Ecological Modeling for the Extrapolation of Ecotoxicological Effects Measured during in Situ Assays in Gammarus

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Ecological modelling for the extrapolation of ecotoxicological effects measured during *in situ* assays in *Gammarus*.

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KEYWORDS

Gammarus fossarum, life-history, biomonitoring, population models, effect upscaling

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ABSTRACT

Evaluating the effects of chemical contamination on populations and ecological communities still constitutes a challenging necessity in environmental management. However the toxic effects of contaminants are commonly measured by means of organism-level responses. Linking such effects measures with ecological models is a promising way to apprehend population-level impacts. In this way, population models are currently increasingly used in predictive risk assessment procedures, but their use in environmental diagnostic framework remains limited due to their lack of ecological realism. The present study with the crustacean *Gammarus fossarum*, a sentinel species in freshwater monitoring, combines a dual field and laboratory experimental approach with a population modelling framework. In this way, we developed an ecologically-relevant periodic matrix population model for *Gammarus*. This model allowed us to capture the population dynamics in the field, and to understand the particular pattern of demographic sensitivities induced by *Gammarus* life-history phenology. The model we developed provided a robust population-level assessment of *in situ*-based effects measures recorded during a biomonitoring program on a French watershed impacted by past mining activities. Thus, our study illustrates the potential of population modelling when seeking to decipher the role of environmental toxic contamination in ecological perturbations.

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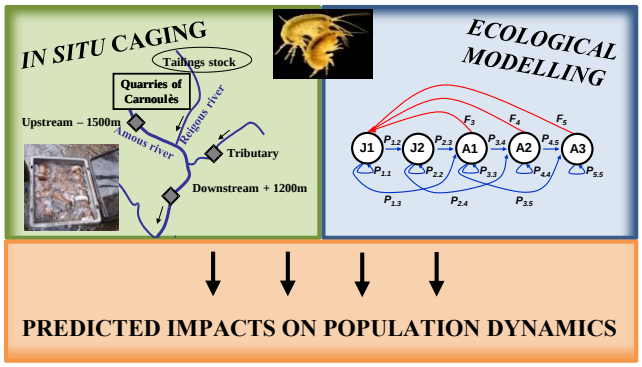
40 **TOC/Abstract graphic**



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1. INTRODUCTION

In order to understand the role of chemical contamination in the degradation of environmental quality, the ecotoxicological approach studies the sub-lethal effects of contaminants by means of sub-individual biomarkers or individual responses. One can thus focus the diagnostic assessment at lower levels of biological organization compared to integrated ecological impact studies on populations, communities and ecosystem functioning [1-4]. These organism-level markers are expected to be specific and sensitive to the toxic effects of contaminants. They are therefore used to gain insights into causal relationships between contamination and biological impacts, an essential step in proposing relevant environmental management programs. Nevertheless, their ecological relevance continues to be debated [5]. In fact, the effects detected by biomarkers and individual responses can be directly related to the presence of contaminants but do not inform on the severity of environmental quality degradation in terms of ecological effects, because they cannot be directly interpreted as predictors of the impacts on populations or communities, which constitute the protection goals of ecosystem management. One promising way to overcome this difficulty is to include the impacts measured on individual demographic parameters (*e.g.* survival, growth, reproduction) in population models to investigate adverse outcomes at the population level [6-8]. Nevertheless, although such population models are currently increasingly used in predictive approaches [9], their use in the diagnostic framework remains limited. In the majority of studies, population models are based on laboratory data with species that are not representative of ecosystems. Consequently, while these models are useful for extrapolating the effects of a specific toxicant observed during a laboratory experiment on a model species to outcomes on the population growth rate for instance [7, 10], the integration of field-based input data in modelling approaches is lacking to really anticipate what would happen in the

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field in native populations [11, 12]. Therefore, to develop the use of population models as predictive tools, we need to develop models based on field data and autochthonous species.

In the present study, we aimed to develop an ecologically relevant population model for the crustacean *Gammarus fossarum*. Gammarids are recognized as tractable organisms for water quality biomonitoring [6, 13]. We have recently contributed to the development of several sub-individual biomarkers [14-17] and individual markers [4, 18] for this sentinel species. Gammarids are considered as very promising species for multi-level assessment schemes from sub-individual to population or community levels [6]. Here, in a first step, we report the implementation of a population modelling approach suited for the life-history and phenology of this invertebrate species. We parameterized a periodic matrix population model [12, 19, 20] based on a 1-year study of a perennial population of *G. fossarum*. We used a combination of laboratory and *in situ* experiments in order to precisely characterize life-history trait dynamics throughout the year. This model allowed us to capture the population dynamics and to understand the particular pattern of demographic sensitivities in *Gammarus*, *e.g.* the relative importance of recruitment processes (fertility) *vs* survival processes, or the temporal variability of population vulnerability. In a second step, we demonstrate the suitability of this population model for a multi-level assessment of water quality based on a case study on the Amous watershed, a French river known to be contaminated by heavy metals due to mine drainage. Finally, we discuss the potential contribution of this type of population modelling approach to environmental monitoring, considering that biomarkers and individual responses could be complemented by population models to decipher the role of environmental contamination toxicity in ecological perturbations.

2. MATERIALS AND METHODS

Biological data

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We conducted our experiments on a long-established population of *Gammarus fossarum* which is present all the year upstream of the Ardières River (04°31′16 E; 46°11′12 N, Rhône, France). We used a dual approach combining laboratory and field experiments to characterize life-history trait variability throughout the year and population dynamics. On the one hand, we conducted a field-based demographic sampling based on a monthly population census to estimate population characteristics (*e.g.* changes in density and size structure, fertility). On the other hand, we quantified the influence of water temperature throughout the year on the growth rate and interlaying interval, by coupling laboratory experiments and *in situ* validation. For all experiments, water temperature was recorded every 2 h using the Tinytag temperature logger Aquatic 2 ®.

Field-based demographic sampling

A demographic sampling was conducted from November 2008 to November 2009. Each month, gammarids were sampled along a 50-m river section using a hand net (25 × 18 cm). Separate samples were taken for each substrate in the river, including favourable substrates for gammarids (*i.e.* bryophytes, leaves) and dominant substrates in the river (*i.e.* gravels). The percentage of each substratum was estimated on six transects shared out along the station. Samples were sieved on site at 1.25 mm to separate juveniles from adults. Juveniles were fixed in alcohol and adults were placed in buckets with river water. Juvenile densities were estimated within the different substrates with a sub-sample corresponding to 20% of the total sample from which we measured the body size of 60 individuals. For this measurement, we used the length of the second segment of the antenna, which proved to be a robust indicator of the body size in juveniles, adult males and females (data not shown). Concerning adults, we counted gammarids within the different substrates and separated *in vivo* gravid females. Then the body size of 60 individuals from the sample of gravid females was measured, and 60

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individuals from the sample of males and non-gravid females were sexed using a binocular microscope and measured. This methodology allowed us to estimate monthly population density, size distribution, sex ratio and percentage of gravid females. By this means, we also determined size at birth, size at maturity and maximum size. In addition, every month we measured size and fertility (number of embryos in the marsupium) on 60 gravid females covering a wide range of sizes sampled randomly in the river section. The detailed fertility measurement procedure is described in Geffard et al. [18].

Quantification of the monthly variability in growth rates and interlaying intervals

Water temperature is known to influence the life-history traits of gammarids [21-25]. Therefore, we expected that the variability in growth rates and interlaying intervals would be mainly explained by water temperature variability during the year. Consequently, in the first step we studied the influence of water temperature on growth and interlaying interval under laboratory conditions. In the second step, we confirmed predictions using *in situ* caging assays.

Laboratory experiments. To estimate the influence of temperature on growth, we tested three temperatures, 7.0 (± 0.05), 12.1 (± 0.01) and 16.4 (± 0.03) °C, as representative of the range observed at the study site (mean monthly water temperature varied from 5.2 to 15.9°C in the year of the study). Because the growth was slow (in particular for low water temperatures), we decided not to investigate growth from neonates to adults but to observe the growth of three size classes of organisms simultaneously: 2.5 (± 0.3) mm, 4.5 (± 0.8) mm and 5.8 (± 0.7) mm. It allowed us to estimate growth from juveniles to adults using relatively short experiments which is an advantage when studying gammarids under laboratory (*e.g.* no nutrient deficiencies, less mortality). Gammarids were exposed to the three temperatures for 56 days. We used four replicates of 15 individuals for the first size class and four replicates of

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10 individuals for the other two. Gammarids were placed in 0.5-L beakers filled with continuously renewed water (four renewals per day; hardness 88 mg L^{-1} of $CaCO_3$ corresponding to the hardness of the river at the study site). A pumping system maintained oxygen in the beakers and a 16 h / 8 h light / dark photoperiod was applied. The organisms were fed *ad libitum* with alder leaves conditioned in groundwater. Twice a week, freeze-dried worms (*Tubifex)* were added. For the first size class, we also added frozen faeces from adult batches. The body size of individuals was measured at days 0, 14, 32, 46 and 56. After having tested different classes of non–linear growth models, we chose to fit experimental data with a logistic model. For each temperature, a logistic model was thus fit simultaneously considering the three classes of gammarids. The model is expressed as follows:

$$L(t)=\frac{Lmax}{1+\left(\frac{Lmax}{Linit}-1\right)e^{(-r\,t)}} \quad (1)$$

where $L(t)$ corresponds to the size of gammarids at time t (in days), $Lmax$ to the maximum size, $Linit$ to the size at the beginning of the experiment and r to the daily growth rates of the logistic model. Considering 99% percentiles in total size distributions of the field-based demographic sampling (see below), we set $Lmax$ at 8.5 mm. For the quantification of interlaying intervals, because laying is synchronous with moult in gammarids [16, 18, 26], we used the relationship established in a study on moulting dynamics (unpublished data). From this, the influence of temperature θ (in °C) on the interlaying interval d (in days) is described by:

$$d = \frac{(30.61-0.39\theta)}{(0.01+0.05\theta)} \quad (2)$$

This hyperbolic relationship is consistent with studies reported for *Gammarus* [22, 27].

In situ assays. We tested the predictive ability of these models of temperature influence on growth rates and interlaying intervals during four campaigns of *in situ* caging experiments at different seasons between November 2008 and November 2009 (*i.e.,* November-December

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2008 for winter caging; April 2009 for spring; June 2009 for summer and October 2009 for autumn). Mean water temperature during *in situ* experiments was ranged from 7.8 to 15.1°C. The gammarids were sampled and calibrated (*i.e.* selection of homogenous size) directly at the study site. To determine growth rates, depending on the availability of gammarids, we used from one to three size classes. For each size class, three replicates of 12 organisms were placed in cylinders capped at their ends with pieces of netting (mesh size, 350 µm). Alder leaves were supplied *ad libitum*. Initial sizes were estimated with a sample of 30 organisms. After one month of exposure, gammarids were counted for survival estimation, placed in alcohol and measured. Concerning the interlaying interval, we adapted the protocol proposed in Geffard et al. [18]. Three replicates of seven pre-copulatory pairs of gammarids (*i.e.* male and female on amplexus, with the female at the end of its reproductive cycle) were placed in cylinders (diameter, 5 cm; length, 10 cm) capped at their ends with pieces of netting (mesh size, 1 mm). Alder leaves were supplied *ad libitum*. Every 2 days, gammarids were checked in order to record the dates of moults (*i.e.* a first moult which occurred a few days after the beginning of the experiment and a second which depended on temperature (between 4 and 8 weeks).

Modelling framework and demographic analysis

Development of the population model

We used a periodic Lefkovitch matrix population model with five size classes [19, 20, 28, 29] with the same structure as the model we previously developed for the gastropod *Potamopyrgus* [12]. We employed a size-class rather than an age-class or a stage-class model because *(i)* a strong correlation exists between body size and life-history traits in gammarids [22, 25, 27] and *(ii)* the life-history of individuals of such birth flow populations from short-living species inhabiting variable environments (seasonality) strongly depends on their date of birth

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190 in the year, which makes age a very weak predictor of biological features. The present model
191 only deals with females and distinguishes two classes of juveniles – J1 (individuals with a
192 size up to 3.5 mm) and J2 (size from 3.5 mm to the size at maturity) – and three classes of
193 adults – A1 (from the size at maturity to 6 mm), A2 (from 6 to 7 mm) and A3 (greater than 7
194 mm). This model can therefore integrate heterogeneity of demographic rates (survival, growth
195 and fertility) between size classes throughout the year.

196 Let $n_i(k)$ be the number of individuals of size class i (i = 1 for J1, i = 2 for J2, i = 3 for A1, i
197 = 4 for A2 and i = 5 for A3) at the beginning of month k. The five $n_i(k)$ variables can be
198 gathered in a population vector $\mathbf{n}(k)$. Then we can define 12 monthly matrices $\mathbf{M}_k$, which link
199 the population vectors $\mathbf{n}(k)$ between months k and k+1 as follows:

200 $$\mathbf{n}(k+1) = \mathbf{M}_k \, \mathbf{n}(k) \qquad (3)$$

201 with:

202 $$\mathbf{M}_k = \begin{bmatrix} s_1(k)\left(1-\sum_{j>1} g_{1,j}(k)\right) & 0 & f_3(k)\sqrt{s_1(k)}\sqrt{s_3(k)} & f_4(k)\sqrt{s_1(k)}\sqrt{s_4(k)} & f_3(k)\sqrt{s_1(k)}\sqrt{s_5(k)} \\ s_1(k)\,g_{1,2}(k) & s_2(k)\left(1-\sum_{j>2} g_{2,j}(k)\right) & 0 & 0 & 0 \\ s_1(k)\,g_{1,3}(k) & s_2(k)\,g_{2,3}(k) & s_3(k)\left(1-\sum_{j>3} g_{3,j}(k)\right) & 0 & 0 \\ s_1(k)\,g_{1,4}(k) & s_2(k)\,g_{2,4}(k) & s_3(k)\,g_{3,4}(k) & s_4(k)\left(1-g_{4,5}(k)\right) & 0 \\ s_1(k)\,g_{1,5}(k) & s_2(k)\,g_{2,5}(k) & s_3(k)\,g_{3,5}(k) & s_4(k)\,g_{4,5}(k) & s_5(k) \end{bmatrix}$$

203 (4)

204 where $s_i(k)$ is the survival rate (proportion) of the size class i during month k, $g_{i,j}(k)$ the
205 transition rate (proportion) between size classes i and j during month k, and $f_i(k)$ the
206 reproductive rate (mean number of juveniles produced per individual) of the size class i
207 during month k. The product of the 12 monthly matrices $\mathbf{M}_k$ leads to an annual periodic
208 matrix $\mathbf{L}$, which links the population vector from year t to year t+1 as follows:

209 $$\mathbf{n}(t+1) = \left(\prod_{k=1}^{12} \mathbf{M}_k\right)\mathbf{n}(t) = \mathbf{L}\,\mathbf{n}(t) \qquad (5)$$

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Parameter estimation

This matrix model encompasses three types of elements corresponding to survival, reproductive and transition rates (see Coulaud et al. [12]). For each month *k*, we estimated the parameters from our laboratory and field experiments. We estimated the reproductive rates $f_i(k)$ of the three adult classes (i = 3, 4 or 5) as follows:

$$f_i(k) = \frac{sr_i(k)\, b_i(k)\, \rho_i(k)\, \Delta t(k)}{d(k)} \quad (6)$$

where $sr_i(k)$ (proportion of females) corresponds to the sex ratio of class *i* during month *k*, $b_i(k)$ to fertility (mean number of embryos carried by gravid female) of class *i* during month *k*, $\rho_i(k)$ to the percentage of gravid females (proportion) in class *i* during month *k*, $\Delta t(k)$ to the number of days of month *k* and $d_i(k)$ to the interlaying interval in days computed for month *k*. Parameters $sr_i(k)$ and $\rho_i(k)$ were obtained with data from the demographic sampling (see above); parameters $b_i(k)$ were estimated for each size class from the relationship between body size (mm) and fertility (number of embryos) obtained from the demographic sampling and parameters $d(k)$ were calculated according to eq. 2 with the mean monthly water temperatures (°C).We calculated transition rates $g_{i,j}(k)$ (proportion of individuals from size class *i* reaching a size embedded in class *j*) using the relationship between growth rate (day^{-1}) and water temperature (°C) (eq. 1) established during the laboratory experiment and validated during *in situ* caging experiment (see above). Lastly, we estimated survival rates $s_i(k)$ for month *k* from the comparison of densities ($gammarid.m^{-2}$) observed during the demographic sampling (see above) in month *k* with densities expected from the observed densities in month *k−1* combined with growth and reproductive rates estimated for month *k−1*.

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Model outcomes and elasticity analysis

The first dominant eigenvalue of the Lefkovitch matrix $\mathbf{L}$, λ, corresponds to the asymptotic growth rate of the gammarid population [28]. The right eigenvector w associated with this first eigenvalue gives the asymptotic stable size structure. According to the matrix used first in the product of the 12 monthly matrices $\mathbf{M}_k$, we obtained the size structure at the end of each month of the year. Elasticities were calculated through the reduction in the asymptotic population growth rate λ induced by 10% reduction on all monthly matrices $\mathbf{M}_k$ in each life-history trait successively and independently (*i.e.* survival of each class, fertility, reproductive cycle duration and growth) before the calculation of the matrix L. We also examined the monthly variability of this elasticity pattern. For this, we applied an episodic but more severe 50 % reduction in juvenile survival (s1 and s2), adult survival (s3, s4 and s5) and fertility successively and independently on only one monthly matrix $\mathbf{M}_k$ before the calculation of the matrix L.

Case study on the Amous watershed

Following the transplantation methodology developed in our group[14-17], a campaign of *in situ* assays was conducted in March 2008 in four stations of the Amous watershed, a French river known to be contaminated by heavy metals originating from mine drainage from the former mine at Carnoulès [4, 30]. The same four stations as in these two previous studies were considered: three stations along the Amous River: *Upstream −1500 m*, *Downstream +1200 m* and *Downstream +3500 m* with different levels of metallic contamination (*i.e., Upstream −1500 m* < *Downstream +3500 m* < *Downstream +1200 m*, for metal bioaccumulation data [30]), and a fourth reference station on a tributary from the same river system (*Tributary*) that is not impacted by metal-loaded mine leachates. Juvenile survival, adult survival and fertility

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were measured in the different stations. We used gammarids from a station displaying good water quality and currently used by our laboratory for *in situ* assays[14-17] located upstream of the Bourbre River. To estimate juvenile survival, four replicates of 12 individuals were placed in cylinders capped at their ends with pieces of netting (mesh size, 350 µm). For adult survival and fertility, three replicates of seven pre-copulatory pairs were placed in cylinders (mesh size, 1 mm). Gammarids were exposed for 21 days and were fed *ad libitum* with alder leaves. At the end of exposure, gammarids were counted and fertility was measured (*i.e.* number of embryos in the marsupium). To estimate population impacts, we integrated these different individual impacts into our population model in terms of percentage of demographic rate reduction in comparison to the control levels observed in the reference station *Tributary*.

Statistical analyses

Statistical procedures and population models were implemented with the R software [31]. Before performing the ANOVA procedure, normality and homoscedasticity were checked using the Shapiro-Wilk and Bartlett tests.

3. RESULTS

Field-based demographic sampling: size structure and fertility

Changes of size distribution in the population, as estimated with the monthly field-based demographic sampling, are presented in Figure S1. The highest densities were observed in autumn and at the beginning of winter, while the lowest densities were observed in winter and spring. We observed highly fluctuating densities of juveniles, with very low densities in spring and high densities during the rest of the year, particularly autumn. Contrasting with juveniles, adults are present throughout the year with little variability and persist during winter. Considering 1% and 99% percentiles in total size distributions, we estimated a size at

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283 birth equal to 2.3 (± 0.2) mm and a maximum size equal to 8.2 (± 0.3) mm. Considering 1%
284 percentiles of reproductive female size distributions, we estimated a size at maturity equal to
285 5.2 (± 0.3) mm. These values appeared constant during the year. The monthly demographic
286 sampling allowed us to estimate the percentage of females in reproduction $\rho_i(k)$ and the sex
287 ratio $sr_i(k)$. We observed substantial between-month and between-class variability of $\rho_i(k)$
288 values (from 0.1 to 95%). For all months, we observed an increase in this percentage between
289 the three size classes. Furthermore, we noted low percentages of reproductive females at the
290 end of autumn and during winter, as well as high percentages during spring and summer. For
291 sex ratio $sr_i(k)$, we generally observed an equal number of males and females. Consequently,
292 we set the sex ratio at 0.5 in the model. A strong positive relationship was detected between
293 body size and fertility without seasonal influence (Figure S2). Size class A1 females had a
294 mean fertility of 3.6 (± 1.3) embryos, A2 females a mean fertility of 5.1 (± 1.6), and A3
295 females a mean fertility of 9.2 (± 2.9) embryos.
296
297 **Monthly variability of growth rates and interlaying intervals**
298 The measurements taken during the laboratory experiment on growth are presented on
299 Figure S3 (A, B, C), showing that individuals grew faster when temperature increased. With
300 the logistic models fitted simultaneously on the three size classes (Figure S3 A, B, C), we
301 estimated daily growth rates for each temperature. Between 7.0 and 16.4°C, they varied from
302 0.008 to 0.021 day^{-1}. In this temperature range, consistent with temperatures recorded at the
303 study site during the year, we fitted a linear model between water temperature θ (in °C) and
304 daily growth rate r (day^{-1}):
305 $$r(\theta) = 0.0014\,\theta - 0.0024 \quad (4)$$
306 During the *in situ* assays performed at different seasons, we observed substantial variability
307 in daily gain in size (*i.e.,* between 0.011 and 0.052 $mm.day^{-1}$ depending on the season and the

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size-class). When the observed sizes were compared for each size class with values predicted from the logistic model established in laboratory exposure (eq. 1) combined with (eq. 4), we observed a good correlation (Spearman rank correlation test, $p < 0.05$, $r^2 = 0.87$) (Figure S3 D), which validates the fact that this variability in growth rates is mainly related to water temperature variability between the different caging experiments.

During the *in situ* experiments, we recorded an interlaying interval of 34 days in spring and 26 days in summer. These values are consistent with model predictions from eq. 2, which predicts durations of 35.5 and 27.1 days, respectively. During winter and autumn, we were not able to follow females up to the end of the reproductive cycle due to the low temperature which induces long reproductive cycles.

Population model analysis

Regarding parameter estimation (Table S1), we observed considerable monthly and between-class variability in reproductive rates $f_i(k)$, which varied from 0.01 to 7.07 in the mean number of juveniles produced per month per female. Concerning transition rates $g_{i,j}(k)$, we also observed high monthly variability, with a majority of individuals staying in their initial size class during the coldest months, whereas during months with a high water temperature, a majority of individuals moved up one or two size classes from month to month. Computed adult survival rates show substantial seasonal variability with low survival rates in summer, whereas survival rates were high in winter and spring. Furthermore, size class A3 showed the lowest survival rates for all months. Juvenile survival rates were generally higher than adult survival rates. For some months, we calculated survival rates higher than 1, in particular when densities were low (*i.e.* the uncertainty in count estimates increased with smaller size samples) or for size class J1 (*i.e.* gammarids of the size class J1 were very small and therefore difficult to sample, which makes density estimation difficult).

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To generate a more robust estimation of survival rates for each month k to implement the population model, we used the same smoothing methodology as in our study on the mollusc *Potamopyrgus* [12]. We calculated the geometric mean of the survival rate estimates for months $k-1$, k and $k+1$.

The asymptotic population growth rate λ was estimated at 1.07. We computed the stable size distribution corresponding to the structure of the population at the end of November, February, May and August (Figure 1). We observed good consistency with field data of the demographic sampling (chi-square tests not significant for the four seasons). The elasticity analysis on matrix L (Figure 2) highlights that the asymptotic population growth rate λ is more sensitive to relative changes in juvenile survival rates than to changes in the other life-history parameters. The life-history traits corresponding to the second highest elasticity are the survival rates of small adults (*i.e.* elasticities of *s3* and *s4*), followed by fertility (*b*), interlaying interval (*d*), growth rate (*r*) and survival rate of larger adults (*s3*). Although the population is generally more sensitive to impacts on juvenile survival, when we applied 50% reductions in juvenile survival, adult survival or fertility rates successively on each monthly matrix $\mathbf{M}_k$, we observed a strong variability of population sensitivity throughout the year (Figure 3). Reduction in juvenile survival rates led to the highest impacts in June, then in autumn. Concerning adult survival, we noted the highest impacts in winter with more than 20% reduction in λ when survival reduction occurred in January, February or March. Finally, concerning fertility, we observed a bimodal pattern with the highest impacts in April and in July-August.

Case study on the Amous watershed

In comparison to the reference station *Tributary*, we observed impacts on juvenile survival rates (s_j) in *Upstream −1500 m* and *Downstream +1200 m* (unilateral proportion test, $p <$

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0.05), on adult survival rates (s_a) in *Downstream +1200 m* (unilateral proportion test, $p < 0.05$) and on fertility (b) in *Upstream −1500m* and *Downstream +3500 m* (Wilcoxon rank sum test, $p < 0.05$) (Table 1 A). In *Downstream +1200 m*, we did not record fertility due to excessively high adult mortality. When we applied simultaneously the effects in term of percentage of reduction in comparison to the reference site *Tributary* on all monthly matrices $\mathbf{M}_k$ before the calculation of the matrix L, we predicted severe impacts on the asymptotic population growth rate in all contaminated stations (Table 1 B, Table S2). We also calculated the specific impacts of the degradation of each life-history trait (Table 1 B, Table S2) with a one-by-one application of the effects on each life-history-trait in term of percentage of reduction in comparison to the reference site *Tributary*. In this way, we noted that in *Upstream −1500 m* impacts on fertility were responsible for the great majority of global impacts on population dynamics, whereas in *Downstream +1200 m*, reduction in juvenile survival rates had the greatest impact at the population level.

4. DISCUSSION

Picturing the *G. fossarum* population biology in the field

We have described the characteristics of one population of *G. fossarum* by means of a combination of a field survey and laboratory or *in situ* experiments. During the field-based demographic sampling, we observed fluctuating densities throughout the year (Figure S1) with high-density peaks (*i.e.* more than 800 gammarids per m² in September and December) and periods of lower densities, in particular at the end of winter and at the beginning of spring . This high seasonal variability in gammarid density agrees with other studies on *G. fossarum* [32-34]. Our population is mainly composed of juveniles, except in spring where adults are more abundant, agreeing with previous data [32]. In this way, the persistence of the population at the beginning of spring when densities are minimal is ensured by the survival of an overwintering

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383 stock of adults. As for the percentage of reproductive females, we observed significant
384 between-month variability with a high percentage in summer and a very low percentage in
385 winter. This pattern is consistent with other studies on *G. fossarum* [32] or other gammarid
386 species [35, 36], which report a higher reproductive activity in warm seasons. The estimation of
387 the seasonal variability of life-history traits confirmed our primary hypothesis that
388 temperature is the main source of variability. This is consistent with previous
389 ecophysiological studies, which have shown that daily growth rates [22, 25, 27, 37] and interlaying
390 intervals [23, 38-40] are mainly modulated by temperature. Density-dependent processes could
391 also be involved in temporal patterns of survival, and they could further be taken into account
392 with an appropriate amendment of our model. But we should dispose from a multi-year
393 demographic monitoring (ongoing study), to decipher whether such effects occur here in
394 *Gammarus* population dynamics.

395 Nevertheless, this complex population dynamics in a natural freshwater environment is well
396 described by the different outcomes of our population model. Indeed, from the analysis of the
397 annual matrix model, a good match was found between the stable size distribution computed
398 for each season and the population structure observed during the field demographic sampling
399 (Figure 1). This highlights that the present modelling approach succeeds in properly
400 describing the dynamics of this *G. fossarum* population throughout the year. It confirms that
401 periodic matrix population models with size classes can be very useful tools to capture the
402 dynamics of such small invertebrate species [12]. We showed that the population dynamics is
403 particularly vulnerable to juvenile mortality (Figure 2). This high sensitivity to juvenile
404 survival reduction is consistent with a study on the estuarine amphipod *Leptocheirus*
405 *plumulosus* [41]. More generally, it also agrees with Forbes et al. [42], who concluded that benthic
406 macro-invertebrate species generally show a high sensitivity to reduction on juvenile survival
407 rates.

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In addition, the sensitivity of the *Gammarus* population studied varies substantially between months (Figure 3). For instance, the population dynamics appeared highly sensitive to adult survival reductions in winter, whereas it is more sensitive to fertility inhibitions in April, July and August. McGee and Spencer [41, 43] also concluded in high monthly variability of population sensitivity, but the monthly sensitivity pattern was different in *L. plumulosus*. These contrasting patterns highlight the role of species life-histories in governing demographic impacts. The pattern of demographic sensitivity in *G. fossarum* is consistent with our understanding of its population biology. We observed a functioning of the population with two renewal time periods in the year. In spring, an overwintering stock of adults provides the main contributors to population renewal, and this explains the high sensitivity to the adult survival rate in winter and a peak of fertility sensitivity in April (Figure 3). The juveniles produced by these surviving breeders in early spring reach maturity in summer and in turn contribute to the production of new individuals in the population. This results in a second peak of sensitivity to fertility inhibitions in July and August, preceded by a peak of sensitivity to juvenile survival rate reductions in April and May. Thereafter, high sensitivity to juvenile survival reduction appears during autumn, because the survivors from this second wave of new individuals constitute the overwintering stock of adults, which mainly ensures the population restarting in the next year. The modelling approach developed in this study is thus suitable to mechanistically understand the complexity of the demographic processes occurring in a wild *G. fossarum* population. Therefore, this approach can be used to anticipate population impacts of life-history trait alterations caused by toxic environmental contaminations, taking into account the influence of life-history and phenology for this effect upscalingat the population level. On a methodological point, our modelling approach would be fully complemented with the challenging possibility to adapt in the present case of a birth

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flow population, the analytical methodology of sensitivity analysis developed for periodic matrix models [20].

Application to improve the ecological relevance of water quality diagnostic

Combining active biomonitoring approaches and population modelling is a promising way to perform an ecologically relevant assessment at the population level for diagnostic purposes [6]. On the one hand, the biomonitoring methodology of *in situ* caging makes it possible to measure the impact of toxicants on the life-history traits of native species in the field [44]. On the other hand, population models, which are recognized as relevant tools for projecting individual effects to consequences on population dynamics [6-8], provide an assessment at an ecologically relevant level of biological organization. We have illustrated this approach herein with the Amous watershed study . At the three contaminated stations, we observed impacts on life-history traits on caged gammarids (Table 1). When introducing these alterations of life-history traits in the population model, in all cases we anticipated severe consequences on the asymptotic population growth rate λ (between 80 and 99% reduction, Table 1). Interestingly, we also separately considered the potential reduction in λ of the impacts observed on the different life-history traits. Thereby, in comparison with the reference station *Tributary*, we observed that a 15% reduction in the juvenile survival rate (*i.e.* in the station *Upstream −1500 m*) impacts λ with the same severity as a 56% reduction in the adult survival rate (*i.e.* in the station *Downstream +1200 m*) or a 58% reduction in fertility (*i.e.* in the station *Downstream +3500 m*). This pattern is explained by the greater sensitivity of population dynamics to impacts on juvenile survival as revealed by the model's elasticity analysis. Of note, accrediting the relevance of the model predictions, a natural gammarid population is present all along the year in the station *Tributary*, while no gammarids can be harvested in the two contaminated *Downstream* stations. In the station *Upstream -1500*m, gammarids can be found

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episodically, but these are mainly adults, with high occurrence of necrotic gills; no embryos are present in females, and juvenile density is quite null. These observations indicate that organisms present in this station do not constitute a durable population, but are rather drifting animals from more upstream pristine areas.

Furthermore, this modelling approach with periodic matrix population models has particular value for the diagnostic of water quality. Indeed, in addition to characterizing the impact of toxicants in persistent chronic pollution contexts, the periodic structure of these models can anticipate the impact of pulsed sources of contamination, such as peaks of pesticides or contaminations with seasonal variability due to water flow, run-off or effluent emission management. This is in fact particularly relevant with regard to significant between-month differences in population vulnerability (Figure 3). Thus the development of population models that integrate seasonality is a relevant way to increase our ability to project toxic effects on individual performance onto population demographic consequences. One question still to be resolved is how much between-population and between-species variability of life-history phenology could condition the relevance of the approach, which presumed a genericity of demographic patterns in *Gammarids*.

Finally, this study contributes to establishing gammarids as sentinel species for European freshwater ecosystems [6, 13]. Indeed, we have already mentioned that numerous biomarkers tracking toxic effects at the molecular level and *in situ* bioassay protocols are available in these species. Here we demonstrate how alterations of life-history traits could be translated in terms of potential population impacts, by means of an ecologically relevant population model. Therefore, gammarids are good candidates to elaborate multilevel assessment schemes for environmental monitoring. Indeed, based on mechanistic hypotheses as proposed for the predictive hazard assessment of chemical compounds [45-47], one could establish quantitative links between sub-individual biomarker responses and individual performance alterations [48-

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[50], and then use population modelling. In the context of environmental management, this could allow combining the sensitivity and the specificity of field ecotoxicological tools with the ecological relevance of a diagnostic of toxicity projected at the population level.

ASSOCIATED CONTENT

Supporting Information

Supporting information provides details on biological data measurement allowing the parameterization of the population model. Figure S1 relates monthly field-based demographic sampling data, Figure S2 relates details on the relationship between body size and female fertility, and Figure S3 relates details on growth experiments. Table S1 gives the different parameters of the population model (reproductive rates, transition rates and survival rates) and Table S2 gives the actual lambda values corresponding to the Table 1 B. This material is available free of charge via the Internet at http://pubs.acs.org.

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Note

The authors declare no competing financial interest.

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659 **TABLES**

660 **Table 1. A)** Juvenile survival (%), adult survival (%) and fertility (embryos per females)
661 values measured in the stations in the Amous watershed. * indicated significant inhibitions in
662 comparison with the reference station Tributary. **B)** Percentage of inhibition of population
663 growth rate λ caused by the different individual impacts observed in the three contaminated
664 stations.

	Stations			
	Tributary	*Upstream −1500 m*	*Downstream +1200 m*	*Downstream +3500 m*
A) Recorded individual traits during *in situ* assay				
juvenile survival (%)	83	71*	19*	95
adult survival (%)	96	92	42*	94
fertility (embryos per female)	18.0	1.7*	/	7.5*
B) Projected population impacts (% reduction in the asymptotic population growth rate λ)				
all traits	/	99	99	80
juvenile survival	/	65	99	no impact
adult survival	/	no impact	81	no impact
fertility	/	98	/	80

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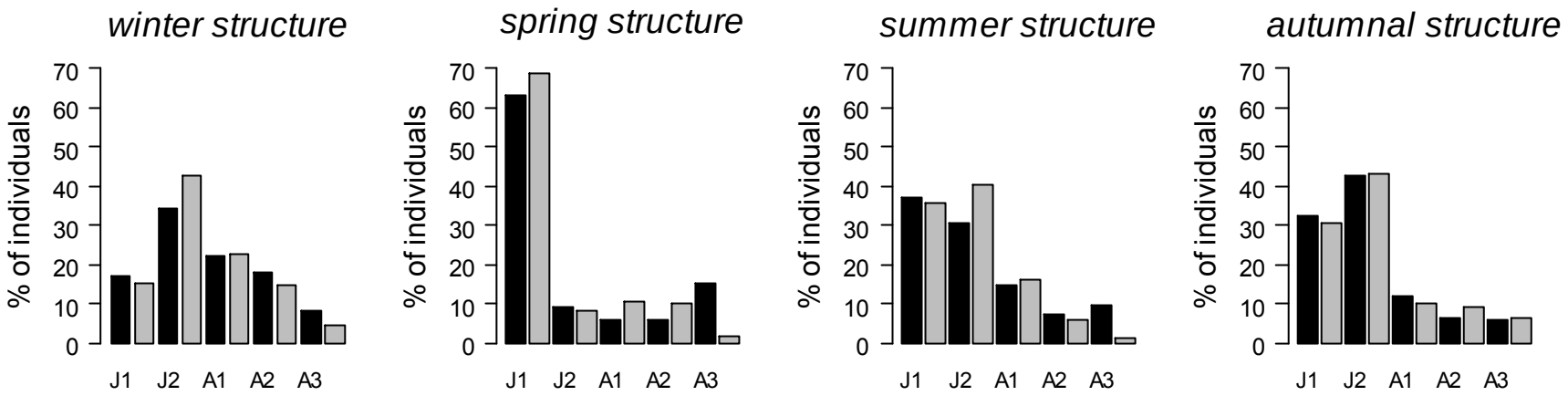
667 **FIGURES**



668

669 **Figure 1**. Stable size distributions computed from the population model (in black) and size-distributions observed during the **field-based**
670 **demographic sampling** of the *Gammarus fossarum* population (in grey). J1 and J2 correspond to the two classes of juveniles of the model; A1,
671 A2 and A3 correspond to the three classes of adults.

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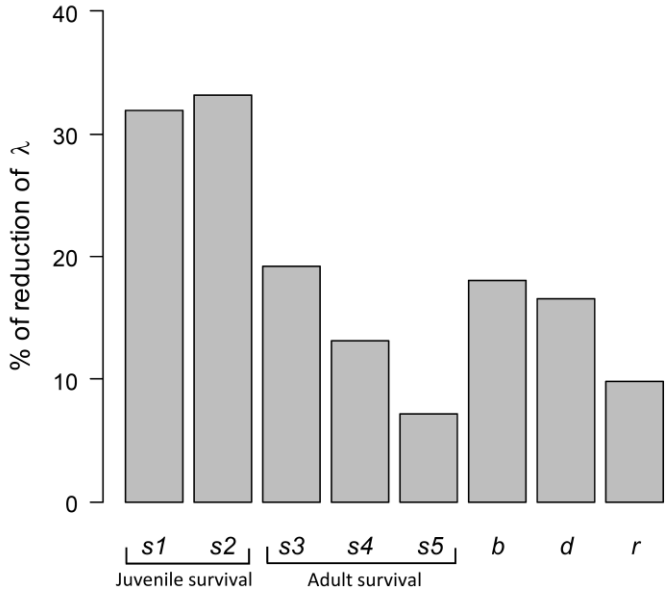

672

673 **Figure 2**. Reduction in the annual asymptotic population growth rate λ induced by a 10%
674 reduction in juvenile survival rates (s1 and s2), adult survival rates (s3, s4 and s5), fertility (*b*),
675 growth (*r*) or a 10% increase in reproductive cycle duration (*d*) applied simultaneously to each
676 month of the year.

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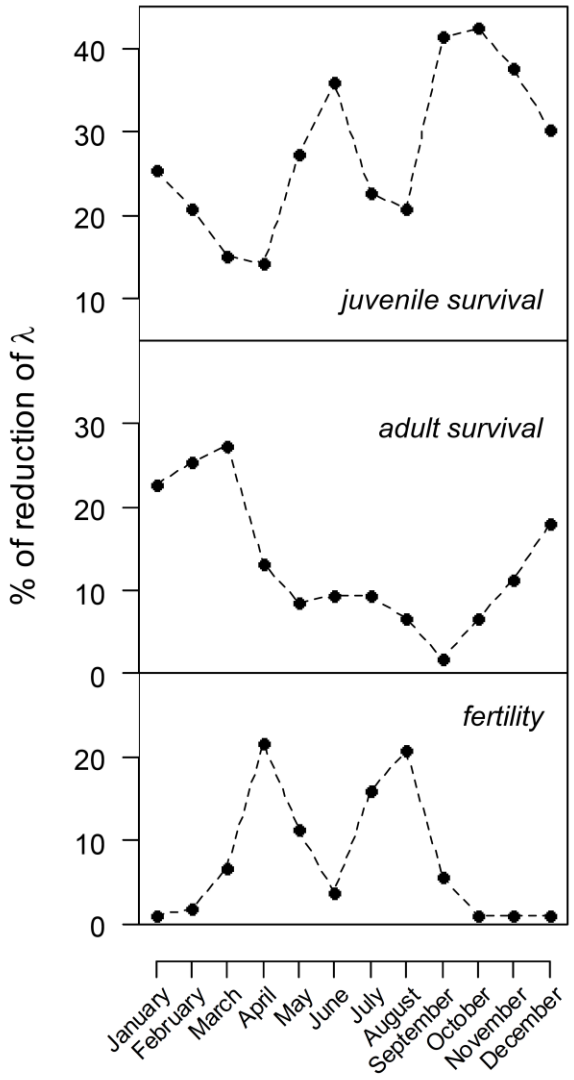

677

678 **Figure 3.** Reduction in annual asymptotic population growth rate λ induced by a 50% reduction
679 in juvenile survival rates (s1 and s2), adult survival rates (s3, s4 and s5) and fertility applied
680 during the different months in the year.

681