Supporting Information

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SI Materials and Methods

Striped Bass Capture. Adult male and female striped bass were collected from the Sacramento River between Knights Landing and Colusa, CA, weekly during the spawning seasons of 1999 and 2001 (April to June) using standard electrofishing methods. Female striped bass were catheterized upon collection to determine the stage of the eggs. Only females with eggs approaching maturation (stage 15) were kept. Collected fish were then injected with 100 international units/kg human chorionic gonadotropin (hCG; Sigma–Aldrich) to induce egg maturation and sperm production. Fish were held throughout the day's collection in a fish transport vehicle equipped for oxygenation. Dissolved oxygen levels were maintained between 5 and 7 mg/L and monitored continually by using a YSI dissolved oxygen meter (Yellow Springs Instruments). Fish were transported at the end of each day's electrofishing from the river to the Professional Aquaculture Services facility in Chico, CA. Adult striped bass were placed in holding facilities at 18 °C until spawning, which occurred in the following 24–48 h.

Hatchery-reared F_2 generation striped bass were used as controls in this study. This domestic striped bass brood stock was created using Sacramento River-captured striped bass. The F_1 generation was produced from striped bass collected using standard electrofishing techniques in 1990. These fish were spawned and reared to sexual maturity (4–5 years) in freshwater in a semiintensive manner at the Professional Aquaculture Services facility. After maturation, these F_1 broodstock fish were spawned and their progeny were reared to maturation to create the F_2 broodstock. The F_2 broodstock (5 years of age in their second season of spawning) were spawned to obtain the eggs and larvae used in this study.

Striped Bass Spawning. Hatchery-reared and river-collected striped bass were spawned by using identical methods. After injection with hCG (100 international units/kg), females were catheterized and an aliquot of eggs was removed several times over a 12- to 36-h period (frequency of egg removal depended on the stage of the eggs and the speed of their development) to determine the proper stage of development, such that spawning could be performed successfully (1). When the eggs were ripe and ovulation was occurring (indicated by both egg stage and eggs flowing freely from the female), females were euthanized by using an overdose of tricaine methanesulfonate (MS-222, 99.5% pure; Argent Chemical Laboratories). The ovaries then were removed, and the eggs were placed in a stainless steel bowl for fertilization. Male striped bass were netted and held ventral side up adjacent to and above the bowl containing the eggs to avoid water from being introduced with the sperm, and the ventral abdomen was squeezed gently to inject sperm into the bowl. Sperm from 3 to 5 male striped bass was used to fertilize each female's eggs. After the addition of sperm, water was added to the egg/sperm mixture for fertilization to occur. The fertilized eggs were placed into 8-L McDonald hatching jars (Aquatic Eco-System) with constant flow-through freshwater at 18–19°C. The eggs hatched in ≈48 h. If eggs are not fertilized properly or the eggs do not develop normally, a white-out (dead eggs turn white and are expelled from the hatching jars) occurs between 10 and 18 h after fertilization. After the white-out occurs, virtually all of the eggs remain viable until hatching. In this study only larvae from successful spawns and hatches were used in the analysis. Egg volumes were marked on the hatching jars 2 h after fertilization, and the criterion for a successful spawn was that 75% or more eggs had to be viable at 24 h after hatching. In addition, at 24 h after fertilization, 10-mL aliquots of eggs were randomly removed and counted 3 times each from each hatching jar to determine egg size/volume of both wild and domesticated females. Egg volumes following are the mean of the 3 counts ± SE, and the aserisk indicates statistical difference of $P \leq 0.05$ between the groups after performing the nonparametric Mann–Whitney U test. Volume measurements for the eggs used in this study were as follows: 1999 hatchery control egg volume = 193.3 ± 1.5 eggs per mL; 1999 eggs from river females volume = 189.0 ± 1.1* eggs per mL; and the egg volume from river females in 2001 = 190.1 ± 1.2* eggs per mL. Egg volume data indicated that the egg volume from the river fish was slightly larger than that of the hatchery controls, which typically means better-quality eggs.

The larvae were expelled from the top of the hatching jars into 2,700-L round flow-through tanks (Red Ewald). Flow rates of ≈10 L/min were used to keep the larvae suspended within the tank throughout the developmental period. Hatchery lighting was adjusted to light levels similar to what larvae encounter in the estuary by using very dim ambient and incandescent lighting.

Sample Collection at Spawning. Both hatchery-reared and river-collected female striped bass were assigned an identification code before spawning. Weight, length measurements, and general gross and behavioral observations were recorded before and at spawning. Duplicate egg samples between 5 and 10 g per sample were collected from each female's ovaries immediately before spawning and analyzed for trace elements and organic chemical analyses. The samples were coded and immediately frozen. Egg samples were stored at −80°C until they were analyzed. Liver and scale samples were also collected from each female at spawning. Liver samples were cut into small pieces no more than 1 cm in thickness by using a razor blade and were preserved in 10% neutral-buffered formalin (Fisher Scientific). The head from each female was removed, coded, and frozen such that otoliths could be removed for subsequent aging and microgeochemical analysis.

Larval Developmental Sampling. Larvae from hatchery controls and river-collected striped bass were reared under the same conditions and sampled for developmental studies using identical methods. Larval samples (>200 larvae per sample) were collected randomly from each rearing tank containing larvae from a single female striped bass at the following intervals for the maternal transfer study: 1, 2, 3, 4, and 5 days posthatching. Larvae representing 3 critical periods of development—early organogenesis (1 day posthatching), midorganogenesis (3 days posthatching), and completed organogenesis just before exogenous feeding (5 days posthatching)—were sampled and used in the morphometric and histopathological analyses. For these developmental analyses, larvae from 1 hatchery control female were used as controls and compared with larvae from 3–5 river-collected females per year ranging in size from 2.25 kg to 20.5 kg. A minimum of 12 larvae per female per developmental period (days 1, 3, and 5 posthatching) were subjected to morphometric and histopathological analyses. Additional samples were collected through day 16 posthatching for other studies. Larvae were preserved in coded glass scintillation vials (Kimble/Kontes) by using 10% neutral-buffered formalin and stored at room temperature. Forty-eight hours after fixation, formalin was removed and replaced with 70% ethanol (Fisher Scientific).

Egg Analysis. Organic chemical analysis of the eggs was performed at the California Department of Fish and Game Marine Pollution Laboratory in Elk Grove, CA, and trace elements were analyzed at the Department of Fish and Game laboratory in Moss Landing, CA.

The following methods were used to analyze for organochlorine pesticides, PCBs, and PBDEs. Tissue (egg) samples were extracted by using pressurized fluid extraction, cleaned by using gel permeation chromatography, and fractionated by using Florisil (Mallinckrodt Baker). Extracts were then analyzed by using Agilent 6890 plus gas chromatographs (Agilent Technologies) equipped with dual 60-m capillary columns (DB5 and DB17) and dual microelectron capture detectors. PCB aroclors (1248, 1254, and 1260) were estimated by using the method devised by Newman *et al.* (2). Total lipid content of the eggs was determined as part of the chemical analysis and found to be: 1999 hatchery control = 18.5% ± 0.9% (n = 3); 1999 eggs from river females = 22.3% ± 0.8%* (n = 11); and 2001 eggs from river females = 23.8% ± 0.9%* (n = 10). Lipid content of the 1999 and 2001 eggs from river females was significantly higher (*, $P \leq 0.05$) than the 1999 hatchery controls. Higher lipid content usually indicates better egg quality, therefore the eggs from the river were similar in quality to or better than hatchery controls as measured by lipid content.

Trace element analysis was performed by using inductively coupled plasma–atomic emission spectrometry (EPA method 200.7). The tissue digestion for all metal analyses was performed by using EPA method 3052M. The metal analyses were performed by using EPA method 200.8M, and mercury analysis was performed by using California Department of Fish and Game's Marine Pollution Laboratory method MPSL-103. The results of trace element analyses were unremarkable.

Developmental Morphometry. Before histological preparation, sectioning, and morphometric analysis, larvae randomly selected for analysis were photographed and measured by using an Olympus SZ40 dissecting microscope equipped with an 1.5× auxiliary objective linked to an Olympus DP11 digital camera (Olympus America). Larvae were photographed in both the lateral and dorsoventral positions. Standard length, area, and mean diameter were measured by using Image Pro Plus 4.0 software (Media Cybernetics). Body and organ volumes were measured by using the Computer Assisted Stereological Toolbox Grid System (CAST Grid; Olympus Danmark). The software package used was CAST Grid version 2.00.04 updated to 2.1.6.0, loaded on a Windows 98 computer system with a 21-inch Sony Trinitron monitor (Sony Electronics). Microscopy was performed by using an Olympus BH2 microscope equipped with 1×, 2.5×, 4×, 10×, 20×, 40×, and 60× objectives. The microscope stage was computer controlled (Prior Scientific), equipped with a microcater gauge (MT12; Heidenhain). Imaging was performed by using a JVC model KY-F58, 3 CCD digital camera (JVC Americas). The system is designed with scientifically validated design-based stereology, incorporating user-defined variables for morphometric analysis of histological samples. The CAST Grid system is used to execute numerous stereological techniques, many of which are described in depth in Howard and Reed (3).

For measurement and analysis of body and organ volumes using the CAST Grid system, 12 larvae were randomly chosen that represented each of the developmental periods of 1, 3, and 5 days posthatching. Larval samples from 5 field-collected and 3 hatchery control females were used in the analysis. Larvae were measured by using the methods described above, embedded in glycol methacrylate (Polysciences.), and serial sectioned at 4-μm thickness using a Sorvall JB-4 Microtome (DuPont). Sections were placed onto coded and numbered glass slides, stained with H&E (Sigma–Aldrich), and coverslipped by using Shandon-Mount (Thermo Fisher Scientific). Using the CAST Grid system, larval whole body, brain, liver, and yolk volumes were calculated by using the histological slides and following the rules of unbiased stereology by the Cavalieri method (4, 5). Volumes were calculated by using the formula: (area/point) (total points counted) (organ thickness) = volume. This procedure was repeated 2 to 3 times for each larval whole body/organ/tissue analyzed to corroborate and verify measurement accuracy. The accuracy of the Cavalieri method is within 5% of true volume. Therefore, repeat measurements where the P value was $\leq$0.05 were deemed acceptable. Average values of the 2 to 3 analyses per larva per tissue were used as the final calculated volumes.

Statistical Analysis. Statistical analyses of organ and yolk sac volumes of larvae from hatchery-reared striped bass and field-collected females were conducted by using the statistical program SigmaStat, version 2.03 (Systat Software). The same program was used to compare contaminant concentrations in eggs from hatchery and field female striped bass. Data were tested for normal distribution and homogeneous variance. Differences between the means of organ and yolk sac volumes from both groups were analyzed for each sampling event by using the parametric t test. The same procedures were applied for comparison of organ volumes normalized to whole-body volumes. Replicates contributed equally to data sets used for statistical analyses. Significance was accepted at $P \leq 0.05$.

PCB, PBDE, and pesticide concentrations in eggs from the 2 groups of river-collected striped bass were compared to contaminant concentrations in eggs from hatchery-reared females by using 1-way ANOVA of the same statistical program. For normally distributed data, a pairwise comparison was performed by using the Tukey test; rank-based ANOVAs (Dunn test) were carried out to account for nonnormal distributed data sets with unequal group sizes.

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