Chemical–environment interactions affecting the risk of impacts on aquatic organisms: A review with a Canadian perspective — interactions affecting vulnerability

Catherine M. Couillard, Simon C. Courtenay, and Robie W. Macdonald

Abstract: Environmental change can increase the vulnerability of aquatic species to toxic chemicals by challenging an organism's aptitude to respond to chemicals or to repair toxic injury or by modifying animal behaviours like migration or predation. On the other hand, xenobiotics may affect the capacity of aquatic species to adapt to environmental challenges that come with change (e.g., pathogens, temperature). Across Canada we have identified a number of circumstances where chemicals and environmental variability have likely worked together to affect vulnerability of aquatic organisms. For example in the Maritimes, exposure to municipal wastewater or bleached kraft pulp mill effluent altered immune function in bivalves and increased their risk of developing haemocytic neoplasia, a disease known to cause high mortality. Northwest Atlantic cod stocks have experienced large-scale changes in environment and exhibit marked seasonal cycles in energy reserves. The risk associated with subsequent redistribution of persistent chemicals in the body together with nutritional deficiency is presently under evaluation since it could affect the recovery of these endangered stocks. In the Great Lakes, the introduction of an invasive fish species, the alewife, modified the diet of salmonids, which led to a deficiency of the vitamin thiamine in eggs causing early mortality. Contaminants may interact with thiamine deficiency and thus critically impair recruitment of salmonids. Viewing the risks presented by toxic chemicals from the point of view of species vulnerability, offers managers opportunities to mitigate such risks, for example, through habitat, ocean and fisheries management. Further research is needed to develop biomarkers of vulnerability, identify most vulnerable life stages and populations, to understand the interactions between global environmental changes, nutritional status, pathogens and toxic chemicals, and to develop integrated approaches to manage vulnerability of aquatic ecosystems to toxic chemicals.

Key words: toxic chemicals, environmental factors, multiple stressors, vulnerability, fish.

Résumé : Les changements environnementaux peuvent accroître la vulnérabilité des espèces aquatiques aux produits toxiques en changeant les capacités des organismes à répondre aux produits chimiques, à réparer les dommages toxiques ou en modifiant leur comportement ou développement. D'autrepart, les xénobiotiques peuvent affecter la capacité des espèces aquatiques à s'adapter aux facteurs de stress qui accompagnent les changements environnementaux (ex. agents pathogènes, température). De part et d'autre du Canada, nous avons identifié un certain nombre de circonstances où les produits chimiques et la variabilité environnementale ont probablement intéragi et affecté la vulnérabilité des organismes aquatiques. Par exemple dans les Maritimes, l'exposition à des effluents municipaux ou de pâtes et papiers ont altéré la fonction immunitaire chez les bivalves et ont augmenté les risques de développement de néoplasie hémocytaire, une maladie pouvant causer de fortes mortalités. Les stocks de morues de l'Atlantique Nord Ouest ont été exposés à des changements environnementaux à grande échelle et démontrent des cycles saisonniers marqués dans leurs réserves énergétiques. Le risque associé à la redistribution subséquente des composés persistants dans le corps combinée à des déficiences nutritionnelles fait l'objet d'une évaluation car il peut affecter le rétablissement de ces stocks en danger. Dans les Grands Lacs, l'introduction d'une espèce de poisson invasive, le Gaspareau, a modifié la diète des salmonidés, ce qui a mené des déficiences d'une vitamine, la thiamine, dans les œufs entraînant des mortalités dans les jeunes stades de vie. Les contaminants peuvent interagir avec la déficience en thiamine et donc avoir un impact important sur le recrutement des salmonidés. L'examen des risques du point de vue de la vulnérabilité des espèces offre aux gestionnaires la possibilité de mitiger ces risques par exemple, par des interventions sur l'habitat, l'océan ou les pêches. Des travaux de recherche sont requis pour développer des biomarqueurs de vulnérabilité, identifier les stades de vie et les populations les plus vulnérables, pour comprendre les interactions entre les changements environnementaux globaux, le statut nutritionnel, les agents pathogènes

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C.M. Couillard.[1] Fisheries and Oceans Canada, Maurice Lamontagne Institute, 850 route de la mer, Mont-Joli, QC G5H 3Z4, Canada.
S.C. Courtenay. Fisheries and Oceans Canada at the Canadian Rivers Institute, Department of Biology, University of New Brunswick, P.O. Box 4400, Fredericton, NB E3B 5A3, Canada.
R.W. Macdonald. Fisheries and Oceans Canada, Institute of Ocean Sciences, P.O. Box 6000, Sidney, BC V8L 4B2, Canada.

[1]Corresponding author (e-mail: Catherine.Couillard@dfo-mpo.gc.ca).

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et les produits toxiques, et pour développer des approches intégrées pour gérer la vulnérabilité des écosystèmes aux produits toxiques.

Mots-clés : produits toxiques, facteurs environnementaux, agents stressants multiples, vulnérabilité, poisson.

Introduction

In Canada, health impairment has been demonstrated in aquatic species exposed to relatively high concentrations of contaminants in urbanized and industrialized areas. For example, in the Great Lakes, early life stage mortality causing reproductive failure in lake trout (*Salvelinus namaycush*) has been attributed to organohalogen compounds (Cook et al. 2003). Alteration in fish reproduction (smaller gonads) and energy storage (fatter, faster growing, with larger livers) has been observed downstream from some pulp and paper mills throughout Canada (Munkittrick et al. 1998; Wilson et al. 2000; Lowell et al. 2005). Fish liver tumours indicative of exposure to polycyclic aromatic hydrocarbons (PAHs) have been reported in the Great Lakes, St. Lawrence River and Estuary and in Vancouver Harbour, BC (Hayes et al. 1990; Couillard et al. 1997; Mikaelian et al. 2002; Stehr et al. 2004). Environmental regulations have focussed on reducing emissions of chemicals resulting in declines of the concentrations of certain persistent, bioaccumulative and toxic compounds (PBTs) such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzo-*p*-furans (PCDFs) in several environmental reservoirs (Yunker and Cretney 1996; Schneider et al. 2001). Improvement in fish reproductive parameters and community assemblages have been observed downstream from pulp and paper mills following pulp and paper effluent regulations (Munkittrick et al. 1997; Kovacs et al. 2002). In the Great Lakes, declines in the prevalence of liver tumours and increased longevity were documented in brown bullheads (*Ictalurus nebulosus*) after reduction of PAH concentrations in the sediments (Baumann and Harshbarger 1995). Despite regulation, fish continue to be exposed to complex mixtures of contaminants including PBTs remaining in the system and newer compounds such as pharmaceutical agents, brominated and perfluorinated compounds.

The number of listed endangered aquatic species increased in North America from 8 in 2000 (Bourdages and Labelle 2000) to 31 in 2004 (COSEWIC 2004). Furthermore, there is evidence of depletion of fish and fisheries, eutrophication (and associated hypoxia or anoxia), habitat alteration and invasions by non-indigenous species and pathogens, all of which can interact with contaminants (Adams 2005). At the 2002 World Summit on Sustainable Development in Johannesburg, South Africa, coastal countries agreed to implement an ecosystem-based approach to fisheries management that would incorporate interactions between fisheries and their supporting ecosystems. Effects of chemicals on aquatic species populations and the effects of natural or anthropogenic changes in the environment have traditionally been evaluated independently (Fig. 1, left hand side). This must change. Chemicals must be viewed as one of many factors with the potential to affect aquatic species populations, and the contribution of such chemicals relative to other factors should be assessed for each species and locality (Fig. 1, right hand side). Even if aquatic species can survive stressors one at a time, a combination of stressors can lead to unexpected impacts on populations. Throughout this paper we will use the term "toxic chemicals" recognizing that, these chemicals are, strictly speaking, *toxic* depending on dose, timing, species and life stage. In this paper, we consider the direct and indirect risks presented by chemicals to shellfish, echinoderms, crustaceans, fish and to marine mammals, which we hereinafter refer to generically as "aquatic species". We focus particularly on how factors like environmental variability and change interact with toxic chemicals and thus alter the risk of deleterious impacts on populations. To illustrate how these interactions can express themselves, we provide several case studies drawn from across Canada.

To assess risks from toxic chemicals appropriately requires consideration of both the *exposure* and *vulnerability*. In a companion paper (Couillard et al. 2008), we have examined how chemical exposure, itself, may be affected by environmental variability (see Table 1). Processes acting outside of the species of interest and affecting exposure were considered (Fig. 2*a*). Processes affecting the diet or the spatiotemporal distribution of the species of interest through an action on predator–prey–competitor, or on the habitat were also discussed. In this paper, we examine how environmental factors affect aquatic species vulnerability. Processes acting inside of the species of interest and affecting its susceptibility or its resilience are considered: toxicokinetics, toxicodynamics, capacity to repair damage or to recover. Processes affecting opportunities for exposure by causing neurobehavioural alterations or a change in the development rate in the species of interest are also considered (Fig. 2*a*).

Vulnerability of fish to toxic chemicals

Identification of subpopulations or populations of aquatic species particularly vulnerable to exposure and toxic effects of chemicals is an essential component of risk assessment. Vulnerability to chemicals is higher for organisms that are more sensitive, less resilient or more exposed to toxic chemicals as a result of their life history or behaviour (USEPA 2003; Turner et al. 2003). Individuals located in the same area with the same general concentrations of contaminants in the environment may exhibit different degrees of toxic injury. Under stable environmental conditions, *vulnerability* of organisms to chemicals varies among individuals and within an individual over time as a result of intrinsic factors (species, gender, age, development, maturation). Environmental variability (Table 1) will also affect the spatiotemporal pattern of vulnerability of aquatic organisms (Table 2; Fig. 2*b*). Consequently, assessing vulnerability becomes the task of assessing which populations are most vulnerable to toxic

Fig. 1. The single chemical approach to assessment of risk from toxic chemicals (left panel) compared to the cumulative risk approach (right panel) (modified from USEPA 2003).

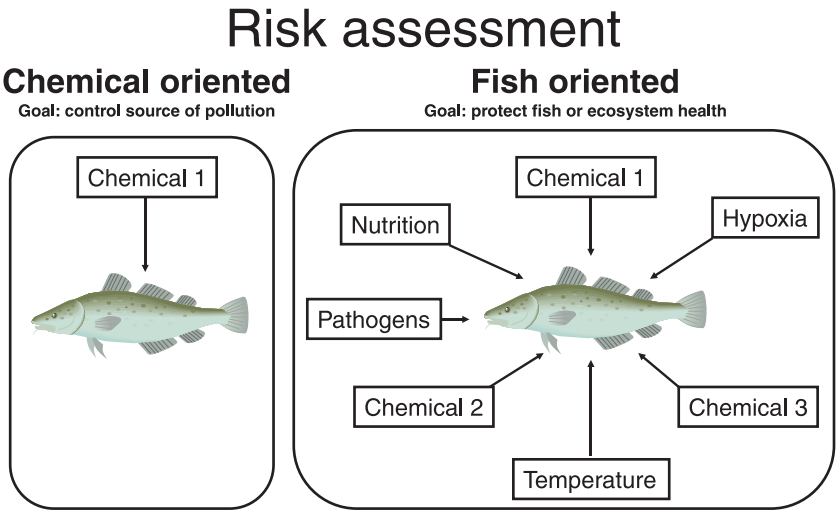


Table 1. Environmental stressors affecting fish populations.

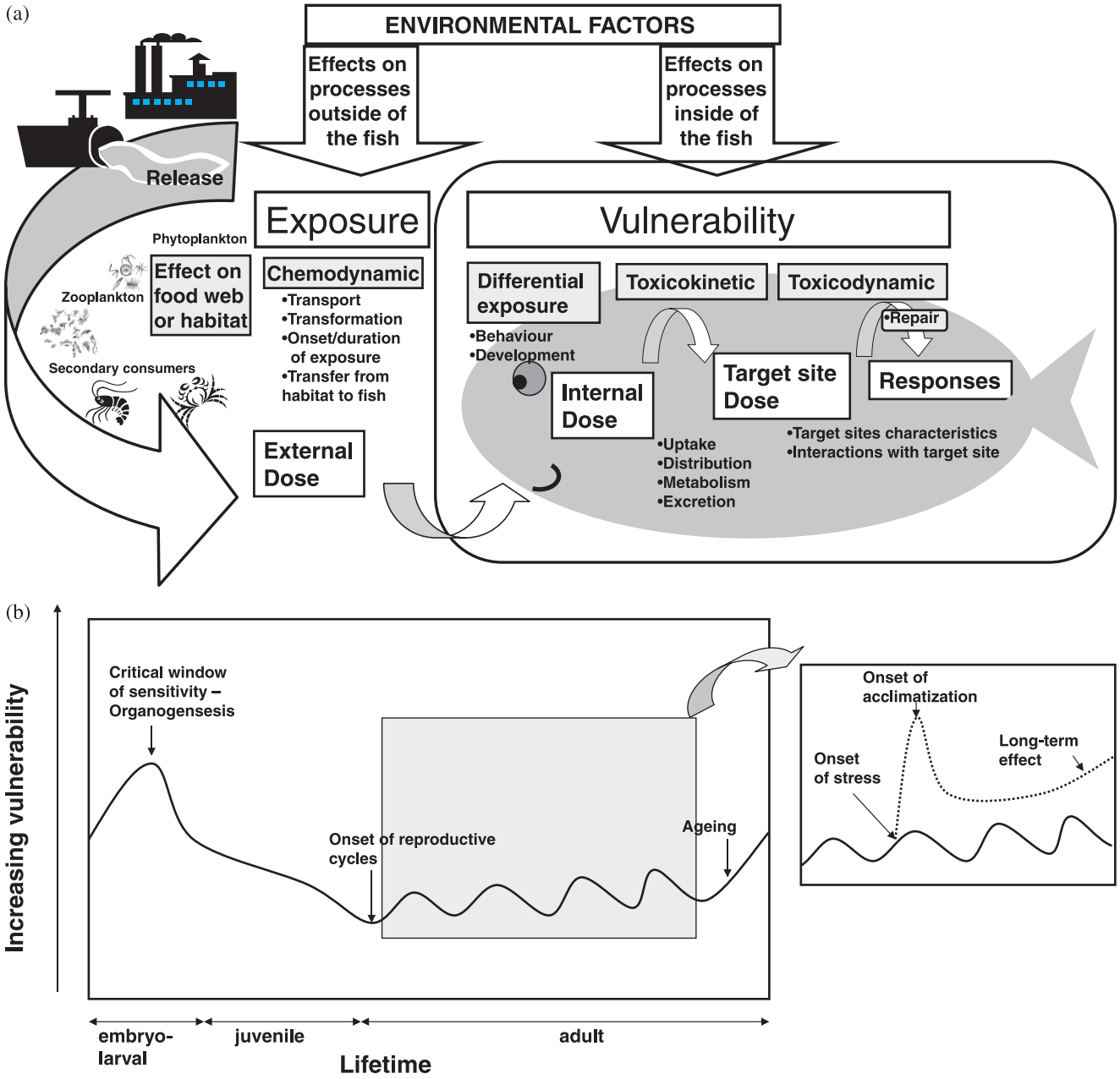
Environmental stressors	
Physicochemical	Toxic contaminants (point- and nonpoint sources)
	Climatic changes (temperature, UV, extreme events, ice cover)
	Alteration of hydrological systems (damming, dredging)
	Hypoxia
	Changes in salinity
	Dissolved gases, pH
	Turbidity, suspended sediments
	Sounds (seismic exploration, navigation)
Biological	Pathogen pollution, toxic algae
	Invasive species
	Intraspecific competition
	Interspecific competition
	Nutritional changes (food web alteration, depletion of food)
Habitat loss and fragmentation	Coastal or riverine development, wetland loss, erosion
Eutrophication	Leading to increased nutrients, hypoxia, proliferation of pathogens
Fishing	Leading to altered food web, changes in habitat, altered intraspecific and interspecific competition
Aquaculture	Leading to changes in habitat, introduction of pathogens, release of toxic chemicals, eutrophication, altered intraspecific and interspecific competition

chemicals, when, where and under what conditions. Surprisingly, little is known about the vulnerability to chemicals of most valued aquatic species populations in Canada and how this vulnerability relates to other environmental factors.

Vulnerability may be assessed over a wide range of ecological scales, from individual fish to population, metapopulations, communities, ecosystems or habitats, and geographical scales, from local to regional and landscape (Smith et al. 2000). The outcome of a toxic event may range from a local temporary effect to a regional extinction depending on the spatiotemporal configuration of the population in the landscape (patchy or continuous, presence of dispersal routes, configuration of habitat) and spatiotemporal distribution of toxic events (single local, multiple local, large-scale etc.). If a toxic event affects a local population that is a critical source of colonists for other local populations, recolonization may not occur after extinction, whatever its cause. Consequently, the effect of a toxic event can extend beyond the area of direct impact and last longer than the toxic persistence of the substance (Fahrig and Freemark 1995).

Life history is an important determinant of vulnerability of aquatic species populations to toxic chemicals. Populations that aggregate in small geographic areas at any stage of their life cycle such as spawning or migration are particularly vulnerable to a single toxic chemical spill. Polar benthic species retaining developing larvae in brood chambers instead of having pelagic larvae are more vulnerable to contaminant-induced impacts on local populations since they have reduced capacity for larval dispersion and recolonization from distant area (Chapman and Riddle 2005). Species that are slow to reach sexual maturity may not be able to recover easily from a toxic insult. Top predators, benthic species and fatty species are more vulnerable to exposure to PBTs than other species (Elliott et al. 2003). Long-lived top predators may be especially vulnerable because they accumulate and maintain PBTs within their bodies over a lifetime that may include early periods of exceptionally high exposure (e.g., Hickie et al. 2007). Marked seasonal cycles in energy reserves such as those observed in high latitude aquatic species make such species particularly vulnerable to the effects of persistent contaminants (see case study No 1). Diadromous species such as eels and salmonids are vulnerable to contaminants because of opportunities for exposure to a wide range of contaminants during their large-scale migrations in the freshwater and marine environment. Furthermore, they have multiple vulnerable life stages such as preadaptation to salinity changes, or migration requiring large

Fig. 2. (*a*) Effects of environmental factors on exposure (action outside the fish) or on vulnerability (action inside the fish) to toxic chemicals. (*b*) Dynamic changes in vulnerability of fish to toxic chemicals over their lifetime. Vulnerability of an aquatic organism changes over time as the organism ages and undergoes seasonal reproductive cycles (solid line). This pattern of vulnerability will vary among different chemicals. In a changing environment, vulnerability is further altered as the individual responds to or is injured by chemicals or other stressors (dotted line in inset, hypothetical example of one possible change in vulnerability in response to environmental stressors).



energetic reserves, and complex behavioural skills (Moore and Waring 1996; Robinet and Feunteun 2002).

The severity of toxic injury depends on duration and concentration of the active toxic compound(s) at the target site, the number of available target sites, and the capacity of the organism to recover. Toxicokinetic processes (uptake, distribution, metabolism, and excretion) control the concentration of the toxic compound at the target sites within an organism whereas toxicodynamic processes (characteristics of the target sites, interaction between chemical and target sites, repair mechanisms) modify the response to a particular concentration at the target sites (Heinrich-Hirsch et al. 2001). Endogenous or exogenous factors affect the sensitivity of aquatic species to toxic chemicals by altering one or more of these processes (Table 2; Fig. 2*a*).

Vulnerability to chemicals depends on species, gender, and genetic makeup. Rates and pathways of biotransformation and critical target sites vary among species and affect their sensitivity to chemicals. For example, English sole (*Parophrys vetulus*) show much higher prevalence of hepatic neoplasms than the closely related benthic starry flounder (*Platichthys stellatus*) when captured from PAH-contami-

Table 2. Examples of processes altered by environmental factors and affecting vulnerability of aquatic species to toxic chemicals.

Process altered	Environmental factors affecting this process	References
Opportunities for exposure		
Olfaction	Chemicals	(Scott and Sloman 2004)
Swimming and Foraging	Diseases, oxygen, temperature, chemicals	(Farrell et al. 1998; Barber et al. 2000; Birtwell et al. 2001; 2003; Scott and Sloman 2004; Riddell et al. 2005)
Migratory clock	Temperature, chemicals	(Cooke et al. 2004)
Development rate	Temperature, oxygen, salinity, chemicals	(Boeuf and Payan 2001; Devlin and Nagahama 2002; Weis and Weis 1987)
Toxicokinetics		
Uptake	Temperature, oxygen, chemicals affecting ventilation rate	(Heugens et al. 2001; Pierron et al. 2007)
	Infectious diseases or chemicals affecting permeability of gills, skin or chorion	(Black and McCarthy 1990; Hallare et al. 2005)
	Salinity affecting drinking rate or integument permeability	(Bervoets et al. 1995; Hall and Anderson 1995; Wood et al. 2004)
Tissue distribution	Climatic and environmental changes affecting metabolic rate and mobilization of fat reserves	(Debruyn et al. 2004, Couillard et al. 2005; Jorgensen et al. 2006)
	Parasites	(Sures 2001; Baudrimont and de Montaudoin 2007)
	Factors affecting partitioning in lipids (e.g., temperature)	(Van Wezel and Jonker 1998)
	Factors affecting growth rate and lipid content of the species of interest (temperature, oxygen, habitat degradation, invasive species, intensive fishing etc.)	(Boeuf and Payan 2001; Gordon 2005; Swanson et al. 2006)
Biotransformation and excretion	Temperature, oxygen, salinity affecting enzymatic activities	(Lange et al. 1998; El-Alfy et al. 2002; Prasch et al. 2004; Paterson et al. 2007)
	Photo-transformation of chemicals accumulated in tissues	Reviewed by (Diamond 2003) and (Pelletier et al. 2006)
	Diseases affecting bile duct or kidney function	(Kirby et al. 1995)
	Other toxic compounds (xenobiotic or natural toxins) affecting biotransformation and excretion	(Van der Oost et al. 2003; Smital et al. 2004)
Toxicodynamics		
Interactions with receptors	Factors causing endocrine disruption (e.g., hypoxia, parasites)	(Wu et al. 2003; Sures 2006)
	Temperature affecting mitochondrial function	(Cherkasov et al. 2006)
	Other chemicals	(Pollenz 2002)
	Genetic adaptation	Reviewed by (Wirgin and Waldman 2004)
Repair or ability to cope	Factors inducing nutritional or antioxidant deficiencies (changes in quality or quantity of food, invasive species, exposure to other toxic compounds, temperature etc.)	(Liess et al. 2001; Hennig et al. 2004; Brown et al. 2005*a*; Gordon 2005; Rooney 2007)
	Factors causing energy expenditure or stress (e.g., extreme environmental conditions, diseases)	(Lemly 1996; Sures 2006)

nated estuaries because English sole has a greater capacity to activate and lower capacity to detoxify PAHs compared to starry flounder (Collier et al. 1992). Male and female differ in vulnerability to toxic chemicals as a consequence of different lifestyles (diet, spatiotemporal distribution, behaviour), different body sizes and composition in sexually dimorphic species, and different hormones affecting opportunities for exposure, toxicokinetic and toxicodynamic (Gochfeld 2007). For example, annual exposure to the highly potent promotor 17-beta-estradiol during sexual maturation and lower detoxifying capacity, promote higher incidence of PAH-induced liver tumors in female flounder (*Platichthys flesus*) compared to male (Koehler 2004).

Vulnerability varies over the life of the organism as it undergoes physiological change associated with sexual maturation, annual reproductive cycle, smoltification, migration, ageing, and exposure to environmental stress factors (Fig. 2*b*). Organisms are most vulnerable to chemicals at critical life stages. Early life stages are generally more sensitive to chemicals than adult stages because they tend to absorb higher concentrations of chemicals and have a lower capacity to detoxify (Weis and Weis 1987; Black 1988; Andersen et al. 2003). Early life stages also have specific developmental processes such as gonad differentiation, which are very sensitive to endocrine disruptors (Devlin and Nagahama 2002), and intense cellular multiplication rates, which make them susceptible to carcinogens (Black 1988; Devlin and Nagahama 2002). Finally, these stages are less capable of avoiding contaminated areas than juveniles or adults.

Effects of environmental factors on vulnerability to toxic chemicals

In a companion paper (Couillard et al. 2008), we have described how major changes in environmental and climate regimes occurring as a consequence of human activity can

affect exposure of aquatic species to toxic chemicals. In this paper, we describe how some of these changes affect their vulnerability to toxic chemicals. Concentrations of chemicals sublethal to organisms living at optimal conditions may become lethal at suboptimal conditions. Furthermore, rapid environmental change, to which organisms are acclimating, will exacerbate the toxic effects of chemicals (Rattner and Heath 1995).

Global environmental and climate changes

Global changes in temperature and precipitation is leading to modifications in water level, degree of eutrophication, ice regime, stratification, acidification, ocean currents and extreme events (Marcogliese 2001; Macdonald et al. 2005). Most aquatic species are ectothermic and their physiology and response to chemicals are markedly affected by temperature. Water temperature affects metabolic rate, locomotion and feeding which in turn affect rates of uptake, bio-activation, detoxification, and elimination of chemicals (Birtwell et al. 2001, 2003; reviewed by Heugens et al. 2001). For example the activity of cytochrome P4501A (CYP1A), an enzyme metabolizing some aromatic hydrocarbons to more toxic forms, depends markedly on water temperature in some fish species (Lange et al. 1998). All kinetic and thermodynamic processes are altered by temperature: e.g., accessibility and sensitivity of the target sites and partitioning of narcotic compounds in different body compartments (Van Wezel and Jonker 1998). For example, PCB elimination was 2.6 times to 7.5 times slower in winter than in summer in yellow perch (*Perca flavescens*) dosed with 2000 ng g^{-1} wet weight of a mixture of PCBs and allowed to depurate under an ambient temperature cycle typical of northern latitudes. Temperature effects are more important for persistent and more toxic congeners of PCBs that will increase in proportion of the body burden over successive cold water periods (Paterson et al. 2007).

Higher temperatures generally increase toxicity indicating that climate change may have greater impacts on aquatic populations in polluted areas (reviewed by Heugens et al. 2001). For example, the mean survival times of freshwater clam, *Pisidium amnicum*, exposed to 100–200 μg pentachlorophenol (PCP) L^{-1} was 5 to 15 times shorter in summer (19 °C) than at winter temperature (5 °C) in Finland, and uptake of PCP was greater in summer (Heinonen et al. 2001). The 24 h LC50 of cadmium (Cd) for *Daphnia magna* was 20-times lower at 32 °C (>2000 μg L^{-1}) than at 10 °C (<100 μg L^{-1}). Two processes explain the observed interaction: an increased uptake of Cd (toxicokinetic) and an increased sensitivity to a given tissue concentration of Cd (toxicodynamic). This latter mechanism became more important as temperature increased (Heugens et al. 2003). In oysters (*Crassostrea virginica*), a more severe mitochondrial dysfunction was observed at higher temperatures, which could not be explained by differential Cd accumulation indicating the importance of toxicodynamic interactions (Cherkasov et al. 2006; Lannig et al. 2006). Low temperature can occasionally increase toxicity. For example, the 48 h LC50 of Cd for zebrafish (*Danio rerio*) embryo was 10-times lower at 21 °C (4.75 mg L^{-1}) than at 33 °C (46.8 mg L^{-1}). Alteration of the chorion at low temperature causing increased permeability to Cd and cold-induced stress could contribute to this observation (Hallare et al. 2005). Exposure to extreme temperature reduces growth and impairs swimming leading to reduced capacity of aquatic species to avoid exposure to toxic concentrations of contaminants (Gordon 2005).

Temperature also alters the duration of early life stage development and the duration and timing of critical life stages most sensitive to toxic chemical injury (Devlin and Nagahama 2002). By modifying water temperature and (or) availability of prey, climate change can affect the duration and (or) the severity of the seasonal fasting period experienced by many high latitude aquatic species (Walther et al. 2002; Macdonald et al. 2005). Seasonal fasting releases and (or) redistributes fat-soluble chemicals stored within the fish and thus increases concentrations at target sites and risk (Debruyn et al. 2004; Couillard et al. 2005; Maule et al. 2005; Jorgensen et al. 2006; see case study No 1). Prolonged fasting can induce a deficiency in antioxidant vitamins or in selenium (Se), which further contributes to enhance sensitivity to certain chemicals (Hennig et al. 2004). For example, Se deficiency enhances the toxicity of Hg (reviewed by Rooney 2007).

Modifications in ice regime and ozone layer depletion increase the intensity of ultraviolet (UV) at sea surface (Macdonald et al. 2005) suggesting greater vulnerability in the future. Warming and acidification cause a reduction of dissolved organic matter that enhances UV penetration in fresh water (Schindler 2001). UV can cause the activation of some petroleum PAHs accumulated in fish tissues into more toxic metabolites and reactive oxygen species (photosensitization). A twofold to greater than 1000-fold increase in toxicity has been reported in the presence of UV compared to standard laboratory lighting conditions (Barron and Ka'aihue 2001; reviewed by Diamond 2003 and Pelletier et al. 2006). However, few studies on photoenhanced toxicity have been conducted under environmentally realistic conditions (McDonald and Chapman 2002; Barron et al. 2005; Diamond et al. 2006). A synergetic effect between TBT (120 ng L^{-1}) and natural ambient UV-B radiation was demonstrated in a planktonic assemblage collected from the St. Lawrence Estuary (SLE) exposed in microcosms (Sargian et al. 2005). Field observations revealed that the mortality rate of the Antarctic amphipod *Paramoera walkeri*, a species clinging underneath the ice during winter and spring, increased at concentrations of metals much lower than the concentrations that were determined to be toxic in the laboratory. Laboratory experiments revealed that the combined effects of UV-B radiation and food shortage increased by more than 30-fold the sensitivity of these amphipods to copper (Cu) toxicity (Liess et al. 2001).

Biological invasions

The rate of introduction of invasive species is increasing in Canada as a result of climate change and global trade (Vander Zanden 2005). Invasive species can affect the vulnerability of aquatic species to toxic compounds by altering the quantity or quality of food available. For example in the Great Lakes, the introduction of alewives (*Alosa pseudoharengus*), a fish species rich in thiaminase, has induced thiamine deficiency in eggs of piscivorous fish (Brown et al. 2005*a*) and enhanced susceptibility of early life stages to or-

ganochlorine compounds (Fitzsimons 1995) (see case study No 2). Invasive species can also affect vulnerability of aquatic species by producing substances altering defence mechanisms. For example, the tropical green alga *Caulerpa taxifolia* introduced in the Mediterranean in the mid-1980s produces substances inhibiting the multixenobotic resistance (MXR) mechanisms in native species such as the marine sponge *Geodia cydonium* or the mussel *Mytilus galloprovincialis* (Schröder et al. 1998; Smital et al. 2004). Consequently, chemicals normally removed by the MXR are accumulated in the organisms and may induce toxic effects at environmental concentrations generally not considered harmful (Bard 2000).

Pathogen pollution

Nutrient enrichment, climate change, reduced water circulation, and shifts in the distribution of either hosts or pathogens can favour pathogen proliferation (Marcogliese 2001; Schuwerack et al. 2007). Pathogens are released in municipal and agricultural effluents or introduced by infected invasive or cultured species. Exposure of aquatic species to novel pathogens or to higher density of existing pathogens increases the risk of development of infectious epidemics. The effect of disease on vulnerability to toxic chemicals is well documented in human and laboratory rodents (Boelsterli 2003) but poorly known in aquatic species. Infectious diseases altering oxygen uptake by the gills, water uptake by the gills and skin, the metabolizing capacity of liver or renal clearance or tissue repair, enable toxicity from some chemicals. Infectious agents causing proliferative lesions could potentiate the effects of carcinogenic agents as documented for biliary parasites promoting liver tumors in fish exposed to PAHs in the Great Lakes (Kirby et al. 1995). Furthermore, pathogens can induce stress or endocrine disruption increasing the susceptibility of aquatic species to chemicals having similar mechanisms of action (Sures 2006). Infectious diseases can alter foraging efficiency, habitat selection, and swimming performance (reviewed by Barber et al. 2000) and thereby change exposure to anthropogenic chemicals. Diseases can induce general stress and low appetite leading to nutritional deficiencies and these factors also contribute to increase sensitivity of diseased organisms to chemicals.

Parasites can affect the bioaccumulation of contaminants in their host. In some instances, parasites accumulate higher concentrations of chemicals than their host. For example, the acanthocephalon *Pomphorhynchus laevis* accumulates higher concentrations of lead than its host, the chub (*Leuciscus cephalus*) and the presence of the parasite reduces the concentration of lead in the fish intestine and liver and the risk of toxic effects (Sures 2001, 2006). In other cases, parasites promote the accumulation of toxic compounds in their host. For example, Cd concentrations are higher in cockles (*Cerastoderma edule*) infected with a digenean parasite than in uninfected cockles, without an associated elevation in protective metallothioneins (Baudrimont and de Montaudoin 2007).

Hypoxia

Hypoxic zones are developing worldwide in water bodies as a consequence of eutrophication, high temperature, and alterations in water circulation (Paerl 2006). Discharge of nutrient-rich domestic sewage, agricultural fertilizers and pulp mill effluents to the aquatic environment leads to a greater production of particulate organic matter in the water column or on the sea bed and greater use of oxygen for its degradation (Gray et al. 2002). Hypoxia increases vulnerability to some chemicals in some aquatic species by increasing the ventilation rate and uptake, in which case acclimation can play an important role. The relationship between ventilation rate and uptake of chemicals is well established for hydrophobic chemicals, which are absorbed by diffusion through the epithelial lipid bilayers, but appears to be more variable among species and exposure concentrations for metals transported by carriers. For example, hypoxia-induced hyperventilation increased Cd accumulation by a factor of two in shrimp *Palaemon longirostris* exposed experimentally to 0.2 and 0.5 μg Cd L^{-1} at low salinity (0–2 ppt) (Pierron et al. 2007). In contrast, Zn uptake was not altered in carp (*Cyprinus carpio*) exposed under hypoxia (25%), despite an enhanced ventilation rate and a three-times higher toxicity under hypoxia than normoxia (96 h LC50 of 55 and 149 μmol Zn L^{-1}, respectively; Hattink et al. 2006).

Hypoxia can also affect metabolic transformation of chemicals and interaction with receptors. For example, it decreases the induction of CYP1A mRNA in zebrafish (*Danio rerio*) embryos exposed to tetrachlorodibenzo-*p*-dioxin (TCDD, 0.0125–4 ng mL^{-1}), and decreases the potency of TCDD to cause edema (Prasch et al. 2004). Hypoxia causes endocrine disruption in fish and therefore could interact with endocrine disruptive chemicals (Wu et al. 2003). For example, hypoxia (0.8 mg O_2 L^{-1}) affects sex differentiation and development leading to a male-dominated population in zebrafish. It downregulates genes involved in the synthesis of sex hormones and increases the testosterone/estradiol ratio (Shang et al. 2006). Hypoxia is expected to augment the toxicity of chemicals that block cellular respiration or reduce respiratory gas exchange by increasing metabolic rate and oxygen demand (Gordon 2005). However, pre-treatment with PCP (20 μg L^{-1}), a mitochondrial uncoupler agent, increased oxygen consumption but ameliorated the impact of hypoxia on swimming performance in sockeye salmon *Oncorhynchus nerka* suggesting the presence of other unknown mechanisms of action (Farrell et al. 1998).

Organisms exposed to diurnal hypoxia associated with eutrophication have little time for adaptation and thus, may be more vulnerable to toxicants (Rattner and Heath 1995). One example of a complex interaction between exposure factors is the photo-enhanced toxicity of anthracene in bluegill sunfish (*Lepomis macrochirus*), which is mitigated by low or high dissolved oxygen concentrations. Hypoxia may reduce photoinduced toxicity which is oxygen-dependant while at high oxygen levels the ventilation volume is lowest and uptake of anthracene is reduced (McCloskey and Oris 1991).

Pre-exposure to contaminants

Organisms that have been exposed to contaminants may become sensitized to further exposure through additive or synergetic interactions. On the other hand, given sufficient time, genetic adaptation or physiological acclimation to chronic exposure may occur, thus reducing population sensi-

tivity. Physiological mechanisms of acclimation include altered inducibility of metabolizing enzymes or enhanced production of antioxidants, stress proteins, metallothioneins, and MXR proteins. With chronic exposure, toxicodynamic factors such as the number of receptors available and the repair mechanisms may change (reviewed by Van der Oost et al. 2003). Interactions among chemicals are difficult to predict because of species-specific variation, poor understanding of mode and mechanisms of action, and influence of dose or of unidentified toxicity modifying factors including environmental factors (McCarty and Borget 2006). For example, co-administration of two coplanar PCBs, PCB126 and PCB77, to Atlantic tomcod (*Microgadus tomcod*) was expected to produce greater CY1A mRNA induction than administration of PCB126 alone, but it did not (Yuan et al. 2006).

Over time, populations may adapt to chronic exposure via genetic selection of resistant individuals. In highly contaminated sites along the Atlantic coast of North America, populations of mummichog (*Fundulus heteroclitus*) and Atlantic tomcod have become resistant to the toxic effects of aromatic hydrocarbon contaminants, including PCBs, PCDDs, PCDFs, and PAHs. In controlled laboratory studies, these populations exhibit resistance to early life-stage toxicities and expression of CYP1A in response to these contaminants (reviewed by Wirgin and Waldman 2004). For these populations, the toxic effects predicted by laboratory tests will not be mirrored in the field. However, genetic adaptation to contaminant stress often entails a cost in ecological performance and genetic diversity with the consequence that these adapted populations may become more vulnerable to other environmental stresses (Hebert and Murdoch Luiker 1996; Van Straalen and Timmermans 2002). For example, mummichog from a highly contaminated site on the Elizabeth River (Virginia, USA) are more resistant to the toxicity of Elizabeth River sediments than are offspring from a reference site but are less resistant to other stressors, both anthropogenic (photoenhanced toxicity) and natural (hypoxia) (Meyer and Di Giulio 2003).

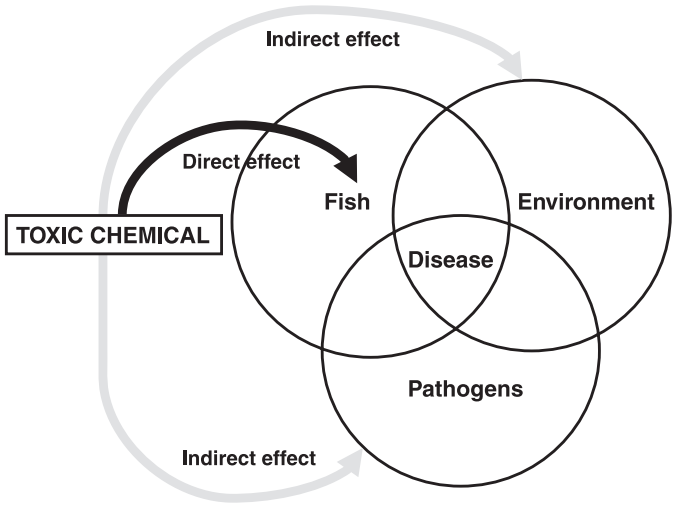
Effect of chemicals on the vulnerability of fish to environmental factors

Exposure to chemicals can alter the ability of aquatic species to survive in sub-optimal environmental conditions by impairing metabolic, hormonal or behavioural adaptive responses. Frequency of extreme environmental conditions is projected to increase with global warming (Weaver 2003) and the capacity of aquatic species to survive an extreme event could be impaired by prior chemical exposure. The influence of chemicals on vulnerability of fish to environmental stress has been demonstrated, mostly in laboratory controlled experiments, in a limited number of model fish species.

Chemicals can increase maintenance energy expenditures as a consequence of energetic costs associated with detoxification, elimination and repair or by causing stress (Beyers et al. 1999). Some contaminants alter the amount of energy available to the organisms by affecting foraging behaviour, digestive processes or by inducing gill damage. Others directly alter metabolic processes (e.g., mitochondrial function; Couture and Rajotte 2003) or their hormonal control (e.g., thyroid; Verreault et al. 2007). All of these chemicals increase the needs of metabolic energy and consequently, less energy is available for acclimation to other environmental stresses such as gill parasites, food limitation or extreme physicochemical water conditions (Lemly 1996). Certain high latitude fish species exhibit reduced activity and food intake during winter. These fish may not be able to respond to the increased metabolic demand induced by contaminant exposure by increasing feeding. If these demands persist, body condition declines and the fish may eventually die. For example, juvenile bluegill (*Lepomis machochirus*) exposed to Se in water (4.8 $\mu g\ L^{-1}$) and in diet (5.1 $\mu g\ g^{-1}$ dw) for 180 days at low temperature (4 °C) experienced higher mortality rate, gill damage, higher oxygen consumption and lower condition factor than fish exposed at the same temperature but lower concentrations of Se (0.8 $\mu g\ g^{-1}$ dw in diet and 0.8 $\mu g\ L^{-1}$ in water; Lemly 1993). This metabolic distress in cold water has been called "winter stress syndrome" (Lemly 1996). However, this syndrome was not observed in young-of-the-year northern pike (*Esox lucius*) and burbot (*Lota lota*) collected before and after winter in lakes receiving a metal mining effluent likely because these species could feed and maintain their energy during winter despite chemical stress (Bennett and Janz 2007). More field studies are needed to identify which aquatic species are most vulnerable to the 'winter stress syndrome' and under which conditions.

Immunotoxic chemicals impair the ability of aquatic organisms to resist infectious agents, therefore putting at risk fish populations facing a microbial challenge (Faisal et al. 1991; Zelikoff et al. 2000) (Fig. 3). Blue mussels (*Mytilus edulis*) caged for 6 months near untreated municipal wastewater or bleached kraft pulp mill effluents showed a significantly greater chance of developing haemocytic leukemia, a fatal disease, than mussels caged elsewhere within the same harbour (St-Jean et al. 2005) (see case study No 3). Fish captured in PAH-contaminated water have high rates of fin, skin, and gill lesions due to opportunistic infections suggestive of immunosuppression. Immunotoxicity and increased susceptibility to bacterial pathogens have also been demonstrated in fish exposed to PAHs in laboratory experiments (Reynaud and Deschaux 2006). In Newfoundland and Quebec, some parasites were more abundant in fish captured in the vicinity of pulp and paper mills compared to fish from reference sites (Couillard et al. 1995; Billiard and Khan 2003), however, gradients in nutrient loading may also have contributed to the observed changes. In the St. Lawrence River near Montreal, Quebec, high abundance of the digenean parasite *Plagioporus sinitsi* was observed in spottail shiner *Notropis hudsonius* at sites receiving urban and industrial effluents. Furthermore, the parasite infection appeared to have a greater effect on fish condition at polluted sites (Thilakaratne et al. 2007). The results from extensive, long-term monitoring programs show that some diseases decreased whereas others increased in fish in the southern North Sea and that, among other factors, contaminants may play a role in the temporal changes recorded in disease prevalence (Hylland et al. 2006). Alteration of immune function was demonstrated in seals ingesting fish contaminated with organochlorine compounds and an epidemic of phocine dis-

Fig. 3. Effect of toxic chemicals on incidence of infectious diseases in fish. Toxic chemicals may have a direct effect on the fish immune system by reducing resistance to infectious disease. Toxic chemicals may also have indirect effects on the environment causing stress in fish or affecting the proliferation or survival of pathogens.



temper was most severe in harbour seals (*Phoca vitulina*) from the highly contaminated Baltic and North Seas (Ross et al. 1996; Van Loveren et al. 2000). Immunotoxicity linked to accumulation of persistent organic and inorganic contaminants in tissues is believed to be responsible for the high rate of infection by opportunistic pathogens observed in the St. Lawrence beluga whale (*Delphinapterus leucas*) population (DeGuise et al. 1995; case study No 4).

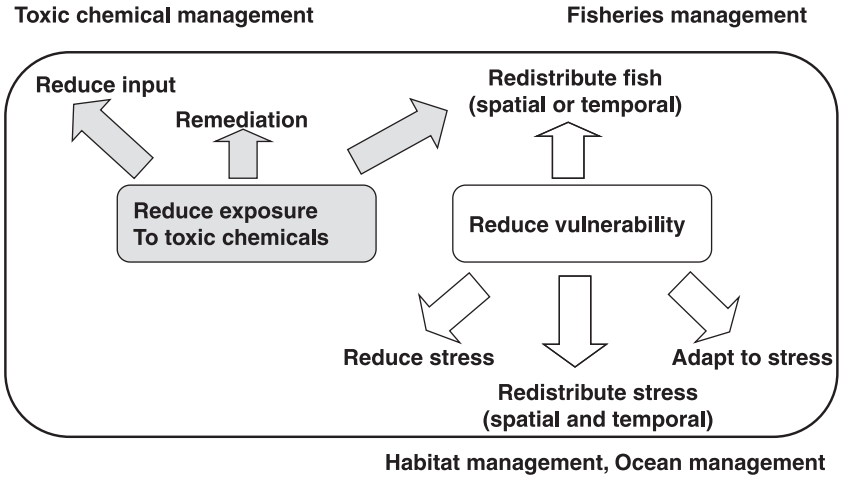
Chemicals can reduce the ability of fish to adapt to abrupt salinity changes by targeting gills, kidney or skin or the hormonal system involved in osmoregulation. Exposures to toxicants and to other environmental stresses do not need to be coincident to interact (Heugens et al. 2001). For example, pre-exposure of Atlantic salmon smolts to sublethal concentrations of the pesticide atrazine (1 μg L^{-1} for 7 days) manifests itself later, when smolts transfer to saltwater, through osmoregulatory dysfunction and mortality (Waring and Moore 2004). Exposure of salmon smolts to sublethal concentrations of nonylphenol or aluminium in their freshwater natal environment has also been associated with osmoregulatory impairment and reduced growth and survival in the marine environment (Fairchild et al. 2002; Kroglund and Finstad 2003). Certain chemicals disrupt the process of adaptation to hypoxic environments by targeting gills or red blood cells or by inducing a dysfunction of the glycolytic processes (Rattner and Heath 1995; Kraemer and Schulte 2004). Some chemicals such as metals and pesticides can disrupt the hypothalamo-pituitary-interrenal axis making fish less resilient to acute stress such as environmental variation. For example, 1+ yellow perch (*Perca flavescens*) captured in a metal contaminated lake had impaired capacity to respond to an acute stress challenge and high whole body Cd, Cu, and Zn concentrations (Gravel et al. 2005).

Chemicals can also alter the capacity to detect or respond to prey or predators raising vulnerability to nutritional or predation stress (Scott and Sloman 2004). For example juvenile brook trout (*Salvelinus fontinalis*) exposed to Cd (0.5 and 5 μg L^{-1}) exhibited lower activity, a lower capture efficiency, and a preference for less mobile prey (Riddell et al. 2005). This modification in feeding behaviour could impair growth, condition and exposure to toxic chemicals. Sufficient exposure to endocrine disrupting chemicals impairs neuroendocrine mechanisms controlling timing of reproduction, with the consequence that spawning becomes unsynchronized with optimal conditions (temperature, availability of food) for larval growth and survival (Scott and Sloman 2004). For example, exposure to low concentrations of pesticides such as diazinon (1 μg L^{-1} for 120 h) affects the capacity of male Atlantic salmon (*Salmo salar*) to detect pheromones emitted by females and may impair spawning success (Moore and Waring 1996). Many chemicals including pesticides and metals have been shown to alter thermoregulatory behaviour in fish and could lead to selection of habitats less favourable for development, growth or reproduction (Gordon 2005). Some toxicants may be detected and avoided by olfaction, even when doing so prevents access to an optimal spawning area (Kitamura and Ikuta 2000).

Integrated monitoring and management

Environmental factors (Table 1; Fig. 2*a*) can markedly influence both exposure and vulnerability of aquatic species to chemicals resulting in variations of risk of toxic effects on spatial and temporal scales. Current monitoring programs are designed to avoid the effects of environmental factors, seen as confounding variables, on reported results. Often, only adult fish, sometimes only males, in the most abundant size class are sampled to reduce inter-individual variability and sample size. Fish are sampled outside of their reproductive and migratory periods and at one time of the year (generally summer). This strategy clearly ignores the influence of environmental factors and underestimates risks from toxic chemicals since fish are not sampled at the time and site of maximal exposure and (or) vulnerability. Environmental variability should, instead, become a research focus since it is a major factor determining the impact of chemicals on local populations. Field and laboratory studies should be designed to identify the conditions of maximal exposure and vulner-

Fig. 4. Integrated management of the risk related to toxic chemicals (adapted from Smith et al. 2000).



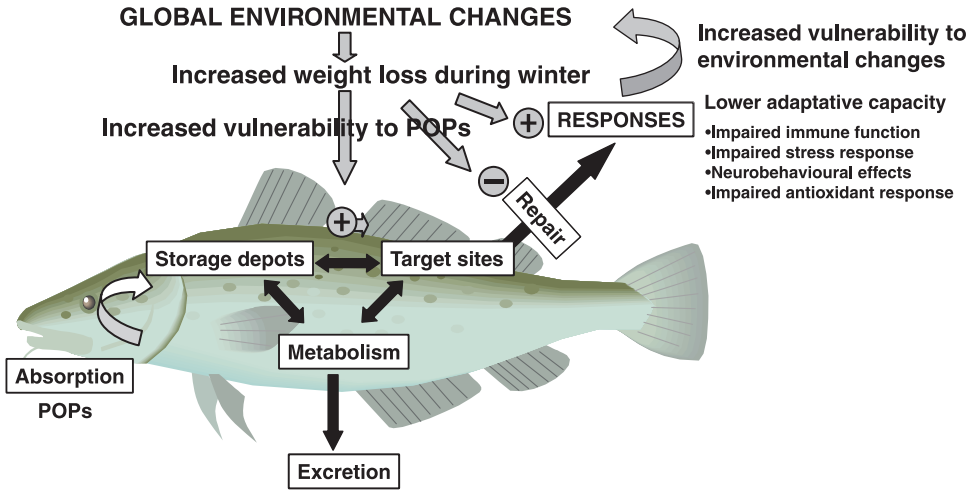
ability to toxic chemicals and this information should be used to design efficient risk management and sensitive monitoring programs adapted to the unique characteristics of each site and species.

Chemical monitoring programs must be integrated with other monitoring programs evaluating physical or biological stressors to assess exposure to multiple stressors and effects. Health of the target species, food availability and habitat status should be evaluated using a suite of bioindicators and biomarkers to identify direct or indirect mechanisms linking exposure to effects (Adams 2005). The most vulnerable receptors (populations, habitats or ecosystems) and the factors affecting their vulnerability should be identified (Smith et al. 2000). Biomarkers of vulnerability may be used to indicate the inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance (e.g., genetic factors, changes in receptors; Van der Oost et al. 2003). The processes of adaptation of populations to chemicals should be further investigated, particularly in species used as sentinels in environmental monitoring programs (Wirgin and Waldman 2004). Multistressor experiments should be conducted to demonstrate the link between the responses of the vulnerability biomarkers and population-level impacts.

Chemical exposure may be assessed by measuring concentrations of chemicals and their metabolites or tracers in tissues. Where practicable, concentrations of the active form of chemicals in blood or target organs should be measured. Correlation between concentrations and specific biomarker responses should be conducted (e.g., Myers et al. 1991; Levin et al. 2005). Because chemical analyses address only a small subset of the toxic chemicals to which fish are exposed, caution should be used when attempting to relate chemical concentrations with biological effects over a large geographic scale since the composition of the mixture of chemicals/stressors may vary spatially. The presence of other chemicals and stressors can modify the toxic response associated with exposure to a particular level of chemical. In vitro bioassays detecting particular toxic responses (e.g., induction of cytochrome P450, estrogenic activity, embryotoxicity) can be applied to tissue extracts and used as a complement to chemical analyses to assess the capacity of chemical analyses of individual compounds to predict the toxicity of the mixture accumulated in tissues (Cizmas et al. 2004; Denison et al. 2004).

Management of the risks from toxic chemicals should consider the potential to manage exposure (through chemical and habitat management) and vulnerability of populations, habitats or ecosystems through fisheries and habitat management (Couillard et al. 2008) (Fig. 4). Environmental factors may be manipulated to reduce exposure to chemicals (e.g., adding nutrients to promote biodegradation of petroleum oils; Lee et al. 1993). The factors responsible for the variations in vulnerability of population to chemicals need to be identified on a site-specific and species-specific basis since these factors could be managed to reduce the risk of toxic impact. For example, the release of pathogens in municipal or agricultural effluents could be controlled in the habitats of marine mammals highly contaminated with PBTs and likely immunosuppressed (see case study No 4). Surveillance and regulation to prevent introduction of thiaminase-rich invasive prey in water bodies would reduce risk associated with the enhanced vulnerability of thiamine deficient predator species to some xenobiotics. Restoration of food resources (e.g., prey species) through habitat or fisheries management is important at polluted sites to improve nutrient status and capacity to cope with toxic chemicals. The ability of aquatic species to cope with Hg contamination in hydroelectric reservoirs could be enhanced by adding Se in the habitat; however, the dose of Se should be carefully monitored since excess Se can cause toxicity (Mailman et al. 2006). Vulnerability could also be controlled by reducing opportunity for exposure through control of spatiotemporal distribution. Dams can be installed to prevent access of diadromous species to contaminated sites. Alternatively, engineered structures or sensory stimuli can be used to favour access to less contaminated sites (Schilt 2007). Finally, vulnerability could be reduced at a population level by maintaining dispersal routes, protecting critical sources of colonists (e.g., through marine protected areas) and managing exposure to stressors over the whole range of geographic distribution to avoid cumulative impacts (Fahrig and Freemark 1995).

Fig. 5. Conceptual model of the interactions between cold water temperature, increased seasonal fluctuations in energy reserves and persistent organic pollutants (POPs) in northern Atlantic cod. Starvation leads to mobilization of lipids with increase in lipid concentrations of POPs at target sites, to nutritional deficiencies causing impaired antioxidant defence mechanisms and to increased vulnerability to POPs. Toxic effects on immune, endocrine, and neurological systems can reduce the capacity of cod to adapt to further environmental changes (e.g., exposure to pathogens or to acute stress; Jorgensen et al. 2006 for Arctic charr) and could impair cod recovery.



Case studies

Across Canada we have identified several examples where exposure to chemical contaminants appears to have interacted with environmental factors affecting fish or marine mammal vulnerability. Below, we describe several of these cases to illustrate how such interactions may operate.

Case study 1. Northern Atlantic cod: possible interaction between seasonal fasting and toxic chemicals

Atlantic cod (*Gadus morhua*) stocks of the Northwest Atlantic, including populations off southern Labrador, eastern Newfoundland and northern and southern Gulf of St. Lawrence declined in the late 1980s and eventually collapsed in the early 1990s. Overfishing has been a popular culprit, but several observations are inconsistent with fishing-only effects and it is clear that other factors have played a role (Dutil et al. 2003; COSEWIC 2005). Northern cod stocks are poorly productive compared to other stocks (Dutil and Brander 2003) and their production on a per capita basis was observed to decline during the collapse. Large-scale changes in the environment throughout the Northwest Atlantic may explain some of these observations and the failure of these stocks to recover following protective measures. In poorly productive stocks, individual fish exhibit a marked seasonal cycle in energy reserves which was aggravated in the mid 1980s when a decline of water temperature was observed (Fig. 5). Growth takes place in summer (Mello and Rose 2005) whereas feeding activity declines in the fall resulting in a decline in condition in the winter. Cod spawn in the spring at which point they become emaciated. This is the period when they are most vulnerable to disease (Dutil et al. 2006) and mortality (Dutil and Lambert 2000). Declining growth rates and declining energy reserves were associated with lower fecundity and swimming capacity, and increased natural mortality in poorly productive stocks (Dutil and Lambert 2000; Lambert and Dutil 2000; Martinez et al. 2003).

On the basis of the information currently available, contaminants cannot be excluded as contributors to the cod recovery problem. As a long-lived top predator, Atlantic cod are vulnerable to exposure from persistent organic contaminants such as PCBs. Approximately 90% of the total load of these contaminants is located in the fatty liver (Bernhoft et al. 1994). Cod from the Gulf of St. Lawrence receive significant inputs of persistent organic contaminants, presumably as a result of long range transport in water and in the atmosphere (Hellou et al. 1992, 1993; Hellou and Payne 1993; Gobeil et al. 1997; Lebeuf et al. 1999). Whereas PCB concentrations have declined during the past two decades, other persistent lipophilic compounds such as PBDEs have been increasing in biological tissues from the Gulf of St. Lawrence (Lebeuf et al. 2004) and elsewhere in Canada (Ikonomou et al. 2002; Ross 2006). Many compounds are endocrine disrupting chemicals that interfere with the normal functioning of endogenous steroids and may affect growth, reproduction, and immune response (Vos et al. 2000). Cod are exposed to complex mixtures of contaminants. There remains relatively little information on the toxicity of individual contaminants and even less on the effects of complex mixtures of contaminants to Atlantic cod.

New information obtained in other high-latitude fish species indicates that a marked seasonal cycle in energy reserves, such as is observed in high latitude cod stocks in the Northwest Atlantic, would make them particularly vulnerable to the effects of PBTs. As fat is mobilized, the body distribution of contaminants changes and concentrations in target tissues increases leading to an increased risk of toxicity. In 2 year old Arctic char (*Salvelinus alpinus*) given a single oral dose of 1 to 100 μg Aroclor 1254 g^{-1} and submitted to fasting for several months at low water temperature, PCBs were redistributed from lipid-storing tissues such as muscle to target tissues such as liver and brain. These elevated toxic concentrations were associated with altered CYP1A activity, increased susceptibility to infectious diseases, altered acute

stress response, and seawater preadaptation (smoltification) disturbances. Effects were observable at environmentally realistic concentrations of as low of 0.1 μg g^{-1} muscle wet weight at the time of the stress treatment, and field studies are underway to demonstrate the environmental relevance of these changes (Jorgensen et al. 2002*a*, 2002*b*, 2004; Aluru et al. 2004; Maule et al. 2005; reviewed by Jorgensen et al. 2006). In large 2–5 year old emaciated Atlantic tomcod (*Microgadus tomcod*) sampled in spring in the Saint Lawrence Estuary (SLE), hepatic PCB concentrations increased as lipid contents decreased and high PCB concentrations (0.5–1.0 μg g^{-1} liver wet weight) were related to suppression of the activity of a CYP1A enzyme, suggestive of hepatocellular injury. Suppression of CYP1A activity was not observed in large-sized tomcod from two less contaminated estuaries, also sampled in spring and having similar low hepatic lipid content but 2.5 to 4 times lower PCB concentrations. Further studies are needed to evaluate if hepatocellular injury is associated with impacts on growth, survival, and (or) reproduction of the SLE tomcod population (Couillard et al. 2004, 2005). In the SLE, Atlantic cod have hepatic concentrations of PCBs (1.3 ± 0.5 μg g^{-1} liver wet weight) of the same magnitude than those reported in tomcod (Lebeuf et al. 1999).

Thus, the combination of unfavourable environmental condition and seasonal fasting could potentiate the effects of persistent toxic contaminants in northern Atlantic cod. A transient increase of contaminant concentrations in target tissues above toxicity thresholds during the fasting season may cause detrimental health effects (such as immunotoxicity and increased rates of fatal infectious diseases) and reduce the capacity of northern cod population to recover (Fig. 5). Enhanced natural mortality rates of unknown origin are currently observed in northern cod populations (Sinclair 2001). To detect these processes, target tissues in cod should be sampled at the most vulnerable time of the year, at the end of the fasting season. This has not been done because the results of the studies conducted by fisheries scientists on the bioenergetic changes observed in the northern Atlantic cod stocks were not considered in the design of the toxic contaminants monitoring studies conducted in these stocks. Multidisciplinary experimental and field studies are needed to assess the significance of these processes in northern cod. The lipid reserve dynamics, the associated changes in the tissue concentrations of persistent pollutants and antioxidants should be investigated in Atlantic cod collected in the field at various seasons or exposed to fast in environmentally-realistic laboratory experiments. The relationships between these changes and the rate of potentially lethal infectious diseases should be investigated. Changes of concentrations of chemicals in the brain and effects on behaviour (e.g., predator–prey interactions) should also be assessed. Toxicity threshold concentrations in target tissues for population-relevant responses should be determined in fasted and fed fish held in the laboratory at different temperatures (e.g., Jorgensen et al. 2006). Mathematical models should be developed to predict changes in chemical concentrations in target tissues under different environmental scenarios and to predict the impact of these changes on growth, survival or reproduction (e.g., Kelly et al. 2007).

Case study 2. Early mortality syndrome (EMS) in Great lakes salmonids

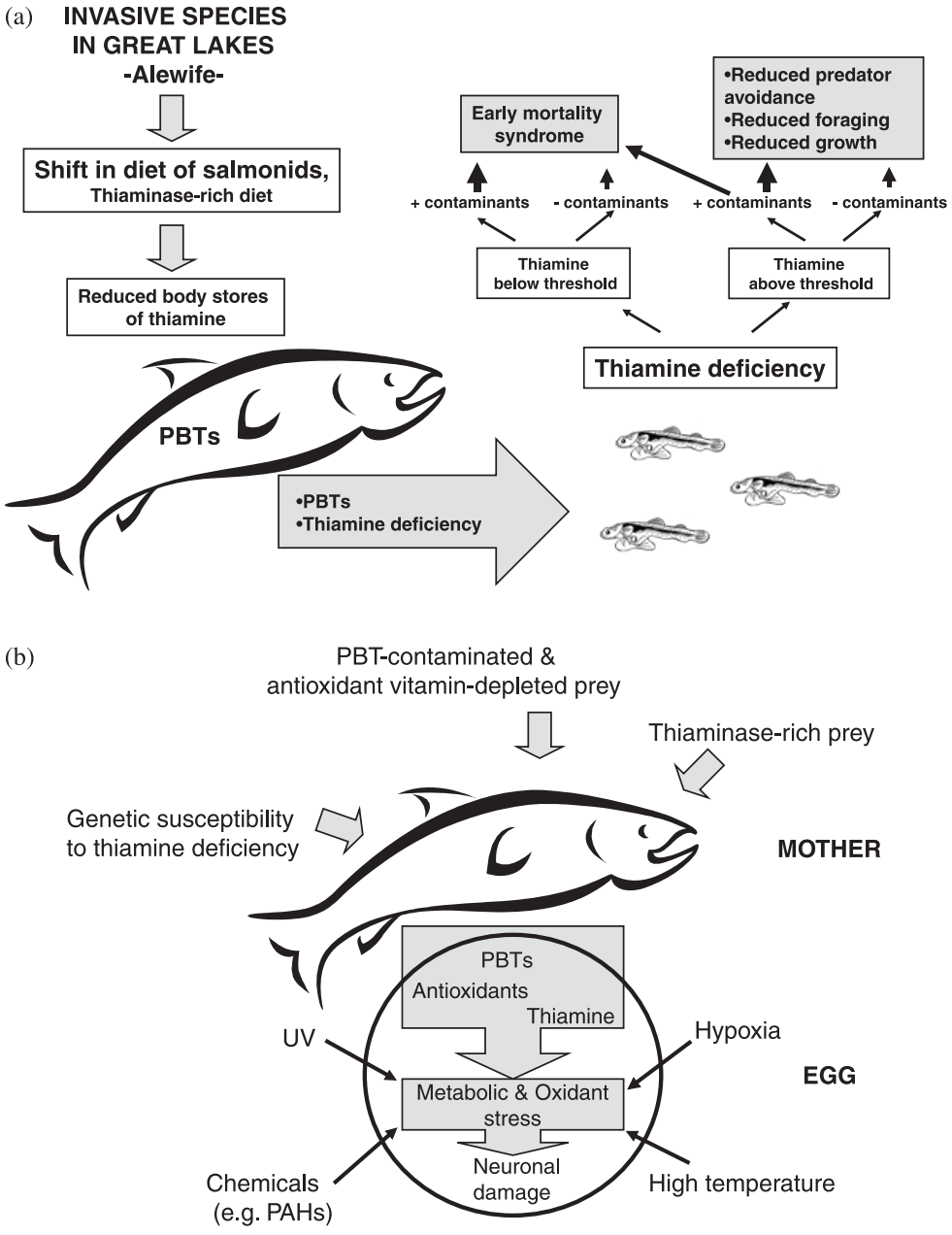
The early mortality syndrome (EMS) is characterized by mortality of offspring of Great Lakes salmonids between hatch and first feeding related to low concentrations of thiamine (vitamin B1) in the eggs. Clinical signs include loss of equilibrium, a spiral swimming pattern, lethargy or hyperexcitability and hemorrhages (Marcquenski and Brown 1997). From 1968 to present, it affected several species of salmonids (coho salmon (*Onchorhynchus kisutch*), chinook salmon (*Onchorhynchus tshawytscha*), steelhead trout (*Onchorhynchus mykiss*), brown trout (*Salmo trutta*), and lake trout (*Salvelinus namaycush*) in Lakes Michigan and Ontario and to a lesser extent in Lakes Huron and Erie. In the mid-1990s, mortality rates due to EMS rose dramatically in coho salmon in Lake Michigan hatcheries (up to 60%–90%) and became more variable (Wolgamood et al. 2005). Early mortality syndrome represents a significant problem for Great Lakes fish stocks because it affects the hatchery stocking program and it has the potential to impair the recruitment of indigenous fish stocks (Fitzsimons et al. 2001).

What causes EMS? Thiamine deficiency is a key factor in the development of the disease. Field studies show consistent association between low concentrations of thiamine in the eggs or fry and the development of the symptoms in wild salmonids (Brown et al. 2005*b*). Treatment of the eggs or yolk sac fry with thiamine can successfully alleviate the symptoms (Fitzsimons et al. 2001). The disease has been induced experimentally by treating healthy offspring with thiamine antagonists or in offspring hatched from females fed diets containing thiamine-degrading enzyme (thiaminases) activity (Honeyfield et al. 2005). Shifts of the diet of Great lakes salmonids from prey low in thiaminase to thiaminase-rich prey, such as alewive (*Alosa pseudoharengus*), appear to be the most important cause of the thiamine deficiency. Thiaminase activity in prey fishes varies among species, seasons, years and size (condition) and this variation may contribute to the observed year-to-year fluctuation of EMS (Fitzsimons et al. 2005; Tillitt et al. 2005).

Are factors other than thiamine contributing to the development of EMS? A large proportion of coho or Chinook salmon eggs developing EMS have thiamine concentrations below a threshold of 1.7 nmol g^{-1} egg and it is clear that at this level, thiamine deficiency alone can induce EMS. However, at thiamine concentrations above this threshold, the incidence of EMS is variable and not related to variations in thiamine concentrations. These findings suggest that factors other than thiamine deficiency may contribute to EMS (Wolgamood et al. 2005). Fry with high contaminant burden and (or) with low concentrations of antioxidant vitamins may be more vulnerable to thiamine deficiency.

Early mortality syndrome is most prevalent in the more contaminated lower Great Lakes. A fry mortality syndrome very similar to EMS, called M74, also occurs in a contaminated area in the Baltic sea. Both EMS and M74 are more common in contaminated ecosystems suggesting an interaction between contaminants and thiamine deficiency. The concentrations of certain dioxin-like compounds (coplanar PCBs and PCDFs) in the muscle of Baltic salmon (*Salmo salar*) in the River Simojoki on the north-eastern coast of the Gulf of Bothnia increased from 1988 to 1992 as the

Fig. 6. Hypothetical interaction between contaminants and thiamine deficiency. (*a*) Toxic contaminants may potentiate the effect of low level thiamine deficiency. At thiamine concentrations below a certain threshold, thiamine deficiency alone can induce early mortality syndrome (EMS). At thiamine concentrations above this threshold, persistent bioaccumulative toxic compounds (PBTs) could make the larvae more vulnerable to thiamine deficiency and contribute to EMS. (*b*) Possible mechanisms of interaction between thiamine deficiency, PBTs and other environmental stressors. In the mother, exposure to PBTs could reduce dietary consumption of thiamine, alter its gastrointestinal absorption or its pathways of utilization or degradation. Exposure to PBTs can also induce a deficiency in antioxidants (e.g., vitamin A). In larvae, chemicals and other stressors causing oxidative or metabolic stress, and nutritional deficiencies can increase the requirements for thiamine and the risk of EMS.



prevalence of M74-induced mortality increased (Vuorinen et al. 1997). Until now in the Great Lakes, no association has been found between concentrations of organochlorine contaminants and EMS-related mortality of swim-up fry (Honeyfield et al. 1998). Nevertheless, sublethal concentrations of contaminants might potentiate the effects of low-level thiamine deficiency (Fig. 6). Interactions between thiamine and chemicals including PCBs (Pelissier et al. 1992), ethanol (Pires et al. 2001), and arsenic (Nandi et al. 2005) have been demonstrated in humans and laboratory mammals. Several mechanisms have been proposed for these interactions. Chemicals can alter foraging behaviour and reduce dietary consumption of thiamine, impair its gastrointestinal absorption, decrease the activity of enzymes responsible for thiamine utilization or increase the activity of phosphatases involved in the breakdown of the active form of thiamine

(Mulholland et al. 2005). Many environmental contaminants cause oxidative stress and therefore increase the requirements for thiamine and induce thiamine deficiency (Gibson and Zhang 2002; Fig. 6). Reduced thiamine concentrations can cause neuronal damage through impaired energy metabolism or oxidative stress. Chemical-induced oxidative stress inhibits mitochondrial dehydrogenases and thiamine treatment attenuates the reactive oxygen-mediated inhibition of these enzymes and the subsequent loss in mitochondrial function and neuronal death (Sheline and Wei 2006). It has also been suggested that thiamine may bind metals covalently and facilitate their excretion (Nandi et al. 2005).

Antioxidant vitamin content of the eggs may change as a function of exposure to toxic contaminants or of the diet (quantity and composition) and may potentially interact with thiamine deficiency (Brown et al. 2005*a*). In a Lake Ontario study, female lake trout whose offspring developed EMS had lower vitamin E (an antioxidant) concentrations compared to those whose offspring did not. Lower total carotenoid concentrations were measured in the eggs of the EMS group (Palace et al. 1998). Lower carotenoids have also been associated with the presence of M74 in Baltic salmon (Lundström et al. 1999). However, in Great Lakes salmon, changes in carotenoid concentrations are not always associated with occurrence of EMS.

Thus, considerable progress has been made in the last decades on the pathogenesis of EMS in Great Lakes salmonids. While EMS is now unequivocally linked to low concentrations of thiamine in the eggs, more work is needed to understand the complex and variable interactions among contaminants, thiamine deficiency and antioxidant vitamins. Understanding these interactions is required to make the link between low-level thiamine deficiency, potential impacts on recruitment of salmonids at different sites in the Great Lakes, and different levels of contamination. Interactions may only be observed in certain species and certain sites since they may not occur at thiamine concentrations below certain species-specific thresholds for neurological alteration or at contaminant concentrations above certain thresholds for toxic effects (i.e., oxidative stress in the brain). The effects of environmental changes such as climate variability, eutrophication, and invasive species on the food webs and consequently, on the content of the diet in chemicals and antioxidants should be investigated. The genetic or environmental factors affecting the gastrointestinal absorption of thiamine in fish should be studied. The cumulative effects of chemical burden, deficiency in thiamine or other antioxidants, temperature, oxygen and UV on the overall level of oxidative stress should be examined in early stages of fish. For certain fish species, cDNA microarrays are available and could be used to reveal molecular pathways of effects and mechanisms of interactions (e.g., Vuori and Nikinmaa 2007). Evaluation of toxicity thresholds in critical life stages of different fish species under different conditions with respect to thiamine deficiency or concentrations of other antioxidant vitamins is required to model these complex interactions and look for them in a complex and variable field situation.

Case study 3. Shellfish disease – contaminants interaction

The health of aquatic animals has become a focus for research in Canada and particularly in the coastal zone. Concerns have been generated both by the steady increase in human activities in bays and estuaries, and by the coincident decline in coastal fisheries. It is often suspected that declines in populations of wild or cultured animals are related, at least in part, to exposure to toxic contaminants. However, demonstrating all the steps linking exposure to a toxicant, through physiological changes and death of individuals, to ultimate changes in population characteristics is a difficult task. Consequently, few good examples exist, but one to which Canadian scientists have contributed concerns haemocytic leukemia in bivalve molluscs (Fig. 7).

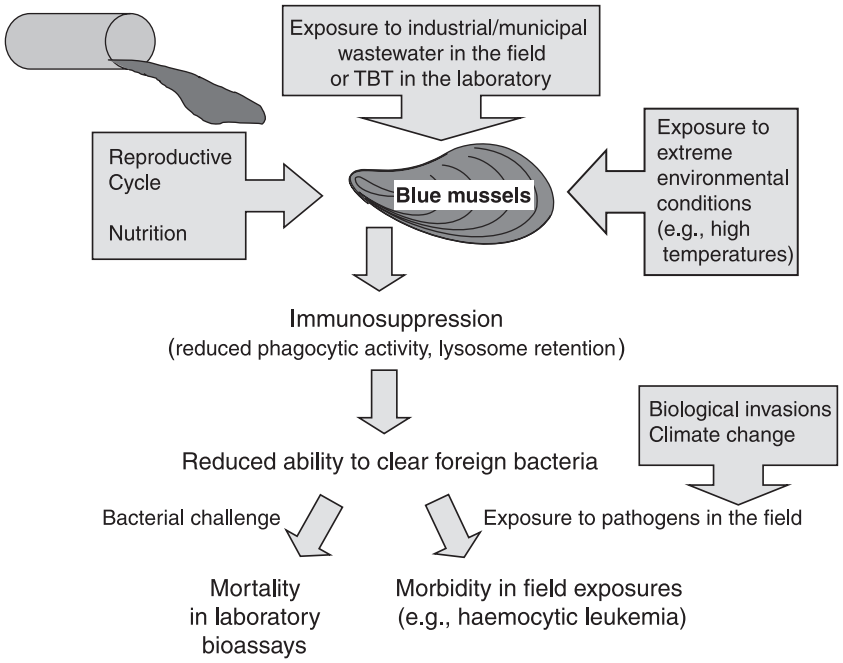
Haemocytic leukemia is a fatal disease of many molluscs around the world including both the Pacific and Atlantic coasts of Canada (Bower 1989; McGladdery et al. 2001). The cause of the disease is unknown but speculations have included exposure to toxicants and infectious agents. A recent study demonstrated that blue mussels (*Mytilus edulis*) caged for 6 months near untreated municipal wastewater or bleached kraft pulp mill effluents showed a significantly greater chance of developing haemocytic leukemia than mussels caged elsewhere within the same harbour (St-Jean et al. 2005).

Interestingly, mussels caged at these same sites in Pictou Harbour, NS, showed significant changes in immune function [reduced phagocytic activity (PA) and lysosome retention (LR)], a reduced ability to clear a foreign bacterium (*Listonella anguillarum*), and increased mortality during bacterial challenge tests (St-Jean et al. 2003*a*). Another study showed reduced levels of PA in wild mussels from the industrialized Pictou site relative to the relatively pristine Richibucto estuary (Mayrand et al. 2005). Furthermore, this immunosuppression was at least partially reversible. When caged in Richibucto for 9 days, PA in Pictou mussels rose to the levels seen in Richibucto mussels, though bacterial clearance rates were still depressed, demonstrating partial recovery of haemocyte responses.

These field results are consistent with results of earlier laboratory studies with tributyltin (TBT) and its degradation product dibutyltin (DBT). Marine animals, including blue mussels, in the coastal zone are exposed to TBT primarily through its use as an antifoulant in boat paint (St-Jean et al. 1999). In addition to affecting developmental and reproductive processes, the butyltins have been shown to modulate immune function in bivalve molluscs. Laboratory bioassays revealed dose-dependent changes in cell membrane injury, PA, LR, and haemocyte count with both TBT and DBT (St-Jean et al. 2002*b*). Most interestingly, these changes were associated with a reduced ability to clear a foreign bacterium. Furthermore, these immunomodulations occurred at concentrations of TBT and DBT found in coastal marine environments of Canada (up to 83 ng Sn g^{-1} dry weight in sediments and 671 ng Sn g^{-1} dw in mussel tissues; St-Jean et al. 1999) and at the very low concentrations found to disrupt reproduction in neogastropod mollusks (1 ng Sn L^{-1}; St-Jean et al. 2002*a*).

More recently, Akaishi et al. (2007) studied immunological responses, histopathological responses, and disease resistance in blue mussels exposed to treated and untreated municipal wastewater. Mussels exposed to 50% or 100% untreated sewage in the lab for 21 days showed a number of

Fig. 7. Blue mussels (*Mytilus edulis*) exposed in Pictou Harbour Nova Scotia to either bleached kraft mill effluent or untreated sewage show similar modulation of the immune system to mussels exposed in the laboratory to tributyltin. The ability of cells to sequester and neutralize foreign bodies through phagocytosis and lysosomal degradation is compromised. These changes are associated with a reduced capacity to clear invasive bacteria in the laboratory and reduced survival following bacterial challenges and an increased incidence of haemocytic leukemia in the field (Mayrand et al. 2005; St. Jean et al. 2002*a*, 2002*b*, 2003*a*, 2005). Other environmental stressors (e.g., extreme physiochemical conditions) and physiological cycles could interact with chemicals to induce immunosuppression and increase vulnerability to pathogens. Exposure to novel pathogens could increase as a consequence of biological invasions and climate change and increase vulnerability to immunotoxic chemicals.



changes in immune function including decreased PA. When challenged with bacteria mussels died at a higher rate than mussels exposed to lower concentrations or no sewage. Similarly, mussels exposed for 90 days to (presumably) low concentrations of untreated sewage in the field showed neither depressed PA nor reduced performance in bacterial challenge tests. Interestingly, those mussels caged near the untreated sewage outfall actually showed elevated PA, relative to mussels caged in clean sites, which mussels caged near a treated sewage outfall did not. These results suggest that low concentrations of untreated sewage were still immunomodulative and that this property was removed by secondary treatment of wastewater.

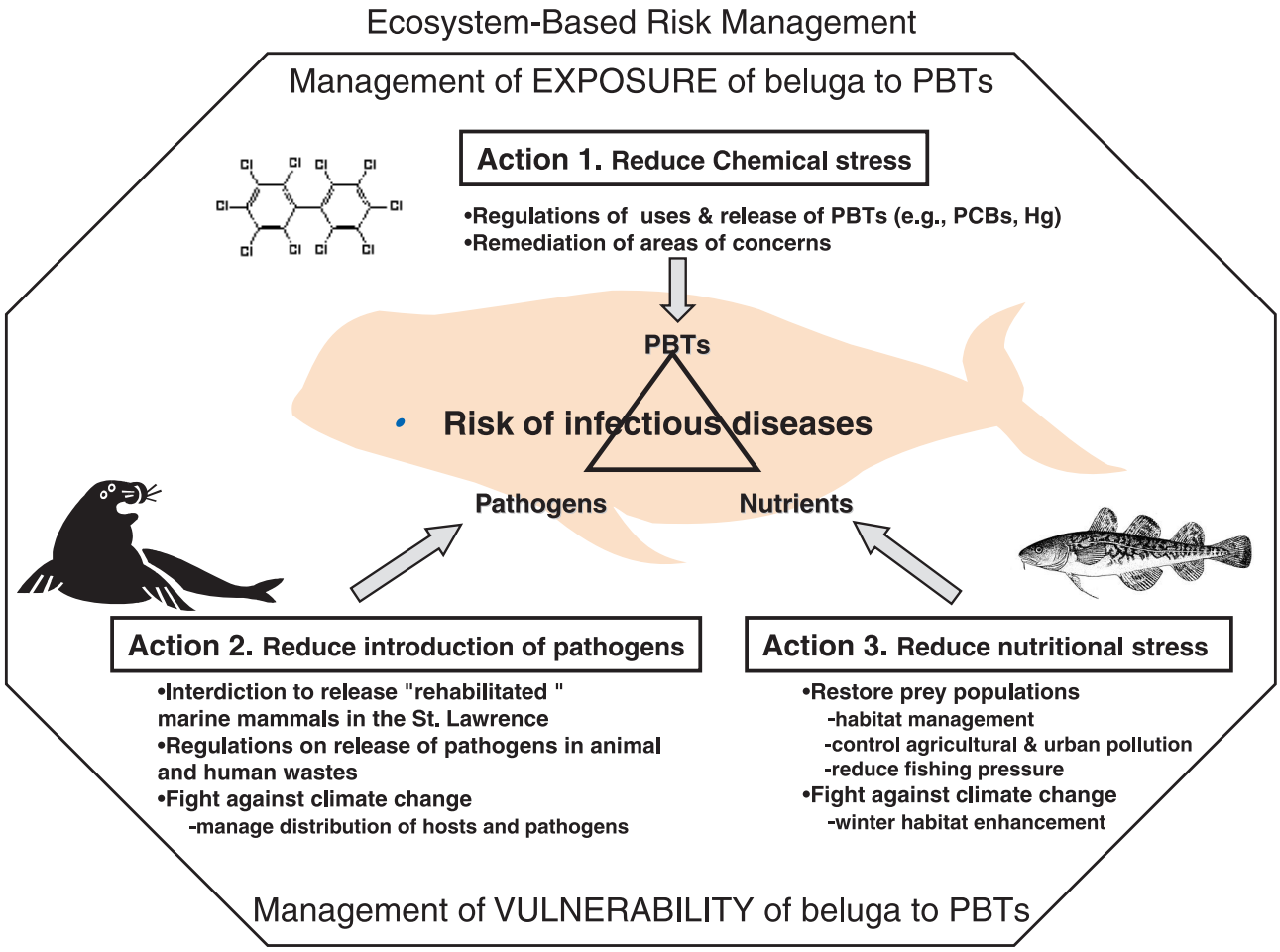
Taken together, the results of these field and laboratory studies indicate that exposure to a toxicant, whether a single chemical or anthropogenic effluent, can cause relatively predictable changes in immune characteristics of bivalves, which are in turn associated with impaired function, and may be associated with increased rates of morbidity and mortality due to infectious diseases (Fig. 7). Development of these indicators is continuing and, because of their replicability and ecological relevance, immunological endpoints are already finding application in aquatic environmental effects monitoring (EEM) programs for pulp and paper mills (e.g., Stantec Consulting Ltd. 2004), municipal wastewaters (e.g., St-Jean et al. 2004; GVRD 2006), and metal mines (e.g., Voisey's Bay NFLD, Dr. S. St-Jean, Jacques Whitford Environmental Ltd, Moncton NB, pers. comm.).

However, complete acceptance of caged bivalves for industrial EEM programs will require the development of measures of energy storage, equivalent to condition factor (K) and hepatosomatic index (HSI) in finfish, and energy investment in reproduction equivalent to gonadosomatic index (GSI) in finfish (St-Jean et al. 2003*b*). In addition, data collection will have to be stratified with respect to sex and reproductive stage as these can affect immune endpoints. Additional areas requiring further research include comparisons of responses among different bivalve species because blue mussels are not found in all locations and depths and responses may differ between species (e.g., Blaise et al. 2002). We also need to better understand how bivalve responses relate to the more commonly measured responses in finfish (St-Jean et al. 2003*c*). As well, it would be helpful to develop shorter term bioassays than the present 60–90 day caging regimes. Immunological endpoints have been shown to vary quantitatively and even qualitatively (i.e., changing direction) over time confounding comparisons among studies of caged bivalves that differ in duration and comparisons of caged to wild bivalves. Finally, research is required into the development of disease because the leukemogenic agents within industrial and municipal effluents have not yet been identified (St-Jean et al. 2005).

Case study 4. Management of the risk presented by toxic chemicals in the St. Lawrence Estuary beluga population

Located downstream from the highly industrialized and contaminated Great lakes, St. Lawrence River, and Saguenay River, the SLE beluga are exposed to a complex mix-

Fig. 8. Different options to manage the risk related to potentially immunotoxic persistent bioaccumulative (PBTs) compounds in the St. Lawrence Estuary beluga.



ture of contaminants. This long lived marine mammal species and top predator is vulnerable to exposure to persistent contaminants. In addition, females download contaminants to their offspring during lactation, ensuring intergeneration contamination. High concentrations of PCBs, organochlorine pesticides, heavy metals and more recently PBDE have been measured in SLE beluga tissues (Muir et al. 1996; Lebeuf et al. 2004). Beluga are also exposed to carcinogenic PAHs as indicated by the detection of benzo[*a*]pyrene diolepoxide, a DNA adduct originating from benzo[*a*]pyrene, in brain and liver (Shugart et al. 1990).

Exposure of SLE beluga to contaminants has been associated with a variety of detrimental effects on the health. High prevalence of gastrointestinal cancer has been attributed to carcinogenic PAHs, possibly originating from aluminium smelters (Martineau et al. 2002). It has also been suggested that high prevalence of infection by opportunistic pathogens may be a consequence of exposure to immunotoxic compounds such as PCBs or Hg (Brousseau et al. 2003). Hermaphroditism and lesions in the thyroid may be related to exposure to endocrine disruptive compounds such as organochlorine contaminants (DeGuise et al. 1995).

Management of exposure

The demonstration of high concentrations of toxic chemicals and of a variety of pathologies in SLE beluga initiated management actions to reduce pollution of the Great Lakes – St. Lawrence River ecosystem and to protect the SLE beluga population (Schneider et al. 2001; Environment Canada 1993). Environmental regulations have been developed to reduce the emission of persistent organochlorine compounds and heavy metals in the environment (Fig. 8). Concentrations of PCBs and of organochlorine pesticides in blubber have decreased by a factor of two between 1987 and 2002, presumably as a result of reduced inputs of these compounds in the habitat of the SLE beluga and its prey, but they are still elevated (around 50 μg g^{-1} lipid weight in blubber; Lebeuf et al. 2007). Other factors could have influenced this trend including biotransformation of these compounds in the habitat and in the beluga and possible changes in the diet of the beluga. In contrast, concentrations of PBDEs, not yet regulated in Canada, have shown an exponential increase during the 1988–1999 time period (Lebeuf et al. 2004). Thus, legacy and new persistent organic chemicals are still found in the habitat of the SLE beluga and are maintained within the beluga food web and in beluga tissue at poten-

tially toxic concentrations. Consequently, management of inputs of toxic chemicals and exposure concentrations, while essential, will not immediately protect the population against the risk of toxic effects, and may show results only after decades of source control. An evaluation of the potential to manage beluga vulnerability to toxic chemicals should be undertaken.

Management of vulnerability

St. Lawrence Estuary beluga have high tissue concentrations of PBTs that have been shown experimentally to disrupt immunological function in a variety of species including marine mammals (Ross et al. 1996). Immunosuppression related to contamination with organochlorinated compounds has been suggested to be a contributing factor in a severe outbreak of morbillivirus infection observed in harbour seals (*Phoca vitulina*) in the North Sea (Van Loveren et al. 2000). Therefore, an epizootic of morbillivirus could be more devastating in SLE beluga than it would be in belugas from less contaminated areas (i.e., Arctic; Anonymous 1997). Furthermore, the restricted range of this population would result in rapid dissemination of the virus to a large proportion of the population. The SLE beluga do not appear to have been exposed to morbillivirus but phocine morbilliviruses have been isolated from several species of seals in the Gulf of St. Lawrence and the SLE (Anonymous 1997; Mikaelian et al. 1999; Nielsen et al. 2000). Thus, for several reasons including high contamination with PBTs, the SLE beluga may be at risk for an outbreak of morbillivirus or for infection by other exotic or novel pathogens. One way to manage this risk associated with exposure to contaminants, would be to reduce potential contact between beluga and novel pathogens (Fig. 8). The release of "rehabilitated" seals held at the Quebec Aquarium into the SLE should not be authorized. Released seals may be disease carriers threatening the beluga population (Measures 2004). The introduction of pathogens released in animal or human wastes into the SLE beluga's habitat should also be controlled (Payment et al. 1988, 2000, 2001). Finally, climate change should also be addressed via international fora promoting beluga conservation such as the Great Lakes Commission (Simmonds and Isaac 2007) since it can alter distribution of hosts and pathogens and promote the contact of the beluga with new pathogens (Harvell et al. 1999, 2002).

Vulnerability of SLE beluga to toxic contaminants may also increase if they face nutritional stress, i.e., starvation and (or) nutritional deficiencies causing changes in the tissue distribution of lipophilic contaminants and impairing defence mechanisms (both immune system and detoxifying systems). Major changes have occurred in the SLE ecosystem in the last 20 years. Many fish species, which were used as prey by SLE beluga, have severely declined including the American eel (*Anguilla rostrata*), Atlantic tomcod (*Microgadus tomcod*), and rainbow smelt (*Osmerus mordax*). The impact of the decline in fish species eaten by beluga on the nutritional condition of beluga and on food web transfer of contaminants should be assessed. Because SLE beluga whales cannot be captured alive, obtaining information on their condition factors, their nutritional status, and their diet is a scientific challenge. Stable isotope ratios have revealed a higher reliance on benthic or demersal prey of beluga whale compared to harbour seal in the SLE (Lesage et al. 2001). Other techniques such as fatty acid signatures could provide further insights on the foraging behaviour and diet of beluga (Thiemann et al. 2007). Biomarkers of nutrient status (e.g., retinol, stable isotopes, fatty acids) could be used in parallel to toxicity biomarkers and measures of chemical concentrations in beluga biopsy samples.

Several factors including contaminants are involved in the decline of these species (Mailhot et al. 1988; Castonguay et al. 1993; Équipe de rétablissement de l'Éperlan Arc-en-Ciel 2003). Thus contaminants not directly affecting beluga but affecting their prey could indirectly affect beluga. Measures taken to restore some prey fish populations (e.g., control of agricultural pollution, restoration of spawning sites, reduction in fishing pressure) may help to reduce the vulnerability of SLE beluga to toxic effects of contaminants and to pathogens (Fig. 8). The impact of climate change on foraging behaviour of beluga and on spatiotemporal distribution of prey should be investigated (Macdonald et al. 2005). Thus, an understanding of the potential interaction of environmental factors (new pathogens, prey availability) and toxic chemicals in SLE beluga is required to manage globally and adequately the risk of toxic chemicals affecting this threatened population.

Summary and conclusions

In this paper and a companion paper (Couillard et al. 2008) we have shown how environmental variability and change alter the exposure and vulnerability of fish to toxic chemicals and thus alter the risk of deleterious impacts on populations. We have provided several case studies drawn from across Canada to illustrate the manner in which such interactions could affect the risk of deleterious impacts in fish exposed to multiple stressors. While considerable evidence has emerged from laboratory experiments showing interactions between environmental factors and chemicals, our understanding of how these interactions operate in the field remains limited partly due to the complexity of environmental processes and partly because the potential importance of such interactions has been overlooked and therefore "not observed". To assess the risks of deleterious impacts to fish populations it is crucial to identify and quantify these interactions and to develop conceptual models for the most valued and endangered fish populations that would allow prediction of the environmental conditions under which fish would be most exposed and (or) most vulnerable to chemicals.

Currently, the effects of contaminants are evaluated independently from fish population dynamics, fish habitat and physical and geochemical oceanography whereas it is clear that these factors interact. There is a need to integrate the various research fields including environmental chemistry and toxicology, fish habitat, fisheries science, eco-physiology, oceanography, hydrography and modeling. The current sectoral approach does not adequately protect marine ecosystems. Important stressor interactions and insidious but critical effects of chemicals on fish populations are overlooked. Current monitoring programs are designed to reduce possible effects of environmental factors, seen as confounding variables, on chemical concentrations and biological re-

Table 3. Future research directions.

Inherent vulnerability of valued aquatic species in Canada to toxic chemicals	Identify the life stages most vulnerable to different chemicals (e.g., smoltification, Waring and Moore 2004)
	Study the effect of life history on vulnerability to different chemicals (e.g., polar species, Chapman and Riddle 2005)
	Study the effect of the spatiotemporal configuration of populations in the landscape on their vulnerability to toxic chemicals (Fahrig and Freemark 1995)
Biomarkers and bioindicators of vulnerability	Develop methods to indicate the inherent or acquired ability of an organism to respond to the challenge of exposure to chemicals or other environmental stress (Van der Oost et al. 2003)
	Develop methods to evaluate the resilience of aquatic species, populations or ecosystems population to further environmental stress (e.g., impaired resistance to infection, Zelikoff et al. 2000; impaired osmoregulation, reduced genetic variability, Wirgin and Waldman 2004)
	Develop multistressor experimental models to investigate the link between the response of vulnerability biomarkers and population-level impacts (e.g., Jorgensen et al. 2006)
Nutrition	Study the effect of nutritional deficiencies or starvation on vulnerability to chemicals and mechanisms of interactions (e.g., Brown et al. 2005*a*; Couillard et al. 2005; Jorgensen et al. 2006; Kelly et al. 2007; Rooney 2007)
	Study the effect of climate change on nutrition and on the severity and duration of the seasonal fasting periods (Walther et al. 2002)
Behaviour	Study the effects of altered foraging behaviour, altered timing of migration or development on exposure to chemicals
	Study the effects of chemicals on the selection of habitat for growth or reproduction (e.g., Kitamura and Ikuta 2000)
Pathogens	Develop environmentally relevant experimental models to study the impact of interactions between pathogens and chemicals on survival, growth and reproduction of aquatic species (e.g Levin et al. 2005; Reynaud and Deschaux 2006; Sures 2006)
	Evaluate the replicability and ecological relevance of immunological biomarkers and study their natural variation
	Study the pathogenesis of diseases potentially associated to environmental stress and contamination (e.g., McGladdery et al. 2001)
Adaptation to extreme environmental conditions	Study the effects of chemicals on the ability to cope with extreme environmental conditions (e.g., Kraemer and Schulte 2004; Lannig et al. 2006; Bennett and Janz 2007)
	Study the effect of genetic adaptation to chemicals or to other environmental stressors on the ability of the organisms to cope with environmental stress (e.g., Meyer and Di Giulio 2003)
Ultraviolet	Evaluate photoenhanced toxicity in environmentally relevant experiments or in the field (Pelletier et al. 2006)
Multiple stressors	Study the effects of the toxins produced by invasive species on the toxicokinetics and toxicodynamics of xenobiotics (e.g., Smital et al. 2004)
	Identify the most important mode of actions involved in the interactions among different chemicals or between environmental stressors and chemicals using cDNA microarrays when available (e.g., Adams 2005; McCarty and Borget 2006; Vuori and Nikinmaa 2007)
Mathematical modeling	Develop and use toxicokinetic and toxicodynamic models capable of predicting effects under a range of environmental conditions. These models require a laboratory-generated understanding of the effects of environmental factors on the relationship between environmental concentrations of chemicals and biologically effective dose including identification of key steps in the mechanisms of toxic action and target sites (e.g., Heugens et al. 2003; Hickie et al. 2007).
Ecological management	Use geographic information system to construct maps of vulnerability of populations (track populations through their life cycles and geographic ranges to evaluate cumulative effects and to identify the locations and timing for maximal exposure and vulnerability, examine the spatiotemporal configuration of populations, source of colonists and presence of dispersal route) (Fahrig and Freemark 1995)
	Develop mitigation measures to reduce vulnerability of aquatic populations to toxic chemicals through modifications of the characteristics of the environment and (or) the fish (e.g., reduce exposure to pathogens, improve food supplies, control behaviour and spatiotemporal distribution) (e.g., Mailman et al. 2006)
	Evaluate the effect of currently applied mitigation measures on the vulnerability of aquatic species to toxic chemicals

sponses. Instead, environmental variability should become a research focus since it is a major factor determining the impact of chemicals on local fish populations. Field and laboratory studies should be designed to identify the conditions of maximal exposure and vulnerability to toxic chemicals to design efficient risk management and sensitive monitoring programs adapted to the unique characteristics of each site and species.

There are major gaps in our understanding of the effects of environmental stressors on risks to aquatic populations exposed to toxic chemicals in the natural environment (Table 3). One challenge is to identify which environmental stressors contribute significantly to the risk related to chemicals in aquatic species. Another challenge is to identify and predict the time of the year, locations, species, and life stages most vulnerable to exposure and effects of chemicals. The consequence of not addressing these issues could be the decline or non recovery of aquatic populations due to misidentifying the underlying mechanisms of toxicity and, therefore, not managing the risk related to toxic chemicals in an appropriate or timely manner. In the context of the acceleration of environmental variations associated with climate change, the need to address the issue of interaction of chemicals with other environmental stressors is all the more urgent. In viewing vulnerability as a contributing or controlling factor in the expression of chemical toxicity in fish and marine mammals, research may bring to light new, practicable approaches to mitigating chemical toxicity. This is particularly important in the case of globally cycling contaminants like PCBs and PBDEs for which controls offer at best a very delayed improvement and, potentially, insufficient protection for long-lived aquatic predators (Hickie et al. 2007). Here, we propose that vulnerability may very likely be decreased through management of fisheries and habitat, the development of marine protection zones and action on other environmental stressors.

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