

Focus articles are part of a regular series intended to sharpen understanding of current and emerging topics of interest to the scientific community.

Are We Going About Chemical Risk Assessment for the Aquatic Environment the Wrong Way?

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Abstract—*The goal of protecting the aquatic environment through testing thousands of chemicals against hundreds of aquatic species with thousands of endpoints while also considering mixtures is impossible given the present resources. Much of the impetus for studies on micropollutants, such as pharmaceuticals, came from the topic of endocrine disruption in wild fish. But despite concern over reductions in fish fertility, there is little evidence that fish populations are in peril. Indeed, fish biologists suggest that many cyprinid populations have been recovering for the past 30 to 40 yr. The central assumption, key to current risk assessment, that effects observed in the laboratory or predicted by models are readily transferrable to the population level, is therefore questionable. The neglect in monitoring wildlife populations is the key weakness in environmental protection strategies. If we do not know whether aquatic wildlife species are declining or increasing, how valuable are our other ecotoxicological activities? Environ Toxicol Chem 2016;35:1609–1616. © 2016 SETAC*

Keywords—Aquatic; Risk; Chemical, Wildlife; Monitoring

The Predominant Role of Laboratory Aquatic Ecotoxicity Data

In 1980 Richard Schoettger of the US Environmental Protection Agency stated, “the US scientific community does not have the time, research facilities, trained personnel, experimental animals, nor financial resources to provide the

additional data needed for comfortable predictions of the possible environmental effects of a broad spectrum of chemical contaminants” [1]. If anything, the problem has worsened since his prescient statement. But despite this warning, scientists have continued to attempt this. This research activity has been stimulated by our awareness of the many thousands of personal care products and pharmaceuticals we now consume and discharge in modern developed societies. If, for example, pharmaceuticals were designed to have a biological effect, surely wildlife exposed to these chemicals will be disrupted by this challenge. A very high number of published studies now report changes in gene expression, protein profiles, and metabolite profiles in a variety of aquatic organisms exposed to chemicals, without linking them to any phenotypic change relevant to the animal. We appear to have become very caught up in detail and to have lost sight of the wider picture [2]. The task of testing thousands of chemicals against hundreds of aquatic species with thousands of endpoints is daunting enough [3], but it has become even more challenging as we realize that chemical mixtures may harm wildlife [4]. Despite what seems a mathematically impossible challenge, much of environmental risk assessment still revolves around trickling chemical A onto species B to examine endpoint C in the laboratory [5].

The vast and growing library of results from laboratory-based studies claiming some effects may now be moving risk assessment to being hazard-based only. In the United States the ToxCast program is testing tens of thousands of chemicals in over 300 different in vitro assays to find out which chemicals possess what biological activities [6]. This very large toxicological project is likely to discover that just about every chemical has some biological activity or other, and

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Published online in Wiley Online Library
(wileyonlinelibrary.com). DOI: 10.1002/etc.3441

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many will possess different types of biological activity. In other words, just about all chemicals will present a hazard. So if risk assessment becomes hazard-based (i.e., concentrations and potency are not factored in), just about every chemical will “fail.”

It has been argued that we should react quicker to concerns about chemicals because the scientific methodology used is actually biased toward false negatives [7]. But there is a counter-argument that many ecotoxicology studies tend to be in the “false-positive” camp because they are poorly designed and, therefore, likely to produce unrepeatable results [5]. These can often be the studies purporting to show effects at very low concentrations [8]. In the preparation of species sensitivity distributions (SSDs) for a chemical, typically all the literature is assimilated in an unbiased way. These false positives could then distort the SSD at the critical point where effects are reported at the lowest concentrations, thereby potentially undermining the whole risk-assessment exercise. Despite these uncertainties, the European Community is edging toward putting some pharmaceuticals on the Priority Substances List of the Water Framework Directive COM (2011)876 [9]. Indeed, some countries are planning to massively improve their sewage treatment to eliminate most micropollutants from effluent [9].

Are Laboratory Ecotoxicology Data Alone a Sound Basis for Action?

The Swiss initiative to upgrade many of its sewage-treatment plants (STPs) with advanced tertiary treatments to eliminate most micropollutants has been taken, presumably, on the premise that the chemicals currently present in STP effluents and rivers are adversely affecting aquatic organisms [9,10]. The assumption being that if all these chemicals are removed, things will improve and the ecology of Swiss rivers will get better—there will be more species and more individuals of each species. But if chemicals are not a major factor in the “poor” current ecology of Swiss rivers, then the initiative will not deliver what the proponents presumably expect it will. This initiative is a “real-world,” and very expensive, test of whether or not chemicals currently pose a serious threat to aquatic organisms. If they do not, then the Swiss are definitely going about protection of their aquatic environment in the wrong way! It was previously noted that the decline of trout across many Swiss rivers recorded by the angling community since 1980 was most closely associated by proliferative kidney disease and declining habitat quality rather than exposure to sewage effluent [11].

Know the Baseline

Unless the baseline is known, it is impossible to know if any factor—chemicals in this case—is affecting the ecology of a river. In nature, the populations of most aquatic species will not be highly stable (i.e., consistent) from year to year but

instead will fluctuate. But by how much? Populations of cyprinid fish, for example, can fluctuate by 17-fold or more over several years in southern UK rivers [12]. Supporting this assertion, there is very strong evidence that populations of non-aquatic species (which are monitored much better) can fluctuate markedly. A good example can be found in butterfly species, whose population variability has been linked to variable weather conditions [13]. Hence, we need to know not only what (biologically) is in a river this year but also population sizes from year to year, perhaps over 10 yr or so, before we can be confident in knowing what “normal” is. Only against such a background can we begin to judge if chemicals are adversely affecting those populations. A recent review of the Gulf of Mexico oil spill made the statement “Baseline environmental data are crucial for understanding the impacts of oil spills” [14], thereby emphasizing that potential effects cannot be determined without prior knowledge of baseline ecological data.

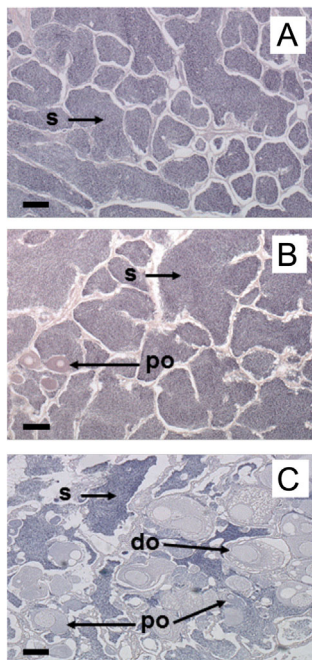
In some instances baseline data can even help unravel the effect mechanism, such as with DDT and eggshell thinning [15]. The author was only able to deduce that the eggs of peregrine falcons were breaking in the nests as a consequence of eggshell thinning because there was a century of data that provided the baseline (how thick is the eggshell of a peregrine falcon normally?). After 1947, the shells of the eggs were thinner than they had been for the previous 100 yr, an effect coinciding with the widespread introduction of DDT as a pesticide.

The Informative Story of Endocrine Disruption

Endocrine disruption in wild fish is one of the most celebrated and apparently well-understood issues of harm to an aquatic species from a domestic micropollutant in modern times [16]. It revealed that minute concentrations of apparently benign steroid estrogen hormones in wastewater were affecting male fish fertility (Figure 1).

With the ubiquity of STPs in developed countries, modeled concentrations of steroidal estrogens indicated that there could be widespread effects across an entire country [17]. We know that the oral contraceptive hormone ethinylestradiol on its own can eliminate fish populations at a concentration of approximately 5 ng/L [18]; but sewage effluent concentrations are typically 0.5 ng/L [19], and 95% of Europe’s rivers have concentrations likely to be less than 0.1 ng/L [20]. Thus, use of oral contraceptives would need to rise by 50 to 500 times in Europe for ethinylestradiol on its own to seriously damage fish populations. However, even if slightly less successful than unaffected fish, to many people’s surprise it was found that even severely intersex fish could still breed [21].

Another argument has been that estrogens may be reducing the genetic diversity of wild fish populations because



Non-intersex male;
intersex index = 0

Mildly intersex fish;
intersex index = 1.8

Severely intersex fish;
intersex index = 4.8

FIGURE 1: Example of ovotestis in male fish that can develop downstream of a sewage-treatment plant. Bar = 100 mm (Photograph by J. Sumpter). s = spermatozoa; po = primary oocyte; do = degenerating oocyte.

infertility or reduced fertility problems could reduce the pool of reproducing males. However, no evidence for this idea has been found [22]. Indeed, fish biologists suggest that UK cyprinid populations have been recovering since reaching a low point in the period from the 1950s to the 1970s [23,24]. What this example illustrates is that what we consider in our laboratories to be the worrying effect of a chemical on an individual organism may be irrelevant in relation to the more important factors controlling reproduction and recruitment in the wild. The central assumption, key to current environmental risk assessment, that effects observed in the laboratory or predicted by models are readily transferrable to the population level [2], is therefore questionable (Figure 2).

Why Is Extrapolating to Population-Level Effects So Uncertain?

Despite it being apparently obvious that severe impacts of a chemical on an individual are likely to lead to population-level effects [2], it has long been recognized that the reality may be different for a range of reasons [25–27]. Much research over the years has been carried out on factors which influence the fluctuations in cyprinid fish populations. Perhaps the most important feature to bear in mind is the demographics of a typical fish population. Unlike our human population, a fish population will often be dominated by the very young, mainly 1-yr-old to 2-yr-old fish (Figure 3).

What this suggests is that if a chemical were to damage such fish only after several years of chronic exposure, then it would be unlikely to have a noticeable effect at the population level (assuming serious impacts occur long after sexual maturity has been achieved). Perhaps we should be more cautious with long-lived migratory species, such as eels and salmonids, which spawn late in life? One of the reasons fish are survivors is the level of redundancy in their reproductive strategy. For example, with many fish species a mature female will reproduce every year and release a prodigious number of eggs, such as approximately 5000 eggs to 30 000 eggs from a roach [28]. Rather surprisingly, chemicals which might harm only the juvenile year 0+ class might not actually be negative for the population because then more juveniles could survive as more resources are now abundant for the survivors [27]. Long-term studies of fish populations typically show rather erratic patterns of boom and bust [29]. This is considered entirely natural, and there is a strong argument that these patterns relate to the environmental conditions in the first few weeks after hatching of the eggs. Negative correlations have been seen with river flow where too much flushes the juveniles out of the river [30] and positive correlations with temperature (juveniles grow faster and stronger and so become better foragers and able to maintain themselves against the current) [31,32]. There is an argument

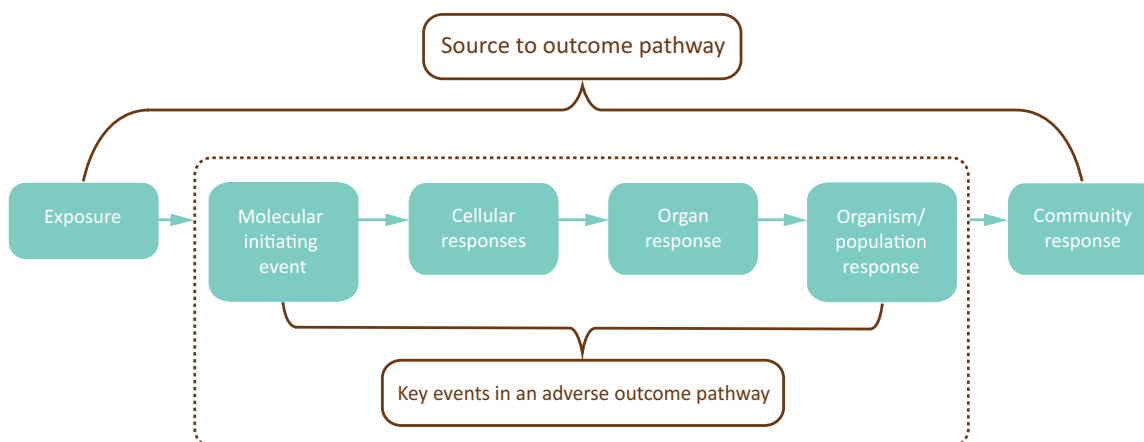


FIGURE 2: Example of an adverse outcome pathway where a molecular event can apparently be used to predict a population impact.

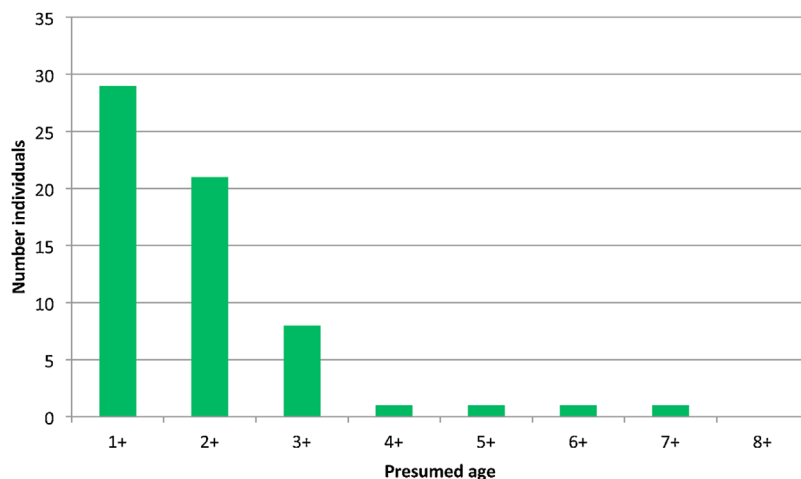


FIGURE 3: A typical cyprinid fish demographic for roach (years of age) from Environment Agency monitoring of the River Aire (United Kingdom) in 2010.

that the apparently erratic fluctuations in population abundance over the years means that trying to find links with chemical exposure is futile. Certainly fish populations do not respond consistently to a particular pressure over time and space. But if the pressure is strong enough, then an impact on populations will become evident no matter how many other variables exist, such as the effects of tributyltin on shellfish [33] or metals in mine-damaged streams affecting fish and invertebrate populations [25,34].

Acknowledging That Real Impacts at Hot Spots Can Occur

It may well be true that for much of the freshwater environment, chemicals are the thing that aquatic organisms should not spend too much time worrying about. Other factors are of more concern [35]. But there are often “hot spots” where chemicals do pose a threat to aquatic organisms. Harbors and marinas were hot spots of the boat antifouling compound tributyltin, and the local shellfish populations suffered accordingly [33]. Back in 1988, internal Yorkshire Water reports indicated that in the downstream River Calder in the United Kingdom very few fish could be found. It is possible that the high discharge of xenobiotic endocrine disrupters such as 4t-nonylphenol from the local textile industry played a role [36]. As Joakim Larsson and others, have suggested, on a river downstream of a STP receiving wastes from pharmaceutical manufacturing companies might well be one such location [37]. Hence, we are not dismissing the potential for chemicals to have adverse effects on fish in local situations where very high exposures have occurred.

Individual Harm Versus Population-Level Effects

Presently, environmental risk assessment is based primarily on whether or not a chemical will have adverse effects on populations. An alternative viewpoint is that harm to an individual from a chemical, even if it does not imperil the

population, is a matter of concern in its own right. After all, that is the standard we apply to humans. Problems come in the definition of harm to an organism. How would we rank levels of harm, particularly, when the effects do not impinge on survival or reproduction? It may be that any chemical, given in sufficient concentration, would evoke some response in an aquatic organism! Where would we draw the line?

Challenging the Argument That When You See Population-Level Effects It Is Already Too Late

One of the strongest arguments for relying on laboratory ecotoxicity studies to assess whether a chemical might be of concern is that if you perceive a population-level effect, it is already too late. In other words, we should focus on pre-emptive laboratory studies to identify potential problems with a chemical at the crucial early stages, before that chemical is widespread in the aquatic environment at concentrations that do harm.

Field Population Monitoring

- The assumption that our laboratory ecotoxicity studies will always identify the sensitive species and critical endpoint for a chemical in question in a timely fashion is doubtful.
- Many argue that if we see populations in decline it is already too late, that they are then unlikely to recover, implying that population monitoring studies are therefore redundant. However, most studies show that populations will indeed recover when the chemical stress is removed (such as with DDT and tributyltin).
- We should consider that if a chemical has been discharged to the environment for many decades and as far as we know the wildlife populations in receiving waters are being maintained, then perhaps there is a message here.

But there are a number of problems with this approach. Firstly, there is the mathematical impossibility of carrying out the required numbers of studies on thousands of chemicals on hundreds of species and tracking thousands of different endpoints (necessitating hundreds of millions of experiments). As the quote from Schoettger in the introduction makes clear, this is simply not possible, for a number of reasons, costs being a major one. Secondly, even if we did pour in massive resources, identifying the critical sensitive species and endpoint might still elude us. Who would have thought to test tributyltin on *Nucella lapillus* reproduction or diclofenac on Asian vulture kidney function?

Populations can recover. For example, populations that disappeared as a result of biological oxygen demand/oxygen stress and/or chemical pollution have recovered when the stress has been removed, such as fish in the United Kingdom in the Trent [23], the Mersey River [38], and the Thames River [39], as well as mollusks after tributyltin use was controlled [40]. Salmonid populations completely decimated by metal pollution fully recovered within 3 generations after the chemical stress was removed [34]. The routine monitoring of wildlife, such as butterflies and bees in the terrestrial environment, alerted us to the fact that populations were declining rapidly [41]. As a result, it has been possible to reverse some of those declines with appropriate management, thereby showing the value of such approaches. Where declines in populations occur, forensic ecotoxicology could be applied (where it has a very good track record).

If a chemical had been discharged into the environment for 30 yr to 40 yr and populations of the exposed species can still function normally, we should be reassured, though some might argue that a population weakened by a harmful chemical might soldier on for years before collapsing. However, if it has withstood the challenge for 30 yr, it probably is not going to collapse in the 31st yr! There are some people who could argue it was an accumulation of lipophilic chemical toxins which has led to the decline of the eel seen in many parts of the world. But the evidence suggests that the eel decline, which started in the early 1980s, occurred in a period of reduced chemical challenge [42]. Eel populations appeared to have done well in the much more polluted postwar period.

Pioneers in Linking Wildlife to Chemical Stressors

There are some environmental scientists who have used the large monitoring data sets collected by regulators to link biodiversity with local pressures [43]. One of the drivers has been the European Water Framework Directive, where attributing the cause of biological decline is required to develop catchment management plans. The approach of using SSDs and then, from chemical measurements, assessing the potentially affected fraction of wildlife for a river location would appear to be logical [44]. However, the degree to which aquatic wildlife is less diverse than it might be in the face of

chemicals from the domestic environment is debatable [45]. What has received less attention from these approaches, however, are the trends in wildlife living in proximity to wastewater over time. Are drier years, when effluent is a more important component of flow, linked to population declines? The observation that macroinvertebrate diversity appears to be generally improving across all parts of the United Kingdom's freshwater system demands an explanation [46], particularly given the relatively high exposure to down-the-drain chemicals in this country [47].

Avoiding Risk-Assessment Failure

We seem to be looking in finer and finer detail at the mechanism of action of chemicals on a wide range of aquatic species without actually attempting to link our findings to population-level effects [48,49]. Even worse, many of the harmful effects that occur with chemicals and wildlife are so unpredictable, or unknowable, that laboratory tests, such as those used in the current regulatory approaches to chemicals, may still miss them (given the tributyltin mollusk reproduction and diclofenac vulture kidney stories). Would it not be advisable, therefore, to put more resources into monitoring wildlife populations as a vital backstop? There would need to be serious consideration of what, where, and how often to monitor [50]; but we are arguing here for a change in philosophy. A valuable addition to this approach is tissue archiving, which can allow retrospective chemical investigation—so you could ask, What is normal? or What happened before [51]? A combination of wildlife monitoring with what might be called forensic or diagnostic ecotoxicology could well be the strongest approach to protecting our natural environment from chemical threats.

It is intriguing that in the field of terrestrial science and ecology monitoring population diversity and abundance has pride of place in assessing the state of the environment and guiding interventions, whereas the terrestrial environmental chemistry community seems to play a modest role. In contrast, in the field of aquatic science and ecology, we do monitor population diversity but pay little attention to abundance, whereas here the aquatic environmental chemistry and ecotoxicology community seems to be very strong and “runs the show.”

The precautionary principle may be a well-intentioned philosophy, but it should not underwrite weak science; nor should it preclude the need to study populations. There is a danger that we become trapped in a perpetual merry-go-round, chasing after an ever-increasing number of chemicals for which no definitive answers on risk will ever be produced. We should be very cautious about advocating the expenditure of huge sums of money in dramatically upgrading sewage treatment to remove trace organics if the chemicals in today's effluent are not currently significantly affecting wildlife populations [52]. If we do not know whether our aquatic wildlife species are declining or increasing, how valuable are



We do not have tests for whether chemicals disrupt molluscan organ development or Asian vulture kidney function. It would take a long time to develop them, by which time another unexpected population crash may have happened! The essential lesson here is surely the need to maintain our vigilance regarding changes to wildlife populations and not put all our faith in bioassays and toxicity tests.

our other ecotoxicological activities? Where we can demonstrate that a species is in decline, we can then ask, Are chemicals involved? Where species and populations that are exposed to the current mix of chemicals discharged in effluent are doing well, then anxieties about chemical effects are likely to be misplaced.

Imaginative Approaches to Monitoring Aquatic Wildlife

Canada is fortunate in having a well-planned environmental monitoring program [53], but such comprehensive approaches seem rare. European countries have had to comply with the Water Framework Directive and monitor macro-invertebrate species diversity every couple of years. This monitoring may now extend to fish, but the number of locations examined and the frequency of examination are limited by resources. Many countries (such as China and Japan) do not carry out even this level of routine wildlife monitoring throughout their river networks. Perhaps, rather than directly counting fish, we might be able to assess aquatic populations by detecting their DNA signatures in the future [54]? Alternatively, could there be a role here for the public? In the United Kingdom a range of charities, dependent on public donations rather than government funding, have organized ongoing bird surveys by citizen volunteers for the last 40 yr. Anglers catch thousands, perhaps millions, of freshwater fish each week. Could these catches be recorded to provide the “missing” picture? Some of these angling data have been collected and used in the past [24,55]. With the proliferation of smart phones, if an app was developed that allowed transfer of these records (as has been created for a range of insects and birds in the United Kingdom), with minimum effort, to a central database, then over time a comprehensive picture of fish populations could be assembled. Although not perfect, these databases might provide valuable background information on the health of fisheries.

Our Strategic Direction

This and many similar journals have borne witness to the great deal of time, money, and expertise that have been devoted to the testing of chemicals for their possible adverse environmental impacts. But outside identifying acute toxicity and persistence characteristics, it is very difficult to say how successful these approaches have been. Our direction of travel seems to be to devise yet more tests or bioassays to reveal possible effects at the molecular/receptor level [56]. We argue that without a good knowledge of populations of aquatic organisms and their natural fluctuations, it is not possible to know if the current approaches are protecting the environment from chemicals. Monitoring aquatic wildlife diversity and abundance over time is probably the single most important activity that we should be doing in freshwater environmental science today and vital to the risk assessment of chemicals.

Acknowledgment

To the British tradition of enjoying wide-ranging discussions over a cup of tea! The authors are grateful for helpful comments and suggestions from the reviewer, R. Shore, and to A. de Polo. The lead author is grateful for CEH Science Budget support provided through NERC.

Data availability

There are no data in this *Focus* article other than for Figure 3. This would be available from the authors on request (ajo@ceh.ac.uk).

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