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Proceedings of a Workshop to Assess Alternatives to the Fish Survey Component of the Environmental Effects Monitoring Program for Canadian Pulp and Paper Mills

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1998

PROCEEDINGS OF A WORKSHOP TO ASSESS ALTERNATIVES TO THE FISH SURVEY COMPONENT OF THE ENVIRONMENTAL EFFECTS MONITORING PROGRAM FOR CANADIAN PULP AND PAPER MILLS

by

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ABSTRACT

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On 17-18 February 1997 approximately 30 participants from Canada and USA gathered in Dartmouth, Nova Scotia to discuss alternatives to the Adult Fish Survey (AFS) component of Canada's Environmental Effects Monitoring (EEM) program for liquid effluents from pulp and paper mills. During the first cycle (1993-1996) of the pulp mill EEM, the AFS performed reasonably well in the freshwater environment for which it was designed, but poorly in the marine and estuarine environments. The objective of the workshop was to assist the AFS Expert Working Group (EWG) in making recommendations for the second cycle by:

1. reviewing difficulties with Adult Fish Surveys in Cycle One;
2. exploring alternative approaches;
3. identifying alternative approaches ready for use in Cycle Two; and,
4. identifying alternatives that are not ready for Cycle Two but that merit further research and development in order that they be available for Cycle Three and beyond.

Presentations were given on experiences and difficulties with the AFS in Cycle One across the country and on potential alternatives to be considered for Cycle Two including: use of alternate sentinel species including macroinvertebrates and forage fish; use of alternate endpoints including health indices, scope-for-growth, behavioural and life history indices; cageing of bivalves and finfish; and mesocosms. In light of the principles for conducting EEM studies and the comments made by the workshop participants, the alternative methods identified as being acceptable for Cycle Two are, in order of preference, surveys of wild forage fish (i.e., small-bodied, sedentary fish), wild bivalves, juvenile fish, caged bivalves, and mesocosm studies with fish. The workshop participants also concluded that there was a need for an increased level of research and development aimed at the provision of better monitoring tools for EEM.

RÉSUMÉ

Courtenay, S.C., W.R. Parker, and G.P. Rawn. 1998. Proceedings of a workshop to assess alternatives to the fish survey component of the environmental effects monitoring program for Canadian pulp and paper mills. Can. Tech. Rep. Fish. Aquat. Sci. 2233: viii + 108 p.

Les 17-18 février 1997, une trentaine de participants du Canada et des États-Unis se réunissaient à Dartmouth, Nouvelle-Écosse, pour discuter d'alternatives au relevé des poissons adultes (RPA), composante du programme d'études sur les effets environnementaux (ÉEE) des effluents liquides des usines pâtes et papier. Lors du premier cycle (1993-1996) des ÉEE des pâtes et papier, le RPA a fonctionné raisonnablement bien dans les milieux aquatiques d'eau douce, en fonction desquels il avait été développé, mais piètrement dans les milieux marins et estuariens. Le but de l'atelier était d'aider au groupe de travail sur le RPA à composer des recommandations en vue du deuxième cycle, en:

1. faisant la revue des difficultés éprouvées dans le cadre du RPA dors du premier cycle;
2. explorant des méthodes alternatives;
3. identifiant les alternatives prêtes à être utilisées au deuxième cycle; et
4. identifiant les alternatives non prêtes pour le deuxième cycle mais qui méritent des recherches additionnelles pour qu'elles soient disponibles au troisième cycle et au-delà.

Des présentations étaient soumises sur les expériences vécues et les difficultés éprouvées à l'échelle nationale dans le cadre du RPA lors du premier cycle et sur les alternatives à prendre en considération en vue du deuxième cycle, y inclus: l'usage d'espèces indicatrices alternes telles les macroinvertébrés et poissons fourragers; l'usage d'indices alternatifs, tels les indices de santé, potentiels de croissance, indices de paramètres naturels et comportementaux; la mise en cage de mollusques et poissons; et mésocosmes. Guidés des principes d'exécution des ÉEE et des commentaires suscités des contributeurs, les alternatives jugées adéquates pour le deuxième cycle sont, en ordre de préférence, les relevés de poissons fourragers sauvages (c.à.d. petits poissons sédentaires), mollusques sauvages, poissons juvéniles, mollusques en cage, et études en mésocosme avec poissons. Les participants à l'atelier ont aussi conclu qu'il existe un besoin d'intensifier le niveau de recherche et développement visant à fournir de meilleurs outils pour les ÉEE.

Introduction

In May, 1992, the federal government passed amendments to the Pulp and Paper Effluent Regulations (PPER) under the authority of the *Fisheries Act*. The amendments included limits on biochemical oxygen demand, total suspended solids and acute toxicity to fish that were more stringent than those contained in the 1971 regulations. As well, the amended regulations applied to every pulp and paper mill in Canada; no mills were exempt. There was provision for a transitional period to allow all mills to make modifications necessary to meet the new requirements. All mills were required to be in compliance with the amended regulations by 31 December 1995.

In addition to the changes in effluent limits, the amended PPER required every mill to conduct an environmental effects monitoring (EEM) program. The pulp and paper industry is the first in Canada to be required to conduct an EEM program and is providing the model for other industries. The objective of the EEM program is to determine if the fish, the fish habitat and the utilization of the fisheries resource are being adequately protected by the requirements of the PPER. The EEM studies were to be conducted on three year cycles with the reports for the first cycle due on April 1, 1996. The requirements for the environmental monitoring studies were set out in Section 28 - 35 of the PPER and in a supporting requirements document (EC/DFO 1992a).

All mills were required to conduct an adult fish survey, an assessment of fish exposure to effluent (a tracer study), a benthic invertebrate survey, sub-lethal toxicity tests, measurements for chlorinated dioxins and furans in fish tissue if the mill used chlorine and some supporting water and sediment quality measurements. As well, if there were any records of public concerns about the tainting of fish, a fish tainting study was required.

Throughout discussions of the fish survey component of EEM, there has been considerable debate on the definition of a "fish". As the EEM program falls under the authority of the *Fisheries Act*, the definition from the *Act* has been adopted for the fish survey. Under the *Act*, "fish" includes finfish, shellfish, crustaceans, marine animals and the eggs, spawn, spat and juvenile stages of finfish, shellfish, crustaceans and marine animals. It is on the basis of this definition that aquatic invertebrates have been included in the discussion on alternatives to the AFS. In addition to being defined as a fish for the EEM program, many invertebrates [e.g., bivalves] are also of economic and ecological importance. The AFS, however, was originally designed for finfish which remain the preferred sentinel species. In those circumstances in which the use of finfish is not appropriate, alternatives such as the use of aquatic invertebrates must be considered.

The fish survey component of the pulp mill EEM was designed to assess aquatic environmental quality by examining morphological and life history characteristics of fish. The first cycle AFS was based on the assumption that a small subsample of a specific fish population is sufficiently representative of the entire population that it can provide a preliminary indication of aberrations in population structure. Requirements were a minimum of 20 adults of each sex of two sentinel species from a near-field area and at least one reference area. Data to be recorded from sentinels included: length, weight, age, gonad weight, egg size, liver weight and external condition. Measurements had to be taken only once during the first cycle.

The original interpretive framework of this method was developed from concepts described by Colby and Nepszy (1981) and Colby (1984) and was used by Munkittrick et al. (1991) to measure the potential effects of a bleached kraft mill effluent on white suckers (*Catostomus commersoni*) living in Lake Superior. The method involved the use of one or two adult "sentinel" fish species as indicators of the health of fish populations and has been described in detail by Gibbons and Munkittrick (1994). In brief, fish characteristics were grouped according to age structure (mean age or age distribution), energy expenditure (growth rate, gonad weight, fecundity, age at maturity), and energy storage (condition, liver weight, tissue lipid levels). Based on comparisons with a reference area, a response characteristic for each grouping was assigned as either an increase (+), a decrease (-), or no change (0) for the sentinel species from the exposure area. The response pattern was then compared against generalized response patterns in order to provide direction and focus for research into the causal factors (Gibbons and Munkittrick, 1994). In the original AFS approach, 3-5 studies (i.e., 10-15 years under the current EEM monitoring requirements) would be required to determine temporal/spatial trends in the data (Hodson et al. 1996). However, one objective of Cycle One was to determine the variability in the measured parameters and to use this information in power calculations to estimate minimum sample size requirements for the second cycle.

According to the study design requirements, Cycle One could be used to evaluate the suitability and capture success of sentinel species, the adequacy of the reference area(s), and the appropriateness of the sampling methods and gear. The mills, consultants and government participants in the EEM program identified difficulties with some of the EEM requirements. To address these concerns, the national EEM Technical Management Committee established Expert Working Groups, including one for the Fish Survey (Munkittrick et al. 1997), to review the results of Cycle One, identify technical difficulties and provide recommendations on modifications or alternatives where existing approaches proved unproductive or did not work. These recommendations will be considered by the Technical Management Committee in the development of the Cycle Two requirements.

Mills discharging effluent into the marine and estuarine environments of British Columbia and the Maritime provinces experienced greater difficulty in implementing the Cycle One Adult Fish Survey than did freshwater mills, for whom the survey had been designed. Some mills discharge effluent at 20-205m depth and the resulting effluent may not surface on a regular basis. In such circumstances, the degree of exposure of sentinel species could not be known with the same degree of certainty as for freshwater mills. In addition, some mills discharge into the same environment as other industries and municipal discharges, the effects of which cannot be discriminated from those of pulp mill effluent. Bidirectional movement of tidal waters in estuaries and physical processes associated with the interaction of fresh and salt water further complicate the situation. The most common problem, however, was the inability to catch sufficient numbers of the selected sentinel species. Nine of 27 marine mills failed to catch the required number of even one sentinel species. Some mills addressed this problem by including species which are less than ideal sentinels. Decapods (crabs and lobsters) cannot be aged and therefore cannot provide information on growth rates from a single sampling (Appendix E). Similarly, the reproductive effort of shellfish such as oysters and mussels can be difficult to assess from a single sample. In summary, the results of Cycle One Adult Fish Surveys at marine and estuarine mills, and to a lesser extent freshwater mills, indicated a clear need for refinements to the existing Adult Fish Survey, and in some cases alternative approaches.

A national workshop was held in Dartmouth, N.S. on 17-18 February 1997 to consider alternatives to the Adult Fish Survey. The workshop was organised and hosted jointly by Environment Canada (Roy Parker, Environmental Protection Branch, Atlantic Region) and DFO (Gary Rawn, Environmental Science Branch, Headquarters; Simon Courtenay, Marine Environmental Science Division, Maritime Region). The objectives of the workshop were to:

1. review difficulties with Adult Fish Surveys in Cycle One;
2. explore alternative approaches;
3. identify alternative approaches ready for use in Cycle Two; and,
4. identify alternatives that are not ready for Cycle Two but that merit further research and development in order that they be available for Cycle Three and beyond.

The workshop program and list of attendees are presented in Appendices A and B respectively. Abstracts of the 15 invited presentations are presented in this report grouped into four categories: review of Adult Fish Surveys during Cycle One (abstracts 1-4), alternative approaches using bivalves (abstracts 5-8), alternative approaches using finfish (abstracts 9-12), and health indices as additional endpoints to be considered in fish surveys (abstracts 13-15). Accounts of discussions held on each day of the workshop are presented in Appendices C and D. Additional information on two issues that arose during discussions - use of crustaceans in surveys, and life-cycle studies of wild salmon - is supplied in Appendices E and F respectively. Detailed analysis of the readiness of alternative approaches to the fish survey for Cycle Two are presented in Appendix G.

Summary and Conclusions

The first three presentations at the workshop provided an overview of the results of the adult fish survey (AFS) that were conducted during Cycle One in Environment Canada's Atlantic Region, the Pacific & Yukon Region and the Ontario Region. Although the AFS was successful at many sites, there were some common problems that occurred among the AFS conducted. These included not catching enough suitable fish, unconfirmed exposure of the fish to the effluent, inability to make all of the required measurements on the sentinel species captured and confounding factors from other discharges. These problems were particularly common in studies at mills discharging to the marine environment. Those attending the workshop agreed that in some cases these failures in the Cycle One AFS could be addressed by improved study designs and field methods in the next EEM Cycles. However, at a number of sites, it was concluded that the AFS approach in Cycle Two, even with improvements based on the Cycle One experience, would not provide useful information. At these sites, other approaches to assess the health of the fish community would be required.

Four presentations at the workshop dealt with approaches that used bivalves to assess environmental impacts. These bivalve monitoring techniques were at various stages of development. C. Hawkins pointed out several good reasons for using bivalves as sentinel species for monitoring studies. These included the well understood biology of many common species, the natural occurrence of many bivalve species in areas of interest and the fact that because the animals were immobile, determining exposure to effluents was not a problem. M. Salazar described the use of caged bivalves to assess environmental impacts and cited many examples of studies where this approach had been successfully used. The primary measurements on these caged bivalves included growth and the bioaccumulation of contaminants. While these refined techniques have not been used in Canadian pulp and paper EEMs, some elements of the caged bivalve methodology have been successfully used at some mills in Canada and several other countries. Caged bivalves appear ready for use in Cycle Two and pilot studies are currently underway. A partnership of government (EC, DFO) and the private sector is participating in these pilot studies to facilitate transfer of this technology to the consulting community. P. Cranford described an apparatus (called a HABITRAP) and a technique that he had developed to assess impacts from offshore energy projects. His technique involved the assessment of bivalve feeding rates, energy transformation and growth. This technique is not ready for use in Cycle Two but it is sufficiently promising to merit development during Cycle Two. Adaptation of the HABITRAP for pulp mill monitoring is being pursued by DFO. Finally, R. Addison described the bivalve Scope For Growth (SFG) approach that has been used in a number of studies of environmental impacts. In general, bivalves are exposed in cages or captured from the wild and their condition and energy transformation are assessed. SFG requires additional work on costs and data interpretation, but given the likelihood of increased use of bivalves in pulp and paper mill monitoring, it would appear worthwhile to pursue additional endpoints such as SFG during Cycle Two.

Four presentations described alternative approaches or modifications to the fish survey. M. Dubé and J. Culp made a presentation about the possible application of a mesocosm (artificial stream) approach to assessing impacts of pulp mill effluents. This technique, which is essentially a bioassay conducted in the field, has been used successfully in many types of studies to assess impacts on fish and invertebrates. The advantage of this approach over laboratory bioassays such as the fathead minnow life cycle assay is that environmental conditions such as salinities, temperatures, photoperiod and test species are more closely simulated. During 1997, a partnership led by Washburn and Gillis Associates Ltd. and including representatives of industry, government (EC and DFO) and academia conducted a mesocosm pilot study to assess potential impacts of the effluent from an east coast pulp mill on a marine forage fish. As for all alternatives, criteria of success (see Appendix G) were adopted from Annex 1 of the PPER (EC/DFO 1992b).

Methods that had been used on the west coast to assess effects of contaminants on caged finfish were described by I. Birtwell. These studies have been conducted on several species of salmonids and involved measurements on survival, behavioural changes and complete life cycle studies. In general, cageing of finfish does not appear to be a useful alternative for pulp mill monitoring. Fish are stressed by confinement, are prevented from moving up and down river with tides and thereby selecting waters of particular salinities, temperatures, etc., and cannot feed naturally. Birtwell also described a life cycle study with salmon in which the effects of exposure to oil in early life were measured in rates of survival and return to the home river as adults. There followed much discussion about such life cycle studies, particularly for Pacific salmon, in situations that preclude a standard fish survey. A more detailed description of how such a study might work was contributed by I. Birtwell and R. Addison (Appendix F). Such an approach is not ready for Cycle Two but merits additional research by a partnership of government and industry.

Two papers discussed the use of small-bodied ("forage") fish for assessing impacts on fish communities from industrial effluents. S. Courtenay described C. Couillard's comprehensive study that had been conducted using the mummichog in the Miramichi River in NB, a marine estuary. K. Munkittrick described studies that his colleagues had conducted in fresh water systems using small species of forage fish including the spoonhead sculpin, lake chub, trout-perch and peamouth chub. Selection of forage fish as sentinels is a refinement of the existing fish survey rather than an alternative *per se*. The main advantage of forage fish over the more usual large, mobile fish, beyond greater abundance, ease of capture and lack of impact from commercial fisheries, is that some of these small-bodied fish are remarkably sedentary, providing assurance of exposure (or non-exposure in reference sites) to effluent without the need of a tracer.

Three presentations dealt with fish health assessment indices (HAI) and approaches. A.-M. MacKinnon provided an overview of a survey of estuarine fish in Atlantic Canada and B. Antcliffe described a major project that had been conducted on the Columbia River in British Columbia. The health assessments used included visual observations, blood chemistry, virology and pathology. S. McGladdery described a histopathological approach that she and co-workers were using to measure effects on invertebrates from industrial discharges in the southern Gulf of St. Lawrence. Inclusion of HAIs in pulp mill EEM generated much discussion. Overall, the opinion of the experts appears to be that health parameters can provide useful supporting information to the standard fish survey, but are not a replacement for it. Furthermore, additional research is required into the relationship between exposure to pulp mill effluent and specific pathologies.

Discussion sessions were held following the presentations on both days and a summary of the questions, answers and comments raised are included following the abstracts (Appendices C and D). In general, the workshop attendees raised issues about the various alternatives to the fish survey that had been presented at the workshop and expressed opinions about the applicability of the various options to become an alternative to the standard fish survey approach that had been used in Cycle One.

Based on the presentations and discussions summarized above, we (the editors) have attempted to evaluate the various alternatives in light of the principles for conducting EEM studies (as published in EC/DFO 1992b) and the comments made by the workshop participants. Each alternative was rated as being either: 1) Ready to be used in Cycle Two; 2) Applicable for EEM but the methods require additional work and confirmation; or 3) Not applicable to pulp and paper EEM. A summary of the results of this evaluation are summarized in Table 1. More detailed evaluations are provided in Appendix G.

Table 1. - Evaluation of the applicability of alternative methods for pulp and paper EEM studies.

Alternative Method or Approach	Ready for Cycle Two	More Work Required	Not Applicable to EEM
Wild bivalve survey	*		
Caged bivalve studies[1]	*		
Habitrap		*	
Bivalve Scope for Growth		*	
Mesocosm with fish[1]	*		
Health Assessment Index		*	
Invertebrate Pathology		*	
Caged Finfish			*
Forage fish - marine	*		
Forage fish - freshwater	*		
Juvenile fish survey	*		
Salmon life cycle study		*	

[1] Ready for Cycle Two contingent on successful completion of ongoing pilot projects

In general, consensus was that if the fish survey in Cycle One was successful, or could be made successful by better capture techniques, timing, sentinel selection, reference site choice, and there are no serious confounds with other effluent discharges or historical damage (e.g., substrate alteration by fibre mats, wood chips), then a similar, standard fish survey should be followed in Cycle Two. To ensure exposure to effluent in the nearfield, species should be sedentary (e.g., the mummichog) or there must be a working tracer. Given the poor record of tracers in Cycle One and their associated expense and logistical problems, it seems preferable to work with a sedentary species if such are available. Where possible, animals exposed consistently to $\geq$ 1% effluent should be sampled but where this is not practical, animals near the discharge point should be sampled. However, effluent discharged into currents that are rapid and/or variable in speed and direction may have most effect some distance from the end of the pipe. This is particularly true in marine and estuarine receiving environments. Mills carrying out fish surveys in such environments should seek advice from a qualified hydrologist or physical oceanographer before establishing impact and reference sites. Where sedentary finfish species are not available, wild bivalves are suitable sentinel species. In general, crabs and lobsters cannot be aged (Appendix E) and should be avoided. Every effort should be made to sample two sentinel species but if only one appropriate sentinel species can be found, the survey might proceed with just the one.

Some areas are not inhabited by adult finfish or macroinvertebrates but are nursery areas for their juveniles. In such cases, it would seem logical to replace the standard fish survey by a survey of juvenile fish. Relevant measures would include: abundance, deformities associated with exposure to pulp mill effluent (e.g., vertebral fusions and compressions, spinal curvatures including lordosis and scoliosis, fin erosion), and growth in juveniles exposed to pulp mill effluent, compared to juveniles in otherwise comparable reference sites. Methods for the collection of juvenile fish in marine environments are well established (e.g., Hanson and Courtenay 1995; Locke and Courtenay 1995; Robichaud et al. 1996) and many juvenile fishes can be aged (e.g., Secor et al. 1995). In some areas, such a survey might be precluded by insufficient knowledge of distributions to establish reference and exposure sites, high temporal and geographic variability, or inability to determine age and therefore growth. Therefore, in these situations, preliminary surveys will be required to assess the feasability of this technique.

If a fish survey cannot be carried out, either because the receiving environment cannot be sampled or because insufficient finfish or shellfish are found there, a suitable alternative would be a caged bivalve exposure. In many cases, this approach will be able to circumvent problems with confounding discharges and historical damage such as substrate alteration. In situations where a caged bivalve exposure cannot be carried out because the receiving environment is inappropriate (e.g., strong currents, fluctuating salinities, confounding effluents, historical damage (e.g., substrate alteration)), a mesocosm exposure is a suitable alternative. Because neither caged bivalves nor finfish in mesocosms have been used previously in EEMs for Canadian pulp and paper mills, demonstration projects are being carried out to transfer these methodologies to consultants.

Recommendations

1. Based on the presentations made at the workshop, the discussion of the various alternatives by workshop attendees and the evaluation of the alternatives provided in this report; it is recommended that the Fish Survey Expert Working Group and the Technical Management Committee (TMC) for the pulp and paper EEM program make provisions for the inclusion of applicable alternatives in Cycle Two. These include: surveys of small, sedentary (forage) fish and bivalves, caged bivalve studies and mesocosm studies with fish. In addition, surveys of juvenile fishes should be an acceptable alternative in receiving environments that are nurseries for fish but do not serve as habitat for adult fish.
2. Where possible, the TMC should encourage and sponsor protocol development and pilot studies to facilitate technology transfer to the consulting community. Pilot studies to

demonstrate the utility of caged bivalves and mesocosms for fish should proceed immediately.

3. The TMC should encourage and sponsor research for those alternatives that require further refinements.

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EXTENDED ABSTRACTS
(addresses of authors provided in Appendix B)

1. Status Report from the Fish Survey Expert Working Group

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The amendments to the *Fisheries Act* passed in May, 1992, included a provision for Environmental Effects Monitoring at pulp and paper mills in Canada. The first cycle reports were due on April 1, 1996. An Expert Working Group (EWG) was formed to review the results of the fish survey component, with representatives from government, the pulp and paper industry and consultants with specialized expertise. The main purpose of the EWG was to review the first cycle objectives and to make recommendations for the second cycle.

The EWG reviewed 112 studies submitted, only 7% of the studies were determined to have been totally successful, and 53% were unsuccessful. The remainder were partially or marginally successful based on numbers of fish caught. The EWG documented generic problems faced during the completion of the Cycle One studies. In marine and estuarine systems, the dominant problems were related to migratory fish and an inability to document exposure. No marine and estuarine surveys were totally successful. In freshwater, the dominant problems were related to an inability to catch fish, uncertain exposure, problems with reference sites, the presence of confounding factors and incomplete or poor reporting of data. The data were reviewed as they applied to the secondary objectives of the first cycle: evaluating species suitability, collection methods and reference sites. A review of variability showed large differences between studies, even those at closely related sites.

It was determined that the generic survey required in the first cycle was not appropriate for all receiving waters, and a decision tree was developed to assist in determining when an alternative approach was warranted. It is felt that the decision tree will be useful for industry, their consultants and the local authorities in defining a suitable study design for Cycle Two on a site-specific basis. Alternative approaches are required in situations where fish were not present, not exposed or the data collected could not be interpreted. The present workshop is the EWG's opportunity to review alternative approaches with regard to their readiness for inclusion in Cycle Two. Approaches deemed promising but not yet proven might be explored on a pilot scale where acceptable to both the mill involved and the regional technical advisory panel.

The EWG will submit a draft report to the National Technical Management Committee (TMC) at the end of April 1997. Following this, it is expected that the EWG will continue work on sections not completed, pending the comments and advice of the TMC. Included in the final report will be a series of recommendations for research and development needs for the fish survey component of EEM.

Questions, Answers and Comments

Q. Has the EWG determined what magnitude of difference we want to be able to detect between areas receiving pulp mill effluent and reference areas (i.e., effect sizes)?
A. The EWG has discussed differences in gonad sizes. In our previous research we have found 25% changes in fish exposed to pulp mill effluent. The EWG is presently looking at variances seen in reference sites.

Q. With regard to alternative approaches, what criteria will be used to determine which alternatives are suitable?
A. The EWG will be discussing criteria immediately following this workshop. From that we also expect to generate a list of research needs.

C. We should include all potential approaches at this stage.

C. We should keep in mind Richard Addison's point that we should be looking at commercially important species.

C. Perhaps, but we don't care only about commercially important species, we care about the effects of pulp mill effluent on the entire biological integrity of the receiving environment.

C. This brings us back to the definition of exactly what question the fish survey is supposed to address which has never been clear.

2. EEM Cycle One Adult Fish Survey Experience: Pacific and Yukon Region

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Before evaluating the EEM Adult Fish Survey (AFS) it is necessary to have a clear understanding of the distribution of mill effluents in the environment. Knowing the dilution and dispersion characteristics for each mill effluent is a key element in study design: it determines the exposure/dose of the effluent to the biological receptors (in this case, the fish sentinel species). Similarly, one of the prime objectives of the Cycle One AFS was the evaluation of the potential sentinel species and the confirmation of the validity of the reference areas.

The 23 pulp and paper mills that conducted an EEM program in British Columbia (Figs. 2.1 and 2.2) discharge to a wide range of receiving environments. Eastern B.C. mills discharge to clear rivers, two of which are downstream of hydro dams (Fibreco CTMP on the Peace River and Celgar kraft mill on the Columbia River). The mill at Crestbrook discharges tertiary treated kraft effluent from a colour clarifier to the glacially clear Kootenay River. The two mills at Mackenzie are located on the Williston Reservoir which also possess unique challenges to conducting an EEM program given the 17 m draw-downs which occur. The Fraser River, which carries a high suspended solids load, directly receives the discharge of four kraft and CTMP mills in the Prince George-Quesnel area, and indirectly receives the effluent from one kraft mill on the Thompson River tributary at Kamloops.

The marine and estuarine mills discharge to environments which range from riverine to oceanic. Two paper mills, Scott and Paperboard, located in the estuarine portion of the Fraser River at Vancouver, and the CTMP mill in the Somass estuary at Port Alberni, have shallow, and in the case of the paper mills, low-volume discharges (8,000-15,000 m^3/d). This is in marked contrast to the large-volume (85,000 to 270,000 m^3/d), deep (73-115 m) sea outfalls of the Port Mellon, Harmac, and Powell River kraft mills. The effluent from these three outfalls have been shown through dye studies to remain trapped at 20-60 m below the sea surface (Table 2.1). At the other mixing extreme is the mill at Elk Falls which discharges effluent at 15-40 m but this effluent is rapidly mixed due to the 6 m/s currents of Discovery Passage. Similarly, well-mixed effluent is found at the Skeena kraft mill discharging to Porpoise Harbour. The mills at Port Alice, Woodfibre, and Gold River have relatively shallow discharges (20-40 m) which allow the effluent to rise to the surface (0-10 m).

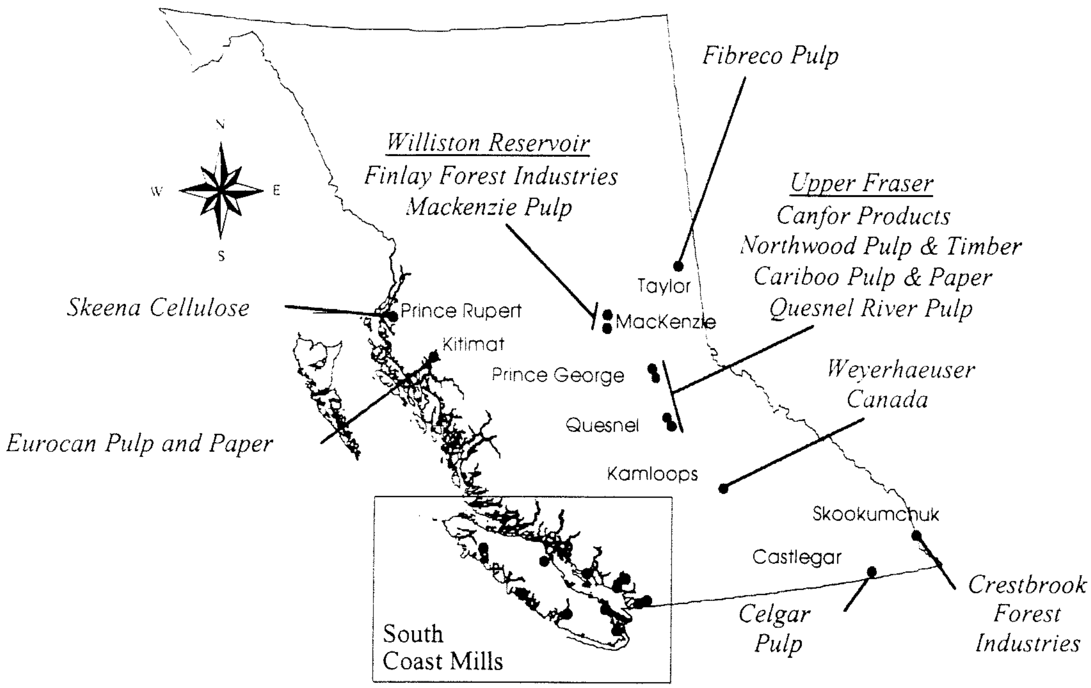

Figure 2.1: Pulp and Paper Mills in North and Interior British Columbia

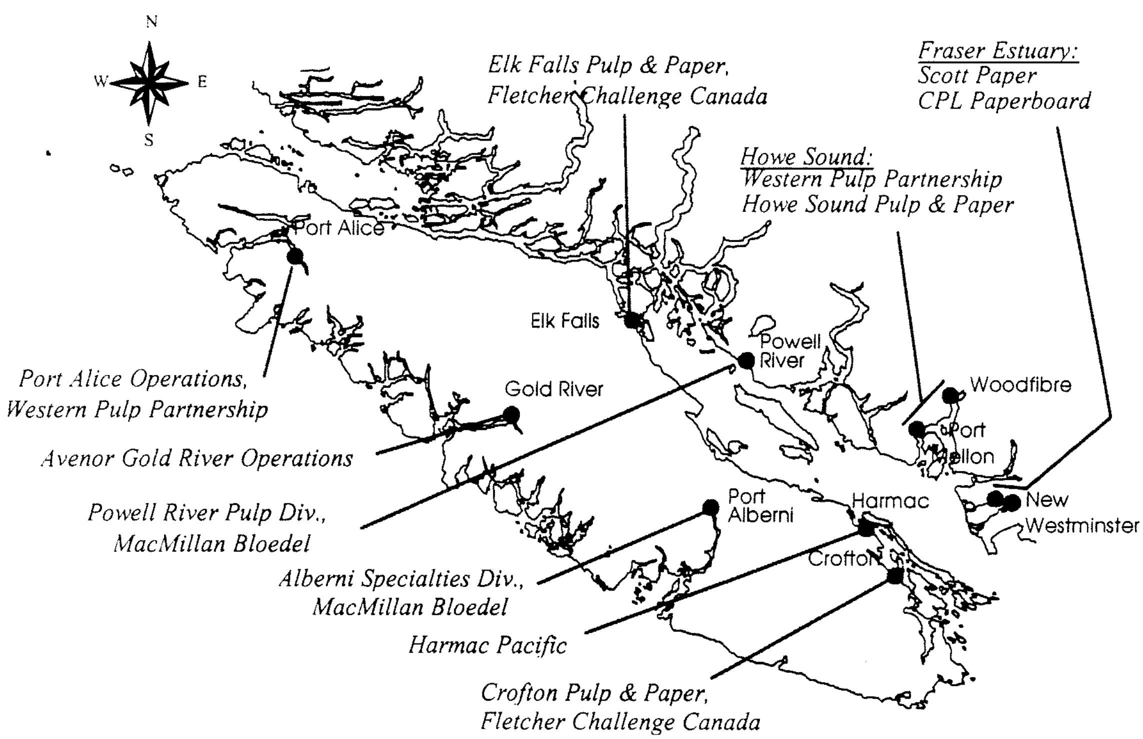

Figure 2.2: Pulp Mills on the South Coast of British Columbia

A range in length of 1% effluent dilution zones is apparent for marine and estuarine mills (Table 2.1). The largest 1% dilution zones are in estuaries where a large volume of effluent enters the surface layer and is transported down-inlet (5 - 24 km). The smallest dilution zones (<500 m) consist of a variety of low and high volume, trapped and surfacing effluents, released into relatively high energy environments. Effluent mixing may be less complicated for freshwater mills, but should not be assumed to occur instantaneously. The 1% dilution zones range from 50 m to 7 km under most conditions, but may reach 85 km below Crestbrook and 500 km below the Upper Fraser mills during periods of high effluent discharge and very low river flows.

For the AFS, mills were required to sample two sentinel species, and were given a minimum sample size target of 20 adult males and 20 adult females for each species. Sampling areas included at least one reference area and an effluent-exposed area, many mills also included a far-field fish sampling area. There was a further requirement to confirm that samples were representative of these areas by looking for an appropriate mill tracer. To this end, ambient water was sampled at 18 mills and fish tissues were sampled at nine mills. In general, water column tracers were more successful than tissue tracers, and tracers were more successful in freshwater environments than in marine environments. At one freshwater mill (Crestbrook) dioxins and furans indicated that reference mountain whitefish had previously been exposed to effluents and were thus not a suitable sentinel species, in contrast to the dioxin monitoring at Celgar which confirmed the integrity of mountain whitefish and large scale suckers species for that mill. (Table 2.2). Most marine mills located exposed and reference sites for AFS crab sampling based on dioxin and furan data from crabs collected for their dioxin monitoring programs. These data are not considered in this table. No marine mills were able to confirm exposure using chemicals in fish and only four of nine marine and estuarine mills confirmed exposure using a water column tracer. A complete analysis of tissue tracers from mills in Canada is reported elsewhere (Ali et al. 1997).

Table 2.3 presents an overview of capture and analysis success for marine/estuarine and freshwater sentinel species. Only one marine and four freshwater mills caught the requisite number samples of two sentinel species at both exposed and reference sites; six mills included a far-field location in addition to the near-field and reference locations. The majority of mills were able to catch at least one sentinel species, though five of the eleven marine mills used a bivalve species to meet this requirement. Only one marine mill was unsuccessful at obtaining the required number of at least one sentinel species (due to the high current speeds in Discovery Passage and the segregation of male and female crabs in the environment). One Fraser River estuary mill caught two species in adequate numbers, but they were predominately juveniles. These findings were reflected at recent regional workshops (Colodey 1996, COFI 1996) and underscore the need for alternative approaches in marine and estuarine environments. The use of wild or caged mussels or oysters may be useful ways to assess the impact of effluents. Caged shellfish offer the opportunity to assess growth and condition changes after periods of known exposure and can provide adequate replication for statistical purposes. Mesocosms may also allow for the controlled exposed of selected species as a substitute for locations which are confounded by multiple discharges.

Table 2.4 compares capture success of finfish and invertebrate species in relation to the number of times they were proposed in study designs. In general, freshwater fish were more often used as proposed in the study design and had a higher rate of successful capture compared to marine fin fish. Large-scale sucker (LSS) was the most common freshwater species (five mills) and blue mussels (three sites), English sole (two sites) and prickly sculpin (two sites) top the list for marine and estuarine mills.

When the performance of various species from marine and estuarine environments are considered (Table 2.5) a number of problems are evident above and beyond the numbers of fish caught: juveniles predominate (starry flounder, prickly sculpin), sexes not equally caught (Dungeness crab, widow rockfish), old fish or difficult to age (Widow rockfish, threespine stickleback), and batch spawners (threespine stickleback). Species which were plentiful in the marine environment at some locations include blue mussels, Pacific oysters, and Dungeness crab. Unfortunately crabs cannot be reliably aged and are subject to commercial and recreational harvesting, thus limiting their usefulness as a sentinel organism. Other shellfish (oysters or mussels) had reduced condition factors in near-field locations compared to reference sites (Crofton, Powell River, Port Mellon, Woodfibre), although in the latter three cases the reference area was not suitable. Fishing success for freshwater mills was higher and sex distribution was more evenly distributed than for the marine and estuarine mills (Table 2.6).

Few conclusions regarding differences between exposed fish in relation to reference sites are possible given the exploratory nature of Cycle One EEM sampling. Further examination of changes in fish will be done on a species and sex specific basis to determine if changes to, e.g., LSS are consistent between locations, although a cursory examination of the data would not seem to confirm this.

CONCLUSIONS

1. It is essential to have a good knowledge of effluent distribution in the environment prior to selecting sentinel species, given the problems associated with the use of tracer compounds.
2. Other discharges and confounding habitat factors need to be considered and underscore the usefulness of coordinated monitoring through a basin approach where other dischargers also monitor the sublethal and environment effects of their effluents. Do not assume because you are "downstream" of a pulp mill that it is the only factor influencing what you are sampling.
3. There is a need for alternatives to the Fish Survey approach as outlined for Cycle One EEM: mesocosms for highly confounded areas; wild and/or caged mussels for areas where finfish cannot be used due to exposure or catch difficulties.
4. There may be instances where a Fish Survey is not practical due to either lack of exposure, confounded exposures, or lack of appropriate sentinel species.

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Questions. Answers and Comments

Q. How can you quantify exposure to effluent in wild fish?
A. This cannot be certain without cageing.

Q. Did any studies do tagging?
A. Mark Spafford did some.

C. Juvenile fishes should not be written off.

C. To help address questions about exposure, some mills used multiple reference sites.

C. We should remember that the mandate of Cycle One was to determine whether existing regulations are adequate to protect the environment. This is much more difficult than simply determining whether effluent has the potential for harming fish. With this in mind, we shouldn't be too hard on ourselves as we review the often imperfect results of Cycle One.

Table 2.1: Characteristics of effluent discharge - B.C. marine and estuarine mills

MILL	Receiving Environment	Effluent Volume (x1000 m3/d)	Diffuser Depth (m)	Trapping Depth (m)	Length 1% Zone max. (km)
Port Alberni	Somass R. Estuary	87	surface	0 - 7	24
Port Alice	Neuroutsos Inlet	136	20 - 40	0-6	10
Eurocan	Kitimat R. Estuary	63	river	none	5
Gold River	Muchulat Inlet	85	24	0-10	4
Powell River	Malaspina Strait	270	57 - 73	15-50	3
Woodfibre	Upper Howe Sound	66	22.5	2 - 8	3
Port Mellon	Lower Howe Sound	85	30 - 115	15-50	2
Crofton	Stuart Channel	154	18 & 28	0 (8-15)	0.6
Skeena	Porpoise Harbour	123	20	none	0.5
Paperboard	Fraser R. Estuary	8	river	none	0.3
Elk Falls	Discovery Passage	168	15 - 40	none	0.25
Harmac	Northumberland Ch.	143	105	20 - 60	0.25
Scott Paper	Fraser R. Estuary	15	river	none	0.1

Table 2.2: Confirmation of fish exposure to effluent at 23 B.C. pulp mills

Mill	Tissue Tracer Used[1]	Species and Tissue Used	Exposure Confirmed	Water Column Tracer Used[3]	Exposure Confirmed
MARINE AND ESTUARINE MILLS					
ALBERNI	None	na	na	Colour	Yes
CROFTON	None	na	na	Colour, CP, R/FA, T/L	No
ELK FALLS	None	na	na	Colour, CP, R/FA, T/L	No
EUROCAN	R/FA	Flounder, sculpins liver, gall bladder	No	Sodium	Yes
GOLD RIVER	Dioxin[2]	Widow rockfish muscle	No	None	na
HARMAC	CPs	English sole liver	No	None	na
PAPERBOARD	R/FA	Staghorn sculpin liver	No	None	na
PORT ALICE	None	na	na	SSL, CP, R/FA	Yes
PORT MELLON	None	na	na	Colour, CP, R/FA	No
POWELL	None	na	na	Colour, CP, R/FA	No
SCOTT	None	na	na	None	na
SKEENA	None	na	na	Colour, CP, R/FA	Yes
WOODFIBRE	None	na	na	Colour, CP, R/FA	No
13 MILLS	**9**		**0**	**9**	**4**
FRESHWATER MILLS:					
CANFOR	None	na	na	Na, CP, R/FA	Yes
CARIBOO	None	na	na	Na, CP, R/FA	Yes
CELGAR	Dioxin/furan	LWF, LSS liver, muscle	Yes	Na, CP, R/FA	Yes
CRESTBROOK	Dioxin/furan	LWF, LSS liver, muscle	Yes	Na, CP, R/FA	Yes
FIBRECO	R/FA	MWF, LNS bile	Males	None	na
FINLAY	CP, R/FA	LWF, LSS liver	No	R/FA	Yes
MACKENZIE	CP, R/FA	LWF, LSS liver	No	6 Cl-Vanillin	Yes
NORTHWOOD	None	na	na	Na, CP, R/FA	Yes
QUESNEL	None	na	na	Na, CP, R/FA	Yes
WEYERHAEUSER	None	na	na	Na, CP, R/FA	Yes
10 MILLS	**5**		**2.5**	**9**	**9**
TOTAL 23 MILLS	**9**		**2.5**	**18**	**13**

NOTES:

1 Dioxin and furan also used at marine mills - See Dioxin and Furan section

2 In addition to Dioxin and Furan Monitoring Program

3 CP - Chorophenols; R/FA - Resin & Fatty Acids; T/L- Tannins & Lignins; Na - Sodium

Table 2.3: Overview of capture and analysis success from 23 B.C. pulp mills

Meets requirements for two sentinel species:

Marine/Estuarine:	**1**	Alberni[1]
Freshwater:	**4**	Canfor/Northwood, Fibreco, Weyerhaeuser[2]

[1] 18 male English sole captured at reference site

[2] 17 male Peamouth chub captured at exposed site

Meets requirements for one species at exposed site:

Marine/Estuarine:	**10**	Eurocan, Gold River, Harmac, Port Alice, Paperboard,Crofton[3], Port Mellon[3], Powell River[3], Skeena[3], Woodfibre[3]
Freshwater:	**6**	Cariboo/Quesnel, Celgar, Crestbrook, Finlay/ Mackenzie[4]

[3] 30 or more unsexed mussels or oysters captured at exposed site

[4] 19 male Lake whitefish captured at exposed site

Requirements not satisfied for either species:

Marine/Estuarine:	**2**	Elk Falls, Scott
Freshwater:	**0**	none

Table 2.4: Capture success in B.C. EEM Cycle One programs

Finfish:

		Proposed	**Captured**	**Successful**
Marine/Estuarine:				
English sole	*Parophrys vetulus*	**9**	**3**	**1**
Prickly sculpin	*Cottus asper*	**3**	**3**	**2**
Starry flounder	*Platichthys stellatus*	**3**	**3**	**0**
Staghorn sculpin	*Leptocottus armatus*	**2**	**2**	**0**
Threespine stickleback	*Gasterosteus aculeatus*	**1**	**1**	**1**
Quillback rockfish	*Sebastes maliger*	**0**	**1**	**0**
Rock sole	*Lepidopsetta bilineata*	**0**	**1**	**0**
Slender sole	*Lyopsetta exilis*	**0**	**1**	**0**
Widow rockfish	*Sebastes entomelas*	**0**	**1**	**0**
Pacific hake	*Merluccius productus*	**1**	**0**	**0**
Shiner perch	*Cymatogaster aggregata*	**1**	**0**	**0**
Freshwater:				
Largescale sucker	*Catostomus macrocheilus*	**10**	**8**	**5**
Peamouth chub	*Mylocheilus caurinus*	**7**	**5**	**2**
Longnose sucker	*Catostomus catostomus*	**3**	**4**	**1**
Mountain whitefish	*Prosopium williamsoni*	**3**	**3**	**2**
Lake whitefish	*Coregonus clupeaformis*	**2**	**2**	**0**
Northern squawfish	*Ptychocheilus oregonensis*	**1**	**0**	**0**

Invertebrates:

Marine:				
Dungeness crab	*Cancer magister*	**5**	**7**	**4 x 0.5**
Blue mussels	*Mytilus edulis*	**1**	**3**	**3**
Pacific oyster	*Crassostrea gigas*	**2**	**2**	**2**
Red rock crab	*Cancer productus*	**0**	**1**	**0**
Two spot prawn	*Lampetra ayresi*	**0**	**1**	**0**

NOTES:

There were **10** mills discharging to marine waters, **3** discharging to estuaries, and **10** discharging to freshwater.

Species **proposed** in the Design Study were not always **captured** and were **successful** only if 20 male and 20 female were collected from both exposed and reference areas.

Table 2.5: Sentinel species captured - B.C. marine mills

		Reference		Near-field		Far-field	
		Male	**Female**	**Male**	**Female**	**Male**	**Female**
ALBERNI	Threespine sickleback	**20**	**20**	**20**	**20**	20	20
	English sole	18	**20**	**20**	**20**		
Two reference areas for Threespine (Nahmint Bay and Effingham Inlet). One for English sole (Barkley Sound).							
CROFTON	English sole	15	**28**	11	15		
	Pacific oyster*	42		63		61	
	Dungeness crab	0	0	**32**	1	54	1
ELK FALLS	Quillback rockfish	12	10	**28**	14		
	Dungeness crab	**37**	1	**41**	2	106	36
EUROCAN	Starry flounder*	76		140			
	Prickly sculpin	**43, 31**	**20**, 7	**20**	**21**	Two reference areas	
	Staghorn sculpin	10	0	4	**20**		
GOLD RIVER	Widow rockfish	11	11	**51**	**34**		
	Red rock crab	3	0	8	3		
HARMAC	English sole	**21**	**24**	**41**	**20**	27	24
	Dungeness crab	**36**	4	**23**	17	35	5
	Two spot prawn	**28**	1	7	10	21	5
PAPERBOARD	Prickly sculpin	**22**	**20**	**21**	**26**		
	Staghorn sculpin	**10**	**11**	**10**	**14**		
	Starry flounder*	46 Juveniles		40 Juveniles			
PORT ALICE	Slender sole	0	0	**20**	**20**		
	Blue mussels*	63		63			
	Dungeness crab	**23**	**20**	12	**27**		
PT. MELLON	Dungeness crab	**31**	6	**42**	13		
	Blue mussels*	30		30			
POWELL R.	English sole	1	1	13	16		
	Pacific oyster*	42		84		42	
	Dungeness crab	2 - not dissected		19	4	10	0
SCOTT	Starry flounder*	97		53		69	
	Prickly sculpin	14	6	15	2	4	7
	Prickly sculpin*	48 Juveniles		39 Juveniles		29 Juveniles	
SKEENA	Rock sole	7	7	7	5		
	Dungeness crab	**55**	**32**	**73**	5	119	33
WOODFIBRE	Dungeness crab	4	0	**42**	7		
	Blue mussels*	30		30			

Bolded numbers indicate that the required number of species were captured; * - not sexed

Table 2.6: Sentinel species captured - B.C. freshwater mills

		Reference		Near-field		Far-field	
		Male	Female	Male	Female	Male	Female
CANFOR	Largescale sucker	**24**	**23**	**24**	**22**		
	Peamouth chub	**25**	**27**	**20**	**20**		
CARIBOO	Largescale sucker	**25**	**23**	**22**	**20**		
	Peamouth chub	**20**	**27**	12	14		
CELGAR	Mountain whitefish	17	15	11	10		
	Longnose sucker	1 - not dissected		**21**	**20**		
CRESTBROOK	Mountain whitefish	**21**	**21**	**21**	**21**		
	Largescale sucker	7	13	8	19		
FIBRECO	Mountain whitefish	**20**	**20**	**20**	**20**	20	20
	Longnose sucker	**20**	**20**	**20**	**20**	20	20
FINLAY	Lake whitefish	**23**	**22**	19	**29**	9	26
	Longnose sucker	**30**	10	14	13	2	2
	Largescale sucker	3	0	11	10	1	26
MACKENZIE	Lake whitefish Longnose/Large-scale sucker	Same fish as FINLAY					
NORTHWOOD	Largescale sucker Peamouth chub	Same fish as CANFOR					
QUESNEL	Largescale sucker Peamouth chub	Same fish as CARIBOO					
WEYERHAEUSER	Largescale sucker	**21**	**21**	**22**	**21**		
	Peamouth chub	**21**	**21**	17	**30**		

Bolded numbers indicate that the required number of species were captured
Starry flounder, juvenile Prickly sculplin, mussels, and oysters were not sexed

3. Experiences with the first cycle EEM adult fish surveys in the Atlantic Region

W. Roy Parker
Environment Canada, Atlantic Region

During the first EEM cycle for the pulp and paper industry in the Atlantic Region, there were nineteen mills operating and subject to the requirements of the Pulp and Paper Effluent Regulations. In total, 17 EEM studies were conducted; there were 2 joint studies. Of the 19 mills, 4 discharge to freshwater, 8 discharge to estuaries and the remaining 7 mills discharge directly to the ocean. At the time of the EEM studies, 8 mills had secondary treatment and the other 11 had primary effluent treatment only.

The EEM studies conducted at mills that discharge to freshwater used three sentinel species: white sucker (2 mills); yellow perch (2 mills) and threespine stickleback (1 mill). At mills that discharged to the marine environment, a total of 10 different sentinel species were used. The most commonly used sentinel species were the Atlantic tomcod (7 mills) and winter flounder (8 mills). Green crab and rock crab were each used at 3 mills. The remaining sentinel species: Atlantic silversides, cunner, longhorn sculpin, shorthorn sculpin, American lobster and mummichog, were each used at one mill. The most common fishing techniques used were gill netting and trap netting. Other methods used to capture fish included baited long lines, minnow traps, electrofishing, otter trawling and SCUBA divers.

Only one EEM study captured the minimum required numbers of 20 males and 20 females for 2 sentinel species at both a reference and effluent exposure site. For 9 other EEM studies, the minimum numbers were captured at both sites for one sentinel species.

The following is an example of an adult fish survey (AFS) at a mill discharging to freshwater. The minimum numbers of targeted sentinel species, white sucker and yellow perch, were captured. Measurement of resin and fatty acids in the bile of a sub-sample of the sentinel fish indicated that the fish from the exposure area, but not reference area, had been exposed to pulp mill effluent. The measurements of these fish and the analysis of the data indicated that there were significant differences between reference area fish and exposure area fish. Overall, this study met the objectives of Cycle One.

At another mill discharging to freshwater, threespine stickleback were the only non-migratory fish in the river and were used as the sentinel species. The minimum number of fish were not captured in both the exposure and reference areas. The reference and exposure areas were separated by a hydroelectric dam so a tracer was not required to confirm exposure. Measurements on the captured fish indicated that they were all of the same age class. The only significant difference between exposure and reference fish was in female length. In summary, the Cycle One objectives were not attained for this mill.

The following is a summary of the adult fish survey for a mill discharging to marine waters. The selected sentinel species were winter flounder and rock crabs. Although the minimum numbers of both sentinel species were not captured, the results indicated that the minimum numbers were almost achieved. The use of tracers was not successful at confirming effluent exposure. The measurements made on the fish and the data analyses indicated many differences between the fish in the reference and exposure areas. Unfortunately, because the crabs could not be aged, the data were incomplete. Overall, the Cycle One adult fish survey was partially successful.

The final example is for a mill discharging to a very dynamic estuary system. For sentinel species, the Atlantic tomcod, green crab and mummichog were selected. The fishing effort was not successful at capturing the minimum number of any of these fish in both the reference and exposure areas. Due to the limited number of specimens, the comparison between fish from the different areas were not possible. Attempts to confirm the exposure of the fish to effluent were not successful. Overall, the Cycle One adult fish survey was not useful at this site.

A review of all of the adult fish survey results indicated that this EEM component provided useful information at two of the freshwater mills and at seven of the marine mills. At one freshwater mill and at the remaining eight marine mills, the first cycle adult fish survey was not very useful. Some of the problems experienced with the AFS during Cycle One included not capturing enough fish, finding suitable sentinel species, using crustaceans which could not be aged, finding suitable reference sites, the confounding of results by other pollution sources and the lack of confirmation of effluent exposure.

For the next cycle of EEM studies, a continuation of an AFS type of approach would be appropriate at about half of the Atlantic Region mills. At the other half of the mills, it does not make a lot of sense from a scientific or cost effectiveness perspective to continue with an AFS approach. If a different method of assessing the health of crustaceans can be developed which does not require ageing, the AFS could be continued for a few more mills. It is obvious that alternate means of assessing fish community health will be required at a number of sites for the next EEM cycle.

Questions, Answers and Comments

Q. Where exposure to effluent was not confirmed, was this because no tracer was detected?
A. Tracers were sometimes detected but no difference was seen between the effluent and reference sites.

Q. Is it possible to age crustaceans and if not, why use them?
A. Ageing decapods is not generally possible though we can still compare sizes, quality etc. Perhaps we have learned during Cycle One that these animals are not the best sentinel species to use in Cycle Two. You can mark individual crabs and know that they have been exposed but if we don't know how they respond to exposure, we can't use them.

Q. Only one mill caught enought fish and only 3 determined exposure so how can you say that 9 were successful?
A. At least partial success was seen at 9 mills and with greater effort and better tracer methodology, we will do better in Cycle Two. Remember that Cycle One was exploratory.

Q. One objective of Cycle One was to determine required sample sizes for Cycle Two. What did you find?
A. Sample size depends on what magnitude of effect you want to detect. Twenty fish may be adequate for detecting a 50% difference in liver size but not a 20% difference for example.

4. Adult Fish Survey in Ontario Region: Experiences from Cycle One Environmental Effects Monitoring.

Susan Humphrey and Nardia Ali
Environment Canada, Ontario Region

Twenty-seven pulp and paper mills in Ontario conducted an adult fish survey during the first cycle of the regulated environmental effects monitoring program. The mills discharge into a variety of unique freshwater receiving environments ranging from small headwater streams and human-made historic canals to large rivers and lakes. One mill discharges its effluent to an enclosed waterlot.

Adult fish were captured mostly in autumn, using a number of techniques, including gill nets, trap nets, seine nets and electroshocking equipment Frequently, the targeted fish species were not present in sufficient numbers during field work, and alternate species were collected and measured instead. In most cases, the level of fishing effort was 7 days or less. The incidental sacrifice of non-target fish was high at some sites and included large schools of migrating lake whitefish and lake trout and also sportfish such as muskie and northern pike. Environment Canada staff conducted field audits at twenty-one mills to verify that collection sites and procedures were in accordance with the authorized study design.

Interpretive reports indicate that the mills and their consulting laboratories had reasonably good success with completing the required fish measurements, including age, length, body weight, liver weight, and external/internal physical condition. Somewhat problematic, but only in a few cases, was the requirement to measure gonad weight and egg size. The requirement to analyze for an effluent tracer in fish tissue also presented problems at some sites.

Data analysis and interpretation revealed that differences between exposed and reference fish from site to site varied considerably. These differences have been difficult to link to any particular influence given the complexity of many Ontario mill receiving environments, including the presence of other effluents and in-water structures; a limited one percent effluent plume; fish mobility; and habitat variability between the reference and exposure zones.

The adult fish survey experience in Ontario has lead to a number of recommendations for the Fish Survey Expert Working Group to consider during their evaluation of Cycle One results, including:

1. examine the adult fish survey data from Cycle One, especially in light of confounding factors;

2. consider passive alternatives to the adult fish survey (e.g., community assessments), especially for receiving environments which support sensitive or limited fisheries; and,

3. provide a decision tree to aid in Cycle Two fish survey negotiations between government/mills, especially for those sites where the adult fish survey did not seem appropriate during Cycle One.

Questions, Answers and Comments

Q. What is meant by community assessments?
A. This is not clear but the Ontario Ministry of Natural Resources recommended this instead of lethal sampling of fish.

C. Community studies are harder to design and execute than studies on single sentinel species.

Q. Can't we do an IBI (index of biotic integrity)? This is simple and fast to do.
Q. Do we want to examine the presence and use of habitat by fish or an IBI or what?
A. We are just asking for alternatives, whatever the EWG decides those are, because the option is dropping the AFS and this is unacceptable.

C. We hardly know enough biology of individual sentinel species, let alone enough to make judgements on whole ecosystems through community surveys.

Q.If we can't catch enough of one sentinel species, how will we sample an entire community?
A. In many cases sufficient sentinels were caught but killing them, and bycatch, is the problem.

C. Remember that community assessments will require adding replicate areas.

5. The Use of Bivalves and Crustaceans as Sentinel Species for the EEM Adult Fish Survey

Christopher M. Hawkins
Triton Environmental Consultants

It has become evident that finfish have not been as suitable a sentinel for the AFS of the Pulp and Paper Industry Environmental Effects Monitoring program as initially thought. Consequently, there has been renewed interest in selecting marine invertebrates for use as sentinel species. A recent literature review of invertebrates common to various substrate types throughout the Maritimes has led to the selection of two groups which offer the best selection of sentinel choices; bivalve molluscs and crustacean shellfish. The bivalve candidates for sentinel species include: the blue mussel, *Mytilus edulis*, the soft shell clam, *Mya arenaria*, and the American oyster, *Crassostrea virginica* and the crustacean shellfish species include: the rock crab, *Cancer irroratus*, the Jonah crab, *C. borealis* and the green crab *Carcinus maenas*. Although either group of invertebrates offer choices for candidate sentinel species; the bivalves are considered a better group from which to select a candidate sentinel species. This is due to a considerable number of advantages offered by their ecology, biology and how well each species satisfies the criteria presently used in the selection of a sentinel species by the AFS Technical Guidance Document. Specifically, all the bivalve species mentioned above meet a substantial number of the criteria which include, substrate exposure, food preference, food chain involvement, abundance, longevity, fecundity growth rate and age to maturation among others. In addition, these bivalve species are common throughout the Maritimes (except for the American oyster which does not occur naturally in Newfoundland) a factor which offers some continuity to the establishment of a general protocol which may be applied across the Maritimes as opposed to specific provinces or regions within provinces.

What to measure as well as an approach strategy to be followed are not trivial problems that must be addressed once a sentinel species has been selected. A tiered or hierarchical approach is preferred. Crucial to the strategy is the determination of gross changes that are statistically significant. The study can then be further expanded to address the cause of the gross changes and how they are manifested within the organism. Relatively simple analytical procedures can be used to quantify gross biological changes such as shell growth and tissue growth. More complex procedures such as measures of contaminant levels in tissues, histological examination of tissues and biochemical enzyme analysis could follow. However a detailed blueprint or strategic plan to follow has yet to be addressed. It is highly unlikely that one approach will surface as a common plan to EEM programs. It is more likely that each mill will develop their own strategy based upon a cost/benefit approach tailored to the guidelines and/or recommendations provided by federal agencies.

Questions, Answers and Comments

Q. Aren't there problems with multiple spawnings and measuring gonads etc. in bivalves?

A. Yes, you must know the biology of the species and population. Therefore preliminary work and multiple field trips will be required.

Q. How many trips do you think are necessary?
A. This would have to be decided with the client and experts.

Q. How ready is this for Cycle Two? Is it ready for monitoring now or does it need further research?
A. This will be an item for discussion a little later.

C. Global "mussel watch" has gathered lots of data on mussels which may be useful.

6. Refined Caged Bivalve Methodologies as Part of EEM: Characterizing Chemical Exposure and Biological Effects

Michael H. Salazar
Applied Biomonitoring

Caged bivalve monitoring is a potentially powerful tool that could be used routinely as part of the Environmental Effects Monitoring (EEM) program at Canadian pulp and paper mills. The approach is simple, yet refined, and the ability to detect significant site-specific differences on various temporal and spatial scales has been demonstrated repeatedly. A number of refinements to the caged bivalve methodology have been made in the last decade that dramatically increased its utility for characterizing chemical exposure and associated biological effects in effluent monitoring. The use of caged bivalves facilitates reducing the uncertainty associated with traditional risk assessments because these manipulative field experiments combine elements of experimental control from laboratory bioassays with the environmental realism of observational field monitoring. Moreover, during the development of those refinements, standard, well-known, and well-established endpoints (e.g., growth and bioaccumulation) have been used by many different investigators, including those in Canada and the U.S. Therefore, the methods are proven, and widely accepted. They are also cost-effective.

Working Hypotheses

Three working hypotheses were developed as a way to critically evaluate whether caged bivalves might represent a reasonable alternative to the adult fish survey approach, particularly in areas where the failure to collect sufficient fish has been most pronounced. The hypotheses are: (1) Tissue chemistry characterizes exposure better than water or sediment chemistry; (2) Tissue chemistry can be used to help predict potential environmental effects; and (3) Bioaccumulation and growth in bivalves can be used to help predict potential effects on fish. Emphasis will be placed on evaluating these three hypotheses.

Characterizing Exposure and Effects

Transplanting caged bivalves along suspected chemical gradients allows direct and integrated measurements of exposure and effects over space and time. This is important since these characterizations have been problematic in many adult fish surveys. The ability to control the supply of test organisms in areas of concern and make synoptic measurements of well-established endpoints like growth, which have been related to bivalve population effects, reduces the uncertainty associated with measuring natural populations of either fish or bivalves. The ability to clearly define the exposure period and test animal position make this field bioassay methodology a viable alternative or perhaps even a supplement to the adult fish surveys in EEM. The exposure-dose-response triad, consisting of water or sediment chemistry, tissue chemistry, and biological effects (Salazar and Salazar, 1997a,b) emphasizes the importance of tissue chemistry in establishing links between the environment and the organism as well as between results from traditional laboratory tests and field monitoring. Caged bivalve monitoring facilitates establishing those links.

Bivalve Sensitivity

Bivalves help quantify exposure by concentrating and integrating chemicals in their tissues. These estimates of biologically available chemicals are not possible with thousands of water samples. There is a perception among many scientists that bivalves are not very sensitive to chemicals because they survive exposure to high concentrations of chemicals and accumulate high concentrations of those chemicals in their tissues. Recent studies however, have shown that bivalves are as sensitive or more sensitive than standard test organisms such as daphnia, fathead minnows, midges, and amphipods to a variety of chemicals. This includes those chemicals in pulp and paper mill effluents from both freshwater and marine environments (Salazar and Salazar, 1997a,b). Growth is one of the more commonly measured endpoints, it is extremely sensitive to a variety of chemicals, and it is easily measured in caged bivalves.

Mill Monitoring History

Caged bivalve monitoring has been used in Canada and other countries to evaluate the effects of pulp and paper mill effluents on the environment. Scientists have been using this approach for mill monitoring in Finland since 1984. Almost all studies reported in the open literature were conducted in freshwater environments and bioaccumulation was almost always the only measured endpoint. Caged bivalve monitoring that included measurements of bioaccumulation and growth in mussels was required by the U.S. Environmental Protection Agency (EPA) as part of the discharge permit at a pulp mill in Alaska. The approach was similar to that used in numerous studies to evaluate the effects of tributyltin and other chemicals using mussels (i.e., minimum size range about 5 mm, compartmentalized cages for individual measurements, large replication with approximately 100 animals/replicate). Results from monitoring in Alaska showed significant accumulation of dioxins and furans (PCDD/PCDF) by caged mussels. Changes in whole-animal wet-weight and end-of-test tissue weights proved to be the most discriminating growth endpoints in these studies and a statistically significant relationship was found between tissue concentrations of PCDD/PCDF and mussel growth.

Linking Mussel and Fish Dose-Response

McCarty (1987) recalculated a series of Quantitative Structure Activity Relationship (QSAR) regressions and combined them with other bioconcentration relationships to predict water concentrations for acute and chronic effects of organic chemicals on fish (Figure 6.1). Although there were seven orders of magnitude difference in the acute and chronic QSARs, there was only one order of magnitude difference between tissue concentrations predicted for acute and chronic effects. Donkin et al. (1989) measured water and tissue concentrations and mussel feeding rates to make similar predictions (Figure 6.2). These results suggested that tissue burdens would be a better predictor of effects than water concentrations. McCarty and Mackay (1993) have also suggested that using body residues as a reference framework could enhance ecological risk assessments. Collectively, these and other studies demonstrate the following: (1) The ability to predict tissue concentrations associated with effects in mussels, fish, and other species (existing data for tributyltin and other chemicals also support this conclusion for a variety of species). (2) The ability to predict dose-response relationships in fish from dose-response relationships in mussels. (3) Tissue burdens may be a better predictor of effects of hydrophobic organic chemicals than concentrations measured in either water or sediment. Another link between mussels and effects on fish could be established by using the P450 RGS (reporter gene system) to simulate effects in fish. Methods have been developed using a human liver cancer cell line engineered to produce luciferase instead of P450 (Anderson et al., 1995). If chemicals associated with mussel tissues are exposed to this test system, they can be used as a surrogate for the mixed function oxygenase (MFO) induction that has been used in other adult fish surveys as an indicator of exposure and the potential for adverse effects. Most of the exposure and effects endpoints that have been measured in fish can be measured in bivalves.

Prospectus

Having demonstrated the utility of various relationships between exposure and effects in previous mill monitoring (Salazar & Salazar, 1997a,b), it appears that caged mussels could be used as an alternative to adult fish in Cycle Two in EEM. Caged bivalves could also be used in concert with fish exposures, in a preponderance-of-evidence approach to demonstrate exposure to pulp mill effluents and associated effects. Other important information can be obtained using this approach: (1) Mapping and monitoring the exposure plume more efficiently than traditional approaches because of the ability of bivalves to quantify bioavailable chemicals. (2) Validation of laboratory bioassays and confirmation that similar exposures and effects have been demonstrated in the lab and the field. (3) Evaluation of results from other EEM elements including, adult fish survey, laboratory bioassays and benthic community assemblages. It is important to note that in the Alaska pulp mill study, caged bivalves accumulated elevated concentrations of PCDD/PCDF in their tissues while clams in laboratory exposures with contaminated sediment did not. Surprisingly, the relationship between PCDD/PCDF tissue burdens in mussels and sediment used in the laboratory bioassays with clams, was much better than the relationship between clams and the sediment to which they were exposed. The mussel growth endpoint also detected more statistically significant differences among sites than the clam survival endpoint. These results suggest that field bioassays with caged bivalves in the water column are a better predictor of exposure to and potential effects of mill-associated chemicals than laboratory sediment bioassays with infaunal bivalves.

Pilot Study

A caged mussel pilot study is currently underway. Mussels were deployed at the Port Alice sulphite mill (Vancouver Island, B.C.) on August 7, 1997 and it is anticipated that they will be retrieved on or about October 15, 1997. The objective of the pilot study is to test the feasibility, scientific value, and applicability of using caged bivalves as an EEM tool at Canadian pulp and paper mills. The pilot study also included elements of a workshop with participation by government, industry, and consultants to facilitate a transfer of this technology to the mills and the consulting community. The process has been an open one that included planning, the field trials, and data analysis. A progress report and data summaries of the initial deployments have already been provided to interested parties. Other data, statistical analyses, and interpretive guidance will be provided as they become available. The final report will be provided to Environment Canada and the Department of Fisheries and Oceans by December 31, 1997 to allow possible inclusion in Cycle Two of EEM, should the pilot study prove successful. Evaluation criteria will be those identified in Annex 1 of the Pulp and Paper Effluent Regulations.

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Questions, Answers and Comments

Q. Are effects on reproduction easy to measure? How?
A. Someone else did this for us as part of a cooperative study but the data were hard to interpret. Others have been more proficient. It is usually done by measuring the weight of the gonad.

Q. What would be the cost for a pulp mill study without the bioaccumulation study?
A. $8-10K (U.S.) for the initial setup, i.e., consultants to come, teach, and run the experiment. Then, $15-20 K if two people are needed, $40K if a whole team of consultants do the whole thing.

Q. How do you deal with confounding point sources?
A. Spatial resolution is fine-tuned by using a gradient design with mussel cages at varying distances away from each source. However, if two different effluents are exactly coincident, then no technique can resolve their separate effects. This technique is ready for Cycle Two and it could be your best alternative. The best way to test it however, is to compare methods side-by-side.

Q. How euryhaline are these bivalves, will this work in the estuarine environment?
A. Different species will be required for different environments and there are data available to help in making selections. There is a substantial amount of data supporting the use of tissue residues; different species are in some cases comparable in this aspect.

Q. What chemicals should we measure in bivalves exposed to pulp mill effluents?
A. I don't know but whatever you choose you'll find it concentrated by mussels. Perhaps Kelly Munkittrick or representatives from the tracer working group could offer advice.

C. There are 5 or 6 compounds in pulp mill effluent which have been associated with biological responses but there are probably dozens or even hundreds of other chemicals that have not yet been identified. In any case though, bioaccumulation is not the point of the fish survey aspect of EEM, we really only need look at biological effects.

C. True, but even without knowing which chemicals are responsible for whatever biological effects are observed, we could measure a series of chemicals with different bioaccumulation characteristics or KOWs so we at least know the range.

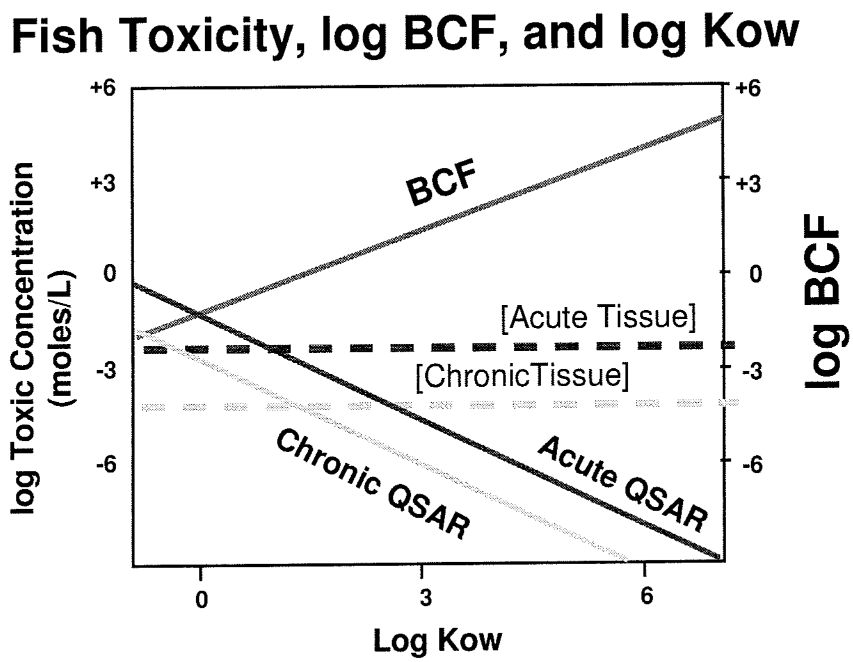

Figure 6.1. The relationship between fish toxicity, log BCF and log Kow. Note how although acute and chronic QSARs span seven orders of magnitude along with BCF, tissue burdens associated with acute and chronic toxicity are constant and separated by about one order or magnitude. (Redrawn from McCarty, 1987)

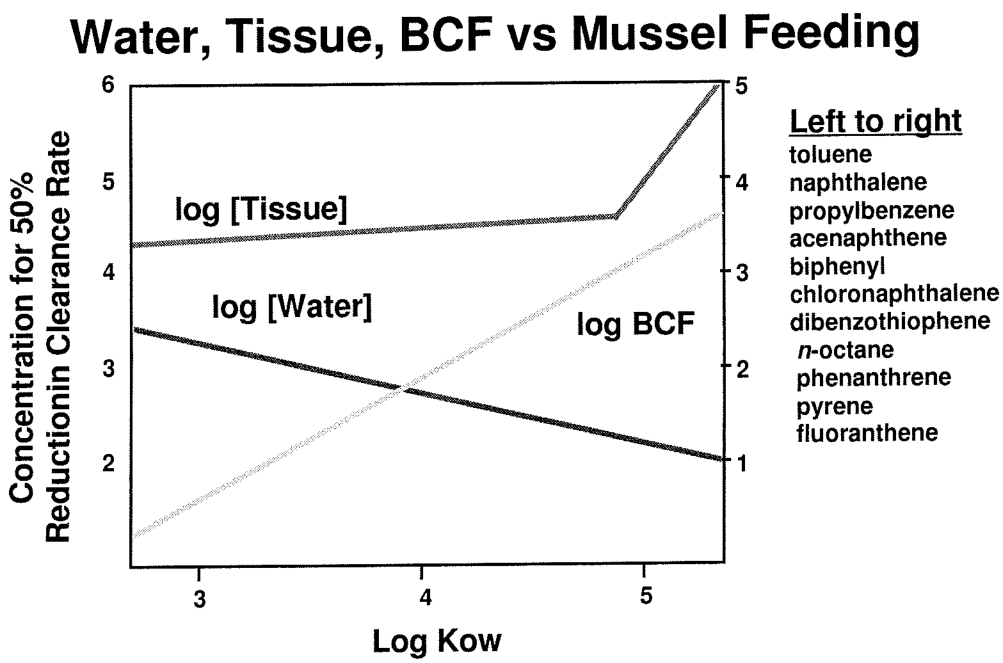

Figure 6.2. The relationship between mussel feeding, log BCF and log Kow. Note how although the water concentration and BCF associated with reduced feeding spans several orders of magnitude, tissue burdens associated with reduced feeding are nearly constant over a wide range of log Kow. These relationships are very similar to those shown for fish in Figure 1. (Redrawn from Donkin et al., 1989.)

7. HABITRAP: A Biomonitoring System using Bivalves for Assessing Environmental Impacts from Chronic Pollution.

Peter J. Cranford
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A Biological Monitoring System, called HABITRAP, was previously developed in response to a need for more practical, sensitive and easily interpreted biological effects indices that can be used in offshore oil and gas Environment Effects Monitoring (EEM) programs. A similar need exists for alternative approaches to pulp and paper mill EEM. The system uses bivalve filter feeders as sentinel organisms for environmental impacts as they are: abundant and widely distributed; commercially and ecologically important; unable to escape pollution; able to process large volumes of water; and can be used to indicate both the biological availability and consequences of a wide range of pollutants.

The most important biological responses of organisms to pollution stress are those that affect their growth, reproductive potential and survival. This is particularly true for a resource species. Tissue growth is clearly related to population effects as it determines the organisms ability to contribute to the gene pool. Pollution stress does not act directly on growth, but on the biochemical and physiological processes controlling growth. Numerous studies have shown that bivalve growth responses to a wide variety of contaminants depend primarily on changes in processes controlling energy acquisition rather than energy utilization. Bivalve feeding behaviour is therefore a sensitive index of the organismal and population effects of contaminants. The HABITRAP Biological Monitoring System is based on the measurement of bivalve feeding responses to ambient changes in environmental and contaminant conditions.

The HABITRAP provides continuous measurements of the clearance, ingestion, absorption and egestion rates of a population of bivalves held *in situ*. The method has been extensively tested, and the results are comparable to those obtained using traditional laboratory methods. However, as the measurements are obtained under conditions of natural food supplies, flow conditions and contaminant levels, the *in situ* responses of bivalves are more reliable than laboratory measurements. The HABITRAP method has the additional advantages of being largely automated and relatively inexpensive.

Bivalve growth rate is a scientifically defensible and useful index of water quality. However, there are two major advantages to measuring bivalve feeding behaviour in EEM programs as a combined index of growth and environmental stress. First, continuous monitoring of feeding rate permits an assessment of the relative impact of short- and long-term environmental changes. Short-term events of importance may include large changes in effluent discharge composition and rate or the resuspension of previously deposited contaminants during storms. Direct growth measurements can only indicate the cumulative stress response over a period of several weeks to months. The ability of the HABITRAP to identify the relative impact of different industry activities would provide regulatory agencies with information on the best practices for the pulp and paper industry to follow for minimizing impacts. Second, feeding behaviour measurements provide a physiological explanation of the mechanism of population impacts and, if used under well defined conditions, permit a diagnosis of the relative impact of natural and anthropogenic stressors. An increased knowledge of cause-effect relationships would enhance our capacity to predict the biological effects of known contaminants.

Questions, Answers and Comments

Q. How do you get ingestion rate from egestion rate?
A. Animals don't absorb any ash so egestion of ash = ingestion rate of ash.

Q. How do you see applying this to Cycle Two?
A. I prefer a gradient approach to an impact vs reference site approach.

Q. Is this ready for Cycle Two?
A. No. The HABITRAP is already developed and proven for offshore oil development monitoring but you would need a pilot project to adapt this for monitoring pulp and paper mill effluents. Therefore, this development should be done during Cycle Two to provide the tool for standard use in Cycle Three.

Q. What would the cost be?
A. Approximately $2K per automated HABITRAP.

Q. What number of animals do you use per collector?
A. Ten scallops or 25 mussels.

8. The Application of the Scope for Growth Approach to Assess Environmental Impacts from Pulp Mill Effluents and Other Point Sources

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"Scope for growth" (SFG) describes the "excess" energy accumulated by an organism after its current energy demands are met, and which is usually directed to somatic growth or reproduction. The rationale for using SFG to monitor environmental quality is that an organism under stress will use energy differently from one not stressed; this difference in energy partitioning is reflected in SFG. Two points about SFG should be obvious: first, stress does not necessarily mean "pollution". In fact, SFG could reflect any change to ambient conditions which results in the organism's processing energy differently; this could include physical disturbances, temperature changes, or changes in food supply, etc. Second, SFG can, in principle, be measured in any organism, though in practice it has been used most in sessile bivalves, where energy balance is not distorted by variations in energy expenditure during movement.

One might ask why growth itself, rather than SFG, is not measured. The answer is that SFG can be measured over a period of a few hours and under controlled conditions, whereas measurements of growth in organisms such as molluscs require at least a few days, or, more usually, weeks; during this interval, sources of stress can change. However, examples exist of changes in molluscan growth being ascribed to a single pollution source (e.g., Black, 1973).

The simplest approach to measuring SFG is to take organisms from their natural habitat and measure feeding, respiration and excretion rates over a few hours under controlled conditions. SFG may then be calculated from the equation:

$$\text{SFG} = \text{Energy absorbed} - (\text{energy respired} + \text{energy excreted})$$

where energy absorbed is measured via filtration or clearance rates (for filter feeders), respiration rate is measured by oxygen consumption, and excretion is estimated from ammonia production (from molluscs) or other forms of nitrogen excretion.

Numerous field studies have illustrated the use of this approach to assessing the impact of pollution. Martin et al. (1984) found SFG in mussel *(Mytilus edulis)* collected from various points in San Francisco Bay to be negatively correlated with several metal and organochlorine concentrations in transplanted *M. californianus*, which was used as a "bioaccumulator" of contaminants. In a similar study, SFG in *M. edulis* from sites along a contamination gradient in an industrialised fjord in Norway declined gradually with increasing contamination by PAH, PCB and (less clearly) metals (Widdows and Johnson, 1988). In a pollution gradient in Hamilton Harbour (Bermuda) a local bivalve *Arca zebra* was transplanted from "clean" sites and caged for 11 - 12 d at suspect sites; this was long enough for contaminants to accumulate through feeding and for the physiological responses to develop. Declines in SFG at contaminated sites were well correlated with increasing tributyltin (TBT) concentrations in the water column and with tissue concentrations of PAH and Pb (Widdows et al., 1990). Sewage discharges may also reduce SFG: at a sewage sludge dump site off Plymouth (UK) transplanted *M. edulis* had lower SFG than animals caged inshore (Lack and Johnson, 1985). The bivalves *M. edulis aoteanus* and *Perna canaliculis* sampled from close to a sewage outfall at Wellington, NZ, had lower SFG, and higher metal concentrations, than those from further away from the source of pollution (Anderlini, 1992). In cockles (*Cerastoderma edule*) from various sites in Southampton Water (UK) SFG (and other indices of health) were generally related to the level of contamination of the site with metals and hydrocarbons (Savari et al., 1991). *M. edulis* caged in the expected pollutant "plume" from an industrial discharge area on the west coast of Sweden had lower SFG than animals caged at a "reference" site (Magnusson et al., 1988). Changes in SFG also explained variations in growth rate of mussels *(M. galloprovincialis*) reared at different sites in an estuary used for aquaculture, though in this case the SFG changes reflected quality and quantity of food, rather than contamination (Navarro et al., 1991). Measurements of SFG are not restricted to bivalves: the invertebrate *G. pulex* showed reduced SFG (resulting from reduced energy assimilation) at a polluted site as compared to a reference site (Maltby et al., 1990). Often, the most obvious impact of stress --- whether from pollution or other causes --- is on the clearance or filtration rate component of the SFG equation (Maltby et al., 1990; Anderlini, 1992; Okomus and Stirling, 1994). Table 8.1 summarises some of the impacts of pollution on SFG in bivalve molluscs.

The measurement of SFG over the short term can easily be related to longer-term changes in growth rate or condition (Savari et al., 1991; Magnusson et al., 1988; Navarro et al., 1991). SFG is therefore attractive as an early warning of potential long term effects.

No "field" studies appear to have been carried out on which the response of SFG to pulp mill effluents. In one lab. study (Sunoko, 1993) *Mytilus edulis* were exposed during 7 day static bioassays to effluent from a pulp mill discharging into Boat Harbour, NS. SFG was reduced significantly in *M. edulis* exposed to 40% v/v effluent in sea-water; the reduction was due to reduced filtration rate by the test animals. Concentrations of effluent greater than 50% were lethal to mussels so could not be tested; 20% effluent had no significant effect on SFG.

There seems to be no reason why SFG should not be used as a field measure of the impact of pulp mill effluents on appropriate "indicator" sessile bivalves.

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Table 8.1. Some examples of changes in Scope for Growth (SFG) in bivalves in response to pollution stress

Site, Species	**Reference**	**Response**
San Francisco Bay, CA (sewage influence) *Mytilus edulis* (resident)	Martin et al. 1984	Decline in SFG with increasing chlordane, dieldrin, Cr, Cu, Hg, Zn, Ag, Al concs.
Industrialised fjord, Norway *Mytilus edulis* (resident)	Widdows and Johnson 1988	Decline in SFG with increasing PAH and PCB gradient
Southampton Water, UK (mixed contaminants); *Cerastoderma edule* (resident)	Savari et al. 1991	Decline in SFG correlated with increasing Cu in sediments
Hamilton Hbr. Bermuda *Arca zebra* (transplanted 11-12d)	Widdows et al. 1990	Decline in SFG with increasing PAH and TBT gradient
Wellington Harbour, New Zealand (sewage impact) *Mytilus edulis aoteanus* *Perna canaliculus*	Anderlini 1992	Decline in SFG with increasing tissue Ni conc.

Questions, Answers and Comments

Q. At what point is it more sensible to measure actual growth than SFG?
A. It's a question of rapidity; SFG is much quicker - virtually instantaneous - to measure. It depends on what question you're asking.

C. Sure, SFG is faster than looking at growth in caged bivalves but it gives poorer information.

C. SFG is easy to measure but hard to interpret. Bivalves have a great ability to acclimate to conditions so you must know your biology. SFG might be a better replacement for the benthic invertebrate survey than for the fish survey.

Q. How many animals are tested in a SFG study?
A. Widdows generally used 8-10 animals per treatment.

Q. Will this be too expensive? Who will do the work?
A. For a pilot project, a graduate student. One idea is to request 40-50K to get a graduate student to look at SFG as an indicator of general health of 6 coastal sites in BC.

C. If SFG is appropriate for graduate theses, then it's a research project and not appropriate for EEM. If it's not research, then a graduate student shouldn't get a degree for doing it. Therefore graduate students are not an answer to cost effectiveness.

C. SFG is a general response to environmental conditions, not specific to pulp mill effluent. Do we want this for EEM or specific responses?

C. Shouldn't we be putting together a suite of endpoints rather than discussing which is best?

9. Development and Testing of a Mesocosm Technology for Environmental Effects Monitoring (EEM) Methods Application

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Mesocosms refer to field-based artificial stream systems which can be used in aquatic Environmental Effects Monitoring (EEM) programs as a tool to assess the effects of industrial and municipal effluents on aquatic biota. Typically, the effects of effluents on aquatic receiving environments are assessed through field monitoring programs or effluent sublethal toxicity tests. In field monitoring programs, water chemistry, benthic invertebrates and/or fish populations are sampled from sites exposed to effluents and these results are compared to samples collected from reference sites. In some cases results from field monitoring programs are inconclusive due to the high natural variability of sampled populations, inadequate reference sites for comparison to exposed sites, lack of understanding of the level of exposure, the presence of confounding effluent discharges, and/or low sample sizes and inadequate statistical replication (pseudo replication). Sublethal toxicity tests are laboratory testing procedures where the effects of an effluent are assessed on a single species under controlled laboratory conditions. The significance of sublethal toxicity test results to the natural environment can be difficult to determine as these tests lack realism, and in many cases use test species which are not endemic to the area being studied. Artificial streams can provide an integral link between field monitoring programs and sublethal toxicity tests by simulating the natural environment while allowing for variable control (i.e., sample sizes, sex ratios, effluent exposure), experimental manipulation, statistical replication, and most importantly, providing cause-and-effect results to determine the effects of specific effluents on aquatic systems. Artificial streams are not intended to replace field monitoring programs or sublethal toxicity tests but are intended to be used as an assessment tool in conjunction with these other EEM methods to strengthen the testability of hypotheses (weight-of-evidence approach) and allow for site-specificity in EEM program design.

An mesocosm system was developed by the National Hydrology Research Institute (NHRI) of Environment Canada in Saskatoon, Saskatchewan to determine the effects of pulp mill effluents on algal and benthic invertebrate communities in freshwater rivers in Prince George, British Columbia and Hinton, Alberta (Culp *et al.* 1996, Culp and Podemski 1995). This system consisted of 16 circular tanks (diameter 1 m) on two trailers. These trailers were set up beside a reference site and ambient water was pumped into a head tank, through a distribution manifold, and then delivered to each stream at a controlled rate (2 L/min). Water depth within each stream was controlled at 25 cm by a central overflow standpipe. This depth and flow rate resulted in a hydraulic residence time of 2 hours in each stream. Effluent was delivered independently and continuously to individual streams using peristaltic pumps. Current velocity within each stream was controlled using a belt-driven propeller system. Each stream was then "seeded" with natural substrates, algae and benthic invertebrates endemic to the river being studied. Experiments were conducted for approximately 4 to 6 weeks and biological end-points measured to determine the effects of the effluent on algal growth, composition, and benthic invertebrate biomass, density, and community structure. In the Fraser River studies three treatments were created; control (no effluent), 1% effluent ("zone-of-influence"), and 3% effluent. Results showed that, at these effluent concentrations, treated bleached kraft pulp mill effluent increased algal biomass (enrichment effect) but did not affect algal community composition (Culp *et al.* 1996). In the Athabasca River studies three treatments were created; control, 1% effluent, and 1% nitrogen + phosphorus (at levels found within 1% effluent) (Culp *et al.* 1996). The objective of this latter experiment was to decouple the effects of nutrients and contaminants within treated bleached kraft pulp mill effluent. Results showed that the effects of the effluent were restricted to nutrient enrichment and caused an increase in algal biomass, benthic invertebrate growth and total benthic invertebrate abundance relative to the control streams.

Mesocosm systems are a suitable monitoring alternative which should be considered for future cycles of the pulp and paper EEM program. The results of the first cycle of the EEM program were submitted by pulp and/or paper mills on April 1, 1996. A review of the first cycle results by Environment Canada and the Department of Fisheries and Oceans, revealed that several program inadequacies existed particularly regarding site specific flexibility in program design, control over environmental variables being monitored and implementation of the adult fish survey (AFS) in marine and estuarine receiving environments. The primary problems with the AFS were related to the migratory habits of the selected sentinel species, the complexity of effluent plume dispersion and lack of suitable tracers to confirm exposure.

The mesocosm system developed by NHRI has effectively demonstrated the utility of using mesocosms for monitoring the effects of effluents on algal and benthic invertebrate communities in freshwater receiving environments. This system has the potential to assess the effects of pulp mill effluents on fish, particularly forage species. This system could also be re-designed for use in marine and estuarine environments. To explore these applications further, Washburn & Gillis Associates Ltd. and NHRI have entered into a collaborative pilot project to re-design the existing artificial stream system and to test and evaluate its effectiveness (technical and cost) as a tool for subsequent cycles of the EEM program.

A phased approach has been designed to meet the project objective. This approach will consist of four phases: 1) stream system design and client requirement assessment, 2) stream system construction, 3) system testing (physical and field), and 4) system evaluation. Results from the first phase will form the basis to plan subsequent phases. Proposals for funding will also follow this phased approach. Funding to complete Phase 1 of the project has been obtained by Washburn & Gillis Associates Ltd. and the National Research Council's Industry Research Assistance Program (IRAP). Phase 1 will be completed by the end of February. Proposals are currently being prepared to obtain funding for subsequent phases of the project. It is anticipated that Phase 2 (construction) will commence in March or early April, Phase 3 in June or July, and Phase 4 in September. Full evaluation of the effectiveness of this mesocosm technology as a tool for EEM programs should be completed by December 1997. A successful evaluation of this mesocosm technology requires the cooperation and participation of industry, Environment Canada and the Department of Fisheries and Oceans to ensure their respective concerns and questions are addressed in the pilot project.

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Questions, Answers and Comments

Q. You need input from BC mills for a west coast perspective. Have you looked into this?
A. Not yet.

Q. There may be problems with suspended particulates. Are your flows high enough to handle levels seen in nature?
A. In Fraser and Athabasca River tests, yes. There was some settling out of sediments in pipes but turbidity etc. in our tanks was still comparable to what was seen in the river. This may depend on particle size with large particles, like sand, being a problem. This will need to be looked at, especially given the importance of particulates in pulp mill effluents.

C. Levels as high as 3000 mg/l suspended material have been recorded in BC rivers so this certainly is an issue.

Q. What test duration will you use and what endpoints will you look at in your 1997 pilot study?
A. Four to 6 weeks duration. Endpoints will include MFO, tissue chemistry, reproductive hormones, liver and gonad weight and growth.

Q. What would be the cost of this approach?
A. The initial capital expenditure for the mesocosm system would be made by a consultant wanting to offer this technology to their clients. The cost of conducting an AFS using this approach is estimated to be approximately \$25K-\$30K depending upon the endpoint measured. Cost effectiveness will be an evaluation criterion.

Q. Is this ready for Cycle Two?
A. This approach has been used for bacteria, invertebrates and algae in freshwater systems but not for fish and not in estuarine and marine environments. The technique is ready for a pilot project in the summer of 1997 and then application in Cycle Two.

Q. So the mesocosm approach follows field surveys, asking more refined questions, rather than replaces the field survey?
A. Yes, no one is suggesting that mesocosms replace field or lab studies, they complement them. Where field studies cannot be carried out, mesocosms provide an alternative. Where field studies can be carried out, you might look at a tiered testing approach with field monitoring being conducted first and mesocosm work afterwards to test more specific hypotheses on effluent efffects.

10. Caged Exposures, Life Cycle and Behavioral Studies with Fish...Examples and Comments.

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The use of cages to assess the effects of sediment exposure on Arctic grayling (*Thymallus arcticus*), and pulp mill effluent on Pacific salmon are presented to exemplify the use of such techniques in impact assessments. Similarly the results of a life-cycle study with pink salmon (*Oncorhynchus gorbuscha*) exposed to the water soluble fraction of crude oil are discussed. The use of a "mesocosm" approach, allied to specific assays is commented on in relation to the exposure of juvenile chinook salmon (*O. tshawytscha*) to an anti-sapstain chemical and pulp mill effluent. These comments are offered in relation to potential use within the EEM program, and accordingly positive and negative aspects of the techniques will be presented.

Arctic grayling held within 50L net cages in placer gold-mined (sediment-laden) and unmined streams in the Yukon survived for 96h in the latter situation: mortalities occurred in the former. However, an increase in gill parasites and serum glucose levels in the caged fish populations in both locations, relative to non-captive fish, indicated that the caged fish were stressed by the confinement. Other studies, that assessed the feeding of Arctic grayling in streams that were subjected to placer mining found that confinement reduced the feeding success of the fish relative to those fish captured from the respective creeks (feeding success was impaired in the placer mined stream; a function of visual impairment and prey availability). The same cages were used to assess the survival of juvenile Pacific salmon adjacent to the effluent discharge from coastal pulp mills in B.C. In these experiments the survival of fish was related to exposure to the mill's effluent and related variables. Confinement of juvenile salmon did not affect short-term (4-12d) survival at reference sites. These cages have value for the short-term holding of juvenile fish in low velocity waters.

Cages which enclosed a 6 x 0.5 x 0.5m volume of surface waters were employed adjacent to effluent discharges from pulp mills and at reference areas in coastal locations. The cages were used to hold juvenile salmon and to examine their volitional vertical distribution. Distribution shifts occurred in relation to effluent and variation in natural factors. The technique proved valuable in revealing behavioral changes, and the use (lack of long-term avoidance) of toxic waters by juvenile salmon despite the potential of such conditions to jeopardize survival. Behavioral adaptive responses to occupy surface waters were considered to override short-term avoidance responses to toxic conditions in these waters, resulting in the death of some fish. Laboratory studies with a Water Column Simulator confirmed the behavioral traits of juvenile Pacific salmon to occupy surface waters and their response to environmental changes. The cages have been validated as a technique to investigate the vertical distribution/behavior of juvenile fish within 6m of the water surface. They are particularly useful in waters that are vertically stratified.

The effect of oil on the survival of juvenile pink salmon to adult was assessed by exposing the juveniles to the water soluble fraction of crude oil for 10d prior to their release to the Pacific Ocean. The subsequent return of fish that had received a low (50ppb), or high (350ppb) dose of the water soluble fraction, or no aromatic hydrocarbons was determined from catches by the fisheries and in their natal stream. A total of approximately 270,000 fish were released (90,000 per year, 30,000 per treatment) with half-length coded wire tags. Despite evidence of some dose-dependent histological changes, and behavioral responses, there was no significant difference among treatment groups in the numbers of fish that were captured as adults. Life-cycle studies of this type rely on population success as an end point thereby integrating the effects of "natural" stressors in addition to those of the chosen contaminant.

The exposure of juvenile salmon to pulp mill effluent at an on-site laboratory provided a controlled situation relative to an in-situ field investigation. Assessments of effects in relation to effluent concentration were quantified. An investigation of the potential for juvenile salmon to be exposed to the effluent was undertaken through an in-situ mark-recovery program. A predator-prey experiment revealed how exposure to sublethal concentrations of the pulp mill effluent rendered the exposed salmon more susceptible to predation. A similar susceptibility response has been determined after exposing juvenile salmon to sublethal concentrations of a lumber anti-sapstain chemical (TCMTB). In complex effluent receiving waters the isolation of effects from one source is fraught with problems. By subjecting test organisms to relevant exposures to contaminants (concentration and duration) at an on-site facility using, for example, fresh effluent from one source, some of the complicating variables in the field location are removed. The subsequent choice of tests to indicate effects should be tiered to facilitate correlations that will permit the consequences to organism survival, health and performance to be assessed. The predator-prey assay we utilized is one such test that has direct relevance to survival.

Questions, Answers and Comments

Q. In your life cycle study with pink salmon exposed to oil in early life, do you think that other sources of mortality masked any effects of the oil on return rates from the ocean?
A. Yes. We saw effects of oil exposure on the juvenile salmon but not the number of adults returning to spawn. You must remember that fishing pressure is enormous and non-selective, unlike natural factors such as predation.

Q. How would you use this life cycle approach for the pulp mill EEM?
A. We still see salmon as a good indicator species for the west coast because of their importance to the commercial, recreational and aboriginal fisheries. One of the difficulties with such work is

the requirement to use large numbers of juveniles, and hold them in conditions that do not stress them unduly. Through the recovery of marked fish from the fisheries and natal stream assessments would be made of the relative survival to adult for each treatment group used. It is imperative that the study design mimicks, as closely as possible, the conditions experienced in nature.

11. The Mummichog, *Fundulus heteroclitus*, as a Sentinel Species for Pulp and Paper Mill EEM Surveys in the Atlantic Coastal Environment

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The Adult Fish Survey component of the EEM program for Canadian pulp and paper mills encountered two major problems in Atlantic Canada: availability and mobility of species chosen as sentinels (Parker 1997). Of 15 pulp and paper mills which discharged effluent into estuaries or the coastal environment, not one met its fish requirements in Cycle One (i.e., 20 males and females of each of 2 species in both nearfield and reference sites). Furthermore, the species selected as sentinels by these mills tended to be migratory. The most common sentinel species chosen were the winter flounder (*Pleuronectes americanus*) and the Atlantic tomcod (*Microgadus tomcod*), selected by eight and seven mills, respectively. Within the southern Gulf of St. Lawrence, winter flounder move tens to hundreds of kilometers between nearshore, summer feeding grounds and overwintering grounds in estuaries (Hanson and Courtenay 1996). Similarly, Atlantic tomcod make seasonal migrations of over 80 km between nearshore, summer feeding grounds and winter spawning sites in freshwater (Klauda et al. 1988; Courtenay et al. 1995). The extent of movement within seasons is less well known but is on the order of kilometers for winter flounder (D. Willis, Bedford Institute of Oceanography, pers. comm.) and is probably similar for tomcod. Furthermore, large fish like tomcod and flounder do not need to migrate actively to be displaced within estuaries. The fact that they live in the deep channel of the estuary means that they are exposed to the full extent of tidal movements of water. Tides can move water in the Miramichi Estuary, for example, 10 km in 6 hours. To put these movements in perspective, the reference stations for the Fish Survey carried out by a paper mill on the Miramichi Estuary were approximately 5 km upstream and downstream from the mill. A tomcod at the downstream reference station might easily be carried to the mill in a single tide. Species such as these are good biosentinels for the health of whole estuaries or sections of the coastal environment but not for specific point sources, such as pulp mill effluents, which may be diluted to less than 1% within 50 m of the discharge point. For the detection of impacts occurring on such a small geographic scale, highly sedentary species are required.

The mummichog, *Fundulus heteroclitus*, is both easy to catch in large numbers, and sedentary. Generally less than 10 cm in length, the mummichog is found in estuaries and tide pools all along the east coast of North America between southern Newfoundland and northern Florida. Within this range it is extremely abundant and easy to catch. It is a very plastic species, tolerating a wide range of salinities, temperatures, and oxygen concentrations. These characteristics have led to its wide use as fishing bait and for laboratory studies including many toxicological endpoints (e.g., Prince and Cooper 1995a,b). Spawning occurs in shallow waters between April and August and fish overwinter in holes of tidal streams or in the mud. A study of mummichogs in one stream showed that they moved less than 2 km upstream to overwinter in lower salinity water (Fritz et al. 1975). Mummichogs can be aged by otoliths and live to a maximum of 5 years. They eat sediment based prey including small crustaceans, polychaetes and insects, and are in turn preyed upon by fish such as striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*) and birds such as herons (*Ardea herodius*) and kingfishers (*Megaceryle alcyon*). Given its numbers, the mummichog is an important part of the Atlantic coastal food web. Most importantly though, it is extremely sedentary during the spawning season, typically moving less than 36 meters along the intertidal shoreline of a creek or river (Lotrich 1975). Because of this sedentary nature, mummichogs are good biosentinels of small scale environmental disturbances. Iannuzzi and Bonnevie (1994) reported an extremely strong correlation between PCB concentrations in sediments and in mummichogs along a 1 km gradient from a PCB discharge site in a small river flowing into Chesapeake Bay.

Between 1992 and 1996, one of us (Couillard) studied the effects of bleached kraft mill effluent on mummichogs living in the Miramichi Estuary. Three populations, living 4 km, 21 km and 39 km respectively downriver from a pulp and paper mill were examined during the breeding season. To control for differences other than BKME along this gradient (e.g., salinity), two populations living in the neighbouring Bouctouche Estuary were also examined. Exposure to contaminants was measured both by direct chemical analyses and also through mixed function oxidase levels (MFO). Health was measured through: fin erosion, radiographic vertebral lesions, parasites, steroids in blood and production in gonads, histology, various blood measures including: hematocrit, concentrations of total proteins, glucose, bilirubin and sorbitol dehydrogenase activity, immunotoxicity measured by macrophage activity from the posterior kidney, genetic variability assessed through electrophoretic examination of hepatic enzymes, and developmental homeostasis assessed through meristic and morphometric counts. In addition to measurement of steroids, reproduction was examined through: temporal variation in gonado-somatic index (GSI), percentage mature females, fecundity of females, egg size, and embryonic malformations.

Mummichogs living nearest the mill showed a 2.5 fold EROD induction over populations living downriver or in the Bouctouche Estuary. By comparison, previous investigations with the more mobile tomcod and flounder had detected no such gradient in MFO activity throughout Miramichi Estuary (Addison et al. 1991; Courtenay et al. 1995). Preliminary chemical analyses of mummichogs revealed levels of PCBs and organochlorine pesticides up to ten times higher at the mill site compared to downstream, dioxins and chlorophenols typical of BKME were 30 - 40 times higher, and pentachlorophenol was 200 times higher. Fin erosion and vertebral deformities, effects that have been previously associated with exposure to BKME (but are not specific), were seen in the mill population (Couillard and Légaré 1994) as were effects on parasite load, hematocrit, blood proteins and glucose. The mill population also showed differences from other populations in number of fin rays and incidence of bilateral asymmetry in pelvic fin ray count. Reproduction was also affected by mill effluent (Leblanc et al. 1997). Gonadal maturation was delayed and egg size was reduced at the mill site. However, once reproduction was underway at the mill site, GSI and fecundity of mature females were greater than elsewhere such that despite delayed gonadal maturation, there was an overall increase in reproductive effort in mummichogs exposed to BKME. Interestingly, this did not result in a decrease in condition factor relative to other sites, suggesting that nutrients were abundant at the mill site.

In summary, the mummichog appears to be an excellent biosentinel species for the Fish Survey component of the EEM program for pulp and paper mills. It is abundant, sedentary and appears to show effects of exposure to BKME on health and reproduction. It should be noted however that even the relatively ubiquitous mummichog will not always be found within the 1% effluent plume of estuarine and coastal mills. In Miramichi Estuary, the nearest population to the mill is located at the nearest marsh, 3-4 km downriver from the 1% effluent plume. It is therefore impossible to establish cause-effect relationships with BKME because of the presence of other sources of contamination close to the mill such as sewage and historic deposits from a wood preservation plant (Courtenay et al. 1995). Where mummichogs are not available, other small, forage fish such as sticklebacks may serve as sentinels. Sticklebacks (Gasterosteidae) have the advantages of being circumpolar in distribution and relatively well studied. However, the four species of sticklebacks found in Maritime estuaries are smaller than mummichogs and are probably more mobile. In fact, we know of no other Atlantic, estuarine or coastal, fish species as well suited to the pulp mill Fish Survey as the mummichog. A recent consideration of fish species suited to field monitoring of exposure and effects of environmental contaminants also concluded that the mummichog was perhaps the best sentinel species (Myers 1996). Finally, in choosing sentinel species and sampling strategies, a thorough knowledge of the species, and population, biology is essential (e.g., Leblanc and Couillard 1995). For example, preliminary work with mummichogs in Miramichi Estuary indicated that there was a higher rate of malformations in embryos from females living near the mill than elsewhere (Couillard et al. 1995). Subsequent work revealed that malformations were more common at certain points in the spawning cycle than at others. When differences between populations in the timing of the spawning were taken into consideration, very little difference was seen in the rate of embryonic malformation. This example highlights the danger of comparing fish between sites in a single snapshot versus examining the entire reproductive cycle.

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<u>Questions, Answers and Comments</u>

Q. Were there any differences in the age structures of mummichogs at different sites?
A. I'm not sure. However, ages were recorded and taken into consideration in other analyses.

Q. How close was the "mill population" of mummichogs to the actual effluent outfall from Miramichi Pulp and Paper Inc.'s bleached kraft mill?
A. The "mill population" was located at Strawberry Marsh, approximately 4 km downstream from the outfall. It is interesting to note that, at least according to plume delineations mapped by Beak Consultants in the early 1990s, the 1% plume is very small on a falling tide and never comes anywhere near Strawberry Marsh. Nevertheless, effects which are consistent with exposure to BKME are seen in mummichogs at that site. That said, it must also be noted that Strawberry Marsh is the site of a former wood treatment operation (Domtar) and despite a comprehensive cleanup, conceivably some contaminants remain. Sewage may also be found in the area. Future work at this site will be complicated by the recent construction of a new bridge and a new sewage treatment plant at Strawberry Marsh.

12. Use of Forage Fish as Sentinel Species for EEM Monitoring

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Small fish species were evaluated for responses to pulp mill effluent, and these studies demonstrated that these species were showing responses to pulp mill effluent. We have used spoonhead sculpin (*Cottus ricei*), lake chub (*Couesius plumbeus*), and trout-perch (*Percopsis omiscomaycus*) (Gibbons, W.N. 1997. Suitability of small fish species for monitoring the effects of pulp mill effluent on fish populations. Ph.D. Thesis, University of Waterloo), and peamouth chub (*Mylocheilus caurinus*), and have new studies which will attempt to use log perch (*Percina caprodes*) and johnny darters (*Etheostoma nigro*) for comparison of responses with larger species.

Spoonhead sculpin exposed to kraft mill effluent in the Athabasca River demonstrated major differences in growth rate within 0.5 km of a pulp mill discharge, and significant differences were seen between exposed and unexposed sides of the river. Spoonhead sculpin demonstrated an overall increase in body and organ size, reproductive commitment, MFO induction and steroid hormone production relative to unexposed fish. Sculpin also showed a graded response with distance, and MFO induction persisted for at least 50 km downstream.

Further studies were conducted on the Moose River system to compare responses between small fish species and larger species collected simultaneously. Effort consisted of a comparison of trout-perch and white sucker whole organism and physiological responses downstream of a TMP mill. Initial evaluation of the data demonstrated differences in the responses of the two species. Trout-perch showed decreased length, weight, size-at-age and gonad size, while white sucker demonstrated increased weight, length, condition factor and size-at-age. A fish interpretation framework was modified and use of the information on age structure, energy utilization and energy storage suggested that the trout-perch and white sucker were responding to similar stressors.

It is obvious that forage fish species offer information suitable for effects monitoring.

Advantages

1. Small species represent the forage base and are more numerous than larger species.
2. Capture efficiency can be very good; it is possible to get all fish at a site in a short time period. These fish (most species) also survive capture better, especially since many of the capture techniques are less harmful (ie. Seining, electroshock).
3. Assumption is that mobility is much less than larger species.
4. There are not subjected to fishing pressure.

Concerns

1. Virtually nothing is known about the biology of most of these species (spawning time, fecundity, reproductive life history, growth rates, ages, etc.); background data on basic biology is needed for interpretation.
2. Samples size requirements are larger than estimated for large species, and due to the short life span, immature individuals are required to look at growth rates, etc. We have been trying to get 35 males and females, as well as immature fish.
3. Reference sites become more difficult to standardize, and more care is needed. There are significant differences between rivers.
4. The responses can be different than those seen with larger species due to a variety of factors including different habitat utilization, shorter life span, different dietary requirements.
5. Reproductive outputs can be difficult to estimate in species which are fractional or multiple spawners.

Questions, Answers and Comments

Q. With regard to the lack of adults trout perch found downstream (i.e., fish downstream of effluent were smaller and younger than fish upstream), has anyone looked at predation?
A. We're trying to secure funding to follow up with community responses such as food web structure, energy flows etc. Also, in recent years mills have been modernized so we would like to see what effects changes have had on fish in the receiving environment.

Q. Trout perch live up to 5 years in the Athabasca River. Also, adults and juveniles occupy different habitats, could that explain the difference in age structure between your upstream and downstream sites?
A. We are aware of these facts, but we sampled as thoroughly as we could in both sites and caught adults upstream so we do not think that the lack of adults downstream was a sampling artifact.

C. We should look at similar endpoints in caged bivalves. Using several approaches together would make a much more convincing case than looking only at 1 species.
A. Agreed, a weight of evidence approach would be helpful.

Q. Spot-tail shiners have been looked at in the St. Lawrence; do you think they'd work in EEMs for the Great Lakes?
A. Any species should work. The limiting factor is lack of knowledge of the biology of the fish.

C. The problem is in finding good reference sites and sampling times. Can we really expect consultants to do this?

C. Researchers can optimize collections by better using whatever human resources are available and especially people with knowledge and familiarity with local fish populations. Remember that consultants generally lack such specific knowledge.

C. One problem for consultants catching forage fish is not necessarily lack of knowledge about fish; there is a learning curve to catching new fish at a new site. A good source for advice is anyone who has collected for museums. They have all kinds of different methods and knowledge.

13. Health Assessment of Finfish collected from Estuaries Receiving Pulp Mill Effluent

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Finfish collected from estuaries in the southern Gulf of St. Lawrence which receive pulp mill effluent were examined to detect fish health bio-markers that could be used to create an index for evaluating the impact of pulp and paper mill effluents on finfish populations. Sampling was conducted in five estuaries located in Nova Scotia or New Brunswick, three of which receive pulp mill effluent (Restigouche River, N.B., Miramichi River, N.B. and Pictou Harbour, N.S.) and the remaining two sites were designated as reference sites (Margaree River N.S. and Kouchibouguac River, N.B.). Fish were collected for examination in both the spring and fall of 1996 and consisted predominately of Atlantic tomcod (*Microgadus tomcod*) and flounder (*Pleuronectes americanus* and *P. putnami*).

All fish were examined for gross abnormalities. A wide variety of media (tryptone soya agar (TSA), blood agar (BA), marine agar, cytophaga agar (CA), Shieh's agar (SH) and *Flexibacter marinus* media, as well as TSA, BA , CA and SH all supplemented to 2% salt content) were used to detect the presence of bacterial agents in major organs and skin lesions. Tissue samples (kidney, spleen, gill and pyloric caeca) from all fish were assayed to detect viral agents using the Canadian Fish Health Protection Regulations (FHPR) virus methodology and a variety of cell lines (chinook salmon embryo, grunt fish and epitheloma papulosum cyprini). All abnormalities detected were forwarded to the Atlantic Veterinary College (AVC) in P.E.I. for a histological examination. During the fall sampling only, liver and gill samples collected from 25 Atlantic tomcod from each site were examined for any abnormal pathology and the presence (i.e., intensity and prevalence) of parasites by AVC.

Gross deformities and other abnormalities were detected in low frequency from both the estuaries receiving pulp mill effluent and the references sites. There were no viruses identified using culture techniques. Although a variety of bacterial species were isolated, with the exception of the pathogen atypical *Aeromonas salmonicida*, isolated from Atlantic tomcod from Miramichi River (spring sampling) and Pictou Harbour (fall sampling), all isolates were the typical, ubiquitous bacteria expected from estuarine/brackish water environments. A variety of parasites were identified either as encysted intermediate life stages or as adults (final hosts) and none were considered as abnormal for the fish species examined.

Unfortunately there were no specific bio-markers of exposure to pulp mill effluent identified with which to formulate a Fish Health Assessment Index. Potential disease agents were identified in both reference and pulp mill sites. Statistical analysis is presently underway to determine whether the incidence of parasites and other pathological conditions differs between estuaries with pulp mills and without. If differences in the expression of disease are found between estuaries, a potentially useful direction for future research will be an examination of the role of immunosuppression in finfish populations exposed to pulp mill effluent.

Questions, Answers and Comments

Q. Did you always get your sample size of 60 fish per site?
A. No, but we did for tomcod.

Q. Did you age fish or a representative sample?
A. Yes for tomcod, which were all 2-4 years old, but other species were not aged.

Q. Are there any data in the literature relating effects that you found to pulp mill effluent?
A. Yes, but most studies were laboratory exposures, not field studies like ours.

Q. Were the fish you sampled actually exposed to pulp mill effluent plumes?
A. Tomcod and winter flounder move around estuaries and the coastal environment a lot and present an integrated signal of contamination in that scale of area. Mummichogs, for example, are less mobile and may better reflect the effects of one particular point source.

Q. So you don't have any direct exposure data at all for your fish?
A. No. But we know from others'work that with a high enough dose of pulp mill effluent you can get some of these effects.

C. You can get such effects with a high enough dose of anything. You would need concurrent lab and field studies to tie health effects of pulp mill effluent.

Q. What do you recommend we do next as far as using Health Assessments in EEM?
A. Start with bringing fish into the lab from the wild and look at whether the immune system shows any signs of suppression. Do fish exposed to BKME show a reduced ability to mount an immune response in tests with a variety of agents?

Q. There is extra stress on fish during spawning which may result in a greater expression of disease, e.g., frequency of lumps and bumps. Did you sample during spawning?
A. No.

Q. With the data you collected, can you do a Health Index on the fish?
A. We will be looking at this on larger sample sizes but we are not sure at this point that we have any pathologies that are the result of exposure to BKME. An overall health index may not be the most helpful measure for pulp mill monitoring.

14. Use of a Fish Health Assessment Index Approach for Evaluating Impacts of Multiple Stressors - A West Coast Perspective.

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A number of fish health and chemical contamination problems have been documented in mountain whitefish, *Prosopium williamsoni*, caught in the Columbia River between the Hugh Keenleyside Dam and the International Border, in British Columbia. Abnormalities, such as external lesions, discoloration, and thin fish were first reported by field a field biologist working on the river in October 1990. A consumption advisory was also in effect for this species from June 1990 to March 1995 due to dioxin contamination. A study conducted by DFO in January 1991 (Boyle *et al.* 1992) suggested that the health of mountain whitefish may be impaired.

The three main stressors on this stretch of the Columbia River are: the bleached kraft pulp mill located near Castlegar, B.C.; the lead/zinc smelter and fertilizer plant at Trail, B.C.; and numerous dams and run-of-the-river reservoirs on the Columbia River system. The Department of Fisheries and Oceans (DFO) conducted a monitoring program in this area to document the recovery of the mountain whitefish following the expansion and upgrading of the pulp mill, and the ongoing process changes at the smelter and fertilizer complex. Pulp mill improvements included 100% chlorine dioxide substitution for chlorine in the bleaching process and an air-activated sludge secondary treatment system. The smelter implemented several projects, which significantly reduced the loading of mercury and other metals to the Columbia River. B.C. Hydro also made changes in relation to facility operation to reduce dissolved gas supersaturation in the Columbia River downstream of the Hugh Keenleyside Dam.

The study consisted of three sampling events, in July of 1992, 1994, and 1996. Adult mountain whitefish between 25 to 40 cm in length were collected from two sites on the Columbia River, at Genelle, approximately 10 km downstream of the pulp mill, and at Beaver Creek, about 12 km downstream of the smelter, and from a reference site on the Slocan River. The total number of fish analyzed for wet weight, fork length, age, and disease survey (i.e., gross external and internal examination, bacteriology, and histopathological examination of gill, liver, spleen, kidney and pyloric caeca/hindgut) was approximately 60 fish per site. A subset of fish at each site were analyzed for dibenzodioxins and dibenzofurans (PCDD/PCDF) and polychlorinated biphenyls (PCBs) in muscle tissue (n=10), and mixed function oxidase (MFO) activities (n=15). Another subset of fish was analyzed for metals in muscle tissue, and metallothionein (n=15). All fish analyzed for chemical contamination or biochemical indicators were also analyzed for stomach contents. A separate set of 10 fish from each sampling location were analysed for parasitology, age, and size.

A cumulative disease severity (CDS) coding system was developed as a method of interpreting the fish health significance of the disease survey results, and quantifying the cumulative effect of all abnormalities present in one fish. The coding system subjectively rates the various types of abnormalities as to their potential impact on fish health. An abnormality such as a parasite cyst was rated light (1), reflecting the impact it is likely to have on fish health. The impact of causative organisms such as myxobacteria was considered to be moderate (2), while the finding of mycobacteria is considered more damaging, and was rated as severe (3). The normal condition as well as any artifacts of sampling or slide preparation were given a rating of (0). The total numerical value of all abnormalities found in one fish at a given location was used to indicate the CDS or health status of that fish. This rating system is based on the quantitative autopsy-based system developed by Adams *et al.* (1993).

Results from the July 1992 sampling phase of this study are documented in Nener *et al.* 1995. In 1992 fish sampled below the pulp mill at the Genelle reach exhibited consistently higher frequencies of abnormalities and significantly greater disease severity than fish sampled from the Beaver Creek reach of the Columbia River, located downstream of the smelter, or the reference site on the Slocan River. Fish sampled from Genelle were also significantly older than fish from Beaver Creek or the Slocan River, and a significant relationship between age and CDS was found at both Genelle and Beaver Creek. The confounding effects of age made it difficult to determine whether the greater frequencies of abnormalities and disease severity in fish from Genelle was related to natural ageing or other environmental conditions. PCDD, PCDF and PCB concentrations were also higher at the two Columbia River sites than at the reference site in 1992. Disease incidence and severity could not be correlated with organic contaminant concentrations. Fish sampled below the pulp mill in 1992 had significantly higher muscle concentrations of mercury than fish from either Beaver Creek or the Slocan River, but all values were below the federal human health consumption guideline.

Results of the 1994 sampling program are documented in Antcliffe *et al.* 1997. In 1994 male and female mountain whitefish sampled from the Genelle and Beaver Creek reaches of the Columbia River had significantly higher condition factors, size-at-age, and gonadosomatic index (GSI) than the similarly-aged fish from the reference site. Liverosomatic index (LSI) was higher in female fish sampled at Genelle, downstream of the pulp mill. The presence of loose eggs in the body cavity of 29% of fish from Beaver Creek and 7% of fish from Genelle may indicate nonspawning with resorption of mature oocytes, possibly in response to stress. PCDDs/PCDFs in muscle tissue declined substantially since the 1992 study, and the consumption advisory for this species was lifted based on these data.

In 1994 mountain whitefish sampled from the Genelle and Beaver Creek reaches of the Columbia River had consistently higher prevalence and severity of abnormalities when compared to the reference site on the Slocan River. Mean CDS was also significantly higher at Genelle than at Beaver Creek or the Slocan River reference site. However, abnormalities commonly associated with exposure to pulp mill effluent or metal contamination were observed at low levels, and several abnormalities appeared to be stress-related. CDS was also not correlated with organic contaminants or MFO activity. Results suggest that the CDS rating likely reflects the cumulative effect of all stressors on the system, including effluent discharge from the pulp mill and smelter, and flow regulation.

Results from the final 1996 study are preliminary, however, they appear to be consistent with those found in 1992 and 1994. Recommendations for further study include assessment of effects of various stressors (e.g., pulp mill effluent) on immunosuppression.

The fish health assessment approach used in this study provided a useful screening tool for evaluating the cumulative effect of all stressors on the health of Columbia River mountain whitefish. In general, application of fish health assessment should be based on site-specific needs. Recommendations for conducting fish health assessment include:

- Evaluation of approaches to determine which health-related variables are worth examining;
- Development of a protocol for field methods, including consistent terminology and practitioner training;
- Examination of summary indices and key abnormalities, as single number indices can mask spatial and temporal changes in individual tissues or organs;
- Tiered assessments, starting with gross external and internal examination followed by more detailed histology, bacteriology, and parasitology, if required;
- Similar age distributions among sampling locations to minimize confounding effects of age (older fish tend to have a higher natural incidence of abnormalities and disease);
- Assessment of natural background rates of disease and abnormalities, including natural variability, in order to interpret fish health assessment results;
- Definition of biologically important effects sizes for health-related variables.

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Questions, Answers and Comments

C. This study is a good example of a basin approach.

C. Although you can make an summary Index from these data, the real value is in comparing individual effects across sites.

C. We need to standardize our field crews, more training is needed to cut down on subjectivity.

Q. Were sites compared statistically?
A. Yes, means and standard deviations were calculated for each site. If confounding factors were significant we factored them into our model.

Q. Did you do an AFS-type survey too with the life history variables you collected, and did you leave enough time to see changes? Older fish may have tumours from long ago and improved effluent would not have made them go away.
A. The life history data are there and we're looking at them. With regard to timing of sampling, some workers feel that we should resurvey every year but I think that's too frequent.

Q. Has anyone looked at health indices in crabs?
A. Not to my knowledge.

C. In fact a health index has been worked out for blue crabs.

15. Invertebrate Pathology Alternatives for Monitoring Paper Industry Effects on Environmental Health

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Rationale for selecting invertebrate species to monitor environmental effects of pulp and paper mill ellfuent

Environmentally induced pathology in aquatic invertebrates is relatively undocumented (Cheng 1993) compared with that in aquatic vertebrates (Overstreet 1993; Harshbarger *et al.* 1993). Invertebrates lower in the food chain would be expected, however, to reflect to a greater or lesser degree the conditions of their environment. In addition, sedentary species would be expected to be especially sensitive to environmental changes which exceed those within the genetically-selected tolerance range of the organism. Species with shorter life-spans would also reflect relatively immediate changes in their microhabitats. Environmentally induced pathology can fall into several different categories, e.g., nutritional deficiencies (destruction of optimum or adequate food sources), genetically induced abnormalities (alteration of reproductive stability), environmental stresses (temperature, salinity, turbidity, oxygen deficiency, hydrogen sulphide excess, anthropogenic or biotic toxicity (e.g., pollutant chemicals or algal biotoxins), as well as others which currently defy identification (classic "unknown causes"). Sedentary and short-lived, lower trophic level, species would show a greater overlap with the environment than longer lived, motile, species which can evade sub-optimal environmental influences. Environmentally influenced pathogenicity can thus be seen to have a greater impact on the former group of organisms. Consequently environmental pollutants have both direct and indirect effects on health. Differentiating between the two spheres of influence is complicated and, probably, unnecessary since environmental parameters are not as "easily" modified as pollutants.

The present preliminary survey was designed to scan readily available invertebrates from five estuarine systems in the southern Gulf of St. Lawrence for any pathological symptoms which could be attributed to pulp and paper industrial effluent. Three of the five estuaries contained effluent input. The numbers and frequency of sampling were limited but considered appropriate in an effort to detect obvious (as opposed to subtle) alternatives to the previous adult fish survey in species which hitherto have not been used in such investigations.

Materials and Methods

In May, 1996, five estuaries (Restigouche, Miramichi, Kouchibouguac (New Brunswick), Pictou and Margaree (Nova Scotia)).were sampled for both invertebrates as well as other multidisciplinary samples. Kouchibouguac and Margaree estuaries have no pulp or paper mill industries on them and were used as negative controls.

The invertebrate species collected initially included sand shrimp (*Crangon septemspinosa*), rock crab (*Cancer irroratus*) and blue mussels (*Mytilus edulis*). Two samples were collected, one in May and the second in September, 1996. Rock crab were dropped from the survey following May collections due to their inconsistent availability in all but one estuary. The remaining two species represented sessile primary feeders (mussels) and relatively non-migratory (compared with estuarine finfish sampled previously), zooplankton feeders (sand shrimp). Sample numbers consisted of 30 individuals per species per estuary (with the exception of shrimp collected in May from the Miramichi: n = 21). See Table 15.1.

	Shrimp			Mussel		
	May	Sept.	Total	May	Sept.	Total
Margaree	30	30	60	-	-	-
Pictou	30	30	60	30	30	60
Kouchibouguac	30	30	60	30	30	60
Miramichi	21	30	51	-	-	-
Restigouche	30	30	60	30	30	60
			291			180

Table 15.1. Sample collections and schedule for invertebrate pathology examinations - 1996.

All specimens were screened for grossly visible lesions, before being weighed, measured and processed for histopathological examination of the soft-tissues.

Gross observations were aimed towards cuticular lesions, exoskeletal (including gill and appendages) deformities and abnormal epibiont (fouling organisms) accumulation in shrimp. Mussels were screened for shell deformities, excessive fouling or perforation by shell-boring sponges and polychaetes and shell erosion.

Histology was chosen for its non-specific screening capacity and provision of a permanent record of specimen tissues. Tissues remaining after sectioning for slide staining were stored for additional histochemical processing or back-up sections, as required. Sections were collected from the mussels as outlined in Howard and Smith (1983) and the shrimp were sectioned as outlined for penaeid shrimp in Bell and Lightner (1988). Routine fixatives (Davidson's and 1G4F) and histological stains (Harris' Haemotoxylin and Eosin) were used for the preliminary examinations.

Soft-tissues were examined for necrosis (cell-death), neoplasia, saprobiont accumulation, abiotic and biotic foreign bodies (non-parasitic/proliferative tissue irritants), microbial and eukaryotic parasites, epithelial vacuolisation, oedema and haemocyte infiltration (systemic or focal = "granulomas"). Abscess lesions (lesions containing necrotic tissue debris), epithelial erosion, metaplasia (change in epithelial cell-shape) and hyperplasia (focal epithelial cell proliferation) were also noted. Signs of arrested or regressive maturation were also screened for in both species.

To date (February 24th, 1997) all specimens collected in May have been processed and examined. The list of preliminary observations from the September samples is also completed, however, prevalence and intensity data are still being compiled. The present report, therefore, constitutes a "near-complete" documentation of preliminary results.

Results

Shrimp (*Crangon septemspinosa*)

Several parasites and pathologies were noted in the specimens collected. None appear to be of direct disease significance, being detected in apparently healthy individuals transported to the laboratory and processed within 24 hours of collection. All specimens were fixed live and no processing artifacts were observed.

At least two types of intranuclear digestive tubule epithelial inclusion body were observed; four species of gill ciliate (Protista, Ciliophora) and one species of digenean metacercarial cyst. See Table 15.2 for May prevalence data.

	Margaree	Pictou	Kouchibouguac	Miramichi	Restigouche
Metacercarial cysts	3.3	6.7	30.0	9.5	16.7
Gill ciliates (total)	3.3	6.7	0.0	52.4	93.3
Hypertrophic nuclear inclusions	0.0	0.0	0.0	33.3	40.0
Sex Ratio (F:M)	25:5	28:2	29:1	19:2	30:0

Table 15.2. Prevalence (% of sample infected) data for histopathology of sand shrimp collected in May 1996.

Blue Mussels (*Mytilus edulis*)

Results from blue mussels were equally inconclusive with respect to overall mussel health. No significant pathogens were detected and no significant differences between estuaries were observed. Given the known sequestering capabilities of mussels for certain chemical pollutants, however, the selection of sampling sites may have been too distant from the putative pollutant hot-spots (Cheng 1993). See Table 15.3.

	Pictou	Kouchibouguac	Restigouche
Ancistrum mytili gill ciliates	63.3	45.0	43.3
Sphenophrya-like gill ciliates	6.7	3.4	3.3
Granulomas (focal granulocyte lesions)	3.3	0.0	16.7
Sex Ration (F:M)	18:2	14:15	15:15

Table 15.3. Prevalence data for histopathology of blue mussels sampled in May 1996.

Conclusions

None of the pathogens or pathologies observed to date give a clear effluent correlation. The reasons for this are manifold, but do not warrent exclusion of invertebrates from environmental monitoring. Their use has been well documented for other studies and merits consideration for their continued inclusion in such effluent surveys (Galtsoff 1964; Gilfillan *et al.* 1977; Goldberg 1980; Sparks 1985; Sindermann 1990; Cheng 1993; Gardener 1993; Lightener 1993; Sindermann 1993).

Future Investigation Suggestion

One of the principal limitations to the preliminary survey was the lack of a negative control, i.e., establishing pathological results from known concentrations of pulp mill effluent. This is an imperative next step to establishing both lethal and sub-lethal influences on the lower trophic levels of estuaries under investigation. With this base-line data, subsequent surveys can distinguish with clarity between influences from inorganic compounds and those from normal physiological responses to estuarine fluxes (temperature, salinity, turbidity, etc.). This will provide a base-reference for histopathology surveys or support services to growth and developmental surveys, e.g., caged mussels.

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<u>Questions, Answers and Comments</u>

Q. Were the sand shrimp sampled at the same times and places as the fish in Anne-Margaret Mackinnon's study?
A. Yes.

Q. What is known of the biology of sand shrimp?
A. Our species of sand shrimp lives 2-3 years, we don't know specific moulting times, and little is known of their general biology such as number of eggs per female.

C. Some work was done on *Crangon septemspinosus* in the Bay of Fundy by Sue Corey. Basic biological studies are planned for the southern Gulf of St. Lawrence starting in 1997. Much work has been done on the brown shrimp, *Crangon crangon*, which is commercially fished in Europe.

C. The reasons that we think that sand shrimp may be a good indicator species are that they: 1) are extremely abundant and easily sampled in the estuarine and coastal marine environments; 2) are a major component in the estuarine and marine coastal food web, being the major prey item for many juvenile and adult fish (and probably birds) in these environments (e.g., striped bass, tomcod); 3) have been shown to accumulate contaminants to different levels in industrialised and undeveloped rivers; and 4) have been used extensively in laboratory toxicology studies.

Appendix A. Workshop Program

A Workshop to Discuss Alternatives to the Adult Fish Survey for the Pulp & Paper EEM Program

Date: 17,18 February 1997

Location: Room 1524, Queen Square
Environment Canada Regional Office
45 Alderney Drive
Dartmouth, NS

Monday 17 February - Agenda:

0900: Welcome & Introductions

0910: Roy Parker & Simon Courtenay - Purpose of Workshop

0920 - 0940: Experiences with the first cycle EEM Adult Fish Surveys in the Atlantic Region - W. Roy Parker*

0940 - 1000: EEM Cycle One Adult Fish Survey experience: Pacific and Yukon Region - Al Colodey* and Mike Hagen

1000 - 1020: Coffee Break

1020 - 1040: Adult Fish Survey in Ontario Region: experiences from Cycle One Environmental Effects Monitoring - Sue Humphrey* and Nardia Ali

1040 - 1100: The use of bivalves and crustaceans as sentinel species for the EEM Adult Fish Survey - Christopher M. Hawkins*

1100 - 1200: Refined caged bivalve methodologies as part of EEM: characterizing chemical exposure and biological effects - Michael H. Salazar*

1200 - 1300: Lunch (provided)

1300 - 1330: HABITRAP: a biomonitoring system using bivalves for assessing environmental impacts from chronic pollution - Peter J. Cranford*

1330 - 1410: Development and testing of a mesocosm technology for Environmental Effects Monitoring (EEM) methods application - Monique G. Dubé*, Joseph M. Culp* and James A. Smith

1410 - 1440: The application of the scope for growth approach to assess environmental impacts from pulp mill effluents and other point sources - Richard Addison*

1440 - 1510: Status report from the Fish Survey Expert Working Group - Kelly R.Munkittrick*

1510 - 1530: Coffee Break

1520 - 1630: Discussion period

Tuesday - 18 February 1997 - Agenda

0830 - 0850: Health assessment of finfish collected from estuaries receiving pulp mill effluent - Anne-Margaret MacKinnon*, A. Boraie, M.I. Campbell, A. Kew, M. McMenemy, P. Swan and B. Nickerson

0850 - 0910: Use of a fish Health Assessment Index approach for evaluating impacts of multiple stressors - a west coast perspective - Bonnie L. Antcliffe*

0910 - 0930: Invertebrate pathology alternatives for monitoring paper industry effects on environmental health - John Ochieng-Mitula and Sharon McGladdery*

0930 - 0950: Caged exposures, life cycle and behavioral studies with fish...examples and comments - Ian K. Birtwell*, J.S. Korstrom, G.M. Kruzynski and G.E. Piercey

0950 - 1010: Coffee break

1010 - 1030: The mummichog, *Fundulus heteroclitus*, as a sentinel species for pulp and paper mill EEM surveys in the Atlantic coastal environment - Simon C. Courtenay* and Catherine M. Couillard

1030 - 1050: Use of forage fish as sentinel species for EEM monitoring - Kelly R. Munkittrick*, W.N. Gibbons and W. Taylor

1050 - 1145: Discussion Period

1145 - 1200: Workshop Wrap-up - Simon Courtenay & Roy Parker

1300 - 1630: Fish Survey Expert Working Group Meeting

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Appendix B. List of Attendees

Alternatives to the Adult Fish Survey Workshop - Attendees

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Appendix C: Discussion Session #1
Monday, 17 February 1997

DISCUSSION, SESSION #1, Monday, 17 February 1997

C. Hawkins: What are you looking for exactly, with alternatives to the adult fish survey (AFS) - replacing finfish or looking for something completely different? What are the goals and objectives of the EEM program - to detect effects on the environment, or on finfish, or on invertebrates?

S. Courtenay: The objective of the existing EEM is to determine whether the existing pulp and paper environmental regulations (PPER) are protecting fish, fish habitat and the usability of the resource.

K. Munkittrick: The EEM seeks to determine whether mills are in compliance with regulations, and for the AFS, whether they are affecting fish. How do we define "protect"?

S. Courtenay: The adverse effects being assessed by the AFS pertain to survival, growth, and reproduction.

R. Addison: Do any of the alternatives discussed so far provide surrogates to answer those questions? Can we answer them from, say, measurements of Scope For Growth (SFG)?

K. Munkittrick: I'm not sure that's the purpose. Where the AFS was successfully carried out in Cycle One and found no effects on fish, we can conclude that the PPER are working. We're looking for alternatives to use at sites where the fish survey used in Cycle One isn't suitable to pick up effects; not necessarily on fish alone, but for the site.

R. Addison: Then isn't the benthic study alone enough to determine that? Do we really need an AFS as well?

S. Courtenay: Aren't there cases where the benthic survey wouldn't pick up changes but fish would?

K. Munkittrick: Yes, there are differences in the response mechanisms; fish respond to benthos and to water chemistry, benthic animals don't necessarily do that.

M. Dubé: The benthic response is often one of nutrient enrichment, as was found in the Athabasca River.

C. Hawkins: If we carry out surveys on invertebrates, at some point we'll have to link results to fish populations won't we?

K. Munkittrick: The purpose of the program was to define how widespread effects were, are effects site-specific or common to many mills? This could lead to site-specific regulations or changes to the overall PPER

R. Addison: There are two different questions: are we looking for measurements to detect general environmental changes, or do we want to see evidence of various kinds of disruption in health manifested in growth, survival and reproduction of finfish? Sometimes these are parallel but sometimes not. I don't think we can answer them both with one question.

K. Munkittrick: For some of the issues we have to deal with, there is no kind of fish survey that could be conducted (e.g. trapped plume), and we have to come up with alternatives for those as well as for other situations where a fish survey is possible.

R. Addison: Are there surrogates that can be predictive of effects on fish?

S. Courtenay: Juvenile fish for instance, are there effects on juvenile fish where mills are located near nursery areas (e.g., Miramichi Pulp and Paper Inc.)?

A. Colodey: We must be able to separate out confounding factors, perhaps by focussing more effort on a bioassay or something like that.

K. Munkittrick: If we find that 10% of mills cause effects and all those mills have confounding factors, how do we deal with the situation?

A. Colodey: Exactly, so how do we get those others dischargers involved so they are monitoring the impacts of their effluents? Do we need assessment tools for municipal sewage for example?

S. Courtenay: I think that we do. Ultimately we are probably heading towards a tiered approach in which we monitor the health of watersheds or basins and then use different tools to apportion effects to particular sources of contaminants. We should keep this model in mind as we make our recommendations for AFS so that monitoring for pulp mills fit into the larger picture later on. Peter, how does this fit into what you've seen in the oil industry?

P. Cranford: All the same thing, trying to figure out what differences mean. But we are working in a more pristine environment on the Atlantic continental shelf than your situation in the coastal environment.

S. Courtenay: Are people comfortable with cageing studies?

K. Munkittrick: Is there a problem with bringing caged animals into areas where they're not normally found?

M. Salazar: It can be a concern; hopefully any permitting systems should take care of this. It is a bigger issue in fresh water than in marine waters.

M. Paine: In the EEM program, the approach taken was to use different approaches: lab toxicity testing on individuals, and field components (population survey of fish, community survey of benthos). When looking at alternatives to the AFS, we need to keep the aspects of the survey that make it distinct from the other surveys. Maybe that'll help up to evaluate alternatives.

M. Salazar: The "TRIAD" approach is included in the *In situ* mussel approach. I think there should be more empasis on tissue residues because that's where I think regulations are going. You have bioassays - single species, semi-controlled experiments. You seem to be going toward site-specific because all so different; one size doesn't fit all. You may need different combinations of approaches on a site-specific basis.

M. Paine: Okay, but what elements of the FS do we want to preserve?

P. Wells: Given the problems of sampling fish, how do obtain the most information from the measurements on a few fish? What is your minimum set of health indicator measurements to make this assessment?

S. Courtenay: Maybe the site could dictate the study. If it is a spawning area, do a spawning evaluation; if it is a nursery area, focus the evaluation on juveniles.

K. Munkittrick: The final structure of our decision tree will mean that if a mill wants to do somethig different from the standard program, they'll be forced to justify it but the program will be able to accommodate alternatives.

R. Addison: Remember how much basic biological information is missing from species we use as sentinels - things like food preferences, migratory habits, breeding information.

A. Colodey: On the west coast we considered this for Cycle One; DFO scientists provided advice to help mills and consultants select suitable sentinel species. But let's be realistic about what can actually be monitored. Effluent doesn't behave nicely so showing exposure is not easy.

P. Wells: Chemical residues in bile are good indicators of exposure.

R. Addison: We simply don't have enough basic biological information to interpret field data and conclude that fish are healthy or unhealthy so I say stick with laboratory tests.

S. Courtenay: Is our problem that we haven't seen dramatic effects on fish in Cycle One? Are fish living near mills all healthy, or at least the fish left to be sampled? For context, in the Hudson River, NY, the incidence of hepatic carcinomas in post-yearling tomcod has reached

90% in some years and fish do not live beyond 2 years compared to 8 in the St. Lawrence River. The problem there is obvious.

I. Birtwell: We showed that exposure compromises migratory performance of adult salmon, that they'll go into contaminated areas and die there. So showing presence of contaminants in the environment is not enough. There are survival aspects that should be considered as well: predator escape, swimming performance, behaviour, disease resistance, etc.

K. Munkittrick: The EEM program was supposed to identify research issues and government would do the research. The problem is that no monies were set aside to address these issues. Research has now been left out of the program. What's the minimum acceptable to conclude that there is no problem? That's our question now.

I. Birtwell: And who decides that acceptability?

K. Munkittrick: A small group of people who designed EEM. Their decisions are being questioned and the EWG is now supposed to figure that out.

I. Birtwell: Growth and survival are important issues and there are bioassays to look at those.

S. Courtenay: What about caged bivalves? If you show effects on growth, is that sufficient?

M. Paine: I've some concerns about cage studies. Mussels caged in a trapped plume at depth is not realistic exposure - it would never happen in the real world.

M. Salazar: The point is that in a situation where you have no data, you can say nothing. It would be better to cage mussels than knowing nothing about the potential effects of that effluent. It would be a worst case scenario which can be made more realistic by looking at plume dispersion etc.

M. Paine: It only makes sense to do cage studies if you know fish are exposed at some point, so if tracers have shown that they are exposed then a cage study might make sense.

K. Munkittrick: But if there was no exposure of fish to the effluent, either due to a lack of habitat, rapid dilution, no fish, or a trapped plume, the AFS would not be an issue.

I. Birtwell: Fish can and will stay in bad environments and die there; we see it in the lab and field. For example, Pacific sockeye salmon key in on 12 degree C water during migration. Even if the waters are polluted, and can be detrimental to their health, they will stay the majority of time in these waters, apparently because of their fidelity to the temperature of the water at this life stage.

M. Salazar: Exposure, behaviour, etc. are all important and we don't understand the interactions among all the different factors. If you cage animals in the plume, at least you get an idea of what the effluent could do. Maybe fish swim into it and die right away and that's why you don't capture live fish that show tracers.

C. Hawkins: I agree; cage studies - in a trapped plume for example, could indicate that there is a potential danger of effluent and we need to see this.

P. Martel: That is covered in the EEM program by bioassays, sublethal toxicity testing.

S. Courtenay: There is a distinction between the laboratory toxicity tests and the fish survey; the former addresses the question: CAN effluent harm biota, while the fish survey addresses the question IS effluent harming fish or macro-invertebrates in the receiving environment?

A. Colodey: Caged mussels are an intermediate between lab tests and the AFS field study.

M. Salazar: If you see effects on mussels, then what?

S. Courtenay: Yes, what if you carry out an AFS which shows no effects on fish but your caged bivalve study says there COULD be an effect if there were mussels there… then what?

P. Wells: Salazar's approach has the advantage that it is linked to the chemicals.

M. Paine: Yes, if you know what chemical to look for.

K. Munkittrick: The caged bivalve option will only be on one Alternative list for a certain kind of situation, it is not intended to be used in all situations. There are different reasons why AFS didn't work at various spots. Where a fish survey was successfully carried out in Cycle One, or was sufficiently successful that with modifications it could be made successful, this would be the preference in Cycle Two. Ultimately, the decision about what kind of survey to do would be up to the mill and regional technical advisory committee. The fish survey EWG is working on a decision tree that might look something like this:

Alternative List A: Fish not available and environment not suitable for sampling
- Caged bivalves
- Mesocosms

Alternative List B: Fish not available but environment suitable for sampling
- Wild bivalve survey
- Wild crab/lobster survey

Alternative List C: Fish available but not in sufficient numbers
- Work with small fish species
- Increase sample size of a single sentinel species

Juvenile fish survey for growth and year class strength
Mesocosm studies

In judging the eligibility of alternatives for these lists, the following criteria will be applied. Alernatives should be:

Scientifically defensible
Cost-effective
Manageable in requirements and time frames
Capable of generating interpretable results
Capable of providing defined decision points
Available in the private sector
Relevant to the detection of adverse effects

Criteria of a suitable sentinel species are that they be abundant, exposed, and have easily-measured life–history variables, especially age and gonad weight. A hierarchy of species preference for Cycle Two would be finfish-bivalve-prawn-crab/lobster. Decapods score lowest on this list because they cannot be aged and therefore cannot easily provide information on growth.

There is a similar program in Sweden - measurements on individuals, populations, tissues etc. Their approach is simpler because all of their mills have similar fish species and habitats. They use an annual program and have developed a better understanding of the biology of their sentinel species.

C. Hawkins: What does "fish not available" mean?

K. Munkittrick: That in Cycle One no fish were caught. The EWG wants to clarify that people need to be open to alternatives. For example, if a consultant comes up with another species they want to use and they can justify it, then the regional technical advisory panel has to be able to listen to them

M. Salazar: The choice of alternative approaches should depend on whether fish are being affected by contaminants in the water column or sediments.

A. Colodey: Approaches will differ with the receiving environment too. For example, marine mills might select one finfish and one bivalve species.

M. Salazar: If we have background data, we could compare data in fish and bivalves and see if effects are happening together. If such data aren't available, I think that it should be a research recommendation to collect synoptic measurements between fish and bivalves to see if there are relationships.

K. Munkittrick: Some synoptic fish/bivalve data do exist. Ian Smith of Ontario MOEE did caged bivalve studies for 2 years in Jackfish Bay, Lake Superior.

S. Courtenay: We should discuss the rationale of the 3-year cycle approach.

A. Colodey: Is it better to do a simple survey annually or a more complex survey every 3 years? This question needs to be resolved at a higher level than the decision trees for the FS or other EEM components

B. Antcliffe: How will we improve measures of effort in Cycle Two, when we couldn't compare among mills in Cycle One? Are we going to discuss standardizing measurement of CPUE (catch per unit effort)? Also, temperature/depth profiles should be included in all fishing programs.

K. Munkittrick: It is difficult to standardize effort unless we standardize the method used to fish; some people took 3 weeks, some 1 night. Effort will go down as people learn how to get fish more efficiently. I'm open to suggestions though.

B. Antcliffe: I just want to know how we can determine if fish are really there; just because the consultant didn't catch any doesn't mean there aren't any there. We need to evaluate effort in order to determine this.

Appendix D: Discussion Session #2
Tuesday, 18 February 1997

DISCUSSION, SESSION #2, Tuesday, 18 February 1997

S. Courtenay: Do we need a synthesis of studies which have examined Health Assessment Indices in fish exposed to pulp mill effluents across Canada?

B. Antcliffe: Many different workers across Canada seem to be using Fish Health Assessment (FHA) - Stella Swanson for example. Maybe right now we need a literature search to bring togther all available knowledge.

S. Courtenay: Those who really enjoy meetings might suggest a workshop.

A. Colodey: Sure, but it would be better to get a consultant to put together a review and circulate it first.

S. McGladdery: ICES has done a review on FHA for North Sea fishes, this would be a good place to start. For the invertebrate side, we should look at experimental work to determine dose/effect relationships before instituting health assessments in field programs.

A. Colodey: That's why a workshop might be useful, you've got all these experienced mill-related sources, bring them all together. Much data and expertise will not have been published.

K. Munkittrick: I want to hear what people think about tumours, lesions etc. They are ugly but there's not a lot of evidence that they are that harmful to fish. If fish have lesions but are performing well with respect to growth, survival and reproduction, are the lesions a problem?

B. Antcliffe: Our data from the Columbia River show that despite a lower Health Assessment Index (HAI) (more lesions and tumours), downstream fish were bigger at age, had higher condition, higher GSI and LSI in some cases than fish upstream, so other than the Cumulative Disease Severity index (CDS), their health didn't seem to be compromised.

S. Courtenay: MFO induction has frequently been shown to occur in response to exposure to pulp mill effluent as well, but again, we have not been able to tie this to effects on growth, reproduction and survival. Maybe we should be going right to the bottom line (growth, survival, reproduction); if we see changes there, then start looking at other things.

M. Salazar: Yes. We compared biomarkers to growth rates in bivalves living in San Diego Bay, and in no case were the biomarkers more sensitive than growth. I think you'll see the same with fish - growth will be the most sensitive in the end.

K. Munkittrick: Another way to ask the question is what is the minimum data set required to accept that there is no problem at the site? The decision for Cycle One was to evaluate gonad size, liver size, growth and age structure. If they were "normal", we would say that the fish population was okay. If we want to change that we'll have to justify it.

I. Birtwell: No one has evaluated the performance of live fish before they are sacrificed, swimming performance for example. Fish with fin rot probably swim less well than healthy fish.

A. Colodey: In the Fraser River, there was no correlation between the HAI and histopathological changes.

K. Munkittrick: We found that goldfish near a steel mill recovered from very bad furunculosis lesions; that was not a big health problem for them.

S. Courtenay: On the other hand, we and other workers have found a strong correlation between expression of atypical furunculosis and mortality in Atlantic tomcod.

A. Colodey: We should synthesize the HAI information collected during Cycle One. If disease is a problem at a given site then look at it, but don't go screaming for it everywhere.

S. Courtenay: Has there been enough information collected during Cycle One?

M. Paine: With HAI, it's very quick to measure and can end up being useful.

K. Munkittrick: Requiring it would also ensure that the consultant actually looks at the liver, gonads, etc.

B. Antcliffe: Right now, consultants have to do the internal exams and are killing the fish. We could provide a checklist to help them do a quick external screening. If problems are detected, they could proceed with a more detailed examination.

S. McGladdery: There are multiple causes of gill and liver lesions too which shouldn't be lumped into one and the actual causes ignored.

A. Colodey: Results will be affected by the level of training workers have. We need standard methods for describing observations.

P. Wells: Are we trying to prepare a guideline to lead to next phases?

B. Antcliffe: Part of the HAI is to look at bile colour but that doesn't mean much. People can go astray if they stick to those protocols too closely. What we basically need is some sort of screeing technique at the beginning.

M. Dubé: Yes, the HAI observations are subjective. Maybe we need a centrally coordinated effort with one group that leads the study and has a standardized program, trained people. They could look at the sites where screening in intital surveys has shown a problem.

S. Courtenay: That's right, initial screening for lumps and bumps requires little training but histology seems to be very time-consuming and requires highly trained personnel.

C. Hawkins: Consistency is still going to be a problem. We could generate a guidebook to standardize observations for consultants.

P. Martel: I've used HAI at 2 sites and I understand that you are not supposed to interpret what you are seeing; just looking for differences from normal. Is the gill pale compared to your experience of normal? The index is not to say whether it is more severe or damaged or whatever, just is there a difference. If there is then you have a basis to go in and look more closely, get a pathologist to look at specific problems, etc.

S. Courtenay: But would 2 or 3 people give the same result?

S. McGladdery: There are guides available for HAI, and perhaps a workshop would be a simple way of settling on standards.

P. Martel: Anyone with some experience will be able to do it easily. We shouldn't get too worried about what differences mean at the early stage.

M. Paine: There is subjectivity but one thing we did is a simple scoring system for fat level. There was no problem, regardless of who was doing the work, we always saw that females had higher fat levels than males, as we should find.

S. Courtenay: We should think about what alternative methods or supplements are available for Cycle Two. What methods need further refinement or testing and could possibly be used for Cycle Three? What methods still require further research before they could be used routinely in an EEM program?

K. Munkittrick: There are criteria listed in the EEM Requirements Document for the acceptability of tools. We should go through all the alternatives discussed here and look at them according to these criteria:

Scientifically defensible
Cost-effective
Manageable requirements and time frames
Generate interpretable results
Provide defined decision points

Let's start with mesocosm studies. These could be used for benthics now. Would we allow someone to try to develop it for the FS during Cycle Two at a site that can't be looked at in any other way? I think so.

R. Addison: I have a general question regarding the fish survey. The experience in Cycle One was that in fresh water, better controls were available, more fish were caught, and better interpretation of data was made than in the marine situation which seems to be much more difficult. For the marine environment, does it make sense to keep trying field observational studies rather than laboratory experiments? All the money spent on observational studies that might lead to ambiguous information could be put into a generic experimental study like Ian Birtwell described this morning, resulting in much less ambiguous information. This approach would depart from the traditional monitoring but do we want science or do we want comfy monitoring? Is it better to spend money at each mill to do a cursory study or is it better to pool the money and conduct a few very comprehensive (broader based) studies?

S. Courtenay: That approach would mean a 3-6 year holiday from fish monitoring.

R. Addison: Not necessarily, we could get results in less than 2 years from Ian Birtwell's study.

A. Colodey: Mills don't spend that much on field studies. Also we'd only be able to answer a couple of questions, or the questions at a couple of sites, but what about the rest? That's why it may be appropriate for some mills to do that but for others to keep the FS where it works. If an issue is identified at a site, then design a specific experiment which meets the program requirements.

K. Munkittrick: We can't force research. We can recommend it, but we can't force mills to put money into research.

R. Addison: This is not a research question per se. We are asking, does mill effluent affect growth, survival or reproduction in fish, or not?

A. Colodey: The distinction between research and monitoring can be tricky. We can suggest a multi-mill, salmon life cycle study, put it down as an option, but I don't know if we can implement it. This is no different from the mesocosm approach in that sense.

K. Munkittrick [agrees]

I. Birtwell: Trying to determine effects in the estuarine environment is fraught with so many problems, it's a waste of money, when no results are ever obtained.

S. Courtenay: Do you think that Colin Levings (DFO, Pacific Region, Marine Environmental Science Division) would agree that the estuarine habitat is so complex that there's no way to answer these questions or perform monitoring there?

I. Birtwell: He has looked at pristine estuaries so far. Looking at estuaries with multiple pollutants is different.

S. Courtenay: One concern that I have in moving from the field to the laboratory is that your question changes from DOES effluent impact biota in the receiving environment, to CAN effluent impact biota.

I. Birtwell: You can at least get inferences from cage experiments.

M. Dubé: Experimental doesn't necessarily mean research, it's an approach.

S. Courtenay: Developing new techniques doesn't fit under a monitoring program though. I personally think that government and industry should be in the business of developing better monitoring tools but possibly not under this EEM program.

I. Birtwell: But how can you tell what's happening in monitoring when you only ever sample live fish, that is, the survivors? You don't know how many fish died. That's what more controlled studies, such as mesocosms, show.

M. Salazar: A dye study doesn't necessarily show where contaminants from the plume go. You need tissue residues to pair with responses of fish in exposed area. If you have similar conditions in a lab setting to compare to field observations, you can get a better understanding of the real pathways of contaminants in the environment. What do these dye studies mean anyway?

S. Courtenay: Good point. The EWG has to take note of that; we have been defining the area impacted by effluent as being that within the 1% (or greater) plume. Effects extend beyond this area in some situations.

C. Hawkins: Exposure can at least be quantified with respect to time and concentration if you use a controlled situation like a mesocosm.

K. Munkittrick: I don't think that we have time in this discussion to screen each potential alternative approach through the criteria listed above. This task should be left to the Expert Working Group.

P. Wells: Any alternative approaches should link to the other study tools, benthic studies and laboratory toxicity tests for example.

S. Courtenay: I learned just last week that the benthic survey component of the pulp mill EEM has experienced similar problems to the fish surveys in the marine and estuarine environments. It is noteworthy that there has been virtually no integration at all of different components of EEM so far.

M. Salazar: Yes, it should be a recommendation of this group that there be more integration between different elements and committees of EEM so that we can learn from the mistakes and successes of the other groups. (General agreement).

P. Wells: It is not useful to polarize discussion into lab and field work. We will likely need both approaches, and integrate them for a proper risk management.

Appendix E. Dungeness crab (*Cancer magister*) Usefulness in Environmental Monitoring Studies

Editors' Note: The following was not presented at the Dartmouth Workshop but was referred to by a number of participants during presentations and discussions. To support the recommendation that decapods not be used in future fish surveys because they cannot be aged, the following memo was kindly contributed by Dr. Glen Jamieson (DFO, Pacific Region).

Dungeness Crab (*Cancer magister*) Usefulness in Environmental Monitoring Studies

Glen Jamieson,
Pacific Biological Station, Nanaimo, BC
December 18/96

Crustaceans, and Dungeness crab (*Cancer magister*) in particular, have been proposed as a sentinel organism for the monitoring of marine environmental consequences of pollution in the marine environment. This is because large crustaceans are relatively high in the food chain, meaning pollution products may be concentrated in crab with consequently presumed greater likelihood that physiological effects will be manifested. Usage of crab has been suggested in two ways: 1) growth, or condition, consequences on individual crab, to be determined from population sampling and detection of general population trends (e.g., reduced growth, unusual population size structure, etc.) and 2) uptake and concentration of toxic compounds in particular organs or tissues.

There are major difficulties in relating possible discerned population effects to potential causes, i.e., pollution. Firstly, when crab moult, they shed all their hard structural parts, meaning that crab of any size cannot be accurately aged. Ages are inferred from looking at modes in size frequency data over a number of years and trying to relate them back to some point in time, representing larval settlement. Problems are that growth rate probably varies annually, dependent on water temperatures, food availability, competition, etc., and definitely seems to vary within individuals of a yearclass. These yearclass differences are compounded as the animals grow, so that a male crab yearclass around legal size has a mean size standard deviation of about 13 mm, meaning that 95% of the yearclass are between ± 2 SDs (140-190 mm) of 165 mm carapace width (CW; spine-to-spine). Growth in crustaceans is discontinuous, with sudden jumps in an individual's size when a crab moults. Growth is the result of two variables: size increment at moulting (fairly well described in the literature and reasonably constant as a percentage of pre-moult size) and frequency of moulting. The latter changes with the onset of sexual maturity, being fairly constant across both sexes before maturity. After maturity, female growth becomes quite complicated by reproduction, with considerable energy devoted to egg production and possible delays in moulting arising from external egg incubation. In males, it had previously been thought that because sperm production had a relatively low energy cost, moulting frequency was relatively predictable and probably annual. However, my research is suggesting that population size structure of mature males, affected by fishing removal of large males (maturity is reached at about 150 mm CW), may be affecting moulting patterns of sublegal, mature males. These smaller, presumably slower growing, sublegal males do most of the mating in the absence of dominant larger males, and most seem to never moult to a larger size later in their lives. For reasons unknown, but possibly related to either physiological consequences of mating or greater energy expenditure and/or loss of feeding opportunity while the males carry the females around in mating embraces, many, if not most, of these males seem to have at least a 2-year intermoult period, with increased shell disease and morbidity evident in their second intermoult year. Many of these crabs seem to die of what I tentatively call natural senescence. The consequence of all this is that unless a large sample size of the population is obtainable and collected, use of "population" characteristics in any meaningful cause/effect sense will be difficult.

A second problem, common to many species in addition to crab, is that the marine environment is not a homogeneous one, but rather consists of a number of discrete "habitats", many of which may not be immediately obvious to us as humans. The top and bottom sides of rocks often have different biological communities, for example, and the presence or absence of other animals (prey or predators) can make specific habitats desirable. Crab in the field are typically segregated by both size and sex as a result of specific habitat needs by different life stages, and this can complicate experimental design. One would normally not expect to find adult male and female crab together for extended periods, and field studies should acknowledge this. Prior to any study around a mill, a broad-brush survey should be undertaken to identify important preferred habitats, at least at that time of year, for the life stages of the species being investigated. Subsequent sampling should acknowledge the pattern determined and be designed with this in mind.

From a toxin uptake perspective, crustaceans are probably useful, as even large crabs such as Dungeness do not move too much and are often confined to specific substrate types. However, crab are often spatially separated by size and sex, so some understanding of spatial habitats preferred by different size crab may be important so that exposure periods can be estimated. General data are available, but some site-specific studies may be necessary at relevant locations.

Appendix F. Life Cycle Studies With Salmon....Effects of Pulp Mill Effluent

Editors' Note: During discussions, considerable interest was generated by the suggestion that Pacific salmon life cycle studies be considered as an alternative to the fish survey for pulp and paper mill EEM. Drs. Birtwell and Addison (DFO, Pacific Region) were asked to prepare a summary proposal for such a study, the result of which follows.

Life Cycle Studies With Salmon....Effects of Pulp Mill Effluent

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Introduction.

Assessments of the effects of pulp mill effluents on economically-important Pacific salmon have most frequently been carried out on juveniles (individuals or groups) under laboratory conditions. Determining the effects on individuals under controlled conditions has much scientific merit, but the application of results to predict the population level response from such an indiscriminate pollutant stressor is extremely difficult, even when the experimental conditions closely mimic those in the wild: the deductions regarding effects on populations in the wild may be misleading. Similarly, attempts to determine effects in the field are severely limited by our inability to assess exposure to contaminants (concentration and duration), losses and debilitation of individuals (irrespective of cause), inefficient capture techniques etc. In addition, for example, chemical, biochemical, and histological assays on a (previously-living) portion of a population provide valuable insights into the potential harm to individuals from exposures to stressors, but provide little information related to performance and population success.

The assessment of effects can be enhanced substantially by combining a controlled exposure to the effluent with the natural challenges of life at sea. Thus both indiscriminate (pollutant exposure) and discriminate (e.g. predation) factors which affect population success may be integrated to provide greater relevance to the prediction of the effects of exposure to pulp mill effluent.

A life-cycle study may be used to determine the survival of populations of salmon exposed to pulp mill effluent at the sensitive, and relevant, juvenile stage. Such an approach has been successfully applied for the first time with respect to the effects of the water soluble fraction of crude oil on pink salmon survival to adult (Birtwell et al. 1996). The controlled exposure of populations of salmon juveniles to pollutants, followed by their release to the Pacific Ocean to complete their life cycle and return as adults, will permit an assessment of the effects at the population level. At the same time, a detailed analysis of effects on individuals can be made during the exposure period to relate to the numbers and health of returning adults.

The following comments are provided to indicate the nature of such a program as it may apply to an assessment of the effects of pulp mill effluent at the population level.

Species.

The choice should be guided by the logistics of the experiment, and the return of information in a timely fashion. Accordingly, species with a long life cycle would be a poorer choice than those with a shorter one. But, because the infrastructure is in place in the Pacific Region to determine the survival of tagged (Coded Wire Tag (CWT), adipose-clipped) individuals it would be possible to assess the effects on a longer-lived species such as chinook salmon. Irrespective of this concern, the experiment would need to be replicated. Pink salmon have the shortest life span, but because they are harvested in an indiscriminate manner, often in substantial numbers, and there is difficulty in providing the infrastructure to recover tagged fish from the fisheries, their utility can be problematic. In contrast chinook salmon could be used, and a good retrieval process is available from the fisheries, albeit over a protracted period because of their long life cycle.

Potential program.

Populations of juvenile salmon would be exposed to environmentally-relevant concentrations of pulp mill effluent for a realistic period of time (an industry-government decision) and control waters (salt or fresh water depending on species chosen, life cycle stage and experimental location).

Tagging.

Prior to the exposure the fish would be tagged (coded wire), and marked by clipping their adipose fin. Tag retention must be assessed prior to release to provide an estimate of the population of tagged fish.

Holding conditions.

The fish must be held in optimum conditions so that loading densities are not stressful. This requirement is a potential limiting factor, for the experiment will require the holding of thousands of fish. Depending on the species, life cycle stage chosen and known survival potential for the stock, holding facilities, and their supply of water and effluent, may be problematic. It is, therefore, necessary to calculate the expected return of control fish from release and determine the numbers of fish to be accommodated in the experiment. In studies with pink salmon fry, three batches of 30,000 individuals were held in separate troughs (control, and two treatment groups, respectively) for 28 days, in each of the three experimental years (Birtwell et al. 1996).

Location.

The location for the exposure must be close to a source of fish (hatchery) and fresh effluent, and have the potential for recovering the adult fish on their return to spawn. It would be possible to imprint the juvenile fish on non-natal stream waters (e.g. use of morpholine), if a source of fish was not close to a pulp mill.

There are few locations that meet all criteria, and the Port Mellon mill seems the most likely candidate, having a limited-length Rainy River adjacent to the outfall which discharges to the waters of Howe Sound. Hatcheries are close by, and the fish could be imprinted on home waters (assuming incubation and rearing opportunities) with or without the use of additional substances added to the waters.

Proximity to the estuary of the Rainy River, and the availability of fresh river water will permit an exposure of juvenile salmon to either fresh water or brackish-water conditions. The former would be more favorable: by using the brackish waters of the Rainey River estuary there would be the potential for dilute effluent to be pumped to the holding and exposure facilities due to mixing conditions and tidal effects in the proximal effluent receiving area.
The release of juvenile salmon exposed to dilute effluent in fresh water (rather than exposed to sea water when larger and older), over part of the time that they would be on their downstream migration, would permit smaller individuals to be used, and thereby reduce the need for extensive holding facilities (through a reduction in loading densities). The fish would continue their life in the ocean after passage through the estuary of the Rainy River and the waters of Howe Sound and into Georgia Straight and the Pacific Ocean.

Ancillary assessments.

During the time that the fish are held in the experimental facilities assessments would be made of their responses (biochemical, histological, physiological, behavioral, health, metabolism etc.), and the effluent to which they were exposed would be characterized.

By adding numerous assessments of the fish during the exposure period it may be possible to reveal the potential causes of mortality once the fish are challenged to life in the ocean. It would also be possible to correlate and assess, in a highly relevant manner, the utility of many of the tests that are currently used to assess sublethal exposure to pulp mill effluents, to survival under natural conditions.

Recovery of adults.

Through the "Head Recovery Program" in the Pacific Region, fish captured without an adipose fin should have their head removed and sent for tag retrieval and reading. Accordingly, fish captured in the fisheries and in their natal stream (alive and dead) would be assigned to the respective treatment they received as juveniles. In turn, an assessment would be made of the effects of treatment, and subsequent natural challenges on survival to adult. Analyses of the returning adults that were captured alive would be made to ascertain differences among treatment groups. The exact nature of analyses, aside from determinations of length and weight, gonad and liver, could be guided by the results of analyses done during the exposure period (e.g. histology, biochemistry) in addition to other valuable measures of health and performance.

Using the returning salmon for brood stock in subsequent experiments is desirable but probably not feasible considering the time frame over which one would need to wait before carrying out another experiment. It would be preferable to expose populations of juvenile salmon each year for at least three replicates. In this way the vagaries of "ocean climate" and other uncontrollable variables may be incorporated into the experiment. At the least, this would mean an experimental duration of four years with pink salmon as the test subjects to possibly eight or nine years if chinook salmon were used. Despite the duration of the work the benefits of environmental relevance outweigh this problem, and the study will provide the most meaningful information on the survival of populations of salmon, exposed to pulp mill effluent, to adulthood. (A compromise could be made on the approach outlined above, and replicate exposures could be undertaken in one year, thus reducing the overall costs but eliminating some variables and increasing the risk of failure due to unforeseen events).

Costs and relevance.

The costs of the replicate pink-salmon life cycle study which spanned six years (experimental and data analysis) was approximately $600,000. This did not include the provision of power to the on-site laboratory, the rearing of fish and the provision of holding troughs, nor the salaries of DFO staff involved with the project (estimate: $120,000).

When one considers the relevance of the experiment to the industry and regulators, aside from the scientific findings, the costs are relatively small. The potential of cost sharing among pulp mill companies should be assessed and perhaps such a program could be considered in lieu of some monitoring requirements under the EEM program. The relevance of the findings to other pulp mills using the same processes would be high. For other mills, the relevance would be lower, but it is likely that from the assessment of the effects of the effluent on juvenile salmon during the exposure period, that certain measured variables will correlate with adult survival and treatment. In this case it could be anticipated that the application of these tests to fish exposed to other effluents would yield good predictive results on population survival.

Reference.

Birtwell, I.K., R. Fink, R. Alexander, W. Bengeyfield, and C.D. McAllister. 1996. Survival of pink salmon (*Oncorhynchus gorbuscha*) exposed to the water soluble fraction of North Slope crude oil. Can. Tech. Rep. Fish. Aquat. Sci. 2095: 49p.

Appendix G. Detailed Evaluation of Alternatives to the Fish Survey

Editors' Note: Tables that follow provide our own assessment of each alternative considered at the Dartmouth Workshop, ranked against the criteria identified in Annex 1 of the PPER, to serve as guiding principles in the evolution of the program. A viable alternative to the AFS should be:

Scientifically Defensible
Cost Effective
Flexible (so that new or improved monitoring techniques can be incorporated)
Built on findings of research (relevant to the detection of adverse effects)
Manageable in requirements and time frames
Capable of generating interpretable results
Suitable for a weight of evidence approach
Capable of providing defined decision points

Proposed Alternative Method: Wild Bivalve Survey

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective	*			
Flexible	*			
Built on Findings of Research	*			
Manageable	*			
Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Caged Bivalve Studies

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible	*			
Built on Findings of research	*			
Manageable		*		
Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Habitrap

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible		*		
Built on Findings of research	*			
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Bivalve Scope for growth

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible		*		
Built on Findings of research	*			
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Mesocosm with Fish

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible	*			
Built on Findings of research	*			
Manageable		*		
Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Fish Health Assessment Index (HAI)

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective	*			
Flexible	*			
Built on Findings of research	*			
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Invertebrate Pathology

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective		*		
Flexible	*			
Built on Findings of research		*		
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Caged Finfish Studies

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective			*	
Flexible		*		
Built on Findings of research		*		
Manageable		*		
Interpretable Results		*		
Suitable for a weight of evidence approach	*			
Defined decision points		*		

Proposed Alternative Method: Fish surveys using small forage fish

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective	*			
Flexible	*			
Built on Findings of research	*			
Manageable	*			
Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Juvenile fish survey

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible	*			
Cost Effective	*			
Flexible	*			
Built on Findings of research	*			
Manageable	*			
Interpretable Results	*			
Suitable for a weight of evidence approach	*			
Defined decision points	*			

Proposed Alternative Method: Salmon life cycle study

Principle	Ready	More Work Required	Not Applicable to EEM	Unknown
Scientifically Defensible		*		
Cost Effective				*
Flexible				*
Built on Findings of research	*			
Manageable		*		
Interpretable Results				*
Suitable for a weight of evidence approach	*			
Defined decision points	*			