Experimental Assessment of the Toxicity of the Mosquito Larvicide Golden Bear Oil (GB-1111): (1) Field Evaluations on Ducklings, and Target and Non-Target Prey Survival; (2) Laboratory Evaluations on Reared Mallard and Bobwhite Eggs, and Wild Redwing Blackbird Eggs.

Draft Final Report

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EXECUTIVE SUMMARY

Field and laboratory studies were conducted to determine the effects of the mosquito larvicide California Golden Bear Oil (GB-1111) on duckling survival, target and non-target avian invertebrate prey, and avian embryo development. Field studies on designated natural ponds located in salt marshes in South San Francisco Bay indicated that GB-1111 had an initial significant impact on potential avian prey that dissipated rapidly 3-days post spray. Over spray, spray drift or treatment of more extensive areas would likely delay recovery of non-target prey. Mallard ducklings held on the ponds over the course of 8 days showed no significant effects of weight loss due to prey depletion. However some initial effects of exposure to GB-1111 were noted, i.e., matting of feathers and possible mild hypothermia. Recommended maximum field application rates were determined to be harmless to developing embryos, but reduced hatching success and subsequently mortality of mallards, red-winged blackbirds, and bobwhites was significant at 3 or 10 times the maximum rate. Malformations, edema, and liver EROD in mallards and bobwhites also occurred at 10 times the recommended application rate of GB-1111. These results signify the importance of avoiding application of GB-1111 during colder times of the spring season, and care in avoiding spray drift, over spray, or overlap spraying of this larvicide.

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INTRODUCTION

Some California Mosquito Abatement Districts (MADs) use golden Bear Oil, or GB-1111, extensively as a larvicide. About 300,000 gallons were used throughout California in 1995, and MADs have recommended its use on federal National Wildlife Refuges as a component of an integrated pest control formula for mosquito suppression. GB-1111 is a petroleum distillate that is used as a last-resort larvicide when larvae pupate before the site can be treated via other methods. Other larvicides (e.g., B.t.i. or methoprene) are ineffective once the larvae have pupated. The oil forms a barrier at the air-water interface that suffocates air-breathing insects such as mosquito pupae. GB-1111 may affect natural predators of mosquitoes, such as predatory beetles and hemipterans (Mulla and Darwazeh 1981); otherwise, there are few published reports of effects on non-target organisms.

The oil is somewhat toxic, and has an unsightly appearance. Although classified as a hydro-treated, light naphthenic (closed-chain alkane) petroleum distillate, which implies minimal presence of known toxic polycyclic aromatic hydrocarbons, the product label warns, "GB-1111 is toxic to fish and other aquatic organisms." GB-1111 had a 24hour, LC^{50} of 2387 ppm for young sheepshead minnows (*Cyprinodon variegates*; Tietze *et al*. 1995), but was not toxic to protozoa and rotifers from a sewage treatment plant after 24 hours of exposure at 2625 ppm (Tietze *et al*. 1993). Because GB-1111 is composed of cycloalkanes, it might be more resistant to microbial degradation and have greater toxicity than aliphatic (open-chain alkane) hydrocarbons (Albers 1994, Curl and O'Donnell 1977).

The avian embryo is contained in a virtually closed system and is subjected to a situation environmentally similar to a fish in a small pond, with only limited recourse to protect itself from exposure to xenobiotics including petroleum compounds, pesticides, and industrial effluents (Hoffman and Albers, 1984; Hoffman, 1990). Certain chemicals that have been toxic to fish have also proven to be quite toxic to bird embryos (Hoffman, 1990). Albers and Heinz (1983) previously reported on a petroleum derived mosquito larvicide, FLIT-MLO (a straight-chain or branched alkane product) that was toxic to mallard (*Anas platyrhynchos*) eggs when applied externally at three times the maximum recommended application rate. GB-1111 is a cycloalkane product; hence toxicity of the hydrocarbons should be greater than those of FLIT-MLO.

GB-1111 has not been scrutinized for toxicity to avian species or their invertebrate prey. Thermoregulation is critical to the survival of ducklings, particularly during the first few weeks after hatching. Young ducklings are less sensitive to cold than gallinaceous chicks, but the young of the most cold-sensitive species, e.g., mallard and green-wing teal (*A. crecca*), have been calculated to require metabolic rates of about five times the basal level to maintain their heat balance at an air temperature of 10° C (Koskimies and Lahti 1964). These metabolic requirements compel ducklings to consume large quantities of invertebrates in order to sustain thermoregulation, and mosquito larvae can form a large component of their diet (Meyer and Swanson 1982). If GB-1111 is toxic to mosquito larvae as well as non-target emergent insects, the prey base may be suppressed sufficiently to affect survival of ducklings if applied during the hatching or rearing season.

The purpose of this study was to evaluate the effects of GB-1111 on: (1) survival of reared mallard (*Anas platyrhynchos*) ducklings held under field conditions, (2) productivity and survival of aquatic invertebrate prey organisms of migratory birds, and (3) eggs of mallards, bobwhite quail (*Colinus virginianus*), and red-winged blackbirds (*Agelaius phoeniceus*).

METHODS

Field Study

Study Area. The field component of this study was conducted from 12 June to 13 July 1998. Experimental sites were established at two adjacent high intertidal, salt marsh wetlands separated physically by a road and railroad levee. The marshes are located at the Don Edwards National Wildlife Refuge (Alameda County, near Fremont) at San Francisco Bay, California (Figure 1). These marshes have a well-developed invertebrate fauna (Wes Maffaei, personal communication), and are utilized by waterfowl and shorebirds. Although mosquitoes occasionally breed at both marshes, they are infrequently treated. Invertebrates sensitive to GB-1111 are more likely to occur in these rarely treated sites than on more frequently treated sites. The marshes contain numerous small ponds that are separate during summer, low-precipitation years, or periods of average to low high-tide cycles. Invertebrate numbers and diversity are highest during the summer.

We established replicates of five treatment- and five control-ponds among the Hetch-Hetchy and West Vaca/Newark Slough marshes of the Refuge (Figure 1). The selected ponds ranged in size from 430 – 1300 m^2. The assignment of treatment and

control was random. The ponds were unvegetated with standing water and separated by a minimum of 20 m of moist ground and vegetation (primarily the pickleweed *Salicornia* spp.) during the study.

Invertebrates. To measure pesticide activity, we reared larval mosquitoes in predator-exclusion cages on each site. The cylindrical plastic cages (15 cm diameter x 12 cm deep) had tops and side panels screened with plankton netting to expose organisms to the pesticides, and were suspended in the water by Styrofoam floats to provide an air space for adults. Cage tops were removed during pesticide application. We placed two cages at each pond, each of which contained 15, second stage mosquito (*Ochlerotatus dorsalis*) larvae. The most abundant invertebrates in the ponds were water boatmen, *Trichocorixa reticulata* Guerin-Meneville, and we used these as non-target 'sentinels' to monitor the effects of GB-1111. We placed two predator-exclusion cages that each held 10 *T. reticulata* on every site. Sentinels were replaced on days 3 and 15 after pesticide application.

On 15 June 1998, Alameda County, California MAD personnel applied GB-1111 at the maximum label rate of 47 L/ha (5 gal./acre) by backpack sprayer (Chapin handcan sprayer # 1 53-09 R.E. Chapin Manufacturing Works, Inc., Batavia, NY USA).

We counted surviving mosquitoes and water boatmen on each sampling day, which were 2 days and 1 day before the oil was applied, and on days 1, 2, 3, 5, 7, 14, and 21 after treatment. We also collected aquatic invertebrates from ponds on these days, using four replicated 1 m sweeps with a 'd-ring' net (1 mm mesh) per site. We subsampled collections by wet weight, enumerated subsamples of at least 500 insects per

sample, and calculated total abundances. Insects were identified to family or species, and other taxa were identified to order.

Ducklings. Seventy-four 1-day old mallard ducklings were obtained from Metzer Farms (Gonzales, California) on 4 June 1998, and maintained for twelve days. During this time, the ducklings were numbered by a coded web punch system and maintained on commercial feed supplemented with live food. Each duckling was evaluated for condition and its ability to recognize and consume live food; also, ducklings hatched in incubators and without a hen lack waterproof oil and may not be able to swim or maintain thermoregulation in water for 2 – 3 weeks. For these reasons, ducklings were held for 2 week prior to experimentation. The ducklings were weighed at age day 2 (received the day after birth), 7, and 12, in order to establish a growth curve before treatment. Those ducklings exhibiting pre-treatment growth different from that reported by Sugden et *al*. (1981) or abnormal behavior were excluded from study.

On 15 June 1998 at two-hours post spray, 5 randomly selected 13-day old ducklings each (at least two males and two females) were placed in each of ten 4.3 m diameter, fully enclosed cages. Five cages were constructed at the Hetch-Hetchy marsh and five at West Vaca/Newark Sewer marsh. The cages were constructed of plastic netting with wood stakes. A fence constructed of chicken wire encircled each cage to deter mammalian predators. Each cage was placed on ponds such that about 2/3 of the inner area was in water, and 1/3 on land during MLLW (mean low-low water). A Styrofoam box was tethered in each cage to provide shelter and a floating platform in the event tidal action completely inundated the cages.

The ducklings were alternated between the cages and warm shelter at 3:00 pm (Pacific Daylight Time) every 24 hours because the nightly low temperature was cool (app. 13^{o} C). This procedure was conducted primarily: to obtain routine weights, to allow time for the cages to replenish with mobile invertebrate prey, and also for humane reasons because a hen was not used to shelter the ducklings. Class I mallard ducklings (days 1 – 18) feed continually; therefore their daily timing on the ponds was not critical (Hunter *et al*. 1984). The ducklings were provided only with fresh water during the sheltering period, and were weighed at 9:00 am every other morning (or the morning that they were sheltered). The duckling experiment was scheduled to provide a reasonable amount of time to observe an effect but to avoid undue suffering or mortality.

Laboratory Evaluation on Avian Eggs

Fertile eggs of mallards and bobwhite were obtained from a commercial vendor (Oak Ridge Game Farm, Gravette, Arkansas). Eggs of red-winged blackbirds (redwings) were collected from several sites in Maryland and Delaware during May and early June 1999. In transit, clutches of field-collected eggs were placed in a portable electric incubator. At the laboratory, redwing eggs were candled to confirm fertility and determine the stage of development; individual eggs of clutches with embryos 3-5 days old were randomly selected and assigned to treatment groups. The redwing eggs were placed in inverted paper egg trays and covered with cheesecloth that was wrapped under the tray before placement on incubator racks. Mallard and bobwhite eggs were placed in standard egg trays. Eggs were incubated at 37.5°C, and 70 % relative humidity for mallards and redwings, and 60 % relative humidity for bobwhites, which are the standards for these species.

Treatments per group contained 60 eggs for mallards, 40 for bobwhite, and 25 for redwings. These species differ in incubation period and surface areas of eggs, which accounts for the following differences in treatments. Mallard and bobwhite eggs were treated on day 4 or day 11 and redwing eggs were treated on day 3 – 5 of incubation. Treatments were external applications of GB-1111 in amounts equivalent to 0, 1/3, 1, 3, or 10 times (redwings and mallards) or 0, 1/10, 1/3, 1, 3, 10 (bobwhites) the dose expected from the maximum application rate of 5 gal/acre (47 l/hectare). This correlated to 0, 0.6, 1.8, 5.4, or 18.0 ul/ egg (redwing), 0, 0.4, 1, 3, 10, or 30 ul/egg (bobwhite), and 0, 3.4, 10, 30, or 100 ul/egg (mallard) of GB-1111. GB-1111 was applied by microliter syringe to the external surface of the eggshell, at the base of the air cell. Redwings eggs were candled every 2 – 3 days and mallard and bobwhites weekly to remove dead embryos. Eggs with dead embryos and those that failed to hatch were opened, examined for external abnormalities, and the age at death was estimated.

Hatchlings were euthanized in an oxygen-carbon dioxide chamber within 24 hours of hatching and examined for abnormalities. Measurements taken were body weight with and without the yolk, and liver weight. Skeletal development was assessed following staining with alizarin red-S, and measurements made for crown-rump length, humerus, radius-ulna, femur, and tibiotarsus. Livers at the time of sacrifice were placed in liquid nitrogen after the gall bladder was discarded; samples were later transferred to a freezer at -70°C. Liver tissue was analyzed for hepatic microsomal, P450-associated, monooxygenase activity (EROD).

Statistical Analyses

All data were analyzed using Systat™ (1992) or JMP™ Version 4 (SAS Institute 2000). Invertebrate sentinel survival data were analyzed with either parametric or non-parametric analysis of variance (ANOVA) depending on whether data could be normalized and variances equalized with an arcsine-square root transformation. Abundance data of invertebrates from sweep net samples were log-transformed and analyzed with repeated measures analysis of variance (RANOVA). We used the average abundances of each two adjacent samples in this analysis, because Systat™ (1992) can only analyze up to 8 dates.

Comparisons between treatment and control of weights of mallard ducklings over time were made using repeated measures multivariate analysis of variance (RMANOVA). The null hypothesis was invertebrate prey of ducklings were not affected by GB-1111, resulting in comparable weight gains of ducklings on treated and control ponds over time. The power of this experiment was determined a priori as the number of ducklings required to detect a difference due to treatment (Zar 1996). We estimated that a minimum of a 50-gram change in weight due to treatment was necessary to detect a difference, based on variability of growing duckling weights observed by Hunter *et al*. (1984). Using 25 ducklings each per treatment and control ($\upsilon_1 = 1$, $\Phi = 1.61$, $\alpha = 0.05$, $\upsilon_2 = 46$) the power of analysis was about 0.96, resulting in a 4 % chance of a Type II error.

In the laboratory study, survival, hatching success, incidence of malformations and edema were statistically compared using contingency table analysis ($p \leq 0.05$). Other measurements were compared among treatment groups using one-way analysis of

variance with Dunnett's multiple comparison to quantify significant differences from the control group ($p \leq 0.05$).

RESULTS

Field Study

Invertebrates. Nearly all of the first set of sentinel mosquitoes and water boatmen died in treated sites, but survival was consistently high in control sites, showing that the pesticide can harm some non-target insects while it controls mosquitoes (Figure 2, Kruskal-Wallis non-parametric ANOVAs for each species had 1 df; chi-square approximation > 7; $P < 0.01$). GB-1111 did not cause detectable mortality in the next two sets (i.e., day 3 and day 15) of sentinel mosquitoes (set 2: ANOVA df 1, 8; $F = 0.319$; $P = 0.59$; set 3: non-parametric ANOVA df 1; chi-square 0.1; $P = 0.75$). There was a trend toward a negative effect of GB-1111 on the second set of water boatmen (ANOVA df 1, 8; $F = 3.804$; $P = 0.087$), but clearly no negative effect on the third set, where survival actually averaged higher in the treated sites (ANOVA df 1, 8; $F = 5.926$; $P = 0.04$; Figure 2b). We noted that the difference in the third set of water boatmen sentinels would not be significant if sequential Bonferroni corrections were applied to the tests to adjust for the inflation of type I error caused by using multiple measurements (= sets of sentinels) from the same sites (critical value for $P < 0.025$; see Sokal and Rohlf 1995). The brief activity of GB-1111 is consistent with some of our informal observations during the study. The oil was somewhat volatile and we did not see or smell it after day 3. Wind swept most of the oil from the water surface by 24 h post-spray.

Sweep net collections yielded approximately 1400 invertebrates per site/day, over 90% of which were water boatmen. Other taxa included marine worms, beetle adults and larvae, fly larvae and amphipods, however these were either too scarce or too patchy among sites for meaningful statistical analysis. In comparison to the predator-exclusion cages, the sweep-net collections of water boatmen were more variable and indicated a lower level of mortality. A RANOVA of water boatmen abundances over the entire time series did not show a significant effect of treatment nor a time by treatment interaction (Table la). However, the variance of our time series increased with its length as is typical of ecological data (Bengtsson et al. 1997), and the high variance of a long series could obscure differences that occurred shortly after treatment, when the largest differences are expected *a priori.* We therefore analyzed a truncated data set consisting of the two pre-treatment samples and the first two post-treatment samples. This analysis showed a significant decrease in water boatmen (Table 1[B]). Figure 3 shows the relative decrease in immature and adult water boatmen after application of GB-1111. Loss of adults could be caused by either death or emigration from treated ponds because adults of this species have wings, whereas loss of immatures is likely to reflect only mortality. However, mortality caused at least some loss of both life stages because we observed many dead adult and immature water boatmen floating on the surface of treated sites and virtually none in untreated sites.

Ducklings. Post-treatment gain or changes in weight of ducklings did not differ between treatment and control sites during the study (RMANOVA; df 1, 31; F = 0.103; P = 0.75; Figure 4). Ducklings were placed in the caged wetlands 2 h post-spray on the afternoon of 15 June, and average weights were 208.5 gm (control; Std. Dev. 17.9) and 209.0 gm

(treatment; Std. Dev. 13.2). We noted that ducklings were exposed immediately to an oily sheen of GB-1111. The ducklings preened continuously, huddled, and appeared agitated in response to this exposure, raising concern about the possibility of hypothermia. However, all ducklings survived overnight and appeared healthy and active the following morning. The weight of the ducklings increased fourfold while fed turkey starter diet prior to the study, but weight gain was static the 7 days following introduction into the cages. On the eighth day, weights of ducklings in both treatment and control cages were 9 % lower than those on the previous day, and 11 (control) – 14 (treatment) % lower than those on the start day, and the experiment was ended. In general, ducklings held in cages at the Hetch-Hetchy site faired better than those from the Vaca site (Figure 5), possibly due to intermittent presence of brine flies (*Ephydra* sp.) from a salt pond close to the Hetch-Hetchy site.

Laboratory Studies

Hatching success was significantly reduced for mallard embryos treated on day 4 or day 11 of incubation at 3 and 10 times the maximum field application, with a calculated approximate LD_{50} of 1.9 times the maximum field application (day 4 treatment; Table 2). Most mortality occurred within a week of treatment. With embryos treated on day 4, malformations included: curved bill, hydrocephaly, enlarged gall bladder, and malformed pelvic girdle. Subcutaneous edema was also apparent. The overall frequency of abnormal embryos including those with malformations and edema combined differed marginally from controls ($p<0.10$).

Red-winged blackbird eggs treated with 10 times the maximum expected application of GB-1111 had reduced hatching success compared to all other groups

(Table 3). Also, a trend of reduced hatching success with increasing rates of eggshell application was indicated. The duration of incubation for hatchlings was not different among groups. Body, yolk, and liver weight, liver/body weight ratio, and four of five skeletal measurements for hatchlings, and age at death for unhatched eggs, were not different among groups (Table 4). The reduction of redwing crown-rump length from exposure to one-third of the maximum recommended application rate (1/3 X) might be an anomaly; crown rump distances for the 1/3 X hatchlings were less than those of the control, X, and 3 X groups, but not less than those of the 10 X group. Hepatic microsomal EROD was not different among groups.

Hatching success of bobwhites was significantly reduced to 55% for embryos treated on day 11 at the highest level (10X; Table 5). Effects at this level of treatment on day 4 in bobwhite included a significant increase in incidence of abnormal embryos/ hatchlings, lower body and liver weights of hatchlings, and a two-fold increase in hepatic microsomal P450-associated monooxygenase activity (EROD) in hatchlings.

DISCUSSION

Proper diet to sustain thermoregulation is critical during the first few weeks of development of Anatinae (Cox *et al.* 1998, Sedinger 1992, Koskimies and Lahti 1964). Aquatic invertebrates comprise 100 to 50 % of the diets of young ducklings from age day 1 to day 25, then taper to 10 % of the diet by the class IIb stage (day 36 – 45), and then to about 1 % by class III (day 44 – 55) (Chura 1961). Any impediment to sufficient foraging probably results in loss of fitness and thus mortality especially during the first

few weeks (Cox *et al.* 1998, Street 1978). Mosquito larvae can comprise a large part of the diet of developing ducklings (Meyer and Swanson 1982); without this component, the remaining prey base becomes much more important. Human resource managers are confronted with controlling mosquitoes for both nuisance and health concerns, while wildlife managers face the dilemma of an impaired prey base for fish and wildlife and potentially toxic effects of chemical mosquito controls.

The results reported herein indicated that GB-1111 at recommended field application rates caused no significant or substantial effects to avian embryos or young ducklings. However, exceeding the maximum recommended rates of field application as much as three times, which might occur under conditions of larvicide drift, spray overlap, or over spray could be potentially toxic to mallard embryos or passerine bird embryos. Strict adherence to recommended rates for field applications of GB-1111 coupled with care to avoid overlap spraying or spraying during windy conditions are unlikely to threaten the survival or development of embryos of ducklings or wetland passerines.

GB-1111 was very effective in controlling caged mosquito larvae. However, It also had a strong negative impact on caged and uncaged water boatmen, which were the most abundant macroinvertebrates at the marshes. The effect of GB-1111 was more pronounced on caged water boatmen, probably because the cages reduced their ability to avoid the oil. Winds may have created openings in the oiled water surface outside the cages, but the insides of cages were sheltered from the wind. Some uncaged individuals may have reduced their contact with the oil by climbing out on vegetation and grooming. Also, the impact of the oil seemed smaller on uncaged water boatmen possibly because they migrated from the untreated areas. However, immature water boatmen are unlikely

to move across land, and only 80 % of immatures disappeared from treated sites in the first days post-spray, in contrast to 100 % mortality of caged. Therefore, it seems likely that behavioral avoidance of GB-1111 slightly ameliorates its effects.

The effects of GB-1111 on invertebrates attenuated rapidly over time. The only significant mortality of caged and uncaged invertebrates occurred within the first 3 days, and we did not detect differences between treated and control sites by one week post spray. The rapid recovery of uncaged invertebrates may have resulted from immigration of adult insects from untreated areas and breeding within treated sites. While the spatial scale of GB-1111 application was typical of some operational pest control activities, GB-1111 drift or overlap spray could result in a higher than recommended application or application to a larger area than planned, and in these cases community recovery could be slower than we observed.

Recommended applications of GB-1111 appeared insignificant to duckling fitness under field conditions, despite the initial impact on prey mosquitoes or water boatmen. Ducklings on the treated ponds may have faired well immediately after the application of GB-1111 because of the abundance of dead or floating insects observed that probably were available for consumption (also observed by Hunter *et al*. 1984). We recognized a potential problem of oiling of duckling feathers immediately following application of GB-1111. Oiled, matted feathers impede the ability of water birds to thermoregulate, and can result in poor condition or mortality in cold weather. Field application of GB-1111 should be avoided in early spring and during peak hatching of waterfowl in wetland situations, particularly if daily low temperatures are below about 15 C. Our ducklings were in good condition at the time of exposure and daytime ambient conditions were

fairly mild (> 15 C), but younger or less fit ducklings might not have survived direct exposure to GB-1111.

Class Ic (days 13-18) ducklings consume about 75 % invertebrates (Chura 1961), thus, the field experimental period was sufficient to determine an effect of GB-1111 on their prey. Other studies have observed a difference in weights of ducklings on treated and untreated areas 2 – 3 days post treatment (McCarthy 1995, Hunter *et al*. 1984). Class IIa (days 19 – 25) ducklings consume about 50 % invertebrates and 50 % plant material, and conceivably the ducklings at this point could switch more to plant material for sustenance. The area of cages was probably sufficient for duckling maintenance, but the confinement of the cages might have inhibited effective capture of highly mobile prey. Brine flies or water boatmen were capable of avoiding capture because the cage had water on both inside and outside, thus allowing the prey to move beyond the reach of the ducklings. Further, water boatmen were abundant but are apparently not the main prey of mallards, possibly because of their quick response to predator avoidance (Batzer et al. 1993).

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Figure 1. Location in south San Francisco Bay area where field component of the study of effects of Golden Bear Oil GB-1111 on young mallard ducklings and their invertebrate prey, June 1998, Alameda County, California. Black dots indicate approximate location of treated and untreated experimental ponds.

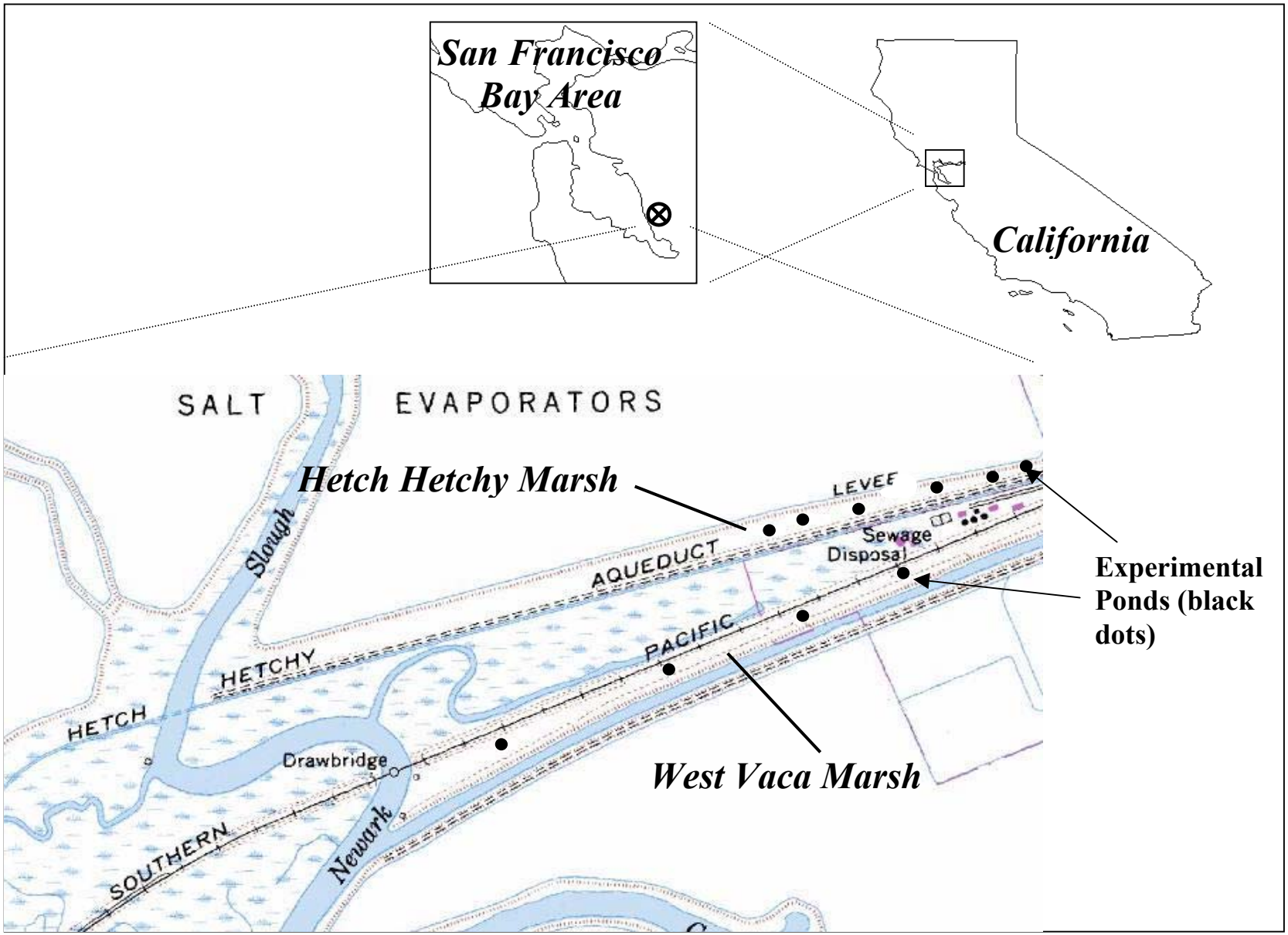
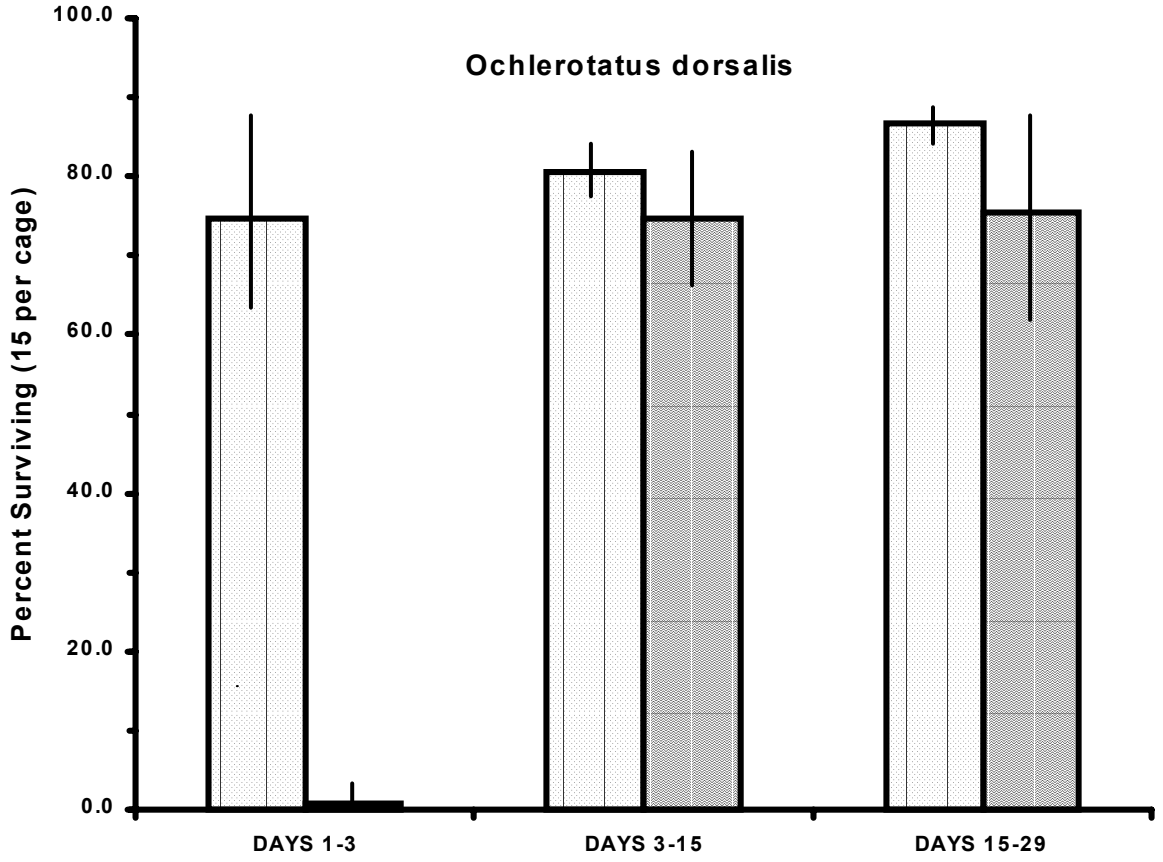

San Francisco Bay Area
California
SALT EVAPORATORS
Hetch Hetchy Marsh
LEVEE
Sewage Disposal
AQUEDUCT
Slough
PACIFIC
HETCHY
HETCH
Drawbridge
Newark
SOUTHERN
Experimental Ponds (black dots)
West Vaca Marsh

Figure 2. Survival of sentinel mosquito larvae and water boatmen enclosed in 2 cages at each of 5 control sites and 5 sites treated with GB-1111 in a salt marsh. Each bar represents the mean and standard deviation.


Ochlerotatus dorsalis
Percent Surviving (15 per cage)
100.0
80.0
60.0
40.0
20.0
0.0
DAYS 1-3
DAYS 3-15
DAYS 15-29

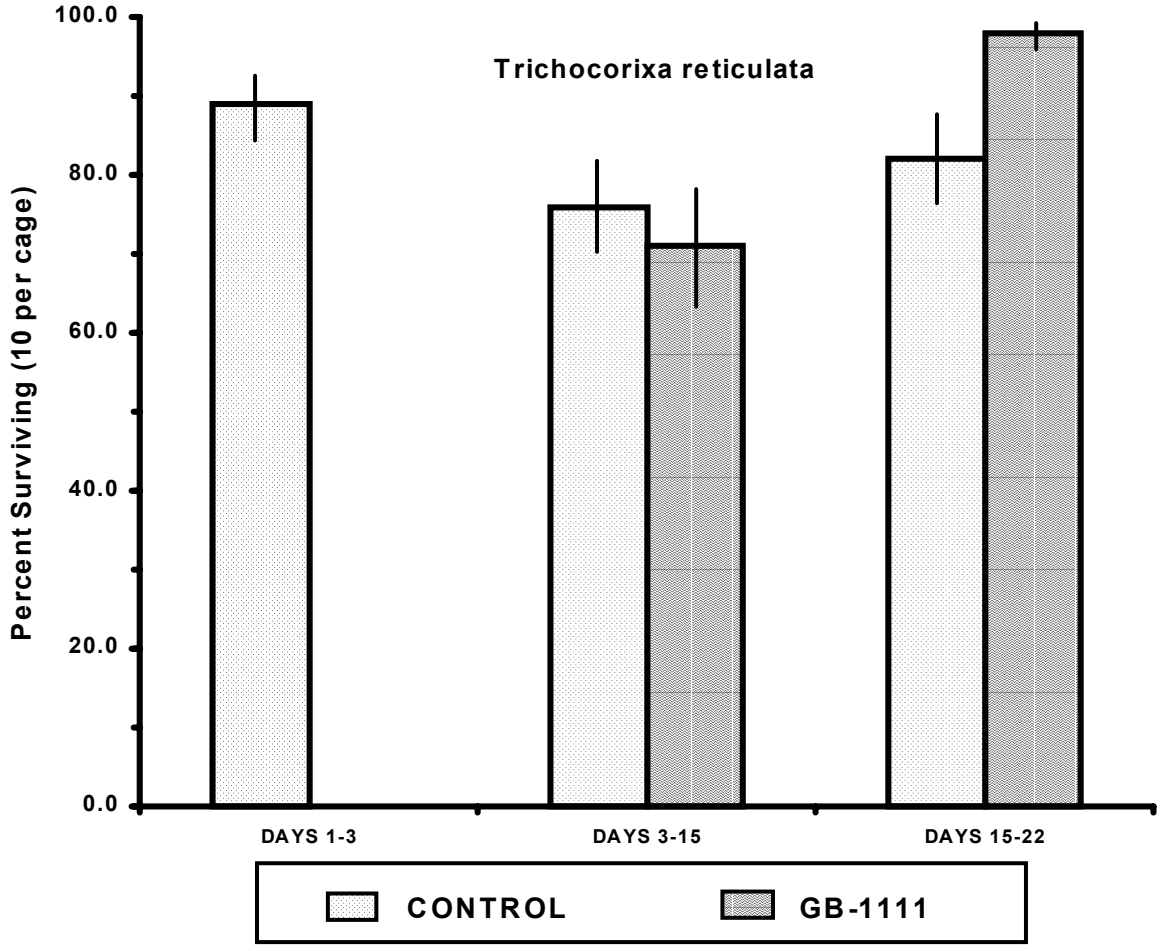

Trichocorixa reticulata
Percent Surviving (10 per cage)
100.0
80.0
60.0
40.0
20.0
0.0
DAYS 1-3
DAYS 3-15
DAYS 15-22
CONTROL
GB-1111

Figure 3. Percent population changes of adult and immature water boatmen in salt marsh ponds treated with GB-1111 and in control ponds, compared 2 days before versus 2 days after the date of pesticide application. Population changes were calculated as [# after application date - # before / # before]. Bars indicate means and standard deviations of 5 sites per treatment and 5 per control.

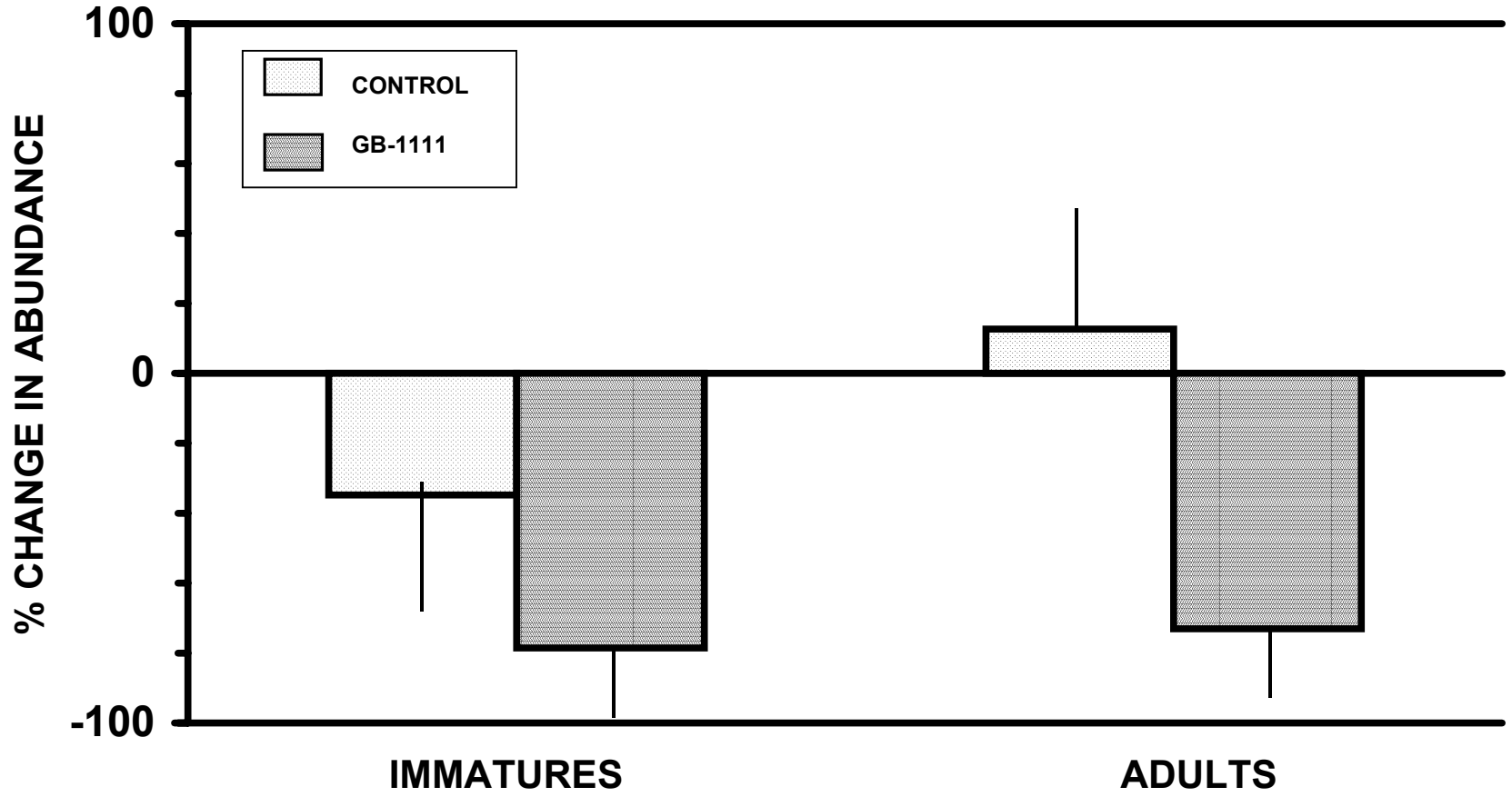

100
0
-100
% CHANGE IN ABUNDANCE
CONTROL
GB-1111
IMMATURES
ADULTS

Figure 4. Pre- and post-treatment and control changes in weight of mallard ducklings held on experimental ponds at the Don Edwards National Wildlife Refuge, San Francisco Bay, California, 5 – 23 June 1998. Treatment ponds were sprayed with the larvicide GB-1111 on 15 June 1998.

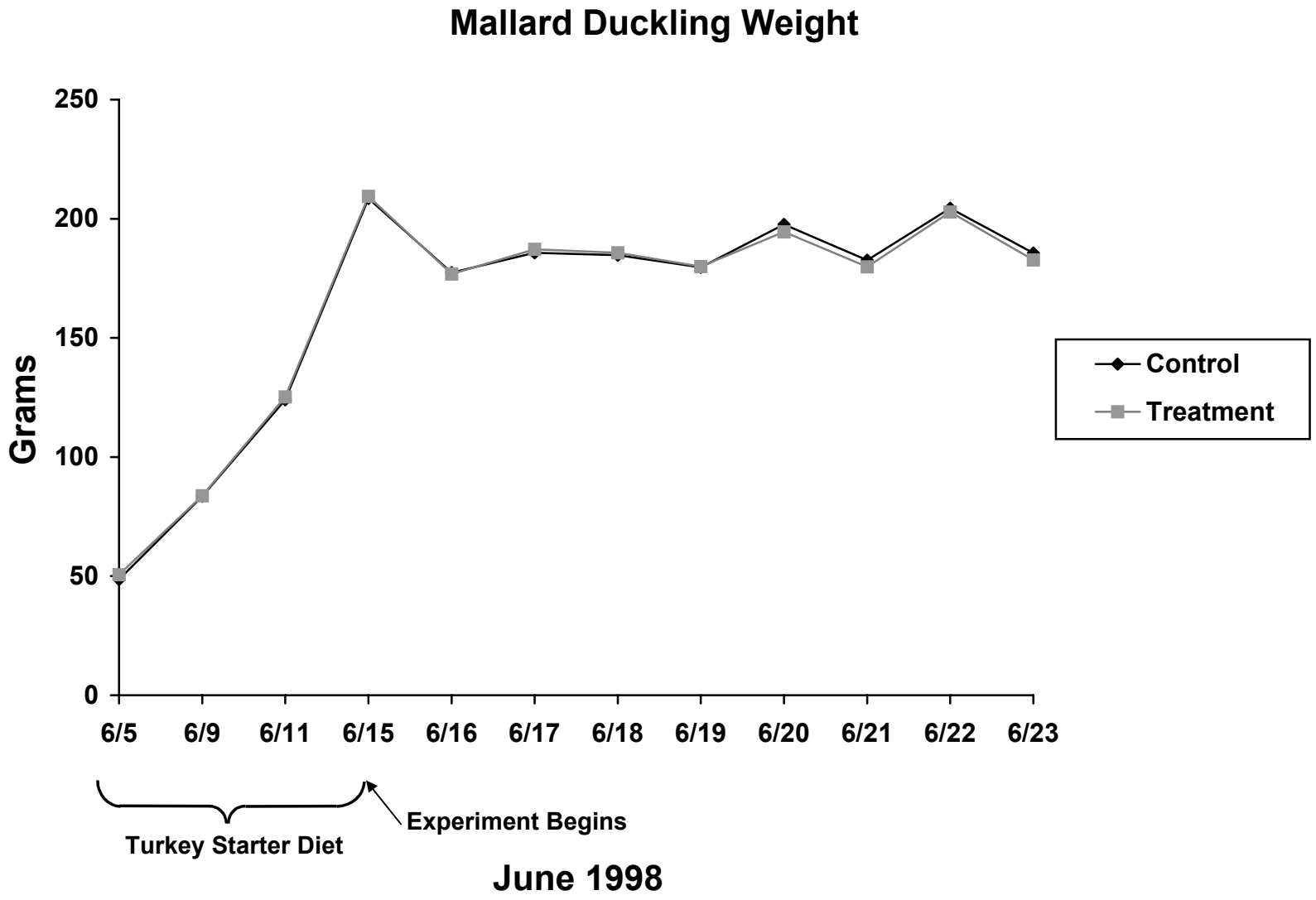

Mallard Duckling Weight
250
200
150
100
50
0
Grams
6/5
6/9
6/11
6/15
6/16
6/17
6/18
6/19
6/20
6/21
6/22
6/23
Control
Treatment
Turkey Starter Diet
Experiment Begins
June 1998

Figure 5. Comparison of weights of ducklings by location of experimental ponds.

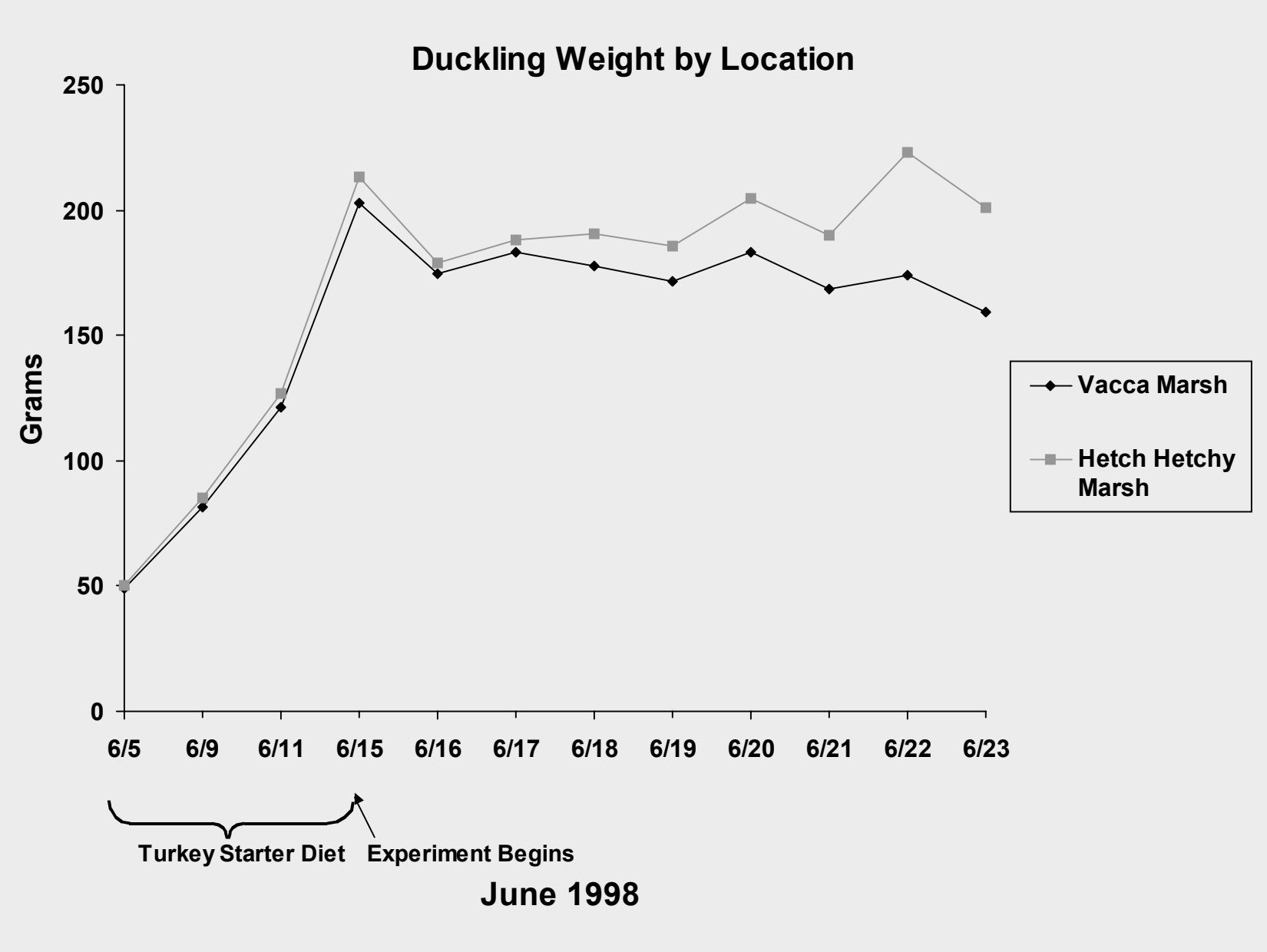

Duckling Weight by Location
250
200
150
100
50
0
Grams
6/5
6/9
6/11
6/15
6/16
6/17
6/18
6/19
6/20
6/21
6/22
6/23
Vacca Marsh
Hetch Hetchy Marsh
Turkey Starter Diet
Experiment Begins
June 1998

Table 1. Repeated measures ANOVA on numbers of Corixidae collected from salt marsh ponds treated with the mosquito larvicidal oil GB-1111versus untreated ponds. Abundances were ln-transformed, and averaged over adjacent pairs of samples. A: Analysis of full data set (pre-treatment days –2 and –2, post treatment days 1, 2, 3, 5, 7, 14, 21); B: Analysis of day days –2, -1, 1, and 2, where the strongest effects were expected.

Source	SS	DF	F	P
A				
Between Subjects				
Treatment	3.65	1	1.60	0.24
Error	18.17	8		
Within Subjects				
Time	11.48	4	6.08	0.001
Time x Treatment	2.09	4	1.10	0.37
Error	15.10	32		
B				
Between Subjects				
Treatment	1.26	1	3.03	0.12
Error	3.33	8		
Within Subjects				
Time	4.32	1	25.02	0.001
Time x Treatment	1.63	1	9.47	0.015
Error	1.381	8		

Table 2. Effects of GB-1111 mosquito larvicide on mallard embryos treated on day 4.

Dose	0	1/3X	X	3X	10X
N	60	60	60	60	59
Survival (%)					
Days 4-12	95	98	93	62[a]	7[a]
Days 12-18	93	98	93	58[a]	5[a]
Hatching success (%)	70	57	63	20[ab]	0[a]
Hatch wt. without yolk sac (g)	33.5 ± 3.3	32.3 ± 3.0	33.3 ± 2.8	32.3 ± 2.6	_
Liver wt. (mg)	950 ± 172	910 ± 133	940 ± 243	1050 ± 212	_
Bone lengths (mm)					
Crown-rump	112.2 ± 4.2	111.1 ± 4.0	112.2 ± 3.7	110.7 ± 2.2	_
Humerus	10.1 ± 0.4	10.1 ± 0.3	10.1 ± 0.4	10.2 ± 0.4	_
Radius-ulna	8.3 ± 0.2	8.2 ± 0.2	8.2 ± 0.3	8.4 ± 0.3	_
Femur	15.9 ± 0.7	15.8 ± 0.5	15.8 ± 0.6	15.9 ± 0.5	_
Tibiotarsus	28.1 ± 1.1	27.7 ± 0.8	28.1 ± 0.8	28.2 ± 0.8	_
Malformations and edema					
Malformed embryos and hatchlings[c] (%)	3	3	5	10	_
Embryos and hatchlings with edema (%)	5	5	3	12	_
Malformed or edema (%)	7	7	7	18	_
Liver EROD activity (pmol/min/mg microsomal protein)	52 ± 7.7	45.8 ± 9.4	46.5 ± 9.3	42.7 ± 5.5	_

[a] Significantly different from controls.

[b] With treatment on day 11, hatching success was 37% for the 3X and 2% for the 10X group.

[c] Malformations included: curved bill, hydrocephaly, gall bladder, pelvic girdle.

Table 3. Number of red-winged blackbird eggs that hatched or failed to hatch after exposure to external applications of GB-1111 mosquito larvicide; n = 25. X = the amount of GB-1111 expected to contact an uncovered egg when the maximum recommended rate of application (5 gal/acre, 47 l/hectare) is used.

Treatment[1, 2]	Eggs hatched	Eggs not hatched
Control	20	5
1/3 X	19	6
X	21	4
3 X	17	8
10 X	5	20

[1] The Jonckeere-Terpstra test was significant ($P \leq 0.05$). This is a test for trend in a contingency table.

[2] Overall Fisher's exact test was significant ($P \leq 0.05$). Fisher's paired tests showed that the 10X group was significantly different from each of the other four groups ($P \leq 0.005$, Bonferroni adjustment of P).

Table 4. Weights and measurements of red-winged blackbird hatchlings and the age at death of embryos that failed to hatch after exposure to external applications of GB-1111. X = the amount of GB-1111 expected to contact an uncovered egg when the maximum rate of application (5 gal/acre, 47 l/hectare) is used. Table entries are means (S.D.)[n]

Treatment	Weight (g) without yolk	Weight of yolk	Weight of liver	Liver/ body wt.	Skeletal measurements (mm)					Age at death
					Crownrump[1]	Humerus	Radius	Femur	Tibiotarsus	
Control	2.66	0.28	0.07	0.03	31.43	2.91	3.44	4.02	5.77	7.60
	(0.29)	(0.10)	(0.01)	(0.01)	(1.04)	(0.16)	(0.18)	(0.21)	(0.26)	(3.21)
	[20]	[20]	[16]	[16]	[20]	[20]	[20]	[20]	[20]	[5]
1/3 X	2.66	0.31	0.07	0.03	30.29	2.97	3.43	4.12	5.86	9.17
	(0.29)	(0.11)	(0.02)	(0.01)	(1.53)	(0.11)	(0.17)	(0.32)	(0.37)	(2.86)
	[18]	[18]	[12]	[12]	[19]	[19]	[19]	[19]	[19]	[6]

Table 4. Continued

Treatment	Weight (g) without yolk	Weight of yolk	Weight of liver	Liver/ body wt.	Skeletal measurements (mm)					Age at death
					Crownrump[1]	Humerus	Radius	Femur	Tibiotarsus	
X	2.66	0.25	0.07	0.03	32.51	2.96	3.39	4.07	5.87	9.25
	(0.26)	(0.12)	(0.02)	(0.01)	(1.07)	(0.15)	(0.21)	(0.32)	(0.44)	(3.40)
	[21]	[21]	[15]	[15]	[21]	[21]	[21]	[21]	[21]	[4]
3 X	2.68	0.31	0.08	0.03	31.62	2.94	3.40	3.96	5.71	10.25
	(0.21)	(0.12)	(0.01)	(0.01)	(1.16)	(0.17)	(0.15)	(0.28)	(0.34)	(2.92)
	[15]	[15]	[13]	[13]	[17]	[17]	[17]	[17]	[17]	[8]
10 X	2.35	0.32	0.08	0.03	31.62	3.02	3.02	4.22	5.84	8.90
	(0.28)	(0.16)	(0.02)	(0.01)	(1.07)	(0.20)	(0.20)	(0.41)	(0.46)	(2.53)
	[5]	[5]	[3]	[3]	[5]	[5]	[5]	[5]	[5]	[20]

[1] Analysis of variance was significant ($P \leq 0.05$). Tukey's HSD Test for pairwise comparisons showed that X was greater than 1/3 X and Controls, and 1/3 X was less than 3 X, X, and Controls.

Table 5. Effects of GB-1111 mosquito larvicide on bobwhite embryos treated on day 4.

Dose	0	1/3X	X	3X	10X
N	40	40	40	40	40
Survival (%)					
Days 4-11	93	88	98	93	95
Days 11-18	88	85	93	93	93
Hatching success (%)	88	85	75	88	70[a]
Hatch wt. without yolk sac (g)	6.5 ± 0.6	6.5 ± 0.7	6.5 ± 0.6	6.7 ± 0.7	5.7 ± 0.7[b]
Liver wt. (mg)	220 ± 35	_	200 ± 31	_	190 ± 36[b]
Bone lengths (mm)					
Crown-rump	52.8 ± 1.7	52.6 ± 2.2	51.3 ± 1.8[b]	52.4 ± 1.8	51.7 ± 2.0
Humerus	5.8 ± 0.2	6.1 ± 0.4	5.9 ± 0.3	6.2 ± 0.4[b]	6.0 ± 0.3
Radius-ulna	5.2 ± 0.2	5.2 ± 0.3	5.1 ± 0.3	5.3 ± 0.3	5.1 ± 0.3
Femur	11.5 ± 0.5	11.9 ± 0.6	11.5 ± 0.5	12.0 ± 0.5	11.4 ± 0.8
Tibiotarsus	16.6 ± 0.7	16.9 ± 0.9	16.7 ± 0.7	17.1 ± 0.7	16.4± 0.9
Malformations and edema					
Malformed embryos and hatchlings (%)	5	5	0	5	20
Embryos and hatchlings with edema (%)	0	0	0	0	8
Malformed or edema (%)	5	5	0	5	28[b]
Liver EROD activity (pmol/min/mg microsomal protein)	19.0± 10.7	_	16.4± 6.7	_	41.6± 14.3[b]

[a] For day 11 treatment, hatching success (55%) was significantly lower than for controls.

[b] Significantly different from controls.

[c] Malformations included: crossed beak, hydrocephaly, gall bladder, tibiotarsus.