From patterns to processes and back: analysing density-dependent responses to an abiotic stressor by statistical and mechanistic modelling

S. Jannicke Moe^{1,2}, Anja B. Kristoffersen³, Robert H. Smith⁴
and Nils Chr. Stenseth^{2,*}

¹Norwegian Institute for Water Research (NIVA), PO Box 173, Kjelsås, 0411 Oslo, Norway

²Department of Biology, Centre for Ecological and Evolutionary Synthesis (CEES), University of Oslo,
PO Box 1050, 0316 Oslo, Norway

³Department of Informatics, University of Oslo, PO Box 1080, 0316 Oslo, Norway

⁴Department of Biology, University of Leicester, University Road, Leicester LE1 7RH, UK

Our knowledge about population-level effects of abiotic stressors is limited, largely due to lack of appropriate time-series data. To analyse interactions between an abiotic stressor and density-dependent processes, we used experimental time-series data for stage-structured populations (the blowfly *Lucilia sericata*) exposed to the toxicant cadmium through 20 generations. Resource limitation results in competition both in the larval and the adult stages. The toxicant has only negative effects at the organism level, but nevertheless, there were positive population-level effects. These are necessarily indirect, and indicate overcompensatory density-dependent responses. A non-parametric model (generalized additive model) was used to investigate the density-dependent structures of the demographic rates, without making assumptions about the functional forms. The estimated structures were used to develop a parametric model, with which we analysed effects of the toxicant on density-dependent and density-independent components of the stage-specific demographic rates. The parameter estimates identified both synergistic and antagonistic density–toxicant interactions. It is noteworthy that the synergistic interaction occurred together with a net positive effect of the toxicant. Hence, the effects of such interactions should be considered together with the capacity for compensatory responses. The combination of the two modelling approaches provided new insight into mechanisms for compensatory responses to abiotic stressors.

Keywords: non-parametric modelling; generalized additive modelling; time-series analysis;
stage-specific density dependence; density–toxicant interaction

1. INTRODUCTION

Factors that regulate an animal population are typically classified as density-dependent or density-independent (Turchin 1995). Abiotic factors are often regarded as density-independent, but may nevertheless interact with density-dependent processes; this may alter the effects of the abiotic factor at the population level compared to the direct effects at the organism level. A classical example is the overcompensatory responses displayed by ‘Nicholson’s blowflies’ (Nicholson 1954a,b), where a density-independent increase in mortality rates resulted in reduced competition, and ultimately in increased population sizes. Hence, in order to understand the possible effects of abiotic factors on population dynamics, an appropriate description of the density-dependent structures under various levels of the abiotic factors is needed (Grant 1998; Sibly *et al.* 2000).

Population models developed in order to investigate density dependence are normally formulated as mechanistic models (i.e. with specified parametric functions representing the density-dependent mechanisms). This approach has many strengths: the mechanistic factors are explicitly formulated, which allows predictions to be

derived and tested; information from other sources may also be utilized; the same underlying dynamical processes may be recognized in different ecological systems (Kendall *et al.* 1999). A main disadvantage with this mechanistic approach, however, is that it requires *a priori* assumptions about the functional forms and therefore solid knowledge of the ecological system. An alternative is to develop non-parametric models, where the density-dependent functions are not specified *a priori*, but estimated from the data using a minimum of assumptions about functional forms. This approach is particularly useful for exploring nonlinearities in time-series data, and has lately been applied for studying density dependencies for a variety of ecological systems (Bjørnstad *et al.* 1998; Ellner *et al.* 1998; Stenseth *et al.* 1998; Wood 2001; Murua *et al.* 2003; Nelson *et al.* 2004).

In this study, we combine non-parametric and parametric modelling, to analyse experimental time-series data on blowfly populations exposed to an abiotic toxicant through 20 generations (Daniels 1994; Smith *et al.* 2000; Lingjærde *et al.* 2001). Although this stressor has only negative effects at the organism-level, there are nevertheless positive population-level responses (increased total biomass), which indicate density-dependent compensatory responses. A non-parametric model is first used to explore time-series data with respect to the

* Author for correspondence (n.c.stenseth@bio.uio.no).

density-dependent structures in the demographic rates. Next, a parametric (mechanistic) model based on the non-parametric functions will be used to analyse interactions between the abiotic stressor and the density-dependent components of the demographic rates. The aim of the study is (i) to identify density–toxicant interactions and to understand how these are related to density-dependent compensatory responses in our study system and (ii) to assess the usefulness of the two modelling approaches for studying such interactions and compensatory responses to abiotic stressors in animal populations.

2. THE DATA

The data (Daniels 1994) used for our analysis are stage-specific counts of 12 laboratory populations of the blowfly *Lucilia sericata* Meigen (1826). The experimental design and protocol are based on an experiment by Nicholson (1954a,b), and described in more detail by Smith *et al.* (2000). The larvae were fed with a blood-based diet in limited amounts, whereas adults were fed with sugar and water *ad libitum*. The blood-based diet was also ingested by adult flies, which need proteins for reproduction, but not for survival. The populations were divided into two treatment groups (six replicates per group), hereafter termed toxicant and control. In the toxicant group, cadmium acetate was added to the diet, and ingested by both larvae and adults. The diet was replenished every two days. The following state variables were recorded every two days for 760 days: number of larvae, number of new pupae (defining a pupal cohort), mean individual weight per pupal cohort and number of adults. In addition, the number of viable pupae (i.e. successfully emerged adults) per cohort was recorded until 20 days after pupation. The mean densities and pupal weight in the two treatment groups are given in table 1. The control populations displayed more or less regular cycles in adult densities (periodicity corresponding to two generations), whereas the toxicant populations had more irregular fluctuations. Larval densities were highly variable in both treatment groups.

3. THE MODEL

(a) Stage structure

The stage structure of the model is based on three life-history stages (L, larvae; P, pupae; A, adults). The larval stage is assumed to last for 8 days and the pupal stage for 10 days. The time-step (t) is 2 days (equal to the census interval). Each stage is divided into 2-day age classes (represented by the superscript of the variable). The assumed stage structure is based on our previous work (Lingjærde *et al.* 2001). Certain assumptions about age structure are modified based on new information from three cohort experiments (Moe *et al.* 2001, 2002a,b), which were designed to test the assumptions and predictions from Lingjærde *et al.*'s (2001) model. Moreover, here we extended the model to include data on pupal weight as both a response and a predictor variable. The estimated demographic rates are shown in table 2a.

It is assumed that larval density (L) may affect both the pupal weight (W) and larval survival. Pupal weight may furthermore affect pupal survival. Adult density (A) may affect adult survival and reproductive rate. The survival of the youngest larvae is assumed to depend on the total larval density (equation (3.1a)). Survival of older larvae is

assumed to be density-independent (equations (3.1b) and (3.1c)). Weight at pupation (equation (3.1d)) is assumed to be determined by the mean larval density experienced by an individual two time-steps before pupation (L_{t-1}). All mortality during the pupal stage (equation (3.1e)) is accounted for in the function for survival from pupa to adult (equation (3.1f)), which is assumed to depend on pupal weight. Another version of the model included also the effects of pupal weight (as a proxy for adult weight) on adult survival and reproduction, but no significant effects were found (see table 4 and figure 5 in Electronic Appendix).

(b) Non-parametric model formulation

The non-parametric model (equation (3.1a–i)) is formulated with unspecified functions $f(\cdot)$ for density- and weight-dependent relationships, and constants $\exp(\alpha_i)$ representing the mean value of the response.

$$L_{t+1}^2 = L_t^1 \exp[\alpha_{L12} + f_{LLL}(\log L_t)], \quad (3.1a)$$

$$L_{t+1}^{i+1} = L_t^i \exp[\alpha_{L24}], \quad \text{for } i = 2, 3, \quad (3.1b)$$

$$P_{t+1}^1 = L_t^4 \exp[\alpha_{LP}], \quad (3.1c)$$

$$W_{t+1} = \alpha_W + f_{LW}(\log L_{t-1}), \quad (3.1d)$$

$$P_{t+4}^5 = P_{t+3}^4 = \dots = P_t^1, \quad (3.1e)$$

$$A_{t+1}^{1,1} = P_t^5 \exp[\alpha_{PA} + f_{WPA}(W_{t-4})], \quad (3.1f)$$

$$A_{t+1}^{M,1} = A_t^{1,2} = A_{t-1}^{1,1}, \quad (3.1g)$$

$$A_{t+1}^{M,i+1} = A_t^{M,i} \exp[\alpha_{AA} + f_{AAA}(\log A_t)], \quad (3.1h)$$

for $i = 1, 2, \dots$,

$$L_{t+1}^1 = A_t^M \exp[\alpha_{AL} + f_{AAL}(\log A_t^M)]. \quad (3.1i)$$

The density- and weight-dependent relationships are defined as follows: f_{LLL} , effect of larval density on larval survival; f_{LW} , effect of larval density on pupal weight; f_{WPA} , effect of pupal weight on pupal-to-adult survival; f_{AAA} , effect of adult density on adult survival; f_{AAL} , effect of mature adult density on reproductive rate. The constant rates are defined as: $\exp(\alpha_{L12})$, mean larval survival for age class 1–2; $\exp(\alpha_{L24})$, mean larval survival for age class 2–4; $\exp(\alpha_{LP})$, mean larva-to-pupa survival; α_W , mean pupal weight; $\exp(\alpha_{PA})$, mean pupa-to-adult survival; $\exp(\alpha_{AA})$, mean adult survival; $\exp(\alpha_{AL})$, mean reproductive rate.

Before estimation, some of the equations are combined so that only observed state variables are used as input for estimation (Electronic Appendix equations (A 1)–(A 5)). Stochasticity terms are added to the equations after log-transformation (Electronic Appendix equations (A 6)–(A 15)), representing environmental stochasticity. The unspecified functions are then estimated with non-parametric spline functions by generalized additive modelling (GAM; see Hastie & Tibshirani 1990; Electronic Appendix equation (A 16)). The estimated functions are shown in figure 2.

(c) Selection of density-dependent and weight-dependent functions

Building upon the estimates from the non-parametric analysis, we proceed to derive a parametric model, with specified density-dependent and weight-dependent functions. The objectives are to replace the non-parametric functions with simple parametric functions with parameters that can represent a biological mechanism, and that will provide ecologically interpretable results (e.g. survival rates

Table 1. Characteristics of real data (average for 6 control and 6 toxicant populations).

(Coefficient of variation (CV)=variance/mean; mean cycle period (T) for the significant cycles, determined by autocorrelation analysis; ACF (the value of the autocorrelation function) for the period T ; and proportion of series with significant autocorrelation (i.e. significant peak and ACF value larger than the 95% CI).)

	control	toxicant
<i>larvae</i>		
mean	368	174
CV	0.66	0.75
ACF	0.09	0.00
T (days)	70	—
proportion	0.50	0.00
<i>pupae</i>		
mean	106	103
CV	0.40	0.40
ACF	0.10	0.09
T (days)	66	73
proportion	0.50	0.33
<i>pupal weight</i>		
mean	17.9	19.7
CV	0.23	0.25
ACF	0.01	0.07
T (days)	—	65
proportion	0.00	0.33
<i>adults</i>		
mean	140	98
CV	0.41	0.44
ACF	0.18	0.12
T (days)	72	102
proportion	0.83	0.50

must lie between 0 and 1) for a wide range of parameter values. For each of the unspecified functions, we fit a set of parametric functions and assess the goodness-of-fit relative to the non-parametric spline function. All equations in the non-parametric model except equation (3.1*d*) were log-transformed before estimation, to obtain an additive relationship between the predictor variables. The goodness-of-fit of each of the different functions is assessed by the cross-validated sum of squares (Hastie & Tibshirani 1990; Bowman & Azzalini 1997; Electronic Appendix). The resulting parametric model is given in equation (4.1*a-i*).

4. RESULTS

(a) Density-dependent and weight-dependent structures

The model-selection procedure provided similar functional responses for the control group and the toxicant group (see figure 5 in Electronic Appendix). We could therefore apply identical parametric functions for both treatment groups in the parametric model (equation (4.1*a-i*)). The parameter estimates are given in table 2*b*.

For the larval survival rate, the non-parametric fit (equation (3.1*a*)) provided a significantly nonlinear function (figure 2*a,b*). The humped shape of the survival function suggests that the individuals benefit from aggregation in low densities, and suffer from competition in high densities. In the parametric model (equation (4.1*a*)), we used a function defined by a density-dependent 'facilitation factor', $(L)^{C_{LL1}}$, and a density-dependent 'competition factor', $\exp[C_{LL2} \cdot L]$.

This function is based on Aviles (1999). The positive facilitation factor will make the survival function increase with density in low densities, whereas the negative competition factor makes it decrease in high densities. A constant (C_{L120}) represents the density-independent component of survival for age group 1, while another constant (C_{L240}) represents the density-independent survival from both age groups 2–3 and 3–4. The fit of this parametric function seems to correspond well with the non-parametric fit (figure 2*a,b*): survival increases with larval density up to an intermediate density, then decreases with density.

The pupal weight function (equation (3.1*d*)) was selected as a linear function of log-transformed larval density (equation (4.1*d*); figure 2*c,d*), which was reasonable within the range of observed densities. The parametric version of the function consists of an intercept (C_{W0}) and a slope (C_{W1}); the intercept represents the maximum potential pupal weight, and the negative slope describes the effect of larval competition on larval growth and resulting pupal weight.

Pupa-to-adult survival (equation (3.1*f*)) was estimated to increase with pupal weight, approaching a threshold for low and high weights (a sigmoid curve; figure 3*e,f*). We applied a function based on the logistic regression model (McCullagh & Nelder 1989), where the logit-transformed survival rate is a linear function of pupal weight: $\log(p/(1-p)) = C_{PA0} + C_{PA1} W = \eta$, where η is the linear link function. Hence, the expression for the survival rate is $p = \exp(\eta)/(1 + \exp(\eta))$. The intercept (C_{PA0}) of the link function is estimated to be negative, which implies that survival rate is low for the lowest values of pupal weight. The positive slope (C_{PA1}) means that pupal weight has a positive effect on survival. The predicted survival rate is well within the confidence interval of the parametric function (figure 3*e,f*).

There was no significant effect of adult density on adult survival (equation (3.1*h*), f_{AAA} ; figure 5 in Electronic Appendix). The reproductive rate (equation (3.1*i*)) was estimated to be a linearly decreasing function of adult density on log scale (f_{AAL} ; figure 2*g,h*). This negative effect of adult density is therefore included as $(A_t)^{C_{AL1}}$ on natural scale in the model. A constant (C_{AL0}) represents the reproductive potential (equation (4.1*i*); figure 2*g,h*). The parametric model overestimates the reproductive rate at low adult densities, otherwise there is a close fit with the non-parametric estimates.

The resulting parametric version of the model is given by equations (4.1*a-i*)

$$L_{t+1}^2 = L_t^1 C_{L120} (L_t)^{C_{LL1}} \exp[C_{LL2} \cdot L_t], \quad (4.1a)$$

$$L_{t+1}^{i+1} = L_t^i C_{L240}, \quad \text{for } i = 2, 3, \quad (4.1b)$$

$$P_{t+1}^1 = L_t^4 C_{LP0}, \quad (4.1c)$$

$$P_{t+4}^5 = P_{t+3}^4 = \dots = P_t^1, \quad (4.1d)$$

$$W_{t+1} = C_{W0} + C_{W1} \log(L_{t-1}), \quad (4.1e)$$

$$A_{t+1}^{1,1} = P_t^5 \exp[C_{PA0} + C_{PA1} W_{t-4}] / (1 + \exp[C_{PA0} + C_{PA1} W_{t-4}]), \quad (4.1f)$$

$$A_{t+1}^{M,1} = A_t^{I,2} = A_{t-1}^{I,1}, \quad (4.1g)$$

$$A_{t+1}^{M,i+1} = A_t^{M,i} C_{AA0}, \quad \text{for } i = 1, 2, \dots, \quad (4.1h)$$

$$L_{t+1}^1 = A_t^M C_{AL0} (A_t^M)^{C_{AL1}}. \quad (4.1i)$$

Table 2. Model estimates for control and toxicant populations: (a) mean demographic rates and weight (common for both the non-parametric and the parametric model) and (b) parameters in weight- and density-dependent functions from the parametric model.

(All replicates per treatment are combined. Ratio T/C is the ratio of mean response values for toxicant group and the control group. Bold values indicate significant difference between control and toxicant rates (non-overlapping 95% confidence intervals (CI)). Confidence intervals are estimated by bootstrapping (1000 runs). A stochastic term $\varepsilon \sim N(0, \sigma)$ is added for each function (equations (A 12)–(A 15) in Electronic Appendix) where σ = standard deviation of the residuals from the parameter estimation of this equation. The standard deviations of these residuals are (for control and toxicant data, respectively): equation (4.1e) (estimated by equation (A 12)): 3.74 and 4.52; equation (4.1f) (estimated by equation (A 13)): 0.580 and 0.693; equation (4.1h) (estimated by equation (A 14)): 0.192 and 0.261; equations (4.1i) + (4.1a) + (4.1b) + (4.1c) (estimated by equation (A 15)): 0.882 and 0.967.)

(a)

			control		toxicant		ratio
equations	response		mean	95% CI	mean	95% CI	T/C
(4.1a) + (4.1b)	larval survival	L_{t+1}^{i+1}/L_t^i	0.58	(0.57, 0.59)	0.40	(0.36, 0.42)	0.69
(4.1c)	larva-to-pupa survival	P_{t+1}^1/L_t^4	0.35	(0.32, 0.39)	0.66	(0.64, 0.69)	1.89
(4.1e)	pupal weight	W_{t+1}	17.9	(17.7, 18.1)	19.7	(19.4, 19.9)	1.10
(4.1f)	pupa-to-adult survival	A_{t+1}^1/P_t^5	0.59	(0.58, 0.60)	0.63	(0.61, 0.64)	1.07
(4.1h)	adult survival	A_{t+1}^{Mi+1}/A_t^{Mi}	0.90	(0.90, 0.91)	0.86	(0.85, 0.87)	0.96
(4.1i)	reproduction	L_{t+1}^1/A_t^{Mi}	1.46	(1.24, 1.70)	1.21	(1.05, 1.43)	0.83

(b)

			control		toxicant		ratio
equations	parameters		coefficient	95% CI	coefficient	95% CI	T/C
<i>larval survival</i>							
(4.1a)	constant	C_{L120}	0.10	(0.06, 0.14)	0.07	(0.06, 0.10)	0.70
(4.1a)	‘facilitation’	C_{LL1}	0.46	(0.40, 0.55)	0.51	(0.45, 0.57)	1.11
(4.1a)	‘competition’	C_{LL2}	−0.0010	(−0.0013, −0.000 77)	−0.0012	(−0.0017, −0.000 75)	1.17
(4.1b)	constant	C_{L240}	0.81	(0.79, 0.82)	0.72	(0.71, 0.72)	0.89
<i>larva-to-pupa survival</i>							
(4.1c)	constant	C_{LP0}	0.35	(0.32, 0.39)	0.66	(0.64, 0.69)	1.89
<i>pupal weight</i>							
(4.1e)	constant	C_{W0}	40.1	(38.1, 42.1)	39.6	(37.2, 42.1)	0.99
(4.1e)	slope	C_{W1}	−3.82	(−4.18, −3.47)	−3.85	(−4.32, −3.39)	1.01
<i>pupa-to-adult survival (odds ratio)</i>							
(4.1f)	constant	C_{PA0}	−2.04	(−2.36, −1.75)	−2.95	(−3.31, −2.63)	1.45
(4.1f)	slope	C_{PA1}	0.12	(0.10, 0.14)	0.16	(0.14, 0.18)	1.33
<i>adult survival</i>							
(4.1h)	constant	C_{AA0}	0.90	(0.90, 0.91)	0.86	(0.85, 0.87)	0.96
<i>reproductive rate</i>							
(4.1i)	constant	C_{AL0}	204	(140, 288)	37.0	(25.6, 52.0)	0.18
(4.1i)	slope	C_{AL1}	−1.20	(−1.08, −1.85)	−0.89	(−0.96, −0.82)	0.74

(b) *Emerging dynamics*

In order to assess the ability of the parametric model (equations (4.1a–i)) to represent the population dynamics, we generated synthetic time-series data (figure 3) and compared various characteristic features of these data with the real data (figure 1). The model was parameterized using the parameter estimates in table 2b. Stochasticity was incorporated into the reproduction function, as described in figure 3. The periodic behaviour of the real and simulated data was described by the autocorrelation function (tables 1 and 3, respectively). The period is estimated by selecting the first true peak in the correlogram that is significant (above the 95% CI). Other models of these data have been evaluated by the

predicted behaviour of adult densities (Smith *et al.* 2000), and the adult densities in our simulations also have a mean period that is close to that observed for the control populations (see tables 1 and 3).

The deterministic part of the model does not produce cycles, which was also observed by Smith *et al.* (2000). This implies that stochasticity is crucial for the cyclic dynamics, and further studies of this system should focus more on the role of demographic stochasticity in combination with density dependence. The GAM-based approach used here, however, is not suitable for detailed analyses of demographic stochasticity. The stochastic terms are added as white noise to log-transformed equations (Electronic Appendix), which is generally

Figure 1. Examples of time-series and autocorrelation functions (ACF) for real data. The first two columns show the time-series for Control and Toxicant populations, respectively, and the last two columns show the corresponding ACF plots. The vertical lines in the ACF plots indicate significant peaks and the corresponding period (number of lag days).

considered to represent environmental stochasticity. Moreover, the dynamics are influenced by the stage structure: if larval density dependence is assumed for the last larval age group (cf. Lingjærde *et al.* 2001) instead of the first (cf. Moe *et al.* 2002a), the simulations will not cycle (with or without stochasticity). This implies that assumptions about the stage structure are critical for identifying the density dependences driving the dynamics.

For the control populations, means for the real data are all within the 95% confidence intervals of the mean simulated densities. The densities of the toxicant populations, however, are somewhat overestimated: the mean real densities of L , P and A are 40, 30 and 14% lower, respectively, than the means from simulated data. Since the real data are characterized by large fluctuations, particularly for the larvae, a more realistic modelling of the demographic stochasticity may be required to obtain a more precise match between real and simulated densities. However, the model generally succeeded in predicting the net toxicant effects for the different stages: reduction in larval and adult densities, and a weak increase in pupal weight. The model also predicted an increase in pupal densities that did not occur in the real data, but the pupal densities do not affect any density-dependent process. We therefore believe that the model represents the density-dependent processes and the toxicant effects well enough to analyse the density-toxicant interactions.

(c) Effects of the abiotic stressor on demographic rates and model parameters

The mean demographic rates for control and toxicant populations are shown in table 2a. The toxicant

populations had lower larval survival, adult survival and reproduction, but also higher larva-to-pupa survival, pupal weight and pupa-to-adult survival. Since the direct effects of the toxicant can only be negative, all positive effects are interpreted as indirect effects, indicating strong compensatory responses.

The estimated effects of the toxicant on the density-dependent and density-independent components of the parametric functions are shown in table 2b. A significant difference between density-dependent components for control and toxicant groups is interpreted as a density-toxicant interaction.

The overall toxicant effect on survival from young larva to pupa is negative (63% of the control survival). The effects on survival within the larval stage are also negative, whereas the estimated larva-to-pupa survival (equation (4.1c), C_{LP0}) is higher in the toxicant group. Note also that the toxicant groups reach higher maximum survival (figure 2b versus a). Effects on the facilitation factor C_{LL1} and the competition factor C_{LL2} (equation (4.1b)) are not statistically significant.

For pupa-to-adult survival (equation (4.1f)), the constant C_{PA0} (intercept of the odds ratio) is lower in the toxicant group, whereas survival increases more rapidly with pupal weight (higher C_{PA1}). Thus there is an antagonistic interaction between the toxicant and the weight-dependent component of the survival function.

The reproductive potential (C_{AL0}) is reduced by the toxicant, but the toxicant has a positive effect on the slope of the reproduction function (C_{AL1}). This means that there is an antagonistic interaction between the toxicant and adult density: the effect of density on reproductive rate

Figure 2. Density-dependent functions estimated by non-parametric functions (equations (3.1*a-i*); dashed line) and by parametric functions (equations (4.1*a-i*); solid line), for control and toxicant populations (left and right column, respectively). Dashed lines around the non-parametric estimate show 95% CI. Rugplot along *x*-axis indicate the data. (a), (b) The larval survival function (equations (3.1*a*) and (4.1*a*)), combined with larval survival (equations (3.1*b*) and (4.1*b*)) and larva-to-pupa survival (equations (3.1*c*) and (4.1*c*)). (c), (d) The pupal weight function (equation (3.1*d*) and (4.1*d*)). (e), (f) The pupa-to-adult survival function (equation (3.1*f*) and (4.1*f*)). (g), (h) The reproductive rate function (equation (3.1*i*) and (4.1*i*)).

is less negative in toxicant populations than in control populations.

5. DISCUSSION

(a) *Density-dependent and weight-dependent structures*

The humped shape of the larval survival function (f_{LLL}) suggests an Allee effect (positive density dependence in low densities; Allee 1938; Stephens *et al.* 1999; Stephens & Sutherland 1999). This nonlinear shape of the survival function was also observed and discussed by Lingjærde *et al.* (2001), and has also been demonstrated experimentally (Moe *et al.* 2002*a*). The most likely explanation is that blowfly larvae may benefit from aggregation, because of feeding facilitation (Ulliyett 1950; Hanski 1987). Larval

survival therefore benefits from facilitation at moderate densities, whereas at too high densities, competition for food results in lower survival. The two density-dependent components (facilitation and competition) of the larval survival function in the parametric model seem to give a good representation of these processes. Mean pupal weight (f_{LW}) is reduced by high larval density, which is a typical response to crowding for blowfly populations (So & Dudgeon 1989; Goodbrod & Goff 1990; Saunders & Bee 1995). Even intermediate densities, which were optimal for larval survival, result in weight reduction at the pupal stage (Moe *et al.* 2002*a*). The adult density is estimated to have a negative effect on reproductive rates (f_{AAL}), which indicates that there is competition also in the adult stage, although not affecting adult survival (Lingjærde *et al.* 2001). Reproduction may have been

Table 3. Characteristics of simulated data (average over 100 runs for each treatment, with 2.5% and 97.5% quantiles in parentheses). (See table 1 for explanations.)

	control	toxicant
<i>larvae</i>		
mean	363 (333, 396)	292 (255, 332)
CV	0.53 (0.45, 0.64)	0.62 (0.55, 0.76)
ACF	0.04 (−0.06, 0.16)	0.07 (−0.05, 0.19)
<i>T</i> (days)	55 (16, 132)	52 (16, 132)
proportion	0.19 (0, 1)	0.31 (0, 1)
<i>pupae</i>		
mean	115 (106, 125)	148 (128, 170)
CV	0.47 (0.41, 0.53)	0.54 (0.49, 0.60)
ACF	0.04 (−0.09, 0.18)	0.08 (−0.04, 0.22)
<i>T</i> (days)	67 (24, 132)	57 (22, 132)
proportion	0.22 (0, 1)	0.37 (0, 1)
<i>pupal weight</i>		
mean	18.0 (17.8, 18.3)	18.3 (17.8, 18.8)
CV	0.11 (0.09, 0.12)	0.12 (0.11, 0.14)
ACF	0.04 (−0.07, 0.16)	0.07 (−0.04, 0.18)
<i>T</i> (days)	61 (17, 144)	57 (20, 156)
proportion	0.16 (0, 1)	0.40 (0, 1)
<i>adults</i>		
mean	140 (130, 148)	115 (104, 126)
CV	0.23 (0.21, 0.26)	0.28 (0.26, 0.31)
ACF	0.09 (−0.10, 0.37)	0.09 (−0.10, 0.33)
<i>T</i> (days)	73 (22, 132)	71 (30, 132)
proportion	0.38 (0, 1)	0.40 (0, 1)

limited by suitable oviposition sites, and by protein resources.

(b) Density-dependent responses to the abiotic stressor

The parametric estimates of the density-dependent functions may now be used as a basis for discussing what causes the compensatory responses to the toxicant: the reduction in densities, density–toxicant interactions, or more complex population-level effects. The indirectly positive effects of the toxicant in the juvenile stages (increased larva-to-pupa survival, pupa-to-adult survival and mean weight; table 2*a*) may in part be explained by reductions in mean larval densities (cf. table 1, rugplot along *x*-axis in figure 2*a,b*). The reductions in larval densities in the toxicant group result from the reductions in mean adult survival, reproduction and young–larval survival, as discussed by Moe *et al.* (2002*b*). Note that reductions in larval density will benefit the toxicant populations only as long as the density is above the optimum for survival (the ‘hump’ in figure 2*a,b*); below the optimum, further reductions in density will on the contrary reduce larval survival.

The reductions in larval densities cannot be the full explanation for the indirectly positive toxicant effects: the estimated survival in toxicant population is often higher even for the same densities (compare figure 2*a* versus *b*). Furthermore, no significant density–toxicant interactions were observed for these rates. An alternative explanation for the positive effect on this density-independent response may be that the toxicant affects the pattern of resource partitioning among the individuals. Blowfly larvae are normally characterized by

‘scramble competition’ (even resource partitioning; Nicholson 1954*a,b*), which implies that some resources are ‘wasted’ on individuals that do not survive. In the present study, the toxicant populations display more efficient use of resources at the population level than the control populations, as the total pupal biomass production is higher (table 1). The toxicant may contribute to more uneven resource partitioning (i.e. more contest-like competition), possibly by reinforcing initial differences in strength among individuals. In this way, the resources may be more efficiently exploited by the population (Parker 2000; Uchmanski 2000). Even if toxicant-exposed individuals at a given density should have lower mean weight than control individuals (as indicated by figure 2*c,d*), a more flattened distribution of individual weights could still allow a higher proportion of the toxicant-exposed individuals to be above the critical weight for survival. Unfortunately this hypothesis cannot be tested explicitly due to lack of data on individual larval weight, but we intend to explore the hypothesis further by individual- and physiology-based modelling.

In the pupal stage, mean survival to adulthood benefits from higher mean weight in the toxicant group than in the control group. There is an antagonistic interaction between the toxicant and the weight-dependent component, C_{PA1} (table 2*b*). However, this pupal weight is a decreasing function of larval density, this should be interpreted as a synergistic interaction between the toxicant and delayed effects of larval density. Although pupae are not directly exposed to the toxicant, a decrease in survival could be expected due to cadmium accumulated during the larval stage (Moe *et al.* 2001). The synergistic interaction implies that the individuals suffer more from the combined density and toxicant stress, but also that the population has stronger capacity for compensation to reduced density stress.

In the adult stage, the estimated density–toxicant interaction for the reproductive rate (C_{AL1}) is antagonistic. This implies that the individual potential for reproduction (represented by C_{AL0}) is reduced by the toxicant (cf. Simkiss *et al.* 1993), while competition among adults is lower. Reduced competition may be a consequence of both lower mean adult density and lower mean fecundity. However, these compensatory responses do not outweigh the negative toxicant effects on survival and fecundity in this stage.

To summarize, through the parametric model we have identified both synergistic and antagonistic interactions between density and the abiotic stressor (see figure 4). Density–toxicant interactions have recently received more attention within ecotoxicology (Forbes *et al.* 2001, Liess 2002), but there are few studies that examine the relationship between such interactions and population-level responses for more than 1–2 generations. In our study, the synergistic interaction in the juvenile stage implies that toxicant groups have higher suffering from combined toxicant and density stress at the organism level, but in the long run this is more than outweighed by a stronger capacity for compensatory responses to density reductions at the population level. Conversely, the antagonistic interaction in the adult stage implies that toxicant individuals suffer less from combined density and toxicant stress than control individuals, but the adult

Figure 3. Examples of time-series and autocorrelation functions (ACF) for simulated data. See figure 1 for explanations. One hundred adults were used as starting values. Stochasticity was added to the reproduction function, because the reproductive rate is the most variable of the demographic rates (see also Moe *et al.* 2002a). We added white noise $\varepsilon \sim N(0, \sigma)$, where σ is the standard deviation of $\varepsilon^{(8)}$ (the residuals for the estimated reproduction function, equation (A 15) in Electronic Appendix). The simulations were run for 400 time-steps, and the first 40 points were removed before analysis (to give a similar length as the data, excluding a transient period).

population also has a weaker capacity for compensatory responses. Hence, the overall effect of a stressor on a particular life-history stage does not depend on whether the interaction with density gives a synergistic or antagonistic effect within one generation, but on how the stressor affects the stage's capacity for density-dependent compensatory responses across several generations. Such compensatory responses cannot be studied by traditional life-history experiments covering 1–2 generations. This underlines the need for time-series data to study compensatory responses to stressors, and for the development of modelling tools suitable for analysing density-dependent processes and interactions in time-series data.

(c) Assessment of the modelling approaches

Models for blowfly population dynamics have been described by several authors (May 1973; Maynard Smith 1974; Brillinger *et al.* 1980; Readshaw & Cuff 1980; Nisbet & Gurney 1982; Stokes *et al.* 1988; Manly 1990; Kendall *et al.* 1999). Most of these models are based on one of the classical 'Nicholson's blowflies' data sets (Nicholson 1954a,b), where adult competition is the main regulating mechanism, rather than larval competition as in our study. A well known model is a mechanistic delay-differential model (Gurney *et al.* 1980; Readshaw & Cuff 1980) that succeeded in predicting several important features of the data. The approach in Gurney *et al.* (1980) was a completely specified mechanistic model, with density-dependent functions based on knowledge or assumptions about the biological system, and parameters estimated

mainly from independent data. In contrast, our model is developed on the basis of statistical (non-parametric) modelling of the time-series data themselves and as little as possible on assumptions; the result (estimated density-dependent structures) was compared with results from independent experiments (Moe *et al.* 2001, 2002a,b). A more recent analysis of Nicholson's data (1954a,b) applied an approach intermediate between these two extremes (i.e. a partly specified model; Wood 2001). This approach came closer to testing for differences between competing mechanisms than the traditional mechanistic approach (Wood 2001). A partly specified (semi-mechanistic) model was also used in Smith *et al.* (2000), and was successful in predicting the dynamics of the population dynamics in Daniels' time-series data (Daniels 1994).

The non-parametric modelling method applied here has proven to be useful for studying density-dependent structures in time-series data. Although the blowfly populations (Daniels 1994) constitute a very simple ecological system, the non-parametric method indicated new features of the population-regulating mechanisms: (i) the nonlinear function for immature survival, (ii) the weight dependence in pupal survival and (iii) the competition among adults for reproduction. These mechanisms have not been included in previous parametric models for this system (Forrest 1996; Smith *et al.* 2000), but may be important both for population persistence and for effects of abiotic factors. The additive form of the non-parametric model on log-scale also makes it useful for analysing effects of several predictor variables

Figure 4. Summary of results: effects of the toxicant on mean densities (boxes), mean demographic rates (arrows connecting boxes), and density-dependent parameters. The net effect on densities and demographic rates are illustrated by the size of the boxes and the width of the arrows, respectively in the right-hand side figure compared to the left-hand side figure. All net positive effects of the toxicant are indirect, and represent density-dependent compensatory responses. The estimated density-toxicant interactions are indicated by grey arrows. Note that the synergistic density-toxicant interaction (i.e. the combined effect of the two stressors is stronger than additive) occurs together with a net positive effect in pupa-to-adult survival.

on demographic rates simultaneously. For example, the analysis suggested a negative correlation between adult weight and the reproductive rate, which was opposite of the expected, but which indicates that density dependence is more important than size dependence in this situation (see figure 5 in Electronic Appendix).

The parametric model provided new insight about stage-specific mechanisms for compensatory responses to abiotic factors (cf. figure 4), and the model estimates also identified a positive relationship between density-toxicant interactions and compensatory responses. We are convinced that a combination of non-parametric and parametric modelling, as used in this study, does provide more insight about interactions between density-dependent and -independent processes in the blowfly populations, than either approach could have done separately.

Identifying the type and strength of density dependence and understanding the compensatory responses to stressors are important for better risk assessment and management of natural populations. For example, identifying an Allee effect may have important implications for population-level risk assessment, since a population might have no capacity for compensatory responses below the optimum density. Nevertheless, this kind of density dependence is rarely considered in studies of population-level ecotoxicology. Since the analyses in this study are based on a single-species system, one might question the relevance of this insight for natural populations. However, Murdoch *et al.* (2002) show that natural populations of many taxa exhibited the same type of 'delayed feedback' cycles as the blowflies. These types of cycles are often promoted by overcompensating density dependence, which indicates a high capacity for compensatory responses. Moreover, Murdoch *et al.* (2002) conclude that a one-species model is a valid representation for generalist-consumer population dynamics in many-species food webs. The phenomena described for this single-species system should therefore be of relevance also for theoretical understanding and for management of natural populations.

This study was supported by the Norwegian Research Council with a doctoral stipend to S.J.M., and by the Nansen Endowment with a grant to S.J.M. A part of this work was

conducted by S.J.M. as a Postdoctoral Associate at the National Center for Ecological Analysis and Synthesis, a Center funded by NSF (grant no. DEB-0072909), the University of California, USA, and the Santa Barbara campus. The blowfly time-series data were collected by Susan Daniels during a series of small grants awarded to Rob Smith and Ken Simkiss by the UK Natural Environment Research Council for research carried out at the University of Reading. We thank Ole Christian Lingjærde, Kyrre Lekve, Bruce Kendall, William Nelson, and four anonymous reviewers for helpful suggestions and comments to the manuscript.

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The supplementary Electronic Appendix is available at <http://dx.doi.org/10.1098/rspb.2005.3184> or via <http://www.journals.royalsoc.ac.uk>.

As this paper exceeds the maximum length normally permitted, the authors have agreed to contribute to production costs.