Article

The Role of Microplastics on Marine Pathogen Transmission: Retrospective Regression Analysis, Experimental Design, and Disease Modelling

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Abstract: Marine wildlife and aquaculture species can accumulate large amounts of microplastic particles (<1 mm), threatening the health of marine populations and ecosystems and posing a risk to food safety and human health. The uptake of chemicals from microplastics seems to decrease the immune capacity of bivalves and corals to fight pathogenic bacteria, thereby increasing their vulnerability to disease. Moreover, major pathogens of bivalves, fish, and humans, including several *Vibrio* species, have been shown to be specifically enriched in the microbial communities adhered to marine microplastic debris (MMD). Microplastics can therefore serve as an important vector for and regulator of pathogen transmission and disease dynamics. Here, we outline a theoretical, three-perspective approach for studying the relationship between MMD and disease. First, we provide a framework for retrospective analysis of MMD and pathogen loads in marine animal tissues to assess the relationships between them, their bioaccumulation over time, and their relationship to other environmental variables. The results from such an analysis can be used to decide whether a compound or pathogen should be considered an emerging substance or organism. Second, we describe an experimental design for testing the effect of a variety of microplastics on *in vivo* pathogen removal (i.e., the phagocytic activity of hemocytes) and infection intensity in two study model species (oysters and zebrafish). Finally, we create a theoretical susceptible-infected microplastic particle and pathogen transmission model for bivalves and fish. Overall, the experiments and models we propose will pave the way for future research designed to assess the role of MMD as a vector for marine and human pathogens. This multi-faceted approach needs to be an urgent priority of the EU Strategic Research Innovation Agenda for addressing marine disease challenges related to MMD.

Keywords: microplastics; pathogens; disease modelling; transmission

1. Introduction

Marine microplastic debris (MMD; plastic particles <1 mm in diameter) is an emerging, human-induced threat to the world's seas and oceans [1]. Annual plastic production continues to rise [2,3], and the continued degradation of larger plastic items [4] further increases the abundance of MMD and therefore the risk of wildlife being exposed to it [5]. Given the small size of microplastics, organisms from diverse trophic levels are capable

 doi:10.20944/preprints202210.0442.v1

of ingesting and accumulating these particles. In the marine food web, microplastics can be found in organisms ranging from zooplankton[6] to fish [7,8], including large pelagic fish [9] and whales [10]. The bioaccumulation of MMD is an emerging risk to the health of marine ecosystems, and, in turn, to food safety and human health [11–13]. Marine invertebrate filter-feeders such as bivalves [12,14,15] are particularly susceptible to MMD accumulation because they process large amounts of water while feeding [16].

In the last decade, large-scale policy recommendations and government-sponsored programs have increased public awareness of marine MMD. At the same time, most scientific investigations have primarily focused on the distribution of MMD in seas and oceans [17,18], its presence in diverse organisms, and its toxicology [19–21]. Nevertheless, little is known about the virulence and disease dynamics of MMD and a comprehensive risk assesment is still far away for marine ecosystems, food safety, and public health [22]. We believe that exploring this knowledge gap should be an important component of future MMD research. In other words, across the diversity of marine biota from zooplankton to bivalves and fish, what is the role of MMD in the transmission of marine and human pathogens?

Answering this broad question requires research on how MMD contributes to the emergence of marine diseases. Marine diseases may emerge as a result of novel introductions, climate change, changes in vector populations, and the introduction of novel vectors. The assessment and management of future disease risks depends on understanding the causes of historical and contemporary disease emergence events [23]. However, because MMD is a newly recognized form of environmental pollution, there is little information on the historical prevalence of MMD. Indeed, MMD monitoring programs were non-existent until recently [24,25], mostly due to the lack of methods for routine MMD quantification [26]. Researchers have recently attempted to quantify MMD in samples collected for zooplankton analysis; the results appear to be promising and could therefore provide low-cost methods for data collection on MMD in the water column [27].

Data from peer-reviewed literature and publicly available repositories, as well as newly emerging data sets, suggest that the abundance and mass of MMD in the North Pacific Subtropical Gyre increased by two orders of magnitude during the period from 1972 to 1987 and again between 1999 and 2010 [28]. Furthermore, North Atlantic and North Sea surface samples collected by a continuous plankton recorder suggest that the frequency with which MMD is encountered during surveys has been steadily increasing alongside the global increase in plastic production [29]. However, researchers have not yet confirmed a corresponding global increase in MMD concentrations in marine organisms.

One approach to obtaining such confirmation would be performing a retrospective study on the occurrence of MMD in biological samples from environmental specimen banks. Retrospective monitoring using archived samples from specimen banks can provide information on past and current trends in the exposure to and consumption of MMD by marine organisms as well as on the prevalence of major pathogens. Such a study would allow for a better evaluation not only of the concrete threat posed by MMD and major pathogens but also of the effect of MMD on disease ecology.

In addition, the role of MMD in the transmission of marine pathogens needs to be addressed by conducting experimental studies that explore both microplastic uptake by different organisms and disease transmission among those same organisms, based on the understanding that microplastics can be carriers of chemicals and pathogens. For example, persistent chronic pollution has been linked to pathological alterations in bivalves and a higher prevalence and intensity of parasites, including *Rickettsia/Chlamydia*-like organisms (R/CLO), in shellfish [30]. Indeed, the uptake of chemicals from ingested MMD has been suggested to decrease the capacity of bivalves to fight off pathogenic bacteria [31]. Microplastic exposure also activates stress responses and suppresses immune function in corals [32,33].

 doi:10.20944/preprints202210.0442.v1

Diverse pathogens have been found in the ocean's plastisphere, which is the microbial community that adheres to microplastics. This microbiome is distinct from the surrounding seawater and can include humans, fish, and bivalve pathogens [34–36]. In laboratory experiments, plastisphere microbial communities form biofilms on poly-vinyl chloride surfaces during lab experiments [34]. The plastic type and particle size affects not only the biofilm formation rate [37] but also the rate at which marine organisms ingest and accumulate the plastic [12] as well the plastic's toxicity [38]. Some of the pathogens within these plastisphere biofilms, including various *Vibrio* species, are extremely virulent. For example, *Vibrio cholerae* can cause cholera when they enter humans through ingested seafood [39], and *V. anguillarum* has been particularly devastating to salmonid populations [40]. *V. parahaemolyticus* causes vibriosis in marine bivalves and fish worldwide as well as sepsis, gastroenteritis, and wound infections in humans [41]. Other human pathogens, including *V. alginolyticus* and *V. vulnificus*, have been found in fish [42] and shellfish samples [43].

These pathogens can be trophically transmitted even if the MMD itself is not. For example, *Vibrio* pathogens have caused extensive epizootics and mass mortalities of oysters [44]. Recent laboratory experiments suggest that MMD does not bioaccumulate in oyster tissues in the short term; however, the microorganisms assimilated via the ingestion of biofilm-coated MMD do seem to be transferred to higher trophic levels and have potential infectious capacity [45]. The transmission of MMD-carried pathogens poses a serious risk to wildlife, food safety, and human health. Understanding the relationship between MMD and bacterial pathogens in commercially harvested bivalve species is particularly important for determining the risks MMD poses to marine ecosystems and human health because (i) several marine pathogens have caused mass mortality events in bivalves [e.g. 44], and (ii) the accumulation of human pathogens in edible bivalve species poses seafood-related health risks to human consumers [46].

Based on these concerns, we have identified three parallel, interconnected, and urgent research objectives for better understanding the role of MMD in the transmission of marine pathogens. First, researchers must explore the past and expected future trends of both MMD exposure and pathogen occurrence in marine ecosystems. Second, field and laboratory experiments should be conducted to determine the effect of the MMD-pathogen interaction on disease transmission. Finally, researchers should develop the quantitative and theoretical basis for modeling disease processes associated with MMD ingestion in marine organisms to better understand the epizootiology of these 'vector-borne' disease. Here, we describe some useful experimental, statistical, and disease modelling methods that can be used to address these three research objectives. We also present some theoretical results that we discussed in relation to the potential mechanisms by which MMD ingestion affects pathogen transmission in marine organisms.

2. Materials and Methods

In this study, we describe the three key approaches outlined above for studying the role of MMD on marine disease transmission: (i) a retrospective analysis of the interaction between MMD and disease in the context of other environmental variables; (ii) an experimental design for studying the uptake of MMD-carrying pathogens by marine organisms and the associated effects on disease transmission; and (iii) a quantitative disease transmission model parameterized by data from retrospective analyses, experiments, and previously published work.

2.1. Retrospective analysis

Data on historical MMD concentrations and pathogen loads in bivalves, as well as data on other environmental parameters (e.g., temperature), can be used to determine the environmental factors that facilitate and limit the exposure of filter feeders to MMD and

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pathogens. In this context, the environmental parameters act as inputs in multivariate regression models predicting MMD or pathogen load.

In a general way, a regression model describes the relationship between a response variable, Y, and some explanatory variables, X = ($X_1, \ldots, X_p$). The explanatory variables are also known as covariates. Such a multivariate model is defined as:

$$Y = m(X) + \varepsilon$$

where $m(\cdot)$ is the mean function and ε is the regression error.

The simplest form of regression analysis is a linear regression, which serves as a good jumping-off point for newer or advanced modeling approaches that generalize or extend this method. In a linear regression, the response variable is assumed to follow a normal distribution, and the effect of the covariates on the response is assumed to be linear. The problem with this simple model is that, in most real-world contexts, including in the study of MMD prevalence, the response variable is not normally distributed. Instead, the response variable might follow a discrete distribution, such as the Poisson. For these situations, generalized linear models (GLMs) [47,48] extend simple linear ones by allowing the use of other distribution families to model the response variable. In a GLM, the relationship between the mean response and the covariates is modeled by:

$$E[Y|\mathbf{X}] = \eta(\beta_0 + \beta_1 X_1 + \ldots + \beta_p X_p),$$

where $\eta(\cdot)$ is a known monotonic function (the inverse of the link function). Once the distribution of the response variable has been determined, we must also determine whether the effect of the covariates on the response is linear. Although simple and generalized linear models have been widely used, their parametric assumption of linear effects is very restrictive and, in certain circumstances, not supported by the data. If the parametric model is inappropriate for the data, the conclusions from the model will be erroneous. In this case, nonparametric regression techniques can be used to model the dependence between Y and X without needing to specify in advance the function that links the covariates to the response. This family of models is called generalized additive models (GAMs) [49] and is defined by:

$$E[Y|\mathbf{X}] = \eta(\alpha + f_1(X_1) + \ldots + f_p(X_p)), \quad (1)$$

where $\eta(\cdot)$ is a known monotonic function (the inverse of link function) and $f_1, \ldots, and f_p$ are smooth, unknown, continuous functions. A large body of literature has been devoted to finding techniques for estimating the regression model in equation 1. Two of most widely used approaches are splines [50,51] and kernel smoothers [52,53]. Spline smoothing involves modeling a regression function as a piecewise polynomial, where the number of pieces is relatively high compared to the sample size. The performance of this technique is governed by the number and position of knots used to calculate the estimator. Despite considerable research effort [54], the difficult problem of knot selection has not been totally solved. Our continued research on the topic of marine microplastics includes the development of a new methodology that will allow us to estimate any type of unknown curve, compare the results with other existing estimation procedures, and use simulations to study the performance of our method in a finite sample.

Another option for fitting GAMs is local regression based on kernel smoothers; this method involves computing the fit at a point x_0 using only the nearby observations. A key advantage of kernel smoothers is their use of binning techniques [55], which greatly reduce the computational time and thus enable the model to be adequately solved in practical situations. However, kernel smoothers require the user to choose the bandwidth parameters, which can have a large effect on the obtained parameter estimates. Different studies have proposed various methods for choosing the optimal

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bandwidth, including generalized cross-validation [56], plug-in methods [57], bootstrap techniques [58].

Variable selection is another important issue when developing a multivariate regression framework, especially when the number of covariates is large enough. Inferences based on models with only a few variables can be biased; conversely, models that use too many variables may result in a lack of precision or false-positive effects. The so-called model selection problem arises from the need to ensure that a model is neither under- nor over-fitted [59]. The literature describes several procedures for solving this problem and choosing the optimal set of variables; these methods can include shrinkage regression (e.g., the Lasso [60,61]), Bayesian approaches [62–64], iterative procedures such as stepwise selection based on the use of some information criteria [65–67], or the use a full information criteria-based approach [68].

The multivariate regression methodology described above can be easily used to investigate the abundance of MMD in bivalves, both at present and over time, with the aim of determining the environmental and food chain-associated human health risks of MMD. For example, such a regression could be applied to retrospective data on microplastic concentrations and pathogen prevalence in bivalve tissue samples from biospecimen banks spanning the last few decades. For this analysis, MMD abundance could be determined in bivalve tissues using polarized light microscopy following the recommendations of recent studies [69]. In addition, the prevalence of shellfish and human pathogens, as well as histopathological alterations, could be scored using either quantitative or semi-quantitative scales [70]. The results of this retrospective study would help assess current and historical trends in the accumulation of microplastics and pathogens in marine filter-feeders as well as the relationship between microplastic accumulation and pathogen prevalence. When combined with information on the ecotoxicology and pathogenicity of a given pathogen, these exposure and prevalence data can be helpful for deciding whether a compound or pathogen must be considered as an emerging substance or organism.

In addition to the multivariate regression modelling approach predicting both MMD and pathogen loads in bivalves based on a suite of environmental variables, some industry evolution data can be included in the predictor data pool. This final model could be evaluated using a specific stepwise method; in this case, we suggest a forward stepwise-based selection procedure that both (i) selects the best combination of variables and (ii) determines the optimal number of covariates to include in the model. This type of analysis would provide valuable information for understanding which factors or variables from the plastic industry, in addition to the physiochemical environment, are involved in the temporal trends of microplastic occurrence and pathogen prevalence in marine animals. The results from such a model would also have important implications for future studies of the ecological and seafood-related risks of microplastics.

In this study, we present an example of this type of analysis by GAMs to analyze (i) the effect of different environmental variables on microplastic abundance (*number of occurrences of microplastics* g^{-1}) and infection intensity (*number of occurrences of pathogens* g^{-1}) in mussels and (ii) the relationship between microplastic abundance and infection intensity. Explanatory variables in the first model included river flow rate ($m^3\ sec^{-1}$), salinity, temperature (°C), dissolved oxygen (%, percent dissolved oxygen saturation), salinity stratification index, and chlorophyll concentrations ($mg\ m^{-3}$). These data were obtained from monthly samplings from 1998 to 2015 in the Basque coast (estuaries of Bilbao and Urdaibai), N Spain (43°24.2'N 2°41.7'W), using the material and methods described in Iriarte *et al.* [71]. The response variables were constructed theoretically. We used the log function as a link and thin plate regression splines as a smoothing basis. The optimal number of degrees of freedom was chosen via (generalised) cross-validation [72], and parameter estimation was performed using the mgcv package [73] in R [74].

 doi:10.20944/preprints202210.0442.v1

2.2. Experimental studies

To better understand the global risks of MMD particles as disease vectors, basic experimental research is needed on how the MMD-pathogen interaction affects emerging marine disease dynamics. Such studies are essential for generating the knowledge needed to mitigate both marine ecosystem degradation and the human health risks of marine pathogens.

2.2.1. Oysters as an experimental model

In the context of MMD, bivalves and other filter-feeders are distinct because they can filter out and therefore accumulate MMD from the water column [75], making them particularly susceptible to pathogens [44]. They are also important vectors for seafood-borne human pathogens [46] that are adhered to microplastics [76]. Due to their tremendous filtration capacity (up to 8 liters of seawater per hour [16]), oysters are one of the best model organisms for experimental studies exploring how marine organisms uptake MMD and the role of microplastics on pathogen transmission.

The experimental setup may emphasize one or more of the following aspects of MMD (Figure 1A): (1) the role of microplastic size or type on its uptake in bivalves; the relationship of this uptake with (2) the *in vivo* accumulation or removal of pathogens (e.g., the phagocytic activity of hemocytes); and (3) the infection intensity of bivalve pathogens. Microplastic types and sizes for the experiments can be chosen from irregular polyethylene and polyethylene terephthalate fragments in the shape of fibres, spheroids, granules, pellets, flakes, or beads. Particle sizes should be in the range of 0.1-5000 μm.

For the study design, oysters should be deployed in tanks and exposed to MMD for 1-5 weeks to obtain stressed oysters for subsequent trials. Stress in oysters can be assessed by studying a variety of stress responses such as tissue alteration, immune alteration, DNA damage, oxidative stress, altered lipid and glucose metabolism, and a reduced clearance rate of pathogenic organisms [77,78]. By comparing MMD-stressed and non-stressed oysters, researchers can evaluate how the uptake of chemicals adhered to the surface of MMD may affect the oysters' capacity to remove (or resist) pathogenic bacteria [31–33]. In this theoretical experimental setting, three important experimental trials can be conducted. First, oysters can be exposed to microplastics of different types and sizes at varying concentrations (e.g., 10 and 1000 μg L^{-1}) (Figure 1A, top panel) and for different periods of time (e.g., 1-5 weeks). This exposure would be performed under static conditions using similar protocols as [78]. Second, oysters can be exposed to different *Vibrio* spp. concentrations in the water column (from 10^3 to 10^7 cells L^{-1}) (Figure 1A, mid panel). By analyzing the bacterial load of oyster samples at the end of the exposure period (e.g., as culturable *Vibrio* counts), researchers can assess the incidence of *Vibrio* in terms of pathogen infection intensity. Third, oysters can be exposed to microplastics with adhered *Vibrio* spp. (Figure 1A, bottom panel) and then assess the incidence of *Vibrio* as in the second experiment. These three trials would ideally be conducted for both stressed and non-stressed oysters at varying temperatures and oyster densities. These trials could also be performed in systems that include non-focal hosts such as tunicates (T in Figure 1B) in order to assess the disease-diluting effect of other filter-feeders in the same ecosystem [79].

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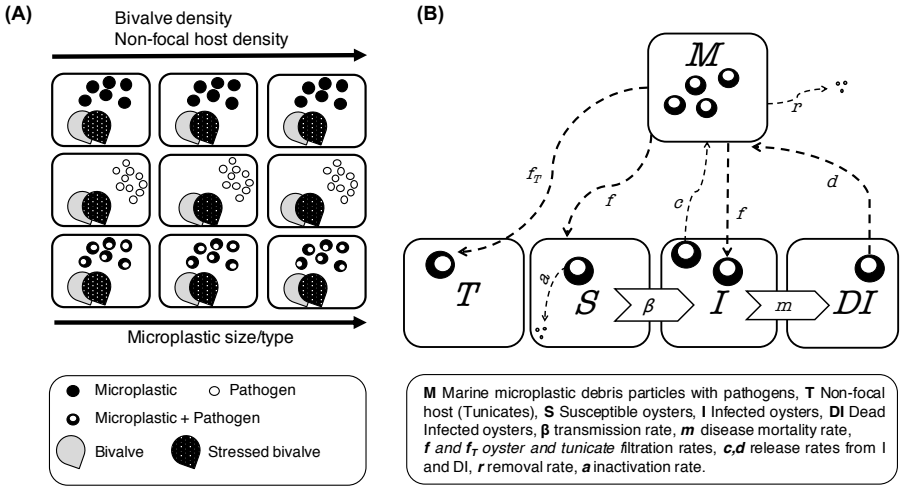

Figure 1. Experimental design for bivalves. The proposed experiment (A) can evaluate different microplastic types/sizes and different oyster and non-focal host densities to determine the effect of these variables on MMD uptake and accumulation in oysters and the relationships between MMD uptake and each of pathogen (i.e., *Vibrio* spp.) occurrence and disease responses. The conceptual disease model (B) represents a simplified scheme of subpopulations, parameters, and processes that will be incorporated into an ordinary differential equation system (as in [80]). This system comprises the 'bivalve-microplastic-*Vibrio*' disease model. B

2.3. *Zebrafish as an experimental model*

Another valuable model system for studying the role of microplastics in pathogen transmission is the zebrafish (*Danio rerio*). Zebrafish are already one of the most important models in environmental toxicology and developmental biology and are rapidly becoming a major model in studies of animal and human health and disease. The zebrafish has a long and extremely successful history as a model organism for many biological processes ranging from development to bacterial pathogenesis [81,82], including the pathogenesis of aquatic pathogens such as *Vibrio* spp. [41,83,84]. Other studies have also investigated the uptake and accumulation of polystyrene microplastics in zebrafish tissue [e.g. 85]. Experimentally studying the role of microplastics as vectors of aquatic pathogens in such a well-established model system is particularly valuable because the biology of zebrafish is already thoroughly understood, allowing researchers to easily identify the risks posed by these various process. Moreover, because zebrafish larvae are transparent, researchers can visualize the *in vivo* uptake and accumulations of microplastics and pathogens using fluorescently labelled pathogens and microplastic particles. These observations may be crucial for studying the behavior of the host-microplastic-pathogen system.

Overall, studies using zebrafish could determine whether the uptake and transmission of pathogens in fish is affected by the presence of microplastics. Future experiments in the zebrafish model should address the basic but unanswered questions about host-microplastic-pathogen dynamics; for example, will microplastics alter the bioavailability, uptake route, or transmission of pathogens like *Vibro* spp.? Will the transmission of pathogens through microplastics be similar in different types and sizes of plastic? Will it be similar in adult fish and larvae?

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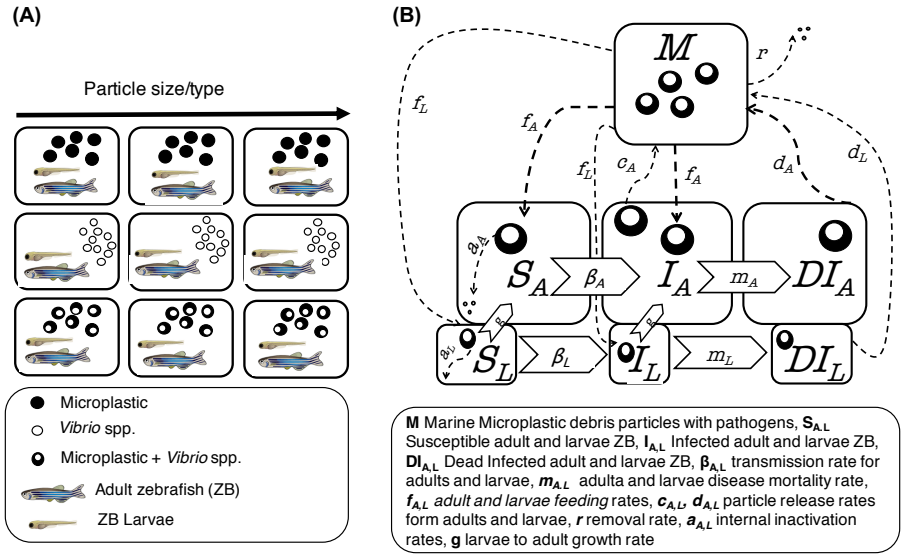

Figure 2. Experimental and model design for zebrafish. The proposed experiment (A) can evaluate different microplastic types/sizes to determine their effect on MMD uptake and accumulation in both adult fish and larvae, as well as the relationship between MMD uptake and each of pathogen (i.e., *Vibrio* spp.) occurrence and disease responses. The conceptual disease model (B) represents a simplified scheme of subpopulations, parameters, and processes that will be incorporated into an ordinary differential equation system referred to as the fish-microplastic-*Vibrio* disease model.

To analyze the behavior, accumulation, and transfer of microplastic-associated pathogens in adult and larval zebrafish, researchers can use different sizes and types of fluorescently labeled microplastics as well as a model pathogen (carrying a plasmid that encodes green fluorescent protein) as representative for aquatic bacterial pathogens. Six-month old zebrafish are sufficient for experiments with adult zebrafish, and zebrafish at five days post-fertilization may be suitable for the larval experiments. Microplastic accumulation could be assessed in the gills, gut, and intestines based on fluorescence intensity. In parallel, pathogen infection levels can be assessed with histological analyses in adults and fluorescence tracking in larvae. By taking advantage of the transparency of zebrafish larvae and using a genetically engineered fluorescent model pathogen, researchers can observe the active uptake and colonization of MMD-associated pathogens.

As in the oyster model, the zebrafish model could use a similar combination of microplastic types/sizes, microplastic concentrations, experimental durations, treatment types, and pathogen concentrations (Figure 2A. As a result, the zebrafish experiment will investigate the role of microplastic size and type on the plastic uptake and accumulation rate as well as the relationships between microplastic uptake, pathogen accumulation, and infection intensity in both adults and larvae. Alternative experiments could investigate the transmission of MMD and pathogens through the food chain by feeding zebrafish with brine shrimp (*Artemia nauplii*) that have already accumulated MMD and pathogens.

2.4. Disease transmission modelling

The results obtained from these controlled experimental studies, in combination with previously published data, would provide the empirical and theoretical information needed to understand the role of microplastics as a transmission vector for bivalve, fish, and human pathogens. Specifically, this data can be used to develop and parameterize epizootiological and epidemiological models. In this study, we use continuous-time compartmental models adapted from previous susceptible-infected-particle-filtration-type disease dynamic models [e.g. 79,86]. Note that, by using a combination of empirical

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data and disease transmission models, researchers can also build relationship models to describe the links between microplastic pollution, microplastic uptake, toxicological effects, and *Vibrio* infections.

2.4.1. Model schemes

Figure 1B and Figure 2B show flow diagrams of the disease transmission models for suspension bivalves and fish, respectively, highlighting the important processes involved in disease transmission. We refer to these models as the bivalve-microplastic-*Vibrio* and fish-microplastic-*Vibrio* disease transmission models, respectively.

In both compartmental susceptible/infected-type models, the pathogen is attached to particles of MMD; these particles are represented by M in the models. The pathogen is then transmitted to the susceptible population S at a rate β through either filtration or ingestion of M at a rate f. Infected animals I die according to a disease mortality rate m. Particles are removed *in vivo* from individuals in each population at a rate a by internal inactivation processes, and particles are removed from the water column at a rate r by diffusion/advection and decay processes. The bivalve model includes a non-target host population (T) that is immune to and importantly inactivates pathogens. The zebrafish model includes adult (subindex A) and larvae (subindex L) subpopulations. A detailed description of the variables, parameters, and units for each model can be found in Table 1 and Table 2.

Variable	Definition	Unit
S, S_A, S_L	Susceptible hosts in the population	Number of individuals
I, I_A, I_L	Infected individuals in the population	Number of individuals
DI, DI_A, DI_L	Dead infected individuals in the population	Number of individuals
M	Marine microplastic debris particles with adhered pathogens	Number of particles
T	Alternate non–competent reservoir hosts	Number of individuals

Table 1: Variables in the bivalve- and fish-microplastic-*Vibrio* models. There is no subindex for the oyster population, whereas the A and L subindexes in the fish model represent adult and larvae subpopulations, respectively. Note that the model has an implicit surface area for the host subpopulations and an implicit volume for the pathogens.

The two theoretical models described here (bivalve and zebrafish) are different from each other because they include the differentiated mechanisms and processes involved in disease transmission in each organism. The main differences are the following: (1) In the bivalve model (Figure 1), an alternative host, tunicates T, competes for waterborne pathogens with the susceptible host. This alternative host is resistant to the disease and does not release particles to the water. Pathogens filtered by T are assumed to be inactivated by the immune system or by diapedesis. (2) In the zebrafish model (Figure 2), populations are subdivided into adults and larvae. The modeled processes are allowed to occur at different rates for fish adults (subindex A) and larvae (subindex L), and larvae mature into adults at a rate g.

2.4.2. Model assumptions

The two disease transmission models track waterborne environmental pathogens attached to microplastic particles. The pathogen-microplastic complex drifts through

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Parameter	Definition	Unit
β	Transmission rate in oysters	individual $^{-1}$day^{-1}
β_A, β_L	Transmission rates in fish	individual $^{-1}$day^{-1}
m	Disease mortality rate in oysters	day^{-1}
m_A, m_L	Disease mortality rate in fish	day^{-1}
g	Growth rate from larvae to adult	day^{-1}
d, d_A, dL	Removal rate of dead individuals by scavengers or decay	day^{-1}
b_I, b_{I_A}, b_{I_L}	Average number of *MMD* per I	*MMD* particles
b_{I_T}	Average number of *MMD* per T	*MMD* particles
b_{DI}	Average *MMD* per DI	*MMD* particles
$c, c_{A,L}$	Release rate of particles from I	day^{-1}
c_T	Release rate of particles from T	day^{-1}
$c_{DI}, c_{DI_A}, c_{DIL}$	Release rate of particles from DI	day^{-1}
r	Loss rate of MMD particles from the local environment	day^{-1}
f, f_A, f_L	Filtration/feeding rate of S and I	m^3 individual $^{-1}$ day^{-1}
f_T	Filtration/feeding rate of T	m^3 individual $^{-1}$ day^{-1}
a, a_A, a_L	Inactivation of pathogens in S and I	day^{-1}
a_T	Inactivation of pathogens in T	day^{-1}

Table 2: Parameters of the bivalve- and fish-microplastic-*Vibrio* disease transmission models. Note that the models implicitly include a surface area (in m^2) for oysters and volume (in m^3) for fish and microplastic particles. In the fish model, the subindex A represents adult fish and the subindex L represents fish larvae.

the water and is either filtered (by bivalves) or ingested (by fish). For simplicity, the model assumes no natural mortality for hosts; infected individuals only die due to disease. Background mortality could be incorporated in more complex models for slow-progression diseases. The model also assumes no natural mortality and total inactivation of particles in the non-focal hosts T,

The models also assume that populations are closed (i.e., demographic turnover processes, like reproduction and migration, are not included in either model). In addition, the models assume that no animals recover from the disease once infected. Indeed, there are only a few examples of disease recovery in the marine realm [87–89]. Finally, parameterization of the model is standardized to represent (i) a square meter of the environment for bivalves and (ii) a cubic meter of the environment for particles and fishes. As a result, units in the bivalve model are individuals per square meter and units for population size are individuals per cubic meter, as in [86]. The variables and parapeters of the model related to the host can be adapted to experimental in formation as the level of stress of oysters in the case these have been exposed to microplastics before microplastic with pathogen exposure.

3. Results

3.1. Retrospective multivariate modeling

Figures 3 - 5 show the response plots from our theoretical GAM examples. The output shown in these figures allows researchers to study the relationship between the various environmental variables and the response variables (either the abundance of microplastics in the organisms (Figure 3) or infection intensity (Figure 4). In our models, salinity, temperature, dissolved oxygen, and especially the stratification index showed a positive relationship with microplastic abundance (Figure 3). River flow rate and chlorophyll concentrations also had an overall positive effect on microplastic abundance, with relative maximums or minimums observed along the measured ranges of the two variables (Figure 3).

With the exception of salinity, all the explanatory variables that we considered also had a positive effect on infection intensity. In the case of dissolved oxygen and

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temperature, infection intensity increased as these variables increased but then reached a maximum beyond which it remained within a range of high values with little oscillation (Figure 4).

Lastly, we analyzed the relationship between the two response variables (microplastic abundance and infection intensity) (Figure 5). This relationship was significantly positive, with infection intensity increasing alongside microplastic abundance, though the relationship was weaker at higher values of microplastic abundance. Continued empirical, retrospective studies of this relationship are critical for gaining further insight into the emergence of diseases due to the transmission of pathogens adhered to MMD.

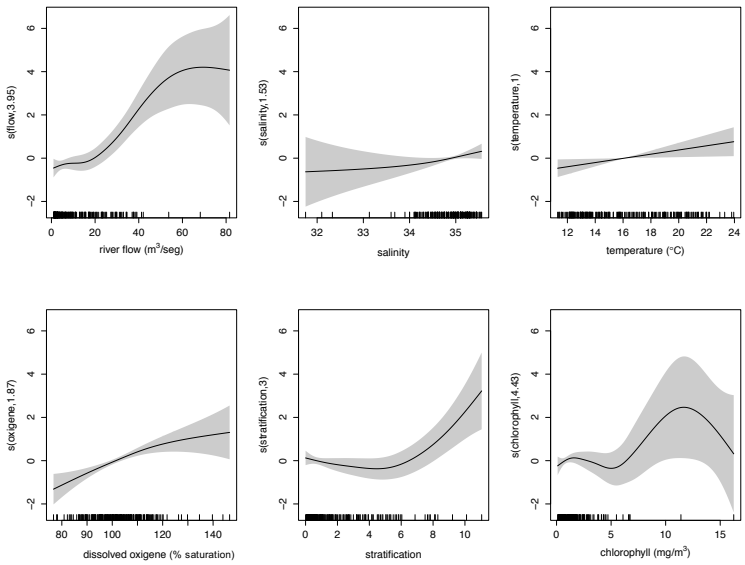

Figure 3. Partial effects from the fitted GAM predicting microplastic abundance (*number of occurrences of microplastics* g^{-1}) in an organism (for example bivalves or oysters) as a function of river flow ($m^3\ sec^{-1}$), salinity, temperature (°C), dissolved oxygen (%, percent dissolved oxygen saturation), salinity stratification index, and chlorophyll concentration ($mg\ m^{-3}$). The degrees of freedom for smoothed fits are indicated in parentheses on the y-axis. Tick marks above the x-axis indicate the distribution of observations. The shaded area represents the 95% confidence intervals of partial effects.

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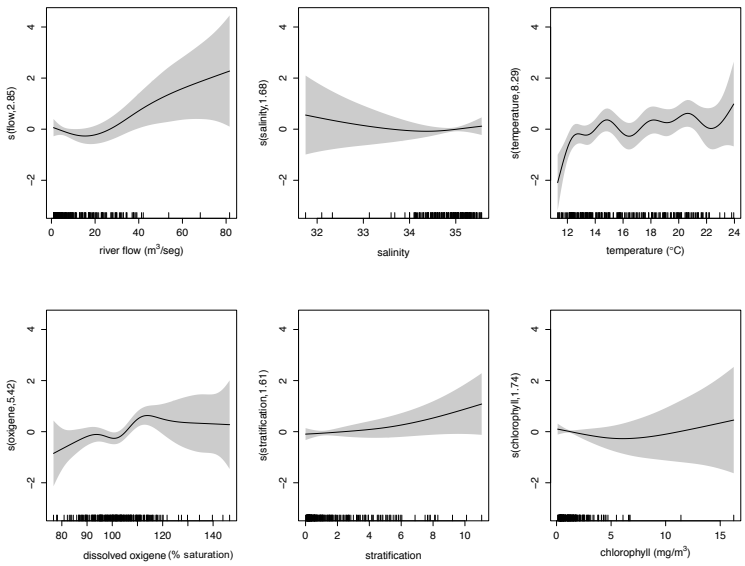

Figure 4. Partial effects from the fitted GAM predicting infection intensity (*number of occurrences of the pathogen* g^{-1}) as a function of river flow ($m^3\ sec^{-1}$), salinity, temperature (°C), dissolved oxygen (%, percent dissolved oxygen saturation), salinity stratification index, and chlorophyll concentration ($mg\ m^{-3}$). The degrees of freedom for smoothed fits are indicated in parentheses on the y-axis. Tick marks above the x-axis indicate the distribution of observations. The shaded area represents the 95% confidence intervals of partial effects.

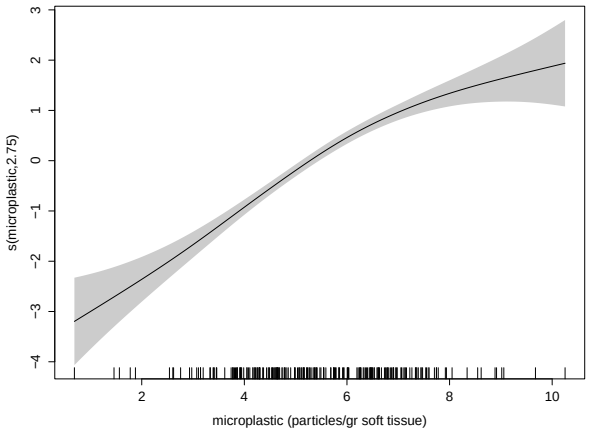

Figure 5. Partial effect from the fitted GAM predicting infection intensity (*number of occurrences of the pathogen* g^{-1}) as a function of microplastic abundance (*number of occurrences of microplastics* g^{-1}). The degrees of freedom for smoothed fits are indicated in parentheses on the y-axis. Tick marks above the x-axis indicate the distribution of observations. The shaded area represents the 95% confidence interval.

3.2. Pathogen transmission modelling

The host and pathogen states or subpopulations (variables) of bivalve- and fish-microplastic-*Vibrio* models satisfy a system of ordinary differential equations describing the dynamics of the host-pathogen association. The variables and parameters for these models are described in Tables 1-2. We programmed the numerical models for these systems in Matlab and solved them with a 4^{th}–order predictor corrector scheme using the

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Adams-Bashforth predictor and the Adams-Moulton corrector. The system of differential equations in each of the two models comprises the following differential equations:

3.2.1. Bivalve-microplastic-*Vibrio* disease model

$$\frac{dS}{dt} = -\beta f M S + SRC_S \tag{2}$$

$$\frac{dI}{dt} = (\beta - a) f M S - m I \tag{3}$$

$$\frac{dDI}{dt} = m I - d DI \tag{4}$$

$$\frac{dM}{dt} = (1-a)\, c\, b_I\, I + (1-a_T)\, c_T\, b_T\, T + b_{DI}\, d\, DI - f M (S+I) - f_T M T - r M + SRC_M \tag{5}$$

$$\frac{dT}{dt} = T + SRC_T \tag{6}$$

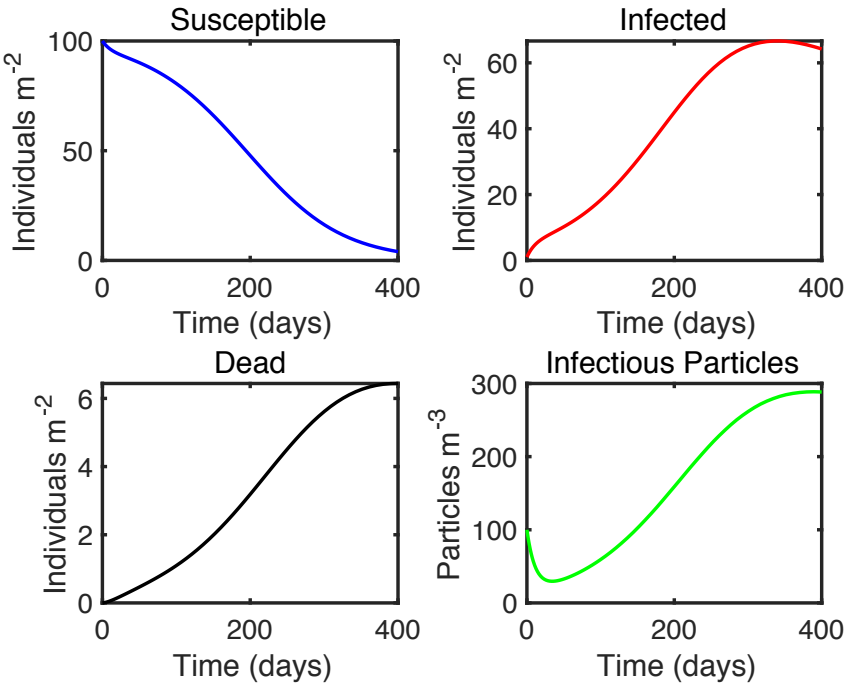

Figure 6. Pathogen transmission simulation involving oysters (as a representative filter-feeder) and microplastics with adhered pathogens (infectious particles). Oysters were divided into three subpopulations (susceptible, infected, and dead/infected), and simulations were run based on an initial population of 100 susceptible oysters, one infected oyster, and 100 infectious particles. Parameter values for the simulations were as follows: β=5 × 10^{-5}, f=2.5 × 10^{-4}, m=2 × 10^{-3}, d=2 × 10^{-2}, c=2.5 × 10^{-2}, r=5 × 10^{-2}, b_I=10, b_{DI}=20, a=0, a_T = 1, SRC_S=0, SRC_T=0, and SRC_M=1. For this example, all rates associated with the non-competent host (T), such as particle uptake and pathogen inactivation, were considered null.

Our bivalve-microplastic-*Vibrio* model simulations (Figure 6) detected the effect of MMD-adhered pathogens on disease transmission. The size of the susceptible sub-

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population decreased as more individuals became infected by filtering infectious MMD, thereby increasing the size of the infected population. The size of the dead/infected subpopulation increased, in turn, as individuals from the infected pool died (Figure 6; S, I, D plots). The number of MMD particles with adhered pathogens initially decreased as the susceptible and infected populations filtered MMD out of the seawater (Figure 6; infectious particle plot); however, this initial decrease was followed by a rapid increase as more MMD particles entered the water column from external water masses and from the infected and dead subpopulations. The overall infection rate for this model (Figure 7) shows an initial decrease as MMD particles are filtered out of the water column, followed by an increase due to the release of particles from infected and dead subpopulations. The infection rate decreases to zero once all susceptible individuals have become infected, and infected individuals continue to die out.

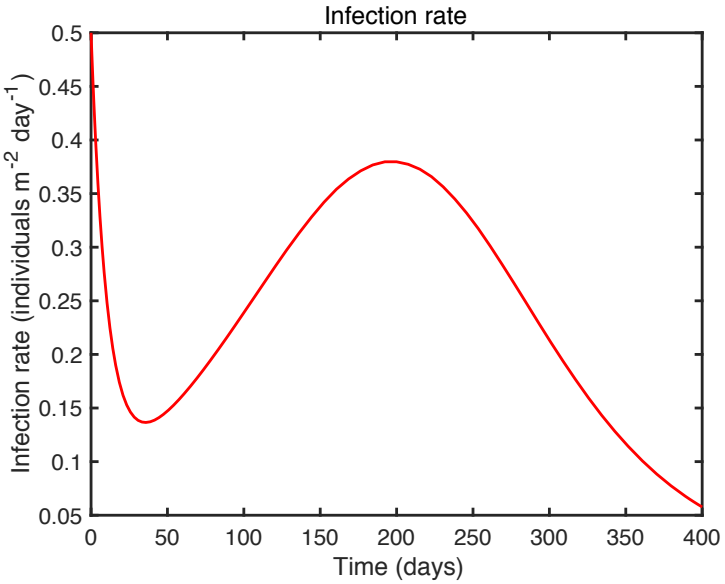

Figure 7. Infection rate dynamics for a simulated oyster population (as an example of filter-feeders) filtering infectious microplastic particles. The simulation began with an initial population of 100 susceptible oysters, one infected oyster, and 100 infectious particles. Parameter values for the simulation were the same as for Figure 6.

3.2.2. Fish-microplastic-*Vibrio* disease model

$$\frac{d\,S_L}{d\,t} = -\beta_L\, f_L\, M\, S_L\ - g\, S_L + SRC_{S_L} \tag{7}$$

$$\frac{d\,S_A}{d\,t} = -\beta_A\, f_A\, M\, S_A\ + g\, S_L + SRC_{S_A} \tag{8}$$

$$\frac{d\,I_L}{d\,t} = (\beta_L\ - a_L)\, f_L\, M\, S_L\ - g\, I_L - m_L\, I_L \tag{9}$$

$$\frac{d\,I_A}{d\,t} = (\beta_A\ - a_A)\, f_A\, M\, S_A\ + g\, I_L - m_A\, I_A \tag{10}$$

$$\frac{d\,DI_L}{d\,t} = m_L\, I_L - d_A\, DI_L \tag{11}$$

$$\frac{d\,DI_A}{d\,t} = m_A\, I_A - d_L\, DI_A \tag{12}$$

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$$\frac{d\,M}{d\,t} = (1-a_L)\,c_L\,b_{I_L}\,I_L + (1-a_A)\,c_A\,b_{I_A}\,I_A + b_{DI_L}\,d_L\,DI_L + b_{DI_A}\,d_A\,DI_A - f_L\,M\,(S_L+I_L) - f_A\,M\,(S_A+I_A) - r\,M + SRC_M \quad (13)$$

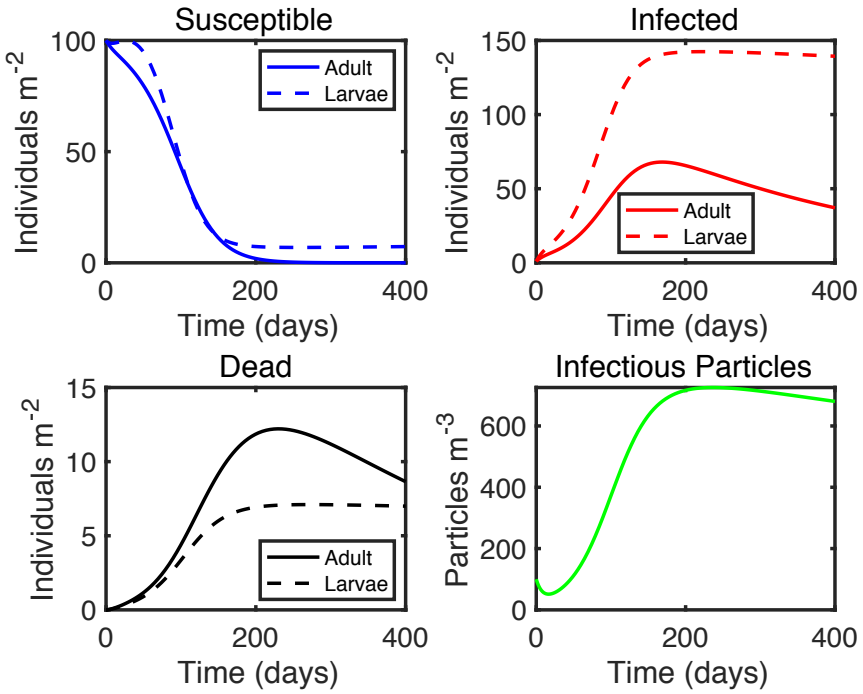

Figure 8. Pathogen transmission simulation involving zebrafish and microplastics with adhered pathogens (infectious particles). Zebrafish were divided into subpopulations that were further divided into adult and larval populations (i.e., susceptible adults and larvae, infected adults and larvae, and dead/infected adults and larvae). Simulations were run based on an initial population of 100 susceptible adults, 100 susceptible larvae, one infected adult, one infected larva, and 100 infectious particles. Parameter values for simulations were as follows: β_A=5 × 10^{-5}, β_L=10 × 10^{-5}, g=0.001, f_A=2.5 × 10^{-4},f_L=1.25 × 10^{-4}, m_A=2 × 10^{-3},m_L=4 × 10^{-3}, d_A=2 × 10^{-2}, d_L=4 × 10^{-2}, c_A and c_L=2.5 × 10^{-2}, r=5 × 10^{-2}, b_{I_A} and b_{I_L}=10, b_{DI_A} and b_{DI_L}=20, a=0, SRC_{S_A}=0, SRC_{S_L}=0.5, SRC_M=2.

Like the bivalve models, the fish-microplastic-*Vibrio* model simulations (Figure 8) also detected the effect of MMD-adhered pathogens on disease transmission in fish adults and larvae. The size of the susceptible adult and larvae subpopulations decreased as individuals became infected by feeding on infectious particles; infected individuals were transferred to the infected subpopulation, causing the size of the infected adult and larval populations to increase. The infected larvae population increased more rapidly due to the higher infection rate for larvae (Figure 8).

The plot for the susceptible population also shows the effect of a continuous source of larvae coming from other regions (SRC_{S_L}=0.5) (Figure 8, S plot, in blue). By day 200, all susceptible adults had become infected, but new susceptible larvae enter the system from external sources. The dead adult subpopulation increased to a higher level than the dead larvae population because the dead larvae decay rate is faster than the decay rate for adults. At the same time, the concentration of MMD particles increased to a maximum as particles are both released from and entering the system from external sources (Figure 8, particle plot, in green). After reaching this maximum, the concentration of MMD

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particles then decreased, as all susceptible individuals had become infected; as infected individuals start dying, MMD particles are removed from the system through decay processes.

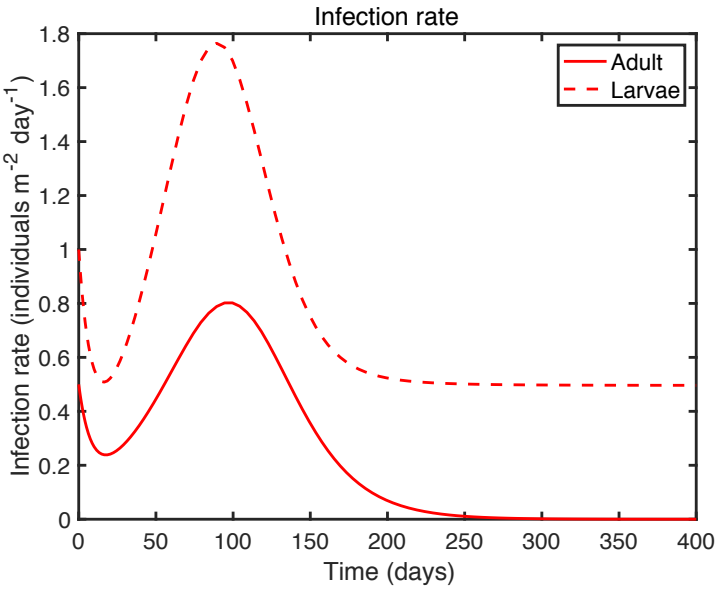

Figure 9. Infection rate dynamics for fish (as an example of filter-feeder) filtering infectious microplastic particles. This simulation used an initial population of 100 susceptible oysters, one infected oyster, and 100 infectious particles. Parameter values for this simulation were the same as for Figure 6.

The behavior of the infection rate for this model is similar to that observed for the bivalve model (Figure 9). In the fish model, the curve of the larvae infection rate is well above the curve of the adult infection rate, adeuately mirroring the higher infection rate considered for the larvae respect to adult fish.

4. Discussion and conclusions

The three-part analytical approach described here (retrospective regression analysis, *in vivo* experiments, and disease modelling) provides a suitable framework for thoroughly exploring the role of microplastics on marine pathogen transmission. Using this approach to further researcher will build a body of knowledge essential for addressing marine disease and food safety challenges related to MMD. This research is an urgent priority of the EU Strategic Research Innovation Agenda [90]. The theoretical results from the retrospective analysis described here demonstrate that a retrospective regression analysis can offer a valuable perspective on the past and expected future trends of MMD exposure in different marine organisms, as well as the relationship between MMD exposure and pathogen prevalence. However, despite the importance of these analysis for understanding the evolution of emerging pathogens, such analyses of microplastic trends remain scarce [91].

Based on the results of retrospective regressions and the relationships among modeled variables, organisms with higher MMD exposure and a higher incidence of pathogens can be considered for *in vivo* experiments and disease modelling. For example, the experimental approach outlined here is designed to determine the effect of the MMD-pathogen interaction on disease transmission. To achieve this, our proposed experimental design includes treatments with non-infectious microplastics, microplastics with adhered pathogens, and free-floating pathogens. Such an experimental approach is particularly timely because recent experiments have used microplastics with adhered

pathogens to assess whether microplastics may facilitate pathogen entry into marine food webs [92].

Together, retrospective analysis and *in vivo* experimental results can provide essential information on the key parameters involved in the mechanisms and processes of disease transmission through microplastic exposure. In this study, as in [80], we developed the theoretical basis for modeling these MMD-pathogen systems for bivalves and fishes. Our models can be parameterized with realistic values obtained from previous retrospective and experimental analyses, and our model results conform to the expectations of mathematical theory and behavior and population dynamics. Most importantly, our models incorporate the effect of microplastic particles with adhered pathogens on disease transmission and mortality. In the age-dependent fish-microplastic-*Vibrio* model, the effect of infectious microplastics could be observed separately for both adult and larval zebrafish. In the future, models based on the experimental design and models described here can be developed to further explore the role of microplastic-derived stress on the transmission of both free-living and MMD-adhered pathogens [32].

Studying the combined risks from microplastic pollution and disease represents a novel approach to the study of marine disease ecology. Future studies along this line of research could involve a linked experimental-disease modelling approach that would allow us to understand the complex organism-microplastic-pathogen system from a predictive and epizootiological perspective. This perspective is inherently interdisciplinary, with research teams possessing a unique mixture of expertise in bivalve and zebrafish microplastic toxicology, histopathology, immunology, and marine disease modelling. Moreover, the interdisciplinary and predictive aspects of this project are essential for making progress towards the long-term objectives of this research, which focus on understanding the rate at which organisms encounter microplastics (e.g., via ocean models) and the physical, chemical, biological, and interactive risks these encounters pose to different organisms at different spatial scales and, through bioaccumulation, different trophic levels. Overall, this proposed study will generate the knowledge needed to guide advanced seafood safety studies in commercial bivalves, and it will be applicable to other ecologically relevant suspension-feeders such as corals.

Author Contributions: Conceptualization, G.B., P.L.L, M.S.; methodology, G.B., P.L.L., M.S., I.U, A.I. and F.V.; software, G.B. and M.S: formal analysis, G.B., M.S., P.L.L., I.U, A.I. and F.V.; original draft preparation, G.B., M.S., P.L.L., I.U, A.I. and F.V.; review and editing, G.B., M.S., P.L.L., I.U, A.I. and F.V.; supervision, G.B, P.L.L.. All authors have read and agreed to the published version of the manuscript.

Funding: This work was conceived under the research framework outlined by the National Science Foundation Evolution and Ecology of Infectious Diseases (EEID) Program Grant, OCE-1216220. Funding was provided by the University of the Basque Country (UPV/EHU, GIU19/059) and the Basque Government (PIBA2020-1-0028). We appreciate this support.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Following the data availability statements in the "MDPI Research Data Policies" https://www.mdpi.com/ethics, data may be available to other researchers under reasonable terms of use. Queries about the terms of use and requests for data should be addressed to the principal investigator of the research team, Dr. Fernando Villate (fernando.villate@ehu.eus).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Cózar, A.; Echevarría, F.; González-Gordillo, J.I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á.T.; Navarro, S.; García-de Lomas, J.; Ruiz, A.; others. Plastic debris in the open ocean. *Proceedings of the National Academy of Sciences* **2014**, *111*, 10239–10244.
2. UNEP. *Emerging issues in our global environment: plastic debris in the ocean.*; United Nations Environment Programme. UNEP Year Book 2014., 2014.
3. *Plastic Europe. Plastics – the Facts 2014/2015: An analysis of European plastics production demand*; Plastics Europe: Brussels, 2015.

4. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: a review. *Marine pollution bulletin* **2011**, *62*, 2588–2597.
5. Shim, W.J.; Thomposon, R.C. Microplastics in the ocean. *Archives of environmental contamination and toxicology* **2015**, *69*, 265–268.
6. Cole, M.; Lindeque, P.; Fileman, E.; Halsband, C.; Goodhead, R.; Moger, J.; Galloway, T.S. Microplastic ingestion by zooplankton. *Environmental science & technology* **2013**, *47*, 6646–6655.
7. Murphy, F.; Russell, M.; Ewins, C.; Quinn, B. The uptake of macroplastic & microplastic by demersal & pelagic fish in the Northeast Atlantic around Scotland. *Marine pollution bulletin* **2017**, *122*, 353–359.
8. Sequeira, I.F.; Prata, J.C.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T. Worldwide contamination of fish with microplastics: A brief global overview. *Marine Pollution Bulletin* **2020**, *160*, 111681.
9. Romeo, T.; Pietro, B.; Pedà, C.; Consoli, P.; Andaloro, F.; Fossi, M.C. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. *Marine pollution bulletin* **2015**, *95*, 358–361.
10. Besseling, E.; Foekema, E.; Van Franeker, J.; Leopold, M.; Kühn, S.; Rebolledo, E.B.; Heße, E.; Mielke, L.; IJzer, J.; Kamminga, P.; others. Microplastic in a macro filter feeder: humpback whale Megaptera novaeangliae. *Marine pollution bulletin* **2015**, *95*, 248–252.
11. Smith, M.; Love, D.C.; Rochman, C.M.; Neff, R.A. Microplastics in seafood and the implications for human health. *Current environmental health reports* **2018**, *5*, 375–386.
12. Van Cauwenberghe, L.; Janssen, C.R. Microplastics in bivalves cultured for human consumption. *Environmental pollution* **2014**, *193*, 65–70.
13. Prata, J.C.; da Costa, J.P.; Lopes, I.; Duarte, A.C.; Rocha-Santos, T. Environmental exposure to microplastics: An overview on possible human health effects. *Science of the Total Environment* **2020**, *702*, 134455.
14. Li, J.; Yang, D.; Li, L.; Jabeen, K.; Shi, H. Microplastics in commercial bivalves from China. *Environmental pollution* **2015**, *207*, 190–195.
15. Van Cauwenberghe, L.; Claessens, M.; Vandegehuchte, M.B.; Janssen, C.R. Microplastics are taken up by mussels (Mytilus edulis) and lugworms (Arenicola marina) living in natural habitats. *Environmental pollution* **2015**, *199*, 10–17.
16. Powell, E.; Hofmann, E.; Klinck, J.; Ray, S. Modeling oyster populations: I. A commentary on filtration rate. Is faster always better? **1992**.
17. Guzzetti, E.; Sureda, A.; Tejada, S.; Faggio, C. Microplastic in marine organism: Environmental and toxicological effects. *Environmental toxicology and pharmacology* **2018**, *64*, 164–171.
18. Zhang, F.; Man, Y.B.; Mo, W.Y.; Man, K.Y.; Wong, M.H. Direct and indirect effects of microplastics on bivalves, with a focus on edible species: A mini-review. *Critical Reviews in Environmental Science and Technology* **2020**, *50*, 2109–2143.
19. Hardesty, B.D.; Harari, J.; Isobe, A.; Lebreton, L.; Maximenko, N.; Potemra, J.; Van Sebille, E.; Vethaak, A.D.; Wilcox, C. Using numerical model simulations to improve the understanding of micro-plastic distribution and pathways in the marine environment. *Frontiers in marine science* **2017**, *4*, 30.
20. Law, K.L.; Thompson, R.C. Microplastics in the seas. *Science* **2014**, *345*, 144–145.
21. Lusher, A. Microplastics in the marine environment: distribution, interactions and effects. In *Marine anthropogenic litter*; Springer, Cham, 2015; pp. 245–307.
22. Vethaak, A.D.; Legler, J. Microplastics and human health. *Science* **2021**, *371*, 672–674.
23. Zeilinger, A.R.; Rapacciuolo, G.; Turek, D.; Oboyski, P.T.; Almeida, R.P.; Roderick, G.K. Museum specimen data reveal emergence of a plant disease may be linked to increases in the insect vector population. *Ecological applications* **2017**, *27*, 1827–1837.
24. Directive, S.F. Guidance on monitoring of marine litter in European seas. *Luxembourg: Publications Office of the European Union, doi* **2013**, *10*, 99475.
25. Galgani, F.; Claro, F.; Depledge, M.; Fossi, C. Monitoring the impact of litter in large vertebrates in the Mediterranean Sea within the European Marine Strategy Framework Directive (MSFD): Constraints, specificities and recommendations. *Marine environmental research* **2014**, *100*, 3–9.
26. Gorokhova, E. Screening for microplastic particles in plankton samples: how to integrate marine litter assessment into existing monitoring programs? *Marine pollution bulletin* **2015**, *99*, 271–275.
27. Frias, J.; Otero, V.; Sobral, P. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. *Marine Environmental Research* **2014**, *95*, 89–95.
28. Goldstein, M.C.; Rosenberg, M.; Cheng, L. Increased oceanic microplastic debris enhances oviposition in an endemic pelagic insect. *Biology letters* **2012**, *8*, 817–820.
29. Thompson, R.C.; Olsen, Y.; Mitchell, R.P.; Davis, A.; Rowland, S.J.; John, A.W.; McGonigle, D.; Russell, A.E.; others. Lost at sea: where is all the plastic? *Science(Washington)* **2004**, *304*, 838.
30. Garmendia, L.; Soto, M.; Vicario, U.; Kim, Y.; Cajaraville, M.P.; Marigómez, I. Application of a battery of biomarkers in mussel digestive gland to assess long-term effects of the Prestige oil spill in Galicia and Bay of Biscay: tissue-level biomarkers and histopathology. *Journal of Environmental Monitoring* **2011**, *13*, 915–932.
31. Browne, M.A.; Dissanayake, A.; Galloway, T.S.; Lowe, D.M.; Thompson, R.C. Ingested microscopic plastic translocates to the circulatory system of the mussel, Mytilus edulis (L.). *Environmental science & technology* **2008**, *42*, 5026–5031.
32. Lamb, J.B.; Willis, B.L.; Fiorenza, E.A.; Couch, C.S.; Howard, R.; Rader, D.N.; True, J.D.; Kelly, L.A.; Ahmad, A.; Jompa, J.; others. Plastic waste associated with disease on coral reefs. *Science* **2018**, *359*, 460–462.

33. Tang, J.; Ni, X.; Zhou, Z.; Wang, L.; Lin, S. Acute microplastic exposure raises stress response and suppresses detoxification and immune capacities in the scleractinian coral Pocillopora damicornis. *Environmental pollution* **2018**, *243*, 66–74.
34. Zettler, E.R.; Mincer, T.J.; Amaral-Zettler, L.A. Life in the "plastisphere": microbial communities on plastic marine debris. *Environmental science & technology* **2013**, *47*, 7137–7146.
35. Kirstein, I.V.; Kirmizi, S.; Wichels, A.; Garin-Fernandez, A.; Erler, R.; Löder, M.; Gerdts, G. Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. *Marine environmental research* **2016**, *120*, 1–8.
36. Kesy, K.; Labrenz, M.; Scales, B.S.; Kreikemeyer, B.; Oberbeckmann, S. Vibrio Colonization Is Highly Dynamic in Early Microplastic-Associated Biofilms as Well as on Field-Collected Microplastics. *Microorganisms* **2021**, *9*, 76.
37. Carson, H.S.; Nerheim, M.S.; Carroll, K.A.; Eriksen, M. The plastic-associated microorganisms of the North Pacific Gyre. *Marine pollution bulletin* **2013**, *75*, 126–132.
38. Koelmans, A.A.; Besseling, E.; Foekema, E.M. Leaching of plastic additives to marine organisms. *Environmental pollution* **2014**, *187*, 49–54.
39. Matte, G.R.; Matte, M.H.; Rivera, I.G.; Martins, M.T. Distribution of potentially pathogenic vibrios in oysters from a tropical region. *Journal of food protection* **1994**, *57*, 870–873.
40. Toranzo, A.E.; Magariños, B.; Romalde, J.L. A review of the main bacterial fish diseases in mariculture systems. *Aquaculture* **2005**, *246*, 37–61.
41. Zhang, Q.; Dong, X.; Chen, B.; Zhang, Y.; Zu, Y.; Li, W. Zebrafish as a useful model for zoonotic Vibrio parahaemolyticus pathogenicity in fish and human. *Developmental & Comparative Immunology* **2016**, *55*, 159–168.
42. Vinh, D.C.; Mubareka, S.; Fatoye, B.; Plourde, P.; Orr, P. Vibrio vulnificus septicemia after handling Tilapia species fish: a Canadian case report and review. *Canadian Journal of Infectious Diseases and Medical Microbiology* **2006**, *17*, 129–132.
43. Lhafi, S.K.; Kühne, M. Occurrence of Vibrio spp. in blue mussels (Mytilus edulis) from the German Wadden Sea. *International Journal of Food Microbiology* **2007**, *116*, 297–300.
44. Lacoste, A.; Jalabert, F.; Malham, S.; Cueff, A.; Gelebart, F.; Cordevant, C.; Lange, M.; Poulet, S. A Vibrio splendidus strain is associated with summer mortality of juvenile oysters Crassostrea gigas in the Bay of Morlaix (North Brittany, France). *Diseases of aquatic organisms* **2001**, *46*, 139–145.
45. Fabra, M.; Williams, L.; Watts, J.E.; Hale, M.S.; Couceiro, F.; Preston, J. The plastic Trojan horse: Biofilms increase microplastic uptake in marine filter feeders impacting microbial transfer and organism health. *Science of the Total Environment* **2021**, *797*, 149217.
46. Cantet, F.; Hervio-Heath, D.; Caro, A.; Le Mennec, C.; Monteil, C.; Quéméré, C.; Jolivet-Gougeon, A.; Colwell, R.R.; Monfort, P. Quantification of Vibrio parahaemolyticus, Vibrio vulnificus and Vibrio cholerae in French Mediterranean coastal lagoons. *Research in microbiology* **2013**, *164*, 867–874.
47. Nelder, J.A.; Wedderburn, R.W.M. Generalized linear models. *Journal of the Royal Statistical Society, Series A* **1972**, *135*, 370–384.
48. McCullagh, P.; Nelder, J.A. *Generalized linear models (Second edition)*; London: Chapman & Hall, 1989; p. 500.
49. Hastie, T.; Tibshirani, R. *Generalized Additive Models*; London: Chapman and Hall, 1990.
50. Eubank, R. Monotone Regression Splines in Action: Comment. *Statistical Science* **1988**, *3*, 446–450. doi:10.1214/ss/1177012763.
51. Wahba, G. *Spline Models for Observational Data*; Society for Industrial and Applied Mathematics: Philadelphia, 1990.
52. Fan, J.; Gijbels, I. *Local polynomial modelling and its applications*; Number 66 in Monographs on statistics and applied probability series, Chapman & Hall, 1996.
53. Wand, M.P.; Jones, M.C. *Kernel Smoothing*; Chapman & Hall: London, 1995.
54. Wand, M.P. A comparison of regression spline smoothing procedures. *Computational Statistics* **2000**, *15*, 443–462.
55. Fan, J.; Marron, J. Fast implementation of nonparametric curve estimators. *Journal of Computational and Graphical Statistics* **1994**, *3*, 35–56.
56. Golub, G.; Heath, M.; Wahba, G. Generalized cross-validation as a method for choosing a good ridge parameter. *Technometrics* **1979**, *21*, 215–223.
57. Ruppert, D.; Sheather, S.J.; Wand, M.P. An Effective Bandwidth Selector for Local Least Squares Regression. *Journal of the American Statistical Association* **1995**, *90*, 1257–1270.
58. Marron, J.S. Bootstrap bandwidth selection. In *Exploring the Limits of Bootstrap*; LePage, R.; Billard, L., Eds.; Wiley-Interscience, 1992; pp. 249–262.
59. Forster, M.R. Key Concepts in Model Selection: Performance and Generalizability. *Journal of Mathematical Psychology* **2000**, *44*, 205–231.
60. Tibshirani, R. Regression shrinkage and selection via the Lasso. *Journal of the Royal Statistical Society B* **1996**, *58*, 267–288.
61. Hastie, T.; Tibshirani, R.; Friedman, J.H. *The Elements of Statistical Learning*; Springer-Verlag, 2003.
62. Green, P.J. Reversible jump Markov chain Monte Carlo computation and Bayesian model determination. *Biometrika* **1995**, *82*, 711–732.
63. Kuo, L.; Mallick, B. Variable selection for regression models. *The Indian Journal of Statistics (Special Issue on Bayesian Analysis)* **1998**, *60*, 65–81.
64. Park, T.; Casella, G. The Bayesian Lasso. *Journal of the American Statistical Association* **2008**, *103*, 681–686.
65. Venables, W.N.; Ripley, B.D. *Modern applied statistics with S-Plus*, second ed.; Springer-Verlag, 1997.
66. Miller, A. *Subset Selection in Regression*; Chapman and Hall/CRC: Boca Raton, FL, 2002.

67. Sestelo, M.; Villanueva, N.M.; Meira-Machado, L.; Roca-Pardiñas, J. FWDselect: An R Package for Variable Selection in Regression Models. *The R Journal* **2016**, *8*, 132–148.
68. Calcagno, V.; de Mazancourt, C. glmulti: An R package for easy automated model selection with (Generalized) Linear Models. *Journal of Statistical Software* **2010**, *34*, 1–29.
69. Wang, R.; Mou, H.; Lin, X.; Zhu, H.; Li, B.; Wang, J.; Junaid, M.; Wang, J. Microplastics in Mollusks: Research Progress, Current Contamination Status, Analysis Approaches, and Future Perspectives. *Frontiers in Marine Science* **2021**, p. 1671.
70. Kim, Y.; Ashton-Alcox, K.A.; Powell, E.N. Histological techniques for marine bivalve molluscs: update **2006**.
71. Iriarte, A.; Villate, F.; Uriarte, I.; Bidegain, G.; Barroeta, Z. Shifts in neritic copepod communities off the Basque coast (southeastern Bay of Biscay) between 1998 and 2015. *ICES Journal of Marine Science* **2022**, *79*, 830–843.
72. Wood, S.N. Stable and efficient multiple smoothing parameter estimation for generalized additive models. *Journal of the American Statistical Association* **2004**, *99*, 673–686.
73. Wood, S.; N..; Pya.; S"afken, B. Smoothing parameter and model selection for general smooth models (with discussion). *Journal of the American Statistical Association* **2016**, *111*, 1548–1575.
74. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, 2022.
75. Ben-Horin, T.; Bidegain, G.; Huey, L.; Nárvaez, D.A.; Bushek, D. Parasite transmission through suspension feeding. *Journal of Invertebrate Pathology* **2015**, *131*, 155–176.
76. Wu, X.; Pan, J.; Li, M.; Li, Y.; Bartlam, M.; Wang, Y. Selective enrichment of bacterial pathogens by microplastic biofilm. *Water research* **2019**, *165*, 114979.
77. Revel, M.; Châtel, A.; Perrein-Ettajani, H.; Bruneau, M.; Akcha, F.; Sussarellu, R.; Rouxel, J.; Costil, K.; Decottignies, P.; Cognie, B.; others. Realistic environmental exposure to microplastics does not induce biological effects in the Pacific oyster Crassostrea gigas. *Marine pollution bulletin* **2020**, *150*, 110627.
78. Teng, J.; Zhao, J.; Zhu, X.; Shan, E.; Wang, Q. Oxidative stress biomarkers, physiological responses and proteomic profiling in oyster (Crassostrea gigas) exposed to microplastics with irregular-shaped PE and PET microplastic. *Science of The Total Environment* **2021**, *786*, 147425.
79. Bidegain, G.; Powell, E.E.; Klinck, J.M.; Ben-Horin, T.; Hofmann, E.E. Microparasitic disease dynamics in benthic suspension feeders: infective dose, non-focal hosts, and particle diffusion. *Ecological Modelling* **2016**, *328*, 44–61.
80. Bidegain, G.; Powell, E.E.; Klinck, J.M.; Ben-Horin, T.; Hofmann, E.E. Marine infectious disease dynamics and outbreak thresholds: contact, transmission, pandemic infection, and the potential role of filter feeders. *Ecosphere* **2016**, p. e1286.
81. Allen, J.P.; Neely, M.N. Trolling for the ideal model host: zebrafish take the bait. *Future microbiology* **2010**, *5*, 563–569.
82. Sullivan, C.; Kim, C.H. Zebrafish as a model for infectious disease and immune function. *Fish & shellfish immunology* **2008**, *25*, 341–350.
83. Rowe, H.M.; Withey, J.H.; Neely, M.N. Zebrafish as a model for zoonotic aquatic pathogens. *Developmental & Comparative Immunology* **2014**, *46*, 96–107.
84. Runft, D.L.; Mitchell, K.C.; Abuaita, B.H.; Allen, J.P.; Bajer, S.; Ginsburg, K.; Neely, M.N.; Withey, J.H. Zebrafish as a natural host model for Vibrio cholerae colonization and transmission. *Applied and environmental microbiology* **2014**, *80*, 1710–1717.
85. Lu, Y.; Zhang, Y.; Deng, Y.; Jiang, W.; Zhao, Y.; Geng, J.; Ding, L.; Ren, H. Uptake and accumulation of polystyrene microplastics in zebrafish (Danio rerio) and toxic effects in liver. *Environmental science & technology* **2016**, *50*, 4054–4060.
86. Bidegain, G.; Powell, E.; Klinck, J.; Hofmann, E.; Ben-Horin, T.; Bushek, D.; Ford, S.; Munroe, D.; Guo, X. Modeling the transmission of Perkinsus marinus in the Eastern oyster Crassostrea virginica. *Fisheries Research* **2017**, *186*, 82–93.
87. Gilmour, J.P.; Smith, L.D.; Heyward, A.J.; Baird, A.H.; Pratchett, M.S. Recovery of an isolated coral reef system following severe disturbance. *Science* **2013**, *340*, 69–71.
88. Paillard, C.; Jean, F.; Ford, S.E.; Powell, E.N.; Klinck, J.M.; Hofmann, E.E.; Flye-Sainte-Marie, J. A theoretical individual-based model of brown ring disease in Manila clams textitVenerupis philippinarum. *Journal of Sea Research* **2014**, *91*, 15–34.
89. Vega Thurber, R.L.; Burkepile, D.E.; Fuchs, C.; Shantz, A.A.; McMinds, R.; Zaneveld, J.R. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. *Global Change Biology* **2014**, *20*, 544–554.
90. Bowley, J.; Baker-Austin, C.; Porter, A.; Hartnell, R.; Lewis, C. Oceanic hitchhikers–assessing pathogen risks from marine microplastic. *Trends in microbiology* **2021**, *29*, 107–116.
91. Alimba, C.G.; Faggio, C. Microplastics in the marine environment: current trends in environmental pollution and mechanisms of toxicological profile. *Environmental toxicology and pharmacology* **2019**, *68*, 61–74.
92. Zhang, E.; Kim, M.; Rueda, L.; Rochman, C.; VanWormer, E.; Moore, J.; Shapiro, K. Association of zoonotic protozoan parasites with microplastics in seawater and implications for human and wildlife health. *Scientific reports* **2022**, *12*, 1–11.