A Pilot Study of the Effects of Contaminants on surfperch (*Cymatogaster aggregata*) in the San Francisco Bay-Estuary

Draft Final Report
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Abstract

In 2005 and again in 2006 field studies of shiner surfperch were carried in San Francisco Bay (Oakland Middle Harbor and near Candlestick Park), Big River (Mendocino County) and in lower Tomales Bay. Various measures of size (surrogate for growth), fitness, reproduction and exposure to contaminants were compared for fish collected from these locations. The reference area for 2005 studies was Big River, Mendocino Co. (BR), but in 2006 Heart's Desire Beach in lower Tomales Bay (TB) was used as it was more suitable for a metric of contaminant exposure (hepatic ethoxyresorufrin-O-dethylase, EROD activity) and more comparable in temperature. Oakland Middle Harbor (OAK) and the Candlestick Park (SFC) locations were collected in both years. In addition, estradiol injection experiments were done to determine if a marker for egg shell proteins (choriogenin) was inducible in shiner surfperch.

There were significant differences between locations in most measures. Chemical contaminants were clearly higher in San Francisco Bay fish than those from Tomales Bay, and the contaminant-inducible hepatic EROD activity in 2006 was lower in TB fish than in the San Francisco Bay fish, confirming the choice of TB as the comparison site. Female wet weights were not different between TB, OAK and SFC, but weight-per-offspring, male weight, and male condition index from TB were greater than from the two San Francisco Bay locations. The female-adjusted condition index (discounting weight of young) of TB and OAK fish were tied but greater than SFC fish. Number of young was greatest in OAK and tied for SFC and TB. Ranking all sites for the best condition of fish using surrogate growth and reproductive measures produced the order of (from best to worst): TB>OAK>SFC>BR. Disregarding BR, which is a different system in many ways, this is also the order of ranking of PCB tissue concentrations. **These data point to a potential for a chronic effect of contaminants on shiner surfperch that may well have contributed to the decline of this species over the past 30 years.**

San Francisco Bay beach seine samples used for a majority of this work captured a preponderance of females, while samples taken from BR had approximately half of each sex and the one TB sample had more males than females. We are hesitant to attribute this to a contaminant effect as it may be due to a preference of females to give birth inshore, however the possible effects of contaminants on sex ratio deserves further examination.

Egg shell proteins were only weakly inducible with injection of estradiol into shiner surfperch and the use of choriogenin as a marker for exposure to estrogenic compounds in this live-bearing fish with few eggs developing each year is not recommended.

Histopathological examination of gills, heart, liver and gonad in 2005 found relatively few lesions. SFC did however have moderate to severe gill lesions (bronchitis: aneurysms, lamellar thickening) in about 25% of the specimens. Gill histopathology appears to be a useful monitoring measure for fish population health as this and another study of other surfperches (Embiotocidae) indicate that it may respond to chronic contaminant exposure. Laboratory studies would need to be carried out to validate this assumption.

A culture was established in June 2006 at the Bodega Marine Laboratory from a collection of shiner surfperch taken at Big River, and in August 2006 some of the females gave birth. So laboratory culture and long-term contaminant exposure experiments are possible.

Introduction

The Regional Monitoring Program for Water Quality in San Francisco Bay (RMP) carries out pilot projects in order to test the suitability of approaches for long-term contaminant and effects monitoring. The RMP is in the process of identifying measures of contaminant effects in the estuary with studies of benthic communities, fish, seabirds and harbor seals. The SFEI funded this 2-year effort to determine if there were effects of contaminants in local declining populations of fish, the shiner surfperch (*Cymatogaster aggregata*) and to determine the suitability of this species for monitoring potential contaminant effects as part of the RMP. There was also supplementary funding for establishing a culture of this species at the Bodega Bay Marine Laboratory. Here the preliminary results of two years of field collections, measurements and an effort to culture this species are reported. Recommendations for future work with this species are also made.

Background

The challenge of determining if there are effects

The San Francisco Estuary has a diverse fish fauna and as many as 90% of species in the commercial and recreational fisheries of California use the Bay at some stage of their life cycle. The Bay is important for Chinook (*Oncorhynchus tshawytscha*) and Coho salmon (*Oncorhynchus kisutch*), Pacific herring (*Clupea harrengus*), Pacific and California halibut (*Hippoglossus stenolepis* and *Paralichthys californicus*), starry flounder (*Platichthys stellatus*), English sole (*Parophrys vetulus*), sturgeon (*Acipenser* spp.), surfperches (Embiotocidae), northern anchovy (*Engraulis mordax*) and Pacific sardines (*Sardinops sagax var. caeruleus*), to mention a few (Baxter et al., 1999). San Francisco Bay also has a large urban recreational fishery (Karpov et al., 1995). In the large estuary watershed there are more than 50 freshwater fishes (Moyle 2002). Many of the species in the estuary and its catchment are in decline (Brown et al., 1994; Moyle 1994 ; Meng et al. 1994; Meng and Moyle, 1994) with contamination, climate change, water diversions, invasive species, habitat alteration and harvesting the primary causes of the declines.

As is the case with many contaminated coastal and estuarine systems, the extent of contaminant effects on fish in this system is not well understood because of insufficient study. There has simply not been enough study of long-term low level exposures to contaminants (e.g., Forrester et al. 2003). However, progress has been made in the last 30 years and based on the available evidence cited below, contaminants are having some effects on fish; it is just that the consequences of these stressors for fish populations are highly uncertain.

Earlier work in San Francisco Bay has provided evidence that reproductive performance of starry flounder (*Platichthys stellatus*) was compromised by organic contaminants, e.g. polychlorinated biphenyls (PCBs) (Spies et al., 1988; Spies and Rice, 1988). Such impairment may have led to poor reproductive success and contributed to population decline. Other work further up the estuary and its watershed has implicated pesticides in genotoxic effects (Whitehead et al. 2005) in the effects on striped bass (Ostrach 2006, in preparation and Bennett et al. 1995) and splittail (*Pogonichthys macrolepidotus*) (Teh et al., 2005), and selenium in splittail (Teh et al. 2004), and in sturgeon (Tashjian 2005).

In general, persistent, low-level contamination affects aquatic organisms in two ways:

1. The direct toxic effects of the contaminants on their biochemistry and physiology (e.g., blocking enzyme activity, alternation of cellular oxidation pathways, production of reactive intermediate metabolites, interference with normal function of receptors), i.e., those regulating organism reproduction, growth and internal homeostasis, either acutely or sublethally.
2. The indirect effects of contaminants on energy allocation, such that the demand on energy from accumulated contaminants (e.g., from repair of cellular damage, enzyme induction, costs of metabolism and excretion) is diverted from growth, reproduction or maintenance of homeostasis.

While there is a great deal of literature on the effects of contaminants on the biochemistry, physiology and structure of fishes based largely on laboratory exposure studies, realistic and rigorous field studies are relatively few. Such studies have been conducted in notably contaminated environments, such as Puget Sound, Washington (Malins et al 1984), English rivers (Sumpter et al., 2005), coastal lagoons of southern California (Forrester et al., 2003), and the Palos Verdes Peninsula (Spies and Thomas, 1997). There are fewer yet that integrate biochemical and physiological alterations by contaminants with higher order processes, that is growth reproduction and maintenance of fitness. For example fish in southern California lagoons have been shown correlations between growth and contamination in field studies and experiments (Forrester et al., 2003).

Choice of species

For many reasons the shiner surfperch (*Cymatogaster aggregata*) is an excellent candidate species for study of potential population-level effects of contaminants on fish in the San Francisco Estuary Regional Monitoring Program:

1. This species accumulates some of the highest concentrations of organic contaminants of any fish species that has been analyzed in the estuary (Davis et al., 2001).
2. Shiner surfperch have high site fidelity (Fritzsche and Collier 2001). Therefore, any health effects are likely to result from local, more identifiable, sources.

3. They are found in shallows and channels of the Bay where most of the chemical monitoring data have been taken for the last 15 years in the Regional Monitoring Program (RMP). Therefore, there is a historical record of contamination for understanding any effects that are found.

4. They are commonly captured by the Interagency Ecology Project (IEP) Bay Study sampling program (Baxter et al. 1999), providing the opportunity for assessing individual health throughout the system as well as providing a historical context for estimating contaminant effects on trends in population abundance.

5. They are also sampled and analyzed by the SFEI sport fish sampling program as well as the Pacific States Marine Recreational Fisheries Statistics Survey (MRFSS) (Karpov et al. 1995), providing additional sources of fishery dependent information.

6. They are a popular recreational fish, caught in large numbers by urban residents for food. Therefore, they are an ideal species for addressing public concerns about estuarine health.

7. Their populations are declining in the estuary and there is concern about their survival (Hieb 2000).

8. They are live-bearers and their entire annual reproductive output (4-36 young annually) can be determined by sampling pregnant females in the late winter and early spring, facilitating study of reproductive effects, such as fecundity, (Fritzsche and Collier 2001) that relate directly to population dynamics.

9. Biomarkers have been successfully applied to other embiotocids around a natural petroleum seep in southern California (Spies et al., 1996).

10. A related but unpublished study found significant abnormalities in incubating juveniles in dwarf surfperch (*Micrometrus minimus*) collected in Mission Bay, San Diego, California (E. Schultz, pers. Comm.).

Objectives

The overall objective of this pilot monitoring project is to determine if contaminants are potentially contributing to the population declines of the shiner surfperch observed in the estuary. Since this objective can not be accomplished directly by empirical measurements of populations, we have approached this question by relating contaminants to possible changes in growth, fitness and reproduction, that all relate directly to the survival and health of individuals and, in turn, to the trajectory of the population. We have a two-phase, long-term strategy. In the first phase, the results of which are reported here, the goal was to determine if fish collected in contaminated environments showed impairment in several measures of individual health, and if such potential impairments were found, to perform laboratory exposures to determine if they could be reproduced under controlled

conditions. Because of unexpected elevation of one biomarker (EROD activity) in the comparison site in year I, the field studies were repeated in year II of this effort using a new control site, delaying the laboratory exposures until further funding could be obtained.

> Objective 1: Determine if shiner surfperch collected in contaminated and less contaminated environments have differences in biomarkers of contaminant exposure, condition, reproductive success and growth and whether such differences, if they exist, affect the population.

In order to carry out the long-term strategy, we needed to establish a population of surfperch in the laboratory, so we had another general objective:

> Objective 2: Establish a population of breeding surfperch in the laboratory for use in long-term exposures.

Conceptual model

Our efforts and strategy were formulated under a conceptual model (Figure 1) that can be modified as more is learned about how contaminants may be affecting shiner surfperch. The graphic representation in Figure 1 shows only some of the main processes that may affect populations, particularly those that we concentrated on in this pilot project. We will relate these processes to the biology and life history of this estuarine species.

The general features of the reproductive cycle of the shiner surfperch are known (Wiebe, 1968; Bane and Robinson, 1970) although some aspects of the reproduction of this species remain to be described. For example, it is not known when and under what circumstances ovulation takes place. The following description is based on the above cited studies and inferred studies of closely related species (Dwarf surfperch; Schultz; 1993a,b; *Hyperprosopon argentum*, DiMartini, 1983; embioticids as a group, Balz, 1984), notes in other published work on the biology of this species, and observations gathered during the course of this study.

Beginning with the production of a new cohort of gametes, which at least in females starts a year before the birth of the young, the gametes pass through the early stages of oogenesis in females in spring and summer. In females this consists of the addition of some yolk and a modest increase in the size of a small number of eggs in the walls of the ovary. But because this species has direct development, i.e., the young are nourished by the mother during development, and is ovoviviparous, the eggs apparently do not need to contain much as stored energy in the form of yolk. Mature oocytes are about 0.1 mm in diameter and are relatively few.

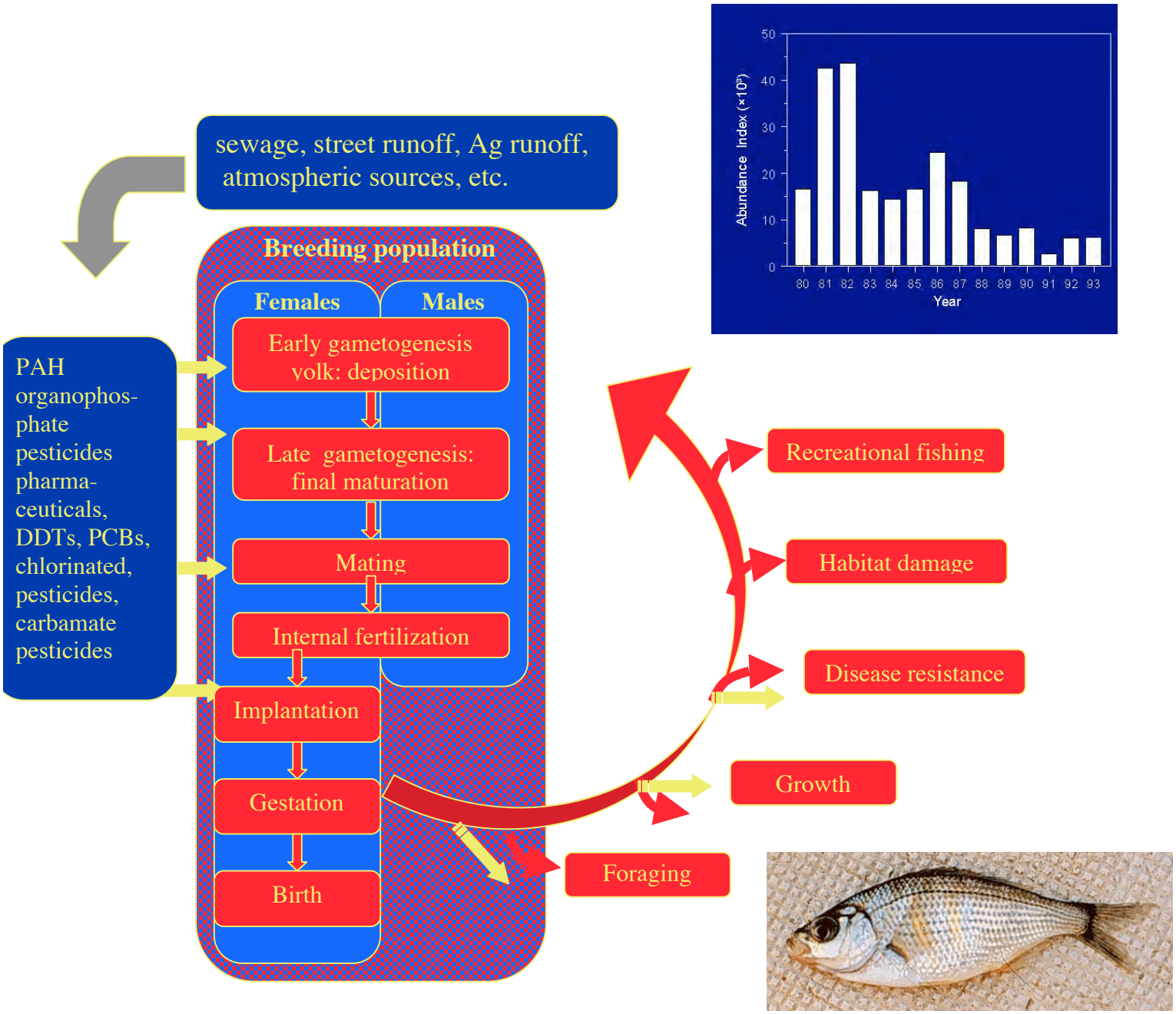

Figure 1. Conceptual model for the effects of contaminants on surfperch populations in the San Francisco Estuary. Yellow arrows are potential influences of contaminants on various processes, such as reproduction, foraging, growth and immune competence. Red arrows are processes that affect the population. Graph insert from Interagency Ecological Program data.

Mating probably takes place in the summer after females give birth. As with other embiotocids (Balz, 1984), males internally fertilize females with a modification of the first fin ray of the anal fin. The sperm are stored in the ovary until, sometime during the winter, fertilization takes place.

Like many nearshore and estuarine fish, numbers decrease in shallow water in Fall and winter and increase again in spring. So, there is probably seasonal inshore-offshore movements.

Successful production of young is key to the continuance of populations and has been shown to be affected by accumulated contaminants (e.g., Spies and Rice 1988). Contaminants have been shown to affect the number of eggs produced (fecundity), successful fertilization, hatching and development of normal larval fish. In the case of embiotocids, development is direct with the fertilized eggs growing inside the lumen of the ovary. The highly vascularized lower intestine of the developing young is interdigitated with the ovary wall, which is well supplied with maternal blood. Females captured in the spring in estuaries have up to about 20 young. The recruitment of just a few individuals to the population occurs after birth and the fully formed young fish are immediately capable of swimming.

Approach

The experimental design was to compare a variety of indicators of condition, reproductive success, growth and contaminant exposure in collections of fish from contaminated sties in San Francisco Bay and a less contaminated comparison site outside the Bay.

Our strategy was to collect at least 20 shiner surfperch at each site in the late spring after the fish had returned to the shallow water of the estuaries and at the time that the females are carrying near-term embryos (in order to estimate fecundity and other measures).

Methods

Locations: Previous work from the SFEI sport fish contaminants survey (Davis et al.. 2001) was used to select locations within San Francisco Bay. Abundance of surfperch and ease of collection with our beach seine were also criteria we used. We settled on two locations in San Francisco Bay that had been sampled previously and from which surfperch had been analyzed for contaminants. The location with the highest concentration of PCBs from the sportfish study, near Candlestick Park in San Francisco, was chosen as was a site with moderately high concentrations of contaminants, Oakland Middle Harbor (Davis et al., 2001). In the first year of the study, 2005, Big River in Mendocino County was chosen as a comparison site. However, elevated EROD values from male fish and colder water temperatures with slower development of the young made this an unsuitable choice. In 2006, we sampled at Heart's Desire Beach, Tomales Bay for the comparison location. The sampling locations are shown in Figures 2-5.

Figure 2. Aerial view of Candlestick Park (SFC) and adjacent beach from Google Earth. Collections taken in area indicated by arrow, approximately 37°42’40.4” N x 122°22’47.48” W.

Figure 3. Aerial view of Oakland Middle Harbor Park beach (OAK) from Google Earth. Collections taken in area indicated by arrow, approximately 37°48’18.18” N x 122°19’31.38” W.

Figure 4. Aerial view of Tomales Bay from Google Earth. Collections taken at Heart's Desire Beach (TB) near area indicated by arrow, approximately 38°06'04.03" N x 122°51'33.01" W.

Figure 5. Aerial view of the mouth of Big River (BR) from Google Earth. Collections taken in area indicated by arrow, approximately 39°18'11.18" N x 123°47'40.97 W.

Collections-Most collections were carried out on slack low tide or flood tide. All collections were done with a 75-ft. beach seine with a pocket located mid-net. The net wings were approximately 5 ft. tall and the mesh openings were 3/8" square. It was deployed in the usual manner by starting close to shore and paying out the net into chest-deep water, and bringing the far end of the net to the beach after towing the net parallel to the beach for a variable length of time. Tows were adjusted for any current, underwater obstacles, free aquatic vegetation and sediment firmness. We did not attempt to sample a fixed area of bottom; our only goal was to collect at least 20 fish from each site. On the beach surfperch were removed from the net as fast as possible and placed in buckets of ambient water and kept cool and well aerated until the seining was complete. Other species of fish were returned to the water. Surfperch were then transferred to 5 gallon buckets in low densities. Each bucket was provided with ice in a plastic bag. Buckets were aerated in 2006 during transport. Transport time to the laboratory varied from 20 minutes to about 1.5 hours.

Dissections-All sample containers (glass vials, cryovials and envelopes) were labeled before the field work was carried out. Fish were removed from their aerated buckets and anesthetized one at a time in a dilute solution of MS-222 in sea water until immobile, except for continued movement of the operula. The excess water was removed from each fish. Whole body weight to the nearest 0.01 gram was determined on a calibrated scale and standard length measured on a ruler to the nearest millimeter. Blood was successfully collected in 2006 by body wall puncture into the pericardium and heart with a glass heparinized micro-capillary tube. The body cavity of each fish was opened with a cut along the ventral line, through the pelvic girdle and then dorsally just posterior to the left pectoral fin. The body cavity was opened by peeling back the resulting flap. Sex was noted: either a single ovary for females or a pair of testes for males. Nearly all females were gravid in our May-June collections and this was also noted. The gonads were removed and weighed wet. With gravid females the young were removed, counted and weighed in total and then the weight of the empty ovary was determined. Portions of the ovary and, in the case of males, a testis, were removed for fixation in 10% buffered formalin. Next, the liver was carefully removed to prevent puncturing the gall baldder and weighed whole. Two sub-samples of liver were removed for flash freezing in liquid nitrogen and the remainder fixed in 10% buffered formalin. In 2005 portions of the gill and heart were also removed and fixed in 10% buffered formalin.

A ventrally-slanting sagital cut behind the eyes was done with a scalpel which usually opened the otic capsule, allowing the largest pair of otoliths to be removed and stored in small labeled paper envelopes.

The remainder of the carcass was wrapped in aluminum foil and frozen at –20°C for later chemical analyses. Liver samples for EROD assay or choriogenin protein assay were transferred to a –80°C freezer.

Samples of liver for EROD assay were shipped in dry shippers saturated with liquid nitrogen to Dr. Peter Hodson's laboratory at Queen's University, Ontario, Canada by Federal Express. All samples arrived frozen at liquid nitrogen temperatures. Likewise,

samples of plasma, liver and ovary were transported to Bodega Marine Laboratory at liquid nitrogen temperatures.

All collection and dissection information were entered in ink in a permanent notebook.

EROD assays- The ethoxyresorufin-*o*-deethylase (EROD) assay, based on an original method by Pohl and Fouts (1980) and modified for a microplate spectrofluorometer (Hodson et al., 1996), was used to measure CYP 1A induction in liver microsomes. Crude activity was calculated by measuring the slope of curves relating fluorescence to time, converting fluorescence to concentration by a resorufin standard curve, and expressing activity as picomole resorufin per minute. Molar specific activity (picomole resorufin per mg protein per minute, or pmol mg-1 min-1) was calculated by normalizing crude activity to S-9 protein concentrations, determined by mixing 50 μl of S-9 fraction with 100 μl Biorad Reagent (Biorad, Hercules, CA, USA) and measuring absorption at 600 nm with a SpectraMax Plus microplate spectrophotometer (Molecular Devices Corporation, Sunnyvale CA, USA). Data were acquired and analyzed with Molecular Devices SOFTMAX PRO software.

Choriogenin assays- Surfperch liver, ovary and blood samples were kept frozen at –80°C until analysis. They were processed as follows:

1. Surfperch liver samples (100 mg/ml) were homogenized on ice in Tris-Hepes EDTA (THE) buffer containing sucrose and protease inhibitor cocktail (Sigma P-2714), then diluted 1:1 with 2X Laemmli sample buffer for electrophoresis..
2. Surfperch ovary samples (100 mg/ml) were homogenized on ice in Tris-Hepes EDTA (THE) buffer containing sucrose and protease inhibitor cocktail (Sigma P-2714), then diluted 4:1 with 5X Laemmli sample buffer.
3. Surfperch blood samples were diluted 1:10 in PBS, then 1:1 with 2X Laemmli sample buffer for electrophoresis.

Electrophoresis and Western blotting:

1. 5-10 ul of sample were run on 16 well 10% PAGR gels (Fisher Scientific) at 160V for @1 hr using a Bio-Rad mini-gel electrophoresis unit.
2. Following electrophoresis, gels were equilibrated in Western blot transfer buffer for 15min.
3. Proteins were transferred to nitrocellulose membranes using a Bio-Rad Trans-Blot semi-dry transfer unit for 1 hr at 10V (250mA/gel).
4. Following transfer, membranes were stained for protein using Ponceau S (0.1%) in 5% acetic acid/95% distilled water
5. Membranes were blocked in PBS/3% non-fat milk for 1 hr at room temperature with rocking.
6. Membranes were incubated in anti-choriogenin antibody (O-146 from Biosense Laboratories in Norway, or anti-herring chorion antibody from Antibodies, Inc. (Davis, CA) overnight at 4^0 C with rocking. Antibodies were diluted into PBS/3% non-fat milk.

7. Membranes were rinsed 2x in distilled water, 1x in PBS/0.05% Tween-20, and 4X in distilled water, 5 min/wash
8. Membranes were incubated in GAR-HRP diluted in PBS/3% non-fat milk for 1 hr at RT with rocking.
9. Membranes were rinsed 2x in distilled water, 1x in PBS/0.05% Tween-20, and 4X in distilled water, 5 min/wash

Proteins were visualized using ECL reagent (Pierce) on a UVP Epi-Chemi II Darkroom (Upland, CA).

Histopathological analyses—These analyses were carried out for 2005 samples by Dr. Mac Law at the Veterinary Pathology Laboratory at the University of North Carolina in conjunction with Dr. David Hinton at Duke University.

Tissues from 2005 were scored for lesions on a scale from 0 to 5, where
0 = no remarkable changes; within normal limits
1 = very mild
2 = mild
3 = moderate
4 = moderately severe
5 = severe (greatest extent of lesions for that particular tissue/condition being described).

This is a semi-quantitative lesion grading scheme commonly used in toxicologic pathology (Hujrty et al., 2002).

In 2006 only ovaries were examined for the occurrence of small oocytes in order to better interpret the reproductive state of the females. Slides were prepared by the UC Davis Veterinary Hospital and interpreted by Dr. Spies.

Statistical analyses- These analyses were carried out using JMP software Version 5.0. Comparison of parameters between sites was done mainly by one-way ANOVA followed by Tukeys HSD for multiple comparisons of site pairs. Data were not transformed, but parallel non-parametric test were run as a check on the validity of test outcomes.

Results

All data analyses, e.g., comparisons between locations, are based on collections carried out in both years. Although Big River collections were included in many of these tests, we generally discount this location as providing useful data for comparing the impacts of contaminants.

Collections-For the main contrasts between parameters at sites, Table 1 shows the numbers of fish collected at each location in the two years of the study with the date of collection in parenthesis.

Table 1. Numbers of shiner surfperch collected at each site by year (month/day).

Location	2005	2006
Big River (BR)	72[a] (5/13)	76[b]
Oakland Middle Harbor (OAK)	3[b] (5/9)	27[a] (5/20)
San Francisco, Candlestick Park Beach (SFC)	24[a] (5/11)	19 (5/24)
Tomales Bay, Heart's Desire Beach (TB)	--------	20 (6/1)

a. only 20 fish were used for comparison of most parameters, but all fish caught in the seine were retained for sex ratio determination.
b. fish used for establishing culture at Bodega Marine Laboratory.

Chemical residues in tissue-The results of organic chemical analyses for the three composite samples from 3 sites in the 2005 collections are presented in Table 2 and Appendix A. Several broad conclusions can be drawn from these data:

1. Tomales Bay fish had the lowest concentrations of nearly all target compounds and the greatest number of "non-detects" (=ND in Table 2).
2. Candlestick Park fish had the highest concentrations of PCBs and a few pesticides (e.g., nanochlor).
3. Oakland Middle Harbor fish had the highest concentrations of DDTs (DDTs, DDDs and DDEs).

Table 2. Contaminant concentrations in 2005 Surfperch carcasses. ($ng^{-1}g$ wet wt.)

Compound/Site	Candlestick Park		Oakland Middle Harbor		Tomales Bay	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
aldrin	ND		ND		ND	
chlordane, cis	2.42	0.54	2.35	0.35	0.72	0.39
chlordane, trans	1.85	0.39	1.82	0.23	1.85	0.39
chlorpyrifos	ND		ND		ND	
dacthal	ND		ND		ND	
DDD, o,p'	0.71	0.10	1.14	0.13	ND	
DDD, p,p'	3.99	1.08	6.81	0.71	0.42	
DDE, o,p'	ND		0.1		ND	
DDE, p,p'	14.73	2.66	21.43	7.56	6.22	3.24
DDMU, p,p'	1.28	0.21	1.77	0.39	ND	
DDT, o,p'	ND		0.2		ND	
DDT, p,p'	0.46		0.7		ND	
diazinon	ND		ND		ND	
dieldrin	1.54	0.29	2.01	0.38	0.37	0.39
endosulfan I	ND		ND		ND	
endrin	ND		ND		ND	
HCH, alpha	ND		ND		ND	
HCH, beta	ND		ND		ND	
HCH, gamma	ND		ND		ND	
heptachlor	ND		ND		ND	
heptachlor epoxide	ND		ND		ND	
hexachlorobenzene	0.16	0.04	0.14	0.02	0.08	
methoxychlor	ND		ND		ND	
mirex	ND		ND		ND	
nonachlor, cis	1.00	0.14	0.97	0.2	ND	
nonachlor, trans	2.31	0.45	1.87	0.12	0.49	0.39
oxadiazon	ND		0.3		ND	
oxychlordane	0.52	0.06	0.37	0.01	ND	
parathion, ethyl	ND		ND		ND	
parathion, methyl	ND		ND		ND	
PCBs (IUPAC congeners)						
8	ND		ND		ND	
18	0.09	0.04	0.12	0.01	ND	
27	ND		ND		ND	
28	0.44	0.12	0.54	0.04	0.08	0.01
29	ND		ND		ND	
31	0.24	0.07	0.31	0.03	0.06	0.004
33	0.02		0.06	0.003	ND	
44	0.59	0.14	0.60	0.06	0.07	0.01
49	1.15	0.20	1.05	0.09	0.09	0.03
52	2.09	0.43	1.76	0.14	0.16	0.04
56	0.06		0.10	0.01	0.02	
60	0.11	0.03	0.13	0.01	ND	
66	0.76	0.19	0.83	0.08	0.11	0.01
70	1.12	0.22	1.17	0.12	0.22	0.02
74	0.73	0.11	0.68	0.06	0.08	0.39

Compound/Site	Candlestick Park		Oakland Middle Harbor		Tomales Bay	
	Mean	St. Dev.	Mean	St. Dev.	Mean	St. Dev.
87	1.75	0.13	1.22	0.16	0.18	0.03
95	3.38	0.75	2.02	0.25	0.18	0.04
97	0.99	0.20	0.75	0.08	0.08	0.01
99	5.98	0.28	4.32	0.44	0.31	0.08
101	10.40	0.26	6.15	0.91	0.46	0.13
105	2.08	0.15	1.38	0.13	0.18	0.03
110	4.39	0.43	3.07	0.42	0.32	0.05
114	0.11	0.03	0.07	0.02	ND	
118	6.84	0.60	4.51	0.51	0.47	0.11
128	1.99	0.12	1.23	0.16	0.07	
137	0.49	0.07	0.32	0.06	ND	
138	15.60	0.70	8.23	1.28	0.51	0.17
141	2.44	0.37	1.00	0.16	0.02	
149	8.20	1.65	4.07	0.73	0.18	0.09
151	4.57	0.48	2.20	0.44	0.22	
153	26.20	2.88	13.67	1.88	0.88	0.31
156	1.29	0.14	0.64	0.09	0.02	
157	0.25	0.03	0.16	0.03	ND	
158	1.72	0.01	0.79	0.08	0.02	
170	4.47	0.30	2.13	0.35	0.15	0.05
174	1.27	0.60	0.59	0.09	0.07	0.02
177	3.72	0.18	2.04	0.35	0.04	
180	13.87	1.63	6.20	0.80	0.25	0.12
183	4.39	0.38	2.19	0.33	0.07	
187	10.06	0.97	5.65	0.81	0.30	0.13
189	0.22	0.03	0.09	0.03	ND	
194	1.83	0.39	0.93	0.12	0.03	
195	0.60	0.09	0.29	0.04	0.07	0.02
200	0.41	0.09	0.28	0.06	ND	
201	1.92	0.35	1.32	0.12	0.02	
203	2.28	0.44	1.21	0.21	ND	
206	0.41	0.14	0.35	0.05	0.07	0.01
209	0.10	0.05	0.14	0.03	ND	
PCB 1248	ND		11.33		ND	
PCB 1254	146.67	5.77	95.00	13.00	6.00	1.00
PCB 1260	72.67	13.58	40.33	5.03	2.67	
∑PCBs (sum congeners)	151.62	4.89	86.55	11.42	5.91	1.78
Moisture	75.30	2.21	73.67	0.99	75.47	1.36
Lipid	2.25	0.78	2.30	0.40	1.99	0.72

Notes: For each site 3 composites of 4 fish each were analyzed. ND values were given a value of 0 when calculating means. Means without standard deviations indicate one or more ND values (0) for the composite samples. Values that fell between the method detection limit and the reporting limit were assigned the value given; these values were given a DNQ designation and flagged in Appendix A.

Morphometrics- The wet weights varied between sites (Table 3). Females were not significantly different between locations (One-way ANOVA; F=0.42, P=0.73), while

there were differences in males, with the larger males being captured at SFC in 2006 and at TB in 2006.

Table 3. Wet weight by location and sex, 2005 and 2006.

Location	Year	Female X±SD (n)	Male X±SD (n)
Big River (BR)	2005	30.8±8.2 (10)	15.2±5.0 (10)
	2006	----------	----------
Oakland Middle Harbor (OAK)	2005	25.6±4.8 (2)	16.6 (1)
	2006	35.5±8.4 (20)	20.6 (1)
San Francisco, Candlestick Park (SFC)	2005	32.2±8.6 (16)	17.7±2.8 (8)
	2006	30.75±9.7 (13)	21.2±1.3 (6)
Tomales Bay, Heart's Desire Beach (TB)	2005	----------	----------
	2006	30.6±7.9 (9)	22.3±3.4 (11)

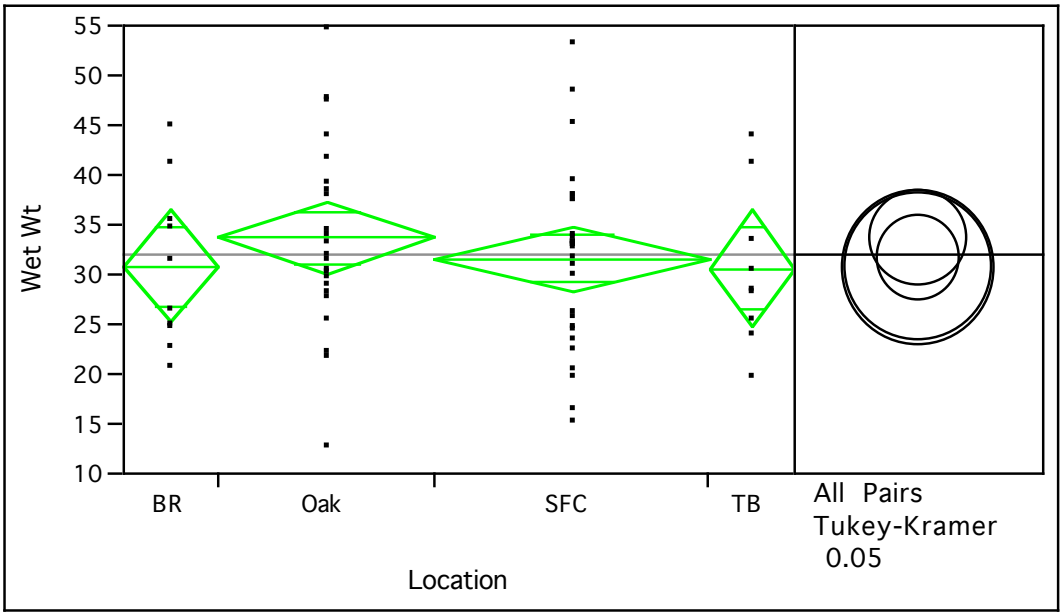

Figure 6. Comparison of wet weights of females between sites. Polygons provide 95% confidence limits, quartiles, means (vertically) and the standard deviations (horizontal). Overlapping circles represent locations that are not significantly different.

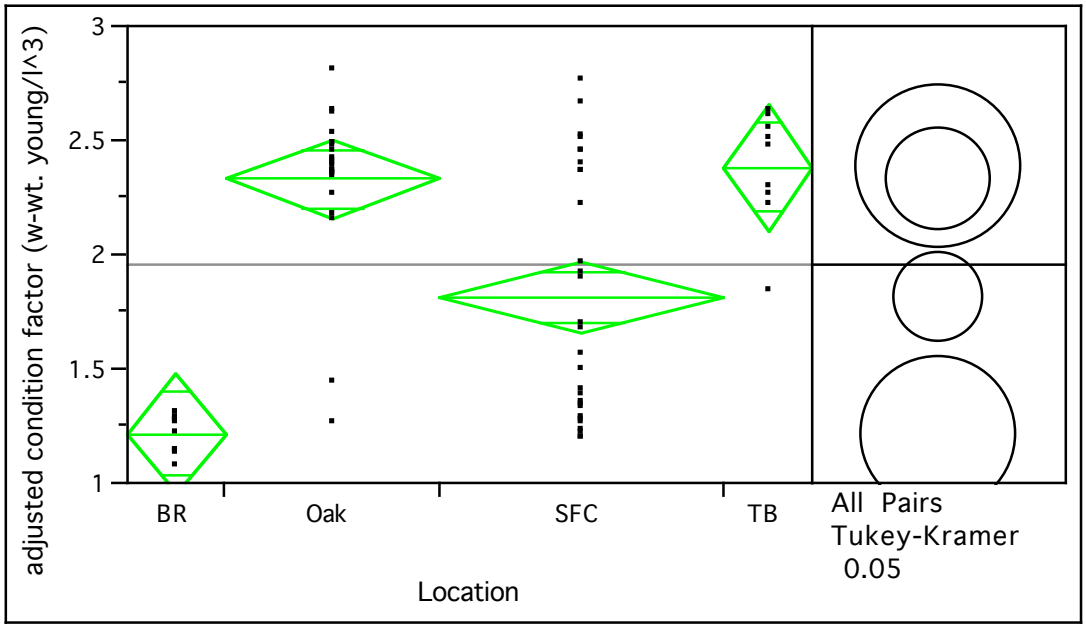

Figure 7. The comparison of adjusted condition factor (wet wt.-weight of young/L^3) between locations for females. Symbols as in previous figure.

While there were no significant differences in wet weight of females from the three locations (Table 3), there were significant differences in the adjusted condition factors. The adjusted condition factor, CF_a= (total wet wt.-weight of young)/L^3). This allows comparison of the weight of females minus the weight of young divided by the standard length cubed. As can be seen in Figure 7 OAK and TB had the greater adjusted condition factors than the other stations. So, Oakland (OAK) =Tomales Bay (TB)>San Francisco, Candlestick (SFC)>Big River (BR) (One-way ANOVA: F=21.19, P<0.0001; Tukey-Kramer, see graphic pair comparison in Figure 8).

Condition factor (wet wt /L^3) for males varied significantly among locations (One-way ANOVA: F=18.45, P<0.0001), and TB=OAK>SFC=BR index (Tukey-Kramer, see graphic pair comparison in Figure 8). This is the same rank order as for females.

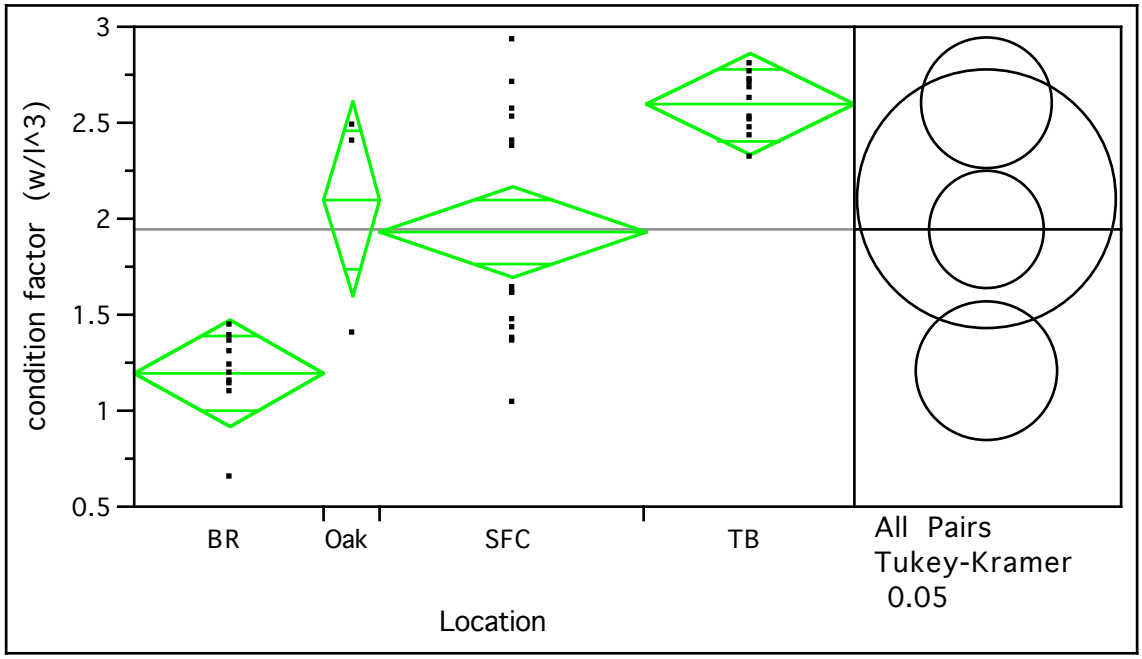

Figure 8. The comparison of male condition factor (wet wt /L^3) between locations. Symbols as in previous figures.

Sex ratios

The sex ratios found in our regular beach seine collections in late spring are presented in Table 4. It can be seen that the beach seines from San Francisco Bay had a predominance of females while those from Big River were close to 50% of each sex. The one collection from Tomales Bay had a preponderance of males.

Table 4. Sex ratios of fish caught in beach seines in late spring (male/female).

Year	Location			
	BR	OAK	SFC	TB
2005	34/38	1/2	8/16	-------
2006	8/8*	1/20	6/13	24/10

* special collection made for estradiol injection experiment.

In addition to the beach seines we have data from two other collections of fish from San Francisco Bay. Both were taken in otter trawls in deeper water. The first collection was taken by Andy Jahn in Oakland Middle Harbor on April 19, 1999 and included 13 males and 12 females. The females included one individual that had both young and a testis, so it was intersex. The second collection was taken on a cruise of the Interagency Ecological Program where shiner surf perch were collected at station 106 on May 10, 2005 and included 8 males and 3 females.

Reproduction-One of the most basic measures of reproductive fitness is gonadosomatic index (GSI), the weight of the gonad divided by the wet weight of the body. We do not compare this measure for females of this live-bearing species, since there are so few eggs, but this is a meaningful comparison for males. As can be seen in Figure 9 GSI rankings were: TB=OAK; TB>BR=SFC and OAK=SFC=BR. (One-way ANOVA: F=10.4.19, P<0.0001; Tukey-Kramer, see graphic pair comparison in Fig. 9). The rank order of stations for this measure is the same as for the condition factor of males and the adjusted condition factor for females.

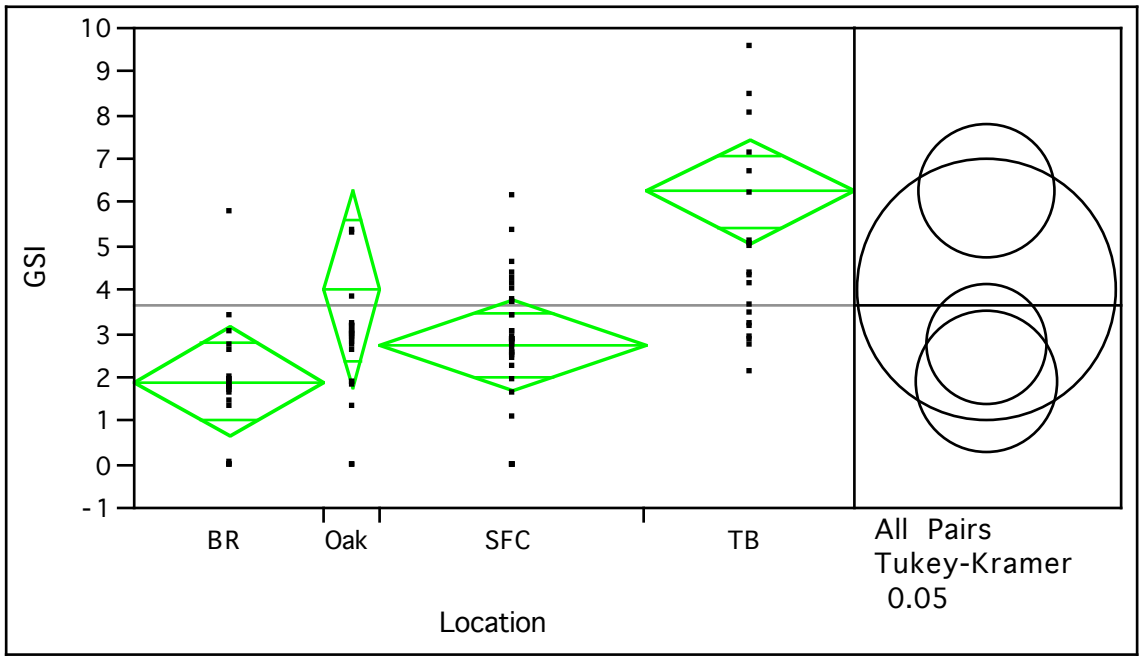

Figure 9. Gonadosomatic indices of males by location. Symbols as in previous figures.

The mean number of young was about 8 except for Oakland, which had a mean of about 12 and the effect of location was significant (One-way ANOVA, F=4.444, P=0.006) (Figure 10). However, a pair-wise comparison of means indicates that none of the pairs were significantly different from one another. Larger, and presumably older, females had more young (Figure 11), with the largest having about 15.

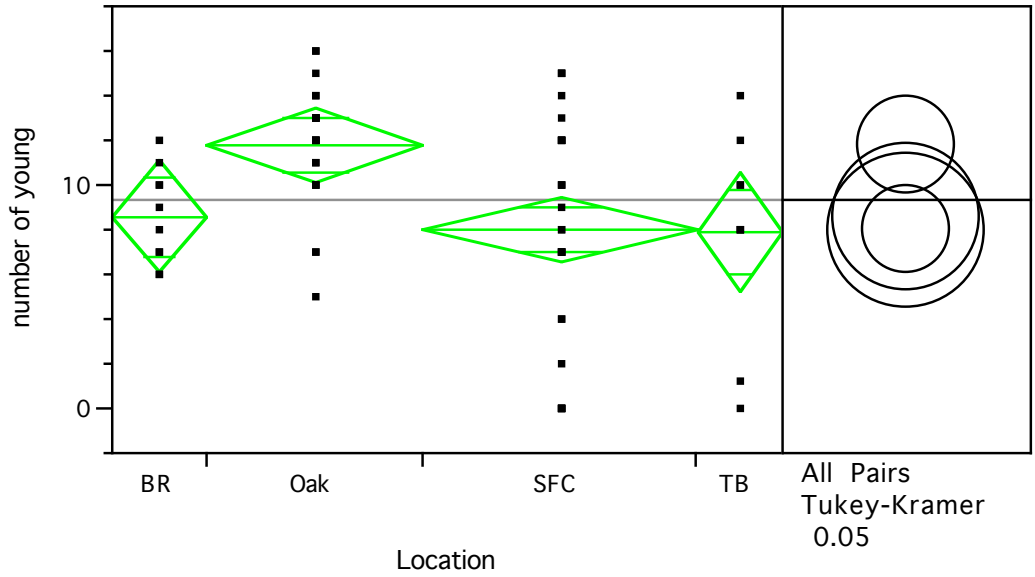

Figure 10. Number of young by site. Symbols as in previous figures.

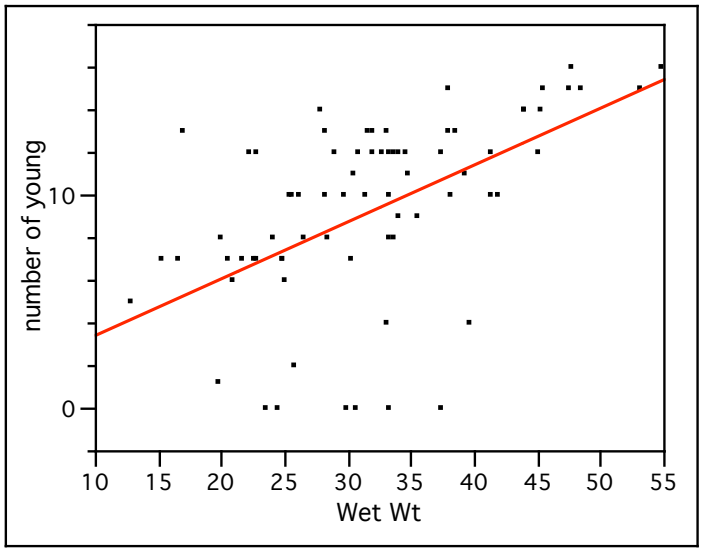

Figure 11. Relationship between number of young and wet weight of females. Number of young=0.716+0.268 wet weight (g) (F=30.1,P<0.0001).

Weight-per-offspring varied significantly with site (One-way ANOVA; F=3.34, P=0.02) with Tomales Bay females having the largest mean weight and Big River the smallest. None of the pairwise comparisons were significantly different (Figure 11).

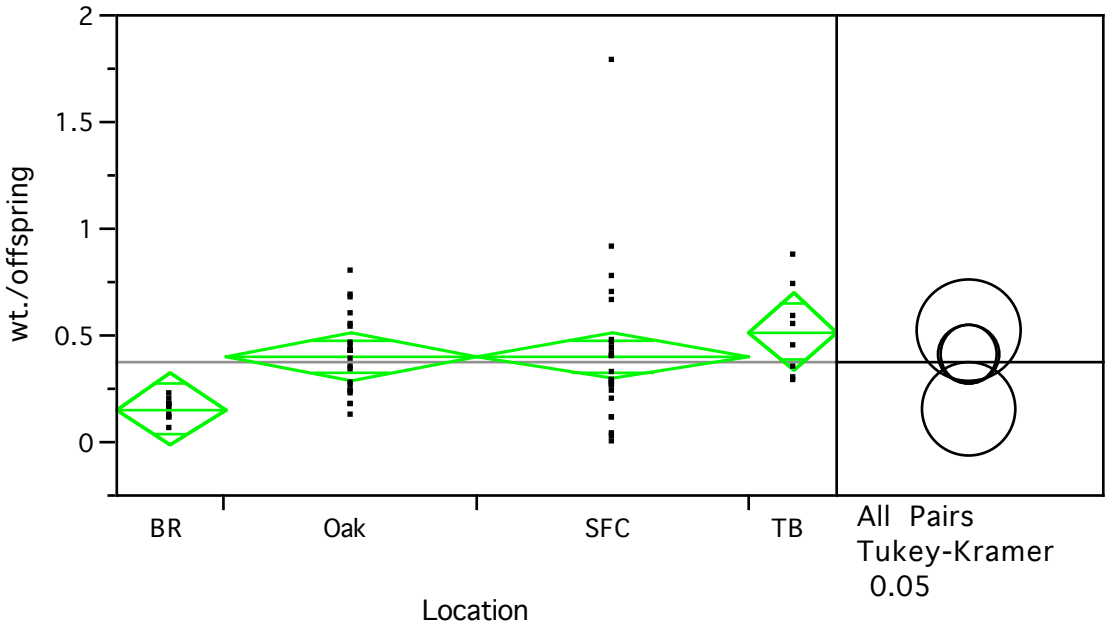

Figure 12. Weight-per-offspring by site. Symbols as in previous figures.

EROD activity- The results of the hepatic EROD assays are provided in Table 5. Both years are presented separately because there were higher values in 2005.

Table 5. Hepatic EROD activities by sex and location (pmol.mg protein-^{1}min^{-1}).

Location	Year	Female X±SD (n)	Male X±SD (n)
Big River (BR)	2005	10.0±9.0 (9)	41.4±49 (9)
	2006	----------	----------
Oakland Middle Harbor (OAK)	2005	0.49±0.002 (2)	54 (1)
	2006	0.57±0.40 (21)	2.7±3.4 (2)
San Francisco, Candlestick Park (SFC)	2005	23.2±30.9 (10)	5.38±3.8 (3)
	2006	1.09±0.81 (13)	4.09±2.3 (6)
Tomales Bay, Heart's Desire Beach (TB)	2005	----------	----------
	2006	0.89±0.63 (9)	1.15±0.4 (11)

There were no significant differences between locations for females (One-way ANOVA; F=2.44, P=0.07). The higher mean value for SFC was due to 2 females without young, which had values greater than 70 pmol.mg protein-^{1}min^{-1}. One of the females was found to have an ovotestis. For males there was significant variation between sites (One-way ANOVA; F=3.96, P<0.019). Oakland (with a single value) and Big River (n=9) had the highest mean values in 2005. The only pair-wise differences between sites was between BR and TB.

Analyzing the years separately indicates that for males, which are generally less susceptible to the effects of the reproductive cycle on EROD activity, there are significant variation between locations (One-way ANOVA; F=6.45, P<0.0095). Site comparisons are as follows: SFC=OAK; OAK=TB; TB<SFC, bearing in mind that there was only one male from OAK in 2006.

Choriogenins

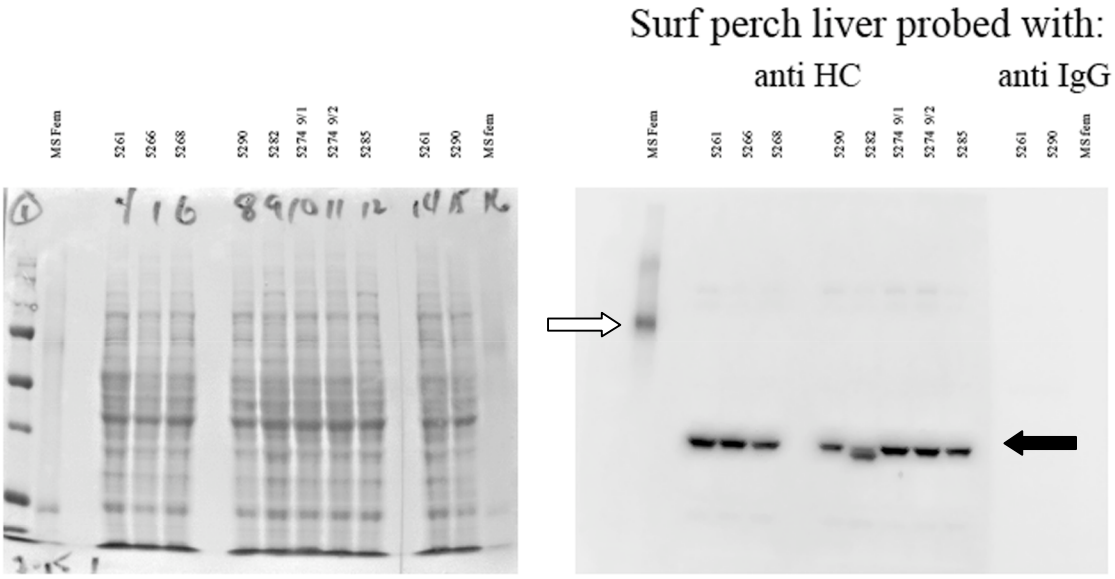

Figure 13. Duplicate electorpohoretic gels of surfperch liver protein. The panel on the left is stained non-specifically for protein by the Ponceau stain. In the Western blot (right panel), plasma from a reproductive mudsucker (positive control) (lane 1 on the left) and liver from surfperch were probed with anti-chorion antibody or with anti-IgG (a control for non-specific staining).

In Figure 13, the mudsucker plasma (right panel, lane 1) shows an expected band at about 84 kDa 9 (white arrow). The surf perch liver produces a strong signal at about 35 kDa (black arrow). However, this molecular weight does not correspond to the known molecular weight for the two isoforms of choriogenins that have been documented in other fish. In addition, this band is found in all of the surfperch tested so far, regardless of sex.

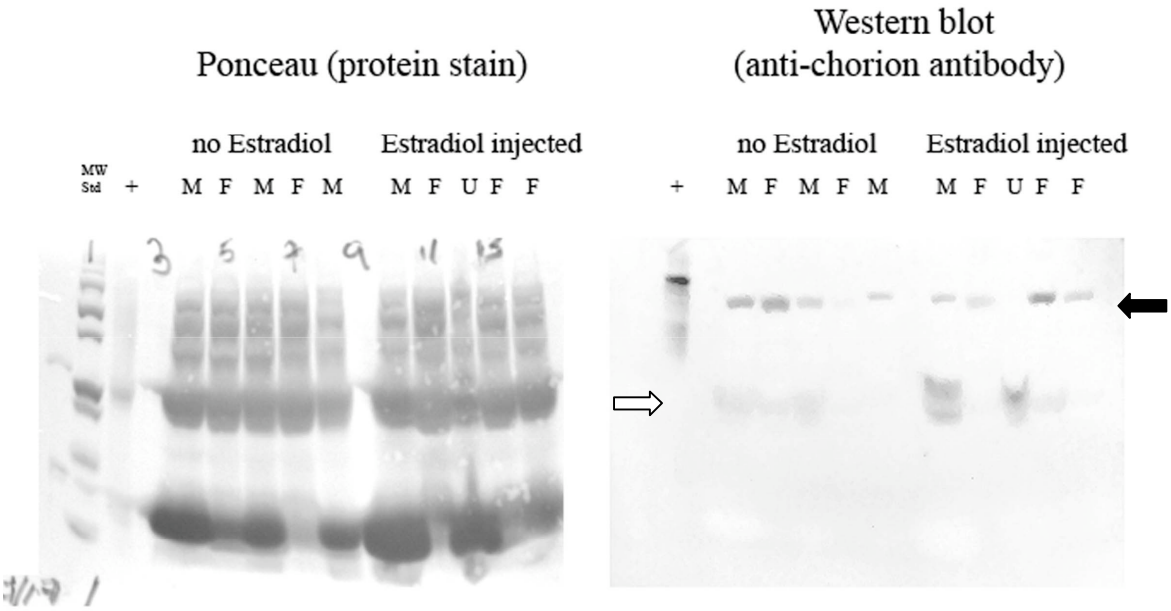

Figure 14. This Western blot shows surfperch plasma samples from fish in the estradiol injection experiment probed with a non-specific protein stain (left) and an anti-chorion antibody (right). Solid black at approximately 60 kDa and whire arrow at approximately 25 kDa.

Plasma from male fish injected with estradiol were subjected to western blots with the anti-chorion antibodies (Figure 14). In the western blot (right panel) there is one protein band (50-75 kDa) that appears in most of the fish, whether injected or not. A 2nd band or pair of bands appears at about 20-25 kDa in some of the estradiol-injected fish. It's possible that this could be choriogenins, but there is some uncertainty.about this It's a very weak signal (a lot of protein was loaded on the blots to even get this signal). Also, it's not at the right molecular weight for known choriogenins in other fish, although the molecular weights can vary from species to species.

Ovarian tissue did not result in a detectable signal. However, the number of oocytes in the ovary was very few and in most fish the few oocytes present were in pre-vitellogenic stages.

Histopathology-A summary of the lesions found in the 2005 samples is presented in Table 6.

Table 6. A summary of histopathological findings from examination of tissues in 2005. Values are individual occurrences/total number of fish examined. Severity scores for individual fish (1, present to 5, severe) in parentheses.

Tissue	Lesion	Location		
		BR	SFC	OAK
Gills	Branchitis	2/20 (1,2)	5/20 (3,3,3,1,2)	0/3
	Lamellar aneurysms	1/20 (2)	2/20 (4,1)	0/3
Liver	Fatty change	1/20	1/20	0/3
Heart	Macrophage aggregates	0/20	0/20	1/3
Gonad	Intersex	1/20	0/20	0/3

The only changes that were particularly notable were inflammatory lesions in the gills (branchitis, 7 of 43 fish). Of these, only 5 were graded as moderate to severe chronic branchitis, characterized by thickening and adhesions of secondary lamellae, infiltration of mixed inflammatory cells, lamellar atrophy, and lamellar aneurysms (dilated capillaries). In no cases were these inflammatory lesions associated with parasites, bacteria, or any other infectious agents apparent in the sections.

The livers contain exocrine pancreatic tissue (hepatopancreas) and showed very few changes from normal (including no pigmented macrophage aggregates, nonspecific indicators of oxidative stress). Some fish had mild to moderate vacuolation, which was either due to fatty change (macrovesicular steatosis) or to increased glycogen content. These lesions are usually nutritionally related, and at these mild-moderate levels are considered reversible changes.

The gonads showed few changes. There was one case of ovotestis (mixed ovarian and testicular tissue) in an SFC fish. A few fish had testes that contained few to no mature spermatozoa (immature vs. empty post-spawn). A few ovaries were pale and devoid of follicles (immature or possibly empty post-spawn).

In summary the most notable changes were branchitis in the gills and this lesion occurred most frequently in fish from SFC.

Discussion

It is useful to look at the outcome of all the measures potentially affected by contamination, ranked from “best” (1) to “worst” (4) for all four locations (Table 6), a sum of ranks procedure. For locations that were not significantly different for a measure tied ranks were given. For the purposes of this exercise it was assumed that the greater the wet weight, condition index, gonadosomatic index, number of young and weight of the offspring, the better the “health” of the local population. While this may be oversimplifying, it does provide a way to look at all the measures for all the sites. Histopathological measures were omitted from this comparison as they were not performed in 2006 and therefore there were no data for Tomales Bay fish.

Table 7. Ranking of measures by location.

Measure	Location			
	BR	OAK	SFC	TB
Female wet wt.	ND	ND	ND	ND
Female condition index	3	1	2	1
Male size	3	2	2	1
Male condition index	3	2	2	1
No. young	2	1	2	2
Weight/offspring	3	2	2	1
Male GSI	2	2	2	1
Male EROD	3	2	2	1
Total of ranks	19	12	14	8

ND= not different

On the basis of this analysis one could rank the sites, from best to worst: TB>SFC>OAK>BR. This is also the order of degree of contamination of PCBs (no PCB data however from the BR fish) (see Table 2). Therefore, these measures of size, weight, condition of fish and offspring, and the EROD biomarker are consistent with an effect of contaminants, in particular PCBs**. These data point to a potential for a chronic effect of contaminants on shiner surfperch that may well have contributed to the decline of this species over the past 30 years.**

Another aspect that arose from data inspection was that there was a group of female surf perch in the 2005 collections from SFC that had a constellation of measures suggestive of contaminant effects, that is they deviated from mean values in a way that identified them as in the poorest health (Table 7). These were all females who had in common gill lesion scores of 2 and greater. As can be seen in the table the mean wet weight was about 10% lower that the average for females from all sites (excluding BR), but the adjusted condition factor was about 32% lower, the number of young 64% lower, and the EROD 403% higher. The trends in these data are very suggestive of an effect on growth and reproduction at this site related to contamination.

Table 8. Comparison of 5 individuals from SFC with moderate to severe gill lesions for growth indicators, reproductive output and contaminant exposure

Acquisition number	Wet Wt. (g)	Cf_a [1]	Number young	EROD ($pg.mgprtn^{-1}.min^{-1}$)	Gill lesion(s) (score)
5272	37.4	1.20	0	83	branchitis(4)
5275	32.8	1.28	0	72	branchitis(3)
5281	32.8	1.36	12	37	branchitis(1) aneurysms (3)
5284	22.6	1.84	7	0.53	branchitis(3)
Mean (above)	28.89	1.40	3.8	48.1	
Mean, all females at all sites but BR	32.42	2.07	9.5	19.4	

1. adjusted condition factor.

In a study of rainbow and rubberlip surfperch, the same type of gill lesions were identified in fish from a petroleum seep near Santa Barbara (Spies et al., 1996). It was speculated then that severe gill lesions might compromise fish health and the findings here are consistent with that possibility.

Our findings are consistent with effects of contaminants on tissue integrity in the gills, the growth and condition of adults, and the size of young either as direct toxicity, probably chronic in nature, or as differences in energy expenditure, energy intake or disease and parasite occurrence (little evidence for this last possibility). While the findings are consistent with the effects of contamination there are other possible explanations as well. For example, forage and feeding opportunities in Tomales Bay may be better than in the 2 San Francisco Bay sites. Or, energy expenditures may be greater at the contaminated sites for a variety of possible reasons related to the nature of the ecosystem there. There could also of course be an interaction between poor conditions in San Francisco Bay (altered habitat, e.g., apparent lack of well developed beds of *Zostera*, a favorite habitat of this species) and contaminants.

We have not measured growth directly but have used size as a surrogate for growth. However, since we have retained the otoliths from the fishes as they were dissected it would be possible to measure growth rates if the resources became available. Such measurements, even daily growth rates, have been done with the closely related dwarf surf perch (Schultz, 1993a,b).

EROD-The EROD activities measured showed a marked difference between locations and sex consistent with previous findings in San Francisco Bay fish. For example, mixed function oxidases have been shown to be elevated in contaminated portions of San Francisco Bay and to be suppressed in female starry flounder during active gametogenesis (e.g., Spies et al., 1988, 1990). Experimental exposure to San Francisco Bay sediments has elevated EROD activities in speckled sanddabs (Gunther et al., 1997). However, in this live-bearing species one would not necessarily expect to find the

suppression during pregnancy. The activity of mixed function oxidases is known to be suppressed by estradiol (e.g., Vacarro et al., 2005), which one would expect to see secreted in large quantities during gametogenesis. On the other hand, in sections of the ovary from these same female surfperch in which there were low EROD activities early stage oocytes were seen and the estradiol titers in these fish are not known. In any case relatively high values found in contaminated sites compared to the control site in males in 2006, as well as the higher values in 2005 males from the SFC and OAK confirm the usefulness of this marker for surfperch contaminant exposure if properly interpreted.

The much lower values for EROD in SFC and OAK fish in 2006 compared to 2005 could be due to the exposure conditions in 2006, with the large amounts of freshwater from late and heavy rains emptying through the estuary in 2006. We sampled about 2 weeks later in the spring of 2006 on account of these conditions, which could have had some effect on these measurements as well. We have also investigated the possibility that somehow the 2006 samples were compromised leading to loss of P4501A catalytic activity, but the samples were frozen immediately at dissection and maintained at –80°C in an ultra-cold freezer at Applied Marine Sciences until transported in a dry shipper at liquid nitrogen temperatures. The samples arrived frozen at Queens University and were maintained at –80°C in the freezer there until analysis. In fact we had saved an additional aliquot of liver tissue at Livermore and sent this second set of liver samples to Queen's University to be analyzed and very similar results were obtained.

Recommendations for further work-

1. Field work-In view of the differences in male size and condition indices, female adjusted index and to some extent the weight of the young between locations, it would be useful to have specific ages for the fish used in the comparisons. The literature suggests that shiner surfperch live for 2-3 years. While nearly all the shiner surfperch caught in SF Bay and TB were sexually mature, ages may have differed by 1-3 years. Ages and growth rates could be derived from existing otoliths and we have otoliths from nearly all fish used in the study.

A second year of field work using Tomales Bay as the reference would also strengthen the data on which to make inferences about contaminant effects. In particular, new EROD analyses, analyzing gill for histopathological analyses, and having more collections for sex ratios. Morphometric analyses of juvenile surfperch taken from their mothers in 2005 and 2006 from all locations, as well as analysis for morphological abnormalities could be very useful.

2. Laboratory experiments—Long-term exposures of surfperch to contamination found at the OAK and SFC sites is needed to determine if they result in negative effects such as found in this field study.

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Appendix A. Chemical concentrations in carcasses of surfperch collected in 2006. Three composites were analyzed from each location. Each composite consisted of four fish.

	Candlestick Comp 1A	Candlestick Comp 1B	Candlestick Comp 1C	Candlestick		Oakland Middle H Comp 2A	Oakland Middle H Comp 2B	Oakland Middle H Comp 2C	Oakland		Tomales Bay Comp 3A	Tomales Bay Comp 3B	Tomales Bay Comp 3C	Tomales Bay	
	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation
aldrin	ND	ND	ND			ND	ND	ND			ND	ND	ND		
chlordane, cis	2.96	1.89	2.42	2.4	0.5	2.06	2.24	2.74	2.3	0.4	0.712	0.81	0.64	0.7	0.4
chlordane, trans	2.30	1.64	1.61	1.9	0.4	1.55	1.95	1.96	1.8	0.2	0.986	1.09	0.974	1.9	0.4
chlorpyrifos	ND	ND	ND			ND	ND	ND			ND	ND	ND		
dacthal	ND	ND	ND			ND	ND	ND			ND	ND	ND		
DDD, o,p'	0.816	0.609	0.711	0.7	0.1	1.15	1.00	1.26	1.1	0.1	ND	ND	ND		
DDD, p,p'	4.13	2.85	5.00	4.0	1.1	7.04	6.02	7.38	6.8	0.7	0.691	0.582	ND	0.4	
DDE, o,p'	ND	ND	ND			0.356	ND	ND	0.1		ND	ND	ND		
DDE, p,p'	17.5	14.5	12.2	14.7	2.7	30.1	16.2	18.0	21.4	7.6	9.11	6.82	2.72	6.2	3.2
DDMU, p,p'	1.50	1.08	1.25	1.3	0.2	1.83	1.49	1.98	1.8	0.4	ND	ND	ND		
DDT, o,p'	ND	ND	ND			ND	ND	0.564	0.2		ND	ND	ND		
DDT, p,p'	1.39	ND	ND	0.5		ND	ND	1.97	0.7		ND	ND	ND		
diazinon	ND	ND	ND			ND	ND	ND			ND	ND	ND		
dieldrin	1.65	1.77	1.21	1.5	0.3	2.44	1.89	1.71	2.0	0.4	0.361	0.403	0.354	0.4	0.4
endosulfan I	ND	ND	ND			ND	ND	ND			ND	ND	ND		
endrin	ND	ND	ND			ND	ND	ND			ND	ND	ND		
HCH, alpha	ND	ND	ND			ND	ND	ND			ND	ND	ND		
HCH, beta	ND	ND	ND			ND	ND	ND			ND	ND	ND		
HCH, gamma	ND	ND	ND			ND	ND	ND			ND	ND	ND		
heptachlor	ND	ND	ND			ND	ND	ND			ND	ND	ND		
heptachlor epoxide	ND	ND	ND			ND	ND	ND			ND	ND	ND		
hexachlorobenzene	0.177	0.197	0.113	0.2	0.04	0.155	0.126	0.129	0.1	0.02	0.159	0.094	ND	0.1	
methoxychlor	ND	ND	ND			ND	ND	ND			ND	ND	ND		
mirex	ND	ND	ND			ND	ND	ND			ND	ND	ND		
nonachlor, cis	1.10	0.84	1.06	1.0	0.1	0.836	0.864	1.21	1.0	0.2	ND	ND	ND		
nonachlor, trans	2.42	1.82	2.70	2.3	0.4	1.9	1.73	1.97	1.9	0.1	0.493	0.613	0.362	0.5	0.4
oxadiazon	ND	ND	ND			0.495	0.467	ND	0.3		ND	ND	ND		
oxychlordane	0.591	0.471	0.494	0.5	0.1	0.371	0.352	0.378	0.4	0.01	ND	ND	ND		
parathion, ethyl	ND	ND	ND			ND	ND	ND			ND	ND	ND		
parathion, methyl	ND	ND	ND			ND	ND	ND			ND	ND	ND		
DDD*, p,p'	74.7	69.9	80.1	74.9	5.1	85.2	80.7	79.3	81.7	3.1	82.5	85.3	84.8	84.2	1.5
DBCE	89.7	85.6	71.5	82.3	9.5	69.0	77.3	30.0	58.8	25.3	80.2	75.9	74.2	76.8	3.1
PCBs															
8	ND	ND	ND			ND	ND	ND			ND	ND	ND		
18	0.085	0.136	0.063	0.1	0.04	0.133	0.111	0.125	0.1	0.01	ND	ND	ND		
27	ND	ND	ND			ND	ND	ND			ND	ND	ND		
28	0.404	0.573	0.339	0.4	0.1	0.512	0.529	0.591	0.5	0.04	0.096	0.067	0.085	0.1	0.01
29	ND	ND	ND			ND	ND	ND			ND	ND	ND		
31	0.207	0.316	0.187	0.2	0.1	0.301	0.291	0.351	0.3	0.03	0.059	0.051	0.056	0.1	0.004
33	ND	0.062	ND	0.02		0.057	0.062	0.062	0.1	0.003	ND	ND	ND		
44	0.555	0.742	0.471	0.6	0.1	0.573	0.566	0.67	0.6	0.1	0.068	0.074	0.054	0.1	0.01
49	1.09	1.37	0.978	1.1	0.2	0.993	0.999	1.16	1.1	0.1	0.115	0.091	0.062	0.1	0.03
52	1.89	2.59	1.80	2.1	0.4	1.68	1.69	1.92	1.8	0.1	0.187	0.179	0.121	0.2	0.04
56	0.077	0.107	ND	0.06		0.093	0.092	0.109	0.1	0.01	ND	ND	0.049	0.02	
60	0.101	0.144	0.083	0.1	0.03	0.121	0.136	0.131	0.1	0.01	ND	ND	ND		

	Candlestick Comp 1A	Candlestick Comp 1B	Candlestick Comp 1C	Candlestick		Oakland Middle H Comp 2A	Oakland Middle H Comp 2B	Oakland Middle H Comp 2C	Oakland		Tomales Bay Comp 3A	Tomales Bay Comp 3B	Tomales Bay Comp 3C	Tomales Bay	
	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation	ng/g Wet	ng/g Wet	ng/g Wet	mean	s.deviation
66	0.667	0.988	0.636	0.8	0.2	0.795	0.777	0.917	0.8	0.1	0.102	0.099	0.121	0.1	0.01
70	0.908	1.35	1.10	1.1	0.2	1.15	1.07	1.3	1.2	0.1	0.237	0.199	0.211	0.2	0.02
74	0.626	0.854	0.719	0.7	0.1	0.658	0.629	0.745	0.7	0.1	0.091	0.071	0.068	0.1	0.4
87	1.63	1.74	1.89	1.8	0.1	1.15	1.10	1.40	1.2	0.2	0.212	0.156	0.163	0.2	0.03
95	3.11	4.23	2.80	3.4	0.8	1.89	1.86	2.30	2.0	0.2	0.215	0.175	0.139	0.2	0.04
97	0.874	1.22	0.873	1.0	0.2	0.693	0.721	0.847	0.8	0.1	0.09	0.067	0.077	0.1	0.01
99	5.90	6.29	5.75	6.0	0.3	4.05	4.08	4.83	4.3	0.4	0.386	0.312	0.227	0.3	0.1
101	10.1	10.6	10.5	10.4	0.3	5.65	5.61	7.20	6.2	0.9	0.609	0.406	0.365	0.5	0.1
105	1.92	2.09	2.22	2.1	0.2	1.38	1.25	1.51	1.4	0.1	0.208	0.172	0.149	0.2	0.03
110	4.05	4.87	4.26	4.4	0.4	2.83	2.83	3.55	3.1	0.4	0.369	0.273	0.315	0.3	0.05
114	0.088	0.107	0.143	0.1	0.03	0.07	0.049	0.085	0.1	0.02	ND	ND	ND		
118	6.35	6.66	7.50	6.8	0.6	4.39	4.07	5.06	4.5	0.5	0.591	0.421	0.384	0.5	0.1
128	2.05	1.86	2.07	2.0	0.1	1.15	1.13	1.42	1.2	0.2	0.076	0.073	ND	0.1	
137	0.509	0.413	0.557	0.5	0.1	0.319	0.272	0.383	0.3	0.1	ND	ND	ND		
138	16.4	15.1	15.3	15.6	0.7	7.80	7.21	9.67	8.2	1.3	0.664	0.547	0.326	0.5	0.2
141	2.61	2.69	2.02	2.4	0.4	0.908	0.897	1.18	1.0	0.2	0.061	ND	ND	0.02	
149	8.46	9.70	6.44	8.2	1.6	3.54	3.77	4.91	4.1	0.7	0.281	0.165	0.102	0.2	0.1
151	4.95	4.74	4.03	4.6	0.5	1.97	1.92	2.71	2.2	0.4	0.135	0.086	ND	0.2	
153	29.4	23.8	25.4	26.2	2.9	13.3	12.0	15.7	13.7	1.9	1.18	0.891	0.569	0.9	0.3
156	1.34	1.13	1.39	1.3	0.1	0.623	0.558	0.731	0.6	0.1	0.051	ND	ND	0.02	
157	0.266	0.214	0.266	0.2	0.03	0.159	0.131	0.196	0.2	0.03	ND	ND	ND		
158	1.72	1.72	1.71	1.7	0.01	0.801	0.712	0.870	0.8	0.1	0.058	ND	ND	0.02	
170	4.66	4.12	4.62	4.5	0.3	2.08	1.81	2.51	2.1	0.4	0.195	0.146	0.098	0.1	0.05
174	1.07	1.94	0.789	1.3	0.6	0.522	0.550	0.694	0.6	0.1	0.096	0.052	0.062	0.1	0.02
177	3.79	3.52	3.85	3.7	0.2	1.95	1.74	2.43	2.0	0.4	0.082	0.049	ND	0.04	
180	15.3	12.1	14.2	13.9	1.6	6.24	5.38	6.98	6.2	0.8	0.351	0.281	0.119	0.3	0.1
183	4.63	3.95	4.60	4.4	0.4	2.22	1.84	2.50	2.2	0.3	0.115	0.098	ND	0.1	
187	11.0	9.07	10.1	10.1	1.0	5.58	4.88	6.49	5.7	0.8	0.415	0.325	0.168	0.3	0.1
189	0.197	0.198	0.251	0.2	0.03	0.06	0.083	0.113	0.1	0.02	ND	ND	ND		
194	2.07	1.38	2.04	1.8	0.4	0.895	0.823	1.06	0.9	0.1	0.09	ND	ND	0.03	
195	0.635	0.499	0.679	0.6	0.1	0.289	0.256	0.332	0.3	0.04	0.089	0.056	0.054	0.1	0.02
200	0.466	0.305	0.450	0.4	0.1	0.273	0.223	0.335	0.3	0.1	ND	ND	ND		
201	2.17	1.52	2.06	1.9	0.3	1.31	1.21	1.44	1.3	0.1	ND	0.054	ND	0.02	
203	2.49	1.78	2.58	2.3	0.4	1.32	0.960	1.34	1.2	0.2	0.119	0.051	ND		
206	0.363	0.301	0.566	0.4	0.1	0.402	0.309	0.330	0.3	0.05	0.061	0.075	0.066	0.1	0.01
209	0.069	0.072	0.164	0.1	0.1	0.168	0.107	0.132	0.1	0.03	ND	ND	ND		
PCB 1248	ND	ND	ND			11	11	12	11.3	0.0	ND	ND	ND		
PCB 1254	150	150	140	146.7	5.8	88	87	110	95.0	13.0	7	6	5	6.0	1.0
PCB 1260	80	57	81	72.7	13.6	41	35	45	40.3	5.0	5	3	ND	2.7	
Moisture [(a)]	75.1	73.2	77.6	75.3	2.2	73.0	74.8	73.2	73.7	1.0	74.4	75.0	77.0	75.5	1.4
Lipid [(a)]	2.24	3.03	1.48	2.3	0.8	2.62	2.43	1.85	2.3	0.4	2.80	1.76	1.42	2.0	0.7
Surrogate %R	%R	%R	%R			%R	%R	%R			%R	%R	%R		

Values that were reported from the laboratory with a DNQ designation, i.e., falling between not detected (ND) and the reporting limit.

(a) **= Percent values**

Appendix B. Data on individual fishes used in this study.

Acquisition No.	Year	Date	Alive/dead	Location	S.L.	Wet Wt.[1]	Sex	Liver Wt.	Testis	Ovary	Gonad +young	Total Young Wt.	Number of young	Wt./offspring	Condition factor (w/l^3)	Adjusted condition factor (w-wt. young/l^3)	GSI	EROD[2]
5236	2005	5/9/05		Oak	10.6	16.64	male	0.11	0.88			0			1.40	1.40	5.29	54.18
5238	2005	5/9/05		Oak	10.8	22.26	female	0.2			4	4	12	0.33	1.77	1.45	0.00	0.492
5243	2005	5/9/05		Oak	12	29.03	female	0.19			7.21	7.21	12	0.60	1.68	1.26	0.00	0.489
5261	2005	5/10/05		IEP 106	14	45.35	female	0.99		0.6	11.89	11.29	15	0.75	1.65	1.24	1.32	0.31
5262	2005	5/10/05	dead	IEP 106	10.7	19.36	male		0.91						1.58	1.58	4.70	
5263	2005	5/10/05	dead	IEP 106	11.2	24.24	male	0.31	0.71						1.73	1.73	2.93	
5264	2005	5/10/05	dead	IEP 106	11.2	16.17	male	0.26	0.63						1.15	1.15	3.90	
5265	2005	5/10/05		IEP 106	11.5	19.56	male	0.27	0.32						1.29	1.29	1.64	28.22
5266	2005	5/10/05		IEP 106	10.9	16.93	female	0.19		0.3	0.92	0.62	13	0.05	1.31	1.26	1.77	37.85
5267	2005	5/10/05		IEP 106	10.6	16.18	male	0.34	0.4						1.36	1.36	2.47	153.29
5268	2005	5/10/05		IEP 106	11.9	22.82	female	0.34		0.87			12	0.04	1.35	1.35	3.81	1.21
5269	2005	5/10/05		IEP 106	10	15.22	male	0.07				0			1.52	1.52	0.00	3.275
5270	2005	5/10/05		IEP 106	8.8	8.52	male	0.17	0.16						1.25	1.25	1.88	2.342
5271	2005	5/10/05		IEP 108	11.8	24.5	male	0.2	0.12						1.49	1.49	0.49	81.556
5272	2005	5/11/05		SFC	14.6	37.4	female	0.53		0.61	0.61	0	0		1.20	1.20	1.63	83.286
5273	2005	5/11/05		SFC	12.7	37.95	female	0.56		0.96	4.46	3.5	15	0.23	1.85	1.68	2.53	5.125
5274	2005	5/11/05		SFC	13	33.23	female	0.48			2.63	2.63	10	0.26	1.51	1.39	0.00	15.75
5275	2005	5/11/05		SFC	12.4	24.39	female	0.26			1.01	1.01	0		1.28	1.23	0.00	72.5
5276	2005	5/11/05		SFC	10.5	18.71	male	0.19	0.8						1.62	1.62	4.28	1.575
5277	2005	5/11/05		SFC	12.4	30.88	female	0.46		0.78	4.67	3.89	12	0.32	1.62	1.42	2.53	0.639
5278	2005	5/11/05		SFC	12.6	33.17	female	0.4		0.91	7.15	6.24	8	0.78	1.66	1.35	2.74	10.87
5279	2005	5/11/05		SFC	13.9	48.45	female	0.62		1.64	15.4	13.76	15	0.92	1.80	1.29	3.38	
5280	2005	5/11/05		SFC	14.3	45.31	female	0.62		1.29	7.12	5.83	14	0.42	1.55	1.35	2.85	4.857
5281	2005	5/11/05		SFC	13.4	32.78	female	0.6		0.84	3.23	2.39	12	0.20	1.36	1.26	2.56	37.111
5282	2005	5/11/05		SFC	10.9	17.56	male	0.16	0.81						1.36	1.36	4.61	5.356
5283	2005	5/11/05		SFC	10.8	15.21	female	0.2		0.16	0.31	0.15	7	0.02	1.21	1.20	1.05	1.738
5284	2005	5/11/05		SFC	10.7	22.58	female	0.29		0.55	3.87	3.32	7	0.47	1.84	1.57	2.44	0.532
5285	2005	5/11/05		SFC	10.6	19.16	male	0.13	0.77						1.61	1.61	4.02	9.224
5286	2005	5/11/05	dead	SFC	11.4	25.72	female	0.2		0.8	3.57	3.57	2	1.79	1.74	1.50	0.00	
5287	2005	5/11/05	dead	SFC	12.8	33.63	female			0.94	6.51	5.57	12	0.46	1.60	1.34	2.80	
5288	2005	5/11/05	dead	SFC	13.7	33.29	female	0.64			0.5	0.5	0		1.29	1.28	0.00	
5289	2005	5/11/05	dead	SFC	12.3	23.49	female	0.36			0.62	0.62	0		1.26	1.23	0.00	
5290	2005	5/11/05	dead	SFC	10.2	11.11	male		0.32						1.05	1.05	2.88	
5291	2005	5/11/05	dead	SFC	12.6	38.11	female	0.66		1.09	5.11	4.02	10	0.40	1.91	1.70	2.86	
5292	2005	5/11/05	dead	SFC	10.8	17.96	male					0			1.43	1.43	0.00	
5293	2005	5/11/05	dead	SFC	10.8	18.63	male					0			1.48	1.48	0.00	
5294	2005	5/11/05	dead	SFC	11	18.31	male					0			1.38	1.38	0.00	
5295	2005	5/11/05	dead	SFC	10.7	20.14	male					0			1.64	1.64	0.00	
5296	2005	5/14/05		BR	13.6	31.42	female	0.53		0.54	1.16	0.62	10	0.06	1.25	1.22	1.72	0.586
5297	2005	5/14/05		BR	11.6	10.2	male	0.13			0.26	0.26			0.65	0.64	0.00	2.259

Acquisition No.	Year	Date	Alive/dead	Location	S.L.	Wet Wt.[1]	Sex	Liver Wt.	Testis	Ovary	Gonad +young	Total Young Wt.	Number of young	Wt./offspring	Condition factor (w/l^3)	Adjusted condition factor (w-wt. young/l^3)	GSI	EROD[2]
5298	2005	5/14/05		BR	11.1	18.92	male	0.24	0.64						1.38	1.38	3.38	6.975
5299	2005	5/14/05		BR	11	17.41	male	0.18	0.53						1.31	1.31	3.04	2.798
5300	2005	5/14/05		BR	8.7	7.26	male	0.12	0.001						1.10	1.10	0.01	108.818
5301	2005	5/14/05		BR	10	11.48	male	0.12	1E-04						1.15	1.15	0.00	101
5302	2005	5/14/05		BR	12.8	24.75	female	0.56		0.45	1.36	0.91	7	0.13	1.18	1.14	1.82	17.7
5303	2005	5/14/05		BR	11.5	18.15	male	0.22	0.24						1.19	1.19	1.32	3.604
5304	2005	5/14/05		BR	10.9	17.61	male	0.67	0.46						1.36	1.36	2.61	
5305	2005	5/14/05		BR	9.5	10.54	male	0.09	1E-04						1.23	1.23	0.00	0.511
5306	2005	5/14/05		BR	14.4	41.3	female	0.82		0.66	2.9	2.24	10	0.22	1.38	1.31	1.60	0.603
5307	2005	5/14/05		BR	15	45	female	0.59		0.86	3.27	2.41	12	0.20	1.33	1.26	1.91	12.25
5308	2005	5/14/05		BR	12.4	25.01	female	0.31		0.48	1.19	0.71	6	0.12	1.31	1.27	1.92	20.375
5309	2005	5/14/05		BR	12	20.82	female	0.23		0.37	1.33	0.96	6	0.16	1.20	1.15	1.78	1.514
5310	2005	5/14/05		BR	13	26.48	female	0.27		0.53	1.84	1.31	8	0.16	1.21	1.15	2.00	1.235
5311	2005	5/14/05		BR	12.7	22.79	female	0.23		0.4	1.2	0.8	7	0.11	1.11	1.07	1.76	21
5312	2005	5/14/05		BR	11.7	23.15	male	0.28	1.34						1.45	1.45	5.79	104.889
5313	2005	5/14/05		BR	13.8	35.53	female	0.59		0.52	2.05	1.53	9	0.17	1.35	1.29	1.46	15.12
5314	2005	5/14/05		BR	13.7	34.72	female	0.45		0.65	2.58	1.93	11	0.18	1.35	1.28	1.87	
5315	2005	5/14/05		BR	11.4	16.84	male	0.15	0.46						1.14	1.14	2.73	42.273
5350	2006	5/20/06		Oak	10.7	34.5	female	0.31		1	6.5	5.5	12	0.46	2.82	2.37	2.90	1.01
5351	2006	5/20/06		Oak	10.7	34	female	0.29		1.03	6.18	5.15	12	0.43	2.78	2.36	3.03	1.34
5352	2006	5/20/06		Oak	10.5	30.5	female	0.49		0.87	3.31	2.44	11	0.22	2.63	2.42	2.85	0.3
5353	2006	5/20/06		Oak	12	47.73	female	0.3		1.46	8.3	6.84	16	0.43	2.76	2.37	3.06	0.49
5354	2006	5/20/06		Oak	11.5	43.88	female	0.26		1.69	12.81	11.12	14	0.79	2.89	2.15	3.85	0.61
5355	2006	5/20/06		Oak	10.5	30.25	female	0.25		0.92	3.4	2.48	7	0.35	2.61	2.40	3.04	0.53
5356	2006	5/20/06		Oak	11	41.79	female	0.23		1.23	7.98	6.75	10	0.68	3.14	2.63	2.94	0.44
5357	2006	5/20/06		Oak	10.5	31.51	female	0.18		0.85	4.43	3.58	13	0.28	2.72	2.41	2.70	0.36
5358	2006	5/20/06		Oak	10.25	33.26	female	0.19		0.98	7.58	6.6	12	0.55	3.09	2.48	2.95	0.93
5359	2006	5/20/06		Oak	11.25	38.5	female	0.25		1.21	4.28	3.07	13	0.24	2.70	2.49	3.14	0.18
5360	2006	5/20/06		Oak	10	28.19	female	0.21		0.78	4.13	3.35	13	0.26	2.82	2.48	2.77	0.18
5361	2006	5/20/06		Oak	12.5	54.73	female	0.56		1.03	7.85	6.82	16	0.43	2.80	2.45	1.88	0.17
5362	2006	5/20/06		Oak	10.5	31.89	female	0.22		1.02	6.62	5.6	13	0.43	2.75	2.27	3.20	0.32
5363	2006	5/20/06		Oak	12	47.45	female	0.23		1.38	8.04	6.66	15	0.44	2.75	2.36	2.91	0.42
5364	2006	5/20/06		Oak	10	27.74	female	0.28		0.72	3.17	2.45	14	0.18	2.77	2.53	2.60	0.63
5365	2006	5/20/06		Oak	10.75	39.3	female	0.31		1.24	5.57	4.33	11	0.39	3.16	2.81	3.16	0.56
5366	2006	5/20/06		Oak	10	25.57	female	0.15		0.47	2.17	1.7	10	0.17	2.56	2.39	1.84	0.4
5367	2006	5/20/06		Oak	11	37.94	female				8.99	8.99	13	0.69	2.85	2.18	0.00	0.37
5368	2006	5/20/06		Oak	9.75	29.73	female	0.34		0.93	6.36	5.43	10	0.54	3.21	2.62	3.13	0.69
5369	2006	5/20/06		Oak	9.5	21.7	female	0.17		0.4	2.04	1.64	7	0.23	2.53	2.34	1.84	1.78
5370	2006	5/20/06		Oak	9.5	20.65	male	0.21	1.11						2.41	2.41	5.38	5.16
5371	2006	5/20/06		Oak	8	12.74		0.12		0.17	0.78	0.61	5	0.12	2.49	2.37	1.33	0.31
5372	2006	5/24/06	dead	SFC	9.75	22.25	male	0.16	0.84						2.40	2.40	3.78	7.33

Acquisition No.	Year	Date	Alive/dead	Location	S.L.	Wet Wt.[1]	Sex	Liver Wt.	Testis	Ovary	Gonad +young	Total Young Wt.	Number of young	Wt./offspring	Condition factor (w/l^3)	Adjusted condition factor (w-wt. young/l^3)	GSI	EROD[2]
5373	2006	5/20/06	dead	SFC	9.5	24.72	female	0.23		0.72	2.56	1.84	7	0.26	2.88	2.67	2.91	0.9
5374	2006	5/20/06		SFC	9.25	21.43	male	0.15	0.79						2.71	2.71	3.69	1.75
5375	2006	5/20/06		SFC	11.5	29.88	female	0.24		1.02	1.02		0	0.00	1.96	1.96	3.41	0.63
5376	2006	5/20/06		SFC	9.25	20.03	male	0.11	0.74						2.53	2.53	3.69	2.69
5377	2006	5/20/06		SFC	9.5	20.31	male	0.21	0.89						2.37	2.37	4.38	2.97
5378	2006	5/20/06		SFC	12	53.2	female	0.4		1.51	11.4	9.89	15	0.66	3.08	2.51	2.84	0.36
5379	2006	5/20/06		SFC	11	34.06	female	0.36		0.91	3.5	2.59	9	0.29	2.56	2.36	2.67	0.4
5380	2006	5/20/06		SFC	11	39.6	female	0.24		2.44	5.26	2.82	4	0.71	2.98	2.76	6.16	1.84
5381	2006	5/20/06		SFC	9.25	23.15	male	0.2	0.66						2.92	2.92	2.85	
5382	2006	5/20/06		SFC	11	33.05	female	0.3		1.01	4.52	3.51	13	0.27	2.48	2.22	3.06	3
5383	2006	5/20/06		SFC	11.75	32.99	female	0.2		1.76	3.51	1.75	4	0.44	2.03	1.93	5.33	1.25
5384	2006	5/20/06		SFC	10.5	31.85	female	0.2		0.89	4.32	3.43	12	0.29	2.75	2.46	2.79	0.83
5385	2006	5/20/06		SFC	11	37.42	female	0.25		0.94	5.74	4.8	12	0.40	2.81	2.45	2.51	0.85
5386	2006	5/20/06		SFC	9.75	26.14	female	0.25		0.79	3.55	2.76	10	0.28	2.82	2.52	3.02	0.87
5387	2006	5/20/06		SFC	9.5	16.54	female	0.12			0.23	0.23	7	0.03	1.93	1.90		2.32
5388	2006	5/20/06		SFC	9.25	20.34	male	0.15	0.84						2.57	2.57	4.13	5.47
5389	2006	5/20/06		SFC	9.25	19.85	female	0.14		0.45	1.33	0.88	8	0.11	2.51	2.40	2.27	0.58
5390	2006	5/20/06		SFC	9.4	20.51	female	0.2		0.4	1.23	0.83	7	0.12	2.47	2.37	1.95	0.37
5391	2006	6/1/06		TB	9.5	25.38	female	0.17		0.69	3.51	2.82	10	0.28	2.96	2.63	2.72	
5392	2006	6/1/06		TB	11.25	43.95	female	0.15		1.28	9.47	8.19	14	0.59	3.09	2.51	2.91	0.31
5393	2006	6/1/06		TB	10	25.2	male	0.29	1.28						2.52	2.52	5.08	1.27
5394	2006	6/1/06		TB	9.75	28.19	female	0.26		0.97	5.46	4.49	10	0.45	3.04	2.56	3.44	0.34
5395	2006	6/1/06		TB	10	25.31	male	0.2	2.15						2.53	2.53	8.49	0.74
5396	2006	6/1/06		TB	8.25	15.26	male	0.1	0.48						2.72	2.72	3.15	1.74
5397	2006	6/1/06		TB	9.5	22.49	male	0.15	1.5						2.62	2.62	6.67	0.72
5398	2006	6/1/06		TB	10	27.57	male	0.15	2.22						2.76	2.76	8.05	0.62
5399	2006	6/1/06		TB	9.25	22.18	male	0.15	1.1						2.80	2.80	4.96	1.47
5400	2006	6/1/06		TB	9.5	23.04	male	0.2	2.2						2.69	2.69	9.55	1.05
5401	2006	6/1/06		TB	11	30.55	female			0.64	0.64		0		2.30	2.30	2.09	1.96
5402	2006	6/1/06		TB	10.75	33.55	female	0.24		1.45	7.39	5.94	8	0.74	2.70	2.22	4.32	0.88
5403	2006	6/1/06		TB	11	41.33	female	0.36		1.5	8.15	6.65	12	0.55	3.11	2.61	3.63	0.37
5404	2006	6/1/06		TB	9	16.89	male	0.14	0.86						2.32	2.32	5.09	1.8
5405	2006	6/1/06		TB	9.5	23.23	male	0.18	1.65						2.71	2.71	7.10	0.94
5406	2006	6/1/06		TB	9.5	24.07	female	0.18		0.68	3.52	2.84	8	0.36	2.81	2.48	2.83	0.92
5407	2006	6/1/06		TB	9.75	22.48	male	0.14	1.4						2.43	2.43	6.23	1.26
5408	2006	6/1/06		TB	10.5	28.42	female	0.14		0.91	7.95	7.04	8	0.88	2.46	1.85	3.20	1.5
5409	2006	6/1/06		TB	9.5	19.75	female	0.19		0.82	1.17	0.35	1.17	0.30	2.30	2.26	4.15	
5410	2006	6/1/06		TB	9.5	21.23	male	0.1	0.93						2.48	2.48	4.38	1.02