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Effects of sedimentation and periphyton communities on embryonic rainbow smelt, Osmerus mordax

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EFFECTS OF SEDIMENTATION AND PERIPHYTON COMMUNITIES ON EMBRYONIC RAINBOW SMELT, *OSMERUS MORDAX*

BY

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EFFECTS OF SEDIMENTATION AND PERIPHYTON COMMUNITIES ON EMBRYONIC RAINBOW SMELT, *OSMERUS MORDAX*

by

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University of New Hampshire, May, 2009

The decline of anadromous rainbow smelt (*Osmerus mordax*) populations has been suspected to be linked to anthropogenic causes. Increased runoff from agriculture and urbanization has led to additional sediment inputs and eutrophying compounds in rivers. The aim of this study was to assess the survival of embryonic rainbow smelt from fertilization through hatching under varying levels of sedimentation (0.00, 0.25, 1.00, and 6.00 g per 45.6 cm^2) and with periphyton communities of different biomass and algal composition. Additionally, embryo survival was 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 sediment. Survival was significantly reduced under the highest sediment treatment and attributed to low oxygen availability to the embryos. Embryonic survival was also significantly reduced on the highest periphyton biomass (251.5 g/m^2 dry weight, 15.7 g/m^2 ash free dry weight), and periphyton containing a high cyanobacteria content (50%). These results suggest that embryonic survival could be reduced in rivers with heavy sedimentation or a high standing biomass of periphyton.

CHAPTER I

INTRODUCTION

Rainbow Smelt

Life History

Rainbow smelt, *Osmerus mordax* (Mitchell), is a small teleost fish (18-23 cm) found in freshwater lakes of northeastern and central United States (Buckley 1989) and in coastal waters of the northwest Atlantic Ocean and northeast Pacific Ocean (Klein-MacPhee 2002). Anadromous smelt populations residing in estuaries and coastal waters (Klein-MacPhee 2002) annually migrate up rivers to spawn when water temperatures reach 4-7°C or higher (McKenzie 1964). Spawning typically occurs in late-February in Massachusetts' rivers (McKenzie 1964) and in mid-March farther north (Lawton et al. 1990).

Smelt become reproductively mature after two years, and males and females broadcast spawn gametes into the water column (McKenzie 1964). Some females can produce up to 75,000 eggs in a single spawning event (McKenzie 1964). Smelt eggs are approximately 1 mm in diameter and are demersal, adhering to the bottom substrate (Klein-MacPhee 2002). Their benthic life stage during incubation leaves them susceptible to changes occurring in the river. Water temperature, velocity, substrate type, and egg density are important factors for embryonic survival (Sutter 1980). Incubation lasts 11-29 days, depending on water temperature (McKenzie 1964). When eggs are deposited on aquatic vegetation rather than gravel, embryonic survival increased from 1.8

to 10% (Rothschild 1961, McKenzie 1964, Sutter 1980). Density is also important, as high mortality, associated with fungal growth, is known to occur in overcrowded egg masses (Rothschild 1961, McKenzie 1964).

Following hatch, smelt larvae utilize the yolk-sac for endogenous feeding. Once the yolk-sac is absorbed smelt begin exogenous feeding on rotifers and other zooplankton in the water column (Gordon 1961, Burbidge 1969, McCullough and Stanley 1981). Juvenile and adult smelt consume amphiphods, mysids, shrimps, marine worms, and small fish (Klein-MacPhee 2002). As adults, smelt are an important forage species for Atlantic salmon (Sayers et al. 1989) and lake trout (Kirn and LaBar 1996) in freshwater lakes, and striped bass and bluefish (Buckley 1989) in marine waters. Smelt have supported both recreational and commercial fisheries on the east coast of the Atlantic Ocean for centuries (Kendall 1927).

Smelt fishery and decline

Historical smelt populations were once abundant and supported large winter fisheries. In addition to food, smelt were also processed for animal feed and used as fertilizer (Fried and Schultz 2006). In recent decades there has been a noticeable decline in the smelt population such that they were listed as a species of concern by the National Marine Fisheries Service (NOAA 2004). Decline is evident by the reduction in their geographical range and landings within this reduced range. On the east coast of North America, rainbow smelt historically inhabited a geographic range extending from New Jersey, USA to Labrador, Canada (Klein-MacPhee 2002), but current populations are estimated to range from Massachusetts to southern Canada (Ross 1991). Population

decline has also been noted within this compressed range from visual estimates and recreational landings (Chase and Childs 2001). In New England landings peaked at 162 million tons in 1966 and have averaged 0.14 mt/year since 1998 (NOAA 2004).

Smelt population decline is likely the result of many factors in freshwater and marine environments. Population decline in freshwater lakes is attributed to ecological causes such as stock size, predation, competition, and disease (Schaefer et al. 1981, Kirn and LaBar 1996), while declines in fully anadromous populations may be more associated with anthropogenic causes. Human influences including eutrophication from urbanization, obstructions to favorable spawning grounds by dams, and degraded water quality have been implicated as causes for the declining stocks (Crestin 1973, Murawski and Cole 1978, Klein-MacPhee 2002). The decline of anadromous smelt may also be linked to spawning habitat degradation from increases in sedimentation and nutrients from runoff.

Environmental influences

Sediment

Sediment deposition may have a negative effect on teleost embryonic survival (Shelton and Pollock 1966, Messieh et al. 1981). For example, sediment infiltration into the gravel bed of salmon eggs may reduce survival by limiting or preventing respiration (Shelton and Pollock 1966). Egg and fry survival increased in cases where there was less siltation or when sediment was manually removed (Shelton and Pollock 1966). Oxygen concentrations also increased when sediment was removed from gravel beds (Wickett

1954). Sedimentation in rivers may act to smother rainbow smelt eggs by reducing oxygen levels and limiting respiration.

Fine sediment (<500 μm) in rivers is likely increased by runoff from agriculture and urbanization (Mayo 1975, Muncy et al. 1979). Every year, 1.9 billion tons of sediment is discharged into lakes and streams in North America (Syvitski et al. 2005). Sediment deposition has been documented in an active smelt spawning tributary of Great Bay, NH (Fuda et al. 2007) and in some Massachusetts rivers (Chase 2006). A preliminary study by Fuda et al. (2007) found high levels of silt deposition and some fungal growth on the rainbow smelt eggs that prevented an assessment of embryonic viability. Chase (2006) examined smelt spawning habitats, and found that some level of sedimentation was present in all rivers studied. Many of the rivers were characterized by moderate or substantial substrate degradation (Chase 2006). A sediment layer may impact embryos by forming a physical barrier that blocks oxygen transfer (Greig et al. 2005). The lowered permeability of oxygen in sediment reduces the diffusion of oxygen and limits its availability to developing embryos (Greig et al. 2005). A thin sediment layer (1 mm) decreased the oxygen consumption in salmon eggs by 41-98% (Greig et al. 2005).

Sediment can also adhere to the chorion of aquatic eggs (Iwamoto 1978), and fine sediment (Bell et al. 1969) can physically obstruct embryonic pores and lead to limited oxygen transport across the egg membrane. Sediment and other organic matter may also consume oxygen and further depress oxygen availability to the egg. Ventlingschwank and Livingstone (1994) recorded a substantial decrease of oxygen from

>6 to 4 mg/L 1.5-2.5 mm above the sediment-water interface; a zone large enough to encompass smelt eggs.

Anthropogenic nitrate and phosphate

In addition to reduced oxygen from sediment, nutrients such as phosphate and nitrate can also be transferred in runoff water and may impact smelt survival. Runoff from fertilizers and agricultural crops has increased the transfer of phosphate and nitrate into watersheds (Schindler 1977, Vitousek et al.1997, Manahan 2000). Nitrate is generally regarded as nontoxic to aquatic organisms (Tomasso 1994) and does not directly influence smelt survival. High embryonic survival (over 95%) has been observed in rainbow smelt exposed to high concentrations of both nitrate and phosphate until hatch (Fuda et al. 2007). While nitrate and phosphate do not directly influence smelt embryos, the increased nutrient concentrations likely promote algal and periphyton growth which may negatively impact smelt egg survival.

Periphyton

Periphyton is loosely defined as the assemblage of organisms which colonize and grow on submersed objects, including algal and bacteria components (Young 1945, Karlström 1978). Periphyton and some algal communities may constitute a substrate that is unsuitable for smelt eggs (Lapierre et al. 1999). Various algal species may secrete mucilage or toxic chemicals that may have a negative impact on embryonic development. The mucilage secreted by some diatom species can trap detritus and sediment particles (Karlström 1978, Hoagland et al. 1982, Roemer et al. 1984), which leads to egg

smothering. The smothering event would most likely be similar to the negative impacts of sediment deposition. Toxins produced by particular genera may also negatively impact egg incubation. In some freshwater lakes, eutrophic conditions favored growth of cyanobacteria that can produce hepatotoxins that may be lethal to adult fish (Chellappa et al. 2008, Wu et al. 2008). Embryonic smelt may also be susceptible to these toxins as concentrated extracts of these toxins were shown to affect embryonic development and hatch in trout (Oberemm et al. 1999) and zebrafish (Keil et al. 2002, Berry et al. 2007). Conversely, toxins had no effect on the embryonic development of other teleost species, such as roach, bream, chub, and stone loach (Oberemm et al. 1999). The chorion may serve as a barrier to these toxins, and the protection the chorion provides may differ among species (Cazenave et al. 2006). Factors that control the toxin content of cyanobacterial strains are still unknown and the effect cyanobacteria could have on smelt egg survival needs to be further investigated.

The combined influence of photosynthesis and respiration can make the micro-environment in periphyton different compared to the surrounding benthic community. Algal cells can alter the oxygen environment surrounding the egg by acting as a source and a sink for oxygen. During daylight hours, photosynthesis increases oxygen concentrations to hyperoxic levels, while in the dark, net respiration can reduce oxygen concentrations to near anoxia (Carlton and Wetzel 1987). The prolonged low oxygen environment during night respiration may negatively impact embryonic survival by limiting the oxygen necessary for metabolic processes.

Periphyton influences on smelt eggs may differ among rivers, and likely depends on geographical variation in light, substrate type, nutrients, and invertebrate grazers all of

which can affect the distribution and biomass of periphyton (Trainor 1978). High periphyton growth during the smelt spawning season has been noted in many Massachusetts rivers (Chase 2006). Several of the rivers studied by Chase (2006) were characterized by substantial substrate degradation, from excessive periphyton growth, with increases in periphyton biomass in April and May. When high periphyton growth occurred simultaneously with smelt egg deposition, increased egg mortality resulted (Chase and Childs 2001, Chase 2006). Reduced survival may be related to substances secreted by certain periphyton genera or from an altered oxygen micro-environment.

Dissolved oxygen

Oxygen is critical for embryonic survival and hatch, and oxygen deficiency during the embryonic period may impact later life stages (Rombough 1988). Reduced survival in salmon embryos has been documented at low oxygen concentrations (Oseid and Smith 1971*a*, *b*, Louhi et al. 2008), and larvae hatched from low oxygen environments are frequently smaller and have more defects than larvae hatched from more oxygen-rich water (Oseid and Smith 1971*a*, *b*, Kaur and Toor 1978, Brooke and Colby 1980, Sawada et al. 2006). The oxygen concentration gradient between the egg membrane and the surrounding boundary layer facilitates oxygen diffusion across the egg membrane, and the amount of oxygen an egg receives depends on this oxygen gradient. High concentrations of oxygen are critical to creating a gradient that moves available oxygen inside an embryo (Daykin 1965, O'Brien et al. 1978, Rombough 1988). When oxygen concentrations are low in the surrounding environment, the reduced availability impacts respiration and metabolism (Hamor and Garside 1979, Rombough 1988).

In addition to sediment deposition, low oxygen conditions may also result from accumulating detritus. Detritus and particulate organic carbon consume oxygen and can place benthic organisms in a lowered oxygen environment (Ventling-Schwank and Livingstone 1994). Unlike some other coldwater species (Araújo et al. 2000, Pientka and Parrish 2002), rainbow smelt are not as sensitive to changes in the concentration of dissolved oxygen. In the laboratory, there was no reduction in embryonic smelt survival from exposure to low oxygen conditions (20% saturation) from 8 days post-fertilization to hatch (Fuda et al. 2007). Complete mortality resulted from lower oxygen conditions (10% saturation) for the same time span, however (Fuda et al. 2007). These results suggest that smelt survival could be lower in rivers where precipitating sediment or detritus creates extremely low oxygen concentrations in the environment.

CHAPTER II

EFFECTS OF SEDIMENTATION AND PERIPHYTON COMMUNITIES ON EMBRYONIC RAINBOW SMELT, *OSMERUS MORDAX*

Introduction

The rainbow smelt, *Osmerus mordax* (Mitchill), is a small anadromous fish found along the Northwest Atlantic and Northeast Pacific coasts that is enjoyed as a food fish, and has supported important commercial and recreational fisheries (Klein-MacPhee 2002). Smelt also serve as an important prey item for many important carnivorous 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 (Klein-MacPhee 2002). As smelt 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, Chase and Childs 2001). Additionally, as smelt spawn in the spring months, the demersal eggs are exposed to runoff from snow melt and spring storms, that 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 of 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 and periphyton communities on oxygen availability and embryonic smelt survival in controlled laboratory conditions.

Materials and methods

Egg collection

During the 2007 and 2008 annual spawning migrations (March – May) adult rainbow smelt were captured by fyke nets in rivers in Massachusetts (MA), New Hampshire (NH), and Maine (ME; Table 1). The smelt were transported to the University of New Hampshire (UNH), Durham, NH, anesthetized with tricaine

methanesulfonate (100 mg/L Tricaine-S; Western Chemicals, Ferndale, WA) and manually spawned (Ayer et al. 2005) using multiple males and females (>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 4, the eggs were less adhesive and were incubated in 3 L polyethylene hatching jars, with vigorous aeration at temperature of 5 or 10°C (± 1°C) and 0 ppt salinity for 2-4 d, prior to assessing fertilization success. Only viable embryos were used in these studies. Embryonic development can be observed using a dissecting microscope, as 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 4, the adhesive eggs were directly transferred to clay bricks and fertilization was assessed 2 DPF.

Sediment collection

Sediment was collected from the intertidal zone of the Oyster River, Durham, NH, at low tide, and sieved though a 300 μm nylon mesh. Sediment was dried at 70°C overnight, sieved again, and sterilized by autoclaving at 123°C for 15 min. Sediment was then sieved though additional sieves (211 and 110 μm) to determine the relative proportions of sediment at a corresponding grain size ($211<X\leq300$, $110<X\leq211$, and $X<100$ μm).

Experiment 1. The effect of sedimentation on embryo survival

Following fertilization, 129-640 embryos per tile were transferred to 16 slate tiles (~104 cm^2) with polyethylene transfer pipettes, and held in 40 L aquaria (10°C ± 1°C, 0 ppt salinity), 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 replicates/treatment). A piece of polyvinyl chloride (PVC) pipe (diameter = 7.6 cm) was used to direct a slurry of sediment over the eggs (area = 45.6 cm^2). Water alone was added to the control treatment (n=4). Sediment was allowed to settle for one hour before the pipe was removed. Embryos were distinguishable in the low and medium treatments (<1 mm cover) but not in the high (~1 mm cover). Following sediment settlement, water was circulated over the covered embryos with small aquarium pumps (~250 L/hr), 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 using a Unisense Clark-type OX50 glass micro-oxygen 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 (created using 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°C ± 1°C. 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 to the 6.00 g treatment described above for Exp. 1) that was aerated for 24 hrs 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 hrs) the embryos were rinsed and examined to confirm viability. Electrodes were re-calibrated prior to each profile. Concentrations were measured every 8.31 sec, and oxygen measurements were averages of 100 consecutive readings. Over a range of high dissolved oxygen concentrations, the oxygen consumption rate was determined to be linear (Torrans 2007). The linear portion of the regression was estimated visually from each profile and the slope of this line was used to calculate the routine metabolic rate (Cech 1990; Fig. 1, Appendix B). The linear portion of the control and sediment treatment regressions were compared using an analysis of co-variance for each day tested (Fig. 1, Appendix C).

To determine the oxygen demand of the sterilized sediment alone, oxygen profiles were recorded in beakers containing sediment but no embryos (n=2). Oxygen measurements were taken 72 hrs after the addition of the sediment (n=2) at various depths above and below the sediment to generate vertical oxygen profiles. Measurements

in increments of 0.05 mm were used from ~2 mm below the sediment to 5.50 mm above the sediment, and increments of 1.00 mm were made from 5.50-19.07 mm above the sediment.

Conditions for periphyton experiments

Embryos were transferred to terracotta clay bricks (n=4/treatment) using polypropylene transfer pipettes 2-4 DPF. The treatment (periphyton cover) and control (no periphyton) bricks were held in 9.5, 18.9 L glass aquaria, or 9.5 L polypropylene plastic bins (Sterilite, Townsend, MA), submerged under 5 cm of well water held at 10°C ± 1°C, 0 ppt, with supplemental aeration, and a 12 Light:12 Dark photoperiod (~1200 Lux light, Milwaukee Instruments, SM700). 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 effect of periphyton communities on embryonic survival

Embryos (77-190/treatment; 4 DPF) were distributed to control or periphyton-covered bricks. Control bricks were collected from the Squamscott River prior to the start of the experiment and air dried for 41 days. Periphyton-covered bricks were collected from the Squamscott River and either used within 9 days ("natural"; representative of the standing community) or "cultured" in nutrient rich water (1000-3000 ppm f/2 media, Guillard and Ryther 1962) under constant high light intensity (800-1150 Lux) for approximately 30 d prior to the start of the experiment. Viability was assessed at 10 DPF.

Experiment 4. 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 from the Squamscott and Crane (Danvers, MA) Rivers. Additionally, the embryos on periphyton-covered bricks collected from the Squamscott River were covered with sediment (0.00, 0.25, and 1.00 g DW) as described above. Viability was assessed at 12 DPF, successful hatching at 20 DPF.

Experiment 5. 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 with either (1) background levels of nitrates (0.4 mg/L NO_3^-, sodium nitrate, Fisher Scientific, Fair Lawn, NJ, USA) and phosphates (0.04 mg/L, Sigma-Aldrich, St. Louis, MO, USA), (2) elevated nitrates (10.0 mg/L and background phosphate), (3) elevated phosphates (0.10 mg/L; background nitrate), and (4) elevated nitrate and phosphate. 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 6. Oxygen concentrations in the embryo micro-environment

Embryos (~20) were plated on bricks (area=~0.0206 m^2) with "cultured" or "natural" periphyton (Squamscott River) as described in Experiments 3 and 4 above, and on control bricks without periphyton (9.5 L glass aquaria, 10°C ± 1°C, 0 ppt salinity). 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 same micro-oxygen probes and recording device described above. Readings were made ~20 cm away from aeration source (Tetra*tec* AP100). A reading was taken every 8.31 sec and recorded oxygen measurements were averages of 100 consecutive readings. Electrodes were calibrated before each profile as described above.

In the trial on the "natural" periphyton, drifting values of oxygen concentration were suspected towards the end of the profile, as negative values were recorded during the last 6 hrs of profiling, nine days after measuring began. The drifting may have been due to electrical noise, slight temperature change, a change in the sensitivity of the probes after standardization, and/or a drift in the calibration. Recalibration is recommended more often than feasible for the continuous DO monitoring in this study. The occurrence of hatch was noted for all treatments.

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 also collected from rocks or bricks from 12 rivers in MA, NH, and ME between

March and May 2008 and processed to estimate the standing periphyton biomass in smelt-spawning rivers (Table 5). DW represents both inorganic and organic material, while ADW represents only inorganic material. To determine the DW, scraped periphyton samples (0.006-0.013 m^2) were transferred to pre-weighed aluminum weigh boats (g/m^2), dried at 105°C, cooled in a desiccator, and then weighed to the nearest 0.0001 g (Mettler Toledo AB54-S) 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 500°C, re-hydrated (~5 mL) and re-dried at 105°C, cooled in a desiccator, and again 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 were 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 (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 counted as a single cell (Smith 1950, Prescott 1978, Weitzel et al. 1979, Wehr and Sheath 2003).

Statistical analysis

Percent 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 there were significant effects. Regressions of oxygen consumption were compared with an analysis of co-variance (Zar, 1999) 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 2). The highest sediment treatment (6.00 g) had a significantly lower survival (53%, $p=0.018$; Table 2) than the control. The sediment was primarily composed of inorganic material (96, 96, and 97% for the 0.25, 1.00, and 6.00 g treatments, respectively). The average DWs, ADWs, and AFDWs for the sediment treatments are presented (Table 2). The relative proportion of sediment at a corresponding grain size index is indicated in Appendix A.

Experiment 2. The effect of sedimentation on embryonic respiration

A linear function described the oxygen consumption by control embryos and those under sediment (Fig. 1, Appendix B) on each of the occasions (DPF) when the measurements were made. An analysis of co-variance on oxygen consumption indicated that embryos under the sediment treatment consumed oxygen at a significantly faster rate

than the controls ($p<0.001$ for all days; Fig. 1, Appendix C). Consumption under the sediment treatment increased with age (Fig. 2, Appendix B) and oxygen concentrations fell below 5 μmol O_2 in 12.1, 4.7, 3.5, and 2.1 hrs for embryos 22, 25, 27, and 29 DPF, respectively. All embryos removed from the sediment and examined after the completion of the experiment were all found to be alive.

Oxygen levels below the sediment without embryos fell below 5 μmol O_2 in 34.9 hrs. The vertical profile indicated levels of unchanging oxygen concentration (45 μmol O_2), 3-7 mm above the sediment-water interface (Fig. 3). Above this area the oxygen concentrations rapidly increased, while below the sediment-water interface the oxygen concentration decreased to near anoxia (Fig. 3).

Experiment 3. The effect of periphyton communities on embryonic survival

Embryos incubated without periphyton had significantly higher survival (99%, $p\leq0.004$) than embryos incubated on either "natural" (95%) or "cultured" (85%) periphyton from the Squamscott River (Table 3). Embryos incubated on the "cultured" periphyton had a significantly lower survival ($p=0.005$) than those incubated on "natural" substratum (Table 3). One cultured sample was calculated to be an outlier and was excluded from further analysis. Both periphyton communities were primarily comprised of inorganic material (ADW >78%) but the "cultured" periphyton had a significantly higher organic (AFDW) component ($p=0.004$; Table 3). "Natural" periphyton was primarily comprised of diatom genera (77%), the genus *Navicula* comprised 58% of the total (Table 4). "Cultured" periphyton was primarily comprised of cyanobacteria (50%) and dominated by *Oscillatoria* (33%) and *Anabaena* (17%). Diatoms comprised (46%)

of the total composition (Table 4). Diatoms were observed adhering to the chorions of live embryos from all periphyton treatments.

Experiment 4. The effects of periphyton and sedimentation on embryo survival

Embryos incubated on periphyton, with or without additional sediment, from the Squamscott River had survival (49-55%) that was not different from the control (61%, $p \geq 0.306$; Table 3), while those incubated on periphyton from the Crane River had significantly lower survival (17%, $p < 0.001$; Table 3). Hatching success did not differ among treatments ($p = 0.117$; Table 3). 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 (DW, ADW) than that from all other sources (Table 3). Periphyton from both rivers were primarily comprised of diatom genera (96%). The genus *Synedra* comprised over 67% of the total (Table 4). 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 surrounded by diatoms, predominately *Cymbella* sp.

Experiment 5. The effects of periphyton and eutrophying compounds on embryo survival

No significant differences were found in survival ($p = 0.967$) or hatch ($p = 0.909$) among treatments containing periphyton or eutrophying compounds compared to controls (Table 3). 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 4). Periphyton was primarily comprised of diatoms (93%), especially *Synedra* (57%; Table 4). As in Experiment 4, diatoms, predominately *Cymbella* sp., were found adhering to the embryos from the Crane River treatments.

Experiment 6. Oxygen concentrations in the micro-environment of embryos

Embryos incubated on both "cultured" and "natural" periphyton experienced oxygen concentrations that cycled during the periods of light and darkness (Figs. 4 and 5). Oxygen levels dropped below saturation (251 μmol O_2) during dark hours but rose considerably during simulated daylight (Figs. 4 and 5). Control embryos remained at or above saturation throughout the light cycle (Figs. 4 and 5). In the "cultured" periphyton the highest average oxygen concentrations of each day ranged 257-304 μmol O_2 in the light and the lowest ranged 204-232 μmol O_2 in the dark (Fig. 4). On the "natural" periphyton the highest average of each day ranged 393-556 μmol O_2 in the light and the lowest ranged 0-243 μmol O_2 in the dark (Fig. 5). Periphyton composition is shown in Table 3. A portion of the embryos were noted to have hatched 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 5). 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 5).

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*b*), lake herring (*Coregonus artedii*; Brooke and Colby 1980), and steelhead trout (*Salmo gairdneri*; 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 and should not hinder hatching success. The presence of dams or other obstructions to water flow, as well as sediment, periphyton, and detritus accumulation, may limit oxygen availability, however. The effects of reduced oxygen availability on embryo survival have not been investigated in natural settings but long-term exposure to poorly oxygenated water was shown to reduce smelt hatching in laboratory studies (Fuda et al. 2007).

In the present study, a covering of sediment (~1 mm) over a 6-day period, significantly reduced embryo survival. Sedimentation has also been shown to negatively impact other teleost species, particularly salmonids, which have been studied extensively (Shelton and Pollock 1966, Olsson and Persson 1988, Meyer 2003, Lapointe et al. 2004). For instance, in laboratory and field studies, large amounts of fine sediment were shown to significantly reduce embryo survival of Atlantic salmon (*Salmo salar*; Peterson and

Metcalf 1981), fall-chinook (*Oncorhynchus tshawytscha*; Shelton and Pollock 1966), and Coho salmon (*Oncorhynchus kisutch*; Meyer 2003). Fine sediment has been shown to be detrimental to survival in these species because it reduces gravel permeability and oxygen delivery to the eggs (Louhi et al. 2008). Furthermore, fine sediment adheres to the chorion (Stuart 1953, Iwamoto et al. 1978) and physically obstructs micro-pores thereby restricting oxygen exchange.

In a study by Grieg et al. (2005), oxygen consumption by embryonic Atlantic salmon (*Salmo salar*) was reduced under a thin layer (<1 mm) of combusted sediment and reduced further with greater amounts of sediment. The decrease in oxygen consumption was due to the sediment creating a zone of reduced oxygen availability which reduced the exchange of oxygen from the macro-environment to the embryo (Greig et al. 2005). In whitefish (*Coregonus* sp.), eutrophication is a major factor influencing egg mortality in lakes, and survival is lowest when eggs are in contact with fine, muddy sediments (Lahti et al. 1979). Winter storms and bottom currents bury the eggs in sediment, which restricts oxygen transfer to the developing embryo (Venting-Schwank and Livingstone 1994). Significant mortality of Atlantic herring (*Clupea harengus*) embryos was also observed following a precipitating phytoplankton bloom (Morrison et al. 1991).

In addition to restricting oxygen delivery through advection, respiration and oxygen uptake by particulate organic carbon (POC) and 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 (Lahti et al. 1979, Wilkonska and Zuromska 1981, Venting-Schwank and Livingstone 1994) embryos in eutrophic lakes. 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 the extent of the fluctuation depended on the periphyton's algal composition. The DO concentration in the proximity of the "cultured" community, which was comprised approximately equally of cyanobacteria and diatoms ranged from 80-120% saturation, while that near the "natural" community, which was comprised almost entirely of diatoms, ranged from near anoxia to 240% saturation. Although greater oxygen depletion was associated with the "natural" periphyton, <36 hr periods of anoxia were not shown to affect embryonic smelt survival in this study.

Toxins produced from cyanobacteria have been shown to adversely affect teleost embryonic development, hatching and larval survival, although the severity of the effects appear to be species dependent. For instance, in one study, the effects of exposure to purified cyanobacterial toxins and cyanobacterial extracts were more pronounced in rainbow trout (*Oncorhynchus mykiss*; Oberemm et al. 1999) and zebrafish (*Danio rerio*; Keil et al. 2002, Berry et al. 2007), than in indigenous species such as roach (*Rutilus rutilus*), bream (*Abramis brama*), chub (*Leuciscus cephalus*), and stone loach (Cobitis taenia; Oberemm et al. 1999). The protective capacity of the chorion to toxins differs among species (Cazenave et al. 2006) and adaptive resistance to some toxins has also been demonstrated (Wirgin and Waldman 2004, Yuan et al. 2006). The effects of cyanobacteria toxicity resulting from harmful algae blooms has been most widely studied on adult fish and other aquatic organisms but fewer studies have focused on short-range, benthic interactions with teleost embryos. In the present study, significantly lower survival was found with smelt embryos cultured in the presence of periphyton containing higher proportions of cyanobacteria, compared to controls (85 vs. 95%). It is unclear, however, if smelt would encounter periphyton with such high cyanobacteria content in the wild, as the algae species composition changed significantly during culture.

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 terms of 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 under a sediment layer, periphyton of high biomass, or periphyton containing significant cyanobacteria populations. Reduced survival may have been due to prolonged exposure to low oxygen conditions resulting from compromised advection and substrate respiration.

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Table 1 Adult rainbow smelt *Osmerus mordax* collection sites in Massachusetts, New Hampshire, and Maine, from the 2007 and 2008 spawning seasons

Year	River (City, State)
2007	Oyster River (Durham, NH)
	Bellamy River (Dover, NH)
	Fore River (Braintree, MA)
2008	Squamscott River (Exeter, NH)
	Winnicut River (Greenland, NH)
	Salmon Falls River (Dover, NH)
	Lamprey River (Newmarket, NH)
	Damariscotta River (Damariscotta, ME)

Table 2 Experiment 1. Mean (±SE) survival (%) rainbow smelt embryos under different levels of sediment and dry weight (DW), ash dry weight (ADW), and ash free dry weight (AFDW) of sediment treatments, expressed as g/m^2 and %. Different letters indicate significant differences among treatments, n=4

Treatment	% Survival	DW	ADW (% inorganic)	AFDW (% organic)
Control	82.4 ± 5.9^{a}	-	-	-
0.25 g	$76.2 \pm 4.6^{a,b}$	54.3 ± 0.2	51.9 ± 0.3 (97.5)	2.4 ± 0.0 (4.3)
1.00 g	$75.5 \pm 5.8^{a,b}$	216.4 ± 0.4	208.1 ± 0.7 (96.2)	8.3 ± 0.3 (3.8)
6.00 g	53.6 ± 4.1^{b}	1296.5 ± 3.7	1261.2 ± 5.4 (97.3)	35.3 ± 2.7 (2.7)

Table 3 Experiments 3-5. Mean (±SE) survival (%) and hatch (%) (rainbow smelt embryos incubated on bricks with and without periphyton), and dry weight (DW), ash dry weight (ADW), and ash free dry weight (AFDW) of periphyton expressed as g/m^2 and %. Lowercase letters indicate significant differences within an experiment. Numbers indicate significant differences in DW, ADW, or AFDW comparing all experiments. n=4. Asterisk indicates n=3. ND = no data

Experiment	Treatment	% Survival	% Hatch	DW	ADW (% inorganic)	AFDW (% organic)
3	Control	99.4±0.2[a]	ND			
	Squamscott - Natural	95.1±0.8[b]	ND	15.3 ± 3.4[1a]	13.9 ± 3.3[1a] (90.3)	1.3 ± 0.2[1a] (9.7)
	Squamscott - Cultured	85.9±2.4[c*]	ND	23.5 ± 6.5[1a]	18.8 ± 5.9[1a] (78.2)	4.7 ± 0.7[1b] (21.8)
4	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 ± 4.8[1a]	32.3 ± 5.0[1a] (91.1)	2.9 ± 0.2[1a] (8.9)
	Crane - Natural	17.8 ± 2.9[b]	74.3 ± 8.1[a]	251.5 ± 22.5[3b]	235.8 ±1 8.2[3b] (94.1)	15.7 ± 5.0[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]			
5	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 ± 17.5[2]	103.5 ± 17.5[2] (82.1)	21.0 ± 1.8[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]			

Table 4 Relative abundance (%) estimate of periphyton cell types by genera from Experiments 3-5. Bottom division combines genera into more inclusive groupings, cyanobacteria, diatoms, green algae, and red algae. Data are from triplicate cell counts of at least 300 cells. [1] indicates periphyton from the "natural" standing community, [2] indicates "cultured" periphyton

	Experiment				
	3	3	4	4	5
Genus	Squamscott[1]	Squamscott[2]	Squamscott[1]	Crane[1]	Crane[1]
Anabaena	0	17.71	0	0	0
Lyngbia	0	0	0.17	0.09	0.30
Oscillatoria	2.25	33.27	1.00	2.94	5.14
Cymbella	0	0	0	1.84	13.65
Eunotia	0	0.59	0.92	4.88	2.57
Fragilaria	0	0	0	0	0
Gomphonema	0	0	0	0.74	2.57
Gyrosigma	0	0	0	0.37	0.10
Melosira	0	0.68	0	0	0
Meridion	0	0	3.34	9.29	6.23
Navicula	58.69	14.97	4.50	6.07	5.24
Surirella	0	0	0	6.16	5.54
Synedra	10.74	2.05	86.99	67.16	57.76
Tabellaria	0.39	2.64	0.50	0.00	0
Asymmetric diatom	2.25	0.29	0	0	0
Pennate diatom	5.47	24.85	0	0	0
Mougeotia	0	2.94	0	0	0
Ulothrix	19.92	0	2.59	0	0
Batrachospermum	0	0	0	0	0.30
Rhodochorton	0	0	0	0.46	0.59
Cyanobacteria	2.25	50.98	1.17	3.04	5.44
Diatoms	77.83	46.09	96.25	96.50	93.67
Green Algae	19.92	2.94	2.59	0	0
Red Algae	0	0	0	0.46	0.89

Table 5 Mean (±SE) dry weight (DW), ash dry weight (ADW), and ash free dry weight (AFDW) of standing periphyton biomass from Massachusetts (MA), New Hampshire (NH), and Maine (ME) expressed as g/m^2. Samples were taken during the smelt spawning season in 2008. n=4. Asterisks indicates n=3

State	River	Date	DW	ADW (% inorganic)	AFDW (% organic)
ME	Tannery Brook	6-May-08	58.8 ± 14.8	41.0 ± 8.7 (72.5)	17.7 ± 7.2 (27.5)
ME	Mast Landing*	9-Apr-08	0.5 ± 0.4	0.4 ± 0.4 (85.7)	0.1 ± 0.1 (14.3)
ME	Deer Meadow Brook*	9-Apr-08	0.2 ± 0.0	0.1 ± 0.0 (44.4)	0.1 ± 0.0 (55.6)
NH	Squamscott	24-Mar-08	15.3 ± 3.4	13.9 ± 3.3 (90.3)	1.4 ± 0.2 (9.7)
NH	Squamscott	5-Apr-08	35.3 ± 4.8	32.3 ± 5.0 (91.1)	2.9 ± 0.3 (8.9)
NH	Winnicut*	5-May-08	7.0 ± 4.8	4.3 ± 2.4 (73.3)	2.7 ± 2.3 (26.7)
NH	Lampery	5-May-08	1.8 ± 1.2	1.5 ± 1.1 (68.5)	0.3 ± 0.1 (31.5)
NH	Bellamy	6-May-08	8.2 ± 4.0	7.1 ± 3.8 (86.3)	1.1 ± 0.6 (13.7)
NH	Oyster	6-May-08	72.0 ± 14.8	64.3 ± 13.9 (89.2)	7.6 ± 1.6 (10.8)
NH	Squamscott	7-May-08	75.7 ± 21.9	69.4 ± 22.5 (88.4)	6.3 ± 0.7 (11.6)
NH	Salmon Falls	7-May-08	179.5 ± 111.2	114.2 ± 50.9 (83.3)	65.2 ± 61.3 (16.7)
MA	Crane	5-Apr-08	251.5 ± 22.5	235.8 ± 18.2 (94.1)	15.8 ± 5.0 (5.9)
MA	Crane	18-Apr-08	124.6 ± 17.5	103.5 ± 17.5 (82.1)	21.1 ± 1.8 (17.9)
MA	Saugus	11-May-08	169.7 ± 34.3	163.2 ± 33.8 (96.5)	6.5 ± 0.7 (3.5)
MA	Crane	11-May-08	120.2 ± 60.1	107.3 ± 25.7 (89.1)	12.9 ± 3.0 (10.9)
MA	Mill	11-May-08	101.5 ± 40.2	86.5 ± 37.0 (79.8)	15.0 ± 3.9 (20.2)
MA	Parker	11-May-08	27.1 ± 9.8	24.5 ± 9.1 (89.1)	2.6 ± 0.7 (10.9)
MA	Little	11-May-08	165.4 ± 57.5	158.9 ± 56.1 (95.2)	6.5 ± 1.4 (4.8)

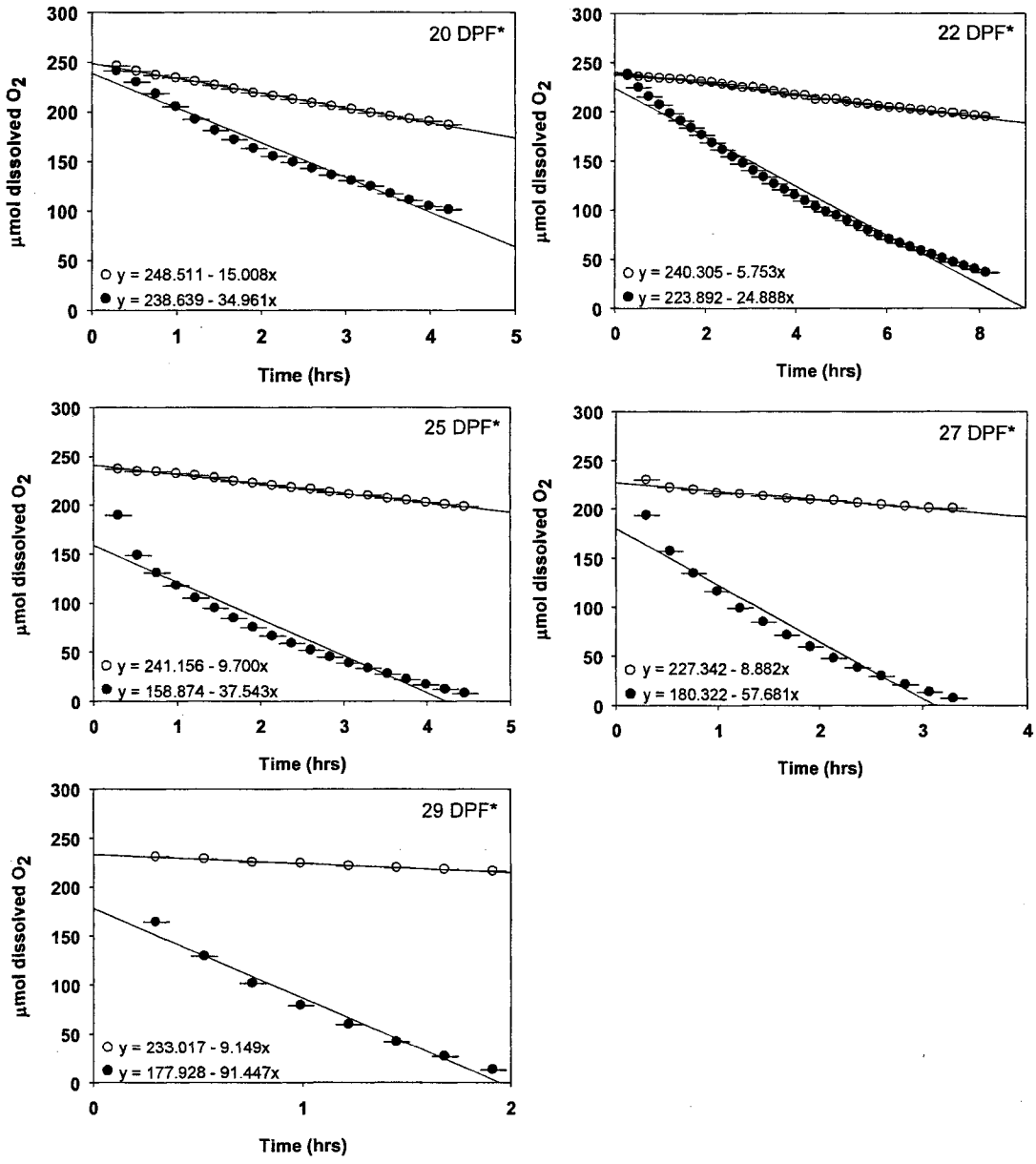

Fig. 1 Regressions of decreasing mean (±SE, n=100) oxygen concentration (µmol O_2) over time from 10 rainbow smelt embryos with no sediment (open circles, control) and covered with 0.45 g sediment (filled circles, treatment), 22, 25, 27, and 29 days post fertilization (DPF). Linear portions of the regressions were estimated visually and regression equations are indicated. Asterisks indicate statistical differences between the controls and treatments, determined by analysis of co-variance

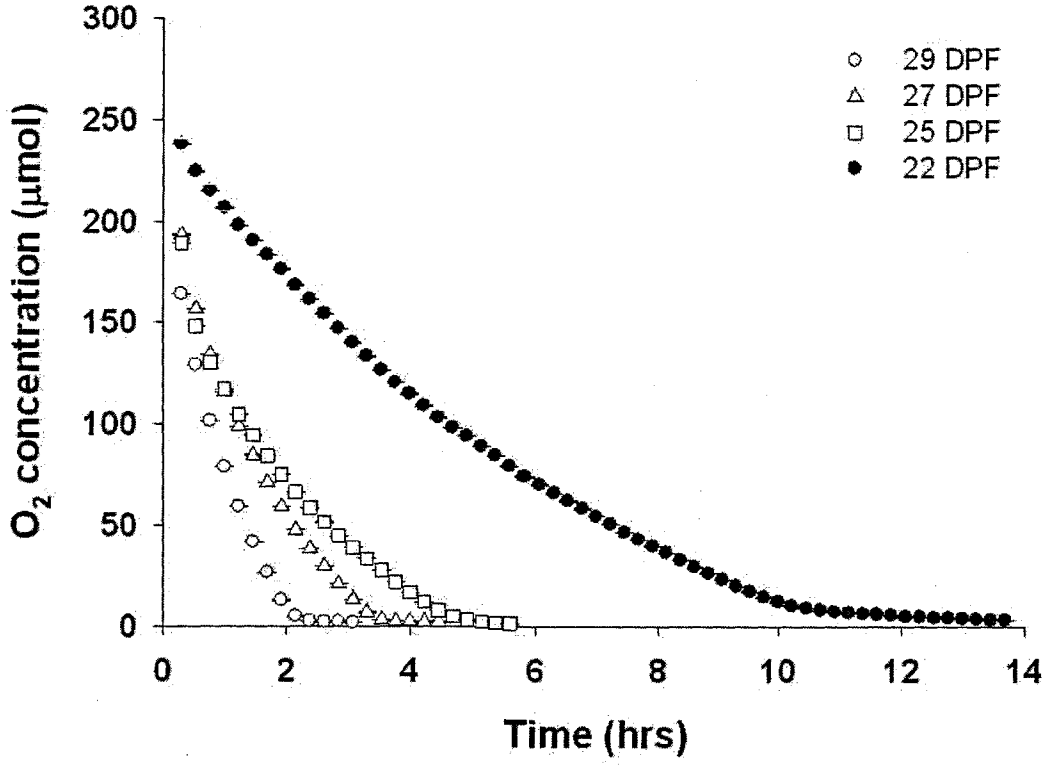

Fig. 2 Regressions of decreasing oxygen concentration (µmol O_2) of 10 rainbow smelt embryos covered with 0.45 g sediment, 20, 22, 25, 27, and 29 (DPF). Each point represents a mean reading (±SE, n=100)

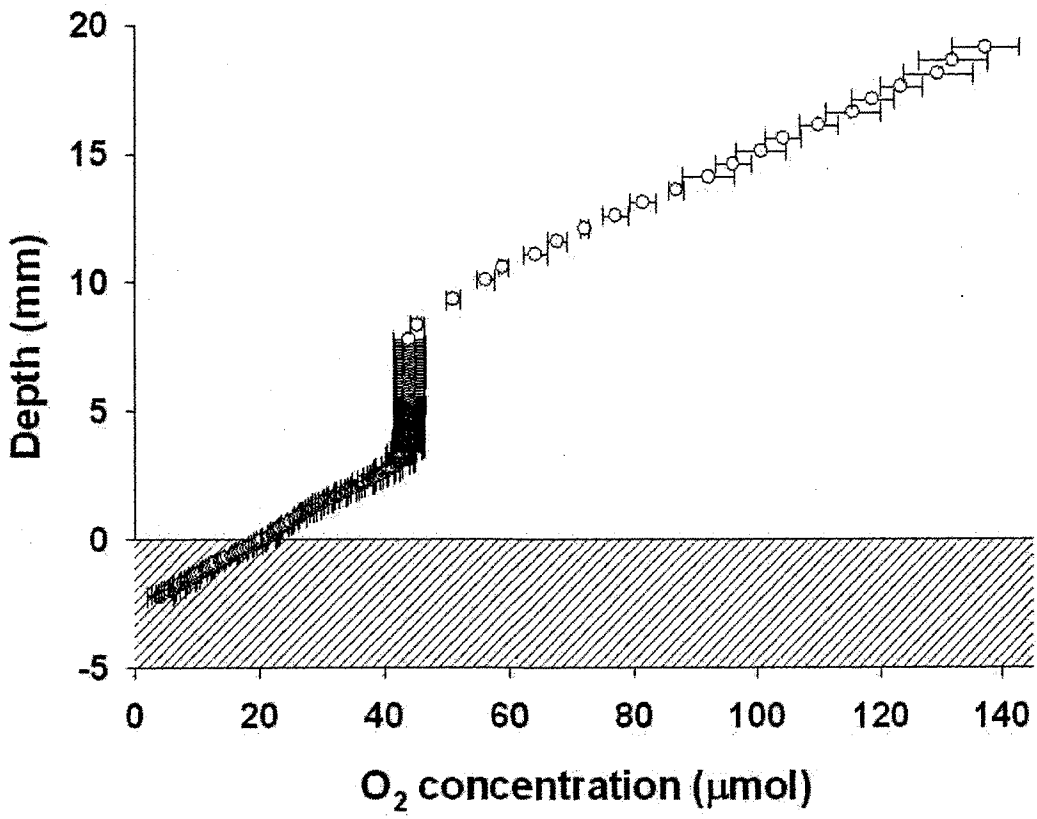

Fig. 3 Vertical oxygen profile (µmol O_2) above and below a sediment layer (0.45 g sediment) with no embryos present (±SE, n=2). Shaded area indicates sediment layer

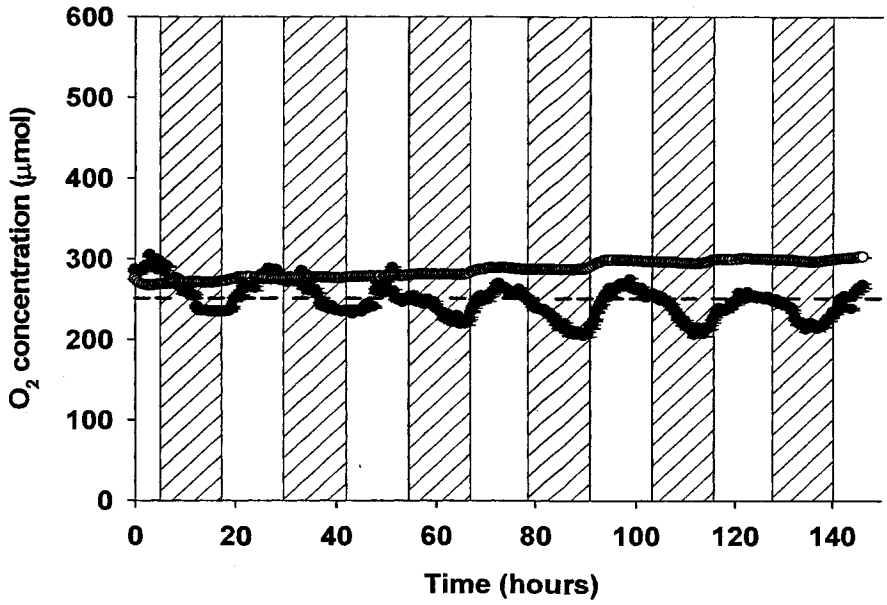

Fig. 4 Mean (±SE, n=100) dissolved oxygen (DO) concentrations (µmol O_2) measured from embryos with (filled circles) and without (open circles) "cultured" periphyton (Experiment 6) during a 12 light (L):12 dark (D) light cycle. Time during L (900 Lux) and D (0 Lux) phases represented by unshaded and shaded backgrounds, respectively. Dashed line indicates 100% saturation, 251 µmol O_2

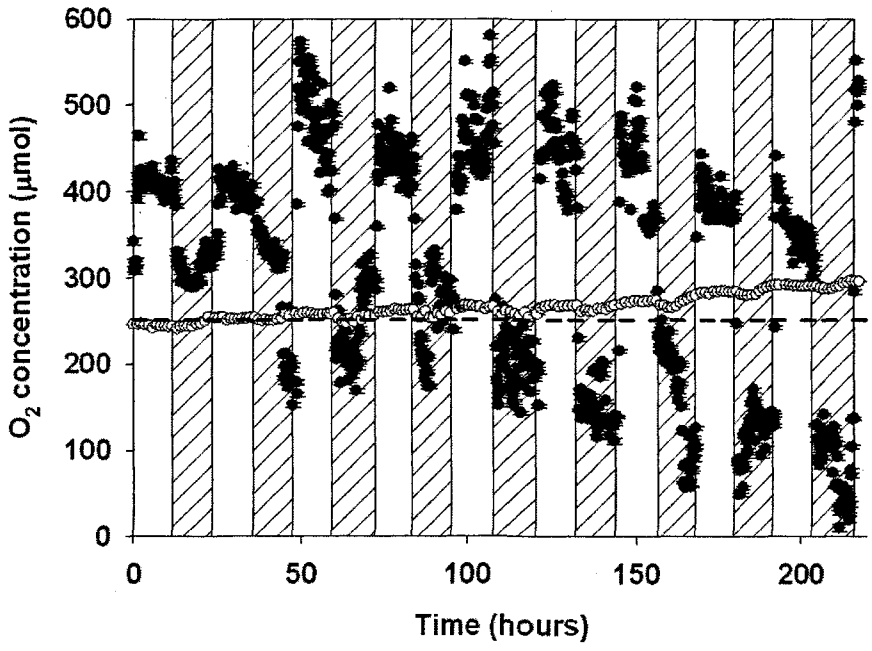

Fig. 5 Mean (±SE, n=100) dissolved oxygen (DO) concentrations (µmol O_2) measured from embryos with (filled circles) and without (open circles) "natural" periphyton (Experiment 6) during a 12 light (L):12 dark (D) light cycle. Time during L (900 Lux) and D (0 Lux) phases represented by unshaded and shaded backgrounds, respectively. Dashed line indicates 100% saturation, 251 µmol O_2

APPENDICES

APPENDICES

Appendix A Sediment grain size (%) distribution estimate

Grain Size (μm)	%
211 < X ≤ 300	29.6
110 < X ≤ 211	29.3
X ≤ 110	41.0

Appendix B Linear regression analysis of oxygen consumption as a function of change in oxygen concentration (y, μmol O_2) per unit time (x, hr), in a 5 mL beaker containing 10 rainbow smelt embryos. The control received no sediment; the sediment treatment received 0.45 g sediment. Experiments were conducted for multiple days post fertilization (DPF) and linear portions were estimated visually (Fig. 1)

DPF	Treatment	Equation	n	R^2	p
20	Control	y = -15.008x + 248.511	18	0.998	< 0.001
20	Sediment	y = -34.961x + 238.639	18	0.975	< 0.001
22	Control	y = -5.753x + 240.305	35	0.994	< 0.001
22	Sediment	y = -24.888x + 223.892	35	0.980	< 0.001
25	Control	y = -9.700x + 241.156	19	0.997	< 0.001
25	Sediment	y = -37.546x + 158.874	19	0.931	< 0.001
27	Control	y = -8.882x + 227.342	14	0.954	< 0.001
27	Sediment	y = -57.681x + 180.322	14	0.952	< 0.001
29	Control	y = -9.149x + 233.017	8	0.989	< 0.001
29	Sediment	y = -91.447x + 177.928	8	0.976	< 0.001

Appendix C Analysis of co-variance comparing the oxygen consumption of 10 embryos (control) and 10 embryos that received a sediment treatment (0.45 g). Oxygen consumption is expressed as a function of change in oxygen concentration (y, μmol O_2) per unit time (x, hr), multiple days post fertilization (DPF)

DPF	Equation	F	df (n,d)	p
20	y = 24.984x – 243.575	666.7	3, 10	< 0.001
22	y = 15.320x – 232.098	497.5	3, 22	< 0.001
25	y = 23.623x – 200.015	741.4	3, 32	< 0.001
27	y = 33.282x – 203.832	1729.0	3, 64	< 0.001
29	y = 50.298x – 205.473	393.2	3, 30	< 0.001