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EFFECTS OF BORON AND SELENIUM ON MALLARD REPRODUCTION AND DUCKLING GROWTH AND SURVIVAL

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Abstract—Boron (B) and selenium (Se) sometimes occur together in high concentrations in the environment and can accumulate in plants and invertebrates consumed by waterfowl. One hundred twenty-six pairs of breeding mallards (*Anas platyrhynchos*) were fed diets supplemented with B (as boric acid) at 0, 450, or 900 ppm, in combination with Se (as seleno-DL-methionine) at 0, 3.5, or 7 ppm, in a replicated factorial experiment. Ducklings produced received the same treatment combination as their parents. Boron and Se accumulated in adult liver, egg, and duckling liver. In adults, B and Se caused weight loss, and B decreased hemoglobin concentration, egg weight, and egg fertility. Both B and Se reduced hatching success and duckling weight, and B reduced duckling growth and duckling production, and caused several alterations in duckling liver biochemistry. Duckling survival was not reduced by B or Se, and neither B nor Se had histopathologic effects on adult or duckling liver, kidney, or spleen. There was little evidence of interaction between B and Se. This study demonstrated that B and Se, in the chemical forms and at the dietary levels administered in this study, can adversely affect mallard reproduction and duckling growth.

Keywords—Boron Selenium Mallard *Anas platyrhynchos* Reproduction

INTRODUCTION

Boron (B) and selenium (Se) sometimes occur together in high concentrations in the environment and can accumulate in aquatic plants and invertebrates consumed by waterfowl [1–3]. In the Central Valley of California, B in widgeon grass (*Ruppia maritima*) ranged from 120 to 780 ppm (dry weight), and widgeon grass seeds contained 430 to 3,500 ppm B [1,4]. Levels of B as high as 1,487 ppm have been found in widgeon grass, and concentrations in brine shrimp (*Artemia* spp.), brine fly cases (Diptera, Ephydridae), and water boatman (Hemiptera, Corixidae) have reached 1,120, 780, and 641 ppm B, respectively [3]. Selenium in aquatic plants can reach mean concentrations of 73 ppm, and in aquatic insects concentrations can average more than 100 ppm [5]. Levels of Se in damselfly larvae (Odonata, Zygoptera), brine fly cases, midge larvae (Diptera, Chironomidae), and beetles (Coleoptera) have been reported as high as 162, 304, 250, and 141 ppm, respectively [3].

Boron and Se can accumulate in waterfowl tissues at sites where environmental concentrations of these elements are high [3,6–8], and both elements have been shown in laboratory studies to have toxicologic effects on waterfowl. In adult mallards (*Anas platyrhynchos*), 1,000 ppm dietary B affected female weight and hatching success of eggs, and in ducklings resulted in reduced hatching weight, weight gain, and survival [9]. Boron has negative effects on duckling blood, brain, and liver biochemistry [10] and can alter duckling behavior [11]. There is some evidence suggesting that dietary B is more toxic to ducklings hatched from B-contaminated eggs than ducklings hatched from uncontaminated eggs [10]. In adult mallards, Se causes weight loss and delays the onset of egg laying [12] and can reduce hatching success [13,14]. Selenium is embryotoxic and teratogenic [1,13–15]; reduces mallard duckling growth, survival, and total production [12,14,16]; and alters duckling liver biochemistry [17].

Toxicity of Se in organisms is well known to often be reduced by the presence of other trace elements, including arsenic, cadmium, copper, mercury, tellurium, tin, and lead [18,19]. In mallards, arsenic interacts antagonistically with Se to reverse Se-induced effects on hatching success and embryo deformities [14] and duckling mortality, growth, and hepatic lesions [20]. Currently, little information on the interactive effects of B and Se on waterfowl is available. In one study on mallard ducklings, B was found to alter the effects of Se on growth and blood chemistry and, under conditions of restricted dietary protein, to decrease survival [21]; ducklings were not exposed to B in the egg and interactive effects of B and Se on adults were not investigated. The objective of the present study was to investigate the main and interactive effects of B and Se on mallard reproduction and duckling growth and survival.

METHODS

Adults and dietary treatments

One hundred twenty-six pairs of 1-year-old mallard drakes and hens were purchased from a game farm (Outdoorsman Hunting Club, Webb, IA, USA) in February 1987. All birds were banded, wing-clipped, segregated by sex, then randomly assigned to indoor holding pens for a 17-day conditioning period. During this period, both sexes received an untreated diet of commercially available developer mash (14.5% crude protein; all feed was supplied by Chesapeake Feed Company, Beltsville, MD, USA), and females were kept under controlled lighting (8 h/d) to delay the onset of egg laying and to synchronize their cycles. Following conditioning, birds were weighed and randomly assigned to one of nine treated diets in a 3×3 replicated factorial arrangement ($n = 14$ pairs per treatment combination). Diets consisted of feed supplemented with B at 0 (B control),

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450, or 900 ppm, in combination with Se at 0 (Se control), 3.5, or 7 ppm. All diets contained about 10% water. Males were placed in 1-m^2 outdoor pens, one bird per pen; females remained in indoor pens under controlled lighting. After 3 weeks on treated diets, females were randomly paired with males on the same treatment combination in outdoor pens. All pens contained a water pan, feeder, and nest box. At pairing, birds were weighed and switched to treated diets prepared with breeder mash (17% crude protein).

Boron, in the form of boric acid (99.9% pure; Fisher Scientific, Fair Lawn, NJ, USA), was mixed into the feed as a dry powder. Selenium, in the form of seleno-DL-methionine (98–99% pure; Bachem Inc., Torrance, CA, USA), was dissolved in distilled, deionized water, and these solutions were mixed into duck feed so that 1% water had been added to each diet. One percent water, by weight, was added to all other diets to ensure that moisture content was consistent across treatments.

Nesting, eggs, and incubation

Nest boxes were checked daily and new eggs numbered sequentially. Cracked or broken eggs were removed from the nest, and eggs laid outside the nest were placed in the nest. The eighth egg was removed from each nest, and whole egg weight and eggshell thickness were determined. Contents of each eighth egg were frozen and retained for chemical analysis. Eggshells were air dried for 30 d before measuring eggshell thickness.

Hens incubated their own clutches, but were not allowed to incubate more than 20 eggs. When a clutch exceeded 20 eggs, successive eggs, beginning with the first egg laid, were removed up until incubation began. From these extra eggs, one egg was randomly selected from each of five hens in the group fed the diet containing 900 ppm B + 0 ppm Se and from five hens fed the diet containing 900 ppm B + 7 ppm Se. With these 10 eggs we separated yolk from albumen and froze the two parts for chemical analysis to determine the distribution of B and Se in the egg.

On day 7 of incubation, all eggs in a nest were candled and dead or infertile eggs were removed. When egg removal exceeded 25% of the clutch, extra eggs (those removed from nests where clutch size exceeded 20 eggs) were marked and added to the nest to bring clutch size up to 75% of its original size. These extra eggs had been stored in a refrigerator and, being at least 1 week behind the female's own eggs in length of incubation, did not hatch. This addition of eggs was done in an attempt to reduce nest desertion in females that had few remaining eggs. Males were removed from the pen on day 7 of incubation and placed in holding pens where they were maintained on treated diets. In nests where the entire clutch of eggs was infertile the male was left in the pen and the female was allowed to recycle. In cases where a female recycled, the eighth egg of the second clutch was removed, measured, and frozen for chemical analysis.

Ducklings and necropsies

On the day of hatching, ducklings were weighed, marked with a web tag, and placed on the same diet as their parents. Eggs that failed to hatch and ducklings that failed to survive the first 12 h were removed from the pen and the embryos and ducklings were examined for deformities. In pens where an incubating hen deserted the nest, eggs were removed and embryos were examined for deformities. Treated diets supplied for ducklings were prepared using starter mash (22.0% crude protein). Ducklings were checked daily for survival, were weighed again at 7 d and 14 d, and at 14 d were euthanized in a CO_2 chamber, as were their parents. In pens where the nest was deserted, adults were euthanized 14 d after nest desertion. At the time of sacrifice, adults were weighed and the liver removed, weighed, and frozen for chemical analysis. Ducklings were also weighed at the time of sacrifice, and one duckling per pen was randomly selected for removal of the liver for weighing and chemical analysis. Samples of liver, kidney, and spleen from 10 randomly selected hens, drakes, and ducklings from the following treatment combinations: 0 ppm B + 0 ppm Se, 900 ppm B + 0 ppm Se, 0 ppm B + 7 ppm Se, and 900 ppm B + 7 ppm Se were saved in 10% buffered formalin for histopathologic analysis (hematoxylin and eosin staining with examination by light microscope by Donald A. Willigan, Germantown, MD, USA). Blood samples from adults and liver tissue from a second randomly selected duckling (one per pen) from the same four treatment combinations were also taken at necropsy for hematologic and biochemical analysis. Hematocrit (packed cell volume) and hemoglobin concentrations (cyanomethemoglobin method) were determined in whole blood.

Liver biochemistry

Aliquots of minced liver were homogenized (1:10 w/v) in ice-cold 1.15% KCI–0.01 M Na K phosphate buffer (pH 7.4), containing 0.02 M EDTA. The homogenate was centrifuged at 10,000-*g* for 20 min at 4°C and the resultant supernatant was used for assays. Liver biochemistry measurements included several indicators of oxidative stress. Glutathione peroxidase (GSH peroxidase, EC 1.11.1.9; coupled reaction at 30°C with glutathione reductase using cumene hydroperoxide) and glutathione reductase (GSSG reductase, EC 1.6.4.2) activities were recorded spectrophotometrically by micromethods using a centrifugal analyzer [22]. Sorbitol dehydrogenase (SDH activity; EC 1.1.1.14) was recorded according to the method of Dooley et al. [23] using the centrifugal analyzer. Crude homogenate and 10,000-*g* supernatant protein concentrations were determined according to the method of Lowry et al. [24] using bovine serum albumin as a standard. Reduced glutathione (GSH) and oxidized glutathione status were determined by the method of Tietze [25] as modified by Griffith [26] for GSSG using vinyl pyridine. The total hepatic sulfhydryl concentration (total SH) was measured according to Sedlak and Lindsay [27]. Thiobarbituric acid reactive substance (TBARS) was measured as an estimate of hepatic lipid peroxidation using the acid method described by Aust [28]. Standard curves were generated for the assay using malondialdehyde tetraethyl acetal.

Chemical analysis

Chemical analyses were conducted at The Research Triangle Institute (Research Triangle Park, NC, USA) under the quality assurance/quality control program of the Patuxent Analytical Control Facility, Patuxent Environmental Science Center (Laurel, MD, USA). All samples were analyzed for B by ICP using a Leeman Labs Plasma Spec I sequential spectrometer, and for Se by graphite furnace atomic absorption using a Perkin Elmer Zeeman 3030 atomic absorption spectrophotometer with an HGA-600 graphite furnace and an AS-60 autosampler. Tissue digestion was by microwave oven for 3 min at 120 W, 3 min at 300 W, and 15 min at 450 W, using a 0.25 to 0.5-g aliquot of freeze-dried tissue in the presence of 5 ml of nitric acid. For B the residue was diluted to 50 ml with 5% HCL, and for Se the residue was diluted to 50 ml with laboratory pure water. Percent recoveries from spiked adult liver, juvenile liver, whole

egg, yolk, and albumen, were 92%, 96%, 93%, 98%, and 91%, respectively, for B; and were 104%, 110%, 108%, 91%, and 96%, respectively, for Se. Limits of detection in adult liver, juvenile liver, whole egg, yolk, and albumen, on a wet-weight basis, were 0.15, 0.13, 0.14, 0.26, and 0.07 ppm, respectively, for B; and 0.09, 0.08, 0.08, 0.15, and 0.04 ppm, respectively, for Se. Boron and Se concentrations reported as below the limit of detection were assigned a value of one-half the limit of detection. Results of all residue analyses are reported on a parts per million wet-weight basis. Percent moisture contents for adult liver, juvenile liver, whole egg, yolk, and albumen were 69%, 73%, 71%, 49%, and 87%, respectively. These percentages can be used to convert wet-weight values to a dry-weight basis.

Statistical methods and data analysis

Data on egg fertility, embryo deformities, hatching success, and duckling survival were analyzed by logistic regression under a mixed effects model with parameters for main and interactive effects between B and Se, and a pen effect. Parameters were tested for significance with *F* tests; multiple comparisons were made using contrasts. Tests for differences in B and Se concentrations between yolk and albumen were made with paired *t* tests.

All remaining response variables were analyzed by analysis of variance (ANOVA) under a model appropriate for the main effects and interactions being tested, and an error term suitable for unbalanced data. Tests of hypotheses that included more than one bird per pen were made with *F* tests under a mixed effects model using an appropriate error term. All other *F* tests assumed a fixed effects model and used the residual mean square as the error term. Normality of residuals was evaluated using the Shapiro–Wilk statistic [29] and normal probability plots. Studentized residuals were plotted to assess homoscedasticity. Data that did not meet normality or homoscedasticity assumptions necessary for ANOVA were transformed so as to meet the assumptions. Consequently, all percentage data were arcsine transformed and all residue data were $\log_e$ transformed before analysis. Multiple comparisons were made using Tukey's multiple comparison procedure (MCP) at $\alpha = 0.05$.

RESULTS

Residue analyses

Boron accumulated in adult mallard liver for sexes combined ($p < 0.001$), females ($p < 0.001$), and males ($p < 0.001$), and accumulated in egg ($p < 0.001$) and duckling liver ($p < 0.001$) (Table 1). The concentration of B in albumen was greater than in yolk ($p < 0.001$). Selenium accumulated in adult mallard liver for sexes combined ($p < 0.001$), females ($p < 0.001$), and males ($p < 0.001$), and accumulated in egg ($p < 0.001$) and duckling liver ($p < 0.001$) (Table 2). Selenium concentrations in albumen and yolk differed among treatment levels ($p < 0.001$ and $p < 0.001$), but did not differ from each other ($p = 0.668$). The average concentration of Se in adult liver differed between sexes ($p < 0.001$). No interactions occurred between B and Se in any tissues analyzed.

Treatment effects on adults and reproduction

Boron had no effect on adult weight at pairing or necropsy, and did not affect adult weight change between pairing and necropsy. Boron at 900 ppm caused weight loss between treatment onset and pairing for sexes combined ($p = 0.001$) and females ($p < 0.001$) when compared to controls, and, for males, B had a significant effect on weight change between treatment onset and pairing ($p = 0.050$) (Table 3). Means for males could not be separated by Tukey's MCP at $\alpha = 0.05$; however, the data suggest that the 450-ppm group gained more weight than both control and 900-ppm B groups. For sexes combined, 900 ppm B reduced liver weight (mean ratio of liver weight to body weight) when compared to the 450-ppm B group but not when compared to the control group ($p = 0.045$) (Table 3). Boron had no effect on female liver weight ($p = 0.201$), but in males

Table 1. Boron concentration (ppm, wet weight) in adult liver, egg, and duckling liver for mallards fed diets supplemented with B (as boric acid)[a]

	B added to the diet (ppm)		
	0	450	900
Days on treated diet (adults only)[b]	120 (92–153)	119 (97–153)	125 (90–168)
Adult liver	0.53 (0.127) A [59]	4.6 (0.18) B [60]	8.5 (0.34) C [60]
Females	0.36 (0.038) A [29]	4.7 (0.26) B [30]	9.2 (0.45) C [30]
Males	0.69 (0.245) A [30]	4.5 (0.27) B [30]	7.7 (0.48) C [30]
Whole egg	0.17 (0.019) A [31]	6.5 (0.25) B [42]	11 (0.4) C [42]
Albumen[c]	—	—	13 (1.1) [10]
Yolk[c]	—	—	6.3 (0.39) [10]
Duckling liver	0.25 (0.015) A [26]	7.6 (0.48) B [33]	13 (0.7) C [26]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.
[b] Range of values is in parentheses.
[c] Levels of B in the albumen and yolk were significantly different ($p < 0.001$).

Table 2. Selenium concentration (ppm, wet weight) in adult liver, egg, and duckling liver for mallards fed diets supplemented with Se (as seleno-DL-methionine)[a]

	Se added to the diet (ppm)		
	0	3.5	7
Days on treated diet (adults only)[b]	120 (90–168)	121 (93–153)	122 (100–153)
Adult liver	0.88 (0.037) A [60]	3.7 (0.16) B [59]	6.2 (0.27) C [60]
Females[c]	0.69 (0.051) A [30]	3.2 (0.17) B [29]	5.1 (0.18) C [30]
Males[c]	1.1 (0.03) A [30]	4.3 (0.23) B [30]	7.3 (0.43) C [30]
Whole egg	0.27 (0.011) A [33]	3.5 (0.10) B [42]	7.1 (0.28) C [40]
Albumen	0.11 (0.010) A [5]	—	6.7 (0.63) B [5]
Yolk	0.60 (0.020) A [5]	—	6.0 (0.65) B [5]
Duckling liver	0.51 (0.016) A [32]	2.8 (0.09) B [29]	5.0 (0.24) C [25]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of B; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.
[b] Range of values is in parentheses.
[c] The average concentration of Se in the liver differed by sex ($p < 0.001$).

Table 3. Main effects of B on weight change, liver weight, hemoglobin, and reproduction in adult mallards fed diets supplemented with B (as boric acid)[a]

	B added to the diet (ppm)		
	0	450	900
Adult weight change, treatment onset to pairing (g)	13 (6.1) A [84]	16 (8.2) A [84]	−14 (7.1) B [84]
Females	23 (7.4) A [42]	−2.6 (9.04) AB [42]	−28 (9.2) B [42]
Males[b]	3.7 (9.66) [42]	35 (13.2) [42]	−0.95 (10.496) [42]
Ratio of liver weight to body weight	0.028 (0.0012) AB [84]	0.029 (0.0011) A [84]	0.026 (0.0011) B [84]
Females[c]	0.036 (0.0016) [42]	0.037 (0.0015) [42]	0.033 (0.0014) [42]
Males[c]	0.020 (0.0006) A [42]	0.022 (0.0005) B [42]	0.020 (0.0006) A [42]
Hemoglobin (g/dL)	15.8 (0.39) A [52]	—	14.6 (0.35) B [54]
Females[c]	14.6 (0.60) [26]	—	13.9 (0.54) [27]
Males[c]	16.9 (0.39) A [26]	—	15.4 (0.39) B [27]
Egg weight (g)	55 (0.7) A [39]	56 (0.6) A [42]	52 (0.7) B [42]
Egg fertility (%)	92 (2.3) A [33]	90 (2.5) AB [32]	84 (3.2) B [31]
Hatching success (%)	66 (5.3) A [33]	58 (5.4) A [32]	38 (5.1) B [31]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.
[b] Means in this row differed by ANOVA ($p = 0.050$) but could not be separated by Tukey's MCP at $\alpha = 0.05$.
[c] Ratio of liver weight to body weight and hemoglobin differed by sex ($p < 0.001$ and $p < 0.001$).

the 450-ppm B group had higher liver weights than both controls and the 900-ppm B group ($p = 0.014$). Hemoglobin concentrations were lower in the 900-ppm B group for sexes combined ($p = 0.022$) and males ($p = 0.007$) when compared to controls, but B had no effect on female hemoglobin ($p = 0.343$) (Table 3). Boron did not affect hematocrit, onset of egg laying, or eggshell thickness, and had no effect on embryo deformities (percentage of fertile eggs having deformed embryos or deformed ducklings failing to survive the first 12 h posthatching). Boron at 900 ppm reduced egg weight ($p < 0.001$) and hatching success (percentage of fertile eggs that hatched) ($p = 0.004$) when compared to controls and the 450-ppm B group, and at 900 ppm reduced egg fertility (percentage of fertile eggs per clutch) ($p = 0.012$) when compared to controls (Table 3).

Selenium had no effect on adult weight at pairing or necropsy, and did not affect adult weight change between pairing and necropsy. Selenium did, however, affect weight change between treatment onset and pairing for sexes combined ($p < 0.001$) and females ($p < 0.001$), but had no effect on males ($p = 0.168$) (Table 4). Selenium did not affect adult liver weight, hemoglobin concentrations, hematocrit, the onset of egg laying, eggshell thickness, egg weight, egg fertility, or embryo deformities. Selenium at 7 ppm reduced hatching success when compared to controls and the 3.5 ppm Se group ($p = 0.010$) (Table 4).

For data pooled over all treatment combinations, weak evidence was found that weight change between treatment onset and pairing differed by sex ($p = 0.069$), and strong evidence was found that liver weight and hemoglobin concentrations differed by sex ($p < 0.001$ and $p < 0.001$). For female weight change between treatment onset and pairing, a synergistic interaction occurred between B and Se ($p = 0.002$) whereby 7 ppm Se caused weight loss in the 450- and 900-ppm B treatment groups, but not in the B controls. No other interactions occurred between B and Se for any of the response variables measured for adults, and neither B nor Se caused histopathologic effects in the liver, kidney, or spleen. No adult mortality occurred during the study.

Treatment effects on ducklings

Analysis of biochemistry data from livers of ducklings showed that, when compared to controls, 900 ppm B decreased total SH ($p = 0.012$), liver protein ($p = 0.013$), and SDH ($p = 0.051$) activity, and increased TBARS ($p = 0.020$) concentrations (Table 5). Boron had no effect on GSH concentration, GSH peroxidase and GSSG reductase activities, or ratio of GSSG to GSH. Selenium had no effect on duckling liver biochemistry, and no interactions occurred between B and Se for any of the biochemistry measurements made.

Ducklings in the 900-ppm B treatment group had lower body weights at hatching than ducklings in the 450-ppm B group ($p = 0.016$), and had lower weights at 7 d ($p < 0.001$) and 14 d ($p < 0.001$) when compared to controls and the 450-ppm B group (Table 6). Boron at 900 ppm reduced duckling growth (measured as weight gain) between hatching and 7 d ($p < 0.001$) and 7 d and 14 d ($p < 0.001$) when compared to controls and the 450-ppm B group, and 450 ppm B increased duckling survival between 7 d and 14 d ($p < 0.006$) when compared to controls and the 900-ppm B group (Table 6). Boron had no effect on duckling survival between hatching and 7 d. Duckling

Table 4. Main effects of Se on weight change, liver weight, hemoglobin, and reproduction in adult mallards fed diets supplemented with Se (as seleno-DL-methionine)[a]

	Se added to the diet (ppm)		
	0	3.5	7
Adult weight change, treatment onset to pairing (g)	22 (7.6) A [84]	11 (6.3) A [84]	−18 (7.3) B [84]
Females	22 (8.1) A [42]	1.1 (7.5) A [42]	−30 (9.8) B [42]
Males	22 (13.1) [42]	21 (10.0) [42]	−4.8 (10.69) [42]
Ratio of liver weight to body weight	0.027 (0.0011) [84]	0.028 (0.0012) [84]	0.028 (0.0011) [84]
Females[b]	0.035 (0.0015) [42]	0.035 (0.0017) [42]	0.036 (0.0013) [42]
Males[b]	0.020 (0.0005) [42]	0.021 (0.0005) [42]	0.020 (0.0006) [42]
Hemoglobin (g/dL)	15.3 (0.39) [52]	—	15.1 (0.37) [54]
Females[b]	14.3 (0.55) [26]	—	14.1 (0.60) [27]
Males[b]	16.2 (0.48) [26]	—	16.0 (0.35) [27]
Egg weight (g)	54 (0.7) [41]	55 (0.8) [42]	54 (0.7) [40]
Egg fertility (%)	88 (2.2) [33]	90 (3.4) [29]	89 (2.6) [34]
Hatching success (%)	62 (5.5) A [33]	61 (4.9) A [29]	41 (5.6) B [34]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of B; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.
[b] Ratio of liver weight to body weight and hemoglobin differed by sex ($p < 0.001$ and $p < 0.001$).

production in the 900-ppm B group was lower than in the control and 450-ppm B groups ($p < 0.001$). Boron had no affect on duckling liver weight.

Selenium had no effect on duckling weight at hatching ($p = 0.346$), but 7 ppm Se reduced duckling weight at 7 d ($p = 0.022$) and 14 d ($p < 0.001$) when compared to the 3.5-ppm Se group (Table 7). Duckling growth between hatching and 7 d was greater in the 3.5-ppm Se group when compared to the 7-ppm Se group ($p = 0.046$), and duckling growth between 7 d and 14 d was greater in the 3.5-ppm Se group when compared to the controls and 7-ppm Se group ($p < 0.001$) (Table 7). Duckling survival between 7 d and 14 d was greater in the 3.5-ppm Se treatment group than in controls ($p = 0.023$), but did not differ from the 7-ppm Se group (Table 7). Selenium had no effect on duckling survival between hatching and 7 d. Duckling production in the 7-ppm Se group was lower than in the 3.5-ppm Se group ($p = 0.042$), but did not differ significantly from controls. Selenium had no effect on duckling liver weight.

For duckling production there was a statistical interaction between B and Se ($p = 0.018$). Addition of 7 ppm Se to diets decreased duckling production in the B control and 450-ppm B groups, but had no effect on the 900 ppm group. No other interactions occurred between B and Se for any response variables measured for ducklings, and neither B nor Se caused observable histopathologic effects in liver, kidney, or spleen.

Table 5. Effects of B on liver biochemistry in ducklings fed diets supplemented with B (as boric acid)[a]

	B added to the diet (ppm)	
	0	900
Total SH (μmol/g)	22.0 (0.6) A [13]	19.9 (0.6) B [12]
GSH (μmol/g)	4.3 (0.2) [13]	4.1 (0.3) [12]
Protein (mg/g)	129 (3) A [13]	119 (2) B [12]
TBARS (nmol/g)	18.2 (0.8) A [13]	22.1 (1.2) B [12]
GSH peroxidase (nmol/min/mg protein)	529 (46) [13]	629 (45) [12]
GSSG reductase (nmol/min/mg protein)	56 (7) [13]	59 (5) [12]
SDH (μmol/min/mg protein)	6.1 (0.6) A [13]	4.0 (0.6) B [12]
GSSG:GSH (×100)	6.0 (0.6) [13]	7.4 (0.8) [12]

[a] Values are arithmetic means (SE) and were computed by pooling data over the Se control and 7 ppm Se treatment groups; bracketed numbers are sample sizes.

DISCUSSION

Interactive effects of boron and selenium

Our main finding in this study was that there were no important interactions between boric acid and seleno-DL-methionine with regard to adult health, reproductive success, duckling growth and survival, and tissue residues of boron and selenium. Hoffman et al. [21] fed mallard ducklings diets containing 1,000 ppm B, as boric acid, with or without the addition of Se, as seleno-DL-methionine, at 15 or 60 ppm; these combinations of B and Se were given in diets containing either 7% or 22% protein. With 22% protein in the diet, 60 ppm Se and 1,000 ppm B interacted to suppress growth more than did either element alone. With 7% protein in the diet, 60 ppm Se, by itself

Table 6. Main effects of B on mallard ducklings fed diets supplemented with B (as boric acid)[a]

	B added to the diet (ppm)		
	0	450	900
Duckling weight (g)			
Hatching	34.1 (0.24) AB [308]	34.5 (0.18) A [356]	32.6 (0.28) B [192]
7 d	66.8 (0.88) A [268]	64.4 (0.79) A [300]	55.4 (0.99) B [137]
14 d	160.1 (2.65) A [262]	146.3 (2.21) A [298]	123.5 (3.46) B [132]
Duckling growth (g)			
Hatching to 7 d	32.2 (0.80) A [267]	29.9 (0.77) A [297]	22.5 (0.97) B [137]
7 d to 14 d	93.1 (1.93) A [262]	81.9 (1.58) A [298]	67.9 (2.60) B [132]
Duckling survival (%)			
Hatching to 7 d	78.2 (5.62) [30]	78.6 (4.67) [35]	62.1 (5.89) [30]
7 d to 14 d	97.7 (0.97) A [27]	99.4 (0.55) B [33]	94.1 (2.88) A [26]
Duckling production	9.7 (0.70) A [27]	9.0 (0.80) A [33]	5.1 (0.74) B [26]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of Se; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.

or with 1,000 ppm B, caused 100% mortality of ducklings by the end of 4 weeks; therefore, more subtle interactions were unobservable. On this same low-protein diet, 15 ppm Se or 1,000 ppm B, alone, killed no ducklings, but in combination, killed 27%. However, restriction of dietary protein to 7% seemed to worsen toxicity of Se more than addition of 1,000 ppm B did.

In studies such as ours and the duckling study by Hoffman et al. [21], in which many measurements are made, a few statistically significant interactions may be found by chance alone. This may have been the case with the interactions we observed. The lack of important interactions between Se and B in our study makes it possible for us to more clearly examine the independent effects of Se and B on mallard reproduction and compare our results to those in other studies in which only B or Se was fed.

Main effects of boron

Boron accumulates rapidly in adult mallard liver and is estimated to take 2.8 d to reach 95% of its asymptotic level; in the presence of arsenic, 12.2 d is required [30]. In our study, adults received treated diets for $\geq$ 90 d, giving them ample time to reach near-asymptotic levels. On a dry-weight basis, B in adult liver in the control, 450-, and 900-ppm B treatments groups was 2, 15, and 27 ppm, respectively. This is comparable to the findings of Smith and Anders [9] who reported 14 and 41 ppm B in the livers of adult mallards receiving 300 and 1,000 ppm dietary B. For mallards collected in the field at a B-contaminated site, liver concentrations in the spring ranged from 1.8 to 7.4 ppm B and in the winter ranged from 1.7 to 23 ppm B [8]. Moore et al. [3] reported liver concentrations of 16 ppm for northern shovelers (*Anas clypeata*) and 26 ppm for ruddy ducks (*Oxyura jamaicensis*). Hence, dietary concentrations of B administered in this study seem close to levels of exposure encountered at some highly contaminated field locations.

Dry-weight concentrations of B in eggs were 0.6, 22, and 38 ppm in the B control, and 450- and 900-ppm treatment groups, respectively. These levels are comparable to levels reported by Smith and Anders [9], but are higher than levels in eggs collected from B-contaminated sites. Moore et al. [3] reported maximum dry-weight concentrations of B in eggs to be 6 ppm for mallards and pintails (*Anas acuta*), and 5 ppm for gadwalls (*Anas strepera*). Despite the fact that eggs are an important means of eliminating B in females, differences in liver concentrations between sexes were not observed in this study. This is probably because female livers were collected well after egg-laying had ceased and because B accumulates rapidly in the liver [30].

Boron in the 900-ppm treatment group caused weight loss in females between treatment onset and pairing (3 weeks) whereas controls gained weight. Weights of females in the 900-ppm group did not differ from the other groups at treatment onset and, despite the differential weight change, weights did not differ at pairing or necropsy. Female liver weights also were not affected by B; however, egg weight and egg fertility were lower in the 900-ppm B group when compared to controls. Effects on egg weight may have occurred because females in the 900-ppm B group had less energy available for egg production, which, on average, began approximately 27 d (median 26; range 11–51 d) after pairing. A similar pattern in female weight change, where the period between treatment onset and pairing was 1 month and females were receiving 1,000 ppm B, was reported by Smith and Anders [9]. Hemoglobin concentrations were lower in the 900-ppm B group for sexes combined and for males when compared to controls, but B had no affect on female hemoglobin. Hoffman et al. [10,21] reported a similar decrease in hemoglobin concentration in ducklings that received 1,000 or 1,600 ppm B in the diet.

Boron did not increase embryo deformities, but at 900 ppm it reduced hatching success by more than 42%. In the 450-ppm B treatment group, hatching success was not affected. Because egg concentrations of B in the 450- and 900-ppm B treatment

Table 7. Main effects of Se on mallard ducklings fed diets supplemented with Se (as seleno-DL-methionine)[a]

	Se added to the diet (ppm)		
	0	3.5	7
Duckling weight (g)			
Hatching	34.0 (0.19) [329]	34.4 (0.22) [323]	33.1 (0.30) [204]
7 d	63.7 (0.88) AB [276]	66.2 (0.81) A [268]	58.9 (1.08) B [161]
14 d	145.1 (2.60) AB [268]	159.4 (2.33) A [266]	130.1 (3.37) B [158]
Duckling growth (g)			
Hatching to 7 d	29.4 (0.85) AB [273]	31.5 (0.75) A [267]	25.6 (1.03) B [161]
7 d to 14 d	81.3 (1.87) A [268]	93.2 (1.67) B [266]	70.8 (2.46) A [158]
Duckling survival (%)			
Hatching to 7 d	77.0 (4.77) [34]	79.7 (4.89) [31]	62.3 (6.42) [30]
7 d to 14 d	96.3 (1.76) A [32]	98.0 (1.74) B [29]	97.8 (1.44) AB [25]
Duckling production	8.4 (0.80) AB [32]	9.2 (0.80) A [29]	6.3 (0.85) B [25]

[a] Values are arithmetic means (SE) and were computed by pooling data over the three levels of B; bracketed numbers are sample sizes. Means in the same row with different letters are significantly different by ANOVA and Tukey's MCP at $\alpha = 0.05$.

groups were considerably higher than residues reported for eggs from B-contaminated sites, it seems unlikely that B would be a significant factor in reducing hatching success of ducks, even at highly contaminated locations in the field.

Duckling weight and growth were reduced by 900 ppm B, but were unaffected by B at 450 ppm. These results agree with Hoffman et al. [10,21] who found that ducklings receiving 1,600 or 1,000 ppm B since 1 d of age had lower growth rates and body weights by 4 to 10 weeks of age than controls, whereas ducklings receiving 400 ppm B did not differ from controls. In contrast, Smith and Anders [9] found that 300 and 1,000 ppm B reduced duckling weight at hatching, and that growth between hatching and 7 d, and 7 d and 14 d, was lower in ducklings receiving 30, 300, and 1,000 ppm dietary B. Despite the fact that ducklings receiving 900 ppm B in our study were 23% smaller at 14 d than controls, duckling survival was not reduced by B. One would expect, however, that, under the stresses present in nature, lighter ducklings might have lower survival rates.

The overall effect of B at 900 ppm in the diet of breeding mallards was a 47% reduction in the number of ducklings produced per female compared to controls; this reduction was due primarily to decreases in egg fertility and hatching success. Other effects, including those for liver biochemistry where there was a decrease in total SH concentration and an increase in TBARS, are indicative of mild oxidative stress. Hoffman et al. [10] observed marginal but not statistically significant effects of this nature in ducklings that were exposed to B. It is possible that the combined in ovo and posthatching exposure contributed to these effects in the present study. No direct field evidence exists that duckling production is reduced at B-contaminated sites, and concentrations of B in eggs, even at the most heavily contaminated locations, tend to be lower than those associated with reproductive problems in the laboratory studies that have been done. However, dietary concentrations of B that apparently can be equaled in nature reduced duckling weights in our study, a result that could, in the wild, cause lower survival. Further, decreases in duckling production observed in our laboratory study might be magnified under the stresses of natural conditions. Therefore, although B does not seem to be as severe a threat to wild birds as Se, high concentrations B cannot be considered innocuous.

Boron is an essential trace element for the growth and development of higher plants, but in animals there is no known requirement for B [31]. There was some evidence in this study, however, that B at 450 ppm acted as a hormetic—an agent that causes stimulation when given in small quantities [32]. For example, at 450 ppm, B significantly increased duckling survival between 7 d and 14 d when compared to controls. Other excitatory effects that were not statistically significant but exhibited a pattern one would expect from hormetics included adult male weight gain, increased egg weight, and increased duckling weight at hatching. In two other studies, excitatory effects consistent with hormesis were observed in mallard ducklings receiving 30 or 300 ppm B [9], and ducklings receiving 100 or 400 ppm B [10]. As in the present study, these effects generally were not statistically significant.

Main effects of selenium

Like B, Se is rapidly accumulated in animal tissues. When female mallards were fed a diet containing 10 ppm Se, as seleno-DL-methionine, concentrations of Se in liver were predicted to reach 95% of their peak in 7.8 d [33]. When female mallards were fed 15 ppm Se, as seleno-DL-methionine, Se levels in their eggs reached a peak in about 2 weeks [34]. Thus, we allowed sufficient time in this study for Se concentrations in adult tissues and eggs to reach harmful levels. Concentrations of Se in adult liver and in eggs were well within the range reported for ducks and other aquatic birds from highly contaminated sites in the wild, and our highest dietary concentration of 7 ppm Se has been far exceeded by concentrations reported in plants and invertebrates at Se-contaminated sites [1,35].

Reproduction was not negatively affected when females were

fed a diet containing 3.5 ppm Se; eggs from these females contained a mean of 3.5 ppm Se, wet-weight. Reproductive success was lower for females fed 7 ppm Se than for controls; eggs from these females contained a mean of 7.1 ppm Se. These results reinforce previous findings that the threshold for reproductive impairment in mallards occurs when selenium concentrations in eggs reach about 5 ppm on a wet-weight basis [16,36]. Prior to our study, the lowest dietary concentration of Se reported to negatively affect reproduction of mallards was 8 ppm, on what was close to a dry-weight basis, and the highest no-effect level was 4 ppm [16]. The harmful effects we observed at a dietary concentration of 7 ppm, also on close to a dry-weight basis, slightly lowers the lowest effect level, and the lack of harmful effects we saw at 3.5 ppm Se in the diet supports the previous no-effect level of 4 ppm. Based on all the data to date, it seems that the dietary threshold of Se for lowering reproductive success in mallards falls somewhere between 4 and 7 ppm, on a dry-weight basis.

Previous studies have reported higher Se concentrations in the liver of male mallards than in females [12,16]. This difference between the sexes is likely due to the ability of females to excrete additional Se in eggs. We found a similar difference between males and females, but it was not as strong, probably because the females in our study were not using eggs as an additional route of excretion during the time when they had stopped laying eggs and were incubating their clutch, whereas in the earlier studies the adults were sacrificed soon after the last egg had been laid.

Selenium affected female weight between treatment onset and pairing but, unlike B, egg weight and egg fertility were not affected. In two similar studies where adult mallards received 10 ppm Se, as selenomethionine, significant weight loss was not observed in females [12,14]. In another study, female mallards fed 16 ppm Se, as selenomethionine, experienced a slight, but statistically significant, weight loss compared to controls between the onset of Se treatment and when the pairs were formed, but weight loss did not occur when the dietary treatment was 8 ppm Se [16]. Dietary concentrations of 7, 8, or 10 ppm Se, as seleno-DL-methionine, negatively affect reproduction but seem to have no serious effects on adult mallards. A small percentage of adult mallards die when Se in the diet, as seleno-DL-methionine, is raised to 15 ppm [36].

Dietary concentrations of less than 7 ppm Se result in increased plasma GSH peroxidase activity in ducklings [13] and in adult mallards [21], whereas 8 ppm for 14 weeks was required to cause an increase in the ratio of hepatic GSSG to GSH, and 16 ppm was required for an increase in TBARS [21]. In the present study, plasma enzyme activities were not measured and 7 ppm Se was not high enough to cause any of the previously observed effects.

In our study, 7 ppm Se had no effect on embryo deformity rates but did reduce hatching success by 33%. Other studies support the idea that hatching success of fertile eggs is the most sensitive measure of selenomethionine poisoning in birds and that teratogenic effects do not appear until dietary concentrations are higher. In another study, when mallards were fed 8 ppm Se, as selenomethionine, 6.8% of the embryos in eggs that did not hatch were deformed, compared with 0.6% for controls; at 16 ppm Se in the diet, 67.9% were deformed [16]. In a study by Stanley et al. [14], 10 ppm Se increased embryo deformities from 1% to 22%. Based on these studies with mallards, all fed seleno-DL-methionine, the dietary threshold of Se that results in teratogenic effects appears to be between about 7 and 8 ppm on what is close to a dry-weight basis (the diets contained about 10% water). As the dietary concentration is raised above 8 ppm, the rate of deformities begins to rise sharply.

Several studies have documented the adverse effects of Se on duckling growth and survival [12,14,21,37]. In the present study, 7 ppm Se affected duckling weight and growth but survival was not significantly reduced. Heinz et al. [16] observed an increase in duckling mortality above that of controls when the parents had been fed 8 ppm Se, as selenomethionine, and both Heinz et al. [12] and Stanley et al. [14] reported increased duckling mortality at 10 ppm Se, as selenomethionine. Thus, like teratogenic effects, the threshold for Se-induced duckling mortality seems to lie between about 7 and 8 ppm dietary Se when the parents have been fed Se.

Although the mean number of ducklings (6.3) produced by females fed 7 ppm Se was not significantly different from the number (8.4) produced by controls (a 25% reduction), it was significantly different from the number (9.2) produced by females fed 3.5 ppm Se. We believe low statistical power was responsible for the lack of significance between the control and 7-ppm Se groups. Hatching success was significantly lower for females fed 7 ppm Se, and fertility and survival of ducklings did not differ from controls. Therefore, the reduction in the final integrated measure of productivity (the number of surviving ducklings produced per female) observed for birds fed 7 ppm Se almost certainly is a biologically meaningful measure. Because plants and invertebrates at Se-contaminated sites in nature often contain well in excess of 7 ppm Se [1,35] and reproductive problems characteristic of Se poisoning have been reported at these sites, our conclusion is probably applicable to contaminated areas in the wild.

Selenium is known to be an essential nutrient for some plants and animals [38]. As was the case with B, the lower dietary concentration of Se (3.5 ppm) may have acted as a hormetic. The diets containing 3.5 ppm Se significantly increased duckling growth between 7 d and 14 d and duckling survival between 7 d and 14 d when compared to controls. Other effects that were not statistically significant but exhibited a pattern one would expect from a hormetic included increased duckling weight at hatching, 7 d, and 14 d; increased weight gain and survival between hatching and 7 d; and increased duckling production. In a separate reproductive study with mallards fed various levels of seleno- DL-methionine, dietary concentrations of 1 and 2 ppm Se may also have had slight, but again not statistically significant, stimulatory effects on reproduction [16].

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