Maternal transfer of xenobiotics and effects on larval striped bass in the San Francisco Estuary

David J. Ostrach[a,b,1], Janine M. Low-Marchelli[c], Kai J. Eder[a], Shaleah J. Whiteman[b], and Joe G. Zinkl[b]

[a]John Muir Institute of the Environment, Center for Watershed Sciences, and [b]Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, One Shields Avenue, Davis, CA 95616; and [c]Department of Pharmacology, University of California at San Diego, 9500 Gilman Drive, La Jolla, CA 92093

Edited by Dixon Landers, U.S. Environmental Protection Agency, Corvallis, OR, and accepted by the Editorial Board October 3, 2008 (received for review March 14, 2008)

Aquatic ecosystems around the world face serious threats from anthropogenic contaminants. Results from 8 years of field and laboratory investigations indicate that sublethal contaminant exposure is occurring in the early life stages of striped bass in the San Francisco Estuary, a population in continual decline since its initial collapse during the 1970s. Biologically significant levels of polychlorinated biphenyls, polybrominated diphenyl ethers, and current-use/legacy pesticides were found in all egg samples from river-collected fish. Developmental changes previously unseen with standard methods were detected with a technique using the principles of unbiased stereology. Abnormal yolk utilization, brain and liver development, and overall growth were observed in larvae from river-collected fish. Histopathological analyses confirmed and identified developmental alterations. Using this methodology enabled us to present a conclusive line of evidence for the maternal transfer of xenobiotics and their adverse effects on larval striped bass in this estuary.

Morone saxatilis | contaminants | biomarkers | histopathology | unbiased stereology

Over the past few decades the world's aquatic environments have been severely impacted by anthropogenic activities. Contaminants from industry, agriculture, urban runoff, and other sources have found their way into these environments, affecting all levels of biological organization, from the individual to the entire ecosystem (1–3). Consequently, fish species assemblages have shifted (4), and major fish population declines have occurred (5, 6). Understanding the role contaminants play in contributing to these declines is essential and will ultimately help elucidate their effects on human populations.

Many classes of contaminants can bioaccumulate in muscle and fat tissue. These compounds can affect physiological processes and the overall health and fitness of the individual, possibly leading to population-level effects (7). Lipophilic compounds bioaccumulate in fish and wildlife, as well as in human tissues, and can be transferred maternally to offspring, generating deleterious effects (8–10).

The striped bass (*Morone saxatilis*) population, along with such other pelagic fish as delta smelt (*Hypomesus transpacificus*), longfin smelt (*Spirinchus thaleichthys*), and threadfin shad (*Dorosoma petenense*), in the San Francisco Estuary has suffered significant declines in the past decades (6, 11). Striped bass were part of a thriving commercial and sport fishery. In 1935 the commercial fishery was closed and striped bass were designated a game fish. The population continued to thrive through the 1960s. The first population crash occurred in the 1970s (6) followed by significant declines in the mid-1980s and mid-1990s (5, 12). The most recent step change raises significant concerns because it has occurred during a period of moderate weather conditions and river flows that typically result in at least minor population recovery (12). The past 3 decades of monitoring data and various studies suggest that some factors causing the striped bass population decline occur in the early life stages and before the 38-mm young of the year index (3, 13, 14), an index that has been used to accurately measure population abundance of striped bass for more than 40 years (15).

During this extended period of population decline, several factors have been identified as possible causes, indicating that multiple stressors are affecting the aquatic fauna in this dynamic and complex ecosystem: entrainment and death of fish due to water diversions for agriculture and human usage, food limitation and larval starvation, introduced species, climate change, and contaminants (5, 6, 11). However, there is evidence that starvation is not occurring (14) and that the combined effects of water diversions and introduced species do not account entirely for the recent declines in the population (12).

On the other hand, contaminant inputs are diverse, and sources such as agriculture, industry, and urban runoff into the estuary have been well documented for several decades (16, 17). Effects of contaminants on the early life stages of striped bass have been documented since the late 1980s (3, 14), but comprehensive studies investigating contaminant concentrations, exposure, and effect have not been conducted to this date.

The study presented here is the first of several ongoing investigations we initiated to determine the contributing effects of timing and route of contaminant exposure, and their physiological and pathological significance on the early life stages of striped bass. The potential routes and timing of exposures considered were: (*i*) maternal transfer of xenobiotics to the egg may affect embryonic and larval development before the onset of exogenous feeding, (*ii*) exposure to xenobiotics located within the spawning grounds *in ovo* immediately after fertilization and before water hardening, (*iii*) larval exposure after hatching to xenobiotics found in the area of hatching and larval development, and (*iv*) larval exposure to xenobiotics via contaminated food sources in the larval nursery grounds. This study focuses on the first potential route of exposure, the maternal transfer of xenobiotics and effects on early development.

Results

Chemical analysis of the eggs from field-collected striped bass revealed significantly higher levels of polychlorinated biphenyls [PCBs; 400–1,000 parts per billion (ppb)], polybrominated diphenyl ethers (PBDEs; 100–150 ppb), and current-use and legacy pesticides (pesticides that are no longer in use but persist in the environment; 5–750 ppb), than found in the eggs of control hatchery-reared females (Table 1). Egg quality and viability were examined and found to be comparable between eggs from the wild and the hatchery. Eggs from field-collected striped bass were slightly larger and in 1 year (1999) had slightly higher total lipid than the hatchery controls. Other studies have reported similar findings,

Author contributions: D.J.O. designed research; D.J.O., J.M.L.-M., and S.J.W. performed research; D.J.O. contributed new reagents/analytic tools; D.J.O., J.M.L.-M., K.J.E., S.J.W., and J.G.Z. analyzed data; and D.J.O. and K.J.E. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission. D.L. is a guest editor invited by the Editorial Board.

[1]To whom correspondence should be addressed. E-mail: djostrach@ucdavis.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0802616105/DCSupplemental.

Table 1. PBEs, PBDEs, and current and legacy pesticide levels (ppb) in unfertilized striped bass eggs

Compound	Hatchery (1999)	River (1999)	River (2001)
PCBs			
Congeners 8–209	93.9 ± 7.5	601.8 ± 107.2*	426.1 ± 54.4*
Aroclors 1248, 1254, and 1260	178.7 ± 15.2	945.2 ± 158.0*	678.3 ± 85.5*
PBDEs	12.9 ± 0.3	138.0 ± 22.0*	134.5 ± 29.3*
Pesticides			
Chlordane, *cis*-	4.9 ± 0.4	15.8 ± 2.4*	15.8 ± 2.5*
Chlordane, *trans*-	3.0 ± 0.1	6.1 ± 1.0	5.0 ± 0.6
Chlorpyrifos	ND	4.5 ± 1.7	0.4 ± NA
DDD, *o,p′*-	ND	6.3 ± 1.5	12.2 ± 4.1*
DDD, *p,p′*-	13.4 ± 1.3	68.9 ± 12.1*	87.3 ± 21.5*
DDE, *o,p′*-	ND	0.4 ± NA	5.4 ± 3.1
DDE, *p,p′*-	41.2 ± 4.8	389.0 ± 60.6*	566.8 ± 195.8*
DDMU, *p,p′*-	ND	16.5 ± 4.3	16.2 ± 6.9
DDT, *o,p′*-	ND	ND	4.3 ± 3.2
DDT, *p,p′*-	4.6 ± 0.3	21.6 ± 2.8*	35.8 ± 18.6*
Dieldrin	10.3 ± 0.5	19.9 ± 2.9	24.7 ± 6.8*
Heptachlor epoxide	1.9 ± 0.1	2.5 ± 0.3	2.3 ± 0.2
Nonachlor, *cis*-	1.9 ± 0.2	11.7 ± 1.9*	10.4 ± 1.7*
Nonachlor, *trans*-	4.0 ± 0.5	24.9 ± 3.6*	23.5 ± 4.4*
Oxychlordane	ND	2.5 ± 0.4*	1.8 ± 0.6
Toxaphene	ND	36.4 ± 27.2	91.8 ± 55.4

A total of 51 PCBs (congeners 8–209, aroclors 1248, 1254, and 1260) and 12 PBDEs (BDEs 17–190) were analyzed. Duplicate samples of eggs (5–10 g per sample) were collected from hatchery control fish (Hatchery 1999, $n = 3$) and river-collected females (River 1999, $n = 11$), and from river-collected females in 2001 (River 2001, $n = 10$) for chemical analysis. Shown are mean contaminant concentrations in parts per billion (ppb) ± standard error. Concentrations below detection limits are indicated with ND. Standard error is not applicable (NA) when only a single sample showed contaminant concentrations above the detection limit. Significant changes ($P \leq 0.05$) in comparison with hatchery control are shown with an asterisk.

indicating that river-collected striped bass eggs were of higher quality than hatchery eggs (18, 19).

Results from the developmental studies showed that control larvae exhibited normal morphology, growth, and development, whereas larvae from field-collected striped bass (subsequently referred to as river larvae) developed abnormally, grew more slowly, and were significantly smaller. River larvae were longer and thinner than hatchery controls at 24 h posthatch, resembling larvae at a later stage (e.g., body morphology, tail fin, and notochord development resembled 3- to 5-day posthatch larvae). Overall growth of these larvae was significantly slower throughout the examined developmental period of 5 days posthatch (Fig. 1). Yolk sac utilization in river larvae was more rapid, with poorer results than in the hatchery control larvae (Fig. 2*A*). At 5 days posthatch, river larvae had significantly less yolk or no yolk sac remaining compared with the hatchery controls. Brain growth in the river larvae was slower throughout the developmental period (days 1 through 5 posthatch; Fig. 3). Liver growth and development in river larvae were accelerated significantly during the first 3 days posthatching; however, growth and development became retarded and regressed between days 3 and 5 posthatch (Fig. 2*B*). In contrast, liver growth in the control group was constant throughout the developmental period, resulting in a significantly larger and better-developed liver at day 5 posthatching.

Histopathological evaluations were performed subsequent to morphometric analysis (Figs. 4 and 5). At day 3 posthatch, yolk sacs from the two groups were similar in appearance (Fig. 4 *A* and *C*) and highly eosinophilic, indicating abundant protein, but there was significantly less yolk remaining in the river larvae. At day 5 posthatch, the disparity between the 2 groups became more pronounced (Fig. 4 *B* and *D*). The hatchery-reared control larvae had a portion of highly eosinophilic yolk sac remaining, whereas the river larvae had very little or no yolk sac remaining. Any residual yolk in river larvae did not stain with eosin, indicating an absence/low concentration of protein remained. In addition, the color and consistency of yolk during the late stages of absorption (days 3 to 5 posthatch) were abnormal. The yolk from river larvae had an inconsistent (bubbly/frothy) appearance, and yolk sac edemas were observed in the majority of these larvae.

Histopathological evaluation of the liver was also performed (Fig. 5). Livers from the control group were beginning to show distinct cellular architecture and glycogen storage 3 days after hatching (Fig. 5*A*). The livers from river larvae at day 3 posthatch were more highly developed, with very distinct cellular architecture and abundant stores of glycogen, and they were significantly larger than those of the controls (Fig. 5*C*). At day 5 posthatch, these changes reversed dramatically. Livers from the control group continued to develop, grow, and exhibit advanced cellular architecture, morphology, and abundant stores of glycogen (Fig. 5*B*). On the contrary, livers from the river larvae had shrunken considerably by day 5 posthatching, cellular architecture became indistinct/regressed, hepatocytes were devoid of glycogen, and nuclear bunching was observed (Fig. 5*D*).

Discussion

This study provides clear evidence of maternal transfer of xenobiotics and their adverse effects on larval striped bass in the San Francisco Estuary. Chemical analysis of unfertilized eggs from Sacramento River-collected striped bass indicated that maternal transfer of biologically significant lipophilic compounds occurred in all 21 females in this study. Contaminants found in these eggs included PCBs, PBDEs, current-use pesticides, legacy pesticides, and their degradation products. Our results indicate that pesticides not in use for decades, such as DDT and its degradation products, are still persistent in the estuary and are being made bioavailable by recycling through the food chain to apex predators. Furthermore, our results show that these contaminants are being transferred to their progeny in biologically relevant levels. Concentrations of

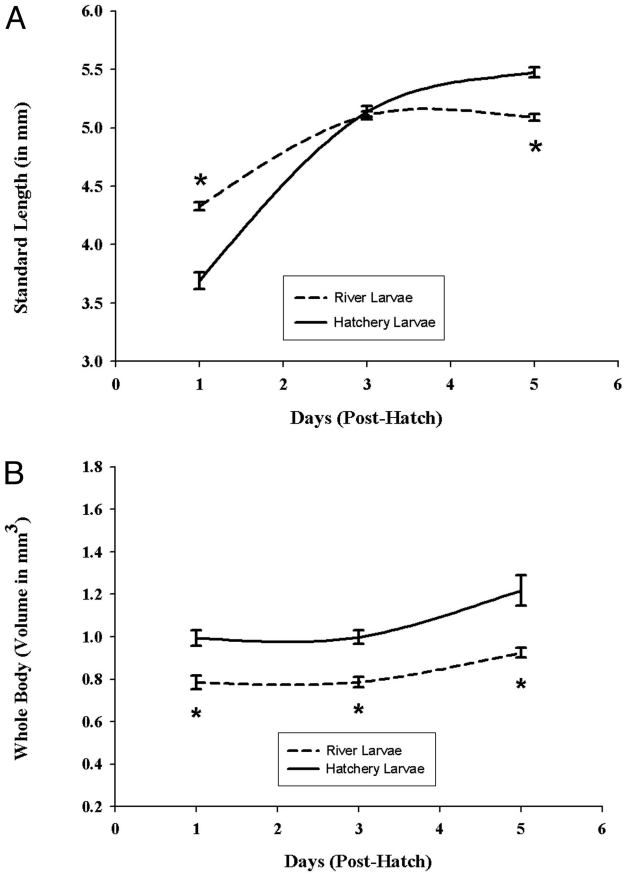

Fig. 1. Comparison of the development of larvae from hatchery and river-collected striped bass. (*A*) Using standard length as a measure of size, this graph represents growth of river and hatchery larvae between day 1 and day 5 post-hatching. Results represent the means ± SE of 12–15 hatchery larvae and 36–75 river-collected larvae. *, Significantly different from hatchery larvae; significance level $P \leq 0.001$. (*B*) Using a technique (Cavalieri method) enabled us to compare whole-body volumes of river and hatchery larvae between days 1 and 5. Results represent the means ± SE of 12–15 hatchery larvae and 36–75 river-collected larvae. *, Significantly different from hatchery larvae; significance level $P \leq 0.001$.

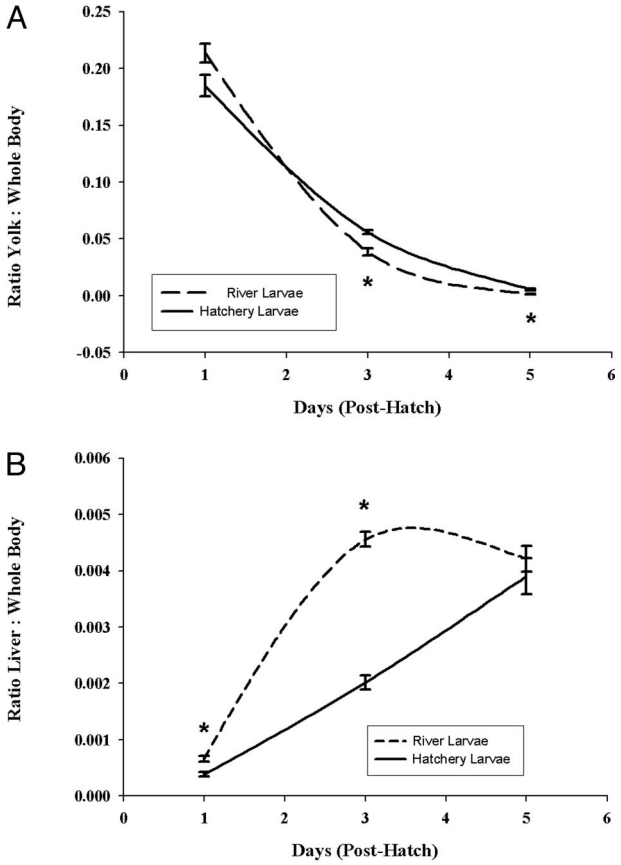

Fig. 2. Comparison of organ development relative to whole-body volume of larvae from hatchery and river-collected striped bass. (*A*) Yolk utilization of river and hatchery larvae between day 1 and day 5. Values are reported as yolk sac volumes relative to whole-body volumes. Results represent the means ± SE of 12–15 hatchery larvae and 36–75 river-collected larvae. *, Significantly different from hatchery larvae; significance level $P \leq 0.007$. (*B*) Comparison of liver growth of river and hatchery larvae between day 1 and day 5. Values are reported as liver volumes relative to whole-body volumes. Results represent the means ± SE of 12–15 hatchery larvae and 36–75 river-collected larvae. *, Significantly different from hatchery larvae; significance level $P \leq 0.016$.

individual contaminants and mixtures determined in this study have been shown to have adverse effects in a wide range of animals, including mammals, reptiles, amphibians, and fish (20–23). Some of the effects described are as follows: alterations of growth and development (24), poor hatching success (22), alterations of the reproductive and nervous system (25, 26), learning and behavioral deficits retained throughout life (27, 28), abnormalities of the liver and other organ systems (29, 30), and endocrine disruption (29, 31). Studies have also shown that these contaminants in combination can increase adverse effects by several orders of magnitude (32, 33). Chemical analysis of hatchery control eggs indicated that fewer compounds are present in comparison with eggs from field-collected females, and that levels of these contaminants are most likely biologically insignificant. Contaminants found in the control eggs are believed to originate from the commercial diet fed to hatchery fish, which is produced from wild fish and other contaminated byproducts (34), and not the hatchery water which is supplied by a deep uncontaminanted well. In addition to current-use pesticides, legacy pesticides, and PCBs, significant levels of PBDEs were also detected in eggs from field-collected female striped bass. This is a reported finding of maternal transfer of PBDEs in fish in the San Francisco Estuary system, a novel group of environmental contaminants that has recently become the focus of many toxicity studies. PBDEs are mainly used as flame retardants and are found in a variety of materials, such as paint, upholstery, carpeting, plastics, textiles, electronic circuits, and insulation (35). PBDEs are now as ubiquitous as PCBs in the aquatic environment, and their levels are rising due to a lack of regulation (35, 36). It is now believed that PBDEs have spread throughout the world's oceans (35, 37), and as a consequence have been found in many aquatic organisms, including fish, where they have bioaccumulated to biologically relevant levels (33). The aquatic environment of the fish we investigated is one of the most contaminated regions worldwide, and studies have shown PBDE levels in breast milk from women living in the San Francisco Bay area up to 100 times greater than those in other regions of the world (8). These compounds have been shown to act as thyroid hormone mimics, induce P450-1A1 and P450-2B2 enzyme systems, have neurotoxic effects, and cause learning and behavioral deficits that remain throughout life in rodents (35). Results presented here support studies indicating PBDE contamination of the San Francisco Estuary (38) and bioaccumulation of these compounds in fish (35). The consequences of PBDE contamination for wildlife and humans are largely unknown (8, 10) and need to be addressed and mitigated.

The study presented here used a method based on the principles of unbiased stereology and the Cavalieri method to measure body and organ volumes of larvae throughout a certain developmental period. This method enabled us to detect developmental changes

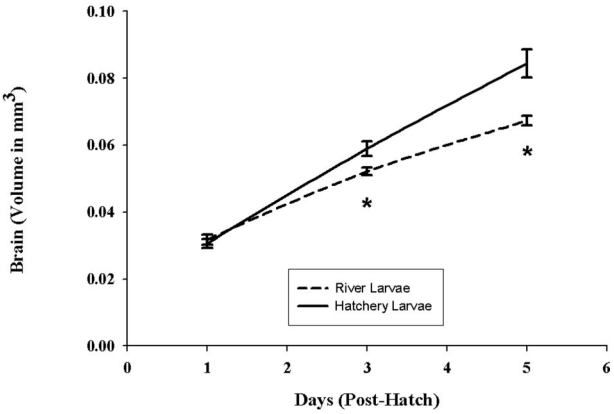

Fig. 3. Comparison of brain development of larvae from hatchery and river-collected striped bass. This graph compares brain volumes of river and hatchery larvae between day 1 and day 5. Results represent the means ± SE of 12–15 hatchery larvae and 36–75 river-collected larvae. *, Significantly different from hatchery larvae; significance level $P \leq 0.021$.

previously unseen using standard methods. Typically, morphological studies of the early life stages of fish use linear measurements, such as standard length, body depth at the pectoral fins, and body depth at the anus, to determine size and growth relationships (14, 39, 40). However, in our opinion these types of metrics do not provide an accurate representation of growth and development. According to the methods in which linear measurements are used, the progeny from river-collected striped bass were significantly larger at day 1 posthatching than the hatchery larvae, similar in size at day 3, and significantly smaller at day 5 (Fig. 1*A*). In contrast, our method revealed that river larvae were significantly smaller and grew slower throughout the developmental sampling period of day 1 to day 5 posthatching (Fig. 1*B*). River larvae at day 1 posthatching, although significantly smaller by volume, were longer and thinner than hatchery larvae, resembling a later and more advanced larval stage. We concluded that development of river larvae is abnormal, with some processes accelerated (tailfin, notochord, and liver) and others retarded (brain and liver from days 3 to 5 posthatching). In addition, standard methods provide data only on exterior body dimensions and do not address the morphometry and condition of developing organ systems. Morphometric and histopathological results of yolk, liver, and brain corroborated findings of larval body development (Figs. 2 and 3). Brain growth was retarded in larvae from river-collected striped bass throughout the developmental period, resulting in a significantly smaller brain than hatchery controls at day 5 posthatching (Fig. 3). The hatchery-reared control larvae at day 5 posthatch just before exogenous feeding had significant yolk remaining as an energy store to aid in finding their first food, as well as a well-developed, glycogen-laden liver (additional energy store) capable of metabolizing food and other exogenous compounds (Figs. 4*B* and 5*B*). In contrast, river larvae had little or no yolk remaining at 5 days posthatching, and any yolk remaining was devoid of protein (Figs. 2*A* and 4*D*). Therefore, just before first feeding, these larvae had virtually no source of energy available to search for food or avoid predators. Abnormal liver growth and development in river larvae were extreme. Liver development and growth were accelerated during days 1 and 3

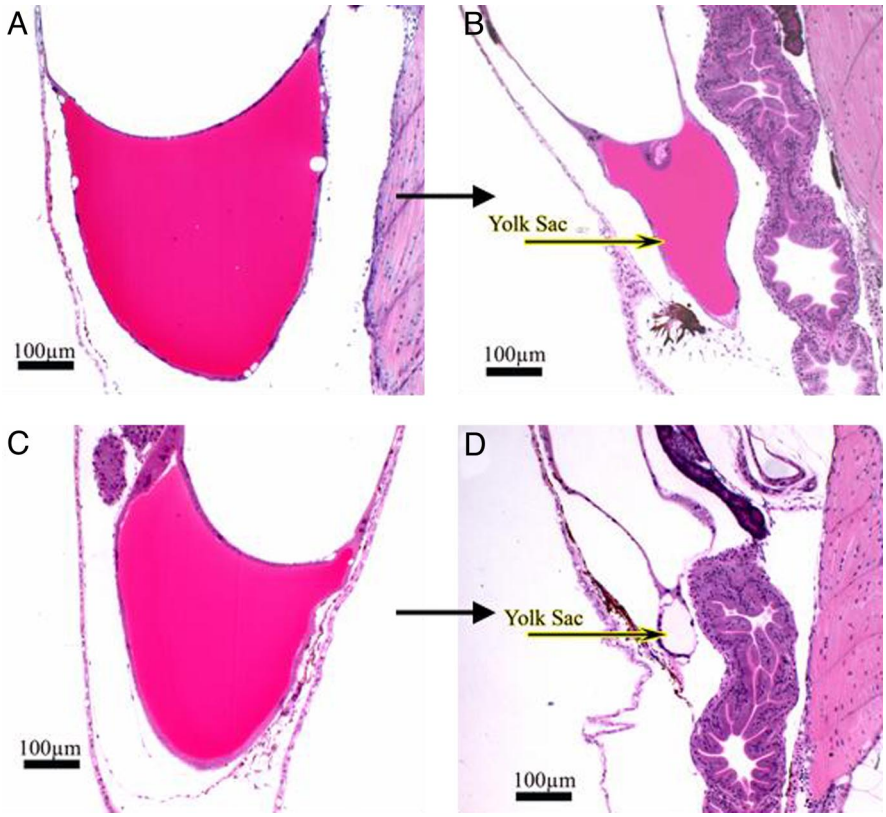

Fig. 4. Histopathological evaluations of yolk sacs of river and hatchery larvae. Striped bass larvae from hatchery females ($n = 15$) and 3–5 river-collected females ($n = 75$) were embedded in glycol methacrylate and serial sectioned at 4-μm thickness by using a Sorval JB-4A Microtome. Sections were placed onto coded and numbered glass slides, stained with H&E, and coverslipped by using Shandon-Mount. (*A* and *C*) Yolk sac appearance on day 3 of larvae from hatchery striped bass females (*A*) and river-collected females' larvae (*C*). (*B* and *D*) Yolk sac appearance on day 5 of larvae from hatchery striped bass females (*B*), and depleted yolk sac from river-collected females' larvae (*D*).

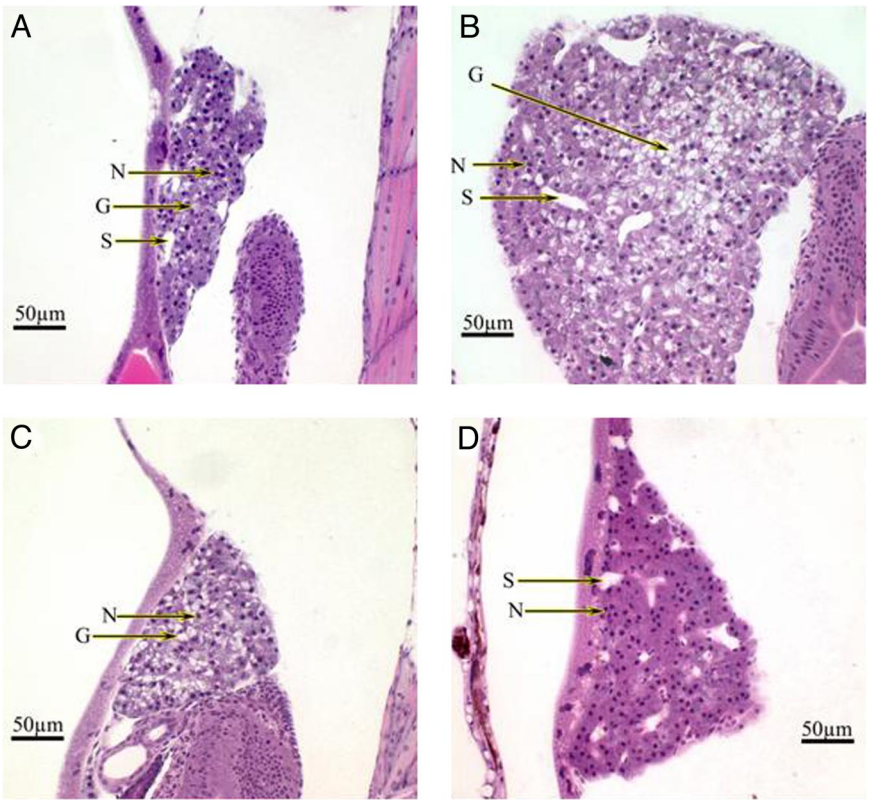

Fig. 5. Histopathological evaluations of livers of river and hatchery larvae. Striped bass larvae from 3 hatchery females (n = 15) and 3–5 river-collected females (n = 75) were embedded in glycol methacrylate and serial sectioned at 4-μm thickness by using a Sorval JB-4A Microtome. Sections were placed onto coded and numbered glass slides, stained with H&E, and coverslipped using Shandon-Mount. (*A*) Liver appearance on day 3 (midorganogenesis) of larvae from hatchery striped bass females. Organ appears normally developed, beginning glycogen storage. (*B*) Normal glycogen-laden liver with distinct cellular architecture of 5-day hatchery larvae. (*C*) Liver appearance on day 3 of larvae from river-collected striped bass females. Liver growth and development are accelerated, as demonstrated by distinct cellular architecture and glycogen storage not normally present during this stage of development. Liver appears developmentally similar to a normal larval liver at day 5. (*D*) Liver appearance on day 5 of larvae from river-collected striped bass females. Growth of the liver is retarded/regressing, cell size is decreased, the cellular architecture is indistinct, glycogen is not detectable, and nuclear bunching can be observed. N, nuclei; G, glycogen storage; S, sinusoids.

posthatching in the river larvae (Figs. 2*B* and 5*C*). The liver deteriorated and regressed between days 3 and 5 posthatching, leading to an extremely small liver devoid of glycogen just before first feeding (Fig. 5*D*). Thus, abnormal liver growth and development in river larvae can be categorized as severe, and the liver is likely much less than optimally functional at day 5 posthatching. Chemical analysis of the eggs coupled with the morphometric and histopathological results from the brain and liver indicate that contaminants are maternally transferred, and that compounds may be causing endocrine disruption during early development of the river larvae. Contaminants found in the eggs of river-collected females are known endocrine disrupters, and effects observed correspond to endocrine disruption during early development (41, 42). We suggest that the combination of the abnormal development, yolk deficiency, and an altered shrunken liver devoid of glycogen at day 5 posthatching adversely affect subsequent growth and survival of larvae from river-caught females. These findings were possible through application of a newly developed morphometric method focusing on whole-body and organ volumes. Coupled with histopathological analysis, this approach overcomes the shortcomings of other, established methods. It provides much more accurate morphometry, representation of body growth, organ growth, organ development, and organ condition in early-life stage striped bass larvae.

The vast majority of maternal transfer studies in fish have been limited to either analysis of eggs from field-collected animals or laboratory studies injecting compounds directly into the developing embryo or female (43, 44). These studies are important sources of toxicological and developmental information, but they are limited to parent compounds, their metabolites, and their effects. These studies fail to reflect the true nature of maternal transfer of complex combinations of xenobiotics affecting fish and wildlife populations. Especially in the aquatic environment today, complex mixtures of xenobiotics, metabolites, and degradation products, not individual compounds, are present and are being bioaccumulated. Accordingly, interactions of complex mixtures detected in the environment may alter effects seen in laboratory studies, and they can cause additional problems not observed when compounds are tested individually or in simple mixtures. To understand the effects of maternally transferred xenobiotics in the environment, it is vital to determine to what extent these real-world complex mixtures are affecting progeny.

The study presented here demonstrates that complex mixtures of contaminants are being maternally transferred to developing progeny, describes developmental alterations detected by using a new technique, and corroborates these findings with histopathological analyses. Laboratory studies can be designed subsequently to investigate the mechanisms involved and to determine which xenobiotics singly or in combination are causing the observed effects. Decisions can then be made to regulate the use of these compounds and their release into the environment to mitigate problems identified by these studies. The results from this study clearly demonstrate that xenobiotics are adversely affecting early-life-stage striped bass in the San Francisco Estuary and need to be considered as one of multiple stressors affecting the continuing population decline.

Methods

Adult male and female striped bass were collected between Knights Landing and Colusa, CA, on the Sacramento River weekly during the spawning seasons of 1999

and 2001 (April to June) by using standard electrofishing methods. Hatchery-reared F_2 generation striped bass were used as controls. This domestic striped bass broodstock was created by using striped bass captured in the same location on the Sacramento River during previous years. Just before fertilization, subsets of egg samples were collected for chemical analysis from both hatchery-reared controls and river-collected fish. River-collected and hatchery-reared females were spawned, and larvae were reared under identical conditions. Larvae were sampled and preserved in 10% neutral-buffered formalin at 3 critical periods during development representing early organogenesis (day 1 posthatch), late organogenesis (day 3 posthatch), and completed organogenesis just before the onset of exogenous feeding (day 5 posthatch). Larvae were embedded in glycol methacrylate and serial sectioned at thicknesses of 4 μm. A technique using the Cavalieri method (45, 46) was used to determine whole-body, brain, liver, and yolk sac volumes during development. Organ and yolk sac volumes were normalized to whole-body volumes such that development and yolk sac utilization between the 2 groups could be assessed. Histopathological analysis of the yolk, liver, and brain was used to describe and corroborate morphometric findings.

Twenty-four duplicate samples of eggs were collected for chemical analysis, with one set processed for organic analysis and the other set for trace element analysis. Eleven samples from river-collected females in 1999, 10 from river-collected females in 2001, and 3 from the hatchery controls were analyzed.

Additional details concerning striped bass capture, spawning, sample collection at spawning and during development of larvae, egg analysis, developmental morphometry, and statistical analysis are provided in supporting information (SI) *Materials and Methods*.

ACKNOWLEDGMENTS. D.J.O. thanks J. Zinkl for his advice, critical scientific input, and support; D. Hyde for his critical scientific input and encouragement to develop the new technique, for providing access to the CAST grid system and his technical staff; W. Bennett for his advice and for providing me the initial opportunity to become involved in striped bass research; J. Low-Marchelli for managing the project during illness; K. Eder and J. Cech for critical discussions, reading, and editing the manuscript; I. Werner for her review of the work; T. Vaught and Professional Aquaculture Services for providing support and the facilities to perform all developmental experiments; D. Crane and the California Department of Game's Fish and Wildlife Water Pollution Control Laboratory for chemical analysis and support; B. Osburn and the University of California, Davis, School of Veterinary Medicine for providing the majority of funding for the project; and T. Sommer, F. Feyrer, and the Interagency Ecological Program Pelagic Organism Decline management team for their support and partial funding under Department of Water Resources Contract 4600004664; and most of all to my father, S. Ostrach, without whose advice and comments on language and content during the writing of this paper, ethical training, and continued support and encouragement, this project and my career in science would not have been possible.

1. Schwarzenbach RP, *et al.* (2006) The challenge of micropollutants in aquatic systems. *Science* 313:1072–1077.
2. Fent K (2004) Ecotoxicological effects at contaminated sites. *Toxicology* 205:223–240.
3. Bailey HC, DiGiorgio C, Doroshov S, Hinton DE (1994) The effect of agricultural discharge on striped bass (*Morone saxatilis*) in California's Sacramento-San Joaquin drainage. *Ecotoxicology* 3:123–142.
4. Matern SA, Moyle PB, Pierce LC (2002) Native and alien fishes in a California estuarine marsh: Twenty-one years of changing assemblages. *Trans Am Fish Soc* 131:797–816.
5. Moyle PB, Williams JE (1990) Biodiversity loss in the temperature zone: Decline of the native fish fauna of California. *Conserv Biol* 4:275–284.
6. Stevens DE, Kohlhorst DW, Miller LW, Kelley DW (1985) The decline of striped bass in the Sacramento-San Joaquin Estuary, California. *Trans Am Fish Soc* 114:12–30.
7. Arkoosh MR, Collier TK (2002) Ecological risk assessment paradigm for salmon: Analyzing immune function to evaluate risk. *Hum Ecol Risk Assess* 8:265–276.
8. She J, *et al.* (2002) PBDEs in the San Francisco Bay Area: Measurements in harbor seal blubber and human breast adipose tissue. *Chemosphere* 46:697–707.
9. Borga K, Fisk AT, Hoekstra PF, Muir DCG (2004) Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* 23:2367–2385.
10. McDonald TA (2002) A perspective on the potential health risks of PBDEs. *Chemosphere* 46:745–755.
11. Bennett WA, Moyle PB (1996) *Where have all the fishes gone? Interactive factors producing fish declines in the Sacramento-San Joaquin Estuary*. San Francisco Bay: The Ecosystem, ed Hollibaugh JT (American Association for the Advancement of Science, San Francisco), pp 519–542.
12. Sommer T, *et al.* (2007) The collapse of pelagic fishes in the upper San Francisco Estuary. *Fisheries* 32:270–277.
13. Chadwick HK, Stevens DE, Miller LW (1977) in *Proceedings of the Conference on Assessing the Effects of Power-Plant-Induced Mortality on Fish Populations* (Pergamon, Gatlinburg, TN), pp 18–35.
14. Bennett WA, Ostrach DJ, Hinton DE (1995) Larval striped bass condition in a drought-stricken estuary: Evaluating pelagic food-web limitation. *Ecol Appl* 5:680–692.
15. Kimmerer WJ, Cowan JH, Miller LW, Rose KA (2000) Analysis of an estuarine striped bass (*Morone saxatilis*) population: Influence of density-dependent mortality between metamorphosis and recruitment. *Can J Fish Aquat Sci* 57:478–486.
16. Moore M, Mulville A, Weinberg M (1996) Water allocation in the American West: Endangered fish versus irrigated agriculture. *Nat Resour J* 36:319–357.
17. Thompson B, Hoenicke R, Davis JA, Gunther A (2000) An overview of contaminant-related issues identified by monitoring in San Francisco Bay. *Environ Monit Assess* 64:409–419.
18. Harrell RM, Woods LC (1995) Comparative fatty acid composition of eggs from domesticated and wild striped bass (*Morone saxatilis*). *Aquaculture* 133:225–233.
19. Gallagher ML, Paramore L, Alves D, Rulifson RA (1998) Comparison of phospholipid and fatty acid composition of wild and cultured striped bass eggs. *J Fish Biol* 52:1218–1228.
20. Jepson PD, *et al.* (2005) Relationships between polychlorinated biphenyls and health status in harbor porpoises (*Phocoena phocoena*) stranded in the United Kingdom. *Environ Toxicol Chem* 24:238–248.
21. Bergeron JM, Crews D, McLachlan JA (1994) PCBs as environmental estrogens: Turtle sex determination as a biomarker of environmental contamination. *Environ Health Perspect* 102:780–781.
22. Spies RB, Rice DWJ (1988) Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. *Mar Biol* 98:191–200.
23. Gutleb AC, *et al.* (1999) Delayed effects of pre- and early-life time exposure to polychlorinated biphenyls on tadpoles of two amphibian species (*Xenopus laevis* and *Rana temporaria*). *Environ Toxicol Chem* 8:1–14.
24. Njiwa JRK, Müller P, Klein R (2004) Life cycle stages and length of zebrafish (*Danio rerio*) exposed to DDT. *J Health Sci* 50:220–225.
25. Waring CP, Moore A (1997) Sublethal effects of a carbamate pesticide on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) Parr. *Fish Physiol Biochem* 17:203–211.
26. Billard R, Roubaud P (1985) The effect of metals and cyanide on fertilization in rainbow trout (*Salmo gairdneri*). *Water Res* 19:209–214.
27. Scholz NL, *et al.* (2000) Diazinon disrupts antipredator and homing behaviors in chinook salmon (*Oncorhynchus tshawytscha*). *Can J Fish Aquat Sci* 57:1911–1918.
28. Sandahl JF, Baldwin DH, Jenkins JJ, Scholz NL (2005) Comparative thresholds for acetylcholinesterase inhibition and behavioral impairment in coho salmon exposed to chlorpyrifos. *Environ Toxicol Chem* 24:136–145.
29. Hinton DE, Klaunig JE, Lipsky MM (1978) PCB-induced alterations in teleost liver: A model for environmental disease in fish. *Mar Fish Rev* 40:47–50.
30. Huggett RJ, Kimerle RAJ, Mehrle PMJ, Bergman HL (1992) *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress* (Lewis, Boca Raton, FL).
31. Donohoe RM, Curtis LR (1996) Estrogenic activity of chlordecone, *o,p′*-DDT and *o,p′*-DDE in juvenile rainbow trout: Induction of vitellogenesis and interaction with hepatic estrogen binding sites. *Aquat Toxicol* 36:31–52.
32. James MO, *et al.* (2004) Increased toxicity of benzo[*a*]pyrene-7,8-dihydrodiol in the presence of polychlorobiphenylols. *Mar Environ Res* 58:343–346.
33. Schmidt K, Steinberg CEW, Staaks GBO, Pflugmacher S (2005) Influence of a xenobiotic mixture (PCB and TBT) compared to single substances on swimming behavior or reproduction of *Daphnia magna*. *Acta Hydrochim Hydrobiol* 33:287–300.
34. Easton MDL, Luszniak D, Von der Geest E (2002) Preliminary examination of contaminant loadings in farmed salmon, wild salmon and commercial salmon feed. *Chemosphere* 46:1053–1074.
35. de Wit CA (2002) An overview of brominated flame retardants in the environment. *Chemosphere* 46:583–624.
36. Rayne S, Ikonomou MG, Antcliffe B (2003) Rapidly increasing polybrominated diphenyl ether concentrations in the Columbia River system from 1992 to 2000. *Environ Sci Technol* 37:2847–2854.
37. Ueno D, *et al.* (2004) Global pollution monitoring of polybrominated diphenyl ethers using skipjack tuna as a bioindicator. *Environ Sci Technol* 38:2312–2316.
38. Oros DR, *et al.* (2005) Levels and distribution of polybrominated diphenyl ethers in water, surface sediments, and bivalves from the San Francisco Estuary. *Environ Sci Technol* 39:33–41.
39. Theilacker GH (1978) Effect of starvation on the histological and morphological characteristics of jack mackerel, *Trachurus symmetricus*, larvae. *US Fish Bull* 76:403–414.
40. Setzler-Hamilton EM, Wright DA, Martin FD, Millsaps CV, Whitlow SI (1987) Analysis of nutritional condition and its use in predicting striped bass recruitment: Field studies. *Am Fish Soc Symp* 2:115–128.
41. Crews D, Willingham E, Skipper JK (2000) Endocrine disruptors: Present issues, future directions. *Q Rev Biol* 75:243–260.
42. Zhou T, Taylor MM, DeVito MJ, Crofton KM (2002) Developmental exposure to brominated diphenyl ethers results in thyroid hormone disruption. *Toxicol Sci* 66:105–116.
43. Miller MA (1993) Maternal transfer of organochlorine compounds in salmonines to their eggs. *Can J Fish Aquat Sci* 50:1405–1413.
44. Westerlund L, *et al.* (2000) Early life-stage mortality in zebrafish (*Danio rerio*) following maternal exposure to polychlorinated biphenyls and estrogen. *Environ Toxicol Chem* 19:1582–1588.
45. Howard CV, Reed M (1998) Volume and surface-area estimation from microscopic images. *J Microsc* 190:291–291.
46. Gundersen HJG, Jensen EBV, Kieu K, Nielsen J (1999) The efficiency of systematic sampling in stereology—Reconsidered. *J Microsc* 193:199–211.

Supporting Information

Ostrach *et al.* 10.1073/pnas.0802616105

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.

1. Howard CV, Reed M (1998) Volume and surface-area estimation from microscopic images. *J Microsc* 190:291–291.
2. Newman JW, Becker JS, Blondina G, Tjeerdema RS (1998) Quantitation of aroclors using congener-specific results. *Environ Toxicol Chem* 17:2159–2167.
3. Howard V, Reed MG (1998) *Unbiased Stereology: Three-Dimensional Measurement in Microscopy* (BIOS Scientific, Oxford).
4. Harrell RM, Woods LC (1995) Comparative fatty acid composition of eggs from domesticated and wild striped bass (*Morone saxatilis*). *Aquaculture* 133:225–233.
5. Gallagher ML, Paramore L, Alves D, Rulifson RA (1998) Comparison of phospholipid and fatty acid composition of wild and cultured striped bass eggs. *J Fish Biol* 52:1218–1228.