CALIFORNIA STATE UNIVERSITY, NORTHRIDGE

THE EFFECTS OF ORGANIC POLLUTANTS ON THE GROWTH, CONDITION, AND REPRODUCTION OF *PARALABRAX NEBULIFER* (BARRED SAND BASS) IN SOUTHERN CALIFORNIA

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By

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ABSTRACT

THE EFFECTS OF ORGANIC POLLUTANTS ON THE GROWTH, CONDITION AND REPRODUCTION OF *PARALABRAX NEBULIFER* (BARRED SAND BASS) IN SOUTHERN CALIFORNIA

By
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Pollutants have the ability to be persistent, circulate worldwide, bioaccumulate, and biomagnify in the tissues of organisms. Pollutants can impact the local economy and human health by altering marine resources such as fisheries. These fisheries can be impacted by both lethal and sublethal effects on fish. Sublethal effects can result from physiological stress to individuals, especially in highly urbanized, polluted areas. *Paralabrax nebulifer* (barred sand bass) supports an important recreational fishery in southern California and the population has declined, possibly due to anthropogenic effects. This study had two main goals: (1) to characterize the levels of organic pollutants in the tissues of barred sand bass from sites across southern California; and (2) to determine if there are detectable sublethal effects of pollution on this species. To evaluate potential sublethal effects on barred sand bass, an array of commonly used physiological indices were used, including hepatosomatic index (HSI), gonadosomatic index (GSI), fecundity, reproductive potential, and growth.

Fish were collected from four sites in southern California: Los Angeles/Long Beach Harbor, Huntington Flats, San Clemente Reefs, and San Diego Harbor. One hundred and seven organic pollutants were tested for and 77 of these were detected in the tissue of barred sand bass. There was a significant difference in tissue pollutant concentration among sites, with fish from LA/LB Harbor having the highest

concentrations of pollutants, followed by Huntington Flats and San Diego Bay, while fish from the San Clemente Reefs had the lowest tissue concentrations. The pollutant with the highest tissue concentrations across all sites was 4,4'-DDE, but tissue burdens of it declined with distance from the Palos Verdes Shelf.

Some metrics of individual performance showed evidence of sublethal effects of pollution, while others did not. Differences in condition (weight-at-length) and growth rate were unrelated to average pollutant concentrations in tissues of fish among sites. The site with the highest tissue concentrations of pollutants, LA/LB Harbor, had a higher proportion of inactive females than the other sites and had the lowest GSI. Fecundity did not differ among the four study sites, suggesting that this metric of reproductive success was not affected by differences in tissue concentrations of pollutants among sites, perhaps because the levels of pollutants were low enough not to impact fecundity in a multi-batch spawning species. Proportional liver size (HSI) was highest in fish from the LA/LB Harbor and Huntington Flats, which had the highest tissue concentrations of organic pollutants. Physiological parameters of individual barred sand bass were compared with the tissue concentrations of toxicants in those individuals. Condition and growth were both negatively related to a multivariate summary of tissue concentrations of pollutants. These results suggest that pollutants can negatively affect growth and body condition in the barred sand bass.

This study provides some evidence that barred sand bass in polluted areas may be in poorer condition and grow at slower rates, which could be detrimental for this population that may already be on the verge of collapsing. This study measured concentration of organic pollutants in liver tissue, which is not typically consumed, but

information about relative differences in pollutant tissue concentrations can be used to estimate and prevent exposure. For example, we now know that LA/LB Harbor and Huntington Flats are sites where barred sand bass have high tissue concentrations of organic pollutants. Huntington Flats is heavily targeted by anglers during the spawning season due to the presence of large aggregations of barred sand bass, making them easy to catch and increasing possible human exposure. It is important to continue to monitor populations in highly urbanized areas in order to understand accumulation rate, sublethal effects, and the persistence of legacy pollutants such as DDTs in southern California.

INTRODUCTION

In the marine realm, pollutants have negatively affected a variety of marine organisms. Many pollutants are environmentally persistent and circulated worldwide by ocean currents. They can have adverse impacts on the local economy and human health by limiting or altering marine resources. Pollutants have impacted fisheries by affecting the health of marine stocks in two ways: by making the product toxic or undesirable (MacGregor 1974, Stull et al. 1987, Marty 2008); or by depleting the population by lethal or sublethal effects (Malins and Hodgins 1981, Sindermann 1986, Cross and Hose 1989, Hose et al. 1989).

In fishes, sublethal effects are due to physiological stress on the individual, which can alter natural growth rates (Munkittrick and Dixon 1989, Farkas et al. 2003, Fang et al. 2009), accelerate mortality (Hilton et al. 1980, Hodson 1990), or alter reproduction (Cross and Hose 1989, Hose et al. 1989, Kime 1995, Scott and Sloman 2004, Marty 2008, Farwell et al. 2012). Physiological stress varies with pollutant load and pollutant type (e.g., metals or organics) (Hilton et al. 1980, Sindermann 1994, Blus 2002, van der Oost et al. 2003).

Other factors related to the health of marine fishes in areas impacted by pollutants include trophic level, with carnivores having higher tissue burdens of pollutants (Mearns and Young 1979, Sindermann 1994, Borgå *et al.* 2004, Blasius and Goodmanlowe 2008), and position in the water column. Benthic associated species are more vulnerable to pollutant impacts due to their direct contact with contaminated substrates (Young et al. 1976a, Sindermann 1994, Schiff and Allen 2000). Age is another factor that influences tissue burden simply because exposure increases with time (Vives et al. 2005).

Organic pollutants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), are known to occur in marine sediments and bioaccumulate and biomagnify in tissues of marine organisms (Mearns and Young 1979, Malins and Hodgins 1981, Blus 2002). PAHs are formed as a byproduct of burning coal, tar, gas, wood, and other fuel oil (ATSDR 1995, Skupinska et al. 2004). PAHs can occur naturally and they are also manufactured for medicines, cigarettes, pesticides, plastics, and asphalt. There are over 100 different types of PAHs that can be found in the air, water, and soil (ATSDR 1995). In living organisms, PAHs can be found in all tissues but are typically stored fats, in organs such as kidneys and liver, where they can reside for a few days to months (ATSDR 1995, US Department of Health and Human Services 1995a).

PCBs are toxic, synthetically manufactured, and have been used as additives in oils for electrical machinery, coolants, hydraulic equipment, plasticizers in paints, sealants, flame retardants, and can be found in plastics (UNEP 1999, ATSDR 2000). There are 209 distinct PCB congers that are characterized by two benzene rings with one to ten of its hydrogen atoms replaced by chlorine atoms (Narquis et al. 2007), the more chlorine atoms the more harmful. The United States stopped the manufacture of PCBs in the late 1970s, but they are still present today. Unlike PAHs, PCBs can remain in the environment for several years after being released (ATSDR 2000). PCBs can accumulate in the tissues of higher trophic level predators at concentrations thousands of times higher than in water (US Department of Health and Human Services 2000). Even at low concentrations, PCBs can affect individual health, with acute and chronic effects (Young

et al. 1976a, Brown et al. 1984, Hodson 1990, Nehlsen et al. 1991, Longwell et al. 1992, Sindermann 1994, Schiff and Allen 2000, Blus 2002, Narquis et al. 2007).

Organochlorine pesticides (OCPs) are synthetic chemicals manufactured to control and manage insect pests for agricultural, residential, and commercial purposes, as well as to prevent the spread of diseases such as malaria (Blus 2002). OCPs also have high solubility in lipids, they are persistent in both the environment and in organisms, and they are linked to toxicity in both animals and humans (US Department of Health and Human Services 1994, 1995b, 1996, 2002a, 2002b, 2002c, 2007, 2013a, 2013b, Blus 2002). OCPs can be present in the environment for months to decades, perhaps even to centuries, as many of the half-lives are not all fully understood. There are five major groups of OCPs pesticides: DDTs (dichlorodiphenyltrichloroethane), hexachlorocyclohexane (HCH), cyclodienes, toxaphene, and caged structures (Blus 2002). Of the five groups of OCPs, cyclodienes are the most toxic, but in southern California the most common are DDTs.

Like many highly urbanized coastal areas, inputs of pollutants into southern California's waters are from aerial fallout, marine vessel activities, runoff from storm drains, sewage, industrial discharge, agricultural runoff, power plants emissions, and water waste discharges (Young et al. 1976b, Bascom 1982, Mearns et al. 1991, Allen 2006, Setty et al. 2012). Some of these discharges and inputs are concentrated in harbors and bays (Mearns and Young 1979, Fairey et al. 1998, Huh and Venkatesan 1998, AMEC Earth & Environmental Inc. 2009, Setty et al. 2010).

DDT is one of the main pollutants in marine waters in southern California. The Montrose Chemical Corporation was the largest producers of DDT worldwide, and is the

principal sources of DDT in southern California. DDT gained its popularity during WWII as a new insecticide that could control malaria (Kehoe and Jacobson 2003). In 1947, Montrose Corporation started manufacturing DDT in Los Angeles and released its discharges directly into the Los Angeles County sewer system (MacGregor 1974, Young et al. 1976a, Kehoe and Jacobson 2003). Millions of tons of DDTs and PCBs (NOAA 2007) were released off the Palos Verdes Shelf. In the early 1970s, after the recognition that DDT and PCB had adverse effects on non-targeted organisms, the manufacture of DDTs and PCBs was banned in the US (Mearns et al. 1991, Kehoe and Jacobson 2003). The Montrose Corporation released DDTs and PCBs into the outfall off of Palos Verdes for almost 25 years, until 1971 (EPA 2007).

The organic pollutants mentioned above have impacted many marine organisms in southern California, especially near shore fishes (Young et al. 1976a, Gossett et al. 1981, Stull et al. 1987, Mearns et al. 1991, Eganhouse and Pontolillo 2000, NOAA 2007, Setty et al. 2012). In 1991, the State of California issued a fish consumption advisory for southern California to protect human health (NOAA 2007). The advisory provides guidelines to the general public for selecting the safest fish to consume and which to avoid based on the level of chemicals found within their tissues (Klasing and Brodberg 2008).

In 2001, the Environmental Protection Agency (EPA) listed Palos Verdes Shelf as a Superfund site in efforts to restore the area (Kehoe and Jacobson 2003, NOAA 2007). The rate of input of pollutants has declined since the 1970s (Kehoe and Jacobson 2003, Allen 2006, NOAA 2007, Setty et al. 2012), but there are still substantial concentrations of organic pollutants in local marine waters, and thus it is still important to monitor the

health of marine fishes in these areas. Historically, the marine environment in California has been impacted and exploited by humans for its resources, which has led to an increase in pollution with the increase of human population. The marine environment in California provides a vital economical resource for the state, which has led to strict regulation in monitoring and managing the health of marine species.

Study Species

Paralabrax nebulifer (barred sand bass) is an important near shore fish that may be impacted by marine pollution. It is of particular interest because it is one of the most frequently caught fish in the multi-million dollar recreational fishery in southern California, with more than a million individuals of this species harvested in many years (Oliphant 1990, CPFV 2000). It is one of three serranid species in southern California, and ranges from Santa Cruz, California to Baja California, Mexico (Miller and Lea 1972, Eschmeyer et al. 1983). It is a benthic associated species that prefers interfaces of sandy and rocky habitat in subtidal water up to 183 m in depth (Love et al. 1996b, Love 2011). This species displays high site fidelity, with home ranges averaging 10,003 m^2 in size (Mason and Lowe 2010). The largest sand bass ever captured was 650 mm in total length, and the oldest was 24 years (Love et al. 1996b). The barred sand bass is a tertiary carnivore with a diverse diet, consisting of benthic associated invertebrates and fish species, as well as midwater species (Limbaugh 1955, Roberts et al. 1984). Since barred sand bass are tertiary carnivores and associated with the benthos they are more susceptible to accumulating pollutants within their tissues both directly and indirectly.

Barred sand bass is a multiple batch, oviparous, broadcast spawner, that spawns from May to August, peaking in July (Demartini 1987, Oda et al. 1993, Hovey et al. 2002). Sexual maturity in females occurs at 2 to 5 years of age (210- 270 mm total length) and at 2 to 4 years in males (190- 260 mm) (Love et al. 1996b, Love 2011). Barred sand bass form large spawning aggregations off the California coast; five known sites include: Ventura Flats, Santa Monica Bay, Huntington Flats, San Onofre, and Imperial Beach (Love 2011).

The sand bass population is in decline in southern California (Allen 1985, 2010, Allen and Hovey 2001, 2002, Erisman et al. 2011). Overfishing is a likely cause of this decline because the sand bass is targeted during the summer spawning periods (Allen and Hovey 2001). A second possibility is that there has been reproductive failure due to high levels of contamination.

Previous studies on barred sand bass have revealed the presence of tumors and deformities on gill rakers, fins, skeleton, and skin, which have been linked to industrial and domestic waste discharge (Valentine and Bridges 1969, Sherwood and Mearns 1977, McCain et al. 1989, Allen and Hovey 2002). Other studies on pollutant concentrations in barred sand bass have focused on potential human impacts due to consumption of fish, but they have had low sample sizes and limited geographic range since they were not specifically targeting this species (Klasing and Brodberg 2008). McCain et al. (1992) compared disease incidence (liver lesions and fin erosion) to tissue pollutant concentrations in barred sand bass from San Diego Bay. Their study was limited to a few pollutants and they did not evaluate OCPs. Another study of barred sand bass focused on mercury accumulation near wastewater outfalls, and found that tissue concentrations of

mercury were not clearly related to discharges, but mercury concentration increased with length and age (Phillips et al. 1997). The Environmental Protection Agency (EPA) added barred sand bass from southern California to the limit your consumption guideline in 2009. Barred sand bass collected from Palos Verdes have an advisory of no consumption (Klasing et al. 2009).

Study Objectives

This study had two main goals: (1) to characterize the levels of organic pollutants in the tissues of barred sand bass from sites across southern California; and (2) to determine if sublethal effects of pollution have impacted barred sand bass. I determined which organic pollutants were present in barred sand bass and compared the concentrations of these pollutants among fish collected from four sites in southern California. I targeted fishes living within impacted areas such as harbors and compared them to those living in less impacted areas outside of harbors. To evaluate potential sublethal effects on barred sand bass, I used an array of commonly used physiological indices, such as hepatosomatic index (HSI), gonadosomatic index (GSI), fecundity, reproductive potential, and growth.

Methods

Study Sites

This study was conducted at four sites: two sites within harbors that are highly impacted by human use and pollution, and two less impacted sites outside of harbors (Figure 1). The highly impacted sites were the Los Angeles/Long Beach Harbor (33º44’628 N, 118º11’956 W) and the San Diego Harbor (32˚42'624 N, 117˚13'520 W). The two less impacted sites were the Huntington Flats (33º39’11 N, 118º05’86 W) and the San Clemente Reefs (33˚23’50 N, 117˚37’5 W). The sites were selected based on the presence of barred sand bass and an expected difference in pollutant load within versus outside of harbors.

The Los Angeles and Long Beach Harbor complex is the busiest port in the United States (US Department of Transportation 2003) and has high concentrations of pollutants due to present day and historical uses (Weston Solutions Inc. 2009a). Los Angeles Harbor has heavy commercial shipping, boat repairs, oil production, ship building, and other activities. Long Beach Harbor has commercial and recreational boating activities and a history of oil production, shipbuilding, canneries, and naval activities. The main contaminants in the LA/Long Beach harbor complex are PCBs, PAHs, heavy metals, waste materials, scrap metals, and runoffs. The Dominguez Channel, Cerritos Channel, and the Los Angeles River are sources of new contaminants in these harbors (Weston Solutions Inc. 2009a, 2009b). The inner harbor habitat is composed of sand and silt, which is suitable for barred sand bass, and is ranked tenth in

total abundance of marine fishes found in the LA/Long Beach harbor complex (MEC Analytical Systems Inc. 2002).

Huntington Flats is one of five known historical spawning sites for barred sand bass in southern California. It is located off of Huntington Beach in Orange County and its substrate is consisted of mostly sandy bottom habitat with sparse, low-relief rocky reefs. Barred sand bass are present there year round, but during the spawning season there is a significant increase in the population, which is targeted by recreational anglers (Allen 2010, Erisman et al. 2011) The Orange County Sanitation District (OCSD) sewage outfall is approximately 13 km away and could impact the Huntington Flats. The OCSD is the third largest publicly owned treatment work in southern California and it releases treated wastewater seven km offshore in 60 m depth off of Huntington Beach (US Geological Survey 2004). Plumes are released offshore and travel north, carried by near shore currents.

The San Clemente Reefs are composed of three kelp bed reefs: two natural reefs (San Mateo Kelp and Barn Kelp) and one artificial reef (Wheeler J. North Artificial Reef). The artificial reef is the northernmost of the three reefs, starting just south and offshore of the San Clemente Pier. San Mateo Kelp is located adjacent to the southern end of the artificial reef, and Barn Kelp is located 12 km south of San Mateo Kelp. All three reefs are composed of low-relief rocky substrate and they support kelp forests. There are no major sources of pollutants near these three reefs.

San Diego Bay is affected by both urban and naval runoff including storm water drains (Unified Port of San Diego 2013) and before the 1960s raw sewage and untreated industrial discharges impacted the harbor (Fairey et al. 1998). San Diego Bay is

influenced by naval activity, which has occurred since the early 1920s (US Navy 2013). San Diego Bay is the homeport to nearly a third of the US Navy's fleet on the Pacific (US Navy 2013). The bay has a sandy bottom with a deep trench at the center and has abundant habitat suitable for the barred sand bass.

Field Methods and the Processing of Fishes

Fish were collected from January to October 2010 using hook and line and also by scuba divers using spears. Fish were euthanized by placing them directly into a cooler that contained slurry of ice and seawater, or by pithing. They were transported to a lab, on ice, for processing, which was normally done within 24 h of capture. The following measurements were taken: standard length (mm), fork length, total length, and whole fish mass (recorded to the 0.01 g). Liver, gonad, and white muscle tissue were dissected and weighed to the nearest 0.001 g. Gonads were macroscopically classified to sex and stage following Wang (2010). Ovaries of mature females were placed in 10% buffered formalin and stored for further processing. Livers and white muscle tissue were wrapped separately in aluminum foil, placed together in a plastic bag, and stored in a freezer until toxicology processing. Sagittal otoliths were removed, cleaned, and placed in trays for storage until age analyses.

Laboratory Methods

Toxicology

Liver tissue samples were used to measure the levels of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons

(PAHs) in barred sand bass. These analyses were done at the Institute for Integrated Research in Materials Environments and Society, Long Beach, California (IIRMES laboratory). Soxhlet extraction was used to extract nonvolatile and semivolatile organic compounds and to isolate and concentrate water insoluble and slightly water soluble organics in preparation of chromatography procedures (EPA 1996a). These protocols were modified by IIRMES using EPA standard methods (3500, 3540, 3541, 8000 and 8270). Whole livers were dissected, wrapped in aluminum foil and stored in a freezer at 20˚C until extractions could take place. Concentrations of 25 PAHs, 53 PCBs and 29 OCPs were measured in each liver sample (Table 1).

Approximately 3 g of homogenized liver tissue and 10 g of sodium sulfate were place in a cellulose thimble, which was then placed inside an extractor that was attached to a condenser. A 10:1 dichloromethane (DCM) to acetone mixture was placed in a flask and then it was attached to the extractor. The Soxhlet extraction system reflux (Figure 2) ran for a minimum of 15 h.

Before the Soxhlet extraction system reflux ran, samples were spiked with chlorohydrocarbon recovery surrogate (CHC-RS) and polycyclic aromatic hydrocarbon recovery surrogates (PAH-RS) that were composed of four known compounds (TCMX, PCB 30, PCB 112, PCB 198) to measure accuracy. A total of 20 study samples were run per batch along with quality control samples. Samples were randomly selected and a total of four batches were performed.

Once cooled each Soxhlet system was broken down and the flask containing the sample was capped and set aside for rotary evaporation (rota-vap). Each flask was then attached to rota-vap machine to evaporate the solvent. Lipid splits were performed on all

the samples, except the two control blanks and the certified reference material (CRM) sample, to ensure that the samples would run properly on the GC/MS machine. The lipid-split protocol was modified by IIRMES based on the method of Bligh and Dyer (1959). Fifty percent of the total sample volume was removed and transferred into a pre-weighed vial and allowed to dry for a minimum of 2 wk to determine lipid weight (0.0001 g). A solvent switch using hexane was performed on the remaining fifty percent of the sample volume. The sample was then rota-vapped again to reduce the solvent in it. The samples were transferred into a vial, capped, and stored in a refrigerator until column cleaning.

Column clean up and chromatographic separation were performed on all the samples except the two controlled blanks following a standard protocol (IIRMES 2009). A sample was loaded to the top of the column and it was allowed to run through until the sample and solvent reached the top of the alumina. Three solvents (hexane, DCM: hexane, and DCM) were then added one at a time until each solvent ran through the column into a flask. The flask was then rota-vap down and transferred into new vial, capped, and stored in a refrigerator until gas chromatography was performed.

To prepare samples for gas chromatography, they were concentrated down using nitrogen, spiked with internal standards, and injected using an autosampler for the Agilent 5975 Inert MSD Gas Chromatography Mass Spectrometer (GC/MS) to identify and quantify contaminants. The GC/MS analysis was performed by IIRMES.

Quality assurance/control

For each batch analyzed, five samples were designated for quality assurance and control and run alongside with the study samples. Precision and accuracy were ensured

by running one blank, two control blank spikes (organics only), one certified reference material (CRM) 1947 (National Institute of Standards and Technology for organics), and a duplicate of a study sample. Blank samples contained recovery surrogates (RS) both CHC-RS and PAH-RS to test for any cross contamination by instruments and methods. Two blank spikes were done with each batch that contained CHC-RS, PAH-RS, and three matrix spikes (MS) of PCB MS, Pesticide MS and PAH MS to measure the precision of the extraction procedures. Three grams of trout tissue CRM 1947 (Lake Michigan Trout tissue 1947) was used for each batch to measure accuracy in the procedure. A duplicate study sample was analyzed for each batch and randomly selected for precision in extraction procedure.

The recovery rates of all the surrogates were in the acceptable range of 70- 130 % (EPA 1996a). OCPs and PCBs had no contamination in the samples. However there was contamination with respect to PAHs with 12 of the compounds appearing in the blank sample. These readings were subtracted out of the study samples to ensure no overestimation of the compounds. All the control blank spikes were within EPA range of 35%. The CRM's samples with respect to OCPs fell with a 17% range and PCBs fell within 2% out of the 35% EPA standard. Duplicate samples for PAHs differed by no more than 9% and OCPs differed by 10% on average, which fell within EPA standards of 35% difference. The duplicate samples for PCBs had some inconsistency but met EPA standards. This was properly due to improper homogenization of the duplicate samples so the duplicates were averaged together and used as references. All samples met precision and accuracy in quality control.

Age and growth

Sagittal otoliths were used to determine age and growth (Figure 3a) (Secor et al. 1990). Love (1996) validated annual formation of bands in otoliths of barred sand bass. Photomicrographs (80- 160× magnification) of whole otoliths were taken and used to age fish under five years of age, following Wang (2010) methods. Otoliths from fish older than four years were aged from photomicrographs (125- 320× magnification) of transverse sections. Otoliths were sectioned after being embedded in epoxy, using a low speed saw with two diamond blades, spaced ~1 mm apart. Sections were cleaned with ethanol, dried, mounted on clear glass slides with Crystalbond (Electron Microscopy Sciences, Hatfield, PA), and polished wet, first with 220 grit and then 1,500 grit lapping paper. Annuli were counted by two separate readers (Figure 3b) and any count disagreement was discussed until agreed upon by both readers.

Reproductive potential: hydrated egg method, mature egg method, and histology

Gonads were classified for maturity macroscopically using the criteria of Wang (2010): F1 (immature/inactive female), F2 (inactive but mature female), F4 (active mature female with ripe eggs, but will not spawn within 24 h), F5 (active, mature female with hydrated eggs, which will spawn within 24 h), M1 (immature/inactive male), M2 (active mature male), and M3 (active mature male with flowing milt). Only active mature females (F4 and F5 stages) were used to estimate reproductive potential (Figure 4a). These active mature females were also used to estimate reproductive failure by comparing the proportion of inactive females above the known size at maturity among

sites. The gonadosomatic index (GSI), which is the ratio of the gonad mass to gonad-free body mass, was calculated for each fish captured during the spawning season. GSI is an index of reproductive function and quantity. GSI increases up to the spawning season peak in reproduction. Having a lower GSI has been associated with exposure to pollutants and can indicate reproduction failure (Sol et al. 2008, Fang et al. 2009, Farwell et al. 2012).

Histological analysis of ovarian tissue was used to confirm macroscopic classification maturity stages and to determine whether postovulatory follicles (POFs) were present in ovaries of mature active females (Figure 4b). POFs are formed after eggs have been ovulated. Cross sections of ovaries fixed in formalin were dehydrated, embedded in parafilm, sectioned at 6μm, and stained using hematoxylin and eosin. The developmental stages of oocytes in these slides were used to determine which method for estimating reproductive potential was used. The mature egg method was used for fish with mature, yolked eggs but no hydrated eggs; whereas the hydrated egg method (Hunter et al. 1985) was used for fish with hydrated eggs or eggs in the migratory nucleus stage.

Hydrated egg method

I estimated fecundity as the total number of hydrated eggs within the ovaries of each female. Hydrated eggs are spawned within a day of being formed. I used a method that was adapted from Hunter et al. (1985) and modified by Wang (2010) for a multiple batch spawning species. A subsample from each ovary was used to estimate the total number of hydrated eggs in the ovaries of each female. Oda et al. (1993) and DeMartini,

et al. (1987) determined that prior to ovulation, hydrated eggs in the ovary were uniformly distributed, so subsamples could be taken from any part of the ovary to estimate total number of hydrated eggs. After air drying for a few minutes to remove excess formalin, the ovaries were reweighed (0.0001 g), and then a cross section was taken from the center of one lobe. From the cross section, a subsample weighing between 0.09- 0.13g was taken and weighed to the nearest 0.0001 g. This was placed into a 50 ml tube, approximately 15 ml of water was added, the tube was capped, and then it was shaken vigorously to separate the eggs from other ovarian tissue. Using a dissecting scope all hydrated eggs in the entire volume of water were counted. The density of hydrated eggs was calculated as the total count of hydrated eggs divided by the subsample mass, which was multiplied by the total mass of the ovary to estimate the total number of hydrated eggs for each female. Ovaries with evidence of recent ovulation (recent POFs) were not used because once ovulation has occurred, eggs are no longer homogenously distributed throughout the ovary, violating an assumption of the hydrated egg method (Hunter et al. 1985). Fish with many oocytes in the migratory nucleus stage were also processed using the hydrated egg method because oocytes in the migratory nucleus stage occurs only within 24 h of ovulation (Hunter et al. 1985, Wang 2010).

Mature egg method

Although counting hydrated eggs is considered the best way to estimate batch fecundity, relatively few females had hydrated or migratory nucleus stage eggs. There were, however, many females that were reproductively active (i.e., F4 stage), and from these I estimated reproductive potential using the “mature egg method” (Wang 2010,

modified from Hunter et al. 1985 as the "size frequency method"). Wang (2010) determine that barred sand bass eggs > 0.4-mm diameter would likely be spawned within the next 2 to 3 batches. The total number of these "mature" eggs was estimated for each F4 female using a method similar to that used with hydrated eggs (Figure 7b). Two small subsamples (0.010- 0.014 g) were taken from the center of a lobe and weighed to the nearest 0.0001 g. These were placed on frosted slides and covered with drops of a 50:50 glycerol-water solution. Under a dissecting scope with a micrometer, a fine probe and forceps were used to tease out all eggs > 0.3 mm. These eggs were arranged in a single layer and a photomicrograph at 0.5x 0.63- 0.8 was taken using a Q Imaging camera (Media Cybernetics, Inc., Bethesda, MD) and transmitted light. Image Pro v. 6.3 was used to measure the size of each egg based on two macro filters for consistency. I then calculated reproductive potential by the total count of mature eggs over the subsample masses multiplied by the total mass of the gonad for each individual.

*Hepatosomatic index (*HSI)

The liver is often use in assessing impact of pollutants due to its lipophilic nature as well as its important role in fish physiology (Mearns et al. 1991, Hinton et al. 2008). Hepatosomatic index (HSI) is an index of liver size as a proportion of body weight. HSI is commonly used as an index to assess fish health and it was calculated to determine liver condition (Fang et al. 2009). HSI has been associated with exposure to pollutants, where high HSI reflects liver toxicity (Klasing and Brodberg 2008, Sol et al. 2008, Bervoets and Campenhout 2009). These physiological indices can give us an insight into stressors of fishes in areas impacted by pollutants as well as determining the pollutant.

Condition

There are a variety of indices that are commonly used to determine the health of animals in the wild. In fish, condition factor is an index of health based on the assumption that having a higher ratio of weight to length should correlate positively with better condition, and as such should lead to higher fitness (Bolger and Connolly 1989, Fang et al. 2009). Condition was evaluated by comparing among site the relationships between weight and length.

Data Analyses

Toxicology

To compare concentrations of pollutants in liver tissue among the four study sites, I used a permutational multivariate analysis of variance (PERMANOVA: PRIMER software v. 6). This analysis compared the concentrations of all 77 pollutants detected in barred sand bass liver tissue among the study sites. I chose a permutational analysis because concentrations of zero were common in the data set, violating the assumption of normality. The concentrations were transformed to log (x + 1) and normalized (the mean subtracted and then divided by the standard deviation) and then a resemblance matrix was calculated using Euclidean distances. There were 999 unique permutations used to calculate the *p* value. The same resemblance matrix was used with principal coordinate analysis (PCO) to summarize pollutants into groups (principal coordinates) that were highly intercorrelated and graphically represent differences among sites.

Analysis of covariate (ANCOVA) was used to compare bioaccumulation (total pollutant concentration at mass) among sites. Sites were the categorical factor and mass (g) was the covariate. Mass was log(x) transformed in order to meet assumptions of ANCOVA. Differences in bioaccumulation rate would be seen by differences in elevations (e.g., total pollutant concentration at mass differing among sites) or slopes (e.g. rate of increase in pollutant concentration with body mass different among sites). One-way ANOVA's with post hoc tests were also used to test for differences in tissue concentrations of specific groups of pollutants (total PAHs, PCBs and OCPs) among sites using SYSTAT 13. OCPs and PAHs concentrations were transformed to log (x + 1) while PCBs and DDTs were transformed to log(x) to meet assumption of normality and homogeneity.

Multiple regressions were used to test if HSI, GSI, condition (weight-length relationship), growth (weight-age relationship), and fecundity (mature eggs-length relationship) were related to pollutant concentrations. Individual fish were used as replicates and pooled among sites. Principal coordinate (PCO) scores from the first two PCOs were used to summarize concentrations of pollutants in the tissue of each fish, and these were the predictor variables. HSI, condition, growth and fecundity were transformed using log (x + 1) and GSI was transformed to square-root (x + 0.5) to meet the assumptions for regression.

Condition

Analysis of covariate (ANCOVA) was used to compare differences in condition (mass at length) among sites. In this analysis, site was the categorical factor and length

was the covariate. Mass was transformed log(x) to in order to meet the assumptions of normality and homogeneity. Differences in condition would be evident as either different elevations of the lines relating mass to length (e.g., lower mass at length in some sites), or different slopes of the lines (e.g., longer fish being lighter than similar length fish at other sites, but shorter fish having similar weights-at-length at all sites).

Growth rates

ANCOVA was also used to determine if growth rates (mass at age) differed among sites. Sites were again the categorical factor and age was the covariate. The slope of the line relating mass to age was the growth rate, and these slopes were compared among sites. Mass was transformed to log(x) to meet the assumptions of ANCOVA.

Reproduction potential

The hypothesis that fish in polluted sites had lower reproductive potential was tested in three ways: first GSI was compared among sites with a one-way analysis of variance (ANOVA), followed by Tukey's post hoc tests to determine which sites differed. Second, ANCOVA was used to compare reproduction potential at size among sites using the hydrated egg method, with sites as the factor and standard length as the covariate. Third, the same ANCOVA model was also used with estimates of reproductive potential from the mature egg method. GSI was transformed to log (x + 1), and the numbers of hydrated or mature eggs were transformed to log(x) to meet assumptions of homogeneity and normality.

I also tested to see if reproduction occurred more frequently at less polluted sites. A test of independence compared among sites the proportion of reproductively inactive, mature females with that of reproductively active mature females. Love et al. (1996) found that the size at which 50% of female barred sand bass were sexually was 239 mm total length. Using a G^2 test of independence, I compared the frequencies of reproductively active and inactive females ≥ 239 mm TL among sites.

Hepatosomatic Index (HSI)

To test if relative liver size differed among study sites, HSI was compared among sites with a one-way ANOVA, followed by Tukey's post hoc test. HSI was transformed to log (x + 1) to meet the assumptions of normality and homogeneity.

Results

A total of 522 barred sand bass was collected: 119 from LA/LB Harbor, 97 from Huntington Flats, 170 from San Clemente Reefs, and 111 from San Diego Bay. The fish collected ranged from 178 - 546 mm in total length (TL) and weighed 71 - 1,996 g.

Evidence of pollutants concentration in the tissue of barred sand bass

Twenty-five PAHs were tested for, but only 16 types were present in the liver tissue of barred sand bass; 29 OCPs were tested for, but only 12 types were detected; and 53 PCBs were tested for and 49 different types were detected (Table 2). Pollutant concentrations in barred sand bass liver tissue differed among study sites with fish from the San Clemente Reefs having the lowest tissue concentrations of all toxicants (PERMANOVA: $F_{3,73} = 17.9$, $p < 0.001$; Figure 5).

Principal coordinate analyses (PCO) revealed that 50% of the variation in tissue concentrations of the 77 detected organic pollutants could be summarized by just two principal coordinates (Figure 6). PCO1 explained 42.3% of the total variation while PCO2 explained 7.4%. The slope of bioaccumulation rate relationship did not differ among sites (ANCOVA: $F_{3,72} = 0.7$, $p = 0.58$, Figure 7). Pollutant concentrations at mass were higher at LA/LB Harbor, Huntington Flats and San Diego Bay ($F_{1,75} = 4.5$, $p = 0.038$). Tissue concentrations were positively associated with fish mass ($F_{3,75} = 28.4$, $p < 0.001$).

Differences among sites in tissue concentrations of specific classes of organic pollutants were evaluated in more detail with one-way ANOVA. PAH tissue

concentrations differed significantly among sites ($F_{3,76} = 12.1$, $p < 0.001$, with fish from LA/LB Harbor having the highest concentrations, those from San Diego Bay having somewhat lower but statistically indistinguishable concentrations, those from Huntington Flats having yet lower concentrations, and those from the San Clemente Reefs having the lowest concentrations (Figure 8a). Tissue concentrations of PCBs also differed significantly among sites ($F_{3,76} = 47.0$, $p < 0.001$), showing the same general pattern as PAHs, but only tissue concentrations in fish from the San Clemente Reefs were significantly lower than the other sites (Figure 8b). DDTs made up 97% of all OCPs detected in barred sand bass tissues, specifically the isomer 4,4'-DDE. Since DDTs were found in such high concentrations, they were analyzed separately from other OCPs. Non-DDT OCPs were found in similar concentrations at 3 of 4 sites, but were much lower in tissues of fish from the San Clemente Reefs ($F_{3,76} = 13.8$, $p < 0.0001$; Figure 9a). Tissue concentrations of DDTs also differed significantly among sites, but the pattern differed from that of non-DDT OCPs, with similar high concentrations in fish from both LA/LB Harbor and Huntington Flats, and lower concentrations in fish from San Diego Bay and the San Clemente Reefs ($F_{3,76} = 29.2$, $p < 0.001$; Figure 9b).

Evidence for effects of organic toxicants on performance of fish

Condition

Condition (mass at length) did not differ among study sites in a manner that suggested any effects of pollutants (Figure 10). Although the mass to length relationship differed in slope among sites (ANCOVA: $F_{3, 488} = 36.5$, $p < 0.001$), the differences in

slope were unrelated to differences in tissue concentrations of pollutants among sites. For example, fish from the least polluted site, the San Clemente Reefs, did not have higher condition.

Growth rates

Effects of pollutants on growth rate were explored by comparing size (mass) at age among the study sites (Figure 11). The mass-at-age relationship (growth rate), did not differ among sites (ANCOVA: $F_{3, 488} = 1.3$, $p = 0.28$). At two sites, however, body mass was higher at any age than at the other two sites ($F_{3, 491} = 84.1$, $p < 0.001$). This result implies that growth rates of young fish, less than 2 years old, differed among the sites. This difference, however, did not reflect any of the differences in tissue concentrations of organic pollutants, since size-at-age was higher at Huntington Flats and San Diego Bay than at the San Clemente Reefs and LA/LB Harbor.

Reproduction potential: Gonadosomatic index (GSI,) fecundity and maturity

To test the hypothesis that fish in more polluted sites would have lower reproductive potential than those in less polluted sites, GSI, batch fecundity, and reproductive potential were compared among sites. GSI (gonad weight expressed as a percentage of body weight) differed among the 4 study sites (one-way ANOVA: $F_{3,233} = 23.7$, $p < 0.001$; Figure 12). Female barred sand bass from the site with the highest tissue concentrations of organic toxicants, LA/LB Harbor, had the lowest GSIs, but GSI of females from the other three sites were quite similar despite differences in tissue concentrations of organic pollutants.

There was no evidence that batch fecundity (hydrated egg method) differed among sites (Figure 13). The slope of the relationship between batch fecundity to length did not differ among sites (ANCOVA: site x length interaction: $F_{3,36} = 0.2$, $p = 0.86$); nor did fecundity-at-size differ among sites (ANCOVA: site: $F_{3,39} = 1.8$, $p = 0.16$). As expected, batch fecundity increasing with fish size (ANCOVA: site: $F_{1,39} = 13.5$, $p < 0.001$).

The slope of the relationship between reproductive potential (as estimated by the number of mature eggs in ovaries of females) differed significantly among study sites (ANCOVA: site x length interaction: $F_{3,117} = 3.7$, $p = 0.014$; Figure 14). The differences in slopes among sites did not correspond to any observed differences in tissue concentrations of organic toxicants, with steeper slopes in San Diego Bay and Huntington Flats, and shallow slopes in the San Clemente Reefs and LA/LB Harbor. The significant difference in slopes among sites precluded any meaningful tests of the main effects of site or mass.

The proportion of mature females that were reproductively active differed significantly among the four sites (Test of independence: $G^2 = 264.4$, df = 3, $p < 0.001$; Figure 15). Females large enough to be mature but reproductively inactive were more common at the LA/LB Harbor site than at the other three sites. The lowest proportion of reproductively inactive females was found at the site with the lowest tissue concentrations of organic pollutants, the San Clemente Reefs.

Hepatosomatic Index (HSI)

HSI differed significantly among sites with fish from the San Clemente Reefs having the lowest HSI (one-way ANOVA and Tukey's post hoc: $F_{3,367} = 6.5$, $p < 0.001$; Figure 16). Thus, fish from the site with the lowest tissue concentrations of organic pollutants had the smallest livers.

Relationships between tissue concentration of organic pollutants and health of individual barred sand bass

Since PCO1 and PCO2 explained 49.7% of the total variation, scores for PCO 1 and 2 were used as predictors in multiple regressions. HSI (liver size index) was not related to PCO 1 or 2 (multiple regression: $r^2 = 0.01$, $p = 0.604$; Table 3). Likewise, there was no relationship between reproductive potential (GSI) and pollutant concentration as summarized by PCO 1 and 2 ($r^2 = 0.02$, $p = 0.354$; Table 3). Condition (weight to standard length) was significantly related to pollutant concentrations ($r^2 = 0.96$, $p < 0.001$; Table 3) with weight-at-length negatively related to PCO1. Growth (weight-at-age) was also negatively related to PCO1 ($r^2 = 0.65$, $p < 0.001$; Table 3). Fecundity (mature eggs-at-length) was not significantly related to pollutant concentrations ($r^2 = 0.29$, $p < 0.001$; Table 3).

Discussion

Characterization of pollutant burdens along the Southern California coast

Highly urbanized areas generally have higher loads of contaminants in the nearby marine environment (Brown et al. 1984, 1998, McCain et al. 1992). Organic pollutant concentrations in barred sand bass followed this pattern. Barred sand bass from Los Angeles / Long Beach Harbor had the highest total concentration of organic pollutants, followed by Huntington Flats, San Diego Bay, and San Clemente Reefs. The pollutants with the highest concentrations in barred sand bass tissue from LA/LB Harbor were 4,4'-DDE, PCB 153, PCB 138, PCB 101, and PCB 118. PCBs and OCPs have historically been present in LA/LB Harbor in both sediment and tissue of marine organisms (EPA 2009). Sources of these pollutants include rivers, storm drains, outfalls, sewer systems, vessel discharges, and the Montrose Chemical Corporation (AMEC Earth & Environmental Inc. 2009, EPA 2009, Weston Solutions Inc. 2009a).

Fish from Huntington Flats had the second highest tissue concentrations of summed pollutants, with OCPs being the major group of pollutants. Concentrations of OCPs were higher at this site than at the other three sites. The top five pollutants in barred sand bass tissue from Huntington Flats were 4,4'-DDE, PCB 153, PCB 138, PCB 101, and PCB 118. This pattern was similar to LA/LBC Harbor's top pollutants, however, Huntington Flats had the highest concentration of 4,4'-DDE overall. The high concentration of 4,4'-DDE in barred sand bass tissue is not surprising given the huge quantities of DDT in the sediments around Palos Verdes, as a result of historical dumping by the Montrose Chemical Corporation (Eganhouse & Pontolillo, 2000; EPA,

2009; Kehoe & Jacobson, 2003). Huntington Flats has both resident and transient barred sand bass; during the spawning season more fish migrate to this area to form large spawning aggregations (Love et al. 1996b, McKinzie et al. 2013). Some of these migratory fish are known to come from Palos Verdes (Jarvis et al. 2010). This is noteworthy because barred sand bass from Palos Verdes are in the EPA advisory for limited consumption (Klasing and Brodberg 2008, Klasing et al. 2009).

Fish from the San Clemente Reefs had the lowest concentrations of total organic pollutants. The main factor that distinguishes this site from the other three study sites is the relatively low human population density on the coast nearby. Thus, it is probably less subject to polluted run-off. Also, compared to the two harbor sites, this site is farther offshore. The top pollutants in barred sand bass liver tissue here were 4,4'-DDE, PCB 153, PCB 138, Phenanthrene, and Naphthalene. Phenanthrene is a PAH that is used in dyes, explosives, plastics, pesticides, and biological research (ATSDR 1995). Napthalene is used as an insectide (e.g., in moth balls), and can also be used in dyes and leather goods (ATSDR 2005). Several outfalls, water waste treatment plants surround the San Clemente Reefs but another possible source of pollutants runoff is from Camp Pendleton Marine Base which is a Superfund site (EPA 1996b).

Barred sand bass from San Diego Bay had the highest tissue concentrations of PCBs. The five pollutants with the highest tissue concentrations were PCB 153, 4,4'-DDE, PCB 138, PCB 101, and PCB 118. Similar to LA/LB Harbor, pollution sources into San Diego Bay include discharges, run offs, spills, and vessel use (Zeng and Vista 1997, Fairey et al. 1998). Historically, San Diego Bay has had high concentrations of PCBs in both the sediment and in marine organisms (Fairey et al. 1998). An earlier

study revealed that barred sand bass in San Diego Bay had high levels of PCBs and liver lesions (McCain et al. 1989); the present study shows that PCB concentrations remain high, decades later. However, concentrations of PCBs and DDTs in the tissue of barred sand bass from San Diego Bay have decreased relative to earlier studies (McCain et al. 1992).

At all four sites, 4,4'-DDE was the most concentrated organic pollutant in the tissue of barred sand bass. This compound may be quite stable in fish, based on a study on brown trout which showed that concentrations of 4,4'-DDE were much higher in older fish than younger fish (Vives et al. 2005). Historically, southern California has been plagued by DDT since the 1940's when it was first manufactured by the Montrose Chemical Corporation. Since the 1960's, there has been decline in DDTs in the environment (Zeng and Venkatesan 1999, Eganhouse and Pontolillo 2000, Lee et al. 2002) due to strict regulations and the prohibition of manufacturing it in the United States (Kehoe and Jacobson 2003, NOAA 2007, Klasing and Brodberg 2008). The Palos Verdes Shelf is still contaminated, and of the total DDTs, 60-70% are DDE isomers (Eganhouse et al. 2000), which can be recirculated into the environment by erosion and biofusion (Sherwood and Drake 2002). DDEs have impacted many marine fishes, including topsmelt, white croaker, kelp bass, anchovy, Dover sole, hornyhead turbot, and northern lampfish (MacGregor 1974, Young et al. 1976a, Cross and Hose 1989, Garcelon 1989, Hose et al. 1989, Klasing and Brodberg 2008); as well as marine birds, such as bald eagle, double-crested cormorant, and brown pelican (Gress et al. 1973, Anderson et al. 1975, Garcelon 1989, Garcelon and Thomas 1997); and marine mammals, including pinnipeds, otters, and whales (Nakata et al. 1998, Hayteas and

Duffield 2000, Blasius and Goodmanlowe 2008). With its persistency in the environment, it is difficult to predict long-term effects without regular monitoring.

Sublethal effects of pollution on barred sand bass populations

This is the first study to characterize possible effects of organic pollutants on barred sand bass. Pollutant concentrations in barred sand bass liver tissue varied among the four study sites with the sites adjacent to high urbanization being the most impacted by pollutant burdens and San Clemente Reefs the least polluted. Comparing pollutant concentrations with physiological parameters is commonly done to assess and monitor the health of marine species (Bolger and Connolly 1989, Hose et al. 1989, Mearns et al. 1991, Scott and Sloman 2004, Gassel and Brodberg 2005, Klasing and Brodberg 2008, Klasing et al. 2009). In the present study, differences among sites in condition (weight-at-length) and growth rate were unrelated to average pollutant concentrations in tissues of fish from those sites. These findings suggest these physiological parameters were not affected by the organic pollutants detected in barred sand bass.

I evaluated possible sublethal effects of organic pollutants on reproduction of barred sand bass in four ways: (1) the proportion of reproductively active mature females; (2) gonadosomatic index (GSI); (3) batch fecundity (hydrated egg method); and (4) reproductive potential (mature egg method). The proportion of reproductively inactive to active mature females was compared among sites, which showed that the site with the highest tissue concentrations of pollutants, LA/LB Harbor, had a higher proportion of inactive females than the other sites during the spawning season. GSI was also lowest in LA/LB Harbor. Batch fecundity did not differ among the four study sites, suggesting that

this metric of reproductive success was not affected by differences in tissue concentrations of pollutants among sites, perhaps because the levels of pollutants were too low to impact batch fecundity. The mature egg method was the last approach used to evaluate reproductive potential. Although this approach provided a larger sample size than did the hydrated egg method, it also did not reveal any pattern consistent with pollutant load impacting reproduction. While the mature egg method may overestimate actual fecundity because mature eggs will not necessarily be spawned (Hunter and Macewicz 1980, 1985, Wang 2010), it probably a reasonable proxy for reproductive output. One possible reason for why pollutants did not impact fecundity in barred sand bass, is maternal offloading, whereby mothers transfer contaminates to their eggs, thus detoxifying themselves (Vives et al. 2005, Daouk et al. 2011). Alternatively, levels of organic pollutants may be too low to affect reproduction of barred sand bass. Total pollutant concentration in hepatic tissue ranged from 0.1-3.4 ppm. However, pollutant concentrations in this range (as low as 1.4 ppm of DDTs) impacted reproduction of white croaker in southern California (Hose et al. 1989). Reproduction of a congener of barred sand bass, the kelp bass, was impacted by slightly higher DDTs levels of 8.3 ppm (Cross and Hose 1989).

Barred sand bass from LA/LB Harbor had lowest GSI, which has been noted in other species where there is reproductive failure in areas with high contaminants loads (Cross and Hose 1989, Fang et al. 2009, Farwell et al. 2012). Fish from Huntington Flats and San Diego Bay also had relatively high tissue concentrations of organic pollutants, but they did not have low GSI or relatively high proportions of reproductively inactive females, as seen in the LA/LB Harbor. A possible cause of reduced reproductive activity

in barred sand bass in the LA/LB Harbor is migration of reproductively active fish out to the harbor to the Huntington Flats to spawn (McKinzie et al. 2013). But there is also evidence that there are year-round residents, which might spawn within the LA/LB Harbor (Jarvis et al. 2010) since some fish captured within the harbor in this study had hydrated eggs present. Barred sand bass hydrate their eggs within the day of spawning (Oda et al. 1993).

Historically, barred sand bass have aggregated to spawn at specific locations across southern California: Ventura Flats, Santa Monica Bay, Huntington Flats, San Onofre and Imperial Beach (Love et al. 1996a, Allen and Hovey 2002, Jarvis et al. 2010, Erisman et al. 2011), but this studied found spawning females (with hydrated eggs) within San Diego Bay, LA/LB Harbor, and at San Clemente Reefs. A spawning aggregation was observed on August 10, 2010 at noon at San Mateo Reef, approximately 5 x 3 meters in size, moving in and out of kelp bed 20 m in depth for about 15 minutes (personal observation). This observation along with the presence of spawning females indicates that barred sand bass can spawn away from well-known spawning aggregation sites in Southern California, possibly due to fishing pressure (Ames 2004).

Proportional liver size (HSI) was highest in fish from the LA/LB Harbor and Huntington Flats, which are the closest sites to Palos Verdes Superfund Site, and the sites where fish had the highest tissue concentrations of organic pollutants. This result is consistent with other studies that found increased liver size in fishes with high levels of pollutants (Mearns et al. 1991, Khan 2003, Hinton, David et al. 2008, Reynders and Bervoets 2008, Sol et al. 2008, Fang et al. 2009). Fish exposed to PCBs and PAHs have also been shown to have increased liver size due to an increase in detoxification enzymes

(Khan 2003, Hinton, David et al. 2008). Metal pollutants can also influence liver size, but generally, high concentrations of metal pollutants coincide with high concentrations of organic pollutants. Due to the widespread response of liver size to pollution, HSI has been used as a biomarker two determine if environmental conditions have improved (Facey and Blazer 2005).

Assessment of sublethal effects on the health of individual barred sand bass

Indices of health of individual barred sand bass were compared with the tissue concentrations of toxicants in those individuals. Certain physiological parameters were related to liver tissue concentrations of organic pollutants. Condition (weight-at-length) and growth were both negatively related to a multivariate summary of tissue concentrations of pollutants. These results suggest that pollutants can negatively affect growth and body condition in barred sand bass, as has been noted in other fishes (Farkas et al. 2003, Fang et al. 2009). In contrast, Loflen (2013) found no effect of pollutants on the age or size of the sister species *Paralabrax maculatofasciatus* but they focused on only two PCBs types, two DDTs, and mercury in their analysis, and all the adult fish exceeded advisory levels in pollutant concentration. Another study that looked barred sand bass with respect to mercury accumulation found that levels of mercury were positively correlated to length and age among sites, so that older and bigger barred sand bass had more accumulation of pollutants within its tissue among sites (Phillips et al. 1997). The present study detected the same pattern, with pollution concentration increasing with size and also having lower levels of contamination in less urbanized areas. Despite negative correlations between pollutant tissue concentrations and condition

and growth of barred sand bass, there was no evidence that HSI, GSI, or fecundity (mature eggs) of individuals were correlated with organic pollutant concentrations.

Caveats

The present study focused on a few indices of health to evaluate how barred sand bass may be affected by organic pollutants. Further studies should evaluate other parameters to assess the health of fish in polluted areas. Regarding reproduction, this studied focused mostly on females, but other studies have shown that pollutants can also impact reproduction of males (Vives et al. 2005) via sperm deformation and fertilization ability (Kime 1995, Wahbi and El-Greisy 2007, Daouk et al. 2011). The present study did not look at egg size or larval deformation as possible responses to pollution (Faulk et al. 1999, Wahbi and El-Greisy 2007, Incardona et al. 2008, Farwell et al. 2012). Furthermore, this study was limited to a single year that focused on one reproductive period. Extending this study or looking at seasonal differences could be beneficial in understanding accumulation rates in tissues. Understanding accumulation and feeding patterns could help limit confounding factors in trying to understand where pollutants were accumulated from in a species that moves large distances (Mason and Lowe 2010, McKinzie et al. 2013).

Human health and the consumption of barred sand bass

The EPA (2009) added barred sand to the "limit your consumption" health advisory, recommending no more than one 8 oz. serving per week of fish captured between Ventura Harbor to Santa Monica Pier (yellow zone); no consumption from Santa

Monica Pier south to Seal Beach Pier (red zone); and no more than one serving from Seal Beach Pier south to San Mateo Point (yellow zone) (Klasing and Brodberg 2008, Klasing et al. 2009). The present study measured the concentration of organic pollutants in liver tissue, which is typically not consumed by humans. There is, however, a positive relationship between pollutant concentration in white muscle and liver tissue, with liver tissue having a higher concentration of pollutants (Reynders and Bervoets 2008, Tapia and Vargas-Chacoff 2012). The information from the present study can still be used to estimate and prevent exposure; for example, we now know that LA/LB Harbor and Huntington Flats were the sites where fish had the highest concentrations of organic pollutants. These two sites are heavily fished and barred sand bass are targeted. In 2013, LA Harbor was re–opened to recreational fishing and barred sand bass are found there in great abundance (MEC Analytical Systems Inc. 2002), which could pose a risk to humans who regularly consume fish from this area. Huntington Flats is also a site of concern because it is heavily targeted by anglers during the spawning season due to the presence of large aggregations of barred sand bass, making them easy to target and also increases human exposure if barred sand bass are consumed.

Conclusions

This study can be used as a baseline for future studies to see if tissue concentrations of organic pollutants are declining in this recreationally important species. Future research should focus on barred sand bass during peak fishing seasons, and measure contaminant loads in both white and liver tissue to determine human exposure, in particular at Huntington Flats, where this species has historically been harvested in

huge numbers. In combination with other factors such as over fishing pressure, this study provides some evidence that barred sand bass in polluted areas may be in poorer condition and grow at slower rates, which could be detrimental for this population that may already be on verge of collapsing. Barred sand bass is a long-lived species so it is important to continue monitoring the population in order to understand accumulation rate, possible future sublethal effects, and the persistency of legacy pollutants such as DDTs in southern California.

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Appendix A

Table 1. List of organic pollutant standards, surrogates and congers used to determine concentration in the tissues of barred sand bass from four sites in California.

Internal Standards			
d10-Anthracene		4,4- Dibromobiphenyl	
d12-Benzo[g,h,i] perylene		2,2',5,5'- Tetrabromobiphenyl	
System Monitoring Compounds			
(d8-Napthalene)	TCMX		
(d10-Acenaphthene)	PCB 030		
(d10-Phenanthrene)	PCB 112		
(d12-Chrysene)	PCB 198		
(d12-Perylene)			
Target Compounds			
PAHs	**OCPs**	**PCBs**	
Naphthalene	BHC-alpha	PCB 003	PCB 114
2-Methylanaphthalene	Heptachlor	PCB 008	PCB 153
1-Methylanaphthalene	Aldrin	PCB 018	PCB 168+132
Biphenyl	Heptachlor epoxide	PCB 031	PCB 105
2,6- Dimethylnaphthalene	Oxychlordane	PCB 028	PCB 141
Acenaphthylene	Chlordane- gamma	PCB 033	PCB 138
Acenaphthene	2,4' –DDE	PCB 052	PCB 158
2,3,5-Trimethylnaphthalene	Endosulfan I	PCB 049	PCB 126
Fluorene	Chlordane- alpha	PCB 044	PCB 187
Dibenzothiophene	trans- Nonachlor	PCB 037	PCB 183
Phenanthrene	4,4'- DDE	PCB 074	PCB 128
Anthracene	Dieldrin	PCB 070	PCB 167
1-Methylphenanthrene	2,4'- DDD	PCB 066	PCB 174
Fluoranthene	Perthane	PCB 095	PCB 177
Pyrene	Endrin	PCB 056(060)	PCB 156
Benz[a] anthracene	Endosulfan II	PCB 101	PCB 199(200)
Chrysene	4,4'- DDD	PCB 099	PCB 157
Benzo[b] fluoranthene	2,4'- DDT	PCB 119	PCB 180
Benzo[k] fluoranthene	cis- Nonachlor	PCB 097	PCB 169
Benzo[e] pyrene	Endrin aldehyde	PCB 087	PCB 170
Benzo[a] pyrene	Endosulfan sulfate	PCB 081	PCB 201
Perylene	4,4'- DDT	PCB 110	PCB 189
Indeno [1,2,3-c,d] pyrene	Endrin ketone	PCB 077	PCB 195
Dibenz [a,h] anthracene	Methoxychlor	PCB 151	PCB 194
Benzo[g,h,i] perylene	Mirex	PCB 149	PCB 206
		PCB 123	PCB 209
		PCB 118	

Table 2. Mean concentrations (± 1 standard error) of pollutants found within the tissue of barred sand bass collected at four sites in California.(LA/LB: Los Angeles / Long Beach Harbor; HF: Huntington Flats; SCR: San Clemente Reefs; and SD: San Diego Bay). Twenty-five PAHs were tested but only 16 were detected; 29 OCPs were tested but only 12 were detected; Fifty-three different types of PCB were tested but only 49 were detected in the tissue of barred sand bass. Twenty fish from each site were sampled.

Pollutants		Sites			
		LA/LB	HF	SCR	SD
OCPs	2,4'- DDD	0.35±0.35	0	0	0.32±0.32
	4,4'- DDD	10.1±1.8	8.9±1.7	1.2±0.6	4.3±1.6
	2,4' -DDE	8.8±1.3	10.4±2.3	0.4±0.4	0.4±0.3
	4,4'- DDE	564.6±91.4	705.3±116.0	124.7±22.3	115.5±28.0
	2,4'- DDT	0.5±0.5	0	0	0.3±0.3
	4,4'- DDT	0.3±0.3	0	0	0
	Chlordane- gamma	0.4±0.3	0	0	0
	Chlordane- alpha	0.3±0.2	0	0	0
	Endosulfan II	0	0.2±0.2	0	0
	Hexachlorobenzene	0.2±0.2	0.2±0.2	0.3±0.3	1.0±0.4
	trans- Nonachlor	10.3±2.1	9.2±1.7	1.6±0.6	8.1±1.5
	cis- Nonachlor	4.9±1.2	3.8±1.0	0.4±0.4	4.9±1.3
PAHs	1- Methylanaphthalene	3.2±0.7	1.9±0.6	0.8±0.3	1.4±0.4
	1- Methylphenanthrene	1.6±1.2	0.3±0.2	0.4±0.3	0.3±0.3
	2- Methylanaphthalene	6.9±1.3	3.8±1.3	2.3±0.5	5.2±1.6
	2,35- Trimethylnaphthalene	0	0	0	1.3±1.3
	2,6- Dimethylnaphthalene	1.1±0.3	0.3±0.1	0.1±0.1	0.8±0.4
	Acenaphthene	10.2±3.1	2.9±1.6	0	0
	Acenaphthylene	0.8±0.3	0.3±0.2	0.1±0.1	0.6±0.5
	Anthracene	2.8±0.6	0.6±0.4	0.3±0.2	1.1±0.5
	Biphenyl	5.0±0.8	2.0±0.7	0.7±0.3	1.9±0.9
	Chrysene	0.2±0.2	0	0.1±0.1	1.0±0.9
	Dibenzothiophene	1.8±0.3	0.8±0.3	1.2±0.3	1.5±0.3
	Fluoranthene	14.1±2.6	3.0±1.8	1.0±0.5	7.1±1.5
	Fluorene	7.3±1.3	2.9±0.9	1.8±0.5	3.2±0.6
	Naphthalene	11.2±1.1	11.0±0.9	5.8±0.6	10.2±1.8
	Phenanthrene	9.6±1.0	7.2±1.1	6.7±0.6	7.8±0.7
	Pyrene	2.0±0.5	0.9±0.4	1.0±0.3	1.6±0.3
PCBs	PCB 003	0	0	0.1±0.1	0
	PCB 008	0	0.1±0.1	0	0
	PCB 028	6.6±1.3	4.6±.8	0	4.4±1.7
	PCB 031	1.9±0.7	0.6±0.3	0	0.8±0.3
	PCB 033	0	0.3±0.2	0.2±0.2	0.3±0.2
	PCB 037	0.8±0.6	0	0.5±0.5	0

Table 2 continued

PCB 044	5.3±1.1	2.6±0.9	0.5±0.5	4.6±1.2
PCB 049	12.9±1.9	8.7±1.2	0.7±0.7	15.6±3.7
PCB 052	18.6±2.7	10.1±1.5	1.0±1.0	16.1±3.9
PCB 056, 060	3.5±0.9	2.1±0.8	0	1.4±1.0
PCB 066	12.6±1.9	8.5±1.2	1.1±0.8	15.0±4.7
PCB 070	4.5±0.7	3.6±0.6	0.2±0.2	3.9±0.9
PCB 074	10.6±1.6	7.3±1.1	0.7±0.4	10.1±3.3
PCB 077	0	0	0	0.4±0.4
PCB 087	14.0±2.3	5.6±1.3	0.5±0.5	12.7±2.9
PCB 095	11.7±2.1	6.0±1.2	1.0±1.0	11.8±2.4
PCB 097	9.2±1.5	5.1±1.0	0.7±0.7	9.7±2.1
PCB 099	31.5±5.3	17.6±2.1	4.0±2.0	43.9±8.4
PCB 101	44.3±7.7	23.0±3.1	4.6±2.5	52.2±11.5
PCB 105	19.4±0.5	12.2±1.8	0.5±0.5	21.3±5.7
PCB 110	16.3±2.9	8.1±1.5	1.3±1.0	13.4±3.1
PCB 114	0.7±0.4	0.4±0.4	0	0
PCB 118	37.0±6.7	21.2±2.3	4.2±2.0	44.1±10.1
PCB 119	0.2±0.2	0	0	0
PCB 123	1.1±0.5	0.6±0.3	0.8±0.5	3.3±0.8
PCB 126	0.4±0.4	0	0	0
PCB 128	13.4±2.3	9.0±1.5	0.7±0.7	15.6±2.9
PCB 138	85.2±3.9	49.4±7.0	10.3±3.9	100.5±19.4
PCB 141	6.4±1.5	2.9±0.7	0.3±0.3	7.3±1.7
PCB 149	21.5±3.7	11.4±2.1	2.4±1.5	25.6±5.2
PCB 151	8.9±1.6	3.8±0.8	0.6±0.5	8.4±1.7
PCB 153	110.6±21.0	62.6±9.4	15.3±5.7	148.2±28.5
PCB 156	5.4±1.5	1.6±0.7	0.3±0.3	6.3±1.8
PCB 157	0.9±0.4	0	0	0.7±0.4
PCB 158	9.8±1.8	8.8±2.6	0.8±0.3	13.2±3.8
PCB 167	4.1±0.8	1.7±0.5	0.3±0.3	4.5±1.2
PCB 168, 132	12.6±2.8	6.7±1.1	1.1±1.1	13.6±2.1
PCB 170	10.8±3.0	7.1±1.3	0.8±0.8	14.9±3.1
PCB 174	4.7±1.6	1.6±0.7	0	5.5±1.2
PCB 177	6.4±1.6	3.7±1.2	0	7.2±1.9
PCB 180	24.3±4.5	13.5±1.9	1.3±0.9	26.0±4.7
PCB 183	13.2±2.3	8.2±1.3	0.5±0.4	14.0±2.5
PCB 187	28.3±4.9	18.2±2.5	2.6±1.8	36.1±6.3
PCB 194	2.2±0.9	1.2±0.6	0	3.5±1.5
PCB 195	1.3±0.5	0.5±0.4	0	1.9±1.2
PCB 199,200	0.4±0.4	0	0	1.1±0.5
PCB 201	5.4±1.4	3.5±1.1	0	8.2±2.0
PCB 206	1.3±0.6	0.8±0.6	0	1.9±0.9
PCB 209	0.4±0.2	0.2±0.2	0	0.7±0.5

Table 3. Results of multiple regressions testing the relationship between physiological indices and principal coordinate (PCO) scores summarizing organic pollutant concentration in barred sand bass liver tissue.

Index	Factors	*n*	Standardized Coefficient	r^2	P-value
HSI		80		0.01	0.604
(log)	PCO 1		-0.078		0.492
	PCO 2		0.083		0.466
GSI		80		0.03	0.354
(sqrt)	PCO 1		0.128		0.259
	PCO 2		0.101		0.372
Condition		80		0.96	0.001
Wt (log)	PCO 1		-0.070		0.003
	PCO 2		-0.012		0.598
	SL		0.969		0.000
Growth		80		0.65	0.001
Wt (log)	PCO 1		-0.156		0.024
	PCO 2		-0.078		0.256
	Age		0.777		0.000
Fecundity		59		0.29	0.001
Mature eggs	PCO 1		-0.191		0.105
(log)	PCO 2		0.112		0.335
	SL		0.476		0.000

Appendix B

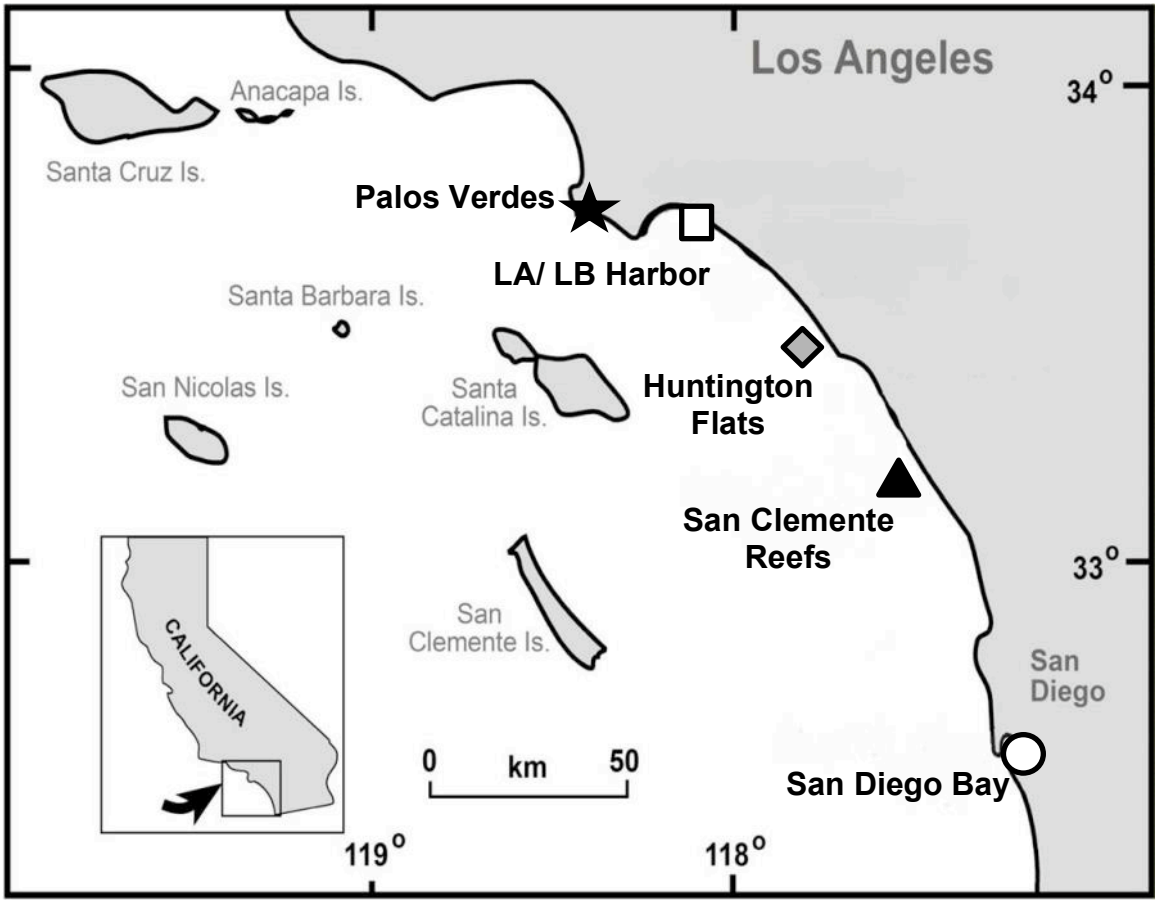

Figure 1. Map showing the four study sites were barred sand bass were collected in southern California. Los Angeles / Long Beach Harbor, Huntington Flats, San Clemente Reefs and San Diego Bay. Los Angeles / Long Beach Harbor are the closet site to the Superfund site off Palos Verdes.

Figure 2. Photograph of Soxhlet tissue extraction system that was used to extract organic pollutants from the liver of barred sand bass.

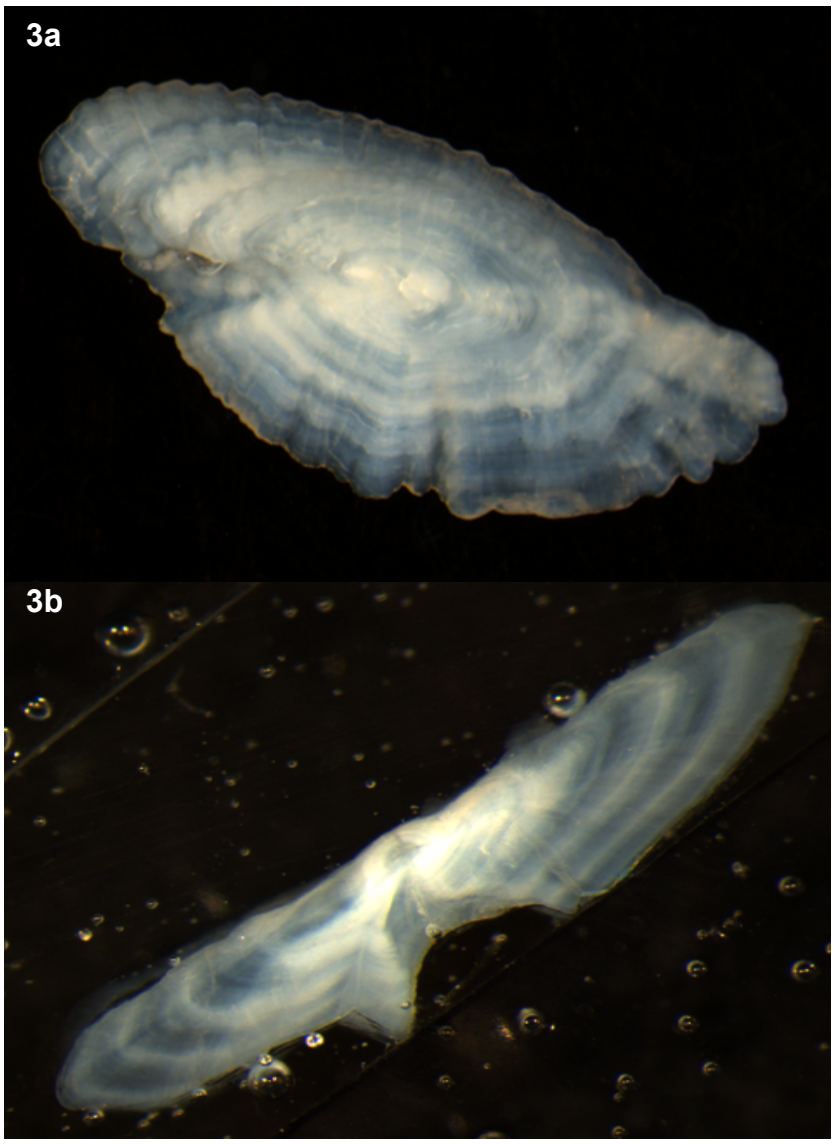

Figure 3. Photograph of a) whole otoliths, which were used to determine age in fish younger than five years; and b) cross sections, which were used in fish older than five years old to confirm whole otolith estimates of age.

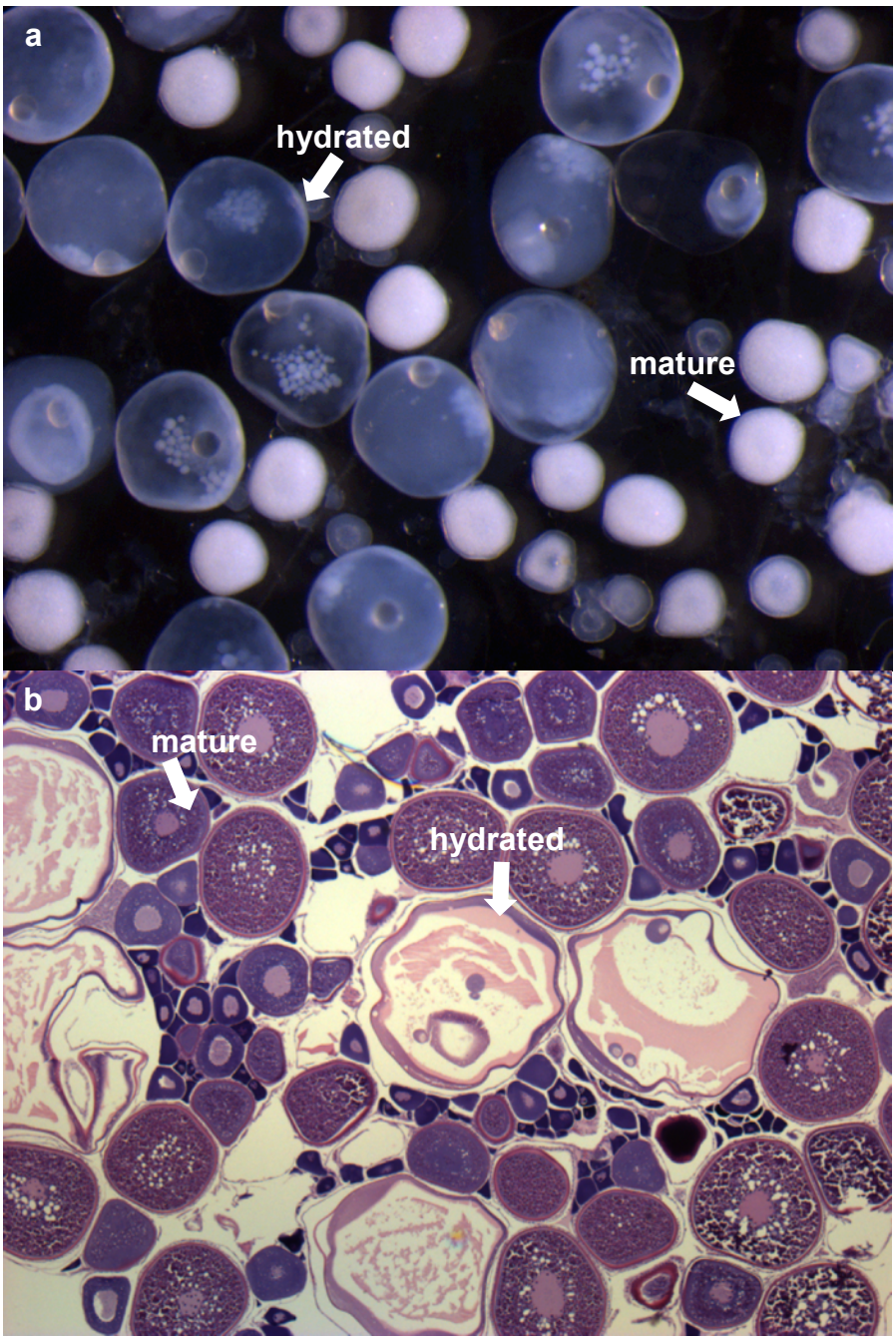

Figure 4. a) Photograph of a) mature and hydrated eggs, which were used to estimate reproductive potential in female barred sand bass. b) Histology analyses were used to confirm classification of ovaries by reproductive stage.

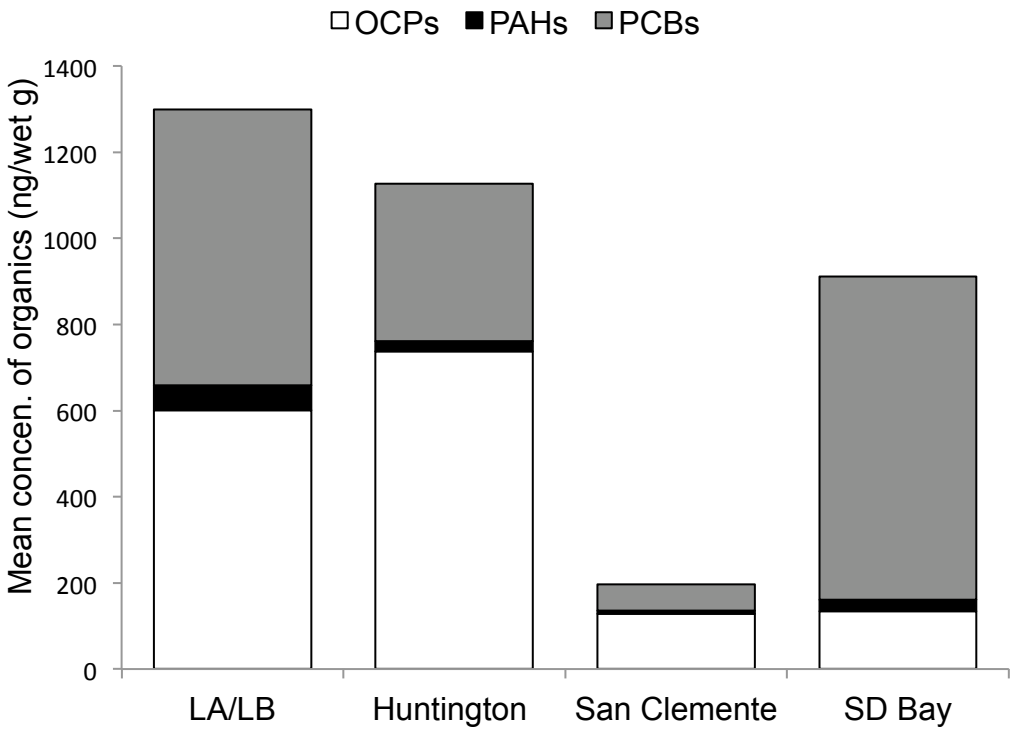

Figure 5. Mean concentrations of all organic pollutants found in the liver tissue of barred sand bass from four sites in southern California. Tissue concentrations of organic pollutants differed significantly different among the 4 sites (PERMANOVA: $p<0.001$), with fish from the San Clemente area having the lowest concentrations. $n = 20$ for each bar.

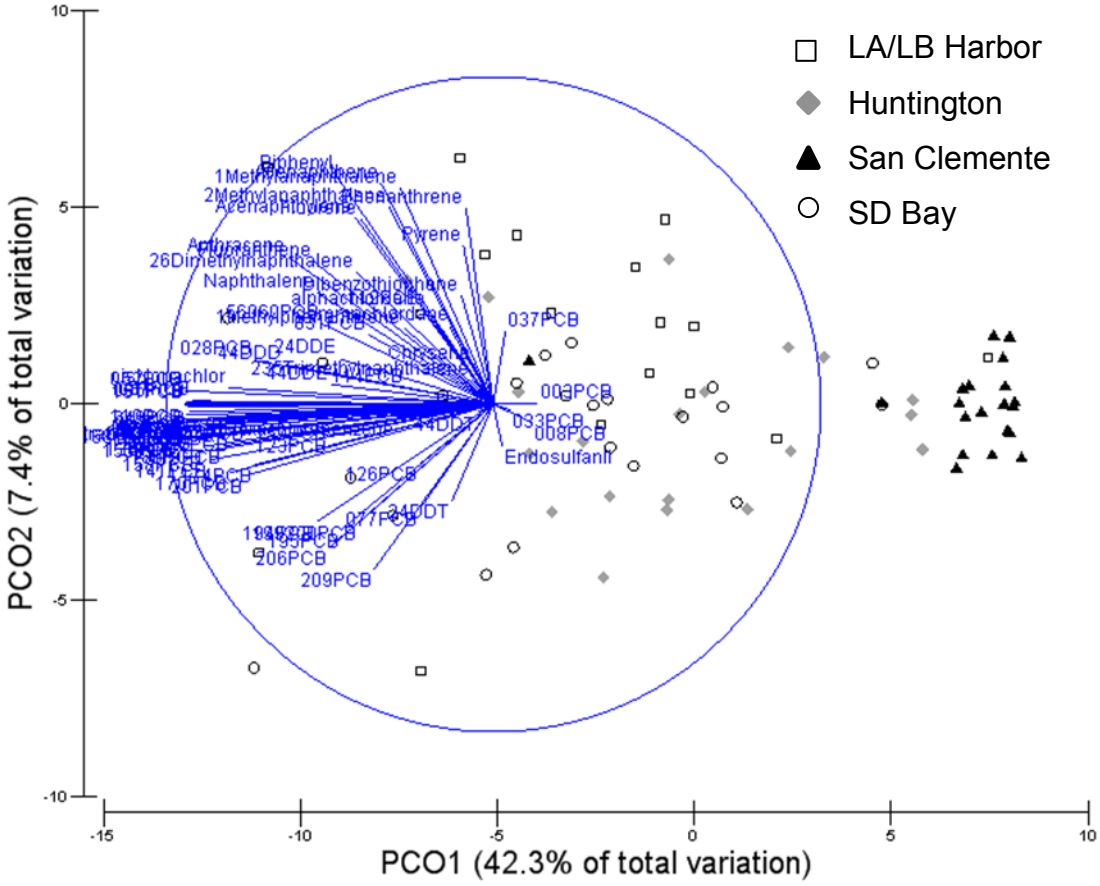

Figure 6. Results of Principal Coordinates analysis using organic pollutants found in the tissue of barred sand bass at four sites in southern California. Principal Coordinates 1 and 2 summarized 49.7 % of the total variance in concentrations. 107 organic pollutants were tested but only 77 were present in liver tissue of individual barred sand bass. PCO were analyzed using detected pollutants only for the analysis. n = 80.

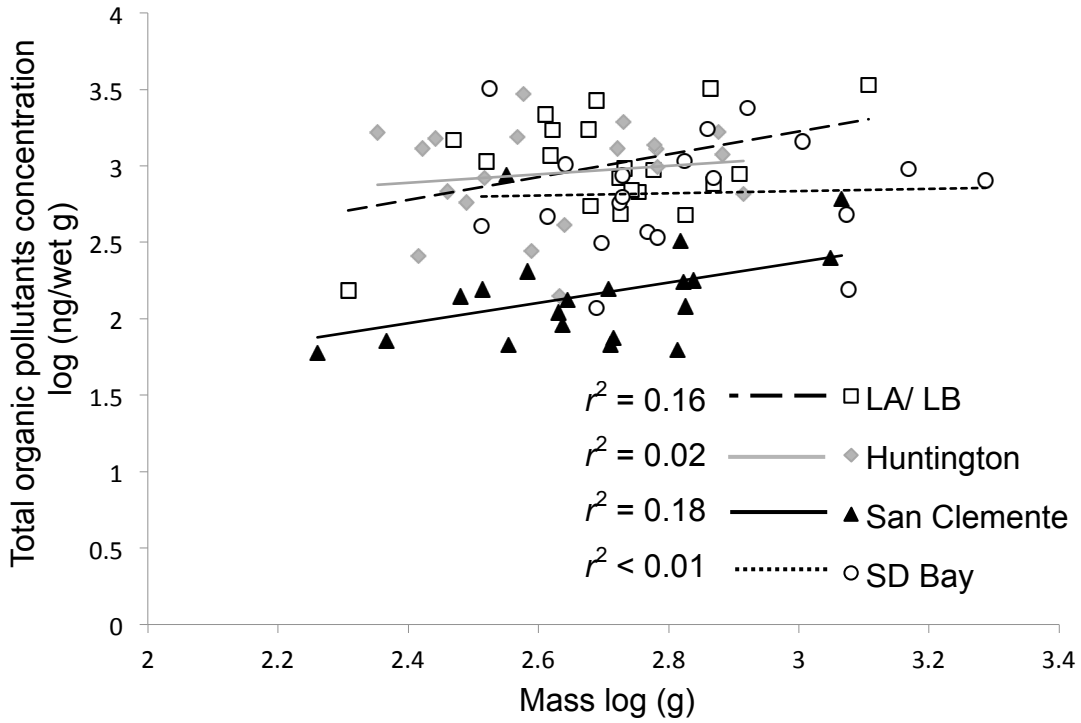

Figure 7. Relationships between total organic pollutant concentration and size of barred sand bass from four sites. The slope of this relationship did not differ among the sites ($p=0.58$), but pollutant concentration at mass were higher at LA/LB Harbor, Huntington Flats and San Diego Bay ($p=0.038$). Bioaccumulation rate was positively associated with fish mass ($p<0.0001$). $n = 80$; 20 per site.

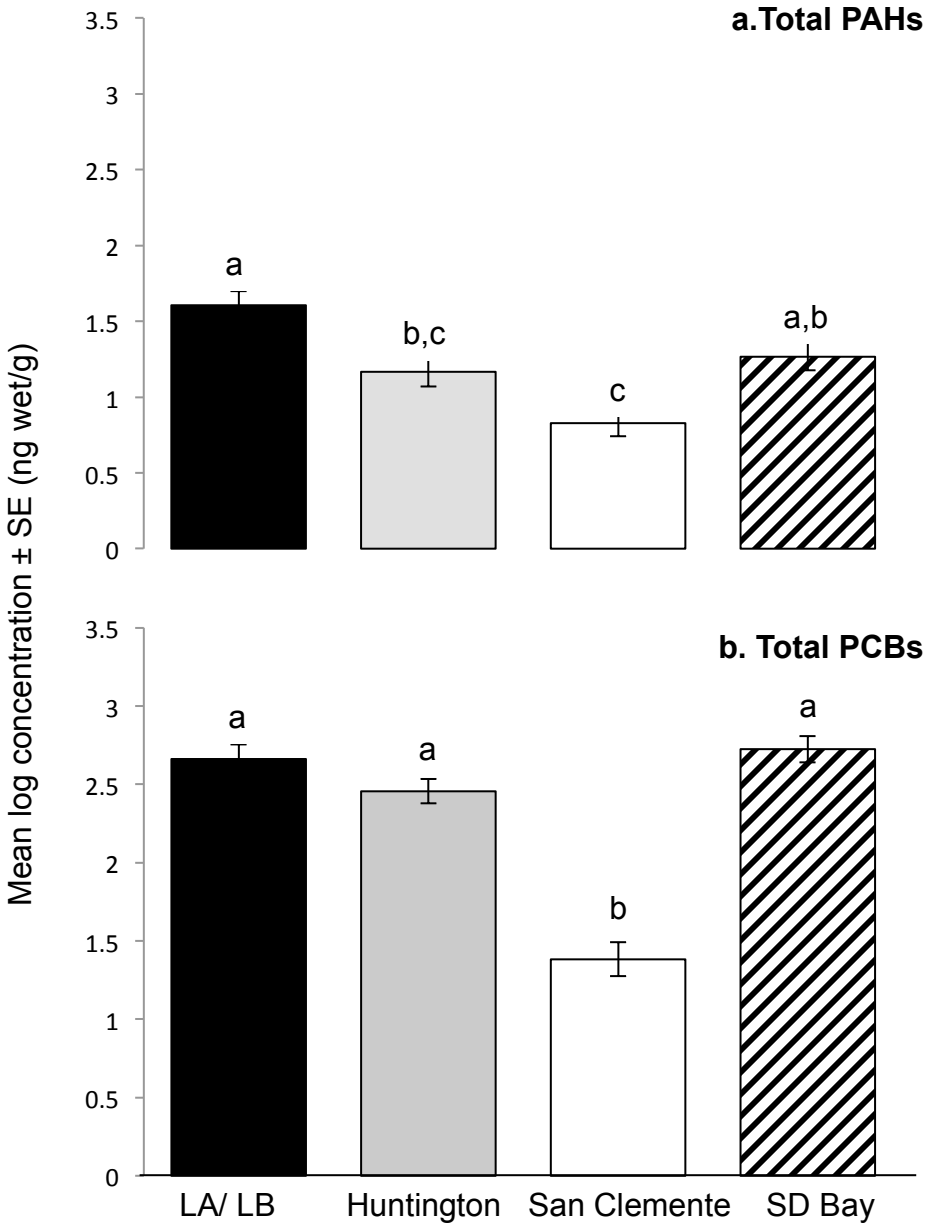

Figure 8. Mean concentrations (± 1 SE) of (a) PAHs and (b) PCBs in liver tissue of barred sand bass from four sites. Concentrations differed significantly among sites (one-way ANOVA with Tukey's post hoc $p<0.001$), with lower concentrations in tissues of fish from the San Clemente reefs. $n = 20$ for each bar.

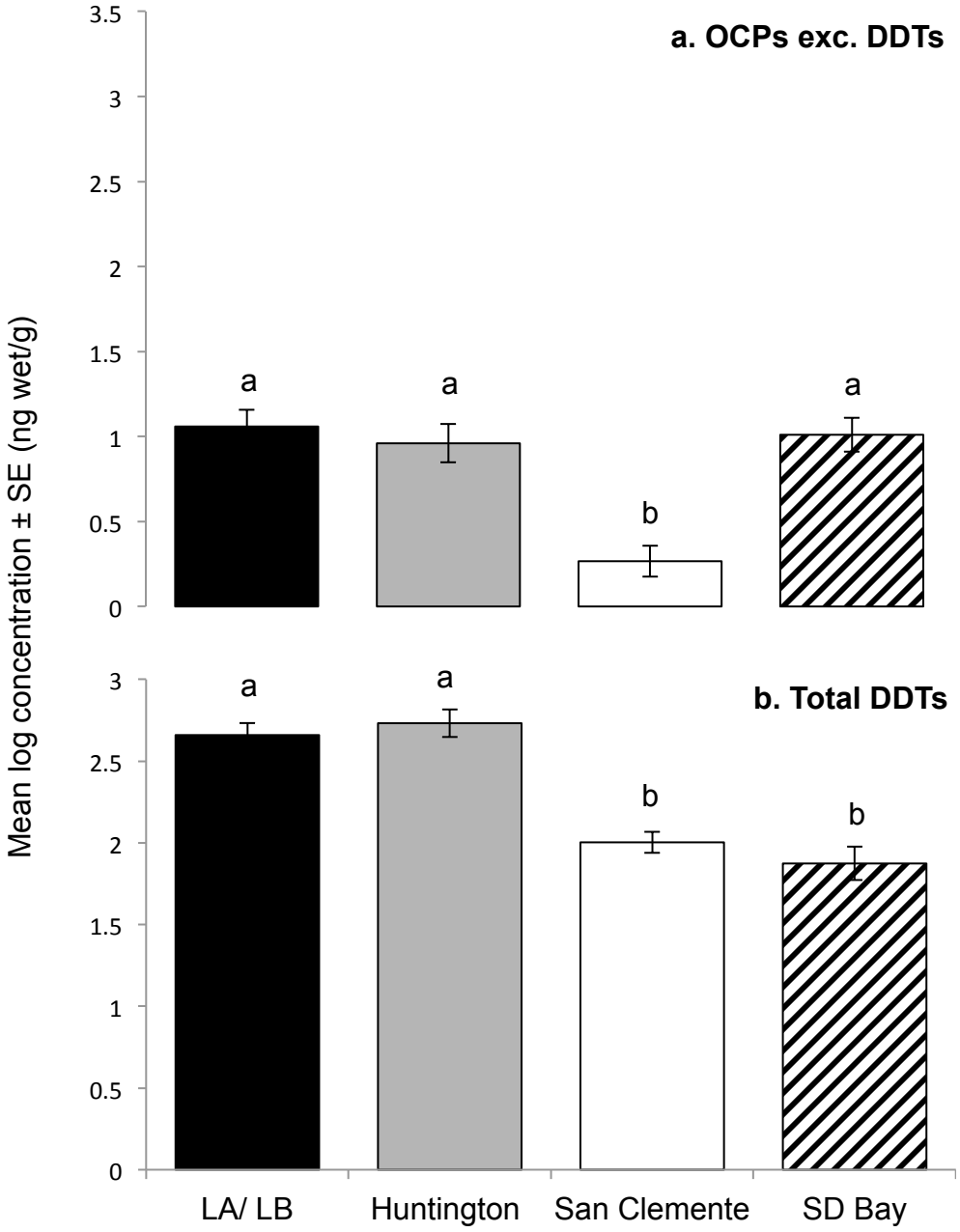

Figure 9. Mean concentrations (± 1 SE) of (a) total OCPs excluding DDTs and DDTs and (b) total DDTs and DDTs in barred sand bass liver tissue from four sites. Total DDTs made up 97% of the total concentration of OCPs. OCPs excluding DDTs differed among sites, with fish from San Clemente Reefs having lower concentrations that those from the three other sites. Total DDTs were lower in fish from the two sites farthest from the Palos Verdes Superfund site (one-way ANOVA with Tukey's post hoc $p<0.001$). $n = 20$ for each bar.

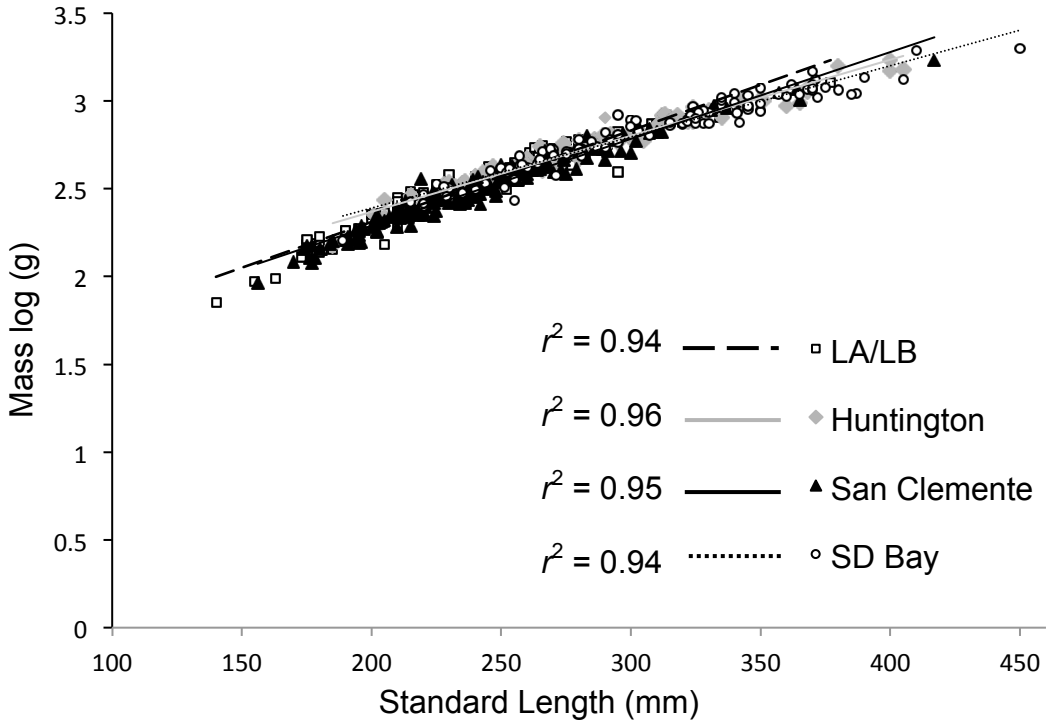

Figure 10. The weight-length relationship for barred sand bass from four sites ($p<0.001$). n = 119, 97, 169, and 111 fish from LA/LB Harbor, Huntington Flats, San Clemente Reefs, and San Diego Bay, respectively.

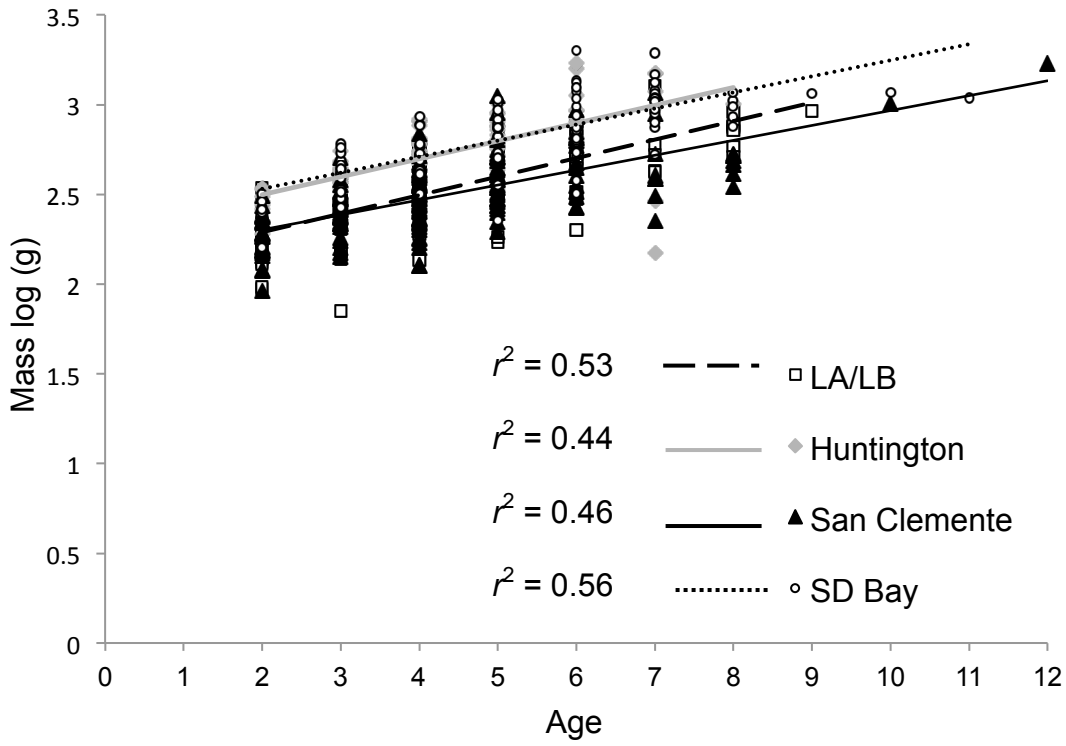

Figure 11. Relationships between body mass and age in barred sand bass collected from four sites. The slope of this relationship did not differ among the sites (p=0.28), but mass at age was higher in fish from the Huntington Flats and San Diego Bay than at LA/LB Harbor or the San Clemente Reefs (p<0.001). *n* = 119, 97, 169, and 111 fish from LA/LB Harbor, Huntington Flats, San Clemente Reefs, and San Diego Bay, respectively.

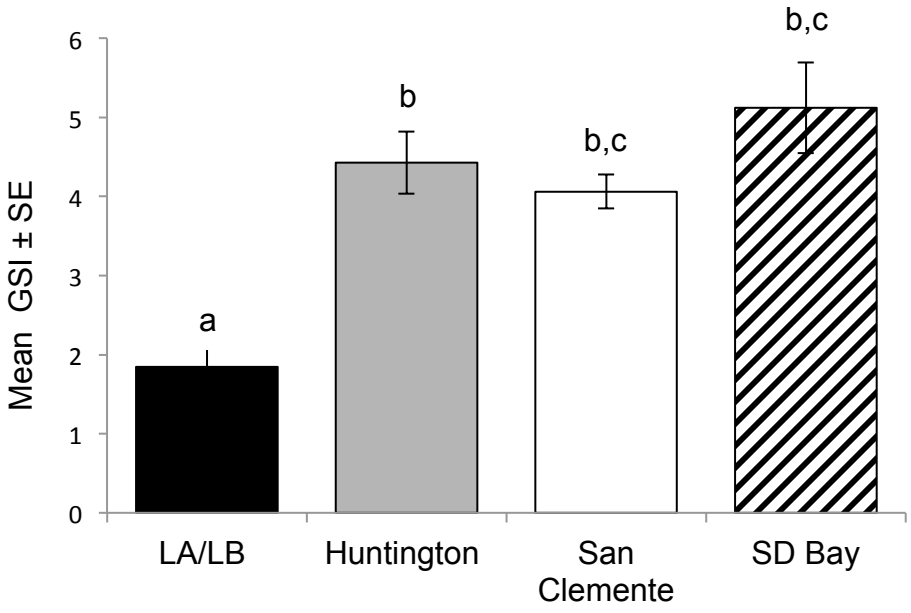

Figure 12. Mean gonadosomatic index (GSI) (± 1 SE) of female barred sand bass from four sites. GSI differed among sites with LA/LB Harbor having the lowest mean (one-way ANOVA with Tukey's post hoc: $p<0.001$). *n* = 53, 52, 77, and 45 fish from LA/LB Harbor, Huntington Flats, San Clemente Reefs, and San Diego Bay, respectively.

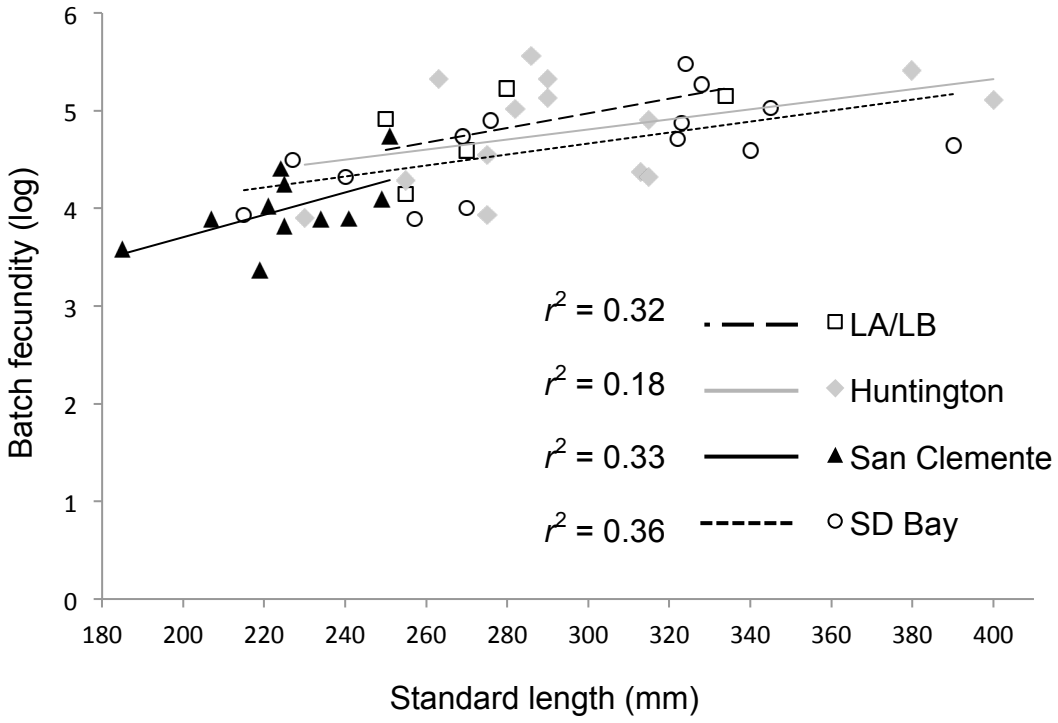

Figure 13. Relationships between batch fecundity and standard length in barred sand bass from four sites. The slopes of this relationship did not differ among sites (p=0.86). n = 5, 14, 11, and 14 fish from LA/LB Harbor, Huntington Flats, San Clemente Reefs, and San Diego Bay, respectively.

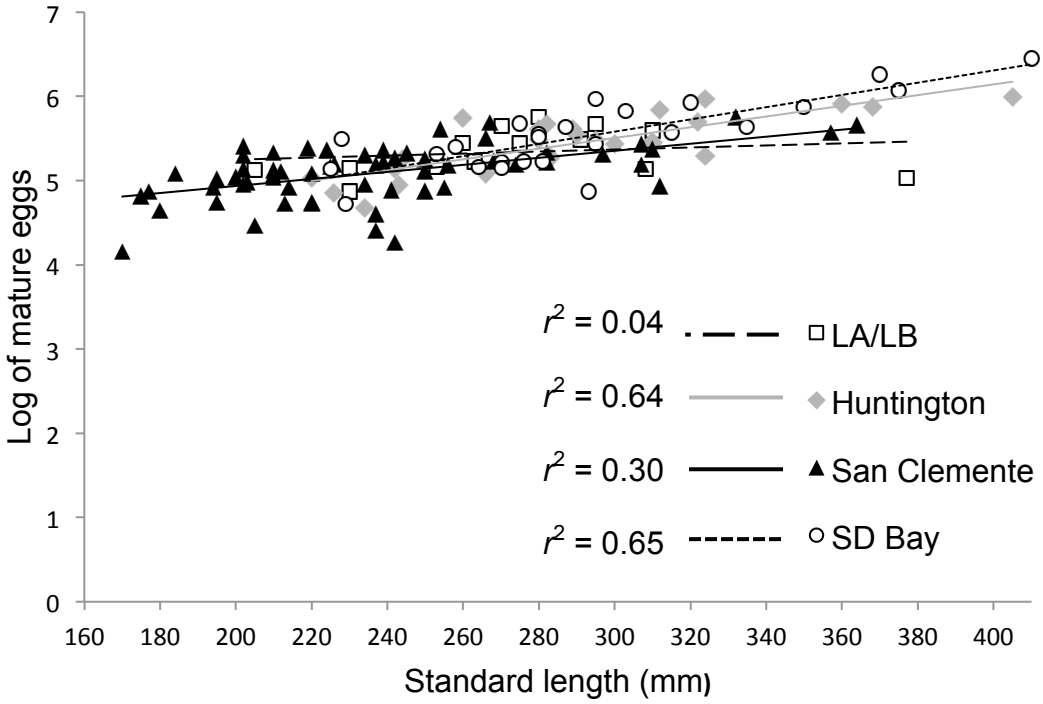

Figure 14. Relationships between reproductive potential (number of "mature" eggs in ovaries) of female barred sand bass from four sites. Slopes differed significantly among sites (p=0.014). *n* = 14, 26, 60, and 25 fish from LA/LB Harbor, Huntington Flats, San Clemente Reefs, and San Diego Bay, respectively.

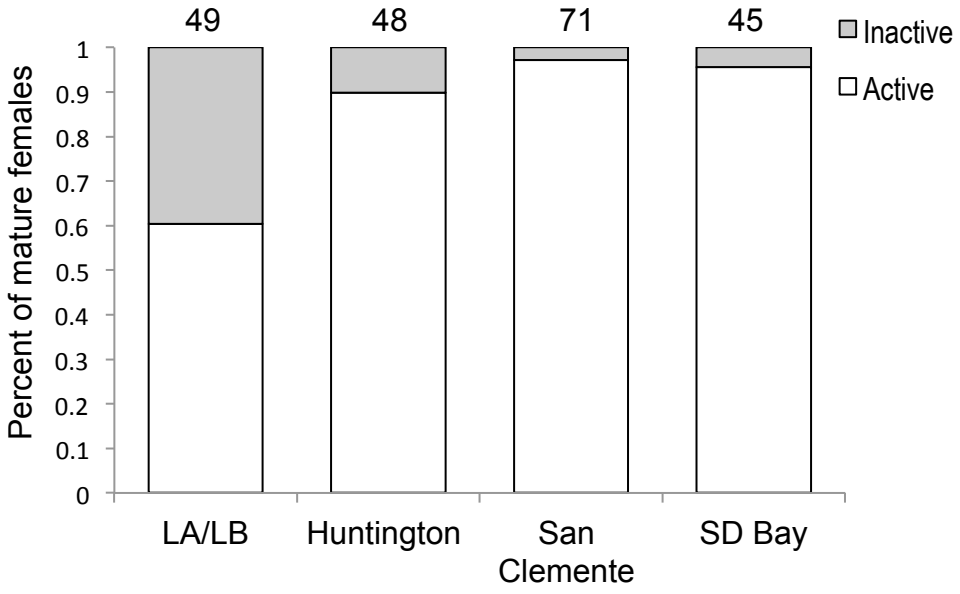

Figure 15. The proportions of mature females that were reproductively inactive or active at four sites. There was a significant different in the proportion of mature active: inactive reproductive females among sites with LA/LB having the highest proportion of inactive females ($p<0.001$). Sample size for each site is given above the bars.

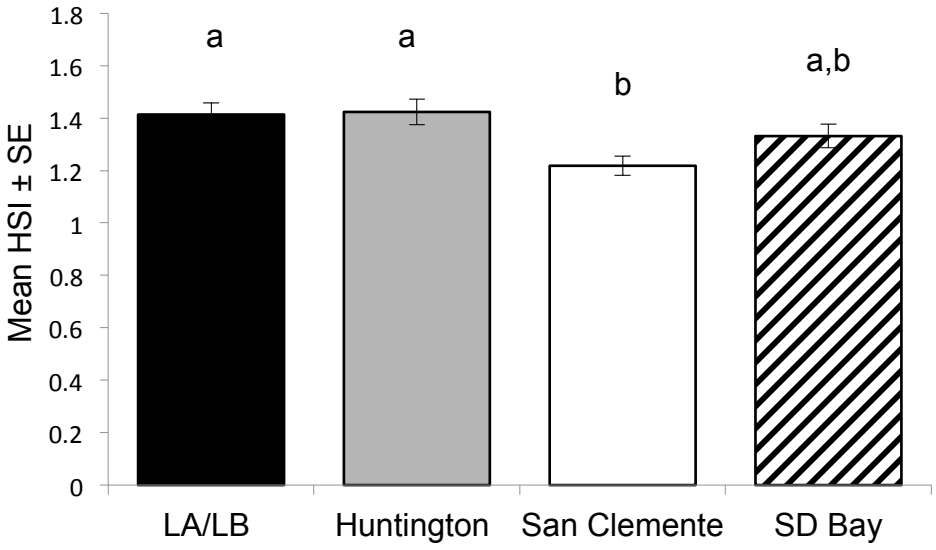

Figure 16. Mean hepatosomatic index (HSI) (± 1 SE) of female and male barred sand bass collected from four sites. HSI was significantly different among the sites, with San Clemente having the lowest HSI (one-way ANOVA: $F_{3,367}$= 6.5, $p<0.001$). Eighty-two fish from LA/LB Harbor, 75 fish from Huntington Flats, 133 fish from San Clemente Reefs and 81 fish from San Diego Bay were sampled for this analysis.