Extinction Risk of Exploited Wild Roach (*Rutilus rutilus*) Populations Due to Chemical Feminization

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A model that assesses risks posed by feminization to wild populations of roach was developed. A population life table matrix model that considered both sexes and a newly developed fertility kinetic function was applied to calculate the intrinsic population growth rate (λ) of roach populations where males had been feminized. The maximum sustainable yield (MSY) was used to quantify the effect of various degrees of feminization on sustainability of exploited fisheries. Risk of extinction was calculated for wild roach populations. The results of the simulations suggested that (a) In the absence of fishing pressure λ would only be decreased 1.5−1.7% even in the presence of a 100% incidence of intersex; (b) in the presence of selective fishing, the occurrence of intersex could significantly increase the extinction risk of local roach populations; (c) the benchmark value for the severity index of intersex and sex ratio required for a sustainable population of roach were estimated to be 1.13 and 0.57, respectively. The approach presented here provides a tool to (1) understand effects of male's feminization on population dynamics; (2) assess extinction risk of wild roach populations from feminization; (3) assist environmental managers in making policy decisions relative to fishery resource conservation.

Introduction

Recently, feminization of male fish due to exposure of endocrine disrupting chemicals (EDCs) has been observed in freshwater and marine environments throughout the world (*1*−*6*). In particular, feminization of male roach was prevalent in lakes and rivers of the United Kingdom, where the proportion of male roach exhibiting intersex was reported to be as great as 100% (*7*). Changes in the structure of the testes results in lesser fertility of feminized male fish (*8*, *9*). In a whole lake experiment conducted in Canada it was found that 17β-ethynylestradiol at 5−6 ng /L caused feminization of males and a subsequent collapse of wild populations of fathead minnow (*Pimephales promelas*) (*10*). However, the 17β-ethynylestradiol concentration was relatively low (0−0.37 ng/L), where the high incidents of intersex in roach but relatively low severity of intersex have been observed in natural fish populations (*3*). Until now, it has been unclear how to quantify the effects of feminization of male fish on the overall fitness of wild fish populations in natural environments, especially in exploited populations. Thus, it has been difficult to assess the potential effects of chronic exposures to these compounds and their risk on ecologically relevant end points.

Because the roach is a species in which feminization of males is often observed in European rivers and adequate information was available on population structure and dynamics, a two-sex, age-classed, population model was developed to assess potential effects of feminization on roach. Specifically, the objectives of this study were to determine (i) the magnitude of effects on population dynamics and demography to clarify whether the intersex could affect persistence of a wild roach population; (ii) the critical parameters (sex ratio, feminization severity (intersex incidence and severity index), etc.) required for the conservation of fishery resources that should be monitored in wild populations. Here we provide a scientific basis for making policy decisions on fishery resource conservation and environmental management in the presence of intersex in males.

Materials and Methods

Procedure of Assessing Potential Effects of Intersex on Roach Populations. As shown in Figure 1, the procedure for assessing potential effects of intersex on Roach populations consisted of two parts: (1) Model establishment and (2) Risk characterization, of which related notations and interpretations are summarized in Table 1. During model establishment, a fertilization kinetic function was developed and incorporated into a two-matrix model (*11*) for simulation of population dynamics under intersex occurrence. The annual survivals except for the survival probability of wild roach from zygotes to first age ($P_{0,1}$) and fecundity rates at different ages were estimated from field surveys. $P_{0,1}$ was calculated by using Newton's iteration method (*12*) to solve a two-sex matrix, after population growth rate (λ, the dominant eigenvalue of the matrix) was estimated from the doubling time (t_d) of small populations, and the other parameters (survival and fertility rates) were estimated above. During risk characterization, we extrapolated individual intersex occurrence to population response by developing the relationship between reductions of fertilization rate with intersex severity which was linked to the two-sex matrix for λ calculation by the fertilization kinetic function. And then, λ values under intersex occurrence were used to calculate the MSY loss and extinction probability of wild roach populations.

Model Development for Extrapolating from Individual Intersex to Population Response. The response of wild populations is often evaluated by using an age-specific two-sex population matrix eq 1 (*11*):

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$$\begin{vmatrix} N_{0,t+1} \\ N_{f1,t+1} \\ \cdots \\ N_{\mathrm{fi},t+1} \\ N_{m1,t+1} \\ \cdots \\ N_{\mathrm{mi},t+1} \end{vmatrix} = \begin{vmatrix} 0 & F_2 & \cdots & F_{i-1} & 0 & 0 & 0 \\ P_{f1,1} & 0 & \cdots & 0 & 0 & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & .. & \cdots & \cdots \\ 0 & 0 & \cdots & P_{\mathrm{fi},i-1} & 0 & 0 & 0 \\ P_{m1,1} & 0 & 0 & 0 & 0 & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & 0 & 0 & 0 & P_{\mathrm{mi},i-1} & \end{vmatrix} \times \begin{vmatrix} N_{0,t} \\ N_{\mathrm{f1},t} \\ \cdots \\ N_{f\mathrm{i},t} \\ N_{m1,t} \\ \cdots \\ N_{\mathrm{mi},t} \end{vmatrix} \quad (1)$$

where $N_{0,t}$ is the total number of zygotes at time t; $N_{\mathrm{fi},t}$ and $N_{\mathrm{mi},t}$ represent the number of males and females at time t and age class i, respectively; $P_{\mathrm{fi},j}$ and $P_{\mathrm{mi},j}$ are the survival rates per year of individual females and males, respectively; and F_i is fertility rate, which is calculated by the fertilization kinetic function.

Sexual reproduction that depends on separated males and females, and can be affected by alterations in reproductive fitness of males and females as well as environmental factors. In fact, sexual dimorphism to maturity and differences in reproductive performance among ages are common in many species. Thus the function describing fertility rate should be age-specific and include both sexes (*11*). Because roach reproduce by external fertilization of eggs with sperm in the water, one male can fertilize the eggs of several females or one female can spawn with several males at the same time (*13*). Therefore, at any given time, there can be some redundant males or females in the spawning population. Considering these conditions, a fertilization function (eq 2) was developed to describe the fertilization process of fish, of which derivation details are given in SI Section I.

$$F = \varphi_f \frac{k\delta(1 - pq)}{0.241 \times (1 - \delta) + \delta(1 - pq)} \quad (2)$$

where φ_f is probability of a female mating relative to the total number of female adults; k is fecundity rate (i.e., average number of eggs per female every year); δ is the sex ratio, which is defined as the proportion of males in the spawning subpopulation; p is the incidence of intersex in the population of spawning males; and q is the reduction of fertilization rate caused by intersex.

European roach spawn from April to June in shallow water, and eggs hatch within 4–10 days. The number of eggs spawned (i.e., clutch size) is directly proportional to the length and weight of the female. Although a local natural population can be divided into three subpopulations: reserve, recruitment, and residue, and only residue subpopulation was reported to attend the spawning troop (*14*). So, it is necessary to consider the mating probability (φ) of individuals in the total population when predicting population dynamics. However, there is no information on mating probability of roach available in the literature. In this study, mating probability was described as the proportion (%) of mating individuals in the total population, and was estimated using the population proportion of spawning in the whole roach population of different ages which has been extensively surveyed in Jelesna Brook, Russia (*14*). Considering that proportions of male ($\varphi_\mathrm{mi}n_\mathrm{mi}$) and female ($\varphi_\mathrm{fi}n_\mathrm{fi}$) roach in the spawning population of different ages (i) can affect the probability of eggs being fertilized, sex ratios (δ) were calculated by eq 3.

$$\delta = \frac{\sum_{i=2}^{18} \varphi_\mathrm{mi} n_\mathrm{mi}}{\sum_{i=2}^{18} \varphi_\mathrm{mi} n_\mathrm{mi} + \sum_{i=2}^{18} \varphi_\mathrm{fi} n_\mathrm{fi}} \quad (3)$$

where n_m and n_f are the stable population age structure vectors at different ages (i) calculated by the two-sex matrix. The magnitude of k can be predicted based on the body length by use of a regression on data collected during a field survey conducted between 1975 and 2000 (*15*). The optimized equation between k and female body length (TL, cm) (eq 4) was selected from different empirical relations, and the details for optimizing and selecting were shown in SI Sections II and III.

$$\log 10(k) = 3.08 \times \log_{10}(\mathrm{TL}) + 0.5637 \; n = 77, \quad r^2 = 0.8331 \; p\text{-value} < 0.05 \; \mathrm{mse} = 0.0505 \quad (4)$$

where n is sample size. The $\log_{10}$ transformation bias was estimated to be the exponent of the normal distribution with a mean of 0 and standard deviation of 0.225 (root square of mse) was used to estimate the predictive error in the uncertainty analysis.

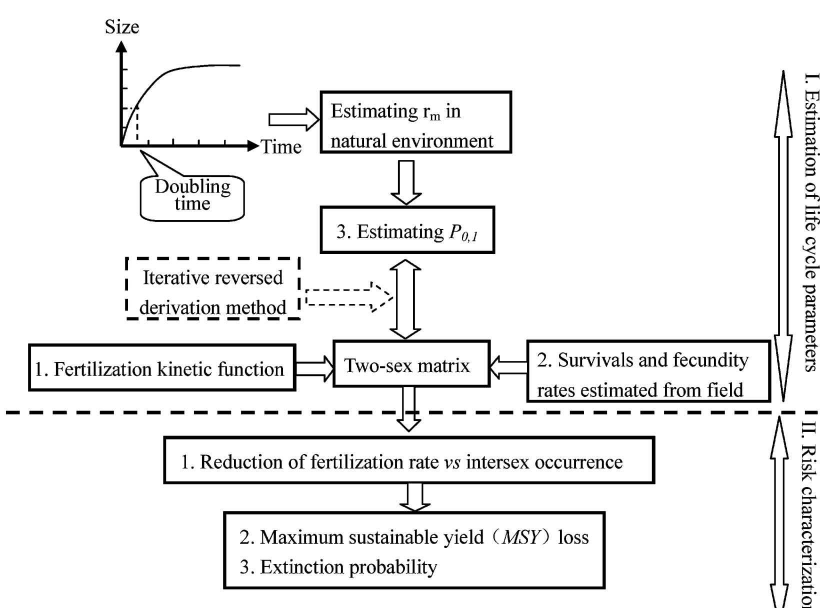

FIGURE 1. Framework for assessing the ecological risk of wild roach population caused by intersex incidents. $P_{0,1}$: survival probability of wild roach from zygotes to first age; λ: populations growth rate; r_m: intrinsic rate of population growth, which is equal to the natural logarithm of λ.

Life Cycle Parameters. *Fertility Rate (F_i).* Roach reproduce by spawning in which gametes are released into the water and fertilization takes place outside of the bodies of the male and female fish. Roach often assemble at spawning sites prior to spawning (*13*), and therefore males and females can still find each other even if the density of the total population is small. Since most populations of roach in Europe are exploited, there is little chance that they would become locally overpopulated. For these two reasons no density-dependent limit was used in the simulation model. Thus, in natural environments in the absence of intersex occurrence fertility rates can be calculated by eq 2 where p and q are 0 and $\delta = 0.5$.

Annual Survival Rates ($P_{i,j}$). The roach (family *Cyprinidae*) is a small freshwater and brackish water fish with a native range that extends across Europe (*15, 16*). The life cycle of roach can be divided into four stages: (1) Zygotes, which is the combination of gametes produced by adult male and female; (2) Larvae, which have no significant sex differentiation characteristics; (3) Adult males and females; (4) Gametes produced by adult males and females (Figure 2). When gametes produced by male and female fish fuse to form zygotes through the fertilization process, the life cycle is repeated. Longevity of roach has been reported to be as long as 18 years. The annual mean mortality rate of the roach populations in the Orava Valley Reservoir is about 0.5, which is nearly identical to that for the populations in the Dyje River and Klicava Reservoir in Czechoslovakia (*14*). The survival rate per year ($P_{i,j}$) except for survival of zygotes to 1 year ($P_{0,1}$) was replaced by the average annual survival rate in a given period of life, which can be estimated based on its catch curve (eqs 5, 6) (*14, 17*).

$$\mathrm{Ln}(N_t) = a - Zt \qquad (5)$$

$$S_{i-j} = e^{-Z} \qquad (6)$$

where S_{i-j} is average annual survival from i to j age; Z and a are the slope rate and intercept of fitted catch curve. N_t is the fish abundance in a given age-t group (t, year).

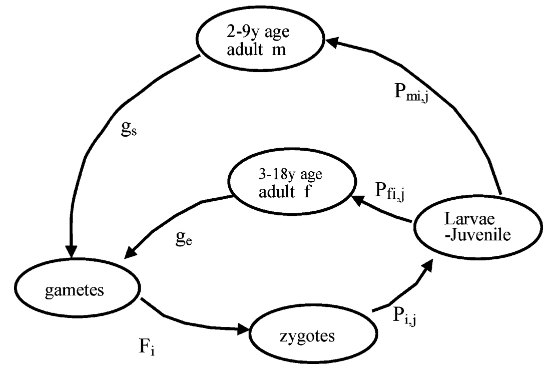

FIGURE 2. Schematic diagram representation of roach (*Rutilus rutilus*) life cycle. P_i: the possibility of survival in age i; Subscript i: the sequence of age (i.e., 1, 2,.. .0.18 year); Up to now, the longest longevity of male was observed to be 9 and female to be 18; So the sequence of adult male is from 2 to 9 years and that of female from 3 to 18 years (*14*). Subscript f (or m): the sex female (or male) g_s: the average clutch size of male gametes every year at age i; g_e: the average clutch size of female gametes every year at stage i; F_i: the fertility rate.

Survival of Zygotes to 1 Year. Because it is difficult to monitor the numbers of zygotes and fry in the field, $P_{0,1}$ have been unknown, which has limited the capability to develop population models. Since the λ of roach populations can be estimated from doubling time (t_d) for roach using eq 7 (*15*), and other life-cycle parameters have been estimated, the survival from zygotes to 1 year was determined by use of Newton's iteration method (*11, 18*).

$$\mathrm{Ln}(\lambda) = \mathrm{Ln}(2)/t_d \qquad (7)$$

Assessing Roach Population Risk from Intersex Occurrence. *Roach Egg Fertilization Reduction due to Intersex.* An experiment evaluating the effects of intersex in male roach on fertilization was conducted with fish taken from the Rivers

TABLE 1. Notations and Interpretations for Assessing Extinction Risk of Exploited Roach Populations due to Chemical Feminization

symbol	description	symbol	description
N_t	fish abundance in a given age-t group (t, year)	Fi	fertility function
Z	slope rate of fitted catch curve	p	incidence of intersex in spawning males
a	intercept of fitted catch curve	q	lost fertilization potential caused by intersex
S_{i-j}	average annual survival from i to j age	α	fertilization rate coefficient
δ	sex ratio	γ	intersex severity index
φ	mating probability	MSY	maximum sustainable yield
n	scalar in vector N_t	B_m	maximum original size of unexploited population
k	average egg clutch size	$a_{i,j}$	traits of matrix M
TL	female body length	w	stable age structure distribution
λ	population growth rate	v	reproductive value distribution
t_d	time required for a quantity to double in roach population size	BMSII	severity index of intersex corresponding to 10% predetermined increase of $\Delta MSY/MSY$.
N_t	population vector of individual roach in different ages	BMSR	sex ratio corresponding to 10% predetermined increase of Ψ from background
M	population transition matrix	MSC	model selection criteria
ΔMSY/MSY	proportion of MSY Loss	mse	mean squared error
r_m	intrinsic rate of population growth	$P_{i,j}$	annual survival rates of individual females and males from i to j age

Aire and Calder in Yorkshire, the River Arun in Sussex, the River Blackwater in Surrey, and the River Lea in Hertfordshire of the U.K. The results of these studies were used to develop a relationship between the rate of fertilization and degree of pathological changes (i.e., intersex) in testes. The q value for the fish with only ovarian cavities in testes was about 21.7%, while that by males containing some testicular ovarian follicles was approximately 28%, and males that were classified as being severely feminized exhibited a 77% reduction (*9*). To quantify the adverse effects on fecundity due to intersex, a severity index of intersex (γ) was defined as a variable to describe the degree of intersex pathological changes in testes. The values of the severity index of intersex that corresponded to the three pathologies were 1.0, 2.5, or 5.5, respectively. The boundary conditions were set such that q was equal to 18.5% when $\gamma = 0$ (normal males), and q was 100% when $\gamma = 7$ (complete feminization of males) (*9*). The optimized severity index of intersex (γ)-response (q) curve (eq 8) was selected from different empirical relations (details are in SI Section IV.

$$q = \exp(0.2534\gamma - 1.743)\, n = 5,\ \text{mse} = 0.0027,\ \text{p-value} < 0.05 \quad (8)$$

where mse is mean squared error.

Extraploating from Individual Intersex to Population. The dominant eigenvalue of the two-sex population matrix was regarded as population growth rate per year (λ) over a unit time period, and the corresponding right eigenvector represented the stable age structure (*11*) which was calculated by use of Matlab *Ver.* 6.5. *MSY* is the largest catch that can be taken from a species' stock over an indefinite period, and reflects a balance between fish harvesting rate (*19*) and its λ, and it can be calculated using eq 9.

$$\text{MSY} = \ln(\lambda)B_m/4 \quad (9)$$

where B_m is the maximum original size of the unexploited population. The quotient of MSY loss (i.e., ΔMSY) due to intersex occurrence under exposure to EDCs and the *MSY* in the natural environment (i.e., ΔMSY/MSY), was defined as the proportion of *MSY* loss, which was calculated by $\Delta\ln(\lambda)/\ln(\lambda)$ and applied to relate the effects of intersex occurrence on the ability of the population to sustain exploitation.

The value of λ determines whether a population is locally sustainable. In this study, the population extinction probability (Ψ) with the stress of intersex occurrence, was defined as the area proportion of $\lambda < 1.0$ to the total area under possible values of severity index of intersex (γ) and incidence (p) corresponding to effects due to exposure to feminizing chemicals and individual sensitivities in different habitats. The Ψ represents the population extinction risk with the stress of intersex occurrence. More details about Ψ are illustrated in SI Section V.

To obtain a criterion for protection of roach populations as a fishery resource, the relation between the ΔMSY/MSY and severity index of intersex at 100% incidence was established, and then applied to estimate the benchmark value by the benchmark dose/level methodology (*20*). The benchmark value is the dose/level referring to some response above background (e.g., 10%) and often regarded to be equivalent with no-observed-effects dose/level, which could be calculated using Newton's iteration method (*12*). In this study, the benchmark severity index of intersex (BMSII) was calculated as the severity index of intersex corresponding to 10% predetermined increase of ΔMSY/MSY. And the benchmark sex ratio (BMSR) was the sex ratio corresponding to 10% predetermined increase of Ψ from background.

Sensitivity Analysis. The sensitivity of λ to changing of life-cycle traits can be calculated using eq 10 which is based on the two-sex matrix (eq 1).

$$\frac{\partial\lambda}{\partial a_{ij}} = \frac{vw}{\langle v, w\rangle} \quad (10)$$

where a_{ij} is the traits of roach life cycle in two-sex matrix (eq 1); w is calculated using the corresponding right eigenvector with the dominant eigenvalue (λ); v is estimated by the corresponding left eigenvector with λ. The vw and $<v, w>$ denotes the cross and scalar product, respectively (*11*). The sensitivity was analyzed using Matlab V6.5 software.

Simulation of Multiple Models and Uncertainty Analysis. Simulation in this study involved multiple models. The sources of uncertainty were partitioned into two components, i.e., predictive errors for predicting fertility rate and value fluctuation of annual survivals. The fertility rates were predicted by use of the fertilization kinetic function (eq 2) which was embedded in the relationship between body length and egg clutch size (eq 4) and the relationship between reduction of fertilization rate and severity index of intersex (eq 8), and their predictive errors were estimated by use of the bootstrapping method. The model describing the relationship between reduction of fertilization rate and severity index of intersex was optimized using model selection criteria (MSC) (*21*) as described in SI Section II (Model Specific Error and Model Selection) and SI Figure S2. The annual survivals were simulated by Monte Carlo methods in which distributions (SI Table S1) were derived from a serial data set which covered different roach natural habitats.

Population responses (ΔMSY/MSY, Ψ) were simulated by use of resampling methods (Matlab version 6.5) (400 trials, a trade-off between the requirement of uncertainty analysis and time cost of simulation). The BMSII and BMSR for each trial were obtained by use of Newton's iteration method, respectively, and then the cumulative probability and density distribution of the BMSII and BMSR values for all 400 trials were analyzed by use of the nonparametric method of Statistic software version 6.5.

Data sets Used for Calibration of Multiple Models. The original data in the multiple models consist of fish abundance in different habitats, average eggs per female, doubling time and reduction of fertilization rate with intersex pathological changes. The abundances of fish at different ages as shown in SI Figures S4−S13 were collected from different habitats in Europe (*13*−*15, 23*), and were used to estimate the probability distribution of annual survival rates. The t_d of roach (1.4−4.4 years) was reported only for the UK (*16*), and its probability distribution was assumed to be uniform. The average egg numbers per female with body length from field investigation (*11*) and reduction of fertilization rate with intersex pathological changes based on field experiment (*9*) were applied to predict the fertility rate of roach under intersex occurrence.

Results and Discussion

Estimation of Roach Life Cycle Parameters. The annual survivals of roach depend on environmental factors such as food abundance, predators, climates, conditions, and so on. Therefore, survival rates among specific habitats are always different. The age composition has been reported for catches of roach from 1930 to 1941 in watersheds of the Norfolk Broads, Rivers Cam, and Shepreth Brook at Barrington, the Old West River, and River Granta in Cambridgeshire, the Grantham Canal, and other locations in Europe (*13, 22*). Based on these age compositions of catches, catch curves were fitted using eqs 5−6 and shown in SI Figures S4−S13, from which the annual male survival rates of age III−XVIII class groups were 0.4975 (SD: 0.1414) and that of female were 0.5291 (SD: 0.1459), and annual survivals of males and females of age classes I−III were estimated to be 0.118 (SD: 0.0343) and 0.123 (SD: 0.0339), respectively (SI Table S1). Using the catch abundance of spawning roach from Jelesna Brook in

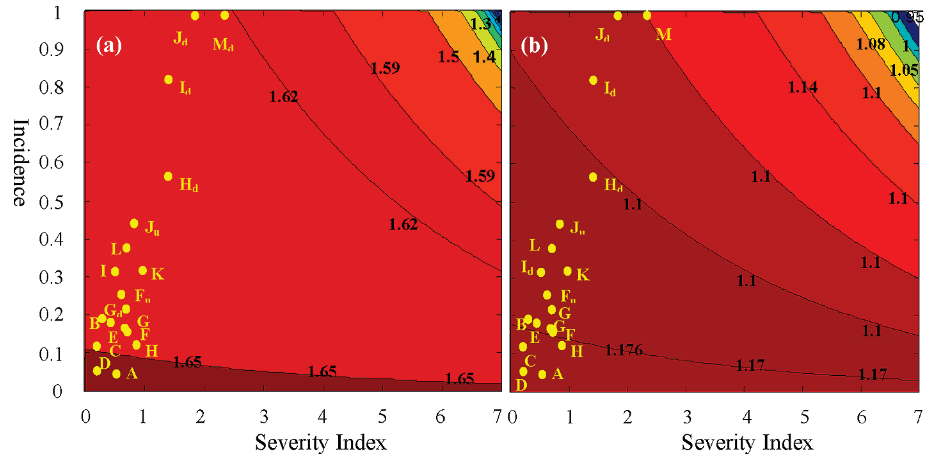

FIGURE 3. Contour of the roach population growth rate (λ) with intersex occurrence under the best condition ($P_{0,1}$ = 0.022, (a)) and the worst condition ($P_{0,1}$ = 0.079, (b)). The circle represents I of wild roach inhabiting in watersheds in England. Sites A: Labor control; B−E: lakes and canals without sewage treatment work in England and southern Ireland; Rivers upstream (subscript u): F_u: Wreake/Eye;G_u:Ouse; H_u: Lea; I_u: Arun; J_u: Nene; Rivers downstream (subscript d): F_d: Wreake/Eye; G_d: Ouse; H_d: Lea; I_d: Arun; J_d: Nene; *K*: Trent; *L*: Rea; *M*: Air.

Russia (*14*), the ranges of φ of male and female were estimated to be 0.268−1 and 0.054−1 by SI eq S-24, respectively (SI Table S2). Thus, the F_i from age classes III−XVII were calculated to be from 2255 to 73829 based on k and φ by fertilization kinetic function (eq 2 where $p = 0$, $q = 0$, and $\delta = 0.5$) as shown in SI Table S1. The details of estimating annual survival rate and mating probability are shown in SI Section VI. The probability of survival of zygotes to age class I ($P_{0,1}$) is unknown for field populations. Using eq 7, the value of λ for wild roach populations was estimated to be between 1.1712 and 1.6405 based on t_d (1.4−4.4 years). Thus, using the λ and other life-cycle parameters, the least and greatest values of $P_{0,1}$ were calculated to be 0.022 and 0.079, respectively by using Newton's iteration method (*12*).

Relationship between λ and Intersex Occurrence and Sensitivity Analysis of Roach Life Cycle Traits. To simulate the population response affected by intersex in males, values of "λ" were calculated (eq 1) under intersex occurrences. Contours of λ were developed across the range of least to greatest values of $P_{0,1}$ with values of the severity index ranging from 0 to 7 and incidences ranging from 0 to 100% were developed (Figure 3). Values of "λ" were more sensitive to severity index than incidence of intersex. When the severity index increased from 0.0 to 6.3 with an incidence of 100% intersex, the value of "λ" changed from 1.17 to 1.0 at the least value of $P_{0,1}$ (Figure 3(b)), whereas λ ranged only from 1.63 to 1.36 within the same changing range at the highest $P_{0,1}$ (Figure 3(a)). These results suggest that the species with the lesser value of $P_{0,1}$ would be more susceptible. The susceptibility of species to pollutants depends on their life- cycle variables, as exemplified by the population persistence analyses for threatened and endangered species in lab (*23*).

Mean values of roach life-cycle parameters (SI Table S1) were used to determine the sensitivity to λ (eq 1). The results of this sensitivity analysis provided profiles of uncertainty sources in the two-sex matrix population model. Fertility rate (F_i) and survival ($P_{0,1}$) from zygotes to age class I contributed for more than 90% of the variation in population growth rate (SI Figure S14).

Effects of Intersex on Wild Roach Populations and Uncertainty Analysis. In recent years, several typical EDCs such as 17β-estradiol, 4-nonylphenol, dioxin, and bisphenol A (*24−27*) have been detected in rivers of England which receive effluents from sewage treatment works. At the same time, relatively great incidences of intersex has been observed in wild populations of roach in eight rivers, the Air, Arun, Lea, Nene, Ouse, Rea, Trent, Wreake/Eye, and some lakes and canals throughout the British Isles (*3, 7, 9*). The incidence (4−18%) and severity index of intersex (0.19−0.50) in the lakes and canals were both less than those in the upstream (11.7−44% for incidence and 0.60−0.95 for severity index) and downstream (16−100% for incidence and 0.68−2.32 for severity index) of the eight rivers. Such intersex conditions resulted in 19.8−21.7%, 20.2−27.5%, and 20.2−31.1% reduction of fertilization rates in lakes and canals, upstream and downstream reaches of the eight rivers according to eq 8

TABLE 2. Prediction of the Intrinsic Population Growth Rate (λ) and Loss of Maximum Sustainable Yield (MSY) of Roach Caused by Intersex in the UK with Average Value of Life Cycle Parameters

rivers/lakes	locations	incidence	severity index	fertilization reduction	predicted λ	MSY loss (%)
Wreake/Eye	river upstream	0.25	0.60	0.198	1.403	0.42
Ouse0.177	0.62	0.199	1.404	0.21		
Lea		0.12	0.86	0.212	1.404	0.21
Arun		0.32	0.95	0.217	1.402	0.63
Nene		0.44	0.81	0.209	1.400	1.05
Wreake/Eye	river downstream	0.16	0.69	0.202	1.404	0.21
Ouse0.22	0.68	0.202	1.403	0.42		
Lea		0.57	1.40	0.244	1.397	1.68
Arun		0.82	1.39	0.244	1.393	2.52
Nene		1	1.85	0.275	1.387	3.79
Trent		0.32	0.49	0.192	1.402	0.63
Rea	lake	0.38	0.68	0.202	1.401	0.84
air		1	2.32	0.311	1.383	4.64

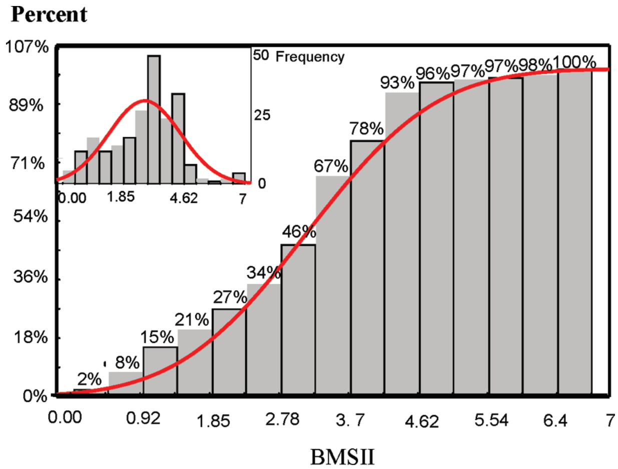

FIGURE 4. Cumulative probability and probability density distribution of the benchmark severity index of intersex (BMSII) which is defined as the severity index of intersex of no-observed-effects on maximum sustainable yield (MSY). The BMSII is the lower 90% confidence interval bounds of severity index of intersex, corresponding to a 10% predetermined increase of ΔMSY/MSY. The cumulative probability curve (red line) was fitted using Matlab version 6.5 software as Percent (%) = 224 × normcdf (BMSII, 3.1, 1.36).

(Table 2), respectively. The corresponding λ of wild roach populations in these watersheds were calculated to be 1.177 to 1.159 (1.52% different) and 1.651 to 1.623 (1.69% different) at the least and greatest values of $P_{0,1}$, respectively (Figure 3). This result suggests that even if a 100% incidence of intersex occurred, it was unlikely to cause the collapse of local roach population due to the low severity index of intersex.

MSY is a common index to assess the effects of many environmental conditions on fishery resource. In this study, the proportion of MSY predicted to be lost (ΔMSY/MSY) was used as an index to assess the ecological effects of intersex occurrence in English watersheds (Table 2). The proportion of MSY loss of roach population with intersex in English watersheds was estimated to be 7.8−9.2% and 3−3.6% at the least and greatest values of $P_{0,1}$, respectively (eq 9).

To calculate a criterion for protection of roach populations as a fishery resource, the ΔMSY/MSY response was simulated by use of resampling methods (400 trials). In each trial, one BMSII would be obtained. From all trials, the cumulative probability and density distribution of BMSII was developed by the nonparametric method using Statistic software version 6.0 (Figure 4). The result indicates that when the mean value of severity index of intersex is less than 1.13, there would be less than 10% probability that intersex occurrence would significantly affect MSY. Alternatively, if the value is more than 4.5, the probability of affecting the MSY would be more than 90%. In the Nene River and Air Lake of England, the probabilities to affect MSY were approximately 20 and 28%, respectively. The BMSII was estimated to be 1.13 and 4.5 at the lower and upper 90% confidence interval bounds, corresponding to a 10% predetermined increase of ΔMSY/MSY (Figure 4). This provides the direct reference value against which to assess the effects of the of intersex occurrence in roach population.

Ecological Significance of Intersex under Selective Fishing and Uncertainty Analysis. Roach, is harvested as a valuable fish across Europe. In most locations, roach populations are managed to achieve MSY by allowing only males to be taken (*28, 29*). Until the mid-1960s, the ratio between male and female roach estimated from seine catches in Europe, was approximately 1:1. Due to the selective fishing for males from the populations of roach in the Volga delta

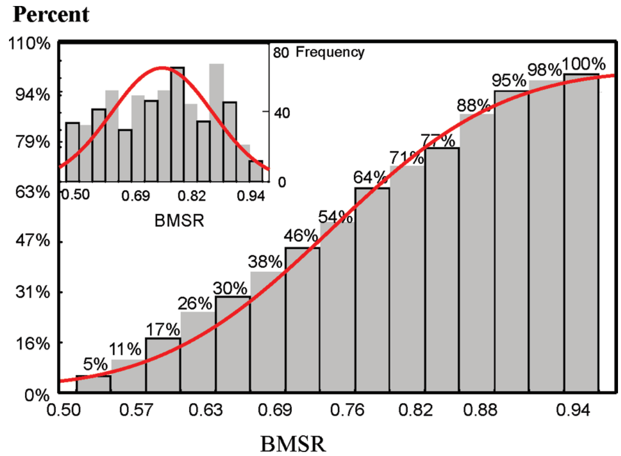

FIGURE 5. Cumulative probability and probability density distribution of the benchmark sex ratio (BMSR) which is regarded as the severity index of intersex of no-observed-effects on increase of extinction risk (Ψ). The BMSR is the lower 90% confidence interval bounds of sex ratio, corresponding to a 10% predetermined Ψ. The cumulative probability curve (red line) was fitted using Matlab version 6.5 software as percent (%) = 635 × normcdf (BMSR, 2.34, 0.119).

and in the Ural region, populations consisted of more than 90% female in the period from 1995 to 1999. Similar sex biases have been observed in commercial catches of roach in Azerbaijan (61−65% females) and Turkmen (57−98% females) (*15*).

To analyze the effects of intersex occurrence under selective fishing on the potential of roach populations to become locally extinct, an index of extinction probability (Ψ) was calculated. To estimate the effects of intersex occurrence in the presence of male-selective fishing, the Ψ value of the wild roach populations was calculated with sex ratios (δ) ranging from 0.5 to 1. The probability of extinction (Ψ) as a function of sex ratio (δ) was simulated by the use of the same methods as above (400 trials) (see SI Section VII). In each trial, one BMSR was calculated, which is the sex ratio corresponding to 10% predetermined increase of Ψ. The cumulative probability and density distribution of the benchmark sex ratio (BMSR) were calculated using nonparametric methods (Figure 5). When the sex ratio bias is less than 0.07, there would be less than 10% probability of sex ratio bias significantly affecting the local extinction under the condition of the intersex occurrence. Alternatively, when the bias is more than 0.39, the probability of affecting local extinction will be more than 90%. Considering the lower 90% confidence interval bound, the intersex would exert significant adverse effects on the extinction risk of local roach populations with the sex ratios skewing beyond 0.87. In the Volga delta and the Ural Rivers, the sex ratio of roach is more than 0.9, especially in the Turkmen River where the population is 98% female (*15*). In these two places, the probabilities of causing local extinction of the roach populations were 91 and 99%, respectively. To further investigate the potential for local extinctions, the occurrence of intersex in roach populations should be surveyed in rivers where selective fishing is applied. From the viewpoint of fishery resource conservation, the current selective fishing policy should be modified in the presence of intersex. The result of our study provides a key reference value for the selective fishing policy to protect the local resource of fishery.

Overall, we developed an approach to illustrate how to incorporate histological status of feminization with life-cycle parameters to extrapolate the response of population and MSY of wild fish population. Specifically, a modeling framework is presented to (1) understand the effects of the intersex occurrence of in male roach on wild populations;

(2) assess extinction risk of wild roach populations under the stress of feminization; (3) help environment manager to make policy decisions on fishery resource conservation and environmental protection due to EDCs exposure.

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Supporting Information Available

Detailed descriptions of development of fertilization kinetic function, model specific error and model selection, optimizing relation between egg clutch size and body length, judgment of intersex diagnose system and optimizing relation between severity index of intersex and reduction of fertilization rate of roach, illustration of the extinction probability (Ψ) due to intersex occurrence, estimating annual survivals of roach and mating probability from field survey, and uncertainty analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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