Marine Pollution Bulletin 106 (2016) 225–235

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Use of the Atlantic nut clam (*Nucula proxima*) and catworm (*Nephtys incisa*) in a sentinel species approach for monitoring the health of Bay of Fundy estuaries

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ARTICLE INFO

Article history:
Received 5 August 2015
Received in revised form 21 February 2016
Accepted 26 February 2016
Available online 16 March 2016

Keywords:
Saint John Harbor
Metals
Estuarine benthic invertebrates
Sediment contamination
Environmental monitoring

ABSTRACT

Designing an effective environmental monitoring system for population responses requires knowledge of the biology of appropriate sentinel species and baseline information on the area's physical and chemical characteristics. This study collected information in Saint John Harbor, NB, Canada, for two abundant marine benthic invertebrates, the Atlantic nut clam (*Nucula proxima*) and the catworm (*Nephtys incisa*) to characterize their seasonal and spatial variability, determine the ideal sampling time and methods, and develop baseline data for future studies. We also evaluated whether contamination is impacting invertebrates by comparing sediment metal concentrations to responses of benthic infauna. Metals were generally below sediment quality guidelines except for nickel and arsenic. Clam densities were variable between sites but not seasons, whereas catworm densities were not significantly different between sites or seasons. Overall, these species show potential for environmental monitoring, although investigation at more contaminated sites is warranted to assess their sensitivity.

1. Introduction

Benthic invertebrates are often used in environmental monitoring, as they live in or on the sediment and are exposed to and affected by sediment-borne contaminants. There have been a variety of approaches for monitoring benthic invertebrates, including traditional community-level indicators of benthic macrofauna, such as richness, diversity, and abundance (e.g. Dowd et al., 2014), and assessments of benthic megafaunal communities via trawl catches (e.g. Jørgensen et al., 2015). Studies focusing on population-level indices, however, are relatively few in number, despite this approach also being an effective means of monitoring (Schafer et al., 1995), and include assessments of stressors, such as sediment contamination, on growth, fecundity, and abundance in marine benthic invertebrate species (Munari and Mistri, 2007; Zajac and Whitlatch, 1988). As for community-level effects, population-level endpoints have also been shown to respond to chemical contamination of the sediment (Moreira et al., 2006) or water column (Munari and Mistri, 2007), or to physical disturbances (Zajac and Whitlatch, 1989).

An ideal sentinel species is one that is resident, abundant, can be easily collected, and which responds to contaminants in a measurable manner (Amiard-Triquet et al., 2012). Detecting changes in a population due to human activities requires an understanding of baseline conditions, natural variability, and the magnitude of change that represents meaningful impairment. This requires sufficient baseline data from reference sites or pre-impact data, the selection of appropriate sentinel species and consideration of their life history characteristics to ensure that sampling is done at the most appropriate time (Barrett and Munkittrick, 2010). This information is not always known, particularly when dealing with species of benthic invertebrates, since the majority of information on these animals stems from inventory assessments or community surveys, which neglect to study specific species in detail. In addition, as population characteristics of benthic invertebrates are variable over time or between locations (Zajac and Whitlatch, 1988, 1989), assessments of their responses to contaminants must have some knowledge of the growth and reproductive cycles to identify when a change or site difference is due to anthropogenic stressors and not natural variability. In addition, the tolerance of benthic invertebrates to pollution varies; for example, in marine ecosystems amphipods such as *Ampelisca agassizi* are generally sensitive whereas the polychaetes *Nephtys incisa* and *Capitella* spp. are considered tolerant to trace metals (Chang et al., 1992). This intraspecific sensitivity needs to be understood before appropriate sentinel species can be chosen.

The objectives of this study in the Saint John Harbor, Bay of Fundy, New Brunswick, Canada, are to develop baseline biological data for two infaunal invertebrate species at sites believed to be low in sediment contaminants, and then to assess growth, abundance and reproduction of these species at more contaminated locations in this estuary. The study was done in two phases: the first sampled benthic invertebrates and sediment metals over time to identify the most appropriate time of year for sampling; and the second assessed benthic invertebrate populations, at the determined time of year, at sites that were known to have historically-high sediment metals. The two species chosen for this study were dominant members of the silt–clay benthic infaunal

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http://dx.doi.org/10.1016/j.marpolbul.2016.02.065
0025-326X/

community, namely the Atlantic nut clam *Nucula proxima* (Say, 1822) and the polychaete *N. incisa* (Malmgren, 1865).

The Atlantic nut clam is a small protobranch bivalve native to the east coast of North America, found from the coast of Nova Scotia down to as far south as Florida (Abbott, 1974). They spawn yearly during the summer, and have lecithotrophic, non-feeding, planktonic larvae (Scheltema and Williams, 2009). While the lifespan of *N. proxima* is not specifically known, the lifespan of other nuclid clams is between 12 and 20 years (Allen, 1954). Average shell size is around 5 mm for an adult clam (Abbott, 1974; Hampson, 1971 as *Nucula annulata*), and they reach maturity at roughly 2 mm shell length (Scheltema and Williams, 2009 as *N. annulata*). *N. proxima* burrows into the surface sediment and feeds on bacteria and organic matter (Drew, 1899; Levinton, 1972) using their labial palps, which grab the food using a set of cilia (Abbott, 1954). Their horizontal distribution is effectively random, with no apparent competition with other nuclids for food or space (Levinton, 1972). *N. proxima* have not been studied in the Bay of Fundy before, nor are their responses to contamination well-known; however, they are known to persist in metal-contaminated sediment and are believed to be moderately tolerant to contamination (Chang et al., 1992).

The second species, *N. incisa*, is a marine polychaete in the catworm family found along the North American coast of the Atlantic Ocean (Rainer, 1990), and is commonly found in the Saint John Harbor, albeit in lower numbers than some of the smaller polychaetes, which can number in the thousands of animals/grab (Hampson, 1971). The most abundant polychaete in Saint John Harbor was *Cossura longocirrata*, however, its small size made length and fecundity measurements very difficult so it was not an ideal species for monitoring programs. *N. incisa*, by contrast, is larger and easier to handle while still common in the infaunal community. The larvae of *N. incisa* are planktonic, reaching up to 550 μm and 9 segments before metamorphosis into a juvenile (Lacalli, 1980). The larval stage lasts for roughly a month, and adults can live for up to three years (Lacalli, 1980; Giangrande, 1997), reaching lengths of 2.5–6.5 cm (Rainer, 1990). *N. incisa* is primarily a facultative deposit feeder, though they may also prey on other invertebrates (Clark, 1962); they thrive in silt/clay sediments and create and abandon burrows as they move in search of food, thereby improving sediment aeration (Davis and Miller, 1979). Populations in Long Island Sound (North of Long Island, NY) were found to be at peak reproduction (based on observations of oogenesis) during spring and fall, with roughly 50% of adults producing gametes at the peak (Carey, 1962; Zajac and Whitlatch, 1988). In addition, a small percentage (roughly 7%) reproduced throughout the year at this site (Zajac and Whitlatch, 1988); however, size and age at maturity have not been clearly determined for this species. *N. incisa* has not previously been studied within the Saint John Harbor, and has only rarely been studied elsewhere. In community-level analyses *N. incisa* is frequently associated with contaminated areas, notably those with high metals, which they appear to tolerate (Chang et al., 1992), but their growth and reproductive responses to sediment contamination are not known.

The current study characterized the variation in invertebrate populations between sites and between seasons, and was part of a broader series of projects aimed at developing a comprehensive baseline against which future samples can be compared to assess changes in Saint John Harbor. Those other studies included benthic community surveys (Van Geest et al., 2015), sediment toxicity assessments (Pippy, 2015), caged bivalve studies (McMullin and Courtenay, unpubl. data), and nearshore sand shrimp surveys (*Crangon septimspinosa*; Power, 2015).

2. Materials and methods

Saint John Harbor, New Brunswick, Canada, is highly industrialized and receives discharges from several industries (brewery, pulp mills, oil refinery) and wastewater treatment plants, in addition to having traffic from local fishing vessels, tankers using the liquid natural gas terminal, oil tankers, and cruise ships. This activity provides a source of contaminants to the harbor, which accumulate in the sediment (Van Geest et al., 2015). In addition, due to the discharge from the Saint John River and the substantial tides in the area (≈8 m), the harbor requires annual dredging to accommodate the passage of larger ships (Envirosphere, 2003); this sediment is deposited at the Black's Point dredge disposal site and has resulted in historically high metals, PCBs, and PAHs at this location (Envirosphere, 2003; Parrott et al., 2002). With the exception of some limited infaunal invertebrate community assessments (Envirosphere, 2002), basic biological information on an appropriate sentinel species is lacking and needed for monitoring long-term changes in this estuary. The information will be relevant for designing monitoring programs more broadly for the Bay of Fundy and for estuaries along the coast of northeastern North America.

2.1. Sampling sites

Six sites known to have low historical metal concentrations (Parrott et al., 2002) were selected as reference locations and sampled across three seasons (October 2011, April 2012, and June 2012). Three inner harbor sites were selected: site 1 (45° 14.939 N, 66° 01.395 W), site 2 (45° 13.895 N, 66° 04.199 W), and site 3 (45° 13.786 N, 66° 01.606 W). The outer harbor sites were site 4 (45° 12.535 N, 66° 03.740 W), site 6 (45° 12.097 N, 65° 58.382 W), and site 13 (45° 12.070 N, 66° 06.382 W). Annual nearshore surface water temperatures range from 0.4 °C to 16.4 °C (Arens, 2003). The ideal time to sample was determined as described in more detail below after the three initial sampling seasons and based on the abundance of animals and their ease of identification (sampling when the majority of animals are small or juvenile makes species-level identification difficult). In October 2012 (the identified ideal time), the six reference sites were sampled as well as three potential hotspots that were identified as having high metal concentrations in previous studies (Parrott et al., 2002). The three potential hotspots were historically high in copper (Cu), chromium (Cr), zinc(Zn), lead (Pb), total mercury (THg), and nickel (Ni), and two of the three were also high in vanadium (V), PAHs, and PCBs (Tay et al., 1997; Parrott et al., 2002; Envirosphere, 2003).

Sediment samples ($n = 5$–10/site) were collected from the Saint John Harbor using a Smith–McIntyre grab sampler measuring 27.9 cm × 11.5 cm, with a surface area of 321 cm^2. For sites where 5 grabs were taken (which represents the majority of dates), the total sampling area was then 1605 cm^2/date. Individual grabs were divided in two, with one half used for contaminant analysis, and the other sieved for invertebrates. The top 5 cm of sediment was collected for contaminant analysis using pre-cleaned core tubes, put in pre-cleaned glass jars, kept on ice and then frozen the same day upon returning to the lab. Invertebrate samples were sieved immediately on the boat and animals were then preserved in ethanol until they could be identified and counted.

2.2. Invertebrate population assessments

Counts for each species were done concurrently with growth measurements, and the number of individuals was tallied to obtain the abundance of *N. proxima* and *N. incisa* per ½ grab. The number of animals was then divided by the surface area of the portion of the grab sampled for invertebrates to yield the number of animals/m^2. Damaged specimens were included in abundance counts if the heads of *N. incisa* were present and if clam tissues were inside the shell; empty shells were not counted.

For *N. proxima* maximum shell length (shell's longest dimension) was measured to the nearest 0.1 mm using a micrometer. Since *N. incisa* are prone to fragmentation during sediment sieving, L3 measurements were taken instead of total body length. L3 refers to the length of the first three segments of an annelid (segmented) worm and is the combined length of the prostomium, peristomium, and first setiger. This measurement is recommended for fragmenting species

(Durou et al., 2008). In both species, badly damaged specimens were not measured. Size frequency distributions were then determined for each species and site by grouping length measurements into bins and representing the number of individuals in each bin.

Reproductive activity and fecundity in *N. incisa* were estimated using egg counts. Worms were stained with Rose Bengal, which stains gonads darker than surrounding tissue. Then, for each worm, one or two segments were removed and dissected to determine the presence or absence of reproductive organs. Segments were randomly removed, since the high degree of fragmentation made removing the same segment from each animal difficult. As *N. incisa* do not have visible gonads outside of periods of active reproduction, each individual with L3 greater than the determined size-at-maturity (adults; see below) was grouped into one of four categories: female (undeveloped eggs), female (developed eggs), adults (no gonads), or male. Males were identified by undifferentiated masses of spermatocytes within the gonads, whereas females had clearly distinguishable eggs. Undeveloped eggs were relatively small — up to ≈80 μm, with the eggs being completely contained within the gonadal sack in each segment. Developed eggs were larger, between 80 and 120 μm, with loose eggs filling the coelom as well as the gonads. Worms with no sign of gonads were labeled as adult (no gonads) if they were above the lowest identified L3 size for sexually mature worms at that site, since there was no way to sex the worms when they are not actively reproducing. Following egg counts, the smallest reproductively-active worms at each site were used to estimate size at maturity and animals smaller than this were then classified as being immature. Because the segments were removed at random, additional segments were removed from non-egg-bearing worms and examined to ensure that eggs were not missed.

Females with developed eggs (and some containing undeveloped eggs) were selected for egg counts. An additional one or two segments were removed for counting and, through careful dissection, non-reproductive tissue was removed and the eggs separated from each other. Depending on the number of eggs, counts were either done in a dish or by mounting the eggs on microscope slides. In rare cases of very large numbers of eggs (>10,000 eggs/segment), eggs were suspended in a known volume of water and aliquots removed and counted to obtain an estimate of the total count. Counts were expressed as the number of eggs/segment.

2.3. Analyses for sediment metals, organic carbon and grain size

Sediments were freeze-dried and homogenized, then subsampled for each analysis. For each sample, 0.500 g of sediment was weighed for metal analysis, and the sample extracted via microwave digestion, as in EPA 3051 A, in 10 mL of nitric acid (US EPA, 2007). Extracts were run on an Inductively Coupled Plasma-Optical Emission Spectroscopy, based on EPA 200.7 (US EPA, 1994), to determine metal content, and Cu, Zn, Pb, As, Cd, Cr, and Ni were quantified using internal standard calibration (the internal standard, Yittrium, was added following digestion). Detection limits differed for each element, but ranged from 0.004 to 6.4 mg/kg dw. Total Hg (THg) was measured from a 0.030 g aliquot of dried, homogenized sediment using a Direct Mercury Analyzer, based on EPA 7473, (US EPA, 1998). The detection limits were calculated as the average plus three times the SD of all method blanks (detection limits for THg were 2.8–4.9 μg/kg dw).

Quality Assurance and Quality Control (QA/QC) for metals consisted of a Method Blank (MB) of nitric acid, a Certified Reference Material (CRM), calibration checks and a sample duplicate for each batch of eleven samples. Instrument Detection Limits (IDLs) were determined after running a blank 20 times (from US EPA, 200.7), and the Limit of Quantification (LOQ) was set as 5× the IDL (Montaser and Golightly, 1992). If the results from the MB exceeded the LOQ, then the LOQ was increased to match the MB. The CRM (National Institute of Standards & Technology Standard Reference Material 2702 Inorganics in Marine Sediment) was used to determine extraction efficiency for each run, and the sample duplicates were compared to obtain a relative percent difference. QA/QC for THg was similar: each batch of ten samples included a method blank, a CRM (NIST SRM 2702 Inorganics in Marine Sediment), calibration standard checks and a sample duplicate.

Sediment composition was determined by the percentage of 20 g of dried and homogenized sediment which was retained on a series of sieves. The different size classes were silt–clay (for particles <0.125 mm), fine and medium sand (0.125 to 0.5 mm), coarse sand (0.5 to 1 mm), and gravel (>1 mm). A Malvern Mastersizer 2000 particle size analyzer was used to analyze clay content in a subset of samples from each site ($n = 3$/site·date). Dried and homogenized sediment was reconstituted in deionized water and an aliquot added to the particle size analyzer, which gave the percentage based on particle size. The sizes of the sediment were then compared to the Wentworth scale to determine the size range of clay particles, and so the clay content of the sample. Total Organic Carbon (TOC) was calculated after measuring loss on ignition (LOI) using a method modified from Wang et al. (2011), with the drying times reduced to 3.5 h. Twenty samples were sent to the Research Productivity Council in Fredericton, New Brunswick, for direct TOC analyses using LECO combustion. The TOC content for all samples was calculated from LOI using a regression model developed from paired measurements ($R^2 = 0.882$, $n = 20$).

2.4. Statistics

Sediment contaminant concentrations and animal densities were analyzed by ANOVA. Assumptions of normality were checked using probability plots, and assumptions of homogeneity of variances were tested using Levene's test. The metal concentrations for Cu had a skewed distribution in October 2011, and Cd was skewed in October 2011 and April 2012. THg concentrations in April also failed the assumption of homogeneity of variances. Log transformation of these metals did not improve their distribution or variance; however, since ANOVA is considered a robust test, and the transformation did not improve the data, parametric tests were used on the untransformed data. The number of eggs/segment/worm was compared between sites and between seasons using ANCOVA, with the number of eggs being used as the dependent variable against the length of the worms. Invertebrate sizes were plotted in Microsoft Excel to form length-frequency distributions for different sites and seasons. Statistical analyses were done in Systat 11™, with an alpha of 0.05.

Multivariate analyses comparing the sites and sampling dates for their physical parameters (namely metals, TOC, sediment composition, and PAHs) were done using PRIMER v6. Prior to analyses, the data were standardized to a maximum value of 1 for each parameter to prevent the results from being dominated by the contaminant which occurred at the highest concentration (Clarke and Warwick, 2001). To visually contrast assemblage structure between sites and sampling dates, non-metric multidimensional scaling (MDS) plots were constructed using similarity matrices based on Euclidean distances of the physical parameters in each sample (Clarke and Warwick, 2001). Analyses of Similarity (ANOSIM) were used to compare the physical parameters between sites and sampling dates.

3. Results

3.1. Seasonal variability at reference sites — phase 1

3.1.1. Population structure of N. proxima

N. proxima was found at all reference sites, although at site 1 only one clam was found across all three seasons (Fig. 1) and therefore this site was excluded from further analysis. The highest and lowest mean densities were 2782 ± 1566 clams/m^2 (site 13, June) and 311 ± 103 clams/m^2 (site 3, October 2011), respectively. Based on a two-factor ANOVA, clam densities did not differ significantly between seasons ($df = 2$, $p = 0.248$) but varied significantly between sampling sites (df = 4, $p < 0.001$), with no interaction between the two (df = 8, $p = 0.722$). The season with the highest density was June for two outer harbor sites (sites 4 and 13) and April for the other three sites (sites 2, 3, and 6). Tukey's test indicated that site 13 had significantly higher densities than all other sites, and that the remaining sites did not differ from one another.

The largest average clam size was 2.09 ± 0.99 mm (site 13, October 2011) and the smallest was 1.05 ± 0.50 mm (site 2, October 2011, Fig. 2). The date of largest average size varied among sites, with October 2011 having the greatest shell length for site 13 but the lowest for sites 2, 3, and 4, while April had the greatest shell length for site 4, and June had the greatest shell length for sites 2 and 3 but the lowest for site 13 (Fig. 2). Shell lengths at site 6 did not change significantly between seasons. All sites had animals with a similar range of shell lengths, with the smallest individuals <0.9 mm and the largest ≥3.9 mm (Fig. 2). The range of clam sizes differed seasonally in the inner harbor. In October 2011, the inner harbor sites (2 and 3) lacked clams in the higher size bins and had a cohort of very small (<1 mm) clams which were likely those subsequently in the 1.4 mm bin

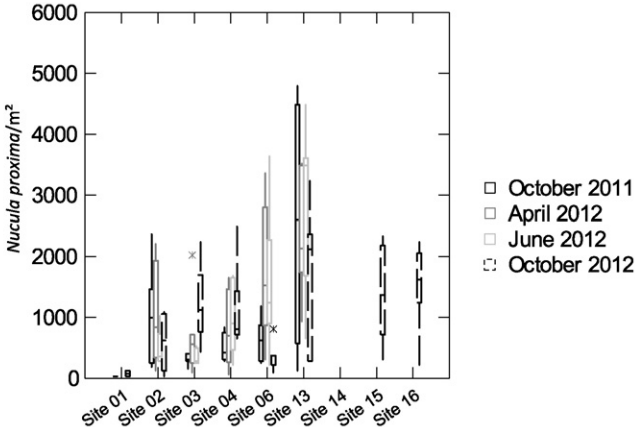

Fig. 1. Average population densities of *Nucula proxima* for six reference sites and three potential hotspots (sites 14–16) in Saint John Harbor, sampled in October 2011, April 2012, June 2012, and October 2012.

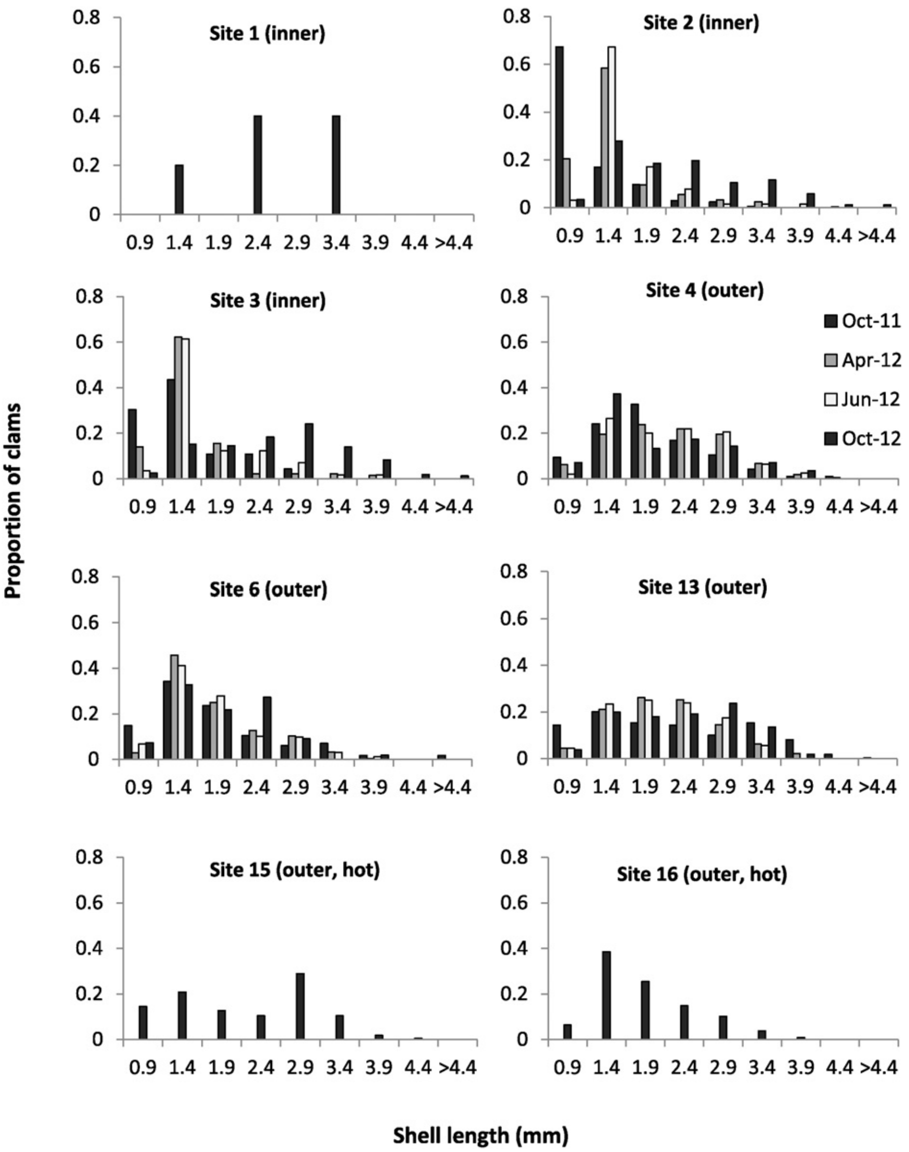

Fig. 2. Length frequency distribution of *Nucula proxima* for six reference sites and three potential hotspots in Saint John Harbor, sampled in October 2011, April 2012, June 2012, and October 2012.

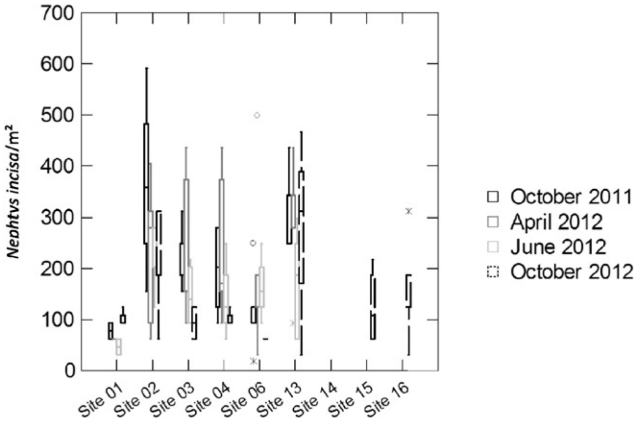

Fig. 3. Density of *Nephtys incisa* for six Saint John Harbor reference sites and three potential hotspots (sites 14–16) sampled in October 2011, April 2012, June 2012, and October 2012.

during April and June (Fig. 2). In contrast, histograms from outer harbor sites had similar size distributions in all three seasons (Fig. 2).

3.1.2. Population structure of N. incisa

All grabs from site 14 had zero *N. incisa*. The average density for *N. incisa* at the other sites ranged from 4.67 ± 2.20 (site 1, June) to 292.6 ± 225.1 worms/m^2 (site 2, October 2011) (Fig. 3). Densities of *N. incisa* did not change significantly between sites (ANOVA, df = 5, p = 0.270) or seasons (df = 2, p = 0.266), nor was there any interaction (df = 10, p = 0.950).

The largest average worm length was an L3 of 0.97 ± 0.23 mm (site 1, October 2011), and the smallest average L3 for worms was 0.34 ± 0.14 mm (site 6, April). The length frequency distributions for *N. incisa* indicated that worm lengths for all sites were generally centered between the <0.25 mm and 1.50 mm size classes, with much larger worms (L3 > 2.00 mm) occasionally appearing in samples (Fig. 4). In addition, length frequency histograms for *N. incisa* suggest that the size structure did not change between seasons in either the inner or outer harbor sites. Because the seasonal variation in body length seemed to be minimal, there was no evidence of distinct cohorts.

3.1.3. Reproductive activity and fecundity in N. incisa

Size at maturity of *N. incisa* was determined separately for each site with one exception. Since site 6 had no reproductively active worms, its size at maturity was estimated using an average of the other sites. Across sites, the average size at maturity was 0.76 mm L3, ranging from 0.56 mm L3 at site 2 to 1.01 mm L3 at site 13. Generally, size at maturity was slightly smaller at inner harbor sites than those from outer harbor sites and in October 2011 than April and June (0.56 mm L3 in October 2011 vs. 0.69 and 0.68 mm in April and June, respectively).

Outer harbor sites were dominated by juvenile worms in all seasons (average 72% juveniles across sites and seasons), while inner harbor sites had a greater proportion of adults (average 33% juveniles across sites and seasons; Fig. 5). Inner harbor sites also had a greater proportion of egg-bearing females (17% on average, across sites and seasons) compared to outer harbor sites (0.02% on average, across sites and seasons). The average proportion of reproducing worms was highest at site 3 (25%, across seasons) and lowest at site 6 (0%, across seasons), which also had the fewest adult worms. Egg-bearing females were most prevalent during the April and June sampling times, and virtually all developed eggs were found during these two seasons (Fig. 5). Actively reproducing males were found sporadically at most sites, the highest proportions of which were also during the April and June months.

The number of eggs/segment in individual worms seemed to be dependent on body size. Larger specimens generally had many more eggs than smaller adults; worms with L3s >2 mm had 2000–

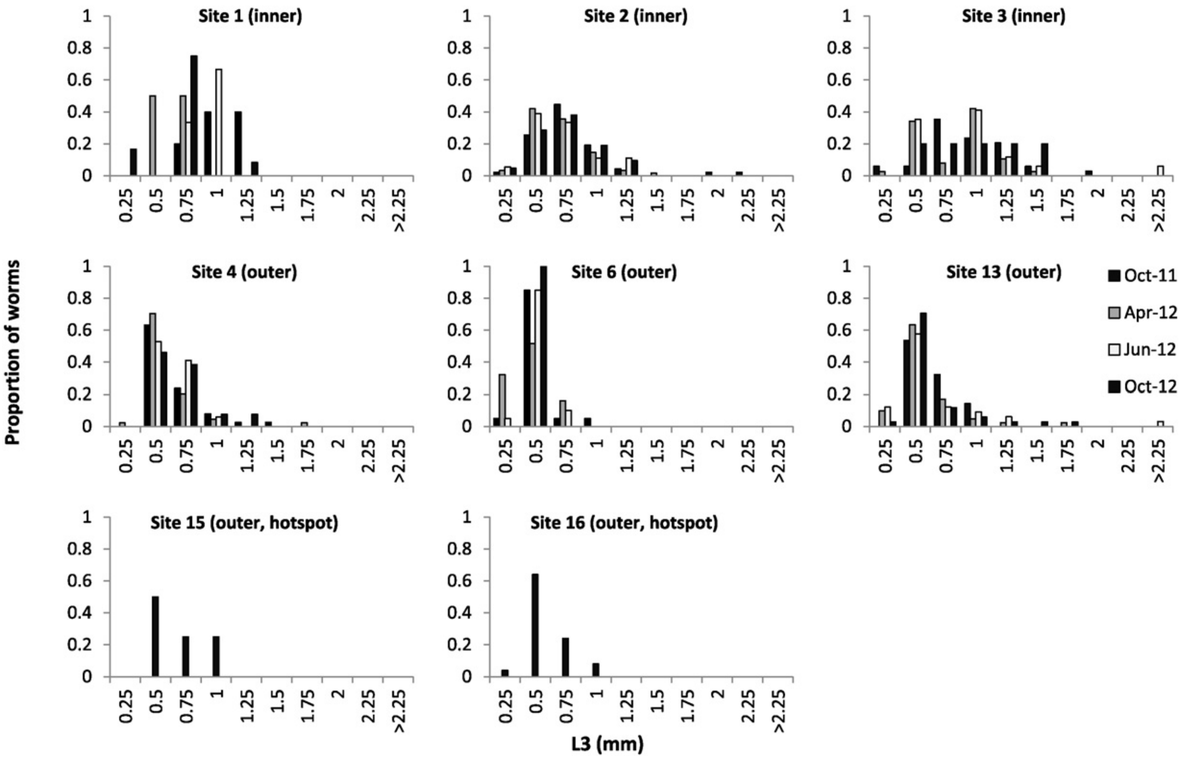

Fig. 4. Length frequency distributions of *Nephtys incisa* at six reference sites and potential hotspots (sites 15 and 16) sampled in October 2011, April 2012, June 2012, and October 2012, in Saint John Harbor.

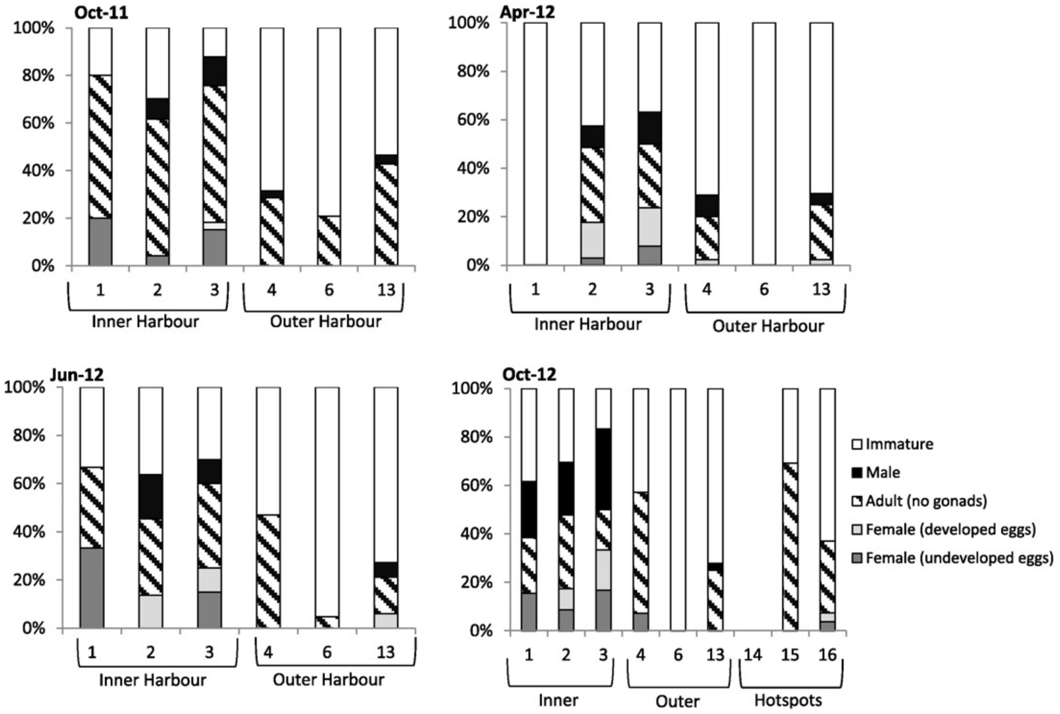

Fig. 5. Proportions of reproductively active adults (females with developed eggs, females with undeveloped eggs, males), adults (no gonads), and immature *Nephtys incisa* for six reference sites and three potential hotspots (sites 14–16) in Saint John Harbor across four sampling dates (October 2011, April 2012, June 2012, and October 2012). Immature worms fell below the size at maturity for a given site, whereas adult (no gonad) worms were large enough to be adults but contained no eggs or sperm.

15,000 eggs/segment, whereas smaller worms had at most several hundred eggs/segment. Linear regression of L3 length against the number of eggs/segment was significant ($p < 0.001$, data not shown).

3.1.4. Sediment chemistry

Of the reported metals, Zn and Cr concentrations were typically highest across all sites and seasons, with means ranging from 34 (SD 10) to 194 (38) mg/kg for Zn, and from 17.6 (4.8) to 29.4 (6.5) mg/kg

Table 1

Concentrations (mg/kg dw) of metals ± SD in sediments collected in October 2011, April 2012, June 2012 (phase 1) and October 2012 (phase 2) at reference and potential hotspots (sites 14–16). CCME/NOAA (for Ni) guidelines are also shown, and results exceeding guidelines are presented in bold. Alphabetical annotations indicate significant differences between seasons within sites (n = 5–10/site/season).

Season	Site		Cu	Zn	Pb	As	Cd	Cr	Ni	THg (μg/kg dw)
CCME		ISQGs	18.7	124	30.2	7.24	0.70	52.3	15.9 (TEL)	130
Oct-2011	1	Inner	5.34 ± 2.68^{b}	34.31 ± 10.13^{b}	7.28 ± 1.39^{a}	5.15 ± 1.28^{b}	$<0.08^{a}$	17.63 ± 4.80^{b}	11.93 ± 3.87^{b}	8.0 ± 3.5^{b}
Apr-2012	1	Inner	10.49 ± 2.07^{a}	52.63 ± 12.00^{a}	9.42 ± 2.06^{a}	8.66 ± 1.97^{a}	0.13 ± 0.05^{a}	26.44 ± 6.09^{a}	$\mathbf{19.38 \pm 2.89^{a,c}}$	$15.7 \pm 6.9^{a,b}$
Jun-2012	1	Inner	9.83 ± 3.71^{a}	50.81 ± 15.28^{a}	10.18 ± 3.79^{a}	8.00 ± 1.99^{a}	0.12 ± 0.03^{a}	27.61 ± 8.26^{a}	$\mathbf{21.51 \pm 4.84^{a}}$	21.8 ± 12.3^{a}
Oct-2012	1	Inner	$6.28 \pm 1.89^{a,b}$	$42.03 \pm 8.34^{a,b}$	9.32 ± 1.95^{a}	$6.10 \pm 0.85^{a,b}$	$<0.08^{a}$	$20.31 \pm 3.74^{a,b}$	$14.60 \pm 3.41^{b,c}$	$15.68 \pm 10.84^{a,b}$
Oct-2011	2	Inner	9.01 ± 1.16^{a}	$51.3\,9 \pm 3.25^{a}$	10.56 ± 0.85^{a}	7.20 ± 0.42^{a}	$0.09 \pm 0.02^{a,b}$	29.35 ± 1.66^{a}	$\mathbf{19.40 \pm 1.16^{a}}$	12.4 ± 1.6^{a}
Apr-2012	2	Inner	$6.58 \pm 1.76^{a,b}$	35.85 ± 4.18^{b}	6.58 ± 1.13^{b}	5.76 ± 0.69^{b}	$<0.08^{b}$	23.95 ± 3.10^{b}	15.57 ± 2.12^{a}	6.1 ± 1.4^{b}
Jun-2012	2	Inner	$7.67 \pm 1.65^{a,b}$	41.35 ± 7.47^{b}	$8.45 \pm 1.55^{a,b}$	$6.73 \pm 0.96^{a,b}$	0.10 ± 0.02^{a}	$25.52 \pm 4.00^{a,b}$	$\mathbf{18.69 \pm 3.54^{a}}$	12.6 ± 4.9^{a}
Oct-2012	2	Inner	6.04 ± 1.55^{b}	$43.82 \pm 6.27^{a,b}$	9.00 ± 1.53^{a}	$6.62 \pm 1.59^{a,b}$	$<0.08^{b}$	22.00 ± 3.55^{b}	15.85 ± 2.43^{a}	$10.50 \pm 2.68^{a,b}$
Oct-2011	3	Inner	4.11 ± 2.82^{b}	51.45 ± 8.39^{a}	10.60 ± 2.33^{a}	7.35 ± 1.44^{a}	0.12 ± 0.03^{a}	27.85 ± 6.03^{a}	$\mathbf{19.67 \pm 3.27^{a}}$	14.7 ± 3.8^{a}
Apr-2012	3	Inner	9.54 ± 2.03^{a}	46.45 ± 9.87^{a}	9.43 ± 1.92^{a}	7.41 ± 1.49^{a}	0.12 ± 0.04^{a}	29.20 ± 6.50^{a}	$\mathbf{19.06 \pm 4.63^{a}}$	9.7 ± 1.4^{a}
Jun-2012	3	Inner	7.62 ± 1.22^{a}	42.32 ± 5.45^{a}	9.03 ± 1.09^{a}	6.67 ± 0.55^{a}	0.10 ± 0.02^{a}	27.10 ± 3.02^{a}	$\mathbf{18.06 \pm 2.53^{a}}$	10.7 ± 4.1^{a}
Oct-2012	3	Inner	$6.31 \pm 0.70^{a,b}$	44.97 ± 6.40^{a}	8.87 ± 0.72^{a}	6.04 ± 0.57^{a}	0.10 ± 0.03^{a}	23.59 ± 1.97^{a}	$\mathbf{15.92 \pm 2.81^{a}}$	10.14 ± 0.65^{a}
Oct-2011	4	Outer	$<0.19^{a}$	34.96 ± 5.65^{a}	7.56 ± 1.11^{a}	5.19 ± 0.48^{a}	$<0.08^{a}$	19.51 ± 4.12^{a}	$13.72 \pm 2.50^{a,c}$	5.7 ± 1.8^{a}
Apr-2012	4	Outer	6.54 ± 2.15^{b}	35.87 ± 8.22^{a}	7.93 ± 1.39^{a}	5.76 ± 0.77^{a}	$<0.08^{a}$	23.48 ± 5.41^{a}	$15.38 \pm 3.55^{a,c}$	5.9 ± 2.3^{a}
Jun-2012	4	Outer	$5.82 \pm 1.14^{b,c}$	40.19 ± 4.92^{a}	9.14 ± 0.88^{a}	5.91 ± 0.66^{a}	0.12 ± 0.02^{b}	22.07 ± 3.51^{a}	$\mathbf{16.23 \pm 1.95^{b,c}}$	5.9 ± 1.5^{a}
Oct-2012	4	Outer	3.62 ± 1.68^{c}	34.81 ± 5.72^{a}	7.50 ± 1.33^{a}	4.80 ± 0.76^{a}	$<0.08^{a}$	19.65 ± 4.30^{a}	11.03 ± 2.46^{a}	7.18 ± 1.54^{a}
Oct-2011	6	Outer	0.33 ± 0.50^{c}	44.94 ± 3.15^{a}	$7.92 \pm 0.77^{a,b}$	$6.57 \pm 0.29^{a,c}$	0.09 ± 0.01^{b}	26.27 ± 2.43^{a}	$\mathbf{17.85 \pm 1.60^{a}}$	5.5 ± 2.2^{a}
Apr-2012	6	Outer	7.25 ± 0.59^{a}	40.33 ± 5.24^{a}	$7.17 \pm 0.59^{a,b}$	$6.06 \pm 0.48^{a,c}$	0.08 ± 0.00^{b}	24.10 ± 2.22^{a}	$\mathbf{16.97 \pm 1.51^{a,b}}$	7.9 ± 2.1^{a}
Jun-2012	6	Outer	6.25 ± 0.64^{a}	46.53 ± 3.87^{a}	8.82 ± 0.54^{b}	8.23 ± 3.76^{a}	0.16 ± 0.03^{a}	24.02 ± 1.87^{a}	$\mathbf{17.86 \pm 1.95^{a}}$	5.7 ± 1.1^{a}
Oct-2012	6	Outer	4.79 ± 1.20^{b}	39.84 ± 7.27^{a}	6.88 ± 1.62^{a}	$4.82 \pm 0.48^{b,c}$	$<0.08^{b}$	22.41 ± 3.38^{a}	13.80 ± 2.67^{b}	9.22 ± 4.72^{a}
Oct-2011	13	Outer	5.07 ± 6.76^{a}	48.54 ± 20.22^{a}	9.19 ± 3.81^{a}	6.89 ± 2.44^{a}	0.11 ± 0.05^{a}	25.57 ± 8.51^{a}	$\mathbf{17.58 \pm 6.85^{a,b}}$	8.8 ± 7.8^{a}
Apr-2012	13	Outer	10.88 ± 3.29^{a}	51.78 ± 9.89^{a}	11.48 ± 2.73^{a}	7.69 ± 1.66^{a}	$0.12 \pm 0.03^{a,b}$	32.41 ± 6.54^{a}	$\mathbf{21.34 \pm 4.18^{b}}$	11.8 ± 4.0^{a}
Jun-2012	13	Outer	8.23 ± 2.58^{a}	50.38 ± 12.04^{a}	11.32 ± 2.62^{a}	7.05 ± 1.43^{a}	0.16 ± 0.03^{b}	25.27 ± 6.08^{a}	$\mathbf{21.49 \pm 4.37^{b}}$	11.0 ± 4.6^{a}
Oct-2012	13	Outer	4.17 ± 1.97^{a}	36.31 ± 8.65^{a}	8.11 ± 2.01^{a}	4.71 ± 1.00^{a}	$<0.08^{a}$	20.63 ± 4.75^{a}	11.98 ± 3.11^{a}	8.74 ± 2.92^{a}
Oct-2012	14	Inner-Hot	$\mathbf{31.14 \pm 4.89}$	$\mathbf{193.94 \pm 37.85}$	$\mathbf{35.66 \pm 5.98}$	4.22 ± 0.34	0.12 ± 0.02	17.76 ± 2.25	$\mathbf{21.75 \pm 3.27}$	16.20 ± 5.71
Oct-2012	15	Outer-Hot	4.10 ± 0.64	43.37 ± 3.08	8.70 ± 0.56	6.36 ± 0.42	0.13 ± 0.01	21.36 ± 2.56	$\mathbf{16.98 \pm 2.05}$	8.88 ± 1.07
Oct-2012	16	Outer-Hot	3.48 ± 1.42	41.05 ± 5.56	8.88 ± 1.50	5.71 ± 0.72	0.11 ± 0.01	21.26 ± 4.01	15.17 ± 2.23	8.78 ± 3.27

for Cr (Table 1). Cd was typically present in the lowest concentrations, ranging from values below the detection limit of 0.08 mg/kg to 0.16 (0.03) mg/kg. Ag was rarely found at concentrations above the detection limit (11 of 148 samples across sites and seasons), and was excluded from the analysis. All metals tested had a significant interaction between sites and season. For this reason, seasonal comparisons were made within sites using separate one-way ANOVAs.

Metal concentrations in the inner harbor varied between sites and seasons (Table 1). Concentrations at site 1 were significantly lower in October 2011 (Oct < Apr, Jun, and Oct < Jun for THg) for all metals except Cd ($df = 5$, $p = 0.059$) and Pb ($df = 5$, $p = 0.104$) when compared to June and April. Cu concentrations at site 3 were also lower in October than in April or June, which did not differ from one another (Oct < Apr, Jun). In contrast, Pb at site 2 was highest during October (Oct > Apr, Jun), and Cu, Pb, and As concentrations were higher in October than in April (Oct > Apr). Lastly, THg at site 2 was high in both October and June (Oct, Jun > Apr). Compared to the inner harbor sites, metals in the outer harbor had fewer significant differences between seasons. Cu was lowest in October for sites 4 and 6, Cd was highest in June at sites 4, 6, and 13, and, with the exception of Cd, metals at site 13 did not differ significantly with season.

Most sites were dominated by silt/clay (average = 72.2 ± 18.4% across sites and seasons), with minor proportions of fine/medium sand (average = 21.0 ± 11.6% across sites and seasons) and pebbles (average = 2.6 ± 5.9% across sites and seasons). Exceptions to this were site 1 (April) and site 6 (October and June) where there was a greater amount of fine/medium sand (average = 39.7 ± 2.7%, site 6, October) and pebbles (average = 14.7 ± 9.4%, site 6, October) or fine/medium sand, coarse sand, and pebbles (averages 19.4 ± 13.5%, 24.7 ± 26.7%, and 12.6 ± 13.4%, respectively, site 1, April).While most replicates were consistent within sites and dates, site 1 (April and June) was an exception, with some grabs >58% silt/clay, and others <35% silt/clay (data not shown). Site 14 was also an exception, being dominated almost entirely by fine/medium sand (92.6%) followed by minimal silt/clay (3.8%) and coarse sand (2.9%). Sediments ranged from 5 to 11% clay across sites and the ratio of clay:silt was generally around 1:10 (data not shown).

TOC values were relatively low at all study sites. The highest mean TOC was 0.97% by weight at site 3 in October 2011, and the lowest was 0.41% by weight at site 4 in October 2011. TOC was significantly higher at site 3 than values at sites 1 and 4 across seasons and was generally consistent within sites, across seasons with the exception of sites 2 and 13. TOC at site 2 was significantly lower in April than October and June (ANOVA, $df = 2$, $p = 0.001$). TOC at site 13 in April had high variability between replicates, the lowest being 0.59% by weight and the highest 1.48% by weight; the differences between seasons at this site were not significant (ANOVA, $df = 2$, $p = 0.111$).

3.1.5. Multivariate statistics — reference sites

Plots of the similarity of physical parameters between samples across sites and sampling dates indicate that the reference sites 1 and 6 differed from other reference sites for some replicates, and that there were some difference between the inner harbor sites (sites 1, 2, and 3) and the outer harbor sites (4, 6, and 13, Fig. 7). ANOSIM indicated a significant difference in the physical properties of the sediment between sites ($R = 0.427$, $p > 0.001$), and between dates ($R = 0.463$, $p > 0.001$), with all pair-wise comparisons of sites and of dates being significant. The physical differences parallel the differences in biological responses at those sites, where abundances of both species were low (especially site 1) and site 6 had no reproducing *Nephtys*, and the difference in populations between inner and outer harbor, where the size distributions of *N. proxima* and reproduction of *N. incisa* differed based on location.

3.2. Sentinel species' responses to contamination — phase 2 at reference and potential hotspots

Data from the three seasons were examined to determine the most appropriate time for sampling within a year. October was chosen as the best time to sample both species because, in contrast to August when worms were often too small for identification, it was possible to identify both male and female catworms (although females with developed eggs were not present), and small individuals of *N. proxima* were found, allowing for an assessment of reproduction.

Sediment metal concentrations from October 2012 were generally similar to those in October 2011, with a few exceptions (Table 1). Cu was lower at site 2 (2011 > 2012) and higher at sites 3 and 6 (2011 < 2012), and Cr and Ni were lower at sites 2 and 6, respectively (2011 > 2012). Sediment composition was generally comparable, however some sites (2, 3, and 6) had slightly lower silt–clay content and higher fine/medium sand in 2011 compared to 2012. TOC generally did not change between 2011 and 2012.

In October 2012, the size distributions of *N. proxima* at the inner harbor sites differed from the pattern observed in October 2011. Whereas previously clams at the inner harbor sites had a high proportion of very small clams at this time of year, the samples from October 2012 had a greater proportion of large clams and fewer clams in the smallest size classes (Fig. 2). Size distributions of *N. proxima* at the outer harbor sites did not change considerably between October 2011 and 2012, which matched the reduced seasonal variation observed for the outer harbor at other sampling times as well. Abundances of *N. proxima* were different at all sites in October 2012 when compared to October 2011 (Fig. 1). Generally speaking, sites with relatively high abundance in October 2011 had low abundance in October 2012, and sites with relatively low abundance in October 2011 were high in October 2012.

N. incisa collected in October 2012 had similar size distributions to those from October 2011, for both inner and outer harbor sites (Fig. 4). Abundances of *N. incisa* in October 2012 were slightly higher than October 2011 for sites 1 and 13, but similar at all other sites (Fig. 3). Reproductive activity in *N. incisa* was also similar in the second year, in that the inner harbor sites had greater proportions of egg-bearing females, and the outer harbor sites had primarily immature worms (Fig. 5). The proportion of reproducing worms at inner harbor sites was, however, much higher than in October 2011, with up to 80% of adult worms actively reproducing at some sites (site 3, Fig. 6). In particular, reproducing male worms were more prominent at this time than in any other sampling season.

Of the three potential hotspots initially sampled during phase 2, only one had elevated metal concentrations when compared to other sites. This site had no animals of either of the two species, and had a very different sediment composition, being primarily fine/medium sand instead of silt–clay. The other two potential hotspots, which surrounded the dredge disposal site, were similar in sediment composition to the reference sites and also had similar metal concentrations. Abundances at these two sites were 1382 ± 885 and 1475 ± 802 animals/m^2 for *N. proxima*, and 100 ± 86 and 168 ± 102 animals/m^2 for *N. incisa* which, for both species, exceeded the abundances at two of the three outer harbor hotspots. Sizes of the two species were 2.01 ± 0.84 and 1.70 ± 0.64 mm shell length for *N. proxima*, and 0.56 ± 0.23 and 0.47 ± 0.18 mm L3 for *N. incisa* at the two sites near the dredge disposal site, which were comparable to animal sizes at the reference sites collected at the same time.

The MDS plot of the October 2012 samples based on physical properties of the sediment indicated that site 14 was different from all other sites in its physical properties, whereas the other two potential hotspot sites (15 and 16) had physical properties similar to the other reference sites (Fig. 8). This aligns with the biological data at these sites; site 14 was the most different in physical properties and was also devoid of the two species, and sites 15 and 16 were comparable

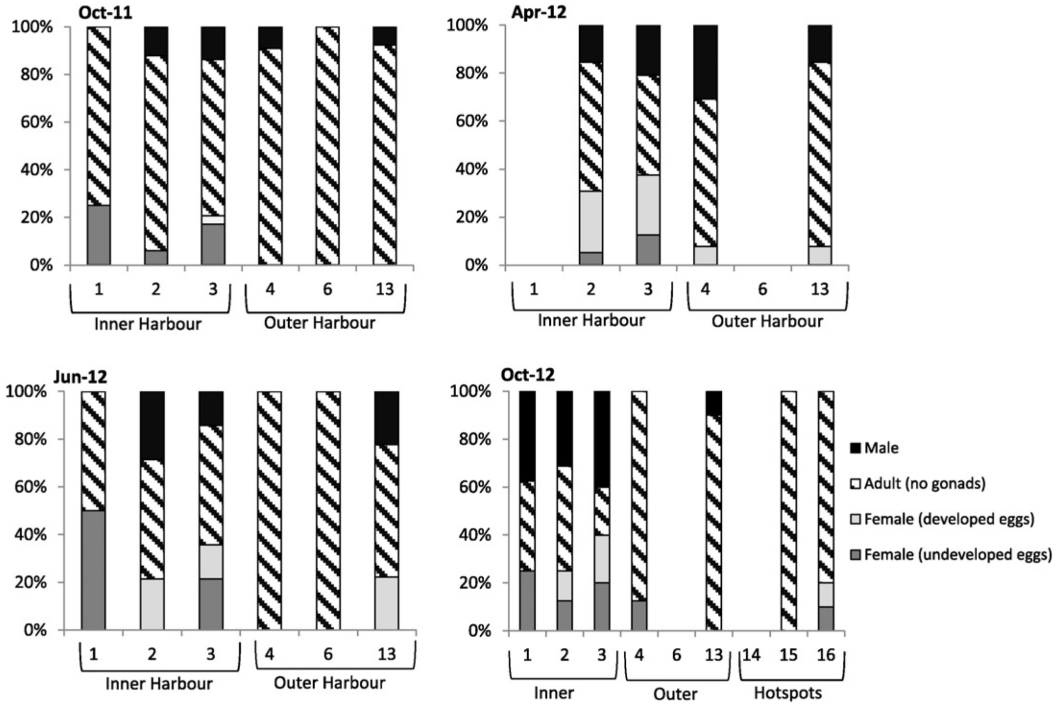

Fig. 6. Proportions of *Nephtys incisa* adults with different reproductive status (male, adult no gonads, female developed eggs, female undeveloped eggs) for six reference sites and three potential hotspots (sites 14–16) in Saint John Harbor in October 2011, April 2012, June 2012, and October 2012.

to the reference sites, both physically and biologically. ANOSIM indicated a significant difference in physical properties between sites ($R = 0.464$, $p > 0.001$). Pairwise comparisons showed that most sites were significantly different from each other ($R > 0.168$, $p < 0.048$), except for site 2, which was not different from sites 1 and 3 ($R < 0.048$, $p > 0.286$), and site 13, which was not different from site 4 or site 16 ($R < 0.032$, $p > 0.278$).

4. Discussion

A main focus of this study was to determine the physical–chemical characteristics of sediments and the biological characteristics of two potential sentinel species, the Atlantic nut clam and the clamworm, at reference sites in the Saint John Harbor, New Brunswick, Canada. In particular, an understanding of how sediment characteristics and benthic infauna vary spatially and temporally was used to develop recommendations on the ideal time to sample in Saint John Harbor for future monitoring studies. Metal concentrations in the harbor varied among sites and sampling dates, and exceeded sediment quality guidelines for Ni and As at all sites across seasons (see below). Sediment was primarily silt–clay with some fine sand at all sites. *N. proxima* were prevalent at all but one sampling site across seasons, and the distribution of size classes differed between inner and outer harbor sites and between seasons within inner harbor sites. *N. incisa* were present at all sites in lower abundances than *N. proxima*, and worms were fairly evenly distributed among the different size classes. There was little seasonal variability in size or abundance of *N. incisa*. Larger worms had a greater number of eggs/segment on average, and inner harbor sites had a greater proportion of eggbearing females. In addition, eggs were more commonly found and better developed during April and June than in October.

Metal concentrations in the Saint John Harbor were low, with the exception of Ni and As. As exceeded the lower limit of sediment quality guidelines (Interim Sediment Quality Guidelines, ISQG; CCME) in 26 of the 103 samples. Ni exceeded the Threshold Effect Level (TEL) from the National Oceanic and Atmospheric Administration's (NOAA) Screening Quick Reference Tables (SQuiRTs) in 68 of the 103 samples,

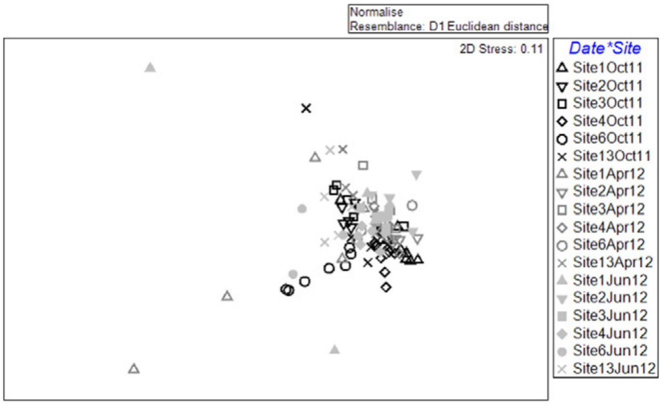

Fig. 7. MDS plot of the sediment samples from reference sites for October 2011, April 2012, and June 2012, based on physical properties of the sediment.

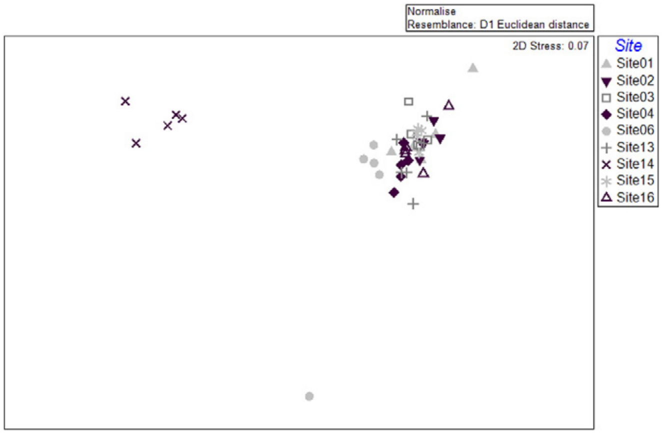

Fig. 8. MDS plot of reference sites and potential hotspots for October 2012, based on physical properties of each site, in Saint John Harbor.

and the NOAA Effects Range Low (ERL) in a further 16 of those 68 samples. Sediment metal concentrations measured in this study were, on average, comparable to reference sites from other regions. As concentrations in this study were two-fold higher than a previous study in Saint John (Parrott et al., 2002). Sediment Cr was two-fold higher than both 2000 levels in the harbor and those sampled in a muddy-sand reference site in Italy (Etiope et al., 2014), but was half the concentration of Cr sampled in other areas of the Bay of Fundy (Loring, 1979) and a harbor in Germany (Beck et al., 2013). Differences between past studies in the Saint John Harbor and the current project may be the result of spatial and temporal variability among locations as the range of values obtained in previous studies were often considerable (Ray and MacKnight, 1984) or it may be attributable to differences in analytical techniques. THg in this study was consistently much lower than in all other studies (Loring, 1979; Ray and MacKnight, 1984; Apeti and Hartwell, 2014).

Clams collected in the harbor had a maximum shell length of 4–5 mm for most sites, and individuals of shell length ≥2.1 mm were found at all sites. The peak of small individuals at inner harbor sites (particularly in October) seems to indicate that *N. proxima* have one major spawning event during the year (periodic spawning) at those sites. *N. proxima* (described as *N. annulata*) collected from Massachusetts to Bermuda reach maturity at 2.1 mm (determined from the shell length where ova first start to appear), and can grow to 5 mm in length (Scheltema and Williams, 2009). In addition, based on observations of oogenesis, reproduction was limited to summer and fall in Scheltema and Williams (2009). Since there were clams >2.1 mm at all sites in the Saint John Harbor, it seems likely that there were reproductively active clams at each site in the harbor. The peak of smaller clams in October in the current study also coincides with evidence from literature that *N. proxima* are breeding during the summer and fall.

There was considerable spatial variation in abundance of clams in the Saint John Harbor (between sites and between grabs), as was also observed in the samples collected by Sanders (1958, 1960) in Buzzard's Bay, Massachusetts, the closest site for which spatial data were available. *N. proxima* from Saint John Harbor had average densities between 31.12 and 2782 animals/m^2, whereas the majority of samples from Buzzards Bay (from sediments of ≥60% silt–clay) had densities ranging from ≈100 to 3500 animals/m^2 throughout a year of monthly sampling at four stations (Sanders, 1958). According to Sanders (1958), abundance in *N. proxima* is partly explained by the grain size of the sediment they inhabit. Specifically, abundances of *N. proxima* collected from Buzzard's Bay, MA, and Long Island Sound were highest in sediments of 10–20% clay, and areas with more or less clay in the sediment had lower abundances. It was speculated that clay particles, being much finer than silt, have a greater surface area and can retain more organic matter for the clams to feed on, yet too much organic matter would reduce the oxygen available for animals, thus resulting in the 'ideal' range of clay content (Sanders, 1958). In the current study sediments ranged from 5 to 11% clay, which was generally lower than the ideal 10–20% clay from Sanders.

Abundance of *N. incisa* in Saint John ranged from 46.8 to 292.6 animals/m^2 on average across sites and seasons, with the highest recorded density being 591.3 worms/m^2. *N. incisa* collected from Buzzard's Bay, Massachusetts, were present in higher densities than those of the Saint John Harbor, ranging from 300 to 900 animals/m^2 in silt–clay dominated areas, with two exceptional samples having densities close to 1500 animals/m^2 (Sanders, 1958). Because *N. incisa* prefer silt–clay sediments (Chang et al., 1992), it is possible that differences in abundance between the Saint John Harbor and Buzzard's Bay sites were the result of differences sediment composition, as with *N. proxima*. However, the highest and lowest abundances of *N. incisa* in Saint John Harbor did not correlate with silt–clay content, nor was there any discernable pattern between the initial clay measurements and average abundance of benthic invertebrates, so other factors seem to play a greater role in determining site variability.

The maximum size of *N. incisa* in the current study was 2.84 mm (L3), and the average size of worms was 0.59 mm (L3) across all sites and seasons. Body length in *N. incisa* is difficult to compare between studies due to the variety of methods used. However, a rough comparison was made by extrapolating the different measurements to total worm length, using the regression of each individual metric to total body length of intact worms. To equate L3 measurements to W10 – the width of the 10th setiger – which was used by Zajac and Whitlatch (1988), the following equation was used:

$$W = 3.0346(L) - 0.3748, R^2 = 0.8868, p = 0.017, n = 5.$$

This equation was derived from the few intact worms collected in Saint John Harbor. The majority of *N. incisa* that were initially collected in Long Island Sound had a W10 <2 mm (Zajac and Whitlatch, 1988), which roughly equates to an L3 of approximately 0.78 mm (or a total length of 20 mm), the same general size as the worms in Saint John Harbor. For animals from Long Island Sound, the maximum size was also similar to Saint John Harbor, with worms occasionally reaching a W10 of 8 or 9 mm, which corresponds to an L3 between 2.76 and 3.08 mm (or a 97–110 mm long worm); the largest worm found in Saint John had an L3 of 2.84 mm. Overall, *N. incisa* in Saint John Harbor appeared to have the same patterns in body size as those in Long Island Sound.

Reproduction of *N. incisa* was greatest during the spring and early summer in the current study, judging by the high proportion of adults with eggs during the April and June sampling periods. The length data did not provide any insight into the spawning time or frequency of *N. incisa*, as there were no discernable cohorts in the size frequency distributions. The difficulty in identifying cohorts may have been the result of *N. incisa* spawning infrequently throughout the year, in addition to at peak times (Carey, 1962). A study in Long Island Sound examined fecundity and the number of reproducing adults in the population; in that region, *N. incisa* spawn biannually, in spring and fall (Zajac and Whitlatch, 1988). As in the current study, fecundity varied both between and within sites. When selecting worms to examine for egg counts, Zajac and Whitlatch (1988) used worms of W10 >2.5 mm (or an L3 of 0.95 mm) to ensure that specimens were adults. The estimated size at maturity in Saint John Harbor was only slightly smaller than this, being generally between 0.70 to 0.80 mm L3, so size at maturity in Long Island Sound is likely similar to that in Saint John Harbor. Reproductive activity in *N. incisa* is otherwise not well studied (Zajac and Whitlatch, 1988; Carey, 1962). During peak reproduction in Long Island Sound (March), roughly 50% of adults were reproductively active, with their proportions dropping to 20–30% in May and to 7% in August. Reproduction peaked at that location early in the spring and dropped off into the summer, and the second peak happened at some point after the August sampling. Reproduction in Saint John Harbor also peaked starting in April at approximately 52% of adults containing eggs or sperm but, unlike in Long Island Sound, the proportion of reproducing animals remained high at the June sampling date, and then dropped down in October. In addition, since 18–35% of *N. incisa* were reproducing in October (compared to 7% in August in Long Island Sound), the timing of the October sampling appears to have been closer to the fall reproductive peak, which may have occurred after this sampling period. This is assuming that the population in Saint John Harbor had two reproductive peaks each year, as in populations further south along the coast.

Within Saint John Harbor there were several differences between animals inhabiting the inner versus outer harbor. *N. proxima* in the inner harbor displayed greater seasonal variability in shell length and lower abundances than outer harbor clams. More specifically, the inner harbor had a high proportion of clams in the smallest size class (<1 mm shell length) in October, while the outer harbor clams were evenly distributed between size classes regardless of season. If the peak in small clams in October is evidence of a discrete settling event in the inner harbor, then it seems that the settling of young *N. proxima* in the outer harbor has different dynamics to those of the inner harbor.

Because *N. proxima* has a planktonic larval stage, larvae settling in a given area may be arriving from other locations, and so the differences between inner and outer harbor sites could be the result of either a difference in spawning within-site at those locations, or from a difference in the source of recruitment. Existing literature describes *N. proxima* as a synchronous, periodic spawner that only reproduces in summer and fall, based on several populations in Massachusetts, USA, (Scheltema and Williams, 2009), however, bivalve populations that live further north may only have one spawning time each year, in contrast (e.g. *Mya arenaria*, Ropes and Stickney, 1965).

Reproductively active adults of *N. incisa* were almost exclusively found at inner harbor sites, while the outer harbor sites were dominated by juveniles. The high proportion of juveniles at these sites indicates that recruitment still occurred, but the scarcity of reproducing adults, and of adults in general, seems to suggest one of two scenarios. First, that *N. incisa* in the outer harbor are spawning at a different time than those in the inner harbor, and that this project sampled when the number of reproducing adults was at a minimum (presumably after a recent spawning event). Second, young *Nephtys* may be carried to the outer harbor sites by the current from elsewhere during their planktonic larval stage, and the outer harbor sites may lack a reproductive, adult community of their own.

To measure and identify changes in the environment, it is important to develop and implement monitoring programs that are tailored to the areas in which they are implemented. One must define what is normal at each site to recognize when and where changes occur, and this is done through the collection of baseline data. In particular, cumulative effects assessment and other studies geared towards measuring long-term changes require data collected over a larger time span. The following are some recommendations for future research on the Saint John Harbor that would both profit from and build upon what has been accomplished herein. The basic sampling methods used in this study are recommended for future monitoring, along with concurrent measures of these invertebrates and the physical and chemical characteristics of the sediments. Similarly, the reference sites from this study can be used as a baseline for future comparisons to detect changes over time. Given the seasonal variability in the abundance, reproduction and growth of the two invertebrate species it is recommended to sample at the same time each year, though exactly when depends on the endpoints of interest. No seasonal effects on abundance were observed in this study, although it was more difficult to identify animals in August than other times due to their small size. October was the best time of year to examine reproduction in *N. proxima* given the presence of smaller size classes, whereas April had the greatest reproductive activity in *N. incisa*. Measurements of fecundity in *N. incisa* were difficult due to the fragile nature of the worms, and the difficulty in removing the eggs from the gonadal tissue without damaging them. In future studies, it may be more effective to simply dissect the worms and note the number that are bearing eggs (or sperm), and how mature the eggs appear to be. This was initially done in this study to find egg-bearing females for egg counts, and it would still give a sense of the reproductive activity across the population while streamlining the processing of samples considerably.

There were two major obstacles to the evaluation of contamination effects in the harbor. The first was the lack of higher sediment contaminants at the potential hotspots. Of the three potential hotspots, only one had contaminant concentrations that were greater than the existing reference sites. Because these potential hotspots were historically higher in contaminants than other sites, the current low metal and PAH concentrations suggest they are now more typical of the sites further removed from industrial activities, and that there have been lower inputs of these contaminants in recent years. Second, when the variability in sediment composition between sites is too great, the responses from the infauna are driven more by habitat type than by contaminants, and any anthropogenic effects are masked. Thus, to understand the effects of sediment contaminants on invertebrates in the harbor, it would be ideal to sample sites with similar sediment composition. Also, the collection of reference sites must encompass any sediment types that are to be sampled at test sites or hotspots to understand how sediment composition affects benthic invertebrates over and above effects of contaminants. This will likely result in an overall increase in the number of sites sampled. Additionally, obtaining a representative sample of each sediment type in the harbor may be challenging because of the difficulty in having the grab sampler penetrate some sediment types. To predict the type of sediments that one will find at different sites, it is recommended to examine results from previous studies on seafloor composition in the harbor. However, just as the historical data on sediment contamination did not directly translate into areas that are currently contaminated, it is possible that the sediments in the harbor will shift in composition over time.

5. Conclusion

Overall, the suitability of the two sentinel species selected in this study to assess sediment contamination requires additional evaluation. Owing to the lack of sites that were more contaminated (i.e., real hotspots), there were no major differences in contaminant concentrations between sites (without the confounding factor of sediment composition) that allowed one to assess their effects on growth and abundance of these two species. Given that the test species are tolerant of metals, and thus presumably accumulate them by residing in contaminated areas, future studies could benefit from measuring metal concentrations in the tissues of *N. proxima* and *N. incisa*, as this would provide evidence as to whether a) the selected species are indeed accumulating contaminants, and b) if any observed responses in growth and abundance are correlated to a higher metal burden. The samples collected during this study provide baseline data that contribute to our understanding of the current state of the Saint John Harbor and provide valuable ecological information on two species for which there is currently little known. The results of this project will be instrumental for future studies that aim to assess changes in the harbor due to new developments or to climate change.

Acknowledgments

The authors wish to thank the crew of the *Fundy Spray*, Ryan Power, Lacey Haddon, Leophane Leblanc, Jordana Van Geest, and Patrick McIntyre for help with field collections, with a special mention to Marie-Josée Maltais and Marie-Josée Abgrall for their invaluable work in the field and in sorting and identifying the invertebrates. Clay content was analyzed in the lab of Katy Haralampides (University of New Brunswick Fredericton). Funding for this research was provided by the Canadian Water Network (grant #WS2011-SJH-2) and the Natural Sciences and Engineering Research Council of Canada (Discovery Grant #312237-2012-RGPIN, CREATE Grant #398003-2011, and Canada Research Chairs #211803 Programs), as part of a larger project seeking to develop a detailed monitoring strategy for the Saint John Harbor.

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