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Ecological modelling and toxicity data coupled to assess population recovery of marine amphipod *Gammarus locusta*: Application to disturbance by chronic exposure to aniline

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ABSTRACT

A population agent-based model of marine amphipod *Gammarus locusta* was designed and implemented as a basis for ecological risk assessment of chemical pollutants impairing life-history traits at the individual level. We further used the model to assess the toxic effects of aniline (a priority hazardous and noxious substance, HNS) on amphipod populations using empirically-built dose-response functions derived from a chronic bioassay that we previously performed with this species. We observed a significant toxicant-induced mortality and adverse effects in reproductive performance (reduction of newborn production) in *G. locusta* at the individual level. Coupling the population model with the toxicological data from the chronic bioassay allowed the projection of the ecological costs associated with exposure to aniline that might occur in wild populations. Model simulations with different scenarios indicated that even low level prolonged exposure to the HNS aniline can have significant long-term impacts on *G. locusta* population abundance, until the impacted population returns to undisturbed levels. This approach may be a useful complement in ecotoxicological studies of chemical pollution to transfer individual-collected data to ecological-relevant levels.

1. Introduction

Assessing the impact of exposure to environmental pollutants, in the framework of ecological risk assessment, requires analytical and modelling tools to examine their biological and ecological effects on target organisms (Rodier and Norton, 1992). In most laboratory studies, toxicity is assessed in bioassays in which organisms are exposed to a concentration range of the selected pollutant, and survival, growth and reproduction endpoints are determined at the individual level. However, effects at higher organisational level, such as population, are more valuable for ecological risk assessment, particularly if we want to infer abundance patterns over space and time, and population-level properties such as persistence and recovery. The challenge, at this point, is the extrapolation from individuals to populations by combining toxicity data from bioassays and population modelling approaches. Agent-based models are a good option for this purpose because population properties emerge from individuals (or agents) and their interaction with the environment, and also because they allow the incorporation of individual variability (Grimm and Railsback, 2005). These models have proven useful in predicting short- and long-term population responses under different scenarios of environmental disturbance, based on individual-level endpoints (e.g. Forbes et al., 2008; Galic et al., 2014; Preston and Snell, 2001), making them valuable tools for ecological risk assessment and management of environmental pollutants (Galic et al., 2010, 2014; Meli et al., 2013; Pastorok et al., 2003).

Chemical pollution is one of the most likely occurring disturbances in estuarine and coastal ecosystems, either by inputs from domestic and industrial sewage or from contaminants delivered by rivers and surface water run-off from the land. In addition, the risk of accidental spills is rising given the increasing shipping of chemicals in the last decades (Purnell, 2009). In particular,

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hazardous and noxious substance (HNS) are contaminants other than oil which, if introduced into the marine environment, are likely to create hazards to human health, to harm living resources and other marine life, to damage amenities and/or to interfere with other legitimate uses of the sea (IMO, 2000). However, the toxicity and ecological impacts of HNS on marine organisms are poorly understood, making it difficult to predict the effects on marine ecosystems (Neuparth et al., 2011; Cunha et al., 2014). Particularly, aniline (also known as phenylamine or aminobenzene; which is categorised as a floater/dissolver) is present in the environment at detectable levels and it is considered one of the priority HNS posing a major risk in the European context (Neuparth et al., 2011). Apart from the risk of spill during its transportation, aniline and its derivatives are pollutants originated from pesticides, dye manufacture and coal liquefaction, so aniline can also be potentially introduced in aquatic environments by industrial inputs to sewage treatment works and by agricultural run-off (Lyons et al., 1985 and references therein).

Most HNS spills are frequently characterised by an initial large scale release of a chemical with a diminishing concentration profile over time. If a HNS is persistent and/or cargoes are lost from vessels to the seabed or when it becomes associated with sediments, exposure may continue for many months or years albeit to relatively low levels, leading to chronic exposure. In these situations, the seriousness of spills is primarily related to the time to recovery of the impacted habitats and populations, that is, the time needed to re-establish the population at the pre-disturbance conditions or to levels that would have prevailed in the absence of the HNS spill. Coupling ecological modelling with toxicological data represents a useful tool for inferring times to recovey of populations under different scenarios of contamination, providing relevant information to natural resource management and ecological risk assessment of toxic chemicals (Forbes and Calow, 2013; Galic et al., 2010, 2014).

Another key challenge in ecological risk assessment is to find appropriate model organisms to efficiently evaluate the complexity of potential environmental effects of pollutants at ecologically relevant levels (Fairman et al., 1998). The amphipod *Gammarus locusta* is a suitable test organism in the evaluation of environmental effects of pollutants and its implications in ecological risk assessment (e.g. Costa et al., 1998). This species is broadly distributed in marine and estuarine ecosystems, it is sensitive to a broad range of aquatic contaminants, and it has an important role in food webs of coastal and estuarine regions (Costa and Costa, 2000). In addition, it is amenable to laboratory bioassays due to its short life cycle, simple handling for brooding, adaptability and testing. In the last decades, procedures using *G. locusta* as a model test organism in ecotoxicology have been developed (Correia et al., 2002; Costa and Costa, 1999; Costa et al., 2005; Neuparth et al., 2002, 2005, 2014a,b), providing essential baseline data on the biology and population dynamics of this species, both in the field and under experimental conditions, making available the foundations to construct an ecological model. Despite the existence of other population models developed for marine amphipods (*Talitrus saltator*, Anastácio et al., 2003 and *G. locusta*, Andersson et al., 2009), they are not designed to include toxicity data. However, a recent agent-based model for the freshwater amphipod *Gammarus pulex* demonstrated the validity of this approach in introducing toxicity at the individual level (Galic et al., 2014).

The general aim of this study was to construct an agent-based model for the marine amphipod *G. locusta* to assess the toxic effects of chemical pollutants on estuarine and coastal ecosystems. In this study, in particular, we additionally aimed at improving the knowledge on the priority HNS aniline by producing ecotoxicological data and combining it with the population model to support ecological risk assessment of HNS spills in marine ecosystems. The specific objectives were to: (1) examine the sensitivity of the marine species *G. locusta* after chronic exposure to aniline; (2) translate those effects to the population level by implementing an agent-based model for *G. locusta* in combination with results obtained from bioassays; and (3) use the population model to estimate times to recovery after disturbance in different scenarios of exposure to aniline.

2. Material and methods

2.1. *Test organism* Gammarus locusta

G. locusta (Linnaeus, 1758) is an epibenthic amphipod with a wide geographical distribution along the North-East Atlantic coast (Costa and Costa, 2000). This species is normally found in coastal and estuarine ecosystems, typically on rocky substrates associated to seaweeds, or in sandy or muddy bottoms of *Zostera* spp. beds (Costa and Costa, 2000). *G. locusta* is amongst the main prey of many fish, birds and invertebrate species, which greatly contributes to its high ecological value (Costa and Costa, 1999; MacNeil et al., 1999). Its life-cycle comprises juveniles and adults (with distinct sexual characteristics) and females reproduce over the whole life-span. Natural populations of *G. locusta* are found in the range of 17–35 of salinity, which is a key factor in its distribution, and 11–24 °C of temperature, the latter modulating reproduction and growth of individuals (Costa and Costa, 1999, 2000; Neuparth et al., 2002). Compiled information used to parameterise the life-cycle processes of *G. locusta* is fully presented in Supplementary material.

2.2. *Agent-based model for* G. locusta

An agent-based model (ABM) was built in which population properties were derived by keeping track through time of the characteristics of each individual and their interaction with the environment (Grimm and Railsback, 2005). The model is implemented using R programming language R 3.0.2 (R Core Team, 2013), and R package "simecol" (Petzoldt and Rinke, 2007). The model is described following the ODD protocol (overview, design, details; Grimm et al., 2010). The model is partly based on a previous ABM built for a conspecific freshwater amphipod (Galic et al., 2014). The full R-code of the model is available on request from the corresponding author.

2.2.1. *Purpose*

The purpose of the model is to translate toxicant-induced changes in life-cycle traits (growth, reproduction and mortality) measured at the individual level, to population dynamics of the marine amphipod *G. locusta*. The model includes seawater temperature as a forcing factor affecting life cycle traits of individuals and it is spatially-explicit. In the present study, the model is specifically applied to evaluate the effects of chronic exposure to the HNS aniline.

2.2.2. *Entities, state variables and scales*

The model comprises two entities: female individuals and square cells in a two-dimensional grid simulating the landscape (5×5 identical square cells of 1 m^2 each). The model assumes that individuals were not previously exposed to aniline. Individual state variables include individual age (days), body length (mm), brood size and embryo age (when applicable, i.e. gravid females) and spatial position (discrete x and y coordinates in the grid). Individuals are categorized as juveniles and adults based on their size. All cells in the grid have the same conditions regarding temperature (°C) and toxicant concentration. Local density of individuals (ind m^{-2}) is specific to each cell and determines the probability of density-dependent mortality in that cell. There is not a direct limitation of number of individuals per cell, but this is indirectly controlled

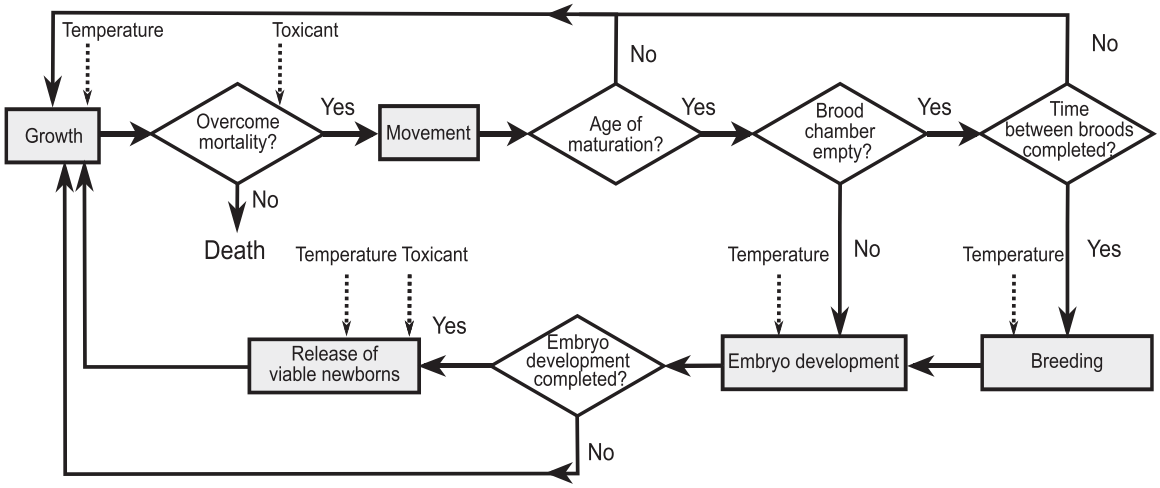

Fig. 1. Conceptual diagram of the modelled life cycle of *Gammarus locusta*. Rectangles indicate processes at the individual level and queries are expressed in diamonds. Dashed lines indicate processes affected by temperature or exposure to toxicant.

by density-dependent mortality. Model runs at one-day time step starting on January 1 and running for 4 years (365 days each). Environmental seawater temperature is updated at each time step according to an external time series based on real observations. The exposure to the toxicant is also given by an external series in which the toxicant concentration and time of exposure are defined according to different scenarios. The transitional population dynamics influenced by the initial conditions, corresponding to the first year, were excluded to avoid skewness in the output analysis.

2.2.3. Process overview and scheduling

At each one-day time step, the state variables of each individual of the initial population matrix are updated according to the four submodels based on sequence of logical queries (Fig. 1): mortality (including background, density-dependent and toxicant-induced mortality), motion (random movement within the grid), reproduction (determination of the brood size in mature females and hatching of any ready-to-hatch embryos in the brood pouch), and growth (increase in age and length). Reproduction and growth are influenced by environmental external factors (temperature).

2.2.4. Design concepts

2.2.4.1. Emergence. Population dynamics (size and structure over time) emerges from the individual life-history (mortality, growth and reproduction) and its interaction with temperature, and aniline concentration and exposure time.

2.2.4.2. Interaction. Three types of individual interaction are included in the model: interaction of individuals with the environmental seawater temperature, interaction of individuals with other individuals through density-dependent mortality, and interaction with the pollutant (aniline) concentration and exposure time. In the latter case, the interaction with aniline is given by a toxicity-induced mortality and toxicity-induced reduction of newborns, both obtained from dose-response functions empirically derived from a laboratory bioassays (see Subsection 3.3).

2.2.4.3. Stochasticity. In order to attain variability amongst individuals, some parameters are randomly drawn from uniform probability distributions (when values are given in a minimum–maximum range) or from normal distributions (when parameters are given as mean and standard deviation). Stochasticity is also included in empirically-obtained equations by adding a variation term from a normal distribution of mean zero and standard deviation equal to the residual standard error of the linear regression in each case.

2.2.4.4. Scheduling. The sequence of the processes is given in Fig. 1. At the onset of a new time step, the individual mortality is determined based on their age, the local density of individuals in the cell they are allocated, and the toxicant exposure (submodel mortality). Then, individuals move randomly in the grid so new local densities are calculated (submodel motion). After that, the brood size is attributed to non-gravid mature females that already overcome the between-broods interval. If any ready-to-hatch females are present, then the new juveniles are added to the population (submodel reproduction). Finally, individuals increase their age and length (submodel growth). The order of the submodels in the sequence did not alter the results (data not shown).

2.2.4.5. Observation. The model yields the population size (total, adults, and juveniles) and individual density (ind m^{-2}) over time and their distribution in the spatial grid. Coefficient of variation (%) of population size from different stochastic runs of the model is also determined. The population growth over time is interpreted according to the empirical increase, decrease or extinction of the population during the simulations.

2.2.5. Initialisation

Initial population in the model is based on field observations. A two-year field survey of *G. locusta* in Sado Estuary (Portugal, Costa and Costa, 1999) recorded a low density of individuals (both females and males <20 ind m^{-2}) in the first two months of the year, for which population structure was not defined. The mean percentage of juveniles along the whole sampling time was 40.5 ± 19.3%, with a minimum value of 3.5% in June and a maximum of 62.1% in July. In January, size classes for juveniles ranged from 4 to 6 mm and for adults from 7 to 9 mm (Costa and Costa, 1999). The model is initiated in January with an initial female population of 10 ind m^{-2}, including 40% of juveniles, randomly assigned in the spatial grid. Size classes for adults are uniformly distributed in the range 7–9 mm and for juveniles in the range 1–6 mm. For each replicate run of the model, the number of individuals and the proportion of juveniles are the same, but their sizes and positions may differ due to stochastic processes.

2.2.6. Input data

The time series of water temperature was obtained for the Mondego estuary (Portugal) in 1995 (Maranhão et al., 2001), which is relatively close to the Sado estuary, from which field data of *G. locusta* is available (Costa and Costa, 1999). The time series consisted of monthly averaged water temperatures, so daily

temperatures were obtained by linear interpolation from the two closest values in time. Exposure to aniline is given by an external time series according to the different scenarios of concentration and exposure time (see Subsection 2.6).

2.2.7. Submodels

Here, we present the most important processes of the submodels, whereas the full description is given in the Supplementary material. The background mortality of *G. locusta* is given by an exponential function of abundance with time from laboratory observations, obtaining a maximum lifespan of 153 days for 90% of the population, in accordance with lifespan ranges reported for this species (112–154 days, Neuparth et al., 2002). Density-dependent mortality refers to mortality due to crowding effects and cannibalism, and it is a linear function of local density which is calculated as the sum of individuals in each cell at each time step. The toxicant-induced mortality is assessed in this model by a concentration-effect function empirically obtained from laboratory assays in which individuals are exposed to different concentrations ($\mu g\,L^{-1}$) and mortality rate is measured at different times during the exposure period (days). This function determines the probability of dying for a given concentration (see Subsection 2.5). Individual growth (increase in length) is given by the Von Bertalanffy growth equation, corrected by a temperature factor. The submodel reproduction determines which females reproduce, the brood size (as a power function of body length) and the occurrence of hatching based on the temperature-dependent embryonic development. Half of the newborns in a hatch event are added to the population (i.e. only females considering a sex ratio of 1) and they are randomly and uniformly allocated in the grid. The number of released newborns is affected by the toxicant through a dose-response function (see Subsection 2.5). After hatching, females pair again after a lapse of time (after moulting). All parameters used in the model are described in Table 1.

2.3. Model validation and sensitivity analysis

For model validation, 5 stochastic model simulations were carried out with the same set of parameters. After exclusion of the transitional population dynamics (first year), simulated density of individuals was compared to field observed density using an independent inventory of monthly population density of *G. locusta* in the Sado estuary (Portugal) in 1995 (Costa and Costa, 1999). Since the model only considers females, the simulated density was doubled (sex ratio of 1:1) to obtain the total population density and compare it with the observed data. The goodness of fit between simulated and observed density was assessed by linear regression, so that a slope equal to 1 and intercept equal to 0 indicate a full match between observed and simulated values.

Sensitivity analysis aims to identify which parameters are the most influential in controlling and structuring the population dynamics of *G. locusta*. Each parameter (reference value) was individually changed by ±10% while keeping the remaining parameters with the reference value. For each parameter, the model was run 5 times for its reference, increased and decreased values. The sensitivity (S^+ and S^-) was then calculated as the relative change in the population annual mean density to the relative change in parameter change (10%).

2.4. Chronic bioassay

Individuals of *G. locusta* were exposed to aniline in a chronic bioassay during 25 days based on Neuparth et al. (2014a,b); Neuparth et al. (2014a,b). Individuals were obtained from a continuous culture maintained at the Interdisciplinary Centre of Marine and Environmental Research (Porto, Portugal). The culture is partially renewed once a year by introducing specimens collected from a clean site in Sado estuary, Portugal (Neuparth et al., 2002). The animals in the culture were fed with *Ulva* sp. that, along with seawater, sediments, and small stones were, collected from coastal areas near Porto, Portugal, in a site without direct contamination sources. Juvenile amphipods (2–4 mm length and 3 weeks old), born within the culture, were selected for the bioassay, thus, making sure that the individuals had not been previously exposed to aniline or to any other contaminant. They were allocated to 5-L glass aquaria with aerated filtered natural seawater at 20–21 °C and salinity 33–35 under a 12 h photoperiod, with a 1 cm deep layer of natural sediment and small stones to provide shelter. Organisms were fed with the macroalgae *Ulva* sp. on an ad libitum basis, assuring that food was never in shortage. The water of each aquarium was renewed daily. Test chambers were inspected daily for aeration and feeding requirements. In a first exploratory trial, amphipods were exposed to six aniline concentrations (0.5, 2.5, 10, 50, 100 and 300 $\mu g\,L^{-1}$) plus control (seawater), obtaining lethal effects in a short period of time at the highest concentrations: 12 h for the 300 $\mu g\,L^{-1}$, 7 days for the 100 and 50 $\mu g\,L^{-1}$, and 9 days for the 10 $\mu g\,L^{-1}$. Therefore, only the two lowest concentrations (nominal concentrations: 0.5 and 2.5 $\mu g\,L^{-1}$) were used in the chronic assay plus the control treatment, with three replicates each. These treatments are in the range of environmental concentrations found in aquatic systems (European Commission, 2004; Okumura et al., 1996). An aniline stock solution was prepared in distilled water aliquoted and stored at −20 °C. At each water renewal, the final aniline concentrations were re-established. One aliquot of the stock solution was employed and serially diluted to achieve the aniline final concentrations in each aquaria. Survival (percentage of alive individuals) and number of hatched newborns were monitored three times over the assay period (days 11, 18 and 25) and, additionally, brood size (number of embryos carried by female) and body length (mm) were measured at the end of the experiment (day 25).

Data from each studied endpoint at the individual level were first checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance criteria (Levene's test), and subsequently assessed by a one- or two-way ANOVA to determine if differences in responses between exposed and control organisms could be attributed to the exposure to aniline. When differences amongst treatments were found, homogenous groups were obtained from Tukey's multiple comparisons tests. Significance level was set at 0.05 for all statistical tests. Data analysis was done using R programming language R 3.0.2 (R Core Team, 2013).

2.5. Incorporation of toxicity data in the model

Experimental data on effects of aniline on *G. locusta* obtained from the chronic bioassay (Section 2.4) were used to model dose-response functions at the individual level for mortality and production of newborns by females. First, the toxicant-induced mortality, given as the probability of dying at a given concentration, was modelled as a sigmoidal function of the concentration of aniline and the exposure time. The function parameters were obtained by a binomial regression analysis. Second, the effects of aniline exposure on newborn production was modelled by a power function. The function parameters were obtained by linear regression analysis on $\log_{10}$-transformed data. To construct this dose-response curve, newborn production values for different treatments and replicates were converted to proportion of the control value, so the response in the dose-response function was displayed as the reduction in newborn production in comparison to the control group, at a given aniline concentration.

Table 1
Model parameters and sensitivity analysis. S: whether parameter accounts for stochasticity or not (y: yes, n: not). *Q*: estimated quality of the empirical knowledge used to set the parameter value: 1 for low certainty (value taken from similar *Gammarus* species), 2 for medium certainty (value taken for *G. locusta* but with some degree of uncertainty), 3 for high certainty (value taken for *G. locusta* supported by laboratory and/or field observations). Relative change in mean annual abundance after parameter change by ±10% (S^+ and S^-) are given from the sensitivity analysis. A value of 1 means that the corresponding model output has changed by 10%. Sensitivities larger than or equal to 1.12% are shown in bold, meaning a change in the model output greater than the coefficient of variation of the baseline model.

Parameter	Definition	Units	Reference value	S	*Q*	Source	+ 10% value	−10% value	S^+	S^-
m_1	Daily background mortality probability	day^{-1}	0.015 ± 0.001	y	2	[1]	0.0165	0.0135	−0.5	−0.1
m_2	Density-dependent mortality probability	$m^2\ ind^{-1}\ day^{-1}$	0.0001	n	1	[2]	0.00011	0.00009	**−1.4**	**+1.1**
d	Total daily distance in the spatial grid	m	1 ± 1	y	1	[3]	1.1	0.9	0	0
L_{mat}	Length of maturation	mm	7	n	3	[4]	7.7	6.3	**−2.3**	**+2.2**
b_1	Parameter brood size equation	–	−1.328	n	3	[1]	−1.4608	−1.1952	0.8	**+1.2**
b_2	Parameter brood size equation	–	2.848	n	3	[1]	3.1328	2.5632	**+2.2**	**−2.2**
b_3	Parameter brood size equation	–	0.1647	y	3	[1]	0.18117	0.14823	+0.2	−0.9
d_1	Parameter brood development time equation	–	9.857	n	2	[1]	10.8427	8.8713	**−9.9**	**+7.5**
d_2	Parameter brood development time equation	–	−2.409	n	2	[1]	−2.6499	-2.1681	**+4.2**	**−7.9**
s_1	Parameter embryo survival equation	–	75.99	n	1	[6]	83.589	68.391	+0.3	**−1.5**
s_2	Parameter embryo survival equation	–	−0.43	n	1	[6]	−0.473	-0.387	+0.3	+0.9
s_3	Parameter embryo survival equation	–	15	n	1	[6][7]	16.5	13.5	+1.0	**−1.9**
L_{ini}	Length at birth	mm	1–1.5	y	3	[1]	1.1–1.65	0.9–1.35	−0.1/0.0	−0.3/−0.3
T_{bet}	Time between broods	day	2–3	y	1	[8][9]	2.2–3.3	1.8–2.7	+0.1/ **+1.1**	+0.3/ **+1.3**
L_{max}	Maximum body length for females	mm	14.59	n	3	[1]	16.049	13.131	**+4.1**	**−4.0**
r	Individual growth rate	day^{-1}	0.012	n	3	[1]	0.0132	0.0108	**+2.6**	**−3.0**
T_{opt}	Optimum temperature for growth	°C	20	n	3	[1]	22	18	+0.4	**−1.8**
T_{max}	Maximum temperature for growth	°C	24	n	2	[4]	26.4	21.6	**+ 1.3**	**−4.9**
T_{min}	Minimum temperature for growth	°C	11	n	2	[4]	12.1	9.9	**−1.5**	**+1.9**

[1] Neuparth et al. (2002), [2] Van den Brink et al. (2007), [3] Elliott (2003), [4] Costa and Costa (1999), [5] Welton and Clarke (1980), [6] Pöckl and Humpesch (1990), [7] Neuparth (1999), [8] Sutcliffe (2010), and [9] Iribarne et al. (1995).

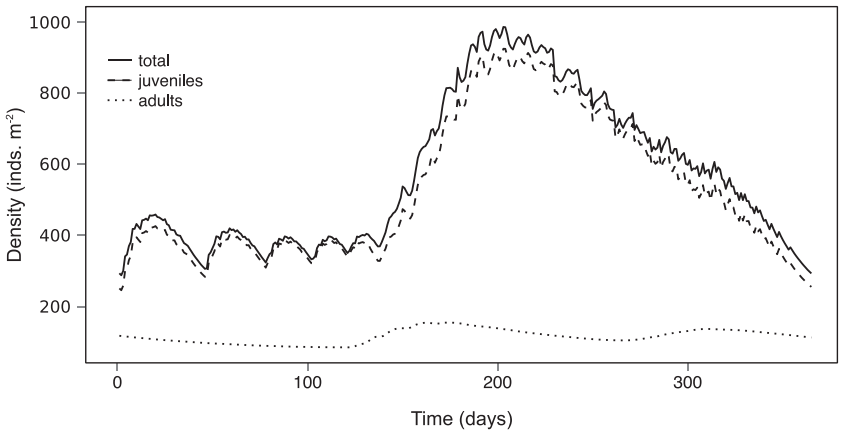

Fig. 2. Simulated density of amphipod *Gammarus locusta* for a year.

2.6. Exposure scenarios and analysis of time to recovery

Ten aniline exposure scenarios were defined by the full combination of two concentrations (0.5 and 2.5 $\mu g\,L^{-1}$) and different exposure times (5, 10, 15, 20, and 25 days), plus the reference or zero-toxicant scenario. These times of exposure were selected based on the persistence (half-life) of aniline, which is ca. 15 days (Cunha and Santos, 2013). Exposure to aniline was initiated on the first day of the year and the population was simulated for 3 years, using 5 stochastic model runs for each scenario.

The return capacity of the population to a pre-disturbance state was taken as the time to recovery by comparing population density in a given toxicant scenario with the non-toxicant scenario, which represented the baseline population state. The time to recovery was defined as the time elapsed from the day after the toxicant exposure until the day on which population overtakes 98% of the baseline density during 5 consecutive days, i.e. the conditions that would have prevailed in the absence of the disturbance. This percentage was chosen considering the coefficient of variation of the mean annual abundance simulated with the model. Time to recovery for each scenario was given as the average for the 5 stochastic simulations. It is important to notice that, as a consequence of the definition of time to recovery in this study, even when post-disturbance population density is within 2% of variation in comparison to the baseline model, the obtained time is 5 days.

3. Results

3.1. Baseline population model and validation

The agent-based model of *G. locusta* was implemented with an initial population density of 10 females m^{-2}, 40% of them being juveniles. The simulated population reached an abundance peak during late spring and summer, with densities close to 1000 ind m^{-2} (Fig. 2). Adults reached two peak densities: in mid-late spring (80 ind m^{-2}) and in early-mid winter (65 ind m^{-2}), while maximum of juveniles was reached during summer (Fig. 2). The coefficient of variation of mean annual abundance calculated for the 5 simulations was 1.12%.

Simulated density of individuals was compared to field observed abundance of *G. locusta* in the Sado estuary (Portugal) in 1995 (Costa and Costa, 1999). A significant regression between simulated and observed abundances was obtained ($R^2 = 0.438$, $p < 0.05$, df = 10, Fig. 3), although the intercept was significantly different from zero (371, 95% confident interval 195–546), indicating that

Table 2
ANOVA table for effects of aniline on individual endpoints of amphipod *Gammarus locusta*.

	Source	df	SS	MS	*F*	*p*
Brood size	Treatment	2	915	457	0.186	0.831
	Residuals	37	91066	2461		
Survival	Treatment	2	0.4650	0.2325	33.76	**<0.001**
	Time	1	0.5371	0.5371	77.98	**<0.001**
	Residuals	32	0.2204	0.0069		
Newborn production per female	Treatment	2	43228	21614	11.5	**<0.01**
	Residuals	6	11281	1880		
Body length (females)	Treatment	2	3.55	1.776	1.639	0.205
	Residuals	49	53.11	1.084		

Table 3
Regression coefficients for the dose-response functions built on the results from the bioassay of exposure of amphipod *Gammarus locusta* to aniline during 25 days.

	Estimate	Standard error	Statistic	*p*
Multiple logistic regression for toxicant-induced mortality ($R^2 = 0.86$)				
Intercept	−4.308	0.351	$Z = -12.262$	**<0.001**
Concentration ($\mu g\,L^{-1}$)	0.579	0.094	$Z = 6.171$	**<0.001**
Time of exposure (days)	0.104	0.015	$Z = 6.854$	**<0.001**
Simple linear regression on $\log_{10}$-transformed data for reduction of newborn production ($R^2 = 0.58$)				
Intercept	−0.014	0.218	$T = -0.064$	0.951
$\log_{10}$(concentration [$\mu g\,L^{-1}$] + 1)	−2.311	0.661	$T = -3.499$	**<0.01**

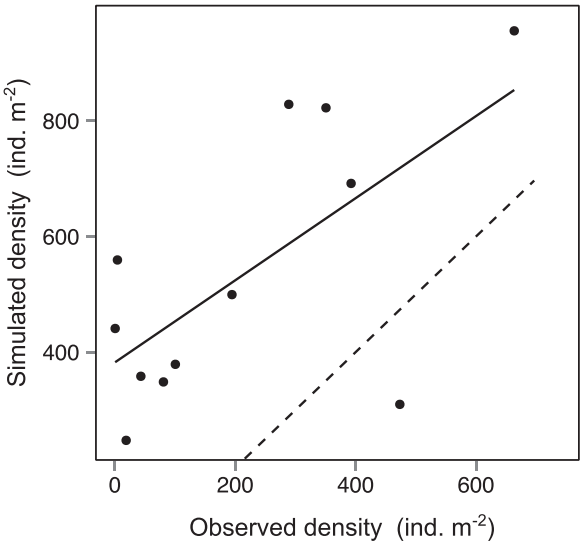

Fig. 3. Linear regression (solid line) between simulated and observed density of *Gammarus locusta* over a year (observed data from Costa and Costa, 1999). Dashed line (1:1) indicates hypothetical full match between simulated and observed values.

the model overestimate the population. However, the slope was not significantly different from the unit (0.731, 95% confident interval 0.147–1.314), indicating that the model simulates satisfactorily the patterns of population dynamics (peak of maximum abundance and seasonal changes) over the year.

3.2. Model sensitivity analysis

Mean annual density was highly affected when density-dependent mortality, reproduction-related parameters (length of maturation, brood size parameters, brood development time parameters), and growth-related parameters (individual growth rate, maximum body length, and temperature for growth) were altered (Table 1). Some of these parameters had previously been sorted in the group of parameters with high or medium uncertainty (Table 1): parameters of the function determining brood development time given the temperature (sensitivities up to 9.9%), the density-dependent mortality (up to 1.4% of change), and the parameters of the growth equation as a function of temperature (sensitivities up to 4.9%). The percentage of change in the rest of the parameters was smaller than the coefficient of variation (1.12%) of the mean annual abundance, so we considered that the observed changes in the sensitivity analysis fall into the baseline variability of the model output, so the changes are not significant.

3.3. Aniline effects on individuals and toxicity functions for the model

After a 25-day exposure, body length of exposed individuals did not differ from those in the control (Table 2). However, significant differences were found in individual survival amongst the control and the treatments, with 77% and 49% of survivorship in the 0.5 and 2.5 $\mu g\,L^{-1}$, respectively (Fig. 4a and Table 2). Brood size did not differ amongst control and exposure treatments (Fig. 4b and Table 2), although total newborn production per female during the exposure period was reduced by 87% at the highest concentration treatment (2.5 $\mu g\,L^{-1}$) (Fig. 4c and Table 2). This means that the brood size was irresponsive to aniline exposure but that the post-hatching survival of embryos was severely affected.

Toxicity functions for the population model were built with the bioassay results. Reduction in the production of newborns was

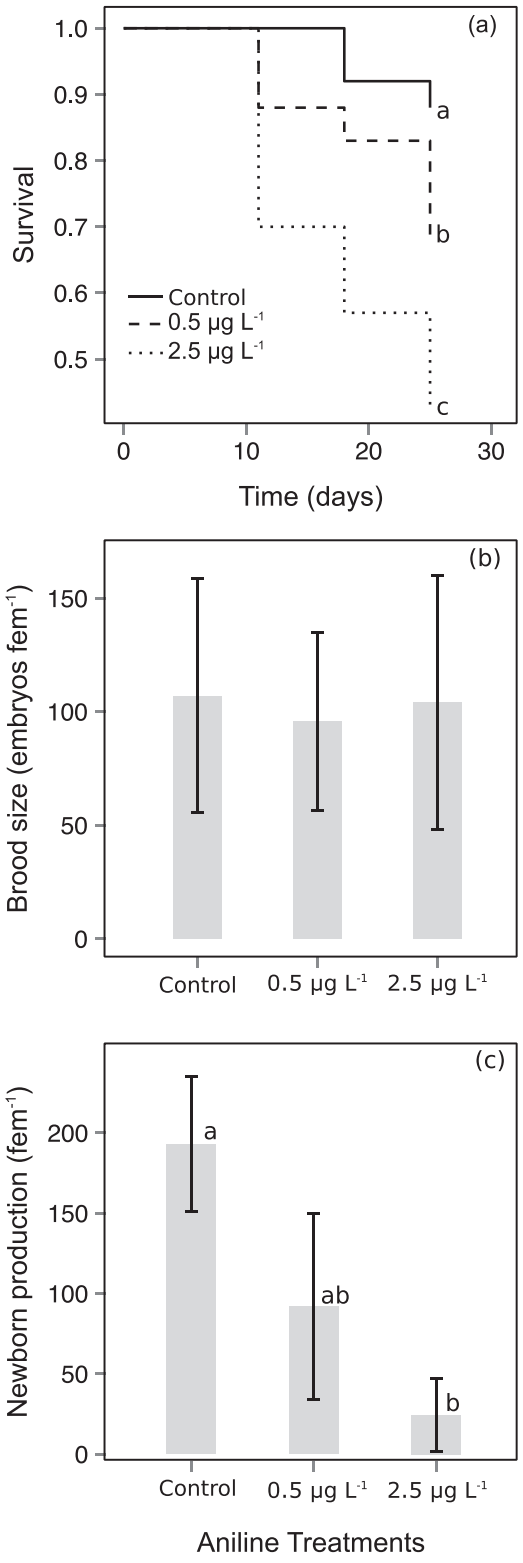

Fig. 4. Effects of 25-day exposure to aniline on survival and reproductive endpoints (brood size and cumulative newborn production) of amphipod *Gammarus locusta*. Lowercase lettering on lines or bars indicates significant differences amongst groups.

modelled as a negative power function of aniline concentration (Fig. 5a and Table 3) and mortality was modelled as a logistic function of aniline concentration and time of exposure (Fig. 5b and Table 3).

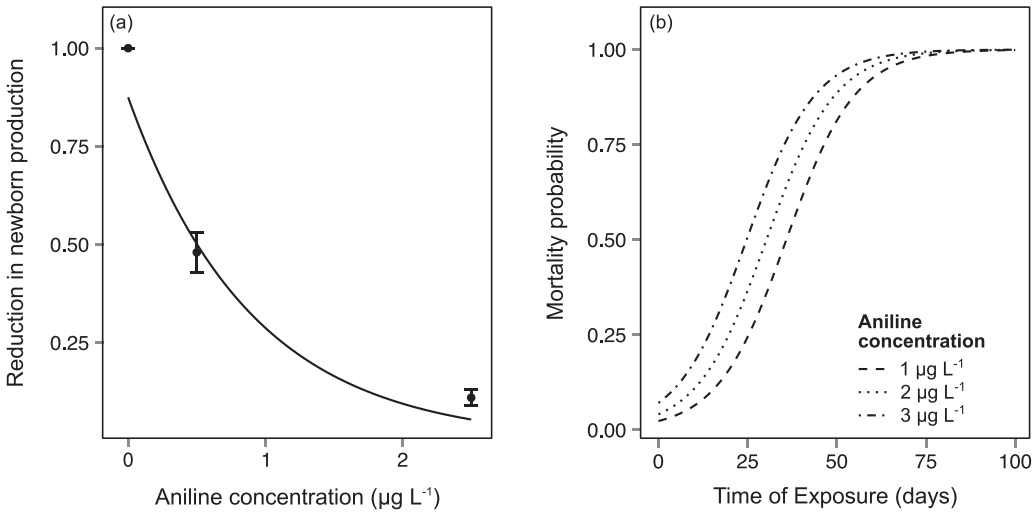

Fig. 5. Dose-response functions built on the results from the bioassay of exposure of amphipod *Gammarus locusta* to aniline during 25 days. (a) Reduction on newborn production as a negative power function of aniline concentration (mean and 1 standard error), and (b) Mortality probability as a sigmoidal function of aniline concentration and exposure time.

3.4. *Aniline effects on population and time to recovery*

The effect of exposure to aniline was simulated by combining two concentrations and five exposure times. After exposure to aniline, a sharp drop in population abundance was observed followed by a linear increase in the abundance for the lowest concentration and short exposure time scenarios, and by an exponential growth for the scenario of the highest concentration and longest exposure time (Fig. 6). Abundance of impacted populations overtook the baseline abundance after recovery on two occasions, with subsequent abundances matching the baseline (Fig. 6). A time to recovery of less than one month was estimated for scenarios of exposure to an aniline concentration of 0.5 $\mu g\,L^{-1}$ during 5 and 10 days (Fig. 7). For the remaining scenarios, the time to recovery increased linearly with exposure time until 15 and 20 days for concentrations of 0.5 and 2.5 $\mu g\,L^{-1}$. The longest time to recovery was 456 ± 5 days after a 25-day exposure to an aniline concentrations of 2.5 $\mu g\,L^{-1}$ (Fig. 7).

4. Discussion

Our main objective was to design and implement a population agent-based model for the marine amphipod *G. locusta*, as a basis for ecological risk assessments of chemical pollutants impairing life-history traits at the individual level. We further used the model to assess the effects of chronic toxicity by aniline (a priority HNS) on the amphipod population using empirically-built dose-response functions derived from a chronic bioassay. We observed chronic adverse effects in *G. locusta* at the individual level in reproductive performance, with a sharp reduction of newborn production, and also a significant toxicant-induced mortality. The coupling of population modelling with toxicological data from the chronic bioassay allowed the projection of the ecological costs associated with exposure to aniline that might occur in wild populations. Model simulations with different scenarios indicated that even low level prolonged exposure to the HNS aniline can have a significant long-term impact (>1 year) on population abundance of *G. locusta*, before the impacted population returns to levels that would have prevailed in the absence of chemical disturbance.

4.1. *Agent-based model of* G. locusta *population dynamics*

Population abundance in our model is an emergent property of the modelled interactions between individuals and water temperature (interaction at the reproduction and growth levels), and amongst individuals (density-dependent mortality). The model output matched well with the population data reported by Costa and Costa (1999) under field conditions (Fig. 2), simulating seasonal changes in population density over the year. However, total density was overestimated by the model, as occurred in a similar agent-based model of a freshwater amphipod (Galic et al., 2014). Model limitations, such as accounting for natural predation, cannibalism-related issues (more accurate rates for this species or effects of patchy distribution), and, particularly, dependency on algal cover (which acts both as shelter and food availability) could explain this mismatch. In fact, seasonality of *G. locusta* abundance in Portuguese coast showed a strong coupling with algal density, mainly *Ulva* spp. (Costa and Costa, 1999). Our model, albeit considering unlimited resources for growth, is sufficiently sensitive to translate effects at the population level based on individual endpoints.

The mean annual abundance in the model was sensitive to changes in parameters related to mortality, individual growth, and brood development, which is in accordance with previous amphipod models, where changes in mortality probability, fecundity (brood size) and recruitment-related parameters, resulted in large changes in the population abundance (Anastácio et al., 2003; Coulaud et al., 2014; Galic et al., 2014; McGee and Spencer, 2001). The modification of the parameters in the sensitivity analysis may also represent the likely effects of exposure to toxic chemicals on individuals. Thus, it can be used to discuss the extrapolation of observations from the individual to the population level. Our results indicate that the pollutants that potentially affect mortality, individual growth, brood size or brood development time, could have high negative impacts at the population levels, even when changes in these endpoints are relatively small (i.e. 10%, Table 1). Analysing the sensitivity of the model parameters can also provide valuable insights in establishing the accuracy at which each parameter should be determined, due to their high influence in the model predictions (i.e. the higher the sensitivity, the higher the accuracy should be). In particular, further research effort should focus on those parameters with high sensitivity and high uncertainty in their reference values (i.e. little information from which to estimate their values). These parameters deserve special attention as calibration parameters and as targets of empirical research to reduce their uncertainty and, consequently, the uncertainty of the model results for ecological risk assessments. In our model, we highlight this attention towards parameters related to

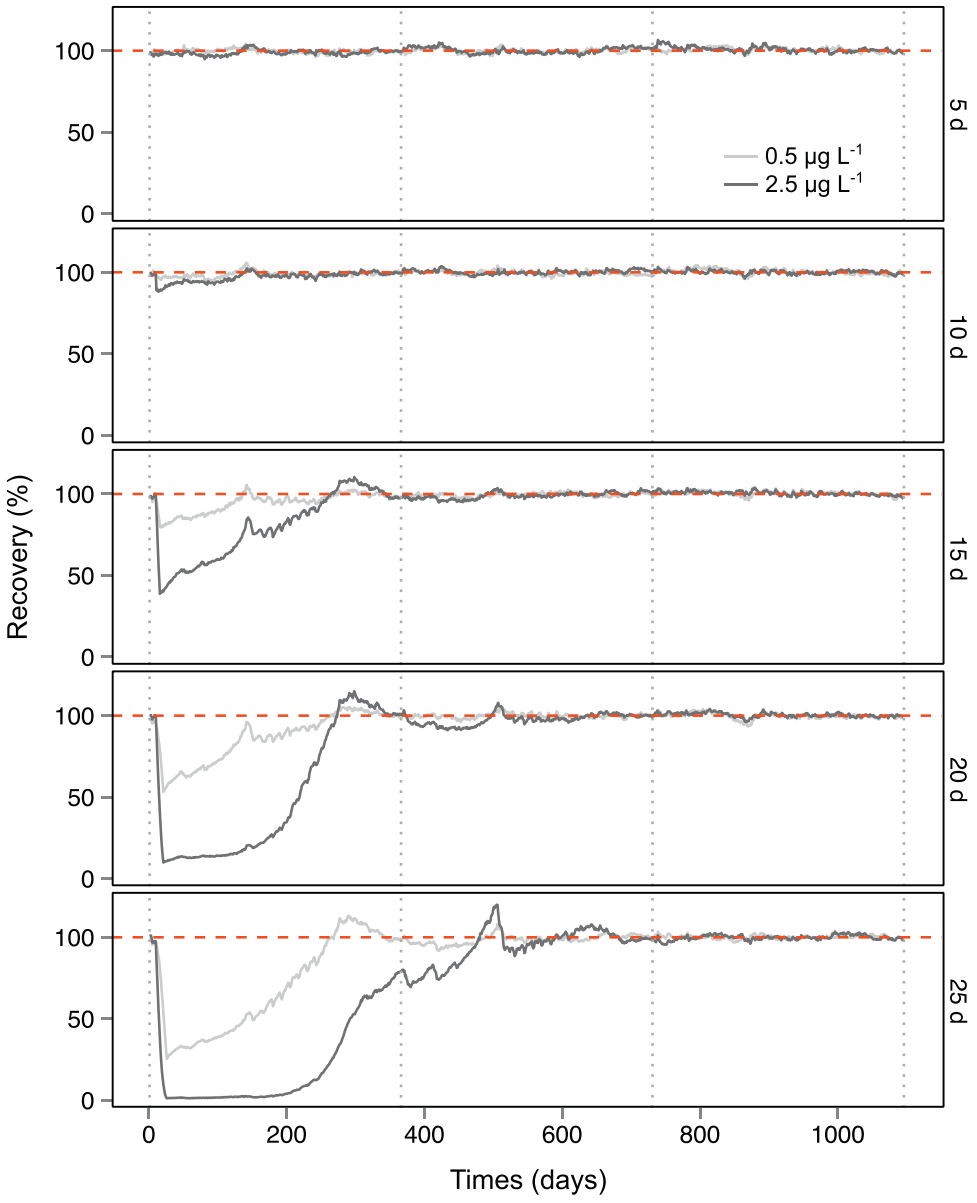

Fig. 6. Recovery pattern of population abundance of amphipod *Gammarus locusta* over time under different scenarios of toxicity by aniline (concentration given by grey lines, and time of exposure given by different panels: 5, 10, 15, 20, 15 days) after coupling the agent-based model with the toxicity data from the chronic bioassay. Showed values are the mean of 5 runs of the model. Red dashed line indicates the baseline population (zero-toxicity scenario). Vertical dotted lines indicate years. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the temperature effect on the brood development time (sensitivities up to 9.9%) and individual growth (sensitivities up to 4.9%), and the density-dependent mortality (up to 1.4% of change).

4.2. Individual and population effects after chronic exposure to aniline

Chronic toxicity bioassays provide a more realistic representation of the type of exposure expected in environmental situations after chemical spills where there are low dispersion rates (e.g. some nearshore locations), where there is a continuous leakage of the chemical or the chemical is relatively persistent. We observed chronic adverse effects in *G. locusta* at the individual level in reproductive performance of females exposed to low concentrations of aniline, with a sharp reduction in the newborn production, but not in the brood size. Individual growth and sex-ratio of *G. locusta* exposed to the HNS *p*-xyleno were also impaired in a similar chronic bioassay (Neuparth et al., 2014b), showing the sensitivity of this species to diverse HNS. Reproduction impairment due aniline toxicity has been previously reported for freshwater Crustacea *Daphnia magna* (Abe et al., 2001; Kühn et al., 1989) and acute toxicity shown for sand shrimp *Crangon septemspinosa* (McLeese et al., 1979). This shortage in juvenile recruitment and the toxicant-induced mortality imply severe costs at the population level, as shown when toxicity data were introduced into the model and upscaled to the population level. The simulations under different scenarios indicated that even low level prolonged exposure to aniline can have significant long term impacts to population abundance of marine organisms. Experimental data on long-term effects of aniline on amphipods are absent, so further investigations using long-term mesocosm studies are needed to reinforce the results derived from this study, including multi-generational effects. Since the exposure to aniline was initiated at the onset of the year in the simulated scenarios, i.e. when abundance is at the lowest values, the estimated

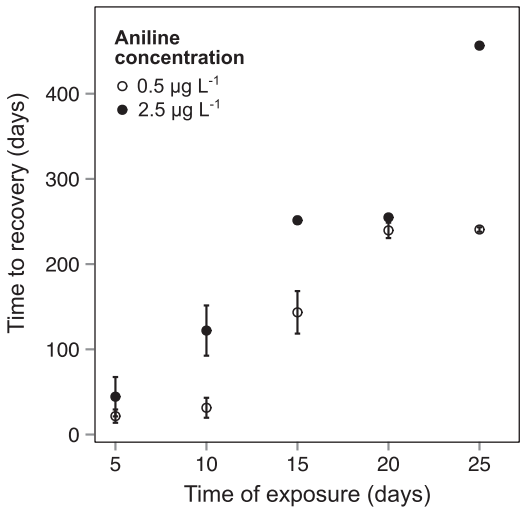

Fig. 7. Time to recovery (mean and 1 standard error, $n = 5$) under different scenarios of toxicity by aniline for amphipod *Gammarus locusta*. Time to recovery was defined as the time elapsed from the day after the toxicant exposure until the day in which population overtakes 98% of the baseline density during 5 consecutive days.

times to recovery could reflect the worst case. However, this corresponds to the most probable scenario since the majority of spill incidents occur during winter due to bad weather or sea conditions. The same magnitude of disturbance at different periods of the year, especially during the peak of maximum abundance, is likely to reduce the negative impacts on the amphipod population. To increase the model robustness in predicting times to recovery of natural populations after exposure to aniline, the modelled patterns should be compared with real scenarios caused by spills or from long-term, low-level aniline-addition experiments in controlled mesocosms. Other applications of the model on the effects of environmental disturbances on estuarine biota may include scenarios of acute toxicity (Neuparth et al., 2014a). In addition, scenarios of multiple stressors could be interesting, since the natural populations are normally exposed to local (e.g. spills) and global pressures (e.g. warming or acidification). The co-occurrence of environmental stress can have unexpected synergistic or antagonistic overall effects. For instance, simulations of water temperature modifications have been reported to affect the population abundance (Galic et al., 2014), given the sensitivity of amphipod growth and reproduction rates to temperature. The co-existence of chemical pollution and eutrophication could be another interesting scenario, since eutrophication-induced changes in benthic algae may lead to a *G. locusta* population increase (Kraufvelin et al., 2006). Finally, the coupling of the present ecological model with a spill trajectory model in which concentration over time and space is predicted based on local conditions and physical–chemical properties of the toxicant (e.g. half-life, fugacity, persistence, solubility, density), could be a powerful tool in predicting in a more realistic way the effects of chemical spills on estuarine biota and to estimate the times to recovery to pre-disturbance conditions. Regarding economic concenrs in the sequence of a spill, the model may be helpful for calculating claim compensations; in the way it allows to calculate time to recovery of a sentinel organism population (Cunha et al., 2014).

5. Conclusions

The model we implemented for an organism widely studied in ecotoxicology may be used to project the ecological costs associated with exposure to environmental stressors, such as chemical pollutants, forming the basis for predicting what effects might be occurring in wild populations. If our study reflects a real case scenario, the impairment produced by aniline at the individual level would lead to important impacts on *G. locusta* natural populations. Such findings highlight the value of population modelling in risk assessment and reinforce the need for remedial action to minimise the likely long-term chronic effects on the marine environment following a HNS incident.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquatox.2015.03.019.

References

Abe, T., Saito, H., Niikura, Y., Shigeoka, T., Nakano, Y., 2001. Embryonic development assay with *Daphnia magna*: application to toxicity of aniline derivatives. Chemosphere 45, 487–495, http://dx.doi.org/10.1016/S0045-6535(01)49-2.

Anastácio, P., Gonçalves, S., Pardal, M., Marques, J., 2003. A model for amphipod (*Talitrus saltator*) population dynamics. Estuarine Coastal Shelf Sci. 58, 149–157, http://dx.doi.org/10.1016/S0272-7714(03)41-6.

Andersson, S., Persson, M., Moksnes, P.O., Baden, S., 2009. The role of the amphipod *Gammarus locusta* as a grazer on macroalgae in Swedish seagrass meadows. Mar. Biol. 156, 969–981, http://dx.doi.org/10.1007/s00227-009-1141-1.

Correia, A., Lima, G., Costa, M.H., Livingstone, D., 2002. Studies on biomarkers of copper exposure and toxicity in the marine amphipod *Gammarus locusta* (Crustacea): I. Induction of metallothionein and lipid peroxidation. Biomarkers 7, 422–437, http://dx.doi.org/10.1080/135475002760413516.

Costa, F.O., Correia, A., Costa, M.H., 1998. Acute marine sediment toxicity: a potential new test with the amphipod *Gammarus locusta*. Ecotox. Environ. Saf. 40, 81–87, http://dx.doi.org/10.1006/eesa.1998.1646.

Costa, F.O., Costa, M.H., 1999. Life history of the amphipod *Gammarus locusta* in the Sado estuary (Portugal). Acta Oecol. 20, 305–314, http://dx.doi.org/10.1016/S1146-609X(99)136-8.

Costa, F.O., Costa, M.H., 2000. Review of the ecology of *Gammarus locusta* (L.). Pol. Arch. Hydrobiol. 47, 541–560.

Costa, F.O., Neuparth, T., Correia, A.D., Costa, M.H., 2005. Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta* II. Organism and population-level endpoints. Mar. Environ. Res. 60, 93–110, http://dx.doi.org/10.1016/j.marenvres.2004.08.005.

Coulaud, R., Geffard, O., Coquillat, A., Quéau, H., Charles, S., Chaumot, A., 2014. Ecological modelling for the extrapolation of ecotoxicological effects measured during in situ assays in *Gammarus*. Environ. Sci. Technol. 48, 6428–6436, http://dx.doi.org/10.1021/es501126g.

Cunha, I., Santos, M.M., 2013. Revision on the Fate, Behaviour and Weathering of Priority HNS. Technical Report ARCOPOLplus. Improving Maritime Safety and Atlantic Regions' Coastal Pollution Response Through Technology Transfer, Training and Innovation, 50 pp.

Cunha, I., Neuparth, T., Moreira, S., Santos, M.M., Reis-Henriques, M.A., 2014. Management of contaminated marine marketable resources after oil and HNS spills in Europe. J. Environ. Manage. 135, 36–44, http://dx.doi.org/10.1016/j.jenvman.2013.12.032.

Elliott, J., 2003. A comparative study of the dispersal of 10 species of stream invertebrates. Freshwater Biol. 48, 1652–1668, http://dx.doi.org/10.1046/j1365-2427.2003.01117.x.

European Commission, 2004. European Union risk assessment report. aniline. In: Munn, S.J., Allanou, R., Aschberger, K., Berthault, F., de Bruijn, J., Musset, C., O'Connor, S., Pakalin, S., Pellegrini, G., Scheer, S., Vegro, S. (Eds.), Environment and Quality of Life Series, vol. 50. European Commission, Luxembourg, 222 pp.

Fairman, R., Mead, C.D., Williams, W.P., 1998. Environmental risk assessment: approaches, experiences and information sources. Monitoring and Assessment

Research centre, King's College, London. Published by European Environment Agency–EEA Environmental issue Report No 4.
Forbes, V.E., Calow, P., Sibly, R.M., 2008. The extrapolation problem and how population modeling can help. Environ. Toxicol. Chem. 27, 1987–1994, http://dx.doi.org/10.1897/08-029.1.
Forbes, V.E., Calow, P., 2013. Developing predictive systems models to address complexity and relevance for ecological risk assessment. Integr. Environ. Assess. Manage. 9, 75–80, http://dx.doi.org/10.1002/ieam.1425.
Galic, N., Hommen, U., Baveco, J., Van den Brink, P.J., 2010. Potential application of population models in the European ecological risk assessment of chemicals II: review of models and their potential to address environmental protection aims. Integr. Environ. Assess. Manage. 6, 338–360, http://dx.doi.org/10.1002/ieam.68.
Galic, N., Ashauer, R., Baveco, H., Nyman, A.-M., Barsi, A., Thorbek, P., Bruns, E., Van den Brink, P.J., 2014. Modeling the contribution of toxicokinetic and toxicodynamic processes to the recovery of *Gammarus pulex* populations after exposure to pesticides. Environ. Toxicol. Chem. 33, 1476–1488, http://dx.doi.org/10.1002/etc.2481.
Grimm, V., Railsback, S.F., 2005. Individual-based modeling and ecology. In: Princeton Series in Theoretical and Computational Biology. Princeton University Press, Princeton.
Grimm, V., Berger, U., DeAngelis, D.L., Polhill, J.G., Giske, J., Railsback, S.F., 2010. The ODD protocol: a review and first update. Ecol. Modell. 221, 2760–2768, http://dx.doi.org/10.1016/j.ecolmodel.2010.08.019.
IMO–International Maritime Organisation, 2000. Protocol on Preparedness, Response and Co-operation to Pollution Incidents by Hazardous and Noxious Substances (OPRC-HNS Protocol).
Iribarne, O., Fernández, M., Armstrong, D., 1995. Precopulatory guarding-time of the male amphipod *Eogammarus oclairi*: effect of population structure. Mar. Biol. 124, 219–223, http://dx.doi.org/10.1007/BF00347126.
Kraufvelin, P., Salovius, S., Christie, H., Moy, F.E., Karez, R., Pedersen, M.F., 2006. Eutrophication-induced changes in benthic algae affect the behaviour and fitness of the marine amphipod *Gammarus locusta*. Aquat. Bot. 84, 199–209, http://dx.doi.org/10.1016/j.aquabot.2005.08.008.
Kühn, R., Pattard, M., Pernak, K.D., Winter, A., 1989. Results of the harmful effects of water pollutants to *Daphnia magna* in the 21 day reproduction test. Water Res. 23, 501–510, http://dx.doi.org/10.1016/0043-1354(89)90142-5.
Lyons, C., Katz, S., Bartha, R., 1985. Persistence and mutagenic potential of herbicide-derived aniline residues in pond water. Bull. Environ. Contam. Toxicol. 35, 696–703, http://dx.doi.org/10.1007/BF01636575.
MacNeil, C., Dick, J.T., Elwood, R.W., 1999. The dynamics of predation on *Gammarus* spp. (Crustacea: Amphipoda). Biol. Rev. 74, 375–395, http://dx.doi.org/10.1111/j.1469-185X.1999.tb00035.x.
Maranhão, P., Bengala, N., Pardal, M., Marques, J.C., 2001. The influence of environmental factors on the population dynamics, reproductive biology and productivity of *Echinogammarus marinus* Leach (Amphipoda, Gammaridae) in the Mondego estuary (Portugal). Acta Oecol. 22, 139–152, http://dx.doi.org/10.1016/S1146-609X(01)1112-2.
McGee, B., Spencer, M., 2001. A field-based population model for the sediment toxicity test organism *Leptocheirus plumulosus*: II. Model application. Mar. Environ. Res. 51, 347–363, http://dx.doi.org/10.1016/S0141-1136(00)133-1.
McLeese, D., Zitko, V., Peterson, M., 1979. Structure-lethality relationships for phenols, anilines and other aromatic compounds in shrimp and clams. Chemosphere 8, 53–57, http://dx.doi.org/10.1016/0045-6535(79)90049-3.
Meli, M., Palmqvist, A., Forbes, V.E., Groeneveld, J., Grimm, V., 2013. Two pairs of eyes are better than one: combining individual-based and matrix models for ecological risk assessment of chemicals. Ecol. Modell. 280, 40–52, http://dx.doi.org/10.1016/j.ecolmodel.2013.07.027.
Neuparth, T., 1999. Gammarus locusta e Corophium Volutator (Amphipoda) em Estudios de Ecotoxicologia Sedimentar: Biologia e Sensibilidade a Ambiestas Sedimentares Perturbados. Master's Thesis, Gammarus locusta e Corophium Volutator (Amphipoda) em Estudios de Ecotoxicologia Sedimentar: Biologia e Sensibilidade a Ambiestas Sedimentares Perturbados. In: Master's Thesis. Universidade Nova de Lisboa.
Neuparth, T., Costa, F.O., Costa, M.H., 2002. Effects of temperature and salinity on life history of the marine amphipod *Gammarus locusta*. Implications for ecotoxicological testing. Ecotoxicology 11, 61–73, http://dx.doi.org/10.1023/A.1013797130740.
Neuparth, T., Correia, A.D., Costa, F.O., Lima, G., Costa, M.H., 2005. Multi-level assessment of chronic toxicity of estuarine sediments with the amphipod *Gammarus locusta* I. Biochemical endpoints. Mar. Environ. Res. 60, 69–91, http://dx.doi.org/10.1016/j.marenvres.2004.08.006.
Neuparth, T., Moreira, S., Santos, M.M., Reis-Henriques, M.A., 2011. Hazardous and noxious substances (HNS) in the marine environment: prioritizing HNS that pose major risk in a European context. Mar. Pollut. Bull. 62, 21–28, http://dx.doi.org/10.1016/j.marpolbul.2010.09.016.
Neuparth, T., Martins, C., de los Santos, C.B., Costa, M.H., Martins, I., Costa, P.M., Santos, M.M., 2014a. Hypocholesterolaemic pharmaceutical simvastatin disrupts reproduction and population growth of the amphipod *Gammarus locusta* at the ng/l range. Aquat. Toxicol. 155, 337–347, http://dx.doi.org/10.1016/j.aquatox.2014.07.009.
Neuparth, T., Capela, R., Pereira, S.P.P., Moreira, S.M., Santos, M.M., Reis-Henriques, M.A., 2014b. Toxicity effects of hazardous and noxious substances (HNS) to marine organisms: acute and chronic toxicity of *p*-xylene to the amphipod *Gammarus locusta*. J. Toxicol. Environ. Health Part A 77, 1210–1221, http://dx.doi.org/10.1080/15287394.2014.921867.
Okumura, Imamura, K., Nishikawa, Y., 1996. Determination of anilines in river water sediment, and fish samples by gas chromatography–mass spectrometry. J. Chromatogr. Sci. 34, 190–198.
Pastorok, R.A., Akçakaya, R., Regan, H., Ferson, S., Bartell, S.M., 2003. Role of ecological modeling in risk assessment. Hum. Ecol. Risk Assess. 9, 939–972, http://dx.doi.org/10.1080/713610017.
Petzoldt, T., Rinke, K., 2007. Simecol: an object-oriented framework for ecological modeling in R. J. Stat. Software 22, 1–31.
Pöckl, M., Humpesch, U., 1990. Intra-and inter-specific variations in egg survival and brood development time for Austrian populations of *Gammarus fossarum* and *G. roeseli* (Crustacea: Amphipoda). Freshwater Biol. 23, 441–455, http://dx.doi.org/10.1111/j1365-2427.1990.tb00286.x.
Preston, B.L., Snell, T.W., 2001. Direct and indirect effects of sublethal toxicant exposure on population dynamics of freshwater rotifers: a modeling approach. Aquat. Toxicol. 52, 87–99, http://dx.doi.org/10.1016/S0166-445X(00)143-0.
Purnell, K., 2009. Are HNS spills more dangerous than oil spills? In: In: Conference Proceedings, Interspill, Marseille, May 12–14.
R Core Team, 2013. R: A Language and Environment for Statistical Computing. Foundation for Statistical Computing, Vienna, Austria.
Rodier, D., Norton, S., 1992. Framework for Ecological Risk Assessment. Technical Report. Environmental Protection Agency, Risk Assessment Forum, Washington, DC (United States).
Sutcliffe, D.W., 2010. Reproduction in *Gammarus* (Crustacea, Amphipoda): basic processes. Freshwater Forum 2, 102–128.
Van den Brink, P.J., Baveco (Hans), J.M., Verboom, J., Heimbach, F., 2007. An individual-based approach to model spatial population dynamics of invertebrates in aquatic ecosystems after pesticide contamination. Environ. Toxicol. Chem. 26, 2226–2236, http://dx.doi.org/10.1897/07-022R.1.
Welton, J., Clarke, R., 1980. Laboratory studies on the reproduction and growth of the amphipod, *Gammarus pulex* (L.). J.Anim. Ecol. 49, 581–592.