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LINKING MECHANISTIC TOXICOLOGY TO POPULATION MODELS IN FORECASTING RECOVERY FROM CHEMICAL STRESS: A CASE STUDY FROM JACKFISH BAY, ONTARIO, CANADA

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Abstract: Recovery of fish and wildlife populations after stressor mitigation serves as a basis for evaluating remediation success. Unfortunately, effectively monitoring population status on a routine basis can be difficult and costly. In the present study, the authors describe a framework that can be applied in conjunction with field monitoring efforts (e.g., through effects-based monitoring programs) to link chemically induced alterations in molecular and biochemical endpoints to adverse outcomes in whole organisms and populations. The approach employs a simple density-dependent logistic matrix model linked to adverse outcome pathways (AOPs) for reproductive effects in fish. Application of this framework requires a life table for the organism of interest, a measure of carrying capacity for the population of interest, and estimation of the effect of stressors on vital rates of organisms within the study population. The authors demonstrate the framework using linked AOPs and population models parameterized with long-term monitoring data for white sucker (*Catostomus commersoni*) collected from a study site at Jackfish Bay, Lake Superior, Canada. Individual responses of fish exposed to pulp mill effluent were used to demonstrate the framework's capability to project alterations in population status, both in terms of ongoing impact and subsequent recovery after stressor mitigation associated with process changes at the mill. The general approach demonstrated at the Jackfish Bay site can be applied to characterize population statuses of other species at a variety of impacted sites and can account for effects of multiple stressors (both chemical and nonchemical) and dynamics within complex landscapes (i.e., meta-populations including emigration and immigration processes). *Environ Toxicol Chem* 2015;34:1623–1633. © 2015 SETAC

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INTRODUCTION

Examining the viability of chemically impacted populations is a primary goal of ecological risk assessment [1–7]. Modeling approaches link individual-level responses recorded in the laboratory or field and risk to the population as a whole and serve as a key component for analysis of population-level impacts of chemical stressors. The application of models offers an effective methodology for addressing the toxicity of an increasing number of chemicals and chemical mixtures for risk assessment [3,4,8]. Furthermore, population modeling, in combination with field-based monitoring assessments, may provide the ability to predict population dynamics over time and across sites in response to remedial actions [5,7,8–12].

Ankley et al. [13] defined an adverse outcome pathway (AOP) as a conceptual construct that portrays existing knowledge concerning the linkage between a direct molecular initiating event (i.e., an interaction between a xenobiotic and a specific biomolecule) and an adverse outcome at a biological level of organization relevant to risk assessment. The AOP concept has received substantial attention in the context of screening and testing single chemicals (see review by Garcia-Reyero [14]) with, for example, international efforts sponsored by the Organisation for Economic Co-operation and Development (OECD) focused on the standardization and implementation of AOPs for regulatory applications [15]. In conjunction with the OECD effort, an open-access AOP Wiki recently was made available for development and archiving of a variety of AOPs for both mammalian and non-mammalian species [16]. Although emphasis on the AOP concept has focused on prospective assessment scenarios, the framework also has significant potential utility in the context of retrospective or diagnostic ecological risk assessments [17]. For example, through the depiction and causal association of events across biological levels of organization, it is possible to link mechanistic (e.g., biomarker) data to apical responses that can be directly incorporated within the computational structure of a population model to examine population status and thus potential ecological risk [13,18].

In the present study, we demonstrate the practical utility of AOPs for ecological risk assessment of chemicals in the field by showing how conceptual pathway depictions facilitate the use of biochemical endpoints for forecasting chemical impacts in combination with a population model for white sucker (*Catostomus commersoni*). Specifically, we apply AOPs in combination with a population model to examine the effects of effluent from a bleached kraft pulp mill located in Terrace Bay, Ontario, Canada on the white sucker population at Jackfish Bay, Lake Superior, Canada.

The white sucker population at Jackfish Bay, a Great Lakes Area of Concern, has been monitored as part of ongoing field studies that collectively span over 20 yr [19–21]. For the present

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analysis, we used effects-based monitoring data from 1998 to 2007 at the Jackfish Bay site in combination with a population model for white sucker developed by Miller et al. [7]. That study described construction and application of a density-dependent population model for white sucker exposed to pulp mill effluent at the Jackfish Bay site to examine the impact on population status for the period of 1988 to 1994. That timeframe is representative of exposure conditions prior to implementation of process changes at the pulp mill associated with modernization, which should conceptually result in partial remediation to the site and, thus, diminished effects. In 1995, the mill at Terrace Bay, Ontario, Canada, underwent a series of major process changes associated with effluent quality, including continual production of elemental chlorine-free pulp and the creation of a spill elimination basin to prevent untreated effluent from being released [21].

In the present study, we build on the work by Miller et al. [7] and consider data from 1988 to 1994 and from 1995 to 2007 as representative of the period following remedial changes at the mill (i.e., before and after the remedial process changes took place). Although the Jackfish Bay site has been researched routinely as part of effects-based monitoring studies, the implementation of population modeling to investigate projected population status under different management scenarios could be envisioned at any of a number of impacted sites. The present study demonstrates how the AOP concept can be used with a population model to investigate a before–after control-impact design analysis, whereby the reduction in effluent toxicity at Jackfish Bay can be correlated with the demographic parameters becoming more similar to an unimpacted control site over time. The modeling construct demonstrated in the present study is useful for investigation at other field locations (or for other fish species) where similar types of effects-based monitoring are being conducted in conjunction with ongoing remediation activities.

METHODS

Study site background

We analyzed data collected over a 20-yr time span that began in 1988 and continued intermittently until 2007 at 2 study sites located in Lake Superior. The Jackfish Bay study site, located at 48°50′N, 86°58′W, receives effluent from the bleached-kraft pulp mill located in Terrace Bay, Ontario, Canada, via Blackbird Creek, a tributary that enters Jackfish Bay on Lake Superior. White sucker are found abundantly in Jackfish Bay and migrate through Jackfish Lake on their way to spawn in Sawmill Creek. The Jackfish Bay site is an unpopulated and isolated site, without permanent residents, industrial activity, or agriculture. The pulp mill effluent is the only known source of contamination to the site. From 1988 through 2007, the mill produced as much as 1200 air-dried metric tons of pulp per day associated with as much as 120 000 m^3 effluent per day [21]. A reference site at Mountain Bay (46°56′N, 87°58′W), located approximately 60 km west of the Jackfish Bay study site, has been used as a reference site for Jackfish Bay monitoring studies. The white sucker population at the Mountain Bay study site is exposed to minimal industrial or municipal pollution [21] and spawns in the Little Gravel River [19]. The Mountain Bay and Jackfish Bay study sites have a comparable fish community structure [19–21]. Although environmental variation (i.e., temperature and food availability) contributes to intra-annual variability in field measurements at each site, the long-term monitoring records for the 2 sites demonstrate concordance in between-year variations across measures between these sites [21].

Over the years of study at these locations, fish length, total weight, age, gonad weight, and liver weight were consistently measured and recorded. In estimating total fecundity for female fish, ovarian tissue was collected, and the number of eggs was counted in the 1-g sample and extrapolated to the entire gonad. Plasma was collected for the measurement of testosterone (T) in both sexes [19–21].

Population model

Miller et al. [7] described a density-dependent logistic matrix model for white sucker exposed to the Jackfish Bay pulp mill effluent for the period of 1988 to 1994. This time period would be representative of maximum exposure conditions before process changes at the mill. This model was used to evaluate the effects of the pulp mill effluent on white sucker population status. Population size was projected under continuous exposure conditions (without remediation) over a 20-yr period and resulted in an estimated equilibrium population size that was limited to approximately 71.0% of carrying capacity [7].

Expanding on the analysis of Miller et al. [7], in the present study we consider recovery after mitigation of the pulp mill effluent in Jackfish Bay. As an initial step, we modeled population recovery scenarios in the post-1995 remediation period. We employed the Leslie projection matrix [22,23] for white sucker developed by Miller et al. [7], which had an intrinsic rate of increase of 0.25 (Figure 1), in combination with previously described population modeling approaches [2,7,24–27]

$$\mathbf{n_{t+1}} = \exp(-rP_t/K)\mathbf{M_1 n_t} \quad (1)$$

In Equation 1, $\mathbf{n_{t+1}}$ is the vector of population age structure at time $t+1$, $\mathbf{n_t}$ is the vector of population age structure at time t, r is the intrinsic rate of increase, P_t is the population size at time t, K is carrying capacity, and $\mathbf{M_1}$ is the Leslie matrix containing vital rates (survivorship and fertility) that have been adjusted to include an age-specific percentage reduction in reproduction or survival rates over the time step t resulting from an exposure (i.e., exposure to pulp mill effluent in the present study). Thus, $\mathbf{M_1} = \mathbf{M} - \mathbf{C_t}$, where $\mathbf{C_t}$ is a matrix representing the discount to vital rates resulting from exposure to the stressor at time t. The matrix $\mathbf{C_t}$ is represented by elements $c_{i,j}$, $i = 1, 2, \ldots, n$; $j = 1, 2, \ldots, n$. Within the matrix $\mathbf{C_t}$, $c_{1,j} = \alpha_j \times F_j$ for $j = 1, 2, \ldots, n$, where α_j is the percentage discount to the fertility rate of age class j (F_j). Furthermore, $c_{i+1,i} = \beta_i \times S_i$ for $i = 1, 2, \ldots, n-1$, where β_i is the discount to the survival rate corresponding to age class i (S_i). All other elements of $\mathbf{C_t}$ are equal to zero. In addition, compensation is achieved by density dependence that acts cumulatively over life stages and generations to stabilize the population [28]. The model (Equation 1) thus provides a factor through which toxic chemicals affect the Leslie matrix elements related to baseline reproduction values.

When applied to field populations for which detailed information has been collected for the effect of density on individual age classes, Equation 1 offers the versatility of replacing the scaling factor exp (–rP_t/K) with age-specific density-dependent functions. This could be accomplished in Equation 1 by replacing exp(–r P_t/K) with a diagonal matrix of age-specific density-dependent functions. Alternatively, when field data exist such that density-dependent functions could be precisely estimated for each vital rate within the matrix $\mathbf{M_1}$, the Leslie matrix in Equation 1 can then be separated into a matrix for births and a matrix for deaths [29–32], and separate density-dependent functions could be applied to each process. A complete

$$\mathbf{M} = \begin{bmatrix}
0 & 0 & 0 & 0.2 & 2.6 & 9.9 & 11.6 & 13.2 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 & 14.8 \\
0.62 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0.62 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0.62 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0.62 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
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0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0.9 & 0
\end{bmatrix}$$

Figure 1. Leslie projection matrix (**M**) for white sucker (*Catostomus commersoni*).

derivation of the population model used in Equation 1 can be found in Miller [27].

The logistic model of population dynamics is commonly used in population ecology as a way to understand the cause and effect relationship between carrying capacity and population size [33–39]. In the absence of limitations on resources (i.e., space and food), populations would grow exponentially. When resources are limited, however, the growth rate begins to decelerate until equilibrium (carrying capacity) is reached. In application of the model presented in the present study, the carrying capacity was held constant for all simulations. The estimate of carrying capacity does not include consideration of chemical stress; therefore, under conditions of chemical stress that affect vital rates, the population reaches equilibrium below carrying capacity. Thus, the model has the ability to project trajectories of population size over time associated with a given change in vital rates, as well as the resulting expected change in equilibrium population size at a threshold below carrying capacity. To provide an indication of relative impact, output of the model can be expressed invariant of carrying capacity by plotting population size proportional to carrying capacity at each time step in the model, as opposed to evaluation on the basis of absolute numbers [7,24,25]. In demonstrating model output in this manner, a value of 1.0 represents a population at carrying capacity, and values between 0 and 1.0 represent fractional population sizes relative to carrying capacity. The population recovery trajectories were estimated under multiple model scenarios whereby the effect of exposure on fish reproduction (fecundity reduction and delay of age at spawning) was altered by 25%, 50%, 75%, and 100% (full recovery) after the exposure period of 1988 to 1994. Remedial process changes were initiated at the mill in 1995, and population recovery would then, in theory, follow. Application of the population model allows for estimation of the extent and trajectory of population recovery. This was addressed in the model by scaling the elements within the matrix $\mathbf{C_t}$ to represent the corresponding reduction in the effect of the exposure on fish reproduction. For all simulations, the population was initiated at carrying capacity and at the stable age distribution, as determined by finding an eigenvector (right eigenvector) associated with the dominant eigenvalue of the matrix [22,23,38,40].

Defining relevant AOPs

In applying AOPs that correspond to effects observed in the field, it is necessary to have an easily monitored pathway-specific endpoint that can be linked, ideally in a quantitative manner, to the adverse outcome(s) of concern. These endpoints typically will be intermediate key events within a given AOP [13]. The first step is to explicitly identify adverse outcomes observed in the field, based on the collected data. In the present study, 2 adverse outcomes were recorded in exposed fish: decreased fecundity (number of eggs produced per female) and delay in maturation of females (as reflected by the difference in the distribution of age structure of breeders at the impacted Jackfish Bay site in comparison with the reference site at Mountain Bay [7]). A subsequent step is to then identify potentially useful data from lower biological levels of organization based on 2 factors: plausible linkage to the adverse outcome(s) of concern and availability of a robust dataset for the population/situation of concern. In the present study, our analysis focused on the steroid T; past studies at Jackfish Bay have shown that exposure to the pulp mill effluents decreases T production in the white sucker, suggesting the presence of chemicals that inhibit sex steroid synthesis [41,42]. Importantly, T was measured consistently as part of the effects-based monitoring work at the Jackfish Bay site over the 20-yr period of 1988 through 2007.

Testosterone concentrations can be associated with both adverse outcomes observed in white suckers from Jackfish Bay. In terms of egg production, previous research with another cyprinid, the fathead minnow (*Pimephales promelas*), has shown that endocrine-active chemicals that decrease steroid synthesis (including T) in females also depress egg production via a relatively well-defined AOP [13]. Briefly, in vertebrates, T serves as the metabolic precursor to 17β-estradiol (E2) via a reaction catalyzed by cytochrome P450 aromatase. An important role of E2 in oviparous females is stimulation of the hepatic production of the egg yolk precursor protein vitellogenin (VTG), through activation of the estrogen receptor. Vitellogenin subsequently is released to the bloodstream, taken up by the ovary, and incorporated into developing oocytes. Consequently, chemicals that reduce steroid synthesis (and, hence, estrogen receptor activation) will reduce VTG synthesis and, subsequently, egg production (fecundity). This relationship serves as a basis for linking biochemical indicators of endocrine function (T, E2, VTG) to a vital life history trait (fecundity) that can then be incorporated into population models to explore the potential higher-level effects [18,24,25]. In the present study, the steroid synthesis inhibition–reproduction AOP developed for the fathead minnow was used to hypothesize a linkage between field observations of decreased T and fecundity in white sucker

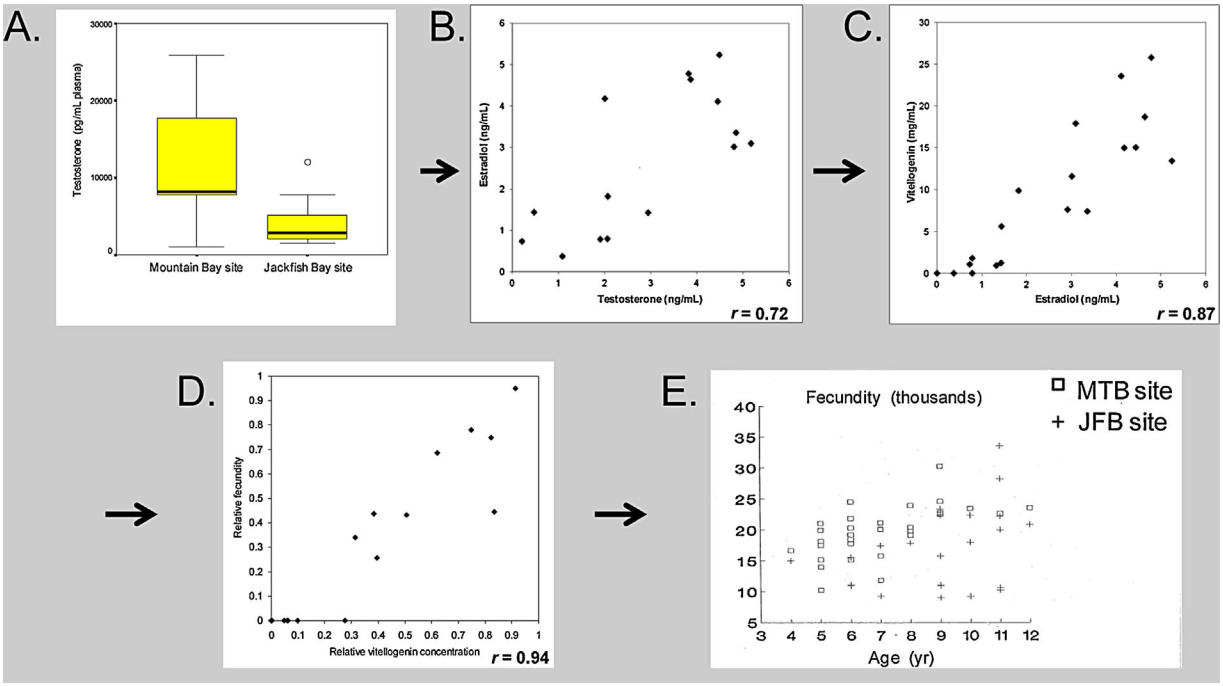

Figure 2. The adverse outcome pathway framework was used as an organizing principle that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome at a biological level of significance relevant to inclusion in the population model. (**A**) We observed decreased testosterone (T) for white sucker at Jackfish Bay (JFB) relative to Mountain Bay (MTB; ON, Canada) [21]. (**B**) A biologically plausible relationship exists, and we have documented in laboratory studies that decreased T is correlated to decreased estradiol [25]. (**C**) A biologically plausible relationship exists, and we have shown in laboratory studies that decreased estradiol is correlated to decreased vitellogenin [25]. (**D**) We have demonstrated in laboratory studies that decreased vitellogenin is correlated to decreased fecundity [24]. (**E**) Decreased fecundity is observed for white sucker at JFB relative to MTB [20].

at Jackfish Bay relative to Mountain Bay [20,21] (Figure 2). This linkage suggested T as a valid pathway-specific endpoint that could be used to formulate a quantitative relationship with fecundity within the population model.

Although no AOP exists that documents a direct linkage between T and age at first spawning (or maturity) of females, there is evidence of a correlation between T and spawning activity in female fish. For example, studies with female salmonids have reported that plasma T concentrations increase with advancing maturity of eggs [43–45], and Scott et al. [46] found that T concentrations in the blood of white suckers were at their highest levels in females that had just spawned. However, most studies evaluating relationships between plasma steroid levels and oocyte development, maturation, and release have shown that other plasma steroids (e.g., E2) also vary depending on maturity of the fish, often in a manner parallel to T. Accordingly, these observations do not establish the sort of causal link between plasma T and sexual maturity in female fish required to be considered an intermediate key event within an AOP. For the purpose of this illustrative example with the Jackfish Bay white sucker data, however, we believe it is reasonable to base the analysis on an established correlative association between T and maturity in female fish.

Incorporating AOP data into the population model

Based on the biologically plausible linkages between T and observed adverse outcomes, we developed quantitative relationships between T in female white sucker and reproduction using field data collected at the Jackfish Bay and Mountain Bay study sites. Then, we linked these quantitative relationships with a population model to forecast population dynamics for the white sucker population at the impacted study site at Jackfish Bay (Figure 3).

We examined measurements for fish collected in the spring of each year at both the Jackfish Bay and Mountain Bay study sites using hoop or trap nets set across the spawning streams to capture the white sucker as they ascended to spawn. Ages of female fish were determined by counting annular rings on dried opercula (a detailed description of field methods can be found in McMaster et al. [19] and Munkittrick et al. [20]), and female breeders were stratified according to study site and year of collection for use in data analysis and construction of the population model [7].

Plasma T concentrations were compared for breeders of each age class between the 2 sites. For each year, the difference in T for each class was expressed as a proportion of T for breeders in the respective age class of the unexposed population (Mountain Bay site). As remedial process changes were implemented at the Jackfish Bay study site in 1995 [21], we stratified the data into preremediation and postremediation periods. The differences in T between age classes for a given year were then averaged over all years for which data were available within either the preremediation or postremediation periods.

Previous analyses for the period of 1988 to 1994 demonstrated site-specific differences in fecundity that occurred across all age classes, with breeding females at the Jackfish Bay site having consistently lower fecundity [7]. We expanded that analysis by comparing age-specific fecundity measurements (eggs/female) for breeders of each age class between the 2 sites for the postremediation time period. For each year, the difference in fecundity for each class was expressed as a proportion of fecundity for breeders in the respective age class of the unexposed population (Mountain Bay site). The differences in fecundity between age classes for a given year were then averaged over all years for which data were available within either the preremediation or postremediation time periods.

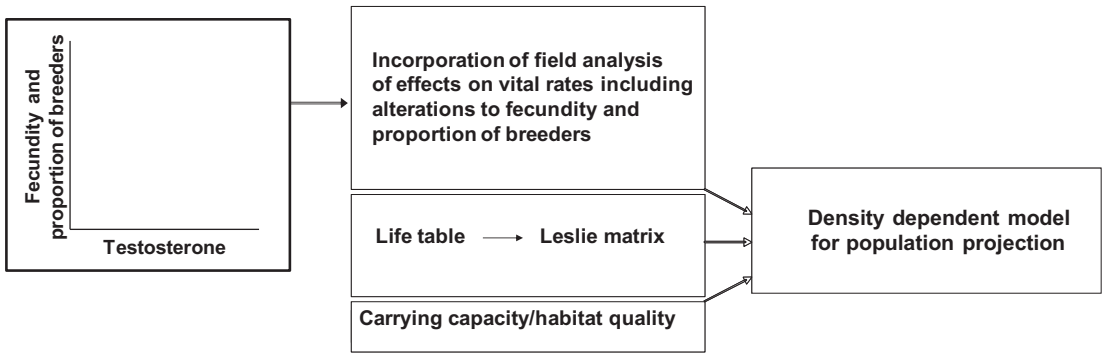

Figure 3. Qualitative overview describing the application of the adverse outcome pathway (AOP) in combination with the density-dependent logistic matrix model of Equation 1 in terms of input parameters and model output. In the present application, the AOP was used to affirm a linkage between field observations for decreased testosterone for white sucker at Jackfish Bay relative to Mountain Bay (ON, Canada) [21] and decreased reproductive capacity observed for white sucker at Jackfish Bay relative to Mountain Bay [20].

We produced a scatter plot of relative fecundity versus relative T corresponding to age classes for which the data allowed for an interannual comparison between the 2 sites. A Pearson correlation coefficient was computed to assess the strength of relationship between relative fecundity and relative T, and the 2 variables were regressed against one another, resulting in a nonlinear regression function with a minimum residual sum of squares ($S_{y,x}$), which allowed for estimation of fecundity of fish at the Jackfish Bay site relative to the Mountain Bay site based on observed T concentrations and fecundity measures at the 2 sites.

Our previous analysis of the 1988 though 1994 data demonstrated a delay in age at reproduction at the Jackfish Bay site compared with the Mountain Bay site for age classes 5 yr through 9 yr. In the present study, we evaluated the postremediation time period for these same age classes. For each year, the difference in breeders of each class was expressed as a proportion of breeders in the age class of the unexposed population. The percentage differences in breeders of each age class were then averaged over all years for which data were available. We produced a scatter plot, a Pearson correlation coefficient, and a nonlinear regression function with a minimum residual sum of squares ($S_{y,x}$), which allowed for estimation of proportion of breeders at the Jackfish Bay site relative to the Mountain Bay site based on observed T levels at these sites.

The model of Equation 1 provides a calculation through which toxicants affect the Leslie matrix elements related to baseline reproduction values. In application of Equation 1, the effect of changes in T concentrations in females on population size can be investigated, given that quantitative relationships between T and fish reproduction can be used in formulation of $\mathbf{C_t}$ to adjust age-specific values of fertility rates over the time step t, resulting from chemical exposure. Using Equation 1 in connection with quantitative relationships between T and fecundity, as well as T and delay in age at reproduction, formulated in the present study, we demonstrated the application of the population modeling construct to investigate population recovery scenarios for white sucker at the Jackfish Bay site relative to the Mountain Bay site for recovery in the post-1995 remediation time period. We estimated how a reduced impact on the T levels at the Jackfish Bay site would result in an improved reproductive capacity and corresponding change in fertility rates and population status. The population recovery trajectories were estimated under multiple model scenarios whereby the effect of exposure on fish T levels at the Jackfish Bay site was completely eliminated (0% effect or total recovery), or impacted by 25%, 50%, or 75% after the exposure period (1988–1994). All simulations were initiated at carrying capacity and at the stable age distribution. The population model was executed over a 20-yr simulation period, and results were recorded using an annual time step. The equilibrium population size for each scenario was determined by running the population model beyond the 20-yr simulation period.

RESULTS

Population recovery trajectories were determined under multiple model scenarios (Figure 4). Recovery was initiated in 1995, corresponding to the remedial process changes at the mill. In comparison with a full recovery that results in an equilibrium population size that approaches carrying capacity, remedial actions that decrease effects on fish reproduction by 25% would be expected to result in an equilibrium population size that approaches 78.0% of carrying capacity. Similarly, if remediation decreased effects on fish reproduction by 50%, the fish population would recover to approximately 85.2% of carrying capacity. Finally, a 75% reduction in impact would result in the population recovering to approximately 92.6% of carrying capacity.

By using the AOPs in conjunction with the population model, we developed statistical relationships between plasma T concentrations in female white sucker and both fecundity and relative proportion of breeders based on field data collected at the study sites (Figure 2). In comparing the Jackfish Bay and Mountain Bay sites, Table 1 describes the difference in T of breeders of a given age class, expressed as a proportion of T of breeders in the respective age class of the unexposed population. As demonstrated in Table 1, there is a large difference between T of the age classes at the Jackfish Bay site compared with the Mountain Bay site during 1988 to 1994. After 1995, there is a notable change in the difference in measurements between the Jackfish Bay and Mountain Bay sites, either with the difference becoming smaller or with some age classes actually having greater T concentrations at the Jackfish Bay site (Table 1). The difference in fecundity of breeders of a given age class, expressed as a proportion of fecundity of breeders in the respective age class of the Mountain Bay site for both the pre-1995 and the post-1995 periods, was analyzed (Table 2). After 1995, there is a notable change in the difference in measurements between the Jackfish Bay and Mountain Bay sites, with improvements in

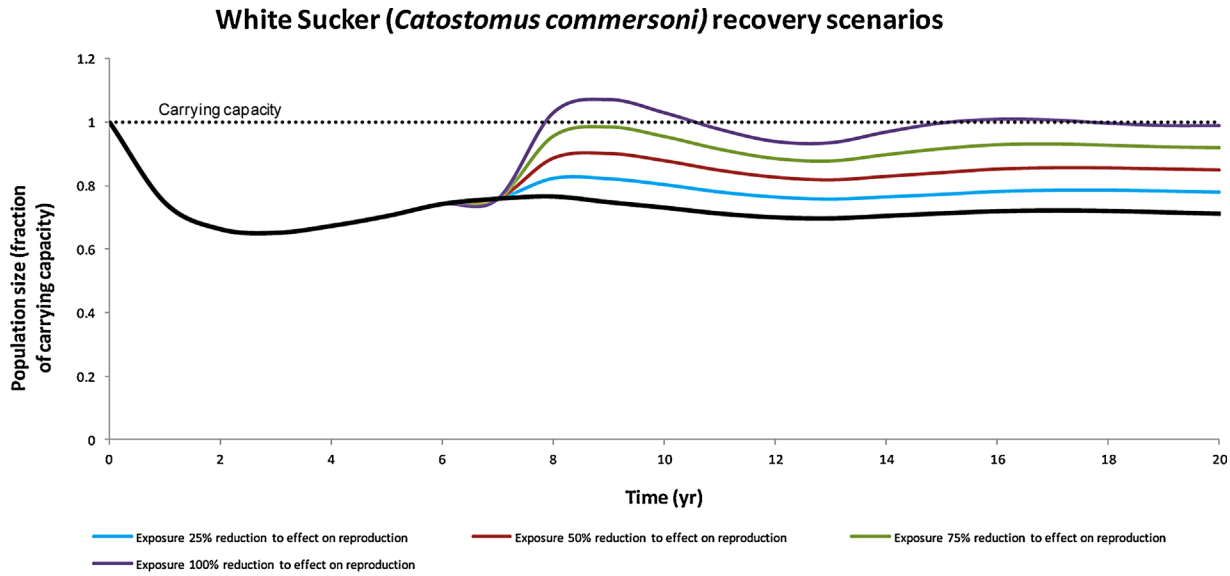

Figure 4. Application of the population modeling construct to investigate population recovery scenarios for recovery in the post-1995 remediation time period. In this figure, the population recovery trajectories are estimated under multiple model scenarios whereby the effect of exposure on fish reproduction was reduced by 25%, 50%, 75%, and 100% (full recovery) after the exposure period of 1988 to 1994.

fecundity across all age classes at the Jackfish Bay site (Table 2). A statistically significant correlation was found between relative fecundity and relative T ($r = 0.60$, $p < 0.01$). Relative fecundity was regressed against relative T (Figure 5), resulting in the nonlinear regression function

$$\text{Relative Fecundity} = (\text{Relative Testosterone})^{0.121} \quad (2)$$

Table 1. Percentage of difference in testosterone (pg/mL plasma) expressed as a proportion of testosterone of breeders in the age class of the unexposed population (Mountain Bay site, ON, Canada)[a]

	Exposure						
	Pre-1995 remediation			Post-1995 remediation			
Age class	1989	1990	Average % difference	1996	2001	2003	Average % difference
4	N/S	N/S	N/S	N/S	N/S	N/S	N/S
5	N/S	N/S	N/S	N/S	N/S	−45	−45
6	N/S	−83	−83	N/S	N/S	N/S	N/S
7	N/S	−86	−86	N/S	−4	N/S	−4
8	−27	N/S	−27	N/S	98	−51	23
9	−67	N/S	−67	−38	N/S	—61	−49
10	−75	−86	−80	−78	N/S	N/S	−78
11	−92	N/S	−92	N/S	−44	N/S	−44
12	N/S	N/S	N/S	N/S	−56	N/S	−56
13	N/S	N/S	N/S	−90	22	N/S	−34
14	N/S	N/S	N/S	−62	N/S	N/S	−62
15	N/S	N/S	N/S	−65	N/S	−29	−47
16	N/S	N/S	N/S	N/S	N/S	34	34
17	N/S	N/S	N/S	N/S	N/S	N/S	N/S
18	N/S	N/S	N/S	N/S	N/S	4	4
19	N/S	N/S	N/S	N/S	N/S	N/S	N/S
20	N/S	N/S	N/S	N/S	N/S	N/S	N/S
21	N/S	N/S	N/S	N/S	N/S	N/S	N/S
22	N/S	N/S	N/S	N/S	N/S	N/S	N/S
23	N/S	N/S	N/S	N/S	N/S	N/S	N/S
24	N/S	N/S	N/S	N/S	N/S	N/S	N/S

[a]N/S refers to no sample comparison. This would include cases where no sample was obtained for at least 1 location for a given age class for a given year.

The nonlinear regression function of Equation 2 exhibited a standard deviation of the residuals ($S_{y,x}$) = 0.12. As part of evaluating the robustness of the relationship between T and fecundity of Jackfish Bay fish, we assessed the association between fish size and fecundity. Fish from the Jackfish Bay site had a statistically different relationship between size and fecundity when compared with fish from the Mountain Bay site. Breeders across all age classes, stratified by site, were examined from the fish collected during the preremediation period for both sites. For breeders at the Mountain Bay site, an analysis of fecundity regressed on weight for all breeders yielded the function

$$\text{fecundity} = -3688.37 + 24.93\,(\text{weight}) \quad (R^2 = 0.48,\ p < 0.000) \quad (3)$$

For breeders at the Jackfish Bay site, the resulting linear regression function for fecundity regressed on weight was

$$(\text{fecundity}) = 9014.91 + 12.11\,(\text{weight}) \quad (R^2 = 0.16,\ p = 0.019) \quad (4)$$

The results demonstrated that the slope of the regression function for fecundity regressed on weight for breeders at the Mountain Bay Site was 206% of the slope of the regression function for regressing fecundity on weight for breeders at the Jackfish Bay site. The coefficient of determination, R^2, for the function for breeders at the Mountain Bay site was 300% of the R^2 for the function for breeders at the Jackfish Bay site. Consequently, we concluded that differences between fecundity for breeders at the 2 sites could not be explained by fish size.

After 1995, there is a notable increase in relative proportion of breeders across all age classes at Jackfish Bay compared with measurements from the Mountain Bay site (Table 3). A Pearson correlation coefficient was computed to assess the relationship between relative proportion of breeders and relative T. There was a statistically significant correlation between the 2 variables

Table 2. Percentage of difference in fecundity (eggs/female) expressed as a proportion of fecundity of breeders in the age class of the unexposed population (Mountain Bay site, ON, Canada)[a]

	Exposure						
	Pre-1995 remediation	Post-1995 remediation					
Age class	Average % difference 1988–1994[b]	1996	2001	2003	2006	2007	Average % difference
4	−10	N/S	N/S	N/S	N/S	N/S	N/S
5	N/S	N/S	N/S	−7	N/S	N/S	−7
6	−36	N/S	N/S	N/S	N/S	N/S	N/S
7	−23	12	−32	N/S	N/S	7	−4
8	−12	N/S	−12	−12	8	N/S	−5
9	−17	−31	N/S	−4	34	15	3
10	−13	−25	N/S	N/S	−5	30	0
11	−8	4	−8	N/S	−7	33	5
12	−16	25	−7	N/S	N/S	9	9
13	−37	−5	−22	N/S	N/S	N/S	−14
14	N/S	−1	N/S	N/S	N/S	N/S	−1
15	N/S	3	N/S	2	N/S	N/S	2
16	N/S	N/S	N/S	23	N/S	N/S	23
17	−16	N/S	N/S	N/S	N/S	N/S	N/S
18	N/S	N/S	N/S	38	N/S	19	29
19	N/S	N/S	N/S	N/S	N/S	N/S	N/S
20	N/S	N/S	N/S	N/S	N/S	N/S	N/S
21	N/S	N/S	N/S	N/S	N/S	N/S	N/S
22	N/S	N/S	N/S	N/S	N/S	N/S	N/S
23	N/S	N/S	N/S	N/S	N/S	N/S	N/S
24	N/S	N/S	N/S	N/S	N/S	N/S	N/S

[a]N/S refers to no sample comparison. This would include cases where no sample was obtained for at least one location for a given age class for a given year.
[b]As taken from Miller et al. [7].

($r = 0.80$, $p < 0.05$). Relative proportion of breeders was regressed against relative T (Figure 6), resulting in the nonlinear regression function

$$\text{Relative Proportion of Breeders} = (\text{Relative Testosterone})^{0.504} \quad (5)$$

Table 3. Percentage of difference in breeders of each age class expressed as a proportion of breeders in the age class of the unexposed population (Mountain Bay site, ON, Canada)[a]

	Exposure						
	Pre-1995 remediation	Post-1995 remediation					
Age class	Average % difference 1988–1994[b]	1996	2001	2003	2006	2007	Average % difference
5	−94	N/S	−100	0	N/S	N/S	−50
6	−58	0	−100	0	0	N/S	−25
7	−61	0	0	N/S	N/S	0	0
8	−22	0	−67	0	0	0	−13.4
9	−26	−75	0	0	0	−34	−21.8

[a]N/S refers to no sample comparison. This would include cases where no sample was obtained for at least one location for a given age class for a given year. A value of 0 indicates that an equal or larger number of breeders occurred at the Jackfish Bay site relative to the Mountain Bay site.
[b]As taken from Miller et al. [7].

The nonlinear regression function of Equation 5 exhibited a standard deviation of the residuals ($S_{y,x}$) = 0.13.

We incorporated the regression functions of Equation 2 and Equation 5 in combination with the Leslie projection matrix for white sucker and the population model to evaluate population recovery scenarios for recovery in the post-1995 period. The quantitative relationships between T and both fecundity and proportion of breeders were employed to estimate how a reduced impact on plasma T concentrations at the Jackfish Bay site would result in a corresponding change in reproductive capacity and population status. Population recovery trajectories were estimated at the Jackfish Bay study site under multiple model scenarios (Figure 7). In comparison with a full recovery that results in an equilibrium population size that approaches carrying capacity, remedial actions that decrease the effect of the exposure on fish T levels by 25% would result in a 37.3% decrease of the effect of the exposure on fecundity, a 31.7%

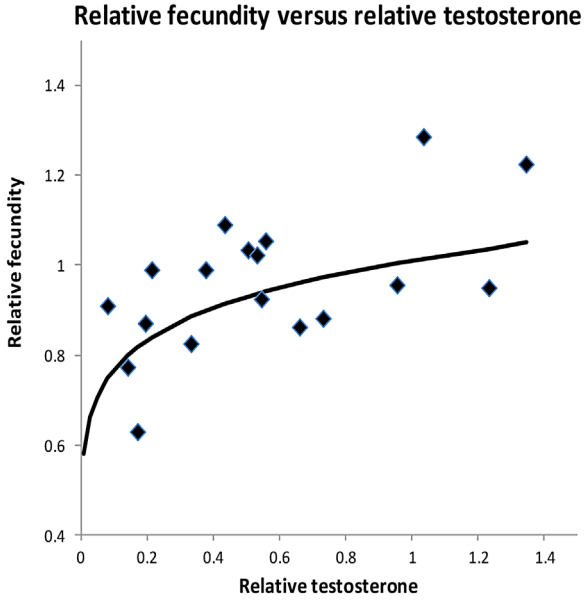

Figure 5. Relative fecundity was regressed on relative testosterone, using a nonlinear regression function: Relative Fecundity = (Relative Testosterone)$^{0.121}$.

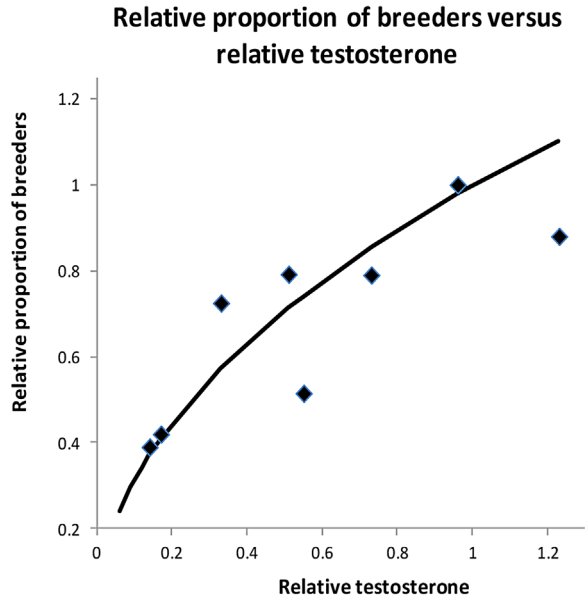

Figure 6. Relative proportion of breeders was regressed on relative testosterone using a nonlinear regression function: Relative Proportion of Breeders = (Relative Testosterone)$^{0.504}$.

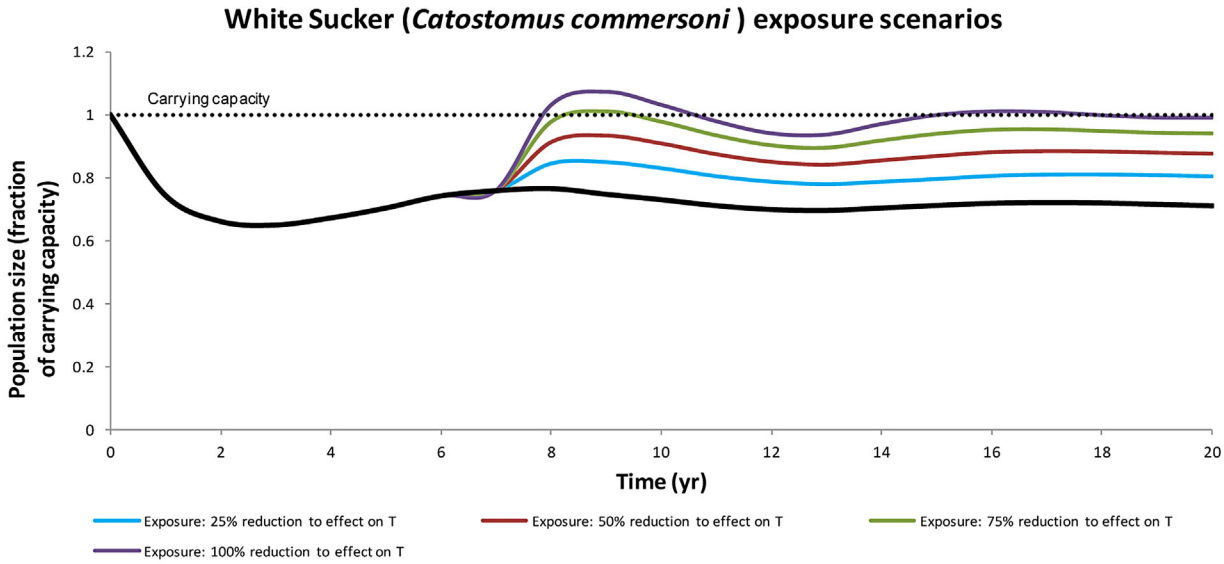

Figure 7. The quantitative relationships between testosterone and both fecundity and proportion of breeders were employed to estimate how a reduced impact on the testosterone levels (resulting in increased testosterone levels for breeders) at the Jackfish Bay (ON, Canada) site results in a corresponding change in reproductive capacity and population status. Population recovery trajectories were estimated at the Jackfish Bay study site under multiple model scenarios, whereby the effect of exposure on fish testosterone levels at the Jackfish Bay site was reduced by 25%, 50%, 75%, and 100% (full recovery) after the exposure period of 1988 to 1994. Population trajectories are based upon the functions: Relative fecundity = (relative testosterone)$^{0.121}$ and Relative proportion of breeders = (relative testosterone)$^{0.504}$ and a relative testosterone of 27.7% observed during the exposure period at Jackfish Bay (1988 to 1994).

decrease of the effect of the exposure on the proportion of breeders in age classes 5 through 9, and an equilibrium population size that approaches 80.5% of carrying capacity. In a similar manner, if remediation decreased the effect of the exposure on fish T levels by 50% (corresponding to an estimated 63.3% decrease of the effect of the exposure on fecundity and an estimated 57.5% decrease of the effect of the exposure on the proportion of breeders in age classes 5 through 9), the fish population would be anticipated to recover to approximately 88.0% of carrying capacity. If T concentrations were reduced by 75% (corresponding to an estimated 83.4% decrease of the effect of the exposure on fecundity and an estimated 80.0% decrease of the effect of the exposure on the proportion of breeders in age classes 5 through 9) at the Jackfish Bay site, this would result in the population recovering to approximately 94.5% of carrying capacity.

DISCUSSION

A population modeling framework such as that described in the present study provides a methodology for estimating population impacts by inference from observed responses at lower levels of biological organization. To be implemented in combination with field-based monitoring assessments, this framework requires either direct measurements of changes to apical endpoints or mathematical expressions relating changes in relevant molecular, biochemical, or histological responses to changes in apical endpoints. Ideally, these biomarkers will be quantitative. The AOP functions as a mapped reference from which to formulate biologically plausible and scientifically defensible relationships between potential biomarkers (e.g., intermediate key events within the AOP) and apical endpoints with relevance to population-level outcomes. Responses that can be experimentally linked to endpoints of regulatory concern as measured in laboratory studies (e.g., fertility, development, growth, survival, and reproduction) can be selected and used in conjunction with effects-based monitoring assessments in the field at impacted sites. The present study illustrates how the AOP framework can be implemented as an organizing tool whereby mechanistic data are translated into endpoints relevant to evaluation of ecological risk (in the present study, reproduction). Furthermore, the present study provides an example of the applicability of AOPs for ecological risk assessment of chemicals by demonstrating how a pathway-based understanding facilitates the selection of biochemical endpoints for monitoring in the context of forecasting chemical impacts in combination with a population model.

The use of molecular or biochemical endpoints to estimate effects of toxic chemicals on populations is advantageous because early key events within an AOP are, by definition, more sensitive to chemical stressors than adverse outcomes in the whole organism, more diagnostic of specific molecular initiating events (and, hence, potential stressors) than apical endpoints, and often more efficient to collect than longer-term responses at higher levels of biological organization [47]. In developing a framework that incorporates AOPs, effects-based monitoring, and population modeling, the key step is to formulate ecologically relevant quantitative relationships between responses at lower levels of organization and vital rates at the individual level that are relevant to and can allow for improved understanding at the population level. Vitellogenesis is one of several examples that illustrate a linkage of molecular initiating events to adverse outcomes at the individual level that subsequently can be evaluated in terms of population-level effects. In addition to the fathead minnow work on which a key AOP considered in the present study was based, Murphy et al. [48,49] developed models of hypoxia, Cd, and polychlorinated biphenyl impacts on Atlantic croaker populations through evaluation of the effects of these stressors on vitellogenesis.

In addition to effects on reproductive output, population status can be affected by survival. Several studies have focused on formulation of quantitative relationships between responses at lower levels of organization, survival at the individual level, and subsequent population-level consequences (see Kramer et al. [18] for review of this work in the context of AOP frameworks). One such example involves acetylcholinesterase, an enzyme that

mediates neurotransmitter-mediated signaling at synapses. Baldwin et al. [50] developed an AOP relating reductions in acetylcholinesterase activity to reductions in feeding behavior, food consumption, growth, and size in outmigration of subyearling chinook salmon (*Oncorhynchus tshawytscha*). Individual size was used to estimate size-dependent subyearling survival during the migration and transition to sea. Another example of using a biochemical response to investigate population status was provided by Cook et al. [51], who formulated a relationship between activation of the aryl hydrocarbon receptor by planar halogenated hydrocarbons and early life-stage mortality in lake trout in Lake Ontario.

Quantifying the relationship between molecular, biochemical, or histological endpoints and adverse effects in individuals is only the first step in using biomarker-type data for population-level risk assessment. To evaluate population-level outcomes that are useful for management decisions, one must be able to consider output from these quantitative relationships under different potential scenarios. In our analysis, we substituted various changes in T concentration into the regression models to formulate corresponding changes in fecundity and proportion of breeders. Inputting this information into the population model allowed for estimation of benefits of remedial actions using the currency of improved T measurements as a surrogate for improved measurements of both fecundity and proportion of breeders. For example, a 25% improvement in T concentrations in female white sucker at the Jackfish Bay site was predicted to result in a new equilibrium population size representing a 31.0% improvement to the exposure-induced deficit to the population carrying capacity (Figure 7). Likewise, a 50% or 75% reduction on the impact of T concentrations in breeding female white sucker would be expected to result in substantial improvements in population size over time and new equilibrium population sizes, representing 58.6% and 81.0% improvements, respectively (Figure 7). In addition to changes in equilibrium population size, trajectories produced by the modeling framework that are associated with a given reduced impact on the population enable consideration of the degree of recovery that would take place over a selected time interval. This would facilitate forecasting recovery of a population at a given point in time, and thereby an estimation of when the population would become reestablished to a defined, desired threshold.

Current research on the sources and characteristics of bioactive substances associated with pulp and paper mill effluents have led to a lack of definitive identification of the responsible compounds [52,53]. Continued investigation into the identities, origins, and environmental fate of these substances and the efficacy of effluent treatment is needed to determine the significance of and relationship to the existing impacts of pulp and paper mill effluents in aquatic ecosystems [54–57].

No monitoring was conducted for total population size over time at the Jackfish Bay site for the period of 1988 through 2007, and so the population trajectories and associated equilibrium population size estimates produced by the model provide an important evaluation of how a reduced impact on the T levels of fish at the Jackfish Bay site would result in a change in population status. In addition to field estimates of total population size, independent data sets collected beyond 2007 at the Jackfish Bay and Mountain Bay sites for T, fecundity, and proportion of breeders could be used to corroborate output from the model. The present study underscores the need for estimating population size over time at impacted sites in addition to demographics and biochemical endpoints of interest. Furthermore, the promotion of long-term studies on natural history and demographics of new species would enhance the ability to investigate AOPs across diverse species. This would, in turn, enable the application of the methodology presented in the present study (of using a biomarker or in situ effects-based measurement in a given local or sentinel species of fish to evaluate toxicity) to be extrapolated at a population scale using population models developed for other species [5,6,8–10,12,58].

The population model construct used in the present study is adaptable to a variety of risk assessment scenarios. Alterations to habitat can be incorporated directly into the estimate of carrying capacity of the population, K. In considering the collection of long-term monitoring data for future applications of the model construct, the model presented in the present study can be linked to studies of habitat suitability analysis and landscape scale analysis, using geographic information systems technology and interpolation approaches. For example, the population model construct was applied to investigate population dynamics for gray wolf populations in the Upper Peninsula of Michigan (USA), using landscape scale habitat suitability analysis and geographic information system technologies for estimation of carrying capacity under different land use scenarios [26,27,59,60]. In situations in which nonchemical stressors (such as competition, habitat loss, or predation by an invasive species) co-exist with chemical stressors, the modeling construct can easily accommodate multiple stressor inputs. Similarly, the model can be adapted to situations in which more than 1 chemical stressor may produce different adverse outcomes (i.e., multiple AOPs) relevant to population status. Multiple stressors, chemical or nonchemical, can be incorporated into the density-dependent modeling framework as additional matrices that discount the vital rates that are reflective of reproduction and survivorship of age classes within the population. The model construct can be written as

$$\mathbf{n_{t+1}} = \exp(-rP_t/K)\mathbf{M_2 n_t} \quad (6)$$

where $\mathbf{M_2}$ is the Leslie matrix containing vital rates (survivorship and fertility) that have been adjusted to include an age-specific percentage reduction in reproduction or survival rates over the time step t resulting from an exposure to multiple stressors $\mathbf{C_t}$ and $\mathbf{C_x}$. Thus, $\mathbf{M_2} = \mathbf{M} - \mathbf{C_t} - \mathbf{C_x}$, and $\mathbf{C_t}$ and $\mathbf{C_x}$ are separate matrices representing separate discounts to vital rates resulting from separate stressors at time t. The matrix $\mathbf{C_t}$ was described in the *Population model* section, and the matrix $\mathbf{C_x}$ is similarly represented by elements $c_{i,j}$, $i = 1, 2, \ldots, n$; $j = 1, 2, \ldots, n$. Within the matrix $\mathbf{C_x}$, $c_{1,j} = \alpha_j \times F_j$ for $j = 1, 2, \ldots, n$, where α_j is the percentage discount to the fertility rate of age class j (F_j). Furthermore, $c_{i+1,i} = \beta_i \times S_i$ for $i = 1, 2, \ldots, n-1$, where β_i is the discount to the survival rate corresponding to age class i (S_i). All other elements of $\mathbf{C_x}$ are equal to zero.

The model described in the present study also can be used with either a closed population or a population that is subject to the forces of influx and outflow via immigration and emigration processes. In the present study, the white sucker population was treated as a closed population without considering the effects of emigration and immigration. This was justifiable because the mouth of Jackfish Bay is greater than 100 m deep, and the white sucker avoid deep-water areas. Extractable organic halogen measured in the liver tissue of white sucker in Sawmill Creek confirmed that the fish spawning there were exposed to effluent, and repeated studies confirm that liver hepatic detoxification enzymes are highly induced, associated with the effluent exposure in Jackfish Bay. Further evidence in support of the assumption of a closed system can be found in telemetric studies on suckers that suggest little movement within a nonspawning

home range [61,62]. However, a metapopulation modeling approach [8] that integrates multi-patch spatial dynamics within Equations 1 and 6 can accommodate both immigration and emigration scenarios using the addition of population vectors to represent individuals of each age class entering or exiting the population at each time step. For example, expanding Equation 6 yields

$$\mathbf{n_{t+1}} = \exp(-\mathrm{rP_t/K})\mathbf{M_2 n_t} + \mathbf{i_t} - \mathbf{e_t} \quad (7)$$

where i_t is a vector of age structure at time t entering the population through immigration, and $\mathbf{e_t}$ is a vector of age structure at time t exiting the population through emigration. Application of the model construct whereby immigration and emigration fluxes are included will affect the resulting population trajectories because of the influence of the age structure and exposure history of fish moving into and out of the population at each time step. For example, if the vector $\mathbf{i_t}$ contains only unexposed breeders that are contained within the age classes exhibiting the highest reproductive capacity, age classes 9 and older (see Figure 1), and the vector e_t is 0 (no emigration), this would result in a maximum effect on population sustainability at the impacted site in that it would result in a scenario of maximum recruitment.

In considering the use of AOPs to link molecular, biochemical, and histological responses to populations within a modeling framework such as that described in the present study, several advancements would be useful. For example, it would be desirable to incorporate an ability to consider multigenerational responses whereby contaminant exposure reduces fitness in exposed populations through effects on survival and reproduction, but adaptation of genetic selection acts on the capacity of individuals to tolerate the toxicant exposure. This would require a better understanding of individuals' resistance to toxicity and of the likelihood of selection processes resulting in future generations with greater resistance over time. Another area of advancement would be the inclusion of genomic data as a more routine endpoint in toxicity studies to allow for a better connection between the expression of genes, proteins, and small molecules with survival and reproduction, which may result in further characterization or verification of key pathways below the level of the individual [63].

In summary, the framework described in the present study can be used to address uncertainties as to what changes in the status of a biomarker of a given fish population may mean relative to apical endpoints, such as reproduction, that directly affect population status and dynamics of the population over a given interval. Biomarkers (for example, sex steroids in the present study) can help identify biological effects and in the context of an AOP can be connected to endpoints of regulatory concern (fish reproduction and population status). The framework illustrated in the present study shows how AOPs serve as a mechanism by which laboratory-developed assays can be applied to the field. A logical subsequent step in continuing beyond the current demonstration is to apply the framework presented, in combination with effects-based monitoring approaches, in before- and after-impact control analysis to examine the impact of remediation activities at sites undergoing remedial activities, such as Great Lakes areas of concern, under conditions representative of preremediation and postremediation efforts.

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Data Availability—Data, associated metadata, and calculation tools are available on request from the authors (miller.davidh@epa.gov).

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