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Environ. Sci. Technol., **2008**, 42 (21), 8108-8114 • DOI: 10.1021/es801030t • Publication Date (Web): 23 September 2008

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A Bayesian Mixture Model for Estimating Intergeneration Chronic Toxicity

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Received April 14, 2008. Revised manuscript received August 4, 2008. Accepted August 7, 2008.

Understanding toxic effects on biological populations across generations is crucial for determining the long-term consequences of chemical pollution in aquatic environments. As a consequence, there is considerable demand for suitable statistical methods to analyze complex multigeneration experimental data. We demonstrate the application of a Bayesian mixture model (with random-effects) to assess the effect of intergeneration copper (Cu) exposure on the reproductive output of the copepod, *Tigriopus japonicus*, using experimental data across three generations. The model allowed us to appropriately specify the nonstandard statistical distribution of the data and account for correlations among data points. The approach ensured more robust inferences than standard statistical methods and, because of the model's mechanistic formulation, enabled us to test more subtle hypotheses. We demonstrate intergeneration Cu exposure effects on both components of reproductive output (1) the ovisac maturation rate, and (2) the number of nauplii per ovisac. Current and parent generation Cu exposures negatively affected current generation reproductive output. However, in terms of reproductive output, there was also some evidence for adaptation to parental Cu exposures, but with an associated cost under Cu concentrations different from the parental exposure. Bayesian mixture and random-effects models present a robust framework for analyzing data of this kind and for better understanding chemical toxicity.

Introduction

There has recently been a great deal of interest in applying multigenerational toxicity tests to evaluate the long-term effects of chemical pollutants on the aquatic environment (1–3). Experimental data have suggested that aquatic organisms chronically exposed to even very low levels of a chemical

pollutant can establish resistance to the chemical in terms of enhanced fitness (e.g., 4). However, since the maintenance of resistance requires energy, this can result in lower fitness, particularly in the absence of the pollutant. This negative effect on fitness can be viewed as a cost (5). One important application of multigeneration toxicity experiments is to investigate and quantify the magnitude of both adaptive responses and fitness costs.

Nevertheless, data derived from multigeneration experiments often do not conform to standard statistical distributions, and complex spatio-temporal experimental designs can also lead to non-independence among data points. For instance, many aquatic crustaceans used in ecotoxicological studies (e.g., the amphipod *Hyaella azteca* and the mysid *Holmesimys costata*) are capable of laying multiple discrete broods of eggs over a period of time (1). Longitudinal data collected on reproductive output in these organisms will be characterized by a high frequency of zeros (known as “zero-inflation”), corresponding to periods between discrete breeding events. Further, multiple data points from each reproductive unit can result in correlations among data points. Conventional statistical approaches, such as standard analysis of variance, or life table analysis, may be inappropriate for modeling this kind of data because of a failure to accurately represent the statistical distribution of the data and account for dependencies among data points (6, 7). Therefore, more flexible approaches are required to robustly test hypothesis about the effects of multigeneration toxicity on aquatic organisms using longitudinal experimental data.

This paper presents a Bayesian mixture model (with random-effects) for analyzing multigeneration experimental data (8). The model accounts for zero-inflation in the data and correlations among data points within individuals. The advantages of this approach, compared to standard methods, are more robust inferences and, because of the mechanistic formulation, the ability to test more subtle hypotheses. The model is mechanistic in that it explicitly models the two components of reproductive output: ovisac development, followed by larvae production. We apply the model to experimental data collected by Kwok et al. (2), who recorded reproductive output in the marine harpacticoid copepod *Tigriopus japonicus* for three consecutive captive generations reared under different copper (Cu) exposure levels. *T. japonicus* is found abundantly in the intertidal rocky shores of Hong Kong waters and is subject to anthropogenic stress resulting from, among other causes, high ambient levels of Cu. Kwok and Leung (9) hypothesized that high resistance to Cu toxicity observed in these copepod populations might be due to an adaptation to high ambient Cu concentrations, which in Hong Kong coastal areas may be up to 50 times higher (7–11 $\mu\text{g L}^{-1}$ (10)) than in uncontaminated areas (0.15 $\mu\text{g L}^{-1}$ (11)). Kwok et al.'s (2) multigeneration experiment was designed to test this hypothesis. However, the reproductive output data obtained are highly zero-inflated and thus do not conform to a standard statistical distribution. In addition, the longitudinal experimental design introduces correlations among data points within individuals. The Bayesian mixture model deals with both zero-inflation and correlations among data points, and we applied it to test a range of hypotheses about the effect of current and previous generation Cu exposures on reproductive output. We discuss the implications and consider the benefits of these statistical methods for analyzing multigeneration experimental ecotoxicity data.

Experimental Section

Tigriopus species are used extensively in many fields of research, including ecotoxicology, environmental tolerance,

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and molecular studies (12). Their experimental popularity follows from small size, high fecundity, short generation time, and tolerance to broad ranges of temperature and salinity conditions, as well as well studied genomes (12). A consecutive three-generation data set of survivorship, fecundity, and timing of reproduction of *T. japonicus* was collected by Kwok et al. (2) for separate cohorts reared in the laboratory under different Cu exposure concentrations. Here, we provide a brief summary of their experimental methods and data, but the reader is referred to Kwok et al. (2) for a complete description of the experiment and life table analysis.

Experimental Methods. Kwok et al. (2) applied three treatment exposures to each generation: 0 $\mu\text{g Cu L}^{-1}$ (control); 10 $\mu\text{g Cu L}^{-1}$ concentration (representative of the Cu concentration in contaminated coastal waters); and 100 $\mu\text{g Cu L}^{-1}$ concentration (as an extreme exposure). The initial cohort (henceforth referred to as generation F0) was sourced from a laboratory culture that was originally collected from supra-littoral rock pools in Cape D'Aguilar Marine Reserve in Hong Kong. The Cape D'Aguilar Marine Reserve is a relatively clean site, with ambient waterborne Cu concentrations well below 1 $\mu\text{g Cu L}^{-1}$ (2). Generation F0 consisted of 81 mating pairs (with females at copepodite stage five), randomly chosen from the laboratory culture and separated into three groups of 27 pairs. Pairs within each group were reared under exposure to the three different Cu concentrations for 25 days, which, for conciseness, are denoted by A (= control), B (= 10 $\mu\text{g Cu L}^{-1}$), and C (= 100 $\mu\text{g Cu L}^{-1}$), respectively. Each pair was kept in a separate 50 mL beaker and males were removed once the female had developed the first ovisacs in order to prevent predation of the nauplii (14). Female *T. japonicus* deposit eggs at discrete points in time in ovisacs, with the eggs reaching maturity 2–3 days after an ovisac is deposited (13). Once the eggs in an ovisac have reached maturity, the nauplii (larvae) hatch, all within a few minutes of each other (13). The number of nauplii produced by each female and the number of ovisacs (broods) from which these came were recorded daily and removed.

The same process was then repeated for the next generation (F1) and the subsequent generation (F2). The first brood of nauplii produced by each F0 female was isolated and simultaneously mixed with the first brood of another F0 female (exposed to the same Cu concentration) to avoid inbreeding. Two mating pairs were then randomly selected from each of these samples and pairs from the same F0 treatment were divided into three equal-sized groups. Pairs within each of these groups were then reared under exposure A, B, or C. This was then repeated for the F2 generation, with nauplii sourced from the F1 generation. Throughout the experiment, environmental conditions were held constant and mortality under all treatments was low (<11%, except the F2 generation from lineage AAA, which had 33% mortality). We defined reproductive output as the total number of nauplii produced per female or, equivalently, the number of nauplii produced per female per day. See the Supporting Information for a schematic of the experimental design.

In referring to intergeneration treatments, we subsequently adopt the term “lineage” to describe the sequence of ancestral exposures encountered in the maternal line of a given cohort. For example, in the F1 generation, the lineage AB represents individuals reared under a 10 $\mu\text{g Cu L}^{-1}$ exposure (B), descended from F0 parents reared under the control exposure (A). In the F2 generation, for example, the lineage CAB represents individuals reared under the 10 $\mu\text{g Cu L}^{-1}$ exposure (B), descended from F1 parents reared under the control exposure (A), and F0 grandparents reared under the 100 $\mu\text{g Cu L}^{-1}$ exposure (C). Further, in interpreting these data, we define “resistance (or adaptation)” as delayed positive effects on reproductive output associated with Cu

exposures to previous generations and define “costs” as delayed negative effects on reproductive output associated with Cu exposures to previous generations. These definitions are based on reproductive output as a measure of fitness.

Data Analysis and Bayesian Mixture Model. The experimental data consisted of the daily number of nauplii produced by each female and the number of mature ovisacs from which these arose. Visual inspection of the average number of nauplii produced per female indicates a consistent downward trend in reproductive output as Cu exposure increases (Figure 1a). Further, there is an indication of resistance to Cu, with those copepods in the F1 generation exposed to 100 $\mu\text{g Cu L}^{-1}$ having higher reproductive output if their parents were exposed to 100 $\mu\text{g Cu L}^{-1}$, than if their parents were exposed to lower concentrations. There is also an indication of an associated cost, with copepods in the F1 generation under the control exposure having lower reproductive output if their parents were exposed to 100 $\mu\text{g Cu L}^{-1}$, than if their parents were exposed to lower concentrations. Similar patterns are also evident for the F2 generation.

Each female deposited a maximum of one ovisac per day (K. Kwok, personal observation), so the maximum number of ovisacs maturing per female per day was also one. On any given day, if an ovisac did not mature, then the number of nauplii was zero. However, the number of nauplii could also be zero if an ovisac did mature, but no nauplii hatched from it (i.e., the ovisac aborted). Hence, the reproductive output data (number of nauplii per day) consisted of (1) many zeros, corresponding to days when an ovisac did not mature, or when an ovisac matured, but no nauplii hatched; and (2) a few positive values, corresponding to days when an ovisac matured and nauplii hatched. The standard distribution for modeling count data is the Poisson distribution (15) but, in this case, the production of nauplii in discrete ovisacs clearly leads to a distribution where the observed frequency of zeros is far greater than expected under a standard Poisson distribution (Figure 1b). Also, data points from the same individual are likely to be correlated. Failure to properly account for the zero-inflation and correlations in the data will lead to underestimated variance components and hence elevated type I statistical errors (6, 7).

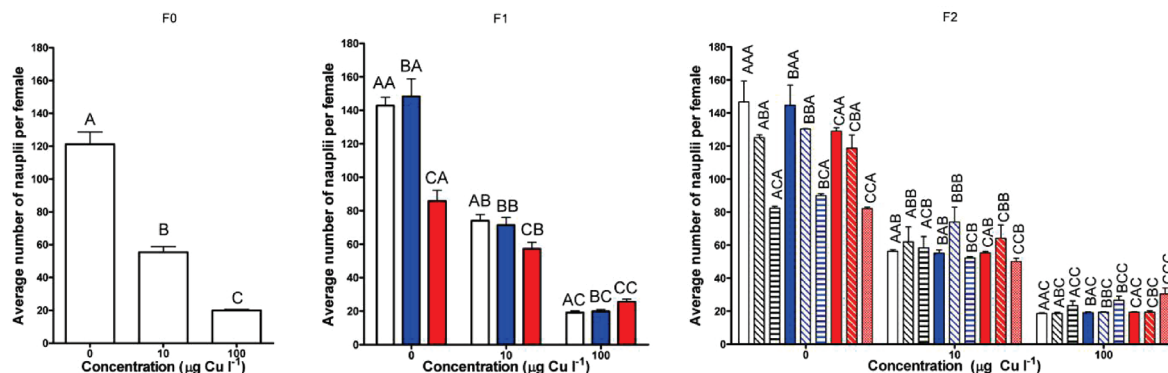
We addressed these issues by modeling reproductive output using a Bayesian zero-inflated Poisson (ZIP) model, with random-effects among individuals (8, 16). By using a ZIP model with random-effects we were able to (1) explicitly model the effect of Cu exposure on both the ovisac maturation rate and the number of nauplii per ovisac; (2), properly account for zero-inflation in the data, leading to more robust statistical inferences (6, 17); and (3) account for correlations among data points within individuals (8, 18). We fitted the models to the data within a Bayesian framework because this provides a more natural specification of parameter uncertainty than frequentist approaches (19, 20).

Based on the ZIP model described by Lambert (16) we modeled reproductive output using a finite mixture distribution (21), such that

$$\Pr(Y = y_{i,t}, Z = z_{i,t} | p_i, \lambda_i) = I(z_{i,t} = 0)(1 - p_i) + I(z_{i,t} = 1)p_i f(y_{i,t} | \lambda_i) \quad (1)$$

where $y_{i,t}$ is the observed number of nauplii produced by female i on day t ; $z_{i,t}$ is the observed number of mature ovisacs for female i on day t (note that the $z_{i,t}$ observations can only take values of 0 or 1); p_i (the mixing probability) is the probability that the observed number of nauplii comes from a Poisson distribution for female i (i.e., the probability that an ovisac reaches maturity on any given day); $f(y_{i,t} | \lambda_i)$ is the probability density function for the Poisson distribution for female i (i.e., the probability density function for the number of nauplii, given an ovisac has reached maturity); λ_i is the shape parameter for the Poisson distribution for female i ;

(a)



(b)

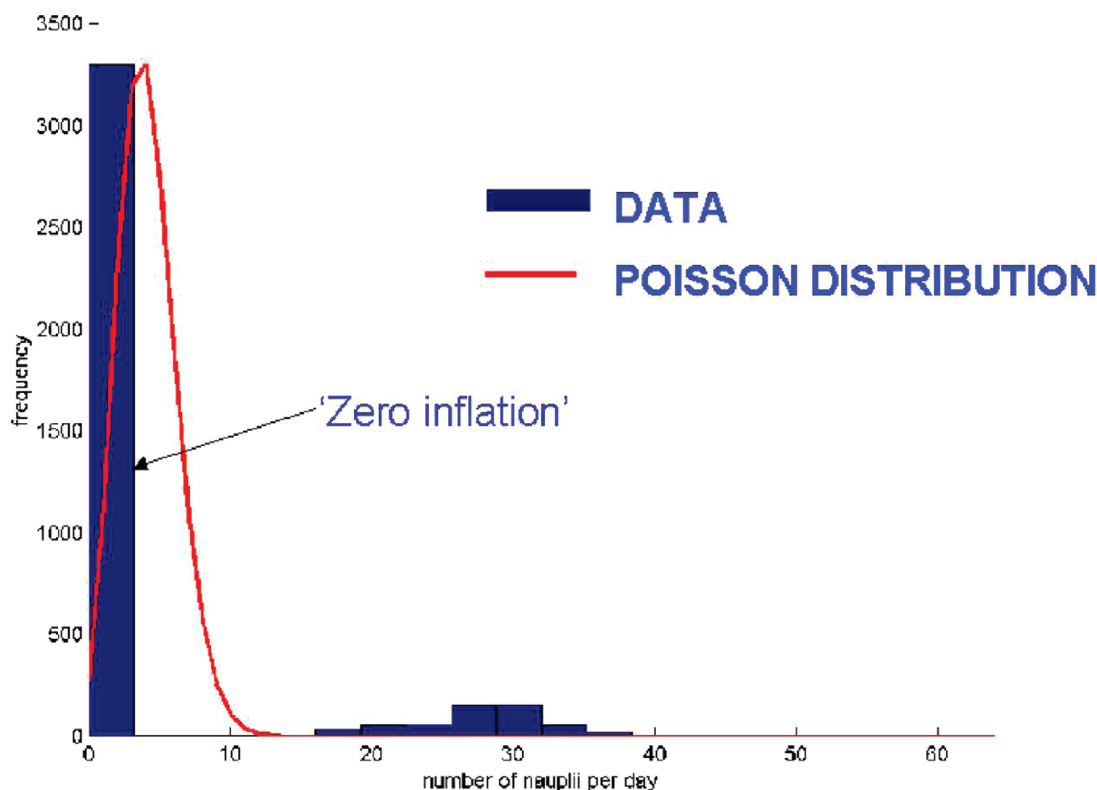


FIGURE 1. The experimental data. (a) Reproductive output (mean + 1 SE) recorded in the 25 day tests for each cohort in successive generations F0, F1, and F2 under exposure to the three Cu treatment concentrations of A ($0 \mu\text{g Cu L}^{-1}$), B ($10 \mu\text{g Cu L}^{-1}$), and C ($100 \mu\text{g Cu L}^{-1}$). Lineages are shown above each bar. (b) Frequency plot of the number of nauplii produced per day per female taken across all generations and exposures. The maximum likelihood estimate standard Poisson distribution is superimposed on the graph (red line). Part a reprinted with permission from ref 2, 2008, Elsevier.

and the indicator function $I(x) = 1$ if x is true and $I(x) = 0$ otherwise. Here, p_i is interpreted as the expected daily probability that an ovisac from female i reaches maturity (the ovisac maturation rate), while λ_i is interpreted as the expected number of nauplii produced per ovisac by female i . The expected number of nauplii produced per day by female i (i.e., female i 's expected reproductive output) is $E(Y_i) = p_i \lambda_i$. The model specified in eq 1 assumes that the reproductive output on each day arises from either a Poisson distribution, with probability p_i , or a distribution consisting of point mass at zero, with probability $1 - p_i$. See the Supporting Informa-

tion for details relating to the calculation of the likelihood function.

We modeled the parameters p_i and λ_i via their logit and log canonical link functions (15), respectively, so that

$$\log\left(\frac{p_i}{1-p_i}\right) = \alpha' \mathbf{W}_i + a_i \quad (2)$$

where α is a vector of regression coefficients; \mathbf{W}_i is a vector of explanatory variables for female i ; and a_i is a random-effect for female i , with $a_i \sim \text{Normal}(0, \tau_a)$, and

$$\log(\lambda_i) = \beta'X_i + b_i \quad (3)$$

where β is a vector of regression coefficients; X_i is a vector of explanatory variables for female i ; and b_i is a random-effect for female i , with $b_i \sim \text{Normal}(0, \tau_b)$. The random-effects accounted for correlations among data points collected from the same female (8, 18). The explanatory variables (W_i and X_i) specified the Cu exposure patterns of each lineage as row vectors of dichotomous variables (0s and 1s). Finally, because we had no prior information, we adopted standard noninformative prior distributions (e.g., 17) for the parameters

$$\begin{aligned} \alpha_k &\sim \text{Normal}(0, 0.001) \\ \beta_k &\sim \text{Normal}(0, 0.001) \\ \tau_a &\sim \text{Gamma}(0.1, 0.1) \\ \tau_b &\sim \text{Gamma}(0.1, 0.1) \end{aligned} \quad (4)$$

where α_k is component k of α and β_k is component k of β . The intercept parameters α_0 and β_0 were assumed to be different for each generation in order to account for any differences in reproductive output among generations that were unrelated to Cu exposure.

If no Cu resistance develops as a consequence of previous generations' exposures, then the effects of current and previous generations' Cu exposures on the current generation's reproductive output will be independent; we call this a "lineage independent" model. In this case, the overall intergeneration effect of Cu exposure on the current generation's reproductive output (on the link function scale) can be expressed as the sum of "main" effects corresponding to each generation's exposure. Here, a main effect is the independent effect of each generation's Cu exposure on the current generation's reproductive output, when all other generations' exposures are control. Conversely, if Cu resistance does develop as a consequence of previous generations' exposures, then the effects of current and previous generations' Cu exposures on the current generation's reproductive output will depend on each other; we call this a "lineage dependent" model. In this case, the overall intergeneration effect of Cu exposure on the current generation's reproductive output (on the link function scale) can be expressed as the sum of main effects and "interaction" effects between each generation's exposure. The interaction effects quantify how much each generation's Cu exposure effect depends on the exposures to other generations. In eqs (2) and (3), main and interaction effects can be specified for either the ovisac maturation rate (p_i), or the number of nauplii per ovisac (λ_i), or both. We measured all effects relative to the control exposure by arbitrarily setting the main effects for the control to zero. We also assumed that, for the F0 generation, parents and grandparents, and for the F1 generation, grandparents, were exposed to the control condition. See the Supporting Information for further information on the specification of the main and interaction effects.

We constructed a range of alternative models representing different hypotheses about the effect of current and previous generations' Cu exposures on the current generation's reproductive output. The first model (the "null" model) assumed that Cu exposure has no impact on reproductive output. The other models, however, all assumed that Cu has an effect on reproductive output. For these models, the overall intergeneration effect of Cu exposure was assumed to depend either (1) only on the current generation's exposure; (2) only on the current and parent generations' exposures and lineage independent; (3) only on the current and parent generations' exposures and lineage dependent; (4) on the current, parent and grandparent generations' exposures and lineage independent; or (5) on the current, parent and grandparent

TABLE 1. Model Rankings, Deviance Information Criteria (DIC), and Δ DICs (Difference between Each Model's DIC and the Model with the Lowest DIC)^a

model	DIC	Δ DIC
LI, current, parent (p, λ)	6544.77	0.00
LD, current, parent (p, λ)	6548.78	4.01
LI, current, parent, grandparent (p, λ)	6552.29	7.52
LI, current (p, λ)	6557.31	12.54
LD, current, parent, grandparent (p, λ)	6617.07	72.30
LI, current, parent (λ)	6699.94	155.17
LD, current, parent (λ)	6704.20	159.43
LI, current, parent, grandparent (λ)	6705.06	160.29
LI, current (λ)	6714.73	169.96
LD, current, parent, grandparent (λ)	6739.14	194.37
LI, current (p)	6817.74	272.97
LI, current, parent (p)	6819.49	274.72
LD, current, parent (p)	6820.59	275.83
LI, current, parent, grandparent (p)	6822.06	277.29
LD, current, parent, grandparent (p)	6855.50	310.73
null	6975.43	430.66

^a LI = lineage independent, LD = lineage dependent, current = current generation effect, parent = parent generation effect, and grandparent = grandparent generation effect (p = effect on ovisac maturation rate, λ = effect on number of nauplii per ovisac). Bold text indicates those models with support from the data, relative to the other models.

generations' exposures and lineage dependent. The lineage dependent models assume there is no development of Cu resistance in response to previous generations' exposures, whereas the lineage dependent models assume that the development of Cu resistance in response to previous generations' exposures is possible. For each of the five possibilities above, Cu exposures could affect either the ovisac maturation rate (p_i), or the number of nauplii per ovisac (λ_i), or both. Therefore, we had a total of 16 alternative models (Table 1). These represented a range of alternative hypotheses about the effect of Cu exposure on the reproductive output of *T. japonicus*.

Prior to fitting the models we removed the first eight days of data from each experiment because this was the minimum amount of time required for mating and subsequent development of the first ovisac (K. Kwok, personal observation). We used Markov chain Monte Carlo (MCMC), in WinBUGS version 1.4.1 (<http://www.mrc-bsu.cam.ac.uk/bugs/>) to fit each model to the data and estimate parameter posterior distributions (see the Supporting Information for example WinBUGS code). Convergence was assessed using the Gelman and Rubin convergence statistic (22), calculated with the package "coda" in R version 2.4.1 (<http://www.r-project.org/>). For each model, we ran three MCMC sequences for 50 000 iterations per sequence (after discarding a burn-in of 20 000 iterations per sequence) to estimate the parameter posterior distributions. We also calculated the deviance information criterion (DIC, see the Supporting Information for details related to the calculating of DIC) for each model (23). DIC is a Bayesian information criterion for model selection consisting of a component for model fit and a penalty for the effective number of parameters. If one model has a lower DIC than another model, it is considered more parsimonious than the other model. DIC can be used in a similar way to Akaike's information criteria (AIC) (24, 25) for ranking alternative models in terms of their support from the data and is particularly useful for hierarchical models (23).

Results and Discussion

The most parsimonious ("best") model (i.e., model with the lowest DIC) was the lineage independent model containing

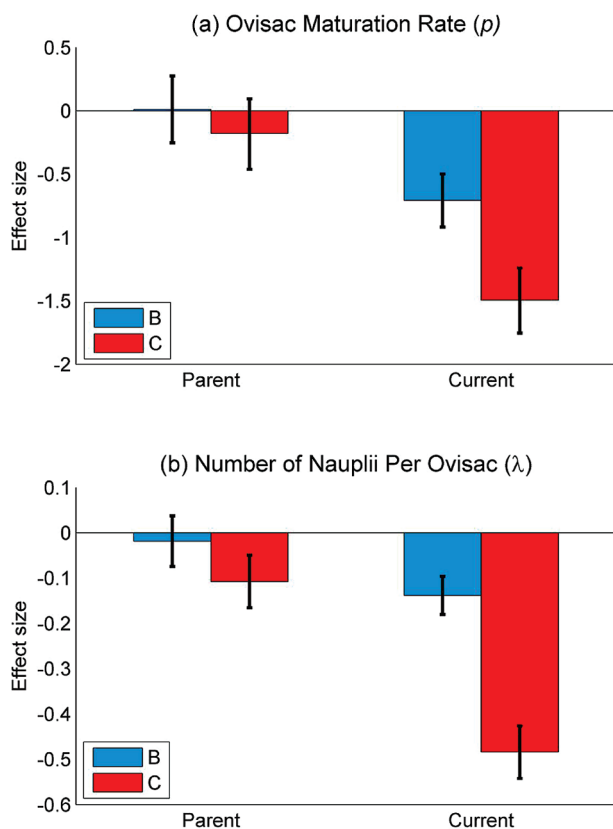


FIGURE 2. Expected posterior current generation and parental Cu exposure main effect sizes and 95% credible intervals for the supported model that was lineage independent (best model) for: (a) the ovisac maturation rate (p); and (b) the number of nauplii per ovisac (λ). Effects are shown relative to the effect of control (A) exposures to both current and parent generations (i.e., lineage AA). Control = 0 $\mu\text{g Cu L}^{-1}$, B = 10 $\mu\text{g Cu L}^{-1}$ treatment (blue bars), and C = 100 $\mu\text{g Cu L}^{-1}$ treatment (red bars).

current and parent generation Cu exposure effects on both the ovisac maturation rate (p) and the number of nauplii per ovisac (λ) (Table 1). Burnham and Anderson (25) suggest (for AIC) that models within seven AIC units of the best model are supported. Spiegelhalter et al. (23) indicate that a similar rule-of-thumb can be applied to DIC. Therefore, the lineage dependent model containing current and parent generation Cu exposure effects on both the ovisac maturation rate and the number of nauplii per ovisac was also supported (Table 1, $\Delta\text{DIC} = 4.01$). All other models had very little support relative to the best model. This indicates limited support for models that included either only a current generation effect, or a grandparent effect. An important additional result was that models that assumed Cu exposure effects either only on the ovisac maturation rate, or only on the number of nauplii per ovisac had substantially less support than those models that assumed a Cu effect on both (Table 1). This suggests that neither component alone can satisfactorily explain the data.

Parameter estimates for the supported model that was lineage independent (best model), indicated that exposure of the current generation to Cu reduced both the ovisac maturation rate (p) and the number of nauplii per ovisac (λ) (Figure 2). Parental exposures to Cu also tended to reduce ovisac maturation rates and the number of nauplii per ovisac in the current generation, although these effects were much weaker than the current generation effects (Figure 2). Further, the 95% credible intervals for the parental effects generally overlapped zero, indicating some uncertainty in their sign. These results show that reduced reproductive output is

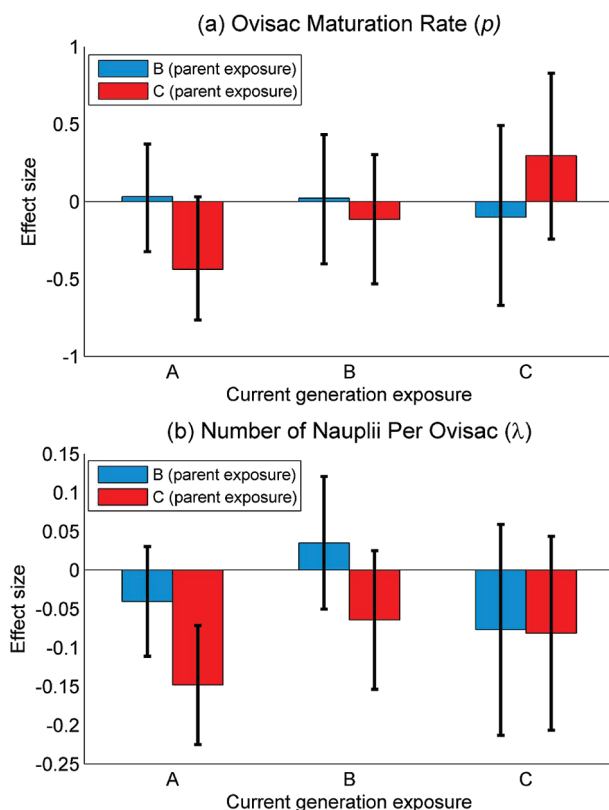


FIGURE 3. Relative expected posterior effect sizes and 95% credible intervals for the supported model that was lineage dependent (second best model) containing current and parent Cu effects on: (a) the ovisac maturation rate (p); and (b) the number of nauplii per ovisac (λ). Effect sizes are shown as differences relative to the current generation's exposure effect, with parents under the control (A) exposure (i.e., lineages BX and CX are shown relative to AX, where X denotes the current generation's exposure). Control = 0 $\mu\text{g Cu L}^{-1}$, B = 10 $\mu\text{g Cu L}^{-1}$ exposure, and C = 100 $\mu\text{g Cu L}^{-1}$ exposure.

associated with current generation exposures to Cu, but also some evidence for reduced reproductive output associated with parental exposures to Cu.

Parameter estimates for the supported model that was lineage dependent (second best model) indicated some evidence for the development of resistance to Cu in response to parental exposures. For this model, parental effects were dependent on the current generation's exposure to Cu (Figure 3). When the current generation was not exposed to Cu, parental effects were negative, as for the lineage independent model. When the current generation was exposed to low Cu concentrations (10 $\mu\text{g Cu L}^{-1}$), parental effects were less negative than when not exposed to Cu. In fact, when both parent and current generations were exposed to low Cu concentrations, the parental effect was actually positive for the number of nauplii per ovisac (λ). When the current generation was exposed to high Cu concentrations (100 $\mu\text{g Cu L}^{-1}$), parental effects were also less negative than when not exposed to Cu. Once again, there was a positive parental effect when both parent and current generations were exposed to high Cu concentrations, but this time for the ovisac maturation rate (p). The positive parental effects indicate some evidence for the development of resistance to Cu when copepods are exposed to the same Cu concentration as their parents. This pattern is also apparent in the expected number of nauplii per day (Figure 4). Whether resistance occurs though changes in the ovisac maturation rate, or the number of nauplii per ovisac seems to depend on the Cu concentration (Figure 3). The observed negative parental

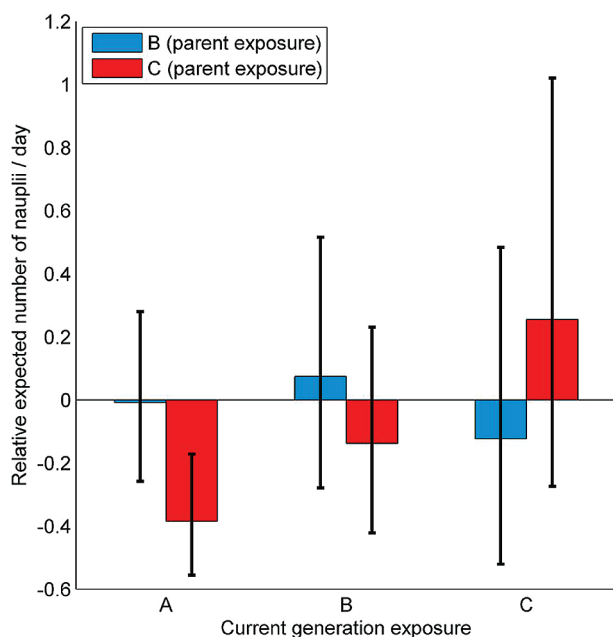


FIGURE 4. Relative expected posterior numbers of nauplii per day and 95% credible intervals for the supported model that was lineage dependent (second best model) containing current and parent Cu effects on the ovisac maturation rate (p), and the number of nauplii per ovisac (λ). Expected numbers of nauplii day⁻¹ are shown as proportional changes relative to the expected number of nauplii per day for the current generation's exposure, with parents under the control (A) exposure (i.e., lineages BX and CX are shown relative to AX, where X denotes the current generation's exposure). Control = 0 $\mu\text{g Cu L}^{-1}$, B = 10 $\mu\text{g Cu L}^{-1}$ exposure, and C = 100 $\mu\text{g Cu L}^{-1}$ exposure.

effects, when copepods were exposed to Cu concentrations different from their parents, suggests a fitness cost that may be associated with the development of resistance (Figure 4). However, most 95% credible intervals overlap zero, indicating some uncertainty in the true sign of many of the effects (Figures 3 and 4).

The theoretical basis underlying fitness centers on physiological mechanisms that are dependent on energy and resource allocation. These imply that maintenance costs for dealing with environmental stress must have associated trade-offs in terms of fitness (5, 26, 27). Evidence from laboratory selection experiments have demonstrated that such fitness costs can be associated with adaptation in many stress-adapted populations (28–30). For *T. japonicus* we found a positive effect on reproductive output if the previous generation was exposed to the same Cu concentration, but a negative effect if the previous generation was exposed to a different Cu concentration. This suggests adaption to the previous generation's exposure, but with an associated cost if exposed to Cu concentrations different from the previous generation. Such adaptations may be caused by genetic selection or physiological adaptation. Given the speed and plasticity of the response, it is thought most likely to be a physiological adaptation, rather than the result of genetic selection. Maternal transfer, where information about the environment is transferred to offspring via cytoplasmic factors, such as enzymes, has been suggested for fish (31, 32) and *Daphnia magna* (33) and is a possible mechanism explaining the patterns we observed in *T. japonicus*. A full understanding of the genetic and physiological mechanisms involved is beyond the scope of this paper, but is an important area for future research.

Our results compare favorably with those of Kwok et al. (2). They show that parental Cu exposures have a significant effect on reproductive output and intrinsic population growth

rates. Kwok et al. (2) sum the nauplii counts within individuals and rely on the central limit theorem to achieve normally distributed data for analysis with a generalized linear model. However, with the data aggregated in this way, it is not possible to partition reproduction output into ovisac maturation rate and numbers of nauplii per ovisac and the ability to model variability through time is also lost. Using a Bayesian mixture model we were able to examine the effects of Cu toxicity on both the ovisac maturation rate and the number of nauplii per ovisac, rather than just overall reproductive output. A consequence is that we were able to identify that, at low Cu concentrations, resistance seems to occur via a change in the number of nauplii per ovisac, whereas at high Cu concentrations, resistance seems to occur via a change in the ovisac maturation rate (Figure 3). These may represent different reproductive strategies arising from pollutant mediated energetic trade-offs and constraints governed by exposure history. Similar trade-offs may be apparent for other species and pollutants. For example, Coors et al. (34) show that daphnids exposed to a xenobiotic produced smaller, but more offspring. It is also possible with our approach to model variation in reproductive output through time (i.e., the timing of reproductive output). This would require the introduction of time-dependent covariates and possibly specification of the temporal covariance structure, but this is readily achievable within our framework. Therefore, compared to standard approaches, the method presented in this paper allows more subtle hypotheses to be tested, which will ultimately lead to better understanding of the long-term effects of exposure to pollutants.

Statistical models based on mixture distributions will be particularly useful in situations where the recorded measure of fitness, or reproductive output, arises from two or more statistical distributions. In the *T. japonicus* data, this occurs because, on a given day, if no ovisacs reach maturity, the data come from one distribution (point mass at zero), while if an ovisac does reach maturity, the data come from another distribution (Poisson). This situation will tend to occur in longitudinal ecotoxicity studies where the study organisms exhibit reproduction in discrete events, such as *D. magna* (35). Many organisms that are used to examine chemical toxicity effects in aquatic systems are characterized by such discrete reproductive events and so the model has broad applicability. Bayesian mixture models also provide a general framework for analyzing data that arise from multiple statistical distributions and are particularly useful when we cannot directly observe which distribution each data point arises from (21). This class of model, therefore, provides a flexible and more mechanistic framework for the analysis of multigeneration toxicity experiments. Importantly, with MCMC software, such as WinBUGS (<http://www.mrc-bsu.cam.ac.uk/bugs/>), now freely available, the routine fitting of these types of models to experimental data is feasible.

Acknowledgments

J.R.R. was supported by a CSIRO Postdoctoral Fellowship and a UQ Postdoctoral Fellowship, and K.W.H.K. was partly supported by the Area of Excellence Scheme under the University Grants Committee of the Hong Kong AR Government (project no. AoE/P-04/2004).

Supporting Information Available

Additional information is available relating to a schematic diagram of the experimental design, calculation of the likelihood function, specification of the main and interaction effects, sample WinBUGS code, and calculation of DIC. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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ES801030T