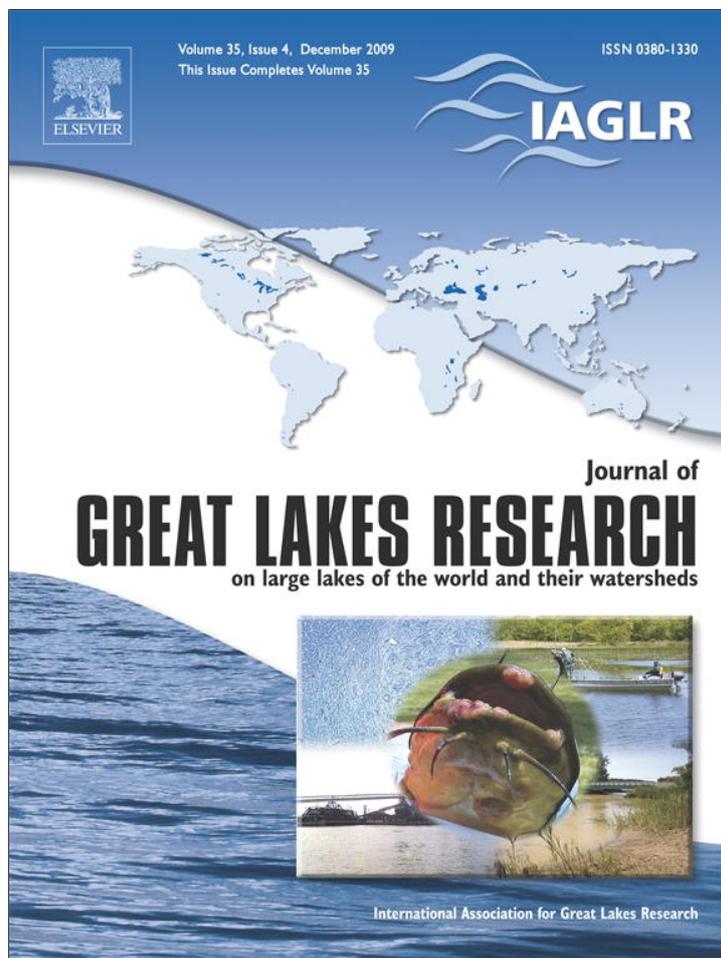


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Journal of Great Lakes Research

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

Movements of brown bullheads in Presque Isle Bay, Lake Erie, Pennsylvania

Michael J. Millard^{a,*}, David R. Smith^b, Eric Obert^c, James Grazio^d, Meredith L. Bartron^a, Colleen Wellington^e, Sara Grisè^c, Sean Rafferty^c, Robert Wellington^f, Shannon Julian^a

^a U.S. Fish and Wildlife Service, Northeast Fishery Center, P.O. Box 75, Lamar, PA 16848, USA

^b U.S. Geological Survey, Leetown Science Center, Aquatic Ecology Lab, 11649 Leetown Road, Kearneysville, WV 25430, USA

^c PA Sea Grant, Tom Ridge Environmental Center, 301 Peninsula Dr., Erie, PA 16505, USA

^d PA Department of Environmental Protection, Office of the Great Lakes, 301 Peninsula Dr., Erie, PA 16505, USA

^e ODNR Office of Coastal Management, 105 West Shoreline Drive, Sandusky, OH 44870, USA

^f 924 Sill Avenue, Erie, PA 16505, USA

ARTICLE INFO

Article history:

Received 2 February 2009

Accepted 6 August 2009

Communicated by M. Evans

Index words:

Brown bullhead
Radiotelemetry
Migration
Presque Isle Bay
Gene flow

ABSTRACT

Presque Isle Bay, Lake Erie, was listed as an Area of Concern (AOC) by the International Joint Commission in part because of the high incidence of external tumor in brown bullheads. Verifying the source of the possible contaminant exposure is critical to addressing the AOC designation. We used telemetry tracking ($n = 49$ fish) to test the hypothesis that adult bullheads captured within the bay during spawning season do not exit the bay during the post-spawning summer and fall months. We analyzed genetic variation at 15 microsatellite loci for 112 adult fish from 5 locations, 4 inside the bay and 1 outside, in order to test for possible differences. Data from fixed-station receivers suggested fish did not leave Presque Isle Bay during the study period. Predicted locations outside Presque Isle Bay were only 0.1% of all predicted locations and were below the 0.2% error rate based on known manual relocations. However, there was evidence for movement within Presque Isle Bay. Most movement was between Misery Bay or Lagoons and the open bay area. Whereas telemetry results showed tendency for adult site fidelity, genetic results showed no differences among locations, indicating that there is a single panmictic population. Our telemetry data suggest that brown bullheads are likely a useful indicator species for environmental conditions in Presque Isle Bay, since adults likely are retained in the system.

Published by Elsevier Inc.

Introduction

Brown bullheads (*Ameiurus nebulosus*) in Presque Isle Bay exhibited elevated incidences of external tumors in 1984, 1985, and 1990, although gross examination showed no liver neoplasms at that time (PADEP 1997). In 1991, four of ten bullheads with external tumors were confirmed to also have liver tumors. The International Joint Commission (IJC) considers incidence rates of “fish tumors or other deformities” as one of its fourteen use impairment listing criteria for Areas of Concern in the Great Lakes (IJC 1989). Presque Isle Bay, Lake Erie, was listed as an Area of Concern (AOC) by the IJC in January 1991. Two beneficial use impairments were cited as justification for the listing: fish tumor rates and dredging restrictions. Subsequent to this listing, the Pennsylvania Department of Environmental Protection

(PADEP) became the lead agency for the investigation of the health of the brown bullhead population in Presque Isle Bay.

In cooperation with the Pennsylvania Fish and Boat Commission (PAFBC) and the Erie County Department of Health, the PADEP initiated a comprehensive study of the resident brown bullhead population beginning in 1992 (PADEP 1997). The mark-recapture analysis estimated a population of 31,715 (95% CI = 24,827–40,476) brown bullheads in Presque Isle Bay and also suggested that they migrated extensively within bay waters but did not typically enter the open water of Lake Erie. This result suggested that environmental stressors responsible for the brown bullhead tumors were present within the confines of Presque Isle Bay. The PADEP study (1997) also explored several lines of evidence (e.g., longitudinal observations of tumor progression in fish removed from Presque Isle Bay, electron microscopic analysis for viral particles, and inoculation of tumor-free fish with tumor homogenate, etc.), which suggested that an environmental contaminant was the likely etiology for observed tumors in brown bullhead rather than a viral etiology (PADEP 1997). Additional studies have suggested a link between exposure to substrate-borne contaminants and liver tumor rates in brown bullheads (Baumann et al., 1987; Pinkney et al., 2001). The possibility of fish routinely leaving the bay and entering the open waters of Lake

* Corresponding author. Tel.: +1 570 726 4247.

E-mail addresses: mike_millard@fws.gov (M.J. Millard), drsmith@usgs.gov (D.R. Smith), eco1@psu.edu (E. Obert), jgrazio@state.pa.us (J. Grazio), meredith_bartron@fws.gov (M.L. Bartron), cwellington@ag.ohio-state.edu (C. Wellington), sng121@psu.edu (S. Grisè), sdr138@psu.edu (S. Rafferty), bobsbluewing@hotmail.com (R. Wellington), shannon_julian@fws.gov (S. Julian).

Erie bears directly upon the AOC designation for Presque Isle Bay with regard to the “fish tumors or other deformities” beneficial use impairment. Verifying bullhead residency is critical to validating the use of tumor rates in bullheads as an environmental monitoring tool (Sakaris et al., 2005). Verifying the source of the possible contaminant exposure which may be causing the elevated tumor rates is critical to addressing the AOC designation.

Presque Isle Bay has a surface area of 1505 hectares and a mean depth of 6.1 m. Fish in Presque Isle Bay, including brown bullheads, have the opportunity to migrate in and out of the bay through the entrance channel to the bay, which is the only access/egress point for the entire bay system (Fig. 1). This configuration facilitates an assessment of fish movement into or out of the bay. Tagging and genetic tools can be used to study movement and migration. Previous tagging of brown bullheads in Presque Isle Bay used operculum tags to study movement between the bay and the lake (PADEP 1997). Although a limited amount of movement was observed, some movement into the lake may have been missed because sampling effort and movement are confounded in standard tagging studies (Gowan et al., 1994). Use of telemetry tags in conjunction with an array of data logging receivers would allow for continuous monitoring of fish movement and reduce the chance of unobserved movements by tagged bullheads out of Presque Isle Bay through the channel.

Analyses of the partitioning of genetic diversity can also be used to evaluate gene flow between populations as an indirect method to estimate migration between populations (Wright 1931). In the absence of gene flow between populations, allele frequencies within populations would vary independently due to genetic mechanisms such as genetic drift, inbreeding, and mutation. Although simplistic in their assumptions (Whitlock and McCauley 1999), general models of gene flow and estimates of allele frequency differences can help understand population interactions (Mills and Allendorf 1996). Estimating allele frequencies of bullhead captured from spawning sites within Presque Isle Bay and in nearby streams draining into Lake Erie could provide information about population structure, and gene flow and migration within and outside the bay.

The objective of this study was to determine whether or not adult brown bullheads are predominantly resident within Presque Isle Bay. We used telemetry tracking to test the hypothesis that adult bullheads captured within the bay during spawning season (April through June) do not exit the bay during the post-spawning summer and fall months. We examined the genetic characteristics of brown bullheads collected within and outside the bay to provide additional insight into possible migratory behavior.

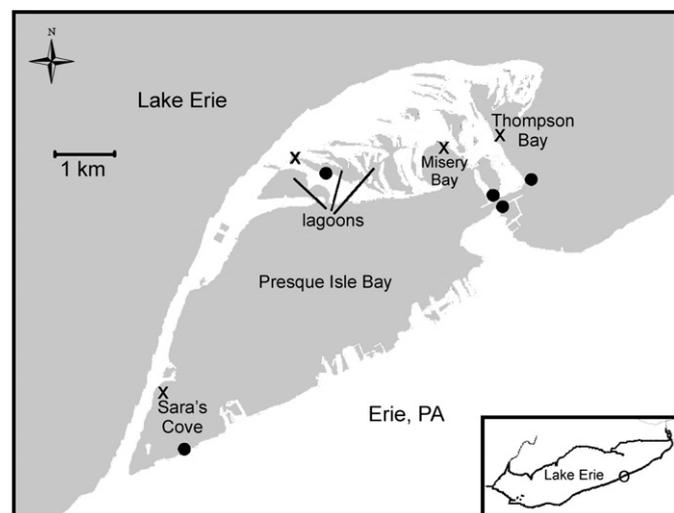


Fig. 1. Map of Presque Isle Bay, Lake Erie. ● = remote radiotelemetry receiver sites and X = site of fish capture and release.

Methods

Radiotelemetry

We monitored the movements of 49 adult brown bullheads in and around Presque Isle Bay using radiotelemetry. In order to track movement or migrations associated with spawning, brown bullheads were captured in early June 2006 from three sites within Presque Isle Bay (Lagoons, Misery Bay, and Sara's Cove) and one site in Lake Erie proximal to the bay's access channel (Thompson Bay) (Fig. 1). Fish were captured from each site by electrofishing using pulsed DC current and held separately, by site, in aerated flow-through tanks at the Presque Isle State Park Marina prior to tag implantation. Mean weight of the bullheads was 527 g (S.D. = 131.5) and mean fork length was 334 mm (S.D. = 22.4).

Radio transmitters (Advanced Telemetry Systems, Inc., Isanti, MN) were surgically implanted in 49 adult brown bullheads from June 19 to 21. Transmitters (44 mm × 15 mm) weighed 10.5 g and had a 300 mm external whip antenna. Each transmitter was individually coded. Transmitter battery life was expected to be at least 180 days. Fish were anesthetized with a 40 mg/l solution of Aqui-S®, a derivative of clove oil, under the authority of an Investigational New Animal Drug (INAD) exemption held by the U.S. Fish and Wildlife Service. A tag was inserted into the abdominal body cavity via a 35 mm midventral incision made just off the ventral center line. The incision was then surgically closed with five or six Ethicon® polydioxinone monofilament sutures. The transmitter's whip antenna was left trailing exterior and ventral to the fish, exiting the body cavity through the posterior terminus of the closed incision. The entire mid-ventrum was swabbed with Betadine® both before and after the procedure. The fish's gills were bathed with water via a recirculating pump system tub throughout the surgical procedure. Mean elapsed time for the entire surgical process was 6 minutes. Fish were then immediately placed in an aerated fresh water holding tub until recovery and then replaced into the appropriate site-specific holding tank before release the next day.

Fifteen fish with transmitters were released into each of the three capture sites within Presque Isle Bay, and four fish with transmitters were released in Thompson Bay (Fig. 1). Fish were released at the site of their initial capture. A sentinel tag was deployed near the access channel as a control signal to validate that remote receivers were operating properly over time.

Five data logging receivers (Advanced Telemetry Systems, Inc., Isanti, MN) were positioned remotely around Presque Isle Bay and the access channel (Fig. 1). The receiver on the east end of the North Pier was located in a U.S. Coast Guard lighthouse and had dual antennas configured to provide coverage for both the access channel and Thompson Bay. The three access channel receivers, designated as lighthouse, range tower, and USCG station, were deployed on June 19 and the receivers in Sara's Cove and Lagoons were deployed on July 13. Data logged by the remote receivers were downloaded and receiver batteries were replaced at least once per week throughout the study.

In addition, manual relocation of fish was accomplished with a portable receiver and handheld YAGI antenna via shoreline monitoring and boat trips. Thirty-two manual relocation trips occurred during daylight and early evening hours between June 26 and November 28. Transmitter codes received during these trips were recorded and the general location of the fish was estimated via an interpretation of the varying signal strengths permitted by the directional YAGI antenna. At a minimum, we assumed manual relocations were accurate enough to determine whether or not the fish was in the bay system at the time of relocation, although more specific locations were generally assigned for each relocated fish.

We used a computer-intensive classification technique, called random forests (Breiman 2001), to predict the location of radio-

tagged fish based on signal characteristics from fixed-station radio receivers. Random forests classification models are an extension of Classification and Regression Tree (CART) models (Breiman 2001). Classification tree models result in a binary tree that branches and splits at values of predictor variables and ends with a category of the dependent variable. Breiman (2001) extended CART models and developed random forests methodology to increase predictive capabilities. In random forests modeling, multiple classification trees are created by selecting a random subset of predictive variables to build the tree model. In this way, a large number of classification trees can be created. Predictive variables from new cases are dropped down each tree and a category is predicted. The majority category among all trees in the forest is the prediction. Random forests have been found to achieve large gains in predictive accuracy over single classification trees (Breiman 2001).

In our application of random forests modeling, the predictor variables are the signal strength and frequency of detection from the fixed-station receivers, and the categories of the dependent variable are the locations of the radio-tagged fish. Each radio receiver records the signal strength whenever a uniquely coded transmitter is within range of one of its antennae. The mean signal strength, maximum signal strength, and detection frequency was summarized over half day periods for each detection of the uniquely coded transmitters. These summary statistics provided the predictor variables in the models to classify fish location. The possible locations were Sara's Cove, Lagoons or Misery Bay, Thompson Bay, and open bay. The open bay designation was used for fish that appeared to be in Presque Isle Bay, either in the middle of the bay or at locations in the bay other than the general stocking sites. Manual tracking locations combined with concurrent signals from the fixed-station receivers served as the training data set to build the classification models. A classification tree was built using the manual tracking locations and concurrent signals. The tree was then used along with receiver signals to predict fish locations at other times. We used the software package Random Forests (Salford Systems, San Diego, CA) to conduct the analysis.

Genetic analysis

Tissue samples were non-lethally obtained from brown bullheads collected from three sites within Presque Isle Bay (Sara's Cove, Lagoons, and Misery Bay) and also from fish collected from two sites outside the bay (Thompson Bay and Elk Creek). Elk Creek is approximately 33.8 km west of the mouth of Presque Isle Bay. Sample sizes for each site varied, with small numbers of fish available from the two sites outside the bay (Table 2).

Genomic DNA was extracted from fin clip tissue using the Purgene DNA extraction kit (Genra Systems, Inc., Minneapolis, MN) following the manufacturer's guidelines. Isolated DNA was resuspended in 100 µl of 10 mM Tris-HCl, pH 8.0, 1 mM EDTA. Microsatellite enriched libraries were prepared by Genetic Identification Systems, Inc., Chatsworth, CA. (www.genetic-id-services.com/). DNA isolation from plasmids, sequencing of clones, and screening of microsatellites for development followed methods described in Julian and Bartron (2007). Of the primers designed, 15 pairs were selected for characterization in this study (Table 1).

Microsatellite analysis was performed using the following 15 loci: *AneB81*, *AneB290*, *AneC337*, *AneD037*, *AneD126*, *AneD143*, *AneD163*, *AneD237*, *AneD303*, *AneD314*, *AneD315*, *AneD344*, *AneD345*, *AneD352*, and *AneD359*. Each 20 µl PCR consisted of 2.0 µl of genomic DNA extract, 1.75× PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl), 3.75 mM MgCl₂, 0.3175 mM each dNTP, 0.07–0.325 µM of each primer (forward primer fluorescently labeled with FAM, NED, or HEX; Applied Biosystems, Foster City, CA), 1.2 U of *Taq* polymerase (Promega Corporation, Madison, WI), and deionized water added to achieve the final volume. The amplification cycle for all loci consisted of an initial denaturing at 94 °C for 2 min; 35 cycles of 94 °C denaturing for 45 sec, 56 °C annealing for 45 sec, 72 °C extension for 2 min; and a 5 min extension at 72 °C. An ABI Prism 3100[®]™ Genetic Analyzer (Applied Biosystems, Foster City, CA) was used for capillary electrophoresis. Genotypic data were analyzed and scored with Genescan 3.7.1 Analysis software and Genotyper 3.7 Fragment Analysis software (Applied Biosystems, Foster City, CA).

Table 1

Characteristics of the 15 brown bullhead (*Ameiurus nebulosus*) microsatellite loci: GenBank accession number, repeat motif, fluorescent primer label, primer sequences (forward and reverse), PCR product size range, number of alleles, observed heterozygosity (H_o), expected heterozygosity (H_e), and sample size (n) screened.

Locus	GenBank accession no.	Repeat motif	Fluorescent label	Primer sequence 5'–3'	Size range	No. of alleles	H_o	H_e	n
<i>AneB081</i>	GQ253899	(CAT) ₁₄	NED	F: GAGGGGTACTACTTTTGTG R: GGTTCACTGTCCTTTCCTTTC	219–234	5	0.396	0.429	111
<i>AneB290</i>	GQ253900	(CAT) ₁₂	NED	F: TACAAGTAAAGGCTCGAAGG R: CATGCTTGAATGTGTTTGTTC	130–154	7	0.571	0.647	112
<i>AneC337</i>	GQ253901	(GGAT) ₁₄	NED	F: TTCTCTGGTTTCTCTACCTC R: ATTCTTGAATGGCGAAACATAC	263–303	8	0.875	0.842	112
<i>AneD037</i>	GQ253902	(CTAT) ₁₃ (CCAT) ₁₀	FAM	F: AAAATGCTACTCCCTTCCTTTG R: TAACCCTGACCAGGATAAAGTG	161–213	14	0.883	0.891	111
<i>AneD126</i>	GQ253903	(CTAT) ₁₅	HEX	F: CACATCCTAACAGTGACACATTG R: TTTTATTTGATTTTCAATGACCG	217–249	9	0.766	0.868	111
<i>AneD143</i>	GQ253904	(CTAT) ₁₇ (CCAT) ₈	FAM	F: GGGTTATAACCAACACACCTGG R: CTGGGGAATATGAGAACAAGC	143–215	15	0.694	0.887	111
<i>AneD163</i>	GQ253905	(CTAT) ₁₄	FAM	F: CGATTCAACTATTTATTCGGTTG R: TACACCCATCACATTTAACAC	250–282	7	0.579	0.790	107
<i>AneD237</i>	GQ253906	(CTAT) ₁₅	HEX	F: GAGTGCAATGCTACTGTTATG R: AAATCTGGTGAATAATTTGATGTG	219–283	15	0.874	0.866	111
<i>AneD303</i>	GQ253907	(CTAT) ₁₄	NED	F: CAGCCTCTTTGCTCATATTTAG R: AGGTGTGTGAGAGTAGAGACCC	252–300	11	0.730	0.802	111
<i>AneD314</i>	GQ253908	(CTAT) ₂₂	NED	F: TTTTCTCTTTTACTGAGAGG R: GAATGAATGAACGATGTGAATG	202–378	19	0.604	0.898	111
<i>AneD315</i>	GQ253909	(CTAT) ₁₄	HEX	F: CTCTCTTTCAAGTGACACGC R: CAACTTAGCGACTTTTCAGACC	200–280	20	0.911	0.919	112
<i>AneD344</i>	GQ253910	(CTAT) ₁₆ (CTGA) ₁₀	FAM	F: ATCCATGCCACAAGAAATTAAG R: AATAAAGCACAGCATTAGAGGG	175–223	12	0.830	0.832	112
<i>AneD345</i>	GQ253911	(CTAT) ₁₄	FAM	F: CGACCACCTTTAAGGTTAAACAC R: TTCATTGGTAGGAAACTGGAAC	262–442	18	0.884	0.887	112
<i>AneD352</i>	GQ253912	(CTAT) ₁₈	FAM	F: ATCATGCATAGCTGTTTCTTC R: ACTGAAGTCCGCAAAGATTAG	197–243	21	0.893	0.910	112
<i>AneD359</i>	GQ253913	(CTAT) ₂₅	HEX	F: TGCAATTAGTAGCATGTTGGAG R: ATTTTGCAGTAGGCATATGCTC	171–279	24	0.865	0.946	111

Table 2
Summary of sample size (*n*), number of alleles per locus (*n_a*), allelic richness (*a_r*), expected and observed heterozygosity (*H_e* and *H_o* respectively), and inbreeding (*F_{IS}*) for all sampling sites and the mean estimate over all locations.

Population	<i>n</i>	<i>n_a</i>	<i>a_r</i>	<i>H_e</i>	<i>H_o</i>	<i>F_{IS}</i>
Elk	12	7.8	7.03	0.804	0.682	0.157
Lagoons	31	11.3	7.75	0.828	0.765	0.078
Misery Bay	30	10.9	7.62	0.822	0.760	0.076
Sara's Cove	30	11.6	7.92	0.824	0.774	0.062
Thompson	9	7.6	7.60	0.831	0.763	0.086
Mean	22.3	9.84	7.58	0.822	0.749	0.091

The number of alleles per locus, allele frequencies, expected and observed heterozygosity, exact tests of Hardy–Weinberg equilibrium, and inbreeding (*f*) estimates were obtained using Genetic Data Analysis (ver 1.1, Lewis and Zaykin 2001) and GENEPOP (ver. 4, Raymond and Rousset 1995). Significance values for exact tests of Hardy–Weinberg disequilibrium were Bonferroni corrected for multiple comparisons (Rice 1989). Allelic richness, the number of alleles per locus standardized for the smallest sample size of the population sampled based on a rarefaction method (El Mousadik and Petit 1996; Petit et al., 1998), was estimated using FSTAT (ver. 2.9.3.2, Goudet 2001). Micro-Checker (ver 2.2.3, van Oosterhout et al., 2004) was used to check for null alleles and genotyping errors. Maximum-likelihood assignment tests were used to assign individuals to the original collection site with GeneClass (ver. 1.0.02, Cornuet et al., 1999). Comparisons between collection sites based on differences in allele frequencies (*F_{ST}* estimates) were calculated using FSTAT (Goudet 2001). Significance values for *F_{ST}* estimates were Bonferroni corrected for multiple comparisons (Rice 1989).

Results

Manual tracking

Fourteen of the fifteen fish captured and released in the Lagoons were relocated by manual tracking, which accounted for 278 individual manual relocations, of which 242 (87%) were located in the Lagoons. Some were relocated in Misery Bay and in the open bay (Table 3). Twelve of the fourteen identified fish were always relocated in the Lagoons. One fish was relocated 16 times in Misery Bay between July 10 and October 2 but was never manually relocated anywhere else. After being initially relocated in the Lagoons on July 10, another fish was manually relocated 20 times in the open bay between July 13 and October 10. One fish was never manually relocated after release in the Lagoons.

All fifteen fish captured and released in Misery Bay were subsequently relocated by manual tracking. These fish accounted for

Table 3
Release and manual relocation sites for brown bullheads in Presque Isle Bay, Lake Erie.

Release site	Manual relocation site					Total
	Lagoons	Misery Bay	Open bay	Sara's Cove	Thompson Bay	
Lagoons (<i>n</i> = 15)	12.5 89%	1 7%	0.5 3%	0	0	14
Misery Bay (<i>n</i> = 15)	0	13 87%	1.5 10%	0.5 3%	0	15
Sara's Cove (<i>n</i> = 15)	0	0	0	14 100%	0	14
Thompson Bay (<i>n</i> = 4)	0	0	0	0	4 100%	4

Values have been weighted in order to sum to one observation per individual fish, i.e. if a fish were relocated in two different sites, it received a weight of 0.5. No individual fish was relocated in more than two different sites. Percentages represent the percentage of fish relocated at various sites for each given release site.

276 individual manual relocations and 252 (87%) of those relocations were located in Misery Bay. Some were relocated in the open bay and Sara's Cove (Table 3). Eleven of the fifteen fish were always relocated in Misery Bay. One fish was never manually relocated in Misery Bay but was relocated 17 times in the open bay between July 13 and November 17 and was also found twice in Sara's Cove on July 24 and July 31. Another fish was manually relocated once in the open bay on August 28 and relocated 24 times in Misery Bay. After being initially relocated in Misery Bay on June 26, a third fish was sporadically relocated three times in the open bay between July 13 and August 7. A fourth fish was only manually relocated twice, once in Misery Bay on June 26 and once in the open bay on September 11.

Fourteen of the fifteen fish captured and released in the Sara's Cove were relocated by manual tracking. These fish accounted for 276 individual manual relocations. All of the fish released in Sara's Cove were manually relocated only in Sara's Cove (Table 3). These results indicate no large-scale movements by any of the fish released into Sara's Cove, although one fish was never manually relocated after release.

All four of the fish captured and released in the Thompson Bay were relocated by manual tracking. These fish accounted for 64 individual manual relocations, and all of the fish released in Thompson Bay were manually relocated only in Thompson Bay (Table 3). These results from manual tracking suggest that the Thompson Bay fish did not enter Presque Isle Bay throughout the summer.

Predicted locations from fixed-station tracking

Random forests classification models with high predictive accuracy were built using known locations from manual tracking and concurrent signals from fixed-station receivers (Table 4). The overall error rate was 0.11 when predicted locations were Misery Bay or Lagoons, open bay, Sara's Cove, and Thompson Bay (Table 4). Most of the errors (103 out of 111 or 93%) came from misclassifying the location of fish in Misery Bay or Lagoons as being in the open bay. The rate that fish in Misery Bay or Lagoons were predicted to be in the open bay was 0.28 (103 out of 373). Only 1 out of 604 (0.2%) of the manually located fish was misclassified as being in Thompson Bay when it was actually inside Presque Isle Bay.

Detections of radio-tagged fish that were logged by the fixed-station receivers were summarized into 6258 records. The random forests model was applied to these 6258 records to predict the locations of radio-tagged fish (Table 5 and Fig. 2). The percent of locations predicted to be outside Presque Isle Bay were 0.1%, which is below the error rate of 0.2% calculated from known manual relocations (cf. Table 4). Among the fish released in Misery Bay or Lagoons, 46% of the predicted locations were in the open bay, which exceeds the error rate of 28% calculated from known manual

Table 4
Predicted locations based on random forest model using signals from fixed-station receivers and known release site as variables to predict location.

Actual location	Number relocations	Percent correct predictions	Predicted location			
			Misery Bay or Lagoons	Open bay	Sara's Cove	Thompson Bay
Misery Bay or Lagoons	373	72	269	103	0	1
Open bay	38	87	5	33	0	0
Sara's Cove	193	99	0	2	191	0
Thompson Bay	52	100	0	0	0	52

The actual location was determined by manual tracking. Overall error rate was 0.105 with 93% of the errors coming from misclassifying Misery Bay/Lagoons and open bay.

Table 5

Predicted location of radio-tagged fish based on random forest model using signals from fixed-station receivers and known release site as variables to predict location (cf. Table 3).

Release site	Predicted location			
	Misery Bay or Lagoons	Open bay	Sara's Cove	Thompson Bay
Misery Bay	1872	1248	1	5
Lagoons	696	899	0	1
Sara's Cove	0	0	1536	0

The number of summarized detections used for prediction was 6258.

relocations (cf. Table 4). Among the fish released in Sara's Cove, 100% of the predicted locations were in Sara's Cove.

Genetics

Primer sequences for the microsatellites developed and utilized in this study are described in Table 1. The number of alleles per locus observed was greater for the collection sites with larger sample sizes (Table 2). Allelic richness (a_r) adjustments for sample size indicated that the collection from Sara's Cove had the most alleles per locus (7.92, Table 2), compared to the Elk Creek collection (7.03, Table 2). Exact tests for Hardy–Weinberg disequilibrium were significant ($p < 0.01$) for locus *AneD314* in Elk Creek, locus *AneD143* and *AneD314* in the Lagoons, *AneD359* in Misery Bay, and at *AneD143* in Sara's Cove. These deviations from Hardy–Weinberg (H–W) equilibrium indicate violations of one or more of the H–W assumptions, such as the presence of null alleles. Micro-Checker indicated possible null alleles at loci *AneD143*, *AneD314*, and *AneD359*, but not across all collections sites. χ^2 tests between the expected and observed heterozygosity for each collection were not significant.

Pairwise comparisons of differences in allele frequency (F_{ST}) between collection sites were not significant (Table 6). Although estimates of F_{ST} were higher between the Elk Creek and the Presque Isle Bay collections, differences in allele frequencies between collection sites were generally low.

Table 6

Pairwise differences in allele frequencies (F_{ST}) between brown bullhead collections for each site.

Population	Lagoons	Misery Bay	Sara's Cove	Thompson
Elk	0.0199	0.0070	0.0160	0.0000
Lagoons		0.0006	0.0047	0.0069
Misery Bay			0.0010	0.0021
Sara's Cove				0.0066

No comparisons were statistically significant.

Assignment of individuals to likely site of origin was also used to examine collection site distinctness. When all collection sites were reclassified separately, low success of reassigning fish to area of origin was observed (Table 7). When the three Presque Isle Bay collection sites were pooled, correct assignment to collection site of origin increased for the Presque Isle Bay sites to 93.4% (Table 8). Neither the pooled or non-pooled assignment tests resulted in any individuals from the Thompson Bay sample being accurately reclassified to the collection site.

Discussion

Our telemetry results suggest that very few if any brown bullheads migrate between Presque Isle Bay and Lake Erie, whereas genetic data suggest they are a single biological population. However, the 1997 PADEP data reported only 3 bullheads to have moved between the bay and lake during a large-scale tagging program that incorporated over 3000 fish, which may indicate that gene flow outside the bay may be relatively low – a result that could not be detected here due to our low sample size outside the bay. Results from fixed-station tracking were consistent with our manual tracking results. Based on the 6258 records of summarized detections from fixed-station receivers, the fish did not leave Presque Isle Bay during the study period. Predicted locations outside Presque Isle Bay were only 0.1% of all predicted locations and were below the 0.2% error rate based on known manual relocations. For only 1 out of 6258 predictions did the probability that the fish was within Presque Isle Bay fall below 0.5 and thus more likely to be outside than inside Presque Isle Bay (Fig. 2).

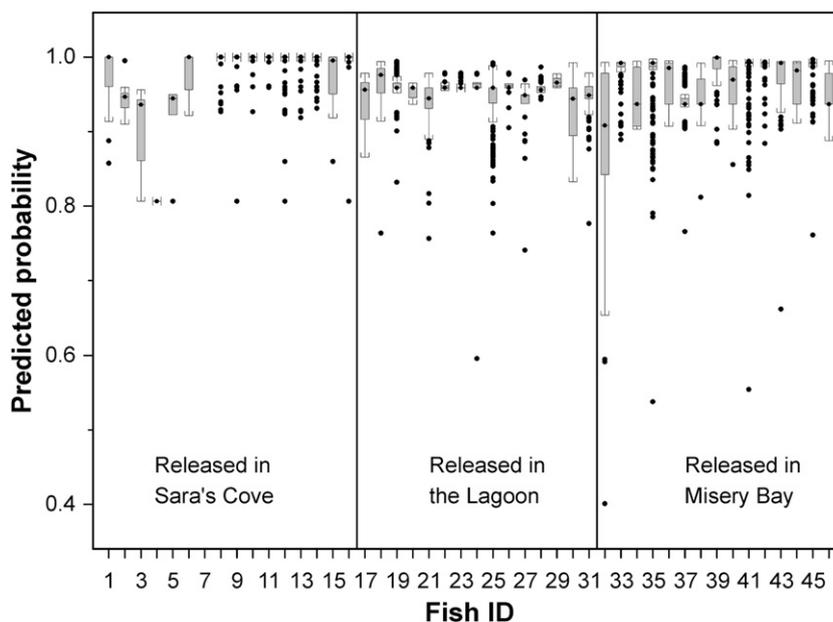


Fig. 2. Box plots showing predicted probability of each radio-tagged fish being located within Presque Isle Bay based on random forests model using signals from fixed-station receivers and known release site as predictive variables. Each box covers the interquartile range from the 25th to the 75th percentiles with the median indicated by a horizontal line. Whiskers extend beyond the box to include all observations or to observations within 1.5*interquartile range. Outlying observations are exceed 1.5*interquartile range and are indicated by the '+' symbol. The percent of locations predicted to be outside Presque Isle Bay was 0.1%, which is below the error rate of 0.2% calculated from known manual relocations (cf. Table 3).

Table 7
Results from individual-based assignment to sample site based on the allele frequencies for each collection.

Collection site	Collection site assigned to					% correct
	Elk	Lagoons	Misery Bay	Sara's Cove	Thompson	
Elk	2	1	5	2	2	16.7
Lagoons	1	14	11	4	1	45.2
Misery Bay	1	11	8	8	2	26.7
Sara's Cove	0	8	9	13	0	43.3
Thompson	2	2	1	4	0	0

Numbers indicate the number of individuals from each collection site re-assigned to one of the five collection sites. Numbers on the diagonal indicate the number of individuals correctly reassigned to the collection site of origin, and the percentages indicate the percentage correct assignment.

Among the fish released in Misery Bay or Lagoons, the percent of predicted locations away from the release areas and in the open bay (46%) exceeded the expected error rate (28%). Thus, there was evidence for movement within Presque Isle Bay. However, most movement was between Misery Bay or Lagoons and the open bay area. The fish released in Sara's Cove tended to be relocated only in Sara's Cove.

Tests for genetic differentiation among sites for potentially sedentary species can be useful to determine the scale of migration and gene flow (Wilmer and Wilcox 2007). Here we found no appreciable genetic differentiation among sites within the bay, indicating substantial gene flow, which may occur during juvenile dispersal. Our low sample size outside the Bay likely obscured our ability to test for gene flow.

Our telemetry results suggest brown bullheads exhibit limited dispersal from within Presque Isle Bay to outside the bay. If migration and thus gene flow was restricted between fish in Presque Isle Bay and those outside the bay, we might expect to discern some genetic divergence, which was not the case. Given the low sample sizes from locations outside the bay, conclusions regarding the genetic divergence among fish from the bay and outside the bay are unresolved. Further sampling of brown bullheads in additional locations outside Presque Isle Bay would be useful to make conclusions about estimates of gene flow among bullhead in the Presque Isle Bay area of Lake Erie.

Telemetry results indicated fish were likely to remain in a particular location during and after the spawning season. Our manual relocations occurred during daylight hours and it may be that bullheads moved more actively at night. Our genetic results indicate that genetically differentiated populations likely do not exist within the bay and that brown bullheads in Presque Isle Bay are panmictic, representing a single genetic population within the bay. The general lack of movement we observed between sites within the bay has apparently not equated to genetic differentiation of localized spawning groups. We do not have location data from the early spring pre-spawn period, prior to any migration to the littoral spawning areas. Larger over-wintering aggregations of fish may randomly disperse to the spawning areas in the spring, which would be consistent with our genetic finding of panmixia.

The validity of using fish tumor surveys as an indicator of environmental health is dependent upon choosing a species which resides almost exclusively in the system being monitored. Sakaris et al.

Table 8
Results from individual-based assignment based on allele frequencies for each collection site when all Presque Isle Bay collected samples are pooled and compared to Elk Creek and Thompson Bay.

Collection site	Collection site assigned to			% correct
	Elk	PIB sites	Thompson	
Elk	3	7	2	25.0
PIB	3	85	3	93.4
Thompson	2	7	0	0

The pooled Presque Isle Bay (PIB) sites are the Lagoons, Sara's Cove, and Misery Bay.

(2005) performed a similar movement study on brown bullheads in the Anacostia River system and concluded that they were resident to the system and thus were useful as an indicator species. We observed movements within Presque Isle Bay as extensive as 6 km, depending on the effective range of our manual detection gear. Although outside the scope of this study, brown bullheads may make these migrations seasonally in search of thermal optima (Richards and Ibara 1978) or in search of spawning habitat (Dedual 2002). The lack of observed movement between the lake and bay observed may be the result of several different influences, including temperature, habitat, water quality, and the relatively small channel that controls egress from the entire bay.

Our telemetry data suggest that most adult brown bullhead individuals stay within the bay. Genetic data reveal no differentiation among sites inside the bay. Gene flow with sites outside the bay should be further investigated with larger sample sizes and additional locations. Gene flow outside the bay could occur via migration of juveniles or occasional straying. Our telemetry data indicate that the brown bullhead is likely a useful indicator species for environmental conditions in Presque Isle Bay, with most adults being long-time residents.

Acknowledgements

The authors would like to acknowledge Mr. Lorne Brousseau from the Cornell University Cooperative Extension and Mr. Dick Riechle from Advanced Telemetry Systems, Inc., for technical assistance with the radiotelemetry array. Jeff Kalie from the U.S. Fish and Wildlife Service assisted with fish collections. This project was jointly funded by Pennsylvania Sea Grant, Pennsylvania Department of Environmental Protection, and the U.S. Fish and Wildlife Service.

Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

Baumann, P.C., Smith, W.D., Parland, W.K., 1987. Tumor frequencies and contaminant concentrations in brown bullheads from an industrialized river and a recreational lake. *Trans. Am. Fish. Soc.* 116, 79–86.

Breiman, L., 2001. Statistical modeling: the two cultures. *Stat. Sci.* 16, 199–231.

Cornuet, J.M., Piry, S., Luikart, G., Estoup, A., Solignac, M., 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153, 1989–2000.

Dedual, M., 2002. Vertical distribution and movements of brown bullhead (*Ameiurus nebulosus* LeSeuer) Motuopa Bay, southern Lake Taupo, New Zealand. *Hydrobiologia* 483, 129–135.

El Mousadik, A., Petit, R.J., 1996. High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic of Morocco. *Theor. Appl. Genet.* 92, 832–839.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from <http://www.unil.ch/izea/software/fstat.html>.

Gowan, C., Young, M.K., Fausch, K.D., Riley, S.C., 1994. Restricted movement in resident stream salmonids: a paradigm lost? *Can. J. Aquat. Sci.* 51, 2626–2637.

International Joint Commission, 1989. Proposed listing/delisting criteria for Great Lakes Areas of Concern. Windsor, ON: International Joint Commission, 14(1).

Julian, S.E., Bartron, M.L., 2007. Microsatellite DNA markers for American shad (*Alosa sapidissima*) and cross-species amplification within the family Clupeidae. *Mol. Ecol. Notes* 7, 805–807.

Lewis, P.O., Zaykin, D., 2001. Genetic Data Analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). <http://lewis.eeb.uconn.edu/lewishome/software.html>

Mills, L.S., Allendorf, F.W., 1996. The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.* 10, 1509–1518.

PADEP, 1997. The 1997 Presque Isle Bullhead Tumor Study. Prepared by Eric C. Obert, Pennsylvania Department of Environmental Protection. <http://www.depweb.state.pa.us/northwestregion>

Petit, R.J., El Mousadik, A., Pons, O., 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* 12, 844–855.

Pinkney, A.E., Harshbarger, J.C., May, E.B., Melancon, M.J., 2001. Tumor prevalence and biomarkers of exposure in brown bullheads (*Ameiurus nebulosus*) from the tidal Potomac River watershed. *Environ. Toxicol. Chem.* 20, 1196–1205.

Raymond, M., Rousset, F., 1995. GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *J. Heredity* 86, 248–249.

- Rice, W.R., 1989. Analyzing tables of statistical tests. *Evolution* 43, 223–225.
- Richards, F.P., Ibara, R.M., 1978. The preferred temperature of the brown bullhead, *Ictalurus nebulosus*, with reference to its orientation to the discharge canal of a nuclear power plant. *Trans. Am. Fish. Soc.* 107, 288–294.
- Sakaris, P.C., Jesien, R.V., Pinkney, A.E., 2005. Brown bullhead as an indicator species: seasonal movement patterns and home ranges within the Anacostia River, Washington, D.C. *Trans. Am. Fish. Soc.* 134, 1262–1270.
- van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Biol.* 4, 535–538.
- Whitlock, M.C., McCauley, D.E., 1999. Indirect measures of gene flow and migration: $F_{ST} \neq 1/4(Nm+1)$. *Heredity* 82, 117–125.
- Wilmer, J.W., Wilcox, C., 2007. Fine scale patterns of migration and gene flow in the endangered mound spring snail, *Fonscochlea accepta* (Mollusca:Hydrobiidae) in arid Australia. *Conserv. Genet.* 8, 617–628.
- Wright, S., 1931. Evolution in mendelian populations. *Genetics* 16, 97–159.