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Recovery of a Wild Fish Population from Whole-Lake Additions of a Synthetic Estrogen

Paul J. Blanchfield,*[,†,‡] Karen A. Kidd,[§] Margaret F. Docker,[‡] Vince P. Palace,[†,∥] Brad J. Park,[†] and Lianne D. Postma[†]

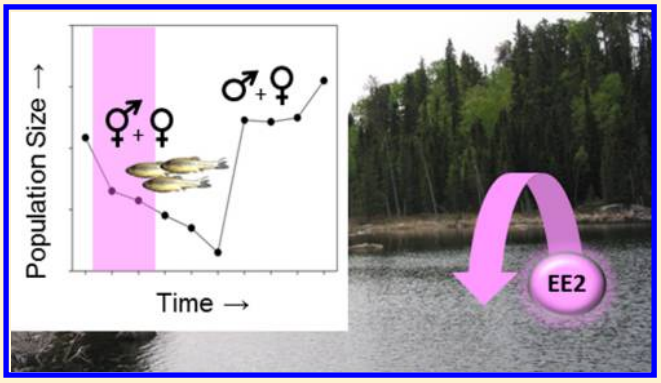
[†]Freshwater Institute, Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, Manitoba R3T 2N6, Canada

[‡]Department of Biological Sciences, University of Manitoba, 50 Sifton Road, Winnipeg, Manitoba R3T 2N2, Canada

[§]Biology Department & Canadian Rivers Institute, University of New Brunswick, Saint John, New Brunswick E2L 4L5, Canada

[∥]Stantec Consulting Ltd., 602-386 Broadway Avenue, Winnipeg, Manitoba R3C 3R6, Canada

ABSTRACT: Despite widespread recognition that municipal wastewaters contain natural and synthetic estrogens, which interfere with development and reproduction of fishes in freshwaters worldwide, there are limited data on the extent to which natural populations of fish can recover from exposure to these compounds. We conducted whole-lake additions of an active component of the birth control pill (17α-ethynylestradiol; EE2) that resulted in the collapse of the fathead minnow (*Pimephales promelas*) population. Here we quantify physiological, population, and genetic characteristics of this population over the 7 years after EE2 additions stopped to determine if complete recovery was possible. By 3 years posttreatment, whole-body vitellogenin concentrations in male fathead minnow had returned to baseline, and testicular abnormalities were absent. In the spring of the fourth year, adult size-frequency distribution and abundance had returned to pretreatment levels. Microsatellite analyses clearly showed that postrecovery fish were descendants of the original EE2-treated population. Results from this whole-lake experiment demonstrate that fish can recover from EE2 exposure at the biochemical through population levels, although the timelines to do so are long for multigenerational exposures. These results suggest that wastewater treatment facilities that reduce discharges of estrogens and their mimics can improve the health of resident fish populations in their receiving environments.



INTRODUCTION

Over two decades of research has unequivocally demonstrated the presence of endocrine disrupting chemicals (EDCs) in municipal wastewater treatment plant (MWTP) effluents, and their detrimental effects on sexual development and reproduction in individual fishes downstream of these discharges.[1] Much of the focus of previous research has been on natural estrogens (e.g., 17β-estradiol, E2) and their potent, synthetic mimics, such as 17α-ethinylestradiol (EE2), because these compounds are detected at low ng·L^{-1} concentrations in municipal effluents and in downstream waters in populated areas.[2,3] Exposure to these chemicals,[4,5] as well as antiandrogens,[6] are linked to the feminization of male fishes and female-dominated sex ratios observed in numerous rivers in the U.K., other parts of Europe, North America, and Asia.[7−14] More specifically, feminized male fishes have elevated concentrations of the egg yolk protein precursor, vitellogenin (VTG), decreased testes size, a loss of secondary sex characteristics, and intersex,[12,15−17] the latter of which has been linked to reduced sperm motility[14] and lower reproductive success (fitness) of individuals.[13,18] In addition, laboratory experiments show that exposure to low ng·L^{-1} concentrations of estrogens alter reproductive behaviors of male fishes that in turn decrease breeding success.[19−21] Whole-lake experimental evidence supports many of the findings of laboratory-based studies, and has also demonstrated that continuous exposures to 5−6 ng EE2·L^{-1} can result in the collapse of a wild fish population.[22,23] Though the literature on the effects of estrogens and estrogen mimics on fishes is considerable, recovery from these exposures has been comparatively ignored.

Recognition of the negative consequences to fishes from releases of EDCs in domestic wastewaters has motivated investigations into treatment methods to reduce estrogenicity of these effluents.[24] However, the ability to predict the response of downstream fish populations to improved treatment is difficult, in part because concentrations of natural and synthetic estrogens are temporally and spatially variable, and are affected

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by characteristics of the influent, the type of wastewater treatment, and dilution in receiving waters.[2] Individual estrogens, mainly natural ones and EE2, contribute to the total estrogenicity (E2 or EE2 equiv) of the effluents, surface waters and sediments, which can be 80 ng E2 equiv·L^{-1}, 71 ng E2 equiv·L^{-1}, and 55 μg EE2 equiv·kg^{-1}, respectively.[25−27] In addition, variability in fish exposure to these EDCs will also be influenced by individual and species-specific mobility and habitat use, with higher exposure expected for less mobile species found in reaches immediately downstream of MWTP outfalls. Despite these complexities, more advanced wastewater treatment generally results in reduced estrogenicity of the effluents and less severe effects in fish experimentally exposed to these wastewaters.[28−30]

Global evidence of endocrine disruption in fishes living downstream of municipal wastewater discharges exists, but studies of their recovery postestrogen exposures are limited to laboratory-based investigations, which collectively suggest that only partial recovery is possible.[31−34] For instance, VTG in male fathead minnow (*Pimephales promelas*) and immature rainbow trout (*Oncorhynchus mykiss*) showed only marginal decreases 8 and 14 days postexposure to EE2 or E2, respectively.[33,34] Adult zebrafish (*Danio rerio*), exposed to EE2 and E2 during only their early life stages, had reduced fertilization success following a 5 month recovery period.[32] To date, laboratory and mesocosm studies show that fish are not able to fully recover from estrogen exposures, though this may be due to the lack of prolonged monitoring during the recovery period. In addition, there is some evidence that EE2 may have transgenerational effects on the offspring of EE2-exposed adult fathead minnow, even after fish have been allowed to recover.[35] A key and outstanding question is whether wild fish populations can fully recover from exposure to estrogen mimics and, if so, how quickly this recovery will occur.

Biological recovery of natural populations is typically preceded by chemical recovery of the perturbed ecosystem, although the timing and magnitude of species returns varies considerably with location, stressor, and human intervention.[36] For example, acidification and recovery of boreal lakes has been well-studied, and the lag between chemical and biological recovery is 10 or more years, even for short-lived organisms such as zooplankton.[36,37] Moreover, recovery from stressors may not occur or can be delayed for isolated populations, where there is limited dispersal from regional species pools[38] or no option for immigration. Not suprisingly, long-term studies of perturbed ecosystems demonstrate the recovery trajectory for species and communities can differ markedly from that of the effects stages.[37,39]

To understand whether estrogenic compounds could affect the sustainability of fish populations, a whole-lake experiment was performed in which EE2 was added continuously to surface waters over three summers. In the first summer of treatment, VTG was detected in male fathead minnow at concentrations up to 22 200-fold higher than fish from reference lakes or pretreatment fish.[22,23] Delayed spermatogenesis and reproductive failure of the fathead minnow were observed in the experimental lake the following year, precipitating a near extinction of the species that persisted for two years post-treatment.[22] In the current study, we examine recovery of the fathead minnow population that collapsed during whole-lake EE2 additions,[22] through an assessment of male VTG production, gonadal histology of male fish, population parameters, and genetic population structure in fish from the experimental and reference lakes over a period of 7 years. The primary objectives were to determine the timing and magnitude of recovery of a wild fish population from exposure to a potent estrogen mimic, and to establish whether the recovered population originated from the remnant population.

EXPERIMENTAL SECTION

Study Site and Fish Sampling. This whole-ecosystem study took place at the Experimental Lakes Area (ELA), a pristine area set aside for scientific research in northwestern Ontario, Canada (49° 39′ 14″ N, 93° 43′ 18″ W). Lake 260 is a small (34 ha, maximum depth of 14 m) boreal lake to which we added EE2 (Schering AG, Berlin, Germany) by boat, 3x weekly during the open water seasons of 2001−2003 to achieve ambient concentrations of ∼5−6 ng·L^{-1} in epilimnetic waters.[22,23] We studied Lake 260 and two nearby reference systems, Lakes 114 and 442, for just over 1 decade, which encompassed the time prior to, during, and following EE2 additions to the experimental lake. The physical, chemical, and biological characteristics of Lake 260 and the reference lakes are reported elsewhere.[22,23,40,41] Here we report on the recovery of the fathead minnow population during the 7 years (2004−2010) after EE2 amendments were discontinued.

Male fathead minnow were collected from Lake 260 and reference Lakes 114 and 442 in the spring and fall when possible for VTG analyses (2006−2010) and for gonad histology (2004−2010). Sampling was done in accordance with procedures approved by the Fisheries and Oceans Canada Animal Care Committee, Winnipeg, Manitoba, Canada. Whole bodies were either flash frozen between slabs of dry ice and analyzed for VTG content or preserved for histology using the same methods as previously reported.[23,40] EE2 concentrations in water were not measured in 2004 or thereafter, but are postulated to have declined rapidly given the half-life of ∼12 d in the water column of Lake 260 (K. Kidd, data not shown); however, it is possible that prolonged EE2 exposures occurred via the sediments.

Fathead minnow populations at the ELA are similar to other northern boreal regions. Spawning occurs from ∼mid-June to mid-August (P. Blanchfield, pers. obs.), and the breeding population is mainly composed of 1- and 2 year old fish, with few older individuals present. For spring sampling of adult populations, 30 baited minnow traps (Gee) were randomly set in littoral regions (<3 m in depth) around the perimeters of Lakes 260 and 442. Traps were made of square galvanized mesh (6.4 mm diameter), modified to have a single 20 mm opening at one end, and allowed for the capture of fish ≥40 mm in length.[42] In spring, the smallest fathead minnow in our study lakes were ∼35−50 mm in length and almost 1 year old;[43,44] therefore, spring sampling would allow for the capture of young-of-year (YOY) fish from the previous year, if they were present. Minnow traps were collected daily and rebaited with a pasta/flour mixture. Fish were placed into holding containers with lake water and transported to shore, whereupon fish were placed into a large holding pen after measuements were taken. To estimate the size structure of the population, a random subsample of ∼500 fathead minnow, when available, were measured for fork length (nearest mm) and mass (nearest 0.1 g) each year. Sampling occurred in May each year, just prior to the breeding season, over a 10-d period from 2001 to 2006, and a 4−5 d period from 2007 to 2010. Daily catches of fathead minnow were variable, and differences in sampling duration among years did not influence estimates of abundance (catch-

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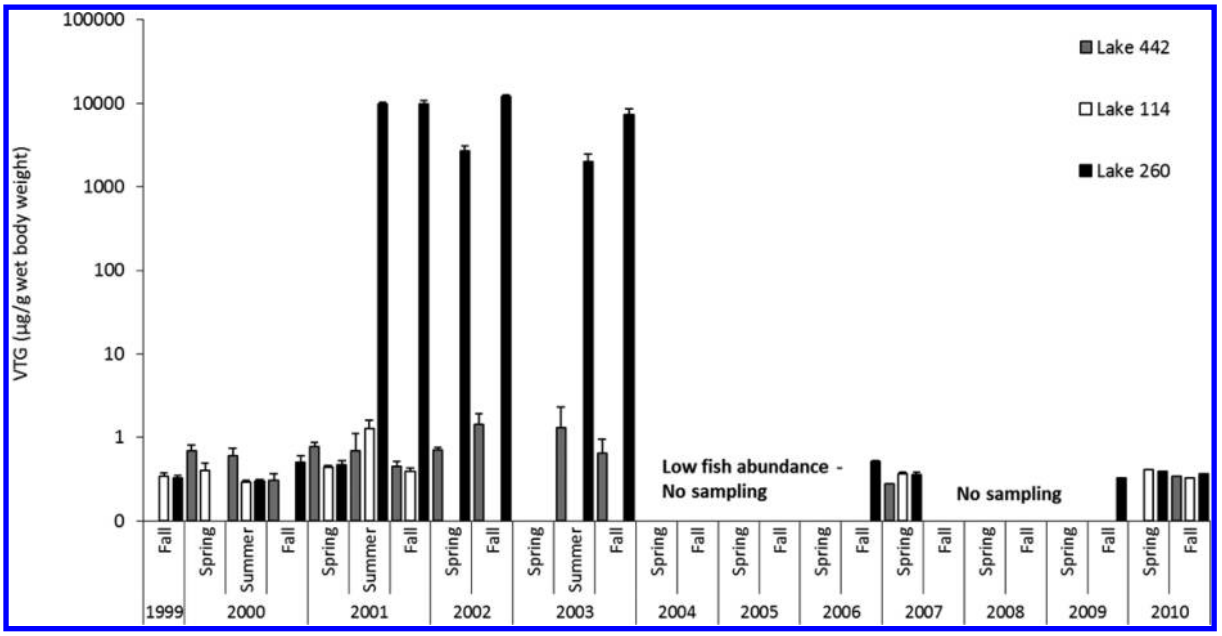

Figure 1. Mean (+SEM) whole-body vitellogenin (VTG) concentrations in male fathead minnow from Lake 260 and reference Lakes 114 and 442 before (1999 to spring 2001), during (summer 2001 to fall 2003), and after (fall 2006 to 2010) additions of EE2 to Lake 260 (n = 4–19 fish/date/lake; there were no VTG samples from 2004 or 2005 due to low abundances, see below; histological sampling was given priority in these years). Early VTG data (up to fall 2003) has been previously reported (refs 22 and 23).

per-unit-effort; CPUE). All fish were returned to the study lakes after the sampling period, except for those taken for VTG and histological analyses. Trap nets used in fall sampling were made from smaller mesh than the minnow traps and able to capture YOY fathead minnow (details in refs 22 and 42).

Population Genetics. Ten microsatellite loci developed for fathead minnow[45,46] were used to determine the genetic source of the fathead minnow collected in 2010 from Lake 260 (n = 59). These specimens were compared to "pre-collapse" genetic samples from Lake 260 (n = 88 pooled from 1999, 2000, and 2001) and to specimens from the two reference lakes (n = 92 and n = 97 in 1999–2001 and n = 63 and n = 41 in 2010 from Lakes 114 and 442, respectively); neither of these lakes is connected to Lake 260. In 2010, specimens were also collected from Lake 660 (n = 62), which appears to be the only lake with fathead minnow that is connected to Lake 260 (although it is downstream of Lake 260 and there are numerous barriers to fish movement between the lakes). Extensive trapping was done in the wetlands between Lakes 260 and 660 and no fathead minnow were found. Lake 112, which is upstream of Lake 260, was also trapped exhaustively for fathead minnow but none were found. A test for null alleles was performed in MICRO–CHECKER v. 2.2.3,[47] and each microsatellite locus was tested for Hardy–Weinberg equilibrium (HWE) and genotypic linkage disequilibrium using GENEPOP v. 4.0.10.[48,49] Two of our original 12 microsatellite loci were found to be out of HWE and were eliminated from subsequent analyses; the remaining 10 were in HWE, and there was no evidence of null alleles or linkage disequilibrium. The genetic distance between lakes and time periods was determined using Arlequin v. 3.5.1.2[50] and GENEPOP v. 4.0.10 to estimate pairwise F_{ST} values and significance levels, and using GenAlEx v. 6.4[51] to estimate pairwise R_{ST} values and significance levels. Population clustering was analyzed using STRUCTURE v. 2.3.4[52] without a priori knowledge of putative populations. Data were analyzed using the admixture model with correlated allele frequencies; runs were performed from K = 1 to 10, with 10 replicates of each K and with an initial burn-in of 50 000 iterations followed by 500 000 Markov Chain Monte Carlo iterations. Using the method of Evanno et al.[53] and the program Structure Harvester,[54] K was chosen based on the greatest change in log-likelihood.

RESULTS AND DISCUSSION

Production of VTG in wild or caged male fishes indicates the presence of estrogens and their mimics in surface waters; rapid production and declines (days to weeks) of VTG are observed during and after estrogen exposures, respectively, the latter of which occurs through improved treatment of MWTP effluents[29] or transfer of fish to clean waters.[31] In the current study, the prolonged and elevated VTG induction in male fathead minnow during EE2 treatment (2001–2003)[22] also disappeared after amendments were terminated. Because of the near extinction of the population in 2002 to 2005, the first postexposure sampling of fish for VTG analyses occurred in fall 2006 and, at this time, there was no evidence of VTG induction in male fathead minnow from Lake 260 (Figure 1). Mean VTG concentrations in male fathead minnow collected from Lake 260 during the post-treatment period ranged from 0.33 ± 0.01 to 0.51 ± 0.09 $\mu g \cdot g^{-1}$; values that were similar to those reported for fathead males from the reference lakes (Lakes 114 and 442) throughout the study (1999–2010), and for fish from Lake 260 prior to the EE2 treatments (1999–2001), indicating full recovery at the biochemical level within 3 years. Additionally, low VTG concentrations in postexposure male fathead minnow leads us to postulate that there was no prolonged EE2 exposure to this species in Lake 260 through sediments; an environmental compartment where EE2 can persist for extended periods.[55]

Intersex and the presence of other gonadal abnormalities in male fishes occurs after laboratory exposures to EE2 (e.g., threespine stickleback, *Gasterosteus aculeatus*),[56] and we have

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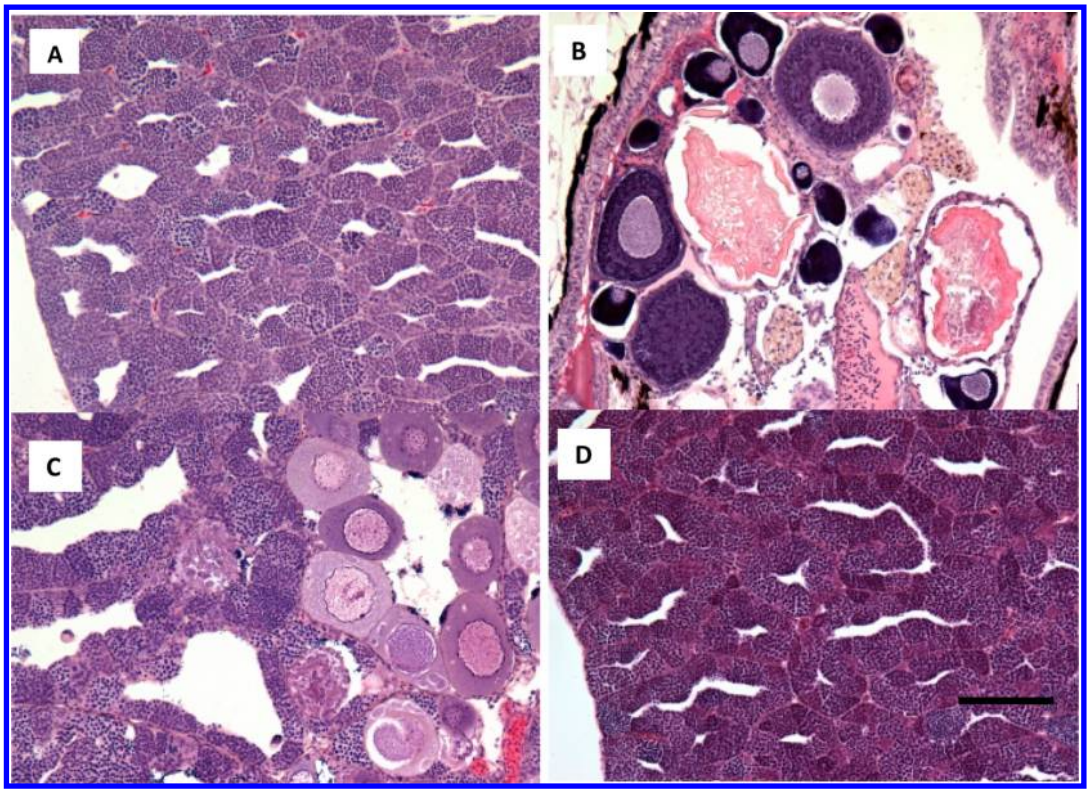

Figure 2. Gonad photomicrographs of male fathead minnow sampled from Lake 260 before, during, and after whole-lake additions of EE2. (A) Spring 2001, prior to EE2 additions; normal male with abundant well-formed spermatogenic tubules. (B) Spring 2004, 7 months after cessation of three consecutive seasons of EE2 additions; spermatogenic tubule structure was absent, whereas fibrotic tissue, edema, pigmented cell accumulations, and primary and atretic oocytes were evident. Males and females could not be clearly discerned. (C) Spring 2005, 18 months after cessation of EE2 additions; feminized male with active spermatogenic tubules interspersed with abundant primary oocytes. (D) Spring 2007, 3.5 years after cessation of EE2 additions; normal male exhibiting typical spermatogenic tubules, comparable to reference lake samples and Lake 260 predose samples (A). Feminization of males and pathological effects were absent; males and females gonads were histologically normal. (Bar = 100 μ, hematoxylin and eosin stain).

previously reported these effects were present in fathead minnow from Lake 260 during the whole-lake addition phase of the study.[22,40] In spring 2004, approximately 7 months after EE2 treatments ceased, all male fathead minnow (100%, n = 10) from Lake 260 contained gonadal abnormalities. No phenotypic males could be readily distinguished, as there was no evidence of active spermatogenesis and a complete lack of seminiferous tubule structure. All specimens contained primary oocytes, amorphous eosinophilic staining (presumed to be due to excess VTG accumulation in extracellular spaces), and abundant pigmented cell accumulations (putative macrophage aggregates; an immune response to chronic tissue injury; Figure 2B). In year two post-treatment (2005), fewer males (54%, 4 of 7) exhibited gonad abnormalities, and these were less severe than in the previous year. Notably, spermatogenic cysts were present in numerous samples, indicating active spermatogenesis, despite the presence of previtellogenic and vitellogenic oocytes (Figure 2C). Sustained testicular abnormalities have also been observed in male zebrafish after 5 months of recovery from exposure to 5 ng·L^{-1} of EE2, and were concurrent with reduced fertilization success.[32] In the current study, fathead minnow collected from Lake 260 in fall 2006 were readily distinguishable as males and females (n = 14 and 9, respectively), and no histological abnormalities were observed (Figure 2D). The histological appearance of the tissues was comparable to that of reference fish and to Lake 260 fish prior to the experimental manipulation (Figure 2A), indicating tissue-level recovery three years after cessation of EE2 additions. Analysis of fathead minnow tissues from subsequent collections (spring 2007, 2009, 2010, and fall 2010; n = 140) confirmed that normal gametogenesis was occurring in the extant population.

The collapse of the fathead minnow population observed during the second and third years of EE2 additions (2002–2003) and for the first two years postadditions (2004–2005) was attributed to reproductive failure that resulted in the near complete absence of YOY fish in Lake 260.[22] The remaining adult population (age 1 and older) continued to decline in abundance from 2003 to 2005, and showed dramatic shifts in size structure each fall toward larger fish. Here we show that effects of the EE2 additions on the adult fathead minnow population persisted for 4 years postmanipulation. Diminished recruitment of YOY to the adult population, indicating reproductive failure, continued up to the spring of 2006, and was followed by recovery of adult size structure by spring 2007 concurrent with an increase in abundance (Figure 3). Recent mesocosm and aquaria studies using similar EE2 concentrations (5.3 ng·L^{-1}) as the whole-lake addition confirm that changes in population structure occur through reduced juvenile production of fathead minnow, but also through lower survival of adult male fish.[35] In the current study, spring catches of adult fathead minnow in the reference lake were variable throughout the study period, but did not show any patterns of changing size structure or abundance. The lowest catches in Lake 442 were

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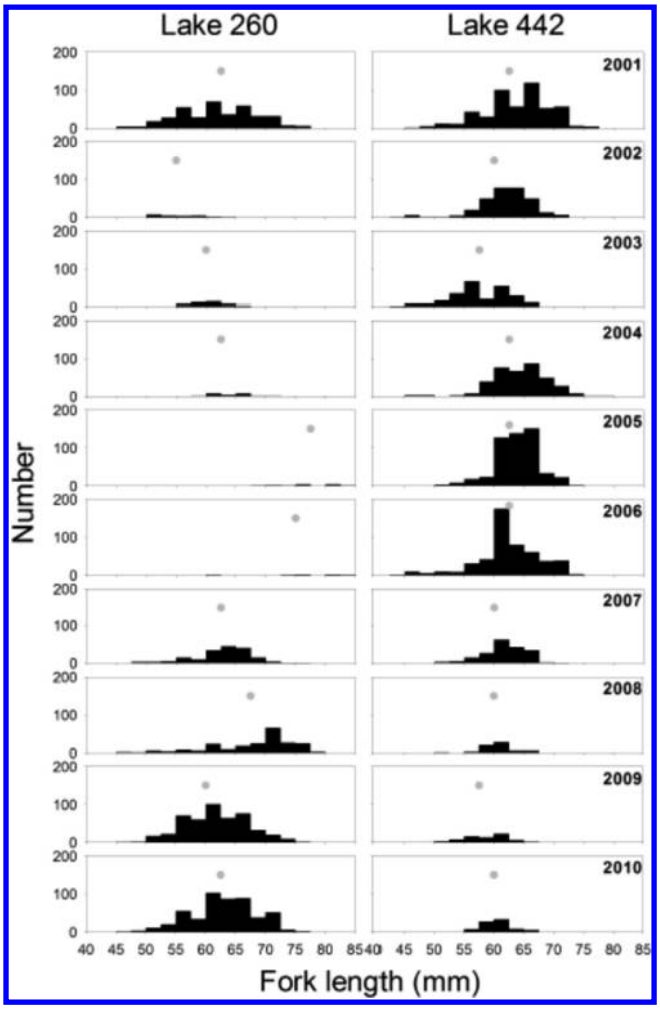

Figure 3. Length–frequency distributions of adult (age 1 and older) fathead minnow in Lake 260 and in reference Lake 442 sampled each spring by minnow traps from 2001 to 2010. Ethynylestradiol (EE2) was added to Lake 260 from May to October of 2001 to 2003; spring sampling in Lake 260 occurred prior to EE2 additions. A total of 500 fish were annually measured in each lake sampling, when available. Mean length of the sampled population is shown (grey circle).

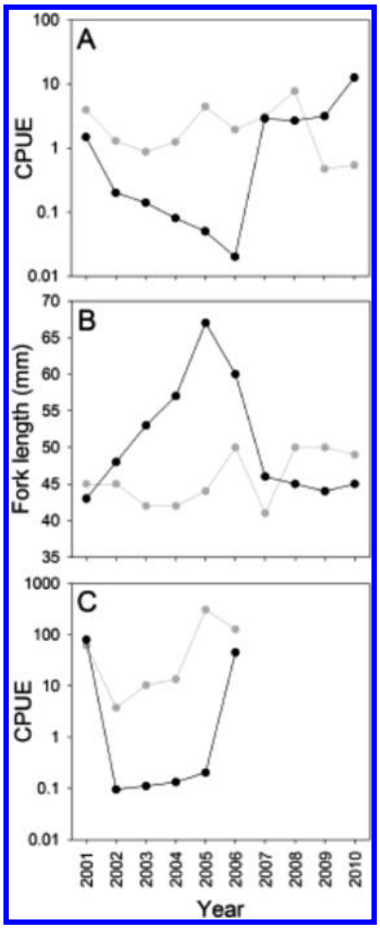

Figure 4. (A) Abundance (catch-per-unit-effort; CPUE) and (B) minimum size of adult (age 1 and older) fathead minnow captured in Lake 260 (black circle) and in reference Lake 442 (grey circle) sampled each spring by minnow traps. (C) CPUE of YOY fathead minnow from fall trap-net sampling (data up to 2005 previously reported in refs 22 and 41).

20-fold greater than the minimum observed in Lake 260 (Figure 3), and highlights that natural variability is much lower for unmanipulated populations than those that experience chemical manipulation (this study) or natural disturbance.[57]

Spring CPUE of adult (age 1 and older) fathead minnow, which is an estimate of the relative size of the breeding population,[42] declined after the first year of EE2 additions, and continued to decline to a minimum of <2% of premanipulation levels by spring 2006 (Figure 4A). The few remaining adults captured in the lake at this time were large (annual mean ± SD; fork length, 74 ± 7 mm; mass, 5.2 ± 1.3 g; n = 7); approximately 25% longer and >2-fold heavier than fathead minnow from 2001, before treatment, and the reference lake (Lake 442 annual mean from 2001–2010; fork length, 59 ± 2 mm; mass, 2.5 ± 0.4 g). Importantly, the minimum size of fathead minnow in Lake 260 increased over time, and was 60 mm in length in 2006, suggesting that little new recruitment of YOY to the breeding population had occurred for some time in this system (Figure 4B). Changes in fish population size-structure have previously not been observed following EE2 exposure, even in multigenerational studies.[32,35] Although fish were not aged in this study, we surmise that the increasing maximum size of fathead minnow during the years of recovery represent a cohort of fish that had initially been exposed to EE2 during whole-lake additions. Fathead minnow typically do not live beyond age 3, but fish up to age 5 are known to occur in populations in northern boreal regions.[57]

By the spring of 2007, almost 4 years after EE2 additions to Lake 260 had ceased, the size structure and abundance of adult fathead minnow recovered (Figures 3 and 4A). Unlike the progressive changes to fathead minnow size structure observed during and for the 3 years following EE2 additions, population recovery occurred rapidly. The abundance of fathead minnow increased >100-fold 1 year (2006–2007); by spring 2007, CPUE was similar to that found prior to EE2 additions (spring 2001), and remained at this level for several years until a further substantial increase in abundance occurred in the final year of sampling (Figure 4A). The presence of smaller size classes of adults (40–50 mm) in the spring of 2007 (Figure 4B) indicates that successful reproduction had occurred the previous summer. Indeed, trap net catches in the fall of 2006 confirmed that YOY fathead minnow were present in similar abundance relative to premanipulation levels (Figure 4C). Of note, the capture of small numbers of YOY fathead minnow during whole-lake EE2 additions and in the initial years of population recovery (2002–2005)[22,41] implies that a low level of

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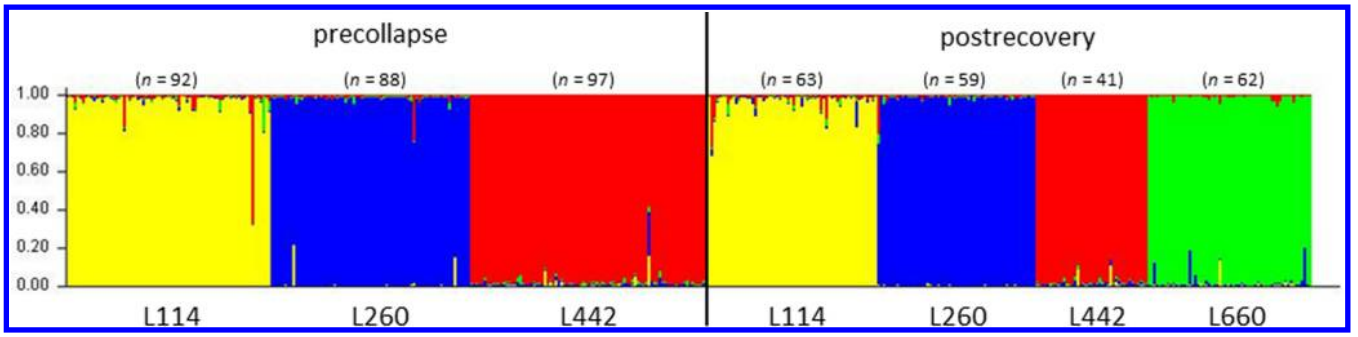

Figure 5. Population assignment from microsatellite data for fathead minnow collected from Lake 260 (L260) and reference lakes (L114 and L442) precollapse (1999, 2000, 2001) and postrecovery (2010), and from Lake 660 in 2010. Individuals within each lake (sample size in parentheses) are represented by a single vertical bar broken into four colored segments. The length of each color within the bar represents the strength of the genetic assignment of that individual to a population cluster. STRUCTURE assigned individual fish to one of four distinct population clusters, which corresponded to the four lakes examined; L114 (yellow), L260 (blue), L442 (red), or L660 (green).

recruitment occurred in Lake 260 throughout the study despite pronounced histological abnormalities in the adults. In laboratory studies, F1 fathead minnow subjected to lifetime EE2 exposure did not produce any viable F2 embryos.[35] Successful reproduction by fathead minnow following multigenerational EE2 exposure may be related to greater breadth of genetic and life history variation in this wild population compared to laboratory-cultured fish, as proposed by others.[35,58] In addition, we suggest individual fish exposure to environmental estrogens in natural settings may be more variable than in aquaria, especially if there are habitats that serve as refugia. For example, during the addition phase of the experiment, the deeper waters of stratified Lake 260 had lower EE2 concentrations (~1−2 ng·L^{-1}) than epilimnetic waters (~5−6 ng·L^{-1}).[23] Because fathead minnow typically inhabit the shallow, nearshore areas of lakes, it is unlikely that this species would have regularly occupied waters >3 m deep (mean depth of the epilimnion in Lake 260 from 2001 to 2005), and therefore would have been exposed to the highest concentrations of EE2. Nonetheless, some variability in EE2 exposure to fathead minnow could have occurred in the nearshore regions of the lake.

Fathead minnow plasticity in life history characteristics, high fecundity, fractional spawning, and a prolonged breeding season (~2 months)[59] collectively allow for rapid population recovery from natural disturbance by small numbers of individuals.[60] In Lake 260, the dramatic increase in the fathead minnow population by spring 2007 from its lowest observed abundance in spring 2006 highlights that population recovery was contingent upon the tissue-level recovery. This is supported by several studies showing that reductions in fecundity, fitness and fertilization success, key predictors of population abundance,[61] occur in fish with testicular and ovarian abnormalities,[13,18] even when VTG concentrations are normal.[32] Furthermore, the rapid population growth of fathead minnow may also have been aided by the altered trophic state of Lake 260 that was an indirect response to EE2 additions.[41] Specifically, the greatest availability of planktonic food for fathead minnow, in conjunction with the lowest predator biomass, occurred in the year preceding rapid population growth (2005) in the experimental lake.[41]

Though unlikely because of physical barriers (see the Experimental Section), recovery of the fathead minnow population in Lake 260 to pretreatment levels may have also occurred through immigration of individuals from the downstream system. Mills et al.[62] reported that fathead minnow from another lake successfully colonized Lake 223 following the extirpation of this species during experimental acidification. In the current study, genotype data (from 10 microsatellite loci) clearly indicated that the fathead minnow collected in 2010 from Lake 260 were descendants of the original EE2-treated population rather than colonizers from downstream Lake 660, the only potential candidate lake (see the Experimental Section). In addition, fish from Lake 260 and downstream Lake 660 were as genetically distinct as those from lakes from which no direct colonization could occur (i.e., Lakes 114 and 442). Fathead minnow from Lake 260 were genetically distinct from those in each of the three other lakes (pairwise F_{ST} values = 0.220−0.315; P = 0.000), and STRUCTURE estimated the most likely number of genetic clusters to be four, with clusters clearly corresponding to each of the four lakes (Figure 5). STRUCTURE showed no distinction between precollapse and postrecovery populations in Lake 260 or the reference lakes; similarly, precollapse versus postrecovery R_{ST} values were not significant in any of the three lakes. Precollapse versus postrecovery F_{ST} in Lake 260 was low but significant (F_{ST} = 0.021, P = 0.000); genetic differentiation pre- versus postcollapse was not significant in either Lake 114 or 442 ($F_{ST} \leq 0.001$; P = 0.228−0.364). Overall, genetic data show that the dramatic recovery of fathead minnow by the fourth year post EE2 additions was the result of in-lake recruitment, rather than immigration from another system. A recent genetic study has shown that in English rivers, populations of roach (*Rutilis rutilis*) that are exposed to estrogens over multiple generations are apparently able to survive without immigration from less polluted sites.[63]

Recovery across all levels of biological organization from exogenous estrogen exposures appears to be related to lifestage, timing, and dose, with much slower recoveries in animals with high or prolonged, especially lifetime, exposures.[31,32] Results from this whole-lake experiment support previous laboratory experiments and modeling, and showed that there is delayed recovery of gonad development and population abundance when EE2 exposures occur over multiple generations of fathead minnow. We demonstrated that for a naturally reproducing population in the absence of immigration, recruitment of new individuals to the adult population at pre-experiment levels took ~4 years following cessation of whole-lake EE2 additions, and occurred when EE2-induced tissue damage was no longer evident. This lengthy recovery is likely due to the lifetime exposure of the offspring (F1 and so on) rather than the adults, because the former lifestage shows more prolonged effects from

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EE2 exposure.[32] In addition, when sexually immature fish are exposed to EE2, their unexposed offspring have reduced survival.[35] This supports the hypothesis that transgenerational effects can occur in fish exposed to estrogens and their mimics. Heritable effects of EE2 were not specifically examined in our whole lake study, so it is possible that other persistent changes in the fathead minnow of Lake 260, such as epigenetic effects (see review in ref 64), have manifested.

Large fluctuations in fathead minnow abundance are a common feature of northern boreal populations that undergo episodic hypoxic winterkill events, demonstrating rapid population growth can occur following natural disturbance.[60] However, unlike during natural disturbances, it appears that population declines from reproductive failures associated with chemical perturbations such as acidification[62] and EE2 (herein) can be sustained for several years after amendments are discontinued. In the latter study, multiple, unexposed generations were a necessary prerequisite for population-level recovery. Notwithstanding the dramatic declines in the fathead minnow from EE2 additions, 7 years of post-treatment monitoring indicate that full population recovery, from the remnant in-lake individuals, can occur. As such, discussions and decisions surrounding predicted no effect concentrations[65] and implementation of wastewater discharge guidelines[66] for compounds like EE2 are warranted to minimize risks to fishes and facilitate recovery of impacted populations.

AUTHOR INFORMATION

Corresponding Author

*P. J. Blanchfield. Phone: (204) 984-4524. Fax: (204) 983-3073. E-mail: Paul.Blanchfield@dfo-mpo.gc.ca.

Notes

The authors declare no competing financial interest.

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