Correlations between pathological changes and chemical contamination in American eels, *Anguilla rostrata*, from the St. Lawrence River

C.M. Couillard, P.V. Hodson, and M. Castonguay

Abstract: American eel (*Anguilla rostrata*) from the St. Lawrence River are heavily contaminated with chemicals that may be associated with increased incidence of diseases and reproductive impairment. The relationship between tissue mirex concentration and body mass was used to separate eels into two groups: the proportion of eels migrating from contaminated areas (Lake Ontario and upper St. Lawrence River) increased as the migration season progressed. Vertebral malformations and basophilic foci in the liver (preneoplastic lesions) were more frequent at the end of the migratory season, when the eels were more heavily contaminated with organochlorine compounds. In contrast, mesenteric nematodes were more common in the first week of the season, when eels were less contaminated. Diameters and percentages of different stages of oocytes, and density and surface area of pigmented macrophage aggregates in the spleen, did not vary among weeks. While basophilic foci are specific biomarkers of exposure to environmental contaminants, vertebral malformations may be caused by a variety of other anthropogenic or natural factors. Further studies are needed to confirm the observed associations between chemical contamination and pathological changes.

Résumé : Les anguilles d'Amérique (*Anguilla rostrata)* du bassin versant du fleuve Saint-Laurent sont fortement contaminées avec des composés chimiques persistants possiblement associés avec une hausse des morbidités et des perturbations dans la reproduction. La relation entre les concentrations tissulaires de mirex et le poids corporel a été utilisée pour séparer les anguilles en deux groupes: la proportion d'anguilles provenant de sites contaminés (Lac Ontario et partie amont du fleuve Saint-Laurent) augmentait à mesure que la saison migratoire avançait. Les malformations vertébrales et les foyers basophiles dans le foie (lésions précancéreuses détectées histologiquement) étaient plus fréquentes à la fin de la saison migratoire, lorsque les anguilles étaient plus fortement contaminées avec des composés organochlorés. À l'opposé, des nématodes mésentériques étaient plus fréquemment rencontrés dans la première semaine de la saison, lorsque les anguilles étaient moins contaminées. Les diamètres et pourcentages d'ovocytes à différents stades de maturation, et les densités et surfaces des agrégats de macrophages pigmentés dans la rate ne variaient pas entre les semaines. Alors que les foyers basophiles sont des marqueurs spécifiques d'une exposition à des contaminants environnementaux, les malformations vertébrales peuvent être causées par une variété d'autres facteurs anthropiques ou naturels. Les associations observées entre contamination chimique et changements pathologiques devront être confirmées par des études ultérieures.

Introduction

Several hundred tons of American eel (*Anguilla rostrata*) are fished each fall in the St. Lawrence Estuary, Québec, Canada, during their seaward migration to spawning grounds in the Sargasso Sea. Most eels captured in the Estuary grow for 12–16 years to sexual maturity in the upper St. Lawrence River and Lake Ontario, highly industrialized areas where they accumulate high loads of persistent contaminants (Hodson et al. 1994). A drastic decline (two orders of magnitude) in the number of juvenile eels migrating upstream to Lake Ontario from the Estuary was observed between 1985 and 1992 (Castonguay et al. 1994*a*). Accumulation of contaminants and toxicity may have contributed to this recruitment failure, although other causes such as oceanographic changes, habitat disturbances, and overfishing are alternative hypotheses (Castonguay et al. 1994*a*, 1994*b*). This study describes pathological lesions and ovarian maturation in migrating American eels and evaluates if there are correlations between chemical contamination and pathological changes.

American eel are benthic, long lived, lipid rich, and reproduce only once in their lifetime, after which they die. In polluted waters, they are likely to accumulate high concentrations of hydrophobic and persistent compounds and to suffer from increased incidence of disease or from reproductive impairment related to toxicity, as do other fish species in the Great Lakes (Baumann and Whittle 1988; Larsson et al. 1991; Mac et al. 1993). In Lake Ontario, several species of benthic fish have developed cutaneous or hepatic tumors associated with exposure to chemicals (Hayes et al. 1990). Significant relationships have been found between concentration of polychlorinated biphenyls (PCB) in eggs and adults and embryonic mortality in Great Lakes lake trout (*Salvelinus namaycush*) (Mac et al. 1993).

Little is known about the health status of American eel in the St. Lawrence drainage basin. Mass mortalities of eels with gill damage and impaired osmoregulation were observed in the

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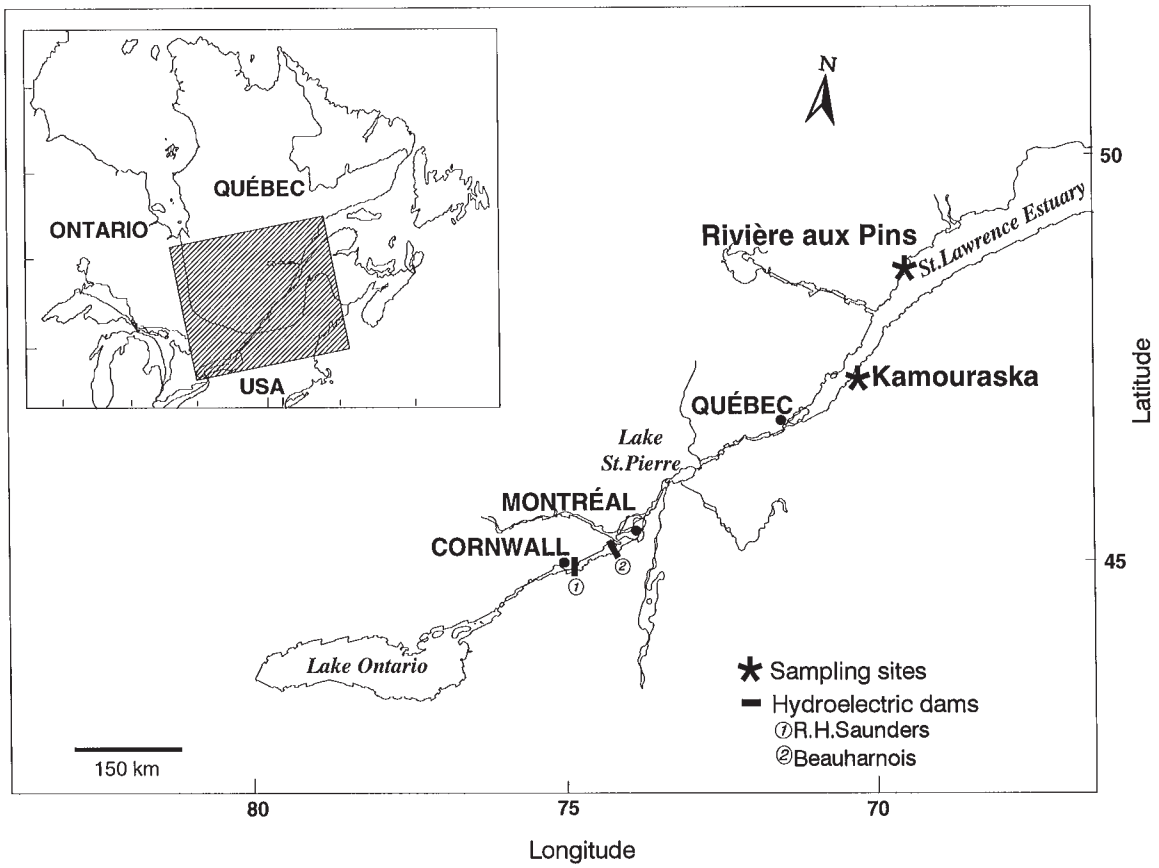
Fig. 1. Map of the study area. Two hydroelectric dams located at Cornwall and at Beauharnois cannot be avoided by American eels migrating from Lake Ontario. Migrating eels were sampled in the St. Lawrence River at Kamouraska and in Rivière aux Pins, a small tributary to the St. Lawrence.



1970s in migrating maturing eels (so-called silver eels) from Lake St-Pierre (river, freshwater) to Kamouraska (estuary, saltwater) (Fig. 1) (Dutil 1984). The cause of these mortalities was not identified, although common bacterial agents were excluded (Dutil and Lallier 1984) and acute toxicity of industrial effluents was suspected (Dutil et al. 1987). Other lesions previously reported include granulomatous parasitic lesions in the gastrointestinal tracts of eels captured in various tributaries of the St. Lawrence River (Cousineau et al. 1977) and vertebral deformities in adults captured by fishermen (Homer 1986). Oocyte diameter increases as silver eels migrate from Lake St-Pierre to Kamouraska but the potential effect of contamination on gonad maturation has never been investigated (Dutil et al. 1985).

In 1990, tissue concentrations of PCB, mirex, and other chlorinated pesticides were 10–100 times higher in silver eels captured at Kamouraska, in the St. Lawrence Estuary than in eels from a reference tributary (Hodson et al. 1994). Samples collected weekly during the 7-week fishing season at Kamouraska (Fig. 1) demonstrated that concentrations of several organochlorine (OC) contaminants increased by more than eightfold with time. This heterogeneous spatiotemporal distribution of contamination gave us an opportunity to determine if levels of contamination were associated with frequencies of gross or microscopic lesions or impairment of oocyte maturation in migrating eels.

The following null hypotheses were tested in migratory maturing American eels sampled weekly at Kamouraska: (*i*) there are no temporal changes in the prevalences of pathological lesions and parasites during the fishing season; (*ii*) prevalences of pathological lesions and parasites are not associated with the level of contamination of the eels; (*iii*) diameter and percentage of vitellogenic oocytes are unchanged in the most contaminated eels; and (*iv*) prevalences of parasites and pathological lesions do not differ between eels from Kamouraska and eels from a reference tributary.

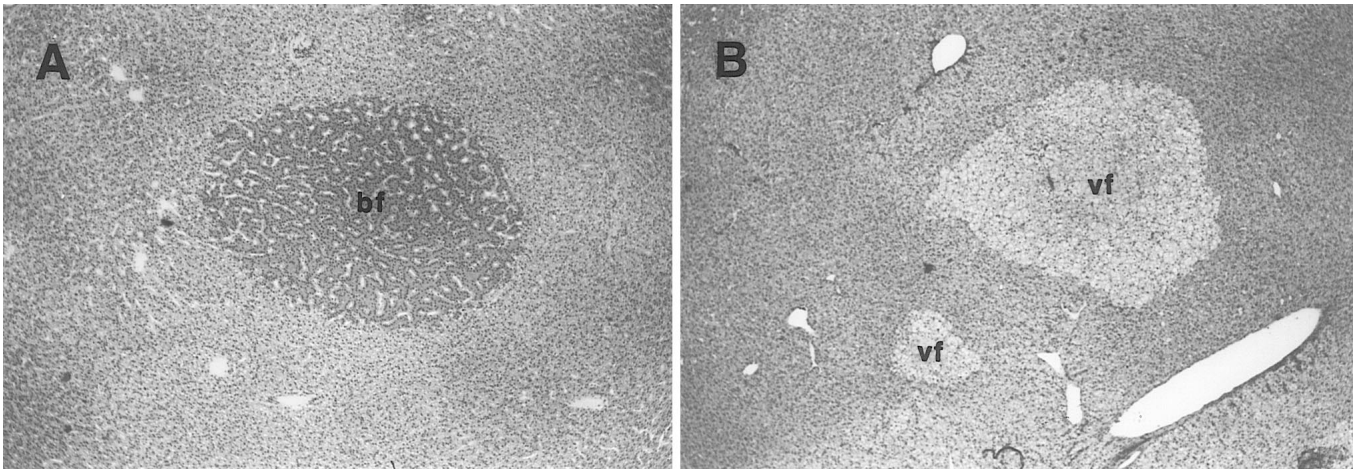
Materials and methods

Sample collection

Maturing female silver eels were trapped in 1990 by a commercial fisherman during their seaward spawning migration using weirs installed at angles to the shoreline at Kamouraska, on the south shore of the St. Lawrence Estuary (Fig. 1). Live eels were purchased weekly during the entire 7 weeks of the fishery, between September 19 and November 1: 100 eels (or less, if fewer eels had been caught) were randomly selected shortly after the fisherman had emptied his traps. Migrating silver eels were also captured in Rivière aux Pins by experimental trap nets on one occasion in the first week of the fishing season. Rivière aux Pins is a reference stream on the north shore of the St. Lawrence Estuary; it drains a forested watershed with no industrial source of chemical contaminants (Fig. 1).

Fish were transported live to the laboratory and held in flowing salt water for 6–48 h before pathological examination. They were anesthetized with MS-222 (tricaine methanesulfonate) and examined

Fig. 2. Foci of hepatocellular alteration in the liver of migrating American eels captured at Kamouraska. (A) Basophilic foci (bf). Hepatocellular cytoplasm is hyperbasophilic and markedly less vacuolated than adjacent hepatocytes. The adjacent tissue is not compressed. Hematoxylin and eosin. 40×. (B) Vacuolated cell foci (vf). Well-delimited zones where the hepatocyte cytoplasm is more vacuolated than that of most hepatocytes. Hematoxylin and eosin. 40×.



for external and internal pathological lesions and parasites. Histological samples were taken from the first 15 eels examined in each sample. Gonads were removed and weighed, and the gutted carcass was frozen for subsequent chemical analyses. Concentrations of PCB, OC pesticides, polycyclic aromatic hydrocarbons (PAH), and mercury were measured in subsamples of 7–16 eels per sample, stratified according to body mass (Hodson et al. 1994). In Kamouraska, only 29 of the 86 eels analysed chemically were also examined histologically but all were examined macroscopically.

Macroscopic pathological examination

We looked for the following lesions previously associated with pollution in fish: vertebral malformations, fin damage, cutaneous ulcers, and cutaneous and liver tumors (Mix 1986; Malins et al. 1988). Prevalences of vertebral deformities, fin damage, and cutaneous lesions were assessed by external examination of each eel. Internal pathological examination was also conducted, and prevalences of nodules in the liver were recorded. A thorough parasitological examination was not performed on each fish, but the presence or absence of commonly occurring and easily- recognized parasites was noted. Presence or absence of the digenea *Azygia longa* in the stomach and of nematodes (species not identified) in the mesentery was recorded.

Histological examination

Liver, gonad, and spleen were excised and fixed in 10% phosphate-buffered formalin. Gonad sections were taken in the middle of the left gonad, and liver sections were taken in the area adjacent to the gall bladder. Tissues were embedded in paraffin, and sections 5 μm thick were stained with hematoxylin and eosin (Luna 1968).

Histopathological examination also focused on the following markers of toxicity: foci of hepatocellular cellular alteration in the liver (preneoplastic lesions) and pigmented macrophage aggregates (PMA) in the spleen (Hinton et al. 1992). In the liver, presence or absence of foci of basophilic hepatocytes was recorded. Basophilic foci are zones of hepatocytes with hyperbasophilic cytoplasm, markedly less vacuolated than adjacent hepatocytes (Fig. 2). These foci, most frequently oval and occasionally with an irregular contour, were not encapsulated and did not compress adjacent tissue. Presence or absence of foci of vacuolation (one or more well delimited zones where the hepatocyte cytoplasm is more vacuolated than that of most hepatocytes) was also determined (Fig. 2).

Pigmented macrophage aggregates (PMA) are focal accumulations of macrophages that may contain four types of brown pigments: melanin, lipofuscin, ceroid, and hemosiderin. Proliferation of PMA has been associated with several natural factors such as aging, starvation, infectious diseases, and parasite infestation (Wolke 1992). Increased density of PMA has also been induced experimentally in fish treated with various toxic compounds and has been observed repeatedly in fish exposed to environmental contaminants (Wolke 1992; Khan et al. 1994; Couillard and Hodson 1996). Density of PMA was evaluated in the spleen by counting the number of PMA at 100× in three randomly selected microscopic fields and dividing this number by the area of the fields examined (2.66 mm^2). The surface area of each PMA counted was measured with an image analysis system (Bioquant, R&M Biometrics®), and mean surface area of PMA was calculated for each eel. PMA were composed of three or more macrophages containing yellow to dark brown pigments within their cytoplasm.

Maturity of oocytes was graded from 1 to 4 with the following morphological criteria: stage 1, no cytoplasmic vacuoles; stage 2, small number of large cytoplasmic vacuoles; stage 3, large cytoplasmic vacuoles forming a complete circle in peripheral cytoplasm and covering less than 50% of the cytoplasm; and stage 4, large and small vacuoles covering more than 50% of the cytoplasm. These criteria have been developed for this study and are based on descriptions of ovarian maturation in European eel (*Anguilla anguilla*) (Colombo et al. 1984). In each gonad section, the proportions of the different stages of oocytes were determined by examining 200 oocytes with a microscope at 160×. The diameters (greatest length through the nucleus) of 10 oocytes of each stage were measured at 160× with the image analysis system.

Statistical analyses

Temporal variation at Kamouraska

Body mass (W), length (L), condition factor ($CF = (W\ (g)/L^{3}\ (cm)] \times 100$), gonadosomatic index, ($GSI = [\text{gonad mass (g)}/(W\ (g) - \text{gonad mass (g)}]) \times 100$, density and surface area of PMA in the spleen, and percentage and diameter of different stages of oocytes were compared among weeks of capture with the Kruskal–Wallis test, followed, when significant, by analysis of variance on ranked data with Tukey's studentized range test (Conover 1980). GSI was based on "gonad-free body mass" to avoid bias due to autocorrelation and the effects of possible variations among fish in the degree of maturity. Prevalences of vertebral deformities, parasites, and of basophilic and vacuolated foci in liver were compared among weeks of capture with

Fisher's exact test (Sokal and Rohlf 1981). Confidence intervals (95%) were determined from a table presented in Scherrer (1984) (Table VIII: confidence intervals for small samples). To evaluate the possible effect of lesions on fish condition and to characterize the fish affected with lesions, body mass, length, CF, and GSI were compared between fish with and without vertebral deformities on week 6 and between fish with and without basophilic foci on week 5 of the fishing season. Weeks 5 and 6 were selected on the basis of maximal prevalences of these lesions.

For each week, linear relationships of gonad mass versus somatic mass (body mass – gonad mass) were assessed by least-squares regression. Analysis of covariance (ANCOVA) was used to compare slopes and intercepts of the lines among weeks. The relationship between fish size and gonadal maturation was assessed by comparing the logarithm of stage 4 oocyte diameters (the largest and most abundant oocytes) to the log of body length. The linear model was assessed by least-squares regression for each week and for all weeks combined. The linear relationship between log(density of PMA) and log(body length) was assessed for each week and for all weeks combined by least-squares regression.

Association with contaminants

To test the association between chemical contamination and pathological changes, two approaches were used. First, for the entire fishing season, prevalences of lesions were compared between eels containing high concentrations of mirex, a pesticide concentrated in the upper and most contaminated part of the St. Lawrence basin (Castonguay et al. 1989), and eels with low concentrations of mirex. Second, a dose–response approach was used among variables assessed weekly: prevalences of pathological changes were correlated with concentrations of chemical contaminants and with percentages of eels heavily contaminated with mirex. However, we did not intend to demonstrate cause–effect relationships here, because numerous confounding variables are associated with mirex contamination.

In migrating eels captured at Kamouraska, a linear relationship between log-transformed mirex concentration and body mass was used to discriminate between eels migrating from contaminated areas (Lake Ontario and upper St. Lawrence River) and eels migrating from less contaminated areas. Previous studies have demonstrated the potential use of mirex as a marker of geographic origin in American eels (Dutil et al. 1985; Castonguay et al. 1989). Because tissue concentrations of OC contaminants are frequently related to fish mass (Borgmann and Whittle 1990; Larsson et al. 1991) as a surrogate for age and lifetime contaminant exposure, plotting concentration of mirex as a function of body mass provides a more accurate means of discriminating geographic origin of migrating eels than unadjusted tissue concentrations of mirex alone. Body mass, length, and prevalences of vertebral deformities, parasites, and basophilic and vacuolated cell foci in liver were compared between mirex-contaminated and less contaminated groups of eels by Kruskal–Wallis and Fisher's exact tests. The linear relationships of percentage of contaminated eels versus prevalences of nematode infection, vertebral lesions, and basophilic foci in liver (parameters showing temporal variations) were assessed by least-squares regression.

Concentrations of mirex, heptachlor epoxide, hexachlorobenzene, endrin, and PCB congener 28 varied significantly among weeks (Hodson et al. 1994). The geometric mean concentrations of these contaminants and total PCB in the subsample of eels analysed chemically were correlated with weekly prevalences of pathological lesions in the whole sample. This procedure assumed that the concentrations of contaminants measured in the subsample of eels analysed chemically were proportional to those in the eels examined for gross and histological lesions. This may not be true because the eels examined pathologically were randomly selected; in contrast, the eels examined chemically were selected to represent the range of masses, not the average mass. Log(body mass) and log(body length) were compared by analysis of variance (ANOVA) between fish analysed chemically and the entire sample of eels. Body mass and length were higher in fish analysed chemically on weeks 4 and 5 of the fishing season (data not shown) indicating that, for these weeks, the subsample of eels analysed chemically may not represent the entire sample of eels. The linear relationships of geometric mean concentration of contaminants versus prevalences of nematodes, vertebral lesions, and basophilic foci were assessed by least-squares regression.

Comparison with the reference tributary

Prevalences of pathological lesions and parasites were not compared statistically between sites because the single sample of eels from Rivière aux Pins was not comparable with the multiple samples covering the complete fishing season in the St. Lawrence Estuary at Kamouraska. In contrast, body mass, length, CF, and the density and areas of PMA in the spleen were compared between these sites because these parameters did not exhibit temporal variations in eels from Kamouraska; comparisons were done with the Kruskal–Wallis test. For the first week of the fishing season only, linear relationships between gonad mass and somatic mass were compared between sites with ANCOVA. Diameters and percentages of oocyte stages were compared between sites with the Kruskal–Wallis test.

Results

Temporal variations at Kamouraska

Characteristics of the whole sample and gonadal maturation

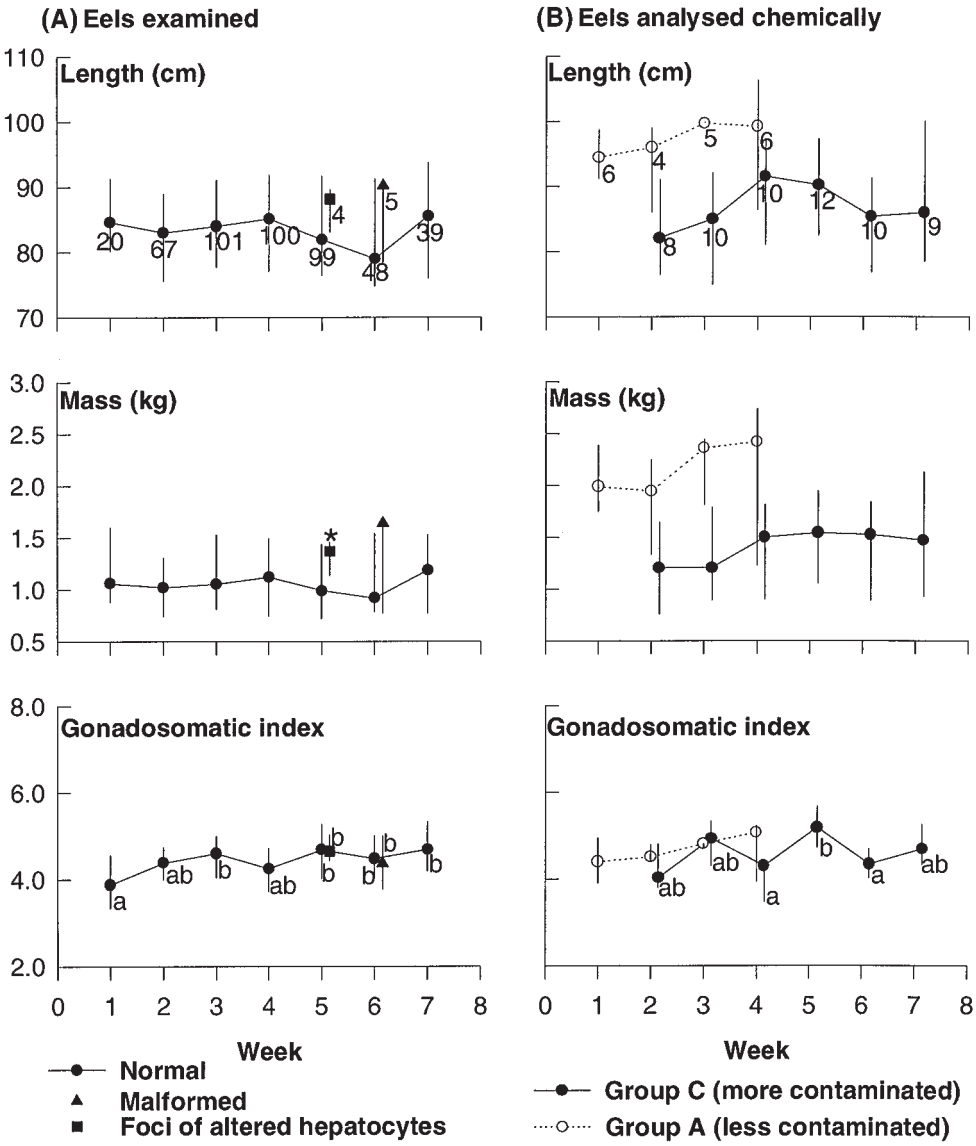
Body mass, length, and condition factor (data not shown) did not vary significantly over time of capture (Fig. 3). All eels sampled ($n = 473$) were mature females with well-developed gonads, as expected for the fall fishery in the St. Lawrence Estuary (Dutil et al. 1985).

The GSI tended to increase with time (Fig. 3). ANCOVA indicated that slopes of the relationship between gonad mass and somatic mass did not differ among weeks but that the intercept was highest on week 5 and lowest on week 1 of the fishing season (Table 1). Diameter of stage 2 oocytes was lower on week 7 compared to week 1 (Fig. 4). Diameters of stages 1, 3 or 4 oocytes and percentages of stage 1, 2, 3 or 4 oocytes did not vary significantly among weeks (Fig. 4). During the entire fishing season, percentages of stage 4 oocytes increased slightly as the percentage of stage 2 oocytes decreased. In the last 2 weeks of the fishing season, however, the ratio of stage 4 versus stage 2 oocytes tended to decrease. Diameters of stage 4 oocytes were not correlated to body length for all weeks combined or taken separately.

Macroscopic lesions and parasites

During the entire fishing season, nine eels with vertebral malformations (9 of 473 = 1.9%) were observed. Two types of vertebral malformations were seen: kyphosis (dorsoventral deviation of the spinal column, humpback), most frequently in the area of the neck (six of nine), and scoliosis (lateral deviation of the spinal column), most frequently in the area of the tail (three of nine). Two-thirds of the malformed eels were observed in the last 2 weeks of the fishing season, particularly in week 6 when 10% of eels were affected (Fig. 5). On week 6, body mass, length, CF, and GSI did not differ between eels with or without malformations (Fig. 3). Radiographic examination revealed that 19% of the eels with no external vertebral malformations had a variety of radiographic vertebral lesions (fusions, compressions, dislocations, deformities) (P.V. Hodson,

Fig. 3. (A) Temporal variation of body length (*L*), body mass (*W*), and gonadosomatic index (GSI) during the 7-week fishing season in Kamouraska. Medians are given for *L*, *W*, and GSI (bars are from the first to third quartiles). GSI with different letters differ significantly (Kruskal–Wallis, $p \leq 0.05$). Numbers of eels examined for macroscopic lesions are indicated. Characteristics of eels were compared between eels with or without basophilic foci in the liver on week 5 and between eels with and without vertebral malformations on week 6. An asterisk indicates a difference between eels with and without lesions (Wilcoxon, $p \leq 0.05$). (B) Temporal variation of body length, body mass and GSI in eels with high or low mirex contamination during the 7-week fishing season in Kamouraska. Numbers of eels analysed chemically are indicated.



unpublished data). There was no obvious temporal pattern of variation of these radiographic vertebral lesions.

Seven eels (1.5%) had an incomplete caudal fin, with no significant variation in prevalence with time. Other lesions such as cutaneous ulcers, broken eyes, malocclusion of the jaws, abdominal masses, and nodules and cysts in the liver were observed at a prevalence lower than 1% with no significant variation in time (data not shown). Cutaneous wounds were observed in many eels, but their prevalence was not recorded because it was difficult to differentiate between the wounds present before capture from those induced by capture and handling.

Prevalence of mesenteric nematodes was significantly higher in the first week of the fishing season but that of the digenean trematode *Azygia longa* did not vary significantly with time (Fig. 5).

Histological changes

Basophilic foci were observed in the liver of 15 (14%) of the 105 eels examined histologically. A peak prevalence was observed on week 5 (27%) but there was no significant difference among weeks (Fig. 5). On week 5, body mass (Fig. 3) and CF were higher in eels with basophilic foci, while length and GSI did not differ between eels with and without basophilic foci.

Vacuolated foci were observed in 17 eels with a maximal prevalence on week 3, but no significant variation with time of capture (Fig. 5).

Density or surface area of PMA in the spleen did not vary

Fig. 4. Temporal variation in the diameters and the percentages of different stages of oocytes during the 7-week fishing season in Kamouraska. Stages of maturation of oocytes are graded from 1 to 4 according to morphological criteria. Diameters and percentages are given as means (with SD given in parentheses). An asterisk indicates a difference compared with the first week of sampling (Kruskal–Wallis, $p \leq 0.05$).

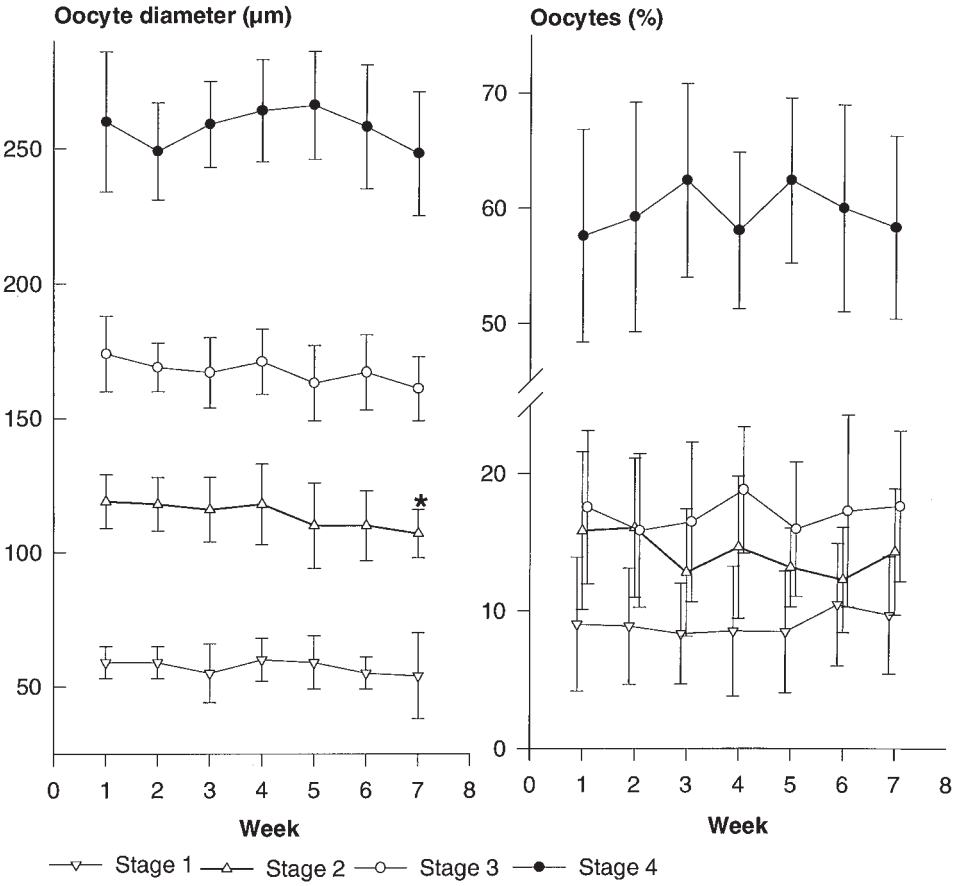


Table 1. Analysis of covariance of gonad mass as a function of somatic mass and time in American eels captured during the 7-week fishing season in Kamouraska.

Week	*n*	Intercept[a]
1	20	–14.8*a*
2	67	–7.6*bc*
3	100	–5.6*cd*
4	99	–10.1*ab*
5	98	–3.8*d*
6	48	–7.1*bcd*
7	39	–5.1*cd*

[a]Gonad mass (g) = 0.05(somatic mass (g)) + intercept. Intercepts with a different letter are significantly different among weeks (ANCOVA, $r^2 = 0.86$, $F = 417$, $p = 0.0001$). Slopes are homogeneous among weeks.

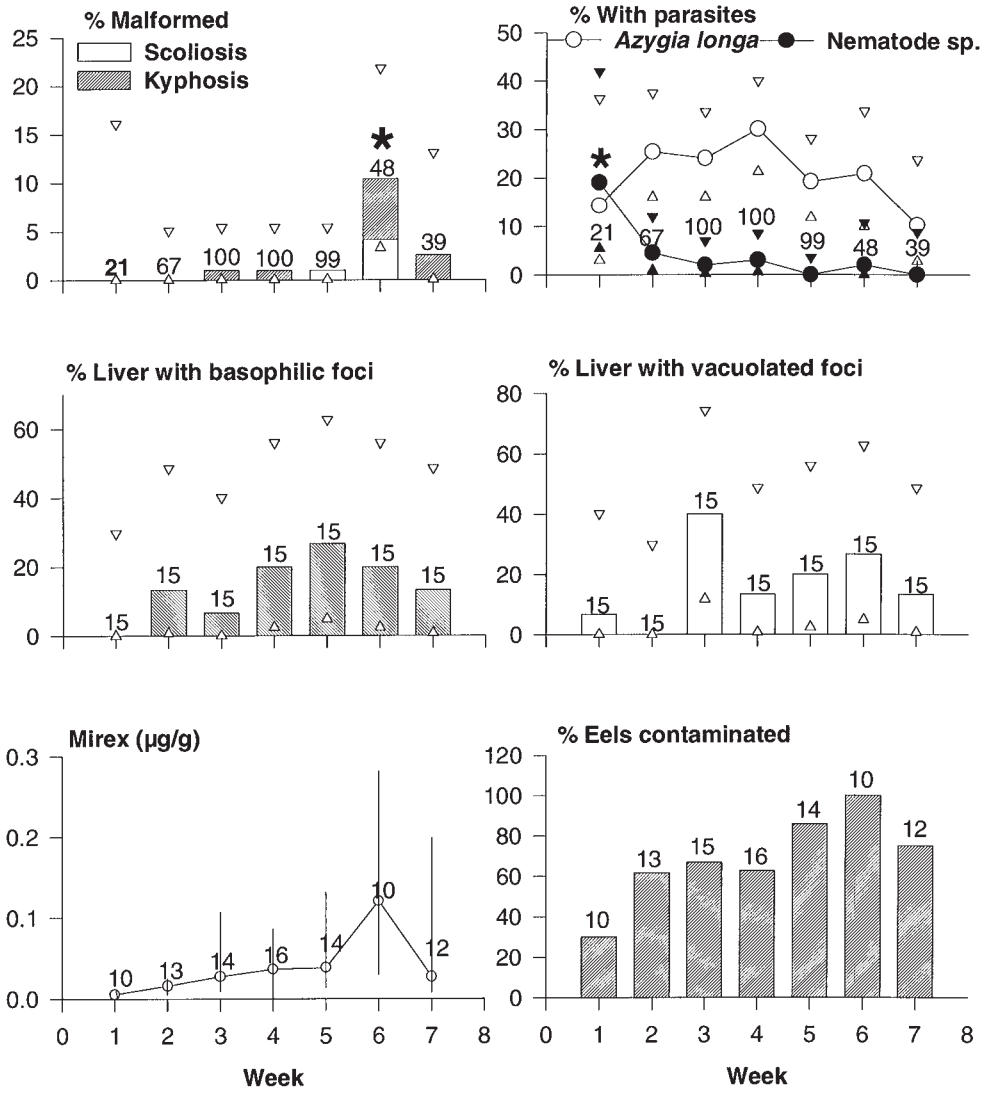
from week to week (Fig. 6). Regressions between density of PMA and body length were not significant for all weeks combined or taken separately.

Association with contaminants

The linear relationship between mirex concentration and body mass successfully separated eels into two main groups: group A, less contaminated (log(mirex) = 0.000 32 W – 3.16, $r^2 = 0.32$, $p = 0.003$, $F = 10.90$, $n = 25$) and group C, more contaminated (log(mirex) = 0.000 71 W – 2.17, $r^2 = 0.55$, $p = 0.0001$, $F = 72.5$, $n = 62$) (Fig. 7). The proportion of group C eels increased with time from 30% in the first week of the fishing season to 75–100% in the last 3 weeks (Fig. 5). Group C eels had lower body mass, length, and CF compared with group A eels (less contaminated) (Fig. 3, Table 2). Group A eels had no vertebral malformations while 6.6% of the group C eels were affected (Table 2). Mesenteric nematodes were found in group A eels but not in group C eels (Table 2). Prevalences of basophilic foci and vacuolated foci were about two times higher in group C than in group A (Table 2). Group C eels also contained higher levels of several other OCs, such as PCB, compared with group A eels (Table 2).

Prevalences of basophilic foci increased, prevalences of nematodes decreased, and the prevalence of vertebral malformations did not vary with the percent of contaminated eels (Table 3). Prevalence of vertebral malformations increased with the concentrations of mirex, endrin, and heptachlor epoxide (Table 3). However, the linear regression was strongly influenced by one data point, a peak of prevalence of malformation on week 6 (Fig. 5). Prevalence of mesenteric nematodes decreased with the concentration of PCB. There was no significant linear relationship between mean concentration of contaminants and prevalence of basophilic foci (Table 3).

Fig. 5. Temporal variations of prevalence (%) of vertebral lesions, parasites, and histological liver lesions and of the contamination with mirex of eels sampled in Kamouraska. Asterisks indicate a significant variation among weeks (Fisher's exact test, $p \leq 0.05$), triangles indicate 95% confidence intervals. The numbers of eels examined macroscopically (malformations and parasites), histologically (liver lesions), and analysed chemically are indicated. Contamination with mirex is illustrated by median concentrations in carcass (first to third quartiles) and by percentages of eels heavily contaminated with mirex (group C). The numbers of eels analysed chemically are indicated.



Comparison with the reference tributary

Eels sampled at Rivière aux Pins had a lower body mass, length, and CF compared with eels sampled at Kamouraska (Table 4). No vertebral malformations and no nematodes were observed in eels sampled at Rivière aux Pins. Prevalences of the digenean trematode *Azygia longa* was about two times lower in eels from Rivière aux Pins compared with eels from Kamouraska. No basophilic or vacuolated foci were found in the liver of the 15 eels from Rivière aux Pins examined histologically (Table 4). However, with a sample size of only 15, it is only possible to detect diseases with a prevalence higher than 18% at a confidence level of 95% (Martin et al. 1987). PMA were more densely distributed and of smaller size in eels from Rivière aux Pins compared with eels from Kamouraska (Kruskal–Wallis, $p \leq 0.05$) (Fig. 6). The intercept of the linear relationship of gonad mass versus somatic mass was lower in Rivière aux Pins compared with Kamouraska (–9.9 compared with –3.4, ANCOVA, $p \leq 0.05$), and the slopes did not differ between sites. On week 1, diameters of stages 2, 3, and 4 oocytes were lower in Rivière aux Pins compared to Kamouraska (Table 5). Percentages of stage 1 oocytes were higher, and percentages of stage 3 oocytes were lower in Rivière aux Pins (Table 5).

Discussion

The first two null hypotheses were rejected. The prevalences of pathological lesions and parasites (*i*) changed temporally in migrating American eels during the 7-week fishing season at Kamouraska and (*ii*) were associated with the level of contamination of the eels. Vertebral malformations and liver basophilic foci were more frequent at the end of the migratory period, when eels were more heavily contaminated with OCs. In contrast, nematodes were more commonly found in the

Fig. 6. Density and surface area of pigmented macrophage aggregates (PMA) in the spleen of eels sampled during the 7-week fishing season in Kamouraska and in the first week of the fishing season at Rivière aux Pins. Density and surface area are expressed as median (first to third quartiles).

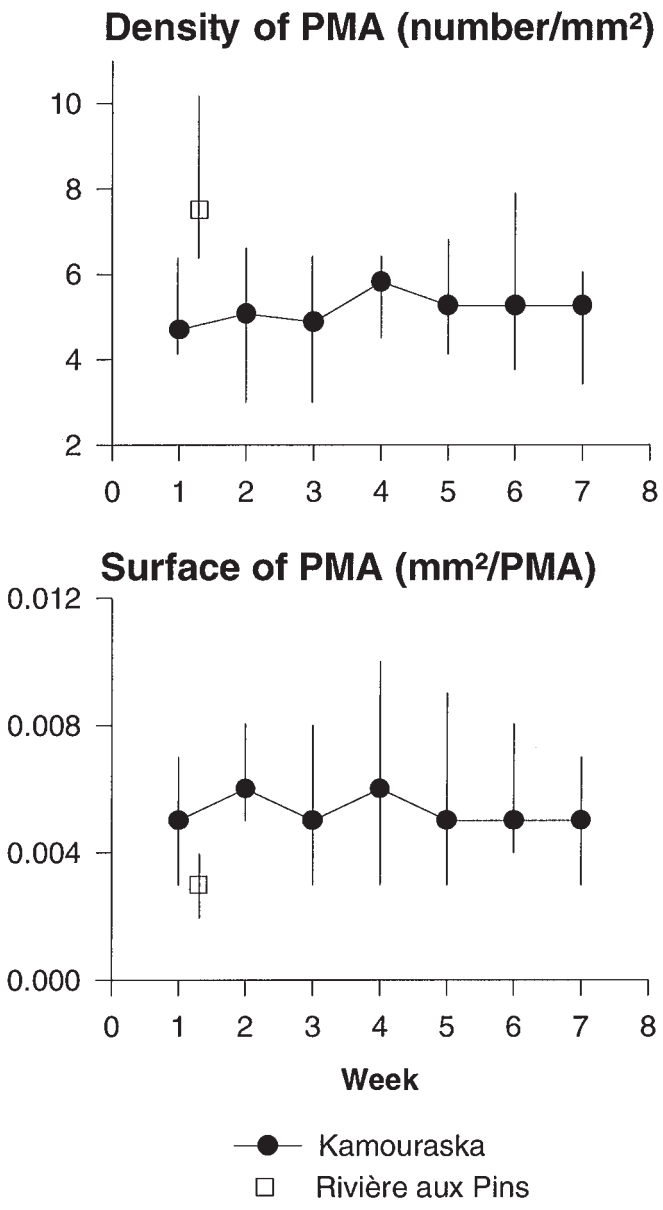
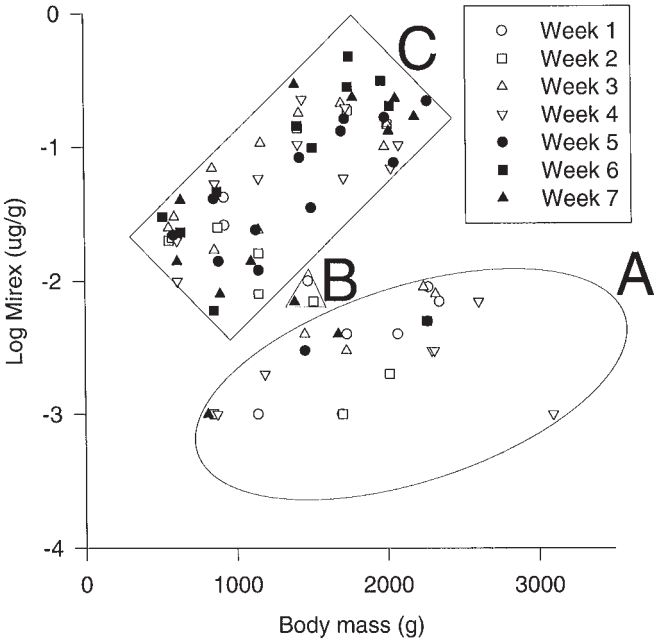


Fig. 7. Concentrations of mirex relative to body mass in eels captured during the 7-week fishing season in Kamouraska, in the St. Lawrence Estuary. Linear relationships between log(mirex concentration) in the carcass and log(body mass) are used to discriminate between eels migrating from contaminated areas (group C) and eels migrating from less contaminated areas (group A).



abdomen of eels captured in the first week of migration, when the eels were less contaminated. Density of splenic PMA did not vary with time. The third null hypothesis was accepted: (*iii*) histological evaluation of the gonads did not reveal any disturbance of ovarian maturation; diameters and percentages of different stages of oocytes did not vary among weeks. Finally, (*iv*) eels sampled in the reference tributary, Rivière aux Pins, had no vertebral malformations, had no basophilic nor vacuolated foci in the liver, and had low prevalences of parasites compared with eels sampled in Kamouraska. However, it was not possible to statistically compare the prevalences of lesions between the single sample at Rivière aux Pins and the multiple samples covering the whole fishing season in the St. Lawrence River, at Kamouraska.

In fish, vertebral malformations may be a consequence of fractures of vertebrae, vitamin deficiencies, altered embryonic development, parasitic infections, electric shock, or exposure to environmental contaminants (Claveau 1989; Hinton 1993). Prevalences of vertebral malformations have been positively correlated with the levels of several OCs and these compounds (and (or) associated contaminants) are one possible cause of the observed malformations (Couch et al. 1977; Mehrle et al. 1981; Bengtsson et al. 1985). However, eels migrating from their most contaminated habitat, Lake Ontario, also go through the turbines of two large hydroelectric dams or their associated ship locks (Fig. 1), that could cause vertebral fractures and spinal deformities, especially in larger eels (Larinier and Dartiguelongue 1989). About 20% (16–24%) of the silver eels injected experimentally in the turbines of the Beauharnois hydrolectric dam died with cutaneous wounds and fractures of the vertebral column (Desrochers 1995). Survivors had no external lesions, but long-term survival and radiographic lesions were not evaluated. In 1992, 12 000 eels captured commercially in five areas of the St. Lawrence River basin including Lake Ontario and Kamouraska were examined for external lesions. Vertebral malformations were about six times more frequent in migrating eels from Kamouraska compared with yellow (resident) eels from Lake Ontario (Dutil 1994; Dutil et al. 1997). Migrating eels were larger and probably older than resident eels, so that the effects of age, size, and migration could not be distinguished. Nutritional deficiencies or contaminant effects on bone metabolism would probably cause

defects in other bones or cartilage, but this was not observed (Hinton 1993). *Myxosoma cerebralis*, a parasite commonly associated with vertebral deformities (Markiw and Wolf 1978), was not found in two malformed eels examined (data not shown). The two leading hypotheses for the causes of vertebral malformations, toxicity and (or) physical damage by the hydroelectric dams, should be tested experimentally and by field investigations. Prevalences of radiographic vertebral lesions should be evaluated in resident eels, at different contaminated sites in the Lake Ontario – St. Lawrence River axis, and in migrating eels as they pass through the R.H. Saunders and Beauharnois hydroelectric dams (Fig. 1).

The arrival of malformed eels in the last weeks of migration could indicate that they have locomotory impairment and slowed migration. Alternatively, eels originating from the most contaminated areas such as Lake Ontario may arrive late because they have a longer distance to cover or a delayed onset of migration. Therefore, while both contaminant concentrations and the frequency of vertebral malformation increase late in the fishing season, no firm conclusion can be reached regarding associations between the two. Contaminants might contribute to vertebral damage, or both responses may simply be indicators of the origin of the eels and the route travelled during the seaward migration.

Hepatic basophilic foci have been described in various fishes exposed experimentally to chemical carcinogens and in wild fish exposed to industrial and urban pollution; they are considered specific biomarkers of exposure to environmental contaminants (Hinton et al. 1992). In rodents and fish, this lesion represents a preneoplastic stage in the histogenesis of hepatocellular neoplasms (Hinton et al. 1988). Basophilic foci, adenomas, and hepatocellular carcinoma have been described in white sucker (*Catostomus commersoni*) captured in Hamilton Harbour, a site on the western shore of Lake Ontario heavily contaminated with PAH and metals (Hayes et al. 1990). Hepatocellular carcinomas have also been induced experimentally in rainbow trout (*Oncorhynchus mykiss*) injected with an extract of sediment from the same site (Metcalfe et al. 1990). In our study, prevalences of basophilic foci were not correlated with concentrations of individual OCs but rather with the proportion of heavily contaminated eels. Other contaminants, such as PAH, may be responsible for the lesions. This is the first report of foci of hepatocellular alteration in American eels, and the prevalences of these lesions and of possibly related liver tumors in resident eels are not known.

Basophilic foci are benign lesions that are not likely to interfere with normal migration, as indicated by the normal CF and gonad development of affected eels. However, the number of eels examined histologically was too small to detect hepatocellular carcinomas and adenomas, and the prevalence and severity of lesions may be underestimated if eels severely affected by carcinomas and adenomas do not migrate. Tumors and preneoplastic lesions may also be more frequent in older eels, and weekly variations in age may influence the variation in prevalences of basophilic foci. Unfortunately, we could not age eels in this study, because there is no published reliable aging method for American eels from the St. Lawrence River from bony structure (Castonguay et al. 1994*a*). Correlations of contamination and pathological changes would also have been more informative if all analyses (chemical, pathological, and histological) had been conducted on the same individuals.

Table 2. Body size and prevalences of pathological changes and parasites in two groups of American eels captured at Kamouraska, characterized by low or high concentration of mirex.

Variables	Less contaminated (group A)	More contaminated (group C)
Length (cm)	97.8 (91.2–99.7)	86.5 (78.6–95.0)*
Body mass (g)	2110 (1533–2418)	1471 (904–1838)*
CF (g/cm^3)	0.23 (0.20–0.24)	0.20 (0.19–0.22)*
n	25	61
Prevalences of lesions and parasites (sample size)		
Vertebral malformations	0 (25)	6.6 (61)
Mesenteric nematodes	20 (25)	0 (61)**
Azygia longa	20 (25)	16 (61)
Basophilic foci in liver	9 (11)	22 (18)
Vacuolated foci in liver	9 (11)	17 (18)
Contaminants		
Mirex (μg/g)	0.003 (0.001–0.005)	0.064 (0.023–0.162)**
PCB (μg/g)	0.25 (0.19–0.43)	0.95 (0.42–1.30)**

Note: Body mass (W), length (L), condition factor (CF = (W (g)/L^3 (cm)) × 100), and tissue concentrations of total PCB congeners are given as medians (with the first to third quartiles given in parentheses).

*Significantly different from group A (Wilcoxon, $p \leq 0.05$).

**Significantly different from group A (Fisher's exact test, $p \leq 0.05$).

Vacuolated foci may appear in the liver during the process of gonadal maturation because of vitellogenesis and transfer of fatty material from the liver to the gonads (Timashova 1981). Vacuolated foci could also be phenotypically altered hepatocytes, also known as clear cell foci, that are frequently associated with basophilic foci in fish exposed to carcinogens (Hinton et al. 1992). It is difficult to differentiate these two types of histological changes in maturing fish. Histochemical markers may be useful in demonstrating altered enzymatic activity within preneoplastic foci (Teh and Hinton 1993).

Density of PMA did not vary temporally as a function of the degree of contamination and did not differ between more and less contaminated eels at Kamouraska. Density of PMA increases with age in several fish species (Wolke 1992). Tagging studies have demonstrated that age may vary from 10 to 20 years in eels migrating from Lake Ontario (Castonguay et al. 1994*a*). It may be necessary to control for this confounding factor to demonstrate a possible effect of contaminants. Density of PMA did not correlate with body length, but length of maturing eels is probably not closely correlated with age (Helfman et al. 1987; Bouillon and Haedrich 1996). Density of PMA was higher in eels captured at Rivière aux Pins, which may begin migration at an older age compared with eels captured at Kamouraska. Factors governing the onset of sexual maturation are not well known in American eels; age and size at maturity appears to vary greatly with latitude and with different habitat characteristics (Helfman et al. 1987; Bouillon and Haedrich 1996). Starvation associated with migration may be another factor affecting the density of PMA in eels since tissue catabolism causes an increased size and (or) number of splenic PMA (Agius and Roberts 1981; Wolke 1992). Without parallel information on individual eel age and feeding status,

Table 3. Regression analysis of the prevalences of pathological changes as a function of the concentration of various contaminants or of the percentage of eels highly contaminated with mirex during the fishing season at Kamouraska ($n = 7$, $y = ax + b$).

	Vertebral malformations					Mesenteric nematodes					Basophilic foci in liver				
	a	*b*	r^2	*F*	*p*	*a*	*b*	r^2	*F*	*p*	*a*	*b*	r^2	*F*	*p*
Pesticides															
Mirex	7.6	14.6	0.59	7.1	0.05	–14.8	–19.6	0.62	8.1	0.04	15.5	39.4	0.41	3.5	0.12
Endrin	10.2	26.5	0.57	6.6	0.05	–8.5	–15.8	0.11	0.6	0.47	14.4	48.5	0.19	1.2	0.33
Heptachlor epoxide	22.0	45.3	0.80	19.9	0.007	–22.9	–40.3	0.23	1.6	0.26	27.4	67.8	0.21	1.3	0.30
Sum of PCB congeners	14.6	5.9	0.34	2.6	0.17	–41.0	–5.8	0.74	14.6	0.01	34.3	22.9	0.32	2.3	0.19
% contaminated eels	0.1	–6.0	0.52	5.4	0.07	–0.3	22.7	0.68	10.9	0.02	0.32	–7.6	0.61	7.8	0.04

Table 4. Body size and prevalences of lesions and parasites in American eels captured at Rivière aux Pins and at Kamouraska.

Variables	Rivière aux Pins (week 1)	Kamouraska (weeks 1–7)
Body length (cm)	60 (56–69)	84 (77–92)*
Body mass (g)	368 (369–570)	1172 (852–1670)*
CF (g/cm^3)	0.17 (0.16–0.19)	0.20 (0.18–0.22)*
n	100	474
Prevalences of lesions and parasites (sample size)		
Vertebral malformations	0 (100)	1.9 (474)
Mesenteric nematodes	0 (100)	2.7 (474)
Azygia longa	9 (100)	23 (474)
Basophilic foci in liver	0 (15)	14 (105)
Vacuolated foci in liver	0 (15)	17 (105)
Contaminants		
Mirex (μg/g)	0.001 (0.001–0.001)	0.024 (0.007–0.107)*
PCB (μg/g)	0.007 (0.005–0.008)	0.54 (0.32–1.10)*
n	7	90

Note: Body mass (*W*), length (*L*), and condition factor (CF = (*W* (g)/L^3 (cm)] × 100) are given as medians (with the first to third quartiles given in parentheses).

*Significantly different from Rivière aux Pins (Wilcoxon, $p \leq 0.05$).

Table 5. Mean (with SD given in parentheses) diameters and percentages of different stages of oocytes in American eels collected in Kamouraska and Rivière aux Pins on the first week of the fishing season.

	Kamouraska	Rivière aux Pins
Diameter (μm)		
Stage 1	59 (6)	55 (6)
Stage 2	119 (10)	106 (11)*
Stage 3	174 (14)	152 (13)*
Stage 4	260 (26)	235 (19)*
Percentages (%)		
Stage 1	9 (5)	14 (6)*
Stage 2	16 (6)	13 (5)
Stage 3	18 (6)	13 (3)*
Stage 4	58 (9)	61 (8)

Note: Fifteen eels were analysed at each sampling location.

*Significantly different from Kamouraska (Wilcoxon, $p \leq 0.01$).

splenic PMA density in eels cannot be used as a reliable biomarker of environmental contamination.

Mesenteric nematodes were encountered only in less contaminated eels captured at Kamouraska and not in more contaminated eels from Kamouraska or in uncontaminated eels from the reference tributary, Rivière aux Pins. Thus, these parasites are probably indicative of the origin of the eels and not of chemical contamination. They could potentially be used to discriminate geographic origins of migrating eels (Williams et al. 1992), but further detailed parasitological studies would be needed to fully evaluate this method of discrimination.

It was not possible to discriminate the freshwater origin of the eels sampled at Kamouraska on the basis of the presence or absence of mirex (Hodson et al. 1994), but the relationship between mirex concentration and body mass provided a very clear separation. We were able to demonstrate that percentages of eels with higher loads of mirex increased with time during the fishing period and that, for the entire fishing season, 71% of the eels probably came from the Lake Ontario – St. Lawrence River axis, assuming Lake Ontario is the primary source. On the basis of presence or absence of mirex, Dutil et al. (1985) estimated that 74% of migrating eels captured between Kamouraska and Lake St-Pierre in 1982 came from Lake Ontario. Between 1982 and 1990, levels of mirex in eel flesh have decreased by 56%, and mirex contamination has spread downstream from its source in Lake Ontario, probably via riverine and atmospheric transport (Castonguay et al. 1989; Sergeant et al. 1993; Hodson et al. 1994); eels from the St. Lawrence Estuary tributaries are now contaminated with low levels of mirex (Hodson et al. 1994).

All gonad sections showed a large proportion of vitellogenic oocytes with diameters within the range of those reported previously for migrating silver American eels (Wenner and Musick 1974). We expected oocyte maturation to progress throughout the migration season, but oocyte diameters and proportions of various stages did not vary significantly among weeks. These parameters were very variable within weeks and larger sample sizes may be necessary to document temporal variations.

The health of any migrating animal population is difficult to evaluate because of complex and variable patterns of spatial and temporal distributions of different cohorts. Intensive daily sampling throughout the migration period may be required to obtain reliable estimates of lesion prevalences (Dutil 1994). However, despite a limited weekly sampling effort, the prevalences of external lesions estimated in this study are comparable with those estimated in eels captured at Kamouraska in 1992 with an intensive daily sampling schedule (Dutil 1994; Dutil et al. 1996). Prevalences of lesions in the migrating population may have been underestimated because diseased eels may die before reaching the Estuary or may be less vulnerable to traps. The apparent good health (low prevalences of

lesions) of the highly contaminated eels captured in Kamouraska may be a consequence of this sampling bias and may not be a reliable index of the health of the whole population.

This study demonstrates an association between contamination and pathological changes in American eel. As a descriptive study, it was not intended to demonstrate cause–effect relationships, because numerous confounding variables are possibly associated with OC contamination. For example, eels that are more heavily contaminated with OCs may also be older, have a longer distance to travel, be exposed to more migratory stresses, pass through the turbines of hydroelectric dams, and be exposed to other contaminants or to habitat deterioration (Couillard et al. 1992). All these factors must be assessed, as covariates, together with OC contamination, to understand potential causes for the observed changes. Our objective was to identify health problems possibly related to contaminants and to indicate priorities for further studies on the health status of American eel in the St. Lawrence basin. These research priorities include studies of (*i*) effects of contaminants on prevalences of vertebral malformations and preneoplastic and neoplastic lesions in resident eels in Lake Ontario and other highly contaminated habitats and (*ii*) effects of hydroelectric dams on long-term survival and vertebral malformations in migrating silver eels. The possible impacts of contaminants on eel reproduction should also be assessed by investigating effects of contaminants (iii) on steroid hormone levels and metabolism and (*iv*) on developing embryos of American eel. These studies are important because both pathological changes and reproductive disturbances may have a deleterious impact on the migratory and reproductive success of the declining population of American eels.

Migrating American eels have been proposed as a vector of contaminants for beluga whales (*Delphinapterus leucas*) and may be a source of specific toxic compound that are present in Lake Ontario but not in the St. Lawrence Estuary (Lum et al. 1987; Hickie et al. 1991; Béland et al. 1993; Hodson et al. 1994; Muir et al. 1996). In the St. Lawrence Estuary, American eels and beluga whales have the following common characteristics: both species are highly contaminated with OCs including mirex originating from Lake Ontario and the upper St. Lawrence River, both have experienced low population sizes and finally, and both have preneoplastic or neoplastic lesions suggesting exposure to environmental carcinogens (this study; Martineau et al. 1994). For both species, it is difficult to establish the link between contamination and the observed health problems because of sampling bias and of multiple factors other than contaminants possibly affecting the population. Additional studies are needed to further our understanding of effects of eel contamination on the health and reproduction of eels and belugas.

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