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Original Article

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A modeling approach to predict population-level impact of silver (Ag) to rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Metal bioavailability plays a pivotal role in determining the toxicity of silver (Ag) to freshwater fish, such as rainbow trout (*Oncorhynchus mykiss*). The current study builds on an existing sodium balance model, a physiological extension of biotic ligand models (BLM). This model mechanistically describes the impact of Ag on Na ionoregulation in rainbow trout and predicts lethal effects under various Ag bioavailability conditions. However, broader implications of Ag toxicity on fish populations and its differential effects across life stages remain underexplored. First, the original extended BLM was improved to account for realistic variability in observed lethal responses of rainbow trout exposed to Ag. Next, a data-driven approach, based on relative survival fractions, was used to account for early life stage (ELS) toxicity. Finally, the physiologically extended BLM approach was integrated into an individual-based model framework to predict environmentally realistic effects of Ag on trout populations (abundance/biomass). Predictions indicate that ELS mainly influence trout population effects (decreased abundance/biomass) when exposed to silver, while effects on adults or juveniles are less critical for population persistence. Juveniles and adults are affected by higher Ag concentrations than embryos and larvae, suggesting greater sensitivity of ELS. Therefore, future development of BLMs for fish should prioritize early life stages to improve understanding of Ag toxicity and its impact on fish populations. This aligns with the goal of reducing animal testing. The presented framework addresses knowledge gaps in ecological risk assessment of Ag on freshwater fish, integrating various modeling approaches—including a physiologically extended BLM—to predict Ag effects on rainbow trout populations.

Keywords: metal toxicity, biotic ligand model, population modeling, rainbow trout, silver

Introduction

Toxicity of metals, such as silver (Ag), to freshwater fish depends on the physicochemical parameters of the receiving water body (McGeer et al., 2000). Water quality criteria for metals are therefore expressed in reference to physicochemical characteristics, such as water hardness, dissolved organic carbon (DOC), or pH (EU Water Directors, 2018; European Commission, 2003). This has led to the development of bioavailability models, such as the Biotic Ligand Model (BLM), which describe the relationship between water quality characteristics, metal bioavailability, and their toxic effects to specific organisms (Di Toro et al., 2001).

Biotic Ligand Models allow for the calculation of interactions between water chemistry and metal toxicity. One of the assumptions of the BLM is that the free metal ion binds to the biotic ligand (BL) after which it can exert toxic effects. For freshwater fish, it is generally assumed that metals bind to the gills, that is, the membrane separating the water phase and the organism's internal vascular system (Webb & Wood, 1998; Wood et al., 1996). For Ag, accumulation at the gill leads to disruption of ionoregulation (Webb & Wood, 1998). This disruption due to Ag exposure can have lethal consequences for freshwater fish: Ag accumulation at the gill disrupts the uptake of sodium (Na) from the environment into the plasma, which leads to a reduction in plasma volume due to osmosis. This eventually leads to hemoconcentration in the vascular system, which increases in blood pressure and blood viscosity, and results in cardiovascular failure and, ultimately, death of the organism (Wood et al., 1996).

Paquin et al. (2002) developed a physiologically based model ("Sodium Balance Model" [SBM]) to further disentangle the relationship between water physico-chemistry, metal accumulation at the gill, and disruption of ionoregulation in freshwater fish. The SBM—a physiological extension of BLM—describes a mechanistic and mathematical relationship between Ag accumulation at the biotic ligand (as calculated with the BLM) and physiological regulation of the Na metabolism in rainbow trout (*Oncorhynchus mykiss*). The predicted endpoint of the extended BLM framework is the median survival time of the population (ET50; the time it takes for 50% of the population to die due to exposure to a specific Ag concentration). Combining both biotic ligand modelling and physiological modeling, the physiologically extended BLM framework allows predictions of rainbow trout survival exposed to Ag under different physicochemical conditions.

Rainbow trout is sensitive to Ag toxicity (Arijs et al., 2021; Davies et al., 1978, 1998). Although the physiologically extended

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BLM can mechanistically explain toxicity of Ag to rainbow trout, some data gaps remain. A first uncertainty is the potential difference in sensitivity to Ag between different life stages. The extended BLM from Paquin et al. (2002) was developed to predict effects of Ag on the ionoregulation of sodium in juvenile and adult rainbow trout. However, standard toxicity tests on fish nowadays often use cell lines or early life stage (ELS) animals (i.e., embryo and larvae) to assess ecotoxicologial effects (e.g., Organisation for Economic Co-operation and Development [OECD], 2019). Using adult animals for vertebrate testing is discouraged due to ethical and animal welfare considerations. Currently, there is thus a discrepancy between the available toxicity data for rainbow trout on ELS and the predictions of the extended BLM for juveniles and adults. Embryos and larvae of rainbow trout have a significantly different physiology than juvenile or adult life stages. An important factor is the development of the gills, which is incomplete in embryos and larvae (Gonzalez et al., 1996). Additionally, there are different Ag effects on ionoregulation between life stages (Brauner & Wood, 2002). These physiological differences and potential contrasts in biotic ligands between life stages suggest that the extended BLM developed for juveniles and adults cannot be used without verification to predict toxic effects of Ag for the earlier life stages of rainbow trout.

This assumption of different toxic mechanisms of Ag for the different life stages of freshwater fish should be assessed by comparing the sensitivities across life stages under comparable physicochemistries. Additionally, there is discussion on the ecological relevancy of effects at different life stages in terms of their consequences at the population level (Janssen et al., 2024). Currently, the physiologically extended BLM only considers effects on juvenile and adult rainbow trout. For assessment of the relevancy of effects at different life stages for rainbow trout survival, an integrated approach needs to be considered at higher levels of organization, such as extrapolating effects to the population level (e.g., effects on population abundance or biomass). Such integrated assessment can be achieved with individual-based modelling (IBM), which can accurately predict population dynamics of fish in the environment (Railsback et al., 2009).

The ET50 has limitations when it comes to prediction and extrapolation to different exposure durations, to different effect percentages, or across levels of biological organization. The ET50 predicted by the SBM represents the timepoint where 50% of the individuals in a population survive at a specific Ag concentration. There is currently no mechanistic relationship in the SBM that allows to predict effects on survival, other than the median effect, as a function of the exposure concentration and duration.

The reasons for the lack of mathematical extrapolation to other effect levels or exposure durations are model structure and the fundamental assumptions of the physiologically extended BLM. The core assumption of the extended BLM is that mortality occurs in rainbow trout when a threshold of 30% decrease in plasma Na is achieved (Paquin et al., 2002; Wood et al., 1996). The predictions of the extended BLM reveal an all-or-nothing response: either the 30% decrease in plasma Na is achieved and the organism dies, or the decrease is less than 30% and the individual survives. Paquin et al. (2002) have originally explained that the point where a 30% decrease in plasma Na is predicted, corresponds to a median survival time, that is, the timepoint where half of the population likely survives. There is thus a clear discrepancy between the deterministic threshold assumed in the extended BLM (i.e., 30% decrease in plasma Na is lethal) and the probabilistic endpoint that is predicted (i.e., probability of 50% survival within the population at a specific Ag concentration). Based on the available ecotoxicological data, however, variability on the lethal threshold of plasma Na decrease has been reported (McDonald et al., 1980; Webb & Wood, 1998). We argue that including variability on the threshold for effects in the extended BLM is necessary to reflect the natural variability that is observed in responses of rainbow trout to Ag exposure. Currently, this realistic variability is lacking in the model developed by Paquin et al. (2002). In addition, further refinement of the extended BLM should allow for easier extrapolation to other effect levels and exposure durations, but also across levels of biological organization, for example, from the individual to the population level.

The goal of the current study was to use the physiologically extension of the BLM from Paquin et al. (2002) combined with the Ag-BLM for rainbow trout (Paquin et al., 2000) and an IBM (Ayllón et al., 2016) to compare sensitivities across different life stages and define the most sensitive and determining life stage for rainbow trout population persistence under Ag exposure. Several steps were considered in the study. First, the extended BLM was refined to reflect variability in the lethal threshold using toxicity data on ionoregulation of rainbow trout. Second, a BLM approach was considered to normalize toxicity data of different studies and calculate the Ag accumulation at the biotic ligand under different physico-chemistries. Finally, an IBM framework was used to predict the impact of Ag exposure on rainbow trout populations, to compare the impact of affected life stages. The InSTREAMgen model (Ayllón et al., 2016) was used to predict population dynamics under Ag exposure. The refined physiologically extended BLM was embedded in the IBM to predict the impact on juveniles and adults. A data-driven approach was considered to predict the effects on ELS. The combined approach of BLM, the physiological extension of BLM, and IBM allows to mechanistically assess how physiological and toxicological processes of Ag exposure affect rainbow trout populations, and identify the life stage driving Ag effects on population abundance or total population biomass.

Material and methods

Modeling overview

The physiologically extended BLM (SBM) from Paquin et al. (2002) predicts the ET50, which has some clear limitations, as argued in the *Introduction*. Here, we first refine the extended BLM, and include a distribution on the lethal threshold for the percentage reduction in plasma Na concentration. However, the refined BLM extension in itself cannot answer the more relevant (ecological) questions on the most sensitive rainbow trout life stage to Ag (under comparable physicochemical conditions) or the most determining life stage for rainbow trout population survival. For that, an integrated modeling approach is needed that includes effects on all life stages and provides an estimate for the impact on rainbow trout populations.

In Figure 1, an overview is provided of the modular design of the modeling framework developed in the current study to assess the relative impact of life stages on population survival under Ag exposure. Different "modules" are considered, comprising BLM modeling, the physiological extension of the BLM (SBM), and a data-driven approach (i.e., the "relative survival fraction" [RSF]). Combining these modules allows for the prediction of effects on all life stages of rainbow trout under specific water chemistry conditions. By combining these effects in an individual-based model (IBM) framework, which takes into account the life history of rainbow trout, the impact on the population can be predicted. The combination of BLM, the physiological extension (SBM), RSF, and IBM thus yields a holistic modeling framework that predicts the effects of Ag on rainbow trout

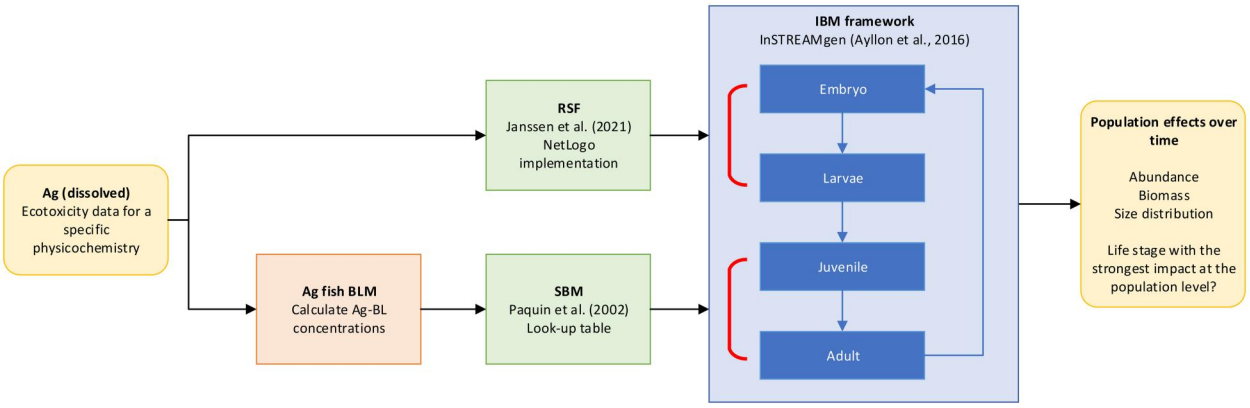

Figure 1. Concept map of the modular design of the integrated modelling framework. *Note*. Ag = silver, BLM = biotic ligand model, RSF = relative survival fraction, SBM = sodium balance model (i.e., a physiological extension of the BLM), IBM = individual-based model. Silver (Ag) ecotoxicity data is normalized using the BLM. RSF is used to calculate embryo and larvae effects, whereas SBM is used for juvenile and adult effects. The IBM (InSTREAMgen) is used to predict effects on population dynamics.

populations for various endpoints (e.g., abundance, biomass, and size distribution; Janssen et al., 2021a).

Data on Ag toxicity to rainbow trout

The original SBM by Paquin et al. (2002) is based on physiological information on Ag toxicity to rainbow trout. Multiple data sources were combined to calibrate and validate the model. For the current study, we further refined the extended BLM by using different data sources for model refinement, validation (internal and independent), and scenario analysis (see Table 1).

To further refine the extended BLM, data from McDonald et al. (1980) and Webb and Wood (1998) were used. These studies investigated the physiological effects of reduced plasma Na levels on the survival of rainbow trout, caused by low pH or exposure to Ag. Parallel measurements of plasma Na levels and rainbow trout mortality were used to derive a lethal threshold distribution. Based on both studies, observed lethal reductions in plasma Na levels varied between 7 and 40% (with a median value of 23%).

The data from McGeer and Wood (1998) as well as Galvez and Wood (1997) were used by Paquin et al. (2002) to validate the predictions of the original SBM. The study of McGeer and Wood (1998) contains plasma Na measurements over time in four different compartments in the organism that are considered in the extended BLM as well. These data were used to directly validate the model predictions of the plasma Na over time. In the current study, these data were used to internally validate the implementation of the SBM equations in RStudio (RStudio Team, 2020; code provided in the online supplementary material Appendix). The data from Galvez and Wood (1997) contain measurements of the ET50 at different Cl and Ca levels (bioassays 1 and 2 mentioned in their study). These data were used by Paquin et al. (2002) to validate the ET50 predictions of the original SBM. In the current study, these data were used to compare the refined model predictions with observations of population ET50-values.

In a third bioassay performed by Galvez and Wood (1997), rainbow trout was exposed to different Ag levels. Mortality as a function of Ag was recorded, for which a dose-response curve (DRC) was established. The data from that experiment were not used for model calibration nor validation by Paquin et al. (2002). In the current study, these DRC data were used only in an independent validation step.

Finally, the data of Davies et al. (1978) were used for scenario analysis with the population model. This ELS toxicity test assesses the effects of Ag exposure on rainbow trout. A 10-week test was performed using freshly hatched rainbow trout larvae, which were exposed until the swim-up phase (i.e., after emergence until the early juvenile life stage). The exposure conditions mentioned in Davies et al. (1978) were used for scenario analysis with the population model (see further).

BLM

The original SBM of Paquin et al. (2002) used the Ag BLM developed by Paquin et al. (2000) for prediction of concentration of Ag accumulated on the biotic ligand. As such, for the present study biotic ligand modeling was also conducted using the methodology proposed by Paquin et al. (2000), which was implemented in the software BLM Ver. 3.41.2.45. Biotic ligand stability constant and gill-site densities are reported in Table 2. For speciation calculations, the amount of dissolved organic matter was assumed to be composed of 100% active substances, with 10% humic acid and 90% fulvic acid (Windward Environmental, 2019). The Ag-BLM was used to translate dissolved Ag concentrations in exposure media to the concentration of Ag accumulated at the biotic ligand, expressed in nanomoles per gram wet weight (Ag: BL; nmol/g_w).

Physiologically extended BLM

Original SBM (*Paquin et al., 2002*)

Paquin et al. (2002) developed the SBM, a physiologically based extension of BLM, that predicts the survival time of rainbow trout (*O. mykiss*) under Ag exposure. More specifically, their model describes an internal mass balance of sodium (Na^+) and how Ag^+ interacts with Na^+-uptake at the gill. The extension is intrinsically connected with a BLM, used to calculate the accumulation of Ag^+ at the biotic ligand, that is, the gill in case of fish. Silver accumulation at the gill will decrease uptake of Na^+ from the environment, leading to disruption of the internal Na balance.

The physiologically extended BLM from Paquin et al. (2002) consists of a set of four ordinary differential equations (ODEs) that describe branchial ionoregulation, and how Na is distributed within the branchial cardiovascular system of the rainbow trout (*Equation 1*). Using these four ODEs, the extended BLM describes the Na balance in four internal compartments: the primary intravascular fluid volume ($IVFV_1$, with internal Na concentration C_1), the secondary intravascular fluid volume ($IVFV_2$, with C_2), the interstitial fluid volume (with C_{IS}), and the intracellular volume (with C_{IC}). Deriviation of the differential equations of the SBM (*Equation 1*) can be found in the online supplementary material (Appendix, Section 2). Explanation of all state variables, parameters, their values, and units are found in Table 3.

Table 1. Overview of the different data sources used in the current study. Studies were selected based on reliability of the test results and specific requirements (*Oncorhynchus mykiss*). Distinction is made between studies used for calibration, internal validation, independent validation, and scenario analysis (extrapolation).

Data used for	Source	Type of data
Model calibration	McDonald et al. (1980)	**Study investigating the influence of Ca on physiological response of rainbow trout to low pH.** Data contain concurrent measurements of decreasing plasma Na concentrations and mortality in adult rainbow trout. Duration: 5 days Life stage: adult Conditions: low pH, soft and hard water Measurements: parallel measurements of plasma Na reduction and survival at different pH and Ca levels
	Webb and Wood (1998)	**Study on the analysis of stress associated with acute Ag exposure in rainbow trout.** Duration: 6 days Life stage: adult Conditions: exposure to $AgNO_3$ Measurements: parallel measurements of plasma Na reduction and survival at different Ag levels
Internal validation	McGeer and Wood (1998)	**Study investigating the protective effects of Cl on physiological responses to Ag exposure in rainbow trout.** Duration: 48 h Life stage: juvenile Conditions: exposure to $AgNO_3$ at different Cl levels Measurements: plasma Na over time at different Ag concentrations
	Galvez and Wood (1997)	**Study investigating importance of water hardness and Cl on acute toxicity of Ag to rainbow trout.** Data from bioassays 1 and 2 Duration: 7 days Life stage: juvenile Conditions: one Ag concentration, four levels of Cl or Ca Measurements: median lethal time (ET50) at different Cl or Ca levels
Independent validation	Galvez and Wood (1997)	**Study investigating importance of water hardness and Cl on acute toxicity of Ag to rainbow trout.** Data from bioassay 3 Duration: 7 days Life stage: juvenile Conditions: 5 Ag concentrations, constant Cl and Ca Measurements: survival at different Ag concentrations
Scenario analysis	Davies et al. (1978)	**Study investigating toxicity of Ag to rainbow trout embryo.** Duration: 10 weeks Life stage: from hatching to swim-up Conditions: 5 Ag concentrations Measurements: survival at different Ag concentrations and different timepoints

Table 2. Overview of parameters of the biotic ligand (BL) model of Paquin et al. (2000) used in the present study.

Parameter	Paquin et al. (2000)	Unit
Gill-site density	3.5	$nmol/g\ dry\ wt$
BL-Ag	7.3	log(L/mol)
BL-AgCl	6.7	log(L/mol)
BL-Ca	2.3	log(L/mol)
BL-H	4.3	log(L/mol)
BL-Na	2.3	log(L/mol)

$$\begin{cases} V_1\frac{dC_1}{dt} = J_M^*\frac{C_w}{C_w+K_M} - P_G(C_1-C_w) + Q_{1,2}(C_2-C_1) + P_{1,IS}(C_{IS}-C_1) - P_rC_1 \\ V_2\frac{dC_2}{dt} = -Q_{12}(C_2-C_1) + P_{2,IS}(C_{IS}-C_2) \\ V_{IS}\frac{dC_{IS}}{dt} = -P_{1,IS}(C_{IS}-C_1) - P_{2,IS}(C_{IS}-C_2) + P_{IS,IC}(C_{IC}-C_{IS}) \\ V_{IC}\frac{dC_{IC}}{dt} = -P_{IS,IC}(C_{IC}-C_{IS}) \end{cases} \quad \text{(Eq. 1)}$$

The maximum Na uptake rate J_M is inhibited by Ag (Paquin et al., 2002). A two-parameter log-logistic equation describes the relationship between the inhibited maximum Na uptake rate (J_M^* in $nmol * (kg_w * d)^{-1}$) and the Ag concentration at the biotic ligand ($BL{:}\,Ag$ in $nmol\ (g_w)^{-1}$) (*Equation 2*). The log-logistic equation has two parameters: the half maximum (50%) effect concentration (EC50 in $nmol\ (g_w)^{-1}$) and the slope (β, no units) of the curve.

$$J_M^* = \frac{J_M}{1+e^{\left(\frac{\ln BL{:}Ag - \ln EC50}{\beta}\right)}} \quad \text{(Eq. 2)}$$

The predicted endpoint of the extended BLM is the *ET50* or the 50% survival time of the population exposed to a specific Ag concentration (*Equation 3*). Due to Ag exposure, Na uptake is reduced (*Equation 2*), leading to a decline in internal Na concentrations in the four compartments described by *Equation 1*. This decrease in internal Na is lethal for rainbow trout once a threshold θ is exceeded. A decrease of 30% is associated ($\theta = 30\%$) with lethal conditions for rainbow trout (*Equation 3*; Wood et al., 1996).

Table 3. Parameters and state variables of the physiological extension of the Biotic Ligand Model (Sodium Balance Model) for silver (Ag) toxicity to rainbow trout (*Oncorynchus mykiss*; Paquin et al., 2002). An asterix (*) indicates the value is case-specific (for parameters) or variable over time (for variables).

Parameter or state variable	Symbol	Value	Unit
Sodium uptake kinetics parameters			
Maximum Na uptake rate	J_M	12	$nmol * (kg_w * d)^{-1}$
Maximum Na uptake rate adjusted for Ag inhibition of J_M	J_M^*	*	$nmol * (kg_w * d)^{-1}$
Active Na uptake rate	J_i	*	$nmol * (kg_w * d)^{-1}$
Half-saturation constant for Na uptake for gill	K_M	0.05	mM
Passive Na gill efflux	J_e	*	$nmol * (kg_w * d)^{-1}$
Passive Na renal efflux	J_r	*	$nmol * (kg_w * d)^{-1}$
Fluid compartment volumes			
Primary intravascular fluid volume	V_1	0.023	$(kg_w)^{-1}$
Secondary intravascular fluid volume	V_2	0.048	$(kg_w)^{-1}$
Interstitial fluid volume	V_{IS}	0.099	$(kg_w)^{-1}$
Intracellular fluid volume	V_{IC}	0.160	$(kg_w)^{-1}$
Fluid compartment sodium concentrations			
External water Na concentration	C_w	0.040	mM
Na concentration at initial condition	C_i	138	mM
Primary vascular system plasma Na concentration	C_1	*	mM
Secondary vascular system Na concentration	C_2	*	mM
Interstitial fluid volume Na concentration	C_{IS}	*	mM
Intracellular fluid volume Na concentration	C_{IC}	*	mM
Permeability coefficients			
Gill permeability of control fish	P_{Go}	0.0386	$(kg_w * d)^{-1}$
Gill permeability of treatment (exposed fish)	P_G	*	$(kg_w * d)^{-1}$
Gill permeability factor	f_{PG}	*	–
Lead coefficient in the expression for f_{PG}	a	$3.09 * 10^{-4}$	–
Exponent for $[Ag^+]$ in the expression for f_{PG}	b	2.05	–
Exponent for $[Ca^{2+}]$ in the expression for f_{PG}	c	−0.22	–
Permeability between primary vascular system and the interstitial fluid compartment	$P_{1,IS}$	0.10	$(kg_w * d)^{-1}$
Permeability between secondary vascular system and the interstitial fluid compartment	$P_{2,IS}$	0.10	$(kg_w * d)^{-1}$
Permeability between the interstitial fluid compartment and the intracellular fluid compartment	$P_{IS,IC}$	0.10	$(kg_w * d)^{-1}$
Renal loss rate permeability	P_r	*	$(kg_w * d)^{-1}$
Primary to secondary plasma skimming flow rate	Q_{12}	0.159	$(kg_w * d)^{-1}$
Sodium uptake inhibition dose-response parameters (2-parameter log-logistic relationship)			
Dissolved silver in exposure water	Ag	*	$\mu g * L^{-1}$
Biotic ligand silver, calculated with the BLM	$BL:Ag$	*	$nmol\ (g_w)^{-1}$
BL: Ag associated with 50% inhibition in sodium uptake	$EC50$	15.8	$nmol\ (g_w)^{-1}$
Slope of the dose-response curve for sodium inhibition	β	0.278	–

$$ET50 = f([Ag^+], \theta, C_1, C_2, C_{IS}, C_{IC}) \quad \text{(Eq. 3)}$$

The physiologically extended BLM was implemented in RStudio (RStudio Team, 2020). The code is available for download (see online supplementary material Appendix). All parameters and state variables from the model extension are reported in Table 3. Original parameter values calibrated by Paquin et al. (2002) were used.

Refinement of the SBM

Paquin et al. (2002) assumed that a 30% decrease in plasma Na concentration is lethal for rainbow trout, based on evidence from physiological measurements of plasma Na reductions in relation to rainbow trout mortality (McDonald et al., 1980; Webb & Wood, 1998; Wood et al., 1996). Despite giving evidence for the lethal reductions in plasma Na, no information is provided by Paquin et al. (2002) on the actual derivation of the strict threshold of 30%. According to data reported by McDonald et al. (1980) and Webb and Wood (1998), there exists variability in the lethal threshold associated with reductions in plasma sodium levels (Table 4).

Our hypothesis is that the threshold of an individual in a population of rainbow trout follows a specific distribution, for example, the normal distribution. The null hypothesis is that the individual threshold θ_i (in %) follows a normal distribution with a mean threshold μ and standard deviation σ (*Equation 4*). To assess normality, the Shapiro-Wilk test for normality was performed, using the *shapiro.test()* function in RStudio (2020). The raw measurements of McDonald et al. (1980) and Webb and Wood (1998) were used, as these contain parallel measurements of plasma Na and mortality. The Shapiro-Wilk normality test demonstrated that the assumption of normality has not been violated ($p = 0.6186$). The mean threshold μ of the fitted normal distribution is 25.6% and the standard deviation σ is 8.9% (see distribution plot in online supplementary material, Appendix, Section 1, Figure S1).

$$H_0: \ \theta_i \sim \mathcal{N}(\mu, \sigma^2) \quad \text{(Eq. 4)}$$

The refined model predicts individual survival times, that is, the timepoint where an individual with a specific threshold would die following Ag exposure. We now define the individual survival time T_i (in days) as a function of the Ag concentration, their individual threshold θ_i (in %; *Equation 4*), and their plasma Na concentrations as predicted by the refined SBM (*Equation 5*).

$$T_i = f([Ag^+], \theta_i, C_1, C_2, C_{IS}, C_{IC}) \quad \text{(Eq. 5)}$$

Internal validation

The refined model was first internally validated by comparing predictions with those of the original model and the data from McDonald et al. (1980). The conditions mentioned by Paquin et al. (2002) were used to predict the Na levels in the different

Table 4. Measurements of lethal decreases in internal plasma sodium (Na) concentrations. For two sources, mortality and a decrease in plasma Na levels were observed in parallel for rainbow trout. The day of death and the percentage decrease in plasma Na concentration are reported. NM = not measured.

Source	Day of death	%Decrease in plasma Na+
Webb and Wood (1998)	5	20%
	5	20%
McDonald et al. (1980)	9	NM
	4	NM
	4	40%
	3	NM
	5	30%
	1	7%
	4	29%
	5	23%
	2	21%
	5	34%
	5	36%
	3	21%

compartments over time, at different Ag concentrations. Predictions were assessed visually by plotting the refined extended BLM predictions with the original predictions and the data from McDonald et al. (1980; see online supplementary material: Appendix, Section 1, Figure S2).

An additional internal validation was performed using the data from Galvez and Wood (1997). They reported ET50-values for different Ca levels. Their conditions were used to predict individual survival times using the refined extended BLM. The range of predicted survival times were plotted for the different Ca levels, together with the ET50 predictions from the original model, in addition to the measurements of ET50 values. The data at different Cl levels from Galvez and Wood (1997) were not used because there was more uncertainty on the physicochemical characteristics of the water for these treatments (i.e., Ca, Cl, Na, and Ag concentrations).

Independent validation

An independent validation was performed using the dose-response data from the third bioassay reported by Galvez and Wood (1997). Their DRC data were used to compare extended BLM predictions with a deterministic threshold (i.e., fixed to 30%) and with a probabilistic threshold (i.e., normal distribution on the threshold). Additionally, the variability of the maximum sodium uptake (J_M) was also assessed separately. The variability on this parameter that was originally reported in Paquin et al. (2002) was used to assess the influence of this parameter on predicting acute 7-day dose-response relationships.

The dose-response relationship was replicated by simulating the 7-day test with the refined extended BLM. For each Ag concentration, the percentage of surviving individuals was calculated at Day 7 of the exposure. A Monte Carlo approach was used, using the refined model ($n = 10^6$). The dose-response relationship was then established by plotting the number of survivors as a function of the Ag concentration.

Integrated population model framework

Population model

The InSTREAMgen model (Ayllón et al., 2016) is an IBM that predicts the life history of trout in streams. The model is an IBM (sometimes called agent-based model) which mathematically describes all life aspects of rainbow trout, that is, growth, feeding, spawning, reproduction, embryo development, life stage transition, ageing and mortality, and so on. The model is implemented in NetLogo (Ver. 5.0.4; Wilensky 1999). InSTREAMgen was a continuation of InSTREAM, a spatially explicit IBM for trout (rainbow and brown trout; Railsback et al., 2009). The InSTREAMgen model was developed for brown trout (*Salmo trutta*) originally. Here, we used the adapted version from Janssen et al. (2021b) that accounts for the life history of rainbow trout, using model parameters for rainbow trout from Railsback et al. (2009; e.g., fish weight-length relationships, spawning-related parameters, and Redd mortality parameters—for details, see the online supplemental material provided by Janssen et al., 2021b).

The InSTREAMgen model simulates population dynamics in discrete, daily timesteps. The model has a spatially explicit structure consisting of different patches that together form a realistic stream (River Belagua, northern Spain). Because the model is individual-based, the individual is the central entity of the model. Individuals have state variables (e.g., length) that describe their condition and life history parameters during a simulation. Interactions between individuals and their environment lead to the emergence of population dynamics—for instance, how the population abundance changes over time due to changes in reproduction and mortality. Different environmental factors are considered, such as the water temperature, food availability and competition, predation pressure, and others.

Ag toxicity effects

The physiologically extended BLM is used to normalize effects on juveniles and adults for specific water physico-chemistries and Ag concentrations. The extended BLM, as implemented in RStudio, was used to generate a look-up table. For a range of Ag concentrations (from 0.1 to 35 nmol/$g_{dry\ wt}$) and a range of internal plasma Na thresholds (from 1% to 90%), individual survival times were calculated with the SBM. The total dissolved Ag concentration is assumed to be below 30 μg/L, so no correction on the gill permeability is assumed (see online supplementary material: Appendix, Section 2, Equation S8). The look-up table is imported in NetLogo. When a new individual is created in the simulation, their individual threshold is drawn randomly from the fitted normal distribution. Based on the environmental Ag concentration, and the individual threshold, the individual survival time of each adult and juvenile individual is determined from the look-up table. Once this survival time is exceeded, the individual is removed from the simulation (i.e., simulated death).

To include effects on ELS, such as the embryonic and larval stage, a data-driven approach is considered, based on the RSF (Janssen et al., 2021b). The RSF (in %) is calculated by dividing the observed percentage survival of a treatment by the percentage survival of the control treatment (*Equation 6*). The percentage of survival is calculated by dividing the number of survivors in a treatment at a specific timepoint (N_t) by the initial number of individuals that are exposed in the treatment (N_0), multiplied by 100. The RSF is directly implemented in the IBM. The RSF at a specific exposure concentration determines the decrease in the number of viable eggs/larvae in a redd at a specific Ag concentration.

$$RSF_t = \frac{Observed\ survival}{Control\ survival} \text{ with } survival = \frac{N_t}{N_0} * 100\% \qquad \text{(Eq. 6)}$$

Figure 2 compares the simulated trout life phases in the population model with the biological life stages. The InSTREAMgen model assumes two model phases: the Redd phase and the free-swimming trout phase. The Redd phase simulates two biological life stages: the egg stage and the larval stage. The egg stage starts

from fertilization and includes the embryo stage and the eyed egg stage. The larval stage starts after hatching, also called the yolk-sac larvae stage or alevins. The free-swimming trout phase corresponds to three different biological life stages: the swim-up fry, parr, and adults. The (swim-up) fry are early juveniles that switch from passive feeding from their yolk sac to active freeding. Fry will mature into parr. Eventually, when sexual maturation is achieved, they become adult trout.

Depending on the modeled life phase, effects are calculated differently. For the Redd phase (embryo and larvae), the effects of Ag are implemented via the RSF method. For a specific dissolved Ag concentration, the RSF will determine how many viable eggs/larvae are present in a Redd, and thus how many juvenile individuals emerge from the Redd. Note that the RSF is calculated for a specific dissolved Ag concentration. No bioavailability correction was performed for multiple reasons: there was only one suitable study with ELS effects (see further in this section), no ELS BLM currently exists, and the RSF can be calculated directly per treatment. The magnitude of the RSF at a specific Ag concentration is dependent on the reliable data on embryo effects that is used (see further). In contrast, for the free-swimming trout phase, the effects of Ag are calculated via the SBM. Based on the predicted individual survival time from the SBM, individuals are removed from the simulation (i.e., simulated death) when their exposure time exceeds this individual survival time at a specific Ag concentration.

Scenario analysis

The combined model framework (BLM, physiologically extended BLM (SBM), RSF, and IBM; Figure 1) was used to assess the impact of Ag on rainbow trout populations and, more specifically, determine what life stage is most sensitive or more determining for population survival of rainbow trout under Ag exposure. First, suitable data were needed for extrapolation to predict effects at the population level. Data were assessed based on several requirements. First, a long-term exposure study on rainbow trout ELS was necessary, preferably covering all stages from hatching to swim-up. Second, the data needed to be of good quality, meaning that reliable methods had to be used. Third, a full dose-response in effects was required. This included at least a control, one concentration resulting in full effects (a 100% decrease in the endpoint), and one concentration with intermediate effects (less than a 100% decrease in the endpoint). Finally, water chemistry parameters had to be provided to support the validity and relevance of the study.

Online supplementary material Table S1 in the Appendix (Section 3) provides an overview of reliable Ag toxicity studies on ELS of fish. Only one study was considered fit for purpose: the ELS toxicity test performed by Davies et al. (1978; Table 1). They exposed ELS of rainbow trout, from hatching to swim-up, to five concentrations of Ag and a control (0, 0.6, 1.2, 2.2, 5.1, and 10 μg dissolved Ag per L) for 10 weeks. Mortality was recorded daily. For each treatment, the RSF at the end of the test was calculated (*Equation 6*). The reported water chemistry parameters were used to normalize the Ag concentrations and calculate the Ag accumulation at the biotic ligand needed to calculate the juvenile and adult effects for those treatments with the SBM (temperature = 6 °C, pH = 7.02, DOC = 1.7 mg/L, hardness = 30.4 mg $CaCO_3$/L, alkalinity = 30.7 mg $CaCO_3$/L).

The Ag-BL concentrations from the BLM were used as input for the extended BLM to predict the percentage mortality in juveniles and adults after 10 weeks of exposure for each respective treatment from Davies et al. (1978). These were then compared to predicted population level effects in a scenario analysis. Four prediction scenarios were considered to assess Ag effects on rainbow trout populations. The first, the control scenario, assumed no exposure to Ag, meaning the exposure concentration was set at 0 μg Ag per liter. The second, known as the RSF scenario, considered only effects on embryos and larvae, with extrapolations made using the RSF approach. The third, the SBM scenario, focused solely on juveniles and adults, extrapolating effects using the extended Biotic Ligand Model (SBM) approach. Finally, the combined scenario accounted for effects on all life stages of rainbow trout by applying both the RSF and SBM approaches.

Considered population endpoints were total population abundance (number of individuals in the population) and total population biomass. The exposure conditions of Davies et al. (1978) were considered (i.e., 0, 0.6, 1.2, 2.2, 5.1, and 10 μg dissolved Ag per L—with the calculated Ag-BL concentrations for the SBM approach). For each scenario, and each tested concentration, 10 simulation iterations were run. The IBM simulates trout population dynamics over 10 years. Of these 10 simulations, the average endpoint for each year (on August 28, and August 27 in leap years) is calculated.

Results

Refinement of the physiologically extended BLM

The ET50 values observed by data of Galvez and Wood (1997) were compared to the predictions of the refined extended BLM and those of the original model (SBM) by Paquin et al. (2002; Figure 3). The same conditions and parameters as mentioned in Paquin et al. (2002) were used to compare predictions. The predictions with the refined extended BLM closely reflect the observations of Galvez and Wood (1997; see solid line in Figure 3). The predicted ET50 values of the refined model are slightly lower than those of the original SBM. This is expected because the

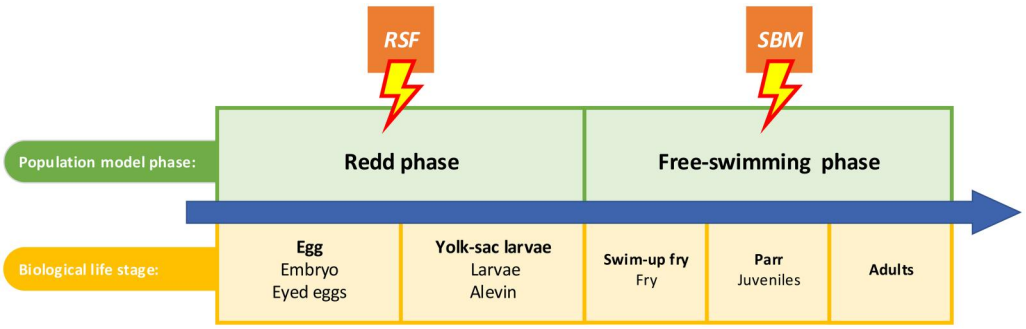

Figure 2. Comparison between the population model phases and the biological life stages of rainbow trout. Effects of silver (Ag) are simulated via either the relative survival fraction-method (for redds) or the sodium balance model method (for free-swimming trout). *Note*. RSF = relative survival fraction, SBM = sodium balance model (a physiological extension of the biotic ligand model).

average of the threshold distribution is lower than the threshold of Paquin et al. (2002): 25.6% in the refined model versus 30% in the original model.

The overall predicted trend of the refined model matches the ET50 observations of Galvez and Wood (1997) and the original model (Figure 3). There is an increase in ET50 predicted with increasing Ca concentration. This increase is explained by the effects of calcium on the gill permeability; the gill permeability decreases as the Ca level in the water increases (Paquin et al., 2002; see online supplementary material: Appendix, Section 2, Equation S9). At low Ca, the gill permeability is high and Na kinetics are fast. This high permeability at low Ca concentrations predicts that the lethal threshold is achieved more quickly, thus leading to lower individual survival times. In contrast, at high Ca concentrations, permeability is low, leading to slow Na kinetics. Consequently, higher individual survival times are predicted at high Ca concentrations.

The refined model predicts a range in individual survival times, which is highlighted by the shaded area in Figure 3. At lower Ca concentrations, the predicted range is narrower than at higher Ca concentrations. The predicted increase in variability is explained as well by the influence of Ca on membrane permeability and Na kinetics. For detailed explanation on the emerged variability and the predicted increase in variability at higher Ca concentrations, we refer to the online supplementary material: Appendix, Section 4. In short: the kinetics follow first an exponential increase in Na concentration due to uptake, followed by an equilibrium phase. At low Ca, Na uptake occurs fast, and the threshold will be in the exponential phase. This is associated with low variability in the predicted distribution in survival times. In contrast, at high Ca and slow Na kinetics, the threshold will be achieved in the equilibrium phase, which leads to high variability in predicted ET50 values (see online supplementary material: Appendix, Section 4, Figure S4A and B).

Compared to the original model, the variability in survival time predicted by the refined model better reflects the survival as a function of the free Ag concentration, observed by Galvez and Wood (1997; Figure 4). The original extended BLM (red line in Figure 4) either predicts population persistence (100% surviving individuals), when the 30% decrease is not achieved, or population extinction (0% surviving individuals), when the 30% threshold is achieved. This leads to a nonlinear dose-response or, more specifically, an all-or-nothing response (red curve in Figure 4), which clearly contrasts the observations from Galvez and Wood (1997). In comparison, implementing variability on the threshold, a more gradual dose-response is predicted. With variability on the threshold implemented, the lethal threshold of some individuals at a specific Ag concentration will be reached on Day 7 of the exposure, meaning some individuals die, while others survive. A gradual dose-response is also predicted assuming variability in the maximum Na uptake parameter (J_M; blue curve in Figure 4), as reported in Paquin et al. (2002). However, the slope of the Galvez and Wood (1997) observations is more gradual compared to the refined extended BLM. In addition, the half-maximum effect concentration of the curve is higher than that of the refined model. Based on the validation and comparison, the model with variability on the threshold better reflects the observed survival as a function of the Ag concentration.

Population predictions

For different lethal thresholds and biotic ligand silver concentrations, individual survival times of rainbow trout were predicted (Figure 5). As the threshold increases, the predicted survival time increases as well. This is expected, because it will take longer for

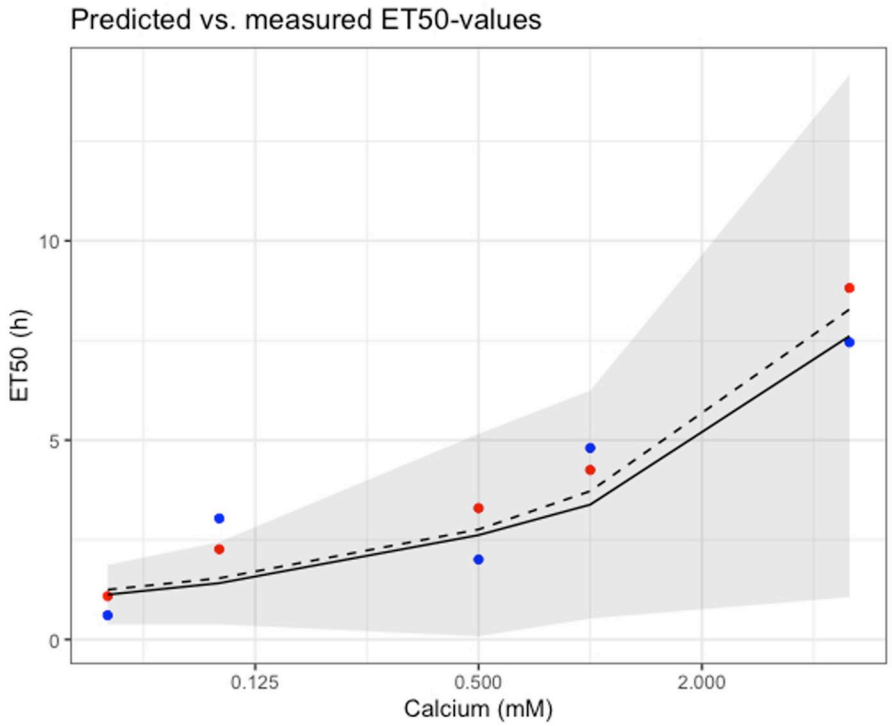

Figure 3. Comparison of predicted (lines) with measured median lethal times (ET50) values for rainbow trout. Data (dots) from Galvez and Wood (1997). The solid line represents the predicted ET50 with the refined sodium balance model (SBM; average of the predicted individual survival times), whereas the dashed line represents the ET50 with the original SBM from Paquin et al. (2002). The gray area represents the 90% confidence intervals on the median of the survival time distribution. This range in predictions is caused by variation on the lethal threshold within the refined SBM. Measured ET50 are from rainbow trout exposed to Ag (±100 μg/L dissolved Ag), under variable treatments of $CaSO_4$ (red dots) and $Ca(NO_3)_2$ (blue dots).

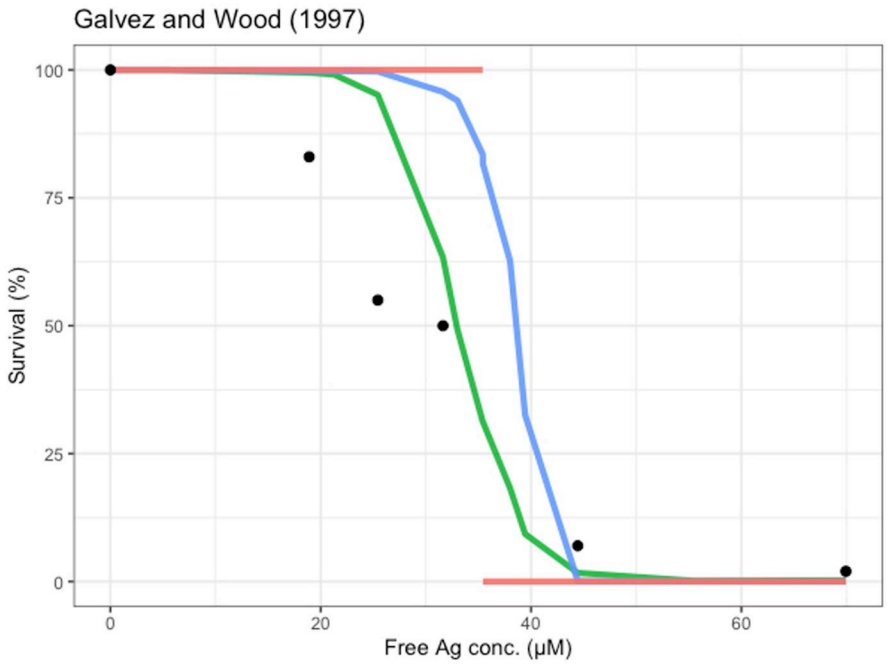

Figure 4. Comparison of predicted (lines) with observed (points) survival of juvenile rainbow trout exposed to Ag (7-day exposure). Data from Galvez and Wood (1997). The red line represents the predicted survival with the physiologically extended biotic ligand model (BLM) of Paquin et al. (2002). The green line represents the predicted survival with the refined extended BLM, assuming variability on the individual lethal threshold for Na decrease. The blue line represents the predicted survival with the refined extended BLM, assuming variability on the maximum Na uptake rate.

a higher threshold of plasma Na reduction to be achieved due to Ag exposure. When the biotic ligand Ag concentration increases, the individual's survival time decreases. This is also expected, because a higher Ag accumulation at the biotic ligand will decrease Na-uptake significantly. A look-up table is generated based on the refined extended BLM predictions (Figure 5), which was implemented in the InSTREAMgen population model to predict the impact on rainbow trout populations.

Figures 6 and 7 show the population predictions with the InSTREAMgen population model for the physico-chemistry conditions of Davies et al. (1978). In Figure 6, predicted population abundance and biomass over time at 1.2 μg dissolved Ag per L and control conditions are plotted. The population abundance of the control predictions (red line in Figure 6—left) fluctuates over time. This is mainly due to yearly variations in environmental conditions, such as water flow, turbidity, food availability, and water temperature, which impacts the population dynamics of rainbow trout (see Ayllón et al., 2016, for details). When Ag effects are implemented, no significant effects on population variables are predicted at 1.2 μg dissolved Ag/L. For all scenarios with Ag exposure (RSF—ELS effects only, SBM—juvenile and adult effects only, and RSF + SBM—all life stages exposed), the range in predicted abundance and biomass completely overlaps with that of the control prediction in Figure 6. There are some minor differences in predicted average abundance and biomass between control and some of the scenarios at specific timepoints. However, these differences can be attributed to stochasticity that arises between simulation iterations. A total of 10 iterations per scenario were simulated, because this is a good tradeoff between computer simulation time and predicting the average behavior of the InSTREAMgen model (similar to Janssen et al., 2021a).

At 2.5 μg dissolved Ag/L, no effects are predicted for the SBM-only scenario (i.e., only effects on juveniles and adults; Figure 7). The implemented effects of Ag on juveniles and adults did not cause a significant impact on either population abundance or biomass. In contrast, when effects on embryo or larvae are considered (the RSF-only scenario), significant effects are predicted. Both abundance and biomass steadily decrease over the years, until the population becomes extinct at the end of the 10-year simulation. The scenario that includes effects on all life stages (the RSF + SBM scenario) predicts a similar trend. At 2.5 μg diss. Ag/L, the population is predicted to become extinct based on the InSTREAMgen.

Table 5 includes a comparison between the implemented individual-level effects for each treatment of Davies et al. (1978) and the predicted effect at the population level (abundance and biomass). The observed relative survival fraction after 70 days of embryo/larvae exposure in Davies et al. (1978) was 36% in the lowest exposed treatment (1.2 μg dissolved Ag/L) and 100% in the higher concentrations. Using the refined extended BLM, at 1.2 μg dissolved Ag/L, no mortality of adults or juveniles was predicted by the SBM. At 2.5 μg dissolved Ag/L, only 2.12% mortality is predicted on juvenile and adult trout. This percentage further increases to 87.2 and 99.7% at 5 and 10 μg dissolved Ag/L respectively.

The percentages of individual-level mortality were extrapolated to the population level using InSTREAMgen (Table 5). At the lowest exposed treatment, no effects were predicted at the population for any of the three scenarios. Even with 36% embryonic and larval mortality, no impact on population abundance or biomass is predicted. In contrast, at 2.5 μg dissolved Ag/L, the population is predicted to become extinct (Figure 7). Note that at this concentration 100% mortality on embryo and larvae is assumed. The 2.2% mortality with the SBM approach did not lead to any predicted impact on population abundance or biomass. At 5 and 10 μg dissolved Ag/L, the population is predicted to become extinct for all three simulated scenarios.

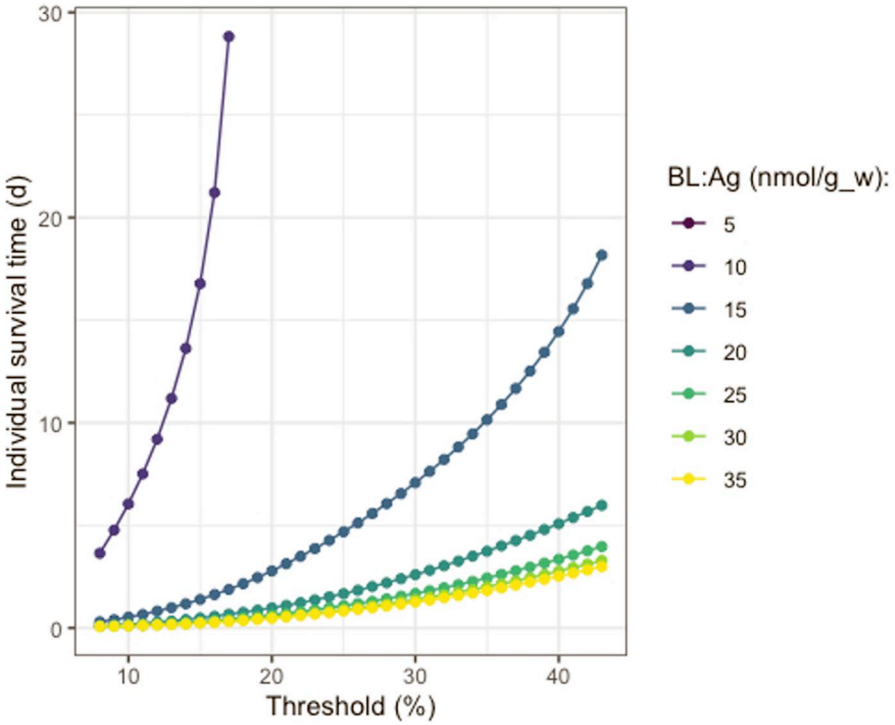

Figure 5. Refined predictions of the physiologically extended biotic ligand (BL) model for individual survival time as a function of the individual threshold and the BL silver concentration. The horizontal axis represents the individual threshold for lethal decrease in plasma Na concentration. The vertical axis depicts the predicted individual survival time. The colored lines connect the predicted individual survival times for discrete BL silver concentrations.

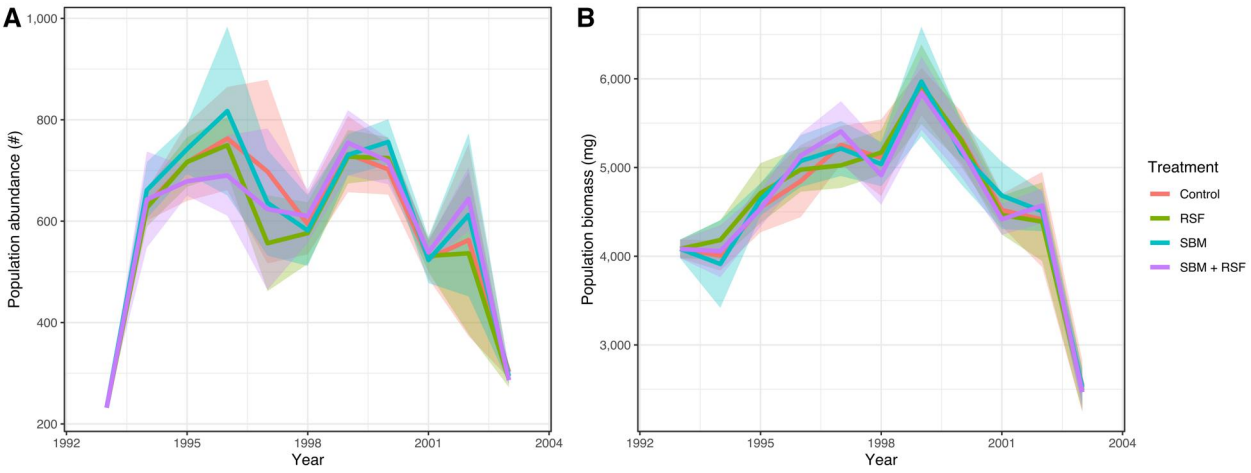

Figure 6. Individual-based model predictions of population-level effects of Ag to rainbow trout populations at 1.2 μg dissolved Ag per L (green, blue and purple lines) compared to the control (red line; no Ag exposure). Population abundance (left) and population biomass (right) is plotted over time. The lines represent the average prediction of 10 simulations; the shaded area represents the minimum and maximum prediction. The green line shows the prediction of the relative survival fraction (RSF)-only scenario, that is, only effects on embryos and larvae are assumed. The blue line shows the prediction of the sodium balance model (SBM)-only scenario, that is, only effects on juveniles and adults. The purple line depicts the combined RSF and SBM scenario, where effects on all life stages are assumed. *Note*. RSF = relative survival fraction (i.e., effects of Ag on embryonic and larval life stage), SBM = sodium balance model (physiologically extended biotic ligand model; i.e., effects of Ag on juvenile and adults).

Discussion

Refined physiologically extended BLM

The physiologically extended BLM, as originally defined by Paquin et al. (2002) was developed to account for the effects of Ag on Na uptake in rainbow trout and how disturbances in the internal Na balance lead to mortality (Wood et al., 1996). Silver binding to the gill causes a net loss of Na due to inhibition of the gill basolateral Na^+/K^+-ATPase (McGeer & Wood, 1998; Morgan et al., 1997). Wood et al. (1996) speculated that death occurs when plasma Na concentration drops by approximately 30%. This was the basis for the lethal threshold set by Paquin et al. (2002) in their model implementation. However, this strict threshold ignores potential inter-individual variability in toxic response of rainbow trout. Bianchini et al. (2002) argue that

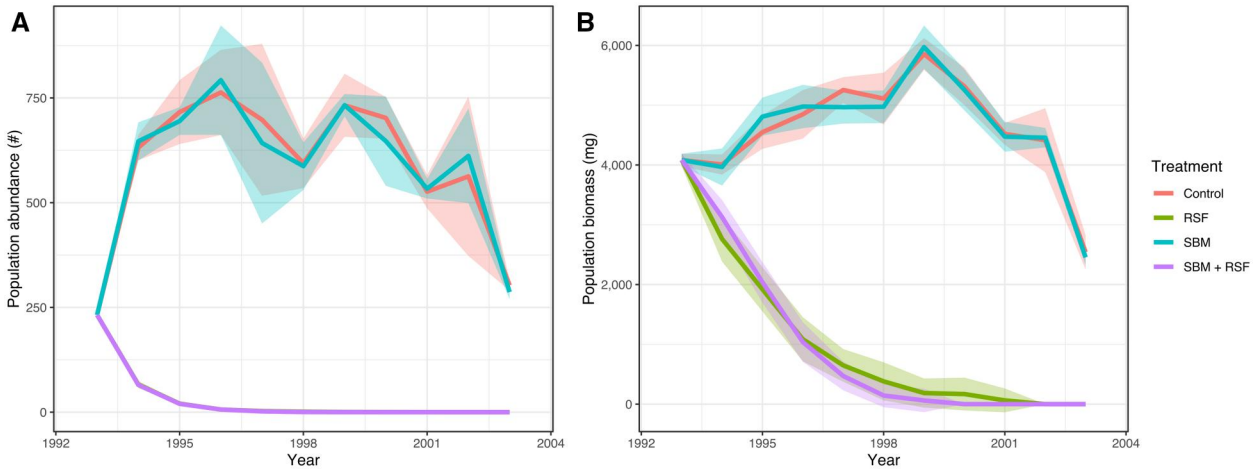

Figure 7. Individul-based model predictions of population-level effects of Ag to rainbow trout populations at 2.5 μg dissolved Ag per L (green, blue, and purple lines) compared to the control (red line; no Ag exposure). Population abundance (left) and population biomass (right) is plotted over time. The lines represent the average prediction of 10 simulations; the shaded area represents the minimum and maximum prediction. The green line shows the prediction of the relative survival fraction (RSF)-only scenario, that is, only effects on embryos and larvae are assumed. The blue line shows the prediction of the sodium balance model (SBM)-only scenario, that is, only effects on juveniles and adults. The purple line depicts the combined RSF and SBM scenario, where effects on all life stages are assumed. *Note*. RSF = relative survival fraction (i.e., effects of Ag on embryonic and larval life stage), SBM = sodium balance model (physiologically extended biotic ligand model; i.e., effects of Ag on juvenile and adults). Note that for population abundance (left), the predictions of the RSF scenario (green) and the combined RSF and SBM scenario (purple) overlap.

Table 5. Implemented individual-level and predicted population-level effects of Ag to rainbow trout. RSF = relative survival fraction (Ag effects on embryo and larvae) SBM = sodium balance model (physiological extension of the BLM; Ag effects on juveniles and adults), AB = abundance, BM = biomass.

Treatment (in μg dissolved Ag/L; Davies et al., 1978)	Individual-level mortality (70 days)		Population-level effect (10 year)					
	RSF	SBM	RSF only		SBM only		SBM + RSF	
			AB	BM	AB	BM	AB	BM
1.2 μg/L	36%	0%	0%	0%	0%	0%	0%	0%
2.5 μg/L	100%	2.2%	100%	100%	0%	0%	100%	100%
5 μg/L	100%	87.2%	100%	100%	100%	100%	100%	100%
10 μgL	100%	99.7%	100%	100%	100%	100%	100%	100%

smaller-sized individuals have a larger mass-specific surface area in the gills, and therefore require higher mass-specific Na uptake rates to balance out ion losses. Inhibition of active ion uptake will therefore affect smaller-sized individuals more strongly than individuals of larger size. However, physiological differences between individuals are not accounted for in the original model.

The original extended BLM predicts internal Na balance and median lethal times (ET50-values) for rainbow trout under various conditions, but it has limitations due to its strict assumption that mortality occurs only when there is a 30% reduction in internal plasma Na. This all-or-nothing approach lacks realistic variability, as experimental data show a gradual dose-response relationship between Ag exposure and trout mortality. Implementing this normal distribution on the individual threshold reflects the observed dose-response of Galvez and Wood (1997) much better (Figure 4). Note that the predictions in Figure 4 are independent, meaning the data were not used to calibrate the model.

Intra-specific response variability of rainbow trout exposed to Ag emerges from two different causes: either due to physiological differences between individuals, or due to chemical variations in Ag-gill binding (Taylor et al., 2003). Physical differences between individuals in the population can lead to different sensitivities toward Ag. An example is the number of Ag binding sites at the gill due to a larger physical gill surface area (Taylor et al., 2003). A relatively higher number of Ag binding sites at the gill will likely increase the potential of Ag to interfere with Na uptake. Taylor et al. (2003) have reported significant intra-specific differences in whole-body Na (a relative standard error up to 12%) in rainbow trout. A larger exchangeable Na pool will likely be associated with more robustness towards disturbances in Na uptake. Taylor et al. (2003) argue that sensitivity to Ag depends on the tolerance and resistance of Na loss. The Na loss was observed to be increased in more sensitive species. Currently, the refined model assumes no physiological differences between individuals, for example, there is a constant Na exchange pool. The volumes of the different compartments in the extended BLM (intravascular, interstitial, and intracellular) are also assumed fixed. This originally made sense because the timeframe within which the extended BLM was applied in the study of Paquin et al. (2002) only comprised a few hours up to maximum two days of exposure to Ag. Within those short timeframes, the total compartment volumes are expected to increase minimally with growth. Additionally, variability was assumed low, because exposed individuals were more or less of the same age, length, and weight. However, in reality, juvenile and adult fish will grow, meaning the compartment volumes and the total exchangeable Na pool will likely increase over time.

Differences in sensitivity can also be caused by variability in Ag-gill binding chemistry. These processes are covered by the

BLM, which calculates the accumulation of Ag at the gill. Variability in BLM-related parameters is currently not considered. These are expected to differ minimally between individuals of the same species. This is mainly because the parameters of the BLM express chemical rates or interactions, which are assumed to be conserved across organisms of the same species (Van Regenmortel et al., 2015). Nonetheless, there are factors related to Ag-gill binding that could slightly differ between individuals, such as the affinity of Ag to bind to the gill (i.e., Ag attraction), the total Ag capacity of the gill (i.e., Ag saturation), or bound Ag that is not reactive. Additionally, the current BLM (as well as the SBM) does not include processes such as detoxification, storage, and elimination of Ag. These processes are not included explicitly in the Ag-BLM, although they are indirectly present due to the calibration of the model using real toxicity data.

Despite the improved predictive capabilities of the refined physiologically based BLM, one of the main limitations of the model framework is the applicability to fish ELS. The extended BLM was originally developed to account for Ag effects on the Na balance in juvenile and adult trout, and the ionoregulation and toxicity data for model development and validation were exclusively based on juvenile and adult rainbow trout (Paquin et al., 2002). The extended BLM cannot be used as such for the embryonic and larval life stage because the physiology between these early versus later life stages is vastly different (Gonzalez et al., 1996). The uptake and related toxicity of Ag to juvenile and adults occurs via the gill (Wood et al., 1996). Contrarily, trout embryos do not possess gills, whereas those of the larvae are underdeveloped. Moreover, there are clear differences in ionoregulatory disturbances between early versus later life stages (Brauner & Wood, 2002). It is also shown that the chorion of embryos plays a protective role against Ag by storing accumulated Ag before it can reach the embryo (Guadagnolo et al., 2000). Additionally, increased sensitivity to Ag has been observed immediately after egg hatching, which could be attributed to the role of ionoregulation during these crucial moments in larval development (Morgan et al., 2005).

The refined model only considers individual variability on the lethal threshold for decrease in Na. However, there are other physiological-related traits that can vary as well. Paquin et al. (2002) already established there is variability in the Na half-saturation constant (K_M) and the maximum Na uptake rate (J_M). Morgan et al. (1997) have identified variability on J_M when assessing ionoregulation of Na under Ag exposure (J_M = 9.55 ± 3.02 mmol kg^{-1} d^{-1}). Moreover, this parameter decreased significantly with Ag exposure, which led to the establishment of sigmoidal dose-response relationship between Ag exposure and inhibition of the maximum Na uptake (J_M; see online supplementary material: Appendix, Section 2, Equation S10). Additionally, variability on the Na affinity of the carrier was demonstrated as well (K_M = 0.348 ± 0.126 mM). However, no attention was given to a possible variability in the lethal threshold of Na reduction in the original model. This variability seems to be crucial when further analyzing the refined model predictions in comparison with observed lethal dose-response relationships. Observations of mortality were better predicted with the extended BLM when variability on the lethal threshold is assumed as well.

Physiologically extended BLM predictions with variability on the maximum Na uptake rate did improve the fit to the dose-response data from Galvez and Wood (1997; see Figure 4). However, these predictions did not yield better results than when the variability on the lethal threshold is assumed. Further refinement could be the inclusion of more sources of variability in the extended BLM, on top of the distribution in lethal threshold. However, based on the limited available dose-response data for Ag toxicity to rainbow trout, this could potentially lead to model overfitting. For now, only one source of variability is assumed. The threshold assumption has a significant impact on the predicted mortality in rainbow trout, and yet no argumentation on the selection of the value of 30% is provided by Paquin et al. (2002), other than cited sources on ionoregulation data (Wood et al., 1996). The physiological extension of the BLM from Paquin et al. (2002) was thoroughly validated when it comes to the predictions of Na plasma over time under various physicochemical conditions. Yet, the lethal threshold was only validated by comparing predicted median lethal times (ET50 values) to data. Additionally, because varying one parameter (the lethal threshold) improves the fit to individual dose-response data significantly, it could be argued it is unnecessary to vary more parameters that would likely improve the fit only marginally (e.g., the maximum Na uptake rate).

Integrated population modeling

This modeling framework further develops the physiologically extended BLM approach by Paquin et al. (2002). A data-driven approach, using relative survival fractions, is used to assess effects on fish ELS, which are not covered by the extended BLM. The exposure conditions from the study of Davies et al. (1978) were used to compare effects across life stages. This study assessed effects on the complete ELS development of rainbow trout, starting from egg fertilization, to hatch, and larval development up to the swim-up phase (when trout transition to free-swimming juvenile). The calculated relative survival fractions over the 70 days of exposure were compared to the extended BLM predications at the same exposure concentrations and within the same timeframe (Table 5). From the observed (embryo/larvae) and predicted (juvenile/adult) mortality, it is concluded that effects on ELS occur at lower Ag concentrations compared to effects on the free-swimming life stages. For example, at 2.5 µg dissolved Ag/L, 100% mortality was observed after 70 days of exposure in the experiment of Davies et al. (1978). With the refined model, only 2.2% mortality is predicted on juvenile and adult rainbow trout. At low dissolved Ag, juvenile and adult trout are seemingly not affected. Similar trends have been reported in literature. Johari et al. (2013) observed a clear trend when exposing different life stages of rainbow trout to colloidal Ag nanoparticles. The juvenile life stage was consistently less sensitive compared to the larval and the eleutheroembryonic life stage. Similarly, Buhl and Hamilton (1991) found lower acute 96 h LC50 values (i.e., lower median lethal concentrations, and thus higher toxicity) for ionic silver in alevins (hatchlings) compared to juvenile (free-swimming) rainbow trout.

The extended BLM and RSF were integrated in an IBM framework, which allows the prediction of population dynamics of rainbow trout under Ag exposure. Based on the scenario analysis with the IBM, it was assessed which affected life stage has a more significant impact on rainbow trout population persistence. An effect of 36% on embryonic and larval survival (at 1.2 µg dissolved Ag/L) causes no significant predicted impact on population abundance or biomass. At that same concentration, no effects on juvenile and adult are predicted with the extended BLM. At 2.5 µg dissolved Ag/L, however, 100% mortality is predicted on ELS. Naturally, when the ELS do not survive, recruitment collapses and the population becomes extinct at the end of the simulation. At that concentration, a low percentage mortality is predicted by the extended BLM. Additional simulations were

performed to assess what percentage of effects per life stage is required to predict population extinction (see online supplementary material: Appendix, Section 5, Figures S5 and S6). Even a high percentage of mortality on ELS (up to 40% after 70 days of exposure) does not lead to any significant impact on population abundance or biomass (see online supplementary material Figure S5). At more than 50% mortality, population decline is predicted. When juvenile and adult trout are affected, the percentage effect required to lead to population extinction is lower (no significant population effects are observed up to 20% mortality after 70 days of exposure to Ag; see online supplementary material Figure S6). Even though the percentage effect leading to population extinction is lower for adults compared to ELS, it should be considered that ELS individuals are affected at lower Ag concentrations than juvenile individuals and adult individuals. As shown by the predictions in Table 5, at 2.5 μg dissolved Ag/L, no effects on juveniles or adults are predicted with the extended BLM, whereas 100% mortality was observed by Davies et al. (1978). Hence, these results stress the importance of ELS mortality in driving population effects to Ag.

Janssen et al. (2021b) predicted a similar impact of ELS mortality when assessing population effects of Zn to trout. Rainbow trout are highly fecund, producing 2,000 to 3,000 eggs per kg body weight per year (Tyler et al., 1996). The mortality in ELS in natural environments is extremely high with survival rates as low as 48% being observed in natural environments (Rye et al., 1990). InSTREAMgen contains several egg mortality sources, including scouring and deposition, low and high temperature stress, dewatering, and superimposition (Ayllón et al., 2016). As predicted with InSTREAMgen, additional mortality due to chemical stress will not affect recruitment in trout populations at first—at least at lower effect percentages. This is mainly due to compensatory density-dependent mechanisms at the population level. An increased embryonic and larval mortality due to Ag exposure, will be compensated by a reduced mortality due to the other sources (Preuss et al., 2009). Additionally, a lower input of new juveniles will increase resource availability for older individuals, reducing intra-specific competition for food and refuge in the local environment (De Roos et al., 2013). Janssen et al. (2021b) predicted similar trends for brown trout when implementing effects of Zn on emergence success (i.e., the rate of success when transitioning from yolk-sac larvae [Redd phase] to juvenile trout [free-swimming phase]). When stronger effects on ELS are assumed, there is a clear negative effect predicted on recruitment (number of young-of-the-year in the population), which causes a decrease in population abundance over time. Here, even though up to 40% of Ag induced ELS mortality is considered, population abundance and biomass are unaffected by Ag exposure (see online supplementary material: Appendix, Section 5, Figures S5 and S6).

The predictions presented here are limited to one bioavailability condition that was extrapolated (i.e., that of Davies et al.,1978). The predictions assume that physicochemical conditions in the environment remain constant over time. With InSTREAMgen, the local water chemistry in the simulated river is assumed constant, even though other variables (water flow, temperature, the amount of refuge, and food availability) change throughout the 10-year simulation. The level of Ag exposure is assumed constant as well. Nonetheless, the modeling framework allows comparison of Ag effects between life stages, while also providing a potential impact at the population level. Currently, extrapolation to other exposure concentrations is difficult due to the lack of good data, and without a mechanistic approach to cover the larval and embryonic life stages. The RSF approach relies on measured survival in ELS. We currently cannot extrapolate to other Ag concentrations, outside the ones tested by Davies et al. (1978). However, we can perform additional simulations, assessing theoretically what the impact of different effect percentages per life stage means for population persistence (see online supplementary material: Appendix, Section 5). Altogether, the modeling framework presented covers effects on all rainbow trout life stages, under specific physicochemical conditions, when exposed to Ag, while also providing a realistic prediction of the impact of Ag on rainbow trout populations.

Janssen et al. (2021a) have developed an IBM framework (InSTREAMgen-based) that includes individual-level effects of Zn and Cu on survival of juvenile trout. Their results imply that individual variation in thresholds affects how the population responds to exposure. To be more precise, they hypothesize a relationship between inter-individual variability of lethal thresholds and population-level effects, further highlighting the advantage of IBM-based approaches over more conventional population modelling approaches (e.g., matrix models of ordinary differential equations). Chaumot et al. (2003) have, for instance, developed a Leslie matrix model to account for the effect of Cd toxicity to rainbow trout. Matrix models represent populations as structured groups based on age, size, or life stage, with demographic transitions between these categories. A common approach to analyzing matrix models is through the dominant eigenvalue (λ) of the transition matrix, which provides an estimate of long-term population growth—indicating whether a population is expected to increase ($\lambda > 1$), decline ($\lambda < 1$), or remain stable ($\lambda = 1$). However, these models can incorporate various levels of complexity, including density dependence, stochasticity, and intra-specific variability, although this makes them more challenging to solve (Accolla et al., 2021). These structure models are generally less suited for explicitly capturing spatial heterogeneity (e.g., Chaumot et al., 2003), individual behavior, and sub-organismal processes, which are more readily incorporated into individual-based models. It lacks implementation of intra-specific variability, important biological processes such as transition between life stages, specific foraging, or reproductive behavior, which are all included in the InSTREAMgen model of Ayllón et al. (2016). Furthermore, the population predictions (without exposure) of InSTREAMgen have been thoroughly validated against trout population measurements in the river Belágua (Spain; Ayllón et al., 2016). Additionally, the predictions of Ayllón et al. (2016) emphasize the strength of an individual-based modelling approach; effects implemented on individuals lead to the emergence of realistic population effects.

The current framework includes lethal effects of Ag on all life stages of rainbow trout. However, sublethal effects of Ag to rainbow trout are currently not accounted for. This is mainly because of the data gap on the sublethality of Ag. Norberg-King et al. (1989) have observed sublethal effects of Ag on the growth of fathead minnow, clearly indicating Ag can have a direct sublethal impact on freshwater fish. Davies et al. (1998) have observed sublethal effects of Ag on growth of rainbow trout as well. However, their data are limited to a qualitative assessment of the sublethal effects (i.e., only a qualitative score is given for the impact on growth at the end of exposure period). Another data gap is the effect of Ag on reproduction of fish. Currently, there are no studies that have assessed reproductive effects of Ag to rainbow trout or freshwater fish in general. InSTREAMgen could be used to theoretically simulate how reproductive impairment affects the population. However, without reliable data it is impossible to assess how reproductive effects compare to the lethal effects and

therefore also to evaluate ecotoxicological questions for silver, such as the concentrations at which the reproductive effects matter, the mathematical or mechanistic relationship best describing sublethal effects, and the impact of reproductive effects on rainbow trout populations. A final data gap is the coverage of bioavailability conditions in the current assessment. As mentioned, only the conditions of Davies et al. (1978) could be evaluated here. Because of the limited data for which the SBM and RSF could both apply, it is currently not possible to compare Ag effects across various bioavailability conditions. Nonetheless, the integrated IBM allows for the comparison of the relative impact of Ag effects on different rainbow trout life stages and how they impact population abundance or biomass.

Based on the InSTREAMgen predictions, and including Ag effects on all rainbow trout life stages, it is clear that the ELS are driving rainbow trout population effects when exposed to Ag. Consequently, it can be concluded that toxic effects of Ag on adults or juveniles are less determining for rainbow trout population persistence. Given these findings, future investments in the development of BLMs for fish should focus specifically on the ELS (ELS-BLM). The physiologically extended BLM performs well in predicting mortality in juvenile and adult life stages under various physicochemical conditions. Prioritizing the ELS in BLM development will enhance our understanding of Ag toxicity and the impact on fish populations. Additionally, integrating an ELS-focused BLM, in contrast to the current data-driven RSF approach, would ensure a more robust framework for assessing ecological risks associated with Ag exposure in aquatic environments.

Conclusion

The current study addressed several uncertainties related to the toxic effects of Ag on rainbow trout. The original physiologically extended BLM developed by Paquin et al. (2002) provides an accurate description of the disturbances of the vascular Na mass balance in rainbow trout when exposed to Ag. In the current study, the original model was further refined by integrating information on Na ionoregulation under Ag exposure. A normal distribution was established to describe the variation of individual lethal threshold for percentage decrease in vascular Na. The refined extended BLM allows for the prediction of individual survival times of juvenile and adult rainbow trout exposed to Ag under various exposure conditions. This model framework is better fit to compare life stage sensitivity between studies, and was therefore integrated in a population modeling framework to predict the impact of Ag on trout population abundance or biomass.

Based on population predictions with an IBM framework, the life stage that is most determining for population persistence is the ELS (embryonic and larval phase). This is mainly explained by the level of Ag concentrations where effects on different life stages are observed or predicted. Based on the extended BLM approach, effects on juveniles and adults occur at higher concentrations compared to observed effective concentrations for embryos and larvae (under the same bioavailability conditions). The modeling predictions are crucial for guiding decisions on future testing strategies for Ag ecotoxicity, which should focus more on the ELS. Additionally, some data gaps were identified as reproductive effects are currently not covered within the integrated population modeling framework. In addition, a limited number of bioavailability conditions were considered due to the lack of data. Nonetheless, this framework fills existing knowledge gaps in assessing the ecological risk of Ag on freshwater fish, by incorporating diverse modeling methods such as BLMs, physiological extensions of BLMs (e.g., SBM), and IBM. This integration ensures a more ecologically realistic evaluation of Ag effects on rainbow trout populations.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

All files containing the software code are available on Figshare data repository: https://figshare.com/articles/software/Vlaeminck_-_2024_-_Electronic_appendix/27232686? file=49807113

The following files are included:

- R-files with the implementation of the original SBM model (Paquin et al., 2002), including the internal validation.
- R-files with the validation of the refined SBM model.
- NetLogo file of the adapted InSTREAMgen model for population-level predictions of Ag toxicity to trout populations.
- R-files with the calculations of population-level effects of Ag toxicity to trout.

Author contributions

Karel Vlaeminck (Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing—original draft, Writing—review & editing), Charlotte Nys (Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing—review & editing), Katrien Arijs (Funding acquisition, Project administration, Resources, Supervision, Writing—review & editing), Jelle Mertens (Funding acquisition, Resources, Supervision, Writing—review & editing), and Karel A.C. De Schamphelaere (Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing—review & editing)

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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