Journal of Shellfish Research, Vol. 22, No. 3, 737–746, 2003.

DIOXIN/FURAN AND POLYCHLORINATED BIPHENYL CONCENTRATIONS IN EASTERN OYSTER (*CRASSOSTREA VIRGINICA*, GMELIN) TISSUES AND THE EFFECTS ON EGG FERTILIZATION AND DEVELOPMENT

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ABSTRACT A 10-mo field study was conducted to evaluate the bioaccumulation of dioxins/furans and polychlorinated biphenyls (PCBs) in transplanted adult eastern oysters (*Crassostrea virginica, Gemlin*) to Newark Bay and the Raritan Complex, New Jersey. Adult oysters (mean size 86.4 ± 14.2 mm) were deployed from September 2000 until June 2001. Oysters transplanted to Newark Bay, Arthur Kill, and Sandy Hook, NJ, accumulated 3.2/2.1, 1.3/1.7, and 0.15/2.3 parts per trillion (pptr) of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)/2,3,7,8- Tetrachlorodibenzo-*p*-furan, respectively. In addition, oysters transplanted to Newark Bay, Arthur Kill, and Sandy Hook, NJ, had bioaccumulation levels of 68.6, 64.5, and 35.3 parts per billion total PCBs, respectively. The number of fertilized eggs (±SD) from strip spawned transplanted oysters from Newark Bay, Arthur Kill, and Sandy Hook, NJ, was 107 (±6.00), 54 (±36.11), and 113 (±13.61), respectively, and the number of unfertilized eggs was 164 (±25.6), 178 (±15.9), and 97 (±39.9), respectively. The number of veliger larvae that resulted from fertilized eggs ($n = 100$) was 3 (±1.7), 4 (±2.31), and 82 (±12.2), respectively, for Newark Bay, Arthur Kill, and Sandy Hook, NJ. Survival data from a laboratory study using an acute static 48-h *in vivo* and *ex vivo* exposure regiment to 2,3,7,8-TCDD showed that exposure to 2 pptr dioxin caused adverse effects on egg fertilization and development. Exposure to dioxin-like compounds at the low parts per trillion ranges can result in altered gonadal development and altered embryonic development.

KEY WORDS: *Crassostrea virginica*, dioxins/furans, egg fertilization, polychlorinated biphenyls, transplant study

INTRODUCTION

Since the early 1970s there has been concern about the impacts of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and related compounds because of their potential hazard to humans and animals. TCDD is a byproduct of anthropogenic processes such as paper and chemical manufacturing, incineration, the manufacturing of pesticides and herbicides, the production of iron and steel, and enzymatic reactions in sewage sludge (Rappe 1992, Alonso et al. 1996, Poland et al. 1982). The most important source of TCDD for humans is food, especially diary products, meat, and fish (Pohjavirta et al. 1994, EPA 2000).

Concern about TCDD stimulated numerous studies to assess its behavior in the environment and its effects on living organisms. Studies conducted in contaminated areas have shown a positive correlation between dioxin levels in animals and their soil contact (Pohjavirta et al. 1994). Studies in aquatic model ecosystems also have shown that TCDD and other organochlorine pollutants bioaccumulate in organisms in concentrations approximately equal to those in the sediment (Isensee et al. 1975, Chen et al. 2002). The effects of TCDD on feeding, growth, and development are most pronounced in young, growing organisms compared with adults (ASTM 1994, Davis & Herber 1969, Calabrese et al. 1973, Capuzzo, 1989, Capuzzo 1996). Because of the lipophilicity of these compounds, they are associated with lipid stores and high lipid-containing tissues (Cooper 1989, EPA 2000). Prior to spawning, bivalves have a high lipid and glycogen content in gonadal tissue. Therefore, the spawning status of the bivalve would affect the amount of dioxin present over the spawning season in a similar fashion to that observed in fish (Capuzzo 1989, Vashchenko et al. 1993, Bayne et al. 1972, Bayne et al. 1978).

Oysters release their gametes into the water column; therefore, planktonic larvae will have limited exposure to TCDD via water due to the low water solubility of dioxin (EPA 2000.). Newly settled bivalve spat and adult bivalve molluscs may be exposed to TCDD through their sediment contact and feeding on resuspended materials, while the developing eggs would receive the majority of exposure from the adult female (Cooper 1989). Bivalve embryos begin to accumulate TCDD at the two-cell embryonic stage (ASTM 1994). This may explain the sensitivity of young, growing organisms to low-level concentrations of dioxins.

There has been limited work on the bioaccumulation of dioxin in the eggs of aquatic organisms. Isensee and Jones (1975) reported no effect of 2,3,7,8-TCDD exposures on snail egg survival, but there was a reduction in the number of viable eggs. There have been several studies on both resident and migratory species of fish and crustaceans in New Jersey. Aquatic organisms in the tidal Passaic River were found to contain elevated levels of TCDD in the edible tissue, ranging from 38 parts per trillion (pptr) in the American eel (*Anguilla rostrata*) to 476 pptr in the blue crab (*Callinectes sapidus*) hepatopancreas (Tucker and Prince 1993). Cooper et al. (1993) found that the TCDD levels in the Arthur Kill organisms accumulated within higher trophic levels. For example, the soft-shell clam (*Mya arenaria*) contained 6.9 pptr TCDD, and the killifish (*Fundulus heteroclitus*) contained 100 pptr TCDD, total body burden.

Changes in the gonadal tissue of bivalves after exposure to a wide variety of pollutants such as oil, heavy metals, and lipophilic organic compounds have been reported (Vashchenko et al. 1993, Capuzzo 1996, Moore et al. 1980, Gardner et al. 1991, Lowe & Pipe 1985, 1986, 1987; Capuzzo & Leavitt 1988; Lowe, 1988; Moore 1988, Widdows & Johnson 1988). For instance, oocyte mass resorption observed in the sea urchin as well as other invertebrates at prespawning is considered to be a reaction to pollution (Vashchenko et al. 1993, Lowe & Pipe 1985, 1986, 1987, Capuzzo 1996). The abnormal development of oocytes, and altered egg shape and size have been correlated with polluted sites (Wintermyer 1998, Lowe & Pipe 1985). The accumulation of pollutants in

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bivalves can cause stress. Capuzzo (1996) reported that pollution-induced sites can lower biochemical reserve, and contribute to poor egg quality and fertilization rates in bivalves. Bayne et al. (1972, 1978) similarity reported that under stressful conditions the mussel (*Mytilus edulis*) produced fewer and smaller eggs, and that larvae that developed from the gametes of stressed adults had a lower growth rate. In a study comparing egg size and larval survival of the hard-shell clam (*Mercenaria mercenaria*) and the bay scallop (*Argopecten irradians*), Kraeuter et al. (1982) reported that for both species, smaller eggs (20–25 μm) had a significantly less than expected survival rate, while larger eggs (35–44 μm) had a significantly greater than expected survival rate. Intermediate size eggs (25–35 μm) showed no difference between the expected and observed survival rates.

The objectives of this study were to transplant adult oysters into sites contaminated with different levels of dioxin and dioxin-like compounds to measure the effects on egg development and fertilization, and to evaluate the potential for restoring oyster populations into the New Jersey bay area.

METHOD AND MATERIALS

Deployment

Adult eastern oysters (n = 180) were purchased from Prince Edward Island, Canada, and were transplanted in September 2000 at three study sites (n = 60 per site): Newark Bay, NJ; Arthur Kill, NJ; and Sandy Hook Bay, NJ (reference site). The oysters were determined to be disease free by histologic examination prior to deployment. Oyster bags (n = 2) were suspended in the water column in Sandy Hook Bay located north of the bridge connecting the Highlands entrance to Sandy Hook State Park. For the Arthur Kill site, oyster bags (n = 2) were suspended in the water column from General Anline Works building dock (longitude 74°12.312W, latitude 40°36.647N) in Elizabeth, NJ. For the Newark Bay site, oyster bags (n = 2) were suspended in the water column from an abandoned dock on Shooter's Island (longitude 74°09.788W, latitude 40°38.482N) in Newark, NJ (Fig. 1).

Each oyster was filed, numbered (1–60), and weighed (in grams), and the dimensions were measured [i.e., length, width, and height (in millimeters)] prior to being placed into marked, mesh polyethylene bags (0.5 × 0.5 inch mesh). Each site was equipped with two bags containing 30 oysters each suspended into the water column 1.8 to 2.4 m (6–8 feet) below the water surface. The depth was selected to avoid low-tide exposure and icing during the winter. Oyster bags were collected in June 2001, terminating the 10-mo field study. Oysters were wet weighed immediately upon collection, and were prepared for tissue chemical analysis, histologic evaluation, and fertilization assays.

Chemical Analysis

Samples of shucked oysters (50 g, n = 7) from each site were sent to Triangle Laboratories (Research Triangle Park, NC) for dioxin, furan, and polychlorinated biphenyl (PCB) tissue analysis. Samples were analyzed by high-resolution chromatography and high-resolution mass spectrometry [method 1613B (9/97) and modified method 680 (11/85), Triangle Laboratories]. Tissues were sent in labeled amber-colored jars and were frozen during shipment.

Histologic Evaluation

Oysters from each site (n = 15) were selected randomly for histologic evaluation. Shucked oyster samples were preserved in a 10% phosphate formalin buffer for several days followed by 70% ethanol. Transverse cuts were made with a scalpel through the mid-visceral region of the oyster to obtain a segment approximately 5 mm thick. Segments were embedded in paraffin after processing (i.e., dehydration and clearance through an alcohol:xylene series). Sections (6 μm) were cut and stained with Harris' hematoxylin and eosin. Histologic grading was based on a scale

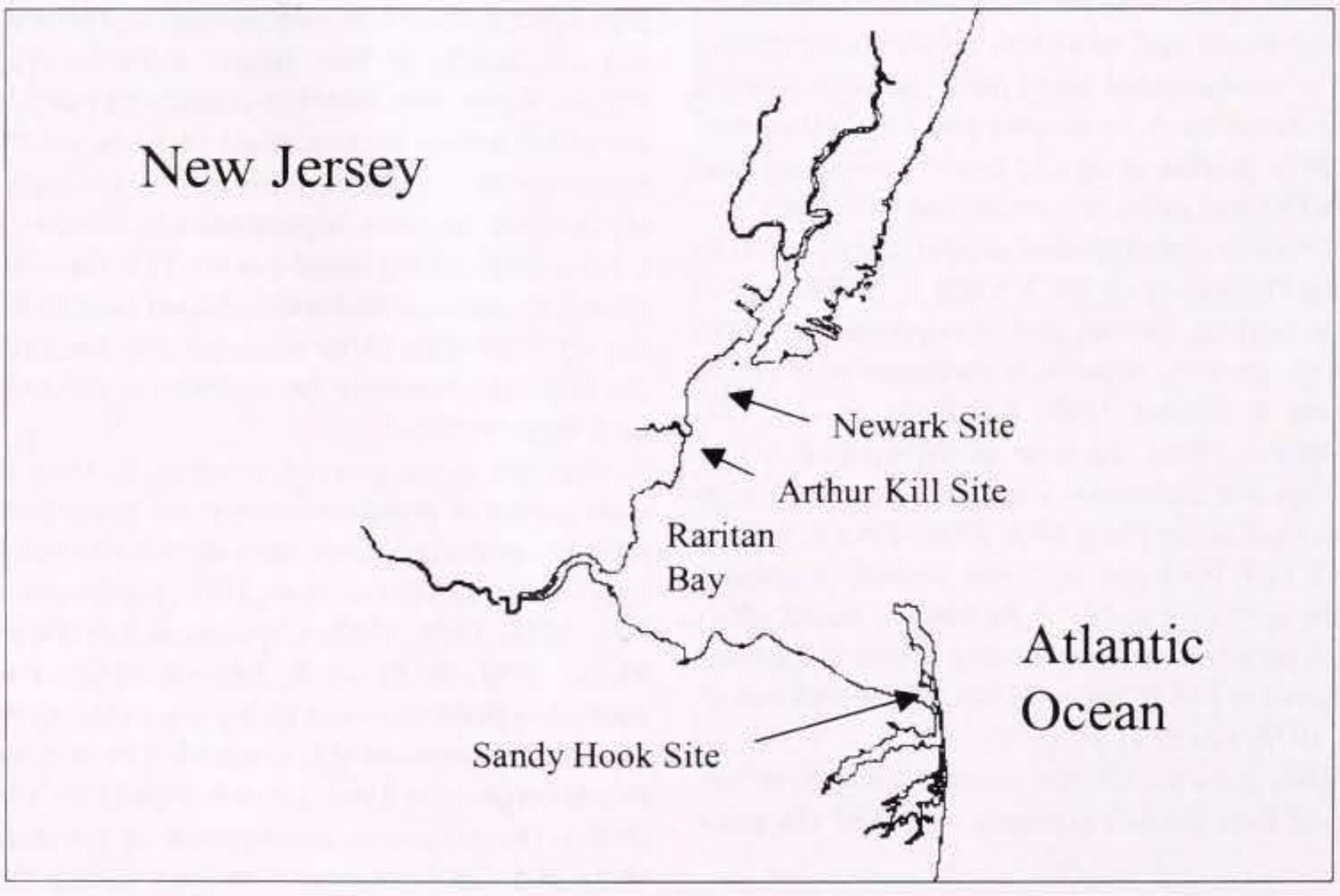

Figure 1. Locations of New Jersey field study sites in the Newark/Raritan Bay Complex.

from mild (↑) to severe (↑↑↑) for lesions, inflammation-like responses, and infectious diseases.

Gonad condition was graded according to Kennedy (1977):
Stage 0 = resting stage
Stage I = early development
Stage II = later development
Stage III = sexual maturity
IIIa = maturity
IIIb = spawning
IIIc = redevelopment
IIId = recently spent

Tissues evaluated were gills, mantle, adductor muscle, kidney/heart, digestive gland, and gonadal condition.

Fertilization Assay: Strip Spawning

Field Study

A total of six ripe oysters from each site were strip spawned (male = 3, female = 3). Eggs and sperm were extracted from the gonadal region using a scalpel and lightly lacerating the gonad (Allen et al. 1989). Collected eggs were sieved on a 25-μm screen and were washed with seawater collected from the respective site. Eggs were viewed under a microscope for maturation before being fertilized with the collected oyster sperm (sperm was diluted to 50 mL). Once sperm (1 mL) was added to the egg suspension (200 eggs per mL), the eggs were set aside for 1 h before being assayed to allow for fertilization. The total number of fertilized and unfertilized eggs, in three 1-mL replicate samples, was ascertained before eggs were dispensed into petri dishes. To each 10-mL glass petri dish (n = 3 per site), 10 mL of the site-collected water and fertilized eggs (n = 100) from each site were dispensed into the appropriate petri dish. Fertilized eggs were allowed to develop for 48 h at room temperature without aeration or food. After 48 h, the larvae were sieved on a 53-μm screen, and the number of larvae that had developed to the straight hinge stage was counted.

Laboratory Study

In vivo. Adult eastern oysters (*Crassostrea virginica*) were purchased from Haskin Shellfish Research Laboratory (Rutgers University, Piscataway, NJ). Oysters (n = 32) were exposed to two treatments of tritium-labeled 2,3,7,8-TCDD via adductor muscle injections. The study was conducted for 28 days to allow the circulation and distribution of dioxin throughout the oyster. This time period was selected based on results obtained from a distribution study using 2,3,7,8-TCDD (Wintermyer 1998). Oysters (n = 48) were weighed (mean weight 50 g), numbered and notched, and their dimensions were measured (i.e., height, length, and width). Oysters were notched on the left side of the valves for access to the adductor muscle. Control oysters (n = 16) were injected (via adductor muscle) with 100 μL (0.1 mL) of 20 parts per thousand filtered seawater. The nominal 2.0 pptr treatment group (n = 16) was injected with 100 μL (0.1 mL) of 0.996 pg/g ^{3}[H]-TCDD. The nominal 20.0 pptr treatment group (n = 16) was injected with 100 μL (0.1 mL) of 27.7 pg/g ^{3}[H]-TCDD. ^{3}[H]-TCDD equivalents were based on radioactivity in 0.1-mL injection volumes in a 50-g oyster (pg/g) (n = 3). All oysters were placed on absorbent paper for 1 h before being put into 76-L aquarium tanks for 24 h. This procedure was performed to allow the discharging and recirculation of dioxin by the oysters. Oysters were not fed 24 h before or 24 h after the injections. Treatment groups (i.e., control, 2.0 pptr, and 20.0 pptr groups) were placed into separate recirculating seawater systems 24 h after the injections. All oysters were reinjected on day 14 of the study according to the procedure described above. This procedure was performed to maintain dioxin concentrations in the oysters over 28 days (Wintermyer 1998). Treatment groups were strip spawned on day 28 according to the procedure described above (field study). Eggs (10 eggs per mL) from each treatment group were fertilized with sperm (1 mL; sperm was diluted to 100 mL) collected from the corresponding treatment group.

Ex vivo. The 48-h static *ex vivo* assay consisted of control eggs (9 eggs per mL) fertilized with control sperm (1 mL; sperm was diluted to 100 mL). Glass exposure beakers (150 mL) (n = 3) consisted of 0.1 mL of nominal 2.0 pptr TCDD, and 0.1 mL of nominal 20.0 pptr TCDD and 0.0 pptr TCDD, respectively. To each treatment beaker, a 10-mL egg suspension and a 2-mL sperm suspension were added, and allowed to set for 2 h for fertilization.

Both 48-h *in vivo* and *ex vivo* assays were conducted in 20-mL glass petri dishes. Fertilized eggs (10 mL) from each treatment group was pipetted into individual petri dishes (n = 20 per group) and were incubated at 22°C for 48 h. After 48 h, each petri dish in both the *in vivo* and *ex vivo* assays was examined for the number of fertilized and unfertilized eggs, as well as for the number of living and dead larvae and their development stages.

Radiolabeled Compounds

^{3}[H] 2,3,7,8-TCDD (34.7 Ci/mM, 98% pure by high-performance liquid chromatography, with carbons 1 and 6 radiolabeled) was purchased from Chemsyn Science Laboratories (Lenexa, KA). Oysters were exposed to 0.996 pg/g (2 pptr) or 27.7 pg/g (20.0 pptr) of ^{3}[H]-TCDD via adductor muscle injection. All ^{3}[H]-TCDD values were based on equivalents.

RESULTS

Deployment and Retrieval

In this study, a total of six bags containing eastern oysters was transplanted to the Newark Bay and the Raritan Bay Complex from September 2000 until June 2001. Oysters transplanted to Newark Bay for 10 mo had the second highest increase in total weight gain (+6 g). Oysters transplanted to Arthur Kill had a decrease in total weight gain (−10.9 g), and oysters transplanted to Sandy Hook Bay had the highest increase in weight gain (+10.3 g). There was not a significant difference in shell growth among the Newark Bay, Arthur Kill, or Sandy Hook transplanted oysters over the 10-mo field study (Table 1).

Tissue Analysis

Oyster tissues were analyzed for dioxin, furan, and PCB analytes. Newark Bay oysters had the highest tissue levels of 2,3,7,8-TCDD (3.2 pptr), total TCDD (16.5 pptr), total TCDF (93.8 pptr), and total PCBs [1.7 parts per billion (ppb)]. Arthur Kill transplanted oysters had the second highest tissue levels of 2,3,7,8-TCDD (1.3 pptr), total TCDD (13.3 pptr), total TCDF (56.7 pptr), and total PCB (64.5 ppb). Sandy Hook oysters had the lowest levels of 2,3,7,8-TCDD (0.15 pptr), total dioxin (2.5 pptr), total furan (47.6 pptr), and total PCBs (35.3 ppb) (Tables 2 and 3).

TABLE 1.

Deployment and retrieval data from *C. virginica* transplanted to Newark Bay, NJ, Arthur Kill, NJ, and Sandy Hook Bay, NJ, field sites.[a]

Sites	Date	No. of Oysters[b]	Temp. (°C)	Salinity (ppt)	Weight (g)	H (mm)	L (mm)	W (mm)
Deployment								
Newark Bay	9/12/00	60	18.5	20	57.5 ± 15.3	81.4 ± 13.6	45.8 ± 5.0	19.8 ± 2.8
Arthur Kill	9/12/00	60	19.5	20	66.8 ± 19.9	88.7 ± 14.0	46.9 ± 7.4	20.5 ± 2.9
Sandy Hook	9/12/00	60	18	23	68.1 ± 25	89.7 ± 15.0	46.6 ± 5.0	20.8 ± 4.0
Retrieval								
Newark Bay	6/1/01	47/13[c] (2 bags recovered)	14.3	16	63.5 ± 18.0	81.7 ± 13.2	45.4 ± 4.8	19.5 ± 2.6
Arthur Kill	6/1/01	45/15[c] (2 bags recovered)	17.3	16	55.9 ± 13	88.2 ± 13.8	46.2 ± 7.7	20.8 ± 3.0
Sandy Hook	6/1/01	25/5[c] (1 bag recovered)	14.6	20	78.4 ± 26	89.4 ± 14.7	46.1 ± 5.5	20.5 ± 4.2

Presented as mean ± Sd, unless otherwise indicated.
[a] H, height; L, length; W, width; ppt, parts per thousand.
[b] Number of oysters per site; two bags per site.
[c] Number of live oysters/number of dead oysters.

Histologic Evaluation

Oysters transplanted to Newark Bay showed moderate signs of epithelial-severe hyperplasia, while oysters transplanted to Arthur Kill showed signs of severe epithelial-severe hyperplasia with some cells (>4) showing mitotic division, and connective tissue displaying areas of focal fibrosis. Oysters transplanted to Sandy Hook showed signs of slight epithelial-severe hyperplasia. Only the transplanted oysters to Arthur Kill were observed to have a *haplosporidium nelsoni* (MSX) infection in the digestive gland and mantle tissues (Table 4). All transplanted oysters showed slight-to-moderate gill hyperplasia ("clubbing"). Oysters transplanted to Newark Bay and Arthur Kill showed an alteration in gill cilia shape, size, and orientation. The cilia had a thickened appearance and an alteration in cilia length resulting in a distinct whip-like appearance (approximately six times the length of normal gill cilia).

Gross Body Evaluation

Oysters transplanted to Newark Bay had semi-developed gonadal tissue. The gonadal area had a slightly cream-colored appearance, and the oysters appeared to be of moderate health and were plump. The shell interior had a white, iridescent color and had no obvious scarring or discoloration. Oysters transplanted to

TABLE 2.

Oyster tissue analysis for dioxins/furans at Newark Bay, NJ, Arthur Kill, NJ, and Sandy Hook Bay, NJ, during a 10-mo water suspension field study.

Analytes	Newark Bay Concentration (pptr)[a]	Arthur Kill Concentration (pptr)[b]	Sandy Hook Concentration (pptr)[c]
2,3,7,8-TCDD	3.2	1.3	0.15[d]
1,2,3,7,8-PeCDD	<DL (0.3)[e]	<DL (0.3)	<DL (0.2)
1,2,3,4,7,8-HxCDD	<DL (0.3)	<DL (0.3)	<DL (0.1)
1,2,3,6,7,8-HxCDD	<DL (0.3)	<DL (0.3)	<DL (0.1)
1,2,3,7,8,9-HxCDD	<DL (0.3)	<DL (0.3)	<DL (0.1)
1,2,3,4,6,7,8-HpCDD	0.59	1.0	0.43
1,2,3,4,6,7,8,9-OCDD	1.8	4.8	2.3
2,3,7,8-TCDF	6.5	4.3	<DL (2.5)
1,2,3,7,8-PeCDF	<DL (0.2)	<DL (0.2)	<DL (0.1)
2,3,4,7,8-PeCDF	0.93	0.73	<DL (0.1)
1,2,3,4,7,8-HxCDF	<DL (0.2)	<DL (0.2)	<DL (0.08)
1,2,3,6,7,8-HxCDF	<DL (0.2)	<DL (0.2)	<DL (0.07)
2,3,4,6,7,8-HxCDF	<DL (0.2)	<DL (0.2)	<DL (0.09)
1,2,3,7,8,9-HxCDF	<DL (0.2)	<DL (0.2)	<DL (0.1)
1,2,3,4,6,7,8-HpCDF	<DL (0.2)	<DL (0.2)	<DL (0.1)
1,2,3,4,7,8,9-HpCDF	<DL (0.4)	<DL (0.3)	<DL (0.2)
1,2,3,4,6,7,8,9-OCDF	<DL (0.5)	<DL (0.4)	**0.47
Total TEFs[f]	4.3	2.2	0.6

DL, detection limit; TEF, total equivalent factor.
[a] Sample size, 25.15 g; 0.3% lipids.
[b] Sample size, 25.17 g; 0.2% lipids.
[c] Sample size; 25.1 g; 0.6% lipids (shown in parentheses).
[d] Concentration is below the calibration curve. Value is an estimate only.
[e] Values are less than the detection limit.
[f] Environmental Protection Agency (1989a).

TABLE 3.

Oyster tissue analysis for total PCBs at Newark Bay, NJ, Arthur Kill, NJ, and Sandy Hook Bay, NJ, during a 10-mo water suspension field study.

Analytes	Newark Bay Concentration (ppb)[a]	Arthur Kill Concentration (ppb)[b]	Sandy Hook Concentration (ppb)[c]
Total MonoCB	<DL (0.09)[d]	<DL (0.1)	<DL (0.08)
Total DiCB	<DL (0.1)	<DL (0.1)	0.54
Total TriCB	5.6	4.5	2.1
Total TetraCB	24.2	23.5	12.2
Total PentaCB	22.8	21.6	14.9
Total HexaCB	14.2	13.0	4.9
Total HeptaCB	1.7	1.9	0.68
Total OctaCB	<DL (0.8)	<DL (0.7)	<DL (0.6)
Total NonaCB	<DL (1.2)	<DL (1.0)	<DL (0.9)
DecaCB (#209)	<DL (2.2)	<DL (1.9)	<DL (1.6)
Total PCB[e]	68.6	64.5	35.3
Total PCB + EMPC[f]	71.6	69.4	35.3

DL, detection limit; EMPC, estimated maximum possible concentration.

[a] Sample size, 30.0 g; 0.3% lipids.

[b] Sample size, 20.0 g; 0.2% lipids.

[c] Sample size, 24.0 g; 0.6% lipids.

[d] Values are below the DL.

[e] Newark Bay and Arthur Kill total PCB was approximately two times that of Sandy Hook.

[f] If matched sets of peaks in the time window do not have the appropriate ion mass ratios for a true PCB, the EMPC is calculated using the sum of the observed peaks (Triangle Laboratories, Inc., modified method 680 (11/85).

Arthur Kill had underdeveloped gonads, and were easily shucked and watery. The gonadal area had a vein-like appearance and a gray coloration. The shell interior had a white, iridescent color and had no obvious scarring or discoloration. Oysters transplanted to Sandy Hook were plump, had a whitish-cream coloration and well-developed gonads, and were in a prespawning state (Table 4).

Field Study Strip Spawning Assay

Results from the strip-spawning assay using oysters transplanted to Newark Bay, Arthur Kill, and Sandy Hook, NJ, showed that the majority of eggs collected from female oysters at the Newark Bay and Arthur Kill sites were not viable. There was not a difference in fertilized egg size (64 μm) among the transplanted oysters, however, oysters transplanted to Arthur Kill had a smaller unfertilized egg size (48 μm) compared with oysters transplanted to Newark Bay or Sandy Hook. This study shows that 60.5% and 76.7%, respectively, of eggs collected from oysters transplanted to Newark Bay and Arthur Kill were not fertilized, and of the eggs that were fertilized (39.5% and 23.3%) only 0.03% and 0.04%, respectively, of the eggs developed to the straight-hinge stage.

TABLE 4.

***C. virginica* histological evaluation for 10-mo field study (September, 2000–June, 2001).**

Sites	Gill	Mantle	Adductor Muscle	Kidney/Heart	Digestive Gland (Midgut)	Gonad[a] Condition
Newark Bay (n = 15)	↑ Hyperplasia (80%) ↑ Inflam. (100%)† ↑↑–↑↑↑ Dysplasia (100%)	↑↑ Epithelial severe hyperplasia (70%) ↑ Inflam. (100%) ↑↑–↑↑↑ Dysplasia (100%)	↑ Brown cell (10%)† ↑ Inflam. (10%) ↑↑–↑↑↑ Dysplasia (100%)	↑↑–↑↑↑ Brown cell (70%) ↑ Inflam. (70%) ↑↑–↑↑↑ Dysplasia (100%)	↑↑ Epithelial severe hyperplasia (80%) ↑ Inflam. (100%) ↑↑–↑↑↑ Dysplasia (100%)	Stage 2 and 3 (80%) Stage 1 (20%)
Arthur Kill (n = 15)	↑↑–↑↑↑ Inflam. (100%) ↑ Hyperplasia (100%) ↑ Brown cell (100%)	↑↑↑ Epithelial severe hyperplasia (100%) ↑↑–↑↑↑ Inflam. (100%) ↑↑–↑↑↑ Dysplasia (100%) ↑ Brown cell (100%) ↑↑–↑↑↑ MSX (70%)	↑ Inflam. (30%) ↑↑–↑↑↑ Dysplasia (100%)	↑ Inflam. (60%) ↑↑–↑↑↑ dysplasia (100%) ↑↑–↑↑↑ Brown cell (60%)	↑↑↑ Epithelial severe hyperplasia (100%) ↑↑–↑↑↑ Inflam. (100%) ↑↑–↑↑↑ Dysplasia (100%) ↑ Brown cell (100%) ↑↑–↑↑↑ MSX (70%)	Stage 1 and 2 (40%) Stage 3 (60%)
Sandy Hook (n = 15)	↑ Dysplasia and filament fusion (100%) ↑ Hyperplasia (100%)	↑ Epithelial severe hyperplasia (40%) ↑ Brown cell (100%) ↑ Dysplasia (100%)	↑ Dysplasia (100%)	↑ Dysplasia (100%)	↑ Epithelial severe hyperplasia (40%) ↑ Brown cell (100%) ↑ Dysplasia (100%)	Stage 3a and 3b (80%) Stage 0 (0.01%)

Numbers in parentheses are % of oysters. Lesion grading definitions: (–), absent; (↑), slight; (↑↑), moderate; (↑↑↑), severe; inflam., inflammatory like response; brown cell, brown cell accumulation.

[a] Gonad grading: stage 0, resting stage; stage 1, early development; stage 2, later development; stage 3, sexual maturity; stage 3a, maturity; stage 3b, spawning; stage 3c, redevelopment; stage d, recently spent (Kennedy 1977).

Most fertilized eggs did not develop beyond the zygote stage. The strip-spawning assay from oysters transplanted to Sandy Hook showed that 53.7% of the eggs were fertilized, and of those eggs 84% developed to the straight-hinge stage (Table 5, Fig. 2).

Acute Static 48-h In Vivo and Ex Vivo Assays

In this study using *C. virginica*, there was an observable decrease in the number of fertilized eggs in the 2 and 20 pptr TCDD groups. In Table 6, controls for the *in vivo* and *ex vivo* assays had high rates of egg fertilization and larvae development to the straight hinge stage (80.3%). The 2.0 pptr *in vivo* assay had 48% egg fertilization, but 100% mortality at the zygote development stage. In the 20.0 pptr *in vivo* assay, to which viable control eggs were fertilized with 20.0 pptr sperm, there was very little fertilization (0.9%), which resulted in a high egg mortality rate (99.%). The 2.0 pptr *ex vivo* and 20.0 pptr *ex vivo* assays also had low fertilization rates (3% and 2%, respectively), which resulted in high egg mortality rates (97% and 98%, respectively). In both the 48-h acute *in vivo* and *ex vivo* studies, there were large decreases in the number of veliger larvae compared with the controls. Within treatment groups (nominal 2.0 pptr TCDD and 20.0 pptr TCDD), there were 52 to 99% unfertilized eggs. Eggs that were fertilized had a 98 to 100% mortality rate and did not develop beyond the zygote stage. In contrast, the control eggs had an 80% survival rate to the straight-hinge stage (Table 6, Fig. 3).

DISCUSSION

PCBs were first commercially produced in 1929 (NJDEP 1993). PCBs were commonly used in transformer oils and electrical products. In 1977, the U.S. Environmental Protection Agency banned the production of PCBs. However, many PCB-laden transformers, capacitors, and other electrical equipment remain in service (NJDEP 1993). PCBs have been and continue to be dispersed throughout the environment through spills, effluent discharges, and incineration.

In the 1970s and 1980s, the levels of TCDD in Newark, NJ, and Arthur Kill, NJ, shellfish approached the no-consumption advisory level suggested by the U.S. Food and Drug Administration of 25 pptr (Belton et al. 1985). The levels of other isomers such as PCBs, polychlorinate dibenzo-p-dioxin (PCDDs), and polychlorinated dibenzo-p-furan (PCDFs) found in aquatic organisms (striped bass and blue crab) in Newark Bay and Arthur Kill resulted in the closing of the waterways to fishing beginning in 1984 (NJDEP 1990). Extensive soil contamination with dioxin, specifically 2,3,7,8-TCDD, discovered at a site adjacent to the Passaic River in Newark, NJ, prompted an intensive study of dioxin levels in sediments and biota in 1983 and 1984 (NJDEP 1990).

In this study, a total of six bags were deployed in the field in September 2000. The field sites were selected based on historical data about the bay system and accessibility via boat. Sandy Hook, NJ, was selected as the reference site, and Arthur Kill and Newark Bay, NJ, were selected as the exposure sites due to the high level of industrialization along the waterways. The approximate distance between the Newark site and the Arthur Kill site is 5 miles. The distance between the Sandy Hook site and the Newark-Arthur Kill site is approximately 32 miles. Oysters were put in the field at the completion of the 2000 spawning season and were collected prior to the 2001 spawning season to ensure bioaccumulation levels prior to and during gametogenesis. Oyster tissues were analyzed for dioxin, furan, and PCB analytes. Newark Bay oysters had the highest tissue levels of 2,3,7,8-TCDD (3.2 pptr), total TCDD (16.5 pptr), total TCDF (93.8 pptr), and total PCBs (68.6 ppb). Oysters transplanted to Arthur Kill had slightly lower tissue levels of 2,3,7,8-TCDD (1.3 pptr), total TCDD levels (13.3 pptr), total TCDF levels (56.7 pptr), and a slightly lower total PCB level (64.5 ppb) than those of the Newark Bay oysters (Tables 3 and 4). Sandy Hook oysters had the lowest levels of 2,3,7,8-TCDD (0.15 pptr), total dioxin (2.5 pptr), total furan (47.6 pptr), and total PCBs (35.3 ppb) (Tables 2 and 3).

Oysters transplanted to Newark Bay showed moderate signs of epithelial-severe hyperplasia, and oysters transplanted to Arthur Kill showed signs of severe epithelial-severe hyperplasia, with some cells (>4) showing mitotic division and connective tissue displaying areas of focal fibrosis. Oysters transplanted to Sandy Hook showed signs of slight epithelial-severe hyperplasia. The epithelial-severe hyperplasia could be interpreted as preneoplastic in nature, however, further research is needed to verify that these lesions can progress to a neoplastic condition. Only the Arthur Kill oysters were observed to have a moderate-to-severe MSX infection in the digestive gland and mantle tissues (Table 4). Sandy

TABLE 5.

Summary of the strip-spawning assay from Newark Bay, NJ, Arthur Kill, NJ, and Sandy Hook Bay, NJ, 10-mo field study (September, 2000–June, 2001).

	Newark Bay, NJ	Arthur Kill, NJ	Sandy Hook, NJ
Weight of oysters at time of deployment (g) (9/00)	57.5 ± 15.3 (n = 60)	66.8 ± 19.9 (n = 60)	68.1 ± 24.9 (n = 60)
Weight of oysters at termination of study (g) (6/01)	63.5 ± 18.4 (n = 45)	55.9 ± 13.1 (n = 47)	78.4 ± 25.6 (n = 25)
% lipid (6/01)	0.3	0.2	0.6
Egg size fertilized vs. unfertilized (μ at 40×) (n = 5)	64 μm fertilized 56 μm unfertilized	64 μm fertilized 48 μm unfertilized	64 μm fertilized 56 μm unfertilized
Total number of fertilized eggs[a]	107 ± 6.00	54 ± 30.11	113 ± 13.61
Total number of unfertilized eggs[a]	164 ± 25.6	178 ± 15.9	97 ± 39.9
Number of veligar larvae after 48 h[b]	3 ± 1.7	4 ± 2.31	82 ± 12.2

Presented as mean ± SD, unless otherwise indicated.

[a] Numbers represent the average of 1-mL replicate samples (n = 3).

[b] Number of veligar larvae resulting from approximately 100 fertilized eggs (n = 3 replicates).

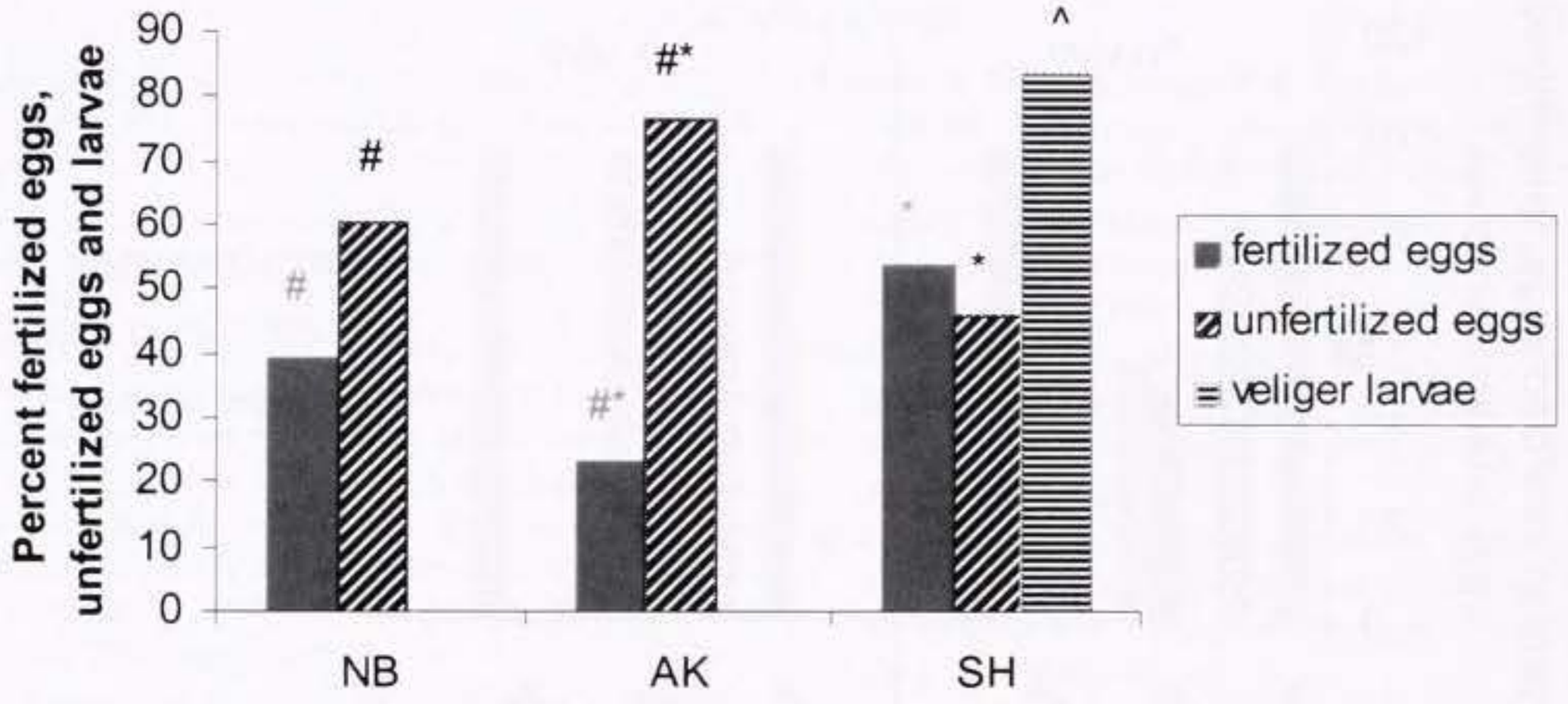

Figure 2. The percentage of fertilized eggs, unfertilized eggs, and veliger larvae resulting from the strip-spawning assay using transplanted oysters from Newark Bay, NJ, Arthur Kill, NJ, and Sandy Hook Bay, NJ (10-mo field study, September 2000–June 2001). Number of fertilized and unfertilized eggs are averages of 1-mL replicates (three per site). Numbers of veliger larvae are those resulting from 100 fertilized eggs after 48 h (three sites). #* (light), fertilized egg groups that are significantly different ($P < 0.05$; ANOVA); #* (dark), unfertilized egg groups that are significantly different ($P < 0.05$; ANOVA); ∧, veliger larvae groups that are significantly different (P < 0.05; ANOVA); NB, Newark Bay; AK, Arthur Kill; SH, Sandy Hook.

Hook oysters had fully developed gonads and were in a prespawning state. Newark Bay and Arthur Kill oysters were slightly moderately underdeveloped due to a lack of gonadal development compared with Sandy Hook oysters at the time of collection (Table 4). All transplanted oysters showed slight-to-moderate gill hyperplasia (clubbing). Oysters transplanted to Newark Bay and Arthur Kill showed an alteration in gill cilia shape, size, and orientation. The cilia had a thickened appearance and an alteration in cilia length resulting in a distinct whip-like appearance (approximately six times the length of normal gill cilia). This alteration in gill cilia could be a result of chronic exposure over time. The lesions observed in the transplanted oysters would be consistent with those resulting from chronic exposure to chemicals. The lesions are not pathoneumonic but are consistent with a wide variety of chemical and physical irritants.

Oysters transplanted to the Newark Bay site had the second highest increase in weight gain (+ 6 g), percentage of lipids (0.3%), egg fertilization (39.5%), and larval development (0.03%). Oysters transplanted to the Arthur Kill site had a decrease in weight over the 10-mo study (−10.9 g), the lowest percentage of lipid content (0.2%), the lowest percentage of egg fertilization (23.3%), and a decrease in larval development (0.04%). Oysters transplanted to the Sandy Hook site had the greatest increase in weight gain (+10.3 g), the highest percentage of lipids (0.6%), the highest percentage of egg fertilization (53.7%), and the highest percentage of larval development (84%) (Table 5, Fig. 2). Weight gain and the percentage of lipid content of the oyster contribute greatly to egg development and production, egg fertilization success, and larval development (Capuzzo 1996, Capuzzo & Leavitt 1988, Lowe 1988, Moore 1988). Oysters transplanted to Sandy Hook had the highest level of fitness followed by oysters transplanted to Newark Bay and Arthur Kill, based on lesion grading, inflammatory-like responses, infectious disease states, weight gain/loss, and the degree of gonadal development.

Results from the strip-spawning assay using oysters transplanted to Newark Bay, Arthur Kill, and Sandy Hook, NJ, showed

TABLE 6.

Summary of an acute static 48-h *in vivo* and *ex vivo* strip-spawning bioassay for *C. virginica* exposed to 2 and 20 pptr 2,3,7,8-TCDD.

	Initial (Egg)		After 48 h (Veliger Larvae)			
	Number of Fertilized Eggs	Number of Unfertilized Eggs	Number Dead after 48 h	Stage of Development	Number Alive after 48 h	Stage of Development[a]
Control *in vivo*	196 ± 143	2 ± 0.63	39 ± 1.45	Trochophore, egg, and D-stage	159 ± 1.66	D-stage
2 pptr *in vivo*	152 ± 3.12	166 ± 3.80	318 ± 3.45	Egg	0	NA
20 pptr *in vivo*[b]	6 ± 0.801	660 ± 16.2	663 ± 17.94	Egg	3 ± 0.52	D-stage
Control *ex vivo*	194 ± 2.17	4 ± 0.84	48 ± 2.10	Trochophore, egg, and D-stage	150 ± 2.36	D-stage
2 pptr *ex vivo*	13 ± 0.489	420 ± 10.8	423 ± 12.0	Egg and D-stage	10 ± 0.513	Trochophore and D-stage
20 pptr *ex vivo*	16 ± 0.410	803 ± 27.3	810 ± 27.6	Egg and D-stage	9 ± 0.510	D-stage

Presented as mean ± SD, unless otherwise indicated. NA, not applicable.

In vivo represents eggs exposed to TCDD during gametogenesis and *ex vivo* represent eggs exposed to TCDD in petri dishes during fertilization (n = 20 for each group). Table taken from Wintermyer (1998).

[a] Stage of fertilized egg (Loosanoff and Davis 1963).

[b] Viable control eggs were fertilized with 20 pptr sperm.

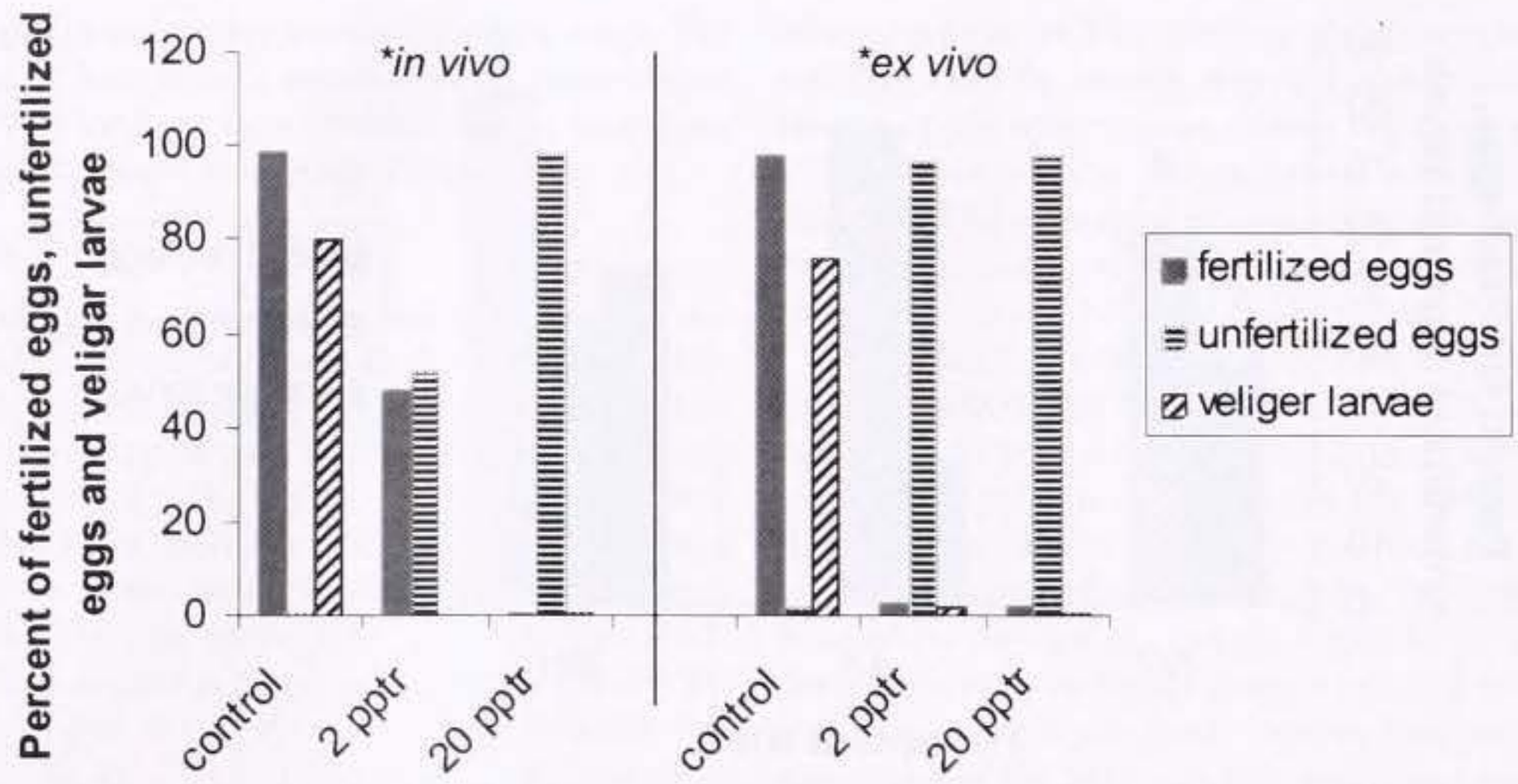

Figure 3. The percentage of fertilized eggs, unfertilized eggs, and veliger larvae resulting from an acute static 48-h *in vivo* and *ex vivo* strip-spawning assay using *C. virginica* exposed to 2 and 20 pptr 2,3,7,8-TCDD. **in vivo*, eggs exposed to TCDD during gametogenesis; **ex vivo*, eggs exposed to TCDD in petri dishes during fertilization (n = 20 for each group). Table from Wintermyer (1998).

that the majority of eggs collected from female oysters at the Newark Bay and Arthur Kill sites were not viable (Fig. 2). This study shows that 60.5% and 76.7%, respectively, of eggs collected from Newark Bay and Arthur Kill transplanted oysters were not fertilized, and of the eggs that were fertilized (39.5% and 23.3%, respectively) only 0.03% and 0.04%, respectively, of the eggs developed to the straight-hinge stage. Most fertilized eggs did not develop beyond the zygote stage. The strip-spawning assay for oysters transplanted to Sandy Hook showed that 53.7% of the eggs were fertilized, and of those eggs 84% developed to the straight-hinge stage (Fig. 2). This study was performed to evaluate the potential for restoring oysters in to the bay area. Based on the field study and strip-spawning assay, transplanting oysters into the Newark bay and Arthur Kill sites at this time would not result in successful recruitment of the bay area. However, the Sandy Hook site would be an ideal area for oyster restoration.

In the laboratory studies, the 2.0 pptr and 20.0 pptr treatment concentrations of 2,3,7,8-TCDD used in the 48-h acute *in vivo* and *ex vivo* studies were based on tissue concentrations that were reported from the soft-shelled clam (*Mya arenaria*) living in Newark, NJ, (11–20 pptr TCDD) and Tuckerton, NJ, (0.1–0.6 pptr) (Brown et al. 1993) and on sediment samples from Newark Bay (20 pptr), Arthur Kill (10 pptr), and Tuckerton (0.5 to 1.0 pptr) (Brown et al. 1993). In this study using *C. virginica*, there was an observable decrease in the number of fertilized eggs within the 2 and 20 pptr TCDD treatment groups. In Fig. 3, controls for the *in vivo* and *ex vivo* assays had high rates of egg fertilization and larvae development to the straight-hinge stage (80.3%). The 2.0 pptr *in vivo* assay had a 47.8% egg fertilization rate, but a 100% mortality rate at the zygote development stage. In the 20.0 pptr *in vivo* assay to which viable control eggs were fertilized with 20.0 pptr sperm, there was very little fertilization (0.901%), which resulted in a high egg mortality rate (99.6%). The 20-pptr treatment group did not have any female oysters remaining due to toxicant-induced stress and mortality by the end of the 28-day period. The 2.0 pptr *ex vivo* and 20.0 pptr *ex vivo* assays also had low fertilization, which resulted in high egg mortality (Fig. 3). In both the 48-h acute *in vivo* and *ex vivo* studies, there was a large decrease in the number of fertilized eggs respective to treatment group compared with the controls. Within treatment groups (nominal 2.0 pptr TCDD and 20.0 pptr TCDD), there were 52 to 99% unfertilized eggs. Eggs that were fertilized had a 98 to 100% mortality rate and did not develop beyond the zygote stage. In contrast, the control eggs had an 80% survival rate to the straight-hinge stage (Table 6, Fig. 3). This laboratory study is important in understanding the effects of 2,3,7,8-TCDD independent of other lipophilic compounds on oyster gametogenesis and egg fertilization. We cannot state that the field study results were solely due to 2,3,7,8-TCDD, but laboratory studies demonstrate that TCDD can result in a significant decrease in gametogenesis and egg viability.

CONCLUSION

In conclusion, this study was designed to investigate two points of interest: (1) the dioxin/furan and PCB concentrations in the eastern oyster during gametogenesis and the effects on egg fertilization and development; and (2) to evaluate the potential for restoring oysters back into the New Jersey bay area. Oysters transplanted to Sandy Hook, NJ, had the greatest weight gain, percentage of lipid content, percentage of egg fertilization, and percentage of larval development to the straight-hinge stage, followed by oysters transplanted to Newark Bay and Arthur Kill, NJ. The laboratory *in vivo* and *ex vivo* strip-spawning assays showed that exposure to compounds such as dioxin can accumulate in animal tissues and can interfere with normal metabolic processes that affect gonadal development and egg fertilization. While we cannot separate the effects of different gonadal development on strip-spawning fertilization and larval development, the laboratory studies support the effect of 2,3,7,8-TCDD on gonadal development at levels observed in the field.

This study demonstrated that dioxins, furans, and PCBs are still bioavailable in the Newark Bay estuary. The levels approach concentrations that in the laboratory result in impacts on gonadal development and egg viability. This study clearly demonstrates that 2,3,7,8-TCDD effects gonadal development and egg viability in the eastern oyster in a similar fashion to fish species.

ACKNOWLEDGMENTS

The authors would like to thank Michael Stringer and the NY/NJ Baykeeper Program for helping with the deployment and retrieval of the oyster bags for the field study reported in this article.

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