelpsiene et al., 2020; Larue et al., 2021; Zhu et al., 2021), benthic macroinvertebrates (Redondo-Hasselerharm et al., 2020) and fish (Mattsson et al., 2017; Pitt, 2018). Moreover, it has been shown that nanoplastics can cross cell membranes (Liu et al., 2021; Yan et al., 2021) and the blood-brain barrier (Mattsson et al., 2017), affect cell metabolism (Cedervall et al., 2012), and can be transferred through the food web from algae to top predators, like fish (Chae et al., 2018; Mattsson et al., 2017, 2015). Particle size is an important feature for toxicity, and, within the nano-scale, the smaller nanoplastics often show higher toxicity than larger ones (Ekvall et al., 2022; Kelpsiene et al., 2020; Mattsson et al., 2017). Particle shape, the surface charge, and the exposure dose are also characteristics that affect their toxicity (Mattsson et al., 2018).

The size of the nanoplastics is below the resolution limit of most analytical techniques (Mitrano et al., 2021), which complicates the discrimination and quantification of nanoplastics in complex matrices. The use of markers embedded into the nanoplastics, as particles with fluorescent aggregation-induced emission fluorogens coated with plastic (called AIEgen-nanoplastics (Wang and Wang, 2023; Yan et al., 2021)) or metal-doped nanoplastics (Mitrano et al., 2019) has promoted considerable advances in the understanding of nanoplastics transport and fate. Metal-doped nanoplastics allow tracing the metal inside them, that can be quantified using standard methods for trace metal analysis. Using this technique, different systems with varying complexity have been studied, examining for instance: nanoplastic uptake and effects on aquatic invertebrates (Redondo-Hasselerharm et al., 2021; Vicentini et al., 2019) and vertebrates (Clark et al., 2023), the distribution and effects of nanoplastics on simplified food chains (Holzer et al., 2022; Tamayo-Belda et al., 2023), and in freshwater mesocosms with longer exposure times (He et al., 2022; Ockenden et al., 2024; Stábile et al., 2024).

Despite all these advances in our knowledge, the complexity of plastic pollution, together with the complexity of natural systems makes our understanding insufficient. The recently reported nanoplastic concentrations for surface inland waters in Sweden (Materić et al., 2022b) provided a primary insight regarding the degree of nanoplastic pollution even in those remote waters (mean for surface waters: 563 µg/L, ranging from 180 to 1588 µg/L, cumulative for all types of plastics found). There is a need to understand nanoplastics fate and effects in natural environments, and there is a call for studies that assess this in more realistic settings, considering environmentally relevant concentrations and materials (Mitrano et al., 2021). Experimental setups at a mesocosm scale allow a higher degree of complexity and realism than laboratory scale experiments, with the advantage that they can be replicated, which is not always possible in natural ecosystems. In parallel, the use of metal-doped nanoplastics to track nanoplastics in complex environmental matrices, appear as a promising tool for assessing this novel pollutant to freshwater organisms and their ecosystem.

The specific aim of this section of the thesis is to evaluate the transport, fate, biological uptake, and effects of nanoplastics in freshwater ecosystems.

Research questions

This thesis is structured around the following research questions:

How does ultraviolet radiation, and its fluctuations, affect the behaviour and fitness of *Daphnia magna*? (**Paper I**).

How are nanoplastics transported and where are they ending up in freshwater ecosystems? (**Paper II**)

Which are the effects of the exposure to nanoplastics in freshwater ecosystems? Which organisms are the most impacted and how does this affect other levels of biological organization? (**Paper III**)

How do nanoplastics interact with phytoplankton species, and what are the effects on their growth and morphology? (**Paper IV**)

Main methodological approaches

This thesis explored how UV radiation and nanoplastics affect freshwater plankton, their growth, morphology, and behavioural responses, including effects at the ecosystem level. All the studies performed were experimental, and two different approaches were followed. In **Paper I** and **IV**, the laboratory scale was used, and the studies were performed using simplified experimental setups. Moreover, this last study (**Paper IV**) included the use of transmission electron microscopy as a method to explore the interaction between nanoplastics and phytoplankton cells. On the other hand, the studies on **Paper II** and **III** were performed at a mesocosm scale, where the complexity and realism of the setup was higher than on the other papers.

Regarding the organisms I worked with, in **Paper I** the zooplankter *Daphnia magna* was the main character. In **Paper IV**, I worked with two species of phytoplankton: the cyanobacteria, *Dolichospermum flos-aquae*, and the green algae, *Tetradesmus obliquus*. **Papers II** and **III**, performed at a mesocosm scale, involved several different organisms and their environment. In these studies, *D. magna* and *Asellus aquaticus* were the focal organisms and were added to the mesocosms in a controlled way.

In the following subsections these components are described briefly. For specific details, please refer to the respective paper.

Daphnia magna and UV radiation experimental setup

Daphnia spp. is a genus of crustacean zooplankton. They inhabit standing fresh- and brackish waters, from small temporary ponds to large lakes, having a near worldwide distribution (Ebert, 2022). *Daphnia* can be a major consumer of phytoplankton through their generalist filter feeding. For example, *D. magna*, can actively feed on particles over a size-range from 0.6 to 40 µm (Geller and Müller, 1981). Further, they are a food source to predatory young fish, but also to adult planktivorous fish (Reynolds, 2011). Therefore, *Daphnia* links the energy transfer between producers and fish in aquatic food webs.

During most of the growing season, *Daphnia* reproduce asexually through parthenogenesis (Ebert, 2005), but they can have sexual reproduction, often due to high population density (Haltiner et al., 2020). When conditions are not favourable, some of the asexually produced offspring develop into males which fertilise females' haploid sexual eggs (Ebert, 2005). Through sexual reproduction, *Daphnia* produce a resting stage (called ephippia) which will stay in the bottom of the water body, in diapause, and can be viable for decades (Ebert, 2022) even hundred years (Frisch et al., 2014). On the other hand, *Daphnia* can exhibit phenotypic plasticity in different traits when exposed to biotic and abiotic factors, as predators (Dodson, 1989; Lampert, 1993) or UV (Hansson et al., 2007; Rautio and Korhola, 2002).

Several characteristics as their ecology, mode of reproduction, and phenotypic plasticity, makes *Daphnia* an interesting model organism, being a model for many disciplines, for example in ecotoxicology (Altshuler et al., 2011; Ebert, 2022), and in ecology and evolution (Ebert, 2022; Gurney et al., 1990; Reynolds, 2011).

In **Paper I**, *D. magna* organisms were presented to three different treatments: control (C), intermittent UV radiation (iUV) and constant UV radiation (UV) (Fig. 3). All the treatments were exposed to the same daylight intensity over the 12 h light part of the photoperiod. The iUV was created by turning the UV lamp on and off every 15 min during daylight, mirroring fluctuating sunlight, and the UV treatment was exposed to constant UVR during 6 h a day (Fig. 3), resembling a sunny day without cloud cover.

Daphnia were isolated from laboratory cultures of three different *D. magna* genotypes, originally isolated from different lakes in southern Sweden. Each treatment had all three genotypes represented, with each genotype replicated at least three times per treatment.

To determine the effects of the UV radiation and fluctuating exposure, *Daphnia* survival and reproduction were monitored during the experiment. To assess *Daphnia* swimming behaviour, the individual position in each aquarium was registered as 'bottom' or 'surface' when the animal was below or above a line drawn at the middle of the aquarium (Fig. 3). This was done periodically during 11 h, on four recording occasions during the experimental time.

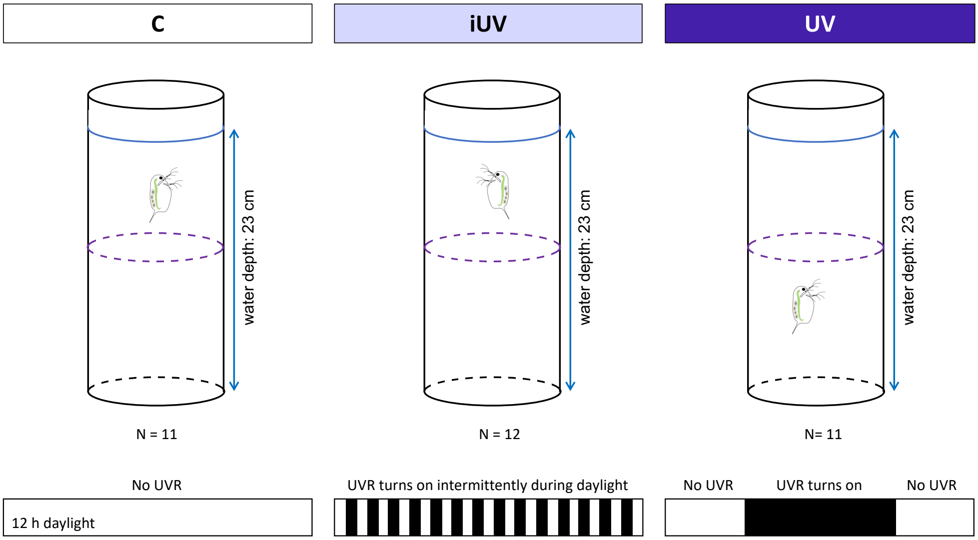

Figure 3. Schematic representation of the experimental setup on Paper I.
Diagram of the experimental design showing the three treatments: control (C, in white), exposed to cool white light and no UV radiation, the intermittent UV radiation treatment (iUV, in lilac), exposed to constant cool white light and fluctuating UV radiation which was turned on and off every 15 min during daylight, and the constant UV radiation treatment (UV, in violet) exposed to cool white light and constant UV radiation during 6 h during daylight. 'N =' denotes the number of replicates per treatment, and the dashed line in the middle of the aquaria represents the criteria for registering *Daphnia* position as 'bottom' or 'surface' during the behavioural recordings. Figure from **Paper I**.

The wetland mesocosms

Wetland ecosystems sustain high biodiversity and provide several ecosystem services, acting as water reservoirs, in flood protection, carbon sequestration and contributing to the improvement of water quality by retention of nutrients and sediments (Zedler and Kercher, 2005). They are at the interface between water and land; therefore, they interconnect, and highly influence, what happens in the adjacent terrestrial and aquatic ecosystems. Understanding how pollutants, as nanoplastics, are transported and distributed in wetlands is highly relevant, both for the ecosystem itself and for the water quality of downstream aquatic ecosystems.

The mesocosm scale (Odum, 1984) allows a degree of realism not possible at laboratory scale studies, and at the same time replication, which is not always possible in nature. In **Paper II** and **III**, wetland mesocosms were used, which were constructed in glass aquaria and set in a greenhouse (Fig. 4 A & B).

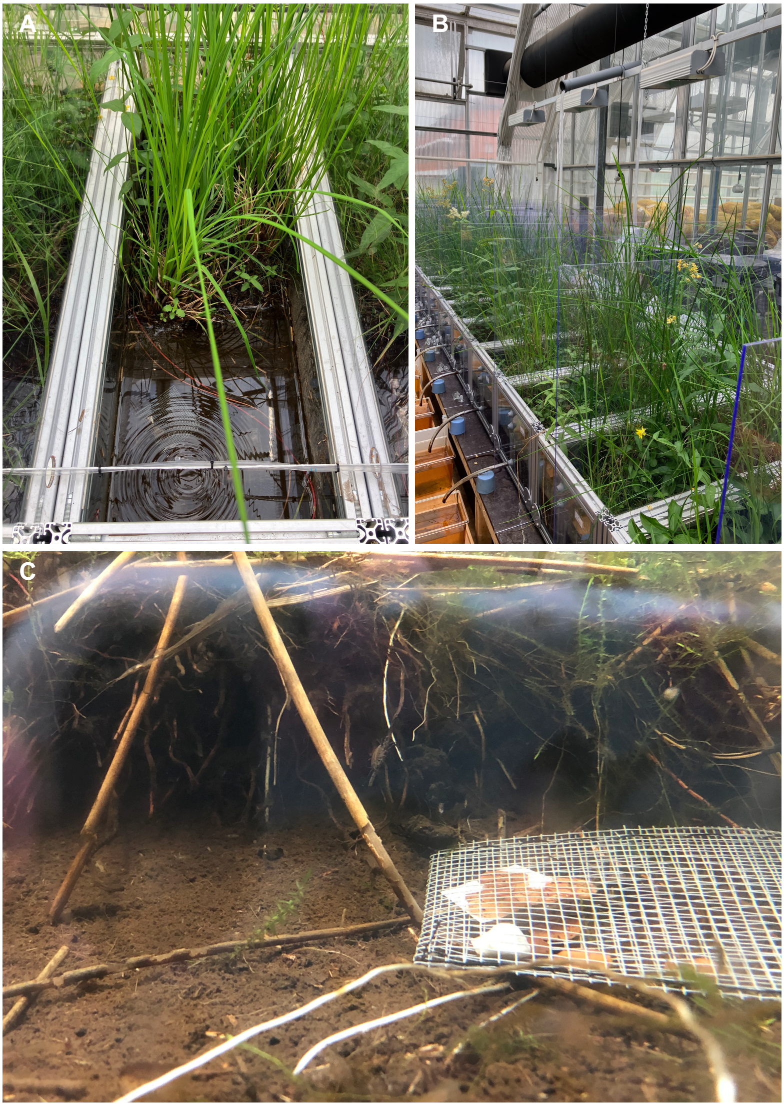

Figure 4. The wetland mesocosms set in the greenhouse at Lund University.
(A) Upper view of the wetland from the lake area, where water dripping from the inlet just fell in the lake. **(B)** The 12 wetland mesocosms seen from the side where the outlet is placed. **(C)** A look inside the lake, where a leaf litter bag to test litter decomposition rate is visible, resting on the bootom. An *Asellus aquaticus* can be seen in the back.

The structure of the wetland mesocosms consisted of an area with water close to the inlet which, despite its small size, carries similar features as a lake and was therefore named “the lake area” (volume = 6.97 ± 0.58 L and 8.4 ± 0.5 L in **Paper II** and **Paper III**, respectively) (Fig. 5). The lake is followed by a sediment area with macrophytes covering approximately three quarters of the aquarium (Fig. 4 A), denoted “sediment and macrophytes area” (Fig. 5). Considering both areas, the total volume of the wetland was, on average, 27.42 ± 0.98 L in **Paper II**, and 23.49 ± 0.68 L in **Paper III**. Each mesocosm was continuously fed with tap water at a constant flow rate. The inlet water was dripped into the small lake area from where the water flowed through the systems to an outlet located at the opposite side of the mesocosm (Fig. 4 & 5).

To create the “sediment and macrophytes area”, sediment tufts with macrophytes were retrieved from a natural wetland and placed in the aquaria three months before the start of the experiment to allow proper establishment. The tufts were divided in smaller pieces and randomly distributed among the 12 wetlands. The “lake area” was initially filled with tap water and no structure or mesh separated this area from the “sediment and macrophytes area” (Fig. 4 C). Before the start of the experiments, each wetland's lake section was inoculated with an algae culture of *Tetradesmus obliquus* and with invertebrates: the benthic detritivores, *Asellus aquaticus*, and the pelagic filter feeders, *D. magna*. Furthermore, as sediment was not frozen after collection, a natural community of organisms came with it, composed by tubificid worms, chironomids, and copepods, from the original wetland (Fig. 4).

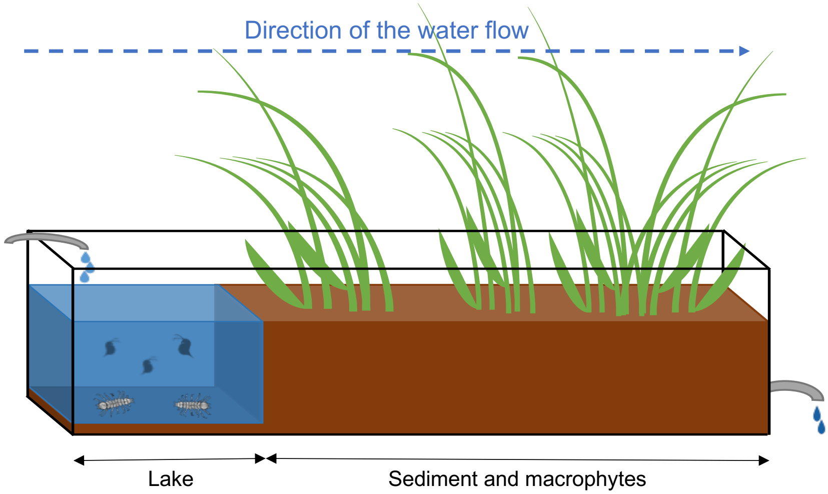

Figure 5. Schematic representation of the wetland mesocosms.
Each wetland has two areas: the lake, and the sediment and macrophytes area. Water continuously flows through the mesocosm, from the inlet (left) towards the outlet (right).

The nanoplastic particles

Two different types of PS nanoparticles were used as a model for nanoplastics. In **Paper III**, spherical, aminated polystyrene (PS) nanoparticles (PS-NH_2) of 53 nm in diameter were used. The surface charge of the particle dispersion (zeta-potential) was positive and was found to be around 27 mV. The other type of nanoplastic used were metal doped nanoplastics, used in **Paper II** and **IV**. Specifically, these were PS nanoparticles (size: 88 ± 11 nm in **Paper II**, and 92.6 ± 9.4 nm in **Paper IV**) with a gold (Au) core (size: 13 ± 1 nm in **Paper II**, and 14.2 ± 1.9 nm in **Paper IV**) surrounded by a silica (SiO_2) layer. The surface charge of the particle dispersion (zeta-potential) was negative (around - 67 mV in **Paper II**, and - 84 mV in **Paper IV**). The particles have a surface with similar properties as a pure PS particle (supplementary information in **Paper II**), since the core is completely incorporated in the polystyrene layer (Fig. 6). At the same time, the gold core allowed us, in **Paper II**, to assess their transport, fate and uptake in the mesocosm wetlands using inductively coupled plasma mass spectrometry (ICP- MS).

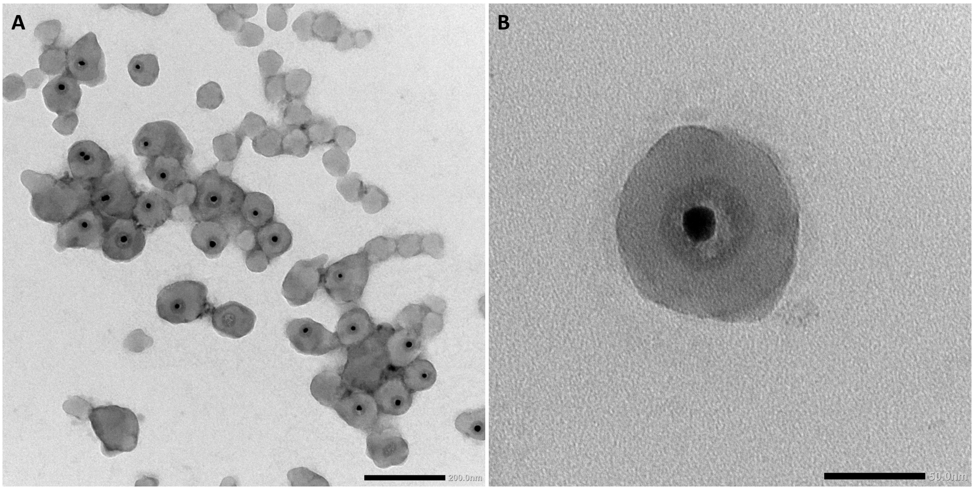

Figure 6. The gold-doped polystyrene nanoparticles used in Paper II and IV.
Transmission electron microscopy images of the Au-doped polystyrene (PS) nanoparticles under x30k magnification **(A)** and x150k magnification **(B)** where the scale bar represents 200 nm and 50 nm, respectively. Note the structure of the particles, with the gold core in the inner part surrounded by a silica (SiO_2) layer and the external PS layer. Some particles without Au core are also visible in image **A**. Figure from **Paper IV**.

The green algae *Tetradesmus obliquus* and the cyanobacteria *Dolichospermum flos-aquae*

Tetradesmus obliquus (Turpin) (Wynne and Hallan, 2015) is also known by the homotypic synonyms *Achnanthes obliqua*, *Scenedesmus obliquus* and *Acutodesmus obliquus* (Guiry, 2022). This species belongs to the class Chlorophyceae, or green microalgae, which includes mostly freshwater species and is usually described as the most abundant of the microalgae groups (Queiroz et al., 2020). These are eukaryotic organisms.

In *Tetradesmus* spp. the individual cells are adjoined to form a colony, also called coenobium. The coenobium usually has 4, 8 or 16 cells in a row, although sometimes they lie in two rows (Hoek et al., 1995). This group is recognised by its high phenotypic plasticity (Lürling, 2003; Peña-Castro et al., 2004) with plastic traits as the coenobium or unicell lifeforms, the number of cells in the coenobium, their sizes, and the numbers of spines (Baudelet et al., 2017). Their cell wall is a complex two-layered structure, described by Baudelet et al. (2017) as a 100–300 nm thick homogeneous layer that surrounds the plasma membrane, and a typical trilaminar sheath on its outer surface.

The asexual reproduction in *Tetradesmus* spp. starts when the contents of the parental cell divide into a certain number of non-flagellate daughter cells. These cells later regroup to form the new daughter coenobium, which is later released from the parental cell through an opening in the parental cell wall (Hoek et al., 1995). In certain nutrients deficiency conditions, sexual reproduction is possible (Trainor and Burg, 1965).

Tetradesmus species occur both in fresh and brackish waters and many species are easy to grow in culture (Hoek et al., 1995). Under culture conditions it is common to find them both colonial and unicellular. The genus *Tetradesmus* is currently highly researched due to its biotechnological potential, as it is able to rapidly produce biomass rich in proteins, carbohydrates, lipids, and bioactives (Do Carmo Cesário et al., 2022).

On the other hand, the species *Dolichospermum flos-aquae* (Bornet & Flahault) (Wacklin et al., 2009), also known by the homotypic synonym *Anabaena flos-aquae*, is a species of cyanobacteria (class Cyanophyceae, order Nostocales) (Molinari Novoa, 2022). Cyanobacteria, or Cyanophyta, are a phylum within the Bacteria domain, therefore prokaryotes, with the ability to perform oxygenic photosynthesis (Hoek et al., 1995). Within the cyanobacteria, unicellular, colonial, and filamentous forms can be found. The species *D. flos-aquae* has both unicellular and filamentous forms (Hoek et al., 1995).

In cyanobacteria, the cell wall consists of four layers. The most internal and strong part of the cell wall is composed of murein (a peptidoglycan), characteristic that is shared with other bacteria. Outside it there are cell wall layers mostly composed by lipopolysaccharides. The cells are often embedded in sheaths of mucilage, predominately composed by polysaccharides (Hoek et al., 1995). Cyanobacteria reproduce only asexually, but genetic recombination can occur via bacterial processes as conjugation (Kumar and Ueda, 1984).

Cyanobacteria occur in marine, freshwater and terrestrial habitats. *D. flos-aquae*, in particular, is a freshwater species with a world-wide distribution (Molinari Novoa, 2022). This species can fix atmospheric nitrogen thanks to a specialized cell called heterocyst. Furthermore, the production of other specialized cells called akinetes, allows this species to survive periods when environmental conditions are not favourable, and even extreme environmental conditions (Hoek et al., 1995). These, and other characteristics, make *D. flos-aquae* a common bloom forming cyanobacteria (Huisman et al., 2018). Moreover, the species can produce different toxins causing problems with water quality worldwide (Huisman et al., 2018). Harmful cyanobacterial blooms have increased on a global scale during recent decades, and likely will continue increasing due to eutrophication and climate change (Huisman et al., 2018; Moss, 2011).

In **Paper IV**, the effects of nanoplastics in the growth and morphology of these two species of phytoplankton were investigated. Furthermore, the interaction between the phytoplankton cells and the nanoplastics at the cellular level was explored using Transmission Electron Microscopy (TEM).

Main results and discussion

"All models are wrong, but some are useful"

George E. P. Box

The main results of this thesis are presented and discussed following each of the research questions. At the end, a brief general discussion is presented.

How does ultraviolet radiation, and its fluctuations, affect the behaviour and fitness of *Daphnia magna*?

In **Paper I**, I together with the co-authors, experimentally investigated individual survival, reproduction, and behaviour within a single generation of *Daphnia magna* when exposed to a constant or fluctuating UV radiation treatment, and a control, not exposed to UV radiation. As explained previously, each treatment had 3 different *D. magna* genotypes, with each genotype replicated at least three times per treatment. The genotypes are identified with 3 different letters: D, N and P, which refer to the location in southern Sweden from where they were originally isolated.

In nature, solar UV radiation varies temporally, during the year, but also over short time scales with the position of the sun and variations in cloudiness. This natural variability could promote different individual responses in zooplankton (Franch-Gras et al., 2017; Sommer, 2020). *D. magna* exhibit a repeated avoidance behaviour in response to UV radiation (Hansson et al., 2016; Storz and Paul, 1998), but responding to short-term fluctuations in UV radiation through avoidance behaviours might imply a cost.

We showed that individuals exposed to fluctuating UV radiation, resembling natural variations in cloud cover, had the lowest fitness (measured as the number of offspring produced during the experimental time of 45 days) (Fig. 7 A). In contrast, individuals exposed to the same, but constant, dose of UV radiation had similar fitness as the individuals not exposed to UV radiation (Fig. 7 A), but they showed a significant reduction in daily vertical movement (Fig. 7 B), being more often in the lower section of the aquarium during the UV exposure period. These results show that the re-occurring behavioural response to the fluctuating UV radiation treatment gave rise to fitness costs for *D. magna*.

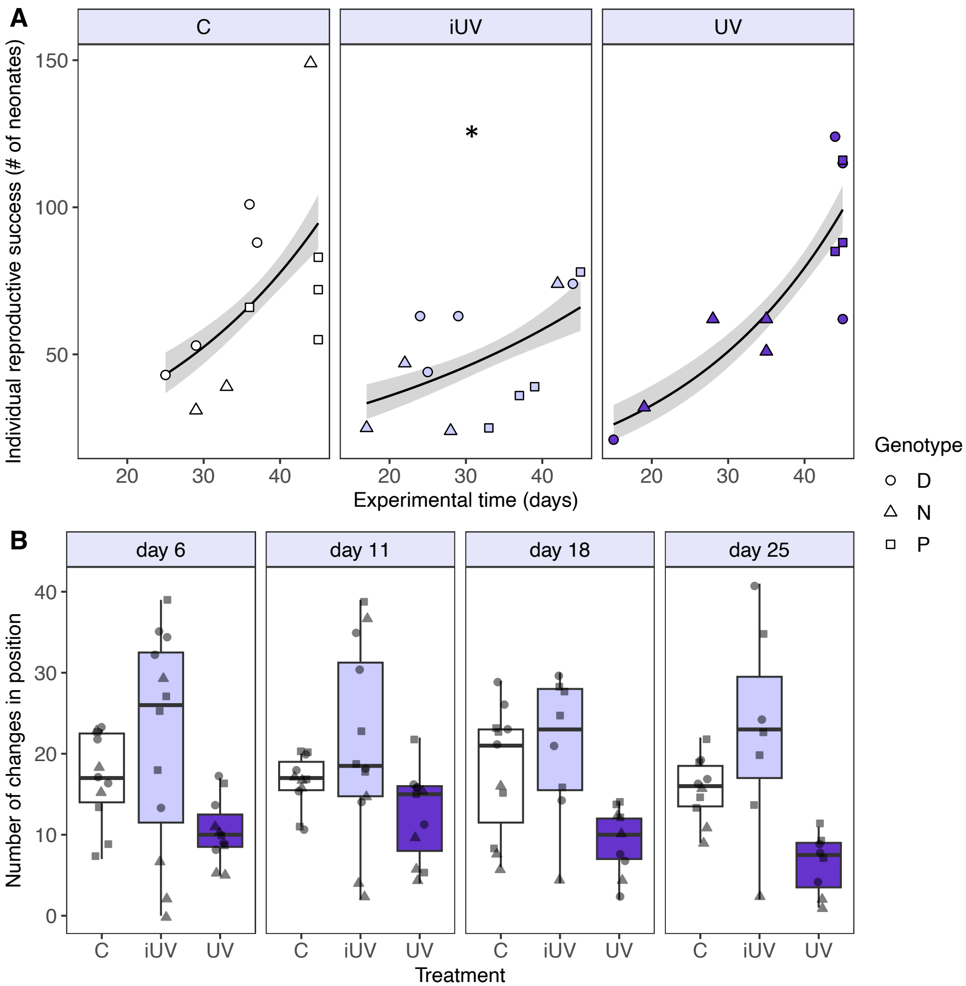

Figure 7. Individual fitness and behaviour of *Daphnia magna* when not exposed to UV radiation (C, in white) or under exposure to a constant (UV, in violet) or fluctuating UV radiation (iUV, in lilac) treatment.

(A) Individual reproductive success (or fitness) during the experimental time for the different treatment groups. The black line represents a Poisson curve adjusted to the data, and the grey shading, the 95% confidence interval. The asterisk indicates significant statistical difference in fitness between the iUV and the other two treatments. **(B)** *Daphnia* up and down behaviour, assesed as the number of changes in position, between treatments for each behavioural recording day. *Daphnia* exposed to the constant UV treatment performed the lowest number of changes in position. The boxplots represent the median as a black horizontal lines, the first and third quartile with the box, and the minimum and maximum with the vertical lines. In both plots **(A & B)**, the symbols represent the data from each individual *Daphnia* and the different shapes indicate the different genotypes (D, circles, N, triangles, and P, squares). Figures rearranged from **Paper I**.

Additionally, we found that the behavioural response of the individuals did not change with the experimental time (Fig. 7 B). Hence, there was no evidence for plastic behavioural responses when continually being exposed to UVR, despite the regular exposure schedule. Interestingly, the analysis showed that *D. magna* genotype was a significant explanatory variable when analysing *Daphnia* survival, reproduction, and behaviour.

It has been shown that predation, an important threat for organisms, can cause rapid local adaptations in *D. magna* (Cousyn et al., 2001), facilitated by the genetic variation that is already present in the population. It is important to mention that our simplified experimental setup only left a restrictive behavioural repertoire since *D. magna* had 23 cm to move in depth and no shelter was provided in the horizontal dimension. Therefore, extrapolation to natural environments should be done with caution. However, our results indicate that depending on how variable a stressor is, *D. magna* populations can adopt different behavioural strategies which imply different fitness cost. These findings, together with the significant effect of *Daphnia* genotype, indicates potential for population differentiation and local adaptation.

How are nanoplastics transported and where are they ending up in freshwater ecosystems?

There is a need to assess the transport, fate, uptake, and effects of nanoplastics in natural ecosystems. In **Paper II**, I together with the co-authors, quantitatively assessed the transport, fate and biological uptake of nanoplastics in wetland mesocosms. For doing this, we exposed 6 freshwater wetland mesocosms during 70 days to polystyrene (PS) nanoparticles doped with a gold core, which allowed us to track the particles over time, and assess their distribution in different compartments of the ecosystem. Additionally, we had six wetlands mesocosms un-exposed to nanoplastic that were used as a control.

We found that most nanoplastics (97% on average) were retained in the wetlands, and only a small fraction (3% on average) left the system through the mesocosm outlet. After 10 weeks of exposure, most of the nanoplastics were found in the sediment of the mesocosm's lake section (Fig. 8 A). Every week during the experiment, nanoplastics were added to the lake section of the mesocosms. It is likely that after being suspended in the water column of the lake section, the nanoplastics aggregated with naturally occurring organic matter, as shown by other studies (Lowry et al., 2010; Pradel et al., 2023) or even biota, such as algae (as shown in **Paper IV**). Associated with these larger aggregates, and in a system with no turbulences like ours, the probability of particles sinking to the sediment substantially increases. Additionally, the filter feeder *Daphnia magna*, also incorporated nanoplastics (Fig. 8 B). Although *Daphnia* faeces were not analysed in our study, some nanoplastics could have been excreted (as shown by Redondo-Hasselerharm et al. (2021) in *Gammarus pulex*) and rapidly sink to the sediment together with the faeces. All these processes likely explain the largest amount of nanoplastics found in the sediment of the lake area, and as proposed by others, when aggregation occurs, the fate of the nanoplastic would be more related to the fate of the bigger aggregate than with the nanoplastic properties (Gigault et al., 2021).

Our result, showing that most of the nanoplastics was found in the sediment of the lake section is in agreement with what was shown by He et al. (2022) and Ockenden et al. (2024), also working with metal-doped nanoplastics. Interestingly, a recent study analysing the fate of microplastics of different size and densities, performed at a mesocosm scale, found that most microplastics ended up in a surface slick and on the bottom (Rochman et al., 2024). These collective results indicate that the current reported concentrations of nanoplastics in freshwater systems, which are analysed in water samples, are not considering the likely much higher concentrations that are associated with the sediments. Moreover, these results evidence that the sediment compartment of lakes could be an important reservoir for micro- and nanoplastics. Therefore, the risks for the benthic organisms and other processes should be more extensively evaluated. We still know very little on how these increasing inputs of plastic-carbon exported to the sediments might impact the communities of decomposers, the carbon fluxes (Stubbins et al., 2021), the oxygen levels in the benthos, or what would happen when sediment resuspension make these pollutants available again to the water column.

Regarding the mesocosm area with sediment and macrophytes, we found nanoplastics in both macrophytes roots and leaves, with higher values in the roots. This result could reflect uptake and incorporation rates, since roots are directly exposed to the water and the sediments, whereas it may take longer for the nanoplastics to be incorporated in leaves. On the other hand, the concentration of nanoplastics in the macrophytes was negatively related with the distance from the point of addition (Fig. 8 C). This could be because, as the water moves through the mesocosm, the nanoplastics are hetero-aggregated or taken up by the biota, and consequently the nanoplastic concentration in water decreases.

In our study, sediment samples were collected and analysed including the biofilm/periphyton layer that could be on top. It has been shown that biofilms and periphyton effectively incorporate nanoplastics (Holzer et al., 2022; Ockenden et al., 2024). Therefore, besides the analysed sediment, the invertebrates and the macrophytes, it is likely that, the biofilm was also responsible for the nanoplastic retention in our wetland mesocosms.

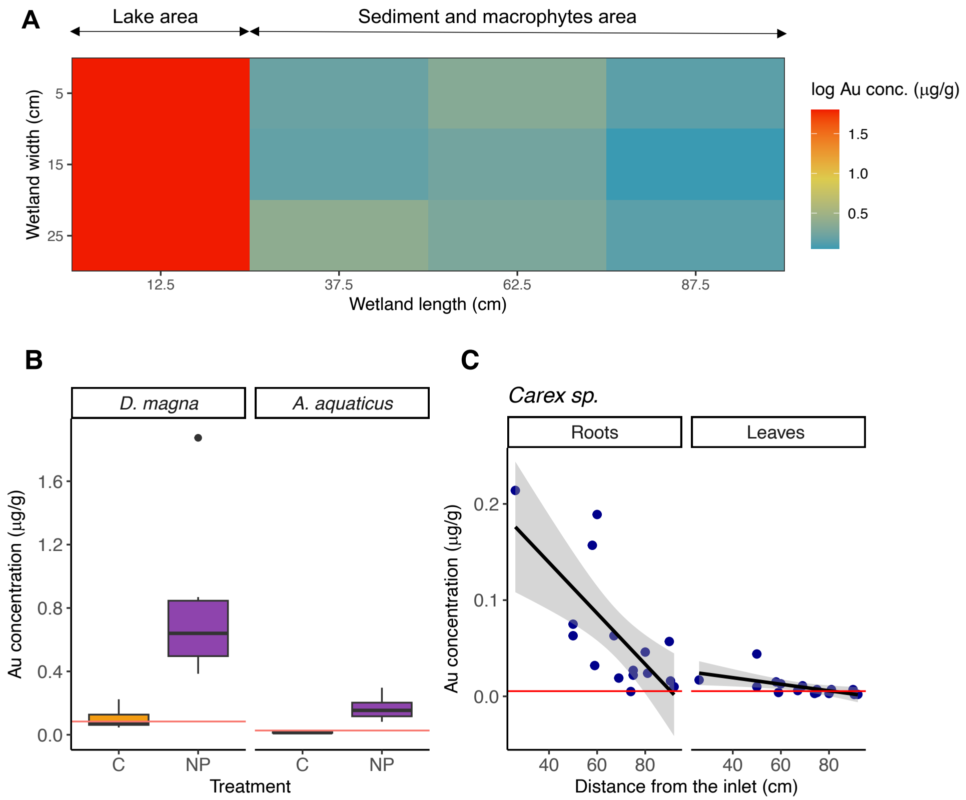

Figure 8. Nanoplastic concentration, measured through the concentration of Au, our proxy, in different environmental compartmentes of the wetland mesocosms at the end of the experiment. (A) Average nanoplastic concentration (µg Au/g dry weight, N=6) measured in sediment samples taken along the nanoplastic exposed wetland mesocosms at the end of the experiment. For the lake area, one representative sediment sample was taken from the bottom of the lake on each nanoplastic exposed wetland. **(B)** Nanoplastic concentration (µg Au/g dry weight, N=12), on the pelagic invertebrate, *Daphnia magna*, and the benthic invertebrate, *Asellus aquaticus*. The invertebrates were collected at the end of the experiment from the lake area of control, not exposed (C, in orange), or nanoplastic exposed (NP, in violet), wetland mesocosms. The boxplot shows the median as a black horizontal line, the first and third quartile with the lower and upper hinges, the extreme values within 1.5× the interquartile range with the whiskers, and data beyond the end of the whiskers are plotted individually. **(C)** Nanoplastic concentration (µg Au/g dry weight) in roots and leaves of the macrophyte *Carex sp.* in relation to the distance from the inlet, where nanoplastics were added. Data is shown for samples taken only from nanoplastic exposed wetlands. The horizontal red line in plots **B & C** indicate the limit of detection of the analytical technique (ICP-MS). In all cases, when samples had a reported Au concentration below the limit of detection of the analytical technique, the value was referred to as the limit of detection (0.02 µg Au/g dry weight for sediments, 0.084 µg Au/g dry weight for *D. magna*, 0.027 µg Au/g dry weight for *A. aquaticus*, and 0.005 µg Au/g dry weight for *Carex sp.*). Figures rearranged from **Paper II**.

An interesting result was that, although at the end of the experiment most of the nanoplastics were in the sediment of the lake area, a significantly higher uptake per unit mass was observed for *D. magna* than for *A. aquaticus* (Fig. 8 B). However, it is important to highlight that, at the end of the experiment the biomass of *A.*

aquaticus was higher in comparison with *D. magna*, and when considering the biomass of invertebrates, the nanoplastic accumulation was similar between the two species. In any case, the higher uptake per unit mass in *D. magna* could be explained due to the exposure route of the nanoplastics, through the lake water, and the feeding behaviour of *D. magna*. The planktonic filter feeder, *D. magna*, has a high filtration capacity (McMahon and Rigler, 1965), which directly expose this organism to the particles in suspension. Moreover, *D. magna* can feed on periphyton as an alternative food source (Siehoff et al., 2009), therefore incorporating nanoplastics through both food sources. On the other hand, the benthic detritivore shredder, *A. aquaticus*, can feed on leaf litter but also on microorganisms that colonise the substrate (i.e., biofilms) (Lafuente et al., 2021). It is possible that in the wetland mesocosm, the higher biomass of *A. aquaticus* "diluted" the nanoplastic exposure. Detoxification rates could also be different between the invertebrate species, but these were not assessed in our study.

Finally, it would be interesting if future studies could evaluate other types of wetland sediments, since other types of sediments could have different efficiencies in nanoplastic retention. In this context, it is important to highlight that in our study, the design of the wetland mesocosms allowed the water to move mostly superficially through the sediment area with macrophytes. Studies that explored nanoplastic transport using columns, (for example as in Pulido-Reyes et al. (2022) or Pradel et al. (2020)), were the aqueous media moved completely through the sediment showed that different filtration media have different retention capacity, and that the presence of a biofilm on the surface of the porous media also influenced the result (Pulido-Reyes et al., 2022).

Which are the effects of the exposure to nanoplastics in freshwater ecosystems?

In **Paper II,** we explored the fate of nanoplastics in freshwater ecosystems, including the biological uptake. In **Paper III**, I together with the co-authors, aimed to study the effects of nanoplastics using a similar setup, again freshwater wetland mesocosms were used, but this time exposed to different concentration of pure plastic (amine-modified PS) nanoparticles, previously proven toxic in small-scale laboratory experiments (Kelpsiene et al., 2020; Mattsson et al., 2017). We exposed the mesocosms during 10 weeks to weekly additions of different concentrations of nanoplastics and assessed their effects on the abundance of different organisms and the leaf decomposition rate. The wetland mesocosms were assigned to four experimental groups: a control group (C, 0 µg PS/L, N=3), not exposed to nanoplastics, and three groups with increasing concentration of nanoplastics, named as “low” (L, 21.41 µg PS/L, N=3), “medium” (M, 214.1 µg PS/L, N=3), and “high” (H, 2141 µg PS/L, N=3) concentrations.

In **Paper III**, we were able to determine a range of nanoplastic concentration that is harmful for *D. magna* (between 214 and 2141 µg/L, Fig. 9) in wetland mesocosms conditions, which are more realistic conditions than simplified experimental setups. *D. magna* population was extinct at the high nanoplastic concentration treatment and affected at the medium (Fig. 9). This species has been shown to be susceptible to the same PS nanoparticles at the laboratory scale (Kelpsiene et al., 2020) and possible explanations for the toxicity are intestine protein depletion and tissue rupture (Kelpsiene et al., 2022). On the other hand, the cyclopoid copepods were unaffected by the tested concentrations of nanoplastics. Cyclopoid copepods have a highly selective predatory feeding (Brandl, 1998) which could have exposed them less to the nanoplastics in suspension, in comparison with the filter feeder *D. magna*.

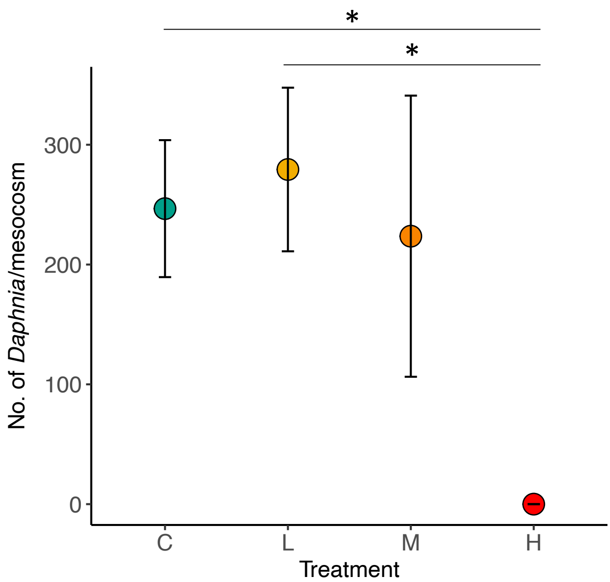

Figure 9. Abundance of *Daphnia magna* in the wetland mesocsosms after being exposed, or not, to nanoplastics during 73 days.
Number of *D. magna* per mesocosm (mean ± SE) at the end of the experiment in the different treatment groups: control (C, 0 µg PS/L, N=3), low (L, 21.41 µg PS/L, N=3), medium (M, 214.1 µg PS/L, N=3) and high (H, 2141 µg PS/L, N=3). Asterisks indicate significant statistical differences. Figure redrawn from **Paper III**.

Furthermore, we found that the negative effects on *D. magna* had consequences at the phytoplankton community with potential consequences for the food web structure in the long term. The extinction of *D. magna* in the highest nanoplastic concentration treatment (2141 µg PS/L) likely allowed the increase in abundance of cyanobacteria and cryptophytes, who seemed unaffected by nanoplastic exposure.

Conversely, nanoplastics negatively affected the abundance of diatoms (**Paper III**). The shift in the composition of phytoplankton towards cyanobacteria and cryptophytes as concentration of nanoplastics increased is particularly worrying. Cyanobacteria can benefit from other anthropogenically induced changes in freshwater ecosystems, as eutrophication and climate warming (Kosten et al., 2012), causing blooms which have negative impacts on water quality and the ecosystem (Huisman et al., 2018).

Regarding the benthic community, the abundance of *Asellus aquaticus*, Chironomids and worms was not affected by any of the exposure concentrations, and neither the leaf-litter decomposition rate. The absence of effects in the abundance of the benthic invertebrates is surprising considering that most nanoplastics would end on the sediment (**Paper II**). It is possible that the time scale assessed in our study was too short to give rise to effects on organism abundance at the benthos. For example, Redondo-Hasselerharm et al. (2020) found changes in the benthic community composition in the long-term, after being exposed to micro- and nanoplastics for 15 months. On the other hand, *A. aquaticus* can be resilient to high levels of organic and chemical pollution (Lafuente et al., 2021), while other benthic species have been shown to be negatively affected by nanoplastics, as caddisfly larvae (Ockenden et al., 2024). Caddisfly larvae were likely highly exposed through their feeding on the biofilm which retained nanoplastics (Ockenden et al., 2024).

In **Paper III**, leaf litter decomposition rates were not affected by the nanoplastics exposure, however, this effect has been found by other studies performed in stream water (Du et al., 2022; Seena et al., 2022). In any case, differences in the methodology used by the studies make it difficult to compare the results, as the different water sources used, or the different species used as leaf litter, could contribute to the different results found.

Finally, our wetland mesocosms in both **Paper II** and **III** did not include fish. It has been shown that fish can accumulate nanoplastics that are exposed through the trophic web (Mattsson et al., 2017). On the other hand, the absence of *D. magna* observed in **Paper III** would have impacts on higher trophic levels, by affecting the food availability for fish. It would be interesting if future studies can be expanded to also include this important component of aquatic trophic webs.

How do nanoplastics interact with phytoplankton species, and what are the effects on their growth and morphology?

In **Paper III**, we found that nanoplastics affect some phytoplankton groups differently. Furthermore, a recent meta-analysis found that changes in freshwater phytoplankton community composition can be expected under nanoplastic pollution scenarios (Guo et al., 2024) due to the differential effects they pose on different phytoplankton species. In **Paper IV**, I together with the co-authors, aimed to understand the responses of two phytoplankton species to nanoplastics' exposure, and explore possible mechanisms behind those responses.

In **Paper IV**, we assessed the growth and morphology of the two species throughout their growth period. We found that nanoplastics did not negatively affect the growth of the cyanobacteria, *D. flos-aquae*, during the exponential phase but did affect the length of the stationary phase for cultures exposed to low and medium nanoplastic concentrations (0.45 and 4.5 mg PS/L, respectively). Furthermore, cultures under a high concentration of nanoplastics (45 mg PS/L) did not reach as high cell numbers as the other treatment groups. In contrast, nanoplastics did affect the growth of the green algae, *T. obliquus*, during the exponential phase. Interestingly, the two species showed contrasting responses regarding the group formation when exposed to nanoplastics: cyanobacteria had a higher proportion of cells in groups during the exponential phase when exposed to the highest concentration of nanoplastics (45 mg PS/L) (Fig. 10 A). For the green algae, the opposite pattern was observed: the formation of groups followed a dose-response pattern and was lower in the presence of nanoplastics (Fig. 10 B).

Moreover, the interaction between the phytoplankton cells and the nanoplastics was studied at the cellular level using transmission electron microscopy (TEM), in cultures exposed or un-exposed to nanoplastics. For doing this, gold-doped PS nanoparticles were used, as in **Paper II**. Gold is an electron dense metal and can aid during the identification of nanoplastics under TEM (the core is clearly visible, Fig. 6) and the complex cellular ultrastructure background. We found nanoplastics attached to the cell wall of the green algae, which seemed damaged. However, we did not find nanoplastics in direct contact with the cells of the cyanobacteria.

To sum up, these results indicate that nanoplastics differently affect these two species of phytoplankton: they interact different at the cellular level and have differential effects in their growth and morphology.

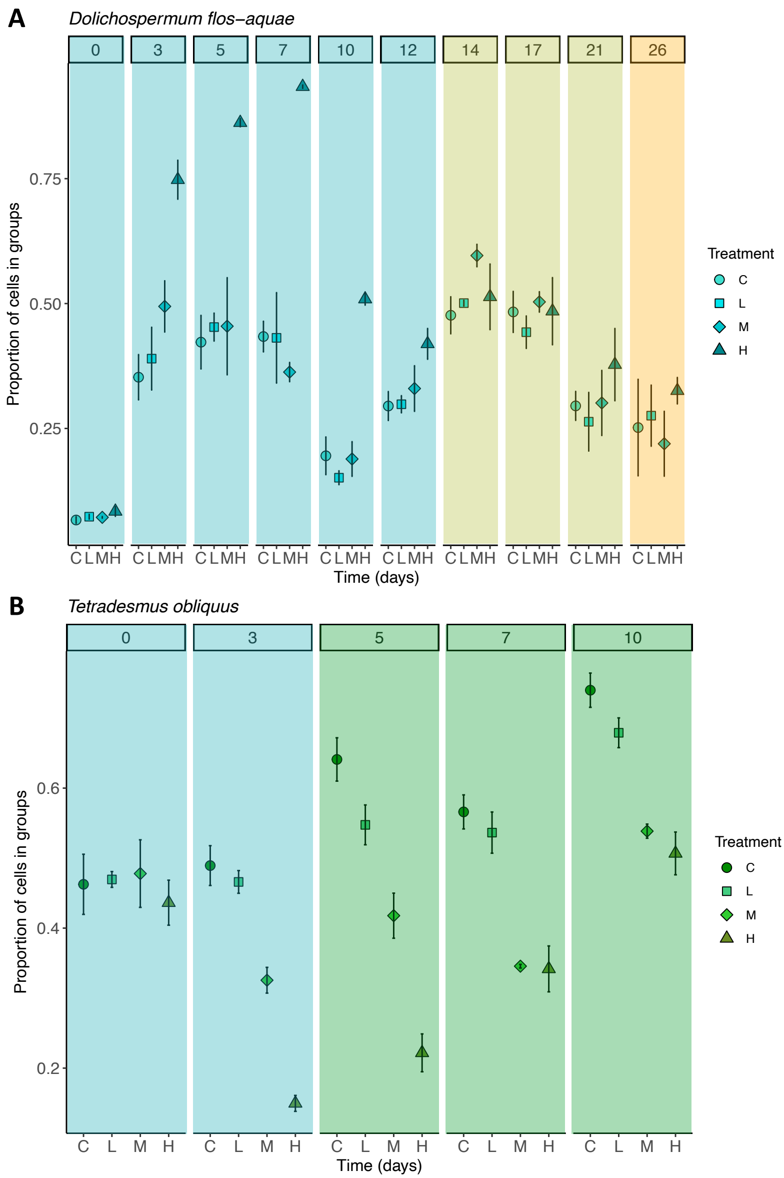

Figure 10. Algae morphology assesed as the proportion of cells in groups during the experimental time when being exposed, or not, to nanoplastics.

The proportion of cells in groups (3 or more cells together) was assessed under different nanoplastic exposure conditions along the experimental time (26 days) on two phytoplankton species: the cyanobacteria, *Dolichospermum flos-aquae* **(A)**, and the green algae, *Tetradesmus obliquus* **(B)**. The different treatments were: control (C, 0 mg PS/L, circles), low (L, 0.45 mg PS/L, squares), medium (M, 4.5 mg PS/L, diamonds) and high (H, 45 mg PS/L, triangles). Blue, green and orange colours in **A** indicate the exponential, stationary and decline phases in the growth of the cyanobacteria, respectively. Blue and green colours in **B** indicate the exponential and declining growth phases in the green algae, respectively. Figures rearranged from **Paper IV**.

General comments and future remarks

The experiments in this thesis were performed using model PS nanoplastics, which were mostly spherical and where most of the particles share similar characteristics in polymer composition, shape, and size. The use of these model particles was needed and beneficial, since it was possible to track them using their gold core (**Paper II**) and study nanoplastic effects (**Paper III**) in complex scenarios as the wetland mesocosms. Including a high particle heterogeneity in a mesocosm study for the first time likely would make the interpretation of results difficult. Furthermore, the gold doped nanoplastics aided in the identification of particles under TEM (**Paper IV**). However, it is important to highlight that model plastic particles do not necessarily represent the type of plastics that are most found in nature. In freshwater ecosystems, the microplastic shapes most commonly found are fibers and fragments (Dusaucy et al., 2021; Nava et al., 2023), and most of the polymers are identified as PE and PP (Dusaucy et al., 2021), and also polyester (Nava et al., 2023). Field studies on nanoplastics occurrence in freshwaters are scarcer, but here also PE and PP were found as the most common polymers (Materić et al., 2022b), with no information regarding shape due to the analytical methods used. This mismatch between natural ecosystems and lab studies have been previously pointed out (see Kukkola et al. (2021)). Very recently, studies including different polymers, sizes, and shapes of microplastics started to provide knowledge regarding their transport, fate and effects on realistic scenarios (Langenfeld et al., 2024; Parrella et al., 2024; Rochman et al., 2024). Regarding nanoplastics, studies analysing nanoplastics created during the breakdown of plastic products started to be performed. For example, a study addressing the effects on *D. magna* of mechanically breakdown nanoplastics of high density PE found different effects depending on the size fractionation, with the more toxic effects found in the fraction containing oligomers and plastic leachates (Ekvall et al., 2022). In future studies it would be interesting to evaluate the effects of breakdown nanoplastics in the experimental mesocosms. This would allow to compare the results using different type of nanoplastics, and more realistic ones, in a similar setup.

Further, the whole field of nanoplastic pollution research needs well-designed controls for the plastic particle additions to experiments, as it is often hard to separate whether the observed effects are caused by the nano-size of the particle irrespective of its chemical composition (see recent method proposed by de Ruijter et al. (2025)), or by chemical and/or surface-physical properties of the intact material, or by leachates. This also concerns the present studies on this thesis and is worth considering in future experiments.

In nature, degradation of plastics is primarily triggered by UV radiation and visible light (Pickett et al., 2008). The interaction between UV light and nanoplastics was not explored in this thesis, nevertheless it is relevant, because nanoplastics toxicity can change in UV exposed nanoplastics (also called aged nanoplastics). Very recently, it was shown that the toxicity to *D. magna* of 53 nm amine-modified PS particles (the same nanoplastics as used in **Paper III)**, was reduced after the nanoplastics were exposed to UV-B (Ekvall et al., 2025). Nevertheless, the study also found that during UV-B induced degradation, new toxic substances are released. On the other hand, a previous study found a reduced cellular internalization of UV aged PS nanoplastics compared to pristine nanoplastics, reducing, as a consequence, the cytotoxic effect (Wen et al., 2022). Although in **Paper IV** we did not find evidence for nanoplastic internalization, it would be interesting to include, in a future study, an additional treatment group of UV aged nanoplastics (which could be the same gold-doped nanoplastics used in **Paper IV**), and assess if the nanoplastic effects and interaction with the phytoplankton species remain the same.

As found in **Paper II**, the highest concentration of nanoplastics trapped in the sediments of the aquatic compartment evidenced that this environmental compartment can be a trap for nanoplastics, but knowledge regarding the effects on the benthic communities and their processes are still scarce. To my knowledge, no studies have evaluated how sediment resuspension make nanoplastics trapped in the sediment available again on the water column, and if the toxicity of these weathered resuspended particles differs. Future studies analysing all these aspects are needed.

The results of this thesis evidenced, in **Paper I**, that *D. magna* changes its behaviour depending on how the stressor is delivered: as a constant exposure or repeatedly fluctuating. Moreover, I found that the organisms exposed to the fluctuating UV radiation reproduced less, which indicates a cost to the organism due to the response to the fluctuating environment. This shows that the repeated vertical movements that *D. magna* performs daily imply a cost, something that has been debated (Hansson et al., 2016).

On the other hand, in **Paper II** it was determined that nanoplastics can be retained by freshwater wetland mesocosms, being mostly accumulated in sediments of the aquatic compartment, but also in organisms as *D. magna*, *Asellus aquaticus* and macrophytes (**Paper II**). *D. magna*, which was the organism that accumulated more nanoplastics in relation to its mass (**Paper II**), was extinct when nanoplastics concentrations in the wetland mesocosms were higher than 2 mg PS/L (**Paper III**). However, effects were not found in *A. aquaticus* or in any of the benthic species or processes studied (**Paper III**). The combination of nanoplastics exposure and the absence of *D. magna* caused by the nanoplastics, changed the community composition of phytoplankton, favouring cyanobacteria over diatoms (**Paper III**). In **Paper III**, the different susceptibilities to nanoplastics of some phytoplankton groups were evidenced, and **Paper IV** aimed to explore this in more detail on two phytoplankton species.

The results on **Paper IV** showed that nanoplastic particles differently affect the two species of phytoplankton. First, they interact different at the cellular level. As detailed in the methodology section, the cell wall structure of cyanobacteria and green algae is different. Future studies can further explore the surface charge on these cells, for example, measuring the zeta potential, to determine if that could be a possible explanation to this result. Second, nanoplastics affected different phases of the growth of these species. It would be interesting to evaluate these results in a community context, because the growth capacity of a population could determine the dominance of certain species on the phytoplankton community (Reynolds, 2006). Third, nanoplastics influenced the group formation differently in these species. The size of the colony or coenobium will likely determine where in the water column the organisms are located (Lürling, 2003), because larger groups tend to sink in the water column unless other mechanisms (as some cyanobacteria have (Reynolds, 2006)) allow them to stay at the surface. Moreover, the size of the group will determine their risk of predation, depending also on the identity of the predator (Colina et al., 2016; Lürling, 2003). It has been shown that Cladocera, as *D. magna,* predated more efficiently on certain phytoplankton groups (Colina et al., 2016), structuring the community. Finally, in **Paper III** the differential effects of nanoplastics and grazing on the phytoplankton community were distinguished. Considering the results from **Paper III** and **IV**, it would be interesting to test how experimental phytoplankton communities are affected by both factors (nanoplastics and *D. magna* grazing) but manipulating *D. magna* grazing pressure in a more controlled way, and considering the morphology of the phytoplankton, such as the group formation.

Although this was not explored in the thesis, the effects of nanoplastics can act in an additive or synergistic way with other current stressors. Combining and comparing the two stressors examined in this thesis, UV radiation exposure and nanoplastic pollution, would be very interesting from the perspective of adaptation. The first one is an ancient pressure that has been part of the evolutionary history of aquatic organisms, while the other is a very novel phenomenon. However, to make general conclusions on the effects of ancient and novel stressors, the stressors themselves need replication, i.e., several stressors of each type should be tested.

Even more relevant, complex stress agents as those combined through climate change, or eutrophication, are pressing even more on freshwater ecosystems and negatively affecting their functioning. It would be interesting to study different stressors in combination and examine interactions of their effects. Experimental settings will however inevitably become very large through the required multifactorial combinations, and the development of well-designed experimental approaches is needed (Pirotta et al., 2022; Rillig et al., 2023). It has been shown that plastic pollution impacts the Earth system, with interactive and amplifying negative impacts between plastic pollution and other processes, as climate and freshwater change, collectively threatening Earth system stability (Villarrubia-Gómez et al., 2024). Plastic pollution is much more than a waste management problem. It is a socio-environmental issue that needs a trans- and interdisciplinary approach to be studied and effectively tackled, with the focus on the full life-cycle of plastics, including the production (Bergmann et al., 2022; Thompson et al., 2024; Villarrubia-Gómez et al., 2024). New regulations but also major social changes are needed to really change the current trend of increasing plastic pollution.

Conclusions

In this thesis, I explored how organisms respond to a natural stressor, as UV radiation, or an anthropogenic stressor, as nanoplastics. Moreover, I investigated the fate of nanoplastics in freshwater wetland mesocosms, and nanoplastic effects on freshwater communities and ecosystem processes as the rate of organic matter decomposition.

In **Paper I**, I found that the exposure to a fluctuating UV radiation environment has a fitness cost for the zooplankter *Daphnia magna*, and this cost is likely due to the repeated behavioural avoidance response to the fluctuating UV radiation. This evidenced that the repeated vertical movements that *D. magna* daily performs can imply a cost, a notion that has been debated.

In **Paper II**, I determined that wetland mesocosms can retain nanoplastics. I performed this using gold-doped PS nanoparticles, with a negative surface charge, which were tracked in the system using standard methods for trace metal analysis. In the wetland mesocosms, most nanoplastics ended up in the sediment of the aquatic compartment besides being taken up also by the aquatic filter feeder *D. magna*, the benthic detritivore *Asellus aquaticus*, and macrophyte species as *Juncus* sp. and *Carex* sp. The first experimental evidences of nanoplastic distributions in more realistic aquatic environments are all very recent (He et al., 2022; Ockenden et al., 2024; Stábile et al., 2024), and include the work done on **Paper II** of this thesis.

Furthermore, in **Paper III**, also using wetland mesocosms, I found that nanoplastics (this time, amine-modified PS nanoparticles) caused the extinction of *D. magna* when concentrations were above 2 mg of PS/L. Likely related with this result, *D. magna*, was the organism that accumulated more nanoplastics in relation to its mass (**Paper II**). In addition, the combination of nanoplastics exposure and the absence of *D. magna* caused by the nanoplastics, changed the community composition of phytoplankton, favouring cyanobacteria and cryptophytes over diatoms (**Paper III**). On the other hand, nanoplastic effects were not found in *A. aquaticus* or in any of the benthic species and the ecosystem process studied (**Paper III**).

Finally, in **Paper IV**, in a laboratory scale experiment, and using similar gold-doped nanoplastics as in **Paper II**, I observed that nanoplastics directly interact with the cell wall of the green algae *Tetradesmus obliquus*, but this was not observed for the cyanobacteria *Dolichospermum flos-aquae*. This could explain the differential effects found in the growth of these species, and the contrasting responses in group formation. While the cyanobacteria exposed to a high concentration of nanoplastics (45 mg PS/L) got more colonial, the green-algae showed a reduced group-formation when exposed to nanoplastics, following a dose-response.

As the results of this thesis indicate, and as shown by others, the organisms' responses to stressors, either natural or anthropogenic, can affect how communities are structured, which ultimate can affect ecosystem function. As shown in **Paper III**, through the direct effect of nanoplastics on the extinction of the key zooplankton species *D. magna*, nanoplastics change the grazing pressure on the phytoplankton community. This indirect effect, together with the different susceptibilities of different phytoplankton species to nanoplastics exposure (as evidenced in **Paper III** and **IV**), can change phytoplankton community structure.

Overall, this thesis analysed the responses of different organisms to both natural and anthropogenic stressors. Despite long-standing adaptations, organisms still face costs associated with their response to natural stressors, as UV radiation. The impacts that humans are causing on natural ecosystems through plastic pollution also press organisms' natural populations. Further, these pollutants can cause shifts on the ecosystem that might be irreversible and harmful to the whole freshwater ecosystem, and to us humans. Therefore, the current trend of increasing plastic pollution is problematic, and actions against plastic pollution should be taken to preserve freshwater ecosystem integrity. New regulations based on scientific knowledge, but also major social changes, are needed to change this path.

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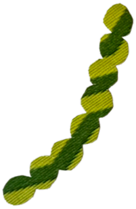
List of Papers

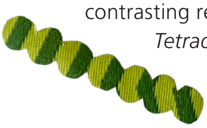


I. Stábile F., Brönmark C., Hansson L.A. & Lee M. (2021) Fitness cost from fluctuating ultraviolet radiation in *Daphnia magna*. *Biology Letters*, 17, 8, 20210261. https://doi.org/10.1098/rsbl.2021.0261.

II. Stábile F., Ekvall M.T., Gallego-Urrea J.A., Nwachukwu T., W.G. Soorasena C.U., Rivas-Comerlati P.I. & Hansson L.A. (2024) Fate and biological uptake of polystyrene nanoparticles in freshwater wetland ecosystems. *Environmental Science: Nano*, 11, 3475–3486. https://doi.org/10.1039/D3EN00628J

III. Ekvall M.T, Stábile F. & Hansson L.A. (2024) Nanoplastics rewire freshwater food webs. *Communications Earth & Environment*, 5, 486. https://doi.org/10.1038/s43247-024-01646-7.

IV. Stábile F., Ekvall M.T., Sjöstedt J. & Hammer E. Unity is strength: contrasting responses in group formation between the green algae *Tetradesmus obliquus* and the cyanobacteria *Dolichospermum flos-aquae* when exposed to nanoplastics. Manuscript.



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