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Effects of Collection, Transport, and Redeployment Methods on Natural Mortality of *Rangia cuneata* (Mactridae) used in Biomonitoring Studies

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ABSTRACT

Sporadic, high mortality in test populations of wedge clams (*Rangia cuneata)* has limited the potential for using this otherwise desirable test organism in biomonitoring studies. To determine whether high mortality was due to ontogenic or experimental variables, a two-phased study was conducted. In phase I, mortality of collected and re-deployed wedge clams, subjected to varying transport conditions, was determined at 7, 14, 21 and 60 days re-deployment. The use of three transport times (1, 2, 3 hr.), two vehicle conditions (open, closed) and three transport treatments (open, closed, iced containers) yielded 18 test groups. Individual test group mortalities were below 10% through the 21 day re-deployment period and peaked at 13% at the 60 day re-deployment point. The low rates of mortality observed in phase I of this study indicate that reasonable collection and transport of wedge clams does not significantly increase natural mortality and suggests other parameters are more strongly correlated to test population mortality. In phase II of this study, percent survival of collected and "acutely" redeployed and "acclimated" redeployed wedge clams was determined. Acclimated re-deployment is the transfer of *R. cuneata* from saline to freshwater in decrements of 3-4 ppt/day in accord with recommendations in Bedford and Anderson (1972). Acute re-deployment is the placement of *R. cuneata* in lower salinity waters or freshwater without acclimation. Although percent survival of clams acutely deployed to the freshwater test site was significantly ($p<0.05$) less than the percent survival at other test sites, mortality was only 3.3 %. No significant differences ($p<0.05$) were recorded in the percent survival of acclimated redeployed wedge clams.

Keywords: *Rangia cuneata*, wedge clam, mortality, bioconcentratable pollutants

INTRODUCTION

Interest in developing biological monitoring systems to monitor pollutant bioaccumulation has increased over the past ten years due in part to the continued emphasis on bioconcentratable pollutants by the U.S. Environmental Protection Agency (EPA)(USEPA 2000, VDEQ 1994, USEPA 1991). The potential for *Rangia cuneata* (wedge clam) to be used as a biomonitoring organism was described approximately 25 years ago (Croonenberghs 1974), and was based on the animal's occurrence in many

oligohaline systems in U.S. and portions of Mexico, it's ease of collection, and it's large tissue yields for analysis (Pfitzenmeyer and Drobeck 1964, Gooch 1971, Pinkney et al. 1995).

Effectiveness of a wedge clam system to monitor pollutant bioaccumulation was recently demonstrated in field studies (Grimes 1992; VDEQ 1994; Pinkney et. al. 1998, 1997, 1995). Notably, it's use in the characterization of bioaccumulation potentials associated with PCB and pesticide discharges from an old leaking landfill provided regulatory agencies the basis for instituting a fish consumption advisory for protection of human health (U.S. Marine Corps Base-Quantico 1996).

However, other wedge clam biomonitoring studies have had varying success rates because of wide ranging mortality in control and test populations in the absence of acutely toxic conditions (Grimes 1996). Causal factors associated with these sporadic high mortalities are unknown, in part because there have been no studies to identify natural mortality rates of *R. cuneata.*

Determination of natural mortality rates, and mortality rates associated with the collection (e.g. dredge), transport [1-3 hr. with varying handling methods (i.e., clams with or without ice in various containers held in or outside vehicle)], and re-deployment of *R. cuneata* was needed to understand these characteristics of the wedge Clam biomonitoring system. Accordingly, objectives of this study were to (1) define natural mortality, (2) determine effects of collection and transport treatment on natural mortality of *R. cuneata*, and (3) test the efficacy of deploying clams acclimated and not acclimated to aquatic environments where water quality parameters differ from those of the collecting site.

MATERIALS AND METHODS

Phase I Collection and transport studies:

A total of 570 *R. cuneata* (size range 33-75 mm) were collected by hand from oligohaline reaches of the James River, VA on 28 October 1999. This collection time was two months later than the latest occurrence of mature gonads as reported by Pfitzenmeyer and Drobeck (1964), and was selected to minimize effects (e. g. low post-spawning biomass) of spawning on mortality. Collected wedge clams were held underwater in plastic mesh baskets while randomly sorted into control and test groups. Each replicate (15 clams) of control and test clams was placed in a 23 x 46 cm, 19.4 mm stretch mesh bag which was tagged for travel time and transport treatment parameters. Control clams were returned to the collection area after zero travel time and no transport treatment. Test clams were exposed to the following conditions (Table 1): 1) transport travel speed=80 km/h; 2) open or closed vehicle conditions (i.e., on top of or inside vehicle, respectively); 3) 1-hr, 2-hr, and 3-hr transport times; and 4) "open" test treatment (bushel baskets), "closed" test treatment (covered Styrofoam coolers), and "iced" test treatment (covered Styrofoam coolers with 1-liter of ice placed on top of clams). These ranges of exposure conditions are based on combinations of conditions that clams experienced in previous studies. Because of the wide distribution of *R. cuneata*, maximum travel times to redeployment test sites have not exceeded 3 hr. in previous studies (pers. obs). Each test treatment was equipped with minimum-maximum thermometers. At the end of each travel time, mortality and minimum and maximum temperatures (C) were recorded from each test treatment.

TABLE 1. Minimum, maximum and ΔT temperatures (C) of containers (open, closed, and closed with ice) of *Rangia cuneata* held inside (initial temperature=13.4 C) and outside (initial temperature=14 C) of vehicle at 1, 2, and 3-hour travel times on 28 October 1999.

Vehicle Placement	Test Condition	Travel Time (hr)	Temp (C)	ΔT
Inside	open	1	16.1	2.7
Inside	open	2	18.6	5.2
Inside	open	3	20.5	7.1
Inside	closed	1	13.5	0.1
Inside	closed	2	16.0	2.6
Inside	closed	3	20.0	6.6
Inside	closed with ice	1	13.9	0.5
Inside	closed with ice	2	10.8	-2.6
Inside	closed with ice	3	13.3	-0.1
Outside	open	1	14.4	0.4
Outside	open	2	15.6	1.6
Outside	open	3	17.8	3.8
Outside	closed	1	13.9	3.9
Outside	closed	2	15.3	1.3
Outside	closed	3	15.6	1.6
Outside	closed with ice	1	11.1	-2.9
Outside	closed with ice	2	15.3[a]	1.3
Outside	closed with ice	3	12.8[b]	-1.2

a-cooler with large crack
b-cooler with small crack

TABLE 2. Air and water quality parameters at study control site in James River, Virginia from October 28 -December 23, 1999.

Date	Temp (C)		pH	Alkalinity	Hardness	DO	Salinity
	Air	Water	(mg/L)	(mg/L)	(mg/L)		(ppt)
10-28	13.9	24.5	7.2	42	280	8.8	3
11-04	11.1	18.0	7.5	90	600	10.5	3
11-11	9.4	17.5	7.4	60	510	10.0	10
11-18	9.4	10.5	7.3	60	560	10.2	3
12-23	3.3	7.5	7.2	80	520	11.8	4

Replicates from each test treatment were placed into 0.2 x 0.3 m square mesh bags and then into one meter square mesh bags, which were tagged for travel time. Travel time mesh bags containing individual "test treatment" mesh bags were then returned to the collection area alongside control clams. Clam mortality, water temperature, pH, dissolved oxygen, salinity, alkalinity and hardness and air temperature were measured at the 0, 7, 14, 21 and 60 day re-deployment intervals (detection levels of: pH ±0.5; dissolved oxygen, ±0.2 mg/L; salinity, ±1 ppt; alkalinity, ±4 mg/L; and hardness, ±4 mg/L (Table 2). Dead clams were measured for maximum shell length (mm) and removed from bags.

Spearman correlation analysis (SAS, 1996) was used to identify variables significantly ($p \leq 0.05$) correlated with mortality. A one-way analysis of variance followed

by Duncan's Multiple Range Test (SAS 1996) was used to determine significant ($p< 0.05$) differences between variables significantly correlated with mortality.

Phase II Acute and Acclimated Redeployment Studies:

A total of 180 *R. cuneata* (size range=35-63 mm), collected by the same methods and in the same locations as used in the phase I studies, were divided randomly and placed in twelve mesh bags (15 clams/mesh bag). Two of these were placed back into the water at the collection site as control replicates. The 10 remaining bags of clams were deployed at three locations in the James River after 0.5, 1, and 2 hour transport times, respectively: James River at mouth of Chickahominy River (two replicates for acute re-deployment and two replicates for acclimation re-deployment; 21 km upstream of control site); Rt. 155 bridge at Jordan Point (two replicates each for acute re-deployment and acclimation re-deployment; 58.5 km upstream of control site); and Heugenot Bridge (two replicates for acute re-deployment; 94.5 km upstream of control site). Clams acutely deployed were left *in situ* for the duration of the 21-day study to compare percent survival between acute and acclimated re-deployment. The two acclimation replicates at the Chickahominy and Jordan Point sites remained at their respective sites for three days based on recommendations in Bedford and Anderson (1972), that transferring *R. cuneata* from saline to freshwater may be less stressful to the animals when made in decrements of 3-4 ppt/day. Acclimation clams at Jordan Point were transferred to the Heugenot Bridge on 27 October 2000. Acclimation clams at Chickahominy River were transported to Jordan Point on 27 October, and then from Jordan Point to Heugenot Bridge on 30 October 2000. Percent survival of clams at each site was determined on October 24, 27, and 30, and November 2, 7 and 14. Water quality parameters measured in acute and acclimation re-deployment studies were the same as those taken in phase I studies (Table 3). A one-way analysis of variance followed by Duncan's Multiple Range Test (SAS 1996) was used to determine significant ($p\leq 0.05$) differences in percent survival between control and test sites.

RESULTS

Phase I Collection and transport studies:

After 60 days re-deployment, total control mortality of *R. cuneata* was 0% while individual test group mortalities ranged from 0-13% ($p\leq 0.05$). Total test group mortality was not significantly different between: vehicle conditions ($F=0.57$; $p>F=0.56$; $df=2$); travel treatments ($F=1.35$; $p>F=0.2553$; $df=3$); or travel treatments by vehicle conditions ($F=1.61$; $p>F=0.1542$; $df=6$). Total mortality of *R. cuneata* in the 1-hr travel time-closed transport treatment test group was significantly greater than other time-transport treatment test groups ($F=1.04$; $p>F=0.4129$; $df=2$)(Table 4). Although there were significant differences in mean clam sizes between some test groups, clam size (mean=48.26 mm, min=33, max=75) was not significantly correlated with mortality ($R=-0.00891$; $p>R=0.67$). Mortality was significantly correlated with pH ($R=-0.1102$; $p>R=0.0001$), hardness ($R=-0.06378$; $p>R=0.0024$), dissolved oxygen ($R=0.1505$; $p>R=0.0001$), and water temperature ($R=0.10676$; $p>R=0.0001$).

Phase II Acute and Acclimated Redeployment studies:

Acute re-deployment survival (96.7%) of *R. cuneata* at the Heugenot Bridge test site was significantly lower ($F=3.67$; $p>F=0.019$; $df=3$) than survival at other re-deployment test locations (Table 5).

TABLE 3. Air and water quality parameters for acute and acclimation re-deployment studies of *Rangia cuneata* at control station and test stations in James River (mouth of Chickahominy River; Jordan Point; and Heugenot Bridge in Richmond, VA) from 24 October – 14 November 2000.

Station	Date		
	10-24	11-7	11-14
Air temp (C)	12.7	10.5	4.4
Control			
Water temperature (C)	21.0	15.0	18.0
pH	7.7	8.0	7.7
Salinity (ppt)	6.0	10.0	16.0
Alkalinity (ppm)	40.0	68.0	82.0
Hardness (ppm)	530.0	520.0	560.0
D.O. (ppm)	9.2	9.2	8.6
Chickahominy			
Water temperature (C)	18.0	15.0	14.0
pH	7.0	7.5	7.3
Salinity (ppt)	3.0	3.5	4.0
Alkalinity (ppm)	40.0	78.0	70.0
Hardness (ppm)	240.0	440.0	400.0
D.O. (ppm)	8.0	10.2	10.0
Jordan Point			
Water temperature (C)	19.5	14.5	14.5
pH	7.7	7.3	7.3
Salinity (ppt)	1.0	1.5	2.0
Alkalinity (ppm)	44.0	80.0	78.0
Hardness (ppm)	100.0	96.0	130.0
DO (ppm)	11.6	9.0	9.0
Heugenot Bridge			
Water temperature (C)	18.0	14.0	13.0
pH	7.7	7.3	7.4
Salinity (ppm)	0	0	0
Alkalinity (ppm)	10.0	100.0	110.0
Hardness (ppm)	18.0	100.0	80.0
D.O. (ppm)	9.6	10.2	9.0

The acclimation re-deployment test groups showed no significant differences in percent survival of *R. cuneata* ($F=5.18$; $p>F=0.60$; $df=2$).

DISCUSSION

Studies of bioconcentratable pollutants typically are conducted over a 28-day period (USEPA and USACE, 1998). Our studies indicate the efficacy of using *R. cuneata* as a biomonitoring tool in studies of up to 60 days. Using protocols outlined in this study, *R. cuneata* can be collected, transported and redeployed from oligohaline (i.e., water temperature range=15-21 C; salinity range=6-16 ppt; pH range=7.7-8.0; alkalinity range=40-82 ppm; hardness range=520-560 ppm; and D.O. range=8.6-9.2

TABLE 4. Control, test group mortality and % mortality of *Rangia cuneata* at 0, 7, 14, 21 and 60 days re-deployment in James River, Virginia from October 28-December 23, 1999.

	Re-deployment Interval (Days)						
Test Group	0	7	14	21	60	Total	Mortality
Control	0	0	0	0	0	0/30	(0%)
Inside Vehicle:							
1 hr. in-open	0	0	1/30	1/29	1/28	3/30	(10%)
1 hr. in-closed	0	0	0	0	4/30	4/30	(13%)
1 hr. in-ice	0	0	0	0	0	0/30	(0%)
2 hr. in-open	0	0	0	1/30	1/29	2/30	(7%)
2 hr. in-closed	0	0	0	0	2/30	2/30	(7%)
2 hr. in-ice	0	0	0	0	0	0/30	(0%)
3 hr. in-open	0	0	0	0	0	0/30	(0%)
3 hr. in-closed	0	0	0	0	0	0/30	(0%)
3 hr. in-ice	0	0	0	0	0	0/30	(0%)
all inside groups	0	0	1/270	2/269	8/267	11/270	(4%)
Outside Vehicle:							
1 hr. out-open	0	0	0	0	1/29	1/29	(3%)
1 hr. out closed	0	0	0	0	4/30	4/30	(13%)
1 hr. out-ice	0	0	0	0	3/30	3/30	(10%)
2 hr. out-open	0	0	0	0	0	0/30	(0%)
2 hr. out closed	0	0	0	0	1/30	1/30	(3%)
2 hr. out-ice	0	0	0	0	1/30	1/30	(3%)
3 hr. out-open	0	0	0	0	0	0/30	(0%)
3 hr. out closed	0	0	0	0	0	0/30	(0%)
3 hr. out-ice	0	0	0	0	0	0/30	(0%)
all outside groups	0	0	0	0	10/269	10/269	(4%)

ppm)(Table 2) to freshwater conditions (i.e., water temperature range=13-18 C; salinity=0 ppt; pH range=7.3-7.7; alkalinity range=10-110 ppm; hardness range=18-100 ppm; and D.O. range=9.6-10.2 ppm)(Table 3) without significant mortality even when acutely deployed to freshwater conditions. Although *R. cuneata* does not inhabit freshwater environments because it requires a minimum salinity of 6 ppt to initiate gamete release (Chanley, 1965, Gainey and Greenberg, 1977), its ability to significantly osmoregulate allows *R. cuneata* to survive in both hypo-osmotic and hyper-osmotic environments (Saintsing, 1979; Saintsing and Towle, 1978a, 1978b). When transferred from 10 ppt to 0 ppt salinity, conditions requiring significant osmoregulation, *R. cuneata* reach equilibrium within 12 hours (Saintsing, 1979; Saintsing and Towle, 1978a, 1978b).

In our collection and transport studies, there were no significant short-term (21 days) effects of collection, travel time or transport conditions, as mortalities were below 0.1 %. Although the 10 % mortality threshold was exceeded in some test treatments 60 days after re-deployment, we suspect this late increase in mortality may have been the result of their inability to escape freezing air temperatures when exposed during periods of unexpectedly low tides. The spatial arrangement of these test treatments in the travel time re-deployment bags during the later stage of the study placed them on

TABLE 5. Control, test group, and percent survival of *Rangia cuneata* at 0, 3, 6, 9, 14, and 21 days deployment and re-deployment in James River, Chickahominy River, Jordan Point, and Heugenot Bridge from October 24 – November 14, 2000.

	Re-deployment Interval (days)						Survival	
Group	0	3	6	9	14	21	Total	%
Control	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Deployment								
Chickahominy	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Jordan Point	30/30	30/30	30/30	30/30	30/30	30/30	30/30	100
Heugenot	30/30	30/30	30/30	29/30	29/30	29/30	27/30	90
Re-Deployment								
Control	30/30							
Chickahominy	30/30	30/30						
Jordan Point			30/30					
Heugenot				30/30	30/30	30/30	30/30	100
Control	30/30							
Jordan	30/30	29/30						
Heugenot			29/30	29/30	29/30	29/30	29/30	96.7

top of other test treatments and thereby precluded significant burrowing into the substrate.

This study addresses some priority issues and research needs for the field of bioaccumulation monitoring as outlined by EPA (2000) (e.g. identification and development of additional species for water quality and sediment bioaccumulation test methods; guidance on how reference sites can be selected; and how this species should be collected and transported for bioaccumulation testing). Based on results of our investigations into the collection, maintenance, transport, and re-deployment of *R. cuneata*, we recommend the following protocol for using *R. cuneata* in bioconcentratable pollutant studies.

1. Collect specimens and conduct bioconcentratable pollutant studies in autumn to minimize effects (e. g. low post-spawning biomass) of spawning on mortality (see Pfitzenmeyer and Drobeck 1964).
2. Maintain specimens in collection site water until ready for transport.
3. Transport specimens in closed containers with ice outside the vehicle; or in open containers inside an air-conditioned vehicle to re-deployment sites within 3 hr. of collection;
4. Maintain specimens in containers specified above while distributing specimens into mesh-bags prior to re-deployment (nylon mesh allows burrowing whereas metal fabrics inhibit burrowing).
5. Deploy 15 clams/2600 cm^3 replicate mesh-bag at sampling sites to safeguard against density limitations to burrowing.

Our future studies include determining rates of bioconcentration in hypo-osmotic and hyper-osmotic environments and ascertaining whether *R. cuneata* continues to feed

after collection, transport, and re-deployment to various sites. Such data are requisite for making comparisons between control and test groups redeployed at sites with different water quality parameters.

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