Published in final edited form as:

Mol Biosyst. 2007 March ; 3(3): 172–177.

Fish ‘n’ chips: the use of microarrays for aquatic toxicology

Nancy D. Denslow^a, Natàlia Garcia-Reyero^b, and David S. Barber^c

^a Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL E-mail: ndenslow@ufl.edu; Fax: +1 (1)352 392 4707; Tel: +1 (1)352 392 4700 Ext. 5563

^b Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL E-mail: nvinas@ufl.edu; Tel: +1 (1)352 392 4700 Ext. 5583

^c Department of Physiological Sciences and Center for Environmental and Human Toxicology, University of Florida, Gainesville, FL E-mail: BarberD@mail.vetmed.ufl.edu; Tel: +1 (1)352 392 4700 Ext. 5540

Abstract

Gene expression analysis is changing the way that we look at toxicity, allowing toxicologists to perform parallel analyses of entire transcriptomes. While this technology is not as advanced in aquatic toxicology as it is for mammalian models, it has shown promise for determining modes of action, identifying biomarkers and developing “signatures” of chemicals that can be used for field and mixture studies. A major hurdle for the use of microarrays in aquatic toxicology is the lack of sequence information for non-model species. Custom arrays based on gene libraries enriched for genes that are expressed in response to specific contaminants have been used with excellent success for some non-model species, suggesting that this approach will work well for ecotoxicology and spurring on the sequencing of cDNA libraries for species of interest. New sequencing technology and development of repositories for gene expression data will accelerate the use of microarrays in aquatic toxicology. Notwithstanding the preliminary successes that have been achieved even with partial cDNA libraries printed on arrays, ecological samples present elevated challenges for this technology due to the high degree of variation of the samples. Furthermore, recent studies that show nonlinear toxic responses for ecological species underscore the necessity of establishing time and dose dependence of effects on gene expression and comparing these results with traditional markers of toxicity. To realize the full potential of microarrays, researchers must do the experiments required to bridge the gap between the ‘omics’ technologies and traditional toxicology to demonstrate that microarrays have predictive value in ecotoxicology.

Introduction

Ecosystems are complex entities, changing on a daily basis due to natural seasonal variations, as well as stressors such as global climate change and anthropogenic contaminants. Many habitats have degraded over the past century with adverse consequences on the populations of some species and in extreme cases, species extinctions.^{1,2} The goal of ecotoxicology is to measure and predict the impact of pollutants on populations, communities, and ecosystems. It is often difficult to define the role of natural stressors *versus* chemical toxicity in these situations. It is even more difficult to determine the contribution of individual contaminants to overall toxicity. While it is clear that exposure of animals to toxicants produces adverse effects that are dose and time dependent, it is also clear that organisms are rarely exposed to single components—rather the exposure is to complex mixtures of potential etiological agents.

Ecotoxicologists have struggled at times to make sense of the health changes observed in exposed animals because they must typically base their work on known mechanisms of toxicity. However, gene expression analyses are changing the ways researchers can approach ecotoxicology. The ‘omics’ technologies (transcriptomics, proteomics, metabolomics) are

discovery technologies that open the door to understanding new mechanisms of action and the interaction among contaminants. Microarray analyses provide the means to rapidly assess the impacts of stressors on all biochemical pathways at the level of mRNA transcription. In this manner it is analogous to a multi-biomarker approach.^{3,4} Microarrays have the potential to provide information about mechanisms and modes of action for classes of chemicals and provide chemical signatures of toxicity.⁵ By simultaneous parallel analysis of multiple biochemical pathways, it is possible to get a *system wide* understanding of the toxicity, touching on pathways that are known and those that are not, finally allowing a better understanding of the observed phenotypic changes.

Fish are likely to be impacted by anthropogenic contaminants since they are continuously exposed to water, the major sink for many contaminants. There are environmental monitoring efforts in most countries to assess potential damage to the environment before it causes irreparable damage. However, these efforts, as well as risk-assessment efforts, are complicated by the presence of complex mixtures in field sites and our lack of knowledge about the mechanism of toxicity for many chemicals or the interaction that occurs when compounds are found as mixtures. Gene expression analysis has the potential to help resolve some of these issues by pointing out biochemical pathways that are altered due to exposure. But simply analyzing for changes in gene expression at a contaminated site is far removed from understanding how a population may be adversely affected, and it is at this level that laws governing the use and disposal of chemicals are based (Fig. 1). For the 'omics' technologies to gain major acceptance in ecotoxicology, they must show how biomarkers identified at the molecular level predict adverse effects at the organismal, population, and even ecosystem levels.

2. The use of gene expression analysis in aquatic toxicology: successes and challenges

Gene expression analyses have been used to great advantage in mammalian toxicology in recent years.^{6–16} Completion of the human and mouse genomes has enabled commercial development of both oligonucleotide based and spotted cDNA microarrays, along with the required bioinformatics support. This has been the basis for the logarithmic expansion of gene expression analyses in mammalian systems.

Gene expression analysis has been much less widely employed in aquatic toxicology primarily because genome sequencing for ecologically relevant species is still in the early stages. To date there are completed genomes for only a few fish species including the green spotted puffer fish (*Tetraodon nigro-viridis*), the Japanese puffer fish (*Takifugu rubripes*) and the zebrafish (*Danio rerio*). Of these, commercial arrays only exist for zebrafish, with a 14 K array available from Affymetrix and a 21 K array available from Agilent. Consequently, many microarray studies have been performed with zebrafish.^{17–23}

Studies using these arrays illustrate their use in understanding physiological responses, determining mechanisms of action for toxicants and other natural stressors and defining modes of action for new chemicals. Van der Meer *et al.*²² examined the expression of over 15 000 genes in the zebrafish gill following 3 weeks of hypoxia. Of the 367 differentially expressed genes, they identified groups that were involved in energy production, protein synthesis, cytoskeletal components and stress response, all expected biochemical pathways were impacted by hypoxia. But, the array study also identified significant changes in lysosomal lipid metabolism in zebra-fish that had adapted to long-term hypoxia. This novel and unexpected adaptive mechanism must now be studied in greater detail to fully understand its impact on higher order physiological levels. In another study, Handley-Goldstone *et al.*¹⁹ used microarrays to examine the mechanism of dioxin (TCDD) induced cardiovascular toxicity in

zebrafish fry. They observed the expected changes in cytochrome P4501A1 (CYP1A1), but they also saw changes in troponin and myosin as well as changes in fatty acid and steroid metabolism and protein synthesis. This work led to several new hypotheses of the mechanisms of TCDD toxicity in fish. Van der Ven *et al.*²³ demonstrated that the antidepressant pharmaceutical mianserin has estrogenic activity in zebrafish by comparing changes in gene expression caused by mianserin to those caused by estradiol. These three studies illustrate the utility of microarrays for a species with a known genome.

For most ecological species, researchers must first obtain gene sequences and construct their own custom arrays, prior to performing the toxicologic study. To accomplish this, researchers have sequenced cDNA libraries for individual tissues or used suppressive subtractive hybridization techniques (SSH)²⁴ or differential display (DD) RT-PCR^{25–28} to develop libraries that are enriched in differentially regulated genes. While not perfect, the SSH approach is superior to un-normalized cDNA libraries which may be populated mostly by highly abundant housekeeping genes that do not change as a result of exposure. While DD RT-PCR is a powerful technique,²⁹ it is extremely labor intensive and often does not generate sufficient gene sequence information to identify responsive genes. All of these techniques have contributed in one way or another to the development of custom arrays for specific purposes containing in the range of 50 to 2000 genes.

Gene sequences obtained in this manner can be used to prepare spotted cDNA arrays or oligo-based arrays. While this is still a far cry from having a whole transcriptome, a number of labs have successfully used this approach.^{30–43} For example, Sheader *et al.*³⁸ used a custom cDNA microarray to identify up-regulation of 27 transcripts, including Cu/Zn superoxide dismutase, thioredoxin, a peroxiredoxin and a glutathione-S-transferase, in European flounder (*Platichthys flesus*) exposed to cadmium. The experiment highlighted a number of candidate genes for further analysis as potential biomarkers for cadmium exposure. In a different study, Tilton *et al.*⁴⁴ used toxicogenomic profiling to demonstrate that the hepatic tumor promoters indole-3-carbinol (I3C) and 3,3'-diindolylmethane (DIM) promoted hepatocarcinogenesis through estrogenic mechanisms in trout liver.

Many of the toxicant exposures for which we have data have been accomplished in laboratories under controlled conditions and usually with single contaminants. The more relevant of these studies use concentrations and routes of exposure that are in the environmental range. While environmental exposures rarely contain a single stressor, these single chemical studies are important because they set the stage against which more complex exposures can be compared. For example, laboratory exposures of rainbow trout (*Oncorhynchus mykiss*) to a series of model toxicants with different modes of action showed unique response patterns.⁴⁵ In this study the chemicals that were tested included ethynylestradiol (a synthetic estrogen), 2,2,4,4'-tetrabromodiphenyl ether (BDE-47, thyroid active reagent), the aquatic herbicide diquat (oxidant stressor), chromium VI, and benzo[a]pyrene (BaP). Each exposure caused between 64 and 222 genes to be significantly altered and the majority of responsive genes were unique to a single chemical, showing a link between gene expression profiles and mode of toxicity. It is not clear whether a mixture of these compounds, as might be found in the environment, would generate gene expression patterns that would remain true to each of the compounds, or whether significant interactions among the contaminants and biological systems would change expression patterns. The problem of accurately predicting toxicological impacts from mixtures is still among the unsolved.

There have been a few studies that have used custom arrays to analyze gene expression patterns for field samples. European flounder (*Platichthys flesus*) have been used to monitor contamination along the coastline in the UK. The Tyne estuary is polluted with polycyclic aromatic hydrocarbons (PAHs) and heavy metals. Williams *et al.*⁴¹ used a custom cDNA

microarray to compare gene expression patterns from fish caught in the Tyne to those caught in the relatively unpolluted Alde estuary (UK). Seven transcripts were found to be significantly higher in the Tyne male fish: cytochrome P450 1A (CYP1A), uridine diphosphate glucuronosyl transferase (UDPGT), α -HSD-glycoprotein, dihydropyrimidine dehydrogenase, copper/zinc superoxide dismutase (Cu/Zn SOD), aldehyde dehydrogenase and paraxonase. Four transcripts were found to be significantly less abundant in the Tyne male fish: elongation factor 1 and 2, Int-6, a subunit of the eukaryotic translation initiation factor and complement component 3. No significant differences were observed in female fish due to the high variation within each site. This study illustrates the potential for micro-arrays to be used on field sampled fish without a sequenced genome. It also illustrates the challenges inherent in the use of microarrays in aquatic toxicology, namely high levels of variation that can mask effects of contaminants.

3. Considerations for the use of microarrays in ecotoxicology

For all of the successes, it is important to recognize the limitations of microarrays. Experiments must be carefully planned and interpreted to obtain accurate information. In most experiments the goal is to compare gene expression in exposed animals to the normal state. However, this is not a simple task as there are many intrinsic sources of variability in expression profiles including the physiological state of the animals, their age, season of analysis, sex and genetic polymorphisms. Temperature and day length are known signals that start the process of reproduction in fish, thus innately changing gene expression.^{46,47} Other pathways not involved in reproduction are also affected by season, such as biochemical pathways involved in freeze resistance in smelts⁴⁸ or chromatin remodelling by specific histones.⁴⁹ It is possible that contaminants may have specific seasons during which they are more potent because they disrupt a particularly sensitive biochemical pathway.

In ecotoxicology there is the added complication of genetically distinct populations that can also affect gene transcription. Oleksiak *et al.*⁵⁰ found extensive variation in both cardiac metabolism and the expression of metabolic genes among *Fundulus heteroclitus* from natural outbred populations raised in a common environment. Clearly establishing normal variations in gene expression is a critical step in being able to determine when a change caused by exposure to a stressor is significant. Being aware of this variability and planning experiments with sufficient statistical power is necessary to obtain useful data. Pooling samples is a cost-effective way to increase statistical power, however, it prevents estimation of individual variation. If assessing individual variation is an important part of the experimental aims, increasing the sample size is the most appropriate way to increase statistical power.

It is critical to determine temporal and dose dependency on changes in gene expression, as these are important considerations when determining mechanisms of action. Transcriptome responses to exposure vary with dose and time and may not be linear, *i.e.* the magnitude of a given response may not increase consistently with the dose (Fig. 2). Within hours after being exposed to a contaminant, gene expression changes begin to occur. At short times or low doses, the genes that are induced tend to be adaptive, protecting the organism from toxicity. Examples of adaptive changes include induction of metallothioneins by metals, or heat shock proteins for a variety of stressors. As the dose increases, the animal tries to compensate for the loss of essential pathways caused by the exposure, and new biochemical pathways are activated resulting in the expression of new sets of genes. Compensatory changes are different from adaptation and are thought to be more clearly associated with adversity. As the dose (or time) increases further, the organism can no longer adapt or compensate, and serious adverse effects occur. Under this circumstance, gene expression may continue to change but homeostasis cannot be achieved. Continued exposure at this level will lead to disease, cessation of growth and reproduction or death. Adaptation, compensation and toxicity form a continuum of responses and the genes associated with each phase of the response will vary. It is important

that gene expression studies be done in the first and second phases of the response, such that changes in gene expression can be linked directly to mechanisms of action for each contaminant and to adverse effect if compensation fails. At the highest dose–duration combination, observed changes in gene expression may no longer relate to the mechanisms of toxicity specific to the contaminant, but rather to general toxicity.

It is imperative that laboratory exposures correlate changes in gene expression with phenotypic changes in the animal, especially pathological changes, reproductive output and growth, as these are well-studied endpoints for which an abundance of toxicological data exists. Changes in gene expression profiles that are anchored to these phenotypic changes will allow investigators to relate changes that occur at each level of biological organization over a range of concentrations that are environmentally relevant. This process is termed “phenotypic anchoring” and allows the investigator to determine the relationship between changes in gene expression and conventional toxicological endpoints.⁵¹ Establishing the relationship between changes in gene expression and traditional markers of toxicity is the next critical step in applying microarrays to aquatic toxicology. Experiments like those performed by Hamadeh *et al.*⁵ and Moggs *et al.*⁵² in rats, which show a clear correlation among gene expression changes, tissue structural changes and contaminants, must be done in ecologically relevant species.

Finally, researchers must also be aware of inaccuracies that are present in micro-array analysis. Microarrays do not provide a truly quantitative assessment of gene expression, but instead show changes in biochemical pathways and processes that are linked together. For true quantitation, real time RT-PCR should be performed to validate changes observed by microarrays.

4. Work in progress

Gene expression analysis holds great promise for understanding basic biology and toxicology and may also help with risk assessment, because it may be able to pinpoint contaminant specific expression patterns,^{5,32} even in complex mixtures. The application of this technology to aquatic toxicology has been hindered by the lack of sufficient sequence information for wild fish and financial support from government agencies for sequencing projects. While researchers have successfully used custom arrays for fish without sequenced genomes, most of the arrays for these models are laboratory-specific and include relatively small numbers of genes, making the arrays useful for specific research efforts but not necessarily for whole ecosystem monitoring. Unfortunately full genome sequencing has been beyond the means of most researchers. However, with the advent of new parallel sequencers it is now possible to obtain substantial sequence information quickly and at much reduced cost. For example, the 454 Life Sciences GS 20 massively parallel DNA sequencer is able to sequence over three hundred thousand DNA fragments in a single four-hour run reducing the time and cost of obtaining a nucleotide sequence. Each DNA fragment generates an average sequence of 100–110 nucleotides which are then assembled into larger overlapping sequences using specialized software. This new sequencer exhibits a quantum leap in sequencing speed and depth, giving not only >20 Mbases of DNA sequence in just a few days, but also allowing the sequencing to be done to great depth to give sufficient sequence information for microarray studies.

Annotation of the genes for non model species continues to be challenge. While many of the genes are similar to annotated genes already in the databases, there are still many genes that cannot be identified. This is confounded by the mistakes in the databases and care must be taken to validate unexpected results. An important resource is the gene ontology (GO) database which categorizes annotated genes into known biochemical pathways.⁸ While not perfect, GO

terms can help identify biochemical pathways affected by toxicant exposure by grouping the changes in multiple genes associated with a specific pathway.⁵³

The biggest challenge for using micro-arrays in ecotoxicology is extrapolating effects from the molecular scale to the cellular level to individuals to populations and finally to various species. In particular the selected biomarkers must predict adverse outcome. Changes in gene expression must be integrated with information on adverse effects in individuals and populations. While these techniques offer the promise of distinguishing mechanisms of action, even in complex mixtures, they still have to be validated in this endeavor. Work in this arena continues across the globe, most of the studies continue to point to the usefulness of this approach, despite the cost of doing the experiments. It is likely that the cost will be reduced as the technology becomes incorporated into standard laboratory approaches. Finally, round robin experiments among established laboratories will be necessary to establish the robustness of the method and to convince the critics that the method is worthy of consideration.

Conclusions

Microarrays can provide a valuable complement to existing ecotoxicologic methods used in evaluating environmental contamination. In the short term, microarrays will allow the identification of novel biomarkers for specific contaminants and may aid in the development of new assays aimed at identifying adverse effects. Indeed, examples of the identification of potential novel biomarkers for classes of toxicants are already emerging from genomic analyses. But the real promise is in the long term, where specific changes in gene expression may be used to unravel the toxicity of mixtures. Their ability to identify novel mechanisms of action and to help understand the toxicity of mixtures should be integrated into risk assessments. While much research is still needed to determine how exactly this approach can contribute to risk management, it should ultimately lead to more efficient decision making. The full application of gene expression studies to the ecological arena will require cooperation among researchers and support from national and international governmental funding bodies.

Acknowledgements

This work has been supported by the Superfund Basic Research Projects from the National Institute of Environmental Health Sciences, NIEHS (P42 ES 07375 and RO1 ES015449) and by the Environmental Protection Agency STAR grant, EPA (R831848). In addition we are grateful for a fellowship to Natàlia Garcia-Reyero from the Spanish Ministry of Sciences and Technology (EX-2004-0986), co-funded by the European Union.

References

1. Lande R. *Res Pop Ecol* 1998;40:259–69.
2. Tanaka Y. *Chromosome* 2003;53:421–5.
3. Galloway TS, Brown RJ, Browne MA, Dissanayake A, Lowe D, Jones MB, Depledge MH. *Environ Sci Technol* 2004;38:1723–31. [PubMed: 15074681]
4. Handy RD, Galloway TS, Depledge MH. *Ecotoxicology* 2003;12:331–43. [PubMed: 12739879]
5. Hamadeh HK, Bushel PR, Jayadev S, Martin K, DiSorbo O, Sieber S, Bennett L, Tennant R, Stoll R, Barrett JC, Blanchard K, Paules RS, Afshari CA. *Toxicol Sci* 2002;67:219–31. [PubMed: 12011481]
6. Akerman GS, Rosenzweig BA, Domon OE, McGarrity LJ, Blankenship LR, Tsai CA, Culp SJ, MacGregor JT, Sistare FD, Chen JJ, Morris SM. *Mutat Res* 2004;549:43–64. [PubMed: 15120962]
7. Bild AH, Yao G, Chang JT, Wang Q, Potti A, Chasse D, Joshi MB, Harpole D, Lancaster JM, Berchuck A, Olson JA Jr, Marks JR, Dressman HK, West M, Nevins JR. *Nature* 2006;439:353–7. [PubMed: 16273092]
8. Harris AJ, Dial SL, Casciano DA. *Mutat Res* 2004;549:79–99. [PubMed: 15120964]
9. Huang Q, Jin X, Gaillard ET, Knight BL, Pack FD, Stoltz JH, Jayadev S, Blanchard KT. *Mutat Res* 2004;549:147–67. [PubMed: 15120968]

10. Kowara R, Karaczyn A, Cheng RY, Salnikow K, Kasprzak KS. *Toxicol Appl Pharmacol* 2005;205:1–10. [PubMed: 15885260]
11. Powell CL, Kosyk O, Ross PK, Schoonhoven R, Boysen G, Swenberg JA, Heinloth AN, Boorman GA, Cunningham ML, Paules RS, Rusyn I. *Toxicol Sci* 2006;93:213–22. [PubMed: 16751229]
12. Sawada H, Takami K, Asahi S. *Toxicol Sci* 2005;83:282–92. [PubMed: 15342952]
13. Seidel SD, Stott WT, Kan HL, Sparrow BR, Gollapudi BB. *Int J Toxicol* 2006;25:57–64. [PubMed: 16510358]
14. van't Veer LJ, Dai H, van de Vijver MJ, He YD, Hart AA, Mao M, Peterse HL, van der Kooy K, Marton MJ, Witteveen AT, Schreiber GJ, Kerkhoven RM, Roberts C, Linsley PS, Bernards R, Friend SH. *Nature* 2002;415:530–6. [PubMed: 11823860]
15. Watanabe H, Suzuki A, Mizutani T, Khono S, Lubahn DB, Handa H, Iguchi T. *Genes Cells* 2002;7:497–507. [PubMed: 12047351]
16. Xie Y, Trouba KJ, Liu J, Waalkes MP, Germolec DR. *Environ Health Perspect* 2004;112:1255–63. [PubMed: 15345372]
17. Carney SA, Chen J, Burns CG, Xiong KM, Peterson RE, Heideman W. *Mol Pharmacol* 2006;70:549–61. [PubMed: 16714409]
18. Corredor-Adamez M, Welten MC, Spaink HP, Jeffery JE, Schoon RT, de Bakker MA, Bagowski CP, Meijer AH, Verbeek FJ, Richardson MK. *Evol Dev* 2005;7:362–75. [PubMed: 16174031]
19. Handley-Goldstone HM, Grow MW, Stegeman JJ. *Toxicol Sci* 2005;85:683–93. [PubMed: 15716485]
20. Lam SH, Gong Z. *Cell Cycle* 2006;5:573–7. [PubMed: 16582610]
21. Mathavan S, Lee SG, Mak A, Miller LD, Murthy KR, Govindarajan KR, Tong Y, Wu YL, Lam SH, Yang H, Ruan Y, Korzh V, Gong Z, Liu ET, Lufkin T. *PLoS Genet* 2005;1:260–76. [PubMed: 16132083]
22. van der Meer DL, van den Thillart GE, Witte F, de Bakker MA, Besser J, Richardson MK, Spaink HP, Leito JT, Bagowski CP. *Am J Physiol* 2005;289:R1512–9.
23. van der Ven K, Keil D, Moens LN, Hummelen PV, van Remortel P, Maras M, De Coen W. *Chemosphere* 2006;65:1836–45. [PubMed: 16750242]
24. Diatchenko L, Lau YF, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD. *Proc Natl Acad Sci U S A* 1996;93:6025–30. [PubMed: 8650213]
25. Carginale V, Capasso C, Scudiero R, Parisi E. *Gene* 2002;299:117–24. [PubMed: 12459259]
26. Denslow ND, Bowman CJ, Ferguson RJ, Lee HS, Hemmer MJ, Folmar LC. *Gen Comp Endocrinol* 2001;121:250–60. [PubMed: 11254367]
27. Denslow ND, Kocerha J, Sepulveda MS, Gross T, Holm SE. *Mutat Res* 2004;552:19–34. [PubMed: 15288539]
28. Lee PH, Goetz FW. *Mol Reprod Dev* 1998;49:112–8. [PubMed: 9444654]
29. Liang P, Pardee AB. *Science* 1992;257:967–71. [PubMed: 1354393]
30. Bayne CJ, Gerwick L, Fujiki K, Nakao M, Yano T. *Dev Comp Immunol* 2001;25:205–17. [PubMed: 11164886]
31. Kanamori A. *Mol Reprod Dev* 2000;55:31–6. [PubMed: 10602271]
32. Larkin P, Folmar LC, Hemmer MJ, Poston AJ, Denslow ND. *EHP Toxicogenomics* 2003;111:29–36. [PubMed: 12735107]
33. Liu L, Fujiki K, Dixon B, Sundick RS. *Cytokine* 2002;17:71–81. [PubMed: 11886174]
34. Miura T, Kudo N, Miura C, Yamauchi K, Nagahama Y. *Mol Reprod Dev* 1998;51:235–42. [PubMed: 9771643]
35. O'Farrell C, Vaghefi N, Cantonnet M, Buteau B, Boudinot P, Benmansour A. *J Virol* 2002;76:8040–9. [PubMed: 12134009]
36. Sagerstrom CG, Kao BA, Lane ME, Sive H. *Dev Dyn* 2001;220:402–8. [PubMed: 11307172]
37. Sangrador-Vegas A, Lenington JB, Smith TJ. *Cytokine* 2002;17:66–70. [PubMed: 11886173]
38. Sheader DL, Williams TD, Lyons BP, Chipman JK. *Mar Environ Res* 2006;62:33–44. [PubMed: 16624402]

39. Shi YH, Liu J, Xia JH, Gui JF. *Cell Res* 2002;12:133–42. [PubMed: 12118939]
40. Soetaert A, Moens LN, Van der Ven K, Van Leemput K, Naudts B, Blust R, De Coen WM. *Comp Biochem Physiol, C* 2006;142:66–76.
41. Williams TD, Gensberg K, Minchin SD, Chipman JK. *Aquat Toxicol* 2003;65:141–57. [PubMed: 12946615]
42. Xie J, Wen JJ, Chen B, Gui JF. *Gene* 2001;271:109–16. [PubMed: 11410372]
43. Xie J, Wen JJ, Yang ZA, Wang HY, Gui JF. *J Exp Zool, Part A* 2003;295:1–16.
44. Tilton SC, Givan SA, Pereira CB, Bailey GS, Williams DE. *Toxicol Sci* 2006;90:61–72. [PubMed: 16192472]
45. Hook SE, Skillman AD, Small JA, Schultz IR. *Aquat Toxicol* 2006;77:372–85. [PubMed: 16488489]
46. Jodo A, Kitahashi T, Taniyama S, Bhandari RK, Ueda H, Urano A, Ando H. *Zool Sci* 2005;22:1331–8. [PubMed: 16462105]
47. Kazeto Y, Trant JM. *J Mol Endocrinol* 2005;35:571–83. [PubMed: 16326841]
48. Liebscher RS, Richards RC, Lewis JM, Short CE, Muise DM, Driedzic WR, Ewart KV. *Physiol Biochem Zool* 2006;79:411–23. [PubMed: 16555199]
49. Pinto R, Ivaldi C, Reyes M, Doyen C, Miettton F, Mongelard F, Alvarez M, Molina A, Dimitrov S, Krauskopf M, Vera MI, Bouvet P. *FEBS Lett* 2005;579:5553–8. [PubMed: 16213499]
50. Oleksiak MF, Roach JL, Crawford DL. *Nat Genet* 2005;37:67–72. [PubMed: 15568023]
51. Waters M, Boorman G, Bushel P, Cunningham M, Irwin R, Merrick A, Olden K, Paules R, Selkirk J, Stasiewicz S, Weis B, Van Houten B, Walker N, Tennant R. *EHP Toxicogenomics* 2003;111:15–28. [PubMed: 12735106]
52. Moggs JG, Tinwell H, Spurway T, Chang HS, Pate I, Lim FL, Moore DJ, Soames A, Stuckey R, Currie R, Zhu T, Kimber I, Ashby J, Orphanides G. *Environ Health Perspect* 2004;112:1589–606. [PubMed: 15598610]
53. Khatri P, Draghici S. *Bioinformatics* 2005;21:3587–95. [PubMed: 15994189]

Biographies

Nancy Denslow is an associate professor in the Center for Environmental and Human Toxicology and the Department of Physiological Sciences at the University of Florida. She earned a BS in chemistry from Mary Washington College, Fredericksburg, VA and a PhD in biochemistry and molecular biology from the University of Florida. Her recent work is on the application of genomics and proteomics technologies to environmental toxicology.

Natàlia Garcia-Reyero is a postdoctoral fellow in the Center of Environmental and Human Toxicology and the Department of Physiological Sciences at the University of Florida. She earned a BS degree in biology from the University of Girona, Girona, Spain. She did her graduate training at the IBMB-CSIC (Institut de Biologia Molecular de Barcelona), under the supervision of Dr B. Piña. She earned MS and PhD degrees in experimental biology and genetics, respectively, from the University of Barcelona, Barcelona, Spain. Her current interests are in applying micro-array analyses to environmental toxicology.

David Barber is an assistant professor in the Center for Environmental and Human Toxicology and the Department of Physiological Sciences at the University of Florida. He earned a BS in Biology at Virginia Polytechnic Institute, Blacksburg, VA and a PhD in Toxicology from the University of Arizona, Tucson, AZ. He did postdoctoral research in toxicology at Virginia Polytechnic Institute, Blacksburg, VA. He is currently using genomic and proteomic approaches to define mechanisms of toxicity in mammals and fish.

Fig. 1.

The biological response varies in proportion to the concentration of the xenobiotic and the time of exposure. Measurements made at the molecular level will be more sensitive and display higher mechanistic relevance than measurements made at the population level. However, measurements made at the population level are more ecologically relevant than those made at the molecular level. Population level effects can translate into population extinction if population sustainability is not maintained. It will be important to tie predictive changes at the molecular level with adverse effects on the organism and population levels, if new molecular methods are to have an impact in ecotoxicology.

Fig. 2.

Stages of toxicity. Adaptation, compensation and toxicity form a continuum of responses and the genes associated with each phase of the response will vary. At low concentration of contaminant and short duration of exposure, organisms will respond with adaptive biochemical pathways, such as increasing the concentration of metabolizing enzymes to maintain homeostasis. As the concentration of contaminant (or duration of exposure) increases, compensatory mechanisms might be triggered by the organism in its quest to maintain homeostasis. At toxic levels of contaminants (or excessive duration of exposure), the organism reaches exhaustion and can no longer respond to the contaminant, but instead goes into terminal toxicity. The promise of “toxico-genomics” methodologies is that changes in the first two stages of toxicity will predict adverse outcomes.