PACIFIC LAMPREY TOXICITY STUDY

PREPARED BY

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AND

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FOR THE

PORTLAND HARBOR
NATURAL RESOURCE TRUSTEE COUNCIL








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Pacific Lamprey Toxicity Study

Prepared for:

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Contents

List of Figures ix
List of Tables xi
List of Acronyms and Abbreviations xiii

Section 1 Introduction 1

1.1 Problem Definition 1
1.2 Sediment and Lamprey Collection 2
1.2.1 Fall 2009 collection of sediment and lamprey 2
1.2.2 Summer 2010 collection of sediment and lamprey 2
1.2.3 Analytical chemistry of sediment samples 4
1.2.4 Ammocoete acclimation and holding 4
1.3 Statistical Analysis 20

Section 2 Task 2a: Depuration Trial 20

2.1 Objective 20
2.2 Methods 20
2.2.1 First depuration trial 20
2.2.2 Second depuration trial 22
2.3 Results 22
2.4 Discussion 28

Section 3 Task 2b: Holding Container (Corral) Trial 28

3.1 Objective 28
3.2 Methods 29
3.3 Results 30
3.4 Discussion 32

Section 4 Task 2c: Feeding Trial 33

4.1 Objective 33
4.2 Methods 33
4.3 Results 34
4.4 Discussion 34

Section 5 Task 3: Contaminated Sediment Exposure Pre-pilot Test and Task 4: Physiology Endpoints 37

5.1 Objective 37
5.2 Methods 38
5.2.1 Sediments 38
5.2.2 Experimental design 38
5.2.3 Ammocoete sampling and preservation 38
5.2.4 Dry weight and lipid and ash content 39
5.2.5 Gill Na^+/K^+ ATPase activity 39
5.2.6 Histology 39
5.3 Results 40
5.3.1 Water quality 40
5.3.2 Survival and behavior 40
5.3.3 Growth 40
5.3.4 Lipid content 44
5.3.5 Histology 44
5.3.6 Gill Na^+/K^+ ATPase activity 44
5.4 Discussion 50

Section 6 Task 5: Behavioral Endpoints 51

6.1 Objective 51
6.2 Methods 51
6.2.1 Multiple sediment trough exposures – preference and burrowing time tests 51
6.2.2 Single sediment exposures – burrowing time tests 51
6.3 Results 54
6.3.1 Multiple sediment trough exposures – preference and burrowing time tests 54
6.3.2 Single sediment exposures – burrowing time tests 56
6.4 Discussion 60

Section 7 Task 6: Induction of Metamorphosis 61

7.1 Objective 61
7.2 Methods 61
7.3 Results 61
7.4 Discussion 62

Section 8 Task 7: Contaminated Sediment Exposure Pilot Test 62

8.1 Objective 62
8.2 Methods 62
8.2.1 Sediments 62
8.2.2 Experimental design 62
8.2.3 Experimental monitoring and sampling 63
8.3 Results 64
8.3.1 Water quality 64
8.3.2 Growth 64
8.3.3 Lipid content 68
8.3.4 Histology 68
8.3.5 Gill Na^+/K^+ ATPase activity 71
8.4 Discussion 72

References 72

Figures

1 Ammocoete holding tank 19
2 Ammocoete in beaker during depuration trial 21
3 Mean wet-weight measurements from first depuration trial (n = 10) 24
4 Mean ash content of non-depurated and depurated (72 hours) ammocoetes from first depuration trial (n = 9) 24
5 Photographs of ash from depurated (left) and non-depurated (right) ammocoetes 25
6 Mean change in wet weight for ammocoetes depurated for 24, 48, and 72 hours from second depuration trial (n = 5) 27
7 Mean ash content in ammocoetes depurated for 0, 24, 48, and 72 hours from second depuration trial (n = 5) 27
8 Holding containers used in corral trials 29
9 Mean change in wet weight in ammocoetes held in corrals of differing sizes (n = 10 for all the “corral” treatments and n =1 for the “no corral” treatment) 33
10 Mean (n = 3) change in wet weight among ammocoetes held in different sediment types, with different water sources and different feeding regimes 37
11 Mean initial wet weight (a) and length (b) in all ammocoetes loaded into each corral (n = 90) 42
12 Mean change in depurated wet weight for individual ammocoetes exposed to each sediment type for 30 days and 60 days (n = 15) 43
13 Mean change in length for individual ammocoetes exposed to each sediment type after 30- and 60-day exposures (n = 15) 43
14 Mean lipid content (n = 3) in individual ammocoetes exposed to each sediment type after 30- and 60-day exposures 44
15 Encysted digenean trematode parasite (arrow) in the gill lamella of an ammocoete exposed to GAS sediment for 60 days 45
16 Encysted digenean trematode parasite (arrow) in the kidney of an ammocoete exposed to GAS sediment for 60 days 46
17 Granuloma (arrow; likely from a degraded encysted parasite) in the liver of an ammocoete exposed to OSU sediment for 30 days 47
18 Encysted digenean trematode parasites (arrows) in the heart of an ammocoete exposed to OSU sediment for 60 days 48
19 Encysted digenean trematode parasites (arrows) in the oropharynx of an ammocoete exposed to SRS sediment for 60 days 49
20 Gill Na^+/K^+ ATPase activity in individual ammocoetes exposed to each sediment type after 30- and 60-day exposures (n = 3) 50
21 Sediment preference experiment using OSU, SRS, and GAS sediments 52

22 Sediment burrowing time experiment, with individual ammocoetes in each exposure container 53
23 Ammocoete sediment preference in paired sediment trials 55
24 Time to initiate burrowing (a) and time to complete burrowing (b) in one uncontaminated (RE2) and three contaminated (GA2, AR2, and SWI) sediments 57
25 Ammocoete mean burrowing times in uncontaminated (RE2) and contaminated (GA2, AR2, and SWI) sediments 58
26 Mean time to initiate burrowing and complete burrowing in RE2 (uncontaminated), GA2, AR2, and SWI (contaminated) sediments used in test series 1 and 2 (see Section 6.2.2) 60
27 Individual lamprey corrals in larger circular exposure tanks 63
28 Mean initial wet weight (a) and length (b) in all ammocoetes loaded into each corral (n = 144) 66
29 Mean change in depurated wet weight for individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 12 in all treatments, except for sediments RE1 and SW1 where n = 11) 67
30 Mean change in length for individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 12 in all treatments, except RE1 and SW1 where n = 11) 69
31 Mean lipid content (n = 6 in all treatments, except RE1, RE3, GAA, and SWI where n = 5) in individual ammocoetes exposed to each sediment type after a 45-day exposure 69
32 Counts of encysted parasites and granulomas on histology slides prepared from individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 3) 70
33 Gill Na^+/K^+ ATPase activity in individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 5 for sediments AR1, GAA, and SWI; n = 4 for sediments RE1, RE3, AR2, ARM, MAR, OST, and SC1; and n = 3 for sediments MSC and GA2) 71

Tables

1 Sediment sampling locations on the Willamette River 3
2 Analytical chemistry results for samples collected in fall 2009 and for MSC 5
3 Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) 8
4 Analytical chemistry results for samples collected in summer 2010 (Willamette River reference samples) 16
5 Water quality monitoring results from ammocoete holding tanks 19
6 Measurements of non-depurated ammocoetes from first depuration trial 22
7 Measurements of depurated ammocoetes from first depuration trial 23
8 Measurements of non-depurated ammocoetes from second depuration trial 25
9 Measurements of depurated ammocoetes from second depuration trial 26
10 Water quality monitoring results from second depuration trial 28
11 Summary of corral trial setup 29
12 Weights and lengths of ammocoetes in corral trials 30
13 Water quality monitoring results from corral trials 32
14 Summary of feeding trial setup 34
15 Weights and lengths of ammocoetes in feeding trial 35
16 Water quality monitoring results from feeding trial 36
17 Average water quality for each sediment type during the 30- and 60-day exposures in Task 3 41
18 Study design and results of multiple sediment preference experiments 55
19 Mean time for ammocoetes to initiate and complete burrowing into different sediments in burrowing test series 1 and 2 and corresponding ammocoete lengths 59
20 Average water quality for each sediment type during the 45-day exposure 65
21 Initial and final wet weight and length measurements of ammocoetes (n = 12 for each sediment type for all analyses, except final measurements for RE1 and SW1 where n = 11) 68

Acronyms and Abbreviations

ADP	adenosine diphosphate
AR1	Arkema 1 sediment sampling site
AR2	Arkema 2 sediment sampling site
ARM	Arco/Mobil sediment sampling site
ATP	adenosine triphosphate
ATPase	class of enzymes that catalyze the decomposition of adenosine triphosphate
CAS	Columbia Analytical Services
COC	chain of custody
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DDx	group of organochlorine pesticides and breakdown products, including dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethylene, and dichlorodiphenyldichloroethane
DO	dissolved oxygen
DRO	diesel range organics
EC	electrical conductivity
EDTA	ethylenediaminetetraacetic acid
FPGL	Fish Performance and Genetics Laboratory
GA2	Gasco 2 sediment sampling site
GAA	Gasco 1 (alternate) sediment sampling site
GAS	Gasco sediment sampling site
GC	gas chromatography
GRO	gasoline range organics
HPLC	high-pressure liquid chromatography
HSD	honestly significant difference
IACUC	Institutional Animal Care and Use Committee
$KClO_4$	potassium perchlorate

LOD	level of detection
LOQ	level of quantitation
MAR	Marcom sediment sampling site
MDL	method detection limit
MRL	method reporting limit
MSC	masonry sand control
OST	Oregon Steel sediment sampling site
OSU	Oregon State University pond sediment sampling site
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PLA	Pacific lamprey ammocoete
RE1	Reference sediment sampling site 1
RE2	Reference sediment sampling site 2
RE3	Reference sediment sampling site 3
RRO	residual range organics
SC1	Schnitzer 1 sediment sampling site
SEI	sucrose, EDTA, and imidazole
SEID	SEI + sodium deoxycholate acid
SOP	standard operating procedure
SRS	Siletz River sediment sampling site
SWI	Swan Island sediment sampling site
TOC	total organic carbon
TPH	total petroleum hydrocarbon
USFWS	U.S. Fish and Wildlife Service

1. Introduction

Contaminants such as chlorinated hydrocarbons, petroleum-related compounds, metals, and other hazardous substances have been released from various sources and have come to be located in Portland Harbor (hereafter, the Harbor) sediments. The concentrations of many of these compounds are elevated in the Harbor compared to upstream locations. Sediments from specific areas in the Harbor have demonstrated toxicity to benthic invertebrates, and sediment-associated biota and fish collected from the area have accumulated contaminants (e.g., Windward Environmental, 2008b; Windward Environmental and Integral Consulting, 2007, 2008). Habitat in the Harbor may be an important resting and foraging area for Pacific lamprey ammocoetes (PLAs; *Lampetra tridentata*) as they transition to the Lower Columbia River and prepare for their marine life stage. Ammocoetes collected from the Harbor have accumulated higher concentrations of some organochlorine compounds than ammocoetes collected upstream.

The Portland Harbor Trustee Council is evaluating potential natural resource injuries to ammocoetes. Insufficient information is available to determine if contaminant exposures to ammocoetes exceed concentrations that could cause injuries or prevent colonization of the Harbor by ammocoetes. In addition, restoration efforts for ammocoetes could be more successful if sediment toxicity to the species were better understood.

1.1 Problem Definition

Problem description: Lamprey ammocoetes are the only detritivorous fish present in the Lower Willamette River (Windward Environmental, 2008a). Their survival, growth, and behavior could be affected from exposure to contaminants in sediment in the Harbor.

Conceptual model of potential hazard: Industrial and municipal sources have released contaminants into the Harbor. Some of these contaminants have come to be located in bed sediments, some contaminants remain near their release points, and others have been transported away from their sources into downstream areas (Integral Consulting et al., 2009). Some of the contaminated sediments are within depositional or other areas where ammocoetes would settle as they move downriver. Ammocoetes readily burrow into sediment at settling areas and filter feed within the sediment or at the sediment surface (e.g., Claire, 2002). They are potentially exposed to contaminants in porewater, transition zone water, surface water, suspended sediment at the interface between surface water and sediment, and by consuming contaminated sediment and detritus. Ammocoetes could be exposed to hazardous substances through dermal, ingestion, and gill uptake pathways. Water toxicity tests conducted on ammocoetes suggest they are moderately sensitive to contaminants (Windward Environmental, 2008a), but their response to contaminants

from exposure in sediments has not been evaluated. Responses in ammocoetes exposed to sample sediments obtained from the Harbor in sediment toxicity tests will be used to determine whether sediment toxicity tests provide a suitable tool to identify and quantify injury in ammocoetes.

Primary study question: Do contaminant concentrations in Harbor sediment cause identifiable and quantifiable injuries to ammocoetes? The specific objective of this study is to evaluate whether measurable adverse effects are observed in controlled laboratory exposures of ammocoetes to Harbor sediments.

1.2 Sediment and Lamprey Collection

1.2.1 Fall 2009 collection of sediment and lamprey

In November 2009, 30 gal of sediment were collected from the Gasco site (GAS) in the Harbor (Table 1; see location in Stratus Consulting, 2009) and approximately 50 gal of sediment were collected from the Siletz River just downstream of the Siletz Highway (229) bridge in Siletz, OR (SRS; see location in Stratus Consulting, 2011a). Approximately 2,000 to 2,500 ammocoetes were collected from multiple locations in the Siletz River. Of these, approximately 500 were longer than 80 mm; the remaining individuals were between 15 and 80 mm. See the *Portland Harbor Pacific Lamprey Ammocoete Study: Sediment Collection and Analysis Plan* (Stratus Consulting, 2009) and *Sampling Plan: Field Collection of Ammocoetes for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011a) for the collection locations and methods that were used to collect sediments and lamprey in fall 2009. The Aquatic Program of the Siletz Indians held the ammocoetes at the Lhuuke Illahee Fish Hatchery on the Siletz River in large circular tanks with at least 6 in. of wood-chip substrate and a continual supply of fresh water from the Siletz River for approximately 5 months. Approximately 400 ammocoetes were transported in April 2010 to the Fish Performance and Genetics Laboratory (FPGL), Oregon State University (Corvallis), for use in this study. The remaining ammocoetes were retained by the Aquatic Program of the Siletz Indians for other purposes.

1.2.2 Summer 2010 collection of sediment and lamprey

Approximately 200 ammocoetes were collected during August and September 2010 from various locations in the Siletz River using electro-fishing gear and delivered to FPGL on September 21, 2010. See the *Sampling Plan: Field Collection of Ammocoetes for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011a) for methods used.

Table 1. Sediment sampling locations on the Willamette River. All sediment was collected in summer 2010 unless otherwise noted.

Sampling site ID	Name	Approximate river mile	Primary contaminants of interest
OST	Oregon Steel	2.1	PCBs,[a] zinc, copper
SC1	Schnitzer 1	3.7	PCBs, phthalates, PAHs,[b] zinc, copper
ARM	Arco/Mobil	5.1	PAHs, TPH[c] (diesel)
MAR	Marcom	5.6	Tributyl tin, zinc, copper, PAHs
GAA	Gasco 1 (alternate)	6.1	Cyanide, PAHs, DDx[d]
GAS/GA2[e]	Gasco 2	6.2	PAHs, DDx
AR1	Arkema 1	7.3	DDx, dioxins/furans, chlordane
AR2	Arkema 2	7.4	Perchlorate, DDx
SWI	Swan Island	8.5	Copper, zinc, PAHs
RE1	Reference site 1	19.1	None
RE2	Reference site 2	23.2	None
RE3	Reference site 3	23.2	None

a. Polychlorinated biphenyls.
b. Polycyclic aromatic hydrocarbons.
c. Total petroleum hydrocarbon.
d. Includes dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD).
e. GAS samples were collected in fall 2009, and GA2 samples were collected in summer 2010.

Approximately 10 gal of sediment were collected from each of 12 locations on the Willamette River during the week of July 26, 2010 (Table 1). Nine locations were areas in the Harbor previously found to contain contaminated sediments; three upstream locations served as reference areas. All sediment was maintained under chain of custody (COC) by U.S. Fish and Wildlife Service (USFWS) personnel who were present during the sediment collection. The sediment was stored at a secure USFWS facility until it was delivered to FPGL on July 30, 2010, by the same USFWS personnel. See the *Sampling Plan: Field Collection of Sediments for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011b) and the *Sampling Report: Field Collection of Sediments for Pacific Lamprey Toxicity Study* (Stratus Consulting, 2011c) for a detailed description of the sediment collection and handling activities.

1.2.3 Analytical chemistry of sediment samples

Following the fall 2009 sediment collection, composite samples of the GAS and SRS sediment were collected from the holding buckets in the field and sent to Columbia Analytical Services (CAS; Kelso, WA) for analytical chemistry and particle size analyses.

Prior to the pre-pilot sediment exposure test (Task 3; Section 5), two additional samples were collected and submitted to CAS for analysis: a sample of Oregon State University pond sediment (OSU) and a second composite sample from the fall 2009 GAS sediment collected using an incremental sampling methodology [PLA standard operating procedure (SOP) P.11; Stratus Consulting, 2011d].

Composite samples from the summer 2010 sampling and the masonry sand control (MSC) sediment were collected and submitted to CAS for analysis at the beginning of the pilot sediment exposure test (Task 3; Section 5) in October 2010. These test results are summarized in Tables 2 through 4.

1.2.4 Ammocoete acclimation and holding

Approximately 600 ammocoetes were transported from the Siletz River to the FPGL in 3 batches of 200 ammocoetes each. The first two deliveries in March and April 2010 consisted of ammocoetes collected in fall 2009. The third delivery in November 2010 consisted of ammocoetes collected in summer 2010.

Ammocoetes were acclimatized to FPGL conditions for at least three weeks before trials began. Approximately 200 ammocoetes at a time were held in each of two 1.5-m diameter by 1.5-m high oval stock tanks filled with a 14.4-cm deep mixture of 80% clean masonry sand and 20% woodchip substrate (Figure 1).[1] Ammocoetes were fed a 1:1 ratio of Baker's yeast and a microencapsulated larval fish enrichment diet (Argent Chemical Laboratories, Redmond, WA; ammocoete diet), suspended in warm water, and delivered at 2% body weight three times per week. Pathogen-free well water was introduced from a spray bar at the top of the tank at a rate of 7.2 L/min. A horizontal effluent standpipe drained water out of the tank at a height of 27 cm above the tank bottom. During holding, dissolved oxygen (DO), temperature, pH, and conductivity of the water were recorded daily, and ammonia was tested weekly.

1. Following the Institutional Animal Care and Use Committee (IACUC) guidelines (IACUC, permit #4022).

Table 2. Analytical chemistry results for samples collected in fall 2009 and for MSC

Component	Unit	Basis	GAS (sampled in the field)		GAS (sampled in laboratory prior to Task 3)		SRS		OSU		MSC	
Total solids	Percent	Wet	51.6		58.4		80		53.1		97.3	
Ammonia as nitrogen	mg/kg	Dry	67.4		87.9		6.89		33.1		1.68	
TOC	Percent	Dry	2.61		3.07		0.5		0.117		0.133	
Gravel (> 2.00 mm)	Percent	Dry	1.26		0.48		0.9		19		0.5	
Sand, very coarse (> 1.00 mm to 2.00 mm)	Percent	Dry	1.99		0.64		1.15		12.5		5.5	
Sand, coarse (> 0.500 mm to 1.00 mm)	Percent	Dry	2.86		2.13		3.73		8.56		19.2	
Sand, medium (> 0.250 mm to 0.500 mm)	Percent	Dry	9.38		4.67		38.4		5.5		54.4	
Sand, fine (> 0.125 mm to 0.250 mm)	Percent	Dry	21.9		17.8		38.4		6.58		0.58	
Sand, very fine (> 0.0625 mm to 0.125 mm)	Percent	Dry	17.4		25.6		10.7		5.46		16.4	
Silt (0.0039 mm to 0.0625 mm)	Percent	Dry	39.1		40.2		4.58		33		0.8	
Clay (< 0.0039 mm)	Percent	Dry	5.11		3.67		0.75		13.4		0.24	
Sulfide, acid-volatile	µmol/g	Dry	0.23	J	0.11		0.11	J	0.254		0.005	U
Cadmium[a]	µmol/g	Dry	NA		NA		NA		NA		0.00047	U
Copper[a]	µmol/g	Dry	NA		NA		NA		NA		0.0076	
Lead[a]	µmol/g	Dry	NA		NA		NA		NA		0.003	U
Nickel[a]	µmol/g	Dry	NA		NA		NA		NA		0.005	
Silver[a]	µmol/g	Dry	NA		NA		NA		NA		0.001	U
Zinc[a]	µmol/g	Dry	NA		NA		NA		NA		0.0657	
Copper	mg/kg	Dry	34.2		39.3		72		36		6.8	
Mercury	mg/kg	Dry	0.148		0.095		0.017	J	0.038		0.007	J
Zinc	mg/kg	Dry	96		125		88.6		66.6		33.9	

Table 2. Analytical chemistry results for samples collected in fall 2009 and for MSC (cont.)

Component	Unit	Basis	GAS (sampled in the field)		GAS (sampled in laboratory prior to Task 3)		SRS		OSU		MSC	
Tetra-n-butyltin	µg/kg	Dry	0.85	U	0.75	U	0.55	U	0.82	U	0.46	U
Tri-n-butyltin cation	µg/kg	Dry	3.6		2.5		0.54	U	0.8	U	0.45	U
Di-n-butyltin cation	µg/kg	Dry	2.9		2.8		0.24	U	0.36	U	0.2	U
n-Butyltin cation	µg/kg	Dry	0.51	U	2.1		0.33	U	0.49	U	0.27	U
DRO	mg/kg	Dry	1,400	DZ	1,700	DZ	3.9	J	12	J	3.5	J
GRO	mg/kg	Dry	7.6	J	81	Y	2.1	U	3.4	U	1.6	U
RRO	mg/kg	Dry	2,200	DZ	3,600	DZ	34	J	99	J	12	J
2,4’-DDD	µg/kg	Dry	13	Ui	33		6.3	Ui	3.3	Ui	0.13	U
4,4’-DDD	µg/kg	Dry	42	PD	62	D	25	PD	0.11	U	0.11	U
2,4’-DDE	µg/kg	Dry	9.7	Ui	0.82	Ui	6.3	Ui	0.16	U	0.16	U
4,4’-DDE	µg/kg	Dry	4.5	Ui	12		3	JD	0.11	U	0.11	U
2,4’-DDT	µg/kg	Dry	6.1	Ui	22		12	PD	0.058	U	0.058	U
4,4’-DDT	µg/kg	Dry	1.7	U	49	D	10	PD	0.17	U	0.17	U
Aroclor 1016	µg/kg	Dry	20	Ui	2.1	U	12	Ui	2.1	U	2.1	U
Aroclor 1221	µg/kg	Dry	61	Ui	2.1	U	44	Ui	2.1	U	2.1	U
Aroclor 1232	µg/kg	Dry	140	Ui	2.1	U	96	Ui	2.1	U	2.1	U
Aroclor 1242	µg/kg	Dry	46	Ui	2.1	U	28	Ui	2.1	U	2.1	U
Aroclor 1248	µg/kg	Dry	12	Ui	2.1	U	5.4	Ui	2.1	U	2.1	U
Aroclor 1254	µg/kg	Dry	97	Ui	110		11	Ui	2.1	U	2.1	U
Aroclor 1260	µg/kg	Dry	15	Ui	2.1	U	12	Ui	2.1	U	2.1	U

Table 2. Analytical chemistry results for samples collected in fall 2009 and for MSC (cont.)

Component	Unit	Basis	GAS (sampled in the field)		GAS (sampled in laboratory prior to Task 3)	SRS		OSU		MSC	
Aroclor 1262	µg/kg	Dry	9.2	Ui	NA	5	Ui	2.1	U	2.1	U
Aroclor 1268	µg/kg	Dry	4.2	Ui	NA	2.1	U	2.1	U	2.1	U

a. These metals are simultaneously extracted metals.

Notes:
DRO: Diesel range organics.
GRO: Gasoline range organics.
RRO: Residual range organics.
TOC: Total organic carbon.
D: The reported result is from a dilution.
J: Estimated concentration. Result is greater than detection limit but less than reporting limit.
P: The gas chromatography (GC) or high-pressure liquid chromatography (HPLC) confirmation criterion was exceeded. The relative percent difference is greater than 40% between the two analytical results.
U: Analyte was not detected at or above the reporting limit.
i: The method reporting limit/method detection limit (MRL/MDL) or level of quantitation/level of detection (LOQ/LOD) is elevated due to a matrix interference. The chromatogram indicated the presence of non-target background components. The matrix interference prevented the adequate resolution of the target compound at the normal limit of detection.
Y: The chromatographic fingerprint of the sample resembles a petroleum product eluting in approximately the correct carbon range, but the elution pattern does not match the calibration standard. This could be due to mixtures of petroleum products and (or) degradation of petroleum products in the field-collected sample.
Z: The chromatographic fingerprint does not resemble a distinct petroleum product standard currently on file at CAS. This could be due to mixtures of petroleum products and (or) degradation of petroleum products in the field-collected sample.

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples)

Component	Unit	Basis	AR1	AR2	ARM	GA2	GAA	MAR	OST	SC1	SWI
Total solids	Percent	Wet	67.2	44.3	52.8	49	41.5	53.1	54.5	52.2	58.5
Ammonia as nitrogen	mg/kg	Dry	72.6	123	154	193	158	92.7	51.1	96.8	58.1
TOC	Percent	Dry	1.46	2.52	2.15	5.02	3.61	2.59	1.97	1.86	1.75
Gravel (> 2.00 mm)	Percent	Dry	9.86	0.71	0.4	0.58	0.03	0.83	2.35	0.14	0.82
Sand, very coarse (> 1.00 mm to 2.00 mm)	Percent	Dry	3.58	0.6	0.71	1.22	0.37	0.93	1.29	7.41	1.78
Sand, coarse (> 0.500 mm to 1.00 mm)	Percent	Dry	7.61	1.29	5.32	9.64	0.89	1.67	5.36	5.22	12.9
Sand, medium (> 0.250 mm to 0.500 mm)	Percent	Dry	20.1	6.32	10.4	6.65	1.22	3.01	29.9	7.52	33.1
Sand, fine (> 0.125 mm to 0.250 mm)	Percent	Dry	7.12	7.5	5.65	9.24	0.49	9.83	10	0.49	10
Sand, very fine (> 0.0625 mm to 0.125 mm)	Percent	Dry	10.1	7.03	7.59	18.3	12.67	30.2	5.72	31.8	8.36
Silt (0.0039 mm to 0.0625 mm)	Percent	Dry	37.9	70.7	63.6	47.8	73.3	50.3	45.8	44.2	27.9
Clay (< 0.0039 mm)	Percent	Dry	2.93	10.3	13.8	5.81	8.79	2.19	5.56	4.79	6.29
Sulfide, acid-volatile	uMole/g	Dry	0.031 J	0.489	0.038 J	0.344	0.095	0.096	0.019 J	0.044	0.036 J
Cadmium	uMole/g	Dry	0.0012	0.0013 U	0.0011 U	0.0013 U	0.0015 U	0.0011 U	0.0011 U	0.0021	0.0021
Copper	uMole/g	Dry	0.157	0.0326	0.0597	0.024	0.024	0.0238	0.0216	0.0954	0.105
Lead	uMole/g	Dry	0.384	0.007 U	0.01	0.013	0.008 U	0.024	0.015	0.046	0.017

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
Nickel	uMole/g	Dry	0.091		0.016		0.016		0.034		0.012	U	0.009		0.009		0.03		0.015	
Silver	uMole/g	Dry	0.002	U	0.0028	U	0.0023	U	0.0026	U	0.0032	U	0.0024	U	0.0024	U	0.0024	U	0.0022	U
Zinc	uMole/g	Dry	0.222		0.157		0.186		0.263		0.13	*	0.281	*	0.27		0.787		0.628	
Copper	mg/kg	Dry	166		92.6		47.6		53.5		52.2		101		37.5		71.6		85.6	
Mercury	mg/kg	Dry	0.086		0.065		0.138		0.259		0.094		0.187		0.079		0.208		0.114	
Zinc	mg/kg	Dry	119		130		112		145		118		193		221		281		256	
Tetra-n-butyltin	µg/kg	Dry	1.3	Ui	1	U	0.84	U	0.9	U	1.1	U	1.5	J	0.81	U	0.85	U	0.76	U
Tri-n-butyltin cation	µg/kg	Dry	6.2		1	J	1.1	J	1.4	J	1.2	J	130		1.3	J	21		70	
Di-n-butyltin cation	µg/kg	Dry	3.7		1.9	J	0.58	J	1.7	J	0.81	J	21		2.4		23		290	D
n-Butyltin cation	µg/kg	Dry	2.1		2	J	0.9	J	1.8	J	1.4	J	11		2		22		63	
DRO	mg/kg	Dry	160	H	85	Z	490	Y	1,300	Z	360	Z	340	Y	48	Z	300	H	210	H
GRO	mg/kg	Dry	11	Z	4.7	J	32	Y	17	Z	6.1	J	5.9	J	3.4	U	4.6	J	5.3	J
RRO	mg/kg	Dry	660	O	570	Z	700	O	2,600	O	1,000	Z	870	O	310	Z	1,400	O	1,000	O
2,4’-DDD	µg/kg	Dry	300	PD	89	D	17	P	26		8.2	Ui	2.4	Ui	2	Ui	3.7	Ui	11	
4,4’-DDD	µg/kg	Dry	470	PD	170	D	25		90	D	19		1.6		1.2	Ui	2.8		0.71	JP
2,4’-DDE	µg/kg	Dry	75	Ui	5.2	P	5.3	P	3.6	P	1.3	Ui	0.68	Ui	2.9	Ui	0.96	Ui	0.86	Ui
4,4’-DDE	µg/kg	Dry	190	D	150	D	16	P	15	P	5.6		1.3	P	1.5	Ui	2.8	Ui	1.8	P
2,4’-DDT	µg/kg	Dry	200	PD	170	D	4.8	Ui	6.8	Ui	2.5	Ui	1.9		4.7	P	11		18	Ui
4,4’-DDT	µg/kg	Dry	1,800	D	1,200	D	7		140	D	2.8	Ui	2.7		7.2		16		70	Ui

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
Aroclor 1016	µg/kg	Dry	910	Ui	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
Aroclor 1221	µg/kg	Dry	3,500	Ui	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
Aroclor 1232	µg/kg	Dry	2,400	Ui	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
Aroclor 1242	µg/kg	Dry	1,500	Ui	12	U	2.1	U	6.9	Ui	13	Ui	5.8	Ui	2.1	U	67		11	U
Aroclor 1248	µg/kg	Dry	850	Ui	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
Aroclor 1254	µg/kg	Dry	1,100	Ui	180	Ui	45	Ui	41	Ui	22	Ui	23	P	100		160		11	U
Aroclor 1260	µg/kg	Dry	960	Ui	13	Ui	43		45		12	Ui	22	Ui	49	Ui	2.1	U	610	D
Aroclor 1262	µg/kg	Dry	210	U	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
Aroclor 1268	µg/kg	Dry	210	U	12	U	2.1	U	2.2	U	2.6	U	2.1	U	2.1	U	2.1	U	11	U
cis/trans-Decalin	µg/kg	Dry	3.2	J	1.4	U	8.3		53	D	8	U	6.9		1.4	U	1.4	U	3.3	J
C1-Decalins	µg/kg	Dry	15	J	3.1	J	25	J	88	JD	26	JD	27	J	1.4	U	7.2	J	11	J
C2-Decalins	µg/kg	Dry	65	J	9.6	J	58	J	350	JD	100	JD	71	J	1.4	U	23	J	37	J
C3-Decalins	µg/kg	Dry	100	J	22	J	82	J	730	JD	170	JD	140	J	1.4	U	62	J	65	J
C4-Decalins	µg/kg	Dry	140	J	33	J	100	J	820	JD	190	JD	230	J	1.4	U	110	J	120	J
Benzo(b)thiophene	µg/kg	Dry	0.9	U	0.88	J	10		280	D	52	D	21		0.87	U	1.4	J	1.5	J
C1-Benzothiophenes	µg/kg	Dry	3.1	J	0.87	U	22	J	560	JD	90	JD	10	J	0.87	U	1.7	J	2.2	J
C2-Benzothiophenes	µg/kg	Dry	6.3	J	0.87	U	68	J	1,400	JD	220	JD	15	J	0.87	U	3.2	J	3.3	J
C3-Benzothiophenes	µg/kg	Dry	6.5	J	1.2	J	70	J	1,300	JD	240	JD	19	J	0.87	U	3.2	J	5.6	J
C4-Benzo(b)thiophenes	µg/kg	Dry	15	J	0.87	U	62	J	1,200	JD	230	JD	41	J	0.87	U	7.7	J	5	J
Naphthalene	µg/kg	Dry	12		6.1		110		2,900	D	790	D	350		9.4		17		23	

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
C1-Naphthalenes	µg/kg	Dry	12	J	4.2	J	72	J	4,200	JD	1,000	JD	96	J	5.3	J	13	J	9.7	J
C2-Naphthalenes	µg/kg	Dry	50	J	6.9	J	370	J	9,000	JD	2,300	JD	120	J	6.3	J	30	J	25	J
C3-Naphthalenes	µg/kg	Dry	100	J	6.8	J	440	J	8,300	JD	2,200	JD	200	J	5.8	J	53	J	50	J
C4-Naphthalenes	µg/kg	Dry	110	J	8.1	J	260	J	4,900	JD	1,200	JD	290	J	6.3	J	41	J	34	J
Biphenyl	µg/kg	Dry	3.8	J	2.1	J	26		370	D	320	D	28		1.8	J	8.9		5.1	
Dibenzofuran	µg/kg	Dry	10		3.9	J	24		670	D	240	D	55		2.7	J	18		8.7	
Acenaphthylene	µg/kg	Dry	6.4		3.5	J	33		830	D	200	D	65		3.1	J	8.8		21	
Acenaphthene	µg/kg	Dry	8.9		6.2		480		7,100	D	880	D	99		4.4	J	27		18	
Fluorene	µg/kg	Dry	20		8		260		5,400	D	2,600	D	110		4.7		34		20	
C1-Fluorenes	µg/kg	Dry	22	J	3.3	J	130	J	3,400	JD	920	JD	82	J	2.7	J	20	J	19	J
C2-Fluorenes	µg/kg	Dry	72	J	11	J	170	J	4,800	JD	1,200	JD	310	J	7.7	J	47	J	43	J
C3-Fluorenes	µg/kg	Dry	110	J	9.9	J	170	J	4,800	JD	1,200	JD	540	J	6.9	J	63	J	62	J
Anthracene	µg/kg	Dry	71		9.2		270		12,000	D	19,000	D	150		9		99		77	
Phenanthrene	µg/kg	Dry	210		50		1,900	D	40,000	D	9,500	D	660		27		400		240	
C1-Phenanthrenes/ Anthracenes	µg/kg	Dry	170	J	25	J	490	J	23,000	JD	5,700	JD	470	J	20	J	200	J	160	J
C2-Phenanthrenes/ Anthracenes	µg/kg	Dry	230	J	34	J	350	J	18,000	JD	4,400	JD	800	J	26	J	180	J	140	J
C3-Phenanthrenes/ Anthracenes	µg/kg	Dry	240	J	33	J	230	J	11,000	JD	2,700	JD	970	J	31	J	160	J	120	J
C4-Phenanthrenes/ Anthracenes	µg/kg	Dry	240	J	43	J	180	J	6,100	JD	1,900	JD	940	J	8.8	J	140	J	130	J

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
Retene	µg/kg	Dry	130		63		160		1,500	D	1,700	D	410		100		38		71	
Dibenzothiophene	µg/kg	Dry	15		3.5	J	250		5,400	D	1,200	D	74		2.8	J	23		27	
C1-Dibenzothiophenes	µg/kg	Dry	33	J	3.9	J	110	J	4,400	JD	980	JD	110	J	3.4	J	26	J	38	J
C2-Dibenzothiophenes	µg/kg	Dry	98	J	9.8	J	120	J	5,300	JD	1,300	JD	280	J	7.3	J	51	J	55	J
C3-Dibenzothiophenes	µg/kg	Dry	110	J	13	J	110	J	4,500	JD	1,000	JD	390	J	9.1	J	74	J	82	J
C4-Dibenzothiophenes	µg/kg	Dry	120	J	32	J	63	J	2,100	JD	480	JD	270	J	0.43	U	76	J	94	J
Benzo(b)fluorene	µg/kg	Dry	150		14		120		7,000	D	1,300	D	170		7.9		82		97	
Fluoranthene	µg/kg	Dry	950		140		1,200		50,000	D	8,900	D	1,300		66		740		470	
Pyrene	µg/kg	Dry	800		130		1,400		61,000	D	10,000	D	1,400		70		680		500	
C1-Fluoranthenes/ Pyrenes	µg/kg	Dry	500	J	61	J	390	J	27,000	JD	5,200	JD	590	J	34	J	350	J	300	J
C2-Fluoranthenes/ Pyrenes	µg/kg	Dry	300	J	45	J	180	J	14,000	JD	3,100	JD	440	J	25	J	210	J	140	J
C3-Fluoranthenes/ Pyrenes	µg/kg	Dry	270	J	34	J	120	J	9,800	JD	2,200	JD	470	J	24	J	160	J	130	J
C4-Fluoranthenes/ Pyrenes	µg/kg	Dry	180	J	32	J	77	J	6,100	JD	1,100	JD	290	J	17	J	96	J	100	J
Naphthobenzo-thiophene	µg/kg	Dry	160		19		85		5,300	D	950	D	120		5.9		82		61	

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Units	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
C1-Naphthobenzo-thiophenes	µg/kg	Dry	140	J	17	J	66	J	5,600	JD	1,100	JD	160	J	9.6	J	94	J	97	J
C2-Naphthobenzo-thiophenes	µg/kg	Dry	160	J	27	J	50	J	4,400	JD	930	JD	170	J	18	J	120	J	170	J
C3-Naphthobenzo-thiophenes	µg/kg	Dry	150	J	41	J	65	J	3,700	JD	870	JD	160	J	25	J	150	J	230	J
C4-Naphthobenzo-thiophenes	µg/kg	Dry	82	J	33	J	35	J	1,200	JD	320	JD	91	J	22	J	99	J	180	J
Benz(a)anthracene	µg/kg	Dry	850		51		350		24,000	D	4,400	D	510		25		350		220	
Chrysene	µg/kg	Dry	1,200		160		480		28,000	D	5,300	D	630		40		450		330	
C1-Chrysenes	µg/kg	Dry	470	J	39	J	180	J	16,000	JD	3,400	JD	310	J	21	J	220	J	150	J
C2-Chrysenes	µg/kg	Dry	330	J	33	J	130	J	12,000	JD	2,700	JD	270	J	20	J	160	J	140	J
C3-Chrysenes	µg/kg	Dry	230	J	32	J	83	J	6,700	JD	1,400	JD	190	J	26	J	120	J	110	J
C4-Chrysenes	µg/kg	Dry	140	J	39	J	55	J	3,900	JD	730	JD	150	J	48	J	85	J	98	J
Benzo(b)fluoranthene	µg/kg	Dry	2,000		120		540		31,000	D	5,800	D	860		49		540		330	
Benzo(k)fluoranthene	µg/kg	Dry	600		33		150		7,900	D	1,700	D	230		15		160		100	
Benzo(a)fluoranthene	µg/kg	Dry	140		13		91		4,800	D	810	D	130		7.3		57		40	
Benzo(e)pyrene	µg/kg	Dry	1,100		65		360		20,000	D	4,000	D	550		34		320		180	
C30-Hopane	µg/kg	Dry	240		96		94		430	D	130	D	400		68		380		470	
Benzo(a)pyrene	µg/kg	Dry	940		73		520		31,000	D	5,500	D	740		42		350		210	
Perylene	µg/kg	Dry	310		35		170		7,700	D	1,400	D	260		40		140		110	

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1		AR2		ARM		GA2		GAA		MAR		OST		SC1		SWI	
Indeno(1,2,3-cd)pyrene	µg/kg	Dry	860		55		450		22,000	D	4,400	D	650		38		290		160	
Dibenz(a,h)anthracene	µg/kg	Dry	200		11		63		3,500	D	700	D	99		6.3		67		32	
Benzo(g,h,i)perylene	µg/kg	Dry	660		57		500		25,000	D	4,800	D	720		44		300		180	
4-Methyldibenzo-thiophene	µg/kg	Dry	12	J	1.2	J	37	J	1,400	JD	370	JD	34	J	1.2	J	8.3	J	13	J
2-Methyldibenzo-thiophene	µg/kg	Dry	11	J	0.93	J	36	J	1,500	JD	350	JD	26	J	1.1	J	7.3	J	11	J
1-Methyldibenzo-thiophene	µg/kg	Dry	3.4	J	0.43	U	11	J	440	JD	89	JD	15	J	0.43	U	2.2	J	3.7	J
3-Methylphenanthrene	µg/kg	Dry	38	J	5.5	J	120	J	4,900	JD	1,200	JD	95	J	4.2	J	46	J	37	J
2-Methylphenanthrene	µg/kg	Dry	47	J	6.7	J	140	J	6,600	JD	1,500	JD	120	J	4.8	J	55	J	38	J
2-Methylanthracene	µg/kg	Dry	16	J	2.5	J	43	J	2,600	JD	1,300	JD	30	J	1.9	J	22	J	18	J
9-Methylphenanthrene	µg/kg	Dry	31	J	4.6	J	91	J	4,700	JD	960	JD	110	J	3.6	J	32	J	32	J
1-Methylphenanthrene	µg/kg	Dry	31	J	4.2	J	88	J	3,600	JD	830	JD	81	J	3.4	J	33	J	31	J
2-Methylnaphthalene	µg/kg	Dry	9.5		3.7	J	66		2,900	D	1,000	D	94		4.7		11		7.5	
1-Methylnaphthalene	µg/kg	Dry	6.6		2.5	J	39		3,500	D	500	D	46		2.4	J	7.6		5.3	
2,6-Dimethylnaph-thalene	µg/kg	Dry	24		2.4	J	120		3,800	D	1,300	D	64		2.8	J	11		9.5	

Table 3. Analytical chemistry results for samples collected in summer 2010 (Harbor sediment samples) (cont.)

Component	Unit	Basis	AR1	AR2		ARM	GA2		GAA		MAR	OST		SC1	SWI
2,3,5-Trimethylnaph-thalene	µg/kg	Dry	26	2.2	J	130	2,500	D	500	D	60	2.1	J	13	11
Carbazole	µg/kg	Dry	9.4	3.2	J	10	1,600	D	4,400	D	35	1.6	J	23	11

Notes:
*: The result is an outlier. See case narrative.
D: The reported result is from a dilution.
H: The chromatographic fingerprint of the sample resembles a petroleum product, but the elution pattern indicates the presence of a greater amount of heavier molecular weight constituents than the calibration standard.
J: Estimated concentration. Result is greater than detection limit but less than reporting limit.
O: The chromatographic fingerprint of the sample resembles an oil but does not match the calibration standard.
P: The GC or HPLC confirmation criterion was exceeded. The relative percent difference is greater than 40% between the two analytical results.
U: Analyte was not detected at or above the reporting limit.
i: The MRL/MDL or LOQ/LOD is elevated due to a matrix interference. The chromatogram indicated the presence of non-target background components. The matrix interference prevented adequate resolution of the target compound at the normal limit of detection.
Y: The chromatographic fingerprint of the sample resembles a petroleum product eluting in approximately the correct carbon range, but the elution pattern does not match the calibration standard. This could be due to mixtures of petroleum products and (or) degradation of petroleum products in the field-collected sample.
Z: The chromatographic fingerprint does not resemble a distinct petroleum product standard currently on file at CAS. This could be due to mixtures of petroleum products and (or) degradation of petroleum products in the field-collected sample.

Table 4. Analytical chemistry results for samples collected in summer 2010 (Willamette River reference samples)

Component	Unit	Basis	RE1		RE2		RE3	
Total solids	Percent	Wet	50		44.5		49.1	
Ammonia as nitrogen	mg/kg	Dry	100		84.2		59.7	
TOC	Percent	Dry	2.16		2.7		1.79	
Gravel (> 2.00 mm)	Percent	Dry	0.16		0		0.19	
Sand, very coarse (> 1.00 mm to 2.00 mm)	Percent	Dry	1.09		0.72		0.41	
Sand, coarse (> 0.500 mm to 1.00 mm)	Percent	Dry	1.01		0.44		0.67	
Sand, medium (> 0.250 mm to 0.500 mm)	Percent	Dry	2.57		0.39		5.85	
Sand, fine (> 0.125 mm to 0.250 mm)	Percent	Dry	14.3		1.24		21.5	
Sand, very fine (> 0.0625 mm to 0.125 mm)	Percent	Dry	31.2		5.25		12.6	
Silt (0.0039 mm to 0.0625 mm)	Percent	Dry	52.1		75.4		52.3	
Clay (< 0.0039 mm)	Percent	Dry	3.58		19		8.84	
Sulfide, acid-volatile	uMole/g	Dry	0.011	U	0.009	U	0.012	U
Cadmium	uMole/g	Dry	0.0012	U	0.00095	U	0.0012	U
Copper	uMole/g	Dry	0.0374		0.0599		0.0311	
Lead	uMole/g	Dry	0.006	U	0.005		0.007	U
Nickel	uMole/g	Dry	0.016		0.012		0.01	U
Silver	uMole/g	Dry	0.0024	U	0.002	U	0.0026	U
Zinc	uMole/g	Dry	0.114		0.0837		0.0806	*
Copper	mg/kg	Dry	34.8		42.9		37.5	
Mercury	mg/kg	Dry	0.046		0.06		0.041	
Zinc	mg/kg	Dry	80		92.5		83	

Table 4. Analytical chemistry results for samples collected in summer 2010 (Willamette River reference samples) (cont.)

Component	Unit	Basis	RE1		RE2		RE3	
Tetra-n-butyltin	µg/kg	Dry	0.88	U	0.98	U	0.9	U
Tri-n-butyltin Cation	µg/kg	Dry	0.86	U	0.96	U	0.88	U
Di-n-butyltin Cation	µg/kg	Dry	0.38	U	0.51	J	0.39	U
n-Butyltin Cation	µg/kg	Dry	0.55	J	0.76	J	0.56	J
DRO	mg/kg	Dry	30	J	56	J	34	J
GRO	mg/kg	Dry	3.7	U	450	O	3.8	U
RRO	mg/kg	Dry	310	Z	4.3	U	260	Z
2,4’-DDD	µg/kg	Dry	0.13	U	0.35	J	0.14	U
4,4’-DDD	µg/kg	Dry	0.27	Ui	1.2	Ui	0.12	U
2,4’-DDE	µg/kg	Dry	0.16	U	0.18	U	0.17	U
4,4’-DDE	µg/kg	Dry	0.81	J	1.1	JP	0.87	J
2,4’-DDT	µg/kg	Dry	0.058	U	0.066	U	0.06	U
4,4’-DDT	µg/kg	Dry	1	Ui	0.61	J	0.18	U
Aroclor 1016	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1221	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1232	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1242	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1248	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1254	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1260	µg/kg	Dry	2.1	U	2.4	U	2.2	U

Table 4. Analytical chemistry results for samples collected in summer 2010 (Willamette River reference samples) (cont.)

Component	Unit	Basis	RE1		RE2		RE3	
Aroclor 1262	µg/kg	Dry	2.1	U	2.4	U	2.2	U
Aroclor 1268	µg/kg	Dry	2.1	U	2.4	U	2.2	U

Notes:
*: The result is an outlier. See case narrative.
J: Estimated concentration. Result is greater than detection limit but less than reporting limit.
O: The chromatographic fingerprint of the sample resembles an oil but does not match the calibration standard.
P: The GC or HPLC confirmation criterion was exceeded. The relative percent difference is greater than 40% between the two analytical results.
U: Analyte was not detected at or above the reporting limit.
i: The MRL/MDL or LOQ/LOD is elevated due to a matrix interference. The chromatogram indicated the presence of non-target background components. The matrix interference prevented adequate resolution of the target compound at the normal limit of detection.
Z: The chromatographic fingerprint does not resemble a distinct petroleum product standard currently on file at CAS. This could be due to mixtures of petroleum products and (or) degradation of petroleum products in the field-collected sample.

Figure 1. Ammocoete holding tank.

Over the course of 8 months (June 2010 to January 2011), only 10 mortalities in the 600+ ammocoetes held were observed in the holding tanks. Ambient temperature at the facility ranged from 12 to 14°C, DO ranged from 7.2 to 9.8 mg/L, pH ranged from 6.4 to 7.0, conductivity ranged from 270 to 320 µS/cm, and total ammonia levels were consistently below 0.3 ppm (Table 5). Stock holding methods demonstrated the ability for ammocoetes to be successfully reared under FPGL conditions and husbandry practices.

Table 5. Water quality monitoring results from ammocoete holding tanks

	Holding tank 1 (6/2/2010–10/28/2010)			Holding tank 2 (6/3/2010–12/15/2010)		
	n	Mean	Standard deviation	n	Mean	Standard deviation
EC (µS/cm)	41	334	147	97	311	96
Temperature (°C)	42	13.1	0.5	96	12.6	0.7
pH	42	6.7	0.1	97	6.7	0.1
DO (mg/L)	42	9.0	0.4	96	8.8	0.5
Ammonia (ppm)	5	0.3	0.1	17.0	0.3	0.2
Unionized ammonia (ppm)	5	0.0004	0.0002	17	0.0004	0.0002

EC: electrical conductivity.

1.3 Statistical Analysis

We conducted statistical comparisons with ANOVA ($\alpha = 0.05$) followed by Tukey HSD (honestly significant difference) post hoc pairwise comparisons using Minitab^{TM} Version 13.31 (Minitab Statistical Software, Minitab Inc.). In some cases, a Bonferroni adjustment of the alpha level was required to compare time series data. Also, some data required a log transformation in order to meet assumptions of equal variance. Cases where a Bonferroni adjustment or a log transformation were necessary are identified in the results section for each task where statistical significance among comparisons is reported.

2. Task 2a: Depuration Trial

2.1 Objective

We performed this trial to evaluate whether a depuration period is necessary before weighing ammocoetes that have been living in sediment to determine if abiotic particles in their gut significantly alter their weight. If a depuration period appears necessary for accurate measurements, depuration would be applied to all subsequent trials and tasks that involve weighing ammocoetes.

2.2 Methods

Two depuration trials were conducted. In the first trial, we tracked individuals over time and compared them to a control group. In the second trial, we measured a subset of ammocoetes at each time interval. In both trials, we attempted to use ammocoetes of similar sizes.

2.2.1 First depuration trial

Prior to the first depuration trial, 20 ammocoetes were held in a 75.6-L aquarium containing approximately 15 cm of OSU sediment for 2 weeks and fed the ammocoete diet described in Section 1.2.4. The water level was approximately 13 cm above the sediment. Water was delivered at the top of the tank through nylon tubing at a rate of 200 mL/min and drained from a cylindrical drainpipe at the opposite end of the tank.

After a 2-week holding period, 19 ammocoetes were recovered from the aquarium (one was lost, presumed deceased) and length and wet weight were measured immediately (0 hour depuration). Wet weight was measured by placing an ammocoete on a dry weigh-boat and recording to the nearest hundredth of a gram (0.01 g) (see PLA SOP P.7, Stratus Consulting, 2011d). Nine fish

were euthanized according to PLA SOP P.3 (Stratus Consulting, 2011d) and stored at -20°C for later analysis of dry weight and ash content.

An additional 10 ammocoetes were placed into separate 1-L beakers with no sediment for depuration. Because ammocoetes are not accustomed to being in open water, cotton balls were placed in each beaker to provide cover, reduce stress, and accommodate for ammocoetes' natural tendency to burrow and avoid light (Figure 2). Each static container was filled with 10 cm of well water, aerated with an air stone, and covered with black mesh to reduce ambient lighting. Ammocoetes were not fed during the depuration period. Wet-weight measurements were taken at 24, 48, and 72 hours from the start of the trial. After 72 hours, the ammocoetes were removed from the beakers, euthanized, and stored at -20°C for later analysis of dry weight and ash content.

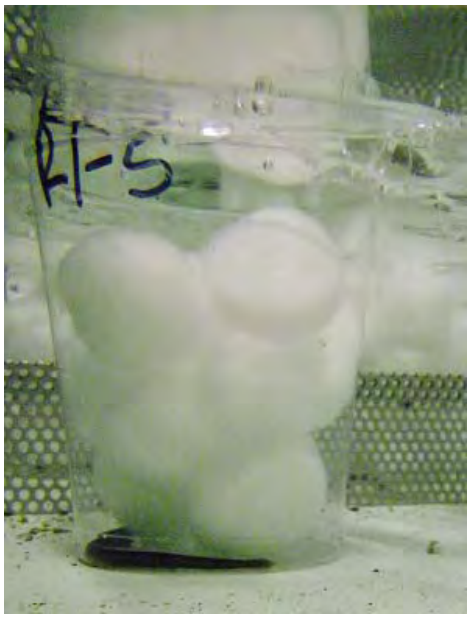


Figure 2. Ammocoete in beaker during depuration trial.

Water quality was measured in the holding tank at the initiation of the trial and in 3 randomly selected beakers at 0, 24, 48, and 72 hours.

Dry and ash weights were determined according to PLA SOP P.14 (Stratus Consulting, 2011d). The frozen samples were thawed and dried in a 60°C oven until their weights were constant. The dry weight of each sample was recorded. The dry samples were then ashed in a muffle furnace at 525°C for 5 hours to burn off all organic content, and the residual material was reweighed.

2.2.2 Second depuration trial

Thirty ammocoetes were removed directly from the holding tanks, and length and wet weight were measured immediately (0-hour depuration). Ten fish were euthanized according to PLA SOP P.3 (Stratus Consulting, 2011d). The remaining 20 ammocoetes were placed into an aquarium containing OSU sediment and held for 2 weeks. After 2 weeks, these 20 ammocoetes were placed into beakers, as in the first depuration trial. At 0, 24, 48, and 72 hours from the start of the trial, 5 individuals were removed from beakers, wet-weight measurements were recorded, and the individuals were euthanized according to PLA SOP P.3 (Stratus Consulting, 2011d).

Dry and ash weights for all ammocoetes were obtained in the same manner as described for the first depuration trial.

2.3 Results

Results from the first depuration trial are presented in Tables 6 and 7. Figure 3 presents the wet-weight measurements of the ammocoetes over time from the first trial. The mean weights of the ammocoetes decreased over time, with the largest decrease occurring in the first 24 hours. After 72 hours, the average decrease in the wet weight of ammocoetes was 29%. The mean wet weight at hour 0 was significantly ($P < 0.01$; Bonferroni-adjusted alpha level of 0.0125 based on time series data with four measurements through time) greater than the wet weights at hours 48 and 72. There were no other significant differences among wet weights (Figure 3).

Table 6. Measurements of non-depurated ammocoetes from first depuration trial

Ammocoete	Length (mm)	Wet weight (g)	Dry weight (g)	Ash weight (g)
A-1	85	1.07	0.12	0.014
A-2	89	1.11	0.12	0.010
A-3	90	1.02	0.10	0.013
A-4	83	0.92	0.10	0.012
A-5	77	0.91	0.07	0.006
A-6	89	1.19	0.14	0.014
A-7	94	1.29	0.16	0.012
A-8	88	0.83	0.08	0.009
A-9	77	0.67	0.06	0.006
Mean	86	1.00	0.105	0.011
Standard deviation	6	0.19	0.034	0.003

Table 7. Measurements of depurated ammocoetes from first depuration trial

Ammocoete	Length (mm)	0 hours wet weight (g)	24 hours wet weight (g)	48 hours wet weight (g)	72 hours wet weight (g)	72 hours dry weight (g)	72 hours ash weight (g)
A-11	86	1.02	0.87	0.77	0.78	0.10	0.006
A-12	83	0.86	0.68	0.66	0.65	0.08	0.005
A-13	90	1.87	1.08	1.07	1.01	0.15	0.007
A-14	89	1.26	1.00	0.92	0.89	0.11	0.007
A-15	87	1.43	1.05	0.86	0.88	0.12	0.008
A-16	84	0.89	0.74	0.76	0.72	0.08	0.006
A-17	83	0.97	0.94	0.85	0.84	0.10	0.006
A-18	78	1.19	0.62	0.62	0.66	0.07	0.005
A-19	90	1.03	1.07	0.99	0.96	0.11	0.006
A-20	74	0.70	0.63	0.56	0.56	0.06	0.004
Mean	84	1.12	0.87	0.81	0.80	0.098	0.006
Standard deviation	5	0.34	0.19	0.16	0.15	0.027	0.001

The depurated ammocoetes from the first trial (72-hour total depuration period) had a significantly ($P < 0.01$; based on analysis of log-transformed data to meet assumptions of equal variance in the data) lower ash content (6.2%, based on dry weight) than non-depurated ammocoetes (10.5%, based on dry weight; Figure 4). This may indicate that the non-depurated ammocoetes had more inorganic material in their gut. This is supported by the visual observation of fecal material in the ash from the non-depurated ammocoetes that was not observed in the depurated ammocoetes (Figure 5).

Results from the second depuration trial are presented in Tables 8 and 9. The second depuration trial resulted in an average loss of 8.2, 8.2, and 7.1% body weight for the 24-, 48-, and 72-hour groups, respectively (Figure 6). In the second trial, percent ash to dry body mass was not significantly different among depuration groups (Figure 7). This indicates that ammocoetes may have had variable amounts of sediment in their guts or that elimination may occur at different rates among individuals. We detected no significant differences among depurated and non-depurated fish, which is contrary to the results in the initial trial.

Water quality monitoring results from the second trial are presented in Table 10.

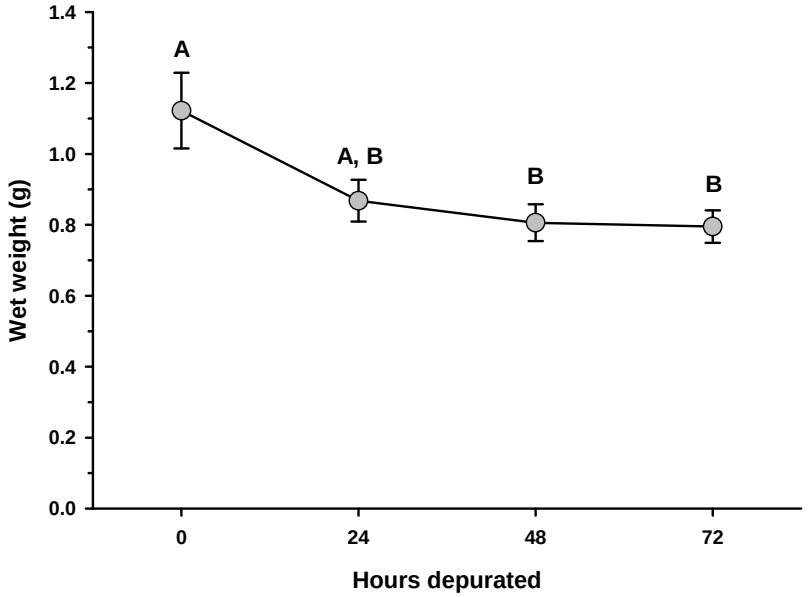


Figure 3. Mean wet-weight measurements from first depuration trial (n = 10). Error bars are ± one standard error of the mean. Treatments that are significantly different ($P < 0.01$) from each other are indicated with different uppercase letters.

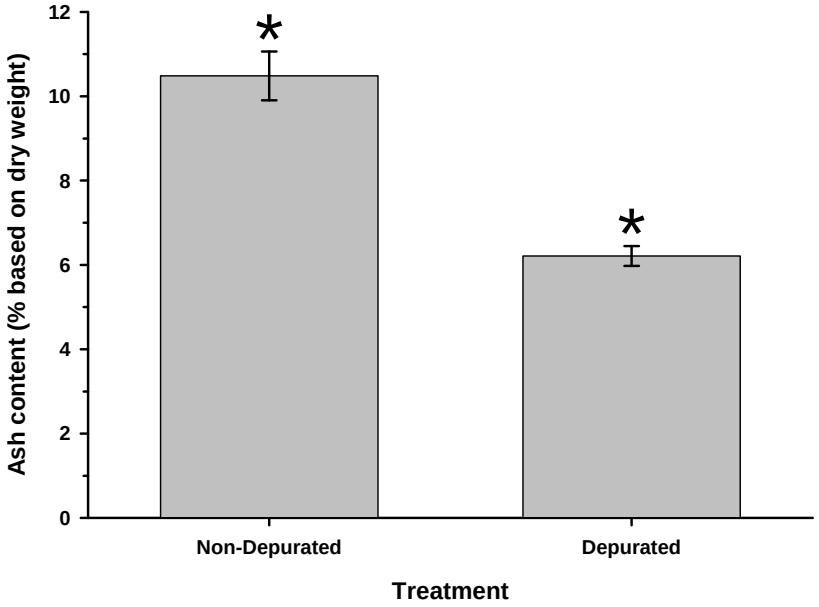


Figure 4. Mean ash content of non-depurated and depurated (72 hours) ammocoetes from first depuration trial (n = 9). Error bars are ± one standard error of the mean. Treatments that are significantly different ($P < 0.01$) from each other are indicated with an asterisk.

Figure 5. Photographs of ash from depurated (left) and non-depurated (right) ammocoetes. A fecal pellet is present in the non-depurated ash (red circle).

Table 8. Measurements of non-depurated ammocoetes from second depuration trial

Ammocoete	Length (mm)	Wet weight (g)	Dry weight (g)	Ash weight (g)
HT-1	90.5	1.39	0.29	0.008
HT-2	71.5	0.64	0.13	0.005
HT-3	68	0.49	0.05	0.004
HT-4	78	0.62	0.10	0.005
HT-5	79.5	0.92	0.15	0.006
HT-6	69	0.53	0.07	0.003
HT-7	86	0.80	0.09	0.006
HT-8	91	1.35	0.16	0.008
HT-9	74	0.69	0.08	0.005
HT-10	76.5	0.82	0.11	0.005
Mean	78	0.83	0.12	0.006
Standard deviation	8	0.32	0.07	0.002

Table 9. Measurements of depurated ammocoetes from second depuration trial

Ammocoete	Hours depurated	Length (mm)	Initial wet weight (g)	Final wet weight (g)	Wet-weight loss (%)	Dry weight (g)	Ash weight (g)
A-1	0	87.5	0.99	0.99	N/A	0.185	0.008
A-2	0	92	1.15	1.15	N/A	0.262	0.010
A-3	0	82.5	0.88	0.88	N/A	0.095	0.007
A-4	0	78	0.86	0.86	N/A	0.171	0.009
A-5	0	93	1.2	1.2	N/A	0.146	0.011
A-6	24	115	2.53	2.43	4.0	0.552	0.017
A-7	24	92	1.12	0.98	12.5	0.124	0.006
A-8	24	95	1.11	1.02	8.1	0.122	0.008
A-9	24	104	1.7	1.58	7.1	0.268	0.011
A-10	24	96	1.05	0.95	9.5	0.108	0.007
A-11	48	105	1.74	1.67	4.0	0.223	0.012
A-12	48	73	0.71	0.64	9.9	0.077	0.003
A-13	48	73	0.62	0.58	6.5	0.099	0.003
A-14	48	97.5	1.2	1.1	8.3	0.125	0.009
A-15	48	73.5	0.49	0.43	12.2	0.046	0.002
A-16	72	90	0.98	0.93	5.1	0.109	0.007
A-17	72	100.5	1.53	1.46	4.6	0.184	0.011
A-18	72	94	0.94	0.85	9.6	0.102	0.017
A-19	72	95	1.29	1.16	10.1	0.148	0.008
A-20	72	97	1.01	0.95	5.9	0.098	0.008

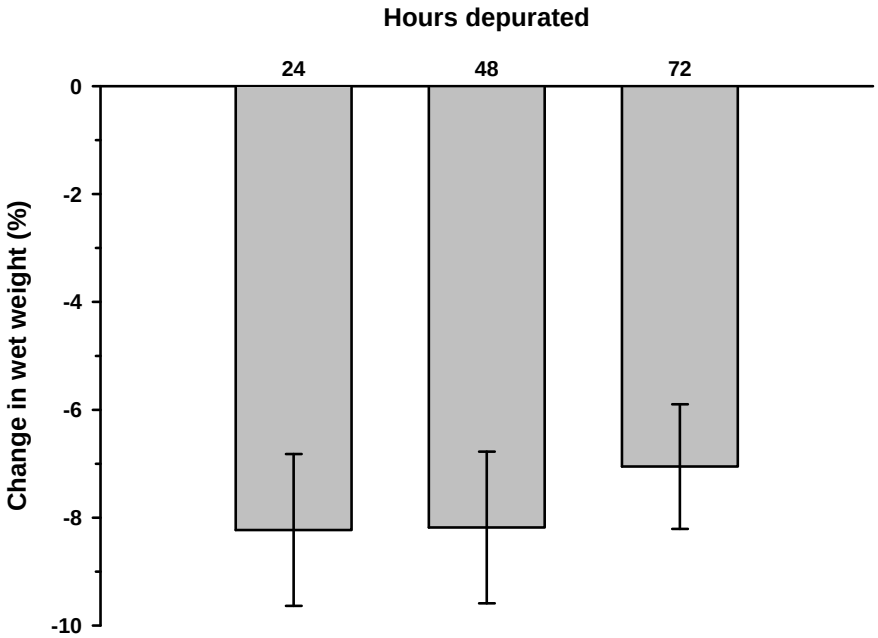


Figure 6. Mean change in wet weight for ammocoetes depurated for 24, 48, and 72 hours from second depuration trial (n = 5). Error bars are ± one standard error of the mean.

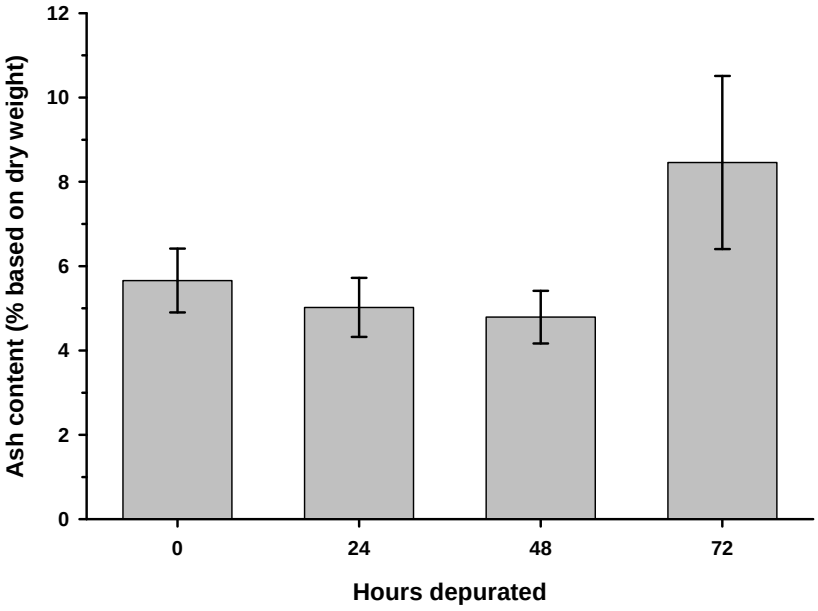


Figure 7. Mean ash content in ammocoetes depurated for 0, 24, 48, and 72 hours from second depuration trial (n = 5). Error bars are ± one standard error of the mean.

Table 10. Water quality monitoring results from second depuration trial

Hour	Container	Temperature (°C)	pH	DO (mg/L)	EC (µS/cm)
0	Holding tank	13.0	6.8	8.5	848
	A-11	15.0	7.3	7.2	836
	A-9	14.9	7.3	7.4	831
	A-15	15.0	7.3	7.3	824
24	A-16	15.0	7.4	7.4	825
	A-15	14.8	7.4	7.5	831
	A-9	15.0	7.4	7.4	829
48	A-17	13.7	7.2	7.3	841
	A-18	13.6	7.4	7.4	842
	A-12	13.6	7.4	7.5	852
72	A-18	15.8	7.3	7.8	848
	A-17	15.8	7.4	7.9	848
	A-19	15.7	7.4	8.0	848

2.4 Discussion

The results of the two depuration trials were variable with the first trial indicating that a depuration period may result in significant weight loss due to the evacuation of inorganic material from the ammocoete gut. Given that the two depuration trials used different methods (repeated weighing of the same individuals in the first depuration trial and weighing of different individuals in the second depuration trial), the results cannot be directly compared. However, because the first depuration trial indicated that the largest decrease in weight occurred over the first 24 hours, we opted to implement a 24-hour depuration period before and after each trial to reduce variability in weights introduced by gut content.

3. Task 2b: Holding Container (Corral) Trial

3.1 Objective

The objective of the holding container trial was to determine if different-diameter holding containers (corrals) significantly affect ammocoete growth.

3.2 Methods

For this trial, we held ammocoetes in several different containers for 30 days and evaluated changes in weight and length. We compared a mixed population (no corrals) with 3 sizes of 1/16-in. mesh isolation corrals (3-in., 4-in., and 6-in. diameter; PLA SOP P.8; Stratus Consulting, 2011d) and two types of tanks (12-in. diameter round and 21.5-gal rectangular). Table 11 summarizes the treatments, and Figure 8 shows the round and rectangular tanks. Each corral and the surrounding tank area were filled with 10.2 cm of OSU sediment. Corrals were suspended from the lip of each tank, with their top portion open to allow for individualized feeding and observation.

Table 11. Summary of corral trial setup

Tank	Subcontainer	Number of tanks	Ammocoetes per tank	Total ammocoetes
12-in. round	None	3	5	15
12-in. round	3-in. mesh bags	2	5	10
21.5-gal rectangle	3-in. mesh bags	1	10	10
21.5-gal rectangle	4-in. mesh bags	1	10	10
21.5-gal rectangle	6-in. mesh bags	2	5	10

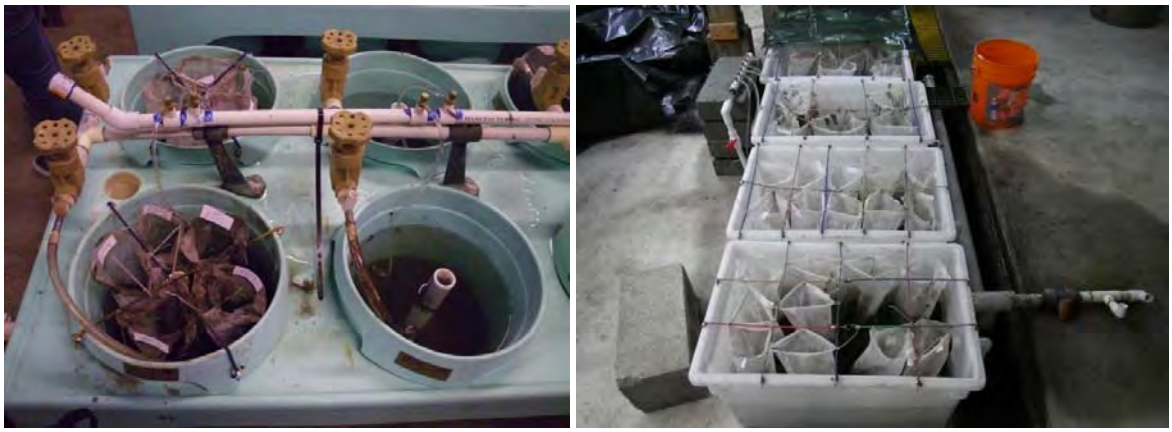


Figure 8. Holding containers used in corral trials. Round tanks are shown in the left-hand photograph (individual mesh bags in tank on the left, mixed population in tank on the right). Rectangular tanks are shown in the right-hand photograph.

All animals received 3 mL of the ammocoete diet (described in Section 1.2.4) used for ammocoetes in the stock tank at the surface three times a week for 30 days. During the trial, well water was delivered to each rectangular tank at a rate of approximately 200 mL/min through nylon tubing and drained from a covered horizontal drainpipe at the opposite end of the tank. Water was delivered to each round tank at a rate of approximately 150 mL/min through nylon tubing placed near the standpipe in the middle of each tank. Water quality testing was conducted on a subset of experimental tanks daily.

All ammocoetes were depurated for 24 hours before the initiation of the trial. Weights and lengths were measured according to PLA SOP P.7 (Stratus Consulting, 2011d) and ammocoetes were placed into their respective containers according to PLA SOP P.9 (Stratus Consulting, 2011d) and held for 30 days. After 30 days, the ammocoetes were removed from the sediments, depurated for 24 hours, and weights and lengths were measured again.

3.3 Results

No mortality was observed in any of the treatments. Table 12 presents the pre- and post-trial weights and lengths of each ammocoete in the mesh corral exposures and the average pre- and post-trial weights and lengths of the five ammocoetes in each 12-in. round mixed population tank. Table 13 summarizes the water quality measurements in each tank over the trial period.

Table 12. Weights and lengths of ammocoetes in corral trials

Tank/ ammocoete ID	Tank	Subcontainer	Initial length (mm)	Initial wet weight (g)	Final length (mm)	Final wet weight (g)
T1-1	21.5-gal rectangle	3-in. corrals	77.5	0.57	76.0	0.54
T1-2	21.5-gal rectangle	3-in. corrals	102.0	1.36	103.0	1.32
T1-3	21.5-gal rectangle	3-in. corrals	102.0	1.57	105.0	1.44
T1-4	21.5-gal rectangle	3-in. corrals	87.5	1.02	83.0	0.59
T1-5	21.5-gal rectangle	3-in. corrals	91.5	1.13	92.0	1.03
T1-6	21.5-gal rectangle	3-in. corrals	79.0	0.60	81.0	0.54
T1-7	21.5-gal rectangle	3-in. corrals	76.5	0.60	74.5	0.56
T1-8	21.5-gal rectangle	3-in. corrals	80.0	0.60	83.0	0.54
T1-9	21.5-gal rectangle	3-in. corrals	86.5	0.95	85.0	0.81
T1-10	21.5-gal rectangle	3-in. corrals	86.0	1.07	85.0	0.91
T2-1	21.5-gal rectangle	4-in. corrals	85.0	0.94	83.0	0.68
T2-2	21.5-gal rectangle	4-in. corrals	87.5	0.97	85.0	0.82

Table 12. Weights and lengths of ammocoetes in corral trials (cont.)

Tank/ ammocoete ID	Tank	Subcontainer	Initial length (mm)	Initial wet weight (g)	Final length (mm)	Final wet weight (g)
T2-3	21.5-gal rectangle	4-in. corrals	101.0	1.64	102.0	1.47
T2-4	21.5-gal rectangle	4-in. corrals	82.5	0.84	85.0	0.75
T2-5	21.5-gal rectangle	4-in. corrals	97.0	1.48	99.0	1.33
T2-6	21.5-gal rectangle	4-in. corrals	90.0	1.09	95.0	1.00
T2-7	21.5-gal rectangle	4-in. corrals	91.5	1.18	92.5	1.05
T2-8	21.5-gal rectangle	4-in. corrals	72.0	0.54	70.0	0.50
T2-9	21.5-gal rectangle	4-in. corrals	98.0	1.40	98.0	1.18
T2-10	21.5-gal rectangle	4-in. corrals	71.5	0.56	70.0	0.58
T3-1	21.5-gal rectangle	6-in. corrals	92.5	1.15	94.0	1.11
T3-2	21.5-gal rectangle	6-in. corrals	103.0	1.58	103.0	1.41
T3-3	21.5-gal rectangle	6-in. corrals	83.5	0.87	85.0	0.83
T3-4	21.5-gal rectangle	6-in. corrals	94.0	1.17	93.5	1.00
T3-5	21.5-gal rectangle	6-in. corrals	100.5	1.50	104.0	1.38
T4-6	21.5-gal rectangle	6-in. corrals	84.0	0.93	82.0	0.75
T4-7	21.5-gal rectangle	6-in. corrals	89.5	1.01	91.0	0.92
T4-8	21.5-gal rectangle	6-in. corrals	91.0	1.16	90.5	1.04
T4-9	21.5-gal rectangle	6-in. corrals	89.0	1.09	89.0	1.02
T4-10	21.5-gal rectangle	6-in. corrals	99.0	1.26	99.0	1.13
R1-1	12-in. round	3-in. corrals	64.0	0.42	63.5	0.40
R1-2	12-in. round	3-in. corrals	85.5	0.91	83.5	0.82
R1-3	12-in. round	3-in. corrals	88.0	0.83	82.0	0.76
R1-4	12-in. round	3-in. corrals	87.0	0.94	84.5	0.80
R1-5	12-in. round	3-in. corrals	85.0	0.90	84.0	0.75
R2-1	12-in. round	3-in. corrals	93.5	1.01	91.0	0.85
R2-2	12-in. round	3-in. corrals	77.0	0.79	75.0	0.57
R2-3	12-in. round	3-in. corrals	83.5	0.99	84.5	0.86
R2-4	12-in. round	3-in. corrals	95.5	0.97	93.0	0.79
R2-5	12-in. round	3-in. corrals	93.0	1.17	91.0	1.00
R3	12-in. round	NA (mean of 5)	86.9	0.98	84.1	0.85
R4	12-in. round	NA (mean of 5)	81.2	0.91	79.4	0.77
R5	12-in. round	NA (mean of 5)	90.9	1.07	86.3	0.89

Table 13. Water quality monitoring results from corral trials

		Temperature (°C)		pH		DO (mg/L)		EC (µS/cm)	
Container	n	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
R1	8	13.5	0.32	6.66	0.08	8.9	0.34	288	4.50
R2	9	13.1	0.30	6.65	0.05	8.8	0.35	288	7.26
R3	6	13.3	0.33	6.64	0.08	8.9	0.27	290	4.27
R4	7	13	0.32	6.64	0.04	9.0	0.40	290	3.09
R5	5	13.1	0.24	6.68	0.06	9.0	0.14	288	2.30
T1	9	13.4	0.74	6.62	0.05	8.5	0.46	289	1.87
T2	8	13.1	0.32	6.63	0.03	8.6	0.49	290	2.53
T3	4	13.3	0.13	6.67	0.03	8.8	0.18	286	8.92
T4	4	13.1	0.56	6.63	0.03	8.9	0.30	289	1.63

Over the 30-day trial, all individuals but one (ammocoete T2-10) in the individual corrals lost weight. The pooled results from each of the round tanks without corrals suggested that these ammocoetes also lost weight (Figure 9). There was no significant difference in the weight lost between ammocoetes held in the corrals in the rectangular tanks and the corrals in the round tanks. The weight loss in the ammocoetes held in a mixed population in the round tanks was very similar to the weight loss in ammocoetes held in 3-in. corrals in the round tanks. However, no statistical comparison can be made because the initial and final weights of ammocoetes in the mixed population are based on the average weights of all the ammocoetes (i.e., we could not track individual ammocoetes in the mixed population).

3.4 Discussion

Although all ammocoetes in this trial lost weight, there were no significant differences among any of the different corral diameters. Also, no differences were seen between ammocoetes that were held in a corral and those that were allowed to roam free in the exposure tank. Therefore, in subsequent trials (Tasks 3 and 7), a 3-in. corral in a round tank was utilized because this configuration maximized the use of space in the laboratory while maintaining the ability to individually track each ammocoete's growth.

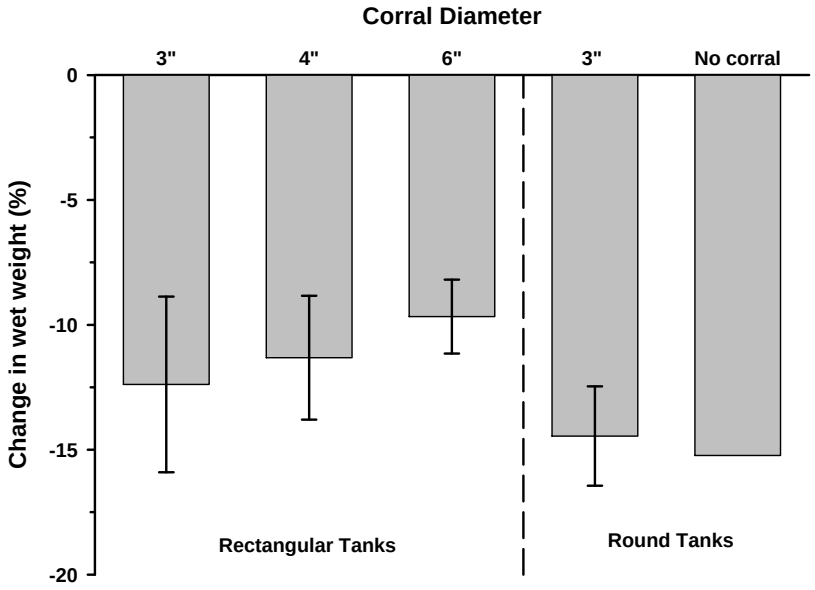


Figure 9. Mean change in wet weight in ammocoetes held in corrals of differing sizes (n = 10 for all the "corral" treatments and n = 1 for the "no corral" treatment). Error bars are ± one standard error of the mean.

4. Task 2c: Feeding Trial

4.1 Objective

The objective of this trial was to determine if ammocoete growth is affected by the type of water delivered to each exposure tank.

4.2 Methods

For this trial, we compared water type and feeding treatments for ammocoetes (Table 14). We evaluated the growth of ammocoetes over a 30-day period in OSU sediment with well water alone, well water plus the ammocoete diet described in Task 2a, and conditioned well water.[2] In addition, we investigated a treatment of SRS sediment where ammocoetes were exposed to well water plus the ammocoete diet.

2. Well water was biologically conditioned in a 1,890-L (500-gal) outdoor flume containing a mixture of cobble/wood chips that was initially seeded with yeast to promote biological activity. Flows in the conditioning flume were minimal. Water was brought within 0.5°C of ambient FPGL temperature before being introduced to treatment tanks at a rate of approximately 120 mL/min.

Table 14. Summary of feeding trial setup

Sediment	Water type	Feeding	Notes
SRS	Well	Ammocoete diet	
OSU	Well	None	
OSU	Well	Ammocoete diet	Results from Task 2b because treatment was duplicative
OSU	Conditioned well	None	

Before being placed into exposure tanks, ammocoetes were removed from the holding tank and depurated for 24 hours. They were measured and weighed according to PLA SOP P.7 (Stratus Consulting, 2011d). Each treatment consisted of three 12-in. diameter round tanks with 5 ammocoetes in each, for a total of 15 ammocoetes per treatment. Water was delivered to each round tank at a rate of approximately 150 mL/min through nylon tubing at standpipe in the middle of each tank. Water quality testing was conducted daily on a subset of experimental tanks. After 30 days of exposure, ammocoetes were removed from the tanks, depurated for 24 hours, and re-weighed and measured.

4.3 Results

No mortality was observed in any of the treatments. Table 15 presents the average pre- and post-trial weights and lengths of the five ammocoetes in each 12-in. round mixed population tank. The pooled weights of ammocoetes in all treatments and replicates indicate that the ammocoetes in all treatments lost weight over the 30-day period (Figure 10). There were no significant differences in weight loss among any of the treatments. Water quality monitoring data for each tank are presented in Table 16.

4.4 Discussion

Conditioning the well water did not significantly affect the change in weight or survival in any of the treatments. Therefore, well water was used in subsequent trials.

Table 15. Weights and lengths of ammocoetes in feeding trial

				Initial length (mm)		Initial wet weight (g)		Final length (mm)		Final wet weight (g)	
Sediment	**Water type**	**Feeding**	**Tank**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
SRS	Well	Ammocoete diet	R13	86.2	5.6	0.91	0.26	83.4	6.6	0.77	0.27
			R14	85.6	8.9	0.94	0.31	83.3	10.2	0.76	0.27
			R15	86.8	16.6	1.13	0.58	84.6	16.9	0.91	0.50
OSU	Well	None	R7	92.1	6.1	1.07	0.20	88.1	6.6	0.86	0.19
			R8	90.3	9.8	1.09	0.35	89.8	9.6	0.91	0.30
			R9	86.8	9.4	0.90	0.30	82.4	10.4	0.75	0.28
	Well	Ammocoete diet	R3	86.9	11.7	0.98	0.38	84.1	11.6	0.85	0.31
			R4	81.2	16.3	0.91	0.47	79.4	15.4	0.77	0.36
			R5	90.9	7.7	1.07	0.31	86.3	8.3	0.89	0.28
	Conditioned	None	R10	82.7	7.3	0.76	0.13	78.3	7.0	0.63	0.10
			R11	84.3	10.8	0.91	0.35	80.2	10.2	0.74	0.29
			R12	80.1	10.4	0.78	0.26	77.0	9.7	0.59	0.25

Table 16. Water quality monitoring results from feeding trial

					Temp. (°C)		pH		DO (mg/L)		EC (µS/cm)	
Sediment	**Water type**	**Feeding**	**Tank**	**n**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**	**Mean**	**Standard deviation**
SRS	Well	Ammocoete diet	R13	11	13.1	0.34	6.64	0.08	9.1	0.25	291	4.66
			R14	11	13.1	0.44	6.64	0.03	8.8	0.50	288	5.71
			R15	10	13.1	0.31	6.65	0.09	8.8	0.36	289	6.65
OSU	Well	None	R7	11	13.5	0.51	6.64	0.08	8.9	0.36	291	4.96
			R8	11	13.1	0.32	6.63	0.05	8.8	0.47	288	5.55
			R9	9	13.1	0.29	6.65	0.05	8.8	0.35	289	6.42
	Well	Ammocoete diet	R3	6	13.3	0.33	6.64	0.08	8.9	0.27	290	4.27
			R4	7	13.0	0.32	6.64	0.04	9.0	0.40	290	3.09
			R5	5	13.1	0.24	6.68	0.06	9.0	0.14	288	2.30
	Conditioned	None	R10	11	14.0	0.86	6.89	0.10	9.7	0.46	288	5.03
			R11	11	13.7	0.81	6.97	0.16	9.6	0.70	286	4.73
			R12	9	13.9	0.73	7.02	0.12	9.6	0.63	288	7.81

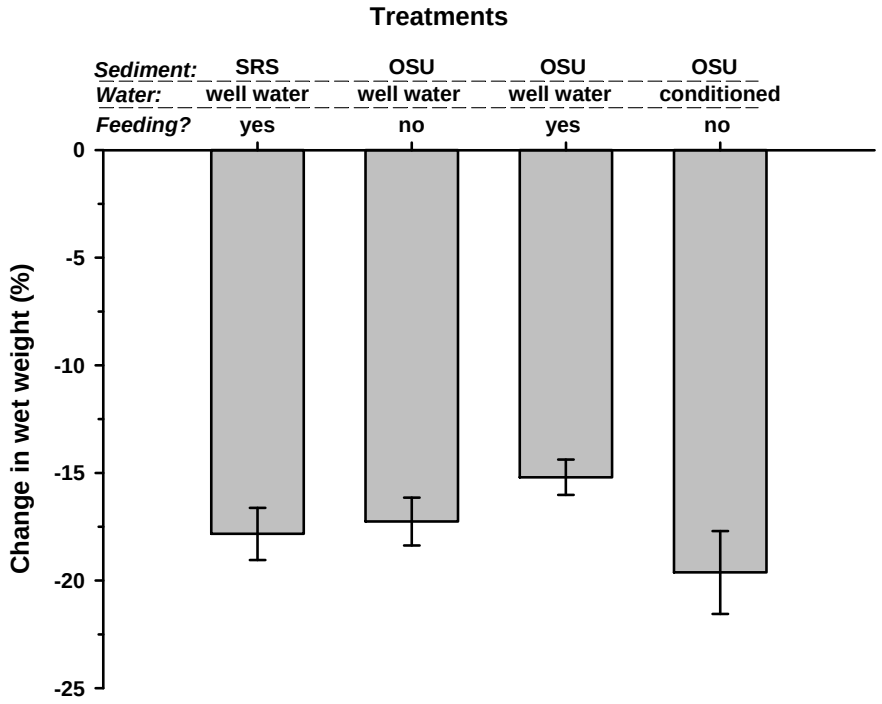


Figure 10. Mean (n = 3) change in wet weight among ammocoetes held in different sediment types, with different water sources and different feeding regimes. Error bars are ± one standard error of the mean.

5. Task 3: Contaminated Sediment Exposure Pre-pilot Test and Task 4: Physiology Endpoints

5.1 Objective

Task 3 involved a pilot assay of a larger-scale sediment bioassay using two uncontaminated sediments and one contaminated sediment. Handling and exposure methods developed in Task 2 were applied to the assay. For Task 3, survival, growth, and behavior of ammocoetes were monitored as bioassay endpoints.

The objective of Task 4 was to expand on the survival, growth, and behavior endpoints included in Task 3 to evaluate the use of alternative measures of physiological fitness in the pilot bioassay.

5.2 Methods

5.2.1 Sediments

Sediment used for this task included SRS, GAS, and OSU. Sediment was collected in 5-gal buckets in the field. To ensure each ammocoete and replicate tank received a representative sample of the total sediment collected in the field, sediment was incrementally loaded into each exposure corral (PLA SOP P.5; Stratus Consulting, 2011d). Eighteen 1-foot diameter tanks, containing five 3-in. diameter corrals, were filled with one of the three sediment types. Representative sediment subsamples were shipped overnight to CAS for physical and chemical analyses (PLA SOP P.11; Stratus Consulting, 2011d).

5.2.2 Experimental design

Water flowed over the sediment-loaded corrals for at least 24 hours before ammocoetes were added. After removal from the stock tank, ammocoetes were depurated for 24 hours, and lengths and weights of individual ammocoetes were measured (PLA SOP P.7; Stratus Consulting, 2011d). Ammocoetes were loaded in random order into each corral. Fifteen ammocoetes (three circular tanks with five corrals per tank) were exposed to each sediment type for 30 days and another 15 ammocoetes were exposed to each sediment type for 60 days. Ammocoetes were fed the ammocoete diet three times per week. Feed was delivered at the surface and was also injected approximately 2 in. into the sediment of each corral.

5.2.3 Ammocoete sampling and preservation

At the conclusion of the trial, ammocoetes were removed, rinsed of sediment using well water, weighed and measured (PLA SOP P.7; Stratus Consulting, 2011d), placed into individual depuration containers for 24 hours, and reweighed. Ammocoetes were then euthanized using an overdose of buffered MS-222 (PLA SOP P.3, Stratus Consulting, 2011d). In a subset of each treatment group, the third and fourth gill pouch was excised and placed in 100 µL of SEI [sucrose, ethylenediaminetetraacetic acid (EDTA), and imidazole] buffer and flash frozen in liquid nitrogen. These gill pouch samples were stored at -80°C for later gill Na^+/K^+ ATPase (a class of enzymes that catalyze the decomposition of adenosine triphosphate) activity measurements. The remaining ammocoetes from each treatment were preserved either in 10% buffered formalin for histological analysis or stored at -20°C for proximate analysis of dry weight and lipid and ash content.

5.2.4 Dry weight and lipid and ash content

In order to compare lipid and ash content in fish reared in each treatment, we used a proximate analysis protocol developed by Reynolds and Kunz (2001). Ammocoetes were thawed and weighed in pre-weighed tins and dried to a constant weight at 60°C. Samples were then ground with a mortar and pestle and placed in pre-weighed thimbles for lipid extraction. Lipids were extracted with a Soxhlet apparatus using a 7:2 ratio by volume of hexane:isopropyl alcohol as a solvent. After the lean mass was obtained, all samples were placed back into aluminum tins and ashed in a muffle furnace for five hours at 525°C. Ash samples were weighed, and contents were retained for archival purposes.

5.2.5 Gill Na^+/K^+ ATPase activity

The third and fourth gill pouch from three ammocoetes out of each treatment were excised and immediately immersed in 100-µL SEI buffer (250 mM sucrose, 10 mM Na2-EDTA, and 50 mM imidazole) and flash frozen in liquid nitrogen, according to a protocol developed for larval and metamorphosizing sea lamprey (*Petromyzon marinus*) by Reis-Santos et al. (2008). Samples were stored at -80°C until the day they were assayed. Gill Na^+/K^+ ATPase activity was determined using a method from McCormick (1993). Samples were partially thawed and then sodium deoxycholate acid was added to make a 1X concentration of SEID (SEI + sodium deoxycholate acid). After homogenization for 15–20 seconds using a Kontes mortar and pestle, samples were centrifuged at 5,000 m/S^2 (i.e., g-forces, or G) for 30 seconds, and the supernatant was retained. Two sets of three subsamples of the supernatant were added to a 96 well plate. Ouabain was added to one set (0.5 nmol at a 1:1 ratio) to inhibit gill Na^+/K^+ ATPase activity, thus making it possible to distinguish this activity from background non-specific ATPase activity. Absorbance was read at 340 nm at 25°C using a Spectra Max 190 absorbance plate reader; absorbance readings were recorded for 10 min. Gill Na^+/K^+ ATPase activity was calculated by subtracting the ouabain-inhibited slope from the uninhibited slope and dividing by the protein content of the homogenate, determined using the Bradford protein assay kit (Pierce Biochemical). Gill Na^+/K^+ ATPase activity measurements were expressed as µmol adenosine diphosphate (ADP)/mg protein/hour.

5.2.6 Histology

Histological analysis was performed to assess the general health of ammocoetes and to identify the presence and prevalence of tissue damage and/or parasites associated with exposure to sediment treatments. Samples were preserved in 10% buffered formalin until processed. Ammocoetes were cut in half longitudinally between the last gill pouch and the mid-gut. Tissues were embedded in paraffin for microtome sectioning and archival purposes. All slides were

stained with hematoxylin and eosin before mounting. A veterinary pathologist assisted with the microscopy.

5.3 Results

5.3.1 Water quality

Overlying water quality in each of the 18 exposure tanks (3 tanks per treatment) remained consistent throughout the 30- and 60-day exposures (Table 17).

5.3.2 Survival and behavior

There were no mortalities during any of the test treatments over the course of the 30- and 60-day exposures. Ammocoetes placed on the contaminated GAS sediment remained on the surface of the sediment and took noticeably longer to burrow into the sediment than ammocoetes placed on the uncontaminated SRS or OSU sediment. This behavior was explored in more detail in Task 5 experiments (Section 6).

5.3.3 Growth

The average initial weight and length of all ammocoetes loaded into each corral was 1.0 ± 0.3 g and 90 ± 8 mm, respectively (n = 90; Figure 11). There were no significant differences among initial ammocoete weights or lengths in any treatment.

On average, ammocoetes in 30- and 60-day exposures to GAS and OSU sediment lost weight, while ammocoetes in the 30- and 60-day exposures to SRS sediment gained weight (Figure 12). Therefore, there was a significant ($P < 0.01$) difference in the change in live weight between the 30- and 60-day SRS treatments and all other treatments. There were no significant differences in weight loss among the GAS and OSU treatments, and there was no significant difference in weight gain between the SRS treatments.

Similar to changes in weight, on average, we observed a reduction in length in ammocoetes in 30- and 60-day exposures to GAS and OSU sediment, whereas ammocoetes in the 30- and 60-day exposures to SRS sediment grew (Figure 13). The change in length in ammocoetes in the SRS 30-day treatment was significantly ($P < 0.01$) greater than in ammocoetes exposed to OSU sediment for 30 days. The change in length in ammocoetes in the SRS 60-day treatment was significantly ($P < 0.01$) greater than in ammocoetes for all GAS and OSU treatments. There were no significant differences between any GAS and OSU treatments, and the difference between the 30- and 60-day SRS treatments was not significant.

Table 17. Average water quality for each sediment type during the 30- and 60-day exposures in Task 3

Sediment	Exposure duration (days)	Flow rate (mL/min)		Temp (°C)		pH		DO (mg/L)	
		Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
GAS	30	NA	NA	13.5	0.03	6.74	0.01	8.7	0.02
GAS	60	209	25	13.5	0.15	6.73	0.02	8.7	0.07
OSU	30	NA	NA	13.4	0.05	6.69	0.01	8.7	0.04
OSU	60	217	9	13.4	0.14	6.71	0.01	8.7	0.08
SRS	30	167	20	13.4	0.07	6.74	0.02	8.8	0.04
SRS	60	195	18	13.4	0.05	6.76	0.07	8.8	0.08

Table 17. Average water quality for each sediment type during the 30- and 60-day exposures in Task 3 (cont.)

Sediment	Exposure duration (days)	EC (µS/cm)		Total ammonia (ppm)		Unionized ammonia (ppm)	
		Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
GAS	30	293	1.3	0.40	0.20	0.0006	0.0003
GAS	60	295	0.6	0.45	0.38	0.0006	0.0005
OSU	30	293	1.5	0.20	0.00	0.0002	0.0000
OSU	60	295	0.9	0.24	0.09	0.0003	0.0001
SRS	30	292	1.0	0.27	0.12	0.0003	0.0001
SRS	60	296	0.9	0.28	0.10	0.0004	0.0002

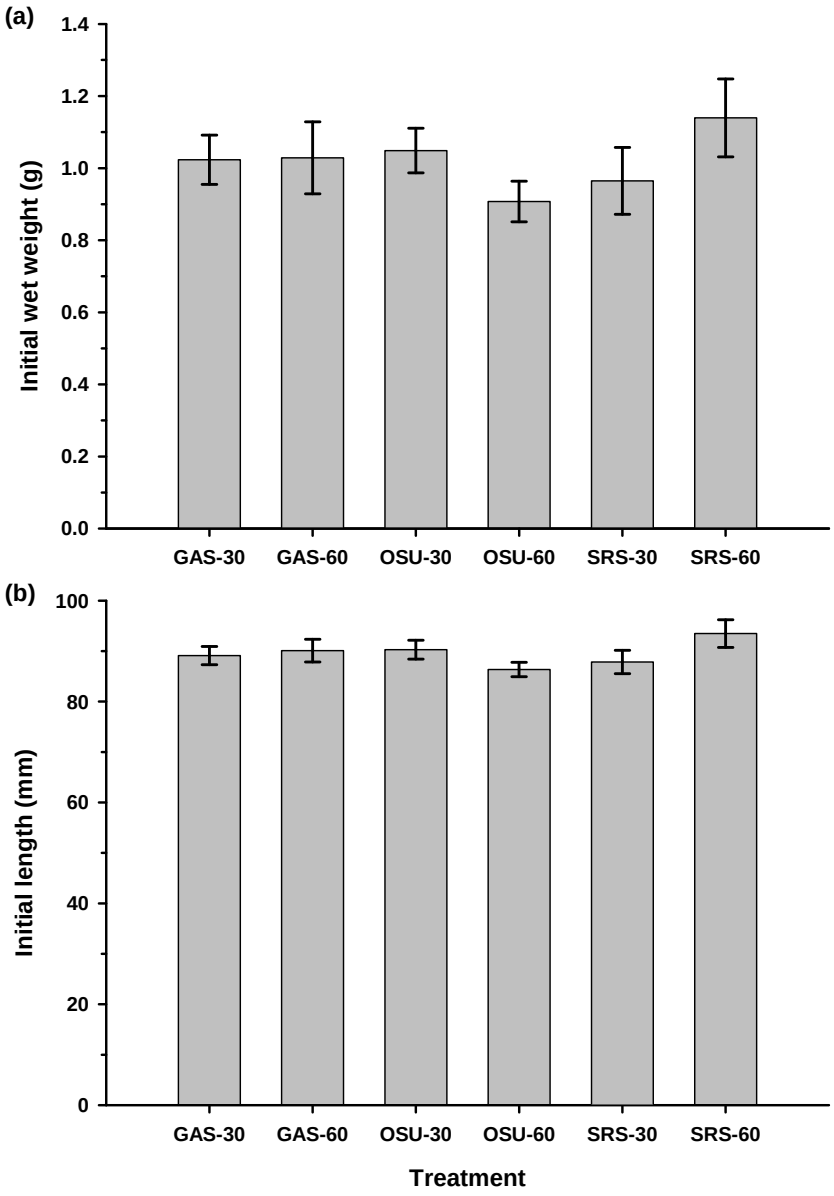


Figure 11. Mean initial wet weight (a) and length (b) in all ammocoetes loaded into each corral (n = 90). Error bars are ± one standard error of the mean.

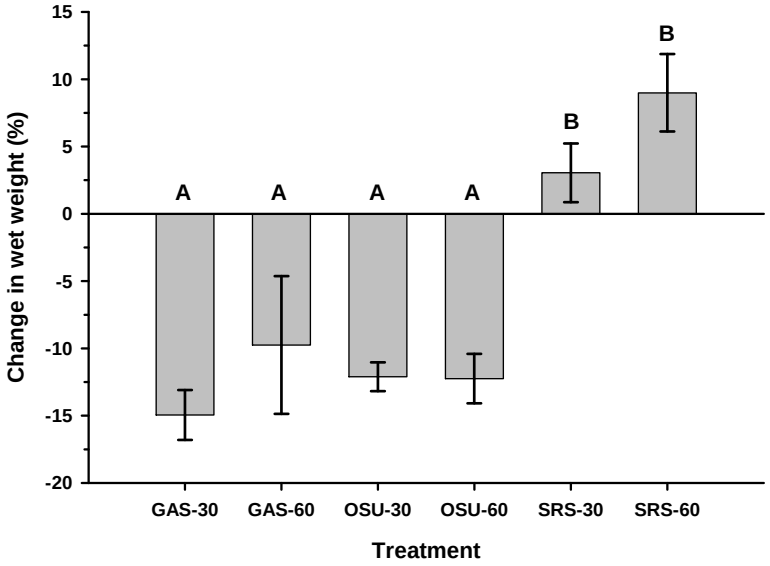


Figure 12. Mean change in depurated wet weight for individual ammocoetes exposed to each sediment type for 30 days and 60 days (n = 15). Error bars are ± one standard error of the mean. Treatments that are significantly different ($P < 0.01$) from each other are indicated with different uppercase letters.

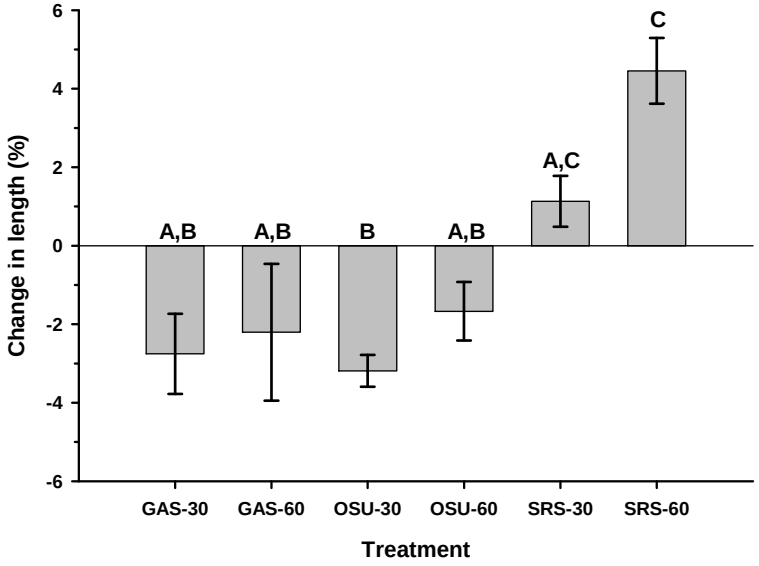


Figure 13. Mean change in length for individual ammocoetes exposed to each sediment type after 30- and 60-day exposures (n = 15). Error bars are ± one standard error of the mean. Treatments that are significantly different ($P < 0.01$) from each other are indicated with different letters.

5.3.4 Lipid content

The mean lipid content was relatively low, less than 1% live weight, in all treatments (Figure 14). There were no significant differences in mean lipid content among treatments. It is likely that the lipid content in these ammocoetes was low when the experiment began because they were held in the stock tank for approximately nine months before the experiment began.

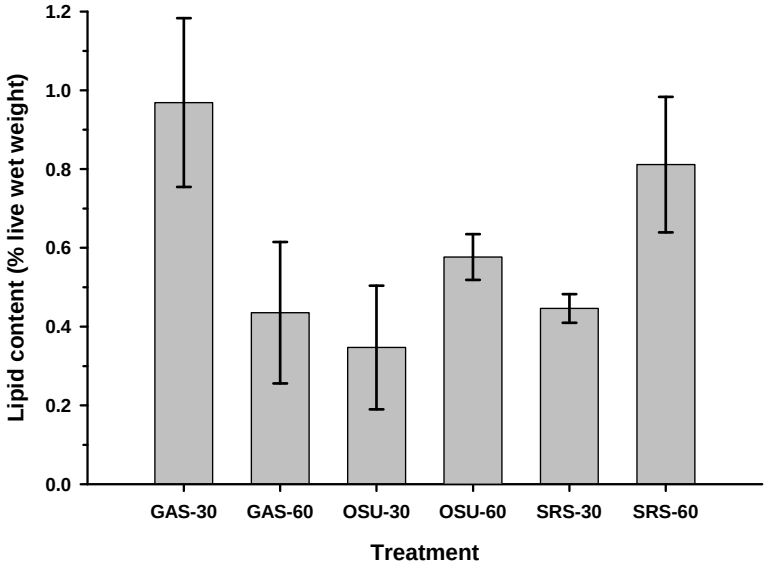


Figure 14. Mean lipid content (n = 3) in individual ammocoetes exposed to each sediment type after 30- and 60-day exposures. Error bars are ± one standard error of the mean.

5.3.5 Histology

The only histological abnormalities observed were encysted digenetic trematodes and granulomas found in various tissues from ammocoetes exposed to all sediment types. Examples of encysted parasites or granulomas in various tissues are displayed in Figures 15–19.

5.3.6 Gill Na^+/K^+ ATPase activity

Gill Na^+/K^+ ATPase was below 1-µmol ADP/mg protein/hour in ammocoetes exposed to all sediments for both 30 days and 60 days. As a comparison, the activity level measured in a larger fish (109 mm, 2.14 g) that was undergoing metamorphosis in the stock holding tank was 51.4-µmol ADP/mg protein/hour. There were some significant ($P < 0.05$) differences in gill Na^+/K^+ ATPase activity among treatments (Figure 20). However, these data appear highly variable, and the statistically significant differences reported here may be an artifact of the preliminary nature of this task, which focused on methods development.

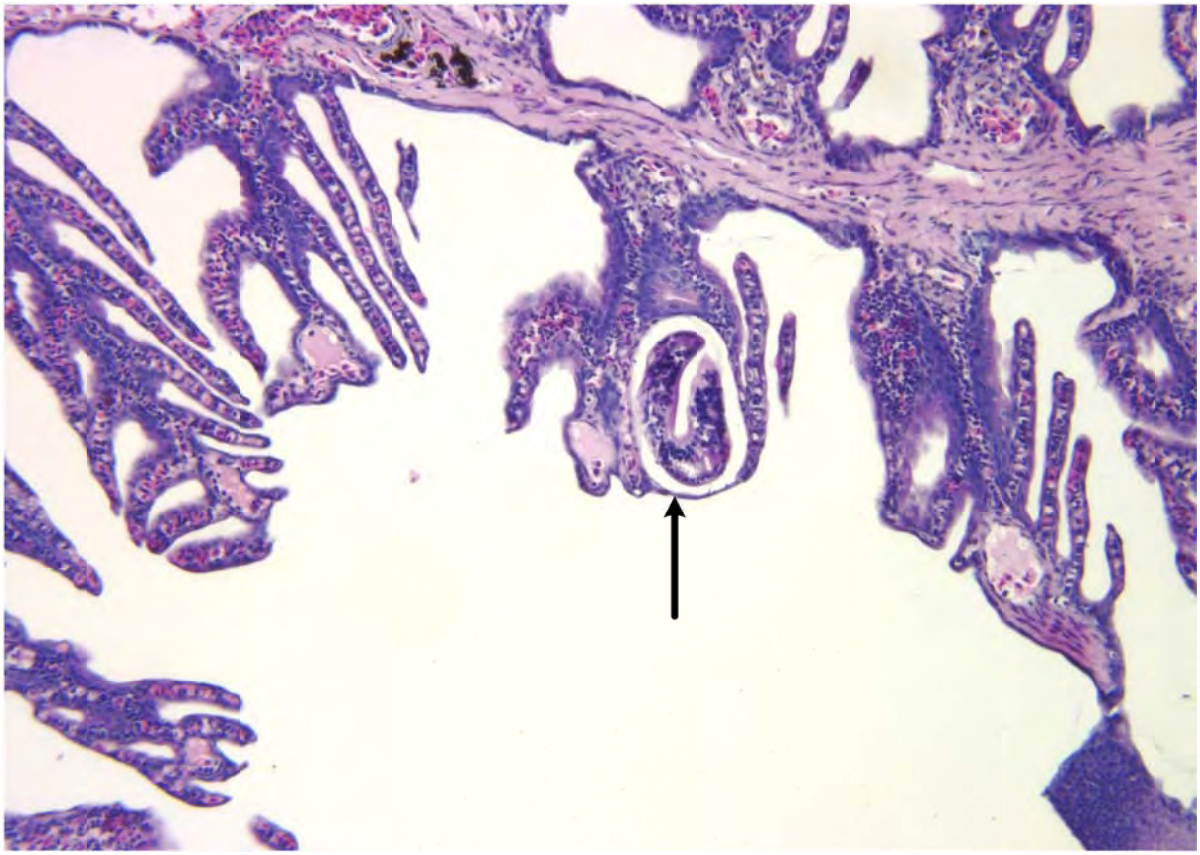


Figure 15. Encysted digenean trematode parasite (arrow) in the gill lamella of an ammocoete exposed to GAS sediment for 60 days. Image taken at 200× magnification.

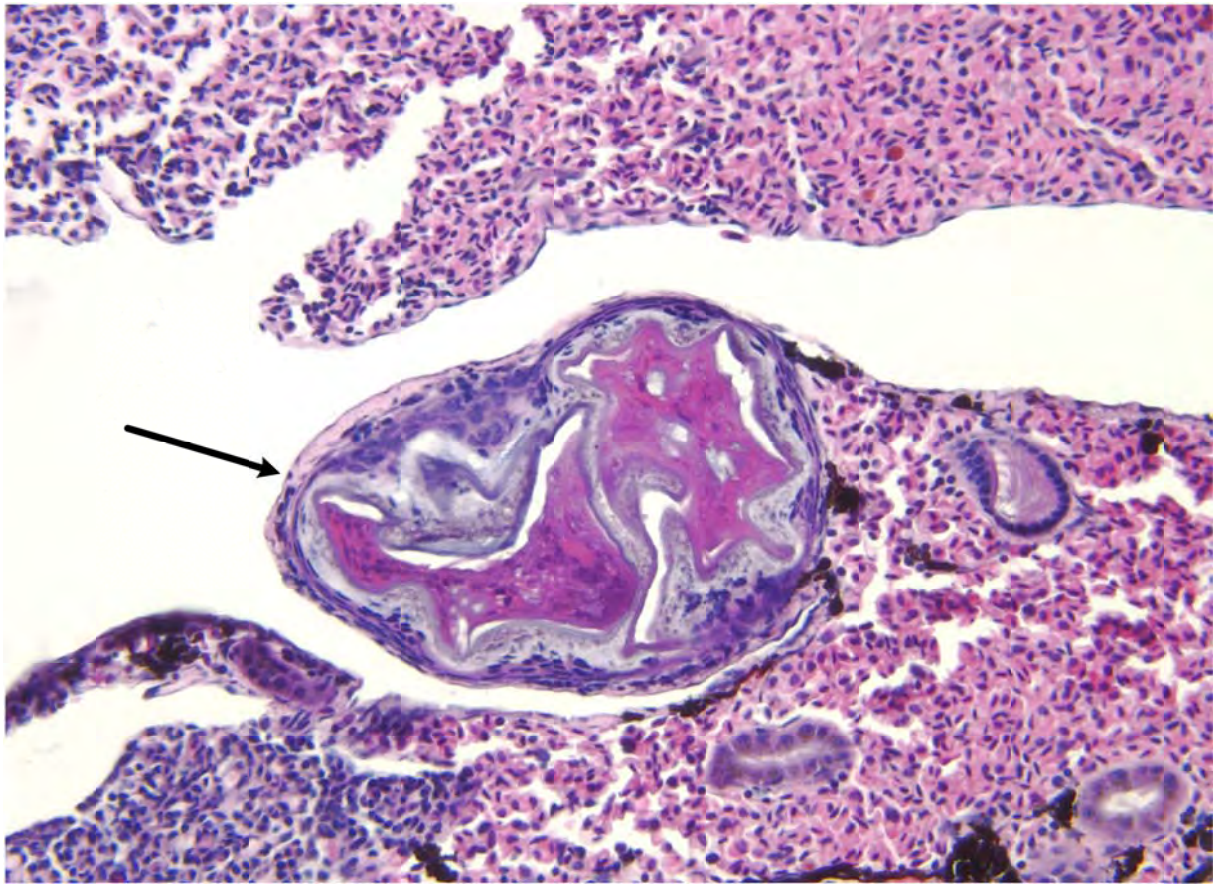


Figure 16. Encysted digenean trematode parasite (arrow) in the kidney of an ammocoete exposed to GAS sediment for 60 days. Image taken at 200× magnification.

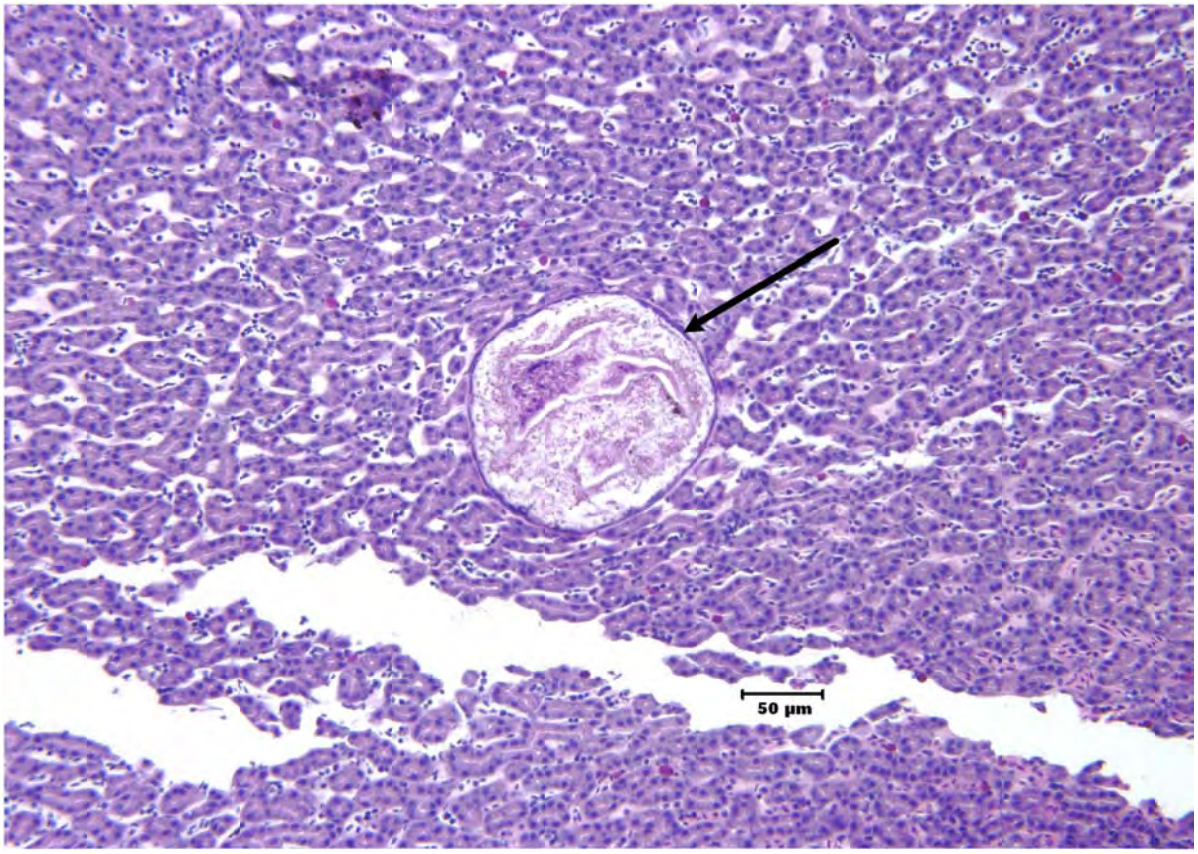


Figure 17. Granuloma (arrow; likely from a degraded encysted parasite) in the liver of an ammocoete exposed to OSU sediment for 30 days. Image taken at 200× magnification.

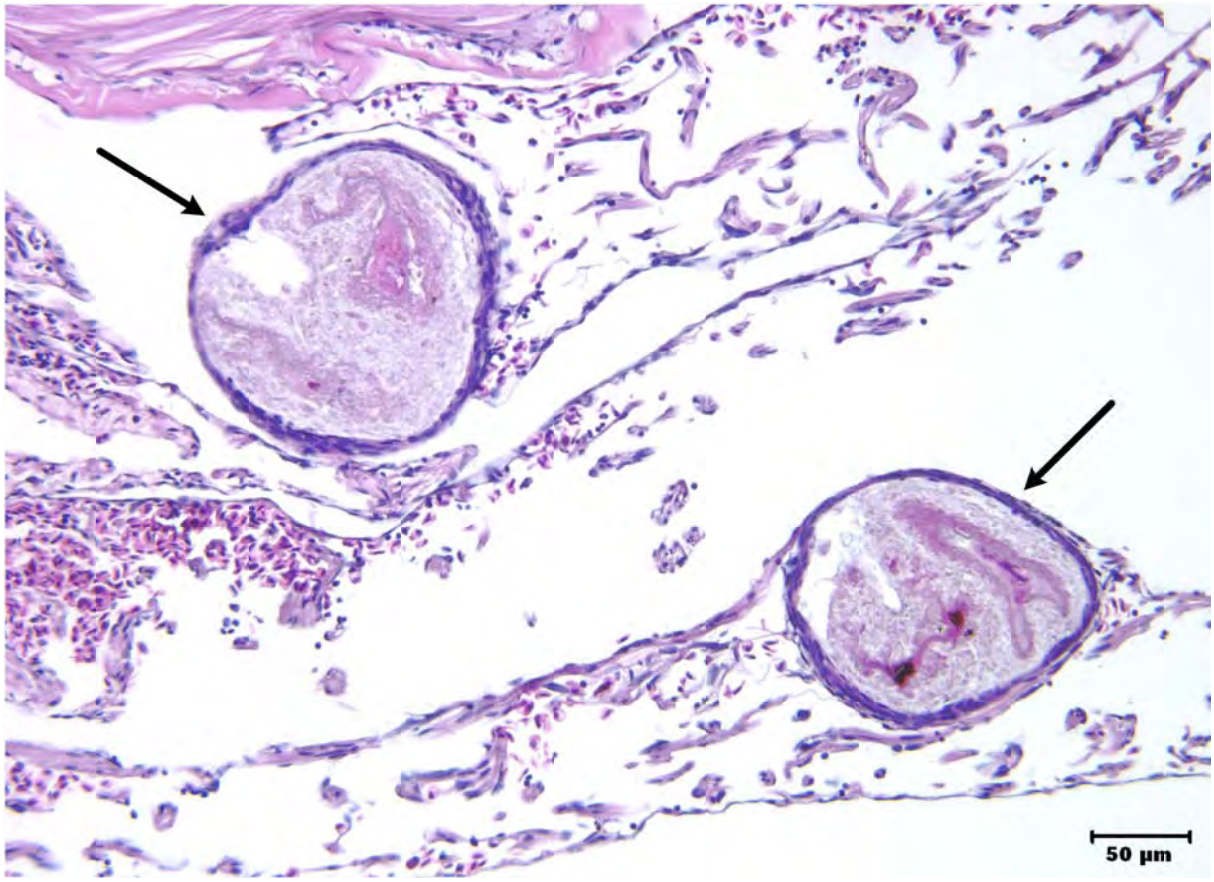


Figure 18. Encysted digenean trematode parasites (arrows) in the heart of an ammocoete exposed to OSU sediment for 60 days. Image taken at 200× magnification.

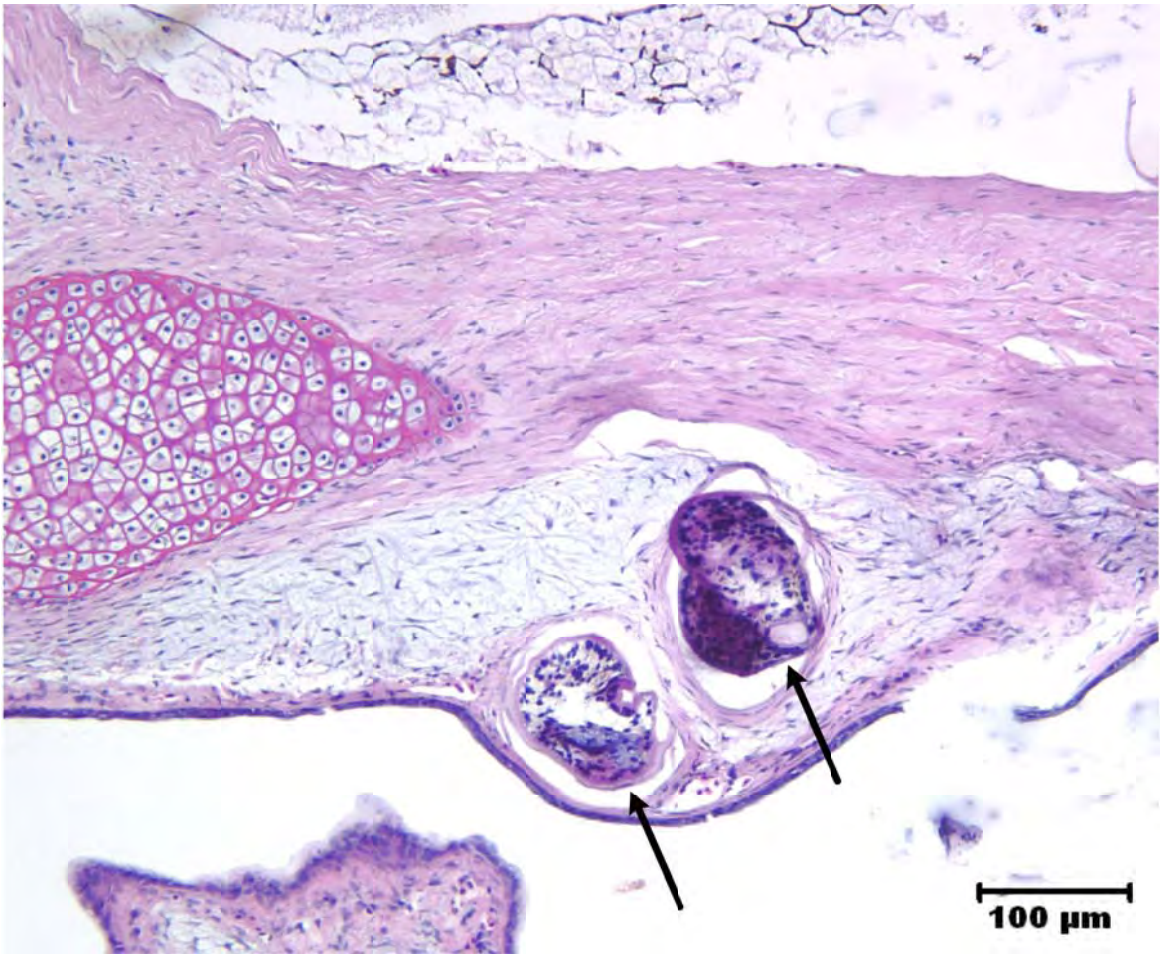


Figure 19. Encysted digenean trematode parasites (arrows) in the oropharynx of an ammocoete exposed to SRS sediment for 60 days. Image taken at 100× magnification.

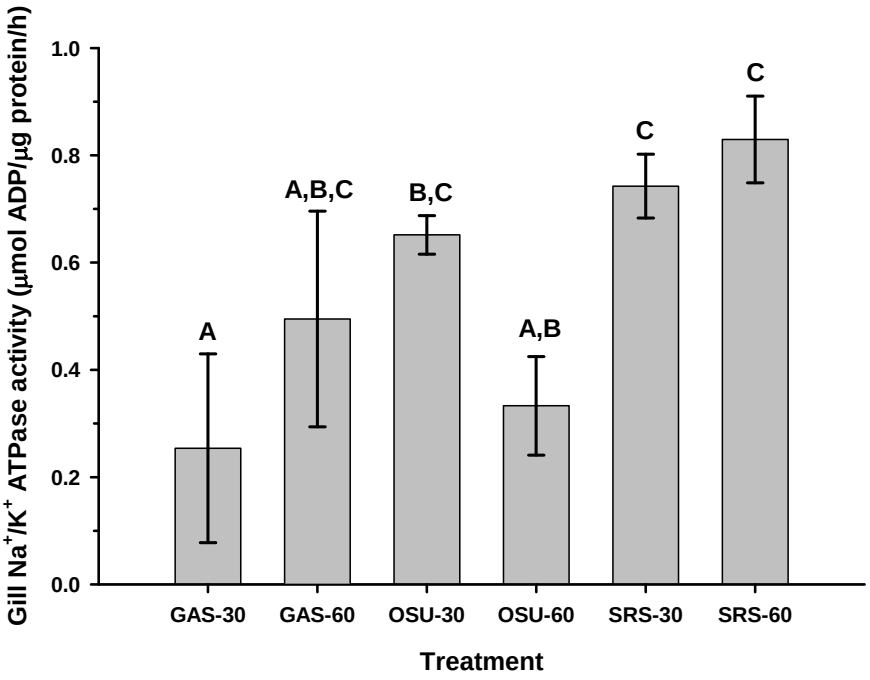


Figure 20. Gill Na^+/K^+ ATPase activity in individual ammocoetes exposed to each sediment type after 30- and 60-day exposures (n = 3). Error bars are ± one standard error of the mean. Treatments that are significantly different ($P < 0.05$) from each other are indicated with different uppercase letters.

5.4 Discussion

The results from Tasks 3 and 4 demonstrate that conducting a long-term sediment bioassay with PLAs is possible in terms of being able to track individual ammocoetes (i.e., corrals) and that control survival was excellent (100%). However, growth appears to be highly variable, with many ammocoetes actually shrinking in size after 30- and 60-day exposures in both contaminated and uncontaminated sediments. Therefore, growth may not be a robust or sensitive endpoint for use in evaluating the effects of sediment contamination on ammocoetes. Analyses of other physiological endpoints, including lipid content and gill Na^+/K^+ ATPase activity and histology, are also possible and were included in pilot testing analysis (Task 7; Section 8).

6. Task 5: Behavioral Endpoints

6.1 Objective

The objective of Task 5 was to determine if ammocoetes actively avoid burrowing into contaminated sediments from the Harbor. If some form of avoidance is observed, the test results may be used to design more in-depth behavior experiments.

6.2 Methods

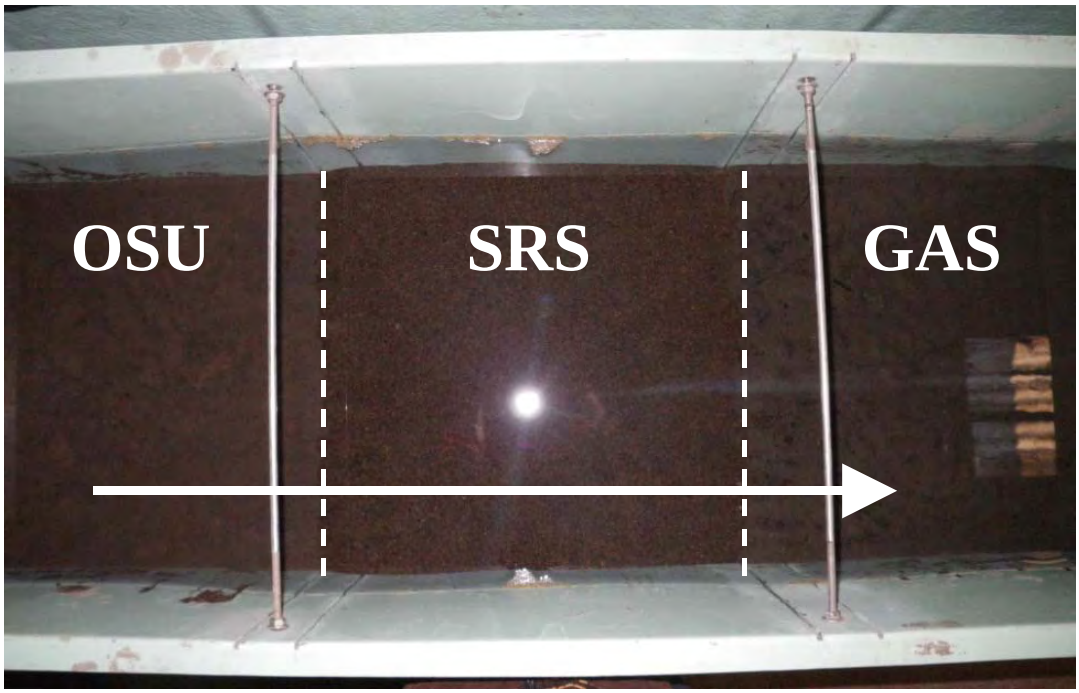
6.2.1 Multiple sediment trough exposures – preference and burrowing time tests

Sediment preference and burrowing time were observed to determine the effects of contaminated sediments on behavior. In the preliminary test, removable partitions were used to create discrete adjacent sediment blocks (30.5 cm wide × 45.7 cm long) in a trough tank with clean water flowing over the sediment. The three sediments used in this preference trial, from upstream to downstream, were OSU, SRS, and GAS (Figure 21). Partitions were used to keep the sediments separated while loading. After loading, the partitions were removed and clean water flowed over the sediment for two days. Twenty-three ammocoetes were introduced, one at a time (randomly over the sediment surface), and the trough was covered with black mesh to diffuse overhead lighting. Partitions were reinserted after seven days, and the number of ammocoetes in each sediment type was recorded.

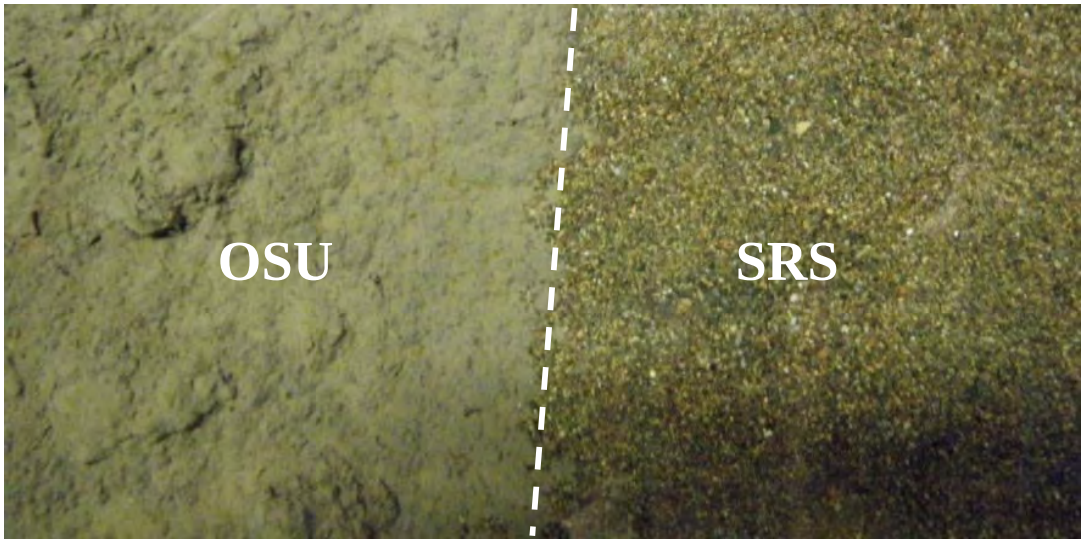
A second round of four sediment preference trials was conducted using a similar design. However, for this trial, only 2 sediments were tested at one time and 15 ammocoetes were exposed in each paired sediment tank for 5 days before the partition was reinserted. The four paired sediment trials, from upstream to downstream were (1) GA2 and RE1, (2) RE3 and SWI, (3) SRS and GA2, and (4) SRS and RE2. In addition to counting the ammocoetes in each sediment type, the time to initiate burrowing was also recorded for each ammocoete during the third of these trials (SRS and GA2).

6.2.2 Single sediment exposures – burrowing time tests

The goal of these tests was to determine if burrowing time was different in four sediment types (RE2, AR2, GA2, and SWI).



(a)



(b)

Figure 21. Sediment preference experiment using OSU, SRS, and GAS sediments. Arrow indicates direction of water flow across all three sediment types (a). Close-up of the interface (dashed line) between OSU and SRS sediments (b).

In the first series of burrowing time tests, one ammocoete was added to each of five 474-mL containers per sediment type (sediments RE2, AR2, GA2, and SWI; 20 total containers). The five containers for each sediment type were then submerged into a 30.5-cm diameter circular tank at a depth of 10.2 cm (Figure 22; four total tanks). Each container held 6.4 cm of sediment and had 0.3-cm holes drilled above the sediment on either side of the container above the sediment to allow water to flow through. To provide water exchange, water was introduced to the circular tanks at a rate of 200 mL/min. After being held for 30 seconds in a small container over the sediment to allow them to orient head down, a single ammocoete was added to each container. When an ammocoete was released, observers recorded the time duration to initiate burrowing and the total time until the animal was completely buried. Observations were made until the animal was completely buried or 120 min after the release. The experiments were video recorded to confirm the observed times.

Figure 22. Sediment burrowing time experiment, with individual ammocoetes in each exposure container.

In the second series of burrowing time tests, three sediment types (RE2, GA2, and SWI) were tested in a slightly different exposure setup. The same small containers were used; however, in this series, they were placed into three 57-L rectangular aquariums filled with clean water, and each had a plastic lid to prevent the ammocoetes from escaping the individual containers. One sediment type was held in four or five small containers per aquaria. Four trials were conducted sequentially over the course of 1 week for a total sample size of 17 per sediment type. To

account for any differences among the aquaria, the type of sediment assigned to each aquarium was alternated in each trial. Unlike the first series of burrowing trials, this series did not utilize flow-through water exchange. Rather, the containers were placed into the aquaria, and allowed to settle overnight. A 50% water exchange was performed just before tests began to siphon out any sediment that settled outside of the containers. A single ammocoete was added to each container using a funnel apparatus with a filter cloth plug at the end that was removed after 1 min of settling time, allowing the ammocoete to enter the container and access the sediment. Observations were made in an equivalent manner as in the first series, and the experiments were also video recorded.

Statistical comparisons of the burrowing times in each sediment type between series one and two were conducted using a Kruskal-Wallis rank sum test ($\alpha = 0.05$; Minitab® version 16.2.2). Comparisons of pooled series of one and two burrowing data for each contaminated sediment type against the reference was conducted using a one-tailed multiple comparison test after a Kruskal-Wallis test using R version 2.15.3 with the "pgirmess" package (version 1.5.7) and "kruskalmc" function (http://perso.orange.fr/giraudoux).

6.3 Results

6.3.1 Multiple sediment trough exposures – preference and burrowing time tests

After 7 days of exposure to OSU, SRS, and GAS, 4 ammocoetes were found in OSU, 15 in SRS, and 4 in GAS sediment.

Of the four dual-sediment preference trials, three trials were conducted with uncontaminated versus contaminated sediment and one was conducted with two uncontaminated sediments. After 5 days of exposure in each of the 3 uncontaminated versus contaminated trials (Trials 1, 2, and 3), the number of ammocoetes found in the uncontaminated sediments was higher than in the contaminated sediments. After 5 days in the exposure trough, the number of ammocoetes in either sediment in the uncontaminated versus uncontaminated trial was nearly even (Table 18; Figure 23). The sediment grain size distribution in the two uncontaminated sediments used in Trial 4 were not similar, suggesting that differences in grain size within this range did not affect sediment preference (Figure 23).

The time to complete burrowing was also recorded during the third trial (SRS versus GA2). On average, ammocoetes completed burrowing significantly faster in the uncontaminated SRS sediment compared to the contaminated GA2 sediment (0.48 min and 1.02 min, respectively; $p < 0.01$, two-sample t-test).

Table 18. Study design and results of multiple sediment preference experiments

		Sediment type		# Ammocoetes after 5 days	
Trial #	**Type[a]**	**Upstream**	**Downstream**	**Upstream**	**Downstream**
1	C vs. U	GA2	RE1	6	8
2	C vs. U	RE3	SWI	9	6
3[b]	C vs. U	SRS	GA2	11	4
4	U vs. U	SRS	RE2	7	8

a. C vs. U: Contaminated versus uncontaminated. U vs. U: Uncontaminated versus uncontaminated.
b. Burrowing time trial conducted.

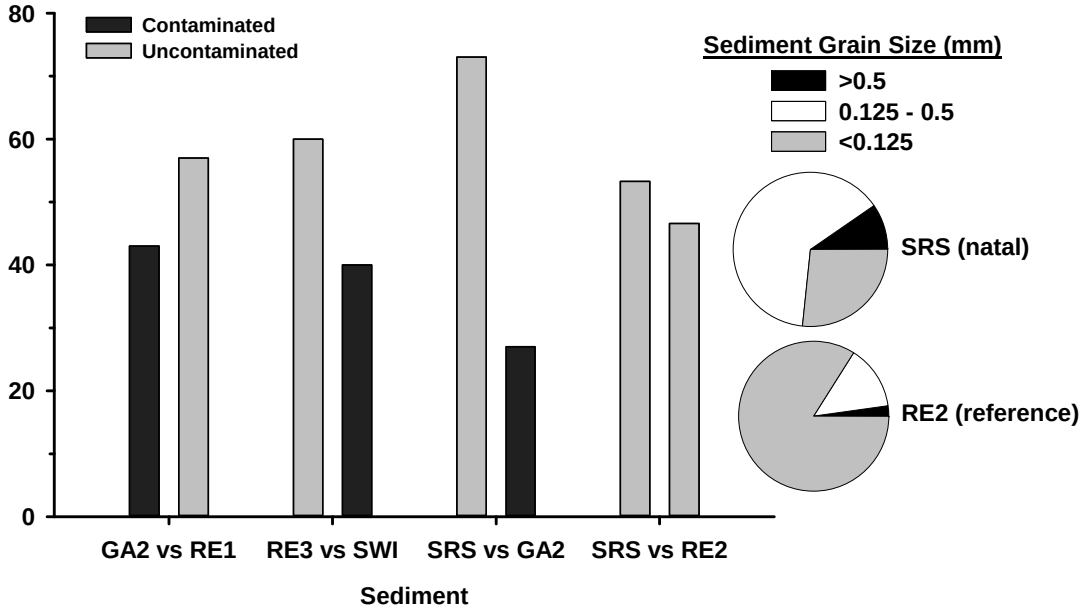


Figure 23. Ammocoete sediment preference in paired sediment trials. For each pair, the upstream sediment is shown on the left and the downstream sediment is shown on the right. Pie charts represent sediment grain size distribution for the two uncontaminated sediments paired in Trial 4 (SRS vs. RE2).

6.3.2 Single sediment exposures – burrowing time tests

Although the exposure systems used in the first and second series of burrowing time tests were slightly different (see Section 6.2.2), three of the same sediments (RE2, GA2, and SWI) were used in each set of test series and there were no significant differences in time to initiate burrowing (Figure 24a) or time to complete burrowing (Figure 24b) among the two exposure series for each sediment type (Kruskal-Wallis test, $\alpha = 0.05$). Therefore, the results of the two exposure series for each sediment type were pooled for further analysis.

Sediment grain size distributions varied among sediments and are displayed in Figure 25. The mean times to initiate burrowing for any of the contaminated sediments were not significantly different than the reference sediment (Table 19, Figure 26). The time to complete burrowing was significantly longer in the GA2 and AR2 contaminated sediments than in the reference sediment. Finally, although the mean time to complete burrowing was much longer in the SWI contaminated sediment than in the reference, this difference was not statistically significant (Table 19, Figures 25 and 26; statistical comparisons conducted using a multiple comparison test after a Kruskal-Wallis rank sum test, $\alpha = 0.05$).

Two ammocoetes exposed to SWI sediment in the second series failed to completely burrow after 120 min (maximum test observation duration). Therefore, the time to complete burrowing was categorized as 120 min for these 2 ammocoetes for all subsequent analyses.

In the second series of tests, all ammocoetes were left in their containers for 24 hours following the 120-min burrowing observation period. After 24 hours, the observers made several additional observations. The observers noted that 1 of the 2 SWI ammocoetes that did not burrow within 120 min was now completely buried and the other was dead. Additionally, observers noted that several ammocoetes had re-emerged from the sediment: Out of 14 total exposure chambers that were quantified for burrowing behavior for each of the 3 sediments, 8 ammocoetes exposed to GA2 sediment and one ammocoete exposed to RE2 sediment had re-emerged. The overwhelming re-emergence of ammocoetes in the GA2 sediment (57%) compared to the RE2 sediment (7%) and the SWI sediment (0%), suggests a possible aversion to the particular types of contaminants in the GA2 sediment. GA2 contains much higher concentrations of petroleum hydrocarbons and PAHs than SWI, and SWI contains higher concentrations of butyltins and PCBs than GA2 (e.g., Table 3).

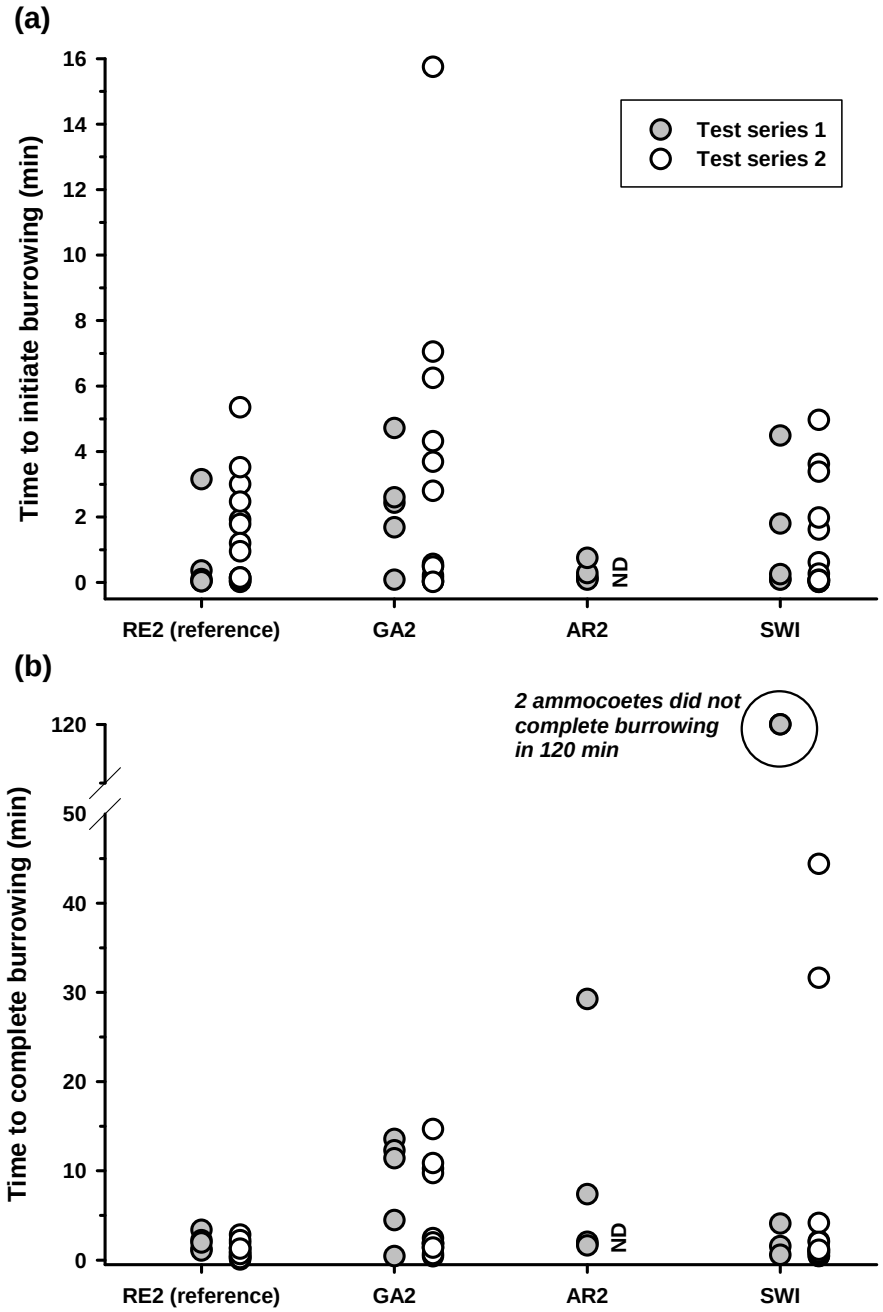


Figure 24. Time to initiate burrowing (a) and time to complete burrowing (b) in one uncontaminated (RE2) and three contaminated (GA2, AR2, and SWI) sediments. The differences in the exposure scenarios for test series 1 (shaded circles) and 2 (open circles) are described in Section 6.2.2. AR2 was not included in test series 2 (ND = no data).

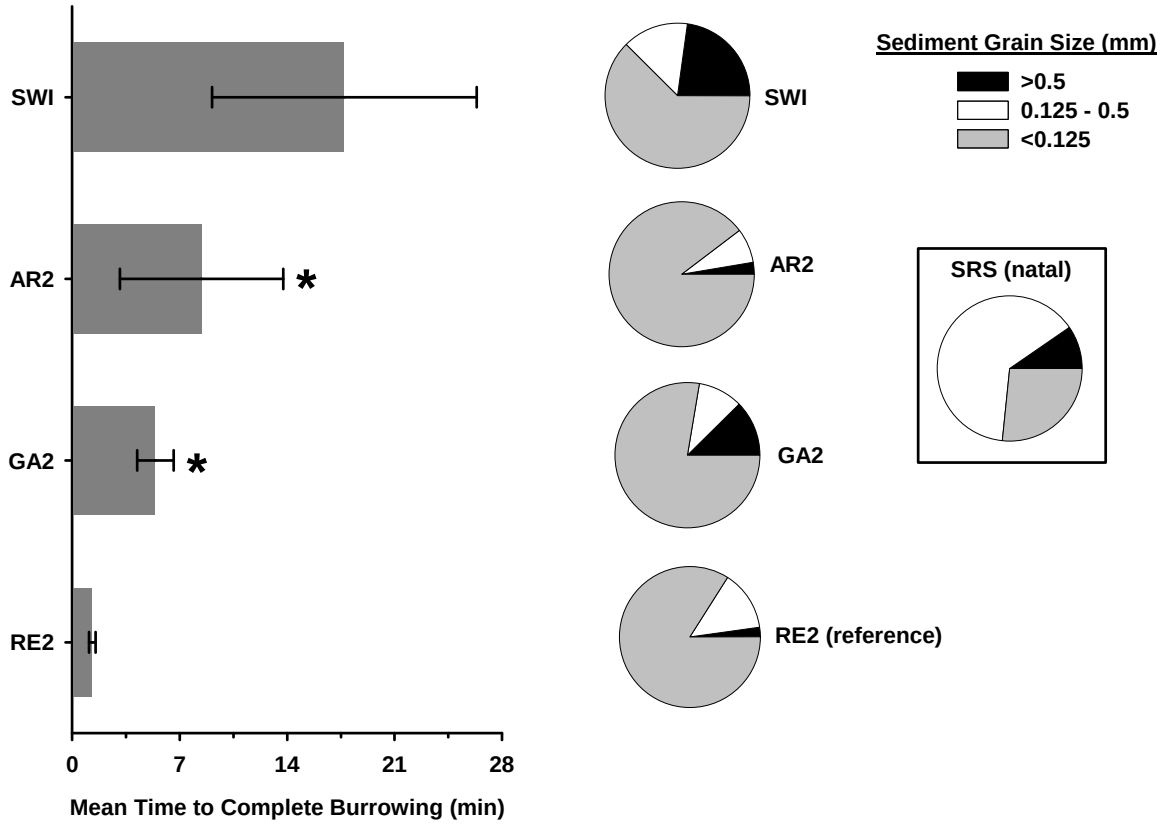


Figure 25. Ammocoete mean burrowing times in uncontaminated (RE2) and contaminated (GA2, AR2, and SWI) sediments. Sample size was 19 for all sediments except AR2 (n = 5). Error bars are ± one standard error of the mean. Asterisks indicate significant differences from the reference sediment (RE2; multiple comparison test after a Kruskal-Wallis rank sum test, $\alpha = 0.05$). Two ammocoetes never completely burrowed in 120 min in SWI. Pie charts represent sediment grain size distributions for each sediment type. Grain size distribution for the SRS natal sediment is presented for reference.

Table 19. Mean time for ammocoetes to initiate and complete burrowing into different sediments in burrowing test series 1 and 2 and corresponding ammocoete lengths

		Time to initiate burrowing		Time to complete burrowing		Ammocoete length		
Sediment	**Burrowing series**	**$\bar{x}$ (min)**	**Standard deviation**	**$\bar{x}$ (min)**	**Standard deviation**	**$\bar{x}$ (min)**	**Standard deviation**	**n**
RE2	1	0.7	1.4	2.0	0.9	91.8	10.5	5
	2	1.5	1.6	1.1	0.8	82.8	13.4	14
	1/2 combo	1.3	1.6	1.3	0.9	84.8	13.1	19
AR2	1	0.3	0.3	8.4	11.9	85.8	14.1	5
	2	ND[a]	ND	ND	ND	ND	ND	ND
	1/2 combo	0.3	0.3	8.4	11.9	85.8	14.1	5
GA2	1	2.3	1.7	8.4	5.7	86.2	19.6	5
	2	3.0	4.4	4.3	4.8	82.0	12.5	14
	1/2 combo	2.8	3.8	5.4	5.2	83.1	14.2	19
SWI	1	1.3	1.9	49.2	64.6	85.4	22.2	5
	2	1.2	1.7	6.6	13.6	81.9	10.8	14
	1/2 combo	1.2	1.7	17.8	37.9	82.8	14.0	19

a. No data collected, test not conducted.

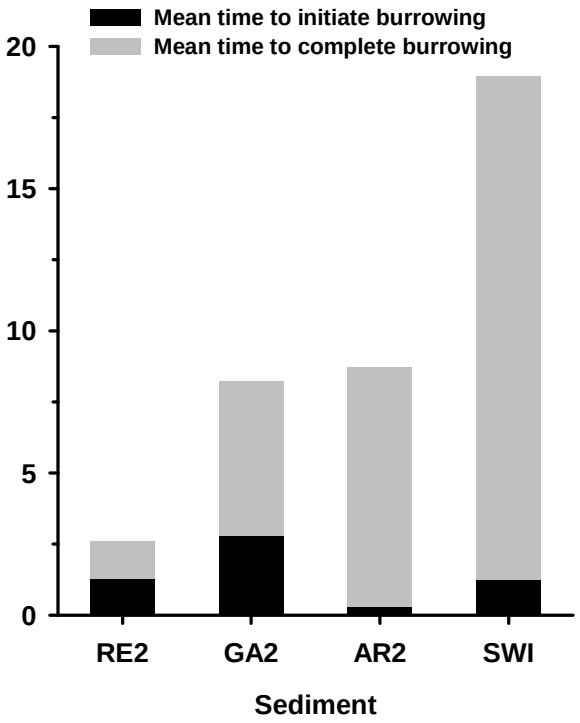


Figure 26. Mean time to initiate burrowing and complete burrowing in RE2 (uncontaminated), GA2, AR2, and SWI (contaminated) sediments used in test series 1 and 2 (see Section 6.2.2). Sample size was 19 for all sediments except AR2 (n = 5).

6.4 Discussion

Results of these preliminary experiments suggest that the presence of contaminants affects ammocoete behavior in terms of sediment preference, speed of burrowing, and aversion to contaminated sediments. Ammocoetes appear to prefer uncontaminated sediment and burrow into uncontaminated sediment faster than they burrow into contaminated sediment.

7. Task 6: Induction of Metamorphosis

7.1 Objective

The objective of this task was to determine if metamorphosis can be induced in PLAs in the laboratory. The results of this trial will help determine the feasibility of conducting future experiments that focus on the effects of contaminant exposure during lamprey metamorphosis, which may be a more sensitive life stage for these organisms.

7.2 Methods

This trial was conducted based on protocols developed by Manzon et al. (1998). These authors demonstrated that potassium perchlorate ($KClO_4$) can induce metamorphosis in larval sea lamprey (*Petromyzon marinus*). Twenty-four large ammocoetes (> 90 mm) were held in four rectangular 5-gal tanks (6 ammocoetes/tank). Each tank was aerated and water was exchanged weekly during the 60-day trial. Two of the four tanks were treated with 0.05% $KClO_4$ with each weekly exchange, and the other two tanks received fresh water only. All tanks were fed a 3% wet-body-weight ration once a week. All animals were weighed and measured (PLA SOP P.7; Stratus Consulting, 2011d), and photographed before and after the 60-day trial. After 60 days, the stage of metamorphosis was assessed visually and gill pouches were removed from each fish to measure gill Na^+/K^+ ATPase activity (see Section 5.2.5 for methods).

7.3 Results

The average initial weight of ammocoetes in the control and $KClO_4$-treated tanks was 1.63 ± 0.71 g and 1.69 g ± 0.62 g, respectively. The average initial length of ammocoetes in the control and $KClO_4$-treated tanks was 105 ± 13 mm and 106 ± 11 mm, respectively. There were no significant differences in initial weights or lengths of ammocoetes between the two treatments. There was no visible evidence that any of the ammocoetes in either treatment group were undergoing metamorphosis before or after the 60-day trial. Furthermore, the mean gill Na^+/K^+ ATPase activity in the control and treatment groups, 0.86 ± 1.31 and 0.60 ± 0.46 µmol ADP/mg protein/hour, respectively, was very low compared to the gill Na^+/K^+ ATPase activity measured in a larger fish (109 mm, 2.14 g) that was undergoing metamorphosis in the stock holding tank (51.4 µmol ADP/mg protein/hour). There were no significant differences in gill Na^+/K^+ ATPase activity among fish from the two treatment groups in this trial.

7.4 Discussion

Although this technique has been used to induce metamorphosis in other lamprey species (e.g., Manzon et al., 1998), it was not successful in this preliminary trial. However, more work is necessary to determine if this technique did not work with Pacific lamprey because the sample size used during this preliminary trial was relatively small and the body condition of these ammocoetes may not have been high enough to begin metamorphosis.

8. Task 7: Contaminated Sediment Exposure Pilot Test

8.1 Objective

The objective of this task was to perform a pilot sediment bioassay to evaluate the toxicity of contaminated sediment from 10 sites in the Harbor to PLAs.

8.2 Methods

8.2.1 Sediments

Nine contaminated sediments collected from the Harbor, two reference sediments collected upstream from the Harbor, and one laboratory control sediment (clean masonry sand) were used to conduct a 45-day sediment bioassay with ammocoetes. The sediment sampling protocol and sampling locations for each sediment type are described in Stratus Consulting (2011b, 2011c).

8.2.2 Experimental design

Each sediment type was loaded into four 7.6-cm diameter lamprey corrals (see Task 3 description in Section 5) in each of three 20.5-cm diameter circular tanks (Figure 27) according to PLA SOP P.5 (Stratus Consulting, 2011d), for a total of 12 lamprey corrals per sediment type. Representative sediment samples were also collected from each sediment type during the loading process according to PLA SOP P.5 (Stratus Consulting, 2011d). These samples were shipped overnight on ice to CAS for subsequent chemical and physical analyses (PLA SOP P.11; Stratus Consulting, 2011d) and archiving.

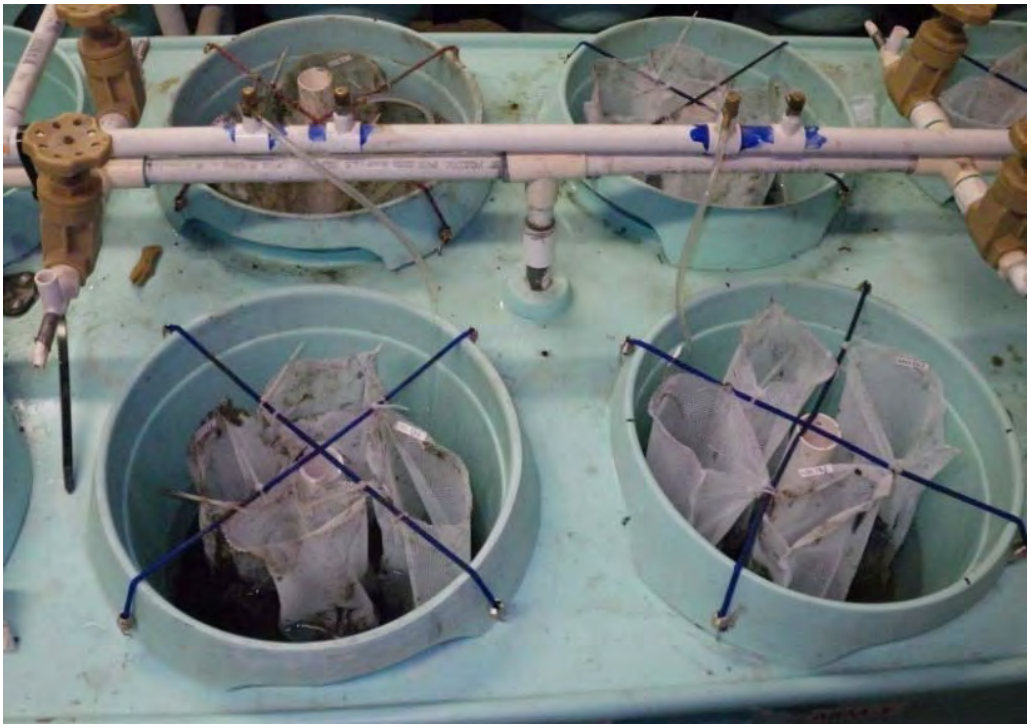


Figure 27. Individual lamprey corrals in larger circular exposure tanks.

Sediment was loaded into corrals on four consecutive days as follows: 10/25/2010 – MSC, SC1, AR1; 10/26/2010 – RE1, OST, GA2; 10/27/2010 – ARM, SWI, AR2; and 10/28/2010 – RE3, GAA, MAR. Water flowed through each tank for 24 hours prior to adding ammocoetes to any of the corrals. One ammocoete was placed at random into each corral (PLA SOP P.9; Stratus Consulting, 2011d) 24 hours after loading sediment into the corral. Ammocoetes were depurated for at least 24 hours, then were anesthetized (PLA SOP P.3; Stratus Consulting, 2011d), weighed, and measured (PLA SOP P.7; Stratus Consulting, 2011d), and randomly loaded into each corral (PLA SOP P.9; Stratus Consulting, 2011d).

8.2.3 Experimental monitoring and sampling

During the 45-day experiment, basic water quality parameters (temperature, pH, DO, EC) and flow rates were monitored in each tank on a rotating schedule so that no tank went more than 3 days without basic water quality measurements and no more than approximately 9 days without flow rate measurements. All corrals were monitored daily and at the end of the 45-day experiment for dead and moribund ammocoetes (PLA SOP P.10; Stratus Consulting, 2011d). At the end of the experiment, live ammocoetes were rinsed to remove sediment, depurated for

24 hours in individual containers containing cotton gauze, weighed and measured (PLA SOP P.7; Stratus Consulting, 2011d), and euthanized (PLA SOP P.3; Stratus Consulting, 2011d). The third gill pouch was excised from half of the ammocoetes removed from each sediment type (six) and immersed in 100 µL of ice-cold SEI buffer before flash freezing in liquid nitrogen. Samples were stored at -80°C for future NA^+/K^+ ATPase activity measurement. The bodies of three of these ammocoetes were preserved in 10% buffered formalin for histology. A section was removed from the abdomen from the remaining three ammocoetes and preserved in RNAlater® for potential future analysis of detoxification enzymes. The remaining ammocoetes (five–six) were stored at -20°C for dry weight and ash and lipid analyses.

8.3 Results

8.3.1 Water quality

Overlying water quality in each of the 36 exposure tanks (3 tanks per sediment type) remained consistent throughout the 45-day exposure (Table 20). The average (n = 27) total ammonia and unionized ammonia concentrations in all treatments were 0.18 ± 0.06 and 0.0002 ± 0.0001 mg/L, respectively.

8.3.2 Growth

The average initial weight and length of all ammocoetes loaded into each corral were 1.4 ± 0.7 g and 93 ± 17 mm, respectively (n = 144; Figure 28). There were no significant differences in initial ammocoete weight or length among any of the sediment types.

On average, ammocoetes in all treatments lost weight during the 45-day exposure (Figure 29, Table 21). Ammocoetes held in MSC lost the most weight (18.7% based on wet weight), which was expected because this treatment was a starvation control to determine maximum weight loss for ammocoetes held in sediment with no organic carbon and without feed. The MSC treatment lost significantly ($P < 0.05$) more weight than the AR2 treatment. There were no other significant differences in change in weight among any treatments.

Ammocoetes in most of the sediment types did not increase in length, and many of the ammocoetes shrunk slightly (Figure 30, Table 21). There were no significant differences in change in length in ammocoetes among any sediment types.

Table 20. Average water quality for each sediment type during the 45-day exposure

	Flow rate (mL/min)		Temperature (°C)		pH		DO (mg/L)		EC (µS/cm)	
Sediment	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
MSC	198	22	12.0	0.06	6.66	0.02	8.2	0.09	293	0.3
RE1	219	15	11.9	0.07	6.68	0.00	8.2	0.05	293	1.3
RE3	212	12	11.9	0.04	6.71	0.04	8.0	0.03	294	1.1
AR1	203	13	12.0	0.02	6.68	0.02	8.1	0.21	293	1.2
AR2	200	9	11.9	0.08	6.69	0.02	8.2	0.03	293	1.4
ARM	203	2	11.9	0.08	6.69	0.02	8.1	0.03	293	2.1
GA2	207	13	11.9	0.04	6.70	0.01	8.1	0.04	292	0.6
GAA	214	5	11.9	0.05	6.70	0.03	8.0	0.05	293	1.4
MAR	195	15	11.9	0.04	6.68	0.03	8.0	0.02	293	1.4
OST	198	12	12.0	0.03	6.67	0.04	8.1	0.05	293	0.7
SC1	198	9	11.9	0.03	6.64	0.03	8.2	0.03	293	1.5
SW1	193	17	11.9	0.05	6.68	0.03	8.1	0.07	294	1.2

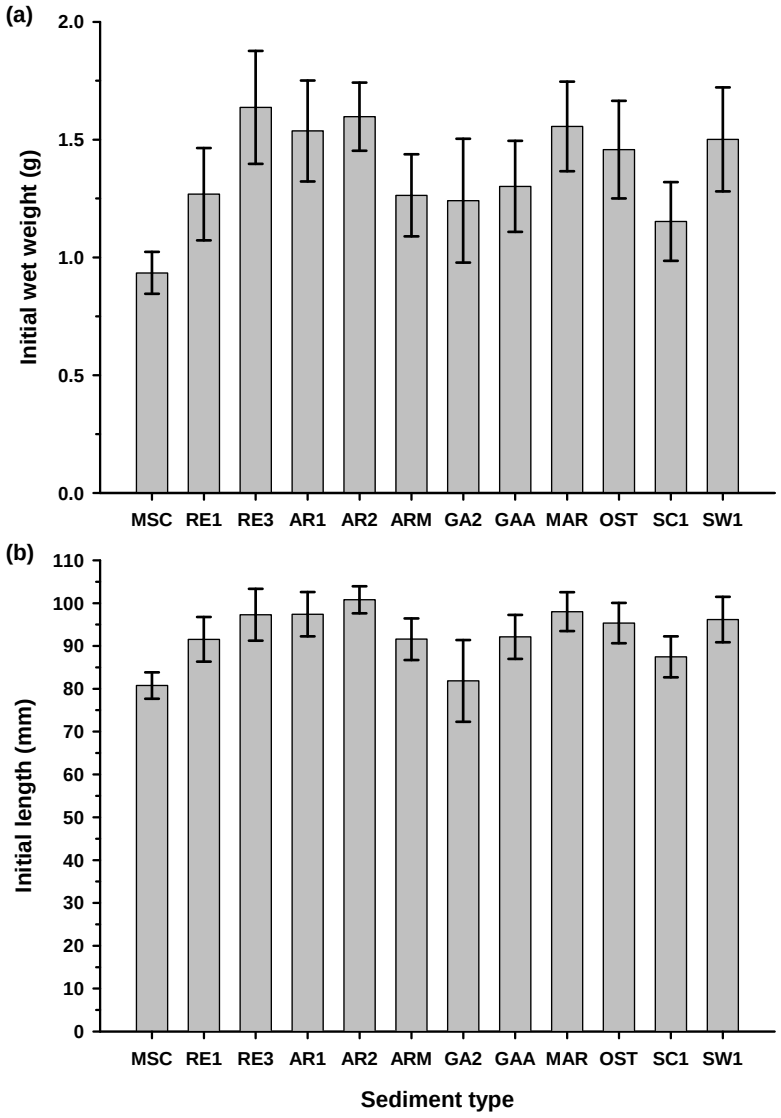


Figure 28. Mean initial wet weight (a) and length (b) in all ammocoetes loaded into each corral (n = 144). Error bars are ± one standard error of the mean. There were no significant differences in weight or length among ammocoetes loaded into corrals containing each sediment type.

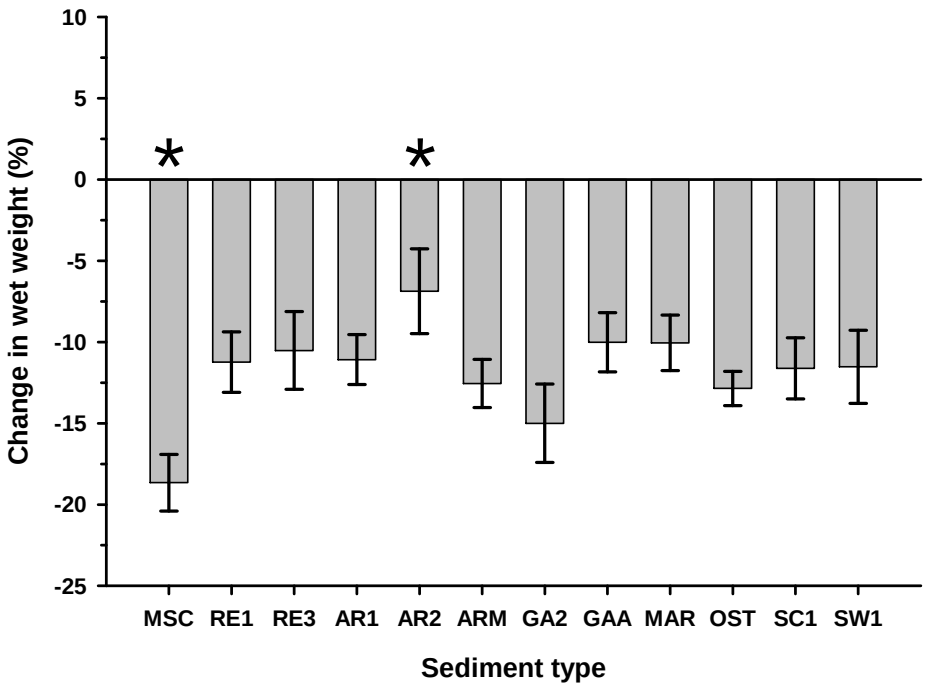


Figure 29. Mean change in depurated wet weight for individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 12 in all treatments, except for sediments RE1 and SW1 where n = 11). Error bars are ± one standard error of the mean. Sediment types with significantly different ($P < 0.05$) changes in mean ammocoete weight are indicated with an asterisk.

Table 21. Initial and final wet weight and length measurements of ammocoetes (n = 12 for each sediment type for all analyses, except final measurements for RE1 and SW1 where n = 11). All ammocoetes were depurated for 24 hours prior to weighing.

	Initial wet weight (g)		Final wet weight (g)		Initial length (mm)		Final length (mm)	
Sediment	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
MSC	0.93	0.31	0.77	0.28	80.8	10.7	78.7	9.8
RE1	1.27	0.68	1.18	0.66	91.5	18.0	91.3	19.8
RE3	1.64	0.83	1.50	0.82	97.3	21.0	97.8	21.7
AR1	1.54	0.74	1.38	0.70	97.4	18.0	96.4	18.4
AR2	1.60	0.50	1.49	0.51	100.8	11.0	101.2	12.4
ARM	1.26	0.60	1.13	0.60	91.6	16.8	90.1	16.4
GA2	1.24	0.91	1.11	0.91	87.3	22.0	85.5	23.4
GAA	1.30	0.67	1.19	0.66	92.1	17.8	91.9	19.3
MAR	1.51	0.67	1.38	0.67	96.8	15.8	96.5	17.1
OST	1.33	0.60	1.18	0.57	93.0	14.8	92.0	15.4
SC1	1.15	0.58	1.04	0.56	87.5	16.6	86.4	16.8
SW1	1.50	0.76	1.41	0.78	96.2	18.4	100.5	26.9

8.3.3 Lipid content

Lipid content in individual ammocoetes at the end of the 45-day exposure was highly variable and ranged from 0.13% ± 0.22 (MSC) to 5.1% ± 3.8 (RE3) based on live weight. There were no significant differences in lipid content among ammocoetes in different sediment types (Figure 31).

8.3.4 Histology

Histological abnormalities were consistent with the observations from ammocoetes examined in Task 3 (Section 5). The only histological abnormalities documented were encysted digenetic trematodes and granulomas likely caused by degraded encysted parasites found in ammocoetes from all sediment types. Counts of parasites and granulomas per slide did not indicate that any sediment type affected the prevalence or severity of infection (Figure 32).

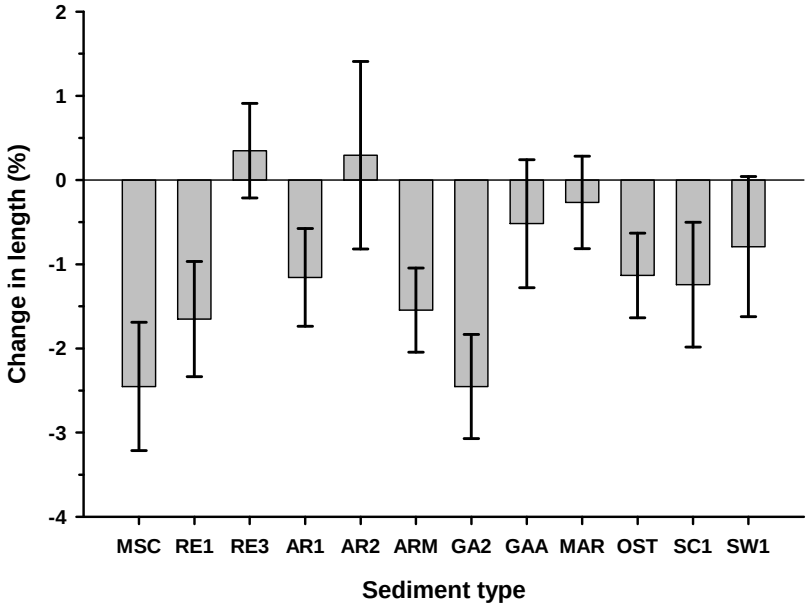


Figure 30. Mean change in length for individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 12 in all treatments, except RE1 and SW1 where n = 11). Error bars are ± one standard error of the mean.

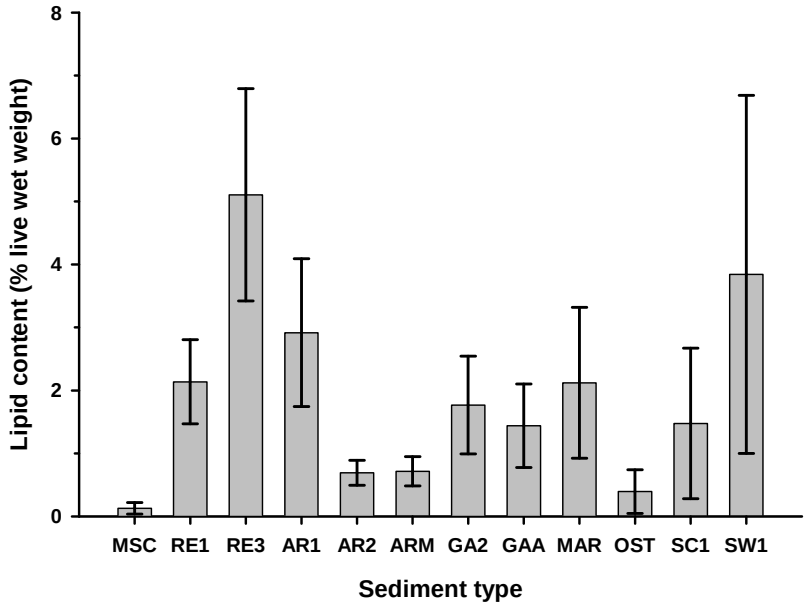


Figure 31. Mean lipid content (n = 6 in all treatments, except RE1, RE3, GAA, and SWI where n = 5) in individual ammocoetes exposed to each sediment type after a 45-day exposure. Error bars are ± one standard error of the mean.

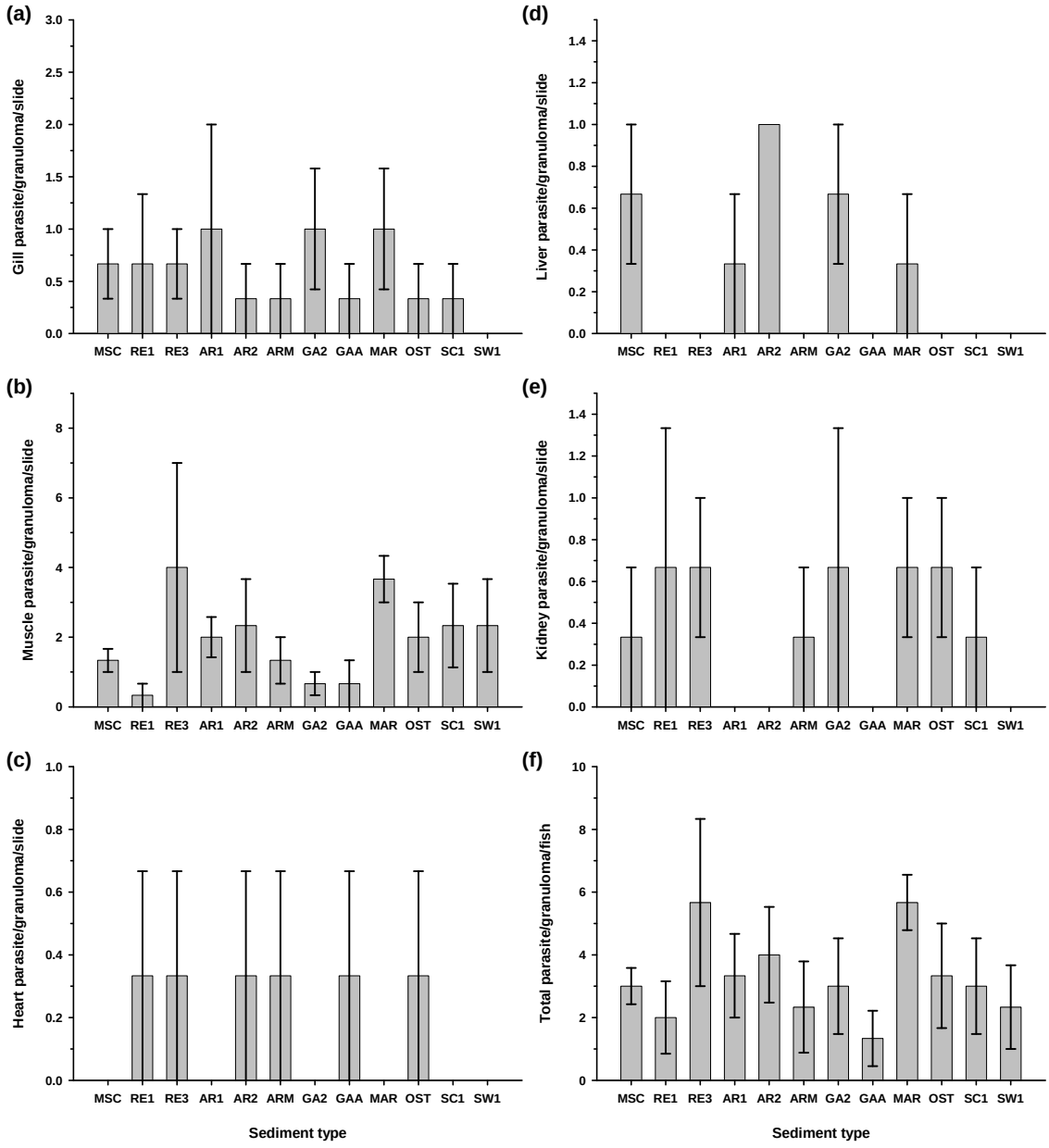


Figure 32. Counts of encysted parasites and granulomas on histology slides prepared from individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 3). Tissues analyzed include gill (a), muscle (b), heart (c), liver (d), and kidney (e). A sum of encysted parasites and granulomas observed on all tissues for each individual fish is also presented (f). Error bars are ± one standard error of the mean.

8.3.5 Gill Na^+/K^+ ATPase activity

The larger ammocoetes utilized in this task compared to Task 3 resulted in a large size range among ammocoete gill pouch samples. Therefore, some samples fell outside the protein standard curve and/or the activity slopes became non-linear as a result of excess protein. Samples with protein concentrations that fell outside the standard curve were not included in this analysis. Of the samples that did fall within the standard curve, gill Na^+/K^+ ATPase activity was below 2 µmol ADP/mg protein/hour in ammocoetes exposed to all sediments, except for one ammocoete from AR2 and GAA, which had activity levels at 5.6 and 12.8 µmol ADP/mg protein/hour, respectively. However, these levels were still much lower than the gill Na^+/K^+ ATPase activity level measured in a larger fish (109 mm, 2.14 g) that was undergoing metamorphosis in the stock holding tank (51.4 µmol ADP/mg protein/hour). There were no significant differences in mean gill Na^+/K^+ ATPase activity in ammocoetes exposed to any sediment types (Figure 33).

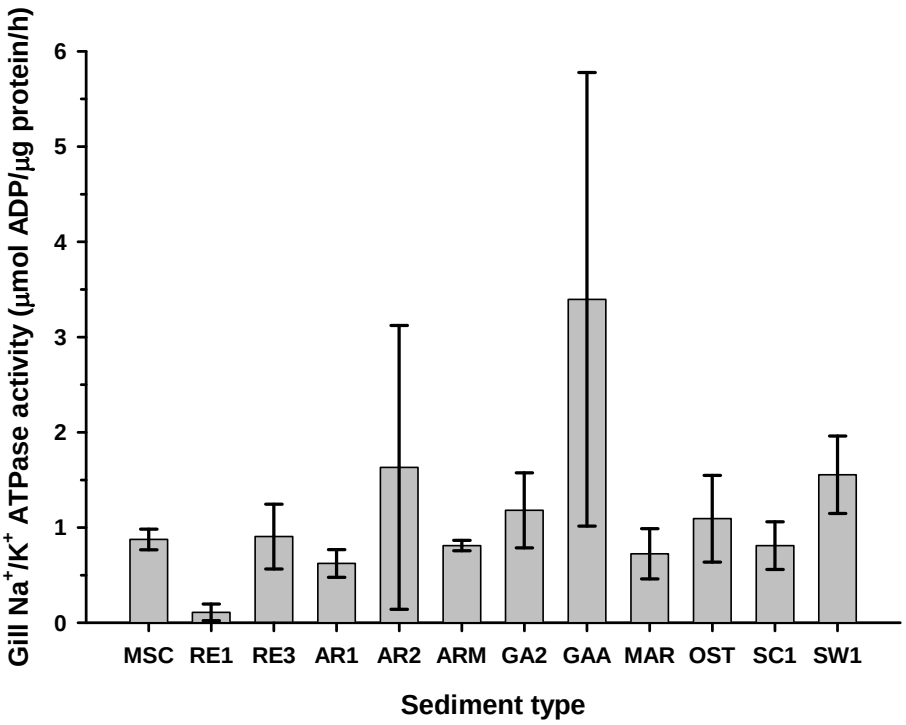


Figure 33. Gill Na^+/K^+ ATPase activity in individual ammocoetes exposed to each sediment type after a 45-day exposure (n = 5 for sediments AR1, GAA, and SWI; n = 4 for sediments RE1, RE3, AR2, ARM, MAR, OST, and SC1; and n = 3 for sediments MSC and GA2). Error bars are ± one standard error of the mean.

8.4 Discussion

PLAs were not measurably adversely affected by any of the contaminated sediments collected from the Harbor compared to control and reference sediments in terms of survival, growth, lipid content, and histology in this 45-day exposure.

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