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Impacts of stage-specific acute pesticide exposure on predicted population structure of the soft-shell clam, *Mya arenaria*

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Abstract

A combined laboratory and modeling approach was used to assess the impact of selected pesticides on early life stages of the soft-shell clam, *Mya arenaria*. Clams were exposed for 24 h as veligers or pediveligers to the broad-spectrum herbicide hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4 (1h,3h)-dione; (Velpar®)], the phenoxyacetic acid herbicide, 2,4-D (2,4-dichlorophenoxyacetic acid; Agway® Super BK 32), or phosmet (Imidan®). In addition, juvenile clams were exposed for 24 h to 2,4-D and their growth monitored for 21 months. Laboratory experiments indicated veligers were more sensitive to acute pesticide exposure than pediveligers, with 2,4-D exposed veligers exhibiting the lowest survival among all treatments. Relative to controls, juvenile clams exposed to 0.5 ppm 2,4-D had enhanced survival following the initial 3 months of grow out. Juveniles exposed to 0.5 ppm, 5 ppm and 10 ppm 2,4-D showed an initial growth delay relative to control clams, but at 21 months post exposure these clams were significantly larger than control clams. Data from the larval and juvenile exposures were used to generate a stage-specific matrix model to predict the effect of pesticide exposure on clam populations. Impacts on simulated clam populations varied with the pesticide and stage exposed. For example, 2,4-D exposure of veligers and pediveligers significantly reduced predicted recruitment as well as population growth rate compared to controls, but juvenile exposure to 2,4-D did not significantly reduce population growth rate. With the exception of veligers exposed to 10 ppm, hexazinone exposure at the both veliger and pediveliger stages significantly reduced predicted recruitment success compared to 0 ppm controls. Hexazinone exposure also reduced modeled population growth rates, but these reductions were only slight in the pediveliger exposure simulations. Veliger and pediveliger exposure to phosmet reduced modeled population growth rate in a dose-dependent fashion. Changes in modeled population stable stage distributions were also observed when veligers were exposed to any pesticide. These results suggest that both the stage of exposure and the specific toxicant are important in predicting effects of pesticide exposure on soft-shell clam populations, with earlier life stages showing greater sensitivity to the pesticides tested.

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Keywords

bivalve; 2,4-D; hexazinone; phosmet; pediveliger; veliger; matrix population model

1. Introduction

Distributed in mudflats along the Maine coast from Casco Bay to Cobscook Bay, soft-shell clams, *Mya arenaria*, have provided an important resource for Maine people for many years. Harvested by both recreational and commercial diggers, soft-shell clams were the 3rd most valuable commercial Maine fishery in 2008 with a preliminary landed value of \$10.9 million dollars (Maine Department of Marine Resources, <http://www.maine.gov/dmr/commercialfishing/recentlandings.htm>). Although apparently stable over the last several years, clam harvests declined precipitously in the 1980s; declines in Hancock and Washington counties were particularly dramatic. No definitive cause for the reduction in clam abundance in eastern Maine has been determined. Because many clam flats in these counties border agricultural lands, roadsides and forests, much speculation has arisen concerning the effects of pesticide run-off on clam flat health (Van Beneden, 2005). Some studies point to lack of larvae arriving at eastern flats (Vassiliev et al., 2000; Congleton et al., 2006). Others note the prevalence of gonadal neoplasia in isolated populations of clams in eastern Maine (Gardner et al., 1991; Barber, 1996, 2004). Data concerning the effects of pesticides on bivalves include reports of organophosphorous pesticide toxicity to *Mytilus galloprovincialis* and *Venus gallina* from the Mediterranean Sea (Serrano et al., 1995), effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on oyster gametogenesis (Wintermyer and Cooper, 2007), genotoxicity of the herbicide 2,4-D (2,4-dichlorophenoxy acetic acid) in mussels (Mičić et al., 2004), and transient effects of potato fungicides on clam hemocytes (Pariseau et al., 2009).

In Maine, several pesticides are applied during the summer when clams are spawning. The herbicide 2,4-D is used for brush control on roadsides and railroad tracks as well as weed control on lawns; it is typically applied during the early and late spring (April–June). Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4 (1h,3h)-dione] is a broad-spectrum herbicide, registered since 1983 for use in Maine lowbush blueberry fields. Sold commercially as Velpar L® and Pronone 10G®, hexazinone is typically applied as a broadcast spray or mixed with fertilizer in the spring after pruning but before blueberries emerge (typically, April–May in Maine). It has a half-life of one month in blueberry soils, primarily degraded by soil microorganisms, and is affected by soil leaching, uptake by plants and breakdown by sunlight. It has been detected in ground water in Eastern Maine at 0.20–10 ppb (Perkins et al., 2006). The organophosphate insecticide phosmet (marketed as Imidan®) has a broader application period in blueberry fields, from April to August, with most concentrated use during July (Yarborough and Drummond, 2009; L. Hicks, pers. comm.). Although rapidly broken down by hydrolysis and photolysis, phosmet has been detected at 0.52 ppb in water samples taken from the Narraguagus River several hours after aerial application (Jackson, 2004).

Although many ecotoxicological experiments focus on individual responses to contaminants (Calow, 1993), pollutants can affect all levels of biological organization from cells to ecosystems (e.g., Moriarty, 1988; Newman and Jagoe, 1996; Galloway and Depledge, 2001; Clements and Rohr, 2009). Studies have described contaminant impacts on marine infaunal growth and reproduction in the laboratory (Pesch et al., 1991; McGee et al., 1993; Fitzpatrick et al., 2008), changes in population abundance (Grassle and Grassle, 1974; Pearson and Rosenberg, 1978), and community composition (Mauer et al., 1991). To link individual exposures to population level impacts, life-stage-specific contaminant effects are sometimes

incorporated into structured demographic models (Caswell, 2001). Such Life Table Response Experiments (LTRE) have been widely used to demonstrate the effect of chronic toxicant exposures on freshwater and marine invertebrates (e.g., Allan and Daniels, 1982; Rao and Sarma, 1986; Green and Chandler, 1996; Levin et al., 1996; Bridges and Carroll, 2000).

Like many other marine invertebrates, soft-shell clams are broadcast spawners; eggs and sperm are shed into the water column, and fertilized eggs develop into free-swimming feeding larvae. The larvae develop through several stages, trochophore, veliger and pediveliger, before settling and metamorphosing into juveniles. In Maine, the progression from spawn to set (juveniles) can take four to six weeks; juveniles grow for 1-2 years before reaching adult size (Newell and Hidu, 1986). Thus, clams may be exposed to pesticides from different routes (waterborne vs. sediment associated) depending on their stage of development. Although recent results suggest that such toxicants can impact adult reproduction (Butler et al., 2003), and presumably have a profound effect on population dynamics, toxicity data for larvae and juveniles are lacking.

In this study, we determined the effects of acute exposure to three pesticides, hexazinone, phosmet or 2,4-D, on survival and growth of larval and juvenile soft-shell clams. The resulting data were used to parameterize a matrix population model and to simulate how pesticide exposure of these early-life history stages could impact population dynamics of soft-shell clams. We hypothesized that veligers would be more sensitive to pesticides than pediveligers or juvenile clams, and that resulting larval mortality rates would be sufficient to reduce clam population growth rates.

2. Materials and methods

2.1 Source, spawning and transport of animals

Soft-shell clam (*Mya arenaria*) larvae were obtained from either the Beal's Island Shellfish Hatchery (Jonesport, ME) or Sandy Cove Hatchery (Harrington, ME). Due to the seasonal nature of the soft-shell clam reproductive cycle, these studies were confined to the summer months (June/July); multiple spawnings (2-3) were typically done each season. Adult clams were induced to spawn by thermal stimulation in filtered seawater (24-25°C). Sperm and eggs were pooled from different individuals. Eggs were filtered (75 µm Nitex filter), suspended, counted and density adjusted to 20-50 eggs/mL. Sperm passed through a 37 µm Nitex filter were counted and added to eggs to a final concentration of 10^5 - 10^7 sperm/mL. Fertilization efficiency was assessed by microscopic examination at 30-60 min post-fertilization. Larvae were either transported immediately to the University of Maine, Orono (approximately 2 h) in aerated carboys or maintained at the hatchery until they reached the desired developmental stage and then transported to the laboratory.

2.2 Pesticide Exposures

Pesticides used in this study were obtained from several sources. Velpar (CAS51235-04-2), a formulation of hexazinone, was obtained from D. Yarborough, University of Maine Extension Blueberry Specialist. Imidan 2.5 EC (containing the active ingredient phosmet) was obtained from B. Perkins (University of Maine, Dept. of Food Science and Human Nutrition). 2,4-D was purchased at the local hardware store (Agway Super BK32 herbicide) and contained 32.15% active ingredient. The concentrations of 2,4-D reported were calculated based on this percentage.

Larvae were treated at the University of Maine, Orono, in environmental growth chambers (Percival Scientific, Perry, IA) operated at 16-18°C, with a 12 h light cycle. D-stage veliger or pediveliger larvae were maintained in suspension by gentle agitation in 4l glass beakers at densities of 15-30/mL. Replicate containers of larvae were exposed to 0, 0.5, 5, 10, or 50 ppm

pesticide in UV-sterilized, filtered seawater for 24 hours (n=3 to 5 replicates per dose). For ethanol carrier controls, all treatments were adjusted to the same final ethanol concentration (15:10000 dilution). This wide range of pesticide levels enabled us to establish a broad dose response curve, with lowest values within the range of environmental relevance and higher levels representing a transient bolus, such as might be experienced during a rain event or spill.

Following the exposure period, larvae were rinsed in UV-treated, filtered seawater, transferred to fresh containers, fed diluted mixed shellfish diet algal paste (Reed Mariculture, San Jose, CA, final concentration $\sim 2.5 \times 10^5$ cells l⁻¹; fed every other day) and observed for 7 days for mortality and developmental delays. Although the population model input required survival data over a 14-day period, the typical duration of veliger and pediveliger stages in the field, larval mortality following laboratory exposure was high after 7 days. Therefore, forecasts of larval survival and proportion remaining in stage over 14 days were calculated using least squares regression and incorporated into the matrix population model.

Due to the apparent relative impact of acute 2,4-D larval exposures, and our ongoing studies on adult clams that suggest reproductive consequences to 2,4-D exposure (Butler et al., 2003), juveniles were exposed for 24 h to 2,4-D under static conditions. Juvenile clams (2-3 mm shell length) were placed in large glass jars (n=3) containing 0.0, 0.5, 5.0, or 10 ppm 2,4-D dissolved in 1 liter seawater, with gentle aeration; clam density was ~ 300 juveniles l⁻¹. After exposure, clams were placed in clean, aerated seawater and observed for two weeks for mortality or developmental delays. Clams were fed during this period (diluted mixed shellfish diet, $\sim 1.6 \times 10^6$ cells replicate⁻¹ every other day). Approximately 400 surviving juvenile clams, pooled from replicate treatments, were transported to the Environmental Protection Agency facility in Narragansett, RI, where clams were maintained in flow-through seawater tanks for an extended grow-out period. Juvenile survival and growth rates for these clams were incorporated into a matrix population model. No juvenile exposures to hexazinone or phosmet were conducted.

2.3 Survival, shell growth and gonadal development in clams exposed as juveniles

Mortality and shell growth of 2,4-D exposed juvenile clams were measured at approximately four-month intervals. At 21 months post-exposure, 20 to 25 reproductively mature clams from each treatment were sacrificed in pre-spawn stage, gonadal and somatic tissue weighed, and gonadal tissue preserved in Dietrich's solution (10% neutral-buffered formalin, 28.5% ethanol, 2% acetic acid) for histology (Mass Histology, Worcester, MA). Preliminary microscopic analysis of gonadal sections from 8 clams from each treatment revealed no significant difference in egg number or size between control and 2,4-D exposed clams. A post-hoc power analysis indicated, however, that a much larger sample size (>100 clams) would be required to accurately detect small differences among treatments (*i.e.*, with power > 65%). Lacking sufficient samples for additional analysis, only 2,4-D effects on juvenile survival and growth were considered in the matrix population model.

2.4 Matrix Population Model

Matrix population models use age- or stage-specific survival, growth and fecundity data to predict the proportion of individuals at different life stages that are present in a given population over time. Of the various types of population models, matrix population models are among the best types for assessing the effects of contaminants on populations (Caswell, 2001). In particular, one can model how the effects of chemical exposures at one life stage might ultimately affect population dynamics. To determine how pesticide effects on larval and juvenile soft-shell clam survival translated into effects on populations, numerous simulations were run using linked larval and adult matrix population models constructed in MATLAB. The transitions considered in each model are diagrammed in Fig. 1. The size classes and stage

durations used in the models were based on the von Bertalanffy growth equation for *M. arenaria* in Georgetown, ME, derived from data of Spear and Glude (1957), as published by Brousseau (1979). The output of the larval model is predicted recruitment success, which modifies the fecundity parameter of clams in the adult population model.

The larval model (Fig. 1A) has five stages with two-week time step intervals up to 10 weeks, approximating the normal development period of the soft-shell clam in Maine (Newell and Hidu, 1986). Forecasts of veliger and pediveliger survival and proportion remaining in stage were calculated with least squares regressions (XLFit 4.1 add-in to Excel) based on data obtained from laboratory exposures of larvae to each pesticide. Transitions from the veliger and pediveliger stages were modeled using three different forecast equations: 1) parameter estimates from the best fitting regression equations, 2) upper 95% confidence limit for parameter estimates of the regression equations, and 3) lower 95% confidence limit for parameter estimates of the regression equations. Detailed data documenting the mortality of newly metamorphosed soft-shell clams (*i.e.*, < 2 mm shell length) in the field were not available. Thus, for transitions between the newly metamorphosed spat stages (S1-S3 in Fig. 1A) we assumed that 50% survived in each stage, and that all surviving clams grew into the next spat stage, except S3, where 50% were retained in stage to be counted as new recruits. These assumptions yielded a cumulative larval survival rate for control treatments that was similar to published estimates of <0.1% larval survival to spat (Newell and Hidu, 1986). Thus, spat stages S1-S3 do not represent discrete sizes between 0 and 1.9 mm shell length, but simply reflect dummy transitions necessary to account for possible early mortality before newly settled clams reach the typical size of newly recruiting “seed clams” (2 mm shell length). For each pesticide exposure level and larval stage combination, we simulated the survival of 10^7 larvae and calculated a cumulative probability of recruitment success. These pesticide and stage exposure-specific recruitment probabilities were used to modify fecundity values of adult clams in the adult population model.

The adult model (Fig. 1B) also has five stages which include juveniles sized 2 to 19.9 mm in shell length, plus four sexually mature adult size classes (20.0 to 29.9 mm, 30.0 to 39.9 mm, 40.0 to 49.9 mm, and 50.0 to 59.9 mm). Stage-specific probabilities of survival and growth into the next size class were calculated based on the life history parameters for the Cape Ann, MA, population of *M. arenaria* (Brousseau, 1978a). As observed in the field, fecundity of adults in the model increased with size; the relationship between shell size and fecundity was based on data of Brousseau (1978b), with the important exception that clams ≥ 20 mm shell length were reproductive, as has been observed for *M. arenaria* in Maine (Newell and Hidu, 1986). For each run of the model, standard fecundity values were multiplied by the appropriate recruitment probability (*i.e.*, the predicted recruitment success given exposure of either veligers or pediveligers to each concentration of pesticide), but all other transitions between adult size classes remained unchanged. Recruitment success has been used to modify fecundity terms in other matrix population models (*e.g.*, Ripley and Caswell, 2006). The matrix corresponding to the adult model diagrammed in Fig. 1B is shown below:

$$\begin{pmatrix} F_1 & F_2 & F_3 & F_4 & F_5 \\ G_{12} & P_2 & 0 & 0 & 0 \\ 0 & G_{23} & P_3 & 0 & 0 \\ 0 & 0 & G_{34} & P_4 & 0 \\ 0 & 0 & 0 & G_{45} & P_5 \end{pmatrix}$$

where F_i indicates fecundity at stage i (stages 1-5; juveniles in stage 1 had $F_1=0$), G_{ij} indicates the probability that an individual survives and grows into the next stage, P_i indicates the probability an individual survives and stays in a stage, and the time step between each stage is

one year. Population growth rate, λ , was calculated as the dominant eigenvalue, and the eigenvector gave the stable age distribution. We used the stable age distribution calculated from the matrices corresponding to the 0 ppm exposures of each pesticide to determine the distribution of clams among stages in an initial population of 100,000 clams. Dynamics of this initial population were simulated for 20 years for each pesticide concentration and stage-exposed combination, assuming that recruitment was affected by exposure to pesticides in each year of the simulation and determining the total population size and number of clams in each size class. For each pesticide and stage combination we ran three models corresponding to the three forecast larval survival values from the laboratory data (best-fitting, upper and lower 95% confidence limit estimates of the regression equation parameters) and also calculated the proportional sensitivities of λ to each element in the adult matrix (*i.e.*, elasticity; Caswell, 2001). For simplicity, only the results of the simulations using the best-fitting larval survival forecast equations are shown, except when noted otherwise.

3. Results

3.1 Laboratory exposures

Pesticide exposure generally reduced larval survival, but these effects varied with pesticide and larval stage exposed. On average, 2, 4-D caused higher veliger mortality than either hexazinone or phosmet (Fig. 2A-C). In all pesticide exposures, cumulative mortality after 7 d was high for veligers (only ~ 4 to 18 % survival depending on pesticide and dose), and forecasts of cumulative survival in 14 d ranged from less than 0.5% survival for veligers exposed to any concentration of 2,4-D, up to 5% survival for veligers exposed to 10 ppm hexazinone (Fig 2A, B). Pediveligers were much less sensitive. Depending on the concentration, pediveligers exposed to 2,4-D had 18-38% survival after 7 d (Fig. 2D, values predicted from best-fitting least squares regression), while 65-67% and 34-57% of pediveligers exposed to hexazinone or phosmet, respectively, survived after 7 d (Fig. 2E-F, values from least-square regressions). Forecast pediveliger survival to 14 d ranged from ~3 % (10 ppm 2,4-D exposure) to 48% (50 ppm hexazinone exposure).

Juvenile clams exposed to 2,4-D had high initial survival (>90% survival at 3 months), but cumulative survival declined to 40% by 9 months for most treatment levels, and remained at that approximate level for 21 months (Fig. 3A). Following the initial 3 month grow-out, juvenile clams exposed to 0.5 ppm 2,4-D had enhanced survival compared to controls and other 2,4-D treatments (Fig. 3A), suggesting a possible hormetic effect. Juvenile percent survival was compared among treatments using Tukey-type multiple comparisons among proportions (Zar, 1984). Compared to all other treatments, the percent survival of clams exposed to 0.5 ppm 2,4-D was significantly higher at both 12 months and 21 months ($\alpha=0.05$). Significantly fewer clams exposed to 5 or 10 ppm 2,4-D survived compared to controls at 12 months ($\alpha=0.05$), and significantly fewer clams exposed to 10 ppm 2,4-D survived compared to controls at 21 months ($\alpha=0.05$). Cumulative survival rates within treatments were not significantly different at 12 and 21 months (chi-square tests, $p>0.05$), thus for simplicity, the effects of 2,4-D exposure on 12-month survival were incorporated in the model.

Juvenile clams exposed to any level of 2,4-D grew slower than control clams for the first 9 months following exposure, but at 21 months, exposed clams had out-grown control clams (Fig. 3B). Repeated measures ANOVA indicated significant effects of exposure level, time, and their interaction on clam shell size (Treatment MS=126.9, $F_{3,699}=22.3$, $p<0.001$; Month MS=28903.5, $F_{4,2796}=4852.8$, $p<0.001$; Treatment X Month MS=54.9, $F_{12,2796}=9.22$, $p<0.001$). In addition to being significantly larger than control clams, clams that were sacrificed 21 months after exposure to 2,4-D had significantly higher gonadal-somatic indices (Table 1, ANOVA $F_{3,87}=7.52$, $p=0.0002$).

3.2 Model Results

Recruitment (survival to spat) is an important parameter for model population predictions, as it reflects input of new individuals into the population. Results of the larval matrix model predicting recruitment success for veligers and pediveligers (Fig. 4) are consistent with the laboratory exposure studies (*i.e.*, veligers were more sensitive to pesticide exposure, resulting in lower predicted recruitment). Simulations of recruitment based on experimental controls predicted survivorship to spat ranging from 0.15% (2,4-D simulations) to 0.28% (hexazinone simulations), comparable to rates observed in the wild, ~0.1% (Newell and Hidu, 1986). Larval exposure to 2,4-D significantly reduced predicted survival to spat compared with controls, regardless of stage exposed (Fig. 4A, Dunnett's tests on proportions, $\alpha=0.05$). Fewer individuals survived to spat when veligers were exposed to either hexazinone or phosmet than when pediveligers were exposed to the same pesticides (Fig. 4B,C). Within each pesticide and stage exposed, however, the proportion of larvae surviving to spat was significantly less for pesticide-exposed larvae than for controls (Dunnett's tests on proportions, $\alpha=0.05$; Zar, 1984), except for veligers exposed to 10 ppm hexazinone.

Predicted recruitment success from the larval model output (Fig. 4) was used to modify the fecundity terms in the adult matrix population model. Dynamics for an initial population of 100,000 adult clams were simulated over a 20-year period. The resulting simulated population size varied depending on the pesticide and developmental stage of exposure (Fig. 5). Simulations using the laboratory-derived control parameters for each exposure resulted in populations 1-3 orders of magnitude larger than the initial population (Fig. 5). Juvenile exposure to 2,4-D did not reduce simulated population size compared to controls; however, exposure of veligers or pediveligers resulted in a 2-3 order of magnitude decrease in total population size compared to controls (Fig. 5A). Veliger exposure to hexazinone had variable effects on population size depending on exposure level (Fig. 5B). In contrast, simulated population size was <1 order of magnitude reduced compared to controls for all pediveliger hexazinone exposures (Fig. 5B). Among the hexazinone exposure simulations, only veliger exposure to 50 ppm hexazinone resulted in a population smaller than the starting population. Both veliger and pediveliger exposure to phosmet decreased population size compared to control simulations, with reductions ranging from <1 order of magnitude in the pediveliger exposure simulations to 4 orders of magnitude in the 50 ppm veliger exposure simulations (Fig. 5C). Only veliger exposure to 50 ppm phosmet yielded a population smaller than the initial population.

The population growth rate (λ) associated with each simulation was calculated from the transition matrices. Simulations that incorporated 14-d larval survival parameters for controls (*i.e.*, 0 ppm exposures) predicted exponentially growing populations ($\lambda > 1$) for each pesticide exposure (Fig. 6). Compared to controls, veliger and pediveliger exposure to any level of 2,4-D decreased population growth rate, and exposure to 5 ppm or 10 ppm 2,4-D yielded exponentially declining populations ($\lambda < 1$). In contrast, population growth rate was positive for all simulations of juvenile exposure to 2, 4-D. In fact, population growth rate in the 0.5 ppm juvenile exposure simulation was greater than that in the control simulation, suggesting a possible hormetic effect of exposure at this life stage (Fig. 6). The results of larval hexazinone exposure on populations were variable. Although some exposure levels reduced population growth rate compared to controls, in all cases, veliger exposure to hexazinone resulted in growing populations ($\lambda > 1$, Fig. 6). Similarly, population growth rates when pediveligers were exposed to hexazinone were consistently high ($\lambda \sim 1.4$ for all treatments). Compared to controls, veliger exposure to any level of phosmet reduced population growth rate in a dose-dependent fashion, but only exposure to 50 ppm phosmet resulted in a declining population ($\lambda < 1$). Pediveliger exposure to phosmet also reduced population growth rates compared to controls, but population growth was positive in all treatments ($\lambda > 1$, Fig. 6).

Pesticide exposure of soft-shell clam larvae also changed the stage structure of the modeled populations. In simulated control populations, juvenile clams (2-19.9 mm) predominated, with progressively fewer large adults. Exposure of juveniles to any level of 2,4-D had no effect on this pattern (Fig. 7). Similarly, when pediveligers were exposed to hexazinone or phosmet, there were no changes to the stable age distribution of the population (Fig. 7). In contrast, when pediveligers were exposed to 2,4-D and veligers were exposed to any of the pesticides, the relative proportion of juvenile clams in the population was significantly reduced, reflecting reduced recruitment demonstrated in earlier results. The effect is most pronounced for 2,4-D exposed veligers (Fig. 7).

Discussion

Although marine mollusks have been the subject of much toxicity research (*e.g.*, reviewed by Hunt and Anderson, 1993), and embryo-larval development assays are widely used as sensitive indicators of toxicity, data concerning the susceptibility of soft-shell clam larvae to toxicants are sparse. Our laboratory experiments demonstrated pesticide and stage-specific differences in clam survival following 24h exposure to different concentrations of 2,4-D, hexazinone or phosmet. Of the developmental stages tested, veligers tended to be most sensitive, with 2,4-D causing the greatest mortality (Fig. 2). In general, pesticide levels <5 ppm caused relatively little mortality in the first 24 h following exposure, but higher doses caused 20-30% mortality during the same period, and 80-90% mortality 7 d following exposure (Fig. 2). In contrast, when pediveligers were exposed to the pesticides, we observed very little early mortality in any treatment (Fig. 2), and only those exposed to 10 ppm or 50 ppm 2,4-D or phosmet showed >50% mortality after 5 days (Fig. 2). Acute hexazinone exposure had comparatively little impact on pediveliger mortality. These results are consistent with reports that even brief exposures to pesticides or other toxicants can reduce larval bivalve survival and growth (Davis and Hidu, 1969; Hunt and Anderson, 1993).

Of the three pesticides tested, 2,4-D seemed to be the most toxic to soft-shell clam larvae. 2,4-D has moderate to low acute toxicity to humans and mammals but the acute toxic level of 2,4-D in aquatic invertebrates has been found between 0.1 and 100 ppm, in amphibians between 8 and 346 ppm and in fish between 0.3 and 2840 ppm (Cox, 1999). Cytogenetic effects have also been observed in fish exposed to sublethal concentrations of 2,4-D (25 to 75 ppm; Ateeq et al., 2002). Toxic effects of 2,4-D on marine bivalves also have been described. Liu and Lee (1975) found that 2,4-D at concentrations in seawater of ~23 to ~183 ppm could adversely affect the growth of mussels (*Mytilus edulis*) at all stages of their life cycle. Phosmet was also very toxic to veligers, but somewhat less toxic to pediveligers (Fig. 2). Conducting acute exposures of adult mussels, *Mytilus galloprovincialis*, Serrano et al. (1995) found a LC-50 (96 h) > 56 ppm for phosmet, but observed no lethal effects on the bivalve *Venus gallina*. In contrast, phosmet is considered very toxic to fish and aquatic invertebrates. The LC-50 (96 h) for rainbow trout is 230 ppb (Solé et al., 2000). Of the pesticides tested, hexazinone was the least toxic to soft-shell clam larvae. This result is consistent with toxicity data for mammals; hexazinone exhibits low toxicity in acute rodent assays (LD50, 1690 mg/kg in male rats; Kennedy, 1984).

The laboratory larval exposure results also suggest that older larval stages are less sensitive to acute toxic exposure (Fig. 2). This is consistent with research demonstrating higher susceptibility of bivalve larvae to toxicants compared to adults (reviewed by His et al., 1999). While they did not consider larval susceptibility, Newell and Hidu (1986) noted that toxic materials generally have less damaging effects as soft-shell clams grow larger. In this study, juvenile exposure to 2,4-D levels > 0.5 ppm had no significant effect on mortality. In fact, juveniles exposed to 0.5 ppm 2,4-D exhibited ~20% higher survival rates than juveniles exposed to 0 ppm, 10 ppm or 50 ppm 2,4-D, translating to a 3 to 4 times larger predicted

population size. The survival advantage of a low-dose exposure may suggest a hormetic effect [a biphasic, dose–response phenomenon characterized by stimulation at low dose and inhibition at high doses (Calabrese, 2008)]. Although the current study was not designed to explore this phenomenon, numerous others have investigated hormetic responses in various aquatic species (Schreck, 2010;Cohen, 2006;Belgers, et al., 2007).

Juvenile soft-shell clam exposed to *any* level of 2, 4-D resulted in an initial growth delay compared to untreated controls. However, by 21 months post exposure, the mean GSI and shell length from all 2,4-D treatments significantly exceeded untreated control means (Fig. 3, Table 1). The physiological mechanism of increased growth and reproductive potential (as measured by GSI) is unknown, but warrants further investigation.

The hypothesis that larval mortality due to pesticide-exposure could impact populations was supported by the results of the matrix population model. Depending on the pesticide and stage exposed, decreased simulated population size and lower population growth rates relative to controls were observed (Fig. 5 and 6, respectively). In addition, lack of recruitment due to pesticide exposure led to shifts in the stable stage distribution of the simulated populations (Fig. 7). Previous studies found that that larval settlement was the most important factor influencing *Mya arenaria* population growth rate, and that survivorship of juveniles was also a significant factor (Brousseau and Baglivo, 1988). Consistent with those calculations, elasticity analysis of our population matrices indicated that population growth rate (λ) was most sensitive to changes in the growth and survival of juveniles (G12) and small adults (G23), as well as the survival of the smaller adult size classes (P2, P3). Brousseau et al. (1982) calculated the equilibrium settlement rate for a *M. arenaria* population in Gloucester, MA, and determined that only 1 egg out of about 68,400 must survive to the 2 mm juvenile stage in order to sustain the population, but noted that settlement in the field was highly variable. In addition, post-settlement mortality can be significant and variable among years and locations (Beal and Kraus, 2002;Beal, 2006). Highly variable recruitment patterns make it difficult to predict population dynamics. Ripley and Caswell (2006) recently incorporated seasonal recruitment stochasticity into a matrix population model for *M. arenaria*, finding that as recruitment variability in the population increased, the survival of adult stages became more important in determining population growth. Our model did not include stochastic recruitment, but the results are generally consistent with the previous models. In simulations where larval mortality was high, as in the 2,4-D exposures, elasticity analysis indicated that population growth rate was more sensitive to adult survival (*i.e.*, matrix element P3, the probability that 30-39.9 mm clams would survive and stay in that size class, elasticity values ranging from 17-21%). In contrast, when larval mortality was low (*e.g.*, 0 ppm controls for all pesticides), population growth rate was most sensitive to juvenile growth and survival (G12, elasticity values 17-20.5%)

The population model used in this study has some important limitations. Notably, the model did not include any effects on adult fecundity that might result from adult exposure to pesticides. In this regard, 2,4-D has the most interesting possible effects. For example, the faster growth of 0.5 ppm exposed juvenile clams is predicted to enhance population growth while another study of adult exposure to ≤ 5 ppm 2,4-D (Butler et al., 2003) demonstrated a disruption in gametogenesis, which could have a significant impact on the population. Similarly, the model relied on modifying the life-history parameters determined for a population of soft-shell clams in southern New England (Brousseau, 1978a); comprehensive life history data for Eastern Maine soft-shell clam populations would improve the model. Although it is possible that we may have under- or overestimated larval survival rates used in the model by extrapolating from 7 day observations to 14 days in the laboratory experiments, predicted recruitment success for the no-exposure simulations was similar to that reported for

field populations. As noted above, incorporating stochastic recruitment is probably more likely to affect the predictions of the model.

Agricultural pesticide, herbicide and fungicide run-off into coastal and estuarine waters can have significant impacts on the organisms that live in these watersheds. For example, excessive run-off from tomato fields has resulted in fish kills in South Carolina (Scott et al., 1990) and may have contributed to the mortality of cultured clams in Virginia (Brady et al., 1999). Arnold et al. (2004) demonstrated that larval clams (*Mercenaria mercenaria* and *Mulinia lateralis*) are strongly sensitive to copper, which is applied as a fungicide on tomato fields, and that controlling run-off from farms can prevent toxic impacts on clams and other non-target organisms. We know of no data on the concentrations of 2,4-D, hexazinone or phosmet in Maine's near-coastal marine waters, but several studies have examined pesticide content in ground water and surface waters near agricultural lands. In a 2005 survey of 17 wells near small grain fields conducted as part of the Maine groundwater sampling program, 2,4-D was detected in water samples only once, at 0.41 ppb (Maine Board of Pesticides Control, 2006a). Hexazinone has been detected in ground water in Maine at 0.20-10 ppb (Perkins et al., 2006). Metabolites of hexazinone have also been detected, but their toxicity is unknown. Maine Board of Pesticide Control drift studies conducted from 2000 to 2004 detected phosmet in the Narraguagus and Pleasant Rivers (or their tributaries) within 24 h of aerial spraying on nearby blueberry fields at values ranging 0.08 to 3.76 ppb (Jackson, 2004). Thus, although best management practices seem to limit the seaward drift of some insecticides applied on coastal waterfront properties (Maine Board of Pesticides Control, 2006b, 2007), coastal run-off of agricultural and residential pesticides may occur. The concentration of pesticides in coastal marine waters is unknown. If it is within the range of pesticide levels detected in groundwater and surface waters in agricultural fields in Maine, then only the lowest pesticide concentration (0.5 ppm) tested in this study would be environmentally relevant. Notably, we found that veliger exposure to 0.5 ppm 2,4-D, hexazinone or phosmet significantly decreased larval survival, resulted in lower modeled population growth rates and reduced predicted population sizes by one to two orders of magnitude compared to those predicted for unexposed veligers. Higher, transient exposures such as those caused by runoff, or accidental spills, may also have an impact on the local clam populations. Considering the potential impact on soft-shell clam populations, further studies of the coastal environment are warranted.

Conclusions

Laboratory exposures to 2,4-D, hexazinone and phosmet caused significant mortality of veliger and pediveliger soft-shell clams. Laboratory results were used to modify parameters of a stage-specific matrix population model. Model results indicate that exposure to pesticides during early larval stages can cause significant changes in predicted recruitment, population growth, and stable-stage distributions. These changes depend on both the larval stage exposed and the pesticide used. Of the pesticides examined, 2,4-D had greatest predicted impact on population dynamics. Historical declines in Maine soft-shell clam populations are likely to be the result of multiple factors. Data for pesticide concentrations in coastal marine waters are necessary to help clarify what levels of agricultural pesticide run-off might influence clam populations. Modeling results suggest that spraying pesticides or herbicides on agricultural fields adjacent to coastal clam flats after new recruits have settled could reduce any negative impact on clam populations.

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Figure 1.

Linked matrix population model structure. A) Larval model used to predict recruitment success with veliger (V), pediveliger (PV), and three spat stages (S1-S3). Recruitment success was determined as the proportion of 10,000,000 larvae surviving to stage S3, and this proportion modified fecundity terms in the adult model. P_i indicates the probability that an individual survives and stays in a given stage from one time step to the next, G_{ij} indicates the probability of surviving and moving into the next stage. B) Transitions in the adult clam model, including juveniles (J), and four size-classes of adult clams (A1 – A4). P_i indicates the probability that an individual survives and stays in a given stage from one time step to the next, G_{ij} indicates the probability of surviving and moving into the next stage, and F_i indicates the fecundity of clams in each adult size class.

Figure 2.

Mya arenaria larval survival following 24-hour laboratory pesticide exposure. Replicate larval exposures [veligers (A-C) or pediveligers (D-F)] to 2,4-D (A, D), hexazinone (B, E) or phosmet (C, F) were monitored for growth and survival for 7 days post-exposure. Lines indicate best-fitting regressions forecasting survival to day 14, the typical duration of either larval stage.

Figure 3.

Survival and growth of *Mya arenaria* exposed to 2, 4-D as juveniles and grown out in ambient sea water conditions; the experiment began with ~400 juveniles per treatment. (A) Cumulative percent of clams surviving. Within a single month, ** indicates percent survival significantly greater than all other treatments and * indicates percent survival significantly less than control (Tukey-type comparisons among proportions, $\alpha = 0.05$) (B) Mean shell length of surviving clams, error bars indicate 95% confidence intervals. Within a single month, means with different letters are significantly different from one another (Bonferroni comparisons, $p < 0.05$). See text section 3.1 for repeated measures ANOVA results.

Figure 4.

Larval matrix model results. Effects of pesticides and stage exposed on *Mya arenaria* predicted survival to spat. (A) 2,4-D exposure, (B) hexazinone exposure, (C) phosmet exposure. Within each pesticide and stage exposed, the predicted proportion of larvae surviving to spat is significantly greater in the 0 ppm treatment than in all other exposure levels except for the 10 ppm hexazinone-exposed veligers (Dunnnett's tests on proportions, $\alpha=0.05$). In the field, survival to spat is estimated to be $< 0.1\%$.

Figure 5.

Adult matrix model results. Effects of pesticides and stage exposed on simulated *Mya arenaria* populations. Predicted population size after 20 years given larval exposure to (A) 2,4-D, (B) hexazinone, or (C) phosmet. Juvenile exposure was modeled for 2,4-D. Dashed line indicates the initial population size in the simulations.

Figure 6.

Adult matrix model results. Effect of pesticide exposure at early life stages on population growth rate of *Mya arenaria*. The stage-specific matrix population model incorporated the effect of veliger (■), pediveliger (▲) or juvenile (●) *Mya arenaria* exposure to 2,4-D, hexazinone, or phosmet on recruitment success. Population growth rate (λ) was determined from the model; values < 1 indicate an exponentially declining population and > 1 indicate an exponentially growing population. Closed symbols indicate λ obtained using best-fit regression equations to predict 14-d larval survival following pesticide exposure (see fig. 2); open symbols indicate the range of λ obtained when upper and lower 95% confidence limits for the regression equation parameters were used to predict larval survival, and include variation in the developmental delay parameter estimates. *N.B.* These ranges are not 95% confidence intervals around a mean value of λ .

Figure 7.

Adult matrix model results. Pesticide-induced alterations in *Mya arenaria* stable stage distributions (*i.e.*, the relative abundance of each size class in the population). In the model, and in Maine, *Mya arenaria* begin reproducing at approximately 20 mm shell length and spend approximately 1 year in each subsequent size class.

Table 1

Gonadal-Somatic Index for clams exposed to 2,4-D as juveniles. Following a 24-hour exposure, clams grew out in ambient conditions for 21 months, then pre-spawn individuals were sacrificed, n=20-24 clams per treatment. 2,4-D exposure level had a significant effect on GSI (ANOVA: MS = 0.03, $F_{3,87}=7.52$, $p=0.0002$). Means followed by different letters are significantly different from one another (Bonferroni multiple comparisons, $p<0.005$).

2,4-D Treatment Level	Mean (95% CI) GSI
0 ppm	0.42 (0.03) A
0.5 ppm	0.48 (0.03) B
5 ppm	0.50 (0.02) B
10 ppm	0.49 (0.02) B