5.0 DISCUSSION

The primary criterion used by DEP for deciding whether or not caged mussels would be a useful monitoring tool for dioxins and furans was to demonstrate a significant difference in dioxin/furan accumulation between upstream and downstream stations. Stations were selected based on areas where fish were previously collected and showed differences in dioxin/furan accumulation, and could also be collected in the current study for comparisons with mussel tissue chemistry. Stations selected by DEP for the caged mussel pilot study were positioned 11 to 13 miles from the suspected source because this was the nearest location where fish could be collected, and because previous fish monitoring had demonstrated significant differences in dioxins and furans when upstream and downstream stations were compared. Accumulation of dioxins and furans in fish tissues could represent exposure to contaminated sediment or food resulting from previous mill discharges and not current discharges. Fish may not be reliable indicators for station selection because of their mobility. Accumulation of dioxins and furans in fish reflects an integration of all exposure conditions encountered during their movement and migration in the river, not just the immediate area selected for upstream and downstream stations.

The decision-making criterion used by DEP was inappropriate for the experimental design used because it is uncertain whether there was really a difference in bioavailable dioxins and furans between the two stations. In addition, the design limited the relative position of the mussel cages and did not provide a true test of the methodology. The approach selected by DEP in this study may have been appropriate for direct comparisons with fish tissue chemistry, but it was inappropriate for a valid test of the caged mussel methodology. In order to evaluate the ability to detect differences between upstream and downstream stations, the downstream station should have been positioned as close as possible to the suspected source in order maximize potential downstream exposures. Similarly, the upstream station should have been as close as possible to the suspected source to eliminate the possibility of contamination from additional upstream sources.

The data require a much more intense analysis and interpretation before reaching conclusions on the utility of caged mussels as a monitoring tool in Maine. More information is provided regarding the utility of the caged mussel approach by evaluating accumulation of individual and lipid-normalized congeners, in addition to the total PCDD/PCDFs. Using only total PCDD/PCDFs in data interpretation (Mower 2001) results in a loss of information, in particular the details associated with bioavailable congeners and potential pathways of exposure. Although statistically significant differences were not found between upstream and downstream stations in the caged mussel dioxin/furan study, the mussels were as effective as fish in accumulating bioavailable dioxins and furans. The mussels accumulated a wider range of congeners than the fish, suggesting uptake from various exposure pathways. DEP also evaluated the utility of SPMDs during the 2000 monitoring study. The data support results from other studies that accumulation of organic chemicals in SPMDs primarily represents aqueous exposures from the water column. As the majority of dioxins, furans, and PCBs available to fish and other aquatic life are probably bound to particles, just measuring the aqueous fraction provides a partial estimate of bioavailability.

The mussels demonstrated their effectiveness as biomonitoring tools because they accumulated many dioxin and furan congeners both upstream and downstream of the mill, showed some differences in upstream and downstream exposures, and identified hotspots of PCB contamination. It is promising that any dioxins and furans were even detected because these stations were situated 13 and 11 miles from the mill, respectively. Although the mussels at the downstream station had a higher mean total PCDD/PCDF concentration, the difference was not statistically significantly. Surprisingly, the data suggest that there is a dioxin/furan source further upstream on the Kennebec River that has affected exposure in the vicinity of the “upstream station.” This is important because it suggests that DEP may require additional monitoring further upstream to identify the sources of these contaminants. If available, dioxins and furans originating from the SAPPI pulp and paper mill may have become too dilute at the downstream station to be statistically and environmentally different than concentrations measured at the upstream station. The downstream station was probably too far from the source to answer this question, and the upstream station was apparently impacted by other sources.

The caged mussel methodology demonstrated its utility by identifying concentrations of PCB as high as 125 ppb and 188 ppb along the stretch of the Kennebec where PCB contamination was suspected, meeting objectives established by FOMB for the PCB study. Concentrations in most other mussel tissues were in the range of 20 to 60 ppb. Since PCBs are in the same class of chemicals (i.e., organochlorines) as dioxins and furans, it would be expected that mussels would accumulate dioxins and furans at proportionately similar concentrations if they were deployed at similar distances away from the sources and the sources were proportional.

5.1 Mussel Tissue Chemistry

5.1.1 Dioxins and Furans

Mussels accumulated many individual dioxin and furan congeners although 10% of reported congeners were present at concentrations slightly below the method detection limit. Most of the total dioxin/furan concentrations in mussel tissues from both upstream and downstream stations were between 2 and 8 ng/kg-wet. This is not surprising since the mussel cages were placed approximately 13 and 11 miles upstream and downstream from the mill, respectively. These results are promising if the test were designed to evaluate the limits of detection at various distances along the suspected chemical gradient of dioxins and furans away from the mill. Assuming an exponential rate of dilution, it might be speculated that concentrations of dioxins and furans in the vicinity of the mill would be one or two orders of magnitude higher if the mill were truly the source of the dioxins and furans measured in this study.

The caged mussels did not meet the primary criterion by the DEP in that there must be a significant difference in dioxin/furan accumulation between the upstream and downstream stations for the mussels to be considered useful. Distance and dilution have likely caused the high variability in the replicates and are attributable to site selection by DEP. One purpose of using caged mussels was to reduce the variability observed in fish studies. Unfortunately, the distance from the suspected source was so great that exposure concentrations were extremely low and near the limit of detection, thus, introducing the variability that was supposed to be avoided. These uncertainties alone provide additional evidence that the caged mussel pilot study should be repeated.

However, the data show that mussels accumulated chemicals that were present, and that there may not be significant differences in exposure at the two locations where mussels were deployed. There may be an additional source of dioxins and furans upstream, and the mussels were sensitive and successful at accumulating these compounds. The mussels, unlike fish, were deployed at a fixed location for a specific duration, and their tissue chemistry reflected site-specific exposure conditions. Although DEP may find statistically significant differences between upstream and downstream concentrations of dioxins and furans in fish, the reasons for those differences are unclear. By looking at accumulation of individual dioxin/furan congeners by mussels, it is clear that they are exposed to and accumulating nearly all congeners. It is the chemical structure and molecular weights of these specific congeners that affect bioavailability, bioaccumulation, and toxicity. The data from this study are consistent with results from other studies suggesting that the mussels are accumulating dioxins and furans from aqueous, particulate, and dietary exposure pathways.

With respect to the utility of using caged mussels as a monitoring tool, the methodology demonstrated its effectiveness by identifying elevated concentrations of dioxins and furans both upstream and downstream of the SAPPI mill at Hinckley, as well as PCBs in the lower Kennebec. The elevated concentrations of the most predominant furan congeners (2378-TCDF and 123789-HxCDF) downstream suggest the SAPPI mill could be a source of these congeners. Although the concentrations of these congeners in the upstream mussels is slightly lower, the concentrations are significantly elevated above T_0, suggesting there is another source of furans upstream of Norridgewock where the mussels were deployed. This is important because it has affected, at least in part, the ability to detect a statistically significant difference between upstream and downstream of the mill. Similarly, concentrations of the most predominant dioxins (1234678-HpCDD and OCDD) were higher upstream than downstream and provide additional evidence of another source of dioxins north of Norridgewock.

5.1.2 PCBs

The objective of the PCB caged mussel pilot study was to help identify specific contaminated areas, or hotspots, along one suspect reach of the Kennebec River. It is important to note the difference between identifying hotspots and monitoring point sources such as pulp mill effluents requires different sampling strategies. Because of the single point source from the pulp mill at Hinckley, caged mussels (and SPMDs) were deployed at one upstream and one downstream station. A more diffuse monitoring design was appropriate for the PCB study because multiple hotspots of PCB contamination were expected. As in the dioxin/furan study, the mussels had high survival, positive growth rates, and accumulated PCBs.

Most of the total PCB concentrations in mussel tissues were between 20 to 60 ug/kg and well above the fish tissue action level (FTAL) of 11 ug/kg. The highest concentrations were more than an order of magnitude above the FTAL. The highest concentration of total PCBs (188 ug/kg) was measured in mussel tissues from midstream just below the Augusta Sewage Treatment plant at South Augusta and in the vicinity of a midstream outfall pipe (Ed Friedman, personal communication). The second highest concentration of total PCBs (125 ug/kg) was measured in mussels deployed on the west side of the Kennebec River, just below the former Williams gravel/asphalt facility (now Ferraiolo) in Farmingdale. This facility contains a large unlined pit of leaky oil and water, leaky asphalt pipe valves, and a number of 3-phase motors (Ed Friedman, personal communication). New transformers are also on-site, but the disposition of the old transformers is unclear. There is an aquifer under this site and a stream that flows through the site which discharges to the Kennebec (Dennis Kinney, personal communication). This facility has been operating at least since the 1940's and is a potential source of PCBs. This information on two potentially significant sources of PCB contamination has been provided to DEP by FOMB and they are both continuing to investigate.

As part of this mussel study, a limited analysis of congener-specific PCBs was conducted because a suite of only 20 congeners were quantified and reported by DEP. None of the reported PCBs have dioxin-like TEQs, which provide a means of estimating potential toxic effects. Certain PCBs are extremely toxic in chronic exposures. The most toxic PCBs are those that closely mimic the potency and mechanism of toxicity of 2,3,7,8-TCDD (one of the most toxic compounds known). These PCBs can cause toxic symptoms similar to those caused by dioxin exposure, including developmental abnormalities, disruption of the endocrine system, impairment of immune function, and cancer promotion.

DEP representatives could not explain the reasons for selecting the 20 congeners measured as part of this caged mussel pilot study and those commonly measured as part of their regular monitoring program. In the future, DEP reports should include the rationale for selecting the particular PCB congeners reported and their potential environmental significance. It seems inefficient to go to the time and expense of congener-specific analysis and not quantify PCB congeners of potentially more environmental significance.

The identification of hotspots of contamination is one of the stated purposes of several mussel monitoring programs that use both indigenous and caged bivalve species (Ontario Ministry of Environment, California Mussel Watch, NOAA mussel watch). Although it could be argued that the use of fish is not appropriate for upstream and downstream comparisons for dioxin/furan monitoring, there is general agreement with respect to the inappropriateness of fish for monitoring isolated and discrete pockets of contamination over small spatial scales on the Kennebec River. Monitoring chemicals in fish tissues is more appropriate for consumption advisories. One of the most obvious advantages of caged mussels (and SPMDs) over fish is the ability to place them along suspected chemical gradients or in the vicinity of suspected sources. The ability to monitor and assess small-scale, microgeographic exposures and effects with caged and indigenous mussels in freshwater and marine environments has been well-documented (Green et al. 1985, Salazar and Salazar 1995). Another reason for deciding to use caged mussels for PCB monitoring in the Kennebec River is that chemical analysis of discrete water samples is generally assumed too variable to be environmentally significant, fish are too mobile, and there are no significant amounts of surficial sediment to collect and measure in this area of the Kennebec. Therefore, caged mussels, SPMDs, or caged fish were the most viable approaches; caged mussels have the longest history of application for these types of assessments.

5.2 Mussel Survival and Growth

The main purpose of measuring survival and growth in this caged mussel pilot study was to demonstrate that the test mussels were in sufficiently good health to accumulate the chemicals of concern; i.e., dioxins, furans, and PCBs. Given the high survival and significant increase in tissue weight, it is concluded that the mussels would have accumulated chemicals at concentrations representative of exposure conditions on the Kennebec. Changes in whole-animal wet-weight and shell weight were not expected due to large size and slow growth rates associated with this species. Nevertheless, for the dioxin/furan study, changes in whole-animal wet-weight were higher at the upstream stations than the downstream stations, but these differences were likely related to differences in physical-chemical factors rather than dioxins and furans because the concentrations of these chemicals were higher upstream than downstream. Although the upstream tissue weights at the end of the test were higher than those at the downstream station, these differences were not statistically significant. As suggested previously, if there were correlations between mussel growth rates and tissue burdens of dioxins and furans, they would be more meaningful if they could have been established along a chemical gradient rather than one discrete location at a distance of 11 to 13 miles from the mill.

For the PCB study, both growth rates and tissue weights were lowest at South Augusta when compared to other stations and highest at Gardiner and South Gardiner. Mussels at all stations except South Augusta had significant increases in tissue weights. The combined low growth rates, low tissue weights, and high PCB concentrations measured in mussel tissues at the mid-river location suggest a correlation between high tissue burdens and decreased mussel growth. A similar relationship between low growth, low tissue weight, and high PCB concentration in mussel tissues was found at Farmingdale. Low growth rates at South Augusta and Farmingdale do not appear to be related to water temperature.

5.3 Water Temperature

Temperature did not appear to be a significant factor that influenced survival, bioaccumulation or growth in this caged mussel pilot study, although there was a significant difference in daily average water temperature between the upstream and downstream dioxin stations. The downstream station was significantly higher that the upstream station, although the means were extremely close. Mean temperatures were even closer among the nine PCB stations.

5.4 Caged Mussels as a Monitoring and Assessment Tool

The data from this study and from hundreds of studies conducted worldwide suggest that caged mussels are a useful and meaningful monitoring tool. The most important concept to remember is that there are no perfect monitoring and assessment tools, and each has its own advantages and disadvantages. The most successful monitoring program integrates elements such as those represented by fish, caged bivalves, and SPMDs. Based on a weight of evidence evaluation of the data from this study, it is concluded that caged mussels are a potentially useful tool for monitoring dioxins, furans, and PCBs in the state of Maine. This is the opposite conclusion to that reached by DEP in their 2000 dioxin monitoring report (Mower 2001).

"Of all the test types (large and small bass, large sucker filets and whole fish, sucker liver composites, freshwater mussels, and SPMDs) tested in 2000, only the fish and livers were able to detect significant differences between stations above and below some bleached kraft pulp and paper mills. Freshwater mussels and SPMDs did not detect any differences. SPMDs were tested again in 2001 with an enhanced sample design that may lead to improved capability to detect differences. Freshwater mussels did not appear to be a useful monitoring device, perhaps because they are at a lower trophic level than fish. MSDs were generally lower for bass than for suckers or livers. Neither liver nor mussel studies were repeated, but studies with fish were repeated in 2001."

While it is true that the total PCDDs/PCDFs were significantly higher in smallmouth bass downstream than upstream, they were lower downstream on a lipid-normalized basis. It is probably most appropriate to compare the concentrations among mussel tissues, SPMDs, and fish using the lipid-normalized data. The DEP interpretation is also opposite the interpretation reached in this report. The possible reason for this discrepancy may be the method used for calculating the mean total dioxin/furan concentrations. DEP only used measured values to calculate the mean whereas "zero" is substituted for undetected values in this report. This latter approach is the one most commonly used in fish and mussel monitoring programs conducted at national, state, or regional levels. Some monitoring programs use half the detection limit, but no other studies could be found where non-detects are completely rejected and not included in calculating the mean. Algorithms are available for estimating the values that might be replaced for non-detects.

Using fish collected at locations upstream and downstream of pulp mills to characterize exposure conditions is complicated by several major factors. Three issues have been identified with respect to using fish to monitor dioxins and furans to satisfy the upstream versus downstream requirement: 1) fish of different ages in same species may contain different concentrations of dioxins and furans, 2) different fish species may bioaccumulate dioxins and furans at different rates and may attain different body burdens at steady state or different stages of reproduction with different lipid levels, and 3) fish are mobile and the source of the accumulated chemicals cannot be guaranteed (Shoven et al. 2001). The Natural Resources Council of Maine (NRCM) explicitly states that a fish monitoring program as currently conducted is not adequate for quantifying differences in dioxin and furan exposures at upstream and downstream locations, primarily for the reasons cited above. The NRCM further suggests that the uncertainty in the fish tissue chemistry data will not be resolved and will lead to future debate regarding the environmental significance of these data. The NRCM concludes that DEP has not yet developed an appropriate fish monitoring program for compliance with the 1997 law (Bennett 2001).

5.4.1 Comparison of Caged Mussels, SPMDs and Fish

The main advantage of using caged mussels as a monitoring and assessment tool is their ability to accurately quantify chemical exposure and associated biological effects over space and time and under environmentally realistic conditions. Another advantage is that they can be strategically placed along suspected chemical gradients to confirm the source of chemical exposure, allowing comparisons to be made, such as those required by regulations regarding pulp and paper mill emissions in the state of Maine. Unfortunately, a gradient design was not tested in this pilot study. The stated purpose of the DEP was to evaluate mussels and SPMDs relative to fish, and not to explore the various advantages of caged mussel biomonitoring. Caged mussel monitoring can also monitor chemical exposure over time and establish the status and trends of dioxin, furan, and PCB contamination in a historical context.

Mussels accumulated more dioxin and furan congeners than either fish or SPMDs. It was surprising to find that fish from the upstream station only accumulated five dioxin/furan congeners and those from the downstream station only accumulated four. In this respect, it could be argued that mussels were actually a better monitoring tool than fish. In terms of total PCDD/PCDFs, the mussels were much more similar to fish than the SPMDs. SPMDs accumulated approximately 2.5 times higher concentrations of dioxins and furans than mussels and 3.8 times higher than fish at the upstream station. At the downstream station, SPMDs only accumulated about 50% more dioxins and furans than either fish or mussels. While it could be argued that this is evidence that SPMDs are superior accumulators, the data from living organisms such as mussels and fish are probably more environmental realistic and relevant. In addition, other studies have shown that SPMDs primarily accumulate lower molecular weight compounds. This interpretation is also consistent with the congener data presented in this study. At the upstream dioxin station, for example, SPMDs accumulated a concentration of 2378-TCDF that was 5.8 and 7.5 times higher than mussels or fish, respectively. The measured concentration of 2378-TCDF in the upstream SPMDs represents 68% of all furan congeners measured. At the downstream dioxin station, SPMDs accumulated a concentration of 2378-TCDF that was 6.1 and 3.7 times higher than mussels or fish, respectively.

Although it may appear that the SPMDs are efficient at accumulating dioxins and furans, it is important to accurately interpret the data in light of the method detection limits. The only congener that was measured at concentrations equal to or greater than the detection limit was 2378-TCDF. Only 12% of all reported concentrations were equal to or greater than the detection limit, with the other 88% reported concentrations less than 1/10 of the detection limit. The general rule of thumb in interpreting “estimated” data is to put more weight on values that are within 50% of the detection limit, and values less than this are considered extremely unreliable. For all practical purposes, the SPMDs only accumulated one congener. If this congener was absent and the others dominated, it is unclear if the SPMDs would accumulate anything.

There are many different monitoring tools, and each tool has appropriate applications and uses, and advantages and disadvantages (Table 16). SPMDs are potentially useful as screening tools for assessing soluble components in the water column. Caged bivalves are useful for characterizing exposure conditions and quantifying bioavailable chemicals. Resident fish are useful for developing fish consumption advisories and monitoring compliance. They are less useful for the upstream/downstream comparisons required by state law. SPMDs and caged bivalves produce complementary data sets, however, they do not appear to be directly comparable on a congener-specific basis (Peven et al. 1996). An integrated monitoring approach using the most appropriate tools provides information on both the bioavailable (bivalves) and water column (SPMD) concentrations of the analytes of interest. The data from the caged mussel pilot study suggests that SPMDs tend to preferentially accumulate the lower molecular weight dioxins and furans while mussels may tend to preferentially accumulate the higher molecular weight congeners.

Table 16. Advantages and disadvantages of caged bivalves, SPMDs and fish as monitoring and assessment tools.

	Advantages	Disadvantages
Caged bivalve transplants	Experimental control	Natural factors can affect responses
	Environmental realism	Effects of caging & transplanting
	Characterization of exposure	Loss of cages (theft, vandalism, nature)
	Characterization of effects	Cost & time of collection
	Status & trends monitoring	Cost & time of measurements
	Large bioaccumulation database	May not be most sensitive species
	Aqueous & particulate pathways	Preferential accumulation of some groups
	Link between lab & field testing	No direct assessment of community
	Integration of bioavailability	Not found in all areas
	Integration of effects	Only conduct tests when not reproducing
	Little or no metabolism of chemicals	Potential effects on indigenous populations
	Large toxicity database	Potential introduction of exotic species
SPMD transplants	Experimental control	Contamination during caging & transplanting
	Characterization of exposure	Loss of cages (theft, vandalism, nature)
	Status & trends monitoring	Little environmental relevance
	Aqueous exposure	No measurements of effects
	Link between lab & field testing	Preferential accumulation of some groups
	Integration of exposure	Effects of fouling and current speed
	Minimal effects of natural factors	Only aqueous exposures
	Commercially available	Relatively small database
	Minimal setup time	
	Minimal labor	
	Easy to transport long distances	
Natural fish populations	Environmental realism	Uncertain exposure due to mobility
	Characterization of exposure	Often difficult to collect in sufficient number
	Characterization of effects	Difficult to collect similar size ranges
	Status & trends monitoring	Dietary exposure may represent previous inputs from mill, not current effluent
	Large database	Can only collect 11 miles from this mill
	Aqueous & dietary pathways	Effects of reproduction on sampling
	Link between lab & field testing	Effects of sampling on populations
	Integration of bioavailability	Time consuming and expensive to collect
	Integration of effects	Different species in different rivers
	Commercial & recreational importance	Metabolism of some chemicals
	Direct human health implications	

5.4.2 Risk Assessment-based Monitoring

There is increasing support for using more integrated approaches in environmental assessment programs (Chapman 1996, Hall 1996). However, this integration should be based on approaches best suited to answer the questions posed by the monitoring model. The risk assessment framework provides a very focused approach to environmental assessment and monitoring of chlorinated hydrocarbons because it includes characterizations of both exposure and effects (Carey et al. 1998). Measuring exposure and effects in natural populations and caged organisms provides a realistic approach to evaluate the success of environmental regulations and resulting mill process changes. However, the issues are complex and appropriate field monitoring methodologies are still being refined. The following have been identified as necessary improvements: 1) the capability to detect effects and establish causal relationships; 2) integration of chemical, biochemical, population-, and ecosystem level measurements; and 3) better sampling designs to account for temporal and spatial variability (Carey et al. 1998).

The recently proposed exposure-dose-response (EDR) triad (Salazar and Salazar 1995, 1998) facilitates those characterizations. With the EDR triad approach, exposure is characterized through the chemical analysis of environmental media (i.e., water and sediment) and biological tissues. Effects are characterized through bioassays and community structure studies, both of which are conducted in the lab and in the field. Using caged bivalves facilitates the field bioassay element of the EDR triad. Through synoptic measurements of bioaccumulation and growth, uncertainties associated with exposure and effects can be reduced. The methods for using field bioassays with caged bivalves have been refined to facilitate synoptic bioaccumulation and growth (ASTM 2001, Salazar and Salazar 1995). Growth is the recommended effects endpoint; in bivalves it is easily measured and understood. Growth represents an integration of all internal biological processes and can be quantified as a dose-response. Bivalve growth data can be readily extrapolated to potential population effects.

Bivalves are commonly used as biological indicators of exposure because of their ability to concentrate and integrate chemicals from water and sediment in their tissues (Metcalfe and Charlton 1990, Phillips and Rainbow 1993) and the utility of caged bivalve transplants in monitoring (de Kock and Kramer 1994). Field bioassays with caged bivalves combine the advantages of experimental control from standard laboratory bioassays with the environmental realism from traditional field monitoring. Strategic placement of caged bivalves along chemical gradients facilitates more environmentally representative descriptions of chemical exposure over space and time than water or sediment samples. The integrating power of bivalve filtration helps to normalize the variability associated with quantifying pulp and paper mill effluents and their receiving waters. These factors include intermittent and variable discharges, variability in the direction and velocity of water currents, and natural factors such as storm events, episodic sedimentation, and runoff. All of these factors affect chemical exposure and associated biological effects and have been addressed previously (Beck 1996, Whitfield and Wade 1996). A single chemical analysis of bivalve tissue provides an integrated record of bioavailable chemicals that cannot be defined with thousands of water or sediment samples. Chemicals in bivalve tissues, which can be referred to as the "dose," provide a direct link between chemical exposure and associated biological effects. It also provides a way to compare the results of bioassays and population or community responses in the field.

In the late 1980s, Swedish scientists were among the first to document that fish collected in the vicinity of bleached kraft pulp mill discharges exhibited chronic sublethal effects such as altered growth rates, carbohydrate metabolism, maturation, recruitment, mortality, and community structure (Servos et al. 1996). It is interesting to note that caged bivalves were already being used on a regular basis for monitoring exposure to chemicals associated with freshwater discharges for several years in Canada (Richman 1997) and Finland (Herve et al. 1996) before this discovery. This is one of the first examples of the dichotomy that still exists today with respect to using bivalves to characterize exposure by measuring bioaccumulation of chemicals of concern in their tissues and using fish to characterize effects by measuring various internal health parameters and community structure. Following those early reports of effects on fish in Sweden, similar effects were reported at a number of pulp mills in the Canada and the US (Servos et al. 1996). Collectively, the potential ramifications of these reported effects on fish led to a series of meetings, increased monitoring and regulations to reduce the discharge of dioxins and furans throughout the world. However, the complexity of these process changes, effluent discharges, and receiving environments have made it difficult to establish a causal relationship between reductions in dioxins and furans and improved fish condition. Many recent studies have shown that altered fish physiology and biochemical composition still occurs, even after elimination of dioxins and furans. These results suggest that it is the natural constituents in wood that are responsible for acute and chronic toxicity as well as biochemical and physiological effects. It has been suggested that low molecular weight PAHs may be causing the observed effects in fish (Hodson 1996). DEP is attempting to structure their dioxin/furan monitoring program against this complex history of exposure and effects monitoring at pulp and paper mills.

Although caged bivalves have been used to monitor organochlorines in countries around the world such as Argentina (Colombo et al. 1997), Australia (Haynes et al. 1995), Brazil (Furley and Oliveira Filho 2000), France (Hayer and Pihan 1996a,b), Germany (Huhnerfuss et al. 1995), Hong Kong (Kannan et al. 1989), Japan (Miyata et al. 1987), New Zealand (Burggraff et al. 1996), Russia (Stepanovaa et al. 2000), and Sweden (Bergkvist et al. 1998), the discussion will focus on studies with the most extensive freshwater monitoring over the longest period of time. In Finland, the emphasis has been on pulp and paper mill monitoring, whereas in Canada monitoring has been used for a variety of sources, and in the US a variety of approaches have been used in marine and freshwater environments.

5.4.2.1 Monitoring in Canada

It is important to remember this distinction in a risk-based monitoring strategy between using fish or bivalves as indicators of exposure, indicators of effects, or both. Caged bivalve monitoring for pulp and paper mills in Canada began in with measuring effects such as growth in oysters (Quayle 1964). Subsequent studies with caged marine mussels deployed adjacent to a pulp and paper mill outfall in Canada measured effects on growth and reproduction (Wu and Levings 1980). These results were correlated with a previous study showing reduced densities of natural mussel populations near the outfall (Levings and McDaniel, 1976).

Canadian scientists were among the first to develop a generic monitoring approach for and justify the use of *Elliptio complanata* as a useful monitoring tool (Curry 1977). Subsequently, several studies have been conducted using *Elliptio complanata* for that purpose (Kauss and Hamdy 1985, Koenig and Metcalfe 1990), as well as comparing accumulation in tissues of mussels and leeches (Metcalfe and Hayton 1989).

Although all caged bivalve monitoring in freshwater in Canada between 1980 and the present was not necessarily associated with pulp and paper mills, organochlorines have been measured in *Elliptio complanata* using the in-situ transplant method for over 20 years as part of a regional monitoring program developed by the Ontario Ministry of the Environment (Hayton and Hollinger 1989a,b, Hayton et al. 1990, Anderson et al. 1991, Richman 1992 1997, Ontario Ministry of the Environment 1996 1999). All of these studies have focused on characterizing exposure by measuring concentrations of organochlorines, such as dioxins and furans, in freshwater mussel tissues.

Initial draft plans for environmental effects monitoring (EEM) at pulp and paper mills in Canada included caged bivalves and measurements of tissue chemistry and growth (Parker et al 1991), but this approach was not required in the first cycles of EEM. Additional studies advocated using caged bivalves to characterize exposure and effects associated with pulp and paper mill effluents and provided the rationale for this approach (Salazar and Salazar 1997). The first integrated monitoring study at a pulp and paper mill with caged bivalves was conducted at Port Alice, Vancouver Island, in 1997 (Applied Biomonitoring 2000). In this study, mussel growth metrics were measured as effects endpoints, as in the Kennebec River study. Several resin acids and plant sterols were also measured in mussel tissues as exposure endpoints. However, in contrast to the Kennebec River study, the Port Alice study used a gradient design, and a significant inverse relationship was established between campesterol in mussel tissues and mussel growth rates. Both of these endpoints were also correlated with distance from the mill. A similar study was conducted in Pictou Harbor by Environment Canada using the same methods (Andrews and Parker 1999). As a result of these two pilot studies and the acceptance of the caged bivalve protocols by ASTM, Environment Canada accepted caged bivalves as an alternate method for the required adult fish survey at all pulp and paper mills in Canada as part of environmental effects monitoring. The caged bivalve methodology is an integrated, risk-based approach that allows simultaneous collection of exposure and effects information.

Adult fish surveys have been required as part of environmental effects monitoring in Canada since 1994 (McMaster et al. 2002). However, only effects endpoints related to fish health are measured as part of this program. Extensive development on fish survey methods has occurred (Munkittrick et al. 2000), and these methods are currently being used throughout Canada. The major shortcoming of measuring only effects endpoints in fish or other species is that there is no confirmation that exposure has occurred or where it has occurred. One reason that exposure endpoints such as bioaccumulation of dioxins and furans in Canadian fish has not been used in association with pulp and paper mill effluents is that, even though effects continue to occur, mill discharges of dioxins and furans are essentially undetectable (reference). It has been suggested that some low molecular weight PAH compound is causing the observed effects in fish (Hodson 1996).

Many people do not understand that invertebrates such as freshwater mussels have endocrine systems that are subject to the same disruption as in fish (deFur et al. 1999). In addition to studying endocrine disruption in fish, Environment Canada is also developing bivalve biomarkers as a complementary monitoring tool (Blaise et al. 2002, Gagne et al. 2000 2001a,b,c). Applied Biomonitoring has participated in these cooperative studies with Environment Canada by helping them sort, distribute, cage, and transplant *Elliptio complanata* upstream and downstream of a municipal effluent in Montreal during 1999, 2000, 2001, and will help in another study planned for May 2002. Other Canadian studies have documented physiological and biochemical changes associated with exposure to organic chemicals (Day et al. 1990).

5.4.2.2 Monitoring in Finland

Caged bivalves and indigenous fish populations have been used in Finland to monitor exposure and effects from freshwater pulp and paper mill effluents since approximately 1985 (Heinonen et al. 1986, Herve 1991, Herve et al. 1988 1996 Koistenen et al 1997, Makela et al. 1992, Pellinen 1994) and some studies have compared accumulation of organochlorines in mussels and SPMDs (Herve et al. 1995). However, most of the bivalve monitoring has been for exposure and most of the fish monitoring for effects. The freshwater unionid mussel (*Anodonta piscinalis*) has proven useful for this type of monitoring because of its ability to survive even under adverse conditions and its high uptake rates of lipophilic persistent pollutants. Altogether, 20 freshwater sites downstream of the pulp and paper industry are included as part of the National Monitoring Program of harmful substances. In studies where bioaccumulation of chlorinated hydrocarbons in caged mussels and natural fish populations have been compared, results have been variable depending on the specific compound being measured as well as the site (Rantio et al. 1996). Most effects monitoring in fish has paralleled the development of endocrine disruption endpoints similar to those developed and routinely measured in Canada (McMaster 2002).

5.4.2.3 Monitoring in the US

Some of the earliest and most innovative caged bivalve monitoring approaches were developed in the state of California and provided important information on the fate and effects of organochlorines associated with an ocean outfall (Green et al. 1986, Young 1982, Young and Heesen 1974, 1977, Young et al. 1976, 1977, 1978, 1988, 1991). One of those findings was a demonstration that contaminated sediments were the primary source of DDT and PCBs and not the water column exposure. This is extremely important relative to DEP being able to make the distinction between exposures associated with current mill discharges versus previously contaminated sediments.

The State of California has been using caged and indigenous marine mussels to monitor chemical exposure since 1977 and is the longest running mussel watch program in the world (Martin and Severeid 1984). Freshwater clams (*Corbicula fluminea*) were added to the monitoring program in the early1990s. The National Oceanic and Atmospheric Administration (NOAA) has been monitoring chemicals in marine mussel and oyster tissues since 1986 (O'Connor et al. 1994). Freshwater mussels (*Dreissena polymorpha*) were added to the program in the late 1990s. The San Francisco Estuary Institute administers a regional monitoring program that includes caged and indigenous freshwater and marine bivalves and has been collecting data since the mid 1990s (Gunther et al. 1999). The USGS conducts regular surveys of chemicals in natural populations of the freshwater clam (*Corbicula fluminea*) tissues at a number of locations throughout the US (Schmitt and Dethloff 2000). All of the above are exposure-based monitoring approaches and do not include effects measurements.

There are also several individual chemical monitoring studies conducted in the US for marine, estuarine, and freshwater environments (Brown et al. 1994). Some of these have been associated with monitoring marine outfalls in Massachusetts (Hall et al. 1995, Massachusetts Water Resources Authority 1993, 1994) and others with freshwater non-point sources (Pereira et al. 1996, Petreas et al. 1992).

In 1994, caged bivalve monitoring was required at a pulp and paper mill in southeast Alaska as part of their NPDES permit (EPA 1994). This resulted in measurements of dioxins and furans in the marine mussel (*Mytilus trossulus*) as well as five different growth metrics in 1996 and 1997 (EVS Consultants 1996 1997).

In addition to these integrated studies combining exposure and effects measurements, there is an increasing trend toward measuring bivalve biomarkers and histopathological changes in marine, estuarine, and freshwater environments associated with organochlorines (Cooper et al. 1989, Cristini and Cooper 1988). Some of these studies have been conducted by scientists at the University of Maine (Butler et al. 2001, Garling and Van Beneden 2001, Harring et al. 2001). USGS also has a program for monitoring freshwater bivalves in the vicinity of pulp and paper mill effluents (Kernaghan et al. 2001). The rarely used estuarine clam *Rangia cuneata* has also been used to monitor organochlorines (Harrel and McConnell 1995, Lunsford and Blem 1982).

Several studies have also compared the utility of caged bivalves and SPMDs for organochlorine accumulation in freshwater (Hayward et al. 1996, Prest et al. 1992) and marine environments (Hofelt and Shea 1997, Peven et al. 1996, Prest et al. 1995, Richardson et al. 2001). Some have even compared mussels, SPMDs, and fish (Bowker et al. 1995)

In addition to new trends in using freshwater bivalve biomarkers to assess potential exposure and effects, there is an increasing trend toward monitoring toxicity in adult, juvenile, and glochidial stages of freshwater unionid bivalves such as *Elliptio complanata* (Keller and Lydy 1997). This is important for DEP because some of these studies are demonstrating the sensitivity of freshwater bivalve toxicity testing. In several cases bivalves have been shown to be among the most sensitive test species and are driving the US EPA water quality criteria for some chemicals. Monitoring freshwater bivalves is also important because they are the most threatened and endangered species in North America (Naimo 1995).

5.4.2.4 Synthesis

A rationale has been presented for a risk assessment-based monitoring approach and examples given based on two of the longest running caged mussel monitoring programs that have focused on the measurement of organochlorines in mussel tissues associated with pulp and paper mills and other industries discharging them. A dichotomy has also been identified between using caged mussels and natural fish populations as indicators of exposure and indicators of effects. Measuring bioaccumulation and growth in caged mussels combines exposure and effects measurements as well as the advantage of experimental control of position, exposure period, and animal size range. Like fish, caged mussels also include the element of environmental realism. The caged mussel methodology has been placed in the context of an exposure-dose-response triad which integrates a variety of monitoring elements. This triad could include caged mussels, SPMDs, and fish. This combination could be used in a weight-of-evidence approach consistent with ecological risk assessment.

In a broader context, caged mussels could also become an integral part of the Surface Water Ambient Toxics (SWAT) Program. This program was developed to document the status and trends of toxic chemicals in Maine's surface waters and to assess the effects of these chemicals on human and ecological health. Caged mussels could fill a needed gap in this monitoring program that currently only includes effects monitoring (Davies et al. 1999). In the context of ecological risk assessment, the missing element is characterization of exposure. The current SWAT approach is based on characterization of effects. The problem with this approach is exemplified in any program that focuses on either exposure or effects; i.e., without the weight of evidence from risk assessment-based monitoring, there is greater uncertainty in the results. An important element in Maine biomonitoring is monitoring benthic community structure, but there is no link to help establish causality. Even without caged bivalves, this element could be improved by measuring bioaccumulation in indigenous bivalves. The SWAT program includes an innovative experimental field approach similar to caged bivalves by using rock-filled baskets, riffle bags, and cones, but no characterization of exposure is included (Davies et al. 1999).

The caged bivalve methodology is consistent with the DEP strategy of assessing water and sediment quality through integrated biomonitoring. Equal emphasis, however, should be placed on developing a program that is more risk assessment based and includes the measurement of biological effects and tissue chemistry. Controlled field experiments with approaches such as caged mussels and riffle bags provide an experimental element to complement the observational monitoring currently emphasized by DEP. The risk assessment-based approach helps characterize and understand processes controlling bioaccumulation and associated biological effects. Routine monitoring without these elements essential to ecological risk assessment cannot establish causality. The opposite dichotomy occurs in the Gulfwatch chemical monitoring program established by the Gulf of Maine council for mussel watch monitoring using the marine mussel *Mytilus edulis* (Environmental Quality Monitoring Committee 1998). This program measures only chemical exposure and not associated biological effects, although caged mussels have been proposed to facilitate the addition of growth and health endpoints as measured in the Kennebec River caged mussel pilot study with *Elliptio complanata*.

Finally, to place the monitoring issues in a perspective of a smaller scale, it is appropriate to consider the dedication in the DEP Biomonitoring Retrospective (Davies et al. 1999): *"This work is dedicated to the smallest creatures, existing at the edges of our awareness. Through them we glimpse intricate realities other than our own, and we are reminded to stay humble."* Similarly, those intricate realities of nature cannot possibly be fully appreciated with characterizing exposure and effects in a risk assessment-based monitoring program such as the one conducted here. It is not the purpose of this report to suggest that one biological indicator is necessarily superior to another, but rather that an integrated risk assessment-based strategy is the most appropriate. This integrated program could include caged mussels, SPMDs, and natural fish populations.