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Evaluation of a macro encapsulated repellent to reduce risk of white phosphorous ingestion by waterfowl foraging in a contaminated marsh

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Abstract

White Phosphorous contamination of a marsh at a U.S. Army artillery training range in Alaska is a causative agent for waterfowl mortality. We developed an encapsulated bird repellent containing the active ingredient, methyl anthranilate, and evaluated the formulation's efficacy in reducing feeding activity by ducks, reducing mortality of ducks feeding in contaminated sediments, and the repellent's ability to move ducks from contaminated areas. The formulation has a limited life span of about 4 days. However, in pen trials feeding activity of mallards can be reduced by up to 80%. Long term exposure to treated sediments confers a survival advantage to mallards tested in pens, and free-ranging mallards can be moved off of treated sections of marsh. This prototype formulation may have utility in the short-term remediation of contaminated sediments for the protection of waterfowl.

1. Introduction

Eagle River Flats (ERF) is an 865 ha estuarine salt marsh on Cook Inlet, Alaska that has been used by the U. S. Army at Fort Richardson as an artillery training range for the past 50 years. In 1990 white phosphorous (WP) was identified as the causative agent responsible for thousands of water bird deaths on the flats (Racine et al., 1992a,b). Although records for the 40 years prior to 1987 are unavailable, between the period of 1987–1990 over 900 kg of white phosphorous contained in smoke munitions was fired into ERF via 105 mm howitzer, or by 60-, 81-, and 107 mm mortars. Firing of munitions containing WP was suspended in 1990.

WP is the most reactive of agents used in smoke projectiles. These munitions can be set to explode above the ground or upon impact. When exploded, exposed WP reacts with oxygen to form various phosphorous oxides and produces a dense white cloud. If the WP is immersed in water it ceases to burn. Approximately 10% (90 kg) of the WP fired into the marsh is estimated not to have burned (Spanggord et al., 1985). Given the undocumented period of training, it is arguable that the level of WP contamination is much higher. Field sampling supports this supposition, and indicates high levels of contamination distributed across large areas of the marsh (Racine and Walsh, 1994; Racine, 1995).

Dabbling ducks are at highest risk to WP poisoning because of their mode of foraging (Reitsma and Steele, 1994). Dabbling ducks strain large quantities of sediment in their bills, filtering out particles (seeds, soft-bodied invertebrates and grit, including WP) for ingestion. Once exposed to even small quantities of WP, e.g., 1 mg (Coburn et al., 1950), death can occur within hours. Observable symptoms of acute poisoning include vomiting and convulsions, with a variety of physiological functions being disrupted to produce these symptoms (Nam et al., 1994a, 1994b; Roebuck and Nam, 1995; Sparling et al., 1994, 1995). Smaller doses may affect long-term survivorship as well as reproduction (Sparling et al., 1995).

Extensive efforts to determine the extent of the problem and develop remediation strategies have been carried out over the past several years (Racine and Cate, 1993, 1995; Racine, 1994). This study describes a single short-term treatability study of the site using the avian repellent, methyl anthranilate (MA) (Clark, 1996, 1998). In 1990, the National Wildlife Research Center was called upon to assist in the development of a repellent that could be applied to a sediment's surface that would prevent ducks from foraging in contaminated areas. Because of the scale

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of the area to be covered, and the environmental conditions where the repellent must exist (a tidal marsh), it was impractical to apply repellent on the water's surface or as a dissolved treatment. To complicate matters further, the marsh contained numerous unexploded munitions that posed a hazard to remediation workers. Therefore any repellent would have to be operationally applied by aircraft. We conceptualized a repellent formulation that would contain the active agent encapsulated in a bead. The repellent bead could be deposited into the water to settle in the sediment. The bead would then be picked up by foraging waterfowl and break under the pressure conditions in the bill of a duck, thus releasing a potent concentration of repellent sufficient to stop foraging behavior of the duck. While the repellent might not eliminate all feeding activity, it should reduce the extent of feeding behavior, and arguably reduce the risk of WP poisoning in a proportional manner. Through individual and observational learning it was hypothesized that waterfowl could learn to avoid treated areas (Mason and Reidinger, 1983). This study reports on the efficacy of a prototype repellent formulation at decreasing feeding activity by ducks in semi-natural and natural settings.

2. Materials and methods

2.1. Study site

The study area is a 865 ha estuarine salt marsh located at the mouth of Eagle River, where it enters the south side of Knik Arm of Cook Inlet, outside of Anchorage, Alaska. The study area is a mix of mud flats, brackish ponds, and marsh with elevations ranging from 1–5.5 m (Racine and Cate, 1993). The elevation of the surfaces and the distance from the river determine how frequently an area floods and therefore, its zonation. The temporary and permanent ponds are the major feeding areas for dabbling ducks during the spring and fall migrations. Two areas within the flats that provided permanent standing water throughout the planned studies were selected for study. Survey studies indicated that Area B was a low risk area to ducks, while Area C sediments were highly contaminated with WP (Racine and Cate, 1995). Area B also contained deeper depressions than Areas C, resulting in deeper pools of water. Prior to establishing study pens and observation blinds, access to the study plots and the study area itself were cleared of unexploded ordinance by military personnel.

Within Area B a large pool was selected and six 9 × 18 m pens were built. Perimeter nylon mesh netting was attached to stakes driven into the sediment. The netting was fixed to the sediment, underneath the water's surface with landscape pins. The height of the netting above the water's surface was 0.9–1.2 m. Elevated board walkways were positioned outside the long axis of the pens. These walkways allowed us to easily herd the ducks toward a funneled exit and into transport cages at the end of observation trials. Water depth varied as a function of tide, but during the course of the study it ranged from 0.45–1.2 m. Area C contained fewer, shallower pools. Six pens of similar dimensions to those used in Area B were built in Area C. The substrate in Area C did not contain vegetation, and was composed entirely of glacial silt and organic debris. The water depth at the site of the pens ranged from 0.03–0.6 m, depending on tidal condition during the period of observation. The studies were conducted during May–June and August–September from 1992–1994.

2.2. Repellent formulation

Methyl anthranilate (Clark, 1998), was dissolved in vegetable oil and encapsulated in a gel-alginate based macro capsule (1–3 mm diameter). Capsules were designed to break under the bill pressure of foraging mallards. During the course of the three year study, a variety of encapsulating compositions were tried. Many of the prototype formulations failed under field conditions either because: (1) the breaking pressure of the capsules was too high, (2) there was insufficient cross linkage of the capsule wall such that there was excessive leakage and diffusion of the active ingredient into the water, or (3) because the capsule wall failed owing to microbial attack (Clark and Cummings, 1994). We only report results for the most promising proprietary prototype formulation, ReJeX-iT WL–05 (R.J. Advantage, Inc., Cincinnati, OH). This formulation was the most stable under field conditions and produced demonstrable responses by bird coming into contact with the formulated repellent.

2.3. Study subjects

Mallards *Anas platyrhyncos*, of mixed sex ($N = 96$), were captured with cannon nets in Denver, CO during the spring of 1992 and 1994, marked with leg bands, quarantined, and subsequently shipped via air to field holding facilities at Fort Richardson, Alaska. Mallards were randomly assigned to groups ($n = 6$/group), with each group housed in outdoor pens (4 × 6 × 6 ft), and maintained on an *ad libitum* diet of mixed poultry chow, whole wheat, cracked corn, and water.

2.4. Experiment 1. Stability of WL–05 under field conditions

The objective of this study was to determine the life expectancy of the WL–05 formulation under conditions prevailing at the sediment level in the marsh. Thirty samples of WL–05 were weighed (15 g/sample), sealed in nylon screen pockets, and randomly placed on the surface of the sediment, under water in Area C. Three samples were removed at 24 hr intervals for each of 10 days. WL–05 beads were gently scrapped into acid-washed amber vials containing 40 ml of 0.1 ppm sodium azide (NaAz) solution, and reserved for subsequent analysis. Methyl anthranilate is highly susceptible to microbial degradation (Aronov and Clark, 1996). Thus, an aerobic poison, such as NaAz, was necessary to preserve samples.

An index of MA content of beads was calculated, but should not be interpreted as the total amount of MA within a bead. Rather the index is a measure of the overall failure of the bead. The equilibrium MA content of the aqueous phase of the sample on day t was normalized to the weight of the field recovered sample and divided by the weight-normalized aqueous phase MA content of beads from time zero. The relationship between MA concentration and time WL–05 beads were left in the sediment was described by the logistic expression, $R_{MA} = a/(1+e^{-(t-t')b})$ where R_{MA} is the amount of MA in the WL–05 field sample at time t relative to the amount of MA in a bead at t', a is the asymptotic maximum amount of MA, b is the slope, and t' is the inflection (the time at which the concentration of the WL–05 bead is 50% of its initial value). The curve was fitted using a Marquardt–Levenberg algorithm (SPSS 1997).

2.5. *Experiment 2. The effect of substrate covering on feeding activity*

This experiment, and the subsequently described experiments, evaluated the effects of manipulation of the sediment on the feeding activity of mallards placed in pens located in the marsh. Because ducks were maintained in terrestrial enclosures without access to natural marsh conditions, and only intermittently introduced into the marsh test system, we were interested in determining what effects deprivation from natural substrates had on feeding activity, as well as monitoring possible habituation effects of exposure to the test system under two experimental substrate conditions: *ad libitum* access to natural sediment, and essentially no access to surface sediments.

Groups of ducks were maintained as described in the general methods. Four groups of six ducks each were randomly assigned to one of two treatment conditions: the control, natural sediment (n = 2 groups), and sediment covered with geotextile landscape fabric (n = 2 groups). Groups were paired (fabric and control) and the matched pairs were tested on alternate days. Briefly, ducks were removed from pens in the morning (0500 hr), transported to the marsh, and placed into a preassigned pen. Ducks were allowed a 15 min adaptation period (the time it took the observer to leave the pen site and settle into the elevated observation blind) before the 2 hr observation period monitoring feeding activity was initiated. The number of ducks feeding on the sediment was recorded every 30 s for each of the pens (fabric and control). At the end of the observation period, ducks were retrieved from the pens and returned to their maintenance housing. The next day the procedure was repeated for the remaining two groups of ducks, with the exception that ducks were introduced into a different set of preassigned pens. Subsequent observations for the matched pairs of groups occurred on alternate days for a total of 4 observation periods of 2 hr/day over the course of 8 days. In each case groups retained their pen assignments. The data were analyzed as a $2 \times 4 \times 8$ fixed effects, repeated measures analysis of variance, with two substrate treatment levels (control v fabric), test day (1–4), and time within an individual test day (1–8). For the time effect, observations were averaged for 15 min bins so as to reduce the number of levels within this factor. The average number of ducks feeding from the sediment out of a total of six per 15 min bin was the dependent variable. Treatment was a between measures effect, while day and time-bin were repeated measures.

2.6. *Experiment 3. The effect of repellent on feeding activity: intermittent exposure to a noncontaminated site*

The objective of this experiment was to determine whether WL–05 beads could reduce the number of ducks engaged in feeding on sediment relative to ducks in control pens. In addition, we determined whether application of WL–05 to the sediment could reduce the feeding intensity of individual ducks relative to individuals feeding in untreated pens.

During the evening prior to the onset of observations a pen was randomly selected and WL–05 beads were dispersed by hand, at a rate of 40 kg/ha (a.i.). The next morning (0500 hr) two groups of ducks ($n = 6$/group) were selected from the maintenance facility and transported to Area B. One group of ducks was placed in the pen treated with WL–05, while the second group of ducks was placed in a randomly selected untreated pen, the negative control. The observer then retreated from the pens to the observation blind. Observations were patterned as follows. An individual patagial tagged duck within one of the pens was arbitrarily selected as a focal animal. Every 10 s, for a total of 10 min, the observer noted whether or not the focal duck was feeding on the sediment or not. At the 5 and 10 min mark the feeding behavior of all the ducks was noted. Timed intervals were marked by tones with the aid of a cassette recorder. At the end of 10 min, the observer switched attention to the other pen and repeated the patterned observation described above. This process was repeated until the total observation time for each pen was 60 min, recorded in alternating 10 min bins between the two pens. This sampling effort also ensured that all six ducks within each pen served as the focal animal at some point during the observation period. At the end of the observation period, the ducks were retrieved and returned to the maintenance housing facility. That same evening, a pen was randomly selected from the remaining four pens and treated with WL–05 as described above. The following morning, two new sets of ducks were selected and tested as described. The process of assignment, treatment, and behavioral observations was repeated on the third day with the remaining two pens. On the fourth day the sequence on behavioral observations was repeated, retaining the duck-pen pairings established during the first 3 days of testing. Pens were only treated once, at the beginning, during a 14-day interval. Thus, each pair of pens (treated and control) was tested at 12, 36, 60, and 84 hrs post treatment.

Chemical analysis of the capsules indicated that MA was not present after 14 days (Experiment 1). Hence, a second set of observations was scheduled, repeating the assignment, treatment, and observation schedule described above, with the exception that assignment of ducks to treatment level (WL–05 v control) was reversed.

The two levels of behavioral observations were analyzed separately. The coarse grained analysis used the 5 min sampling intervals for feeding behavior of all ducks within the pens. The fine grained analysis used the 10 s sampling intervals for feeding intensity of individual focal animals within the pens. We analyzed the coarse grained data using a $2 \times 2 \times 4 \times 12$ fixed effects, repeated analysis of variance. Treatment was a between measures effect (WL–05 application v control). Period (first and second application periods), day (sampling sequence: 12, 36, 60, and 84 hrs post treatment), and time within a pen during a single observation period were all repeated measures. We only report details of the analyses for the highest order effect achieving $P < 0.05$. Scheffe's tests were use to identify post hoc differences among treatment levels. Least significant differences were used for *a priori* contrast comparisons of treatment effect within day and period.

2.7. Experiment 4. The effect of repellent on feeding activity and mortality: intermittent exposure to a contaminated site

The objective of this experiment was to determine whether application of WL–05 capsules to the surface of the sediment could reduce the number of ducks feeding at a contaminated site relative to ducks feeding in untreated control pens, and if so, whether the reduction in feeding activity lowered the risk of WP poisoning. In addition, we determined whether application of WL-05 could reduce the feeding intensity of individual ducks relative to that observed in untreated control pens, and whether a reduction in feeding intensity by individual ducks was related to a lower risk of WP poisoning.

Pen construction, assignment to treatment condition, application procedures, observations, and analyses followed the methods outlined for Experiment 2. Sediment samples were collected at each of the pen sites to verify that WP was present. Any ducks that died were assayed to verify that WP was the causative agent of death (Racine and Cate, 1993).

2.8. Experiment 5. The effect of repellent on mortality: continuous exposure

Experiments 3 and 4 evaluated feeding behavior of mallards that were intermittently introduced to natural foraging substrates. The intervening 3 day deprivation period between observations may have resulted in a greater motivation for the mallards to feed. It is conceivable that the repellent effect of the formulation may have become swamped under this higher motivational state. The objective of this experiment was to determine whether application of WL–05 could confer a survival advantage to ducks that were continuously exposed to natural substrates.

Six 7×20 m test pens were located in Area C, and sediment samples ($n = 5$/pen) were taken to verify that the pens contained WP. Pens were randomly assigned to one of two treatments (WL–05 v control), and similarity for WP content of the sediment samples was verified using a fixed effects 2-way ANOVA. Mallards were captured and maintained as described in Experiment 2. Six groups of mallards ($n = 6$/group) were selected and randomly assigned to pens in Area C. In contrast to previous experiments, once introduced into the pens, the mallards remained in the pens until the end of the test, or until they died from WP poisoning. Mallards were free to feed upon the sediment. They were also provided with supplemental food on a floating platform. Food was replaced every other day. Prior to introducing mallards to the pens, the designated pens were treated with WL–05 following the procedures outlined in Experiment 2. Ducks were checked every 2–3 hrs for the first 48 hrs of the test, and at 24-hr intervals thereafter. Dead or dying ducks were removed from pens if symptoms of WP poisoning were observed. Carcasses were frozen and tissue samples were taken to verify cause of death. The combined cumulative mortality rate across replicates was compared for treatment effect using a simple comparative estimate for differences between proportions (Fleiss, 1973).

2.9 Experiment 6. Effect of WL–05 on distribution of free-ranging ducks

The objective of this experiment was to determine whether ducks could be moved off of areas treated with WL–05. Five plots were identified in Area C (Fig. 1) and the number of ducks within each area was counted every 15 min for a 2-hr period according to a randomly determined time-stratified design. Although counts were made

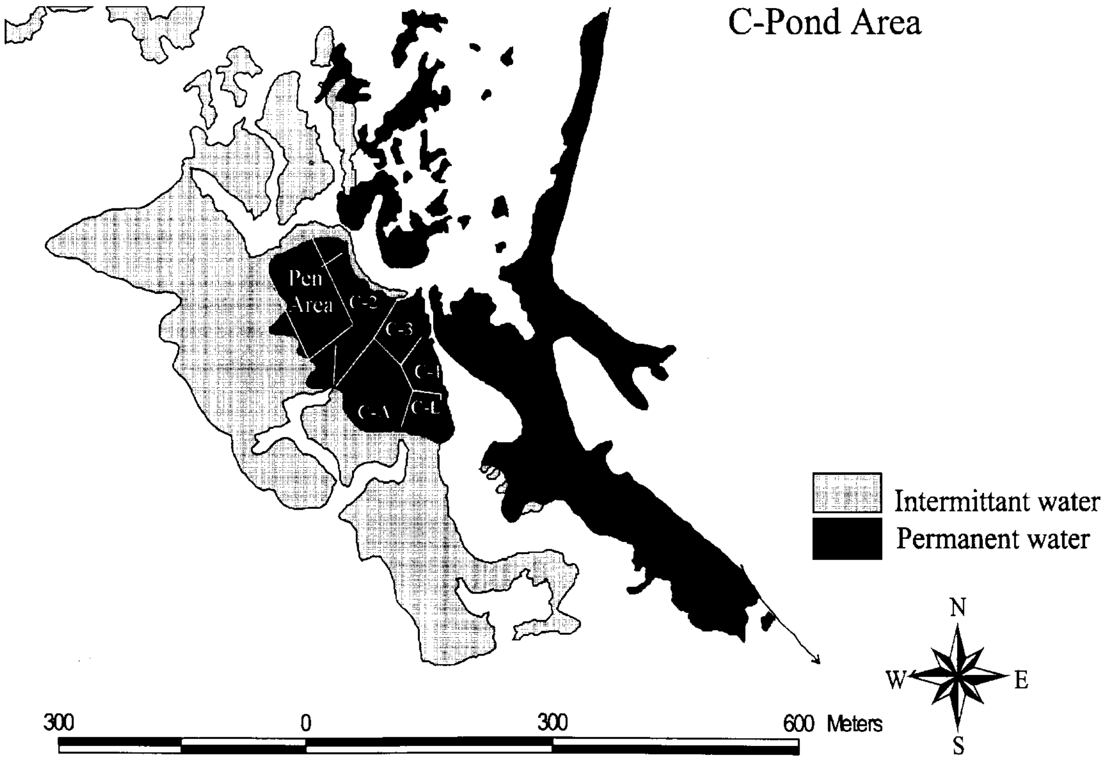

Fig. 1. A map of the study site at Eagle River Flats.

by species, for the purposes of this analysis, counts consisted of total number sediment feeding ducks: mallards, northern pintails *A. acuta*, and green-winged teal *A. crecca*. These species were previously shown to be at highest risk to WP poisoning (Reitsma and Steele, 1994). Counts were made from the elevated tower-blind. The observer would enter the tower one hour prior to initiating the observations. This time period allowed ducks to return to the area if disturbed. The daylight hours were divided into three time periods: morning 0600–1000, midday 1000–1600, and evening 1600–2200 hr.

Once baseline use of the designated areas was established, plots were established and treated with WL–05 in a serial manner to determine if ducks could be moved off a given section of marsh. Area C-L (15 × 46 m), at the southern end of the C-pond was treated with 61 kg of WL–05 (0.108 kg/m^2 a.i.) on 12 August 1994. Subsequently, the treated area was expanded using the same application rate. Plot C1 (30 × 30 m), north but adjacent to plot C-L was treated on 14 August; Plot C3 (30 × 30 m), north but adjacent to plot C1 was also treated on 15 August. All other plots within the C-pond served as untreated controls.

Two ponds in the Area C-D transition were observed from an overlook to the marsh. Initially ducks appeared to be using only one of the ponds. This pond (30 × 30) was selected for treatment on 17 August. The pond was slightly larger than the treated area, but no attempt was made to treat the entire pond.

Treated and control ponds within each area were compared using a Birnbaum–Hall statistic. This procedure is a multilevel statistic of the Kolomogorov–Smirnov type and compares the cumulative probability distributions for use of an area by ducks, addressing the question whether use patterns are the same for each of the plots (Conover, 1980). For example, if the treatment is effective, use of a plot is anticipated to decrease post treatment. Thus, the cumulative distribution for use in these plots should be near one shortly after treatment. In contrast, use of the control plots is anticipated to be random resulting in a linear cumulative probability plot, or increase as ducks switch activity to untreated areas. In either case, treatment effect is measured as a difference in the profile of the cumulative probability plots.

3. Results

3.1. *Experiment 1. Stability of WL–05 under field conditions*

WL–05 capsules were stable for up to 4 days after being deposited in the marsh. Beyond 4 days there appeared to be a catastrophic failure of the capsule, resulting in significant loss of MA (Fig. 2). Physical inspection of beads over time indicated that on days 0–4, the capsules remained firm. Beyond 5 days capsules took on a mushy texture.

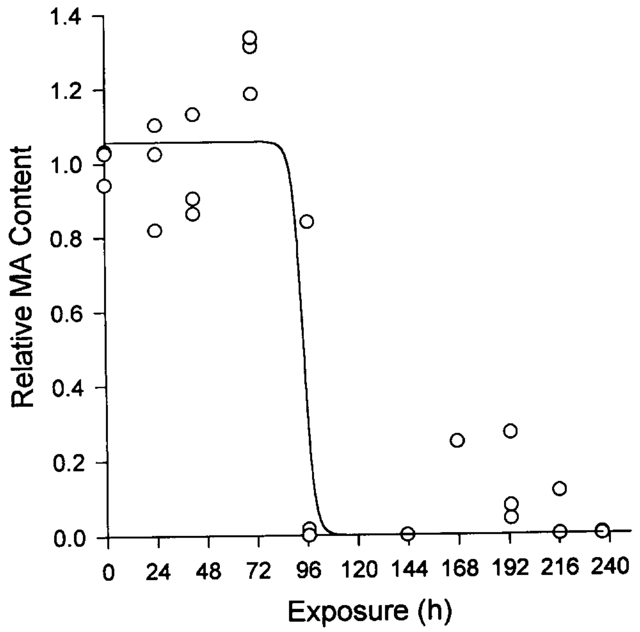

Fig. 2. Methyl anthranilate content of WL-05 beads as a function of exposure to *in situ* marsh sediment (relative to content of beads at time zero). Coefficients for the 3-parameter logistic fitted curve (see text) are: a = 1.057 (±0.067, SEM), b = −2.458 (±2.826), t' = 94.050 (±28.26). R^2 = 86.3%.

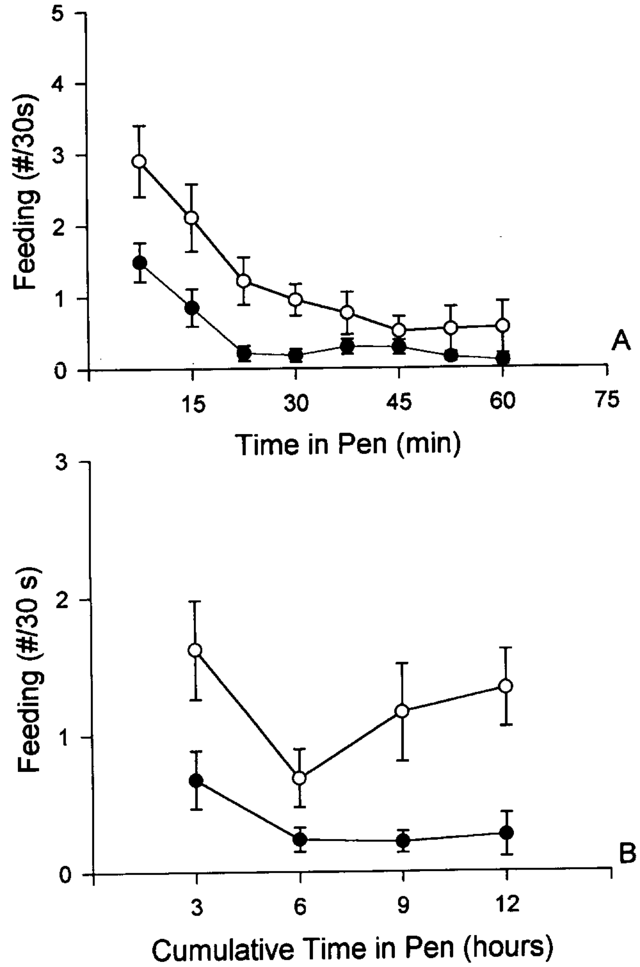

Fig. 3. A. 5 min survey data for the number of ducks observed feeding from the sediment as a function of time spent within a pen and treatment type. B. 5 min survey data for the number of ducks observed feeding from the sediment as a function of the intermittent cumulative time spent in a pen. Solid symbols = pens with geotextile covered sediment, open symbols = pens with exposed sediment. Vertical capped bars depict ± SEM.

3.2. Experiment 2. The effect of substrate covering on feeding activity

There was a tendency for ducks to decrease feeding activity as a function of time in the pens (Fig. 3A; F = 11.72, df = 7, 16, $P < 0.001$).

Mallards also decreased feeding activity as a function of the cumulative exposure to the pens over the course of 4 days (Fig. 3B; F = 4.42, df 3, 48, $P = 0.008$). For both treatments, ducks fed more during the first 30 min within an observation period, and during the first exposure to a pen. Although the treatment-time interactions were not significant, covering the substrate decreased the amount of sediment feeding 63% relative to ducks feeding in the control pens (F = 29.18, df = 1, 16, $P <$ 001).

3.3. Experiment 3. The effect of repellent on feeding activity: intermittant exposure to a noncontaminated site

The number of ducks observed feeding varied as a function of treatment, time spent in the pen, and test period (high tidal conditions v low tidal conditions) (Fig. 4A; F = 2.106, df = 11,552, $P = 0.018$). During the second test period there was no difference in the number of mallards feeding as a function of treatment. In this case the feeding activity paralleled that seen in Experiment 2, with more mallards feeding at the beginning of the trial. However, during the first period the pattern of feeding differed between treatments. The control pens had the same pattern as described above, but the number of ducks feeding was lower in the treated pens, irrespective of the length of time the ducks were in the pen.

Observations on focal mallards indicated that individuals fed less intensely (Fig. 4B, F = 23.32, df = 1,4, $P < 0.001$). During the first observation period, the repellent effect lasted up to 7 days, and the feeding intensity of mallards in treated pens was reduced 78% relative to the controls. We did not see an effect on feeding intensity during the second observation period, primarily because of higher water levels. The higher tides even prevented feeding by mallards in the control pens. There was an 80% decrease in feeding intensity of the mallards from control pens from the first to second observation period. This difference is attributable to their inability to access the substrate owing to the deep water (~0.9 m) Under these circumstances the observed feeding activity most

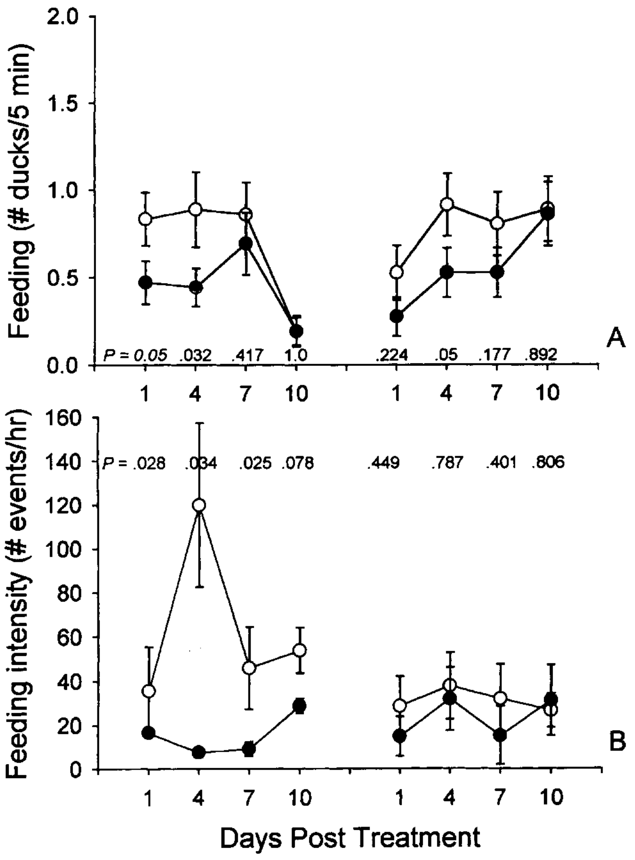

Fig. 4. Feeding behavior of ducks in experimental pens in Area B, uncontaminated sediment. (A) 5 min survey data for the number of ducks observed feeding from the sediment as a function of treatment type, days post treatment, and treatment block. (B) 10 s survey of feeding intensity of individual focal ducks as a function of treatment type, days post treatment, and treatment block. Solid symbols depict pens treated with WL–05. Open symbols depict pens with untreated, exposed sediment (negative control). Probability values for *a priori* contrasts between treated pens and controls for each day are indicated. Vertical capped bars depict ± SEM.

likely represents baseline activity. As such, the ability to discriminate a treatment effect during this period was diminished. However, the data also suggest that the suppressive effect owing to the repellent seen during the first period is at or near the baseline, i.e. sampling, activity level for mallards. This was also similar to the feeding activity level observed in Experiment 2, where substrate was obstructed with fabric.

3.4. Experiment 4. The effect of repellent on feeding activity and mortality: intermittant exposure to a contaminated site

The proportion of ducks observed feeding was unrelated to the time the ducks spent in a pen, i.e., none of the effects involving minutes spent in a pen achieved a $P < 0.2$. Moreover, the pattern for the proportion of ducks observed feeding within pens was similar across period, days, and treatment category ($P = 0.726$). Indeed, none of the *a priori* comparisons by contrasts of treatment effect for paired observations, i.e., within the same period and day, yielded a $P < 0.3$ (Fig. 5A) despite a tendency for fewer ducks in the WL 05 treated pens seen foraging relative to controls.

There was a tendency for the feeding intensity of individual ducks to be suppressed in WL–05 treated pens relative to controls, but in general, there was only a statistical effect of treatment for the first day of the second application period (Fig. 5B, comparison by contrasts p = 0.019). Because WL–05 failed to suppress feeding behavior under the test conditions prevailing in Area C, it is not surprising that mortality between the treatment and control pens did not differ (Fig. 5A, inset, $P = 0.765$). A general failure of the application may be attributed to the water depth within Area C. Depths ranged from 0.05–

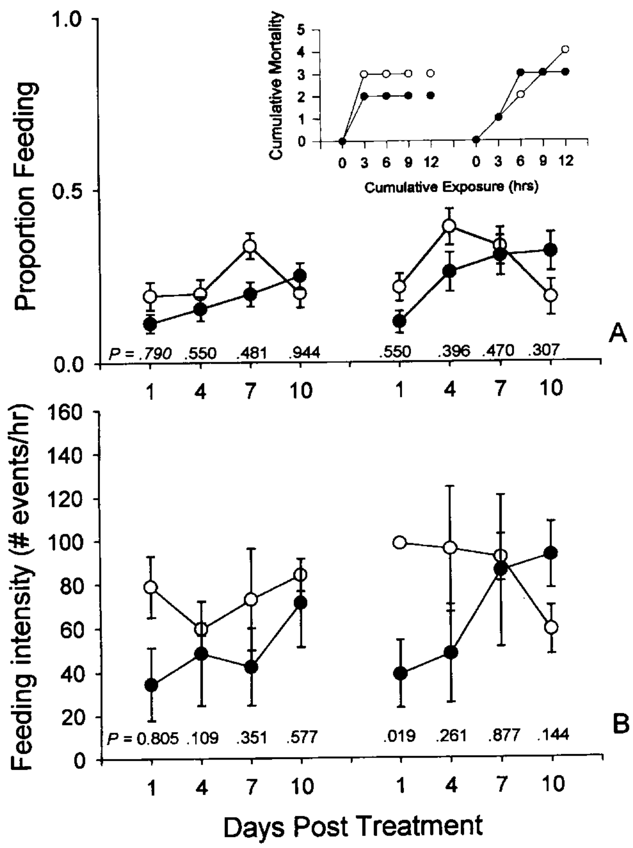

Fig. 5. Feeding behavior of ducks in experimental pens in Area C, WP contaminated sediment. A. 5 min survey data for the number of ducks observed feeding from the sediment as a function of treatment type, days post treatment, and treatment block. B. 10 s survey of feeding intensity of individual focal ducks as a function of treatment type, days post treatment, and treatment block. (Inset) The cumulative mortality associated with each pen type as a function of time. Solid symbols depict pens treated with WL 05. Open symbols depict pens with untreated, exposed sediment (negative control). Probability values for *a priori* contrasts between treated pens and controls for each day are indicated. Vertical capped bars depict ± SEM.

0.25 m. A possible consequence of such shallow water depths was that there was extensive disturbance of the sediment attributable to feeding activity and paddling through the mud. This activity caused a substantial redistribution of WL–05 beads outside the penned areas.

3.4. Experiment 5. The effect of repellent on mortality: continuous exposure

Overall, the mortality rate between treated and control pens was similar for the first 3 days of continuous exposure. Thereafter the mortality rate of mallards from treated pens was lower than that for control pens (Fig. 6). At the end of 6 days, mallards in WL–05 treated pens was reduced by 35.3% relative to controls.

3.5. Experiment 6. Effect of WL–05 on distribution of free-ranging ducks

At the onset of observations, ducks concentrated their feeding activity along the pond's edge at the southern end of the study area. Use of the larger untreated areas of the C pond was much lower at the onset of the study. However, once areas were treated with WL–05, the ducks abandoned these plots as favored feeding areas and shifted their activity to untreated sections of the pond ($P < 0.05$, Fig. 7). Displacement of duck activity as a function of treatment also occurred in the C-D transition area ($P < 0.05$, Fig. 8).

4. Discussion

Developing a bird repellent suitable for application into an aquatic habitat presented several logistical obstacles. First, the repellent had to be applied to a substrate that maximized its likelihood of being encountered by the target species. Because filtering feeding ducks were at highest risk to WP poisoning, the goal became reducing waterfowl exposure to sediment. However, injecting repellent directly into the sediment was impractical because of the scale of the substrate. Thus, the repellent

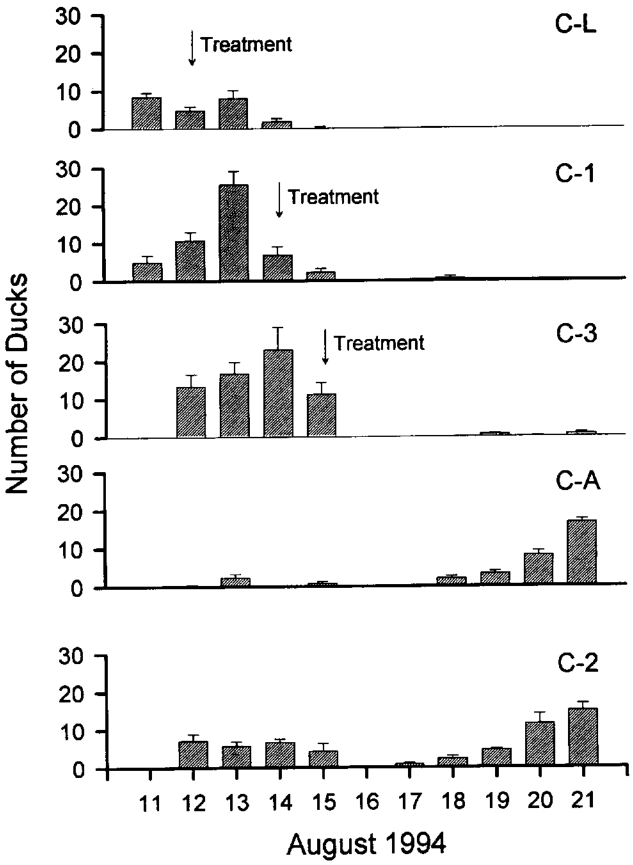

Fig. 7. The number of ducks observed feeding in WL–05 treated and control ponds in Area C.

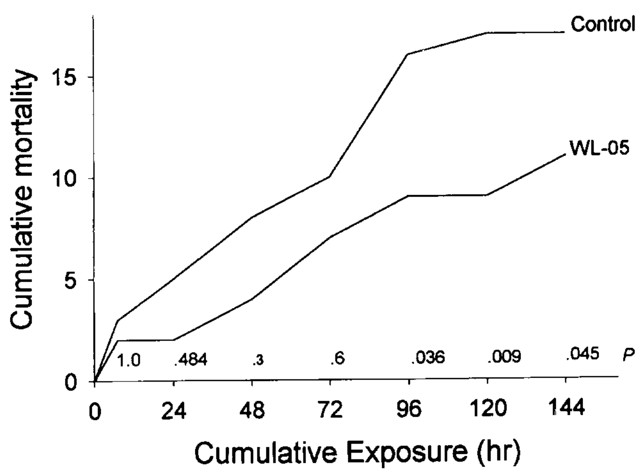

Fig. 6. Cumulative mortality of mallards as a function of treatment type and cumulative time spent in a pen. Probability values for comparison of cumulative probabilities between treated pens and controls for each day are indicated.

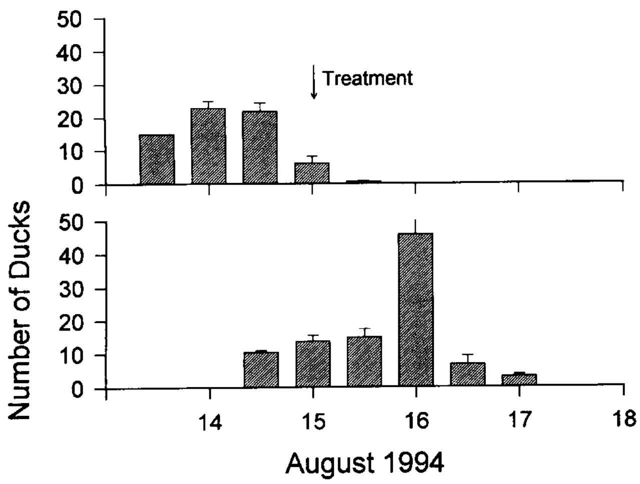

Fig. 8. The number of ducks observed feeding in WL–05 treated and control ponds in Area C-D transition.

had to be placed into discrete packets, and the distribution of the packets would have to be sufficiently high to ensure an effective encounter rate by foraging ducks. The packaging of the repellent would also have to minimize likelihood of biodegradation (Aronov and Clark, 1996) and dissipation into the environment. Yet the engineered characteristics needed to minimize these destructive processes were in direct opposition to the properties of a capsule that would allow ready release of the repellent into the buccal cavity of a filter feeding duck. Release into the mouth was critical because that is the location of the receptors that mediate the avoidance response to primary repellents (Clark, 1998). After a series of formulation attempts, a compromise product was achieved. Finally, the materials to be used would have to be biodegradable, such that the formulation would not leave behind any residues, that themselves might be cause for environmental concern (Clark et al., 1993; Aronov and Clark, 1996). Thus, the selection of gel alginate coatings and vegetable oil carrier. Given the organic nature of the capsules' shell (i.e., gel-alginate), and the catastrophic failure of capsules, we suggest that the integrity of the bead is attacked by microbes as a nutrient source. This attack may render the membrane sufficiently permeable so as to increase the rate at which MA is lost from the capsule. The field failure rate for all beads tested to date is about 4 days, and cannot be generally improved upon so long as a biodegradable gel alginate capsule is used. Efforts to increase the longevity of the capsules by increasing the strength of the capsule wall also prevented ducks from rupturing the capsule when feeding (Clark and Cummings, 1994).

Experiment 2 illustrated that even though the quality of a habitat may be poor, or in terms of foragable sediment, virtually nonexistent as was the case for sediment covered with fabric, eliminating the natural foraging behavior of ducks is difficult. Nonetheless, the data illustrate the potential for depressing feeding behavior in a semi-natural environment. Relative to controls we found that eliminating access to sediment decreased the feeding activity of mallards by about 63%. These data also illustrate that the first entry into a feeding area poses the largest risk to feeding ducks. Circumstances that minimize this initial exposure and moves ducks to unprotected areas may arguably limit the risk to ducks from WP poisoning.

The presence of chemical repellent reduced the feeding behavior of ducks by about 80% from that seen for controls, a value similar to that observed for geotextile inhibition of feeding behavior when water depth was between 0.25–0.45 m. This depth allowed mallards easy feeding access to the sediment without having them disturb the substrate with their feet. The importance of water depth to the formulation's efficacy is illustrated in the second observation period in Experiment 3, where the water depth did not allow mallards easy access to the sediment. The deeper water essentially prevented mallards from feeding from the substrate. In Area C (Experiment 4) the water was shallow 0.1–0.45 m. In this case there was a large scale disturbance of the sediment causing the capsules to be redistributed or broken. In any event, the capsules were not evident on the surface of the sediment as they were for Area B. Thus, at first glance, it appears that the general utility of using this repellent strategy might be highly restricted given the formulation and environmental constraints imposed upon the system.

Ultimately, the use of an encapsulated repellent must reduce risk of WP to ducks. It is clear that when the specified environmental conditions were suboptimal the goal was not achieved. Because we saw no suppressive effect in feeding behavior under shallow water conditions in Area C, it is not surprising that we also did not observe a protection against the probability of WP poisoning when mallards were intermittently exposed to test conditions. However, when mallards were exposed to experimental conditions in Area C for an extended period of time, we did observe a reduction in WP poisoning (Experiment 5). These data suggest that prolonged exposure to treated sediment may promote a learned avoidance response for feeding on the substrate, despite shallow water conditions prevailing in Area C.

Curiously, the cumulative effect of differential mortality did not occur until after a time when it was anticipated that the WL–05 formulation would disintegrate. It is possible that during the first four days, while the beads remained intact, the ducks apparently learned to avoid the sediment, and concentrated their feeding on the alternative food source. The unusual behavior of the dying ducks in combination with the unpalatable substrate may have formed the basis of a learned avoidance of the treated substrate. No such salient cue was available to the control ducks, hence their continued utilization of the untreated substrate. Mason and Reidinger (1981, 1983) showed that blackbirds demonstrate strong observational learning, avoiding patterns and colors associated with illness and unusual behaviors of demonstrator birds (conspecifics as well as interspecifics). The latter mechanism implies that not all birds in a local population need experience the repellent. So long as ancillary salient cues, e.g. visual targets, chemosensory stimuli, are available, demonstrator birds (those experiencing the repellent) can train observers to avoid the ancillary and associative stimuli. In addition, the reduced feeding activity by demonstrators may convey information to observers that the energy return of a particular site is reduced (sensu optimal foraging theory, e.g. Charnov, 1976; Lima, 1985). As a consequence of reduced residency time in a patch by demonstrators, the recruitment opportunities to that patch by observers are reduced (Krebs, 1974).

Regardless of the details of the mechanism, in Experiment 6 we showed that application of WL–05 into the marsh can move free-ranging ducks off treated areas. These data are consistent with the reported repellent properties of the active agent to geese in terrestrial situations (Cummings et al., 1992; Mason and Clark, 1995, 1996). Thus, we conclude that the fundamental strategy that has been shown to be successful for goose repellency in terrestrial situations can be extended to aquatic environments if the appropriate delivery systems are worked out. These results are promising as a first step in the short-term remediation and treatability phase of contaminated wetlands.

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