Do infections with parasites and exposure to pollution affect susceptibility to predation in johnny darters (*Etheostoma nigrum* Rafinesque, 1820)?

Est-ce que les infections parasitaires et l'exposition à la pollution affectent la susceptibilité à la prédation chez raseux-de-terre noir (*Etheostoma nigrum* Rafinesque, 1820)?

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Abstract: Johnny darters (*Etheostoma nigrum* Rafinesque, 1820) were collected from five localities in the St. Lawrence River in southwestern Quebec to test the effects of natural parasite infections and exposure *in situ* to pollution on their anti-predator behaviour. Three measures of antipredator behaviour were made: capture time, capture order and flight initiation distance. Capture time, the time taken to catch individual fish, was used as a proxy for ability to evade predation, capture order was the order in which fish kept in a single tank were taken from the tank, and flight initiation distance was the distance at which the fish moved when approached by a model predator. Only capture time showed a significant correlation with parasitism or pollution status. A non-parametric permutational multivariate ANOVA showed that capture time was significantly correlated with capture location and the abundance of the brain-encysting trematode *Ornithodiplostomum* sp. Infection with *Ornithodiplostomum* sp. may have led to an increase in activity, which would be maladaptive for this cryptic, benthic fish under natural predation conditions. Pollution may have an indirect effect on predator susceptibility in johnny darters, by reducing the abundance of a behaviour-modifying parasite.

Résumé: Des raseux-de-terre noir (*Etheostoma nigrum* Rafinesque, 1820) ont été récoltés à cinq stations le long du fleuve Saint-Laurent (sud-ouest du Québec), dans le but d'examiner l'effet conjoint de l'infection parasitaire naturelle et de l'exposition à des niveaux réalistes de pollution sur le comportement anti-prédateur des poissons. Trois mesures de comportement anti-prédateur ont été réalisées : 1) le temps de capture, soit le temps nécessaire à la capture d'un poisson donné à l'aide d'un filet, 2) l'ordre de capture,

soit l'ordre dans lequel les poissons d'un bassin étaient capturés et 3) la distance d'amorce de la fuite, soit la distance à partir de laquelle un poisson se déplaçait lorsque approché par un prédateur factice. Seul le temps de capture a montré une corrélation significative avec le parasitisme ou le niveau de pollution du milieu d'origine. Cette mesure a donc été utilisée comme témoin de la capacité d'un poisson d'échapper à un prédateur. Une analyse de variance non-paramétrique multidimensionnelle avec tests par permutations a montré que le temps de capture était significativement corrélé à la station d'échantillonnage et à l'abondance d'*Ornithodiplostomum* sp., un trématode enkysté dans le cerveau. L'infection par *Ornithodiplostomum* sp. pourrait conduire à une hyperactivité, un comportement potentiellement mésadapté dans des conditions de prédation naturelle pour ce poisson benthique au mœurs cryptiques. Par ailleurs, la pollution pourrait avoir des effets négatifs indirects sur la susceptibilité aux prédateurs chez le raseux-de-terre noir, en réduisant l'infection par un parasite capable de modifier le comportement de son hôte.

Introduction

Parasites and pollution are common stressors in aquatic ecosystems, and both may affect fish behaviour. The effects of parasites on fish behaviour may be adaptive or simply reflect pathology caused by the parasite (Poulin 1995; Barber et al. 2000; Moore 2002; Barber and Rushbrook 2008). Larval stages of trophically transmitted parasites commonly manipulate behaviour in fish intermediate hosts. They may increase their transmission success by modifying their host's behaviour to increase its susceptibility to predation by the downstream host in the life cycle. Such changes include increased flashing and surfacing, reduced schooling, and altered habitat use. Pathological changes include lethargy, increased or decreased foraging activity, and altered social interactions (reviewed in Barber et al. 2000; Moore 2002; Barber and Rushbrook 2008).

Chronic exposure to sublethal levels of pollutants can also cause changes in fish anti-predator behaviour. Studies of direct effects of exposure to metals, organic chemicals and pesticides show that exposed fish may be more susceptible to predation (reviewed by Atchison et al. 1987; Clotfelter and Levering 2004; Scott and Sloman 2004), because of impaired physiological performance, sensory perception or information processing (Sloman 2007). Pollution can also indirectly affect fish behaviour by eliminating, decreasing or increasing the abundance of behaviour-modifying parasites (Lafferty 1997; Sures 2004; Marcogliese 2005).

Parasitism and pollution stress may have combined effects on fish health. Juvenile Chinook salmon (*Oncorhynchus tschawytscha*) experimentally infected with metacercariae of the digenetic trematode *Nanophyetus salmincola* and exposed to PCBs had lower immune function and were more susceptible to infection by the bacterium

Listonella anguillarum than fish exposed to only individual stressors (Jacobson et al. 2003). Yellow perch (*Perca flavescens*) exposed to environmental levels of industrial and agricultural pollution and naturally infected with larvae of the nematode *Raphidascaris acus* had higher oxidative stress levels than fish exposed to only one of these stressors (Marcogliese et al. 2005). Spottail shiners (*Notropis hudsonius*) exposed to environmental levels of urban and industrial effluents and naturally infected with the trematode *Plagioporus sinitsini* had more pigmented macrophages in their spleens (a general indicator of stress), and lower condition indices than fish exposed to either stressor alone (Thilakaratne et al. 2007).

Behaviour is an important indicator of stress in fish, linking the physiological effects of parasites and pollution with ecological processes (Scott and Sloman 2004; Barber and Rushbrook 2008). Changes in antipredator behaviour are of particular ecological relevance because they have direct consequences for future host fitness. Although parasitism and pollution both have the potential to affect fish behaviour, no studies published to date have considered the combined effects of these two stressors. In this study, we test the combined effects of parasitism and pollution on the antipredator behaviour of johnny darters (*Etheostoma nigrum* Rafinesque, 1820), using fish from contaminated and reference localities in the St. Lawrence River. Johnny darters are small, cryptically coloured benthic fish commonly found in the St. Lawrence River in southwestern Quebec, Canada. They inhabit both relatively pristine and polluted areas of the river, and are host to a diverse community of parasites. The parasite communities of johnny darters from the St. Lawrence River show differences that are correlated with pollution status of sampling localities, as well as the type of pollution (Krause et al.

2010). Here we specifically examine whether pollution and parasitism have a combined effect on fish behaviour, and whether changes in fish parasite community assemblages related to pollution have additional effects on fish behaviour. This study uses field-collected specimens to examine the combined effects of natural parasite communities and mixtures of contaminants, both of which are more relevant to understanding natural fish populations than simplified laboratory experiments that focus on single species and chemicals (Jobling 1995; Marcogliese 2005; Bordes and Morand 2009). While the nature of the study location precludes perfect replication of particular pollution mixtures or parasite community assemblages, we expected to see differences between polluted and reference localities, based on other studies using different indicators of pollution and parasite stress in fish collected from the same localities (e.g. Marcogliese et al. 2005; Thilakaratne et al. 2007; Marcogliese et al. 2010).

Materials and Methods

Study localities

Fish were collected in June 2008 from five localities in the St. Lawrence River in southwestern Quebec, Canada. These included two reference localities, Îles de la Paix (IPA; 45°20.022' N; 73°51.362' W) and Île Dorval (IDO; 45°26.016' N; 73°44.234' W), and three polluted localities, Beauharnois (BEA; 45°19.051' N; 73°52.020' W), Îlet Vert (IVT; 45°42.230' N; 73°27.143' W) and Île Beauregard (IBE; 45°44.965' N; 73°24.910' W) (Fig. 1). Localities were characterized in previous studies, based on concentrations of metals, polychlorinated biphenyls (PCBs), and other contaminants in the sediments (Loiselle et al. 1997; Marcogliese et al. 2005, 2006; Dautremepuits et al. 2009). These

measures are considered an accurate representation of pollution status because sediment contamination is relatively stable over time in this system (Dautremepuits et al. 2009). They are also biologically significant, because johnny darters are benthic organisms that spend their lives in close contact with the sediment and feed on benthic invertebrates (Strange 1991). The reference localities, IPA and IDO, are located upstream of the Island of Montreal in Lake St. Louis. No contaminants surpassing the Canadian Environmental Quality Guidelines Probable Effects Level (PEL) (http://ceqg-rcqe.ccme.ca/for aquatic life) were detected at either locality (Marcogliese et al. 2006). One polluted locality, BEA, is also located in Lake St. Louis, at the mouth of the St. Louis River. It is primarily affected by industrial and agricultural activity upstream in the St. Louis River. BEA has high levels of PCBs, orgnanochlorines, and several metals, particularly mercury, which surpass the PEL (Loiselle et al. 1997; Marcogliese et al. 2005; Dautremepuits et al. 2009). The other polluted localities, IVT and IBE, are located downstream of Montreal in the plume of the Montreal sewage treatment plant outfall. They both have high levels of organochlorines, PCBs and some metals. PCB levels at IBE and chromium levels at IVT surpass the PEL (Marcogliese et al. 2006; M. Pelletier, personal communication, 2009).

Study organisms

One hundred and seventy-eight johnny darters were collected using a beach seine (22.6 × 1.15m; 3mm mesh) and transported live to the laboratory. Fish from each locality (n = 35-36 per locality) were kept in separate tanks (90cm × 45cm × 35cm). Tanks were lined with aquarium gravel, filled with 60L of dechlorinated tap water, and were continuously aerated. Tanks were covered on three walls with opaque black plastic to

prevent fish from seeing those in neighbouring tanks. Fish were kept at 20°C, in a 14:10 light:dark regime and were fed with Nutrafin™ fish flakes *ad lib*. The sex ratio of the fish was approximately 1:1, and all fish were presumed to be from the 1+ age class, as demonstrated by the length frequency distributions from each locality (Bagenal and Tesch 1978).

Behaviour experiments

Fish were acclimated in the laboratory for at least six days prior to testing (Smith 1979). Behaviour experiments for fish from each locality were conducted over two to three consecutive days, within 14 days of collection. Behavioural metrics were chosen based on results from preliminary experiments on johnny darters from two localities in the St. Lawrence River, IPA and IVT, in September 2007. Two experiments were conducted. The first experiment measured capture time of each fish (n = 178), defined as the time taken to catch individual fish, and was considered a proxy for susceptibility to predation. Capture time was tested in the same tanks used for acclimation, to minimize unnecessary handling of the fish. The experiment consisted of catching fish one by one from the large tank using a hand-held dip net (43cm long, 13cm × 16cm opening). During the experiment, the dip net was placed in the middle of the water column in the centre of the tank and shaken vigorously to alert the fish to the net "predator." The net was then moved in a regular manner counterclockwise along the walls of the tank, at an approximately constant speed of 20cm/s around the tank until a fish was caught. This method of capturing the fish, including the capture speed of 20cm/s, was optimized during a pilot study. This procedure was repeated until all fish were caught. The order in

183 which fish were removed from each tank was recorded as “capture order” and examined
184 as an additional behaviour measure.
185 After fish were caught in the capture time experiment, they were transferred into
186 test tanks for the second behaviour experiment, a measurement of flight initiation
187 distance. Fish were paired in narrow test tanks (90cm × 30cm × 35cm, 50L), which were
188 covered on three walls with black, opaque plastic to hide the experimenter from view of
189 the fish. They were left to acclimate in the tanks for two hours before beginning the trial,
190 during which time two carbon water filters were run in the test tanks to remove any
191 chemical cues left by fish previously tested in the tanks. The water filters were turned off
192 during the trial. Flight initiation distance was measured by moving a model of a predatory
193 fish towards the two fish at an approximate speed of 10 cm/s, starting from the end of the
194 tank farthest from the fish. The speed of approach was identified during preliminary trials
195 as the optimal speed for the experiment. The predator model used was a semi-realistic
196 plastic model of a fish, approximately five times larger than the johnny darters. Flight
197 initiation distance was measured for the “focal” fish (n = 89), the fish closest to the
198 approaching predator; the second “dither” fish was placed in the tank to reduce the stress
199 level of the focal fish (Brown et al. 2006). The experiment was filmed and flight
200 initiation distance, defined as the distance from the predator model at which the fish
201 initiated movement, was measured from the video recording.
202 Following the behaviour experiments, fish were killed with an overdose of clove
203 oil solution (50 mg/L) and frozen for later necropsy. All animal collection and
204 experimental procedures were in accordance with guidelines of the Canadian Council on
205 Animal Care in effect at the time of the study.

206 **Examination for parasites**

207 Frozen mass (mg) and standard length (mm) were measured for each fish and
208 followed by a complete necropsy. Parasites from tissues and organs, including fins, skin,
209 gills, eyes, brain, body cavity, gastrointestinal tract, liver, heart, spleen, gonads and
210 muscle were collected following standard parasite examination protocols (Marcogliese
211 2002). During the necropsy, all parasites were enumerated and identified to genus, with
212 the exception of acanthocephalans, non-gyrodactylid monogeneans, and a few rare
213 trematodes, which could only be identified to higher taxonomic levels. Representative
214 samples of parasites recovered from each locality were preserved in 70% ethanol for later
215 identification. Trematodes, cestodes, acanthocephalans and some monogeneans were
216 stained with acetocarmine, cleared with xylene and mounted in Permount or Canada
217 balsam. Other monogeneans were mounted unstained in Hoyer's medium. The remaining
218 monogeneans and all nematodes and copepods were cleared in glycerine alcohol and
219 examined in temporary mounts. Identifications were made using keys in Beverly-Burton
220 (1984), Kabata (1988), Caira (1989), Moravec (1994), Gibson (1996), Scholz (1997) and
221 Hoffman (1999).

222

223 **Statistical analysis**

224 Mean total parasite number, infracommunity species richness, standard length,
225 capture time and flight initiation distance of fish were tested among localities and
226 between polluted and reference localities. Comparisons among localities and between
227 treatments were made using ranked data by one-way ANOVAs (Scheirer and Hare 1976)
228 followed by Tukey-Kramer HSD tests, except for standard length, which was tested using

untransformed data. Because capture order of individual fish was dependent on capture order of other fish from within the same tank (i.e. locality), it could not be compared among localities or between fish pooled by pollution status. Separate regression comparisons for each locality were made between capture order of individual fish and their total parasite number and infracommunity species richness (15 comparisons total). All univariate tests were conducted using JMP® 7.0.1 (© 2007 SAS Institute Inc.).

Multivariate analyses were conducted using the PERMANOVA+ add-on for PRIMER (© 2006 Plymouth Routines In Multivariate Ecological Research, Plymouth, UK). A stepwise regression of capture time with abundances of all parasite species was performed with a distance-based linear model (DISTLM). This test allows for a stepwise test of continuous variables that are not normally distributed. Species that significantly correlated with capture time were included as covariates in a permutational multivariate ANOVA (PERMANOVA) of capture time. PERMANOVA is a nonparametric test analogous to a multivariate ANOVA. It gives the test statistic Pseudo-*F*, which is analogous to the *F* statistic in measuring the among-group to within-group variation. The initial model also included mean total parasite number, mean infracommunity species richness, mean standard length, locality, and interactions between variables. The final model included only terms that significantly explained capture time.

Mean abundances of parasite species included in the PERMANOVA were individually compared among all localities using ANOVAs on ranked data, followed by a Tukey HSD tests. Tests between polluted and reference localities were performed with nonparametric Wilcoxon tests.

Terminology

Parasite terminology adheres to definitions of Bush et al. (1997). Prevalence is the percentage of hosts infected with a given parasite species in a sample. Abundance is the number of parasites of a given species infecting a given host, whether the host is infected or not. Mean abundance is the number of parasites of a given species averaged over the whole host sample, and includes hosts with and without infections. Intensity is the number of parasites of a given species infecting a host, and mean intensity is the number of parasites of that species averaged across infected hosts in a sample. An infracommunity refers to all the individuals of all the parasite species within an individual host. Locality refers to the geographic area from which the host was collected, and site refers to the specific host tissue or organ from which the parasite was collected.

Results

Twenty-four species of parasites were identified in the 178 darters examined. The prevalence and mean intensity of each parasite species at each locality are presented in Krause et al. (2010). Mean total parasite number was highest at BEA and IDO, two upstream localities, and lowest at IVT and IBE, both downstream polluted localities ($F_{4,173} = 31.73$, $p < 0.0001$; Table 1). Mean infracommunity species richness was greatest at BEA, second highest at IPA and IDO, and lowest at the downstream polluted localities, IVT and IBE ($F_{4,173} = 38.48$, $p < 0.0001$). Standard length was significantly larger for fish from BEA than those from IDO ($F_{4,173} = 3.62$, $p = 0.007$), but did not differ among fish from other localities.

Capture time differed significantly among localities ($F_{4,173}$ = 6.20, p = 0.0001), with the longest capture time at IDO and the shortest capture time at IVT (Fig. 2). Capture time did not differ significantly between fish from polluted and reference localities ($F_{1,176}$ = 1.57, p = 0.12). Flight initiation distance did not differ significantly between localities ($F_{4,173}$ = 0.33, p= 0.85) or between polluted and reference localities ($F_{1,176}$ = 0.65, p = 0.42). There was no correlation between capture order and standard length, total parasite number or parasite species richness (all p values ≥ 0.07).

Mean total parasite number and mean infracommunity species richness was weakly, but significantly correlated with capture time (total parasite number: R^2 = 0.03, n = 178, p = 0.03; species richness: R^2 = 0.03, n = 178, p = 0.03). The only parasite species that was related to capture time was *Ornithodiplostomum* sp. A nonparametric DISTLM analysis of pooled data showed that the relationship between *Ornithodiplostomum* sp. abundance and capture time was significantly positive (R^2 = 0.15, n = 178, p = 0.0001; Fig. 3), suggesting that fish with higher intensity infections might be less susceptible to capture than fish with low or no infection. *Ornithodiplostomum* sp. mean abundance was highest at IDO, followed by IPA, and was lowest at BEA, IVT and IBE ($F_{4,173}$ = 46.6, p < 0.0001; Fig. 4). It was significantly higher at reference than polluted localities ($F_{1,176}$ = 119.40, p < 0.0001). Capture time was best explained by a PERMANOVA model including *Ornithodiplostomum* sp. abundance (Pseudo-F = 18.82, p = 0.002, df = 1) and locality (Pseudo-F = 2.45, p = 0.039, df = 4).

Discussion

Johnny darters with high intensities of the brain-encysting parasite, *Ornithodiplostomum* sp., had longer capture times than fish with low or no infections, perhaps reflecting an increase in activity of infected fish. Johnny darters normally exhibit a cessation of movement when they detect a predator (Smith 1979). Stressors that induce hyperactivity may disrupt adaptive anti-predator behaviour in this species. In this study, darters exhibiting typical antipredator behaviour appeared to be more susceptible to capture, while fish behaving abnormally, by moving quickly and erratically, and swimming to the surface, were more difficult to catch. In natural systems, however, predators such as mergansers and other piscivorous diving birds, the definitive hosts of *Ornithodiplostomum* spp., typically depend on visual cues such as movement to capture their prey, and an increase in activity may make cryptic fish such as johnny darters more susceptible to predation (Ydenberg and Dill 1986). These results should be interpreted with caution, because our sampling design does not allow us to consider parasitism and pollution separately; however, deviations from typical, cryptic anti-predator behaviour of johnny darters caused by high intensities of *Ornithodiplostomum* sp. may reflect an adaptation of the parasite to increase its transmission success. Alternatively, the increased activity observed could simply be a pathogenic by-product of infection (Poulin 1995).

Neither parasitism nor pollution could statistically explain observed differences in either of the other two behavioural measures, capture order or flight initiation distance. Capture time has not been used in previous studies; however it was measured because it showed a significant correlation with parasitism in a pilot study. Flight initiation distance is a measure commonly used to assess fish reactions to predation risk (Ydenberg and Dill

1986). The lack of response in this study suggest that it may be an inappropriate measure of anti-predator behaviour in a species such as the johnny darter that typically exhibits a cessation of movement in response to perceived predators.

Studies of fathead minnows (*Pimephales promelas*) with infections of *Ornithodiplostomum ptychocheilus* suggest that behavioural changes may be caused by adaptive manipulation by the parasite or pathology of parasite development in the host. Fathead minnows with mature infections of *O. ptychocheilus* exhibited less compact shoaling behaviour and swam higher in the water column, which may make them more susceptible to predation (Radabaugh 1980). Alternatively, minnows with new infections of *O. ptychocheilus* showed reduced standard optomotor response (OMR), likely due to damage caused at the site of infection, the optic tectum (Shirakashi and Goater 2001, 2002). The greatest decrease in OMR occurred during parasite development and subsided after they reached infectivity, reflecting damage to the optic lobes during parasite growth (Shirakashi and Goater 2005). Behavioural changes induced before a parasite becomes infective are considered pathological, while those that ensue following development to the infective forms may be evidence of adaptation (Poulin 1995). The present study does not explore the specific physiological mechanisms of the observed behavioural change, nor does it measure actual predation rates of infected and non-infected fish. However, evidence from other *Ornithodiplostomum*-fish systems, as seen above, suggests that both scenarios are possible. In our study, parasites were encysted and presumably infective, lending support to the idea that the behavioural changes may be adaptive. Further experiments to test the fitness consequences for both the parasite and host are necessary

342 to determine whether the behaviour change seen here is an adaptive modification by the
343 parasite or merely a pathological side effect (Poulin 1995).

344 Locality was also significantly correlated with differences in fish behaviour. This
345 may reflect a tank effect in the experimental design, because fish from each locality were
346 kept and tested in a single tank. However, it may also be due to a parasite effect that was
347 not statistically detectable. Mean capture time of fish from different localities showed
348 patterns similar to patterns of parasite community parameters: fish from BEA and IDO
349 had higher capture times than fish from IVT, and also higher mean species richness and
350 mean total parasite number. Only *Ornithodiplostomum* sp., the parasite in the highest
351 abundance, was significant in the model of capture time, however failure to detect effects
352 of other species may be due to low infection intensities and species richness. However,
353 the fact that species richness was weakly correlated with capture time lends some support
354 to the idea that parasite diversity may have impacts on individual hosts (Bordes and
355 Morrand 2009). There was no interaction between locality and mean abundance of
356 *Ornithodiplostomum* sp., suggesting that the effects of the parasite on behaviour were
357 independent of pollution exposure.

358 A direct, general effect of pollution on fish behaviour was not detected, nor could
359 we detect an interactive effect of pollution and parasitism. However, pollution appears to
360 have a negative effect on *Ornithodiplostomum* sp. infections in johnny darters in this
361 system, through reducing the abundance of this parasite (Krause et al. 2010). Free-living
362 cercariae of digenetic trematodes are sensitive to a variety of types of pollution, including
363 metals, acidification, chemical fertilizers and pesticides, which can reduce their survival,
364 longevity, encystment and infectivity (Morley et al. 2003; Pietrock and Marcogliese

2003). Cercariae of *O. ptychocheilus* exposed to cadmium showed decreased infectivity to fish (Pietrock and Goater 2005). Therefore, metal pollution may indirectly affect johnny darter behaviour at contaminated localities, through the reduction of survival and/or infectivity of cercariae of *Ornithodiplostomum* sp.

Previous studies of effects of pollution and parasite stress on fish behaviour have focused primarily on single pollutants and single parasite species, and have not tested both stressors together. This study considers these stressors in combination, and tests naturally-infected fish obtained directly from polluted localities. This approach can limit the interpretive power of the study because it does not allow hypotheses regarding effects of specific pollutants to be tested. However this observational approach is nonetheless valuable because it can provide important information about the effects of actual conditions and mixtures. In nature, pollution stress is often due to combinations of many chemicals (Jobling 1995; Lafferty 1997; Marcogliese 2005) and fish are commonly infected with communities of parasites (Barber et al. 2000; Barber and Rushbrook 2008; Bordes and Morand 2009), conditions that are difficult to replicate in laboratory experiments.

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Table 1. Mean total parasites, parasite infracommunity species richness and total length ± SD of johnny darters from five localities in June 2008 in the St. Lawrence River in Quebec, Canada: Beauharnois (BEA), an upstream polluted locality, Île Beauregard (IBE) and Îlet Vert (IVT), downstream polluted localities, and Île Dorval (IDO) and Îles de la Paix (IPA).

Locality	Mean total parasites ± SD	Mean parasite infracommunity species richness ±SD	Mean total length (mm) ± SD
BEA	45.6 ± 30.5	7.7 ± 1.9	51.0 ± 6.9
IVT	14.17 ± 11.7	3.3 ± 1.3	47.9 ± 4.3
IBE	13.5 ± 10.8	3.9 ± 1.5	50.0 ± 4.4
IPA	32.0 ± 28.5	5.6 ± 2.0	46.9 ± 5.3
IDO	54.6 ± 41.4	5.2 ± 1.5	48.1 ± 5.1

Fig. 1. Map of the St. Lawrence River in southwestern Quebec, Canada, showing the five localities sampled in June 2008: one upstream polluted locality, Beauharnois (BEA); two downstream polluted localities, Îlet Vert (IVT) and Île Beauregard (IBE); and two reference localities, Îles de la Paix (IPA) and Île Dorval (IDO).

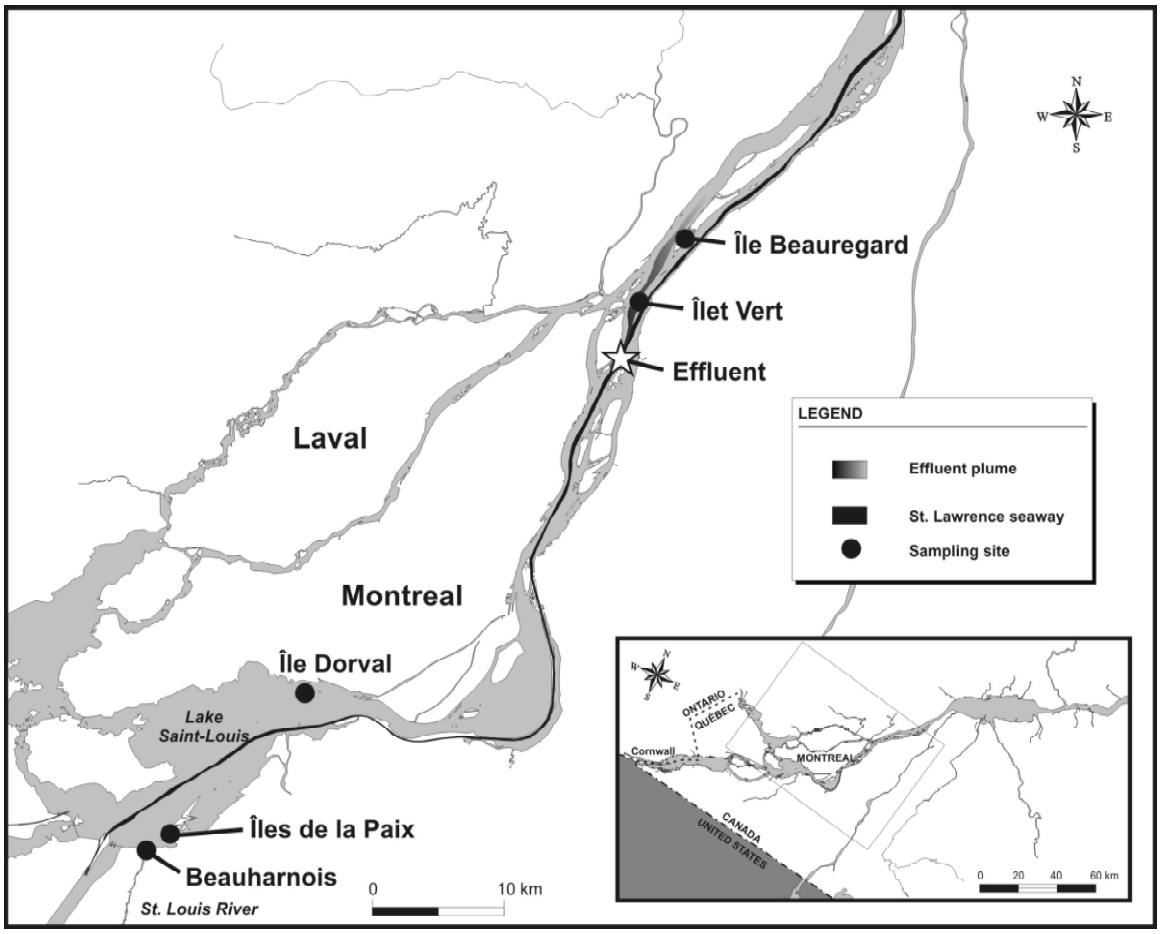

Fig. 2. Mean capture time (s) ± standard error of johnny darters from five localities in June 2008 in the St. Lawrence River in southwestern Quebec, Canada: one upstream polluted locality (light grey), Beauharnois (BEA); two downstream polluted localities (dark grey), Île Beauregard (IBE), Îlet Vert (IVT); and two reference localities (white), Île Dorval (IDO) and Îles de la Paix (IPA). Different letters indicate significant differences between localities.

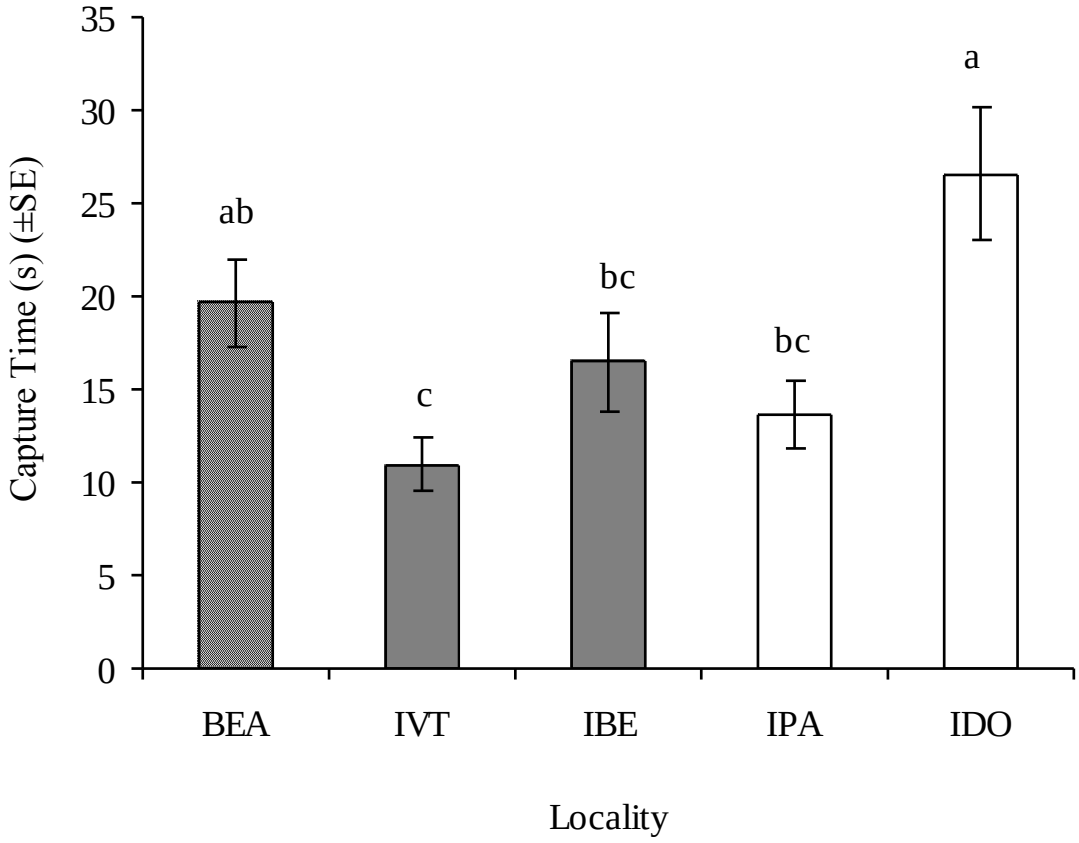

Fig. 3. Scatter plot of capture time (s) versus *Ornithodiplostomum* sp. abundance for johnny darters from five localities in June 2008 in the St. Lawrence River, Quebec, Canada.

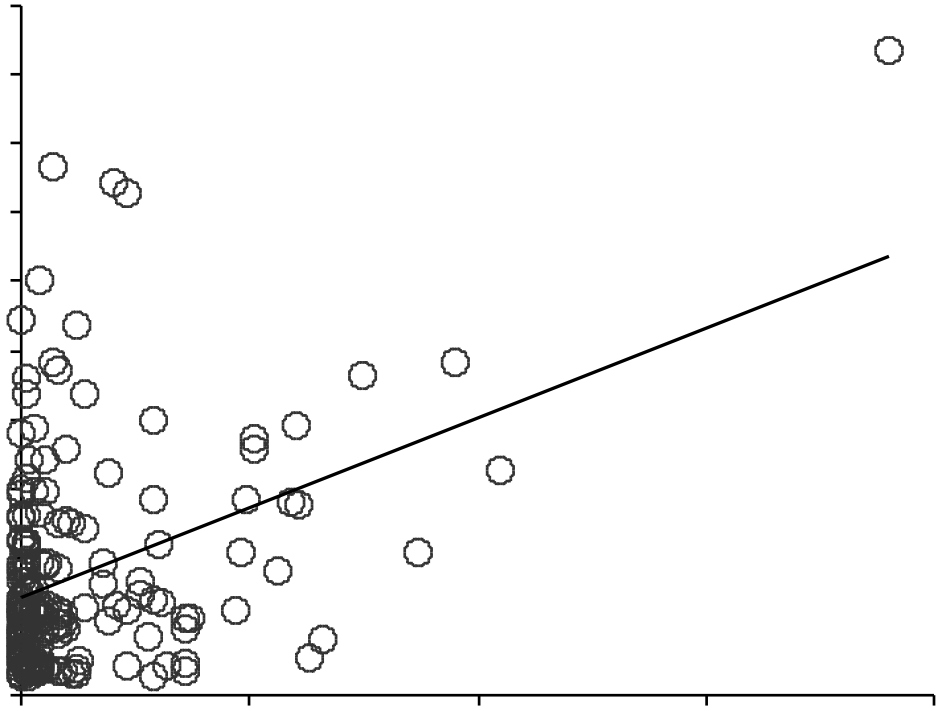

Fig. 4. Mean capture time (s) and mean *Ornithodiplostomum* sp. abundance in johnny darters from five localities in June 2008 in the St. Lawrence River in Quebec, Canada: one upstream polluted locality (grey circle), Beauharnois (BEA); two downstream polluted localities (black circles), Île Beauregard (IBE) and Îlet Vert (IVT); and two reference localities (white circles), Île Dorval (IDO) and Îles de la Paix (IPA). Error bars represent standard errors.