

Dangers for Unprotected Embryos on the Benthos

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Larval Ecology

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Abstract

One of the arguments for the persistence of planktonic larval stages in the life cycles of benthic marine invertebrates is that pelagic larvae experience a release from predation when they move off the benthos and into the water column. However, it is difficult to substantiate this hypothesis because we lack comparative data on mortality rates of unprotected (e.g., not in egg capsules or gelatinous masses) embryos and larvae on the benthos and in the water column. The aim of this study was to provide some of this missing information, by comparing development success in benthic and pelagic environments. *Dendraster excentricus* embryos were placed in enclosed development chambers under three treatment conditions: 1) directly on the substratum (unscreened benthic), 2) on a screen on the substratum (screened benthic), and 3) on a screen raised 3 cm above the substratum (screened pelagic). Development success for pelagic embryos was consistently higher than those on the benthos, substantiating the hypothesis that embryos experience a release from predation as they move off the benthos. In addition, the screened benthic treatment had significantly higher development success than the unscreened benthic treatment, indicating that macro-predators (>55 μm) may significantly reduce development success for unprotected larvae. Higher development success of protected pelagic treatments may have been due to lower abundances of pelagic micro-predators or anoxic conditions on the benthos.

Introduction

The functional advantage of a pelagic larval stage in the life cycle of many benthic marine invertebrates and the processes that lead to the persistence of these pelagic stages are central concepts in marine ecology. Fossil evidence suggests that marine larval stages arose in the Late Cambrian (Muller and Walossek 1988; Walossek

and Muller 1989; Signor and Vermeij 1994) and were likely planktotrophic (reviewed by Freeman and Lundelius 2007). In the early Cambrian few organisms were pelagic, thus escape from benthic predators may have been a driving force in the evolution of planktonic larvae (Signor and Vermeij 1994; Peterson 2005). Although planktonic predators are now present and prevalent, release from predation may still be a driving force in maintaining pelagic larval stages (Strathmann 1985, 1993, 2007).

Many organisms that live on the benthos have encapsulated embryos and larvae, while most pelagic embryos hatch quickly from envelopes and swim as freely swimming larvae (Strathmann 1985; Rumrill 1990). Mortality rates for encapsulated or aggregated benthic embryos are typically less than 0.1 day^{-1} (Strathmann 1985; Rumrill 1990), while those estimated for pelagic larvae are generally much higher (Strathmann 1985; Rumrill 1990; Allen and McAlister 2007; Allen 2008). Despite the high mortality rates in the plankton, benthic mortality of unprotected embryos and larvae is likely to be higher still due to the high concentration of benthic predators (Pechenik 1979; Caswell 1981; Strathmann 1985; Strathmann et al. 2002). Despite this inference, mortality of unprotected embryos and larvae on the benthos is poorly understood for marine invertebrates. When the benthic embryos of the Peacock wrasse were left unguarded mortality was four times higher than with parental care (Warner et al. 1995). In addition, tethering studies on large zooplankton have shown higher mortality at the benthos than the pelagic zone (Acosta and Butler 1999; Motro et al. 2005; Allen and McAlister 2007). Mortality rates on the benthos for small larvae ($< 1 \text{ mm}$) and embryos of benthic invertebrates are unknown, even though benthic embryos are released by members of diverse phyla, such as ascidians and echinoderms (Strathmann 2007)). Limited evidence

from holoplankton demonstrates that benthic predators can decrease survival of resting eggs. Some copepods have resting eggs that can survive up to 10 years in the sediment (Katajisto 1996). However, presence of a deposit feeding amphipod can decrease the survival of these eggs, especially when they are nearing the time of hatching (Albertsson and Leonardsson 2001).

Knowledge of mortality rates and sources for unprotected, benthic embryos and larvae of benthic marine invertebrates will help substantiate or refute the hypothesis that pelagic larvae experience a release from predation when they move from the benthos to the relatively safer planktonic zone (Strathmann 1985, 1993, 2007). I measured development success *in situ* from the early embryonic stage to the newly hatched larval stage for unprotected embryos in the following three conditions: 1) unscreened benthic (embryos were directly on the substratum), 2) screened benthic (embryos were on the substratum, but protected by a 55 μm Nitex mesh), and 3) screened pelagic (embryos were suspended above the substratum on an 55 μm Nitex mesh). By comparing survival to an early larva under these three conditions I partitioned the dangers for unprotected embryos on the benthos. Comparisons between screened and unscreened embryos directly on the substratum provide information on the dangers embryos experience from macro-predators ($> 55 \mu\text{m}$; e.g., crustaceans, cnidarians, Platyhelminthes, annelids, nemerteans) versus micro-predators ($< 55 \mu\text{m}$; e.g., small animals, single celled eukaryotes, bacteria, fungi, viruses). In contrast, comparisons between screened benthic and screened pelagic development success can elucidate how survival is affected by benthic and pelagic micro-predators or physical factors (e.g., toxic sulfides, abrasion by sediment). I hypothesize that unprotected embryos will develop most successfully when

they lack contact with the benthos completely. This will exclude macrofauna and meiofauna benthic predators, as well toxic compounds in the substratum that could negatively impact developing embryos.

Methods

Unprotected embryos: I used *Dendraster excentricus* embryos to represent typical unprotected embryos produced by free-spawning organisms. *D. excentricus* were obtained from Crescent Beach in East Sound, Orcas Island, Washington and held in flowing seawater tanks at Friday Harbor Marine Lab, San Juan Island. Spawning was induced by injecting 1 ml of 0.5 M KCl through the oral opening. Injections were made in three locations to maximize the spread of KCl. To obtain gametes, *D. excentricus* were inverted over a beaker filled with filtered sea water so that the gonopores were immersed. If eggs were observed, spawning was allowed to continue into the beaker. If sperm was observed the individual was placed over a concentrated petri dish to collect concentrated sperm. A dilute sperm mixture was created by mixing concentrated sperm (enough to fill 5 mm up the tip of a pasture pipet) with 50 ml of filtered sea water. Six drops of dilute sperm were added to the eggs produced by three *D. excentricus* suspended in 800 ml of seawater. Eggs were kept in chilled seawater for no more than 2 hours before fertilization and concentrated sperm was kept covered and refrigerated and used within two days. Embryos were deployed in the field within 2 hours of fertilization. To estimate embryo density and fertilization rate, 0.25 ml aliquots were counted ($n = 10$) each deployment period. For the first and second setting periods the fertilization rate was 47.6% (SD = 4.9) and 99.6% (SD = 0.4) and embryos suspended in seawater had a density of 191.2 ml^{-1} (SD = 12.5) and 583.6 ml^{-1} (SD = 13.1), respectively.

Field Site: Field deployments occurred in Argyle Lagoon, on the eastern side of San Juan Island, Washington (N 48°31', W 123°36'). Argyle Lagoon is a shallow, almost enclosed tidal lagoon, which is connected to Argyle Bay at its northwest corner via a narrow stream-like channel, Argyle Creek. Water only enters the lagoon near high tide and its narrow opening prevents a rapid outflow of water; water continues to exit Argyle Creek until the next rising tide. The majority of Argyle lagoon is continuously sub-tidal and it lacks high, turbulent flow conditions. Argyle lagoon is primarily composed of fine sediments with a high organic content, except near the entrance of Argyle Creek where gravel and sand flats occur.

Development chambers: After hatching *D. excentricus* larvae immediately swim upward. Thus, development chambers were constructed that allowed embryos to lie on or suspended above the substratum (depending on treatment); when embryos hatched they swam upward into a collection vial, which could be removed and counted to measure development success.

Development chambers consisted of 473 ml funnels with Nitex mesh attached to the large ends and collection vials at the small ends (Fig. 1). Collection vials were 15 ml centrifuge tubes, with the pointed ends removed. They were attached to the funnels via Tygon® tubing 15 mm in diameter and 7 cm long. Four pairs of 2 mm holes were drilled around the rims of all funnels and U-shaped wire stakes were inserted through these holes to anchor development chambers. To allow water flow, funnels had four evenly spaced holes located 5.5 cm above the base of the funnel. Holes were 2.4 cm in diameter and openings were covered with 55 µm Nitex mesh, attached using cyanoacrylate glue and hot glue. Inside the funnel a PVC ring 10.7 cm in diameter and 3 cm high, was attached

with hot glue. Different Nitex meshes were attached to the PVC ring for the 3 disparate treatments. For the screened pelagic treatment 55 μm mesh was attached at the top of the PVC ring (shown in Fig. 1) so that the mesh was raised 3 cm above the bottom of the funnel. For the screened benthic treatment 55 μm mesh was attached to the bottom of the PVC so that it was flush with the base of the funnel. For the unscreened benthic treatment 1 mm mesh was attached at the top of the PVC ring; embryos fell through this mesh to rest directly on the substrate.

Field Deployment: Twelve development chambers ($n = 4$ for each treatment) were deployed for 24 hours on two occasions in August, 2014 (15th/16th and 16th/17th). Chambers were placed so that they were submerged for the entirety of their deployment on the sand flat near the entrance of Argyle Creek into Argyle lagoon. Chambers were deployed in clusters, with each cluster containing one of each treatment type (screened pelagic, screened benthic, and unscreened benthic). Treatments were clustered so that all treatments were exposed to any changes in micro-environmental conditions. In a cluster chambers were placed within 20 cm of each other and clusters were at least 1 m from each other. Clusters were deployed in locations that had a uniform sandy substrate, lacking shells, bivalve siphons, large crustaceans, and algae.

To deploy development chambers they were first allowed to fill with water through their Nitex mesh bottoms. To help with air release the lids were removed from the collection vials, but openings were kept just above the surface to avoid the entrance of unfiltered water. Once the chambers were filled with water the Tygon® tubing was crimped and 2 milliliters of suspended embryos of *Dendraster excentricus* were added to the collection vials. Development chambers were then placed so that they were flush

with the substrate and the four stakes were pushed by hand into the sand to secure the development chamber. After each chamber was in place the tubing was uncrimped and embryos were allowed to fall down into the funnel and onto or through the Nitex mesh.

To retrieve development chambers after a 24 hour deployment, Tygon® tubing was crimped and chambers were removed from the water. The tubing was then detached from the funnel and collection vials, with tubing attached, were transported back to the laboratory, where all larvae were counted under a dissecting microscope.

Control: To determine if differences observed among treatments were due to biological factors or an artifact of the development chamber construction for each treatment, I ran an additional trial under laboratory conditions. Sand that had been dried in the sun was added to a laboratory tank and all water entering the tank was filtered with a 1 µm bag filter. For each treatment condition four development chambers were deployed for 24 hours on August 17/18, 2014. All methods were consistent with those explained above.

Data Analysis: Statistical analyses were conducted in R (version 2.13.1; <http://www.R-project.org>). I used a linear model to test for the effect the treatment and setting day. Data were arcsine cube root transformed to meet assumptions of normality and equal variance. Equality of variances and normality were tested using Levene's Test and a Shapiro-Wilk Normality Test, respectively. Post-hoc pairwise comparisons among treatments were performed using Ryan's Q-test (recommended in Day & Quinn, 1989); when Q was less than the difference between a pair of treatments, treatment means were considered different. Daily instantaneous mortality (M) rates were calculated as $M = \ln(N_t/N_0)/-t$ (Rumrill 1990), where N_0 is the number of embryos placed in

development chambers and N_t is the number of larvae reared after a certain amount of time (t , in days).

Results

For the field treatments development success of *Dendraster excentricus* embryos was 83.2%, 41.6%, and 0.5% for screened pelagic, screened benthic, unscreened benthic treatments, respectively (Fig. 2). There was a significant difference among treatments, but not between days set or their interaction (Table 1). All treatments were significantly different ($Q < \text{difference between means for each pairwise comparison}$), with screened pelagic embryos having the highest survival and unscreened benthic embryos having the lowest survival. Mortality rates were 5.79, 0.86, and 0.12 day^{-1} for unscreened benthic, screened benthic, and screened pelagic treatments, respectively.

For the laboratory treatment development success of embryos was 60.2%, 38.3%, and 38.1% for screened pelagic, screened benthic, unscreened benthic treatments, respectively (Figure 3). There was no significant difference among treatments (Table 2).

Discussion

Consistent with my hypothesis, *D. excentricus* embryos had the lowest development success in the unscreened benthic treatment. This low development success is consistent with life history theories that predict lower survival on the benthos than in the water column for unprotected embryos (Strathmann 1985, 1993, 2007). Since the unscreened benthic treatment had significantly lower development success than the screened benthic treatment, macro-predators ($> 055 \mu\text{m}$) that were excluded from the screened treatment are a likely cause of benthic mortality. A physical interaction with the substrate, such as abrasion or burial, is another possible cause of this differential benthic

mortality. However, there was no significant difference between screened and unscreened benthic treatments in the laboratory control, indicating that physical disturbance is unlikely.

Higher development success in protected pelagic treatments, compared with protected benthic treatments, could have multiple causes. Macro-predators were excluded from both of these treatments, but micro-predators ($< 0.55 \mu\text{m}$) possibly had higher density on the benthos, reducing development success. For example, bacterial infection could primarily affect eggs directly on the benthos; many benthic egg masses are at risk of bacterial infection and have evolved chemical defenses to deal with infection (Benkendorff et al. 2001). In addition, anoxia within development chambers may have curtailed development success. Oxygen availability is an important limitation on embryo development in egg masses (Moran and Woods 2007). Boundary layers can limit O_2 flux (Moran and Woods 2010), thus the benthic boundary layer combined with limited flow within development chambers may have created a low O_2 environment that prevented embryo development on the benthos, but not raised above the benthos.

To decrease the possibility of anoxia, future studies should use larger Nitex mesh on the sides of the development chambers and have a greater area covered by mesh. To help elucidate whether anoxia reduced benthic development success, an attempt could be made to track the fate of undeveloped embryos from the base of development chambers.

To further understand pelagic versus benthic predation for unprotected embryos, future studies should add an additional “unscreened pelagic” treatment. Rather than allowing water to enter the chamber through the Nitex mesh on the bottom of the chambers, removing macro-predators, water from the deployment location could be

poured into the development chamber, capturing a natural component of the pelagic predator community.

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Table 1: Linear model results for the test of treatment, day, and their interaction on survival to hatching of *Dendraster excentricus* under field conditions. Treatments included embryos placed directly on the substrate, embryos on a screen on the substrate, and embryos raised above the substrate on a screen. There was a significant difference among these treatments, but not between days set or their interaction.

	df	SS	MS	F	P-value
Day	1	0.002	0.0019	0.03	0.864
Treatment	2	5.098	2.549	40.319	<0.001
Day: Treatment	2	0.071	0.035	0.56	0.56
Residuals	18	1.138	0.063		

Table 2: Linear model results for the test of treatment on survival to hatching of *Dendraster excentricus* for a laboratory control trial. Treatments included embryos placed directly on the substrate, embryos on a screen on the substrate, and embryos raised above the substrate on a screen. There was a significant difference among treatments.

	df	SS	MS	F	P-value
Treatment	2	0.112	0.056	2.392	0.147
Residuals	9	0.212	0.024		

Figure Captions

Figure 1. Development chambers used to rear *Dendraster excentricus* embryos at Argyle Lagoon, San Juan Island, Washington. Size and location of Nitex mesh on PVC ring varied among 3 treatments (screened pelagic, screened benthic, and unscreened benthic). Figure depicts screened pelagic treatment, where 55 μm mesh is on the upper side of the PVC ring. Unscreened benthic treatment had a 1 mm mesh on the upper side of the PVC ring, which embryos fell through to rest directly on the substrate. Screened benthic treatment had a 55 μm mesh on the lower side of the PCV ring, so that the mesh lay directly on the substrate.

Figure 2. Mean development success for *Dendraster excentricus* embryos at Argyle Lagoon, San Juan Island, Washington. Embryos were placed directly on the substrate (unscreened benthic), on a screen on the substrate (screened benthic), and on a screen raised 3 cm above the substrate (screened pelagic). All treatments were significantly different, error bars ± 1 SE, $n = 8$ for each treatment.

Figure 3. Mean development success for *Dendraster excentricus* embryos under laboratory control conditions. Embryos were placed directly on the substrate (unscreened benthic), on a screen on the substrate (screened benthic), and on a screen raised 3 cm above the substrate (screened pelagic). Treatments were not significantly different.

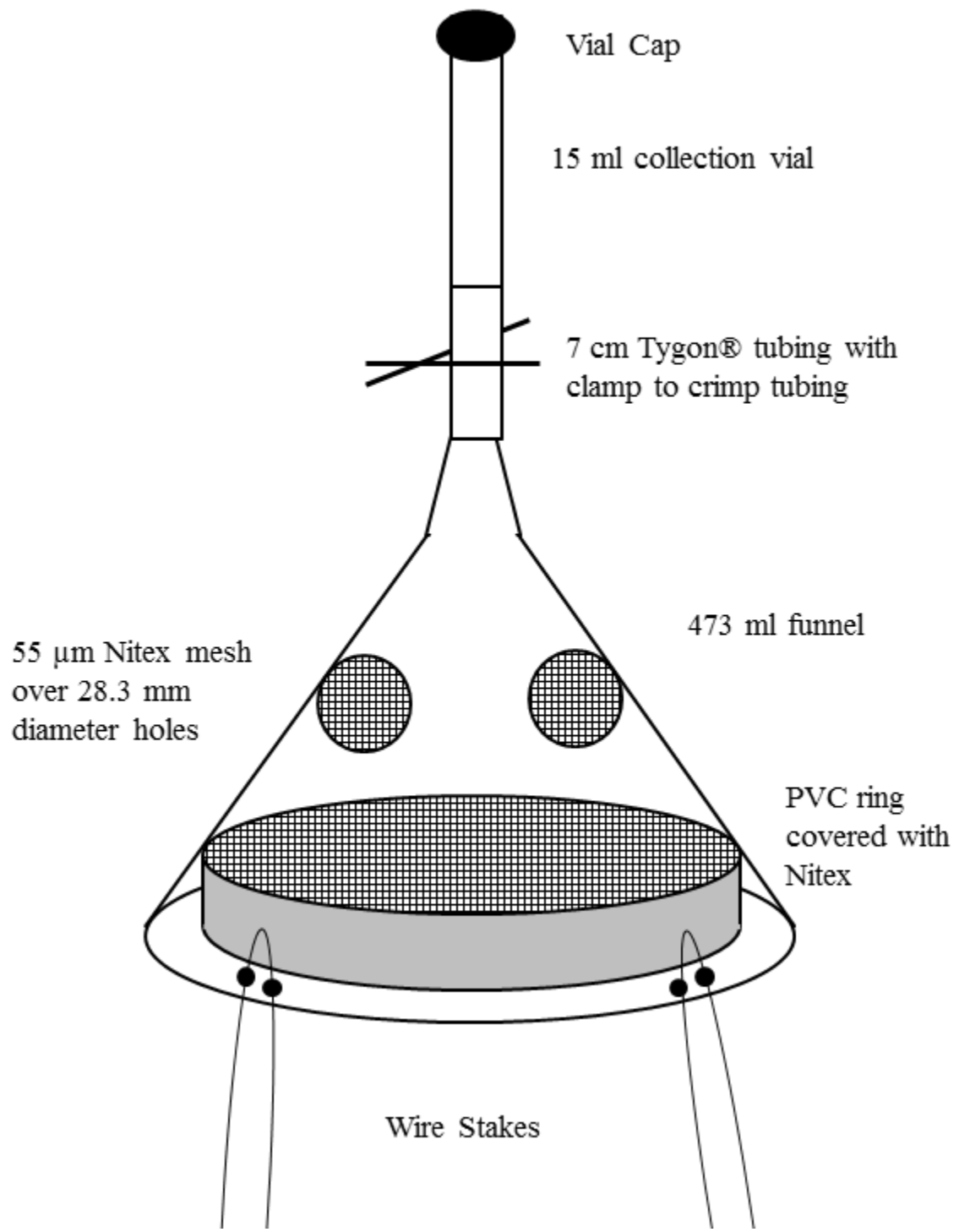


Figure 1

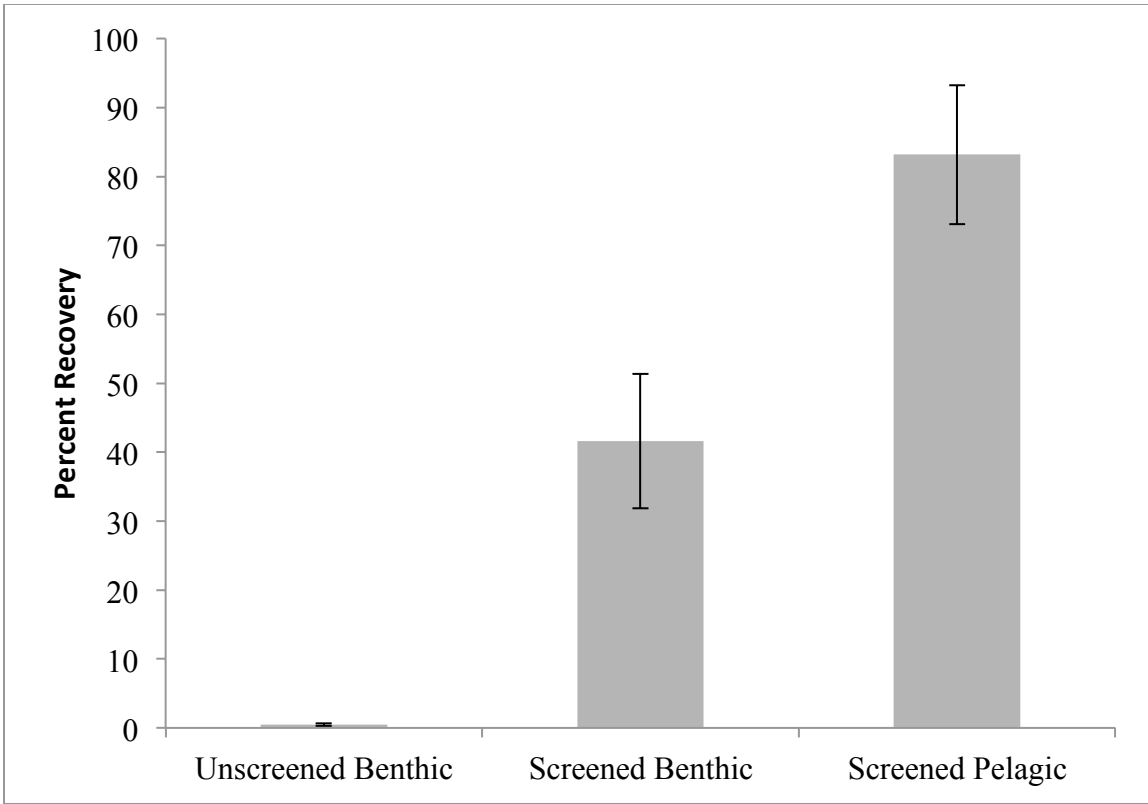


Figure 2

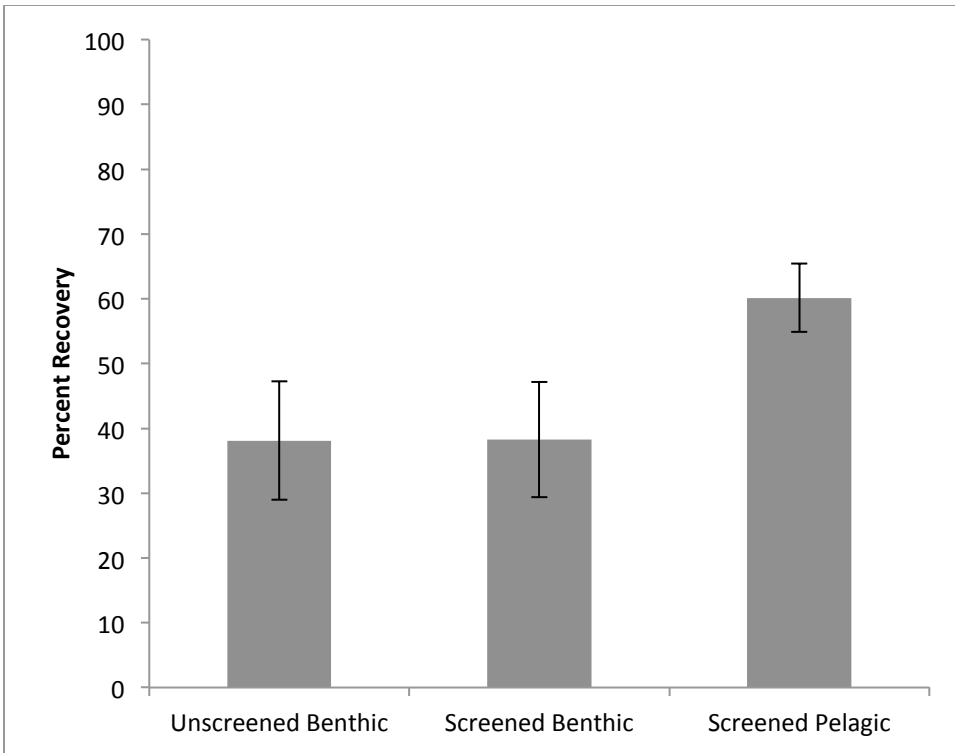


Figure 3