Monitoring of the Environmental Effects of Pulp Mill Discharges in Chilean Rivers: Lessons Learned and Challenges

Gustavo Chiang,1 Kelly R. Munkittrick,2 Rodrigo Orrego,3 Ricardo Barra1*

1 Aquatic Systems Research Unit, EULA Chile Environmental Sciences Centre, University of Concepción, Chile
2 Canadian Rivers Institute, University of New Brunswick, Saint John, New Brunswick, Canada
3 Faculty of Science, University of Ontario Institute of Technology, Oshawa, Ontario, Canada

Environmental monitoring activities in Chile are relatively new and have traditionally relied on physicochemical measurements. The pulp mill industry in Chile is highly competitive in the global market and several new large mills have recently opened. Early studies on fish in the receiving environments revealed lower species richness and an increase in the abundance of introduced species relative to native ones near pulp mill discharges. Even though changes were observed, their relationship with the discharges was unclear. Several difficulties related to small body sizes and the unavailability of basic biological data for native Chilean fish species were found during initial field studies. One of the main challenges is the standardization of monitoring methods (including fish species selection, sampling sizes, indicators, reference sites, etc.) and consensus about the responses that should be considered in a river monitoring program in the Chilean context. This paper summarizes major findings from a series of studies looking at impacts on fish at different levels of biological organization and the current approach used in Chile for monitoring impacts of pulp mill effluents on wild fish populations.

Key words: Chile, pulp mill, fish responses, biomonitoring, reproduction, monitoring design

Introduction

Pulp production began in Chile in the late 1950s and has grown exponentially during the 1990s and the present decade due to favourable conditions offered by the international market and certain competitive advantages in Chile (rapid growth of forest species used to produce pulp, lower labour costs, technological improvements, etc.). The Chilean pulp mill industry is presently within the top ten producers in the global market, annually producing more than 4.79 million tonnes of pulp. There are eight different mills in Chile (Table 1), and most mills discharge waste directly into freshwater river environments.

Three new mills built in the last 10 years were associated with several public controversies related to the sites chosen, an absence of information related to the potential impacts in Chilean ecosystems, and the potential impacts of development on other related economic activities (Parra and Acuña 2005). Public concerns raised by the new greenfield projects included some of the issues commonly addressed by the pulp mill industry worldwide, including the acute toxicity of wastewaters (Gaete et al. 2000; Mulsw and Grandjean 2006), the presence of dioxins and furans in the effluents, and more recently the potential to cause reproductive problems in fish as reported in recent Canadian and Swedish studies. The concerns in Canada and Sweden led to the development of environmental effects monitoring (EEM) programs for pulp mills in both countries (Swedish EPA 1997; Environment Canada 2005), but no such monitoring programs existed in South America.

In Chile, the environmental regulatory process, administered by national and regional offices of the Comision Nacional de Medio Ambiente (CONAMA), addresses pulp mill discharges through separate emission regulations and environmental quality regulations. Both regulations have different spatial application: the emission regulations are at a national scale, while the environmental quality regulations have local specific applications (CONAMA 2004).

The concerns about pulp mills in Chile increased in profile in 2004 after a greenfield mill started to discharge to a river draining into a large wetland. The subsequent disappearance of a submerged aquatic macrophyte (considered a weed) was correlated with mortality in swans inhabiting the wetland. Although no cause-effect relationships have been established, a series of scientific research publications pointed out several potential causes, such as natural changes (Marin et al. 2009), the impact of heavy metals (Jaramillo 2005), and potential effects of ultraviolet radiation (Ramírez et al. 2006). However, from a toxicological point of view, no specific chemical cause-effect relationships were established (Palma et al. 2008). In this context, during the environmental impact assessment for that new mill in Chile, a monitoring program for dioxins and furans in the effluent and the receiving environment was required by the regulatory agency (CONAMA). Our previous experience in the evaluation of those chemicals in a different Chilean watershed impacted by pulp mill discharges were unable to correlate concentrations in fish liver—total TEQs [toxic equivalents] for PCDD/Fs [polychlorinated


<table>
<thead>
<tr>
<th>Name</th>
<th>Region</th>
<th>Company</th>
<th>Kraft type</th>
<th>Tonnes per year (1,000)</th>
<th>Receiving environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Licancel</td>
<td>Maule</td>
<td>Arauco</td>
<td>BSKP/BEKP</td>
<td>145</td>
<td>Mataquito River</td>
</tr>
<tr>
<td>Celco</td>
<td>Maule</td>
<td>Arauco</td>
<td>UKP</td>
<td>350</td>
<td>Sea</td>
</tr>
<tr>
<td>Laja</td>
<td>Biobio</td>
<td>CMPC</td>
<td>BSKP</td>
<td>260</td>
<td>Biobio River</td>
</tr>
<tr>
<td>Arauco</td>
<td>Biobio</td>
<td>Arauco</td>
<td>UKP</td>
<td>80</td>
<td>Sea (Arauco Gulf)</td>
</tr>
<tr>
<td>Santa Fe</td>
<td>Biobio</td>
<td>Arauco</td>
<td>BSKP/BEKP</td>
<td>260</td>
<td>Biobio River</td>
</tr>
<tr>
<td>Santa Fe Línea II</td>
<td>Biobio</td>
<td>CMPC</td>
<td>BEKP</td>
<td>380</td>
<td>Biobio River</td>
</tr>
<tr>
<td>Pacífico</td>
<td>Araucania</td>
<td>CMPC</td>
<td>BEKP</td>
<td>780</td>
<td>Biobio River</td>
</tr>
<tr>
<td>Valdivia</td>
<td>Los Rios</td>
<td>Arauco</td>
<td>BSKP/BEKP</td>
<td>500</td>
<td>Biobio River</td>
</tr>
<tr>
<td>Nueva Aldea</td>
<td>Biobio</td>
<td>Arauco</td>
<td>BSKP/BEKP</td>
<td>685</td>
<td>Cruces River</td>
</tr>
<tr>
<td>Nueva Aldea</td>
<td>Biobio</td>
<td>Arauco</td>
<td>BSKP/BEKP</td>
<td>856</td>
<td>Itata River</td>
</tr>
</tbody>
</table>

**Total**: 4,791

*a* BSKP: bleached softwood kraft pulp; BEKP: bleached eucalyptus kraft pulp; UKP: unbleached kraft pulp.

*b* Empresas CMPC S.A.

*c* Started august 2006.

*d* Started september 2006.

Strategies for Evaluating Environmental Effects

There are a variety of approaches used in environmental monitoring programs that include fish. When designing a monitoring program, the advantages and disadvantages of the different responses and species that can be used should be considered. While many programs within the United States focus on monitoring at the fish community level (Yoder and Rankin 1998), in Canada they focus on the population level (Environment Canada 2005), and the EEM program in Sweden requires information at the biochemical, individual, population, and community levels (Swedish EPA 1997). According to our experience in Chilean environments, a useful approach uses the responses at different levels of biological organization. In this sense, the use of individual organisms is essential as it allows follow-up studies to develop at both lower organizational levels to understand mechanisms (e.g., tissue, physiological, biochemical), and at upper organizational levels to understand ecological relevance (e.g., population, community) (Munkittrick et al. 2000).

Basic Design Following the International Experience.

The most common monitoring design for evaluating new discharges in Chile has been the classical “Before-After Control Impact (BACI) design,” which is based on evaluating the potential impacts prior to project establishment (also called baseline evaluation) as well as after. Attempts are made to increase the number of reference sites to avoid misinterpretation, as recommended by Keough and Mapstone (1997) for Australian EEM programs. Existing mills have been examined using a control impact or gradient design when predevelopment conditions were not known.

Fish have played an increasing role in monitoring programs dedicated to detecting and assessing the potential impacts of industrial effluents in industrialized countries such as Canada (Munkittrick 2004; Lowell et al. 2005; McMaster et al. 2006), the United States (Sepúlveda et al. 2002; Theodorakis et al. 2006; Yeom and
Adams 2007), Sweden (Larsson et al. 2000; Sandström and Neuman 2003), Finland (Donald 2003; Karels and Oikari 2000), and New Zealand (van den Heuvel et al. 2007). However, the use of environmental monitoring programs within a governmental framework is not frequently used. Indeed, the present review found that biological monitoring programs existed in only three of the four countries that are the largest producers of pulp and paper worldwide (Table 2), establishing high cost effectiveness as the primary objective when designing a program to monitor effects in fish.

In Sweden, the Swedish EPA (1997) and Sandström and Neuman (2003) established a national pulp and paper effluent monitoring program to assess the potential changes in a top-down approach by measuring biological parameters at multiple biological levels to discriminate enrichment effects from toxicity/hormonal disturbance. Monitoring parameters included the community structure of fish, and population to individual and biochemical responses in one of two species (Zoarces viviparus or Perca fluviatilis), depending on the receiving body of the discharge. The scale and effects were not defined specifically, but varied according to the receiving environment characteristics. In Canada, the critical effect size was based on historical data review (Munkittrick et al. 2009), but in Sweden, this size was determined by a multistakeholder consensus review (government, industry, and academia). This process had initially set the level of change thought to be unacceptable (Table 2). Any changes detected later in monitoring were evaluated on an acceptable to unacceptable scale, according to responses in various functional groups, from population characteristics to physiological functions and contaminants in fish. Since these criteria were determined by collective bargaining, their environmental relevance remained unclear.

In comparison, the EEM program in Canada was based on a legal mandate under the Fisheries Act to protect fish, fish habitat, and the use of fisheries resources. This program evaluates population and individual level responses in at least two fish species on a cyclical basis (Environment Canada 2005). The results of the first cycle of monitoring were reviewed and discussed by a panel of scientists and government and industry representatives. This panel ultimately defined a 25% difference in size of the gonads and liver, and a 10% difference for condition factor between fish. This program included a sampling design, with highly comparable results (α 0.05 and β 0.20) for pulp and paper mills at the national level (Table 2). More recently, a decision has been made to set α = β, and a review of international approaches to setting critical effects sizes has validated the effects sizes chosen (Munkittrick et al. 2009).

A different philosophy is used for monitoring pulp mills in the United States where there are few legislated field monitoring programs for fish, and the U.S. EPA (1998) supports the development and use of BAT (best available technology) in their pulp and paper industry. This approach only provides limited physical and chemical parameter information on effluent discharge.

### TABLE 2. International pulp mill effluent monitoring programs

<table>
<thead>
<tr>
<th>Country</th>
<th>Monitoring strategies</th>
<th>Endpoints</th>
<th>Effect magnitude</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>MBACI-SCI: *</td>
<td>No specific sentinel species or biological endpoints due to “high variability of the receiving systems in Australia.”</td>
<td>Scalable magnitude from a multiple stakeholder negotiation; Type I and Type II error rate (0.5); 50–100% of change unacceptable.</td>
<td>Kough and Mapstone 1997</td>
</tr>
<tr>
<td>Canada</td>
<td>Legal mandate.</td>
<td>Individual level responses in at least 2 fish species, data distribution between populations exposed and nonexposed to effluents</td>
<td>Gonad and liver size &lt;25%; Condition factor &lt;10%.</td>
<td>Environment Canada 2005; Lowell et al. 2003</td>
</tr>
<tr>
<td>Sweden</td>
<td>Stakeholder negotiation; Nationwide monitoring program.</td>
<td>“Top-down” strategy; Multiple endpoints, from community structure to biochemical indicators in two sentinel species: Perca fluviatilis and Zoarces viviparous.</td>
<td>Division of endpoints in functional groups establishing: unacceptable perturbation of function, unacceptable perturbation in fish health, evident risk to population, and need of confirmation of results.</td>
<td>Swedish EPA 1997, Sandström and Neuman 2003</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>No biological monitoring strategy, EPA supports the development of BAT. *</td>
<td>Nonbiological endpoints; Concentration thresholds for certain harmful components in effluents.</td>
<td>Emission standards below detection levels</td>
<td>U.S. EPA 1998</td>
</tr>
</tbody>
</table>

* MBACI-SCI: Multiple Before-After Control Impact-Scalable Decision criteria.  
* BAT: best available technology.
in water environments (Table 2). However, there have been a number of studies that have used fish to assess the effect of some pulp mill effluents (Sepúlveda et al. 2002; Theodorakis et al. 2006).

Pulp mill activity has expanded in Brazil and Uruguay, and is predicted to increase rapidly in the future throughout South America (Villalonga 2006). Uruguayan studies associated with a new pulp mill have adopted the Canadian EEM model to evaluate the effects of effluent discharges, in addition to the traditional physicochemical monitoring of water quality in a highly controversial development (Joutsenvirta and Vaara 2009). However, since this is a preliminary evaluation, no major conclusions for this monitoring strategy for this newly developed industry have been made.

In the design of monitoring programs, sample size calculations are possible given knowledge of the standard deviation of the endpoint, acceptable type I and type II error rates, and preset critical effect sizes for the endpoints of choice (Munkittrick et al. 2009). There are recommendations for setting type I and II error rates in Canada (Environment Canada 2005), and in Australia, they should be set at a 0.5 ratio (Keough and Mapstone 1997). Although biological monitoring programs exist in Australia, Canada, and Sweden, they all have a different background design frameworks (Table 2).

The Triad Approach for Chilean Environments.

From a Chilean standpoint, a good starting point for the evaluation of effects is a “triad approach” (Chapman and Hollert 2006), which considers: 1) monitoring of field populations, 2) semicontrolled experiments using standard species as bioindicators (i.e., caging the same fish species at different sites with similar environments), and 3) laboratory bioassays using both effluent and sediment samples. Each approach answers different questions but looks for the same responses in order to determine if the effects observed in the field are correlated to effluent exposure. The fish monitoring program helps with understanding the general state of health of the wild fish populations. In some locations however it is only possible to conduct manipulative experiments controlling exposure time and location. This type of experiment can also be affected by other factors such as vandalism and cage destruction, as previously reported (Oikari 2006; Orrego et al. 2006). While field observations provide valuable information, their interpretative power can be reduced because of confounding factors. Thus, laboratory-specific effluent exposure and the evaluation of receiving environment matrices emerge as an appropriate complementary strategy to assess the specific effect with a greater capacity to establish the causal relationships.

Multivariate analysis of laboratory results, field evaluation experiments, and chemical evaluations should help establish specific relationships that can help define environmental thresholds to evaluate the impact of industrial activities on aquatic systems. This strategy can provide important information to be used to improve environmental management and regulation of aquatic ecosystems, particularly in Chile, where threatened and endangered species are often involved. Much of the research conducted by our research group has been designed to accomplish these objectives (Orrego 2006; Orrego et al. 2005, 2006, 2009a). However, implementation has been complicated due to difficulties when conducting the experiments, scarce access to funding, copyright issues associated with proprietary data in private contracting research, and challenges in communication with private companies. This has made it difficult to establish a significant environmental threshold for the pulp and paper mill industries that discharge into Chilean environments.

Research from a recent experiment compared lab exposure of pulp mill effluent to the results observed in caging experiments using the control impact design. Results showed a correlation between lab exposure and field caging studies (Fig. 1). The analysis allowed the identity of two different indicators in rainbow trout—plasma vitellogenin (Vtg) production and gonadosomatic index (GSI)—that were associated with a gradient in dilution in both cases. A clear distinction could be drawn
The design of EEM programs for pulp mill effluents must be cost effective; this is especially important in developing countries compared with other countries with existing regulations. Hewitt et al. (2008) pointed out that most of the published effects of pulp mill effluent identify alterations in fish reproductive and metabolic rates, which have also been identified by our group in Chile under laboratory and field conditions using rainbow trout (Oncorhynchus mykiss) (Orrego 2006; Orrego et al. 2005, 2006), as well as at the molecular level (Chamorro et al. 2010). The main effects associated with Chilean pulp and paper mill effluent exposure to date are consistently described as estrogenic responses, which differ from the mainly androgenic and antioestrogenic effects reported in Canada and New Zealand (Orrego et al. 2009a). Recently, the same estrogenic effects seen in rainbow trout have also been observed in native Chilean fish species, with a possible stimulation of the reproductive system resulting in the consequent disruption of normal sexual maturity. These changes have resulted mainly in a stimulation of in vitro estradiol production by the gonads, induced maturation during recrudescence, and smaller female gonads during spawning seasons, all disrupting the population dynamics of native fish populations exposed to effluents (Chiang et al. in press–a). These reproductive endpoints (molecular, cellular, individual, and population dynamics) must form part of any specific Chilean monitoring program.

The adoption of parameters and strategies from other programs already in place in other parts of the world is also recommended, but should be adapted to the reality of local systems (Munkittrick et al. 2009). Consequently, using a strategy similar to that of the EEM program in Canada (Environment Canada 2005) appears to be adequate to evaluate reproductive patterns in fish populations, but it should be complemented with the laboratory and semicontrolled field evaluations already developed by our research group.

Critical effect sizes for gonad and liver sizes (2.5%) as well as condition factor (10%) have been evaluated in native Chilean fish species (Chiang et al. in press–b), although these parameters have not been correlated with the changes detected under laboratory conditions using introduced species. The design of cost-effective monitoring programs should assess these reproductive abnormalities and metabolic capacities. It is important to incorporate assessment tools that link the information obtained in the triad approach without compromising accuracy, effectiveness, and reproducibility for detecting possible alterations (Table 3).

Monitoring Challenges in Chilean Rivers

Chilean rivers are characterized by short distances, steep slopes, and low biodiversity (Habit et al. 2006a). These rivers face natural changes in chemical and physical stressors along the river, and have adapted over time to such drastic changes. Additionally, the low biodiversity in fish fauna, the sometimes narrow geographic range of unique species, and the widespread introduction of exotic species has affected the availability of native fish. Thus, these characteristics are important to consider during the implementation of monitoring programs to assess impacts of industrial and urban discharges in Chilean rivers.

Since native species have only been studied in the last 25 years, their biology is in general poorly understood. Consequently, the principal challenge when developing standardized monitoring programs in Chilean environments is a better understanding of the biology of native fish species. Unless we can document the natural variability within defined values, we cannot compare responses of native fish populations exposed to pulp mill effluents. Additionally, these gaps in basic biological data for Chilean fish increases the difficulty of evaluating responses at a very low level of biological organization, such as biochemical or molecular biomarkers (e.g., EROD, Vtg). Therefore, detection limits and methodologies have to be improved for native species, and must be calibrated with other species' responses.

Another factor that has to be considered when designing a pulp mill monitoring program is the chemical complexity of pulp and paper mill effluent (Hewitt et al. 2008). The chemicals present relate to the wood source material, the industrial process used, and the type of effluent treatment employed. The environmental fate of these chemicals and potential impacts will also depend on the receiving water morphology and physical and chemical characteristics, as well as the species' sensitivity. The selection of a fish species for the monitoring program is a complex task since there is no one single criterion for the selection. Under our strategic approach, several factors should be considered when making this choice, especially those related to industry and the local environment (Fig 2).
The selection of the spatiotemporal scale of analysis is also highly relevant. Changes in community-based measures are useful to establish the ecosystem's condition and to reveal damage, especially in a highly endemic environment such as found in Chilean river systems (Habit et al. 2006a, 2006b). However, these indicators are difficult to use for establishing potential causal relationships, and understanding the causes is crucial for potential restoration/protection of that aquatic ecosystem. Furthermore, the community structure is influenced by processes operating at different spatial and temporal scales, which makes it more difficult to correlate with the effects of pulp and paper effluents and to implement standard process and/or effluent treatment changes to prevent deleterious effects (Maltby and Burton 2006). Long-term studies in the United States have shown a healthy and diverse community structure in aquatic systems that have received a variety of industrial discharges for more than 100 years (Burton and Hall 2009), and have been unable to show any change at the community level by using biotic condition indices (Hilsenhoff Biotic Index) in a multivariate analysis (Flinders et al. 2009). Impacts at lower levels may not translate to the community level, or the variability at the community level between seasons and sites may be unable to show potential impacts (Orrego et al. 2009b).

The use of population parameters, individual responses, and tissue level effects (physiological and biochemical variables) have been demonstrated to be suitable tools that provide evidence on the effects of industrial discharges. Such studies have been done in independent monitoring programs in several countries: Canada (Munkittrick 2004; Lowell et al. 2005), the United States (Sepúlveda et al. 2002; Theodorakis et al. 2006), Sweden (Larsson et al. 2000; Sandström and Neuman 2003), Finland (Donald 2003; Karels and Oikari 2000), New Zealand (van den Heuvel et al. 2007), and Chile (Orrego et al. 2005, 2006a&b). Responses at these levels happen sooner than community-level responses, are more easily reversible, and are easier to link to effluent exposure. In this way, our approach supports identifying the ecological significance of the impact as well as providing a warning of environmental risk for the fish fauna by establishing causal relationships, while at the same time providing a protective value to monitoring programs (Fig 3). The advantages of a combined approach are obvious in terms of obtaining the benefits of ecologically relevant changes, with changes in reproductive performance, energy storage (i.e., condition factor and lipids), and survival as indicators of ecosystem quality (Munkittrick et al. 2000), and the short-term specific biochemical changes as early indicators of the causal variables (Orrego et al. 2009a, 2009b). However, the identification of an indicator that links both levels is certainly necessary, where the potential evaluation of a reproductive biomarker, such as Vtg in native species, appears to be the key. That objective is possible in Chile using the alkali-labile protein phosphorous method (Kramer et al. 1998; Pollino et al. 2009), which can be applied for several species.
The use of sentinel species gives us useful information on the state of the environment and the modulation of abiotic variables on the responses or effects observed in individuals captured (Munkittrick et al. 2000; van der Oost et al. 2003), but several stressors could alter certain biological endpoints outside of natural variability. The caging bioassays offer advantages in terms of the ability to use them in aquatic systems or seasons when you cannot perform successful environmental monitoring, and allows controlled effluent exposures. These in situ bioassays are more realistic than the traditional laboratory bioassays because they include natural exposure routes, compensation mechanisms, and environmental factors, and also allow the control of fish size, age, reproductive status, sex, number of exposed individuals, spatial gradients, replicates, and time of exposure (Oikari 2006; Orrego et al. 2006; Crane et al. 2007). When using exotic species under semicontrolled conditions, extrapolation to the population level should be avoided (Spromberg and Meador 2005). In contrast, the use of native species for in situ tests allows direct extrapolation to higher levels (Chiang et al. unpublished data). These can be...
verified directly through biological monitoring in the same area (Baird et al. 2007). Additionally, the use of native species minimizes stress during exposure because they are adapted to spatial and temporal fluctuations of environmental variables that occur in the exposed area (e.g., temperature, pH, conductivity, dissolved oxygen) (McWilliam and Baird 2002, Baird et al. 2007).

Since the upper trophic levels in the river systems reflect the energy flow through the ecosystem, and knowledge of the natural variability of the responses tested is required, reference sites are important to consider. The assumption is that the sentinel species used reflects factors or characteristics of the site where they are collected (Bowman and Somers 2005). Habitat changes that result in a change in the energy flow at this level vary their performance characteristics, and therefore it is assumed that changes in performance reflect impacts (Munkittrick et al. 1992). Moreover, one of the primary considerations is the residence time and exposure of the individual or population to the effluent. Large fish present a clear advantage when collecting the samples required for biochemical analysis, but they may have high mobility which can take them outside the effluent exposure area (Katano et al. 2006). In contrast, smaller fish have proved very helpful as a model for assessing the effects of pulp mill effluents in river systems (reviewed in Munkittrick et al. 2002), mainly due to their lower mobility and relatively short time to reach sexual maturity.

Therefore, monitoring programs using small fish can integrate responses over one or more generations more easily (Ankley and Johnson 2004), although there can be problems with sample requirements for biochemical analyses.

Finally, laboratory tests help to isolate the effects of the compound or group of compounds and allows additional control of the size, age, reproductive status, sex, and number of exposed individuals, as well as time of exposure and doses, all adding to a reduction in variability (Crane et al. 2007; Orrego et al. 2009a). However, there are a number of artefacts in this type of analysis associated with the low environmental realism with laboratory bioassays (Baird et al. 2007; Crane et al. 2007). It is possible that two or more species can have the same physiological response and sensitivity to a chemical substance, but the life history strategies (i.e., differences in sexual maturation time, survival rates at different stages of life cycle, reproductive frequency) may alter the significance of the responses and consequences to the population (Schaaf et al. 1993; Caswell 2001 in Spromberg and Meador 2005). Consequently, there are questions about the ecological significance of laboratory studies since they offer limited ability to extrapolate to field conditions (Crane et al. 2007). Their use should be restricted only to the different lines of evidence used in the evaluation of effects of chemical stressors in the aquatic environment or to identify compounds

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**Fig. 3.** Ideal responses of biological endpoints without stress (natural variability range) and exposed to one or multiple stressors (Modified from van der Oost et al. 2003).
responsible for the alterations observed in the field, and to examine mechanisms associated with demographic characteristics and toxicological endpoints (Spromberg and Meador 2005). This reduces the uncertainty in risk assessments, and the order of significance for an accurate determination of the dominant stressor and its ecological significance should be laboratory studies < in situ bioassays < field studies (Burton et al. 2003; Oikari 2006).

Data interpretation is a key issue since there is currently no agreement in considering how big the deviation of certain endpoints should be to consider the change an effect, and what should be done when an effect is observed. The standardization of the monitoring programs should discuss these issues since the definition of an effect could be based on: 1) statistical considerations, based on the variability of the endpoints used, or 2) quantal responses expressed as a percentage of change (Munkittrick et al. 2009). A good starting point could be to establish a threshold from laboratory experiments, and use these thresholds as surrogates to set up when an observed response during the pulp mill effluent monitoring is considered an adverse effect. It is important to evaluate the relationships between effects observed in laboratory bioassays and in controlled experiments in the field. Even though differences in magnitude between laboratory and semfield experiments can be observed, a good correlation between responses at different levels of biological organization will help to establish critical correlations in the responses.

Although endpoints measured at the individual level seem to be appropriate for monitoring purposes, some biomarker responses, such as production or plasma levels of hormones, could be considered in studies where reproductive impairments are observed. This would also be the case with molecular endpoints such as detoxification enzymes, plasma vitellogenin production, and or gonadal production of hormones. For monitoring purposes, according to the international experience, suborganism level endpoints, including condition factor, relative organ sizes (liver somatic index, gonadosomatic index), gonadal histology, and egg production, may be used, with two or more species being used (Table 3). Clearly, a lot of work will need to be performed to be able to extrapolate results from the laboratory to the field in order to establish quantitative relationships and reduce the confounding factors related to the variability within species and between species. Ideally, the work should be conducted with the same species, although this is not an easy task.

Conclusions

It is obvious that programs based only on chemical or biological endpoints do not sufficiently ensure proper environmental protection. Indeed, a program that incorporates both chemical and biological monitoring strategies, such as the experiences in Canada, Sweden, and Australia, are a step ahead. These approaches have been shown to be widely beneficial and accepted not only by the government but also by the public and the industry. Even though reproductive effects have been consistently identified in the Canadian program, the chemical compounds causing those effects still need to be identified (Hewitt et al. 2008). This fact reinforces the idea that good monitoring programs must consider complementary responses in the biota and chemical evaluations.

Even though the Chilean industry has put a lot of effort into implementing the best available technologies and improving the final effluent treatment systems, reproductive effects continue to be observed in fish in our receiving environments. Further research is required to better understand the significance of these effects, especially at the fish population and community levels. It is clear that in the next few years, the dialogue between stakeholders needs to improve, and more independent research should be performed in order to address these important issues. Fortunately, some of the industry has been proactive, but more work is needed to improve the collaboration and compromise between industries, researchers, and the government in order to develop specific environmental monitoring strategies that should be seen as opportunities to improve industrial and environmental sustainability.

Important challenges remain. Strong monitoring programs require a better agreement between regulatory authorities, academia, and industry. These programs need to consider economic and scientifically relevant criteria. Stakeholders need to understand that more monitoring requirements (e.g., physicochemical measurements) do not necessarily lead to better environmental protection, and that the public discussions initiated by the Chilean National Commission on the Environment (the future Ministry of the Environment) should continue. As scientists, we must understand that sometimes the discussion is not limited only to technical aspects, and an effort needs to be made to find the right technical answers which all stakeholders can accept.

In environmental impact evaluations of future and existing pulp and paper mill industrial activity in Chile, it must be recognized that pulp and paper mills are highly dynamic (as reflected in production increases and technology changes) and that the Chilean industry is moving away from discharging into rivers with plans to discharge into the sea. Thus, there is the need to begin analyzing effects in the marine environment. A logical issue to address is the relevance of the effects observed in rivers to these in the marine environments. The Canadian EEM program has identified difficulties in studying marine environments, including tidal action, floating effluent plumes, and movement of species (Boyd et al. 2002). Additionally, further study is required to determine if the new treatment technologies are able to remove the chemicals causing reproductive impacts. To respond to these critical questions, we need to advance the monitoring
requirements by ensuring that adequate sample sizes are collected, the right endpoints are measured, and the analytical capabilities are improved. Additionally, since many Chilean species are listed as “protected,” we need to make progress in nonlethal sampling strategies and nondestructive techniques (such as population size structure dynamics/recruitment and molecular methods for using mucus or blood where possible) for analyzing reproductive and metabolic endpoints. All these advancements should help us determine whether or not the existing effluent and environmental quality standards effectively protect the environment for us and for future generations.

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