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POP-CURE

The use of population models for copper (Cu) risk assessment: Improving ecological relevance

Final report – Partim *Acipenser transmontanus*
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TABLE OF CONTENTS

LIST OF ABBREVIATIONS

DEB	Dynamic energy budget
dph	Days post hatching
ECx	Effect concentration resulting in a x% effect
IBM	Individual-based model
LCx	Lethal concentration resulting in x% mortality
MVP	Minimum viable population size
PVA	Population viability analysis
SSD	Species sensitivity distribution
YOY	Young of the years (age-0 individuals)

1 INTRODUCTION

1.1 Copper risk assessment

The PopCure collaborative project has the goal to promote the use of population models as valuable alternatives for conventional metal risk assessment. Metal risk assessment nowadays consists of assessing data on metal toxicity and constructing a species sensitivity distribution (SSD). However, more and more publications on sensitive species are being published, pushing the environmental safe threshold in either a negative or positive direction. Additionally, conventional toxicity endpoints are only considered at the individual level. Population (and by extension community and ecosystem) models are more and more frequent in ecotoxicology, as they are powerful tools which are less expensive and less time-consuming compared to population or mesocosm experiments.

The first part of the PopCure project involved the development of a population model for the copper sensitive species *Lymnaea stagnalis* (the great pond snail) (Vlaeminck et al. 2017a). A DEB-IBM for *L. stagnalis* model was developed and parameterised based on individual-level, experimental data on copper toxicity for *L. stagnalis*. Population-level effect assessment was carried out, and a comparison was made between (traditional) individual-level EC_x concentrations reported in literature and population-level EC_x values predicted by the model.

In the second part of the PopCure project another copper sensitive species is investigated, *Acipenser transmontanus* (the white sturgeon). The white sturgeon is the largest freshwater fish in North America. It inhabits the major river systems and estuaries in the North American west coast (Israel et al. 2009). Just as with the great pond snail, the white sturgeon seems to be particular sensitive to copper pollution. Low copper LC_x values have been reported for developing white sturgeon larvae and young-of-the-years (YOYs, the age-0 individuals).

The life cycle of white sturgeon is much more complex than that of the great pond snail. Not only do they live longer, but they undergo seasonal migration and inhabit large areas of (multiple) river systems (Israel et al. 2009). In a previous report within the PopCure project the different fish population models in literature were discussed (Vlaeminck et al. 2017b). Based on the model structures, available data, uncertainties and the assumptions of each type the most optimal model structure for the white sturgeon case was selected. Based on the selected model type and available literature, an individual-based population model (IBM) was thus developed for *Acipenser transmontanus*. In addition, experimental data on copper toxicity was integrated to predict copper effects at the population level.

1.2 *Acipenser transmontanus* biology and habitat

White sturgeons have a long lifespan (over 100 years) and on average experience maturity at 10 to 12 years of age (Israel et al. 2009). The white sturgeon is the largest of the *Acipenser* genus with a reported maximal size of 610 cm (816.0 kg) (Froese and Pauly 2017). Different white sturgeon life stages can be discerned: eggs, larvae (up until 20 to 30 dph – days post hatching), YOYs (0 – 1 years), juveniles (2 – 10 years), and adults (i.e. sexually mature individuals, >9 years of age). Additionally, the transition from larvae to YOY occurs in different phases: still having a yolk sack, swim-ups (after completely absorbing the yolk sack), transition to feeding (when exogenous feeding begins), and eventually YOYs (juveniles in the first year of life, i.e. age-0 individuals).

White sturgeons inhabit four major river systems on the west coast of North America: the Sacramento River, the Columbia River, the Snake River, and the Fraser River. The Snake River and the Kootenai River (both part of the Columbia River system) contain naturally-landlocked populations. Because of dams and natural obstructions (e.g. waterfalls) these populations have been cut off from the river estuary and lost connection to the ocean. White sturgeon are anadromous fish (i.e. when they reach maturity they migrate to brackish estuaries, but still require freshwater to reproduce) (Israel et al. 2009). Not all populations migrate to reproduce as some populations are not able to reach estuaries because of dams, natural obstructions (e.g. waterfalls), or large migration distances (e.g. the Snake River and Kootenai River populations) (KTOI 2007).

Although the white sturgeon is classified as 'least concern' by the IUCN Red List of Threatened species (Duke et al. 2004), recruitment failure of juvenile white sturgeon has been reported (Israel et al. 2009; BC Hydro 2016). Recruitment here is defined as the amount of fertilized eggs that successfully hatch and develop in healthy juveniles (>1-year-old). The main cause for the decline in *Acipenser transmontanus* recruitment is the large-scale habitat and ecosystem changes (KTOI 2007; Israel et al. 2009). Because of their declining status in the Fraser and Kootenai River, the government of British Columbia (Canada) has listed white sturgeon as endangered under the Species at Risk Act (2006). Likewise, in the United States of America the white sturgeon is listed as endangered by the United States Department of Agriculture (2011). Monitoring campaigns are critical to manage and monitor the white sturgeon populations. Several monitoring and conservation programs have been set up by governmental instances from both Canada (e.g. BC Hydro) and the United States of America (e.g. US Fish and Wildlife Services). These programs monitor populations in the wild to estimate if densities are increasing or decreasing. Additionally, white sturgeon larvae and juveniles are cultured in conservation areas and are released in the wild once they passed a certain, critical age.

2 MATERIAL AND METHODS

2.1 Data on *Acipenser transmontanus* copper toxicity

In literature, copper toxicity data on *Acipenser* species is almost exclusively mortality on early life stages of the white sturgeon (Table 1). Only Wang et al. (2014) reported effect concentrations for sub-lethal effects on YOY growth.

Table 1. Overview of the literature on copper toxicity in sturgeon species (*Acipenser*). The investigated life stage and corresponding age are reported. The effective concentration (EC_x) are shown with their confidence limits. The corresponding exposure duration are given as well.

Reference	Species	Exp #		Effect	Life stage	Age [dph[a]]	EC_x	Conc [µg/L]	Exposure duration
USASK (2011)	*Acipenser transmontanus*	1	Chronic	Mortality	Larvae	0	LC_{20}	6.76 (-)	60 d
				Mortality	Larvae	0	LC_{50}	11.1 (-)	60 d
Vardy et al. (2011)	*Acipenser transmontanus*	1	Chronic	Mortality	Larvae	0	LC_{20}	3.4 (3.1 – 3.7)	19 d
				Mortality	Larvae	0	LC_{50}	9.9 (9.3 – 10.6)	19 d
		2	Chronic	Mortality	Larvae	0	LC_{20}	5.5 (4.9 – 6.3)	58 d
				Mortality	Larvae	0	LC_{50}	12.4 (11.2 – 13.7)	58 d
Little et al. (2012)	*Acipenser transmontanus*	1	Acute	Mortality	YOY	38	LC_{50}	4.1 (3.4 – 4.8)	96 h
		2	Acute	Mortality	YOY	40	LC_{50}	4.7 (3.9 – 5.4)	96 h
		3	Acute	Mortality	YOY	26	LC_{50}	4.5 (3.4 – 6.1)	96 h
		4	Acute	Mortality	YOY	27	LC_{50}	6.8 (5.2 – 8.9)	96 h
		5	Acute	Mortality	YOY	123	LC_{50}	268.9 (204.8 – 352.9)	96 h
		6	Acute	Mortality	YOY	167	LC_{50}	130.7 (79.4 – 135.5)	96 h
		7	Acute	Mortality	YOY	450	LC_{50}	269 (205.0 – 353.0)	96 h
Vardy et al. (2013)	*Acipenser transmontanus*	1	Acute	Mortality	Larvae	8	LC_{50}	22 (20 – 25)	96 h
		2	Acute	Mortality	Larvae	15	LC_{50}	10 (8 – 12)	96 h
		3	Acute	Mortality	YOY	40	LC_{50}	9 (7 – 12)	96 h
		4	Acute	Mortality	YOY	45	LC_{50}	17 (14 – 21)	96 h
		5	Acute	Mortality	YOY	100	LC_{50}	54 (47 – 62)	96 h
Calfee et al. (2014) as reported in Ingersoll and Mebane (2013)	*Acipenser transmontanus*	1	Acute	Effective mortality[b]	Larvae	2	EC_{50}	1.51*	4 d
		2	Acute	Effective mortality	Larvae	16	EC_{50}	2.59*	4 d
		3	Acute	Effective mortality	YOY	30	EC_{50}	4.2*	4 d
		4	Acute	Effective mortality	YOY	44	EC_{50}	>34.1*	4 d
		5	Acute	Effective mortality	YOY	61	EC_{50}	21.98*	4 d
		6	Acute	Effective mortality	YOY	72	EC_{50}	58.96*	4 d
		7	Acute	Effective mortality	YOY	89	EC_{50}	17.25*	4 d
Little et al. (2014)	*Acipenser transmontanus*	1	Acute	Mortality	YOY	35	LC_{50}	6.3 (4.3 – 8.3)	96 h
		1	Acute	Mortality	YOY	35	NOEC	1.1 (-)	96 h

Vardy et al. (2014)	*Acipenser transmontanus*	1	Acute	Mortality	Larvae	8	LC_{50}	4.5*	96 h
		2	Acute	Mortality	Larvae	8	LC_{50}	8.1*	96 h
		3	Acute	Mortality	YOY	40	LC_{50}	4*	96 h
		4	Acute	Mortality	YOY	40	LC_{50}	4.36*	96 h
Wang et al. (2014) as reported in Ingersoll and Mebane (2013)	*Acipenser transmontanus*	1	Chronic	Mortality	Larvae	2	LC_{10}	1.83 (1.71 – 1.96)	14 d
				Mortality	Larvae	2	LC_{20}	2.24 (2.12 – 2.37)	14 d
		2	Chronic	Mortality	YOY	25	LC_{10}	3.11 (1.71 – 5.63)	28 d
				Mortality	YOY	25	LC_{20}	3.32 (3.01 – 3.67)	28 d
		3	Chronic	Mortality	YOY	27	LC_{10}	3.72 (3.44 – 4.02)	28 d
				Mortality	YOY	27	LC_{20}	4.21 (3.95 – 4.49)	28 d
		1	Chronic	Growth (length)	Larvae	2	EC_{10}	1.12 (0.65 – 1.95)	53 d
				Growth (length)	Larvae	2	EC_{20}	1.80 (1.28 – 2.51)	53 d
		3	Chronic	Growth (length)	YOY	27	EC_{10}	3.85 (3.65 – 4.06)	28 d
				Growth (length)	YOY	27	EC_{20}	4.44 (4.23 – 4.65)	28 d
		1	Chronic	Growth (dry weight)	Larvae	2	EC_{10}	1.19 (0.89 – 1.59)	53 d
				Growth (dry weight)	Larvae	2	EC_{20}	1.55 (1.25 – 1.91)	53 d
		3	Chronic	Growth (dry weight)	YOY	27	EC_{10}	2.04 (1.19 – 3.48)	28 d
				Growth (dry weight)	YOY	27	EC_{20}	2.85 (1.94 – 4.19)	28 d
				Growth (biomass)	YOY	27	EC_{10}	2.03 (1.33 – 3.11)	28 d
				Growth (biomass)	YOY	27	EC_{20}	2.67 (1.95 – 3.66)	28 d
Zahedi et al. (2014)	*Acipenser persicus*	1		Mortality	Juvenile	420	LC_{50}	502	96 h

[a] dph: days post hatching.

[b] Effective mortality: percentage of individuals that show loss of equilibrium or immobilisation (i.e. not moving or showing a lack of hiding) added to the percentage mortality (based on the number of deceased individuals).

* Values normalized to the US-EPA standard water

The results in Table 1 indicate early developmental life stages of white sturgeon are most sensitive to copper, up to a factor 25 lower in LC_{50} compared to older juveniles (USASK 2011; Little et al. 2012; Vardy et al. 2013). The reason for this increased sensitivity during early developmental life stages is still speculative (Calfee et al. 2014). The lowest LC_x values are found in developing sturgeons around the age of 20 to 30 dph. The critical age for white sturgeons seems to be when they completely used their yolk sack and switch to exogenous feeding (i.e. the transition from larvae to swim-up) in addition to a transition from passive gas exchange to active gill gas exchange (Israel et al. 2009; Calfee et al. 2014). This metamorphosis from sturgeon larvae to YOYs is accompanied by a change in ion regulation in the body (Fu et al. 2010). Since copper toxicity is often related to ion regulation disruption, this might explain the increased sensitivity to copper during these early developmental life stages (Calfee et al. 2014).

This finding is also substantiated by the data of Vardy et al. (2011). In the study of Vardy et al. (2011) mortality for different life stages was compared (yolk sack, swim-up, transition to feeding, and YOYs). Freshly hatched embryos were exposed for 66 days to copper. Their results show that 43% of all mortality occurring between 21 and 34 dph during the 66-day experiment. Vardy et al. (2011) appoint the increased mortality to the transition of larvae to exogenous feeding. No concentration-dependent mortality was observed after complete transformation from larvae to YOY. In addition to the above, the results from both Calfee et al. (2014) and Little et al. (2012) suggest white sturgeon become less sensitive to copper with age. Calfee et al. (2014) reported that juvenile sturgeons older than 72 dph were relatively insensitive to copper, even at concentrations 30 times higher than the effect concentrations for early life stages.

In Figure 1, the LC_{50} values are given as a function of the starting age of the sturgeons used in the experiment, expressed as days post hatching (dph). The lowest LC_{50} values are found with experiments where the starting is 40 dph or lower. After that, when transformation from larvae to juvenile is completed, the sensitivity of the sturgeon decreases (i.e. increasing LC_{50} values). Comparing these LC_{50} values is, however, complicated by the fact that every value is was obtained at different experimental conditions (i.e. medium composition) and exposure duration.

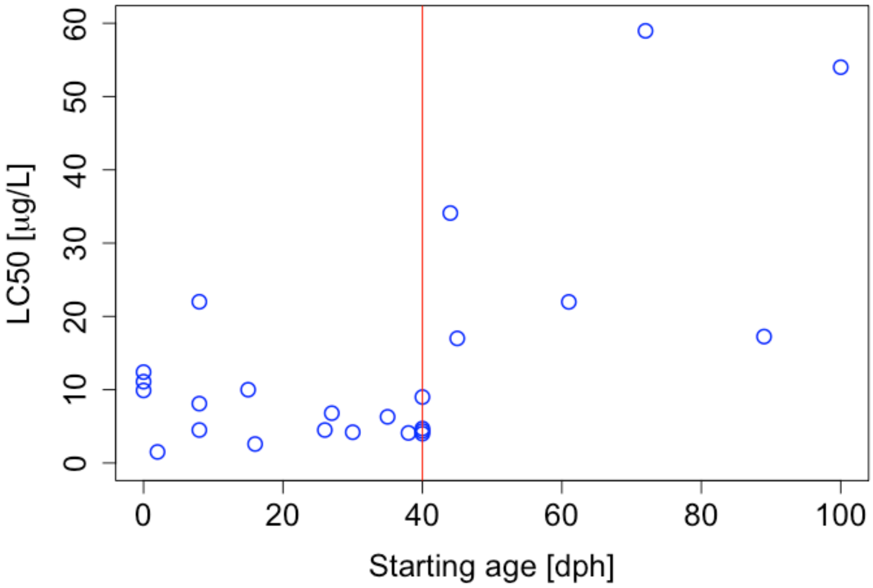


Figure 1. Visualisation of reported LC_{50} values as shown in Table 1. The values are plotted as a function of the starting age (in dph) of each experiment.

It must be noted that relatively high mortality in juvenile fish is commonly observed. Test requirements in ecotoxicology usually demand more than 70-80% control survival. However, Ingersoll and Mebane (2013) observed very low control survival during a 53-day chronic, ranging from about 30% mortality to a maximum of 60%. This is a reason why the second experiment of Ingersoll and Mebane (2014) was not included in the publication of Wang et al. (2014). Yet, the results of the experiment were included in the report of the USGS (Ingersoll and Mebane 2013). High control mortality is not uncommon in *A. transmontanus* and can depend on the experimental set-up. Additionally, there is no standard test guideline on how to handle these animals for ecotoxicological testing. Hence, there is some remaining uncertainty in extrapolating results of studies with high control mortality to the population level.

2.2 Software for model development and effect assessment

The *Acipenser* population model was developed in NetLogo (Wilensky 1999), a software platform used for agent-based modelling. Data processing was performed in R-Studio (R Foundation for Statistical Computing, Vienna, Austria).

3 ACIPENSER POPULATION MODEL

3.1 Model structure

Model selection based on several criteria was discussed in the previous report of PopCure (Vlaeminck et al. 2017b). For the *Acipenser* population the model was based on an individual-based model (IBM) developed by Jager (2000). The implementation follows an individual-based age-class model, meaning the individuals are divided in groups defined by their age (in years), albeit retaining some individual traits (i.e. variability amongst individuals from the same age class group). Originally, the model was used to assess the effect of river fragmentation on the Snake River population in the context of a population viability analysis (PVA). The IBM developed by Jager (2000) has been used and adapted in a couple of articles, all by the same authors (Jager et al. 2001a; Jager et al. 2001b; Jager et al. 2010).

3.1.1 State variables

Since the model is individual-based, every fish within the population is simulated individually, having its own traits and parameters. The IBM from Jager (2000) is an age-class based model, meaning the population dynamics flow from the survival rates between different life stages (or age classes). The most important state variable of each individual within the population is the age of the individual (expressed in years). The age of the individual will define other attributes, such as its weight and size, as well as fecundity and survival rates.

Next to age (expressed in years), other state variables of the individuals include size (in cm), weight (in kg), fecundity (amount of eggs produced – only for females), life stage (YOY, juvenile, or adult), and location (which river segment). Individuals also have a set age of maturity (in years) and spawning interval (also in years).

The model is individual-based, indicating the presence of variability between individuals of the population. Variability can be found in the age of maturity. On average females become sexually mature at the age of 18 years, whereas for males this is 14 years. Variability on the age of maturation has been quantified by Jager (2001a) based on monitoring data. Similarly, the spawning interval shows some variability as well. On average females spawn every 8 years, whereas males reproduce (i.e. release sperm cells) every two years.

Every individual that is introduced in the population will draw their age of maturity and their spawning interval from a normal distribution with a given mean and standard deviation (see parameters in Table 2). The model works with discrete time steps of one year. Once the age of an individual surpasses its age of maturity, the individual can start reproducing. Another state variable will keep track of the amount of years since last reproduction. Once this surpasses the time between spawning events, the individual can reproduce and this parameter is set to 0.

3.1.2 Size, weight and fecundity relationships

As an individual sturgeon grows older, so will its size and weight increase. The growth curve of sturgeons is described by the Von Bertalanffy equation (Equation 1). Jager (2000) estimated the parameters of the Von Bertalanffy equation based on Snake River data (Lepla and Chandler 1995).

Equation 1. The Von Bertalanffy Equation (Von Bertalanffy 1935). The function describes the fork length *L* (cm) of a white sturgeon as a function of its age *t*.

$$L_t = L_\infty * (1 - e^{k*(t+t_0)})$$

Beamesderfer (1993) estimated a standard weight equation for the white sturgeon based on monitoring data. The weight of a white sturgeon can be calculated based on its fork length (Equation 2).

Equation 2. Size-weight relationship for white sturgeon. The weight *W* at age *t* is given as a function of its fork length L_t.

$$W_t = w_a * (L_t)^{w_b}$$

The fecundity of a fish increases with its size (Chapman et al. 1996; Cochnauer 1983; Van Eenennaam et al. 1996). In a similar fashion as for size and weight, the fecundity (i.e. the amount of eggs produced) of female sturgeon has been quantified as a function of its fork length (Equation 3).

Equation 3. Fecundity-size relationship for white sturgeon. The fecundity (#eggs) is described as a function of its length L_t.

$$F_t = f_a * (L_t)^{f_b}$$

3.1.3 Simulated river habitat

The white sturgeon model is spatially structured. As Jager (2000) indicated, the model is a one-dimensional stepping-stone model (Kimura and Weiss 1964). The environment is represented as serially linked river segments separated by dams. These river segments are then further divided into patches (standard implemented in the NetLogo environment), each representing a part of the river of 1 km long. The total length of the river segment as well as the number of river segments separated by dams can be specified when initiating the simulation. As a standard scenario, Jager (2000) used a river length of 200 km with three river segments separated by two dams (Figure 2).

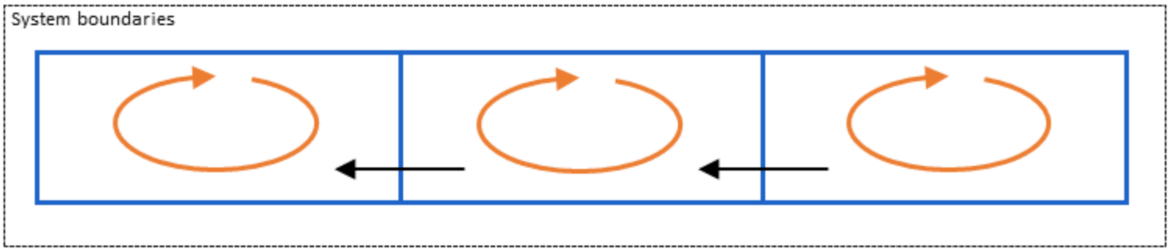


Figure 2. Spatial scheme of the model. Three river segments are considered each separated by dams. Individuals can move freely within each river segment. Downstream migration is assumed between the fragments (unidirectional).

Two different types of movement are considered within the model's spatial boundaries: migration between river segments (1) and movement within river segments (2).

Dams are considered obstructions which impede the ability of the sturgeons to freely move about. However, migration to other segments across dams is possible. Based on the upstream and downstream migration rates (Table 2), some individuals will move to adjacent river segments over the course of each year. The number of individuals emigrating from a specific river segment depends on the migration rates (Table 2) and will be proportional to the population size in that segment. In the case of Jager (2000) only downstream migration was considered. Dams impede the ability of the fish to swim upstream. Usually dams contain downstream passages guiding fish away from the turbines, and allowing them to pass down the river (Larinier 2001). However, these are not suited for upstream migration. Hence only downstream migration is considered.

For every time step, some individuals in the population will migrate to an adjacent river segment. The migration rate determined by Jager (2000) can be interpreted as a probability of migrating, since it is expressed as a fraction per year. A random number will be generated for each individual. If this number is lower than the probability of migrating (i.e. the migration rate) the individual will be moved to the next river segment.

If individuals are not migrating to other segments, movement within river segments is considered. This type of movement is considered random. Over the course of each year (each time step) the individual can move freely within the river segment. The segments are divided into patches of 1 km of length. If the time step increases, all individuals will be moved to a random patch within the same river segment. Since only the position of a fish during reproduction is of importance, this random movement is only calculated once. Reproduction is a timed event that happens once every year (i.e. once every time step).

The NetLogo implementation follows the same standardised spatial structure as proposed by Jager et al. (2001a). The simulated river habitat stretches over a distance of 200 km and contains 2 dams (Figure 3).

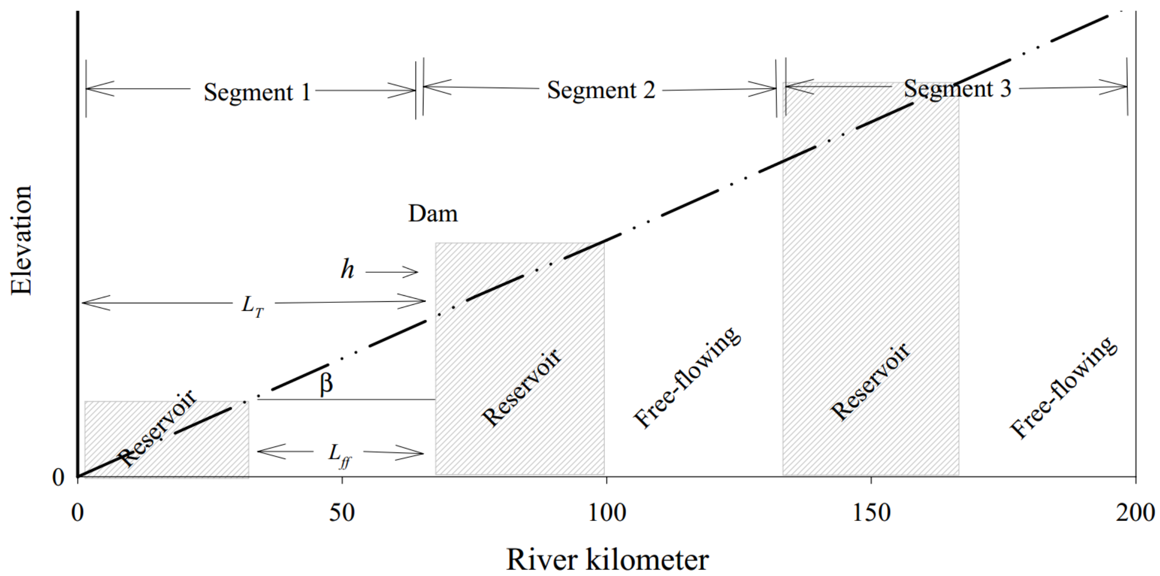


Figure 3. Structural representation of the model (Jager 2001a). The model creates a river with a length of 200 km divided into 3 segments. There are 2 dams, at 1/3rd and 2/3rd of the river distance.

Depending on the height of the dam *h* and the river slope *β* the free-flowing river length L_{ff} (i.e. the part of the river where water is actually flowing) is calculated per river segment (Equation 4). The rest of the river segment is considered reservoir. The free-flowing river length L_{ff} is important as it determines the amount habitat available for spawning and foraging (Jager 2000).

Equation 4. Length of the free-flowing river distance L_{ff} depends on the total river length L_T, the height of the dam *h*, and the slope of the river *β*.

$$L_{ff} = L_T - \frac{h}{\beta} * \sqrt{1 - \beta^2}$$

3.1.4 Density dependent age-0 survival

To predict the density-dependent effects associated with the lack of suitable refuge habitat, mortality was implemented on top of the baseline mortality for age-0 individuals (i.e. larvae and YOYs). Jager et al. (2001a) determined that the amount of refuge with good water quality per river segment is limited. As described by Jager et al. (2001a), it is assumed that the free-flowing river habitat L_{ff} serves as refuge from anoxic and poor water quality conditions. Based on the amount of available refuge (i.e. free-flowing habitat), a fraction of the age-0 individuals will be killed. This fraction depends on the total number of individuals within the river segment *N*, the carrying capacity per km river segment K_{pop}, and the free-flowing river habitat L_{ff} (Equation 5). The value of the carrying capacity K_{pop} has been determined by Jager et al. (2001a).

Equation 5. Yearly surviving fraction of the age-0 population due to the lack of suitable refuge habitat. This fraction depends on the actual population density *N*, the amount of free-flowing river habitat L_{ff}, and a carrying capacity parameter K_{pop}.

$$s_w = \min\left(1, \frac{K_{pop} * L_{ff}}{N}\right)$$

Equation 5 describes a truncated equation (Figure 4). At low population densities (< $K_{pop} * L_{ff}$), there is enough suitable habitat for spawning. All age-0 individuals will thus be able to forage in suitable habitats. The fraction killed will thus be 0 (i.e. a fraction surviving equal to 1). At higher population densities, the amount of suitable habitat will be limiting. The density-dependent effect will be larger. The effect is inversely correlated with the population density (Figure 4). A larger fraction of the population will be affected at higher densities.

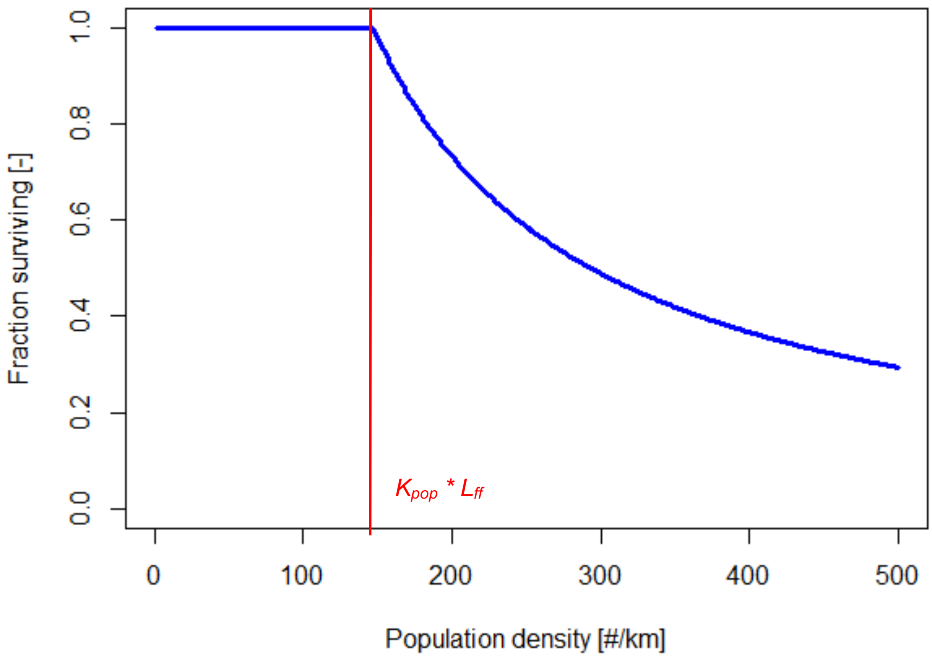


Figure 4. Density-dependent survival. The fraction of individuals surviving is shown as a function of the population density. The density-dependent effect will be larger as the number of individuals increases.

3.1.5 Processes

Each time step (i.e. one year) the model will go through several processes updating the individuals' state variables and simulating *Acipenser transmontanus* population dynamics. A flow chart of the individual-based processes can be found in Figure 5. For every time step each individual will go through all the processes eventually predicting population dynamics. Note that the model uses time steps of one year.

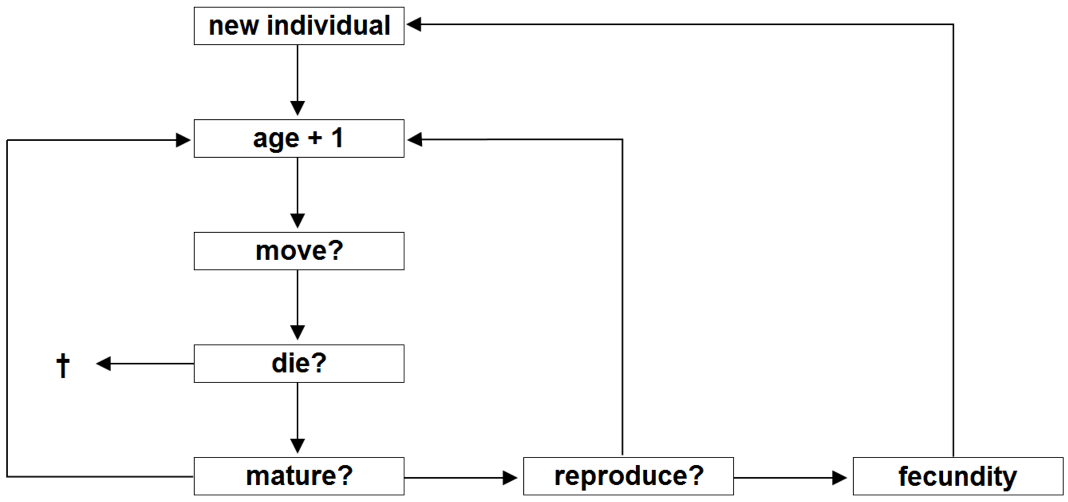


Figure 5. Flow chart of the individual-based population viability model. Scheme based on the IBM scheme from Viaene (2016). For each time step (i.e. one year) every individual will go through all the processes. First their age will increase with one year. Then they will move to a different patch or river segment. Then it will be assessed which individuals die, according to the survival rates. Then for mature individuals it will be assessed if they can reproduce and what their fecundity is. New individuals are then created.

First, each time step (i.e. yearly time steps) the age of all the individuals will increase with one year. Depending on the age, the life stage (YOY, juvenile, adult) will also be changed. Individuals in the first life year are YOYs. Juveniles are older than one year but have not reached their age of maturity yet. Adults surpassed their age of maturity. The embryo life stage is not modelled explicitly.

Each time step, the size and weight of each individual is determined as a function of its age (Equations 1 and 2).

Next, the individuals will move. As explained, according to the migration rates, some individuals will move to an adjacent river segment. All of the other individuals will move randomly within their river segment.

After movement, the model will determine which individuals will die. According to the survival rates of each life stage (see Table 2) it will calculate how many individuals survive. Also, individuals who surpass the maximum age limit will be removed from the simulation.

YOYs who turn one year of age will be subjected to a variability sub-model. This sub-model creates individual variability between the individuals. First, each individual is given a gender (with a male-female gender ratio of 50/50). After that, the age of maturity and the spawning interval are sampled for each individual. Both age of maturity and spawning interval are drawn from a normal distribution with a given mean and standard deviation (Table 2).

A maturity process will determine which individuals reach adulthood. Depending on the age of maturity each individual will start reproducing at a different age. If a juvenile surpasses its age of maturity, it can start reproducing.

Reproduction is a timed event that happens once a year (i.e. every time step). Each adult individual is given a variable determining the time since last reproduction. This variable increases each time step, and eventually if the value is larger than the spawning interval the individual can reproduce in that year. First, the fecundity of each female is calculated (Equation 3). As mentioned earlier, the embryo life stage is not modelled explicitly. Since the fecundity of white sturgeon is very high (sometimes surpassing 4 million eggs per female adult sturgeon; Israel et al. 2009), the model would have to simulate all the embryos individually, which would require immense computational effort. To solve this, in this implementation only the YOYs are simulated. The amount of YOYs are calculated from the fecundity, taking into account the baseline survival of embryos (s_y) and the density-dependent survival (s_w). Second, it is assessed if reproduction can take place in a specific patch. The criteria for this is that there needs to be at least one male adult (able to reproduce in that year) in proximity of a female (i.e. the same patch – river section of 1 km). The timing of reproduction depends on the spawning interval of male and female sturgeons.

3.1.6 Parameters

Table 2 gives an overview of all the parameters used in the equations and processes described above.

Table 2. Parameters of the IBM model, with their respective values and units.

Parameter	Symbol	Value	unit	Source
Maximum age	t_{max}	120	y	Fixed
Baseline adult survival rate	s_a	0.74	y^{-1}	Jager (2000)
Baseline juvenile survival rate	s_j	0.89	y^{-1}	Jager (2000)
Baseline age-0 survival rate	s_y	0.00053	y^{-1}	Jager (2000)
Spawning interval females		8	y	Jager (2000)
Spawning interval males		2	y	Jager (2000)
Age of maturity females		18	y	Jager (2000)
Age of maturity males		14	y	Jager (2000)
Rate of change in fork length	k	-0.045		Jager (2000)
Initial age	T_0	-0.795	y	Jager (2000)
Maximum size	L_{inf}	275	cm	Jager (2000)
Carrying capacity of refuge from anoxic conditions	K_{pop}	210	#/km	Jager et al. (2001a)
Annual upstream migration rate	M_{up}	0	y^{-1}	Jager (2001b)
Annual downstream migration rate	M_{down}	0.02	y^{-1}	Jager (2001b)
Fecundity intercept	f_a	0.072		DeVore (1995)
Fecundity exponent	f_b	2.94		DeVore (1995)
Intercept for weight – length relationship	W_a	$1.925 * 10^{-6}$		Beamesderfer (1993)
Exponent for weight – length relationship	W_b	3.232		Beamesderfer (1993)
Length of the river	L_{river}	200	km	Fixed
Width of the river	W_{river}	0.2	km	Fixed
Number of river segments	N_{segm}	3	#	Fixed

3.1.7 Model overview

Figure 6 gives a structural representation of the *Acipenser* IBM model. The model divides the population in three age classes: young-of-the-years (YOYs, i.e. age-0 individuals), juveniles (older than 1 year), and adults (fully mature and able to reproduce). The eggs/embryos are not modelled explicitly. According to the age-dependent survival rates (s_t) the individuals will grow older. Eventually, when an individual reaches maturity it can reproduce. The fecundity of a female (F_t) individual depends on its size. The amount of produced eggs that successfully hatch into YOYs depends on baseline survival rate for embryos (s_y) and the environment (density-dependent survival, s_x).

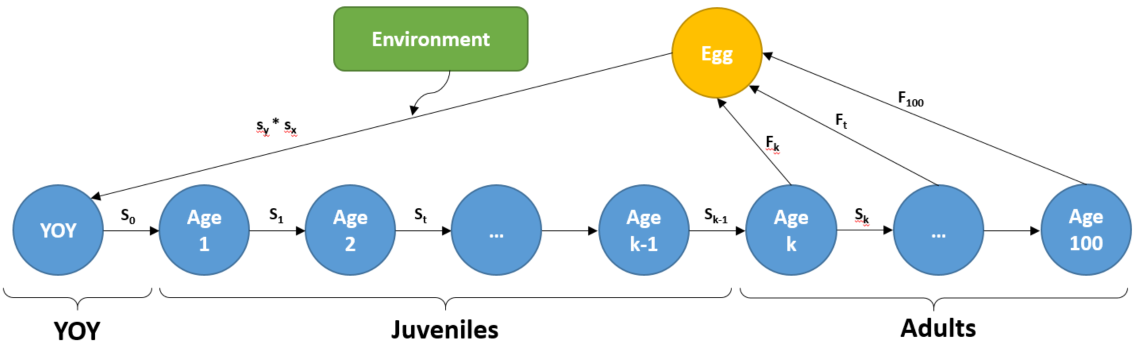


Figure 6. Overview of the *Acipenser* IBM model structure based on the scheme of Jager et al. (2001b)

3.2 Implementation of copper effects

3.2.1 Copper effects on mortality

As seen in literature (Table 1), effects of copper toxicity on white sturgeon are mainly visible on early developmental life stages. Observed effects are almost exclusively increased mortality in developing YOYs (effects on growth will be discussed in Section 3.2.2).

In the *Acipenser* IBM, effects on YOY mortality were implemented by decreasing the chances of survival in age-0 individuals. In addition to the baseline survival rate in age-0 individuals s_y and the density-dependent related survival s_w an extra concentration dependent survival rate s_{Cu} was implemented describing the effect of copper. This survival rate is dependent on the ambient copper concentration, and will decrease with increasing copper concentration.

Since data on effects on YOY survival is available (Table 1), the chance of survival of age-0 individuals exposed to copper can be determined. Via the formula of the De Laender et al. (2008) the concentration-dependent mortality rate *mort* of a given survival experiment can be calculated (Equation 6).

Equation 6. The concentration-dependent mortality rate *mort* [d^{-1}] for a given survival experiment. The mortality rate is given as a function of the copper concentration *Cu*, and depends on the LC_{50} and the slope β of the dose-response curve (log-logistic). This equation also takes into account the exposure duration t_{exp} of the experiment.

$$mort(Cu) = \frac{1}{t_{exp}} * \ln\left(1 + \left(\frac{Cu}{LC_{50}}\right)^{\beta}\right)$$

From the daily mortality rate, the chance of dying can be calculated (Equation 7).

Equation 7. The stochastic chance of dying after one day of exposure.

$$p_{Cu} = (1 - e^{-1*mort(Cu)})$$

Equation 7 calculates the chance of dying during one day of exposure to a certain copper concentration. However, the data shows that YOY sturgeons are most sensitive to copper 20 to 30 dph. Furthermore, the data in Table 1 suggests that their sensitivity decreases significantly after 100 dph. Hence, the period over which the juveniles are actually sensitive to copper pollution must be taken into account. Equation 8 thus calculates the chance of survival of age-0 individuals, taking into account the sensitive period t_{sens} (in days) of developing white sturgeons.

Equation 8. The survival chance when being exposed to a certain copper concentration. The chance of survival depends on the stochastic chance of dying p_{Cu} and the sensitive period t_{sens}.

$$s_{Cu} = (1 - p_{Cu})^{t_{sens}}$$

Based on Table 1, for each survival experiment the concentration-effect relationship could be determined. The slope (if available) and EC_{50} can be put in the model directly. The question is then which of the data must be used, or which of the performed experiments gives the most accurate representation of the sensitivity of developing white sturgeons. Indeed, the calculated survival chance s_{Cu} should take into account the complete period over which the juveniles are sensitive. For instance, the data of Vardy et al. (2011) gives the lowest EC_{50} values of all experiments (Table 1), although the duration of the experiments was only 19 days (with a starting age of 19 dph). A value for the sensitive period t_{sens}, other than 19 days, could be implied since the data in Table 1 indicates that the sensitivity only decreases after 100 dph. However, their most sensitive point seems to be around the age of 20 to 30 dph.

A value larger than 100 days could be chosen for the sensitive period (e.g. 120 days). It is then assumed that during the first year of life the juveniles are sensitive to copper for the first 120 days. However, this might overestimate the effects on the whole population. To overcome the uncertainty on the sensitive period, a sensitivity analysis of the sensitive period t_{sens} was performed (see section 4.2.2).

The available survival data are not all described as full dose-response curves (i.e. an observed LC_{50} and the corresponding slope). For some the slope is missing. Based on given LC_{10}, LC_{20} and/or LC_{50} values the slope of the dose-response curve can be calculated. With the drc package R dose-response curves (two-parameter log-logistic model, see Equation 10 further on) were fitted for each dataset. (Table 3). For some datasets, it was not possible to calculate the full dose response relationship, as only one LC_x value was given without the raw survival data (e.g. Vardy et al. 2013).

Table 3. Mean effect parameters derived for each dataset.

Reference	**LC_{50} [µg/L]**	**β [-]**	**t_{exp} [d]**
USASK (2011)	11.1 (-)*	2.80 (± 3 * 10^{-5})[b]	60
Vardy et al. (2011)	9.9 (9.3 – 10.6)[a]	1.45 (± 0.01)[b]	19
	12.4 (11.2 – 13.7)[a]	1.72 (± 0.003)[b]	58
Wang et al. (2014)	3.25 (± 4 * 10^{-4})[b]	4.30 (± 0.001)[b]	14
	3.72 (± 0.002)[b]	12.25 (± 0.06)[b]	28
	5.54 (± 0.001)[b]	5.37 (± 0.005)[b]	28

[a] 95% confidence intervals as reported in the original paper. The confidence intervals note the variance on the value.

[b] Standard error notes the uncertainty on the fit of log-logistic model.

* No standard error or confidence intervals were given.

3.2.2 Copper effects on growth

Next to effects on mortality in early life stages, effects on growth have been reported by Wang et al. (2014). EC_{10} values on growth as low as 1.12 µg Cu per L are reported. EC_x values on growth have been determined for larvae at 2 dph, with an exposure period of 53 days.

The experiment of Vardy et al. (2014) was further extrapolated in time to observe the effects on growth and reproduction in later life stages. Since the fecundity (amount of produced eggs) is dependent on the size of the fish, later effects in life might be observed, e.g. decrease in fecundity, reproduction starts at a later time in life, etc. To examine this effect, the growth curve for white sturgeon was recalculated with the copper effects implemented. For instance, Vardy et al. (2014) determined a 53d-EC_{10} value of 1.12 µg Cu per L, meaning a 10% decrease in growth was observed between 2 and 55 dph at that copper concentration. Growth is here defined as the increase in length (in cm) relative to the initial length.

In excel the growth curve was calculated for the first life year. The growth curve was recalculated by discretizing the Von Bertalanffy equation (Equation 1). The Von Bertalanffy equation is a continuous relationship between age and size. Discretizing the equation allows us to decrease the growth per time step. The daily change in growth is calculated as the difference between two time-steps (Equation 9).

Equation 9. The change in growth between two time-steps.

$$L(t+1) - L(t) = L_\infty * \left(1 - e^{k*(t+1+t_0)}\right) - L_\infty * \left(1 - e^{k*(t+t_0)}\right) = L_\infty * e^{k*(t+t_0)} * \left(1 - e^{k}\right)$$

By decreasing the change in growth during the exposure period the growth curve for sturgeons exposed to copper can be calculated (Figure 7). This was done assuming copper exposure from to 2 to 55 dph. Note that on Figure 7, EC_{100} concentrations were imposed, meaning no growth is observed during exposure.

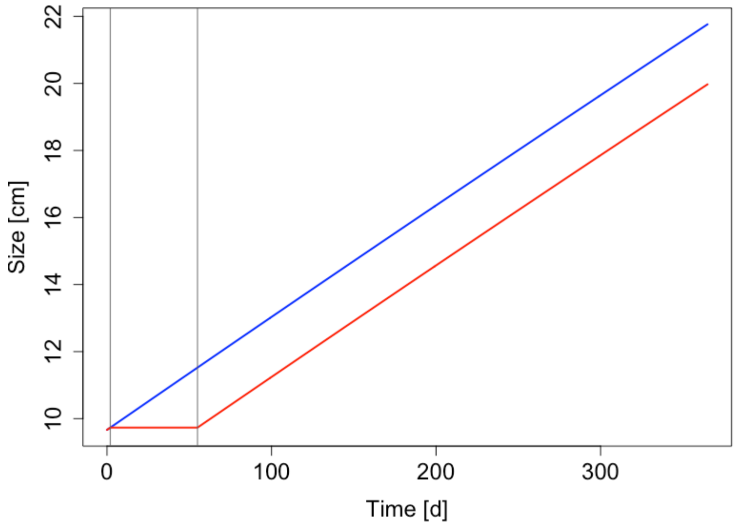


Figure 7. Growth curves for the experiments of Vardy et al. (2014). Length (in cm) is shown over time (in days). The blue curves represent the control growth (no copper exposure). The black vertical lines indicate the start and end of the exposure. The red line shows the growth curve at EC_{100} concentrations.

Clearly from Figure 6 there is a significant effect of copper on the size after the first life year. This trend can be further projected in time to observe the effects on later life stages (Figure 8).

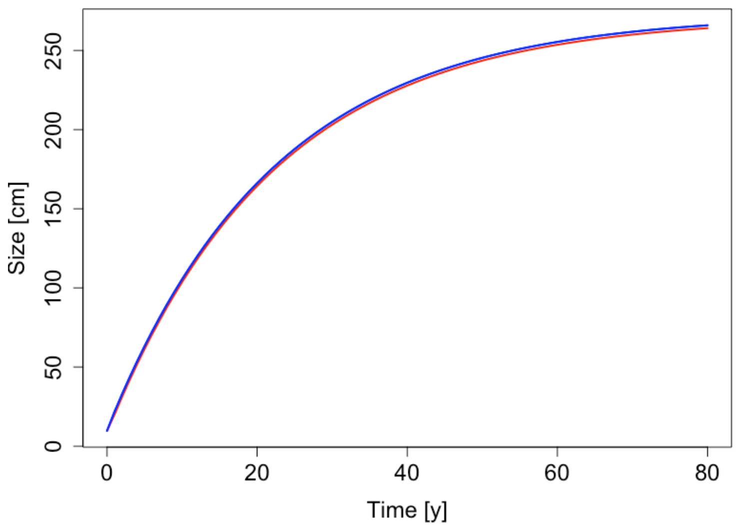


Figure 8. Growth curves for the experiments of Vardy et al. (2014). Length (in cm) is shown over time (in years). The blue curves represent the control growth (no copper exposure). The red line shows the growth curve at EC_{100} concentrations. Note that both lines are situated close to each other.

The affected and unaffected growth curves lie very close to each other. However, the affected growth curve still lies slightly under the control growth curve. Knowing that there is a slight decrease at later life stages, the effect on reproduction can be estimated. Since the relationship between fork length and fecundity is known (Equation 3), the (theoretical) decrease in reproduction can be calculated. The results of the theoretical calculations with the model can be found in Table 4.

Table 4. Effect of growth on the life history of *Acipenser transmontanus* for the data of Wang et al. (2014). The length at the end of the first life year (L) is shown for both control and the copper treatments, as well as the fecundity (F) in the first year of maturity. The effect of copper is expressed as decrease in growth relative to the control. With growth, the increase in length starting at 0 dph until the end of the exposure duration is indicated.

EC_x	L_0 (initial) [cm]	L (control) [cm]	L (copper) [cm]	Decrease in growth	F (control) [#eggs]	F (copper) [#eggs]	Decrease in reproduction
EC10	9.66	21.76	21.58	1.54%	202431	201723	0.35%
EC20	9.66	21.76	22.39	3.07%	202431	201018	0.70%
EC50	9.66	21.76	20.83	7.68%	202431	198910	1.74%
EC90	9.66	21.76	20.09	13.82%	202431	196123	3.12%
EC100	9.66	21.76	19.97	14.80%	202431	195683	3.33%

A maximum (theoretical) reduction of 3.33% in reproduction at EC_{100} concentrations is calculated. The reduction in reproduction decreases even more as the sturgeon ages (less than 0.5% decrease in reproduction at EC_{100} concentrations). EC_{90} concentrations result in a (maximum) decrease of 3.12% in reproduction. At these concentrations effects on mortality would be much larger than the growth effects. To support this statement, a theoretical calculation with the model was performed where mortality effects were compared to growth effects (Table 5). From the experiment performed by Wang et al. (2014), the percentage of mortality during the first life year was calculated at the given copper concentrations and compared to the effects on growth.

Table 5. Comparison between growth and mortality effects observed by Wang et al. (2014). A theoretical calculation with the model was performed to predict the decrease in growth in the first life year, and the decrease in reproduction at the first age of maturity.

EC_x	**Concentration [µg Cu per L]**	**Decrease in growth in first life year**	**Decrease in reproduction at first age of maturity**	**Mortality in first life year**
EC10	1.12	1.54%	0.35%	1.01%
EC20	1.80	3.07%	0.70%	7.31%
EC50	3.98	7.68%	1.74%	70.50%
EC90	13.91	13.82%	3.12%	99.81%
EC100	-	14.80%	3.33%	100%

At concentrations above the EC_{20} for growth, effects on mortality are at least tenfold higher than effects on reproduction. The effects on growth and reproduction in later life stages are thus relatively small compared to mortality during the first life year. Since the effects growth and reproduction are only marginal, these effects were not included in the *Acipenser* IBM, for now. However, in the future, a mathematical method could be developed to integrate the effects of copper on YOY growth. By making the increase in length concentration-dependent, these effects could be predicted as well by the model. Nonetheless, a clear method for implementing these effects is unclear and requires additional research.

3.3 Population level effect assessment

3.3.1 Model output and population level endpoints

The *Acipenser* IBM model will thus be used to simulate a white sturgeon population. Information is given on population demographics throughout the simulations. For every simulated time step (i.e. year) information is given on:

- Population density in each segment (upper, middle, or lower segment)
- Population density of each life stage (embryos/YOYs, juveniles, adults)
- Cumulative reproduction (number of produced eggs)
- Size and weight distribution

The model output can be linked to population level endpoints. By describing the relationship between population level endpoints and the copper concentration, a population level effect assessment of copper on *Acipenser transmontanus* populations can be performed. Naturally, the copper concentration will have an effect on white sturgeon populations. However, other factors can also have an impact, such as the fraction of the river that is polluted, (upstream and downstream) migration rates, the distribution of the copper pollution (homogeneous or heterogeneous), or the location of the pollution (downstream or upstream).

Population level EC_x values were determined based on the fitting of a two-parameter log-logistic model (*llogistic2* function in the drc package of R) (Equation 10). Eventually from the extrapolated individual-level EC_x values, the mean population-level EC_x value was calculated for *Acipenser transmontanus*.

Equation 10. The two-parameter log-logistic model (*llogistic2*). The parameters *b* and *e* are respectively the slope of the dose-response curve and the natural logarithm of the half maximal effect concentration.

$$f(x) = \frac{1}{\left(1 + \exp\left(b * (\log(x) - e)\right)\right)}$$

3.3.2 Research scenarios

A couple of research scenarios were simulated with the *Acipenser* IBM model. The simulated scenarios follow a logical structure, starting with a default scenario, and evolving towards a more representative scenario for the Snake River. Additionally, the effect of certain parameters is assessed in several scenarios. Table 6 gives an overview of the scenarios.

Table 6. Overview of the simulated scenarios with the *Acipenser* IBM.

Nr	Scenario	N_segments[a]	t_sens[b]	L_river[c]	m_up[d]	m_down[e]	f_exposed[f]	Location[g]	Distribution[h]
1	Default 1	1	t_exp	200 km	0 y^{-1}	0 y^{-1}	100%	-	-
2	Default 2	3	t_exp	200 km	0 y^{-1}	0 y^{-1}	100%	-	-
3	Default 3	3	t_exp	200 km	0 y^{-1}	0.02 y^{-1}	100%	-	-
4	t_sens	3	**VAR**[i]	200 km	0 y^{-1}	0.02 y^{-1}	100%	-	-
5	F_exposed	3	t_exp	200 km	0 y^{-1}	0.02 y^{-1}	**VAR**[j]	Random	Heterogeneous
6	Migration	3	t_exp	200 km	0 y^{-1}	**VAR**[k]	100%	-	-
7	Heterogeneous	3	t_exp	200 km	0 y^{-1}	0.02 y^{-1}	33.33%	Random	Heterogeneous
8	Homogeneous upstream	3	t_exp	200 km	0 y^{-1}	0.02 y^{-1}	33.33%	Upstream	Homogeneous
9	Homogeneous downstream	3	t_exp	200 km	0 y^{-1}	0.02 y^{-1}	33.33%	Downstream	Homogeneous

[a] N_segments: number of river segments considered in the spatial setup of the model.

[b] t_sens: the sensitive period parameter. In most scenarios, this is equal to the exposure duration of the experiment (t_exp).

[c] L_river: length of the river considered.

[d] m_up: yearly upstream migration rate.

[e] m_down: yearly downstream migration.

[f] f_exposed: fraction of the population exposed to copper pollution.

[g] Location: location of the pollution (either randomly distributed, upstream, or downstream).

[h] Distribution: distribution of the pollution source (either heterogeneous or homogeneous).

[i] In scenario 4, the sensitive period (t_sens) is varied from 20 to 120 days.

[j] In scenario 5, the fraction exposed (f_exposed) is varied from 0 to 100%.

[k] In scenario 6, the downstream migration rate is varied from 0 to 0.1 y^{-1}.

3.3.2.1 Scenario 1: default 1

The first default scenario considers one river segment of 200 km in length (Figure 9). Since only one river segment is simulated, no migration between segments is considered. The individuals will only move randomly within this segment. A copper concentration range is simulated for this scenario, and for all effect parameters listed in Table 3 population-level effects are assessed. It is assumed that the whole population within this 200-km river stretch is exposed to copper. By varying the copper concentration (from 0 to 100 µg Cu per L) the effects of copper on *Acipenser transmontanus* populations were examined.

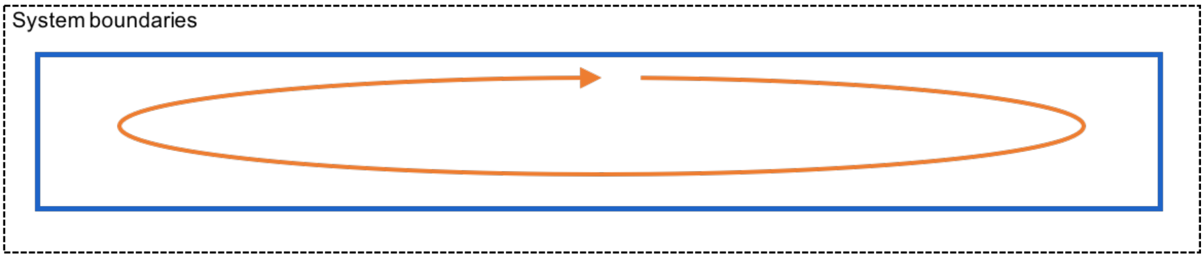


Figure 9. Scenario 1. Schematic representation of the default_1 scenario. One river segment is simulated wherein the individuals can move freely about.

3.3.2.2 Scenario 2: default 2

The second default scenario is very analogous to the default_1 scenario, except that here three river segments are considered instead of one (Figure 10). As described by Jager (2000) the rivers where white sturgeon live, are often fragmented in river segments divided by dams or waterfalls. The Snake River contains about 20 dams over the whole watershed. In the simulations here, the river stretch of 200 km is divided into three equal parts through dams. No migration between the segments is considered however. Only movement within each of the river segments is simulated in this scenario. In a similar fashion as the default_1 scenario, a copper concentration range was tested for this scenario. The fragmentation of the river segment due to the dams might influence the sensitivity of the population.

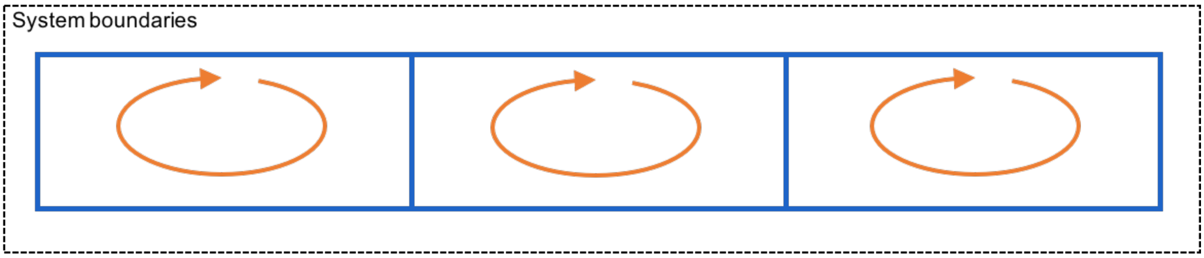


Figure 10. Scenario 2. Schematic representation of the default_2 scenario. Three river segments are simulated wherein the individuals can move freely about.

3.3.2.3 Scenario 3: default 3

The third default scenario simulates an even more representative scenario for the Snake River population. Next to the three river segments, migration between these segments is considered (Figure 11). Since dams are obstructions impeding the ability of sturgeons to migrate upstream, only downstream migration is considered (unidirectional). Again, in a similar fashion as for the default_1 and default_2 scenario, population level effects were investigated.

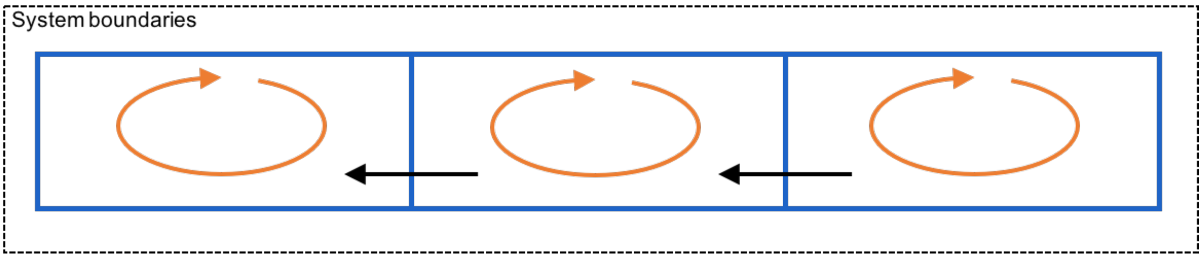


Figure 11. Scenario 3: Schematic representation of the default_3 scenario. Three river segments are simulated wherein the individuals can move freely about. Downstream migration is considered.

3.3.2.4 Scenario 4: t_sens

With this scenario, model sensitivity to the sensitive period parameter (t_sens) was investigated in a sensitivity analysis. For the different effect parameters (Table 3) the effect of the sensitive period was investigated by increasing it from the default value (t_{exp}) to a larger value (120 days). This allows to examine the effect of the sensitive period on the population level in a sensitivity analysis.

3.3.2.5 Scenario 5: f_exposed

All previous scenarios assumed a fraction exposed of 100% (Table 5), meaning that the whole population within the 200-km river stretch is exposed to copper. In a real-life situation, this is not necessarily the case. Some parts of the river will not be polluted, hence leaving part of the population unaffected. The effect of the fraction exposed $f_{exposed}$ is thus investigated. By varying both the fraction exposed and the copper concentration, population response to $f_{exposed}$ is assessed. The default_3 scenario was performed with values for $f_{exposed}$ ranging from 0 to 100%.

3.3.2.6 Scenario 6: migration

Jager (2000) has investigated the effect of (upstream and downstream) migration on the viability of white sturgeon populations. A range from 0 to 0.1 y^{-1} for the migration rates was investigated. Similarly, in this research scenario the migration rates were varied and the effect on the population was examined in combination with copper stress. The default_3 scenario was followed again, but now with varying values for the downstream migration rate m_{down}. Additionally, further downstream migration is allowed in this set-up as well (Figure 12).

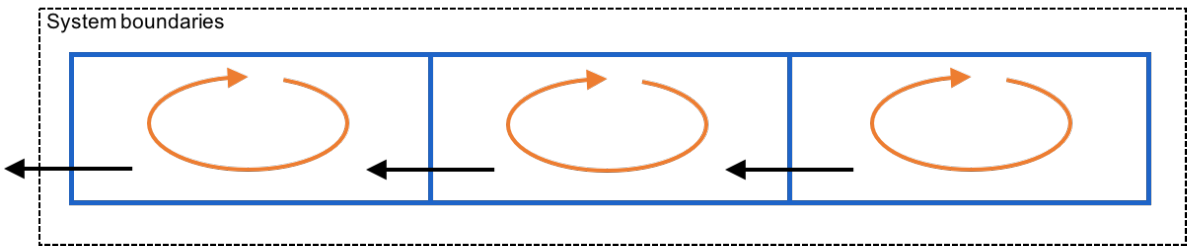


Figure 12. Scenario 6: Schematic representation of the migration scenario. Note the extra downstream migration in the lower segment. Individuals in this system can migrate downstream to lower river parts.

3.3.2.7 Scenarios 7, 8 and 9: heterogeneous, homogeneous upstream and homogeneous downstream

In this batch of research scenarios, the effect of the location and distribution of copper pollution in the river was investigated. With location, the position of the pollution within the river segment is indicated. This can either be upstream or downstream, or somewhere in between. The pollution source can also be distributed either homogeneously (in one place) or heterogeneously (randomly across the river segment). Note that in this research scenario only one third of the river is polluted with copper ($f_{exposed}$ of 33.33%) (Figure 13).

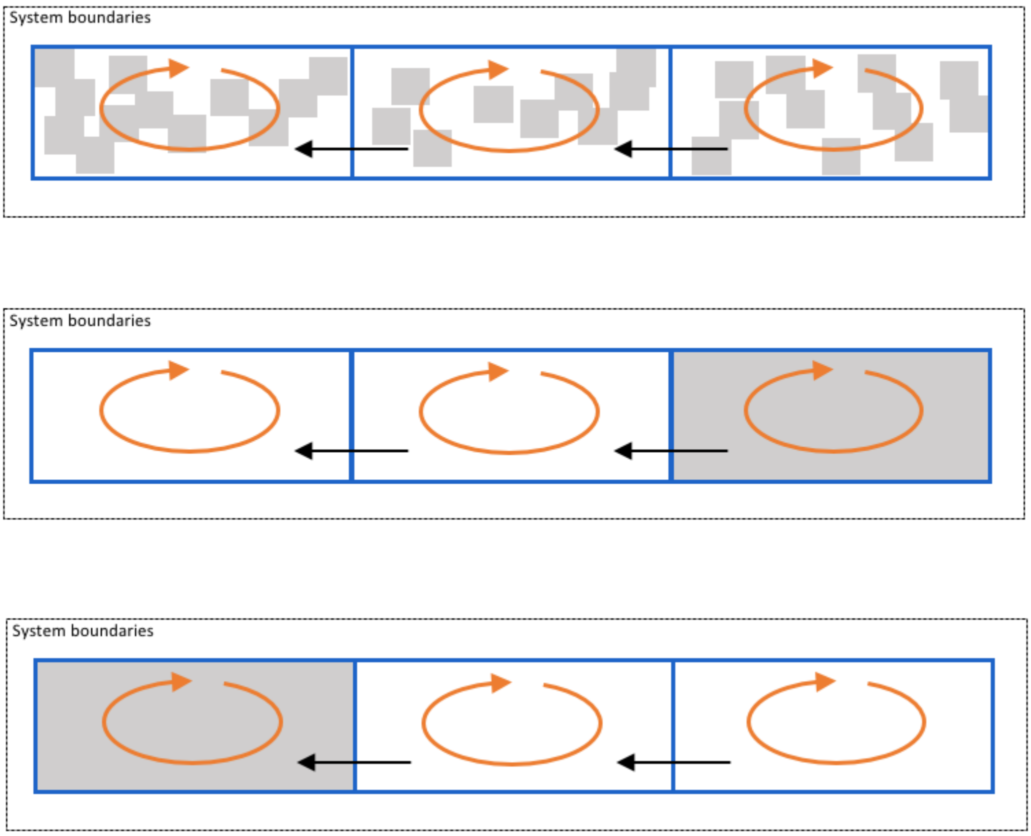


Figure 13. Scenarios 7, 8 and 9. Schematic representation of the simulated scenarios. The polluted areas are indicated in grey. The top scheme shows the situation when a heterogeneous pollution is imposed to the model (i.e. random polluted patches in the river system). The middle scheme shows a homogeneous, downstream pollution. The bottom scheme shows a homogeneous, upstream pollution source.

4 RESULTS AND DISCUSSION

4.1 *Acipenser* IBM model performance

To assess the model performance of the *Acipenser* IBM, model predictions were compared with field monitoring data of white sturgeon demographics. Jager et al. (2001b) have reported population densities from field studies in different river segments of the Snake River. In three of the river segments mentioned, the population densities reported were around 6 individuals per km. In some segments, the densities got lower than 1 individual per km. But in three segments the densities of white sturgeon were as large as 27, 47 and 64 individuals per km river. These large densities were possibly the results of excellent habitat quality and quantity. BC Fisheries (2000) observed densities between 1.5 and 17.1 fish per km in the Fraser River. In one case, even densities as high as 230 individuals per km were observed, although these high densities could be explained by the sampling location. The high abundance was found close to the river mouth, where the Fraser River starts becoming wider and eventually enters the ocean. Lower river parts and river deltas in general contain plenty of habitat for the white sturgeon to spawn and forage (Israel et al. 2009).

Executing the default scenario (default_3), the model predicts average densities of 15 individuals per km river (without exposure to copper). A value of around 15 individuals per km is a realistic value for the population density compared to observed abundances of white sturgeon populations. The value for the carrying capacity parameter K_{pop} was set to 210 individuals per km free-flowing habitat of the river (Table 2), restricting the population to a maximum of 210 individuals per km. Note that a standardised river was considered here, following the spatial representation of Jager (2000) and Jager et al. (2001a). The environment is represented as a uniform river segment, with a fixed river width, free-flowing habitat, etc. Increasing the amount of available free-flowing habitat and the river width is expected to result in higher densities.

4.2 Population level effect assessment

As explained above, several simulation scenarios were performed with the *Acipenser* IBM model. By varying the copper concentration, population level effects were observed. As exposure to copper increases mortality in age-0 individuals, it is obvious that high copper concentrations will lead to population extinction. Figure 14 gives an example of a simulation with the effect parameters of Vardy et al. (2011) (Table 3). Indeed, as indicated on Figure 14, a copper concentration of 10 µg Cu per L decreases the population density significantly as a consequence of the increased recruitment failure.

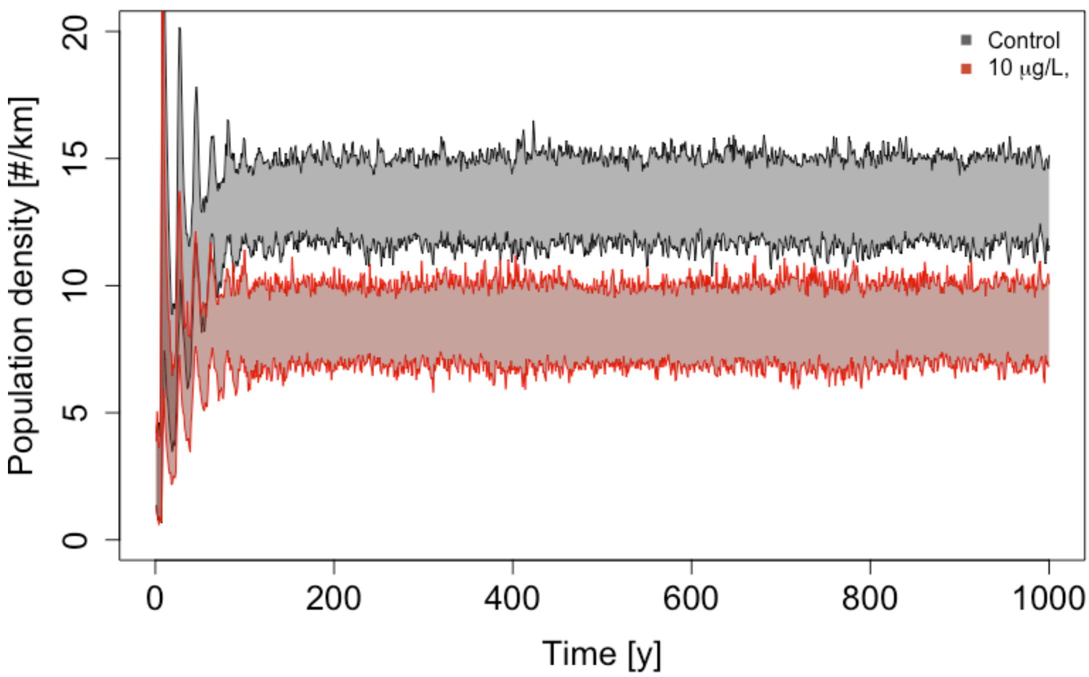


Figure 14. Example of a simulation with the *Acipenser* IBM. White sturgeon population density over time for two copper concentration (0 and 10 µg Cu per L). The error bands on the population density indicate the variability (related to spawning parameters) and stochasticity (stochastic movement and death) included in the model.

From the population dynamics, the equilibrium population density can be determined. The equilibrium population density is considered as the mean population density between 500 and

1000 years of simulation (expressed in number of individuals per river km). It is clear from Figure 14 that the equilibrium population density is lower at 10 µg Cu per L compared to the control simulation (i.e. no copper exposure).

The equilibrium population density can be expressed as a function of the copper concentration (Figure 15). A concentration-response curve can be fitted through the data to establish a relationship between the equilibrium population density and the level of copper pollution. On Figure 15, a two-parameter log-logistic curve (Equation 10) was fitted to the data using the drc-package in R. Based on the fitted dose-response model, population-level EC_x values were determined for the different scenarios and the different data available.

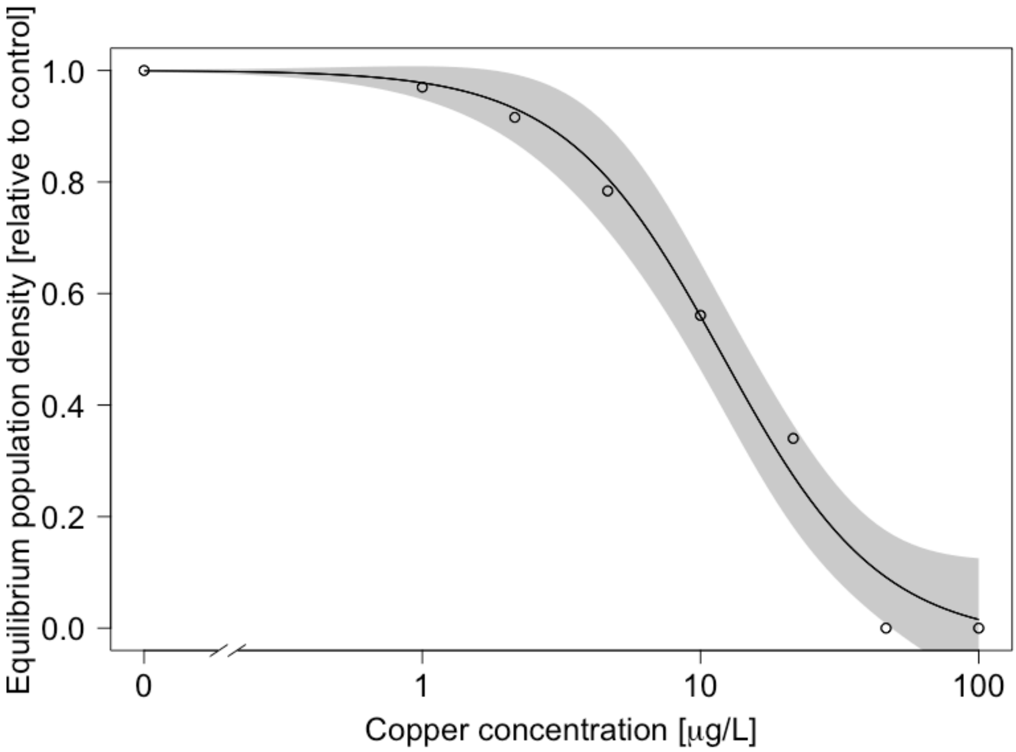


Figure 15. The equilibrium population density (relative to the control) as a function of the copper concentration. The data points represent the mean equilibrium density of 100 simulation iterations. The line represents the fitted dose-response curve. The grey area indicates the 95% confidence intervals of the fitted model (i.e. uncertainty on the model fit).

4.2.1 Effects of copper on *Acipenser transmontanus* populations

First, three different default scenarios were simulated, ranging from a basic scenario to a more realistic approach of the Snake River watershed. The first scenario assumes one river segment of 200 km in which the individuals can move freely (Figure 9). The second assumes the same river stretch of 200 km but divided in three segments separated by dams (Figure 10). No migration between the river segments is considered. The final default scenario does take into account (downstream) migration between the different river segments (Figure 11).

Table 3 shows the different effect parameters derived from literature. For each of the studies mentioned in Table 3, the LC_{50} value and the slope β can be used to extrapolate and determine the effects on the population level. Using the method explained above, EC_x values on population equilibrium density were determined for each set of parameters and for each default scenario (Table 7). A critical note must be added to these effect concentrations. The predicted EC_x values in Table 7 assume that there is 100% exposure, meaning that every individual in the population within this 200-km river stretch is exposed to copper and will experience the same effects. Additionally, it is assumed the effects are induced immediately, without considering uptake and elimination rates. All copper concentrations are considered the same over this 200-km river segment without variability that may be caused by river water chemistry (i.e. no bio-availability corrections are considered).

Table 7. Predicted population-level EC_x values. Values are derived from 100 simulation iterations for the three default scenarios and for each data set. The standard error (between parentheses) indicates the error on the goodness of fit of the log-logistic function.

Population EC_x	Reference	Exp #	Default 1[a]	Default 2[b]	Default 3[c]
EC_{50} [µg/L]	USASK (2011)	1	11.0 (1.0)	11.0 (1.0)	11.0 (1.0)
	Vardy et al. (2011)	1	12.0 (1.1)	12.0 (1.1)	11.7 (1.1)
		2	14.3 (1.1)	14.3 (1.1)	14.1 (1.1)
	Wang et al. (2014)	1	3.6 (1.0)	3.6 (1.0)	3.0 (1.0)
		2	3.1 (1.0)	3.3 (1.1)	3.0 (1.0)
		3	5.2 (1.0)	5.2 (1.0)	5.2 (1.0)
EC_{20} [µg/L]	USASK (2011)	1	8.5 (1.1)	8.5 (1.0)	8.6 (1.0)
	Vardy et al. (2011)	1	4.9 (1.2)	4.9 (1.2)	4.9 (1.1)
		2	6.7 (1.1)	6.9 (1.1)	6.8 (1.1)
	Wang et al. (2014)	1	2.5 (1.1)	2.5 (1.0)	2.4 (1.0)
		2	2.9 (1.0)	3.1 (1.1)	2.7 (1.0)
		3	4.5 (1.0)	4.5 (1.0)	4.5 (1.0)
EC_{10} [µg/L]	USASK (2011)	1	7.3 (1.1)	7.2 (1.1)	7.3 (1.1)
	Vardy et al. (2011)	1	2.9 (1.2)	2.9 (1.2)	3.0 (1.2)
		2	4.5 (1.0)	4.5 (1.2)	4.4 (1.2)
	Wang et al. (2014)	1	2.0 (1.0)	2.0 (1.0)	2.1 (1.0)
		2	2.8 (1.0)	3.0 (1.1)	2.6 (1.0)
		3	4.2 (1.0)	4.2 (1.0)	4.2 (1.0)

[a] Scenario 1: the default_1 scenario assumes one river segment.

[b] Scenario 2: the default_2 scenario assumes three river segments separated by dams.

[c] Scenario 3: the default_3 scenario assume three river segments separated by dams. Migration between the river segments is assumed.

A comparison between conventional LC_x values and population EC_x here will be discussed further in Section 4.3 No major differences in sensitivity are observed between the default scenarios. The maximum difference in EC_x concentrations is 20% (EC_{50} for the 2nd experiment of Wang et al. 2014). All effect concentrations from the default scenarios are situated in a similar concentration range. The fragmentation of the river into three segments separated by dams does not seem to influence the sensitivity of the population. The same accounts for the migration. With or without migration between segments, the effect concentrations are not significantly increasing or decreasing, indicating this has no influence on population sensitivity.

4.2.2 Sensitivity analysis of the model parameter "sensitive period"

In section 4.2.1, population-level effects were determined for various default scenarios. The effects that we integrated into the models were based on observed individual-level data as reported in the original studies. However, as mentioned earlier, those effect concentrations only account for the duration of the experiment. The original experiments were not necessarily performed over the whole period during which the developing YOYs are sensitive to copper. For instance, a sensitive period of 120 days could be used for the simulations to take into account the whole period over which the YOYs are sensitive. The effect of the chosen value of the sensitive period parameter (t_{sens}) on the population level EC_x values was thus investigated in more detail. For the effect parameters of Vardy et al. (2011) and Wang et al. (2014) population sensitivity was assessed as a function of the sensitive period t_{sens} by increasing it from the exposure duration of the experiment to 120 days. EC_{50} values on equilibrium population density were assessed as a function of the sensitive period t_{sens} (Figure 16).

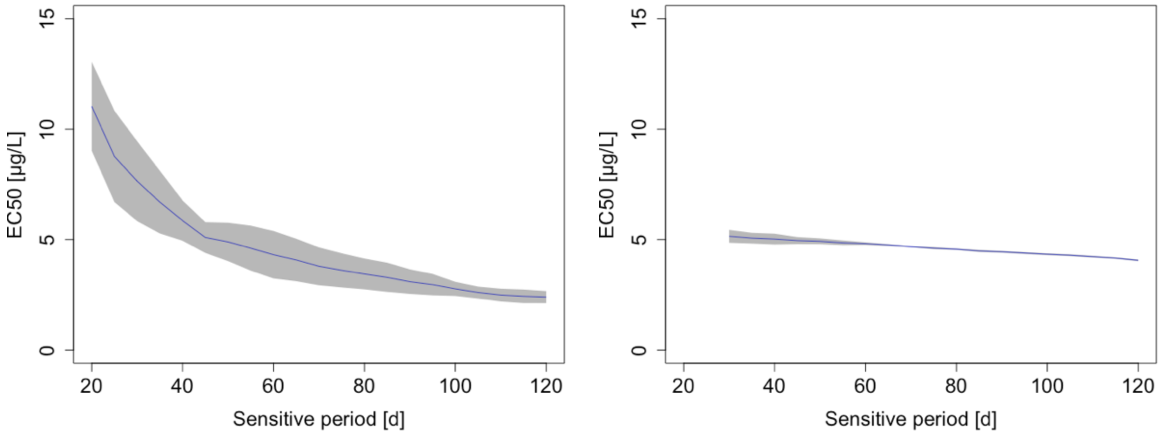
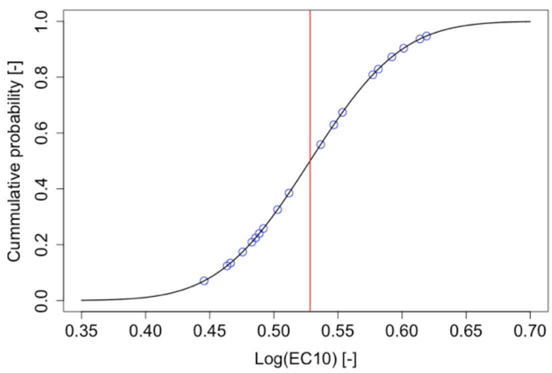


Figure 16. Effect of the sensitive period (t_{sens}) on population sensitivity to copper. The EC_{50} on equilibrium population density is shown as a function of the chosen value of the sensitive period parameter (t_{sens}). The left graph shows the effect of the sensitive period for the first experiment by Vardy et al. (2011), the right graph the third experiment of Wang et al. (2014). The grey area indicates the 95% confidence interval on the determined EC_{50} values.

The parameter t_{sens} influences the model-predicted population-level sensitivity. As the sensitive period increases, the EC_{50} decreases almost 5-fold for the first experiment of Vardy et al. (2011) (from 11.0 µg/L to 2.40 µg/L), whereas for Wang et al. (2014) the effect is less pronounced (from 5.1 µg/L to 4.1 µg/L) (Figure 16). It has not been determined precisely how long juveniles are sensitive to copper, but the experimental data (Table 1) suggest that white sturgeon become less sensitive with age. Based on Little et al. (2010), the sensitive period is between 20 and 120 dph. Altogether, choosing a higher value for t_{sens} results in lower predicted population-level EC_x values. The more conservative case would be to use the duration of the experiment t_{exp} as the sensitive period t_{sens}, since it cannot be known what the effect of copper on the sturgeons is outside the investigated duration of the experiment. But on the other hand, extrapolating the observed effects beyond the duration of the experiment may overestimate the effects on population level. For example, Vardy et al. (2011) observed that most mortality seems to occur between 21 and 34 dph. Extrapolating the effects observed in this study beyond 34 dph might not be necessary, since the most sensitive individuals might have already died in that period. Furthermore, the current risk assessment framework does not take into account the duration of the experiment nor the sensitive period. The LC_x/EC_x values are compared and considered independent of the duration of the experiment, meaning that the current system does not take into account the sensitive period.

Considering the uncertainty about the sensitive period, a detailed sensitivity analysis was carried out on the parameter t_{sens}. For each study, the value for t_{sens} was varied from the nominal value (the experimental exposure duration – t_{exp}) to a value of 120 days (as in Figure 16). The default 3 scenario was executed for each study, and population EC_{10} and EC_{50} values on population equilibrium density were determined. A log_{10}-normal distribution was fitted to the model-predicted EC_x values (Figure 17). Parameter values for the sensitive period t_{sens} were chosen from a uniform distribution, ranging from the experimental exposure duration to a maximum value of 120 days. The values were selected equidistant from each other from the distribution.



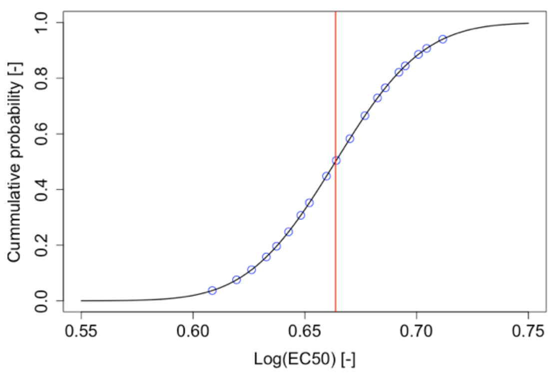


Figure 17. Example of a log-normal distribution fitted to predicted EC_{10} (left) and EC_{50} (right) values for the study of Wang et al. (2014) (third experiment). Each data point (blue dots) represents a population EC_x for a value of the sensitive period for a specific study (sampled from a uniform distribution). The black curve shows the fitted cumulative log-normal distribution. The red line indicates the 50^{th} percentile of the distribution.

From the log-normal distribution on the predicted values, the 50th percentile on the EC_x is calculated and considered as the estimated population-level EC_x (Table 8). A comparison between these values and their individual-level counterparts will be discussed further on (see Section 4.3).

Table 8. Sensitivity analysis for the sensitive period t_{sens}. For each study, the value of the sensitive period was varied from the experimental duration of the experiment ($t_{sens} = t_{exp}$) to a maximal sensitive period (t_{sens} = 120 days). From the log-normal distribution on the EC_x values, the 50th percentile is considered the population EC_{10} value of that study. The corresponding 95% confidence intervals are given between parenthesis (i.e. the 2.5th and 97.5th percentile).

Reference	**Exp #**	**50th percentile of the EC_{50}**	**50th percentile of the EC_{10}**
USASK (2011)	1	10.0 (8.9 – 11.0)	5.9 (4.8 – 7.0)
Vardy et al. (2011)	1	4.1 (2.9 – 5.3)	1.7 (0.5 – 2.8)
	2	10.1 (9.0 – 11.2)	4.3 (3.2 – 5.3)
Wang et al. (2014)	1	2.2 (1.8 – 3.3)	1.6 (0.5 – 2.7)
	2	3.1 (2.0 – 4.1)	2.7 (1.7 – 3.8)
	3	4.6 (3.6 – 5.6)	3.4 (2.3 – 4.4)

4.2.3 Minimum viable population density

The default scenarios assume that a river stretch of 200 km is completely polluted with copper. In a more realistic situation, this would not be the case. Only some parts of the river would be polluted, often with different concentrations depending on the location. In a scenario, the effect of the fraction exposed $f_{exposed}$ (i.e. the percentage of the population actually exposed to copper) is investigated. Logically, if only a small part of the population is exposed to copper, effects would be less grave.

Figure 18 shows the relationship between the exposed fraction (% of the population) and the population density at very high copper concentrations (at LC_{100} concentrations). The population density here is expressed relative to the control (i.e. exposed fraction of 0).

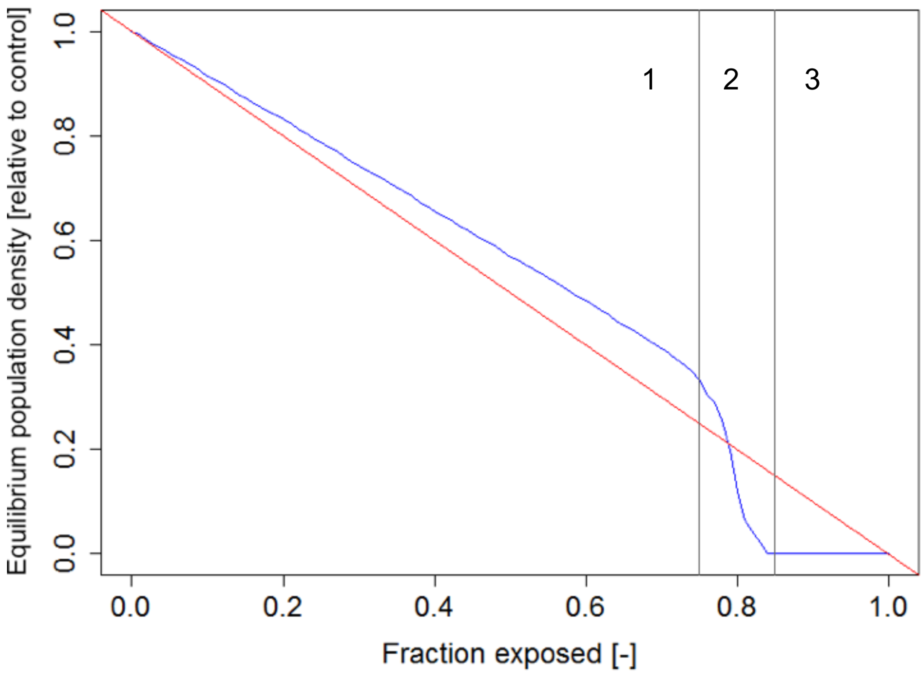


Figure 18. Equilibrium population density (relative to the control) as a function of the exposed fraction (i.e. the fraction of the population that is actually exposed to copper). Lethal copper concentrations are assumed (LC_{100} concentrations). The red line indicates a negative 1:1 relationship. The grey, vertical lines indicate an exposed fraction of respectively 0.75 and 0.85.

If 100% of the population is exposed (i.e. fraction exposed equal to 1) a 100% decrease in equilibrium population density is observed, indicating extinction of the population at high (lethal) copper concentrations. The effect of the fraction exposed on the maximum decrease in population density can be divided into three areas, as shown in Figure 18. First there is a negative, linear relationship between the equilibrium density with an increasing exposed fraction. Second, at higher exposed fractions the population has difficulties to sustain itself. At even higher exposed fractions white sturgeon recruitment is not high enough to sustain a population.

At fractions lower than 0.7 a linear relationship is seen between the fraction exposed and the maximum decrease in population density. If only 10% of the population is exposed to copper, logically only a maximum decrease in population density of 10% can be seen. The linear relationship is not a perfect 1:1 relationship (indicated by the red line). Rather, a fraction exposed of 20% will lead to little less than 20% of the undisturbed equilibrium density. This can be explained by the density-dependent process. If the recruitment decreases because a higher fraction of the population is exposed, the overall population density in the population will decrease as well. In turn, more suitable habitat is available for spawning, increasing the survival chances of age-0 individuals. This eventually compensates the initial loss of recruitment.

At exposed fractions higher than about 75% the population seems to have difficulties sustaining itself, as the relative population density rapidly decreases. Eventually, at fractions higher than 85% the population cannot sustain itself and becomes extinct. A too large portion of the population is affected causing the recruitment to be too low to sustain a healthy population. This trend is also demonstrated in Figure 18. When lethal concentrations are assumed and the fraction exposed is higher than 85% the population will become extinct, as indicated by the blue curve. Over time the population density becomes zero. If less than 85% of the population is exposed, the population persists (red curve). Note that on Figure 19 the red curve shows a simulation performed in area 2 of Figure 18. The blue curve is situated in area 3 of Figure 18.

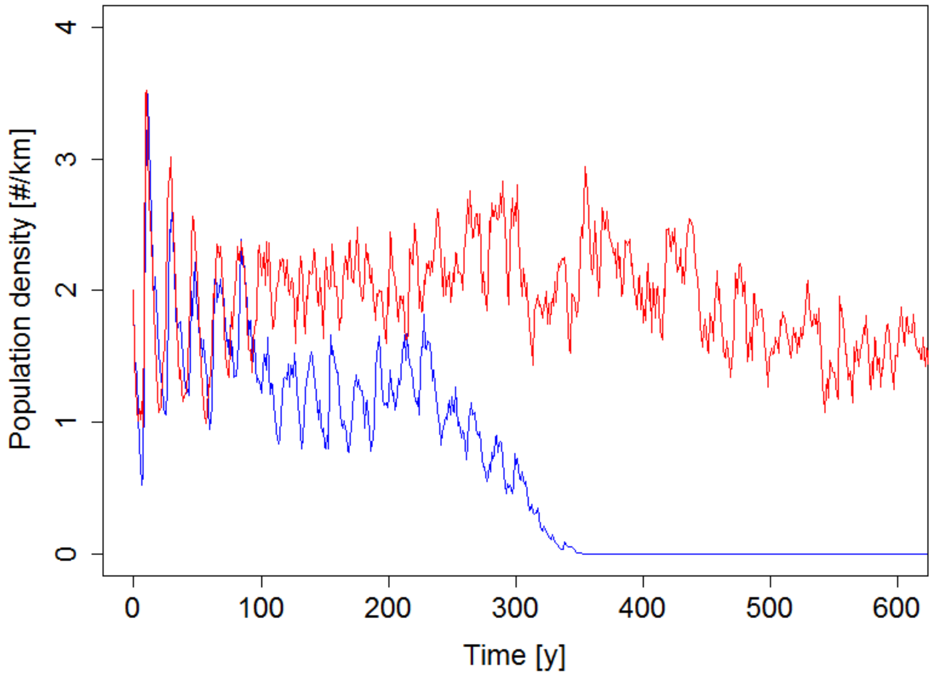


Figure 19. White sturgeon population dynamics over time. The blue curve indicates a scenario where the population becomes extinct (fraction exposed of more than 85%). Because the population density becomes too low, there is not enough recruitment to sustain the population, hence why the population density becomes 0. The red curve indicates a situation where the population density is just high enough to predict population persistence (fraction exposed of less than 85%). Note that the figure the red curve shows a simulation performed in area 2 of Figure 18. The blue curve is situated in area 3 of Figure 18.

The tipping point is around a population density of 2 individuals per km river. The recruitment of a population with less than 2 individuals per km is too low to sustain the population further, eventually causing population extinction. This indicates that there is a certain threshold above which the population can persist and grow, and below which populations are in decline. Jager et al. (2010) identified this threshold for white sturgeon as the minimum viable population size (MVP). From the simulations (Figures 18 and 19) it can be concluded that the minimum viable population size is around 2-2.5 individuals per km. Population effects are irreversible under this threshold and the population does not persist. If copper pollution affects the population in such a magnitude that the density falls below this threshold, population extinction is irrevocable.

Jager et al. (2010) investigated what the minimum viable population size of white sturgeon is (i.e. the population size that still has an adequate recruitment to sustain a population). The Acipenser IBM was used by Jager et al. (2010) to investigate the threshold at which white sturgeon recruitment is insufficient. The results from Jager et al. (2010) indicated that the minimum population size lies around 50 individuals per 10 km of free-flowing habitat (or thus 5 individuals per km of free-flowing habitat). This value can be compared to the predictions of the *Acipenser* IBM model in the present study. The spatial structure of the model's default scenario predicts that every km of river has 0.55 km or free-flowing river habitat (see Figure 3). The minimum viable population size of the present study's model should then be around 2.5 individuals per km of river. Maximum densities of around 15 individuals per km have been observed with the default scenario (without copper exposure) (Figure 14). At a population density of 2.5 individuals per km of river, the density is about 15% of its undisturbed population density. This threshold was observed in the simulations as well (Figures 15 and 19) and is around 2-2.5 individuals per km of river. The implementation of the *Acipenser* IBM in the present study thus predicts a similar threshold as determined by Jager et al. (2010).

4.2.4 Influence of migration rate

Movement behaviour is a very important aspect in the life history of many fish species. Especially in the case of the white sturgeon, where they undergo seasonal migration for spawning and have the ability to swim upstream or downstream. Population sensitivity to copper might be dependent on the migration rate. The effect of both upstream and downstream migration has already been investigated by Jager (2000). However, the effect of the migration rate in combination with exposure to copper might give more insight in the dependence of movement behaviour of white sturgeon populations while being under toxic stress.

From the simulated scenarios, a comparison is made between high and low migration rates in combination with copper stress. Note that for all the simulations performed here only one third of the river is contaminated (either one river segment when the pollution source is homogenous, or either a random 33.33% of the river length is polluted). The migration rate is varied from 0 to a maximum of 0.1 y^{-1}. Results for two copper treatments can be seen in Figure 20.

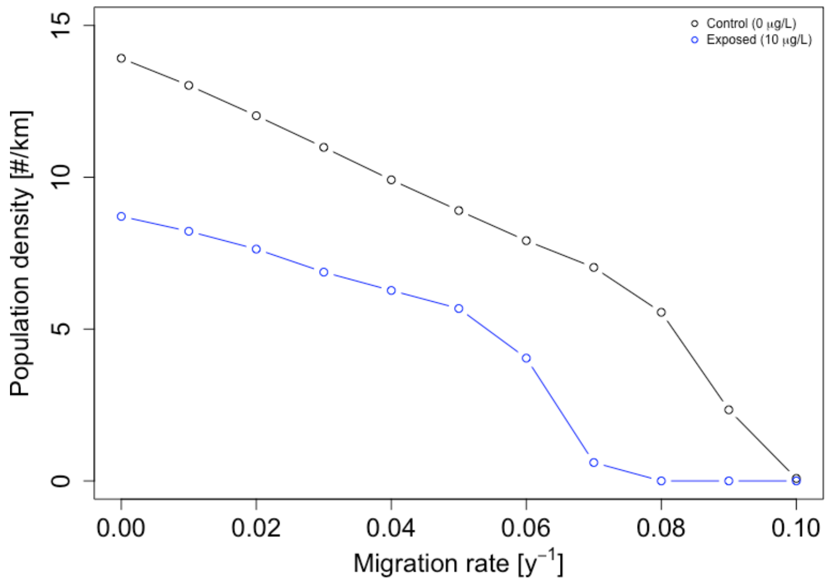


Figure 20. Population density as a function of the migration for two copper treatments (0 and 10 µg Cu per L). Effect parameter of USASK (2011) were used.

Clearly, for the control treatment a decrease in population density with increasing migration rates is observed. Since a higher migration rate means individuals moving faster downstream, eventually more individuals will leave the spatial boundaries of the river system in the model. At migration rates, higher than 0.1 y^{-1} the downstream migration has become too large to sustain a population in the river considered here. As more individuals leave the river system to move to downstream parts of the river (only downstream migration is considered), the overall population density will indeed be lower. Eventually the density falls below the threshold for sufficient recruitment, and the population no longer persists.

As explained previously, the population density decreases with increasing copper concentration. This trend was observed with the simulations here as well. The exposed densities consistently lie under the control curve, which shows the effect of copper on the population. Since only one third of the river is polluted with copper, a maximum decrease in population density of about 34% can be observed. Indeed, at a migration rate of 0, the exposed population density lies at around 66% of the control density. Higher migration rates decrease the population density. Very high migration rates will decrease the population density in such a way that the population gets close to the critical density, increasing the chances of extinction (Jager et al. 2010). The combination of exposure to copper and high migration rates will increase the chances of the population becoming extinct in certain parts of the river.

4.2.5 Influence of the location and distribution of the pollution

Next to the factors investigated above, environmental factors will also influence white sturgeon population dynamics in response to copper pollution. More specifically the location of the copper pollution and how it is distributed over the river stretch will impact population response. Especially since spatial movement is an important part of the life cycle of white sturgeons.

For three different scenarios, the effect of the location and distribution of copper pollution on the population is assessed (Figure 21). In each situation only one third of 200-km river stretch is polluted with copper (Figure 13). The first situation assumes a heterogeneous distribution (i.e. random polluted patches across the river). The second scenario assumes a homogeneous pollution (i.e. pollution in one location), that is located downstream (i.e. in the lower segment). The last scenario also assumes a homogeneous pollution, but located upstream (i.e. in the upper segment). The results for each scenario are shown in Figure 21.

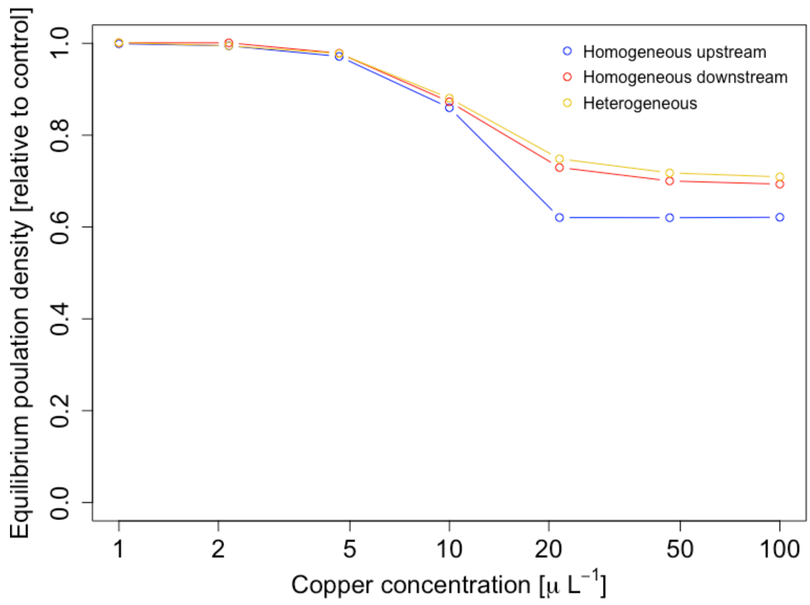


Figure 21. Equilibrium population density (relative to the control) as a function the copper concentration, for different pollution configurations: the blue line for homogeneous upstream source, red line for a homogeneous downstream source, and yellow for a heterogeneous source. Effect parameter of USASK (2011) were used. The mean of 100 simulation iterations is shown.

Since only one third of the river stretch is contaminated, a maximum effect of about 33% is observed in the decrease of equilibrium population density in Figure 21.

A homogeneous upstream pollution (blue curve) has the largest effect on population level. This curve reaches the lowest (relative) equilibrium density. Based on the performed scenarios, if an upstream pollution source is considered, the population will have the tendency to move away from the contaminated areas. Since recruitment in the upstream polluted area will be low, and only downstream migration is considered, the density in the upper part will decrease over time. Eventually, the whole population will shift to the lower, uncontaminated parts. The amount habitat for the population will have decreased, decreasing the overall population size. At higher copper concentrations, no effect is observed anymore (i.e. the curve becomes a horizontal). The population already became extinct in the contaminated areas, and the individuals in the uncontaminated areas remain unaffected.

For a homogeneous downstream source, a different trend is observed. The (relative) population densities are higher than for a homogeneous upstream source. Since only downstream migration is considered older individuals (juveniles and adults) can still migrate to the downstream contaminated areas. Older individuals are not influenced by the copper concentrations and can still thrive in contaminated areas. No reproduction will take place there, however. Under those circumstances, population density will be slightly higher, and the effect of copper will be of a lesser magnitude.

The densities for a heterogeneous pollution are slightly higher than those of the homogeneous pollution. In each river segment, there are both contaminated and uncontaminated areas. Since the contaminated areas are surrounded by uncontaminated patches, there will still be reproduction in each river segment. The effect of copper in this situation will be the least. Next to the migration rate, the location and distribution of the pollution source will influence population response, although the effect is only minor. Since only one third of the river stretch is polluted, intuitively one would expect to see an effect of 33%. However, the relationship is not completely linear (Figure 21). Nonetheless, the effect of a different spatial distribution of the pollution is slight.

4.3 Comparison of population-level effects with conventional effects from lab tests

Predicted population-level EC_x values were determined for several scenarios. A comparison can now be made between the observed effect concentrations of the traditional endpoints (LC_x), and the predicted population-level effect concentrations predicted (Table 9).

Table 9. Comparison between observed, traditional LC_x values and the predicted, population-level EC_x values (50th percentiles as in Table 8) extrapolated with the *Acipenser* IBM model. The last column shows the ratio of population EC_x to traditional LC_x.

Effect concentration	Reference	Exp #	Apical level (traditional LC_x)	Population level (IBM-predicted EC_x)	Ratio (EC_x/LC_x)
LC_{50}/EC_{50} [µg/L]	USASK (2011)	1	11.1	10.0	0.90
	Vardy et al. (2011)	1	9.9	4.1	0.41
		2	12.4	10.1	0.81
	Wang et al. (2014)	1	3.1*	2.2	0.71
		2	3.7*	3.1	0.84
		3	5.2*	4.6	0.88
LC_{10}/EC_{10} [µg/L]	USASK (2011)	1	5.1*	5.9	1.16
	Vardy et al. (2011)	1	1.8*	1.7	0.94
		2	3.4*	4.3	1.26
	Wang et al. (2014)	1	1.8	1.6	0.89
		2	3.1	2.7	0.87
		3	3.7	3.4	0.92

* Values calculated from the log-logistic model fit (i.e. not reported in the original papers).

All EC_{50} values are slightly lower than their traditional LC_x counterpart, by on average 24% (range 10-59%). For the studies of Vardy et al. (2011) and the USASK (2011) the predicted EC_{50} values are between 4.1 to 10.1 µg Cu per L. For the study of Wang et al. (2011) predicted EC_{50} values are somewhat lower, between 2.2 to 4.6 µg Cu per L. However, the concentration range of the predicted population-level EC_x values is similar to the observed conventional lab LC_x values. The ranges are 3.1 to 12.4 µg Cu per L for lab LC_{50} values, and about 2.2 to 10.1 µg Cu per L predicted for the population-level EC_{50} values. The EC_{10} values are largely similar between observed lab values (1.8 to 5.1 µg/L) and IBM-predicted population-level values (1.6 to 5.9 µg/L). Across all studies, the average ratio between population EC10 and lab LC10 was 1.01 (range: 0.87-1.26). Thus, the sensitivity at 10% effect level of *A. transmontanus* at population-level, as predicted with the IBM, is on average the same as the sensitivity in lab (based on larval mortality).

The similarity in sensitivity could be explained by the model structure. Since the basis of the *Acipenser* IBM are the survival rates between the age classes, it is expected that a 10% decrease in age-0 survival will lead to a 10% decrease at the population level (not considering the density-dependent process). This is a logical consequence of the empirical behaviour of the model. The *Acipenser* IBM model does not have a mechanistic foundation (such as DEB-IBMs). Rather, the main driver of the model (and thus the population dynamics) is the survival rates between ages. The *Acipenser* IBM does, however, contain some mechanistic elements, such as the relation between age and size, size and reproduction, etc.

A critical note must be added though to the results in Table 9. These results were determined based on a default scenario. As mentioned previously, it is assumed that the whole population within a 200-km river segment is exposed to copper. In reality, other factors will come in play. There is a large influence of the exposed fraction of the population. If only 30% of the population is exposed, a maximum decrease of 30% in equilibrium density can be seen. If the copper contamination is for instance very local, the local population will react very sensitive to it. However, the effect on the total population will be marginal. Additionally, the location (upstream or downstream) and distribution (homogeneous or heterogeneous) of the copper pollution also plays a role. Other river configurations (e.g. longer river segments, variable river width, more dams, etc.) might thus predict other patterns on the population level.

5 CONCLUSIONS

5.1 Population-level effect assessment with the *Acipenser* IBM

The goal of this study was to develop an *Acipenser transmontanus* (white sturgeon) model that describes population-level effects of copper pollution. Based on available data in literature, *Acipenser transmontanus* indeed is very sensitive to copper pollution. Especially early developmental life stages (20 to 30 dph) are very sensitive, as low LC_x values are observed around that age.

An IBM for the white sturgeon was developed in NetLogo. The model structure follows an individual-based age-class model from Jager (2000). Individuals within the population are divided in groups according to their age. Population dynamics flow from the survival rates between age classes. Individual variability is implemented through variability on parameters amongst individuals within each age class. Based on available toxicity data on *Acipenser transmontanus*, the effects of copper were integrated in the model. Only copper effects on survival on early developmental life stages have been integrated. By increasing the mortality rate in age-0 individuals with the environmental copper concentration effects of copper were integrated.

Copper effects on growth were observed in literature as well, although these were not integrated in the model. A theoretical calculation extrapolating the growth effects revealed minor effects on later life stages. A (theoretical) maximum decrease in fecundity of 3.33% was predicted at EC_{100} concentrations. Since these effects are only marginal compared to the mortality on developmental life stages at those exposure concentrations, the growth effects were not integrated. Nonetheless, a mathematical method could be developed to integrate these effects as well.

Simulations with the *Acipenser* IBM were performed to assess population-level effects of copper pollution. Equilibrium population density was investigated as a function of the copper concentration under various conditions (i.e. different fractions of exposure, migration rates, distribution and location of the pollution). Overall, the equilibrium density of the population revealed a concentration-dependent relationship with the copper concentration. Predicted population-level EC_x values were similar compared to their observed traditional endpoint. The concentration range is quite similar. Traditional LC_{50} values were situated in a concentration range between 3.1 to 12.4 µg Cu per L. Whereas on the population level, the 50% effect concentration range was between 2.1 to 10.1 µg Cu per L. Similarly, the EC_{10} values range from 1.8 to 5.1 at the individual level (lab tests), and 1.6 to 5.9 at the population level (IBM predictions), with the average ration between population EC_{10} and conventional lab LC_{10} being 1.01 (range: 0.87 – 1.26 across six experiments analysed).

The effect of copper on the population highly depends on several factors. If a higher percentage of the population is exposed, the population response will be more sensitive. Furthermore, migration rates between different river segments also determine the magnitude of the effect of the population. Higher migration rates will lead to individuals migrating faster downstream, decreasing the overall population density in more upstream river parts. If on top of the higher migration rates the population is exposed to copper, the risks of the population becoming extinct in a specific river segment will increase even more. The location and distribution of the copper pollution source are also important. An upstream, homogeneous pollution source has the largest effect on the pollution. At very high copper concentrations, the population in the upper river stream parts will migrate completely downstream.

Although the model predicts population-level effects of copper on white sturgeons, there is no explicit validation against real population data. Because of practical constraints, population experiments with white sturgeons are impossible to perform. However, the model does not predict unrealistic population densities. In contrast, there are some inherent assumptions and uncertainties that need further investigation.

5.2 Extensions and future opportunities

This study assessed effects of copper on *Acipenser transmontanus* populations. The results reported here are based on a model from literature that best describes white sturgeon populations. However, there are some assumptions involved in the model that need further investigation and fine-tuning. Additionally, there are some future points of interests and opportunities regarding this model.

A first, and a very important aspect, is the concept of the *sensitive period*. In the implementation of the *Acipenser* IBM here, the sensitive period is the main driver for mortality in age-0 individuals. Mortality due to exposure to copper, is based on the daily mortality calculated from acute and chronic mortality experiments. Because there is uncertainty on the period when the juveniles are actually sensitive, a sensitivity analysis on this parameter was performed. Moreover, data shows that after 20 to 30 days post hatching most mortality occurs. When exactly this process stops, or when they become less sensitive to copper, is unsure. By better understanding how mortality impacts these age-0 individuals, this sub-model could be improved. Finding the raw mortality data could give more insight on the mortality "pattern". Contacting the authors or investigating instances that generated the data could give an opportunity to improve the IBM. For now, the uncertainty on the sensitive period was integrated through a parameter sensitivity analysis. Based on a log-normal distribution the 10^{th} percentile was determined as the population level EC_x value of that study.

To better improve the mortality sub-model, ideally, the mortality over time at different copper concentrations is needed. In the implementation of the *Acipenser* IBM as it is now, the formula of De Laender et al. (2008) is used as a general method to implement increased mortality.

However, other models, such as the General Unified Threshold theory of Survival (GUTS) might predict other patterns. But, again, a prerequisite is to understand when most mortality occurs or what the pattern over time is when larvae/juveniles are exposed to copper. By better investigating existing (or new) data on early life-stage mortality, the mortality sub-model could be optimized.

The density-dependent sub-model (Section 3.1.4) implemented here is rather general and does not have a good scientific support. The equation follows a logical rationale but is not supported by any evidence or data. In addition, the parameter for the carrying capacity K_{pop} seems very empirical, although it is based on monitoring data from white sturgeon populations. The authors indicate that the sub-model is based on assumptions that are derived from monitoring data, but without substantiating or providing actual data to support their equations (Jager et al. 2001; Jager 2000). The shape of the density-dependent model (Figure 4) is also not that of classical and more commonly used density-dependent model (e.g. Ricker, Beverton-Holt). Nonetheless, the density-dependent process is important as it limits population growth. Without density-dependence, population growth would be unlimited. This sub-model needs some further investigation and fine-tuning, since currently it is not optimally reliable. A sensitivity analysis could be performed to determine what the effect of the carrying capacity K_{pop} is on population sensitivity. Moreover, ecological studies have shown that stressors can affect density-dependence in populations, impacting the population dynamics as a whole (Sibly et al. 2000; Vonesh and De La Cruz 2002). In addition, other density-dependent sub-models that are more mechanistic and better supported by evidence could predict different patterns closer to the real situation.

Another aspect for future research, as mentioned previously, is an explicit validation against real-time data. Model performance now is based on average predicted densities and comparing those densities to (averaged) observed densities. An explicit validation will improve reliability of the model and might reveal processes that were previously unaccounted for. One of the major issues of course is the practical restrictions in performing population experiments with *Acipenser transmontanus*. This fish species can grow very old (sometimes more than 100 years) and uses a wide area for habitat (Israel et al. 2009). For practical reasons population experiments with white sturgeons are not performed. On the other hand, white sturgeon monitoring data could be used. The problem with monitoring data is the number of factors that come into play. Factors such as sampling method, sampling location, but also environmental factors. Several monitoring programs for white sturgeon already exist, although the data they provide on population densities and dynamics is limited.

The simulations performed here are based on a default scenario approaching conditions of the Snake River. The simulated scenarios are based on research by the developers of the *Acipenser* IBM model (Jager 2000; Jager et al. 2001a; Jager et al. 2001b; Jager et al. 2010). However, the model itself and the NetLogo environment are flexible. The *Acipenser* IBM could be used to assess effects in other spatial configurations. The whole Snake River could be simulated with the model. This would, however, require some computational effort, and enough data should be available to completely simulate the Snake River.

In addition to the above, using realistic copper monitoring could give more insight in realistic patterns concerning copper pollution as it is now in the Snake River. Especially, since the importance of the distribution of pollution has been shown here (heterogeneous vs homogenous pollution). Using measured concentrations and implementing the spatial distribution of copper pollution, will lead to a very case-specific scenario.

The NetLogo environment is very flexible. A higher resolution spatial scale could be used to simulate more detailed spatial behaviour of the sturgeons. For instance, the zebrafish model from Beaudouin et al. (2015) uses a much more detailed spatial structure, assigning different functionalities to the NetLogo patches. Patches were either assigned to be vegetation cover (for feeding), breeding grounds (for spawning) or open water. Something similar could be achieved for the white sturgeon model, although this might overcomplicate the situation. In addition to a more detailed spatial scale, a smaller time scale could be investigated as well. Now the model assumes time steps of one year. If more data on movement behaviour would be available, a monthly (or even daily time) step could be used where processes such as seasonal migration are implemented directly. However, data on migration and movement behaviour of white sturgeons is limited. In addition, a more detailed spatial structure and a smaller time scale will significantly increase simulation times.

The model now integrates the effects of the environment in a general density-dependent process (Section 3.1.4). However, several environmental factors influence population behaviour of white sturgeons (Jager et al. 2001b). The temperature of the water greatly influences the incubation and hatching success of fertilised eggs (Parsley et al. 2002). A similar situation is seen with the water flow of the river. The hydrological year type (dry, normal or wet year) will have an impact on the population (Jager et al. 2001b). The flow of the river determines egg hatching success since eggs might be dragged along or transported to unsuitable habitats (Jager et al. 2001b). Next to these environmental factors, other external factors include fishing, angling mortality, and turbine mortality (Jager et al. 2001b). Including all of the above would give more realistic and case-specific results.

A last future research point is acclimation and adaptation to copper. Potentially, if the population is exposed over a longer period of time, the sensitivity of the individuals might decrease. Individual sturgeons may exhibit physiological changes that allow them to better cope with the stresses they encounter. Moreover, eggs and embryos exposed to copper during incubation may already prepare the new-born for copper stress, decreasing their sensitivity to copper as well. Such patterns have already been observed for zinc (De

Schamphelaere and Janssen 2004). In addition to acclimation, genetic adaptation can decrease copper sensitivity over multiple generations of white sturgeons. Natural selection may cause juveniles in the population to become on average less sensitive to copper than in the previous generations. Such effects are not included in the current *Acipenser* IBM but could be implemented in the future. The main issue would be the lack of data on copper acclimation in white sturgeon itself. To overcome this issue, extrapolation from data on acclimation in other fish species could be used.

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7 APPENDICES

7.1 NetLogo code

The NetLogo code of the *Acipenser* IBM model will be added electronically. The model consists out of three files, a main *~.nlogo* file and supportive *~.nls* files:

- IBM_Acipenser.nlogo: the main NetLogo code containing all the processes to run the simulation. Also contains the interactive model interface.
- plot.nls: code to create graphs and write output files.
- setup.nls: set-up procedure to create the environment and the initial population. Also contains the parameter values to run the mode.