Final Report

Population Genetic Structure of Anadromous Rainbow Smelt in US Waters

A Project of the NMFS Proactive Species Conservation Program

Principle Investigator: David Berlinsky, Associate Professor of Biological Sciences, University of New Hampshire

Additional Project Participants: Adrienne Kovach, Research Assistant Professor of Natural Resources, University of New Hampshire
Timothy Breton, Biological Sciences, UNH
Lauren Wyatt, Biological Sciences, UNH
Al Baker, Biological Sciences, UNH

Report Submission Date: February 28, 2011

Objectives:

1) Determine the relative amount of periphyton in spawning habitat for anadromous rainbow smelt in selected coastal rivers in Maine, NH, and Massachusetts
2) Determine potential impacts of periphyton growth and sediment deposition on smelt egg hatch
3) Identify dominant species of organisms in the periphyton community associated with rainbow smelt spawning substrate in the gulf of Maine Region
4) Determine the amount of genetic variation in Rainbow smelt within and among various New England estuaries.

<u>Objective 1</u>. Determine the relative amount of periphyton in spawning habitat for anadromous rainbow smelt in selected coastal rivers in Maine, NH, and Massachusetts

Periphyton samples were collected by biologists during the spring of 2009 in Maine, NH, and Massachusetts and transferred to UNH. In the laboratory, periphyton samples were transferred to pre-weighed aluminum weigh boats (using distilled water) to determine dry weight (DW), ash dry weight (ADW), and ash free dry weight (AFDW) by the methods of American Public Health Association, APHA, (1992). To determine DW (g/m^2/day), the samples were dried at 105ºC, cooled in a desiccator, and then weighed to the nearest 0.0001 g (Mettler Toledo AB54-S) multiple days in succession until the weights differed by no more than 0.0008 g. Samples were then ignited for 1 hr in a muffle furnace at 500ºC, re-hydrated (~5 mL) and re-dried at 105ºC, cooled in a desiccator, and again weighed to determine the ADW (g/m^2/day). The DW represents both inorganic and organic material ADW, represents only inorganic material. The AFDW (ADW subtracted from the DW) represents the organic portion and is also expressed as g/m^2/day.

Table 1. Dry weight, ash dry weight and ash-free dry weight of periphyton samples collected from smelt spawning Rivers.

Sample ID	AFDW (g/m^2/day)	Comments
4-15 LC Rock1	0.0239	
4-15 LC Rock2	0.0406	
4-15 LC Rock3	0.012	
4-15 LC Rock4	0.0418	
4-15 LC Rock5	0.0095	
4-15 LC T1	0.0074	
4-15 LC T2	0.0056	
4-15 LC T3	0.0048	
4-15 LC T4	0.0034	
4-15-LC T5	0.0049	
5-29-09 EB Rock 1	0	No detectable difference in DW & ADW
5-29-09 EB Rock 2	0.0032	
5-29-09 EB Rock 3	0.0013	
5-29-09 EB Rock 4	0	No detectable difference in DW & ADW
5-29-09 EB Rock 5	0	No detectable difference in DW & ADW
5-29-09 EB Tile 1	0.0004	
5-29-09 EB Tile 2	0	No detectable difference in DW & ADW
5-29-09 EB Tile 3	0.0008	
5-29-09 EB Tile 4	0	No detectable difference in DW & ADW
5-29-09 EB Tile 5	0	No detectable difference in DW & ADW
5-9-09 Chan Tile 1	0.002	
5-9-09 Chan Tile 2	0.0008	
5-9-09 Chan Tile 3	0	No detectable difference in DW & ADW
5-9-09 Chan Tile 4	0.0007	
5-9-09 Chandler Rock 1	0.0045	
5-9-09 Chandler Rock 2	0.0107	
5-9-09 Chandler Rock 3	0.0087	
5-9-09 Chandler Rock 4	0.0077	
5-9-09 Chandler Rock 5	0.0147	
5-9-09 East Bay Rock 5	0	No detectable difference in DW & ADW
5-9-09 EB Rock 1	0	No detectable difference in DW & ADW
5-9-09 EB Rock 2	0	No detectable difference in DW & ADW
5-9-09 EB Rock 3	0	No detectable difference in DW & ADW
5-9-09 EB Rock 4	0	No detectable difference in DW & ADW
5-9-09 EB Tile 1	0	No detectable difference in DW & ADW
5-909 EB Tile 2	0	No detectable difference in DW & ADW
5-9-09 EB Tile 3	0.0002	
5-9-09 EB Tile 4	0.0034	
6/25/09 DM R1	0.003	
6/25/09 DM R2	0.0001	
6/25/09 DM R3	0	No detectable difference in DW & ADW
6/25/09 DM R4	0.0003	
6/25/09 DM R5	0.0002	
6/25/09 DM T2	0.0005	
6/25/09 DM T3	0.0002	
6-12-09 LC R2	0.0141	
6-12-09 LC R3	0.0163	
6-12-09 LC R4	0.0197	
6-12-09 LC R5	0.0251	
6-12-09 LC Rock1	0.0398	
6-12-09 LC T4	0.0372	
6-12-09 LC T5	0.0175	
6-12-09 LC Tile 1	0.0575	
6-18-09 East Bay Rock 1	0	No detectable difference in DW & ADW
6-18-09 East Bay Rock 2	0	No detectable difference in DW & ADW
6-18-09 East Bay Rock 3	0	No detectable difference in DW & ADW
6-18-09 East Bay Rock 4	0	No detectable difference in DW & ADW
6-18-09 East Bay Rock 5	0	No detectable difference in DW & ADW

Sample ID	AFDW (g/m^2/day)	Comments
6-18-09 East Bay Tile 1	0	No detectable difference in DW & ADW
6-18-09 East Bay Tile 2	0.0009	
6-18-09 East Bay Tile 3	0	No detectable difference in DW & ADW
6-18-09 East Bay Tile 4	0.0007	
6-18-09 East Bay Tile 5	0.0005	
6-25-09 Chandler Rock 2	0.0106	
6-25-09 Chandler Rock 1	0.0136	
6-25-09 Chandler Rock 3	0.0115	
6-25-09 Chandler Rock 4	0.0074	
6-25-09 Chandler Rock 5	0.0189	
6-25-09 Chandler Tile 1	0.0166	
6-25-09 Chandler Tile 2	0.0049	
6-25-09 Chandler Tile 3	0.0113	
6-25-09 Chandler Tile 4	0.0059	
6-25-09 Chandler Tile 5	0.0086	
6-4-09 Chandler Rock 1	0.0089	
6-4-09 Chandler Rock 2	0.0052	
6-4-09 Chandler Rock 3	0.0124	
6-4-09 Chandler Rock 4	0.0056	
6-4-09 Chandler Rock 5	0.012	
DM 4-27-09 Rock1	0.0007	
DM 4-27-09 Rock2	0.001	
DM 4-27-09 Rock3	0.0013	
DM 4-27-09 Rock4	0	No detectable difference in DW & ADW
DM 4-27-09 Rock5	0	No detectable difference in DW & ADW
DM 6-1-09 R5	0.0014	
DM 6-1-09 Rock1	0.0011	
DM 6-1-09 Rock2	0.0056	
DM 6-1-09 Rock3	0.003	
DM 6-1-09 Rock4	0.0009	
DM 6-1-09 T1	0.0005	
DM 6-1-09 T2	0.0005	
DM 6-1-09 T3	0.0002	
DM 6-1-09 T4	0	No detectable difference in DW & ADW
DM 6-1-09 T5	0.0023	
DMB-Rock1-5/11/09	0.0178	
DMB-Rock2-5/11/09	0.0159	
DMB-Rock3-5/11/09	0.0185	
DMB-Rock4-5/11/09	0	No detectable difference in DW & ADW
DMB-Rock5-5/11/09	0	No detectable difference in DW & ADW
DM-T4-6/25/09	0.0003	
DM-T5-6/25/09	0.0004	
DM-Tile1-6/25/09	0.0008	
FR 0609 - 2	0.0006	
FR 0609 - 5	0	No detectable difference in DW & ADW
FR 091-3	0.0002	
FR0609-1	0	No detectable difference in DW & ADW
FR0609-1	0	No detectable difference in DW & ADW
FR0609-3	0.0004	
FR0609-4	0	No detectable difference in DW & ADW
FR0609-5	0	No detectable difference in DW & ADW
FR0609-R1	0.0007	
FR0609-R2	0.0038	
FR0609-R3	0.001	
FR0609-R3	0	No detectable difference in DW & ADW
FR0609-R4	0.0047	
FR0609-R5	0.0007	
FR091-1	0	No detectable difference in DW & ADW

Sample ID	AFDW (g/m^2/day)	Comments
FR091-10	0.0087	
FR091-2	0	No detectable difference in DW & ADW
FR091-2	0	No detectable difference in DW & ADW
FR091-4	0	No detectable difference in DW & ADW
FR091-5	0	No detectable difference in DW & ADW
FR091-6	0.0109	
FR091-7	0	No detectable difference in DW & ADW
FR091-8	0.0061	
FR091-9	0.006	
JF1009-2	0	No detectable difference in DW & ADW
JR0609-2	0	No detectable difference in DW & ADW
JR0609-3	0	No detectable difference in DW & ADW
JR0609-4	0	No detectable difference in DW & ADW
JR0609-R1	0	No detectable difference in DW & ADW
JR0609-R2	0.0012	
JR0609-R4	0.0045	
JR0609-R5	0.0105	
JR091-01	0	No detectable difference in DW & ADW
JR091-10	0.0034	
JR091-3	0.0012	
JR0913-1	0.0135	
JR0913-2	0.0128	
JR0913-3	0.0114	
JR0913-4	0.0025	
JR0913-5	0.0092	
JR0913-R1	0.0069	
JR0913-R2	0.0029	
JR0913-R3	0.0143	
JR0913-R4	0.0202	
JR0913-R5	0.0681	
JR091-4	0.0004	
JR091-5	0.0006	
JR091-6	0.0385	
JR091-7	0	No detectable difference in DW & ADW
JR1009-1	0.0012	
JR1009-3	0	No detectable difference in DW & ADW
JR1009-4	0.0022	
JR1009-5	0.0021	
JR1009-R1	0.0027	
JR1009-R2	0.0053	
JR1009-R3	0.0015	
JR1009-R4	0.0014	
JR1009-R5	0	No detectable difference in DW & ADW
LC 5-19-09 Rock1	0.0206	
LC 5-19-09 Rock2	0.0479	
LC 5-19-09 Rock3	0.0266	
LC 5-19-09 Rock4	0.0358	
LC 5-19-09 Rock5	0.0109	
LC 5-19-09 Tile1	0.0065	
LC 5-19-09 Tile2	0.0079	
LC 5-19-09 Tile3	0.007	
LC 5-19-09 Tile4	0.0082	
LC 5-19-09 Tile5	0.008	
LC-ROCK1-4-29-09	0.0258	
LC-ROCK2-4-29-09	0.0244	
LC-ROCK3-4-29-09	0.0412	
LC-ROCK4-4-29-09	0.0035	
LC-ROCK5-4-29-09	0.0196	

Sample ID	AFDW (g/m^2/day)	Comments
LC-T1-4-29-09	0.0026	
LC-T2-4-29-09	0.003	
LC-T2-6/12/09	0.0099	
LC-T3-4-29-09	0.0096	
LC-T3-6/12/09	0.0773	
LC-T4-4-29-09	0.0021	
LC-T5-4-29-09	0.0055	
MA-CR-03-R1-09	0.0267	
MA-CR-03-R2-09	0.0183	
MA-CR-03-R3-09	0.0212	
MA-CR-03-R4-09	0.0101	
MA-CR-03-R5-09	0.0208	
MA-CR-03-T1-09	0.0028	
MA-CR-03-T2-09	0.0086	
MA-CR-03-T3-09	0.0028	
MA-CR-03-T4-09	0.0034	
MA-CR-03-T5-09	0.0072	
MA-CR-06-R1-09	0.0159	
MA-CR-06-R2-09	0.0046	
MA-CR-06-R3-09	0.0168	
MA-CR-06-R4-09	0.0241	
MA-CR-06-R5-09	0.0025	
MA-CR-06-T1-09	0.0135	
MA-CR-06-T2-09	0.0587	
MA-CR-06-T3-09	0.0079	
MA-CR-06-T4-09	0.0058	
MA-CR-06-T5-09	0.0055	
MA-CR-09-R1-09	0.0274	
MA-CR-09-R2-09	0.0123	
MA-CR-09-R3-09	0.0244	
MA-CR-09-R4-09	0.0161	
MA-CR-09-R5-09	0.0252	
MA-CR-09-T1-09	0.0326	
MA-CR-09-T2-09	0.0309	
MA-CR-09-T3-09	0.0263	
MA-CR-09-T4-09	0.0248	
MA-CR-09-T5-09	0.033	
MA-CR-12-R1-09	0.0324	
MA-CR-12-R2-09	0.0347	
MA-CR-12-R3-09	0.0069	
MA-CR-12-R4-09	0.013	
MA-CR-12-R5-09	0.0111	
MA-CR-12-T1-09	0.0137	
MA-CR-12-T2-09	0.017	
MA-CR-12-T3-09	0.014	
MA-CR-12-T4-09	0.0009	
MA-CR-12-T5-09	0.0175	
MA-FR-09-R1-09	0.0137	
MA-FR-09-R2-09	0.0099	
MA-FR-09-R4-09	0.0111	
MA-FR-09-R5-09	0.0032	
MA-FR-09-T1-09	0.008	
MA-FR-09-T2-09	0.019	
MA-FR-09-T3-09	0.0093	
MA-FR-09-T4-09	0.0118	
MA-FR-09-T5-09	0.0045	
MA-FR-12-R1-09	0.0284	
MA-FR-12-R2-09	0	No detectable difference in DW & ADW

Sample ID	AFDW (g/m^2/day)	Comments
MA-FR-12-R3-09	0.0038	
MA-FR-12-R4-09	0	No detectable difference in DW & ADW
MA-FR-12-R5-09	0.0158	
MA-FR-12-T1-09	0.0094	
MA-FR-12-T2-09	0.0011	
MA-FR-12-T3-09	0	No detectable difference in DW & ADW
MA-FR-12-T4-09	0.0081	
MA-FR-12-T5-09	0.0025	
MA-MR-03-R1-09	0.0247	
MA-MR-03-R2-09	0.0219	
MA-MR-03-R3-09	0.0323	
MA-MR-03-R4-09	0.0343	
MA-MR-03-R5-09	0.0304	
MA-MR-03-T1-09	0.0018	
MA-MR-03-T2-09	0.0018	
MA-MR-03-T3-09	0.001	
MA-MR-03-T4-09	0	No detectable difference in DW & ADW
MA-MR-03-T5-09	0	No detectable difference in DW & ADW
MA-MR-06-R1-09	0.0132	
MA-MR-06-R2-09	0.0349	
MA-MR-06-R3-09	0.0188	
MA-MR-06-R4-09	0.0061	
MA-MR-06-R5-09	0.0299	
MA-MR-06-T1-09	0.0206	
MA-MR-06-T2-09	0.0286	
MA-MR-06-T3-09	0.0398	
MA-MR-06-T4-09	0.0222	
MA-MR-06-T5-09	0.0328	
MA-MR-09-R1-09	0.0151	
MA-MR-09-R2-09	0.0008	
MA-MR-09-R3-09	0.0126	
MA-MR-09-R4-09	0.0525	
MA-MR-09-R5-09	0.0375	
MA-MR-09-T1-09	0.0798	
MA-MR-09-T2-09	0.0549	
MA-MR-09-T3-09	0.0413	
MA-MR-09-T4-09	0.0507	
MA-MR-09-T5-09	0.0411	
MA-MR-12-R1-09	0.0113	
MA-MR-12-R2-09	0.0037	
MA-MR-12-R3-09	0.005	
MA-MR-12-R4-09	0.0051	
MA-MR-12-R5-09	0.0013	
MA-MR-12-T1-09	0.0041	
MA-MR-12-T2-09	0.0062	
MA-MR-12-T3-09	0.0045	
MA-MR-12-T4-09	0.0013	
MA-MR-12-T5-09	0.0062	
MA-NR-03-R1-09	0.0756	
MA-NR-03-R2-09	0.0335	
MA-NR-03-R3-09	0.0508	
MA-NR-03-R4-09	0.0286	
MA-NR-03-R5-09	0.0239	
MA-NR-03-T1-09	0.0087	
MA-NR-03-T2-09	0.0126	
MA-NR-03-T3-09	0.0042	
MA-NR-03-T4-09	0.0049	
MA-NR-03-T5-09	0.0089	

Sample ID	AFDW (g/m^2/day)	Comments
MA-NR-06-R1-09	0.0296	
MA-NR-06-R2-09	0.0491	
MA-NR-06-R3-09	0.034	
MA-NR-06-R4-09	0.044	
MA-NR-06-R5-09	0.0439	
MA-NR-06-T1-09	0	No detectable difference in DW & ADW
MA-NR-06-T2-09	0	No detectable difference in DW & ADW
MA-NR-06-T3-09	0	No detectable difference in DW & ADW
MA-NR-06-T4-09	0	No detectable difference in DW & ADW
MA-NR-06-T5-09	0	No detectable difference in DW & ADW
MA-NR-09-R1-09	0.0681	MA-NR-09-R1-09
MA-NR-09-R2-09	0.049	
MA-NR-09-R3-09	0.021	
MA-NR-09-R4-09	0.019	
MA-NR-09-R5-O9	0.0249	
MA-NR-09-T1-09	0.0133	
MA-NR-09-T2-09	0.0114	
MA-NR-09-T3-09	0.0078	
MA-NR-09-T4-09	0.0044	
MA-NR-09-T5-09	0.0192	
MA-NR-12-R1-09	0	No detectable difference in DW & ADW
MA-NR-12-R2-09	0	No detectable difference in DW & ADW
MA-NR-12-R3-09	0.0041	
MA-NR-12-R4-09	0	No detectable difference in DW & ADW
MA-NR-12-R5-09	0.0112	
MA-RF-09-R3	0.0077	
ML 5/19/09 Rock1	0	No detectable difference in DW & ADW
ML 5/19/09 Rock2	0	No detectable difference in DW & ADW
ML 5/19/09 Rock3	0	No detectable difference in DW & ADW
ML 5/19/09 Rock4	0	No detectable difference in DW & ADW
ML 5/19/09 Rock5	0.0011	
ML-Rock1-4/29/09	0.0002	
ML-Rock2-4/29/09	0	No detectable difference in DW & ADW
ML-Rock3-4/29/09	0.0009	
ML-Rock4-4/29/09	0.0005	
ML-Rock5-4/29/09	0	No detectable difference in DW & ADW
SQ 01	0.0078	
SQ 02	0.0045	
SQ 03	0.0043	
SQ 04	0.0037	
SQ 05	0.0057	
SQ 06	0.0146	
SQ 07	0.0193	
SQ 08	0.0126	
SQ 09	0.0138	
SQ 10	0.0158	
SQ 12	0.0035	
SQ 13	0.0026	
SQ 14	0.0034	
SQ 15	0.0051	
SQ 16	0.0076	
SQ 17	0.0026	
SQ 17	0.0053	
SQ 18	0.009	
SQ 19	0.004	
SQ 20	0.0043	
SQROCK 01	0.0032	
SQROCK 02	0.0114	

Sample ID	AFDW (g/m^2/day)	Comments
SQROCK 04	0.0573	
SQROCK 05	0.0087	
SQROCK 06	0.0016	
SQROCK 07	0.0063	
SQROCK 08	0.0021	
SQROCK 09	0.0091	
SQROCK 10	0.0099	
SQROCK 11	0.0089	
SQROCK 12	0.003	
SQROCK 13	0.0055	
SQROCK 14	0.0049	
SQROCK 15	0.0073	
SQROCK 16	0.0157	
SQROCK 17	0.0022	
SQROCK 18	0.0095	
SQROCK 19	0.0053	
SQROCK 20	0.0118	
TB 4-26-09 Rock1	0.0093	
TB 4-26-09 Rock2	0.0337	
TB 4-26-09 Rock3	0.0056	
TB 4-26-09 Rock4	0.0034	
TB 4-26-09 Rock5	0.0079	
TB 4-26-09 T1	0.0149	
TB 4-26-09 T2	0.0054	
TB 4-26-09 T3	0.006	
TB 4-26-09 T4	0.0058	
TB 4-26-09 T5	0.0031	
TB 6-10-09 R2	0.001	
TB 6-10-09 R3	0.0029	
TB 6-10-09 R5	0	No detectable difference in DW & ADW
TB 6-10-09 T1	0.0091	
TB 6-10-09 T2	0.007	
TB 6-10-09 T3	0.0165	
TB 6-10-09 T4	0.0084	
TB 6-10-09 T5	0.0035	
TB-R1-5-15-09	0.0076	
TB-R1-6/10/09	0.0021	
TB-R1-7-6-09	0.0011	
TB-R2-5-19-09 5/15?	0.0046	
TB-R2-7-6-09	0.0007	
TB-R3-5-12-09 5/15?	0.0021	
TB-R3-5-15-09	0.0039	
TB-R3-7-6-09	0.0017	
TB-R4-6-10-09	0.0073	
TB-R4-7-6-09	0.0049	
TB-R5-5-15-09	0.0018	
TB-R5-7-6-09	0.0024	
TB-T1-7-6-09	0	No detectable difference in DW & ADW
TB-T2-5-15-09	0.0067	
TB-T3-5-15-09	0.0023	
TB-T5-5-15-09	0.0039	
TB-Tile4-5-15-09	0.0024	
WIN 01	0.0036	
WIN 02	0.0031	
WIN 03	0.0047	
WIN 04	0.0054	
WIN 05	0.0042	
WIN 06	0.0167	

Sample ID	AFDW (g/m^2/day)	Comments
WIN 07	0.0062	
WIN 08	0.005	
WIN 09	0.0047	
WIN 10	0.0204	
WIN 11	0.0206	
WIN 12	0.0137	
WIN 13	0.0166	
WIN 14	0.0142	
WIN 15	0.0113	
WIN 16	0.0024	
WIN 17	0.0084	
WIN 18	0.0028	
WIN 19	0.0215	
WIN 20	0.1725	
WINROCK 01	0.0049	
WINROCK 02	0.0107	
WINROCK 03	0.007	
WINROCK 04	0.0093	
WINROCK 05	0.0062	
WINROCK 06	0.0028	
WINROCK 07	0.0166	
WINROCK 08	0.0039	
WINROCK 09	0.0026	
WINROCK 10	0.0115	
WINROCK 11	0.0177	
WINROCK 12	0.0062	
WINROCK 13	0.0197	
WINROCK 14	0.0149	
WINROCK 15	0.0111	
WINROCK 16	0.0091	
WINROCK 17	0.0289	
WINROCK 18	0.0063	
WINROCK 19	0.0053	
WINROCK 20	0.0087	

Objectives 2 and 3.

1) Determine potential impacts of periphyton growth and sediment deposition on smelt egg hatch
2) Identify dominant species of organisms in the periphyton community associated with rainbow smelt spawning substrate in the gulf of Maine Region

The following text was included in a manuscript published in the journal "*Aquatic Sciences*".

Abstract

The decline in anadromous rainbow smelt (*Osmerus mordax*) populations may be due to anthropogenic causes including spawning habitat degradation. The purpose of this study was to assess the survival of rainbow smelt embryos incubated under sediment layers of different depths (0.00, 0.25, 1.00, and 6.00 g/45.6 cm^2) and in contact with periphyton communities of different biomass. Embryo survival was also assessed when cultured on periphyton in combination with sterilized sediment or eutrophying compounds (nitrates and phosphates). Oxygen consumption was monitored from embryos cultured alone, on periphyton layers, and under a sediment layer. Survival was significantly reduced under the highest sediment treatment and attributed to low oxygen availability to the embryos. Embryonic survival was also significantly reduced when incubated on the highest periphyton biomass. Embryos under the sediment layer consumed oxygen at a significantly greater rate than the controls, and the dissolved oxygen concentration below the sediment-water interface decreased to near anoxic. These results suggest that embryonic survival could be impacted in rivers with heavy sedimentation or a high standing biomass of periphyton.

Introduction

The rainbow smelt, *Osmerus mordax* (Mitchill), is a small anadromous fish found along the Northwest Atlantic and Northeast Pacific coasts of North America that is enjoyed as a food fish, and has supported important commercial and recreational fisheries (Buckley, 1989; Klein-MacPhee, 2002). Smelt also serve as an important prey item for many piscivorous fish and bird species. On the Atlantic coast, the southern-most portion of its range has contracted, such that spawning populations are only found in rivers north of Cape Cod, and significant population declines have also been reported in specific rivers within their extant range (Chase and Childs, 2001; Klein-MacPhee, 2002). In response to declining Atlantic populations, rainbow smelt were listed as a "species of concern" by the US National Marine Fisheries Service in 2004 (NOAA, 2004).

The reasons for these population declines are not entirely clear, but human activities in the coastal zone have been implicated in the decline of many anadromous species, including smelt (Murawski and Cole, 1978). Declines in smelt abundance in Massachusetts have been linked to declining water quality from industrial pollution, loss of eelgrass beds, and obstructions in rivers that may prevent upstream migrations (Chase and Childs, 2001; Klein-MacPhee, 2002). As smelt are weak swimmers and are unable to traverse fish ladders, dam construction may also be detrimental to smelt populations, as they prevent spawning smelt from reaching desirable spawning habitats and may expose embryos and larvae to saline environments prematurely (Crestin, 1973). Additionally, as smelt spawn in the spring, the demersal eggs are exposed to runoff from snow melt and spring storms, which may be acidic and/or contain silt and contaminants from anthropogenic activities, such as urbanization (Geffen, 1990; Walling, 1995; Lapierre et al., 1999).

The developing embryos and larval stages of the teleost life cycle are considered to be the most sensitive to environmental stressors (Geffen, 1990; Swanson, 1996) and concern has been raised about the possible effects that degraded water quality has had on rainbow smelt populations. In a previous study, Fuda et al. (2007) demonstrated that smelt are tolerant to a wide range of abiotic environmental factors including salinity, ultraviolet radiation, dissolved oxygen (DO), nitrates, phosphates, and pH during their early developmental stages. In that study, however, smelt embryos incubated in natural spawning rivers became covered with silt, debris, and fungi that impacted hatching success. The purpose of the present study was to investigate the effects of silt, periphyton communities, and eutrophying compounds on oxygen availability and embryonic smelt survival in controlled laboratory conditions (Fig. 1).

Materials and methods

Egg collection

During their annual spawning migration (March-May 2007-2008), adult rainbow smelt were captured with fyke nets in New Hampshire (NH) rivers that are tributaries of the Great Bay estuary. The smelt were transported to the University of New Hampshire (UNH), Durham, NH, anesthetized with tricaine methanesulfonate (100 mg L^{-1} Tricaine-S; Western Chemicals, Ferndale, WA) and manually spawned (Ayer et al., 2005) using multiple males and females (n > 6). While no agents were used to remove egg adhesiveness, the degree of egg adhesion was variable among spawning events. In all studies except Experiments 1 and 3, the eggs were less adhesive and were incubated in 3 L polyethylene hatching jars, with vigorous aeration (5 or 10 ± 1ºC, salinity 0) for 2-4 days, prior to assessing fertilization success. Only viable embryos were used in those studies. Embryonic development can be observed using a dissecting microscope because viable embryos are translucent while non-viable embryos are opaque. In Experiment 1, the eggs were very adhesive and were transferred directly to slate tiles after manual spawning. Fertilized and unfertilized eggs on each tile were enumerated 8 days post fertilization (DPF). In Experiment 3, the adhesive eggs were directly transferred to clay bricks and fertilization was assessed 2 DPF. Directly pouring the embryos onto the tiles and bricks introduced some variability in the numbers among replicates, but variation among treatments was not significant as determined by ANOVA.

Sediment collection

Sediment was collected from the intertidal zone of the Oyster River, Durham, NH, at low tide, and sieved through a 300 µm nylon mesh. Sediment was dried at 70ºC, sieved again, and sterilized by autoclaving at 123ºC for 15 min.

Experiment 1. The effect of sedimentation on embryo survival

Following fertilization, embryos were gently poured to form a uniform monolayer (129 - 640 embryos) on 16 slate tiles (~104 cm^2) and were held in 40 L aquaria (10 ± 1ºC, salinity 0), with supplemental aeration. After determining fertilization success (8 DPF), the embryos were covered with low, medium, and high sediment levels (0.25, 1.00 and 6.00 g dry weight; DW, n = 4/treatment). A piece of polyvinyl chloride (PVC) tube (diameter = 7.6 cm) was used to direct a slurry of sediment over the eggs (area = 45.6 cm^2). Well-water alone was added to the control treatment (n = 4). Sediment was allowed to settle for one hour before the tube was removed. Embryos were distinguishable in the low and medium treatments (< 1 mm cover) but not in the high treatment (~1 mm cover). Following sediment settlement, water was circulated over the covered embryos with small aquarium pumps (~250 L hr^{-1}), that were placed ~26.7 cm vertically and ~22.3 cm horizontally away from the embryos. Prior to hatching (14 DPF), a stream of freshwater was used to gently remove the sediment, and live and dead embryos were enumerated. Survival was assessed as the number of live embryos remaining from the initial number of live plated.

Experiment 2. The effect of sedimentation on embryonic respiration

Oxygen consumption by sediment-covered embryos was measured with a Unisense Clark-type OX50 dissolved oxygen (DO) glass micro-electrodes with guard cathode (50 µm diameter, Unisense, Aarhus, Denmark), connected to a Unisense PA2000 picoammeter (Unisense, Denmark). The electrodes (stirring sensitivity < 2%; response time, t_{90} < 5 s) were calibrated linearly at experimental temperature and salinity using air-saturated water (atmospheric O_2) and oxygen-free water (gaseous N_2).

Ten embryos were transferred to each of two 5 ml borosilicate glass, aluminum foil-covered beakers, with a transfer pipette and maintained at 10 ± 1ºC. The oxygen probe and a slurry of sediment were introduced through two holes (~3 mm diameter) made in the foil. The micro-oxygen electrodes were then lowered to the bottom of the beakers, and positioned < 1 mm from the embryos. Sterilized sediment (0.45 g, equivalent on a g/cm^2 basis to the 6.00 g treatment described above; Expt. 1) that was aerated for 24 hr to remove a portion of the chemical oxygen demand was added to one beaker using a pasture pipette. Well-water was then added to fill both beakers.

Oxygen concentration profiles were recorded (Unisense Profix 3.10; Unisense, Denmark) for 15-26 hr periods, after which embryos, water, and aerated sediment were replaced. Following each experiment (21-36 hr), the embryos were rinsed and examined to confirm viability. Electrodes were re-calibrated prior to each profile. DO concentrations were measured every 8.31 s, and recorded measurements were averages of 100 consecutive readings. Across a range of high DO concentrations, the linear portions of the oxygen consumption regressions were estimated visually from each profile and the slopes of these lines were used to calculate the routine metabolic rates (Cech, 1990; Torrans, 2007).

To determine the oxygen demand of the sterilized sediment alone, DO profiles were recorded in beakers containing sediment but no embryos (n = 2). The oxygen consumption rate between embryos covered and not covered with sediment were compared after correcting for oxygen consumed by the sediment alone. The slopes of the two regressions were compared using a Student's t-test for each day tested (Zar, 1999).

To obtain a vertical oxygen concentration profile, oxygen measurements were taken 72 hr after the addition of the sediment (n = 2) at various depths above and below the sediment. Measurements in increments of 0.05 mm were taken from under the sediment to 5.50 mm above the sediment, and increments of 1.00 mm were measured from 5.50-19.07 mm above the sediment.

Conditions for periphyton experiments 3-4.

Embryos were transferred to terracotta clay bricks (n = 4/treatment; area = ~0.0206 m^2) with polypropylene transfer pipettes 2-4 DPF. The treatment (periphyton cover) and control (no periphyton) bricks were held in 9.5 L glass aquaria, submerged under 5 cm of well-water held at 10 ± 1ºC, salinity 0, with supplemental aeration, and a 12 Light:12 Dark photoperiod (~1200 lx light; Milwaukee Instruments, SM700, Rockymount, NC, USA). Periphyton biomass and composition were determined as described below. Viability was assessed (10-12 DPF) by enumerating the live and dead embryos and hatching success was determined 18-20 DPF.

Experiment 3. The effects of periphyton and sedimentation on embryo survival

Embryos (36-89/treatment, ~80% fertilization; 2 DPF) were distributed to bricks without periphyton, or to bricks with natural periphyton collected from the Squamscott and Crane (Danvers, MA) Rivers. The Crane River was selected because high periphyton loads were observed on submerged substrate. Additionally, the periphyton-covered bricks collected from the Squamscott River were covered with sediment (0.00, 0.25, and 1.00 g DW) as described in Experiment 1 above. Viability was assed at 12 DPF and successful hatching at 20 DPF.

Experiment 4. The effects of periphyton and eutrophying compounds on embryo survival

Embryos (64-126/treatment; 2 DPF) were plated on periphyton-covered bricks collected from the Crane River as described above. Eggs were reared under one of four conditions: (1) background levels of nitrates (0.4 mg L^{-1} NO_3^-, sodium nitrate, Fisher Scientific, Fair Lawn, NJ, USA) and phosphates (0.04 mg L^{-1}, Sigma-Aldrich, St. Louis, MO, USA), (2) elevated nitrates (10.0 mg L^{-1} and background phosphate), (3) elevated phosphates (0.10 mg L^{-1}; background nitrate), and (4) elevated nitrate and phosphate. Well-water was used in all treatments and embryos plated on bricks with no periphyton and background levels of nitrates and phosphates served as controls. Daily water changes (2/3 volume) with the target nutrient levels began 6 DPF. Viability was assessed at 10 DPF and hatching success at 18 DPF.

Experiment 5. Oxygen concentrations in the embryo micro-environment

Embryos (~20) were plated on bricks with natural periphyton (Squamscott River) as described in Experiment 3 above, and on control bricks without periphyton. Bricks were maintained in 9.5 L glass aquaria with well-water at 10 ± 1ºC and salinity 0. Slight aeration was added to the system to simulate an oxygenated river. Oxygen concentrations were recorded continuously in the micro-environment of a single embryo (< 1 mm) from 4 DPF until hatch was observed (10-12 DPF) using the micro-oxygen probes and recording device described above. Readings were made ~20 cm from aeration source (Tetra*tec* AP100). A reading was taken every 8.31 s and recorded oxygen measurements were averages of 100 consecutive readings.

Sediment and periphyton organic content

The dry weight, ash dry weight (ADW), and ash free dry weight (AFDW) of sediment and periphyton samples from each experiment (n = 4) were determined using the methods of the American Public Health Association (APHA, 1992). Periphyton samples were collected from rocks or bricks from 12 smelt-spawning rivers in Massachusetts, New Hampshire, and Maine between March and May 2008 and processed to estimate the standing periphyton biomass (Table 2). DW represents both inorganic and organic material, while ADW represents inorganic material only. To determine the DW (g m^{-2}), scraped periphyton samples from measured areas (0.006-0.013 m^2) were transferred to pre-weighed aluminum weigh boats, dried at 105ºC, cooled in a desiccator, and weighed to the nearest 0.0001 g (Mettler Toledo AB54-S) over multiple days (3-4 days) in succession until the weights differed by no more than 0.0008 g. Samples were then ignited for 1 hr in a muffle furnace at 500ºC, re-hydrated (~5 ml), dried at 105ºC, cooled in a desiccator, and weighed to determine the ADW (g m^{-2}). The AFDW (DW-ADW) represents the organic portion and is also expressed as g m^{-2}. Relative organic (AFDW/DW x 100) and inorganic (ADW/DW x 100) matter content was also calculated (Thomas et al., 2006).

Periphyton Taxonomic Composition

A measured area of periphyton from each experiment (0.006-0.011 m^2) was scraped and preserved in 2% "M^3" fixative (5 g potassium iodide, 10 g iodine, 50 ml glacial acetic acid, 250 ml formalin in 1 L distilled water) to determine taxonomic composition to the genus level (APHA, 1992). Using a light microscope (Olympus CH-2 Melville, New York, 40X, 100X, and 400X magnification) at least 300 algal cells were counted in triplicate from a preserved sample to determine a relative abundance estimate, where each algal or diatom filament was recorded as a single cell (Smith, 1950; Prescott, 1978; Weitzel et al., 1979; Wehr and Sheath, 2003).

Statistical analysis

Percentage data were arcsine transformed. ANOVA at a significance level of $p < 0.05$ was performed using SYSTAT 10 (Systat Software, Inc., San Jose, California, USA). A Tukey-Kramer test was used to determine differences between treatments when significant effects were observed. A Student's t-test (Zar, 1999) was used to determine differences between oxygen consumption using SigmaPlot 11 and SYSTAT 10 (Systat Software, Inc., San Jose, California, USA).

Results

Experiment 1. The effect of sedimentation on embryo survival

There were no significant differences in survival among the control (83%) and 0.25 and 1.00 g sediment treatments (75-76%, $p > 0.678$; Table 1). The highest sediment treatment (6.00 g) had a significantly lower survival (53%, $p = 0.018$; Table 1) than that of the controls. The sediment was primarily composed of inorganic material (~96%). The average DW, ADW, and AFDW for the sediment treatments are presented in Table 1.

Experiment 2. The effect of sedimentation on embryonic respiration

Embryos under the sediment layer consumed oxygen at a significantly greater rate than the controls at 22, 25, 27, and 29 DPF ($p < 0.001$; Figs. 2b-e). Consumption under the sediment treatment increased with age (Fig. 2f), and DO concentrations fell below 5 µmol O_2 in 12.1, 4.7, 3.5, and 2.1 hr for embryos at 22, 25, 27, and 29 DPF, respectively. All embryos removed from the sediment and examined after the completion of the experiment were viable. DO levels below the sediment without embryos fell below 5 µmol in 34.9 hr. The vertical profile indicated levels of unchanging DO concentration (45 µmol O_2), 3-7 mm above the sediment-water interface (Fig. 3). Above this area, the DO concentrations increased, while below the sediment-water interface the DO concentration decreased to near anoxia (Fig. 3).

Experiment 3. The effects of periphyton and sedimentation on embryo survival

Embryos incubated on periphyton from the Squamscott River, with or without additional sediment, had survival (49-55%) that was not different from the control (61%, $p \geq 0.306$; Table 1), while those incubated on periphyton from the Crane River had significantly lower survival (17%, $p < 0.001$; Table 1). Hatching success did not differ among treatments ($p = 0.117$; Table 1). The periphyton from both rivers was primarily composed of inorganic material (>91%) but the periphyton from the Crane River had a significantly higher ($p < 0.001$) biomass (AFDW) than that from all other sources (Table 1). Periphyton from both rivers were primarily composed of diatom genera (96%), specifically the genus *Synedra* comprised over 67% of the total. Diatoms were observed adhering to the chorions of live embryos from all periphyton treatments. This was especially true of those from the Crane River, some of which were completely covered by diatoms (predominately *Cymbella* sp).

Experiment 4. The effects of periphyton and eutrophying compounds on embryo survival

No significant differences in survival ($p = 0.967$) or hatch ($p = 0.909$) were found among embryos grown in the presence of periphyton, with or without nutrient enrichment, compared to controls (Table 1). Periphyton was primarily composed of inorganic material (> 82%) and had a biomass (DW, ADW) that was significantly lower ($p < 0.001$) than the sample from the Crane River collected a week earlier (Experiment 3). Periphyton was primarily composed of diatoms (93%), especially *Synedra* (57%). As in Experiment 3, diatoms, predominately *Cymbella* sp., were found adhering to the embryos from the Crane River treatments.

Experiment 5. Oxygen concentrations in the micro-environment of embryos

Embryos incubated on natural periphyton experienced DO concentrations that cycled during the periods of light and darkness. DO levels dropped below saturation (251 µmol O_2) during darkness but rose considerably during simulated daylight. Embryos in the control treatment remained at or above saturation throughout the experiment. DO in the natural periphyton treatment ranged from 393-556 µmol and 0-243 µmol during the light and dark phases, respectively (Fig. 4). Some embryos were observed hatching following culture on both periphyton communities.

Standing periphyton biomass

Periphyton biomass (DW, ADW, and AFDW) was variable among rivers in the three states and within rivers sampled temporally (Table 2). The highest periphyton biomass was recorded from the Crane River (MA), while low levels were present in Mast Landing (ME) and Deer Meadow Brook (ME) Rivers (Table 2).

Discussion

The importance of sufficient oxygen levels for normal development and embryonic survival has been demonstrated for a number of fish species, including Walleye (*Stizostedion vitreum*; Oseid and Smith, 1971), lake herring (*Coregonus artedii*; Brooke and Colby, 1980), and steelhead trout (*Oncorhynchus mykiss*; Rombough, 1988). The effects of low DO levels are often most evident during the more advanced stages of embryonic development, when oxygen demands are highest (Rombough, 1988; Louhi et al., 2008). The developing embryo acts as an "oxygen sink" so that even at relatively high water velocities, the partial pressure of oxygen at the embryo surface may be much less than that of the surrounding water (Daykin, 1965). In pristine settings, the cold, fast moving, river water in which smelt spawn would be fully oxygen-saturated, but the presence of dams or other obstructions to water flow, as well as sediment, periphyton, and detritus accumulation, may limit oxygen availability. Although the effects of low DO on embryonic smelt survival have not been investigated in natural settings, long-term exposure to poorly oxygenated water was shown to reduce hatching in laboratory studies (Fuda et al., 2007).

In the present study, a sediment covering (~1 mm) over a 6 day period significantly reduced embryo survival. These results are similar to those reported in several other teleost species, such as Atlantic salmon (*Salmo salar*) and whitefish (*Coregonus* sp.) where fine sediment deposits reduced embryo survival by restricting oxygen exchange from the macro-environment (Venting-Schwank and Livingstone, 1994; Greig et al., 2005). Significant mortality was also observed in Atlantic herring (*Clupea harengus*) embryos following a precipitating phytoplankton bloom (Morrison et al., 1991). In laboratory and field studies with several salmonid species such as Atlantic salmon (Lapointe et al., 2004), fall-chinook (*Oncorhynchus tshawytscha*; Shelton and Pollock, 1966), and Coho salmon (*Oncorhynchus kisutch*; Meyer, 2003), fine sediment was shown to reduce embryo survival by restricting gravel permeability and oxygen delivery to the redds. Sediment adhesion can also impact embryonic development by restricting oxygen exchange through the micropores of the chorion (Louhi et al., 2008).

In addition to restricting oxygen delivery through advection, respiration, and oxygen uptake by particulate organic carbon (POC), sediment can deplete DO in riverine systems and generate near anoxic levels at the substrate water interface (Jorgensen and Revsbech, 1985). Reduced embryonic survival may result if developing embryos are deposited on, or covered by, a layer of this respiring material, as oxygen transport to the embryo will be diminished by the low DO concentration gradient in the microenvironment. Both advection and sediment respiration are believed to be responsible for low oxygen conditions experienced by whitefish embryos in eutrophic lakes (Lahti et al., 1979; Wilkonska and Zuromska, 1982; Venting-Schwank and Livingstone, 1994). The sediment used in the present study, although dried, sterilized, and aerated, depleted oxygen in the micro-environment directly above the sediment. In natural settings, smelt embryo survival may be impacted under thinner sediment layers than found in the present studies because un-sterilized sediment would likely harbor respiring microbes that would further deplete oxygen availability.

Periphyton communities can also affect the DO concentration in an embryo's micro-environment, as the assemblage of microorganisms that comprise the periphyton (algae, protozoans, and bacteria) can act as both a source and sink for oxygen (McIntire, 1966; Carlton and Wetzel, 1987). Due to photosynthesis, water can be supersaturated with oxygen during the daylight hours, but approach anoxia in the dark from net respiration (McIntire, 1966; Carlton and Wetzel, 1987). Diurnal DO fluctuations were found in the present study, but it is unlikely this would affect embryo survival because 36 hr periods of anoxia were not shown to affect embryonic smelt survival in this study.

The standing biomass of periphyton among and within smelt-spawning rivers in New England appears to be highly variable and temporally unstable. Periphyton distribution can be affected by light intensity, substrate type, temperature, nutrient levels, and grazing invertebrates (Trainor, 1978). Although no organized sampling protocol was followed in the present study, periphyton samples collected 7 days apart from the same general location in the Crane River differed greatly in biomass. The high biomass from the Crane River samples was comprised primarily of inorganic matter but it is not known if this was from silica comprising the diatom walls or sediment and detrital matter trapped by mucilage and mucilaginous stalks secreted by the diatoms (Karlström, 1978; Hoagland et al., 1982; Roemer et al., 1984). Embryo survival was significantly lower only when incubated on periphyton with the highest biomass, but was unaffected by the presence of lower amounts of similar periphyton, or samples to which sediment or eutrophying compounds (nitrates, phosphate) were added. The reasons for the increased embryo mortality are unknown and representative periphyton availability prohibited direct comparisons among these samples. Additional studies are required to examine the quantity and composition of periphyton communities in smelt spawning rivers and to determine their possible impacts on smelt survival.

In summary, survival of rainbow smelt embryos was lower when cultured with sediment cover or periphyton of high biomass. Reduced survival may have been due to prolonged exposure to low oxygen conditions resulting from compromised advection and substrate respiration.

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Figure Legends

Figure 1 Summary of Experiments

Figure 2 Regressions of decreasing mean (± S.E., n = 100) oxygen concentration (μmol O_2) over time from 10 rainbow smelt embryos with no sediment (○, control) and covered with 0.45 g sediment (●, treatment), a) 20, b) 22, c) 25, d) 27, and e) 29 days post fertilization (DPF). Linear portions of the regressions were estimated visually and regression equations are indicated. Asterisks (*) indicate a statistical difference ($p < 0.0001$) in slope (oxygen consumption) between the control and corrected sediment treatment on days specified post-fertilization. f) Uncorrected consumption regressions of embryos (only) covered with sediment 22 (△), 25 (□), 27 (x), and 29 (○) (DPF) and sediment (only) (●, ± S.E., n = 2)

Figure 3 Mean (± S.E., n = 2) vertical oxygen profile (μmol O_2) above and below a sediment layer (0.45 g sediment) with no embryos present (Experiment 2). Shaded area indicates sediment layer.

Figure 4 Mean (± S.E., n = 100) dissolved oxygen concentrations (μmol O_2) measured next to an embryo on a brick covered with (●) or without (○) "natural" periphyton (Experiment 5) during a 12 light (L):12 dark (D) light cycle. Time during L (900 lx) and D (0 lx) phases represented by unshaded and shaded backgrounds, respectively. Dashed line indicates 100% saturation, 251 μmol O_2.

Table 1 Mean (± S.E., n = 4) embryonic survival (%) and hatch (%). Mean (± S.E., n = 4) DW, ADW, and AFDW of sediment (Experiment 1) and periphyton (Experiments 3 and 4) treatments, expressed as g m^{-2} and % of DW. Significant differences (ANOVA, Tukey's test: $p < 0.05$) between treatments within an experiment are indicated by different superscript letters within a column and comparisons between periphyton biomass among experiments by different superscript numbers within a column.

Experiment	Treatment	% Survival	% Hatch	DW	ADW (%)	AFDW (%)
1	Control	82.4 ± 5.9^{a}	ND	-	-	-
	0.25 g	$76.2 \pm 4.6^{a,b}$	ND	54.3 ± 0.2	51.9 ± 0.3 (97.5)	2.4 ± 0.0 (4.3)
	1.00 g	$75.5 \pm 5.8^{a,b}$	ND	216.4 ± 0.4	208.1 ± 0.7 (96.2)	8.3 ± 0.3 (3.8)
	6.00 g	53.6 ± 4.1^{b}	ND	1296.5 ± 3.7	1261.2 ± 5.4 (97.3)	35.3 ± 2.7 (2.7)
3	Control	61.5 ± 6.5^{a}	92.4 ± 4.8^{a}			
	Squamscott - Natural	55.6 ± 4.2^{a}	68.3 ± 6.7^{a}	35.3 ± 4.81^{1a}	32.3 ± 5.01^{1a} (91.1)	2.9 ± 0.21^{1a} (8.9)
	Crane - Natural	17.8 ± 2.9^{b}	74.3 ± 8.1^{a}	251.5 ± 22.53^{2b}	$235.8 \pm 1\ 8.23^{2b}$ (94.1)	15.7 ± 5.02^{2b} (5.9)
	Squamscott + 0.25 g	49.5 ± 3.3^{a}	75.3 ± 8.2^{a}			
	Squamscott + 1.00 g	50.4 ± 2.3^{a}	77.4 ± 6.5^{a}			
4	Control	81.1 ± 5.8^{a}	95.5 ± 1.8^{a}			
	Crane - Natural	77.5 ± 5.4^{a}	89.9 ± 2.8^{a}	124.6 ± 17.52^{3}	103.5 ± 17.52^{3} (82.1)	21.0 ± 1.82^{2} (17.9)
	Crane + N	82.1 ± 3.6^{a}	92.5 ± 1.7^{a}			
	Crane + P	80.0 ± 4.6^{a}	93.2 ± 1.9^{a}			
	Crane + N + P	81.4 ± 4.8^{a}	88.6 ± 2.5^{a}			

ND = no data.

Table 2 Mean (± S.E, n = 4). DW, ADW, and AFDW of standing periphyton biomass taken during the 2008 smelt spawning season from Massachusetts (MA), New Hampshire (NH), and Maine (ME) expressed as g m^{-2} and % of DW.

State	River	Date	DW	ADW (%)	AFDW (%)
ME	Tannery Brook	6 May	58.8 ± 14.8	41.0 ± 8.7 (72.5)	17.7 ± 7.2 (27.5)
ME	Mast Landing*	9 April	0.5 ± 0.4	0.4 ± 0.4 (85.7)	0.1 ± 0.1 (14.3)
ME	Deer Meadow Brook*	9 April	0.2 ± 0.0	0.1 ± 0.0 (44.4)	0.1 ± 0.0 (55.6)
NH	Squamscott	24 Mar	15.3 ± 3.4	13.9 ± 3.3 (90.3)	1.4 ± 0.2 (9.7)
NH	Squamscott	5 April	35.3 ± 4.8	32.3 ± 5.0 (91.1)	2.9 ± 0.3 (8.9)
NH	Winnicut*	5 May	7.0 ± 4.8	4.3 ± 2.4 (73.3)	2.7 ± 2.3 (26.7)
NH	Lampery	5 May	1.8 ± 1.2	1.5 ± 1.1 (68.5)	0.3 ± 0.1 (31.5)
NH	Bellamy	6 May	8.2 ± 4.0	7.1 ± 3.8 (86.3)	1.1 ± 0.6 (13.7)
NH	Oyster	6 May	72.0 ± 14.8	64.3 ± 13.9 (89.2)	7.6 ± 1.6 (10.8)
NH	Squamscott	7 May	75.7 ± 21.9	69.4 ± 22.5 (88.4)	6.3 ± 0.7 (11.6)
NH	Salmon Falls	7 May	179.5 ± 111.2	114.2 ± 50.9 (83.3)	65.2 ± 61.3 (16.7)
MA	Crane	5 April	251.5 ± 22.5	235.8 ± 18.2 (94.1)	15.8 ± 5.0 (5.9)
MA	Crane	18 April	124.6 ± 17.5	103.5 ± 17.5 (82.1)	21.1 ± 1.8 (17.9)
MA	Saugus	11 May	169.7 ± 34.3	163.2 ± 33.8 (96.5)	6.5 ± 0.7 (3.5)
MA	Crane	11 May	120.2 ± 60.1	107.3 ± 25.7 (89.1)	12.9 ± 3.0 (10.9)
MA	Mill	11 May	101.5 ± 40.2	86.5 ± 37.0 (79.8)	15.0 ± 3.9 (20.2)
MA	Parker	11 May	27.1 ± 9.8	24.5 ± 9.1 (89.1)	2.6 ± 0.7 (10.9)
MA	Little	11 May	165.4 ± 57.5	158.9 ± 56.1 (95.2)	6.5 ± 1.4 (4.8)

Asterisk (*) indicates n = 3.

Figure 1.

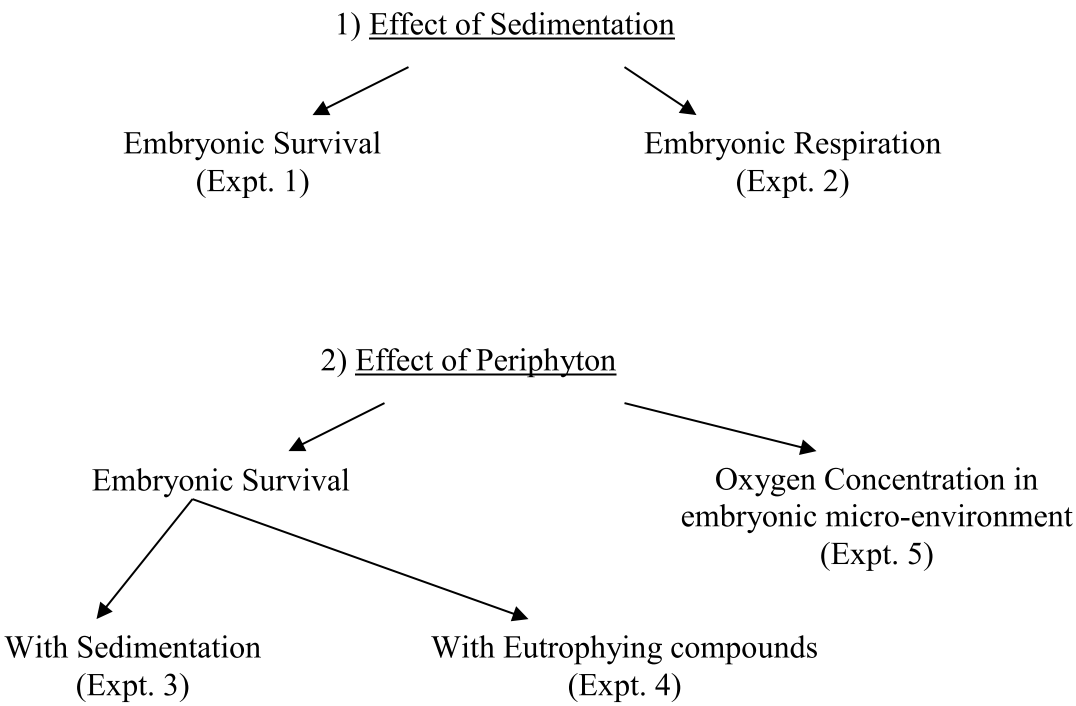

1) Effect of Sedimentation
Embryonic Survival
(Expt. 1)
Embryonic Respiration
(Expt. 2)
2) Effect of Periphyton
Embryonic Survival
Oxygen Concentration in
embryonic micro-environment
(Expt. 5)
With Sedimentation
(Expt. 3)
With Eutrophying compounds
(Expt. 4)

Figure 2.

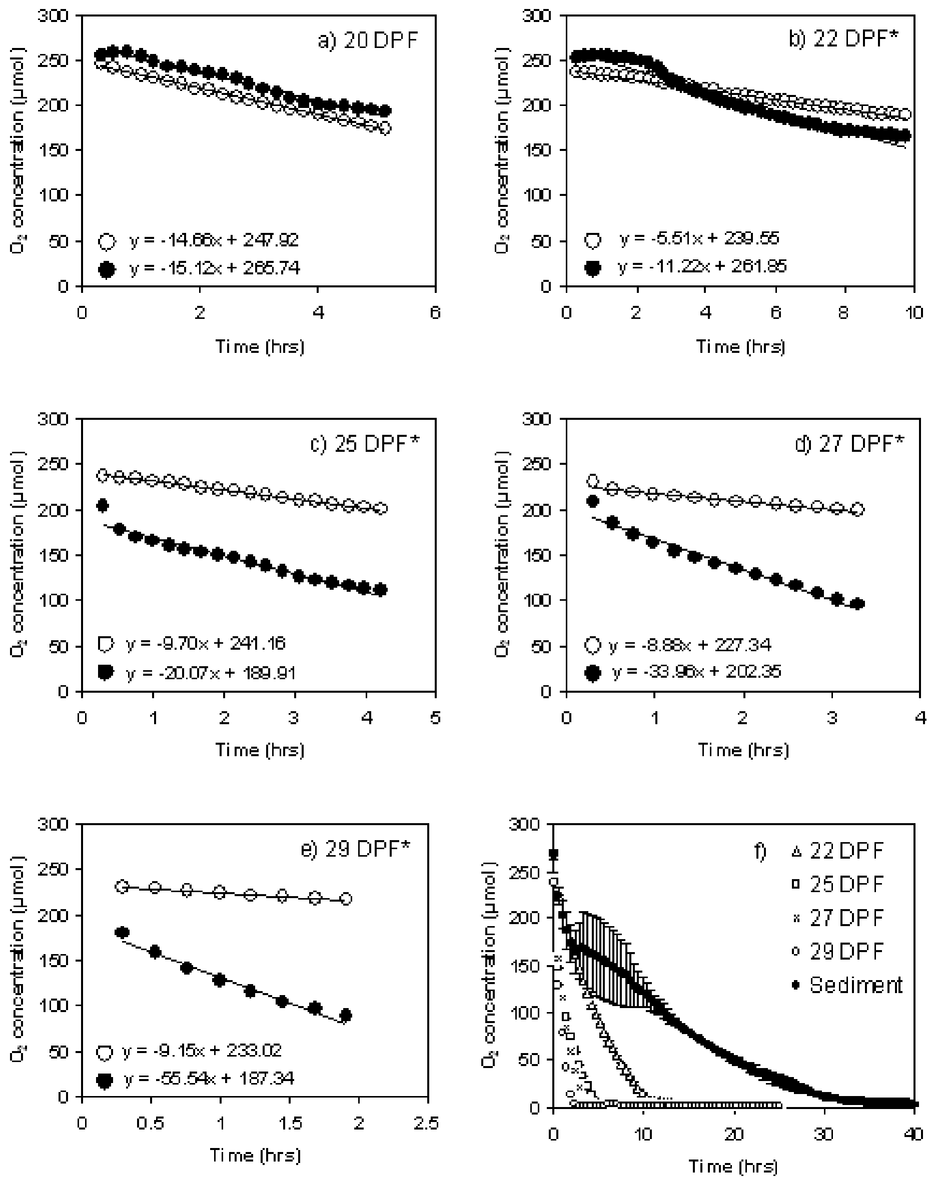

a) 20 DPF
y = -14.66x + 247.92
y = -15.12x + 265.74
b) 22 DPF*
y = -5.51x + 239.55
y = -11.22x + 261.85
c) 25 DPF*
y = -9.70x + 241.16
y = -20.07x + 189.91
d) 27 DPF*
y = -8.88x + 227.34
y = -33.96x + 202.35
e) 29 DPF*
y = -9.15x + 233.02
y = -55.54x + 187.34
f)
22 DPF
25 DPF
27 DPF
29 DPF
Sediment
O_2 concentration (µmol)
Time (hrs)

Figure 3.

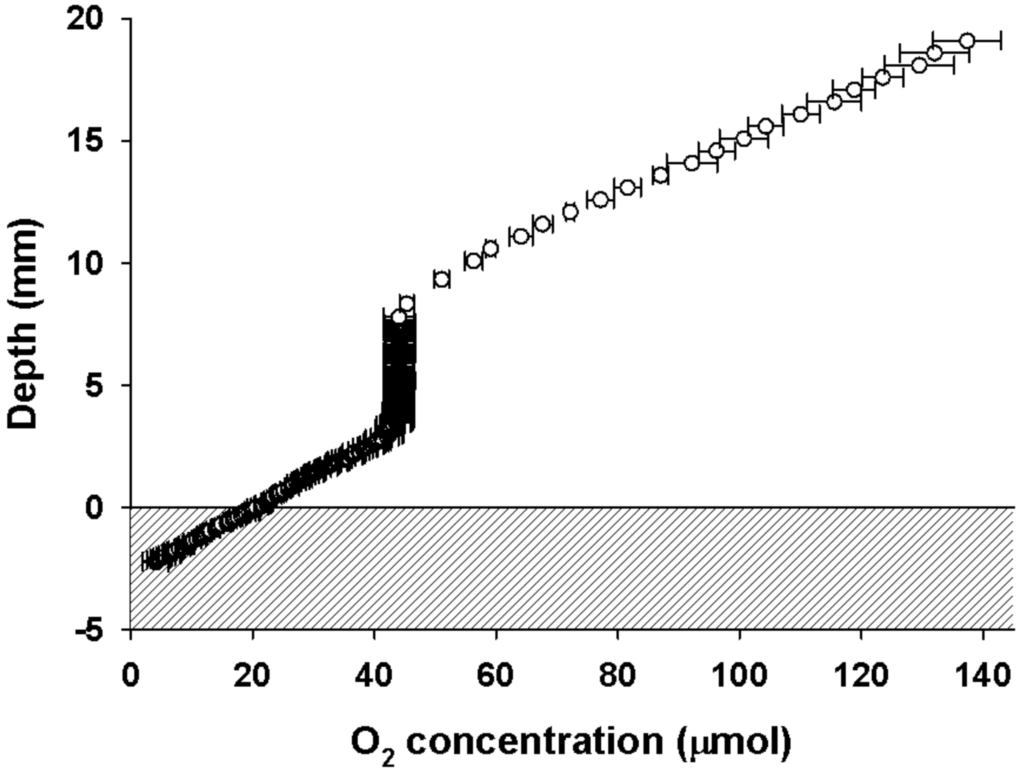
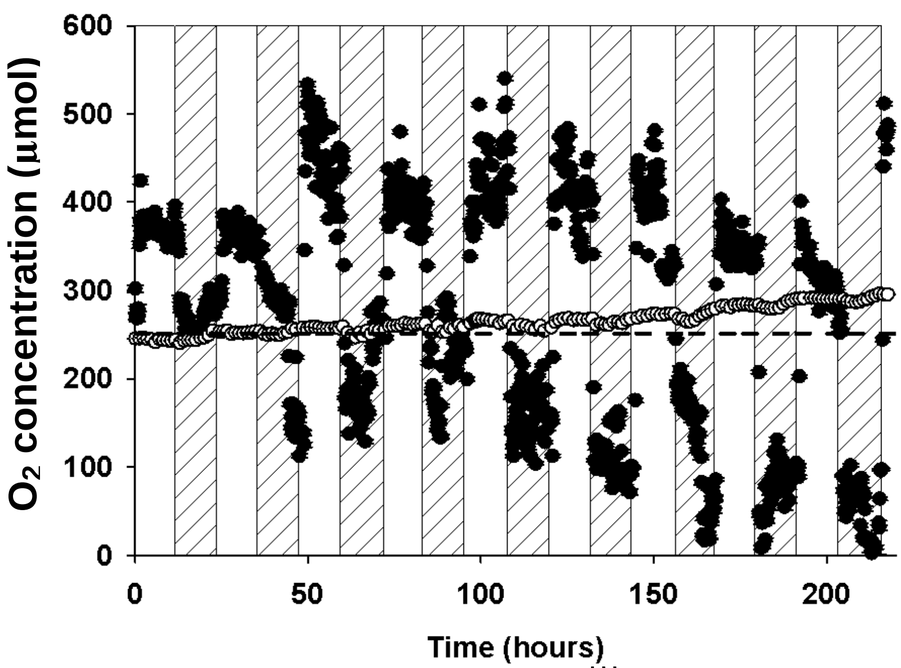


Figure 4.



Objective 4: To determine the genetic variation among rainbow smelt (*Osmerus mordax*) from multiple river systems in New England.

Purpose

In response to the Species of Concern status of rainbow smelt in the Northeast, a collaborative Proactive Species Conservation Program was launched with grant funding by NMFS. Program goals included increasing our understanding of the population status, ecology and structure of smelt in river systems in the Northeast. Prior to this effort, no studies had been conducted on the population genetic structure of rainbow smelt in this region. Knowledge of population genetic structure is critical for informing conservation management.

The objective of this project was to determine the genetic variation among rainbow smelt (Osmerus mordax) from multiple river systems in New England.

Methods

Fin clip samples of adult smelt were obtained from New Hampshire Fish & Game, Maine Division of Inland Fisheries & Wildlife, and Massachusetts Division of Marine Fisheries, collected during spawning runs. A total of 2748 samples were collected from 18 rivers during 2006-2010 (Table 1). Four additional small collections from the Winnicut River and Cascade Brook were not used in analyses due to insufficient sample size (<30 individuals).

DNA was extracted from fin clips using the Qiagen DNeasy Blood and Tissue kit (Qiagen, Valencia, California). Genotyping was performed using a suite of 11 microsatellite loci (Coulson et al. 2006), following published protocols optimized for 3 sets of multiplex PCR amplifications. PCR products were electrophoresed using an automated DNA sequencer (ABI 3130; Applied Biosystems) and alleles were scored manually using PEAKSCANNER software (ABI). Two loci, *Omo3* and *Omo16* were found to be linked in all populations and were dropped from further analyses. Multilocus genotypes for the remaining 10 loci were compiled for individuals and population genetic analyses were performed using multiple individual and population-level analyses.

Descriptive statistics, including observed and expected heterozygosties, allelic richness (a measure of within population genetic diversity), and tests of Hardy Weinberg equilibrium and linkage disequilibrium were conducted in GENEPOP (Raymond and Rousset 1995) and FSTAT (Goudet 1995). Population differentiation was evaluated by analysis of pair-wise population FST, calculated in FSTAT, and chord distances (Cavalli-Sforza and Edwards 1967). We tested for temporal stability in the population genetic structure by AMOVA, using the program ARLEQUIN (Schneider et al. 2000). We further evaluated the level of population structuring and connectivity among rivers using individual-based Bayesian clustering methods of STRUCTURE (Pritchard et al. 2000) and BAPS (Corander et al. 2008). We ran STRUCTURE using the LOCPRIOR model (Hubisz et al. 2009), which is suited to perform for systems with weak genetic structure. We ran BAPS using the group clustering algorithm. We also used the predefined clustering algorithm in BAPS to evaluate evidence of structuring at the river level. This analysis was followed by an assignment test approach, in which we used the genotype data to assign individuals back to their most likely population of origin. We report the percentage of correct self-assignments (percent of individuals correctly assigned to the river in which they were sampled), as a measure of river-level structuring (following Waples and Gaggiotti 2006). Lastly, we evaluated the spatial extent of the observed genetic structure using spatial autocorrelation analysis in GENALEX (Peakall and Smouse 2006).

Table 1. Rainbow smelt fin clip samples collected from 18 rivers in Maine, New Hampshire and Massachusetts 2006-2010.

Collection by river and year	Sample size
Cobscook Bay 2008	91
Cobscook Bay 2009	95
Chandler River 2009	36
Chandler River 2010	96
Pleasant River 2010	96
Penobscot River 2008	95
Penobscot River 2009	95
Marsh River 2008	79
Marsh River 2009	96
Kennebec River 2009	82
Harraseeket River 2008	90
Harraseeket River 2009	96
Long Creek 2009	96
Salmon Falls 2008	51
Oyster River 2007	95
Bellamy River 2007	67
Bellamy River 2008	76
Lamprey River 2008	95
Squamscott River 2007	48
Squamscott River 2008	94
Squamscott River 2009	96
Parker River 2008	99
Parker River 2009	96
Saugus River 2006	37
Saugus River 2007	81
Saugus River 2008	82
Fore River 2006	94
Fore River 2008	100
Jones River 2008	108
Jones River 2009	96
Weweantic River 2008	95
Total:	2748

Results & Interpretation

Multilocus genotypes with no more than 4 missing loci were obtained for 2572 samples. Observed heterozygosities were similarly high for all rivers (mean $H_o = 0.859$), except the Weweantic, in which they were slightly reduced ($H_o = 0.765$). Observed and expected heterozygosities did not deviate from Hardy-Weinberg expectations. Allelic richness (the sample-sized adjusted number of alleles per locus) was significantly reduced in the Weweantic samples relative to all other rivers, except the Cobscook, which was only significantly reduced relative to the Squamscott River collection (ANOVA blocked by locus; Figure 1). These findings suggest that smelt populations in the Weweantic have slightly lower genetic diversity relative to smelt in the other rivers, which may be consistent with the status of these populations at the most southern extent of the current range of the species. Populations at the edges of species' ranges often have reductions in population size or diversity.

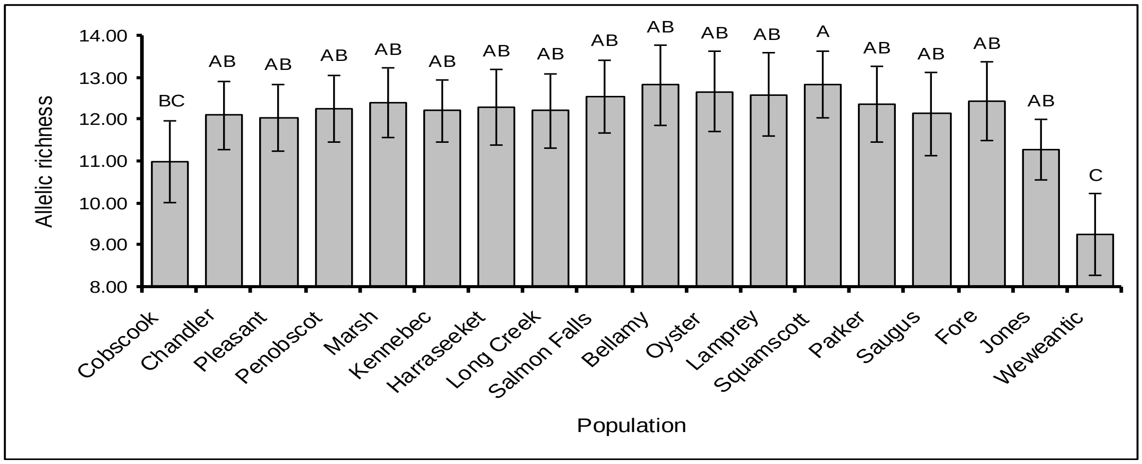


Figure 1. Mean allelic richness across 10 loci for rainbow smelt from 18 rivers in Maine, New Hampshire and Massachusetts. Bars with different letters are significantly different (P<0.05, ANOVA).

To follow up on our findings of reduced genetic diversity in Weweantic, we tested for signatures of population bottlenecks (severe reductions in population size in the recent past) using two complementary approaches, BOTTLENECK (Piry et al. 1999) and M-RATIO (Williamson-Nateson 2005). We found no evidence, with either approach, that any of the smelt populations had experienced a genetic bottleneck, suggesting that either the observed reductions in genetic diversity were not associated with a severe population decline, or that a population reduction was very recent or potentially ongoing (these 2 approaches are not designed to detect slow or currently ongoing population reductions).

For population genetic structure to be meaningful, it must be demonstrated that the differences among rivers/sites are significantly greater than the differences between years within the same rivers/sites (Waples 1998). To evaluate the annual variability in population genetic structure, we conducted an AMOVA (molecular analysis of variance, which partitions genetic variation hierarchically, similar to an ANOVA) using 10 rivers that were sampled in >1 year. We found no significant variation among annual samples from individual rivers, but highly significant differences among different rivers (P<0.001), suggesting that the genetic variation we observed among rivers was very stable over time. Therefore, yearly samples from the same rivers were pooled for further analyses.

We found highly significant differentiation among the 18 rivers overall, with a global F_{ST} of 0.015. This level of differentiation is very similar to that found for other anadromous fish in the region, including salmon in Maine (King et al. 2001, Spidle et al. 2003) and smelt in New Brunswick, Nova Scotia and Prince Edward Island (Bradbury et al. 2006). Interestingly, Bradbury et al. (2006), found an order of magnitude higher differentiation (F_{ST} = 0.11) for smelt in Newfoundland, with structuring on the scale of estuaries and bays. The higher divergence in this system is likely a function of the topography of the Newfoundland coastline, which is much more structured with geographically distinct bays, relative to the more uniform coastline of the Northeast US.

Many pairs of individual rivers were also differentiated, with pair-wise F_{ST} s ranging from 0 (for geographically proximate rivers that shared the same estuary in Great Bay, NH) to 0.08 (for the most geographically separated rivers of Cobscook Bay in ME and Weweantic in MA. The Weweantic River, followed by the Cobscook Bay collection, showed the strongest divergence and both were significantly differentiated from all other rivers. Overall, genetic variation followed an isolation by distance pattern, such that were was a significant correlation between genetic and geographic distance (Mantel test, $r^2 = 0.467$, $p<0.0001$). Spatial autocorrelation analyses indicated significant fine-scale spatial genetic structure extended to approximately 180 km. Similarly, Bradbury et al. (2006) found the spatial extent of genetic structure in Newfoundland was approximately 150 km, although an order of magnitude greater.

Despite these trends for isolation by distance and large and fine-scales, genetic differentiation was not consistent across geographic distances for the whole study area, and several rivers from northern Massachusetts to coastal Maine were genetically quite similar. To evaluate the genetic similarities among rivers, we used the results of Bayesian clustering analyses from STRUCTURE and BAPS. These analyses use the genetic data to cluster the populations (rivers) together into genetically similar groupings. Results of STRUCTURE suggested strongest support for the presence of 5 genetically distinct groups (top bar graph in Figure 2), consisting of 1) Cobscook, 2) Penobscot, 3) Chandler, Pleasant, Marsh, Kennebec, Harraseeket, Long Creek, the NH rivers of the Great Bay estuary, and Parker River, 4) Saugus Fore and Jones, and 5) Weweantic (top bar graph in Figure 2). Within these groupings, Parker River is a mixture of the NH-ME grouping and the Saugus-Fore grouping, and Jones is a mixture of the Saugus-Fore and Weweantic groupings. There was also some evidence to support 6 groups, similar to the 5 above, but with some differentiation of Harraseeket and Long Creek (bottom bar graph in Figure 2). The 6 groupings showed higher admixture than the 5 groupings, especially within the ME and NH rivers. Analyses with BAPS yielded similar results, but did not suggest as fine-scale structuring, with only 4 genetically similar groups detected: 1) Cobscook, 2) Chandler River south to Parker River, 3) Saugus, Fore and Jones Rivers, and 4) Weweantic River (Figure 3). A synthesis of these results is presented in Figure 4, which depicts on a map the geographic composition of each of the genetically distinct groupings. Assignment test results supported the 5 STRUCTURE groupings with 60-85% correct self-assignments (highest for Cobscook and Weweantic and lowest for Penobscot).

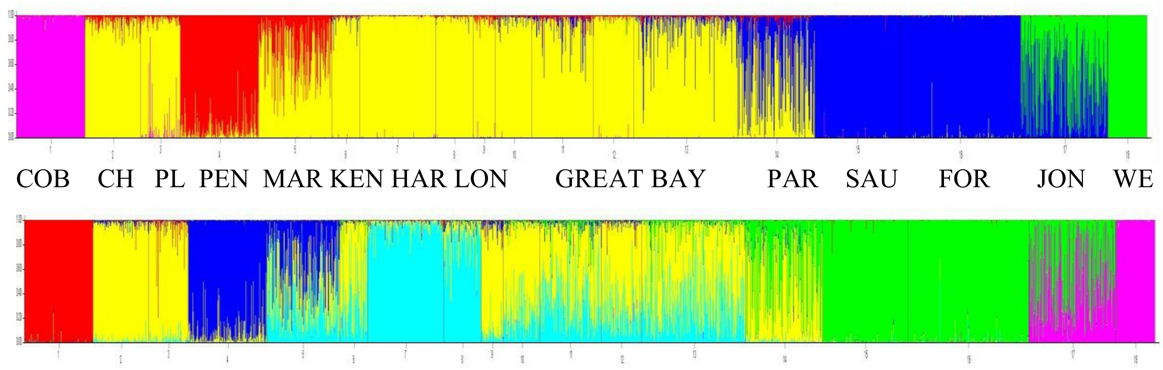


Figure 2. Results of genetic clustering analysis with STRUCTURE for smelt from 18 rivers, with k=5 genetically similar groupings in the top panel and k = 6 in the bottom panel. Colors depict the genetic cluster membership; rivers that are comprised of >1 color are admixed between groups.

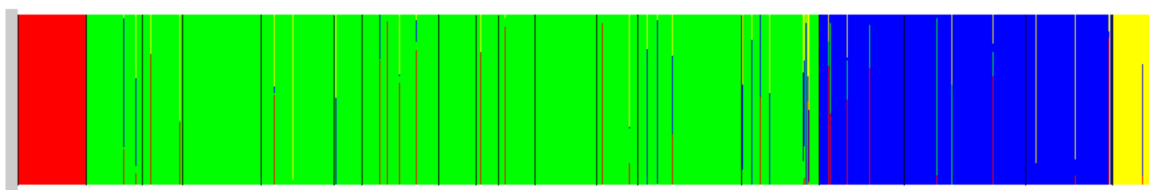


Figure 3. Results of genetic clustering analysis by group (river) with BAPS for smelt from 18 rivers. K= 4 (shown) was the most likely number of genetic groupings.

We also found evidence of finer-scale genetic structure at the scale of individual rivers, although much weaker than at the level of the groups described above. The predefined clustering method of BAPS partitioned the samples by river or estuary (in the case of the Great Bay, NH samples), although admixture among rivers was evident (Figure 4). Results of the assignment tests supported the river level structuring, but indicated it was highly variable among rivers, with 10% - 84% of individuals per river assigned correctly to the river in which they were sampled (Table 2). With 16 rivers, only 6% of individuals would be expected to be correctly assigned by random chance alone. Nonetheless, self-assignments in the 10-20% range suggest only a weak river-specific genetic signal.

Table 2. Percentage correct self-assignments for smelt from 18 rivers (the 5 NH rivers from the Great Bay estuary were combined for this analysis).

River	% correctly assigned
Cobscook	73
Chandler	22
Pleasant	10
Penobscot	41
Marsh	20
Kennebec	16
Harraseeket	27
Long Creek	18
Great Bay, NH	15
Parker	23
Saugus	31
Fore	36
Jones	57
Weweantic	82

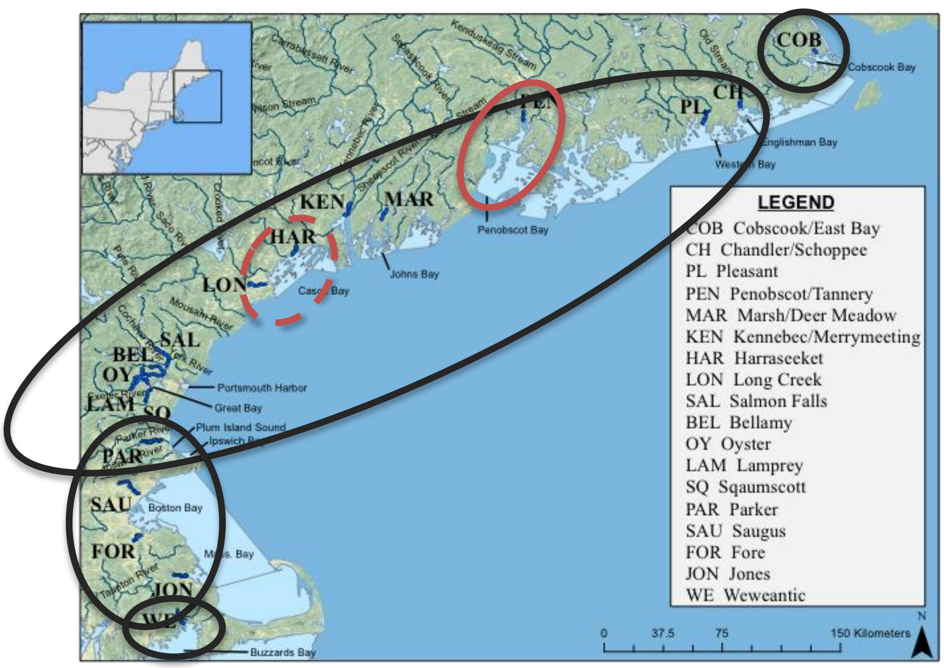


Figure 4. Map depicting the genetic groupings of smelt from 16 rivers, based on a synthesis of genetic clustering analyses from STRUCTURE and BAPS. Black circles indicate the 4 most genetically distinct groupings, with red circles indicating two additional weakly differentiated groups. Overlapping circles (around Parker River and Jones) indicate admixture between 2 groups.

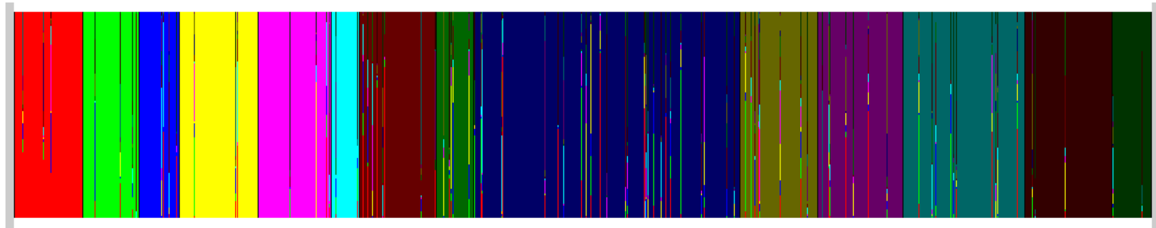


Figure 5. Results of predefined clustering analyses in BAPS, indicating fine-scale structure at the level of individual rivers or estuary (in the case of Great Bay, NH rivers).

Summary and Conclusion

Genetic diversity was high for smelt from the 18 rivers overall, with a reduction in Weweantic and a slight reduction in Cobscook. There was no evidence that the populations had undergone a recent population bottleneck, although an ongoing bottleneck could not be ruled out. Smelt from most rivers were significantly differentiated from each other, with the exception of the most geographically proximate ones. Smelt from the 5 rivers in the Great Bay estuary were genetically homogenous, suggesting smelt did not home strongly to individual rivers. Straying among rivers beyond the level of the estuary was also evident, as gene flow was relatively high among many rivers in the NH- coastal ME region. Overall, genetic differentiation was highly correlated with geographic differentiation, supporting an isolation by distance model. The level of differentiation in the system (global $F_{ST} = 0.015$) was similar to that of other anadromous fish in the region. Genetic structuring was not apparent on an estuarine or bay-scale level, but rather was explained by 4-6 genetic groupings, which differentiated the Weweantic and Cobscook rivers most strongly, and combined the Saugus, Fore and Jones rivers into one grouping, and the remaining rivers from Parker River, MA to Chandler River, ME into another grouping. Weaker divergence was evident in the Penobscot River and a grouping of the Harraseeket and Long Creek samples. On a finer-scale, we found evidence for weak river-level structuring, suggesting widespread straying among most adjacent rivers. We attribute the observed patterns of genetic structuring to the topographic features of the coastline. The most differentiated rivers were located near topographically distinct features, such as capes (Cape Cod, Cape Ann) or enclosed bays, (Cobscook and Penobscot), which may serve as barriers to dispersal or function in larval retention. Areas of highest gene flow corresponded to a stretch of the NH-ME coastline that is topographically unstructured. Our findings give important new insight into the population structure of smelt in US waters.

Recommendations for Future Study

Based on the findings of this study, we recommend additional sampling be conducted in rivers located in and near the enclosed bays (Penobscot, Cobscook) and surrounding the Harraseeket and Long Creek sampling areas. A finer-scale sampling effort focused around the topographically structured areas will increase our understanding of the scale of larval retention and the influence of topography on gene flow and straying among rivers.

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