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REPRODUCTIVE ECOLOGY AND BODY BURDEN OF RESIDENT FISH PRIOR TO DECOMMISSIONING

MMS U.S. Department of Interior
Minerals Management Service
Pacific OCS Region

REPRODUCTIVE ECOLOGY AND BODY BURDEN OF RESIDENT FISH PRIOR TO DECOMMISSIONING

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TECHNICAL SUMMARY

Study Title: Reproductive Ecology and Body Burden of Resident Fish Prior to Decommissioning

Report Title: Reproductive Ecology and Body Burden of Resident Fish Prior to Decommissioning

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Background and Objectives:

All of the 27 oil and gas platforms off southern and central California have finite economic lives and some may be nearing the end of that life span. Regarding platform disposition, there are a number of options, ranging from complete removal to some forms of structure retention. All options have effects on the lives of organisms living on and around these structures. The process by which decisions are made as to the disposition of these platforms is called decommissioning and, in federal waters, these decisions involve detailed environmental reviews by the MMS.

Among the outstanding questions still remaining is that of contamination and body burden in resident fishes residing around oil and gas platforms. The most common contaminants discharged at platforms are hydrocarbons and trace metals. For instance, given the recent mercury alarm concerning this element in organisms around Gulf of Mexico platforms and its potential harm to humans when concentrated in edible fishes, it is timely to determine what contaminants are in fishes that reside at Pacific platforms. However, the contaminant load in platform fishes must be seen against the background levels of fishes in the region. That is, the contaminants present in platform fishes must be compared to that of the fishes in nearby natural areas, in order to establish the relative importance of such contaminants to the region.

Knowledge of the contaminant levels in platform-inhabiting fishes is of importance as it may affect reproduction. In order to analyze the environmental consequences of platform decommissioning on local or regional fish populations, the fate of young fishes recruiting to those populations and the general pollution load carried by fishes at platforms need to be known. This is especially true when the platforms are known to harbor large numbers of juveniles and resident reproducing adults of species that are regionally depleted by fishing and being considered for listing under the Endangered Species Act. Adults observed at the platform are reproductively mature and potentially produce larvae that ultimately replenish populations in natural areas. Adults, themselves, may migrate between platforms and natural areas. Any assessment of the impacts of platform removal on local and regional fish populations must consider the reproductive ecology of the species of interest, the general body burden of contaminants that the fish are carrying and their reproductive capability.

Thus, the goal of this study was to fill gaps in information about the heavy metal body burden and potential reproductive impairment of fishes living around oil and gas platforms. This goal was met through three tasks. Task 1 was to compare heavy metal contaminant levels in several important fish species living around platforms and natural coastal areas. Task 2 determined whether otoliths (fish ear bones) carried heavy metal signatures derived from platforms. Unique signatures of elements (including heavy metals) incorporated in otoliths potentially can be used to measure the extent to which platforms function as nurseries and contribute to the replenishment of regional populations in natural areas. If once a platform "fingerprint" is identified, otolith microchemistry can also be useful for identifying adult fish that have moved between platforms and natural areas. Task 3 was to contrast the reproductive capabilities (as measured by egg atresia) of fishes living around oil platforms and natural areas. Reproductive impairment has direct implications for regional populations. If there is no apparent impairment but contamination is present, there may be indirect implications for larval development and survival of the subject species.

Description:

Issues relating to possible contaminants in fishes living around oil and gas platforms were addressed through three tasks.

Task One: Whole-body Concentrations of Elements in Kelp Bass (*Paralabrax clathratus*), Kelp Rockfish (*Sebastes atrovirens*), and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

There are concerns that fishes living around oil and gas platforms may be more heavily polluted than those from natural sites. This study examined element concentrations in three species of fishes living around platforms and natural sites.

Task Two: Otolith Elemental Signatures of Kelp Rockfish (*Sebastes atrovirens*) and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

Fish may be exposed to trace metal contaminants discharged into the environment from platform activities, and the element composition of otoliths reflects environmental conditions. This study compared the concentration of elements in otoliths of fishes residing at platforms and natural areas and examined otolith composition in relation to seawater chemistry of the environment where fishes reside.

Task Three: A Histological Examination of the Ovaries of Pacific Sanddab, *Citharichthys sordidus*, Captured at Two Oil Platforms and Two Natural Sites in the Southern California Bight

Pollution can reduce the reproductive potential of fishes. This study examined the amount of egg atresia in females from platforms and natural sites to assess their reproductive health.

Significant Results:

Task 1: In this study we collected 18 kelp bass (*Paralabrax clathratus*), 80 kelp rockfish (*Sebastes atrovirens*), and 98 Pacific sanddab (*Citharichthys sordidus*) from five offshore oil platforms and 10 natural areas during 2005-2006 for whole-body analysis of 63 elements. Forty-two elements were excluded from statistical comparisons for one of three reasons: they consisted of major cations that were unlikely to accumulate to potentially toxic concentrations under ambient exposure conditions; they were not detected by the analytical procedures; or they were detected at concentrations too low to yield reliable quantitative measurements. The remaining 21 elements consisted of aluminum, arsenic, barium, cadmium, chromium, cobalt, copper, gallium, iron, lead, lithium, manganese, mercury, nickel, rubidium, selenium, strontium, tin, titanium, vanadium, and zinc. Statistical comparisons of these 21 elements indicated that none consistently exhibited higher concentrations at oil platforms than at natural areas. Eight comparisons yielded significant interactions between total length (TL) of fishes and the two habitat types (oil platforms and natural areas). This implied that relative differences in concentrations of several elements (e.g., titanium, vanadium, copper, rubidium, tin, and selenium) between each habitat type varied with TL of the fish species. To better understand these interactions, we examined elemental concentrations in very small and very large individuals of affected species. Although significant interactions were detected for rubidium, tin, and selenium in kelp rockfish, the concentrations of these elements did not differ significantly between oil platforms and natural areas over the TL range of sampled fish.

Task 2: The otolith composition of kelp rockfish and Pacific sanddab differed between the two habitat types—the sampled platforms and natural sites—and among geographic areas although there were significant interaction (habitat x area) effects. Kelp rockfish collected from platforms tended to incorporate higher concentrations of Mg and Ba and lower concentration of Sr in otoliths compared to fish from natural habitat. In comparison, Pacific sanddab collected from platforms tended to have lower concentrations of Ba and Fe in otoliths compared to fish from natural habitats. Sr/Ca in otoliths of kelp rockfish tended to decrease eastward along the coastal shelf from Point Conception through the east Channel. Mn/Ca in otoliths of sanddabs from both habitat types was lower in the three westernmost geographic areas (Point Conception, west Santa Barbara, and east Santa Barbara) than in the eastern and southern areas (east entrance of the channel, offshore east channel, and Southern California Bight). Mn/Ca was not detectable in kelp rockfish otoliths. The success rate of correctly assigning fish to habitat type (platform or natural habitat) using a canonical discriminant function based on otolith element signatures differed between species. Ninety-four percent of kelp rockfish individuals were correctly assigned to habitat type based on the otolith signature of Mg/Ca, Ba/Ca, and Sr/Ca. A lower proportion, 64%, of Pacific sanddab correctly grouped into habitat type based on the otolith signature of Mg/Ca, Sr/Ca, Mn/Ca, Cu/Ca, and Ba/Ca—otolith element concentrations that individually varied between sanddab habitat types and/or areas. Success in discriminating otoliths of either species from the two different habitat types was not associated with seawater element concentrations. Results from this study imply that platform habitat “fingerprints,” unique microchemical markers, may be identifiable along the time-keeping, growth trajectory of the otolith to reconstruct the history of platform habitat use during lifetime of fish. Such reconstructions will require more study on the spatial variability of platform elemental signatures from all existing platforms and the temporal stability of platform markers.

Task 3: If fishes living around oil and gas platforms are being significantly impacted by pollution, it would be expected to impair their reproductive abilities. Atresia, the abnormal reabsorption of oocytes that are destined to be spawned, is one form of reproductive impairment. Atresia has been widely used as an indicator of pollutant-related reproductive impairment in fishes. We examined the occurrence of atretic oocytes in Pacific sanddab, *Citharichthys sordidus*, collected from two offshore platforms in the Santa Barbara Channel (B and Gilda) and from two natural reference sites (off the east end of Santa Cruz Island and in mid-channel off Rincon). While pronounced atresia was observed in a few fish at one natural site and one platform, there was no evidence of widespread pronounced atresia at any of the four sites. This study implies that fishes that lie on the bottom around platforms and consume prey that lives in that sea floor are not reproductively impaired.

STUDY PRODUCTS

PAPER

Love, M. S. and S. R. Goldberg. A histological examination of the ovaries of Pacific sanddab, *Citharichthys sordidus*, captured at two oil platforms and two natural sites in the southern California Bight. Bull. S. Calif. Acad. Sci.

Nishimoto, M. M. 2009. Effects of coastal circulation on the distributional patterns of pelagic juvenile fish and otolith chemistry, and on the timing of juvenile reef fish settlement. Ph.D. Dissertation, University of California, Santa Barbara

REPRODUCTIVE ECOLOGY AND BODY BURDEN OF RESIDENT FISH PRIOR TO DECOMMISSIONING

EXECUTIVE SUMMARY

Information Needed

All of the 27 oil and gas platforms off southern and central California have finite economic lives and some may be nearing the end of that life span. Regarding platform disposition, there are a number of options, ranging from complete removal to some forms of structure retention. All options have effects on the lives of organisms living on and around these structures. The process by which decisions are made as to the disposition of these platforms is called decommissioning and, in federal waters, these decisions involve detailed environmental reviews by the MMS.

The possibility of contamination in resident fishes residing around oil and gas platforms remains unanswered. Contamination may occur in several ways. Drilling muds or fluids used in drilling operations are specially formulated mixtures of freshwater or seawater with clays, minerals, chemicals, and other materials used for cleaning drill bits, transporting cuttings, reducing friction, and stabilizing the borehole. Drill cuttings are particles of crushed sedimentary rock produced in the process of drilling. Drilling muds and drill cuttings may contain elevated levels of such elements as barium, chromium, lead, and zinc. These and other metals also originate from formation solids and mud additives. Produced waters are natural geological formation water or introduced seawater recovered with the extraction of oil. Most produced water is brine that may contain dissolved solids, metals, sulfur, and organic compounds at substantially higher concentrations than in seawater. Produced water can contain trace elements, radionuclides, and various petroleum hydrocarbons, including monoaromatic compounds, polycyclic aromatic hydrocarbons, and polar compounds. Nickel and vanadium are components of crude oil and sanitary and other domestic wastes are generated by platform personnel, and include sewage, garbage, and wastewater, and any associated contaminants. However, the contaminant load in platform fishes must be seen against the background levels of fishes in the region. That is, the contaminants present in platform fishes must be compared to that of the fishes in nearby natural areas, in order to establish the relative importance of such contaminants to the region.

A knowledge of the contaminant levels in platform-inhabiting fishes is of importance as it may affect reproduction. In order to analyze the environmental consequences of platform decommissioning on local or regional fish populations, the fate of young fish recruiting to those populations and the general pollution load carried by fish at platforms need to be known. This is especially true when the platforms are known to harbor large numbers of juveniles and resident reproducing adults of species that are regionally depleted by fishing and being considered for listing under the Endangered Species Act. Adults observed at the platform are reproductively mature and potentially produce larvae that ultimately replenish populations in natural areas. Adults, themselves, may migrate between platforms and natural areas. Any assessment of the impacts of platform removal on local and regional fish populations must consider the reproductive ecology of the species of interest, the general body burden of contaminants that the fish are carrying and their reproductive capability.

Thus, the goal of this study was to fill gaps in information about the heavy metal body burden and potential reproductive impairment of fishes living around oil and gas platforms. This goal was met through three tasks. Task 1 was to compare heavy metal contaminant levels in several important fish species living around platforms and natural coastal areas. Task 2 determined whether otoliths (fish ear bones) carried heavy metal signatures derived from platforms. Unique signatures of elements (including heavy metals) incorporated in otoliths potentially can be used to measure the extent to which platforms function as nurseries and contribute to the replenishment of regional populations in natural areas. If once a platform "fingerprint" is identified, otolith microchemistry can also be useful for identifying adult fish that have moved between platforms and natural areas. The goal of Task 3 was to contrast the reproductive capabilities (as measured by egg atresia) of fish living around oil platforms and natural areas. Reproductive impairment has direct implications for regional populations. If there is no apparent impairment but contamination is present, there may be indirect implications for larval development and survival of the subject species.

Research Summary

Task 1: Whole-body Concentrations of Elements in Kelp Bass (*Paralabrax clathratus*), Kelp Rockfish (*Sebastes atrovirens*), and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

In this study we collected 18 kelp bass (*Paralabrax clathratus*), 80 kelp rockfish (*Sebastes atrovirens*), and 98 Pacific sanddab (*Citharichthys sordidus*) from five offshore oil platforms and 10 natural areas during 2005-2006 for whole-body analysis of 63 elements. Immediately following capture, fishes were weighed and measured for total length (TL), assigned a unique identification number, then wrapped and bagged in clean plastic and held on wet ice until returned to the laboratory. In the laboratory, fish samples were stored frozen (-10°C) for roughly 1 week to 6 months until shipment to the USGS Columbia Environmental Research Center (CERC), Columbia, Missouri, for further processing and analysis.

At CERC, frozen fishes were minced and lyophilized. After lyophilization, each dried fish sample was reduced to a coarse powder. This final product was then transferred to a 40-ml glass vial that was sealed and stored in a desiccator until further processing. To prepare digestates from dried samples for semi-quantitative scans of multiple elements and quantitative determinations of arsenic by inductively coupled plasma mass spectrometry (ICP-MS), an aliquant of each sample (~0.25 g) was heated with 6 ml of nitric acid in a sealed low-pressure Teflon vessel by using a microwave oven. The cooled digestate liquid was transferred into a 125-ml polyethylene bottle with ultrapure water (>10 megOhm/cm) to a final weight of 101.5 g (100 ml). Final acid matrix was 6% nitric acid.

For quantitative determination of selenium, an additional tissue aliquant (~0.5 g) of each dried sample was subjected to a magnesium nitrate–nitric acid dry ashing procedure, followed by hydrochloric acid reduction. The dry ashing procedure consisted of three steps: boiling with nitric acid for solubilization and partial oxidation, ashing at 500°C with magnesium nitrate to complete the oxidation and decompose any remaining organic matter, and heating with hydrochloric acid to dissolve the ash and reduce all selenium to the selenite (Se^{+4}) oxidation state required for hydride generation. Following reduction, the digestates were diluted to ~100 ml with deionized water, yielding a final acid matrix of 10% hydrochloric acid. To perform a scan of elements (excluding mercury and selenium), samples were analyzed by ICP-MS using the semi-quantitative scan mode. All samples were diluted 10 times with a CETAC ASD-500 autodiluter as part of the analytical sequence. Internal standards were scandium (10 µg/l), rhodium (10 µg/l) and thorium (10 µg/l), and the external standard consisted of a traceable reference solution to which five elements (gold, praseodymium, tantalum, terbium, and thulium) were added for improved calibration in the rare earth region of the mass spectral range.

Quantitative arsenic determinations were conducted using an inductively coupled plasma dynamic reaction cell mass spectrometer. The ICP-MS quantitative method was designed to determine the monoisotopic arsenic mass, ^{75}As. Mercury was determined with a direct mercury analyzer in which a dried sample (50-60 mg) was combusted in a stream of oxygen. All mercury in the sample was volatilized and trapped by amalgamation on a gold substrate and was thermally desorbed and quantitated by atomic absorption spectrophotometry. The determination of selenium in dry-ashed samples was accomplished by flow injection hydride generation atomic absorption spectroscopy. In this procedure, the digestate was mixed with a hydrochloric acid carrier solution and then reduced by sodium tetrahydridoborate which had been stabilized with sodium hydroxide. The resulting volatile hydrogen selenide was transferred with argon carrier gas into a heated quartz cell mounted on an atomic absorption spectrophotometer for decomposition and measurement.

Of the 63 elements analyzed, a total of 21 were deemed suitable for statistical comparisons (Table 4). These included aluminum, arsenic, barium, cadmium, chromium, cobalt, copper, gallium, iron, lead, lithium, manganese, mercury, nickel, rubidium, selenium, strontium, tin, titanium, vanadium, and zinc. These 21 "quantitative" elements were compared between fishes living around oil platforms and natural areas.

Results from mixed models ANCOVA or ANOVA indicated that none of the 21 quantitative elements consistently exhibited higher concentrations in fishes from oil platforms or from natural areas. Out of 63 possible comparisons (21 elements measured in three fish species), 44 did not reveal significant differences between the habitat types, whereas 11 comparisons indicated that elemental concentrations were significantly higher at natural areas than at oil platforms. The remaining 8 comparisons exhibited significant habitat type-total length interactions, indicating that relative differences in elemental concentrations between habitat types varied with the length of the fish species.

Task 2: Otolith Elemental Signatures of Kelp Rockfish (*Sebastes atrovirens*) and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

In this study, we sampled 98 kelp rockfish and 108 Pacific sanddab collected from six offshore oil platforms and 12 natural areas during 2005 and 2006 for microchemical analysis of 19 elements in otoliths. The majority of specimens included in this study were used in the whole-body analysis of elements (Task 1). Kelp rockfish were collected by spear using SCUBA at four platforms at midwater depths and at six kelp forest habitat sites. Pacific sanddab were collected by hook and line at six platforms, including the platforms where kelp rockfish were collected, and six natural habitat sites. At each collection site, fish were bagged together in a plastic bag and kept on ice for up to ten hours. The fish were either processed in the laboratory on the collection day or the whole body samples were frozen until they were thawed and processed up to several weeks later.

Sagittal otoliths were extracted from 8-10 fish specimens per site, rinsed in DI water, and stored dry in a clean plastic snap cap vial until the time of preparation for otolith microchemistry. The otoliths were measured along the axis of the sulcus to the nearest 0.05 mm and weighed to the nearest 0.001 mg. We measured whole fish total length and standard length and weighed whole specimens. Preparation of the otoliths for microchemical analysis included sectioning, polishing, and decontamination procedures.

We used laser-ablation inductively coupled plasma mass spectrometry (LA ICP-MS) to sample a discrete portion of the sectioned otolith nearest the edge. Otolith material was vaporized by a 213 nm solid state Nd:YAg laser ablation system (New Wave UP213), and the sample was analyzed on a Finnigan MAT Element 2-sector field ICP-MS. An imaging system was used to focus the laser on the surface of the otolith and to delineate a sample line target, 100 um long x 40 um wide, following the contour of the edge of the sectioned otolith. This method of sampling the outermost otolith material corresponding to recent growth before capture reduces the uncertainty that the trace elements were incorporated into the otolith when the individual did not occupied the locality were it was collected.

The isotopes assayed from otolith samples were Aluminum-27 (^{27}Al), Magnesium-24 (^{24}Mg), Calcium-48 (^{48}Ca), Vanadium-51 (^{51}V), Chromium-52 (^{52}Cr), Manganese-55 (^{55}Mn), Iron-56 (^{56}Fe), Cobalt-59 (^{59}Co), Copper-63 (^{63}Cu), Zinc-66 (^{66}Zn), Strontium-87 (^{87}Sr), Silver-109 (^{109}Ag), Cadmium-114 (^{114}Cd), Cesium-133 (^{133}Cs), Barium-138 (^{138}Ba), Cerium-140 (^{140}Ce), Mercury-202 (^{202}Hg), Lead-208 (^{208}Pb), and

Uranium-238 (^{238}U). The isotope intensities of each sample were blank-corrected by subtracting isotope intensities of a 1% nitric acid (HNO_3) instrument blank preceding the sample sequence. The abundance of an element was expressed as a ratio relative to the amount of calcium to control for the amount of material analyzed per sample spot. Of the 19 elements analyzed, a total of six elements—Mg, Sr, Mn, Fe, Cu, and Ba—were deemed suitable for statistical comparison.

Seawater samples were collected from the 10 sites where kelp rockfish were collected and 11 of 12 sites where Pacific sanddab were collected. Three seawater samples were collected near the depth where the fish were collected from each site on the day fish samples were collected. Within 12 hours of collection, each seawater sample was filtered and acidified. The acidified filtrate samples were individually stored in airtight plastic bags. Sample filtering and acidification steps were performed under HEPA-filtered Class 100 laminar flow hoods. Methodological procedures minimized contamination of the samples.

Different methods and instruments were used to determine the concentrations of a 19 elements in seawater, the same elements measured in otolith samples. Seawater Ba and U analyses were performed using the method of isotope dilution with all samples run on the Element 2 ICP-MS. Mg, Ca, and Sr analyses were performed using the method of standard additions with all samples run on a Varian AA240FS fast sequential atomic absorption spectrometer. All seawater samples also were run on the Element 2 ICP-MS to collect ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn, ^{109}Ag, ^{114}Cd, ^{133}Cs, ^{140}Ce, ^{202}Hg, ^{206}Pb. Our statistical examination of the seawater data focused on the six elements—Mg, Sr, Mn, Fe, Cu, and Ba—deemed to be suitable of statistical comparisons from the otolith microchemical analysis.

Results from univariate two-factor analysis of variance and multivariate canonical discriminant analysis indicate that the elemental composition of otoliths differ between the two habitat types—the sampled platforms and natural sites—and geographic areas, although there were significant interaction effects (habitat x area) on single element abundances in otoliths. Kelp rockfish collected from platforms tended to incorporate higher concentrations of Mg and Ba and lower concentration of Sr in otoliths compared to fish from natural habitat. In contrast, Pacific sanddab collected from platforms tended to have lower concentrations of Ba and Fe in otoliths compared to fish from natural habitat. Sr/Ca in otoliths of kelp rockfish tended to decrease eastward along the coastal shelf from Point Conception through the east Channel. Mn/Ca in otoliths of sanddab from both habitat types was lower in the three westernmost geographic areas (Point Conception, west Santa Barbara, and east Santa Barbara) than in the eastern and southern areas (east entrance of the channel, offshore east channel, and Southern California Bight). Mn/Ca was not detectable in kelp rockfish otoliths. Variability in otolith element concentrations between habitat types and among areas differed between the two species.

The success rate of correctly assigning fish to habitat type (platform or natural habitat) using a canonical discriminant function based on otolith element signatures differed between species. Cross-validation of the discriminant function showed a very high proportion, 94%, of kelp rockfish individuals were correctly assigned to habitat type based on the otolith signature of Mg/Ca, Ba/Ca, and Sr/Ca. A lower proportion, 64%, of Pacific sanddab correctly grouped into habitat type based on the otolith signature of Mg/Ca, Sr/Ca, Mn/Ca, Cu/Ca, and Ba/Ca—otolith element concentrations that individually varied between sanddab habitat types and/or areas.

Success in discriminating otoliths from the two different habitat types was not associated with seawater element concentrations. Regression analysis showed variability in otolith element concentration among sites was not related to the concentration of the elements in our seawater samples from kelp rockfish and sanddab habitats.

Task 3: A Histological Examination of the Ovaries of Pacific Sanddab, *Citharichthys sordidus*, Captured at Two Oil Platforms and Two Natural Sites in the Southern California Bight

In this study, we collected Pacific sanddab at four sites in the Santa Barbara Channel, southern California. These sites include two platforms, B and Gilda, and two natural comparison sites, one located about 8 km offshore and southeast of Platform B "Rincon") and the other off the northeast corner of Santa Cruz Island.

After capture, the fish were placed on ice and several hours later were measured (total length, cm) and the ovaries fixed in 10% formalin. In the laboratory, a section of each ovary was removed, dehydrated in ethanol of ascending concentrations, embedded in paraffin and serial sections were cut at 5µm using a rotary microtome. Sections were mounted on glass slides, stained with hematoxylin followed by eosin counterstain and each section was examined for the presence of: (1) hydrated eggs for upcoming spawning; (2) vitellogenesis (yolk deposition) in mode of smaller eggs for subsequent spawning; (3) postovulatory follicles (evidence of recent spawning); (4) follicular atresia (degenerating oocytes). Atresia was characterized as minor (<10% of eggs atretic) or pronounced (≥10% of eggs atretic).

Egg hydration, which occurs just prior to spawning, was noted in between 100% (Platform Gilda) and 50% (Rincon) of fish at each site. Smaller yolked eggs were found in between 100% (Platform Gilda) and 55% (Rincon) of fish. Postovulatory follicles are the remnants of the granulosa layer of the previously spawned egg that hypertrophies after the oocyte is released and thus their presence demonstrates that a female has spawned earlier in the season. Postovulatory follicles were found in between 65% (Santa Cruz Island) and 5% (Rincon) of fish. At least some fish with minor atresia were noted at all of the sites, with levels ranging from 60% (Platform Gilda) to 15% at Santa Cruz Island. Pronounced atresia was relatively rare. No fish at Platform Gilda and Santa Cruz Island were identified as having pronounced atresia, while 6% of the sanddabs at Platform B and 16% at Rincon exhibited this condition.

In general, we observed relatively little evidence of conspicuous reproductive impairment in Pacific sanddab from both platforms and natural sites. Overall, most fish contained hydrated oocytes and were about to spawn and most contained oocytes with smaller yolks and thus were likely to spawn again. Many fish exhibited minor atresia. Atresia is the spontaneous degeneration of an oocyte at any stage in its development and it occurs at low frequency throughout the ovarian cycle. We observed relatively few instances of pronounced atresia, a condition that is only occasionally observed in unstressed populations.

It is possible that there was some reduction in the reproductive capacity of fish from Rincon as fewer fish from that site harbored 1) hydrated oocytes, 2) those smaller oocytes destined for later spawning, and 3) post-ovulatory oocytes. Furthermore, 16% of female Pacific sanddab from Rincon contained ovaries with pronounced atresia. However, our sample size from Rincon was relatively small, encompassed only one year, and the factors responsible for the reduction in ovarian output in these females remain to be determined.

Conclusions

During the platform decommissioning process regulators examine a wide range of biological and sociological issues. Among these are questions regarding the possible contamination of platform-dwelling fishes by pollutants that may be present in drill muds, produced water, or otherwise released during platform operations. In the current study, we found no evidence to support the hypothesis that fishes living around southern California oil and gas platforms are more heavily contaminated or suffer higher reproductive impairment than fishes living on nearby natural sites.

We approached the contamination issues in three ways.

First, we examined elemental metal concentrations both in platform-dwelling fishes and those living in natural sites, focusing on a large suite of elements likely to be released during platform operations. Although there was substantial variability in concentrations of a number of elements among fishes, there was no consistent pattern of higher concentrations of any element at either platforms or natural sites.

Second, we examined the otoliths of these fishes to determine if there might be a distinctive "signature" created when platform fishes are bathed in the hypothesized polluted waters. Our results indicate that the elemental composition of kelp rockfish otoliths and Pacific sanddab otoliths differ between the two habitat types—the sampled platforms and natural sites—and geographic areas. However, we found that elemental concentrations in otoliths were not consistently higher from platform habitats. Barium, a residue released into the environment from platform activities, occurred in higher concentrations in otoliths of kelp rockfish collected from midwater depths at platforms than from natural rocky reef areas. In contrast, the concentration of barium in benthic-dwelling Pacific sanddab was lower from platforms than from natural soft sediment habitat sites. We found that variability in otolith element concentration among sites was not related to the concentration of the elements in our seawater samples from kelp rockfish and sanddab habitats.

Third, we characterized the reproductive capabilities of Pacific sanddabs living around platforms and natural sites to assess for possible indirect effects of the hypothesized contaminants on reproduction. We found no consistent pattern of significantly higher levels of severely atretic eggs among either platform or natural site sanddabs.

Task 1: Whole-body Concentrations of Elements in Kelp Bass (*Paralabrax clathratus*), Kelp Rockfish (*Sebastes atrovirens*), and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

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Abstract

Resource managers are concerned that offshore oil platforms in the Southern California Bight may be contributing to environmental contaminants accumulated by marine fishes. To examine this possibility, 18 kelp bass (*Paralabrax clathratus*), 80 kelp rockfish (*Sebastes atrovirens*), and 98 Pacific sanddab (*Citharichthys sordidus*) were collected from five offshore oil platforms and 10 natural areas during 2005-2006 for whole-body analysis of 63 elements. The natural areas, which served as reference sites, were assumed to be relatively uninfluenced by contaminants originating from platforms. Forty-two elements were excluded from statistical comparisons for one of three reasons: they consisted of major cations that were unlikely to accumulate to potentially toxic concentrations under ambient exposure conditions; they were not detected by the analytical procedures; or they were detected at concentrations too low to yield reliable quantitative measurements. The remaining 21 elements consisted of aluminum, arsenic, barium, cadmium, chromium, cobalt, copper, gallium, iron, lead, lithium, manganese, mercury, nickel, rubidium, selenium, strontium, tin, titanium, vanadium, and zinc. Statistical comparisons of these 21 elements indicated that none consistently exhibited higher concentrations at oil platforms than at natural areas. Eight comparisons yielded significant interaction effects between total length (TL) of the fish and the two habitat types (oil platforms and natural areas). This indicated that relations between certain elemental concentrations (i.e., copper, rubidium, selenium, tin, titanium, and vanadium) and habitat type varied by TL of affected fish species. To better understand these interactions, we examined elemental concentrations in very small and very large individuals of affected species. Although significant interactions were detected for rubidium, tin, and selenium in kelp rockfish, the concentrations of these elements did not differ significantly between oil platforms and natural areas over the TL range of sampled fish. However, for selenium, titanium, and vanadium in Pacific sanddab, small individuals (average TL, 13.0 cm) exhibited significantly lower concentrations at oil platforms than at natural areas, whereas large individuals (average TL, 27.5 cm) exhibited higher concentrations at oil platforms than at natural areas. For copper in Pacific sanddab, small individuals did not exhibit differences between oil platforms and natural areas, whereas large individuals exhibited significantly higher concentrations at oil platforms than at natural areas. On the other hand, for tin in Pacific sanddab, small individuals did not exhibit differences between oil platforms and natural areas, whereas large individuals exhibited significantly lower concentrations at oil platforms than at natural areas. Although concentrations of arsenic, cadmium, chromium, lead, mercury, and selenium in fishes from some platforms and natural areas equaled or exceeded literature-based toxicity thresholds for fish and fish-eating wildlife, studies are still needed to document evidence of toxicity from these elements. When estimates of elemental concentrations in skinless fillets were compared to risk-based consumption limits for humans, the concentrations of arsenic, cadmium, mercury, and tin in fish from a mix of oil platforms and natural areas were sufficiently elevated to suggest a need for further study of inorganic arsenic, cadmium, mercury, and tributyltin.

Introduction

Twenty-seven active and seven decommissioned offshore oil-and-gas production platforms (henceforth referred to as "oil platforms") are located within the Southern California Bight between Point Arguello in the north and the Mexican border in the south. Over the next few decades, many active platforms are expected to undergo decommissioning as their economic profit margins decline. Decommissioned platforms in Southern California have historically undergone complete removal. However, recent ecological studies indicate that platforms provide artificial structure for marine life, including many fish species of recreational and commercial importance (Love et al. 2003). Consequently, a Select Scientific Advisory Committee on Decommissioning was convened to explore several scenarios for reducing the ecological impact of decommissioning the platforms (Holbrook et al. 2000). The decommissioning options range from leaving the entire intact structure in place to removal of part or all of the structure. Regardless of the option selected, resource managers are concerned that environmental contaminants associated with the drilling process (i.e., contaminated drilling mud and cuttings), the produced water, and other platform-related activities (e.g., sanitary and domestic wastes) could pose toxic risks to fishes and consumers of tainted fishes, including marine birds and mammals, and humans.

Drilling muds or fluids are specially formulated mixtures of freshwater or seawater with clays, minerals, chemicals, and other materials used for cleaning drill bits, transporting cuttings, reducing friction, and stabilizing the borehole (Raco 1993). Drill cuttings are particles of crushed sedimentary rock produced in the process of drilling. Barium sulfate is added to drill muds as a weighting agent, and the appearance of barium in cuttings is due to drilling mud adhering to the cuttings (Raco 1993). Chromium can originate from chrome or ferrochrome lignosulfonate, which are drilling mud additives (Phillips et al. 2006). Drill cuttings can contain elevated concentrations of lead and zinc, possibly as a result of using pipe-thread compound to lubricate the threads of drill pipes (Raco 1993; Phillips et al. 2006). These and other metals also originate from formation solids and mud additives. Produced waters are natural geological formation water or introduced seawater recovered with the extraction of oil (Raco 1993). Most produced water is a brine that may contain dissolved solids, metals, organic compounds, and sulfur at substantially higher concentrations than in seawater. Produced water can contain trace elements, radionuclides, and various petroleum hydrocarbons, including monoaromatic compounds, polycyclic aromatic hydrocarbons, and polar compounds. Nickel and vanadium are components of crude oil (Phillips et al. 2006). Sanitary and other domestic wastes are generated by platform personnel, and include sewage, garbage, and wastewater, and any associated contaminants (Raco 1993).

Previous surveys of inorganic contaminants in liver, kidneys, gonads, and axial muscle of marine fishes from the Southern California Bight included measurements of aluminum, antimony, arsenic, barium, beryllium, cadmium, chromium, copper, iron, manganese, mercury, molybdenum, nickel, lead, selenium, silver, thallium, tin, vanadium, and zinc (e.g., Stapleton 1968; McDermott et al. 1976; Pollock et al. 1991; SCCWRP 1994; Allen et al. 2004; IEc 2007; Groce and Gartman 2008). However, these early studies generally focused on runoff from urban areas or discharge from nearshore sewage outfalls. Relatively little attention has been given to documenting elemental body burdens in fishes from oil platforms (see Bascom et al. 1976, and McDermott-Ehrlich and Alexander 1976).

The purpose of our study was to determine if offshore oil platforms in the Southern California Bight represented a major source of contamination by trace elements in fish species of economic (commercial and recreational) importance. Natural areas (hard substrates such as rocky outcrops or reefs, and soft substrates such as mud, sand, or both) located close to selected platforms were intended to serve as relatively uncontaminated reference sites. Specific objectives were (i) to document body burdens of elements in representative midwater and bottom-dwelling fish species, (ii) to determine if elemental concentrations in fishes from platforms differed from those at natural areas, and (iii) to determine if any elements approached or exceeded potentially harmful levels to fishes or consumers of fishes.

Study Area and Methods

The study area included five oil platforms that extend over a distance of 186 km from roughly southwest of Santa Barbara to south of Long Beach (Figure 1). To facilitate comparisons of elemental concentrations, each platform was grouped with two nearby natural areas (predominant bottom substrate characteristic given in parentheses) as follows: Platform Holly with Devereux Reef (hard) and Campus Point (soft); Platform Hogan with Horseshoe Rock (hard) and Rincon (soft); Platform Gilda with Santa Cruz Island—Coche Point (hard) and Northeast Santa Cruz Island (soft); Platform Gina with Anacapa Island—Arch Rock (hard) and Hueneme Submarine Canyon (soft); and Platform Edith with Catalina Island—Blue Cavern (hard) and an unnamed locality off San Pedro (soft). The five groups of oil platforms and natural areas were collectively referred to as habitat types, or "HabTypes," with oil platforms progressively coded in a roughly northwest to southeast direction as PL1, PL2, etc., and their corresponding natural areas coded as NA1, NA2, etc. (see Table 1 for codes and additional information).

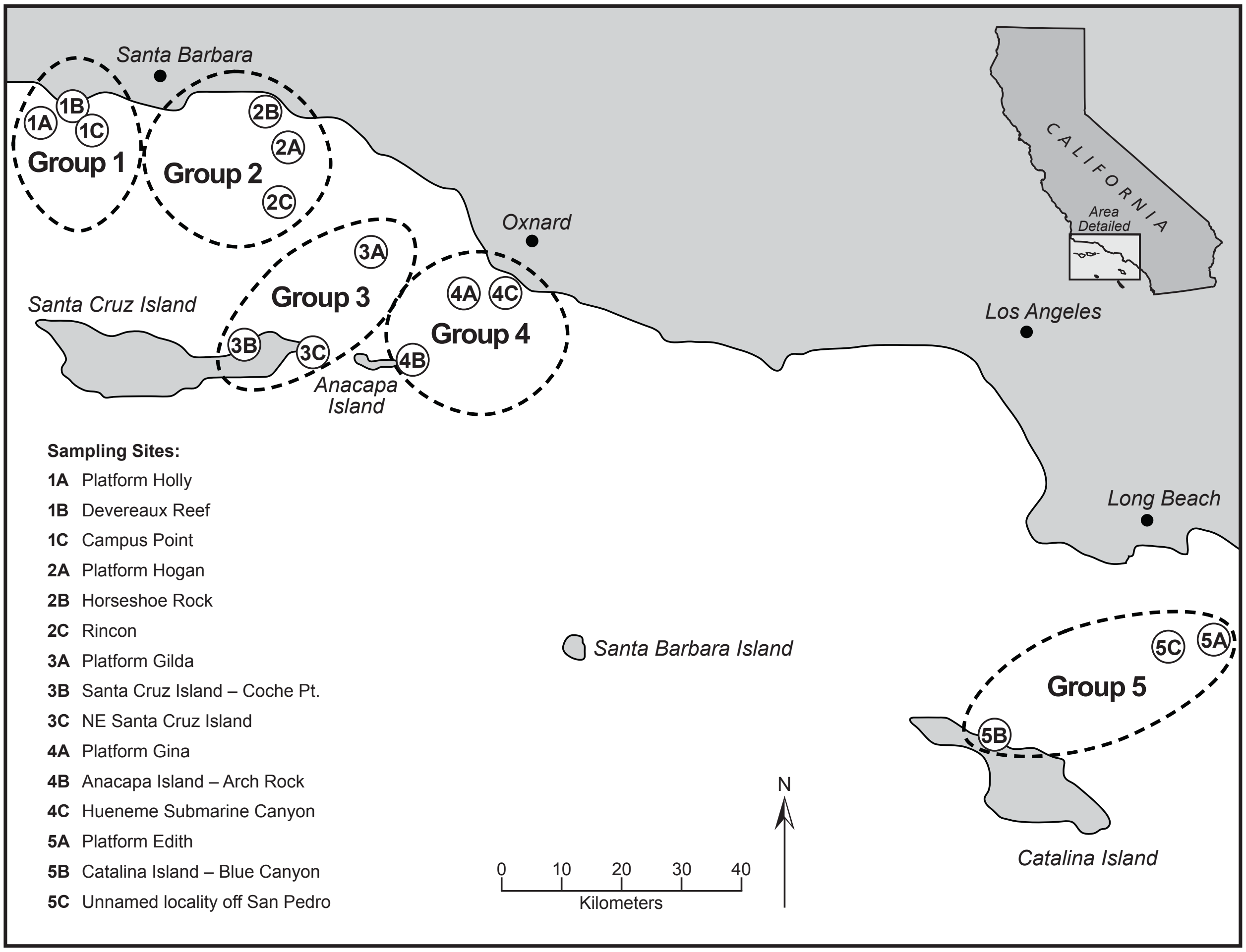

Figure 1. Map of the study area showing locations of five oil platforms and 10 natural areas. Each platform is grouped with two nearby natural areas.

Table 1. Characteristics of the 15 sampling sites, plus sample sizes of kelp bass (KBA), kelp rockfish (KRO), and Pacific sanddab (PSD). Each of five oil platforms is grouped with two nearby natural areas (reference sites).

Group	Site	Habitat type	Site code	Geospatial coordinates	Distance to nearest shore (km)	Water depth (m)	Year in-stalled	Fish sample size		
								KBA	KRO	PSD
1	Platform Holly	Platform	PL1	34°23.366’N, 119°54.406’W	3.1	64	1966	---	10	10
	Devareux Reef	Natural area	NA1 (if kelp rockfish)	34°24.100’N, 119°51.560’W	0.8	15	---	---	10	---
	Campus Point	Natural area	NA1 (if Pacific sanddab)	34°22.365’N, 119°50.168’W	3.6	67	---	---	---	10
2	Platform Hogan	Platform	PL2	34°20.275’N, 119°32.539’W	6.1	46	1967	---	10	8
	Horseshoe Rock	Natural area	NA2 (if kelp rockfish)	34°23.610’N, 119°33.629’W	1.7	9	---	---	10	---
	Rincon	Natural area	NA2 (if Pacific sanddab)	34°15.355’N, 119°33.230’W	15.1	87	---	---	---	10
3	Platform Gilda	Platform	PL3	34°10.932’N, 119°25.150’W	14.1	62	1981	---	10	10
	Santa Cruz Island—Coche Point	Natural area	NA3 (if kelp rockfish)	34°02.233’N, 119°36.600’W	0.1	17	---	---	10	---
	NE Santa Cruz Island	Natural area	NA3 (if Pacific sanddab)	34°01.759’N, 119°30.362’W	1.5	55	---	---	---	10
4	Platform Gina	Platform	PL4	34°07.059’N, 119°16.625’W	6.3	28	1980	8	---	10
	Anacapa Island—Arch Rock	Natural area	NA4 (if kelp bass)	34°00.980’N, 119°21.316’W	0.2	12	---	10	---	---
	Hueneme Submarine Canyon	Natural area	NA4 (if Pacific sanddab)	34°07.100’N, 119°12.730’W	3.0	23	---	---	---	10
5	Platform Edith	Platform	PL5	33°35.726’N, 118°08.501’W	15.5	49	1983	---	10	10
	Catalina Island—Blue Cavern	Natural area	NA5 (if kelp rockfish)	33°26.693’N, 118°28.552’W	0.1	26	---	---	10	---
	Unnamed locality off San Pedro	Natural area	NA5 (if Pacific sanddab)	33°35.006’N, 118°12.770’W	14.1	60	---	---	---	10

Sample Collection

Fish species consisted of kelp bass (*Paralabrax clathratus*), kelp rockfish (*Sebastes atrovirens*), and Pacific sanddab (*Citharichthys sordidus*), which were collected either by spearing or with hook-and-line during July-December 2005 and July-August 2006. Brief species accounts (based on Boschung et al. 1983, Eschmeyer et al. 1983, Love 1996, and Love et al. 2005) are as follows: Kelp bass grow as large as 72 cm, with a maximum weight of about 6.6 kg. This species is substrate-oriented and inhabits both the water column and sea floor within or near kelp beds, rocky reefs, and such human-made structures as oil platforms. It is most often found at depths of 2.4-30 m although large specimens occur as deep as 61 m. Juveniles feed on benthic invertebrates (especially crustaceans), whereas adults feed primarily on fishes and cephalopods. This is an excellent food fish and an important game fish. Kelp rockfish attain a maximum length of about 42 cm, and usually occur on or near the bottom in kelp beds or rocky areas. Although commonly found at depths of 9-12 m, this species can occur as deep as 58 m. Foraging occurs mostly at night on organisms living on the substrate, such as crabs, bottom-dwelling shrimps, fish, and cephalopods. This is an important recreational and minor commercial species. Pacific sanddab grow to about 41 cm, and inhabit sandy and muddy bottoms at depths of 9-550 m. It is an opportunistic predator, feeding on a variety of crustaceans, as well as smaller fish, squid, and octopus. Sanddabs are a popular food fish in California.

Immediately following capture, fish were weighed and measured for total length (TL), assigned a unique identification number, then wrapped and bagged in clean plastic and held on wet ice until returning to the laboratory. In the laboratory, fish samples were stored frozen (-10°C) for roughly 1 week to 6 months until shipment to the U.S. Geological Survey (USGS) Columbia Environmental Research Center (CERC), Columbia, Missouri, for further processing and analysis.

Sample Preparation

Frozen samples held in coolers with dry ice were transported by overnight air freight to CERC. At CERC, frozen fish weighing <100 g were minced with a titanium meat cleaver and placed in a chemically clean 118-ml jar equipped with a filter lid to enable lyophilization. Frozen samples >100 g but <300 g were initially cut into small cubes with a Hobart food-service band saw, then further processed in a KitchenAid meat grinder containing a titanium blade and extrusion plate. Samples >300 g were also cut into pieces with the Hobart band saw and homogenized with a Hobart meat grinder. All samples were processed through the meat grinder three times, collected in a plastic bag, then hand-kneaded to facilitate mixing and produce a homogenized mass. A portion of the resulting ground product was placed in a 118-ml cleaned glass jar and archived for future organic contaminant analysis. A second ground portion was placed in a 118-ml jar equipped with a filter lid for lyophilization. All filter-lid aliquots were lyophilized in a Virtis Genesis 35EL freeze dryer, with moisture content determined during the lyophilization process. After lyophilization, each dried fish sample was transferred to a plastic bag and further reduced with a Teflon® roller pin to a coarse powder. This final product was then transferred to a 40-ml glass vial that was sealed and stored in a desiccator until further processing. To prevent cross-contamination, all equipment used to process a fish sample were cleaned before processing the next fish sample.

Chemical Preparation

To prepare digestates from dried samples for semi-quantitative scans of multiple elements and quantitative determinations of total arsenic by inductively coupled plasma mass spectrometry (ICP-MS), an aliquant of each sample (~0.25 g) was heated with 6 ml of nitric acid in a sealed low-pressure Teflon® vessel by using a microwave oven. The cooled digestate liquid was transferred into a 125-ml polyethylene bottle with ultrapure water (>10 megOhm/cm) to a final weight of 101.5 g (100 ml). Final acid matrix was 6% nitric acid.

For quantitative determination of total selenium, an additional tissue aliquant (~0.5 g) of each dried sample was subjected to a magnesium nitrate–nitric acid dry ashing procedure, followed by hydrochloric acid reduction. The dry ashing procedure consisted of three steps: boiling with nitric acid for solubilization and partial oxidation, ashing at 500°C with magnesium nitrate to complete the oxidation and decompose any remaining organic matter, and heating with hydrochloric acid to dissolve the ash and reduce all selenium to the selenite (Se^{+4}) oxidation state required for hydride generation. Following reduction, the digestates were diluted to ~100 ml with deionized water, yielding a final acid matrix of 10% hydrochloric acid.

For quantitative determination of total mercury, there was no chemical preparation (digestion) because the dried sample was thermally decomposed during analysis (see below).

Instrumental Analysis

To perform a scan of elements (excluding mercury and selenium), samples were analyzed by ICP-MS using the semi-quantitative scan mode (TotalQuant®, Perkin-Elmer Sciex; Orazio et al. 2007). This scanning mode has a manufacturer's reported accuracy of ±30% to ±50%. All samples were diluted 10 times with a CETAC ASD-500 autodiluter as part of the analytical sequence. Internal standards were scandium (10 µg/l), rhodium (10 µg/l) and thorium (10 µg/l), and the external standard consisted of a National Institute of Standards and Technology (NIST) traceable reference solution (Trace Metals in Drinking Water; High Purity Standards, Charleston, South Carolina) to which five elements (gold, praseodymium, tantalum, terbium, and thulium) were added for improved calibration in the rare earth region of the mass spectral range.

Quantitative arsenic determinations were conducted using a PE/SCIEX Elan 6000 inductively coupled plasma dynamic reaction cell mass spectrometer (ICP-DRC-MS), which was operated in "Standard Mode" and optimized according to the manufacturer's specifications (May et al. 2001). Samples were automatically delivered to the ICP-MS with a software-controlled CETAC ASD-500 autosampler/autodiluter system. All sample digestates were analyzed with 10 times predilution by autodiluter. The ICP-MS quantitative method was designed to determine the monoisotopic arsenic mass, ^{75}As. The internal standard was rhodium (10 µg/l), which was metered into the sample line via peristaltic pump. Calibration standards for arsenic were 1.5, 3.0, 6.0, 12, and 24 ng/ml. During the analysis, any digestate concentration exceeding the upper calibration standard was automatically diluted 10 times in serial fashion until its concentration was below this concentration.

Mercury was determined with a direct mercury analyzer in which a dried sample (50-60 mg) was combusted in a stream of oxygen. All mercury in the sample was volatilized and trapped by amalgamation on a gold substrate and was thermally desorbed and quantitated by atomic absorption spectrophotometry (USEPA 2007). The entire sequence was conducted with a Milestone DMA-80 analyzer equipped with an automated sample carousel.

The determination of selenium in dry-ashed samples was accomplished by flow injection hydride generation atomic absorption spectroscopy (May et al. 2008). In this procedure, the digestate is mixed with a hydrochloric acid carrier solution and then reduced by sodium tetrahydridoborate which has been stabilized with sodium hydroxide. The resulting volatile hydrogen selenide is transferred with argon carrier gas into a heated quartz cell mounted on an atomic absorption spectrophotometer for decomposition and measurement.

Quality Control

Calibration. Runs of a laboratory control solution (Trace Metals in Drinking Water; High Purity, Charleston, South Carolina) after every 10 samples served to monitor calibration throughout each of seven semi-quantitative scans. In addition, separate calibration solutions (Spex ClaritasPPT Standards) were also analyzed during each run, which exhibited recoveries ranging from 75% to 130%, with the exception of potassium, which exhibited recoveries ranging from 102% to 167% (average, 130%). A continuing calibration blank (CCB) and an independent calibration verification standard (ICVS) were analyzed every 10 samples to confirm the calibration status of the ICP-MS during quantitative instrumental analyses of sample digestates for arsenic. All CCB blanks were within ±3 times the instrument detection limit, and ICVS recoveries ranged from 92% to 101%. In addition, NIST SRM 1640 Trace Elements in Natural Water was analyzed for arsenic in each ICP-MS run; recoveries were 100%. Mercury calibration was checked during each instrumental run using two reference tissues: International Atomic Energy Agency (IAEA) SRM 407 whole-body fish and National Research Council Canada (NRCC) SRM DOLT-3 dogfish liver; measured concentrations deviated <8% from the certified mean for each material. For selenium, a calibration check solution was analyzed at the beginning and end of each analytical run, with measured concentrations deviating <10% from actual, except for one case of 11.1%.

Control Materials. Two tissue control materials were analyzed with each group of semi-quantitative ICP-MS samples: NIST SRM 1566b oyster tissue and NRCC SRM DORM-2 dogfish muscle. Recoveries of aluminum, cadmium, calcium, cobalt, copper, iron, lead, magnesium, manganese, nickel, potassium, silver, sodium, vanadium, and zinc from NIST 1566b (N=6) ranged from 71% to 128%, except for three measurements of iron (149%, 176%, and 224%); and one measurement each of aluminum (53%), nickel (146%), and potassium (166%). Recoveries of arsenic from four reference tissues—IAEA SRM 407 whole-body fish (N=2); NRCC DORM-2 dogfish muscle (N=3); NRCC SRM TORT-1 lobster hepatopancreas (N=5); NIST 1566b oyster tissue (N=5)—ranged from 100% to 109%. Recoveries of cadmium, calcium, copper, iron, lead, magnesium, manganese, molybdenum, potassium, rubidium, silver, sodium, strontium, and zinc from NRCC DORM-2 (N=6) ranged from 77% to 132%, except for three measurements of iron (155%, 160%, and 184%). Accuracy of mercury measurements by thermal combustion amalgamation atomic absorption spectroscopy was determined with five reference tissues analyzed with the samples (NIST RM50 tuna fillet, N=10; NRCC DORM-2, N=9; NIST SRM 2976 mussel tissue, N=10; IAEA SRM 407 whole-body fish, N=9; and NRCC SRM DOLT-3 dogfish liver, N=9); mercury recoveries ranged from 95% to 103%. Similarly, three reference tissues were analyzed for selenium along with the samples (IAEA SRM 407 whole-body fish, N=8; CERC whole-body striped bass research material, N=7; NRCC DORM-2 dogfish muscle, N=1); selenium recoveries ranged from 94% to 105%.

Analytical and Method Precision. Analytical precision for the semi-quantitative scan on digestates of whole-body fish samples was estimated by repeated runs of a reference solution, which exhibited percent relative standard deviation (%RSD) values of ≤11%, except for one measurement each of aluminum (21%) and strontium (14%). Internal standards (rhodium, scandium, and thorium) differed by ≤33% from the beginning to the end of the analytical runs. Instrumental precision for selenium, determined by repeated analysis of a standard throughout each run, was ≤3% RSD. Duplicate analysis of fish digestates (N=30) for arsenic by quantitative ICP-MS produced relative percent differences (RPD) <3%. Method precision for whole-body fish tissues determined from triplicate digestion and analysis of fish samples (N=13) by ICP-MS semi-quantitative scan exhibited %RSDs <30% for most elements, but higher %RSDs were observed in some measurements of aluminum (44% and 47%), barium (36%), lead (33%), and strontium (39%). Replicate (N=3) digestion and analysis of samples for arsenic (N=6), mercury (N=4), and selenium (N=8) produced %RSDs <15%.

Spikes. For samples analyzed for metals by semi-quantitative ICP-MS, spiking elements were selected that represented the entire mass spectral range (barium, beryllium, cadmium, chromium, cobalt, copper, lead, manganese, molybdenum, nickel, terbium, thulium, vanadium, and zinc). Recoveries of low and high concentrations of these elements spiked into fish samples (N=13) prepared for the semi-quantitative scan ranged from 84% to 121%, except for one instance of a higher recovery of barium (147%). The average recovery was 104%. Samples of fish spiked with arsenic (N=26), mercury (N=22), and selenium (N=32) exhibited recoveries ranging from 89% to 120%, and averaged 104%. Recoveries of arsenic and selenium in analysis (post-digestion) spikes (N=42) ranged from 91% to 109%, and averaged 101%.

Interference Checks. As a check for potential interferences on arsenic in quantitative ICP-MS, dilution percent differences (DPDs) based on 5X dilutions of the fish digestates were determined (N=30). The DPDs were <6% for this element in the whole-body fish matrix. A synthetic solution containing high concentrations of aluminum, calcium, carbon, iron, magnesium, molybdenum, phosphorus, potassium, sodium, sulfur, and titanium was analyzed to observe the effects of these potential interfering elements on the determination of arsenic concentrations in this matrix. Recoveries of arsenic in the matrix ranged from 110% to 130%; recoveries for two analytical runs exceeded the ±20% allowance by 8% and 10%, indicating a slightly positive potential interference on the element, at least when analyzed within this synthetic matrix. It is unlikely that this interference would translate to the whole-body fish matrix (T.W. May, personal communication).

Blank Equivalent Concentrations. Blank equivalent concentrations (BECs) were computed for digestion blanks analyzed with each set of fish samples. For most elements determined by the ICP-MS semi-quantitative scan, BECs were generally below reporting limits of the semi-quantitative scan. (Note: The reporting limits for semi-quantitative "scan" data were set at 0.04 µg/g dry weight for most elements, based on a solution level of 0.1 ng/ml, a dry weight of 0.25 g, and a final digestate volume of 100 ml; exceptions were barium, copper, nickel, strontium, titanium, and zinc [0.4 µg/g dry weight], iron [4 µg/g dry weight], and calcium, magnesium, potassium, and sodium [40 µg/g dry weight]; T.W. May, personal communication). The BEC concentrations above reporting limits were most often observed for aluminum and iron. Mean BECs for arsenic, mercury, and selenium were at or below their respective method detection limits (MDLs) except for one measurement of selenium (BEC of 0.06 µg/g dry weight, MDL of 0.008 µg/g dry weight).

Instrument Detection, Method Detection, and Method Quantitation Limits. The instrument detection limit (IDL) for arsenic by quantitative ICP-MS was 0.006 ng/ml, the IDLs for mercury by thermal combustion/amalgamation atomic absorption spectroscopy were 0.001 ng and 0.003 ng, and the IDL for selenium determined by flow injection hydride generation atomic absorption spectroscopy was 0.020 ng/ml. The MDLs were computed as $3^*(SD_b^2 + SD_s^2)^{1/2}$, where SD_b = standard deviation of a blank (N=3) and SD_s = standard deviation of a low-concentration sample or spiked sample (N=3). The MDLs were established for each analytical run and ranged as follows: arsenic, 0.008 to 0.047 µg/g dry weight; mercury, 0.015 to 0.047 µg/g dry weight; and selenium, 0.004 to 0.022 µg/g dry weight. Method Quantitation limits (MQLs) were computed as 3.3*MDLs. The MQLs established for each analytical run ranged as follows: arsenic, 0.026 to 0.16 µg/g dry weight; mercury, 0.050 to 0.16 µg/g dry weight; and selenium, 0.013 to 0.071 µg/g dry weight.

Overall, the quality control results were acceptable based on specifications established by CERC (T.W. May, personal communication).

Data Analysis

Computerized databases were created as Excel spreadsheets. Raw data were analyzed by using SAS/STAT® and SAS/GRAPH® (SAS Institute Inc., Cary, North Carolina). Characteristics of fish samples were assessed by conducting one-way analysis of variance (ANOVA) to compare mean values for TL, weight, and moisture content among oil platforms and natural areas. To facilitate statistical comparisons of elemental concentrations that fell below reporting limits for semi-quantitative elemental scans by ICP-MS or that fell below detection limits for quantitative determinations of arsenic, mercury, and selenium, we substituted values that were one-half the reporting limit or detection limit. However, statistical comparisons that use substituted values are referred to as "left censored data sets," and are likely to yield biased estimates of the mean and standard deviation (Newman 1995). Thus, interpretations based on censored data should be viewed with caution.

When comparing elemental concentrations in various fish species from the two habitat types (oil platforms and natural areas; also referred to as "HabTypes"), we assumed that fish size might account for some variation in elemental concentrations. This assumption is based on general observations by several investigators that concentrations of at least some elements can occasionally vary with fish age (or TL and weight, which are proxies of age; e.g., Phillips and Russo 1978, Mance 1987). Additionally, we assumed that elemental concentrations could vary locally among each grouping of oil platform and two nearby natural areas ("Group" variable nested in HabType; see Table 1 for Group designations). Therefore, our strategy was to use analysis of covariance (ANCOVA) with mixed effects, with HabType and TL (the continuous covariate) as fixed effects, and Group and Group*TL interaction as random effects (Littell et al. 1996). We estimated all effects using restricted maximum likelihood as the fitting algorithm, which yields unbiased estimates of covariance and variance, and we tested these effects using F-tests based on Satterthwaite's formula for degrees of freedom (Littell et al. 1996). Although we assumed that any of these effects or interactions could be present, the occurrence of a TL effect complicated our interpretations of elemental concentrations at oil platforms and natural areas. Thus, we retained the TL effect only if it was significant at the 0.05 level. We first determined if the interaction term was significant, and removed it from the ANCOVA model if significance was not achieved. We then tested the TL effect for significance, and removed it if not significant (at which point, the ANCOVA simplifies to an ANOVA with random effects). Least squares means and 95% confidence intervals were also computed for each HabType regardless of whether a statistically significant difference was detected.

We interpreted results from the linear mixed models analysis of elemental concentrations for each fish species as follows: a significant HabType*TL interaction indicated that the effect of HabType on elemental concentration varied with fish TL and, conversely, the effect of fish TL varied between oil platforms and natural areas. If HabType*TL interaction was significant, we calculated separate mean concentrations of the element at each HabType for small fish and large fish (the selected TLs representing small and large fish were chosen from the ranges of minimum TLs and maximum TLs common to both HabTypes). If HabType*TL interaction was not significant but TL was significant, we omitted effects from the interaction and calculated the mean concentration of the element within each HabType for fish of average TL. If both HabType*TL interaction and TL effects were not significant, we omitted both effects, thus reducing the model to an ANOVA that estimated only the HabType effect. In this instance, the mean concentration of an element at each HabType applied to all fish regardless of TL.

Additional statistical summaries of data including computation of Pearson product-moment correlation analysis. Moreover, to ensure normality, raw data were routinely subjected to standard transformations (e.g., angular transformation for moisture percentage; logarithmic transformation for TL, weight, and concentrations of various elements).

Results and Discussion

A total of 196 whole-body fish samples (18 kelp bass, 80 kelp rockfish, and 98 Pacific sanddab) was analyzed for 63 elements during this study (Table 2). Geometric mean TLs and weights of fishes were as follows (95% confidence limits in parentheses): kelp bass, 34.3 cm (32.9-35.8 cm), 533 g (466-610 g); kelp rockfish, 25.1 cm (24.2-26.1 cm), 264 g (234-299 g); and Pacific sanddab, 19.4 cm (18.6-20.2 cm), 82 g (72-94 g). Although mean TLs and weights did not vary over sampling sites for kelp bass, they varied significantly for kelp rockfish and Pacific sanddab (Table 3).

Moisture content averaged 67.9% (95% confidence interval, 66.9-68.9%) in kelp bass, 74.2% (95% confidence interval, 73.0-75.4%) in kelp rockfish, and 80.1% (95% confidence interval, 79.7-80.4%) in Pacific sanddab (Table 2). Although moisture content did not vary among sampling sites for kelp bass, significant differences were detected for kelp rockfish and Pacific sanddab (Table 3). With one exception, moisture content was not significantly correlated with TL or weight in all three species (data not shown). Moisture content in kelp bass was exceptional because a significant inverse relation was detected with fish weight (r=-0.53, P=0.0244). Numerous investigators have reported inverse relations between moisture content and fish size or age (e.g., Groves 1970; Reinitz 1983; Degani et al. 1986; Tidwell and Robinette 1990; Lange et al. 1994; Saiki et al. 2005, in press), possibly due to an increase in accumulated lipids as fish transition from the rapidly growing juvenile life stage to slower-growing adults. Reinitz (1983) indicated that energy stored as fat by fishes simply replaces body water. Conversely, as lipids are metabolized, they are replaced with water (Love 1980, cited by Jacobs et al. 2008). Due to variable moisture content of the sampled fishes, elemental concentrations were routinely expressed in terms of dry weight unless indicated otherwise. (Note: Elemental concentrations reported as dry weight can be converted to wet weight by using the following equation: wet weight=dry weight x ((100-moisture content)/100), where wet weight concentration and dry weight concentration are µg/g and moisture content is %.)

The elements measured during this study included calcium, magnesium, potassium, and sodium, which are major cations that must be present in fish tissues at required concentrations for proper functioning of cellular membranes and organ tissues, and for nerve conduction, muscle contraction, and other biochemical and physiological processes. In our study, mean concentrations of these cations varied as follows: for calcium, 22,000-43,000 µg/g; for magnesium, 1,000-1,900 µg/g; for potassium, 13,000-19,000 µg/g; and for sodium, 4,000-13,000 µg/g (Table 2). However, healthy unstressed marine fish held in natural seawater are generally efficient at regulating internal concentrations of major cations (they operate hyposmotically, losing water continually by diffusion to the more saline environment, and must compensate by ingesting seawater and excreting excess salts through chloride cells in gill filaments and opercular skin epithelia; Moyle and Cech 1996, pp. 78-79) so these elements are rarely monitored as part of routine contaminant surveys. For this reason, other than to report the measured concentrations, no further assessments of major cations were attempted. The remaining 59 elements are often described as trace elements because they usually occur in animal tissues at much lower concentrations, and some (e.g., copper, iron, selenium, zinc) can be toxic to fishes when present above required concentrations. Still other trace elements (e.g., lead, mercury) have no known biological function. At least one of these elements (mercury) can be extremely hazardous because, when present as methylmercury in aquatic food webs, it has a propensity to biomagnify especially in predatory fishes so that fish-eating birds and mammals can be at risk of mercury toxicity (Wiener et al. 2003).

Eighteen trace elements exhibited concentrations in all samples that were below the ICP-MS reporting limit of 0.04 µg/g (Table 2). These undetected elements were as follows: beryllium, bismuth, germanium, gold, hafnium, holmium, indium, iridium, lutetium, osmium, palladium, platinum, rhenium, ruthenium, tantalum, terbium, thulium, and ytterbium. Another 12 elements yielded concentrations equal to or greater than the reporting limit in one or more samples, but mean concentrations within fish species and site were <0.04 µg/g (data not shown). These elements included dysprosium, erbium, europium, gadolinium, niobium, praseodymium, samarium, silver, tellurium, thallium, tungsten, and zirconium. Still another eight elements—antimony, cerium, cesium, lanthanum, molybdenum, neodymium, uranium, and yttrium—contained mean concentrations in one or more fish species and sites that exceeded 0.04 µg/g (data not shown), but none of the means exceeded 0.12 µg/g or 3 times higher than their reporting limit (a computation procedure that we roughly equated to method quantitation limits of elements determined by quantitative procedures). Although these eight elements were detected in fish samples, we judged their concentrations to be too low for statistical comparisons.

A total of 21 trace elements were deemed suitable for statistical comparisons (Figs. 2-30). Eighteen of these elements, which were measured by semi-quantitative ICP-MS, included aluminum, barium, cadmium, chromium, cobalt, copper, gallium, iron, lead, lithium, manganese, nickel, rubidium, strontium, tin, titanium, vanadium, and zinc. The three remaining elements, which were measured by quantitative procedures, consisted of arsenic, mercury, and selenium. These 21 "quantitative" elements were compared between the two major HabTypes (oil platforms and natural areas).

Table 2. Summary of fish vital statistics, moisture content, and total concentrations of 63 elements measured in whole-body samples of kelp bass, kelp rockfish, and Pacific sanddab. Except for moisture content, values are geometric means (ranges in parentheses). For moisture content, values are back-transformed angular means (ranges in parentheses). With three exceptions, all measurements of elements are semi-quantitative estimates made by ICP-MS. The exceptions are arsenic, mercury, and selenium, which were measured with quantitative techniques.

Variable	Kelp bass, N=18	Kelp rockfish, N=80	Pacific sanddab, N=98
Total length (cm)[a]	34.3 (27.9-40.2)	25.1 (18.0-34.6)	19.4 (11.9-28.5)
Weight (g)	533 (288-874)	264 (86-790)	82 (14-289)
Moisture (%)	67.9 (62.9-71.5)	74.2 (66.8-87.9)	80.1 (70.9-84.2)
Aluminum (Al, µg/g dry weight)	2.8 (0.50-30.)	7.9 (0.70-100.)	16. (2.0-700.)
Antimony (Sb, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.20)
Arsenic (As, µg/g dry weight)	5.57 (3.40-13.1)	5.43 (2.38-36.4)	7.73 (3.94-23.5)
Barium (Ba, µg/g dry weight)	0.85 (0.40-4.0)	0.72 (<0.40-2.0)	5.0 (0.50-1,000.)
Beryllium (Be, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Bismuth (Bi, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Cadmium (Cd, µg/g dry weight)	0.35 (0.20-0.90)	0.28 (0.07-0.80)	1.2 (0.30-5.0)
Calcium (Ca, µg/g dry weight)	22,000. (12,000.-38,000.)	43,000. (22,000.-77,000.)	26,000. (11,000.-62,000.)
Cerium (Ce, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.07)	<0.04 (<0.04-2.0)
Cesium (Cs, µg/g dry weight)	0.04 (<0.04-0.06)	0.04 (<0.04-0.08)	0.05 (<0.04-0.10)
Chromium (Cr, µg/g dry weight)	0.55 (0.30-0.80)	0.86 (0.30-4.0)	1.2 (<0.04-8.0)
Cobalt (Co, µg/g dry weight)	<0.04 (<0.04-0.04)	0.04 (<0.04-0.60)	0.05 (<0.04-0.30)
Copper (Cu, µg/g dry weight)	1.0 (0.80-2.0)	1.9 (1.0-4.0)	2.7 (2.0-7.0)
Dysprosium (Dy, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.10)
Erbium (Er, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.05)
Europium (Eu, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.20)
Gadolinium (Gd, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.10)
Gallium (Ga, µg/g dry weight)	<0.04 (<0.04-0.10)	<0.04 (<0.04-0.10)	0.15 (<0.04-0.50)
Germanium (Ge, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Gold (Au, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Hafnium (Hf, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Holmium (Ho, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Indium (In, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	(<0.04 (<0.04-<0.04)
Iridium (Ir, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Iron (Fe, µg/g dry weight)	120. (20.-300.)	140. (8.0-300.)	120. (30.-1,000.)
Lanthanum (La, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.80)
Lead (Pb, µg/g dry weight)	0.07 (<0.04-0.20)	0.13 (<0.04-2.0)	0.23 (0.06-2.0)
Lithium (Li, µg/g dry weight)	0.28 (0.20-0.40)	0.52 (0.20-1.0)	0.88 (0.20-30.)
Lutetium (Lu, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Magnesium (Mg, µg/g dry weight)	1,000. (900.-2,000.)	1,400. (1,000.-2,000.)	1,900. (1,000.-3,000.)
Manganese (Mn, µg/g dry weight)	1.8 (1.0-3.0)	1.8 (0.90-4.0)	5.7 (2.0-10.)
Mercury (Hg, µg/g dry weight)	0.378 (0.150-0.900)	0.156 (0.048-1.14)	0.276 (0.072-1.12)
Molybdenum (Mo, µg/g dry weight)	<0.04 (<0.04-0.09)	<0.04 (<0.04-0.10)	0.04 (<0.04-0.30)
Neodymium (Nd, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.80)
Nickel (Ni, µg/g dry weight)	0.46 (<0.40-3.0)	0.80 (<0.40-2.0)	0.65 (<0.40-3.0)
Niobium (Nb, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.20)
Osmium (Os, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Palladium (Pd, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Platinum (Pt, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Potassium (K, µg/g dry weight	13,000. (10,000.-16,000.)	14,000. (10,000.-20,000.)	19,000. (14,000.-30,000.)

Table 2 (continued)

Variable	Kelp bass, N=18	Kelp rockfish, N=80	Pacific sanddab, N=98
Praseodymium (Pr, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.20)
Rhenium (Re, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Rubidium (Rb, µg/g dry weight)	2.0 (2.0-2.0)	2.0 (1.0-3.0)	3.1 (2.0-5.0)
Ruthenium (Ru, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Samarium (Sm, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.10)
Selenium (Se, µg/g dry weight)	1.26 (0.910-1.81)	1.56 (1.01-2.30)	1.68 (1.15-4.72)
Silver (Ag, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.06)	<0.04 (<0.04-0.10)
Sodium (Na, µg/g dry weight)	4,000. (3,000.-5,000.)	6,000.(4,000.-9,000.)	13,000. (7,000.-22,000.)
Strontium (Sr, µg/g dry weight)	88. (60.-100.)	150. (60.-400.)	110. (50.-300.)
Tantalum (Ta, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Tellurium (Te, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.10)
Terbium (Tb, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Thallium (Tl, µg/g dry weight)	<0.04 (<0.04-0.04)	<0.04 (<0.04-0.04)	<0.04 (<0.04-0.05)
Thulium (Tm, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Tin (Sn, µg/g dry weight)	2.7 (1.0-5.0)	0.99 (<0.04-20.)	<0.04 (<0.04-4.0)
Titanium (Ti, µg/g dry weight)	32. (20.-50.)	57. (10.-200.)	41. (10.-400.)
Tungsten (W, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.06)	<0.04 (<0.04-<0.04)
Uranium (U, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.04)	<0.04 (<0.04-0.20)
Vanadium (V, µg/g dry weight)	0.11 (0.06-0.20)	0.53 (0.04-9.0)	0.45 (0.10-40.)
Ytterbium (Yb, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)
Yttrium (Y, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.40)
Zinc (Zn, µg/g dry weight)	43. (30.-60.)	60. (40.-80.)	53. (40.-80.)
Zirconium (Zr, µg/g dry weight)	<0.04 (<0.04-<0.04)	<0.04 (<0.04-0.10)	<0.04 (<0.04-0.50)

[a] Total length (TL) can be converted to standard length (SL) by the following equations: for kelp bass, $\mathrm{Log}_{10}\mathrm{SL}=-0.11813+1.01623*\mathrm{Log}_{10}\mathrm{TL}$, $r^2=0.9953$; for kelp rockfish, $\mathrm{Log}_{10}\mathrm{SL}=-0.11657+1.01570*\mathrm{Log}_{10}\mathrm{TL}$, $r^2=0.9948$; and for Pacific sanddab, $\mathrm{Log}_{10}\mathrm{SL}=-0.10058+1.02399*\mathrm{Log}_{10}\mathrm{TL}$, $r^2=0.9970$.

Table 3. Variation in total length, weight, and moisture of kelp bass (KBA), kelp rockfish (KRO), and Pacific sanddab (PSD) sampled from various sampling sites.[a]

Site[b] or F-statistic	**Total length (cm)**			**Weight (g)**			**Moisture (%)**		
	KBA	**KRO**	**PSD**	**KBA**	**KRO**	**PSD**	**KBA**	**KRO**	**PSD**
PL1	---	22.9 C	17.8 AB	---	206 BC	59 AB	---	71.1 B	81.2 A
NA1	---	26.3 ABC	17.0 AB	---	284 BC	53 AB	---	72.1 B	80.5 A
PL2	---	28.0 AB	17.9 AB	---	376 AB	70 AB	---	70.7 B	80.4 A
NA2	---	23.3 BC	19.7 AB	---	200 C	83 AB	---	72.5 B	79.9 AB
PL3	---	22.4 C	21.7 A	---	214 BC	119 A	---	69.8 B	81.8 A
NA3	---	25.1 ABC	20.4 AB	---	234 BC	92 AB	---	72.5 B	80.0 AB
PL4	34.8 A	---	21.9 A	585 A	---	110 AB	67.3 A	---	79.9 AB
NA4	33.9 A	---	16.0 B	495 A	---	47 B	68.3 A	---	79.7 AB
PL5	---	30.2 A	21.7 A	---	546 A	124 A	---	82.3 A	79.6 AB
NA5	---	23.8 BC	20.7 AB	---	199 C	106 AB	---	81.5 A	77.8 B
$F_{(df1,df2)}$[c,d]	0.39	5.39****	4.04***	1.76	6.63****	3.43 **	1.12	65.65****	4.77****

[a] Within a column, geometric means (total length or weight) or angular-transformed means (moisture) followed by the same capital letter are not significantly different (P>0.05) according to Tukey's studentized range test.

[b] Names and locations of sampling sites are given in Table 1.

[c] For KBA, df1=1, df2=16; for KRO, df1=7, df2=72; for PSD, df1=9, df2=88.

[d] Codes: *, P≤0.05; **; P≤0.01; ***, P≤0.001; ****, P≤0.0001.

Comparisons of Quantitative Elements at Oil Platforms and Natural Areas

Results from mixed models ANCOVA or ANOVA indicated that none of the 21 quantitative elements consistently exhibited higher concentrations in fish from oil platforms than from natural areas. Out of 63 possible comparisons (21 elements measured in three fish species), 44 did not reveal significant differences between HabTypes, whereas 11 comparisons indicated that elemental concentrations were significantly higher at natural areas than at oil platforms. The remaining 8 comparisons exhibited significant HabType*TL interactions, indicating that relative differences in elemental concentrations between HabTypes varied with TL of the fish species. Overall, our findings generally fail to support the hypothesis that oil platforms are major sources of trace element contamination in resident marine fishes. Natural hydrocarbon seeps, which are plentiful in northern portions of the Southern California Bight, could expose fish in natural areas to trace elements and other contaminants, thus obfuscating spatial patterns that might otherwise be attributable to oil platforms. Hornafius et al. (1999) noted that methane and non-methane hydrocarbon emissions from natural seeps are largely responsible for air pollution in Santa Barbara County, and that natural seeps produce oil slicks that evaporate to create abundant "tar balls" found on southern California beaches. Moreover, in the Southern California Bight, some investigators have suggested that urban runoff might contribute more trace elements (chromium, copper, lead, and zinc) and nutrients (nitrate and phosphorus) than all other sources combined (Schiff et al. 2000).

Statistical results for individual elements are summarized below.

Aluminum. None of the three fish species exhibited significant HabType*TL interactions for aluminum (data not shown). However, aluminum concentrations were significantly associated with TL in kelp bass (F1,15=7.82, P=0.014) and kelp rockfish (F1,28.6=16.04, P<0.001), but not in Pacific sanddab (data not shown). In kelp bass, aluminum concentrations decreased by 18.5% (95% confidence interval, -30.4% to -4.8%) for each 1.0-cm increase in TL. In kelp rockfish, aluminum concentrations decreased by 10.1% (95% confidence interval, -14.8% to -5.1%) for each 1.0-cm increase in TL. Nevertheless, after controlling for TL, aluminum concentrations did not differ significantly between HabTypes in both kelp bass and kelp rockfish (Figure 2a). Aluminum concentrations in Pacific sanddab also did not differ significantly between HabTypes (Figure 2).

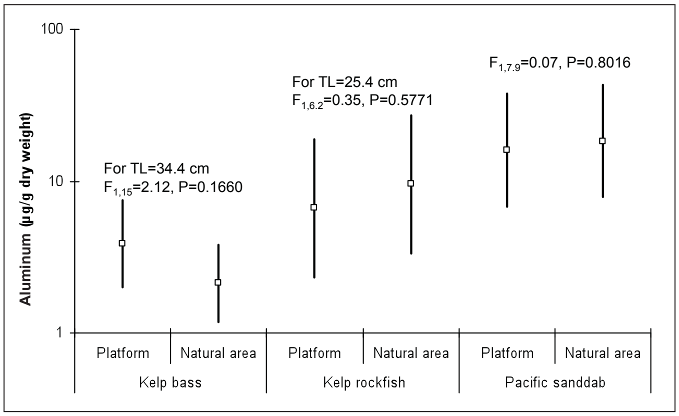

Figure 2. Comparisons of aluminum concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

Arsenic. Arsenic concentrations in the three fish species did not exhibit significant HabType*TL interactions (data not shown). Moreover, arsenic concentrations were not significantly associated with TL in all three fish species (data not shown). However, arsenic concentrations differed significantly between HabTypes for kelp bass and kelp rockfish, but not for Pacific sanddab (Figure 3). In general, arsenic concentrations in kelp bass were lower at oil platforms (mean, 4.33 µg/g; 95% confidence interval, 3.38-5.55 µg/g) than at natural areas (mean, 6.82 µg/g; 95% confidence interval, 5.46-8.52 µg/g). Arsenic concentrations in kelp rockfish were also lower at oil platforms (mean, 3.69 µg/g; 95% confidence interval, 2.37-5.76 µg/g) than at natural areas (mean, 8.14 µg/g; 95% confidence interval, 5.26-12.6 µg/g).

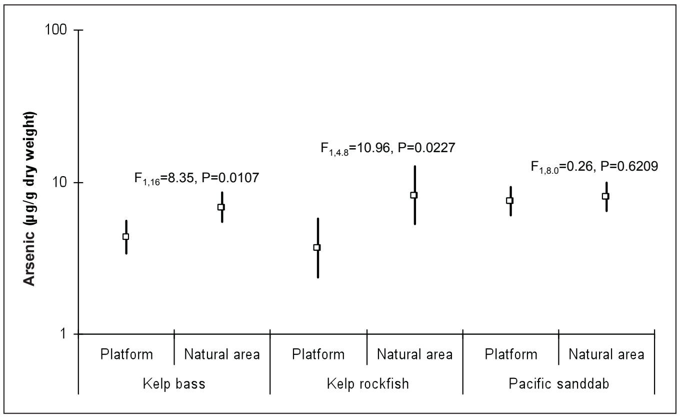

Figure 3. Comparisons of arsenic concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

Barium. Barium concentrations in the three fish species did not exhibit significant HabType*TL interactions (data not shown), nor did they exhibit significant associations with TL (data not shown). Furthermore, barium concentrations in the three fish species did not exhibit significant differences between HabTypes (Figure 4).

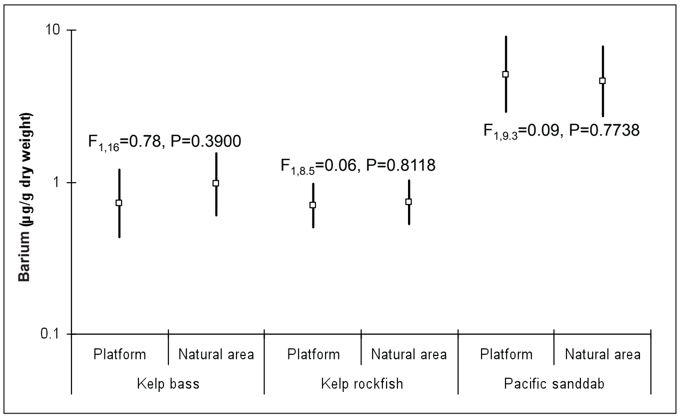

Figure 4. Comparisons of barium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

Cadmium. Cadmium concentrations in the three fish species did not exhibit significant HabType*TL interactions (data not shown). However, cadmium concentrations were significantly associated with TL in kelp bass ($F_{1,15}$=11.74, P=0.004) and Pacific sanddab ($F_{1,95.0}$=6.71, P=0.011), but not in kelp rockfish (data not shown). In kelp bass, cadmium concentrations increased by 9.4% (95% confidence interval, 3.5% to 15.7%) per 1.0-cm increase in TL. In Pacific sanddab, cadmium concentrations increased by 4.1% (95% confidence interval, 0.9% to 7.3%) per 1.0-cm increase in TL. Nevertheless, after controlling for TL, cadmium concentrations did not differ significantly between HabTypes in these two species (Figure 5). Cadmium concentrations in kelp rockfish also did not differ between HabTypes (Figure 5).

Chromium. Among the three fish species, chromium concentrations did not exhibit significant HabType*TL interactions nor significant associations with TL (data not shown). However, chromium concentrations differed significantly between HabTypes for kelp bass and Pacific sanddab, but not for kelp rockfish (Figure 6). In both kelp bass and Pacific sanddab, chromium concentrations were lower at oil platforms than at natural areas. Kelp bass averaged 0.47 µg Cr/g (95% confidence interval, 0.40-0.56 µg/g) at oil platforms, and 0.62 µg Cr/g (95% confidence interval, 0.54-0.72 µg/g) at natural areas, whereas Pacific sanddabs averaged 0.71 µg Cr/g (95% confidence interval, 0.36-1.4 µg/g) at oil platforms, and 2.1 µg Cr/g (95% confidence interval, 1.1-4.3 µg/g) at natural areas.

Cobalt. Cobalt concentrations in the three fish species did not exhibit significant HabType*TL interactions (data not shown), nor significant associations with TL (data not shown). Moreover, cobalt concentrations in the three species did not differ significantly between HabTypes (Figure 7).

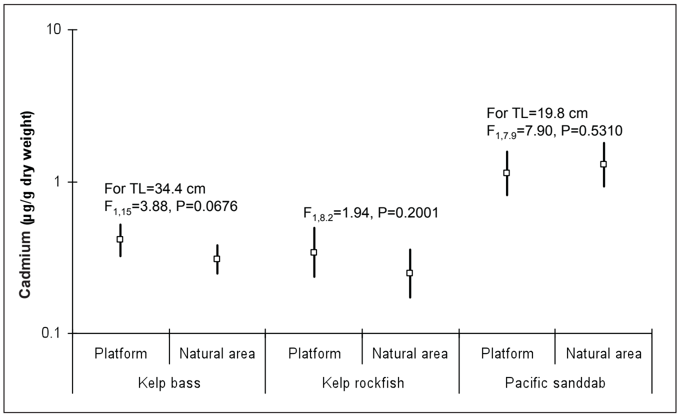

Figure 5. Comparisons of cadmium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

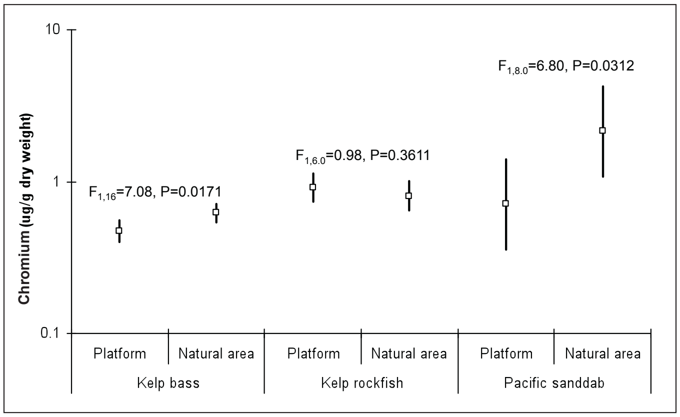

Figure 6. Comparisons of chromium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

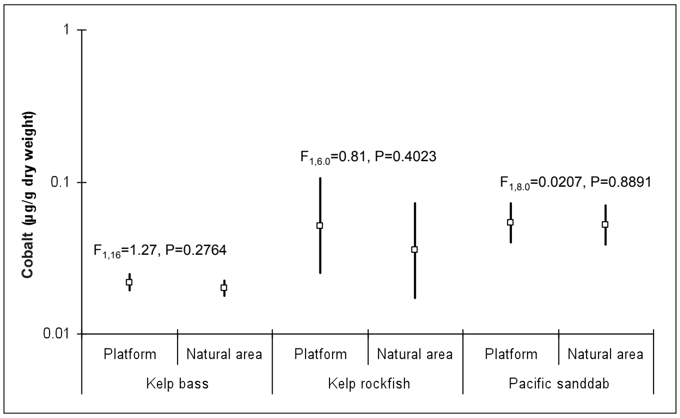

Figure 7. Comparisons of cobalt concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

Copper. Copper concentrations in kelp bass and kelp rockfish did not exhibit significant HabType*TL interactions (data not shown). Copper concentrations were not significantly associated with TL in kelp bass (data not shown), although this association was significant in kelp rockfish ($F_{1,62.2}$=16.98, P<0.001). Copper concentrations in kelp bass did not differ significantly between HabTypes (Figure 8) In kelp rockfish, copper concentrations decreased by 3.3% (95% confidence interval, -4.8 to -1.7%) for every 1.0-cm increase in TL. After controlling for TL, copper concentrations in kelp rockfish did not differ significantly between HabTypes (Figure 8). Copper concentrations in Pacific sanddab exhibited a significant HabType*TL interaction effect (Figure 9), indicating that the relation between copper concentrations and HabType differed by TL. On average, copper concentrations in Pacific sanddab increased by 1.4% (95% confidence interval, -0.79% to 3.7%) for every 1.0-cm increase in TL at oil platforms, but decreased by 1.9% (95% confidence interval, -4.0% to 0.30%) per 1.0-cm TL at natural areas. Although copper concentrations in small Pacific sanddab averaging 13.0 cm TL did not differ significantly between HabTypes ($F_{1,17.7}$=0.05, P=0.8303), copper concentrations in large Pacific sanddab averaging 27.5 mm TL were significantly higher at oil platforms than at natural areas ($F_{1,11.4}$=7.53, P=0.0186; also see Figure 9).

Gallium. None of the three fish species exhibited significant HabType*TL interactions for gallium (data not shown). In addition, gallium concentrations in the three fish species were not significantly associated with TL (data not shown). Moreover, gallium concentrations in the three fish species did not differ between HabTypes (Figure 10).

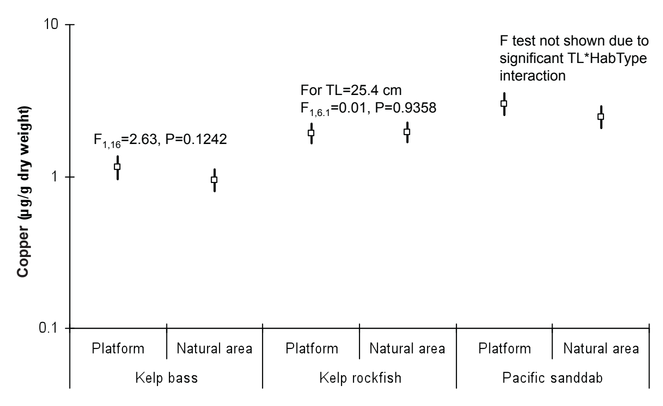

Figure 8. Comparisons of copper concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

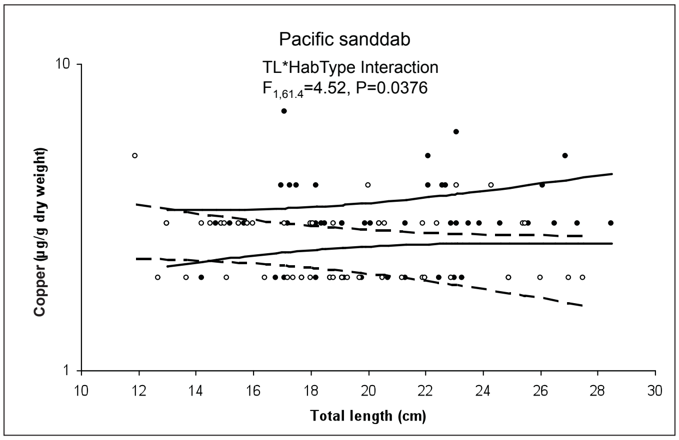

Figure 9. Relation between total length and copper concentration in whole-body samples of Pacific sanddab from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

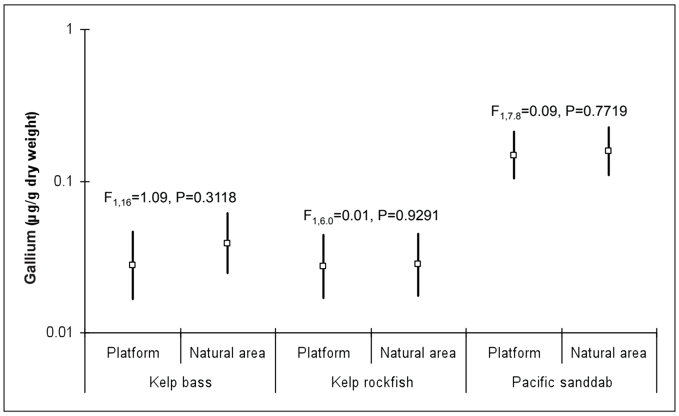

Figure 10. Comparisons of gallium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

Iron. Iron concentrations in the three fish species did not exhibit significant HabType*TL interactions (data not shown) nor significant associations with TL (data not shown). However, iron concentrations differed significantly between HabTypes for kelp bass, but not for kelp rockfish and Pacific sanddab (Figure 11). On average, iron concentrations in kelp bass were lower at oil platforms (mean, 49. µg/g; 95% confidence interval, 29.-83. µg/g) than at natural areas (mean, 240. µg/g; 95% confidence interval, 150.-390. µg/g).

Lead. None of the three fish species exhibited a significant HabType*TL interaction for lead (data not shown). In kelp bass, lead concentrations were not significantly associated with TL (data not shown). However, lead concentrations were significantly associated with TL in kelp rockfish ($F_{1,22.0}=4.93$, $P=0.037$) and Pacific sanddab ($F_{1,94.5}=11.79$, $P<0.001$). In kelp rockfish, lead concentrations decreased by 3.9% (95% confidence interval, -7.4% to -0.3%) for each 1.0-cm increase in TL. In Pacific sanddab, lead concentrations decreased by 5.7% (95% confidence interval, -8.9% to -2.5%) for each 1.0-cm increase in TL. Lead concentrations in kelp bass did not differ significantly between HabTypes (Figure 12). After controlling for TL, lead concentrations in both kelp rockfish and Pacific sanddab also did not differ significantly between HabTypes (Figure 12).

Lithium. The concentrations of lithium did not exhibit significant HabType*TL interactions or TL effects in the three fish species (data not shown). Lithium concentrations did not differ significantly between HabTypes for kelp bass and Pacific sanddab (Figure 13). However, in kelp rockfish, a significant difference was found for HabType (Figure 13). On average, lithium concentrations in kelp rockfish from oil platforms (mean, 0.39 µg/g; 95% confidence interval, 0.35-0.43 µg/g) were lower than those from natural areas (mean, 0.68 µg/g; 95% confidence interval, 0.61-0.76 µg/g).

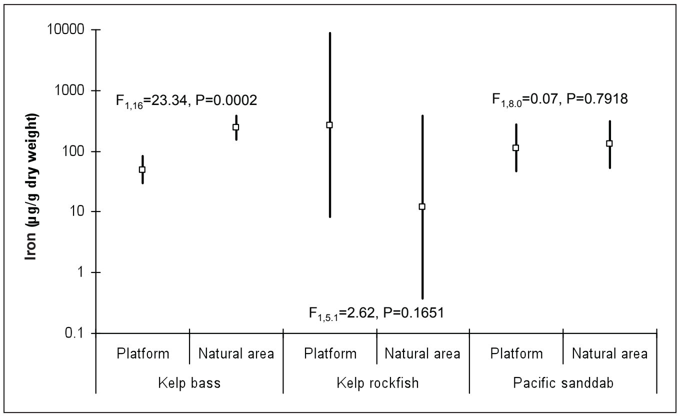

Figure 11. Comparisons of iron concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

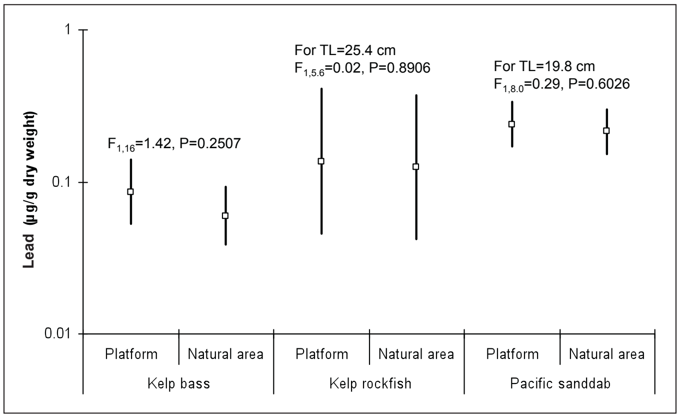

Figure 12. Comparisons of lead concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

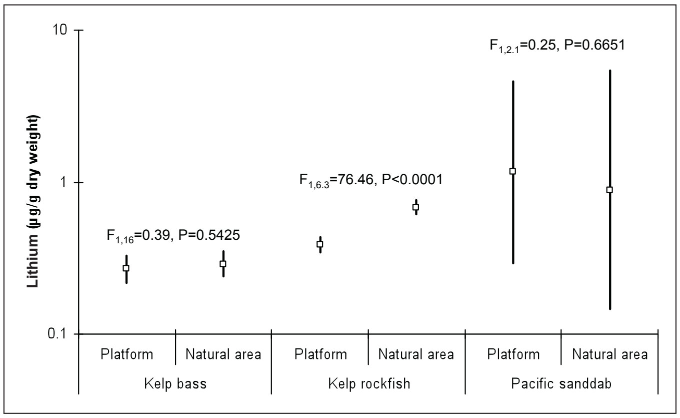

Figure 13. Comparisons of lithium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

Manganese. Among the three fish species, manganese concentrations did not exhibit significant HabType*TL interactions (data not shown), and a significant TL effect was detected in kelp rockfish ($F_{1,74.1}$=8.51, P=0.004) but not in the other two species (data not shown). For kelp rockfish, manganese was inversely associated with TL, with concentrations generally decreasing by 2.4% (95% confidence interval, -4.1% to -0.8%) for each 1.0-cm increase in TL. Manganese concentrations did not differ significantly between HabTypes for kelp bass and Pacific sanddab (Figure 14). However, after controlling for TL, manganese concentrations in kelp rockfish exhibited a significant difference for Habtype (Figure 14), with lower concentrations occurring in fish from oil platforms (mean, 1.4 µg/g; 95% confidence interval, 1.2-1.8 µg/g) than from natural areas (mean, 2.3 µg/g; 95% confidence interval, 1.9-2.8 µg/g).

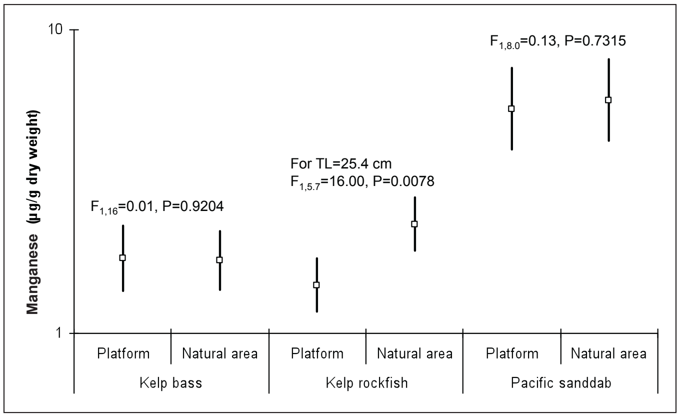

Figure 14. Comparisons of manganese concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

Mercury. None of the three fish species exhibited a significant HabType*TL interaction for mercury (data not shown). However, mercury concentrations in all three species varied with TL (for kelp bass, $F_{1,15}$=5.08, P=0.040; for kelp rockfish, $F_{1,38.0}$=19.58, P<0.001; for Pacific sanddab, $F_{1,95.0}$=15.53, P<0.001). In kelp bass, mercury concentrations increased by 8.3% (95% confidence interval, 0.4% to 16.9%) for every 1.0-cm increase in TL. In kelp rockfish, mercury concentrations increased by 5.7% (95% confidence interval, 3.1% to 8.4%) for every 1.0-cm increase in TL. In Pacific sanddab, mercury concentrations increased by 5.1% (95% confidence interval, 2.5% to 7.8%) for every 1.0-cm increase in TL. After controlling for TL, mercury concentrations exhibited significant differences between HabTypes for kelp bass and kelp rockfish, but not for Pacific sanddab (Figure 15). In general, mercury concentrations in kelp bass were lower at oil platforms (mean, 0.268 µg/g; 95% confidence interval, 0.195-0.369 µg/g) then at natural areas (mean, 0.495 µg/g; 95% confidence interval, 0.372-0.658 µg/g). Similarly, mercury concentrations in kelp rockfish were lower at oil platforms (mean, 0.109 µg/g; 95% confidence interval, 0.062-0.190 µg/g) than at natural areas (mean, 0.227 µg/g; 95% confidence interval, 0.131-0.396 µg/g).

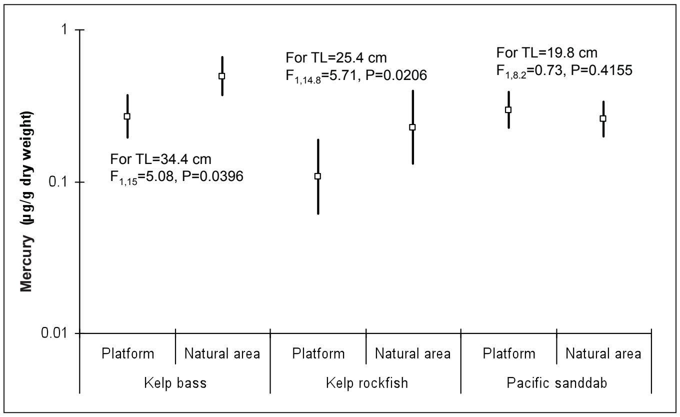

Figure 15. Comparisons of mercury concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

Nickel. None of the three fish species exhibited a significant HabType*TL interaction for nickel (data not shown). Moreover, nickel concentrations were not significantly associated with TL in kelp bass (data not shown) and kelp rockfish (data not shown). However, an inverse association between nickel concentrations and TL was observed in Pacific sanddab ($F_{1,91.9}$=9.91, P=0.002). On average, nickel concentrations in Pacific sanddab decreased by 4.1% (95% confidence interval, -6.7 to -1.6%) for every 1.0-cm increase in TL. Nickel concentrations in all three species (including Pacific sanddab, after controlling for TL) did not differ significantly between HabTypes (Figure 16).

Rubidium. Kelp bass and Pacific sanddab did not exhibit significant HabType*TL interactions for rubidium (data not shown). In both fish species, TL was not significantly associated with rubidium concentrations (data not shown). Moreover, rubidium concentrations did not exhibit significant differences between Habtypes for kelp bass and Pacific sanddab (Figure 17). On the other hand, rubidium concentrations in kelp rockfish exhibited a significant HabType*TL interaction effect (Figure 18), indicating that the relation between rubidium concentrations and HabType differed by TL. On average, rubidium concentrations in kelp rockfish decreased by <0.1% (95% confidence interval, -0.8% to 0.8%) for every 1.0-cm increase in TL at oil platforms, and decreased by 1.4% (95% confidence interval, -2.3% to -0.5%) per 1.0-cm TL at natural areas. However, over the TL range of sampled individuals, rubidium concentrations in kelp rockfish did not differ significantly between oil platforms and natural areas (for small rockfish averaging 18.3 cm TL, $F_{1,21.6}$=3.82, P=0.0637; for large rockfish averaging 34.0 cm TL, $F_{1,30.5}$=3.61, P=0.0668; see Figure 18).

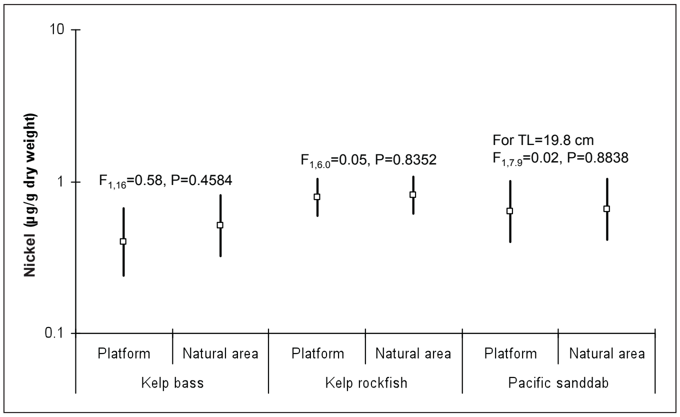

Figure 16. Comparisons of nickel concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

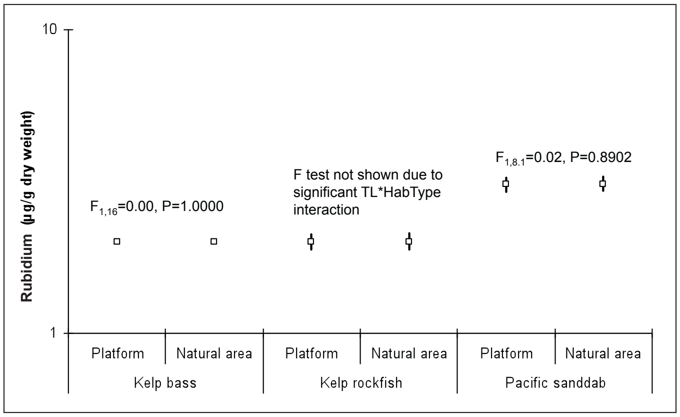

Figure 17. Comparisons of rubidium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

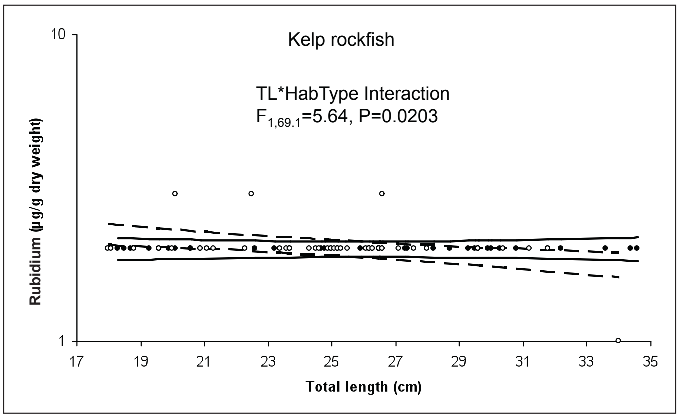

Figure 18. Relation between total length and rubidium concentration in whole-body samples of kelp rockfish from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

Selenium. Kelp bass did not exhibit a significant HabType*TL interaction (data not shown) for selenium. Moreover, selenium concentrations in kelp bass were not significantly associated with TL (data not shown). In addition, selenium concentrations in kelp bass did not exhibit a significant difference between HabTypes (Figure 19). On the other hand, kelp rockfish and Pacific sanddab both exhibited significant TL*HabType interaction effects (Figures 20-21), suggesting that relations between selenium concentration and HabType in these two species differed by TL. In kelp rockfish, selenium concentrations decreased by 0.9% (95% confidence interval, -2.2% to 0.3%) for every 1.0-cm increase in TL at oil platforms but increased by 0.9% (95% confidence interval, -0.4% to 2.3%) at natural areas. In Pacific sanddab, selenium concentrations decreased by 0.9% (95% confidence interval, -2.2% to 0.5%) for every 1.0-cm increase in TL at oil platforms, and decreased by 5.4% (95% confidence interval, -6.6% to -4.1%) at natural areas. Nevertheless, in kelp rockfish, selenium concentrations did not differ between HabTypes at least over the TL range of sampled fish (for small rockfish averaging 18.3 cm TL, $F_{1,4.5}$=1.72, P=0.2522; for large rockfish averaging 34.0 cm TL, $F_{1,3.8}$=0.25, P=0.6444; see Figure 19). In Pacific sanddab, small individuals averaging 13.0 cm TL exhibited significantly lower concentrations at oil platforms than at natural areas ($F_{1,18.6}$=13.99, P=0.0014), whereas large individuals averaging 27.5 cm TL exhibited significantly higher concentrations at oil platforms than at natural areas ($F_{1,22.1}$=6.37, P=0.0193; see Figure 21).

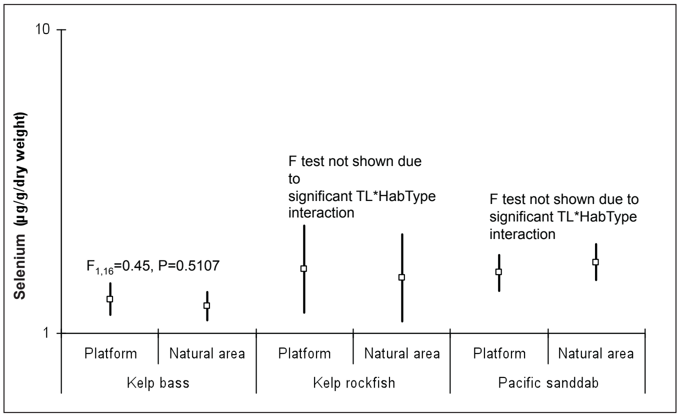

Figure 19. Comparisons of selenium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

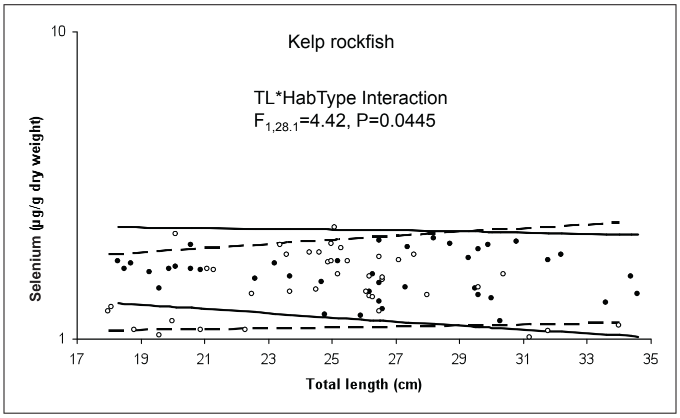

Figure 20. Relation between total length and selenium concentration in whole-body samples of kelp rockfish from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

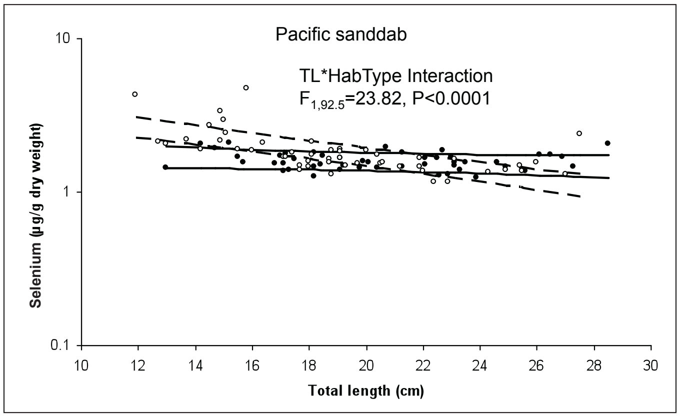

Figure 21. Relation between total length and selenium concentration in whole-body samples of Pacific sanddab from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

Strontium. None of the three fish species exhibited a significant HabType*TL interaction for strontium (data not shown). Moreover, strontium concentrations were not significantly associated with TL in kelp bass (data not shown) and kelp rockfish (data not shown). In kelp bass, strontium concentrations did not differ significantly between HabTypes (Figure 22). However, in kelp rockfish, strontium concentrations differed significantly between HabTypes (Figure 22), with lower concentrations occurring in fish from oil platforms (mean, 120. µg/g; 95% confidence interval, 92.-150. µg/g) than from natural areas (mean, 200. µg/g; 95% confidence interval, 160.-260. µg/g). In Pacific sanddab, a significant inverse association was found between strontium concentrations and TL ($F_{1,94.6}$=11.30, P=0.001). On average, strontium concentrations in Pacific sanddab decreased by 3.8% (95% confidence interval, -5.9% to -1.6%) for every 1.0-cm increase in TL. Nevertheless, after controlling for TL, strontium concentrations in Pacific sanddab did not differ significantly between HabTypes (Figure 22).

Tin. Kelp bass did not exhibit a significant HabType*TL interaction for tin (data not shown). Moreover, in kelp bass, TL was not significantly associated with tin concentrations (data not shown). On average, tin concentrations in kelp bass did not vary between HabTypes (Figure 23). By comparison, tin concentrations in kelp rockfish and Pacific sanddab exhibited significant HabType*TL interaction effects (Figures 24-25), and significant associations with TL (for kelp rockfish, $F_{1,53.6}$=119.42, P<0.001; for Pacific sanddab, $F_{1,80.6}$=6.01, P=0.016). Tin concentrations in kelp rockfish increased an average of 36.8% (95% confidence interval, 25.1% to 50.8%) for every 1.0-cm increase in TL at oil platforms, and increased an average of 60.4% (95% confidence interval, 43.4% to 79.4%) for every 1.0-cm increase in TL at natural areas. By comparison, tin concentrations in Pacific sanddab decreased an average of 1.5% (95% confidence interval, -9.0% to 6.6%) for every 1.0-cm increase in TL at oil platforms, and increased an average of 16.4% (95% confidence interval, 7.7% to 25.7%) for

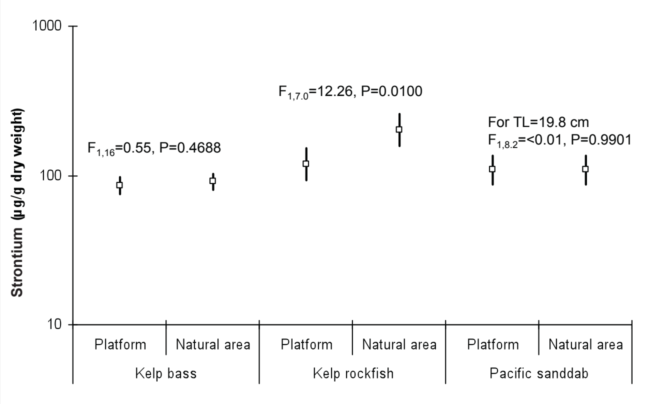

Figure 22. Comparisons of strontium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

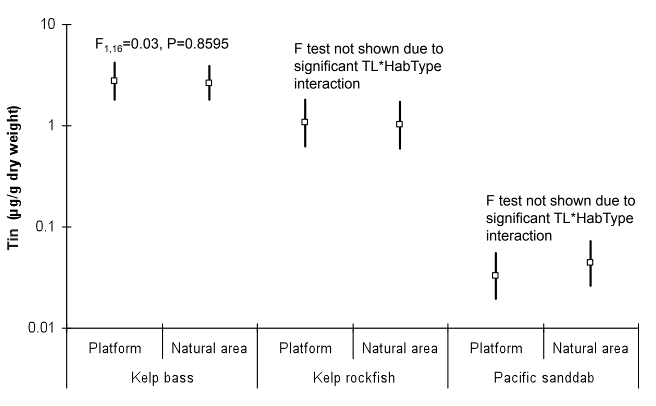

Figure 23. Comparisons of tin concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

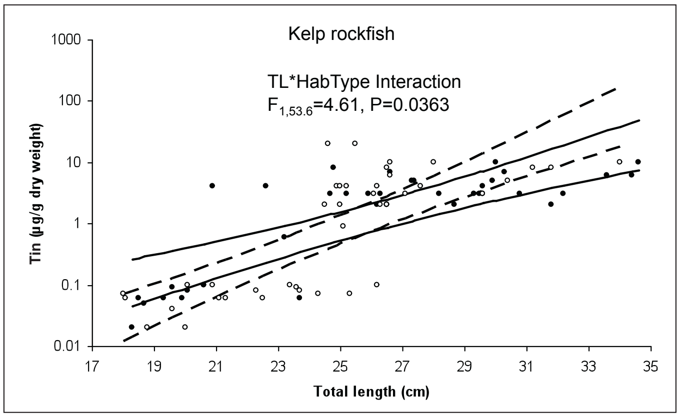

Figure 24. Relation between total length and tin concentration in whole-body samples of kelp rockfish from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

every 1.0-cm increase in TL at natural areas. Nevertheless, over the TL range of kelp rockfish sampled during our study, tin concentrations did not differ significantly between oil platforms and natural areas (for small rockfish averaging 18.3 cm TL, $F_{1,23.8}$=3.87, P=0.0610; for large rockfish averaging 34.0 cm TL, $F_{1,33.7}$=3.30, P=0.0781; see Figure 24). Although tin concentrations in small Pacific sanddab averaging 13.0 cm TL did not differ significantly between HabTypes ($F_{1,62.2}$=3.69, P=0.059), tin concentrations in large Pacific sanddab averaging 27.5 cm TL were significantly lower at oil platforms than at natural areas ($F_{1,19.7}$=7.27, P=0.0140; see Figure 25).

Titanium. Kelp bass and kelp rockfish did not exhibit significant HabType*TL interactions for titanium (data not shown). Moreover, titanium concentrations in kelp bass did not vary with TL (data not shown) or with HabType (Figure 26). By comparison, titanium concentrations in kelp rockfish were significantly associated with TL ($F_{1,68.6}$=34.33, P<0.001), with concentrations decreasing by 8.8% (95% confidence interval, -12.% to -5.9%) for every 1.0-cm increase in TL. However, after controlling for TL, mean concentrations of titanium in kelp rockfish did not differ significantly between HabTypes (Figure 26). On the other hand, titanium concentrations in Pacific sanddab exhibited a significant HabType*TL interaction effect (Figure 27), indicating that the relation between titanium concentrations and HabType differed by TL. Titanium concentrations increased by 8.7% (95% confidence interval, 3.9% to 13.8%) for every 1.0-cm increase in TL for Pacific sanddab from oil platforms, but decreased by 3.9% (95% confidence interval, -8.1% to 0.4%) for every 1.0-cm increase in TL for Pacific sanddab from natural areas. Titanium concentrations in Pacific sanddab were significantly lower at oil platforms than at natural areas among very small individuals averaging 13.0 cm TL ($F_{1,33.1}$=5.49, P=0.0253), but significantly higher at oil platforms than at natural areas among very large individuals averaging 27.5 cm TL ($F_{1,38.2}$=14.81, P=0.0004; see Figure 27).

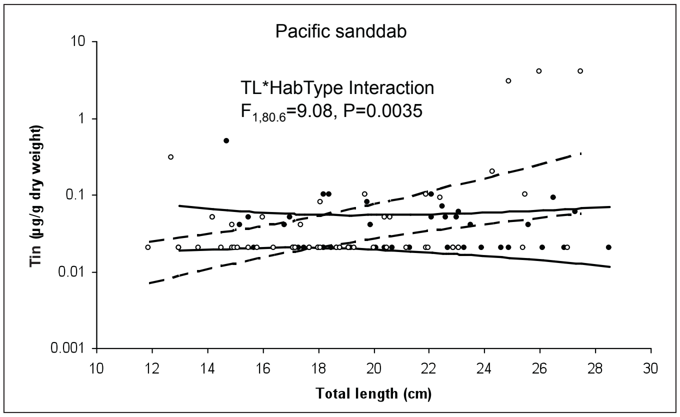

Figure 25. Relation between total length and tin concentration in whole-body samples of Pacific sanddab from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

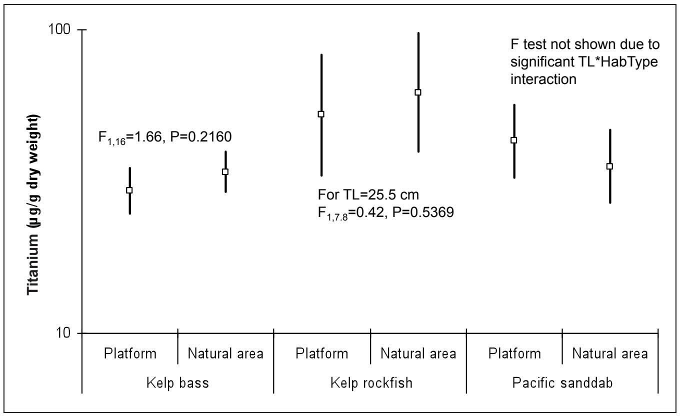

Figure 26. Comparisons of titanium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

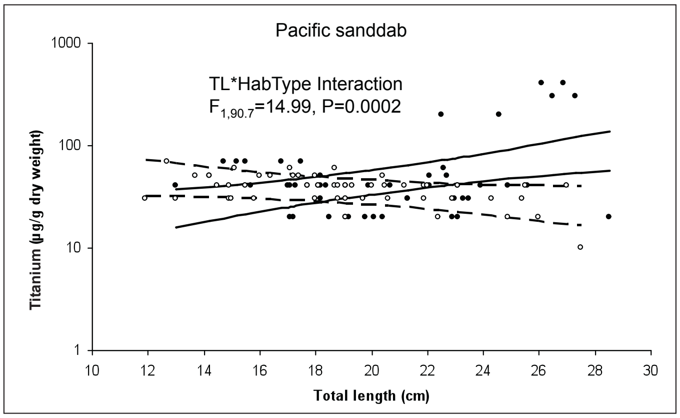

Figure 27. Relation between total length and titanium concentration in whole-body samples of Pacific sanddab from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

Vanadium. Kelp bass and kelp rockfish did not exhibit significant HabType*TL interactions for vanadium (data not shown). Vanadium concentrations in kelp bass were also not significantly associated with TL (data not shown) and between HabTypes (Figure 28). Vanadium concentrations in kelp rockfish were inversely associated with TL ($F_{1,\ 56.1}$=38.93, P<0.001), with concentrations decreasing by 21.4% (95% confidence interval, -27.3% to -15.1%) for every 1.0-cm increase in TL. However, after controlling for TL, vanadium concentrations in kelp rockfish did not differ between HabTypes (Figure 28). This finding contrasts with those by Bascom et al. (1976) who reported higher concentrations of vanadium in liver, kidney, and muscle of two rockfish species (*Sebastes auriculatus* and *S. caurinus*) sampled from Platforms Hazel and Hilda, and lower concentrations in rockfish from a nearby rocky natural area. Nevertheless, Bascom et al. (1976) believed that vanadium concentrations were not sufficiently elevated to harm the rockfish. Vanadium concentrations in Pacific sanddab exhibited a significant HabType*TL interaction effect (Figure 28), indicating the relation between vanadium concentrations and HabType differed by TL. Vanadium concentrations increased by 13.8% (95% confidence interval, 5.6% to 22.8%) for every 1.0-cm increase in TL at oil platforms, and decreased by 8.3% (95% confidence interval, -14.6% to -1.3%) per 1.0-cm TL at natural areas. Vanadium concentrations in Pacific sanddab were significantly lower at oil platforms than at natural areas among very small individuals averaging 13.0 cm TL ($F_{1,59.7}$=6.24, P=0.0153), but significantly higher at oil platforms than at natural areas among very large individuals averaging 27.5 cm TL ($F_{1,19.0}$=13.15, P=0.0018; see Figure 29).

Zinc. Zinc concentrations in all three fish species did not exhibit significant HabType*TL interactions (data not shown), nor did they exhibit significant associations with TL (data not shown). Moreover, zinc concentrations did not differ significantly between HabTypes in kelp bass and Pacific sanddab, but a significant dif-

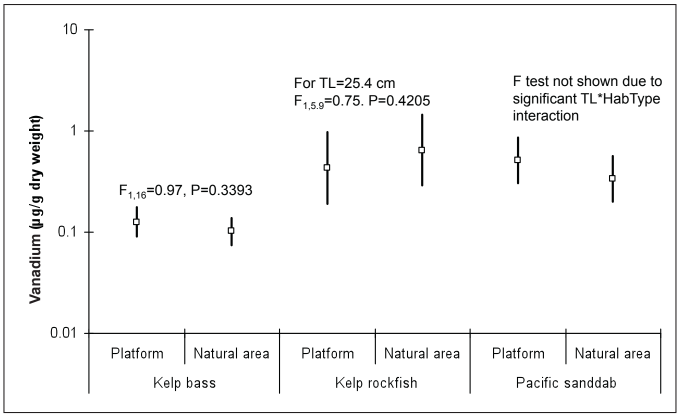

Figure 28. Comparisons of vanadium concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels. Total length is reported if the F statistic was computed from analysis of covariance.

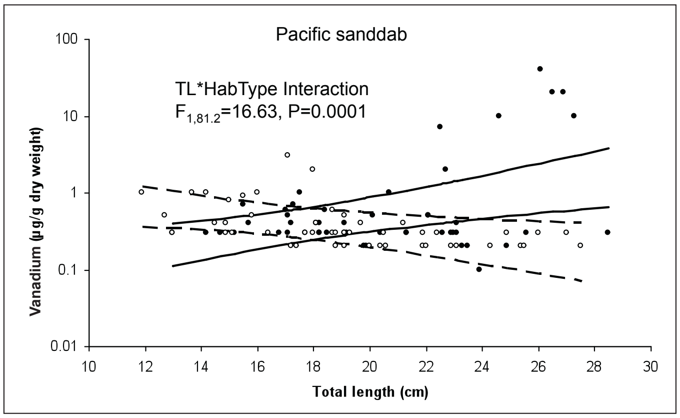

Figure 29. Relation between total length and vanadium concentration in whole-body samples of Pacific sanddab from oil platforms (closed circles & solid 95% confidence band) and natural areas (open circles & dashed 95% confidence band). Also shown are the F statistic and significance level for the TL*HabType interaction.

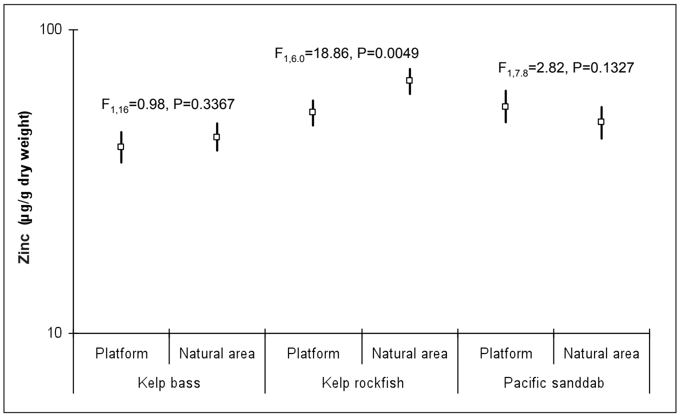

Figure 30. Comparisons of zinc concentrations (least-squares means and 95% confidence intervals) in whole-fish samples of kelp bass, kelp rockfish, and Pacific sanddab from two HabTypes (oil platforms and natural areas). Also shown are F statistics and significance levels.

ference was detected in kelp rockfish (Figure 30). In general, kelp rockfish exhibited lower concentrations of zinc at oil platforms (mean, 53. µg Zn/g; 95% confidence interval, 49.-59. µg/g) than at natural areas (mean, 68.µg Zn/g (95% confidence interval, 62.-74. µg/g).

Relation of Elemental Concentrations to Toxicity Thresholds for Fish and Piscivorous Wildlife

Concentrations of copper and zinc in whole-fish samples from our study were well below literature-based toxicity thresholds for fishes, wildlife consumers of fish, or both (see Table 4). By comparison, arsenic, cadmium, chromium, lead, mercury, and selenium in whole-fish samples equaled or exceeded the toxicity thresholds, raising the possibility of adverse effects.

Arsenic concentrations (measured as total arsenic) in 4 kelp bass from a natural area (NA4, 4 of 10 fish), 14 kelp rockfish from three natural areas (NA1, 1 of 10 fish; NA2, 4 of 10 fish; and NA3, 9 of 10 fish), and 7 Pacific sanddab from an oil platform (PL2, 1 of 8 fish) and a natural area (NA3, 6 of 10 fish) equaled or exceeded the toxic threshold of 2.2-11.6 µg As/g wet weight. Although arsenic concentrations as high as 10.2 µg/g wet weight were recorded during our study (i.e., a sample of kelp rockfish from NA3), the likelihood of toxicity was probably negligible because <10% of total arsenic generally occurs in the highly toxic inorganic form (Attar et al. 1992; U.S. EPA 2000). A recent study by Peshut et al. (2008) determined that inorganic arsenic in marine fish from American Samoa typically constituted less than 0.5% of total arsenic. Eisler (1988a) reported that inorganic forms of arsenic are generally much more toxic than organic forms. In addition to being very toxic to aquatic biota, including fishes, inorganic arsenic can cause poor growth, food avoidance, and metabolic failure (Eisler 1988a). In mammals, arsenic is a teratogen and carcinogen that can traverse placental barriers and produce fetal death and malformations (Eisler 1988a).

Cadmium concentrations approached or equaled the toxic threshold of 0.23-15.6 µg/g wet weight in 1 of 18 kelp bass, 2 of 80 kelp rockfish, and 30 of 98 Pacific sanddab. The kelp bass was collected from an oil platform (PL4, 1 of 8 fish), as were the two kelp rockfish (PL2, 2 of 10 fish). Although 17 Pacific sanddab containing high concentrations of cadmium came from oil platforms (PL2, 3 of 8 fish; PL3, 7 of 10 fish; PL4, 3 of 10 fish; and PL5, 4 of 10 fish), 22 highly contaminated sanddabs also came from natural areas (NA1, 2 of 10 fish; NA2, 5 of 10 fish; NA3, 7 of 10 fish; NA4, 1 of 10 fish; and NA5, 7 of 10 fish). Toxic responses from excessive exposure to cadmium in fishes may include decreased growth, respiratory disruption, altered enzyme levels, and abnormal muscular contractions (Eisler 1985). Although mammals and birds are comparatively resistant to biocidal properties of cadmium, sublethal effects include growth retardation, anemia, and testicular damage (Eisler 1985).

Mean concentrations of chromium exceeded the toxicity threshold of 1.0 µg/g wet weight in 22 of 98 Pacific sanddab, all from three natural areas (NA3, 7 of 10 fish; NA4, 10 of 10 fish, and NA5, 5 of 10 fish). Toxic effects from excess exposure to chromium may include decreased growth and body size, and also significant reduction in the rate of reproduction and survival of progeny (Moore and Ramamoorthy 1984).

Lead concentrations equaled the toxicity threshold of 0.4-8.8 µg/g wet weight in a single kelp rockfish from an oil platform (PL1, 1 of 10 fish). According to Eisler (1988b), lead is neither essential nor beneficial to living organisms, and all measured effects are adverse. Toxic effects in aquatic biota from excessive lead exposure include reduced survival, impaired reproduction, and reduced growth (Eisler 1988b). In birds and mammals feeding on lead-contaminated prey, sublethal effects can include impaired learning and abnormal social behavior (Eisler 1988b).

Mercury concentrations in 12 kelp bass, 11 kelp rockfish, and 10 Pacific sanddab equaled the toxic threshold of 0.1-3.0 µg/g wet weight for methylmercury. Although total mercury was measured during our study, several investigators (Grieb et al. 1990; Bloom 1992; Wiener et al. 2003) reported that approximately 95-99% of mercury in fish muscle tissues occurs as methylmercury. Nevertheless, Mason et al. (2006) reported methylmercury concentrations averaging only 28 ± 14% in axial muscle of planktivorous white perch (*Morone americana*) and 65 ± 22% in axial muscle of mostly piscivorous striped bass (*Morone saxatilis*) from tidal portions of Chesapeake Bay. During our study, kelp bass with high concentrations of mercury came from an oil platform (PL4, 3 of 8 fish) and a natural area (NA4, 9 of 10 fish), kelp rockfish came from an oil platform (PL2, 1 of 10 fish) and two natural areas (NA3, 8 of 10 fish; and NA5, 2 of 10 fish), and Pacific sanddab came from four oil platforms (PL2, 2 of 8 fish; PL3, 1 of 10 fish; PL4, 3 of 10 fish; and PL5, 1 of 10 fish) and a natural area (NA3, 3 of 10 fish). Although mercury is a potent neurotoxin capable of causing outright mortality in fish and wildlife, sublethal effects may include suppression of sex hormones, altered reproductive behavior, and impaired reproduction, along with maternal transfer of potentially toxic doses of mercury to embryos during oogenesis (Wiener et al. 2003; Scheuhammer et al. 2007).

Selenium concentrations in only two Pacific sanddab, both from a natural area (NA2, 2 of 10 fish), equaled or exceeded the toxicity threshold of 0.75-1.0 µg Se/g wet weight. According to Maher et al. (1997), selenium recovered from fish muscle tissues are associated with proteins and consist mostly of selenocysteine. Lemly (2002) reported that adverse effects from excessive selenium concentrations in fishes can include severe tissue pathology, teratogenic deformities, and reproductive impairment, along with high mortality.

High concentrations of total DDT and total PCB have been implicated as the probable cause of reproductive impairment in white croaker (*Genyonemus lineatus*) sampled from San Pedro Bay in the Southern California Bight (Schiff et al. 2000). Damage to DNA and other subcellular evidence of contaminant exposure in marine fishes have also been associated with high concentrations of chlorinated hydrocarbons (Schiff et al. 2000). However, to our knowledge, no one has linked adverse ecotoxicological responses in fishes, marine birds, or marine mammals from the Southern California Bight to elevated elemental concentrations such as those measured during our study.

Relation of Elemental Concentrations in Fish to Human Health

The USEPA (2000) provided guidance on development of risk-based meal consumption limits for inorganic arsenic, cadmium, methylmercury, selenium, and tributyltin in fishes for use in generating fish consumption advisories (Table 4). However, these consumption limits for humans are based on skinless fillets (axial muscle tissue), and not whole fishes. We estimated fillet concentrations from our data on whole fishes by using average ratios of fillet and whole-body concentrations for arsenic, mercury, and selenium in three marine fish species from the Salton Sea in southern California (Saiki 1990). We also estimated fillet concentrations for cobalt, copper, iron, lead, nickel, vanadium, and zinc even though USEPA has not provided guidance for these elements.

We were unable to find conversion factors for cadmium and tin, so we compared USEPA risk-based meal consumption limits using whole-fish data. These comparisons indicated that cadmium concentrations in 14 of 18 kelp bass, 31 of 80 kelp rockfish, and 96 of 98 Pacific sanddab exceeded 0.088 µg Cd/g wet weight, which indicates that fish consumption should be restricted to 16 or fewer meals per month. Judging from data for whole fishes (Figure 5), cadmium concentrations in fillets are not expected to differ substantially between oil platforms and natural areas. None of the measured concentrations exceeded 5.6 µg Cd/g wet weight, which corresponds to no consumption of fishes. Stapleton (1968) reported that muscle tissue in kelp bass from the Scattergood Steam Plant in Santa Monica Bay contained average concentrations of 3.0-4.0 µg Cd/g dry weight (about 0.75-1.0 µg Cd/g wet weight, assuming 75% moisture), whereas kelp bass from Catalina Island contained 2.0-3.0 µg Cd/g dry weight (about 0.50-0.75 µg Cd/g wet weight). Although tin concentrations in 18 of 18 kelp bass, 53 of 80 kelp rockfish, and 4 of 98 Pacific sanddab exceeded 0.088 µg Sn/g wet weight, indicating that fish consumption should be limited to 16 or fewer meals per month, the risk-based consumption limit was based on highly toxic tributyltin, whereas we measured total tin concentrations that possibly included relatively non-toxic inorganic tin. We did not attempt to estimate the proportion of tributyltin in our fish samples because it can vary widely in marine fishes (organotins may represent as little as 3-6% or as much as 18-52% of total tin; Eisler 1989).

Arsenic concentrations estimated for fillets were seemingly elevated in fishes sampled from oil platforms and natural areas during our study, with the lowest concentration (0.674 µg As/g wet weight in kelp rockfish) equating to 4 or fewer meals per month according to the noncancer health endpoint (Table 4). Moreover, two samples of kelp bass, seven samples of kelp rockfish, and two samples of Pacific sanddab exceeded 5.6 µg As/g wet weight, the concentration at which no fish should be consumed. However, these risk-based consumption limits are based on inorganic arsenic, which is highly toxic to mammals, whereas total arsenic was measured during our study. Several investigators (e.g., Attar et al. 1992; U.S. EPA 2000; Peshut et al. 2008) reported that <10% of total arsenic occurs in the inorganic form in fish tissues (most organic forms of arsenic are relatively nontoxic), which would lower our range of concentrations to 0.067-1.61µg As/g wet weight or less. Even with these adjustments, arsenic concentrations may be sufficiently elevated in some fishes to warrant restricted consumption limits. However, Peshut et al. (2008) cautioned that use of assumptions to define the toxic fraction for arsenic can be excessively conservative, and could lead to inappropriate public health determinations or unnecessary government regulatory actions.

Mercury concentrations estimated for fillets were sufficiently elevated to warrant restricted consumption of kelp bass (18 of 18 samples), kelp rockfish (64 of 80 samples), and Pacific sanddab (94 of 98 samples), ranging from 16 meals per month to 2 meals per month or fewer (Table 4). Judging from whole-fish samples (Figure 15), mercury concentrations in kelp bass and kelp rockfish are expected to be higher at natural areas than at oil platforms, whereas mercury concentrations in Pacific sanddabs may not exhibit such differences. The estimated concentrations in kelp bass fillets from our study (range, 0.06-0.35 µg Hg/g wet weight) were similar to concentrations reported in 69 samples of fillets from this species collected from Ventura southward to Dana Point by the 2002-2004 Southern California Coastal Marine Fish Contaminants Survey (range, 0.071-0.550 µg Hg/g wet weight; IEc 2007).

Measured concentrations of selenium in whole fish or estimated concentrations in their fillets were uniformly low in all fishes sampled during our study. Even the maximum whole-body concentration of 0.903 µg Se/g wet weight in Pacific sanddab (about 1.2 µg Se/g wet weight in fillet) fell below 1.5 µg Se/g, the threshold value for restricted consumption of fishes (Table 4). The SCCWRP (1994) tabulated data for selenium in edible muscle tissue of white croaker (*Genyonemus lineatus*) sampled from various localities in September 1990. Average concentrations ranged from 0.250 µg Se/g to 1.216 µg Se/g, wet weight basis, which are similar to estimates from our study.

Table 4. Comparisons of selected trace element concentrations measured in whole fish and estimated concentrations in their skinless fillets (values are minima-maxima for combined samples from oil platforms and natural areas) with toxicity criteria for fish and consumers of fish.

Element	**Whole-fish concentrations (µg/g dry weight)**	**Whole-fish concentrations (µg/g wet weight)**	**Fish and wildlife toxicity threshold (µg/g wet weight, whole fish)**	**Conversion factor (from whole fishto fillet)**	**Estimated fillet concentrations (µg/g wet weight)**	**USEPA risk-based consumption limit for human health (µg/g wet weight)**
Aluminum	0.50-700.	0.11-140.				
Arsenic	2.38-36.4	0.430-10.2	2.2-11.6[a]	1.57X[b]	0.68-16.	0.088-5.6[c,d]
Barium	0.20-1000.	<0.05-190.				
Cadmium	0.07-5.0	0.01-0.96	0.23-15.6[a]			0.088-5.6[c]
Chromium	<0.04-8.0	<0.01-1.7	1.0[a]			
Cobalt	<0.04-0.60	<0.01-0.18		0.01X[b]	<0.01-<0.01	
Copper	0.80-7.0	0.15-1.3	11.1-42.0[a]	0.66X[b]	0.10-0.86	
Gallium	<0.04-0.50	<0.01-0.10				
Iron	8.0-1,000.	2.2-200.		0.24X[b]	0.53-48.	
Lead	<0.04-2.0	<0.01-0.58	0.4-8.8[a]	1.43X[b]	<0.01-0.83	
Lithium	0.20-30.	0.40-8.7				
Manganese	0.90-10.	0.11-2.1				
Mercury	0.048-1.14	0.008-0.298	0.1-0.3[a]	1.27X[b]	0.010-0.378	0.029-1.9[c,e]
Nickel	0.20-3.0	0.05-1.0		0.54X[b]	0.03-0.54	
Rubidinum	1.0-5.0	0.24-1.2				
Selenium	0.910-4.72	0.221-0.903	0.75-1.0[a]	1.32X[b]	0.29-1.19	1.5-94.[c]
Strontium	50.-400.	7.3-100.				
Tin	<0.04-20.	<0.01-5.6				0.088-5.6[c,f]
Titanium	10.-400.	1.6-87.				
Vanadium	0.04-40.	0.01-8.7		0.67X[b]	0.01-5.8	
Zinc	30.-80.	4.8-23.	40.-64.[a]	0.40X[b]	1.9-9.2	

[a] From literature compiled by Hinck et al. (2009).

[b] Computed from average concentrations reported in composite samples (whole body and skinless fillet) of three marine fish species (bairdiella, *Bairdiella icistia*; orangemouth corvina, *Cynoscion xanthulus*; and sargo, *Anisotremus davidsonii*) collected from the Salton Sea (Saiki 1990).

[c] From USEPA (2000) for noncarcinogenic health endpoint. The values refer to as many as16 fish meals per month (lower value) or no more than 0.5 meals per month (higher value), where the assumed meal size is 227 g.

[d] Based on total inorganic arsenic (not total arsenic, as measured during the present study).

[e] Based on methylmercury (not total mercury, as measured during the present study).

[f] Based on tributyltin (not total tin, as measured during the present study).

Conclusions and Recommendations

Statistical comparisons indicated that most of 21 trace elements measured in samples of whole fishes from oil platforms were either similar to or lower than those from natural areas, suggesting that fishes contaminated by high concentrations of trace elements are unlikely to have originated from the platforms. In eight instances, significant interactions between the effects of HabType and TL suggested that elemental concentrations at oil platforms and natural areas varied with TL of the fish species. Although interactions were detected for rubidium, selenium, and tin in kelp rockfish, the concentrations of these elements did not differ significantly between oil platforms and natural areas over the TL range of sampled fish. For selenium, titanium, and vanadium in Pacific sanddab, small individuals exhibited significantly lower concentrations at oil platforms than at natural areas, whereas large individuals exhibited higher concentrations at oil platforms than at natural areas. On the other hand, for copper and tin in Pacific sanddab, small individuals did not exhibit measurable differences between oil platforms and natural areas, whereas large individuals exhibited either significantly higher concentrations at oil platforms than at natural areas (copper) or significantly lower concentrations at oil platforms than at natural areas (tin). For combined samples of fishes from both platforms and natural areas, the concentrations of arsenic, cadmium, chromium, lead, mercury, and selenium equaled or exceeded literature-based toxicity thresholds of fish, wildlife, or both. However, we are not aware of studies that linked elevated concentrations of trace elements in fishes from the Southern California Bight to adverse effects in fish or fish-eating wildlife. The concentrations of arsenic, cadmium, mercury, and tin in fishes from our study area were sufficiently elevated to possibly warrant restricted consumption by human consumers. Nevertheless, before management actions are contemplated, we strongly recommend additional study be conducted that includes chemical determinations of these trace elements in skinless fish fillets to verify crude estimates from whole-fish samples used in our study. Moreover, the follow-up study should specifically include measurements of inorganic arsenic and tributyltin, the chemical species targeted by risk-based consumption limits for humans.

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Task 2: Otolith Elemental Signatures of Kelp Rockfish (*Sebastes atrovirens*) and Pacific Sanddab (*Citharichthys sordidus*) from Offshore Oil Platforms and Natural Areas in the Southern California Bight

Mary M. Nishimoto and Milton S. Love

Abstract

The goal of this study was to determine if fishes, residing in the mid-water habitat or bottom habitat of platforms, possess unique otolith microchemical signatures. The specific objectives of this study were: 1) to determine if otolith microchemistry can be used to distinguish fishes that reside at platforms from those that reside at natural habitat; and 2) determine whether otolith chemistry is related to the water chemistry of the environment where fishes reside. We sampled 98 kelp rockfish and 108 Pacific sanddab collected from six offshore oil platforms and 12 natural areas during 2005 and 2006 for microchemical analysis of 19 elements in otoliths. The otolith composition of kelp rockfish and Pacific sanddab differed between the two habitat types—the sampled platforms and natural sites—and among geographic areas. Kelp rockfish collected from platforms tended to incorporate higher concentrations of Mg and Ba and lower concentration of Sr in otoliths compared to fish from natural habitat. In comparison, Pacific sanddab collected from platforms tended to have lower concentrations of Ba and Fe in otoliths compared to fish from natural habitat. Sr Ca^{-1} in otoliths of kelp rockfish tended to decrease eastward along the coastal shelf from Point Conception through the east Channel. Mn Ca^{-1} in otoliths of sanddab from both habitat types was lower in the three westernmost geographic areas (Point Conception, west Santa Barbara, and east Santa Barbara) than in the eastern and southern areas (east channel entrance, offshore east channel, and Southern California Bight). Mn Ca^{-1} was not detectable in kelp rockfish otoliths. The success rate of correctly assigning fish to habitat type (platform or natural habitat) using a canonical discriminant function based on otolith element signatures differed between species. Ninety-four percent of kelp rockfish individuals were correctly assigned to habitat type based on the otolith signature of Mg Ca^{-1}, Ba Ca^{-1}, and Sr Ca^{-1}. A lower proportion, 64%, of Pacific sanddab correctly grouped into habitat type based on the otolith signature of Mg Ca^{-1}, Sr Ca^{-1}, Mn Ca^{-1}, Cu Ca^{-1}, and Ba Ca^{-1}—otolith element concentrations that individually varied between sanddab habitat types and/or areas. Success in discriminating otoliths of either species from the two different habitat types was not associated with seawater element concentrations. Results from this study imply that platform habitat "fingerprints," unique microchemical markers, may be identifiable in kelp rockfish (although not for Pacific sanddab) along the time-keeping, growth trajectory of the otolith to reconstruct the history of platform habitat use during the lifetime of individuals. Such reconstructions will require more study on the spatial variability of platform elemental signatures from all existing platforms and the temporal stability of platform markers.

Introduction

This study investigates the utility of using microchemical "markers" possessed by fishes to identify individuals that have resided at platforms. Fishes possess otoliths (ear stones), calcareous structures that form part of the hearing/balance system. These are faithful recorders of age and growth histories of individuals. Otoliths grow incrementally; daily and annual growth zones are discernable on a cross-sectioned otolith. Calcium carbonate material closest to the core of the otolith represents the earliest life history (e.g., larval period), and material at the surface of the otolith represents the most recent growth of the fish. Recent breakthroughs in microchemical techniques show that an otolith holds a permanent, historical record of trace element concentrations that reflect changes in environmental conditions over the lifetime of an individual fish. Discrete samples of otolith material can be assayed across the growth increments of a cross-sectioned otolith to obtain the chemical composition of the otolith at known ages of the individual. Laboratory studies show that a detectable concentration of trace elements incorporated into a fish otolith in less than 24 hours reflects the immediate surrounding water chemistry. This study compares the otolith microchemistry of fish residing at platforms and natural areas to determine whether the microchemical marker might be a useful tool for establishing the source and patterns of connections between platforms and local or regional populations.

Platform operations produce trace amounts of heavy metal residues such as vanadium, chromium, nickel, zinc, lead, and barium that enter the water environment or settle on the bottom substrate during release and can become incorporated in sediments in the vicinity of the platform (Science Applications International Corporation, 2003). Trace metals discharged at platforms may contaminate resident fishes. For instance, some investigations done during the 1960's and 1970's indicate rather high levels of zinc in tissue from fish collected at platforms. Fish are likely exposed to contaminants from platform activities (e.g., produced water discharge, solid drill cuttings), either through food habits and/or physical contact with water or sediments. Exposure to elevated environmental concentrations of elements may be reflected in the composition of the otolith. During the time that the fish resided at the platform, it is possible they have incorporated a detectable elemental "fingerprint" of the environment that can be used to identify the site.

There are 26 offshore oil and gas platforms in California waters, many of which may be nearing the ends of their economic life spans (Fig 1). Following the decision by an operator to cease production, the process by which a decision is made as to the disposition of a platform is called decommissioning. This procedure may take into consideration the biological, sociological, and economic consequences of various decommissioning options, which range from leaving the entire structure in place through partial or total removal (Schroeder and Love 2004). At this time there are several key issues in the Pacific OCS platform decommissioning and reefing debate. Included is defining the ecological performance and role that platforms off California may play in the recovery of important groundfish populations in southern California. The Department of Commerce in January 2000 declared the West Coast rockfish fishery a disaster with extremely small populations remaining. A recent MMS-funded study (Love et al., 2003) has revealed that some of the platforms hold large numbers of both juvenile and reproductively mature rockfishes in numbers far greater than any natural reefs that have been surveyed. The observed rockfish species include bocaccio that has been proposed as a candidate for listing under the Endangered Species Act and the cowcod, which is a candidate for special concern to the State of California. The Pacific Fishery Management Council and the State of California began to severely restrict targeted fishing for these species in 2002 and 2003. In addition, the State of California banned the spot prawn trawl fishery in order to eliminate all by-catch of bocaccio. Populations of rockfishes at platforms, and the platforms as habitat for specific life history stages (e.g., nursery habitat for juveniles), may prove to be vital for recovery of the regional rockfish fisheries.

An assessment of the impacts of platform removal on local and regional fish populations must consider the ecology of species of interest that utilize the platform including the degree of connectivity between platform and natural habitat. Juvenile fish recruiting to a platform may utilize the platform for a period of time and emigrate to other habitat or remain at the platform throughout adult life. Adults may migrate between platforms and natural areas. A "platform fingerprint," an otolith microchemical marker, ultimately can be used to estimate the degree to which adult fish or juvenile fish that have used platforms as nursery habitat migrate to local and perhaps regional populations in natural areas off California. This goal of this study is to determine whether fishes, residing in the mid-water habitat or bottom habitat of platforms, possess unique otolith microchemical signatures—platform fingerprints.

The specific objectives of this study were:

1. To determine if otolith microchemistry can be used to distinguish fishes that reside at platforms from those that reside at natural habitat; and
2. To determine whether otolith chemistry is related to the water chemistry of the environment where fishes reside.

Methods

Fish and seawater sample collection

Adult and subadult specimens of kelp rockfish (*Sebastes atrovirens*) and Pacific sanddab (*Citharichthys sordidus*) were sampled from six platforms and twelve natural areas (Table 1). Both species reside at the platforms and natural sites during all seasons and were sufficient in number to be selected for this study. We collected kelp rockfish on the platform structure at midwater depths and in kelp forests of rocky reef habitat. We collected the bottom-dwelling Pacific sanddab on soft sediment within 100m of the platforms and at natural sites at least 3 km away from the platform. Given that circulation patterns in the Santa Barbara Channel region are complex and different water masses with unique element composition are expected to be distributed within the study region at any one time, we (1) selected platforms located in several different water masses; (2) selected natural habitat sites that were outside of the sphere of influence of discharges from platform operations; and (3) paired each platform and natural site within the same water mass to partition variability due to the effect of natural water mass differences within pairs that might confound our test for a platform effect on otolith elemental signatures (Figure 1, Table 1). Platforms and natural habitat sites were located in six geographic areas that we designated: Point Conception (PTC), west Santa Barbara (WSB), east Santa Barbara (ESB), east entrance of the Santa Barbara Channel (EEC), offshore east Channel (OEC), and Southern California Bight south of the Channel (SCB).

Kelp rockfish were collected by spear using SCUBA at four platforms at midwater depths at six kelp forest habitat sites (Table 1). Pacific sanddab were collected by hook and line at six platforms and six natural habitat sites. At each collection site, fish were bagged together in a plastic bag and kept on ice for up to ten hours. The fish were either processed in the laboratory on the collection day or the whole body samples were frozen until they were thawed and processed several weeks later. We measured fish total length (TL) and standard length (SL) to the nearest millimeter (mm), and weighed fish to the nearest gram (g). Sagittal otoliths were extracted from 8-10 fish specimens per site, rinsed in DI water, and stored dry in a clean plastic snap cap vial until the time of preparation for otolith microchemistry. The otoliths were measured along the axis of the sulcus to the nearest 0.05 mm and weighed to the nearest 0.001 mg.

We collected seawater samples at the study sites to examine the relationship between otolith chemistry and seawater chemistry. Seawater samples were collected at the time of sampling fish for the otolith microchemistry analysis. Three seawater samples were collected near the depth fish were sampled from separate casts of a Niskin sampler. We sampled seawater at 25 m depth at platforms where kelp rockfish were collected. Seawater was sampled 2 m above the benthic substrate where kelp rockfish were collected at natural sites and at the platform and natural habitat sites where Pacific sanddab were collected. Seawater collections were within 50 m of the platforms. The Niskin sampler was rinsed with fresh water between collection days. Before collecting each seawater sample, the acid-leached 50 ml sample vial and cap were rinsed three times with seawater from the Niskin bottle. The seawater sample vials were immediately placed in airtight bags and kept on ice in the dark until laboratory processing no more than 12 hours after collection.

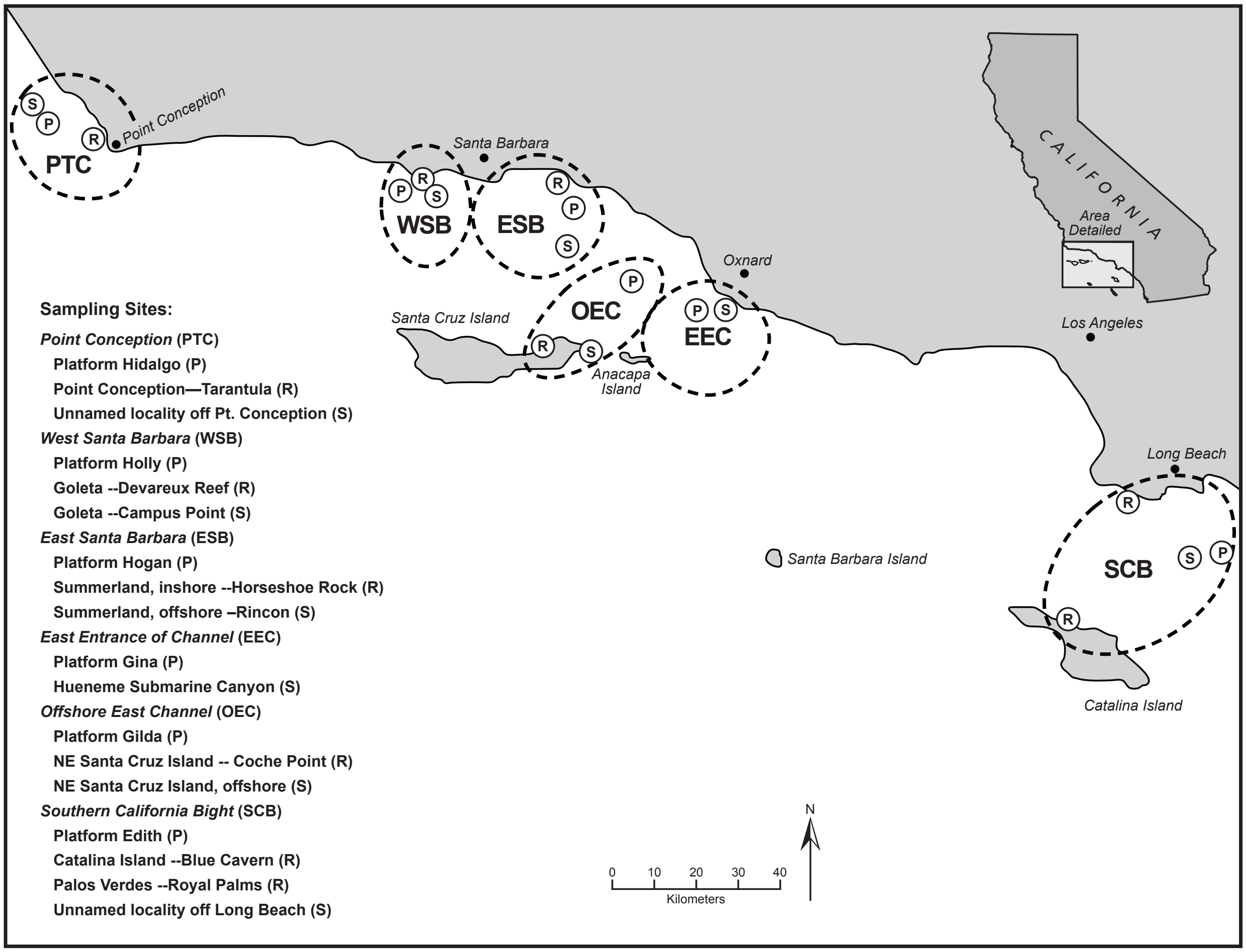

Figure 1. Map of the study area showing locations of six oil platforms and 12 natural areas. Each platform is grouped with natural areas where kelp rockfish and Pacific sanddab were collected in designated geographic areas for comparisons of otolith elemental signatures.

Table 1. Characteristics of the 18 sampling sites where one or both species of kelp rockfish and Pacific sanddab were collected with seawater samples.

Area	**Site**	**Habitat type**	**Species (n)**	**Geospatial coordinates**	**Water depth (m)**
Point Conception (PTC)	Platform Hidalgo	Platform	Pacific sanddab (10)	34°29.721’N, 120°42.227’W	126
	Point Conception, (Tarantula)	Natural area	Kelp rockfish (8)	34°29.563’N, 120°30.307’W	20
	Unnamed offshore locality off Point Conception	Natural area	Pacific Sanddab (8)	34°33.934’N, 120°42.625’W	73
West Santa Barbara (WSB)	Platform Holly	Platform	Kelp rockfish (9) Pacific sanddab (10)	34°23.366’N, 119°54.406’W	64
	Goleta (Devareux Reef)	Natural area	Kelp rockfish (10)	34°24.100’N, 119°51.560’W	15
	Goleta, offshore (Campus Point)	Natural area	Pacific Sanddab (8)	34°22.365’N, 119°50.168’W	67
East Santa Barbara (ESB)	Platform Hogan	Platform	Kelp rockfish (10) Pacific sanddab	34°20.275’N, 119°32.539’W	46
	Summerland, inshore (Horseshoe Rock)	Natural area	Kelp rockfish (8)	34°23.610’N, 119°33.629’W	9
	Summerland, offshore (Rincon)	Natural area	Pacific Sanddab (9)	34°15.355’N, 119°33.230’W	87
East Entrance of Channel (EEC)	Platform Gina	Platform	Pacific sanddab (9)	34°07.059’N, 119°16.625’W	28
	Hueneme Submarine Canyon	Natural area	Pacific Sanddab (9)	34°07.100’N, 119°12.730’W	23
Offshore East Channel (OEC)	Platform Gilda	Platform	Kelp rockfish (10) Pacific sanddab (10)	34°10.932’N, 119°25.150’W	62
	NE Santa Cruz Island (Coche Point)	Natural area	Kelp rockfish (10)	34°02.233’N, 119°36.600’W	17
	NE Santa Cruz Island, offshore	Natural area	Pacific Sanddab (9)	34°01.759’N, 119°30.362’W	55
Southern California Bight (SCB)	Platform Edith	Platform	Kelp rockfish (10) Pacific sanddab (9)	33°35.726’N, 118°08.501’W	49
	Catalina Island (Blue Cavern)	Natural area	Kelp rockfish (10)	33°26.693’N, 118°28.552’W	26
	Palos Verdes (Royal Palms)	Natural area	Kelp rockfish (10)	33°42.791’N, 118°28.552’W	7
	Unnamed offshore locality off San Pedro	Natural area	Pacific Sanddab (9)	33°35.006’N, 118°19.510’W	60

Otolith preparation and microchemical analysis using laser-ablation ICP-MS

Each otolith was soaked in deionized water for 1-3 hours, cleaned with a soft nylon brush in a deionized water bath, rinsed, and air dried in clean, covered plastic multiwell trays. A transverse section was cut from each otolith using a low-speed rotary diamond blade saw and mounted on a plastic slide with metal-free epoxy resin (Epo-Thin, Buehler). Plastic slides were cleaned in metal-free soap and rinsed in distilled water (resistivity>2 M cm). Each section was polished to the center of the otolith core using a lapping wheel and 9, 3, and 1 µm 3M diamond polishing films. The sections were broad, 0.5-1mm between cuts, to keep the polished surface, which was to be assayed, free of the epoxy used to bond the base of the section to the slide.

After polishing, decontamination steps described by Warner et al. (2005) were performed in a clean laboratory. Each otolith section mounted on a slide was individually soaked in equal parts of 30% H_20_2 and 0.1 mol l^{-1} NaOH for 30 minutes, rinsed with ultrapure water (N-pure, resistivity >18.1M cm), individually soaked in 5 separate 5-minute baths of ultrapure water, rinsed again in ultrapure water, and air-dried in a HEPA-filtered Class 100 laminar flow hood. The otoliths were placed separately in multi-welled tissue culturing trays and metal-free airtight bags until the microchemical analysis. All materials that came into contact with the otoliths during the decontamination process (e.g., glassware, plastic trays, plastic instruments) were cleaned in metal-free soap, rinsed five times in distilled water (resistivity>2 M cm), soaked in 1 N mol l^{-1} trace metal grade HCl overnight, and rinsed 10 times with ultrapure water.

We used laser-ablation inductively coupled plasma mass spectrometry (LA ICP-MS) to sample a discrete portion of the sectioned otolith nearest the edge. Otolith material was vaporized by a 213 nm solid state Nd:YAg laser ablation system (New Wave UP213). Helium carrier gas swept the sample of ablated particles into a spray chamber where Argon gas was mixed with the aerosol before entering the ICP-MS torch. The sample was analyzed on a Finnigan MAT Element 2-sector field ICP-MS. An imaging system was used to focus the laser on the surface of the otolith and to delineate two sample targets, 100 µm long x 40 µm wide, following the contour of the edge of the sectioned otolith (Figure 2). This method of sampling the outermost otolith material corresponding to recent growth before capture reduces the uncertainty that the trace elements were incorporated into the otolith when the individual did not occupied the locality were it was collected.

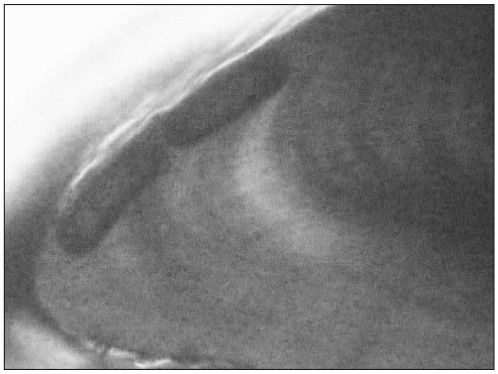
Figure 2. Image of otolith with ablated sample lines.

Otoliths were run as blocks with one specimen per site per species randomly assigned to each block. One to five sequences were run per day. A sequence was comprised of one to seven otoliths. The two targeted lines on the sectioned otolith were pre-ablated as a precautionary cleaning measure.

We initially assayed a suite of isotopes: Magnesium-24 (^{24}Mg), Calcium-48 (^{48}Ca), Strontium-87 (^{87}Sr), Silver-109 (^{109}Ag), Cadmium-114 (^{114}Cd), Cesium-133 (^{133}Cs), Barium-138 (^{138}Ba), Cerium-140 (^{140}Ce), Mercury-202 (^{202}Hg), Lead-208 (^{208}Pb), and Uranium-238 (^{238}U) were determined from the sample of the first line target by using low resolution mode (R=300); Aluminum-27 (^{27}Al), Calcium-48 (^{48}Ca), Vanadium-51 (^{51}V), Chromium-52 (^{52}Cr), Manganese-55 (^{55}Mn), Iron-56 (^{56}Fe), Cobalt-59 (^{59}Co), Copper-63 (^{63}Cu), and Zinc-66 (^{66}Zn) were determined from the sample of the second line target in medium resolution mode (R=4000). The isotope intensities of each sample were blank-corrected by subtracting isotope intensities of a 1% nitric acid (HNO_3) instrument blank preceding the sample sequence. The abundance of an element was expressed as a ratio relative to the amount of calcium to control for the amount of material analyzed per sample spot.

We eliminated undetectable isotopes from our assay panel after 21 of 40 sample sequences were run (50 kelp rockfish and 63 sanddab otoliths). We considered a negative blank-corrected intensity value to be an undetectable amount of an isotope in a sample. The undetectable elements were Ag, Cd, Ce, Hg, U, V, and Co. The isotopes of these elements were detectable in 10% or less of the otolith samples of each species assayed in 21 sequences (Appendix 1). The twelve isotopes remaining on the assay panel were detectable in 36% to 100% of the otolith samples assayed.

Average detection limits for each element per sequence, calculated as 3 x SD of the intensity of 1% HNO3 blanks and expressed as ratios of the isotope intensity and mean otolith Ca48 intensity, are given in Appendix 1. We found that as few as 19% of the measurements of a single element in otoliths of one species were above the detection limit.

We evaluated the suitability of the abundance datasets of each of 12 elements for inclusion in our statistical analysis. We deemed Mg Ca^{-1}, Sr Ca^{-1}, Fe Ca^{-1}, Cu Ca^{-1}, and Ba Ca^{-1} suitable for our statistical comparisons of kelp rockfish otolith element abundances. All of these elements were detectable in at least 80% of the kelp rockfish otolith samples. In addition to these five element Ca^{-1} ratios, we included Mn Ca^{-1} in the sanddab otolith element dataset for statistical analysis. In contrast to Mn Ca^{-1} which was detectable in 99% of the otolith samples, Mn Ca^{-1} was excluded from the kelp rockfish otolith element dataset, because Mn Ca^{-1} was detectable in only 36% of the samples, and only 12% of the samples were above the detection limit. Although otolith Fe Ca^{-1} was above the calculated detection limit in only 34% and 31% of the otolith samples of kelp rockfish and sanddab, respectively, we included the element in our statistical comparisons because of the high proportion (>80%) of detectable measurements (positive-valued, blank-corrected estimates).

Seawater preparation and microchemical analysis

Within 12 hours of collection, each seawater sample was run through a 0.22µm polyvinylidene fluoride (PVDF) membrane (Millipore Steriflip vacuum filter unit). Each disposable, single-use filter unit and acid-leached receptacle vial were rinsed three times with ultrapure water (N-pure, resistivity>18.1 M cm) before running a seawater sample through it. The filtrate was acidified with 1µm l ml^{-1} trace metal grade 12 mol L^{-1} HCl to a target pH of 2-3. The acidified filtrate samples were individually stored in airtight plastic bags. Sample filtering and acidification steps were performed under HEPA-filtered Class 100 laminar flow hoods, except for seawater samples from sanddab collection sites in the Point Conception area and from sanddab and kelp rockfish collection sites in the Southern California Bight area that were prepared under ordinary indoor conditions without flow hoods.

Seawater samples were analyzed by staff at the Marine Science Institute Analytical Laboratory at University of California, Santa Barbara. Different methods and instruments were used to determine the concentrations of a suite of elements in seawater. Seawater Ba and U analyses were performed using the method of isotope dilution with all samples run on the Element 2 ICP-MS. Enriched ^{135}Ba and ^{235}U standards of known isotopic abundance and concentration were added to each sample. ^{135}Ba/^{138}Ba and ^{235}U/^{238}U ratios were measured, mass bias corrected, and used to calculate the amount of natural Ba and U present in the samples at natural isotopic abundances. Mg, Ca, and Sr analyses were performed using the method of standard additions with all samples run on a Varian AA240FS fast sequential atomic absorption spectrometer. Spiked samples served as standards, because it is difficult to matrix match seawater. A calibration curve based on samples spiked to three levels of analyte concentration was used to estimate the element concentration in the seawater samples with the assumption that the matrix of the seawater samples and spiked seawater are the same. All seawater samples also were run on the Element 2 ICP-MS to collect selected isotopes using the method of standard additions and internal standard to correct for instrument drift. Indium-115 (^{115}In) was used as the internal standard for the following isotopes (run in low resolution): ^{109}Ag, ^{114}Cd, ^{133}Cs, ^{140}Ce, ^{202}Hg, ^{206}Pb. Scandium-45 (^{45}Sc) was used as the internal standard for the following elements, which were run in medium resolution (isotopes collected are listed): ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{56}Fe, ^{59}Co, ^{63}Cu, ^{66}Zn.

Statistical analysis of otolith and seawater microchemistry

We used univariate and multivariate statistics to test for differences in the concentration of particular elements and multi-elemental signatures in otoliths and seawater among platforms and natural habitats (SPSS, Inc. 2002). Before the statistical analysis, otolith and seawater element Ca^{-1} data were log_{10}-transformed to better fit assumptions of the models. Log (x+1) transformation was used on all otolith element Ca^{-1} data to account for zero values. Prior to the analyses, we assessed the normality and homoscedacity of the otolith data among water mass groups (SPSS, Inc. 2002).

We employed a two-way fixed analysis of variance (ANOVA) to simultaneously test for the effects of habitat and geographic area on the abundance of an element (element Ca^{-1}) in otoliths of a single species and to test for interaction of these two factors. We also used a one-way ANOVA to test the effects of site (all platforms and natural sites) on element abundance in otoliths. We employed the Tukey *post hoc* multiple comparison test (or the Tamhane test when equal error variances could not be assumed) to examine differences between all possible pairs of group (e.g., area, site) means (SPSS, Inc. 2002).

Canonical discriminant function analysis (DFA) was used to test whether the elemental signature of otoliths identified the habitat types occupied by fish (SPSS Inc. 2002). We used the results from our univariate analysis to determine the elements that were the best discriminators of either habitat types or geographic areas, and included those elements in the DFA. The procedure generated a single discriminant function (DF) for two groups (e.g., two habitat types, platforms and natural sites) or a set of DFs for more than two groups based on linear combinations of the predictor variables (log (element Ca^{-1} +1) transformed) that provide the best discrimination between the groups.

The classification accuracy of the DFA was evaluated by leave-one-out cross-validation, also called jack-knife reclassification (SPSS Inc. 2002). The prior probabilities of group membership were assumed to be uniform.

We used the Spearman's rho correlation to determine whether the abundance of different elements in otoliths co-varied and whether the abundance of an element in otoliths varied with the element concentration in seawater (SPSS, Inc. 2002). We estimated the correlation between the average otolith element abundance and average seawater concentration at sampling sites using the Pearson product-moment correlation coefficient, r. If the correlation was significant, we used least squares regression to model the relationship between seawater element concentration, the environmental predictor, and otolith element concentration, the dependent variable, by fitting a linear equation to the observed data (Systat Software Inc. 2006).

Results

Kelp rockfish otolith elemental signatures

The variability in the concentration of individual elements in kelp rockfish otoliths differed between habitat types and geographic areas. The univariate two-way ANOVA model (habitat x geographic area) showed that Mg Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1} differed between platforms and natural sites, and Sr Ca^{-1} and Ba Ca^{-1} differed among geographic areas (Table 2).

Mean otolith Mg Ca^{-1} of kelp rockfish was higher at platforms compared to natural reefs, except in the ESB area (Fig. 3 A, B). Two-way ANOVA showed a significant difference in Mg Ca^{-1} between habitat types (Fig. 3A) but not among areas (Table 2). There was a significant interaction (habitat x area) effect (Table 2) as indicated by the inconsistency of otolith Mg Ca^{-1} being higher from the natural site in ESB rather than the platform as in other areas. One-way ANOVA showed that otolith Mg Ca^{-1} differed among sites (Table 3), and otolith Mg Ca^{-1} was significantly higher from Platform Gilda (OEC) than from natural sites in the same area and the three sites in SCB (Fig. 3B; Tamhane tests, $p<0.05$). Otolith Mg Ca^{-1} tended to increase eastward along the coastal shelf from PTC to the ESB and was relatively low from natural sites in the OEC and SCB (Fig. 3).

Table 2. Two-way ANOVA comparing trace element concentrations (expressed as Element Ca^{-1}, log (x+1) transformed) in otoliths of kelp rockfish between habitats (platforms and natural) and five geographic areas. The two factors were fixed in the analysis. SS, Sum-of-squares; df, degrees freedom, F approximation, P, significance of test.

Me Ca^{-1}	**Source**	**SS**	**df**	**F**	**Sig.**
Mg Ca^{-1}	Habitat	0.0004	1	9.42	0.003
	Area	0.0002	3	1.52	0.216
	Habitat x area	0.0009	3	6.30	0.001
	Error	0.0032	71		
Fe Ca^{-1}	Habitat	0.4000	1	1.94	0.168
	Area	0.5000	3	0.81	0.494
	Habitat x area	0.4830	3	0.78	0.509
	Error	14.6540	71		
Cu Ca^{-1}	Habitat	0.1240	1	0.93	0.338
	Area	1.0430	3	2.60	0.059
	Habitat x area	0.7970	3	1.99	0.123
	Error	9.4770	71		
Sr Ca^{-1}	Habitat	0.2220	1	86.60	<0.001
	Area	0.0390	3	5.08	0.003
	Habitat x area	0.2340	3	30.40	<0.001
	Error	0.1820	71		
Ba Ca^{-1}	Habitat	0.0950	1	20.40	<0.001
	Area	0.1640	3	11.69	<0.001
	Habitat x area	0.2050	3	14.59	<0.001
	Error	0.3320	71		

Table 3. One-way ANOVA comparing trace element concentrations (expressed as Element Ca^{-1}, log (x+1) transformed) in otoliths of kelp rockfish between collection sites.

Me Ca^{-1}	**Source**	**SS**	**df**	**F**	**Sig.**
Mg Ca^{-1}	Site	0.002	9	4.23	<0.001
	Error	0.004	87		
Fe Ca^{-1}	Site	1.498	9	0.79	0.625
	Error	18.285	87		
Cu Ca^{-1}	Site	2.838	9	2.30	0.023
	Error	11.908	87		
Sr Ca^{-1}	Site	0.557	9	15.40	<0.001
	Error	0.350	87		
Ba Ca^{-1}	Site	0.577	9	13.45	<0.001
	Error	0.414	87		

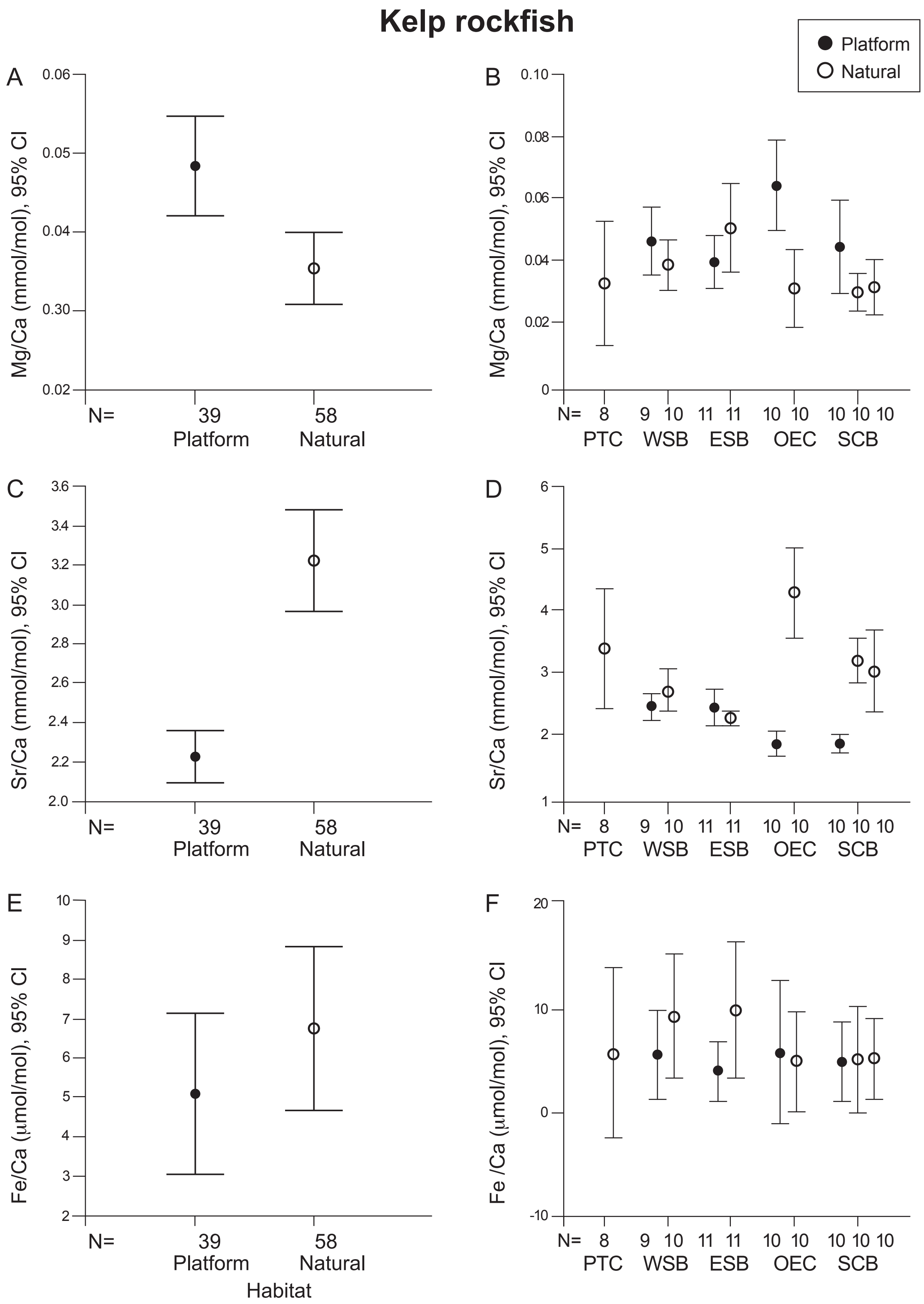

Figure 3. (A, C, E, G, I) Concentrations of five elements, Mg, Sr, Fe, Cu, and Ba (element Ca^{-1}, 95% confidence interval), in otoliths of kelp rockfish from platforms and natural areas (n= number of specimens); (B, D, F, G, H) and from each platform (closed circle) and natural site (open circle) in six geographic areas: Point Conception (PTC), west Santa Barbara (WSB), east Santa Barbara (ESB), east entrance of the Santa Barbara Channel (EEC), offshore east Channel (OEC), and Southern California Bight south of the Channel (SCB).

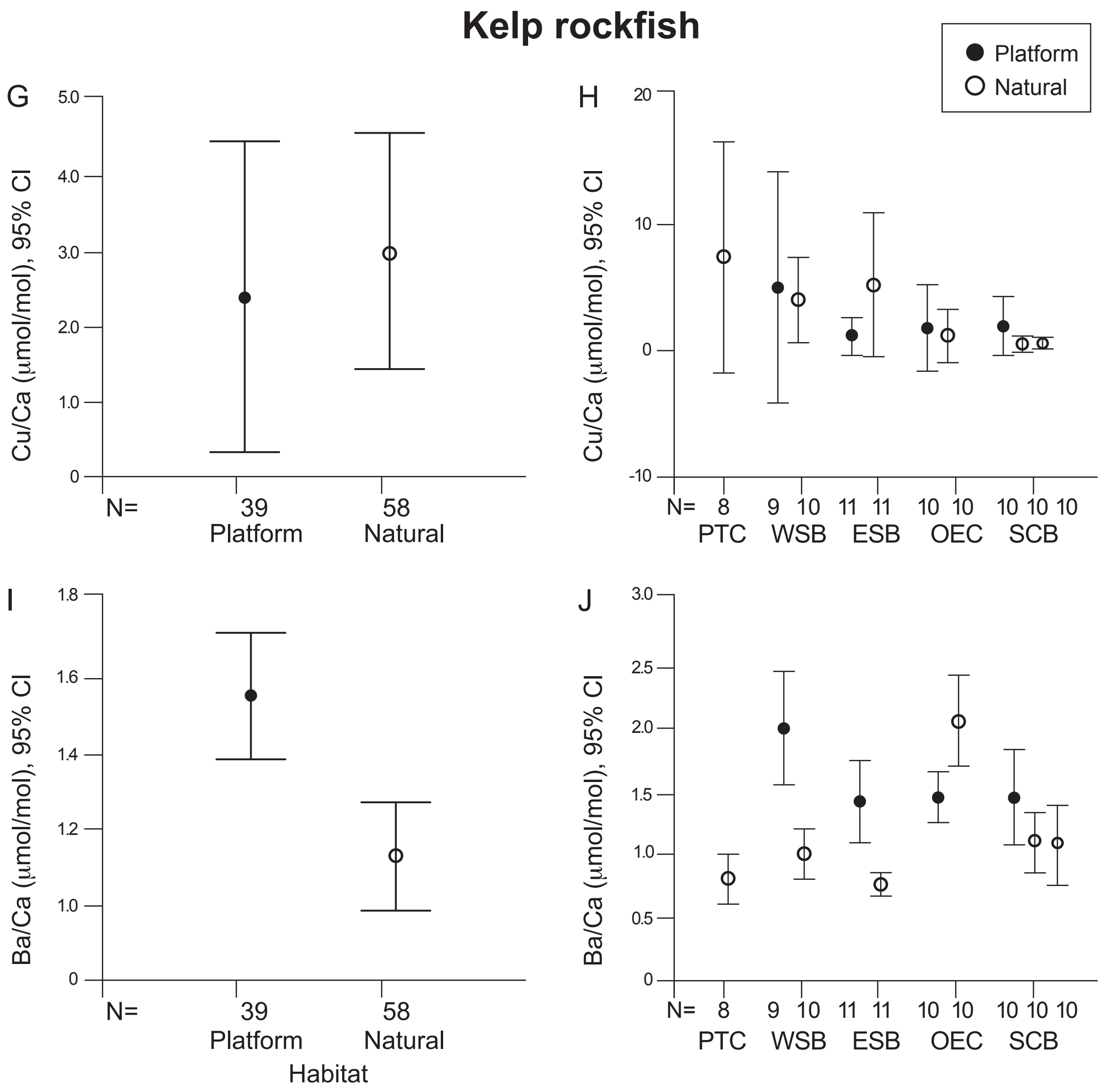

Figure 3. (Continued)

Kelp rockfish otolith Sr Ca^{-1} significantly differed between habitats and among areas; however, there was a significant interaction between the two factors (Table 2) and the Tamhane test did not detect differences among areas. In contrast to otolith Mg Ca^{-1}, Sr Ca^{-1} was lower at platforms than natural areas (Fig. 3C). One-way ANOVA found differences among sites (Table 3). Otolith Sr Ca^{-1} was lowest from two platforms, Gilda and Edith in the areas OEC and SCB, respectively, compared to all other sites (Tamhane test, $p<0.05$) with exception of the natural site in PTC area which did not significantly differ from the two platforms. In contrast to otolith Mg Ca^{-1}, Sr Ca^{-1} tended to decrease eastward along the coastal shelf from PTC through the ESB, and otolith Sr Ca^{-1} was higher from natural sites than from platforms with exception of the ESB.

Kelp rockfish otolith Fe Ca^{-1} did not differ between habitat types (Fig. 3E) nor among areas (Table 2). Mean otolith Fe Ca^{-1} was highest from the two natural reef sites on the mainland shelf of the channel (WSB and ESB areas) compared to all other sites; however, a one-way ANOVA did not detect any site differences (Table 3; Fig. 3F).

Kelp rockfish otolith Cu Ca^{-1} did not differ between habitat types (Fig. 3G) and the difference among areas was marginally significant (Table 2). No between-area differences were detected using tamhane tests. The one-way ANOVA showed a significant difference among sites (Table 3); however, the Tamhane test was not sensitive enough to detect between-site differences. Otolith Cu Ca^{-1} from natural sites along the coastal shelf from PTC through the ESB tended to be higher than otolith Cu Ca^{-1} from all platforms and the other natural sites (Fig. 3H).

Otolith Ba Ca^{-1}, like Mg Ca^{-1}, was higher at platforms than at natural habitat (Fig 3I). Kelp rockfish otolith Ba Ca^{-1} significantly differed between habitat types and among areas, and the interaction (habitat x area) effect was significant (Table 2). Kelp rockfish otolith Ba Ca^{-1} was higher from OEC compared to the ESB and SCB (Tukey HSD test, $p<0.001$; Fig. 3J). One-way ANOVA showed significant difference among sites. Otolith Ba Ca^{-1} was higher at platforms than at natural reefs with exception of the OEC area where otolith Ba Ca^{-1} from the natural site and platform did not differ (Table 3).

With one exception, the site-averaged element concentrations in kelp rockfish otoliths were not correlated with one another. Mean kelp rockfish otolith Mg Ca^{-1} per site was negatively related to mean otolith Sr Ca^{-1} per site ($n=10$, $\log(1+\text{otolith Mg Ca}^{-1})= -0.0471*\log(1+\text{otolith Sr Ca}^{-1})+0.044$, $r^2=0.641$, $F=17.043$, $p=0.003$).

Given our findings of significant platform and/or area effects on Mg Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1} in otoliths, we used the three elements in the CDA to test whether otolith elemental signatures can be used to distinguish fish from different habitats. The model discriminated between habitat groups (Wilk's Lambda=0.382, $X^2=90.023$, df=3, $p<0.001$); and the correlation between the DF and groups was moderate (canonical correlation=0.789). Canonical loadings indicate Sr Ca^{-1} was the best predictor (i.e., most closely related to the DF) and Mg Ca^{-1} was the least important predictor for grouping fish by habitat (Table 4A). Figure 4 shows the natural distribution of habitat and area groups in relation to the abundance of the three element abundances in otoliths. Cross-validation of the discriminant function showed a very high proportion, 94%, of kelp rockfish individuals were correctly assigned to habitat type (Table 4B).

Table 4A. Correlations of predictor variables (Sr Ca^{-1}, Ba Ca^{-1}, and Mg Ca^{-1} in otoliths; log (x+1) transformed), with the discriminant function (canonical loadings) used to classify kelp rockfish to habitat of capture (platform or natural).

Element Ca^{-1}	**Function 1 Loadings**
Sr Ca^{-1}	0.524
Ba Ca^{-1}	-0.349
Mg Ca^{-1}	-0.282

Table 4B. Cross-validated classification success (count and %) in predicting group membership (platform or natural area) of individual kelp rockfish.

Classification Results					
			Predicted Group Membership		
		Habitat	**Platform**	**Natural**	**Total**
Cross-validated	Count	Platform	36	3	39
		Natural	3	55	58
	%	Platform	92.3	7.7	100.0
		Natural	5.2	94.8	100.0

93.8% of cross-validated grouped cases correctly classified.

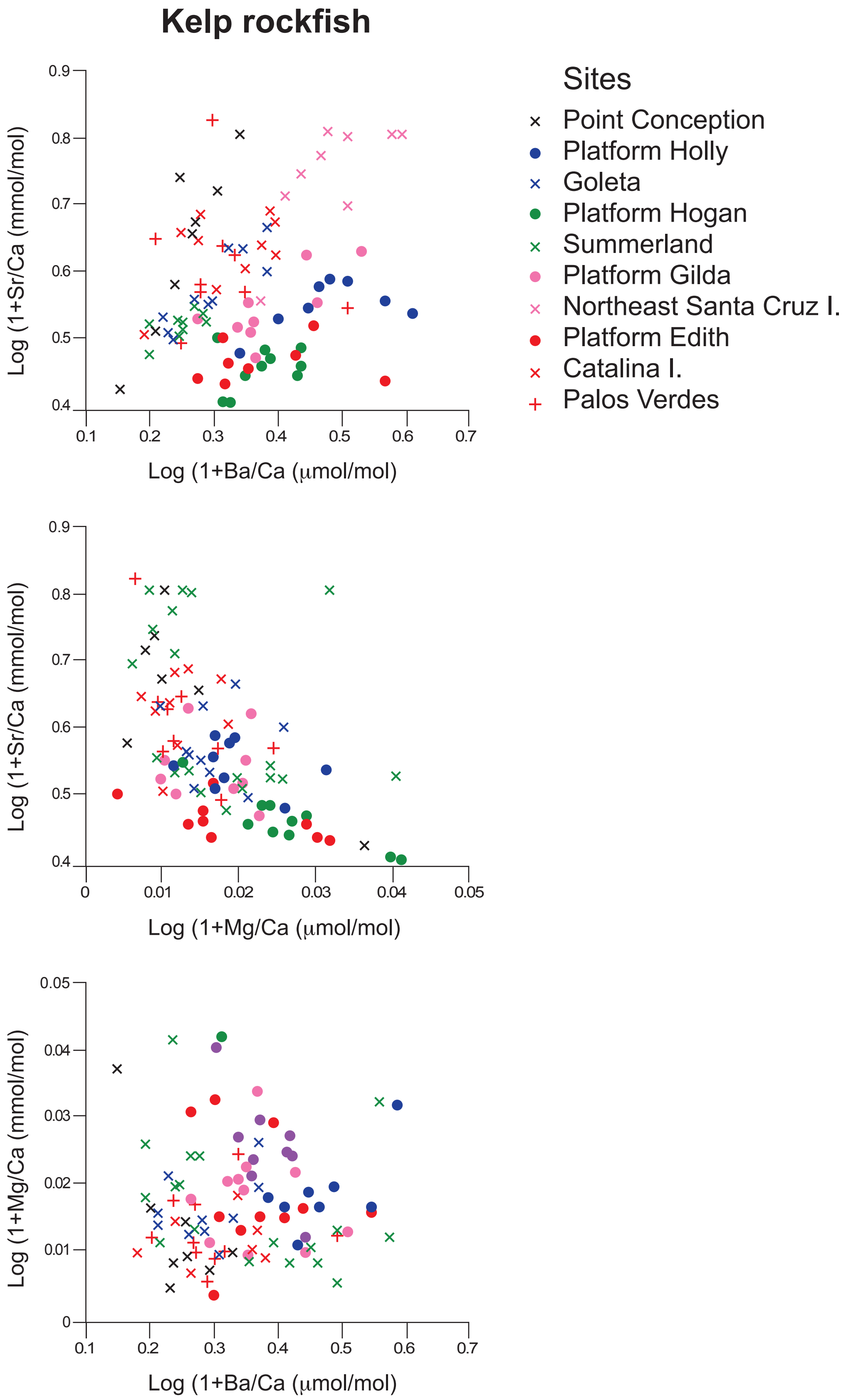

Figure 4. Relationships between log (x+1) transformed concentrations of Mg, Sr, and Ba in otoliths of kelp rockfish. Closed circles are samples from platform sites and x are natural sites. Color designates area: black, Point Conception; blue, west Santa Barbara; green, east Santa Barbara; pink, offshore east Channel (OEC); and red, Southern California Bight.

CDA generated a model discriminating site groupings of otoliths based on the concentrations of the three elements (Wilk's Lambda=0.118, X^2=191.08, df=27, p<0.001). DF1 and DF2 accounted for 66.4% and 29.9% of the discrimination power of the model (Fig. 5). DF1 was closely associated with the site groupings (canonical correlation=0.845). The canonical correlation between DF2 and the site groupings was 0.727. Sr Ca^{-1} and Ba Ca^{-1} were identified by canonical loadings in DF1 and DF2 to be more important than Mg Ca^{-1} for distinguishing fish from the sites (Table 5A). Cross-validation of all samples classified by three discriminant functions showed 49% of kelp rockfish individuals were correctly assigned to the site where they were collected; specifically, 40-80% of the individuals were correctly assigned to the platform where they were collected (Table 5B).

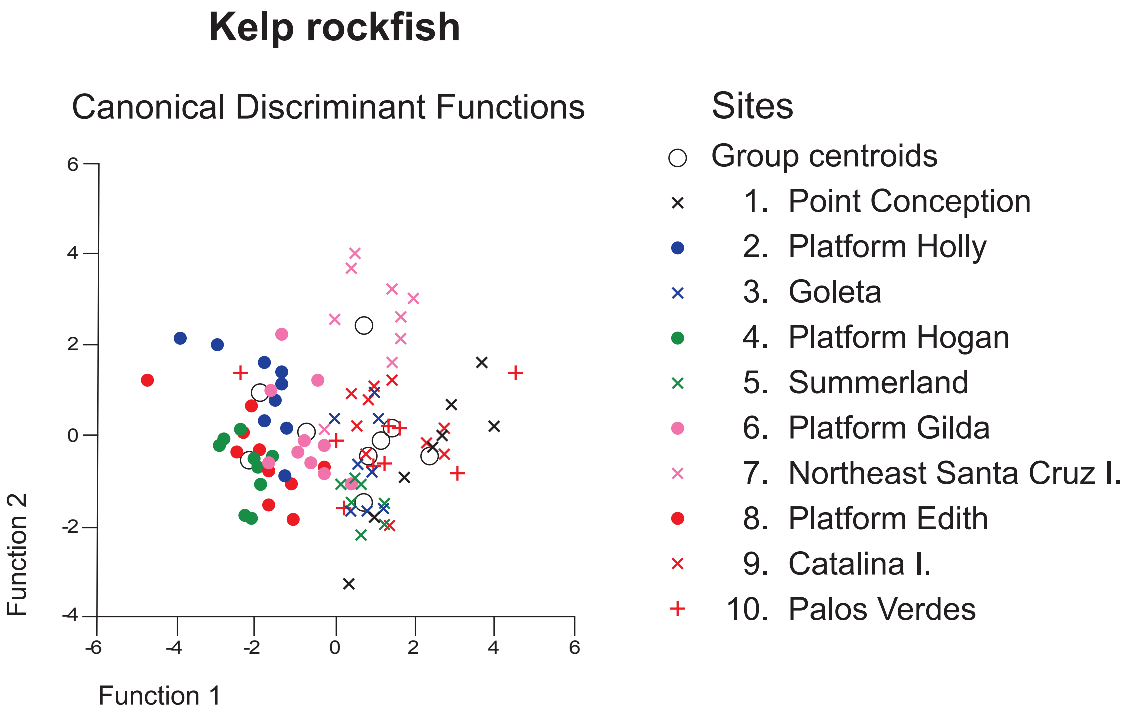

Figure 5. Distribution of kelp rockfish samples in the multivariate space of the first two canonical discriminant functions (DFs) generated from 10 groupings of otolith signatures comprised of Mg Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1}. Closed circles are samples from platform sites and x are natural sites. Color designates area: black, Point Conception; blue, west Santa Barbara; green, east Santa Barbara; pink, offshore east Channel (OEC); and red, Southern California Bight.

Table 5A. Correlations of predictor variables (Sr Ca^{-1}, Ba Ca^{-1}, and Mg Ca^{-1} in otoliths; log (x+1) transformed), with discriminant functions, DF (canonical loadings) used to classify kelp rockfish to ten collection sites.

	Canonical loadings		
Element Ca^{-1}	**DF1**	**DF2**	**DF3**
Ba Ca^{-1}	-0.445	0.895	0.040
Sr Ca^{-1}	0.592	0.800	0.099
Mg Ca^{-1}	-0.309	-0.272	0.912

Table 5B. Cross-validated classification success (count and %) in predicting group membership (collection site) of individual kelp rockfish.

Classification Results													
			Predicted Group Membership										
		Site	**3**	**4**	**6**	**7**	**9**	**12**	**14**	**15**	**17**	**18**	**Total**
Cross-validated	**Count**	1. Pt Conception	4	0	0	0	2	0	0	0	1	1	8
		2. Plat Holly	0	5	0	2	0	1	0	1	0	0	9
		3. Goleta	0	0	3	1	3	0	0	0	3	0	10
		4. Plat Hogan	0	3	0	5	1	0	0	1	0	0	10
		5. Summerland	0	0	2	0	8	0	0	0	0	0	10
		6. Plat Gilda	0	1	0	0	0	8	0	1	0	0	10
		7. NE Santa Cruz I	0	1	0	1	0	0	8	0	0	0	10
		8. Plat Edith	0	2	0	1	0	3	0	4	0	0	10
		9. Catalina I	3	0	1	0	1	0	1	0	1	3	10
		10. Palos Verdes	2	1	3	0	1	0	0	0	2	1	10
	%	1. Pt Conception	50.0	.0	.0	.0	25.0	.0	.0	.0	12.5	12.5	100.0
		2. Plat Holly	.0	55.6	.0	22.2	.0	11.1	.0	11.1	.0	.0	100.0
		3. Goleta	.0	.0	30.0	10.0	30.0	.0	.0	.0	30.0	.0	100.0
		4. Plat Hogan	.0	30.0	.0	50.0	10.0	.0	.0	10.0	.0	.0	100.0
		5. Summerland	.0	.0	20.0	.0	80.0	.0	.0	.0	.0	.0	100.0
		6. Plat Gilda	.0	10.0	.0	.0	.0	80.0	.0	10.0	.0	.0	100.0
		7. NE Santa Cruz I	.0	10.0	.0	10.0	.0	.0	80.0	.0	.0	.0	100.0
		8. Plat Edith	.0	20.0	.0	10.0	.0	30.0	.0	40.0	.0	.0	100.0
		9. Catalina I	30.0	.0	10.0	.0	10.0	.0	10.0	.0	10.0	30.0	100.0
		10. Palos Verdes	20.0	10.0	30.0	.0	10.0	.0	.0	.0	20.0	10.0	100.0

48.5% of cross-validated grouped cases correctly classified.

Pacific sanddab otolith elemental signatures

The variability in the concentration of individual elements in sanddab otoliths differed between habitat types and geographic areas. Univariate two-way ANOVA model (habitat x geographic area) showed that Ba Ca^{-1} and Fe Ca^{-1} differed between platforms and natural sites; and Ba Ca^{-1} Mg Ca^{-1}, Sr Ca^{-1}, Mn Ca^{-1}, Cu Ca^{-1} differed among geographic areas (Table 6).

Table 6. Two-way ANOVA comparing trace element concentrations (expressed as Element Ca^{-1}, log (x+1) transformed) in otoliths of Pacific sanddab between habitats (platforms and natural) and six geographic areas. The two factors were fixed in the analysis. SS, Sum-of-squares; df, degrees freedom, F approximation, P, significance of test.

Me Ca^{-1}	**Source**	**SS**	**df**	**F**	**Sig.**
Mg Ca^{-1}	Habitat	0.0000	1	2.15	0.146
	Area	0.0003	5	3.23	0.010
	Habitat x area	0.0001	5	1.51	0.195
	Residual	0.0018	96		
Mn Ca^{-1}	Habitat	0.0090	1	0.44	0.509
	Area	1.4980	5	13.90	<0.001
	Habitat x area	0.1760	5	1.63	0.158
	Residual	2.0690	96		
Fe Ca^{-1}	Habitat	2.1570	1	13.63	<0.001
	Area	0.7590	5	0.96	0.447
	Habitat x area	0.5160	5	0.65	0.660
	Residual	15.1950	96		
Cu Ca^{-1}	Habitat	0.0531	1	0.95	0.332
	Area	0.8301	5	2.97	0.015
	Habitat x area	0.4894	5	1.75	0.130
	Residual	5.3669	96		
Sr Ca^{-1}	Habitat	0.0030	1	0.81	0.371
	Area	0.0720	5	3.63	0.005
	Habitat x area	0.0370	5	1.87	0.107
	Residual	0.3820	96		
Ba Ca^{-1}	Habitat	1.6640	1	34.61	<0.001
	Area	3.8800	5	16.14	<0.001
	Habitat x area	1.7080	5	7.11	<0.001
	Residual	4.6170	96		

Sanddab otolith Mg Ca^{-1} did not significantly differ between habitat types (Fig. 6A); however, there was a significant difference among the six areas (Table 6). The difference in otolith Mg Ca^{-1} between the PTC and ESB areas was significant (Tukey test, $p<0.001$). The one-way ANOVA showed that otolith Mg Ca^{-1} differed among sites (Table 7), and although most sites did not significantly differ from one another, otolith Mg Ca^{-1} was significantly lower from the platform and natural site off Point Conception than from the natural site off east Santa Barbara (Fig. 6b; Tukey HSD, $p<0.003$).

Sanddab otolith Sr Ca^{-1} did not significantly differ between habitat types (Fig. 6C); however, there was a significant difference among the six areas (Table 6). Mean sanddab Sr Ca^{-1} was higher from the PTC and WSB areas compared to the ESB area (Fig. 6D; Tukey test, $p<0.04$). The one-way ANOVA showed that otolith Sr Ca^{-1} differed among sites (Table 7); however, the Tamhane test did not detect any pairwise site differences.

Sanddab otolith Mn Ca^{-1} significantly differed among areas, but did not differ between habitat types (Fig. 6E; Table 6). Sanddab Mn Ca^{-1} was significantly lower in the region of the three westernmost areas (PTC, WSB, ESB) compared to the region of the two easternmost channel areas (EEC and OEC) and SCB (Tukey HSD, $p<0.020$). The one-way ANOVA found significant differences in otolith Mn Ca^{-1} among sites (Table 7), and all pairwise site differences crossed between the two regions (Fig. 6F, Tukey HSD, $p<0.05$). There were no significant differences between sites, including between platform and natural habitats, within each region. Otolith Mn Ca^{-1} from Platform Gilda in the OEC area did not differ from any platform or natural site.

Sanddab otolith Fe Ca^{-1} significantly differed between habitat types (Fig. 6G) but did not differ among areas (Table 6). Mean sanddab Fe Ca^{-1} was consistently higher from natural areas compared to platforms with exception of the WSB area (Fig. 6H). The one-way ANOVA found a significant difference in otolith Fe Ca^{-1} among sites (Table 7); however, the Tamhane test did not detect any pairwise differences.

There was a significant difference in Sanddab otolith Cu Ca^{-1} among areas but not between habitat types (Fig. 6I; Table 6). Sanddab Cu Ca^{-1} was relatively low from both platform and natural areas in the PTC and SCB areas (Figure 6J). The one-way ANOVA found a significant difference in otolith Cu Ca^{-1} among sites (Table 7); however, the Tamhane test did not detect any pairwise differences.

Sanddab otolith Ba Ca^{-1} significantly differed between habitat types and among areas with a significant interaction effect (Table 6). In contrast to kelp rockfish Ba Ca^{-1} that was higher from platforms than from natural sites, sanddab Ba Ca^{-1} was higher from natural areas than from platforms (Fig. 6K). Sanddab Ba Ca^{-1} was highest from the SCB compared to the other five areas (Tamhane, $p<0.038$; Fib. 6L). The one-way ANOVA found a significant difference among sites (Table 7).

We found relationships between different element concentrations in sanddab otoliths. Sanddab Otolith Sr Ca^{-1} was negatively correlated with otolith Mn Ca^{-1} (Spearman's rho, $p<0.05$). Sanddab otolith Cu Ca^{-1} was correlated with Mg Ca^{-1} and Fe Ca^{-1} (Spearman's rho, $p<0.05$); however, otolith Mg Ca^{-1} was not correlated with Fe Ca^{-1}. Sanddab otolith Ba Ca^{-1} was not correlated with any otolith element Ca^{-1} (Spearman's rho, $p<0.05$).

Mean otolith Fe Ca^{-1} increased with mean otolith Mg Ca^{-1} among sites. Mean otolith Mn Ca^{-1} was negatively related to mean otolith Sr Ca^{-1} among sanddab collection sites. Variability in otolith element concentrations was high within sites.

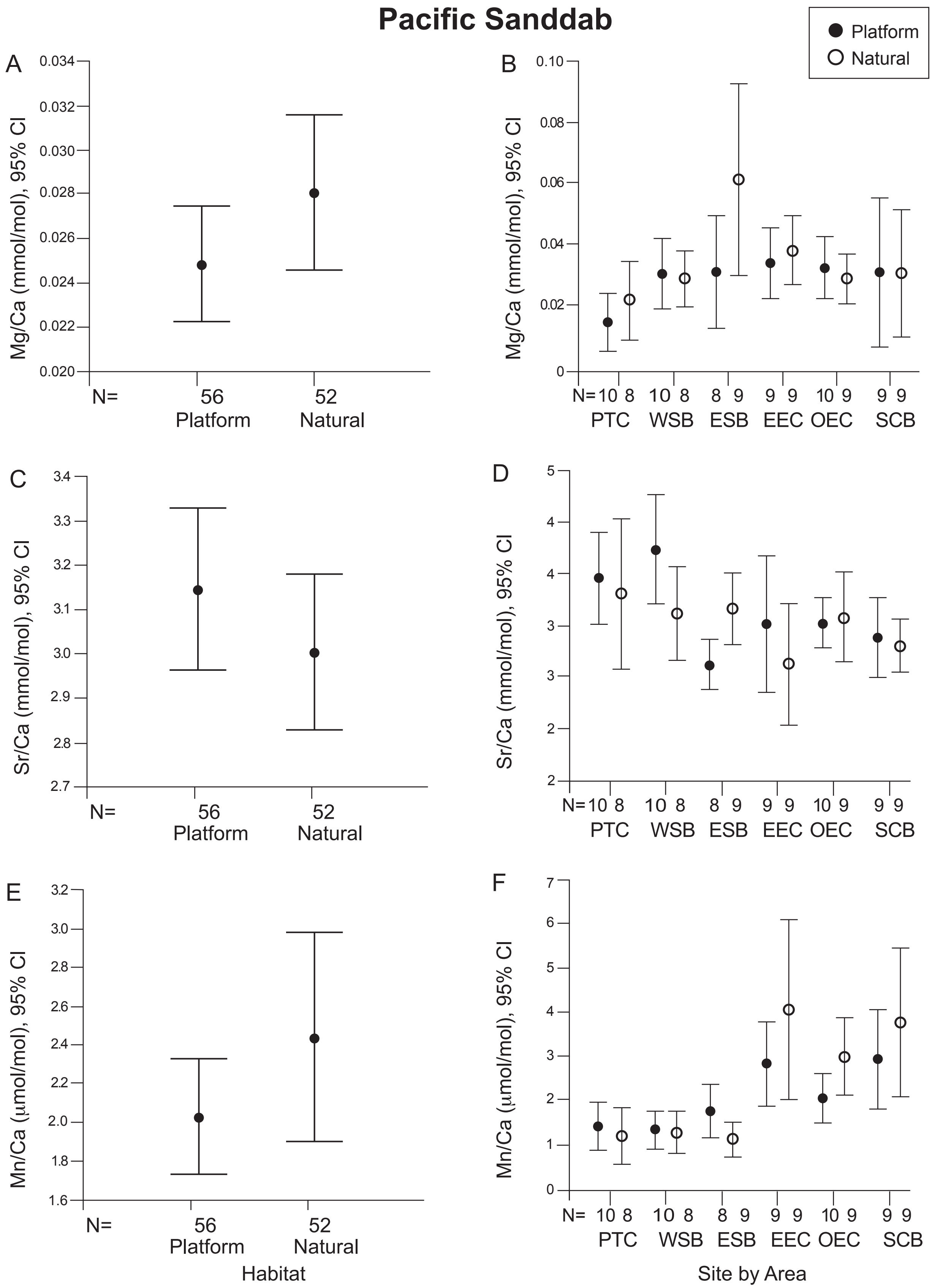

Figure 6. (A, C, E, G, I, K) Concentrations of six elements, Mg, Sr, Mn, Fe, Cu, Ba (element Ca^{-1}, 95% confidence interval), in otoliths of Pacific sanddab from platforms and natural areas (n= number of specimens); (B, D, F, G, H, J) and from each platform (closed circle) and natural site (open circle) in six geographic areas: Point Conception (PTC), west Santa Barbara (WSB), east Santa Barbara (ESB), east entrance of the Santa Barbara Channel (EEC), offshore east Channel (OEC), and Southern California Bight south of the Channel (SCB).

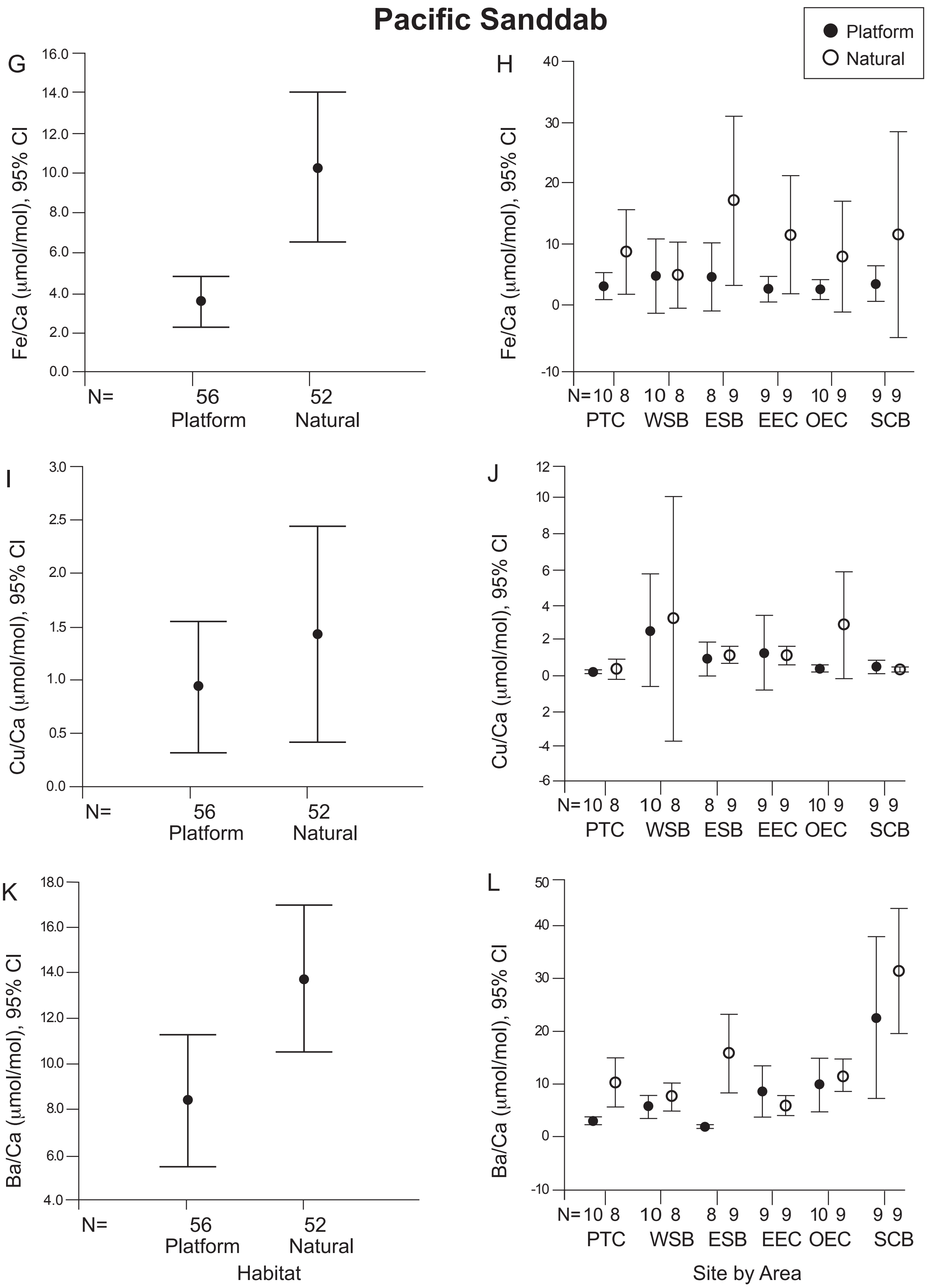

Figure 6. (Continued)

Table 7. One-way ANOVA comparing trace element concentrations (expressed as Element Ca^{-1}, log (x+1) transformed) in otoliths of Pacific sanddab between collection sites.

Me Ca^{-1}	**Source**	**SS**	**df**	**F**	**Sig.**
Mg Ca^{-1}	Site	0.001	11	2.47	0.009
	Residual	0.002	96		
Mn Ca^{-1}	Site	1.685	11	7.11	<0.001
	Residual	2.069	96		
Fe Ca^{-1}	Site	3.541	11	2.03	0.033
	Residual	15.195	96		
Cu Ca^{-1}	Site	1.412	11	2.30	0.015
	Residual	5.367	96		
Sr Ca^{-1}	Site	0.118	11	2.70	0.005
	Residual	0.382	96		
Ba Ca^{-1}	Site	7.226	11	13.66	<0.001
	Residual	4.617	96		

Given our findings of significant platform and/or area effects on the concentrations of Ba Ca^{-1} Mg Ca^{-1}, Sr Ca^{-1}, Mn Ca^{-1}, Fe Ca^{-1}, Cu Ca^{-1} in sanddab otoliths, we used CDA to test whether otolith elemental signatures, defined by the six element Ca^{-1} ratios, can be used to distinguish fish from different habitats (platforms and natural sites) and sites. The model discriminated between habitat groups (Wilk's Lambda=0.752, X^2=29.332, df=6, p<0.001); however, the relationship between the DF and groups was not strong (canonical correlation=0.489). Ba Ca^{-1} and Fe Ca^{-1} were identified by canonical loadings to be most important for distinguishing fish from platforms and natural areas (Table 8A). Cross-validation of the discriminant function showed 64% of Pacific sanddab correctly grouped into habitat type (Table 8B).

Table 8A. Correlations of six predictor variables (Mg Ca^{-1}, Mn Ca^{-1}, Fe Ca^{-1}, Cu Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1}; log (x+1) transformed) with discriminant functions (canonical loadings) used to classify Pacific sanddab to habitat of capture (platform or natural).

Element Ca^{-1}	**Function 1 loadings**
Ba Ca^{-1}	0.705
Fe Ca^{-1}	0.644
Mg Ca^{-1}	0.256
Sr Ca^{-1}	-0.183
Cu Ca^{-1}	0.167
Mn Ca^{-1}	0.128

Table 8B. Cross-validated classification success (count and %) in predicting group membership (platform or natural area) of individual Pacific sanddab.

Classification results					
			Predicted Group Membership		
		Habitat	1	2	**Total**
Cross-validated	Count	Platform	40	16	56
		Natural	23	29	52
	%	Platform	71.4	28.6	100.0
		Natural	44.2	55.8	100.0

63.9% of cross-validated grouped cases correctly classified.

The CDA of the six elements divided site groupings of samples into a multidimensional space defined by six DFs. The model discriminated between sites (Wilk's Lambda=0.090, X^2=235.987, df=66, p<0.001). DF1 and DF2 accounted for 61.1% and 17.2% of the discrimination power of the model (Fig. 7). DF1 was closely associated with the site groupings (canonical correlation=0.835). The canonical correlation between DF2 and the site groupings was 0.627. The canonical loadings suggest that Ba was the best predictor of site groupings in DF1 (Table 9A). Figure 7 shows habitat groupings of sites along DF1. The canonical loadings show the strongest correlation between Mn and DF2. Figure 7 shows some regional separation within each habitat type. Cross-validation of all samples classified by three discriminant functions showed that the CDA poorly discriminated among sites: only 32% of Pacific sanddabs individuals were correctly assigned to the site where they were collected; specifically, 30% or less of the individual from four of the six platforms were correctly assigned to their site of collection (Table 9B).

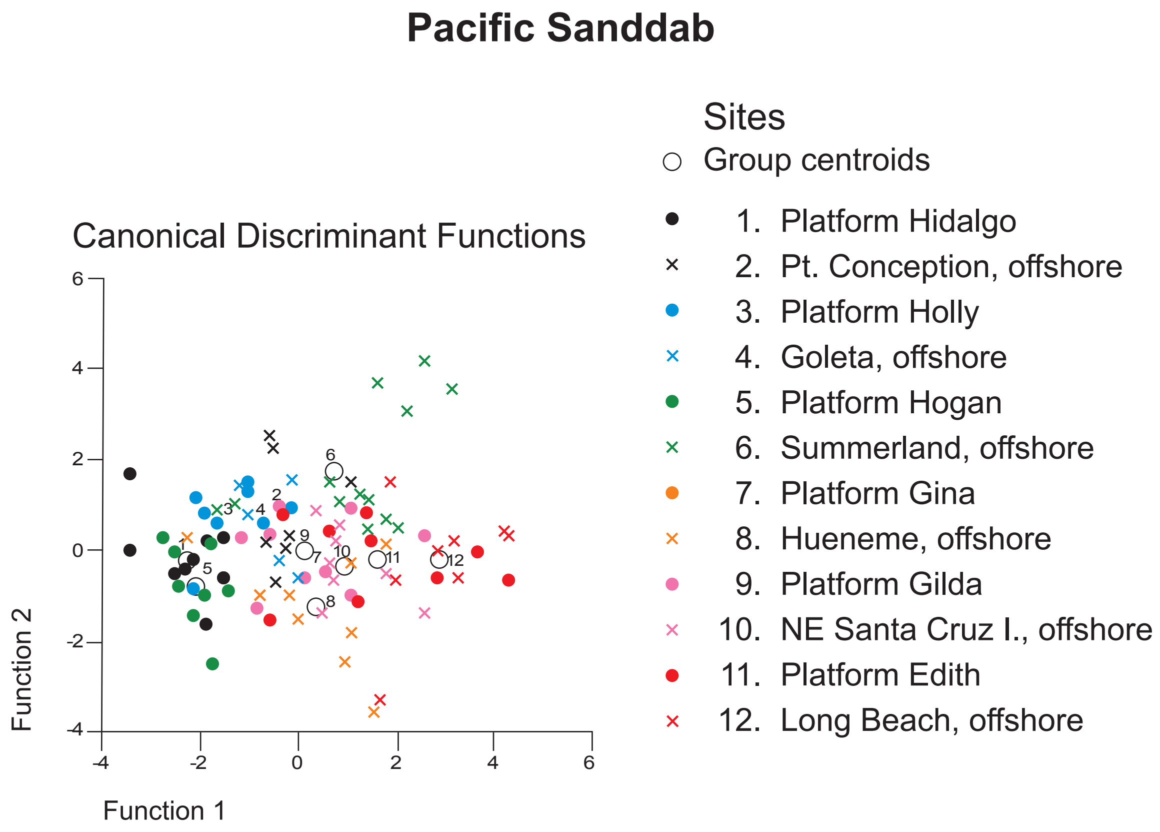

Figure 7. Distribution of Pacific sanddab samples in the multivariate space of the first two canonical discriminant functions (DFs) generated from 12 groupings of otolith signatures comprised of Mg Ca^{-1}, Mn, Ca^{-1}, Fe Ca^{-1}, Cu Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1}. Closed circles are samples from platform sites and x are natural sites. Color designates area: black, Point Conception; blue, west Santa Barbara; green, east Santa Barbara; pink, offshore east Channel (OEC); and red, Southern California Bight.

Table 9A. Correlations of six predictor variables (Mg Ca^{-1}, Mn Ca^{-1}, Fe Ca^{-1}, Cu Ca^{-1}, Sr Ca^{-1}, and Ba Ca^{-1}; log (x+1) transformed) with discriminant functions, DF, (canonical loadings) used to classify Pacific sanddab to 12 collection sites.

Element Ca^{-1}	**Canonical loadings**					
	DF1	**DF2**	**DF3**	**DF4**	**DF5**	**DF6**
Ba Ca^{-1}	0.777	0.444	-0.332	-0.022	0.286	0.087
MnCa^{-1}	0.383	-0.837	0.091	0.230	0.042	0.300
Mg Ca^{-1}	0.132	0.261	0.638	0.133	-0.559	0.421
Cu Ca^{-1}	-0.015	0.062	0.389	0.815	0.072	-0.419
Fe Ca^{-1}	0.100	0.220	0.564	-0.119	0.774	-0.102
Sr Ca^{-1}	-0.157	0.423	-0.414	0.363	0.413	0.568

Table 9B. Cross-validated classification success (count and %) in predicting group membership (collection sites) of individual Pacific sanddab.

Classification Results															
			Predicted Group Membership												
Site			**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Cross-validated	**Count**	1. Plat Hidalgo	6	0	0	1	3	0	0	0	0	0	0	0	10
		2. Pt Conception	1	1	0	2	0	1	0	1	2	0	0	0	8
		3. Plat Holly	2	2	3	1	1	0	0	0	1	0	0	0	10
		4. Goleta	0	4	2	0	0	0	0	1	1	0	0	0	8
		5. Plat Hogan	2	0	1	0	5	0	0	0	0	0	0	0	8
		6. Summerland	0	1	0	1	1	6	0	0	0	0	0	0	9
		7. Plat Gina	0	0	1	0	0	0	0	1	5	1	1	0	9
		8. Hueneme	1	0	0	0	0	1	3	3	0	1	0	0	9
		9. Plat Gilda	0	1	1	1	2	0	2	0	0	0	2	1	10
		10. NE Santa Cruz I	0	0	1	1	0	0	1	0	2	2	1	1	9
		11. Plat Edith	0	0	0	1	0	1	1	0	0	1	2	3	9
		12. Long Beach	0	0	0	0	0	0	0	2	0	0	1	6	9
	%	1. Plat Hidalgo	60.0	.0	.0	10.0	30.0	.0	.0	.0	.0	.0	.0	.0	100.0
		2. Pt Conception	12.5	12.5	.0	25.0	.0	12.5	.0	12.5	25.0	.0	.0	.0	100.0
		3. Plat Holly	20.0	20.0	30.0	10.0	10.0	.0	.0	.0	10.0	.0	.0	.0	100.0
		4. Goleta	.0	50.0	25.0	.0	.0	.0	.0	12.5	12.5	.0	.0	.0	100.0
		5. Plat Hogan	25.0	.0	12.5	.0	62.5	.0	.0	.0	.0	.0	.0	.0	100.0
		6. Summerland	.0	11.1	.0	11.1	11.1	66.7	.0	.0	.0	.0	.0	.0	100.0
		7. Plat Gina	.0	.0	11.1	.0	.0	.0	.0	11.1	55.6	11.1	11.1	.0	100.0
		8. Hueneme	11.1	.0	.0	.0	.0	11.1	33.3	33.3	.0	11.1	.0	.0	100.0
		9. Plat Gilda	.0	10.0	10.0	10.0	20.0	.0	20.0	.0	.0	.0	20.0	10.0	100.0
		10. NE Santa Cruz I	.0	.0	11.1	11.1	.0	.0	11.1	.0	22.2	22.2	11.1	11.1	100.0
		11. Plat Edith	.0	.0	.0	11.1	.0	11.1	11.1	.0	.0	11.1	22.2	33.3	100.0
		12. Long Beach	.0	.0	.0	.0	.0	.0	.0	22.2	.0	.0	11.1	66.7	100.0

31.5% of cross-validated grouped cases correctly classified.

Relationship between otolith element signatures and seawater element concentrations

We found no significant relationship between the concentration of an element in kelp rockfish or sanddab otoliths (mean otolith element Ca^{-1} per site) and its concentration in seawater (mean seawater element Ca^{-1}) among sites (Figs. 8 and 9). The highest correlation between element concentrations in kelp rockfish otolith and seawater was for Cu Ca^{-1} (r=0.535, p=0.111, n=12). Similarly, the highest correlation between element concentrations in sandab otolith and seawater was for Cu Ca^{-1} (r=0.567, p=0.069, n=11). Appendix 2 and Appendix 3 list element Ca^{-1} (mean, SD) in seawater sampled from sites where kelp rockfish and Pacific sanddabs, respectively, were collected

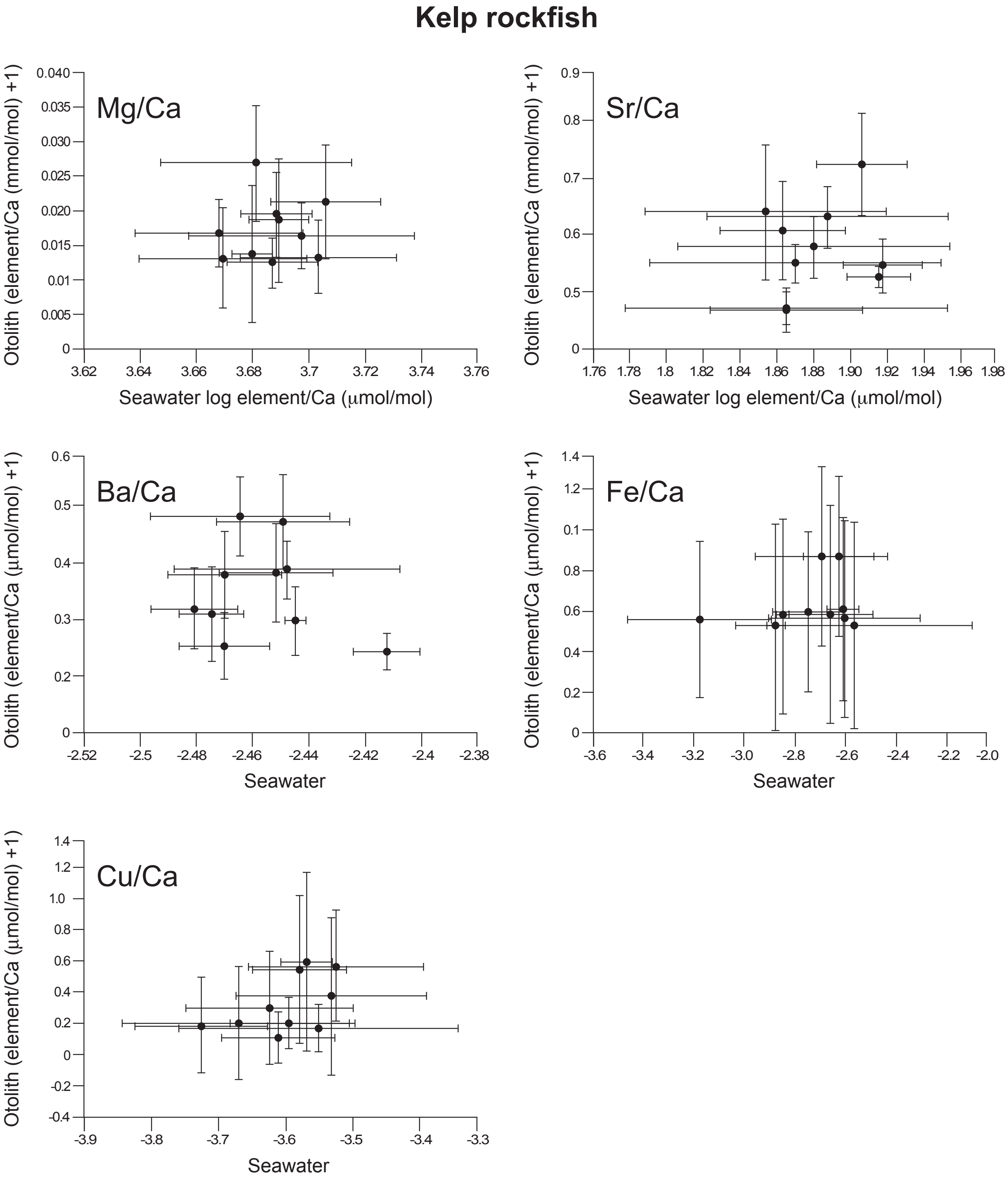

Figure 8. Mean concentration of each element, Mg, Sr, Fe, Cu, and Ba in kelp rockfish otoliths (log (x+1) transformed element Ca^{-1} ±1 SD) in relation to the element concentration in seawater from the fish collection sites.

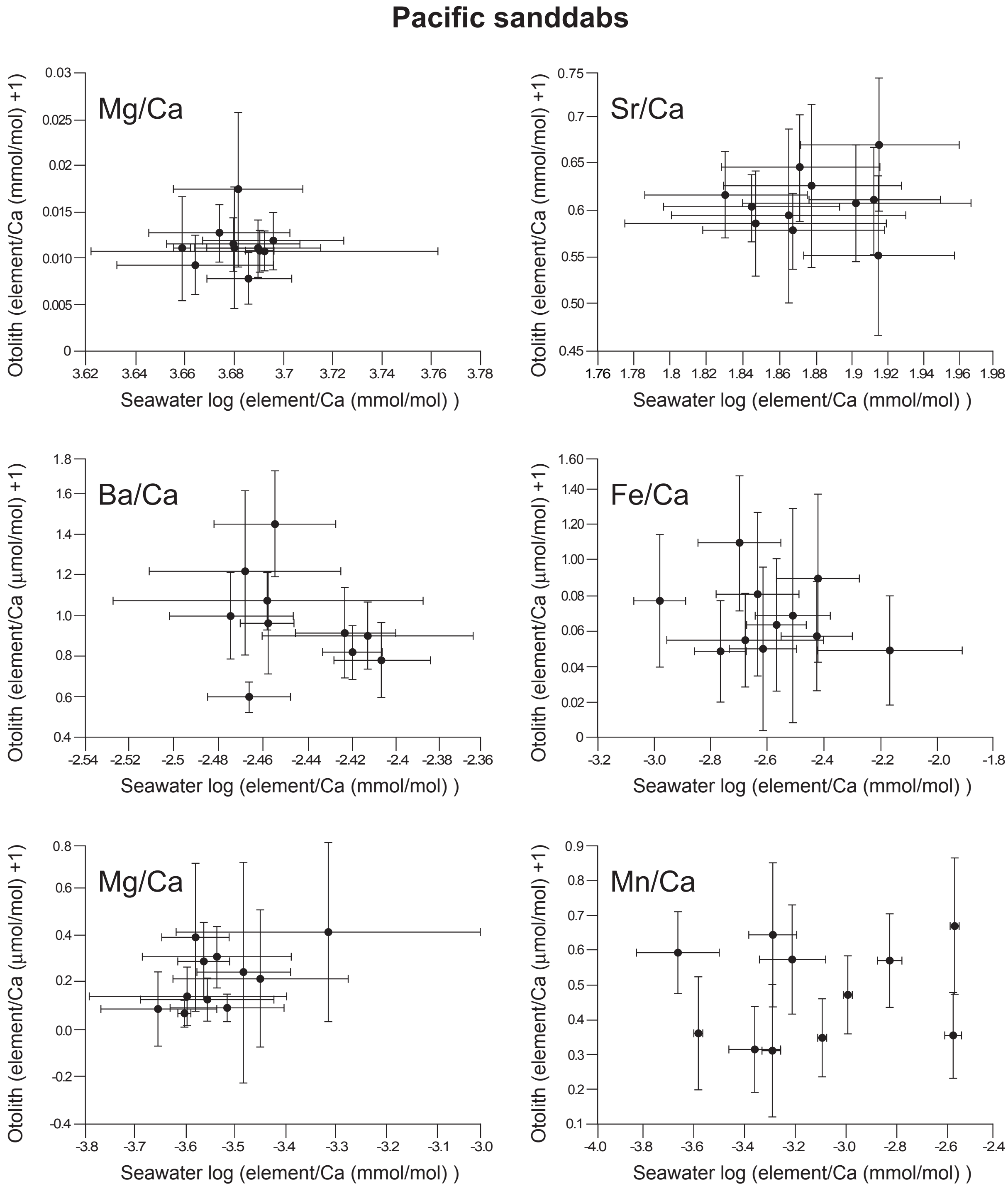

Figure 9. Mean concentration of each element, Mg, Sr, Mn, Fe, Cu, and Ba in Pacific sanddab otoliths (log (x+1) transformed element Ca^{-1} $\pm$1 SD) in relation to the element concentration in seawater from the fish collection sites.

Discussion

Results from univariate two-factor analysis of variance and multivariate canonical discriminant analysis indicate that the elemental composition of otoliths differ between the two habitat types—the sampled platforms and natural sites—and geographic areas, although there were significant interaction effects (habitat x area) on single element abundances in otoliths. Kelp rockfish collected from platforms tended to incorporate higher concentrations of Mg and Ba and lower concentration of Sr in otoliths compared to fish from natural habitat. In comparison, Pacific sanddab collected from platforms tended to have lower concentrations of Ba and Fe in otoliths compared to fish from natural habitat. Sr Ca^{-1} in otoliths of kelp rockfish tended to decrease eastward along the coastal shelf from Point Conception through the east Channel. Mn Ca^{-1} in otoliths of sanddab from both habitat types was lower in the three westernmost geographic areas (Point Conception, west Santa Barbara, and east Santa Barbara) than in the eastern and southern areas (east channel mainland shelf, offshore east channel, and Southern California Bight). Mn Ca^{-1} was not detectable in kelp rockfish otoliths. Variability in otolith element concentrations between habitat types and among areas differed between the two species.

The success rate of correctly assigning fish to habitat type (platform or natural habitat) using canonical discriminant functions based on otolith element signatures differed between species. Cross-validation of the discriminant function showed a very high proportion, 94%, of kelp rockfish individuals were correctly assigned to habitat type based on the otolith signature of Mg Ca^{-1}, Ba Ca^{-1}, and Sr Ca^{-1}. A lower proportion, 64%, of Pacific sanddab correctly grouped into habitat type based on the otolith signature of Mg Ca^{-1}, Sr Ca^{-1}, Mn Ca^{-1}, Cu Ca^{-1}, and Ba Ca^{-1}—otolith element concentrations that individually varied between sanddab habitat types and/or areas.

The usefulness of otolith chemical tags relies on detectable differences between habitats of interest, such as platforms and natural areas that are spatially and temporally consistent (Campana et al. 2000). We observed adequate spatial consistency of the kelp rockfish otolith multi-element signatures to discriminate platforms from natural sites across areas characterized by different water masses and currents (Nishimoto and Washburn 2002, Nishimoto 2009). Uncertainty in the spatial consistency of platform and natural habitat differences beyond our sampling of five platforms and five natural areas could be reduced by examining the otolith microchemistry of fish from a broader sampling of the 23 platforms in the Pacific Outer Continental Shelf region where kelp rockfish reside as juveniles, for example, and natural areas that fish might utilize later during their life history.

In comparison with our findings, a "platform fingerprint" in otoliths from the Gulf of Mexico was identified by Nowling et al. (2005). They investigated the concentration of 15 isotopes of the heavy metals, V, Co, Ni, Zn, Cu, Ag, Cd, Pb, and U, in whole otoliths of the red snapper (*Lutjanus campechanus*) from platforms and artificial reefs off Louisiana and artificial reefs off Alabama. The isotopes were identified as products of historic drilling activities and natural weathering processes of the platform structure and typically are not analyzed in otolith microchemistry. Ba was not included in the assay panel. Isotopes of V, Zn, Ag, U, and Pb were found to be good discriminators between platforms and reefs. Concentrations of ^{51}V, ^{107}Ag, ^{109}Ag, ^{206}Pb, ^{207}Pb, and ^{208}Pb were higher and ^{64}Zn and ^{66}Zn were lower in fish otoliths from platforms than from Louisiana or Alabama artificial reefs. U was higher at platforms and artificial reefs in Louisiana than artificial reefs in Alabama. The elements assayed using solution-based ICP-MS in the Nowling et al (2005) study were either below detection limits or undetectable using LA ICP-MS in this study.

Different combinations of the elements in otoliths that we examined were useful discriminators of habitat type and geographic areas in other studies of rockfishes of the California Current region. Warner et al. (2005) found that the concentrations of Sr and Ba from the outermost growth layer of adult kelp rockfish differed and Mg concentrations did not differ among mainland and island sites in the Santa Barbara

Channel region. Miller and Shanks (2004) found that Mg concentrations, and Zn and Pb concentrations to a lesser extent, in otoliths of black rockfish (*Sebastes melanops*) were good discriminators of open coast habitats along the Washington and Oregon coasts. Nishimoto (2009) examined the variability in otolith chemistry in relation to temperature and salinity and determined that Ba, Mg, and Fe concentrations in otoliths distinguished shortbelly rockfish (*Sebastes jordani*) from geographically distant regions with contrasting oceanographic conditions.

Brown (2006) found that Pacific sanddab living in estuaries and sandy coastal habitats along the central California coast had different elemental signatures defined by Sr, Li, Ba, and Mn. The signatures defining the two habitats for Pacific sanddab were similar for English sole (*Pleuronectes vetulus*), another flatfish species that resides in the same habitats. Sr was consistently higher and Li consistently lower in estuarine fish. Ba and Mn differed between habitats in only some years.

Forrester and Swearer (2002) compared Cu, Sr, Ba, and Pb concentrations in otoliths of California halibut from bay and open coast habitats in the southern California Bight including the Santa Barbara Channel. In contrast to Brown (2006), Sr and Ba showed no significant difference in concentration in juveniles from bays and open coast habitats. Mn, Cu, and Pb were higher in otoliths from bays than from coastal habitats in the single year, 1998, when fish were collected from both kinds of habitats. Forrester and Swearer (2002) also compared the otolith element concentrations from one year of sampling open coast habitats with four previous years of sampling fish from bays. Mn was ruled out as a good marker, because otolith concentrations from the open coast habitat in 1998 did not differ from otolith Mn from bays the four previous years. Although Ba concentrations in otoliths did not differ between bay and open coast habitats in 1998; Ba in otoliths from bay habitats in some years were significantly higher than coastal habitats sampled in 1998. Otolith concentrations of Pb varied less than Cu between years among the bay sites. Findings from these studies indicate that some elements may be more temporally stable than others in otolith elemental signatures.

Success in discriminating otoliths from the two different habitat types in this study was not associated with observed seawater element concentrations. Regression analysis showed variability in otolith element concentration among sites was not related to the concentration of the elements in our seawater samples from kelp rockfish and sanddab habitats.

The variability in seawater sample concentrations of most elements within sites (platform or natural habitat) was substantial (Fig. 8) indicating a source of error may have been our seawater collection method. The seawater samples were taken from aboard an unanchored boat at the platforms and natural sanddab habitat sites. Although the boat was repositioned between casts to adjust for drift particularly when near a platform, and movement may have been (tens of meters at platforms; tens of meters to several hundred meters from natural sanddab habitat collection sites). Although care was taken to minimize contamination of the seawater collected from the Niskin bottle sampler, we cannot fully rule out contamination from the bottle which was not acid-leached. For some trace elements (e.g., Ba, V, As, Se) contamination is relatively easy to control, whereas for other elements (e.g., Pb, Zn, Fe, Hg, and Sn) more painstaking procedures are required to collect uncontaminated samples (Donat and Bruland 1995).

Another source of error in estimating the relationship between element concentrations in otoliths and seawater is that seawater was sampled only during a single visit to each site; whereas, the otolith elemental concentration was measured from ablated samples integrating months of recent growth preceding capture of the fish. We suspect that the element concentrations from the ablated otolith sample is affected by temporal variability in seawater element concentrations over multiple time scales ranging from hours to months due to changes in the rate of produced water discharge from a platform and oceanographic variability.

Produced water is the primary discharge associated with oil and gas recovery and is the second largest non-drilling associated discharge from oil platforms. Cooling water discharges comprise the largest volume of non-drilling associated discharges from offshore oil platforms, but along with sanitary/domestic waste, this discharge is only a source of residual chlorine (Steinberger et al. 2005). Produced waters are natural geological formation water and includes introduced seawater recovered with the extraction of oil (Raco 1994). The produced water is separated from the oil and gas mixture on site at an offshore platform or is sent via pipeline to an onshore facility. After separation, the produced water is treated and discharged to the ocean. If processed onshore, the produced water is transferred by pipeline back to the source platform and discharged. The release of produced water is intermittent over hours or days and discharge rate and volume varies over time and among platforms (D. Panzer, U.S. Minerals Management Service, pers. comm.).

Over the life of the well as the amount of oil in the well decreases, the quantity of produced water extracted with oil and gas increases as the process of water-flooding with seawater is used to enhance hydrocarbon production from an aging well. Consequently, the concentrations of constituent elements, such as Ba, in the discharge often decreases over time. Eleven platforms were discharging produced water in 1990; 14 platforms in 1996, and14 platforms in 2000. Steinberger et al (2005) reported that discharges of produced water were 2.3% higher in 1996 than in 1990, and 7.3% higher in 2000 than in 1990. All of the platforms in federal waters included in our study (the exception is Platform Holly in State waters) presently discharge produced water.

Ba is the largest metal constituent in mass emissions from produced water discharged from platforms (Steinberger et al. 2005). Higashi et al (1997) reported that Ba and Sr were the only elements among Al, As, Cd, Co, Cu, Cr, Fe, Mn, Mo, Pb, Sn, Zn that were consistently in high concentration in produced water from an oil processing facility in Carpinteria. Only one element, Cu, among Mg, Sr, Mn, Fe, and Ba included in our otolith chemistry statistical analysis, is monitored and reported in Discharge Monitoring Reports (DMRs) submitted by individual platforms in compliance with NPDES permit requirements. Metals included in the DMRs are Cd, Cr, Cu, Pb, Hg, Ni (Nickel), Se (Selenium), Ag, and Zn. Steinberger et al. (2005) estimated 1734 kg of Ba was released from federal Southern California platforms in 1996; in comparison, 0.19 kg of Cu was released in the same year. Estimated flow-weighted concentrations were 323 µg l^{-1} (2.352 µM) for Ba and 0.04 µg l^{-1} (6.295×10^{-4} µM) for Cu in produced water discharges in 1996 (Steinberger et al. 2005). The general platform permit limit for Cu discharge is 20 µg l^{-1} (0.315 µM).

Ba enrichment in otoliths of kelp rockfish from platforms may have been related to the discharge of produced water. Kelp rockfish inhabiting the midwater structure of the platform may be exposed to elevated Ba Ca^{-1} which we were unable to detect from our water samples. Higashi et al (1997) reported that *M. californianus* showed a clear decreasing trend in Ba accumulation levels in newly grown shell material with increasing distance from a produced water outfall off Carpinteria in some but not all outplanting experiments conducted over the course of a year suggesting that seasonal currents patterns may have affected dispersal of the discharge.

As in our study, Warner et al. (2005) found no relationship between Ba from the outermost material of adult kelp rockfish otoliths and Ba Ca^{-1} from seawater collected at the time fish were sampled in 2002 from rocky reef sites off west Santa Barbara, northeast Santa Cruz Island, and south Santa Cruz Island. Laboratory and field studies have determined that otolith Ba correlates with ambient Ba in seawater (Bath et al. 2000, Elsdon and Gillanders 2003, Elsdon and Gillanders 2005). The field studies compared estuarine and coastal habitats where differences in otolith Ba are more extreme than open coastal site comparisons (e.g., Brown 2006).

If found to be temporally consistent, unique signatures of elements incorporated in the structure of otoliths of kelp rockfish potentially can be used as a tool to reconstruct an individual's history of movement among habitats. However, we suspect that otolith Ba is temporally variable. For example, Warner et al. (2005) reported the mean Ba Ca^{-1} in seawater was 3.97 (S.E.=0.05, n=12) µmol/mol from the vicinity of our kelp rockfish natural site in the west Santa Barbara area and 3.75 (SE=0.107, n=6) µmol/mol from a northeast Santa Cruz Island site (Pelican) in 2002. In comparison, our seawater Ba Ca^{-1} values from 2005 were relatively lower; Ba Ca^{-1} was 3.573 (SD=0.030, n=3) from our kelp rockfish natural site in the west Santa Barbara area and 3.424 (SD=0.240) from northeast Santa Cruz Island. Interestingly, otolith Ba Ca^{-1} from fish collected at all sites sampled in our study were substantially lower than Ba Ca^{-1} sampled from the outermost material of kelp rockfish otoliths by Warner et al. (2005). This suggests that interannual differences in ambient Ba may affect otolith Ba on a regional scale (Santa Barbara Channel), and further study is needed to examine the temporal consistency of a platform "fingerprint."

We did not expect to find lower concentrations of Ba in otoliths of Pacific sanddab from natural areas compared to platforms. Pacific sanddab at the platforms are in proximal contact with drilling discharges in sediments and are opportunistically preying on such benthic infauna as polychaetes, as well as such benthic, epibenthic, and water column organisms including shrimps, gammarid amphipods, mysids, euphausiids, squids, and fishes (Allen 1982; Allen et al. 2002). Individuals of this species probably do not make large-scale movements as implied from regional surveys of chlorinated hydrocarbon contamination in the Southern California Bight (Schiff and Allen 2000).

Drilling discharges consist of muds and cuttings that are generated during the drilling of a new well. Barite is a major constituent of the water-soluable drilling muds that are used on platforms off Southern California (Raco 1994, Steinberger et al. 2005). Drill cuttings are particles of crushed sedimentary rock produced in the process of drilling. Of the total estimated metals discharged, barium accounted for 96%, lead 1.9%, and zinc 1.5% (Raco 1994). Well drilling and the associated discharges are intermittent from individual platforms and vary in quantity. For example, drill cuttings and mud were discharged by Platforms Gail, Habitat, Irene, Gilda, C, and B in 1990 (Raco 1994); Platforms Harmony, Heritage, Gail, Eureka, Gilda, C and Hermosa in 1996; and Platforms Hondo, Heritage, Hermosa, Harvest, Hidalgo, and Hillhouse in 2000 (Steinberger et al. 2005).

The barium load in sediments is a sensitive and persistent indicator of drilling activities at platforms. Barium was the only element among Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, V, and Zn that changed significantly during a study conducted at Platform Hidalgo before during and after drilling had stopped (Steinhauer et al. 1994). During drilling, barium concentrations in surface sediments increased 30-40% above background. After drilling stopped, barium in sediments decreased, but after 1.5 years at the end of the study, surface sediments were approximately 20% higher than pre-drilling concentrations.

If Ba has accumulated in sediments from historic drilling activities, then our results indicate that the Ba concentration in otoliths of Pacific sanddab residing at platforms is not a sensitive indicator. A possible explanation for the higher concentration of Ba in otoliths of Pacific sanddab from natural areas than from platforms are difficult to infer. The availability of Ba in seawater will decline due to the precipitation of Ba as barite in the presence of sulfate in high concentrations. If sulfate concentrations in bottom waters were higher at platforms than natural sites, then it is plausible that ambient Ba available for uptake by fish was more concentrated at the natural sites, and we were unable to detect this difference using our seawater sampling method.

We found that sanddab otolith Mn significantly differed among areas; otolith Mn Ca^{-1} was higher among the two easternmost areas within the Channel and the Southern California Bight area than the three westernmost areas. We did not find a significant relationship between otolith Mn Ca^{-1} and seawater Mn Ca^{-1}; however, Campana (1999) suggested that the Mn concentration in otoliths is likely to reflect Mn in ambient waters, because Mn is a trace metal that probably is not regulated to any significance in fishes. Studies indicate that differences in otolith Mn Ca^{-1} among locations may be related to the unconservative behavior of Mn in ambient waters due to redox reactions.

Dissolved Mn (Mn^{+2}) rapidly can become unavailable when it is oxidixed and scavenged onto particles in the aquatic environment (Sunda and Huntsman 1987). In laboratory studies testing effects of temperature and salinity on otolith elemental uptake rates, Elsdon and Gillanders (2002) and Martin and Thorrold (2005) suggested that uncontrolled variation of Mn in experimental treatments may have confounded results. Martin and Thorrold (2005) suggested that bacterial growth in the treatments may have served as microbial catalysts forming manganese oxides that were scavenged onto particles lowering dissolved Mn concentrations in ambient waters. It is possible that the particle scavenging rate of Mn by bacteria in the bottom waters of the westernmost areas of our study area may have been higher than the other areas.

In contrast, under anoxic aquatic conditions, the availability of dissolved Mn Ca^{-1} for uptake by fish can increase. Dorval et al. (2007) found that Mn Ca^{-1} in otoliths of spotted seatrout (*Cynoscion nebulosus*) was positively correlated with Mn Ca^{-1} in Chesapeake Bay waters but the relationship was not significantly linear. Dorval et al. associated the field observations of concentrations of biologically available Mn (Mn^{+2}) in ambient waters peaking in particular locations due to the reduction of oxidized Mn during summer when anoxic conditions dominate bottom water and surface sediment. Another explanation for our observations of otolith Mn Ca^{-1} differences among areas may be that the Mn reduction rate may be higher in bottom waters of the eastern Channel and Southern California Bight if waters are relatively anoxic compared to the western areas of our study.

Forrester and Swearer (2002) found no relationship between Mn concentration in otoliths and sediments within bays of the Southern California Bight; however, we cannot rule out differences in the Mn load in sediments in our study that may have contributed to the differences otolith chemistry among areas that we observed.

In summary, our results showed that the elemental concentrations of some elements in the otoliths of kelp rockfish and Pacific sanddab differed between platforms and natural sites sampled in the Santa Barbara Channel and Southern California Bight; however, the differences were not always consistent among geographic areas. Kelp rockfish collected from platforms tended to incorporate higher concentrations of Mg and Ba and lower concentration of Sr in otoliths compared to fish from natural habitat. In comparison, Pacific sanddab collected from platforms tended to have lower concentrations of Ba and Fe in otoliths compared to fish from natural habitat. Mn Ca^{-1}, in otoliths of Pacific sanddab, was the only element in otoliths of either species that showed a well-defined pattern in its distribution among collection areas. Mn Ca^{-1} was not detectable in kelp rockfish otoliths. Variability in otolith element concentrations between habitat types and among areas differed between the two species.

The usefulness of otolith chemical tags relies on the spatial and temporal consistency of the otolith elemental signatures that discriminate between habitats of interest. We observed adequate spatial consistency of the kelp rockfish otolith multi-element signatures to discriminate platforms from natural sites across five geographic areas. There was less certainty in identifying the type of habitat where Sanddab were captured using otolith signatures.

Uncertainty in the spatial consistency of otolith signatures that discriminate platform and natural habitat beyond our sampling sites could be reduced by examining the otolith microchemistry of fish from a broader sampling of the 23 platforms in the Pacific Outer Continental Shelf region and natural areas that fish might utilize later during their life history. Future study should include a multiyear sampling effort to examine the temporal stability of a "platform fingerprint."

Results from this study imply that platform habitat "fingerprints," unique microchemical markers, may be identifiable along the time-keeping, growth trajectory of the otolith to identify adult fish that have moved between platforms and natural areas during the life history of the individuals. Such reconstructions would benefit from more study on the spatial variability of platform elemental signatures from all existing platforms and the stability of platform markers in populations over time. Otolith microchemistry, along with genetics and acoustic tag telemetry, might be used to estimate the degree of connectivity between platforms and local or regional populations.

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Appendices

Appendix 1. Statistics for detection limits (DL) of 18 element (Me) concentrations (Me Ca^{-1}) calculated from n LA ICP-MS sequences (n seqs), and the proportion (%) of kelp rockfish and sanddab otoliths that were detectable (Me Ca^{-1}>0) and above DL (Me Ca^{-1}>DL) with note of the inclusion (Incl.) of the element in the statistical comparisons.

Detection Limit (DL)							Kelp rockfish						Pacific sanddab					
Me Ca^{-1}	n seqs	Mean	SD	Min	Max	Median	n	Me Ca^{-1} >0 (%)		Me Ca^{-1} >DL		Incl.	n	Me Ca^{-1} >0 (%)		Me Ca^{-1} >DL		Incl.
Mg Ca^{-1}	40	4.1005	3.7733	0.1089	14.7603	3.1462	97	97	(100%)	97	(100%)	yes	108	108	(100%)	108	(100%)	yes
Sr Ca^{-1}	40	0.8212	0.5726	0.1099	2.2148	0.6786	97	97	(100%)	97	(100%)	yes	108	108	(100%)	108	(100%)	yes
Ag Ca^{-1}	21	0.0078	0.0055	0.0008	0.0230	0.0075	50	0	(0%)	0	(0%)	no	63	2	(3%)	1	(2%)	no
Cd Ca^{-1}	21	0.4116	0.2095	0.0635	0.9661	0.4053	50	0	(0%)	0	(0%)	no	63	2	(3%)	1	(2%)	no
Cs Ca^{-1}	40	0.0296	0.0185	0.0002	0.0763	0.0258	97	60	(62%)	38	(39%)	no	108	60	(56%)	44	(41%)	no
Ba Ca^{-1}	40	0.0273	0.0167	0.0024	0.0586	0.0253	97	97	(100%)	97	(100%)	yes	108	108	(100%)	108	(100%)	yes
Ce Ca^{-1}	21	0.0026	0.0015	0.0003	0.0068	0.0024	50	1	(2%)	1	(2%)	no	63	1	(2%)	0	(0%)	no
Hg Ca^{-1}	21	8.1983	8.8886	0.1065	26.2905	4.2743	50	5	(10%)	1	(2%)	no	63	5	(8%)	1	(2%)	no
Pb Ca^{-1}	40	0.0238	0.0151	0.0012	0.0713	0.0198	97	46	(47%)	33	(34%)	no	108	41	(38%)	29	(27%)	no
U Ca^{-1}	21	0.0002	0.0001	0	0.0006	0.0001	50	0	(0%)	0	(0%)	no	63	1	(2%)	1	(2%)	no
Al Ca^{-1}	40	5.3199	5.7419	0.6140	28.8881	3.6650	97	57	(59%)	25	(26%)	no	108	62	(57%)	21	(19%)	no
V Ca^{-1}	21	0.0469	0.0383	0.0084	0.1491	0.0311	50	1	(2%)	1	(2%)	no	63	0	(0%)	0	(0%)	no
Cr Ca^{-1}	40	0.1711	0.2313	0.0099	0.9863	0.0692	97	48	(49%)	27	(28%)	no	108	50	(46%)	27	(25%)	no
Mn Ca^{-1}	40	0.2154	0.3034	0.0149	1.3569	0.1151	97	35	(36%)	12	(12%)	no	108	107	(99%)	104	(96%)	yes
Fe Ca^{-1}	40	6.1209	16.7374	0.0600	96.3993	1.0118	97	82	(85%)	33	(34%)	yes	108	102	(94%)	34	(31%)	yes
Co Ca^{-1}	21	0.0223	0.0172	0	0.0696	0.0198	50	0	(0%)	0	(0%)	no	63	0	(0%)	0	(0%)	no
Cu Ca^{-1}	40	0.1935	0.1884	0.0252	0.8697	0.1327	97	80	(82%)	68	(70%)	yes	108	93	(86%)	76	(70%)	yes
Zn Ca^{-1}	40	0.7037	0.5070	0.0000	2.5592	0.6505	97	58	(60%)	45	(46%)	no	108	65	(60%)	50	(46%)	no

Appendix 2. Seawater element-to-calcium ratio (element mmol Ca mol^{-1}; mean, standard deviation, n=3) from platform and natural habitat sites where kelp rockfish were collected.

Site	**Statistic**	**Mg Ca^{-1}**	**Al Ca^{-1}**	**V Ca^{-1}**	**Cr Ca^{-1}**	**Mn Ca^{-1}**	**Fe Ca^{-1}**	**Co Ca^{-1}**	**Cu Ca^{-1}**	**Zn Ca^{-1}**
Tarantula	Mean	4786.33	3.36E-02	2.68E-03	4.01E-04	1.10E-03	3.72E-03	1.53E-05	2.71E-04	1.32E-02
	SD	77.24	3.62E-02	1.41E-04	1.28E-04	9.20E-05	2.82E-03	2.64E-05	2.54E-05	4.68E-03
Platform Holly	Mean	4880.67	2.93E-01	3.02E-03	4.31E-04	6.63E-04	2.31E-03	2.39E-05	3.05E-04	6.35E-03
	SD	144.88	4.51E-01	3.97E-04	9.56E-06	1.37E-04	8.86E-04	2.24E-05	1.07E-04	2.47E-03
Goleta	Mean	4994.33	2.43E-02	2.78E-03	3.19E-04	3.48E-03	2.44E-03	5.80E-05	3.08E-04	6.26E-03
	SD	449.82	1.09E-02	2.04E-04	3.94E-05	3.12E-04	7.61E-04	3.44E-05	9.93E-05	2.43E-03
Platform Hogan	Mean	4663.33	1.10E-02	2.78E-03	3.20E-04	7.18E-04	7.59E-04	0.00E+00	2.57E-04	1.29E-02
	SD	314.29	4.26E-03	1.96E-04	3.28E-05	5.51E-05	4.31E-04	0.00E+00	5.13E-05	1.32E-02
Summerland	Mean	5084.33	2.39E-02	2.85E-03	3.65E-04	2.07E-03	2.28E-03	1.84E-05	2.66E-04	3.71E-03
	SD	233.89	6.63E-03	1.91E-05	9.83E-05	4.35E-05	1.46E-03	3.18E-05	4.07E-05	7.32E-05
Platform Gilda	Mean	4811.33	1.18E-02	2.86E-03	3.53E-04	1.44E-03	1.44E-03	1.70E-05	2.25E-04	1.72E-02
	SD	372.25	1.77E-03	2.61E-04	6.78E-05	5.46E-04	2.96E-05	1.51E-05	8.97E-05	2.35E-02
NE Santa Cruz I.	Mean	4675.67	4.82E-02	2.81E-03	2.73E-04	7.40E-04	1.86E-03	0.00E+00	1.91E-04	5.34E-03
	SD	259.72	6.58E-02	2.26E-04	2.02E-05	4.68E-05	6.53E-04	0.00E+00	4.06E-05	2.05E-03
Platform Edith	Mean	4890.67	1.14E-02	2.83E-03	4.06E-04	2.04E-04	2.90E-03	7.75E-06	2.44E-04	4.87E-03
	SD	116.15	2.37E-03	1.36E-04	1.33E-04	7.97E-05	1.83E-03	1.34E-05	7.49E-05	1.69E-03
Catalina I.	Mean	4867.00	1.25E-02	2.76E-03	3.79E-04	1.85E-04	1.34E-03	9.09E-06	2.48E-04	4.32E-03
	SD	182.45	4.07E-03	1.95E-04	9.10E-05	6.31E-05	1.16E-04	1.57E-05	4.77E-05	1.01E-03
Palos Verdes	Mean	5057.33	4.81E-02	2.71E-03	3.98E-04	1.05E-03	2.48E-03	3.11E-05	3.04E-04	5.47E-03
	SD	323.95	2.77E-03	1.69E-04	5.84E-05	5.17E-05	3.41E-04	3.39E-05	1.54E-04	2.26E-03
Total	Mean	4871.10	5.18E-02	2.81E-03	3.64E-04	1.16E-03	2.15E-03	1.80E-05	2.62E-04	7.96E-03
	SD	265.54	1.46E-01	2.01E-04	8.10E-05	9.69E-04	1.32E-03	2.52E-05	7.73E-05	8.58E-03

Appendix 2. (Continued)

Site	**Statistic**	**Sr Ca^{-1}**	**Ag Ca^{-1}**	**Cd Ca^{-1}**	**Cs Ca^{-1}**	**Ba Ca^{-1}**	**Ce Ca^{-1}**	**Hg Ca^{-1}**	**Pb Ca^{-1}**	**U Ca^{-1}**
Tarantula	Mean	72.05	8.04E-06	4.98E-05	1.82E-04	3.38E-03	3.89E-06	3.35E-07	6.41E-05	1.12E-03
	SD	11.37	4.51E-06	6.15E-06	1.05E-05	1.20E-04	2.18E-06	5.80E-07	3.92E-05	5.36E-05
Platform Holly	Mean	74.96	4.49E-06	5.62E-05	1.97E-04	3.54E-03	5.07E-06	6.67E-07	5.13E-05	1.19E-03
	SD	13.29	1.96E-06	7.97E-06	1.72E-05	1.89E-04	3.56E-06	1.16E-06	1.69E-05	7.22E-05
Goleta	Mean	76.60	6.13E-06	6.63E-05	1.89E-04	3.57E-03	5.50E-06	0.00E+00	4.10E-05	1.18E-03
	SD	12.77	2.89E-06	1.09E-05	6.94E-06	2.97E-05	1.53E-06	0.00E+00	2.30E-05	1.72E-05
Platform Hogan	Mean	82.84	5.34E-06	4.63E-05	1.88E-04	3.38E-03	2.80E-06	0.00E+00	4.71E-05	1.14E-03
	SD	3.99	4.81E-07	8.82E-06	8.87E-06	1.53E-04	5.55E-07	0.00E+00	1.44E-05	4.17E-05
Summer-land	Mean	82.38	6.69E-06	5.66E-05	1.97E-04	3.84E-03	5.41E-06	0.00E+00	6.05E-05	1.19E-03
	SD	3.20	1.90E-06	1.18E-05	2.54E-06	1.00E-04	4.22E-07	0.00E+00	4.53E-05	3.19E-05
Platform Gilda	Mean	73.56	7.52E-06	6.29E-05	1.90E-04	3.56E-03	3.61E-06	0.00E+00	4.18E-05	1.15E-03
	SD	6.87	1.68E-06	3.90E-06	1.59E-05	3.14E-04	1.25E-06	0.00E+00	1.06E-05	9.19E-05
NE Santa Cruz I.	Mean	80.72	4.53E-06	5.23E-05	1.91E-04	3.42E-03	3.64E-06	0.00E+00	3.93E-05	1.14E-03
	SD	4.47	1.92E-06	9.63E-06	1.57E-05	2.40E-04	3.87E-06	0.00E+00	2.29E-05	8.30E-05
Platform Edith	Mean	74.29	4.66E-06	4.86E-05	1.92E-04	3.52E-03	3.47E-06	3.43E-07	5.39E-05	1.17E-03
	SD	15.05	1.39E-06	4.52E-06	9.14E-06	1.61E-04	3.07E-06	5.95E-07	1.74E-05	5.87E-05
Catalina I.	Mean	77.78	4.38E-06	5.42E-05	1.79E-04	3.30E-03	1.89E-06	0.00E+00	6.08E-05	1.09E-03
	SD	11.09	2.82E-06	1.72E-05	7.43E-06	1.10E-04	9.07E-07	0.00E+00	2.13E-05	2.74E-05
Palos Verdes	Mean	73.16	5.16E-06	5.35E-05	1.81E-04	3.34E-03	3.97E-06	0.00E+00	6.55E-05	1.12E-03
	SD	5.70	2.66E-06	4.45E-06	8.36E-06	8.58E-05	7.33E-07	0.00E+00	3.49E-05	6.49E-05
Total	Mean	76.83	5.69E-06	5.47E-05	1.88E-04	3.49E-03	3.92E-06	1.35E-07	5.25E-05	1.15E-03
	SD	8.92	2.40E-06	9.84E-06	1.10E-05	2.08E-04	2.12E-06	4.37E-07	2.43E-05	5.90E-05

Appendix 3. Seawater element-to-calcium ratio (element mmol Ca mol^{-1}; mean, standard deviation, n=3) from platform and natural habitat sites where Pacific sanddab were collected.

Site	Statistic	Mg Ca^{-1}	Al Ca^{-1}	V Ca^{-1}	Cr Ca^{-1}	Mn Ca^{-1}	Fe Ca^{-1}	Co Ca^{-1}	Cu Ca^{-1}	Zn Cav^{-1}
Platform Hidalgo	Mean	4853.67	1.07E-02	2.86E-03	3.81E-04	2.62E-04	2.43E-03	0.00E+00	2.50E-04	2.20E-02
	SD	190.01	9.88E-04	1.80E-04	6.56E-05	1.16E-05	1.64E-03	0.00E+00	6.98E-06	2.90E-02
Pt. Conception, offshore	Mean	4623.33	2.40E-02	2.68E-03	3.34E-04	5.14E-04	2.41E-03	0.00E+00	2.28E-04	5.92E-03
	SD	331.40	1.32E-02	1.57E-04	7.66E-05	4.98E-05	7.54E-04	0.00E+00	6.48E-05	1.70E-03
Platform Holly	Mean	4900.67	1.64E-02	2.83E-03	3.13E-04	2.67E-03	2.48E-03	8.43E-06	2.66E-04	5.42E-03
	SD	294.68	1.53E-03	1.57E-04	1.63E-05	2.05E-04	6.95E-04	1.46E-05	4.18E-05	1.34E-03
Goleta, offshore	Mean	4903.00	2.85E-02	2.99E-03	3.71E-04	8.07E-04	2.75E-03	0.00E+00	3.33E-04	1.35E-02
	SD	64.37	4.06E-03	6.85E-05	4.55E-05	4.03E-05	6.50E-04	0.00E+00	6.74E-05	1.49E-02
Summerland, offshore	Mean	4812.00	1.10E-02	2.94E-03	3.37E-04	4.46E-04	2.08E-03	1.54E-05	3.01E-04	6.44E-03
	SD	297.06	7.65E-03	1.59E-04	9.35E-05	9.69E-05	6.12E-04	2.67E-05	9.66E-05	2.61E-03
Platform Gina	Mean	4971.67	2.99E-02	3.04E-03	3.42E-04	1.50E-03	7.47E-03	2.35E-05	3.73E-04	2.29E-02
	SD	338.17	7.85E-03	2.30E-04	5.01E-05	1.55E-04	4.64E-03	2.17E-05	1.40E-04	3.01E-02
Hueneme Canyon	Mean	4730.00	3.11E-02	3.04E-03	3.01E-04	2.70E-03	3.89E-03	8.45E-06	2.75E-04	6.64E-03
	SD	311.74	8.15E-03	1.83E-04	2.82E-05	1.32E-04	1.31E-03	1.46E-05	3.36E-05	2.81E-03
Platform Gilda	Mean	4790.33	8.12E-03	2.82E-03	3.14E-04	1.02E-03	1.74E-03	1.53E-05	2.86E-04	4.58E-03
	SD	298.47	1.66E-03	1.63E-04	2.82E-05	4.49E-05	1.23E-04	2.64E-05	8.03E-05	8.52E-04
NE Santa Cruz I, offshore	Mean	4965.67	8.49E-03	2.88E-03	3.03E-04	2.28E-04	1.08E-03	1.53E-05	5.76E-04	5.57E-03
	SD	775.08	1.70E-03	5.15E-04	5.94E-05	8.34E-05	2.22E-04	1.37E-05	4.38E-04	9.55E-04
Platform Edith	Mean	4790.33	5.75E-02	2.68E-03	3.19E-04	6.32E-04	3.82E-03	0.00E+00	2.71E-04	6.22E-03
	SD	126.52	4.56E-03	2.68E-04	2.54E-05	1.69E-04	9.90E-04	0.00E+00	1.22E-04	1.63E-03
Palos Verdes, offshore	Mean	4559.67	6.85E-02	2.84E-03	3.25E-04	5.25E-04	3.16E-03	7.32E-06	3.12E-04	7.71E-03
	SD	35.22	3.01E-02	1.46E-04	2.49E-05	1.08E-04	8.56E-04	1.27E-05	8.33E-05	3.98E-03
Total	Mean	4809.12	2.68E-02	2.87E-03	3.31E-04	1.03E-03	3.03E-03	8.51E-06	3.16E-04	9.72E-03
	SD	306.65	2.14E-02	2.25E-04	5.01E-05	8.70E-04	2.13E-03	1.51E-05	1.57E-04	1.30E-02

Appendix 3. (Continued)

Site	**Statistic**	**Sr Ca^{-1}**	**Ag Ca^{-1}**	**Cd Ca^{-1}**	**Cs Ca^{-1}**	**Ba Ca^{-1}**	**Ce Ca^{-1}**	**Hg Ca^{-1}**	**Pb Ca^{-1}**	**U Cav^{-1}**
Platform Hidalgo	Mean	74.84	8.54E-06	8.27E-05	1.83E-04	3.42E-03	3.44E-06	2.99E-07	5.68E-05	1.11E-03
	SD	10.30	4.99E-07	1.19E-05	9.07E-06	1.43E-04	1.39E-06	5.18E-07	3.48E-05	3.96E-05
Pt. Conception, offshore	Mean	75.78	6.45E-06	7.76E-05	1.77E-04	3.36E-03	2.35E-06	2.88E-07	4.36E-05	1.09E-03
	SD	8.70	2.94E-07	6.75E-06	9.17E-06	2.12E-04	8.77E-07	4.98E-07	1.84E-06	7.76E-05
Platform Holly	Mean	82.53	5.19E-06	8.57E-05	2.00E-04	3.92E-03	3.85E-06	3.24E-07	3.97E-05	1.22E-03
	SD	8.05	1.47E-06	9.59E-06	1.30E-05	1.90E-04	9.34E-07	5.61E-07	1.74E-05	5.31E-05
Goleta, offshore	Mean	81.91	6.96E-06	8.13E-05	1.92E-04	3.87E-03	5.34E-06	6.41E-07	5.73E-05	1.16E-03
	SD	6.83	1.18E-06	1.48E-05	7.60E-06	4.29E-04	4.37E-07	5.55E-07	4.22E-05	3.20E-05
Summerland, offshore	Mean	67.93	5.54E-06	8.79E-05	1.87E-04	3.50E-03	2.50E-06	3.23E-07	4.17E-05	1.14E-03
	SD	7.16	9.13E-07	3.44E-06	9.11E-06	1.64E-04	6.53E-07	5.59E-07	1.25E-05	4.10E-05
Platform Gina	Mean	73.86	8.13E-06	9.05E-05	1.95E-04	3.77E-03	3.72E-06	0.00E+00	7.38E-05	1.18E-03
	SD	10.53	1.44E-06	1.80E-06	1.34E-05	1.91E-04	6.72E-07	0.00E+00	1.89E-05	7.03E-05
Hueneme Canyon	Mean	82.35	7.29E-06	7.98E-05	1.94E-04	3.80E-03	4.94E-06	0.00E+00	7.22E-05	1.18E-03
	SD	7.73	2.45E-06	7.55E-06	4.85E-06	1.17E-04	6.43E-07	0.00E+00	1.33E-05	4.31E-05
Platform Gilda	Mean	70.26	5.97E-06	7.94E-05	1.82E-04	3.49E-03	3.42E-06	3.05E-07	5.70E-05	1.13E-03
	SD	7.58	3.83E-07	7.10E-06	8.73E-06	9.79E-05	6.05E-07	5.28E-07	8.84E-06	3.02E-05
NE Santa Cruz I, offshore	Mean	80.38	8.91E-06	9.18E-05	1.85E-04	3.51E-03	1.52E-06	0.00E+00	1.33E-04	1.12E-03
	SD	11.29	1.50E-06	1.26E-05	3.29E-05	5.29E-04	5.99E-07	0.00E+00	8.80E-05	1.68E-04
Platform Edith	Mean	70.97	6.93E-06	5.60E-05	1.72E-04	3.42E-03	4.95E-06	0.00E+00	3.60E-05	1.04E-03
	SD	12.17	4.32E-07	2.45E-06	9.40E-06	3.26E-04	1.46E-06	0.00E+00	6.50E-06	7.96E-05
Palos Verdes, offshore	Mean	74.04	7.66E-06	7.52E-05	1.89E-04	3.52E-03	5.33E-06	0.00E+00	4.32E-05	1.13E-03
	SD	8.28	1.17E-06	5.91E-06	1.50E-05	2.16E-04	2.13E-06	0.00E+00	2.12E-05	7.41E-05
Total	Mean	75.90	7.05E-06	8.07E-05	1.87E-04	3.60E-03	3.76E-06	1.98E-07	5.95E-05	1.14E-03
	SD	9.09	1.55E-06	1.19E-05	1.41E-05	2.97E-04	1.53E-06	3.88E-07	3.85E-05	7.77E-05

Task 3: A Histological Examination of the Ovaries of Pacific Sanddab, *Citharichthys sordidus*, Captured at Two Oil Platforms and Two Natural Sites in the Southern California Bight

Milton S. Love and Stephen R. Goldberg

Abstract

A number of the 26 offshore oil and gas platforms off California may be nearing the end of their economic lives. Decisions as to the disposition of these platforms will be based on a number of parameters, including the biological role of the structures. One issue that has arisen is the possible contamination of fishes living around platforms resulting from contaminants released during drilling and production. If significant contamination is occurring, it would be expected to impair the reproductive abilities of impacted fishes. One form of reproductive impairment is atresia, the abnormal reabsorption of oocytes that are destined to be spawned. Atresia has been widely used as an indicator of pollutant-related reproductive impairment in fishes. We examined the occurrence of atretic oocytes in Pacific sanddab, *Citharichthys sordidus*, collected from two offshore platforms in the Santa Barbara Channel (B and Gilda) and from two natural reference sites (off the east end of Santa Cruz Island and in mid-channel off Rincon). While pronounced atresia was observed in a few fish at one natural site and one platform, there was no evidence of widespread pronounced atresia at any of the four sites.

Introduction

There are 26 offshore oil and gas platforms in California waters, many of which may be nearing the ends of their economic life spans. Following the decision by an operator to cease production, the process by which a decision is made as to the disposition of a platform is called decommissioning. This procedure may take into consideration the biological, sociological, and economic consequences of various decommissioning options, which range from leaving the entire structure in place through partial or total removal (Schroeder and Love 2004).

Among the issues that need to be addressed in this process is the possible contamination of platform-dwelling organisms. A number of activities associated with the production of oil and gas result in the creation of contaminants. Drilling muds (used to lubricate drill bits), drill cuttings (the hard material brought up by the drill bit), the water associated with the petroleum produced ("produced water"), and water used as coolant contain a variety of elements and compounds, including petroleum hydrocarbons and metals (Utvik 1999; Neff 2002; Steinberger et al. 2004). At least some of these elements and compounds are released into the water and may be deposited on the sea floor around these structures (Grant and Briggs 2002; Phillips et al. 2006). While the acute toxicity of these materials varies considerably among both platforms and organisms (Utvik 1999; Grant and Briggs 2002), less is known about their potential chronic effects (Stephens et al. 2000).

One issue to be addressed during decommissioning is the possible damage to fish reproduction if a structure is left in place. Pollutants such as heavy metals and chlorinated hydrocarbons have been linked to a range of physiological problems in fishes, including reproductive impairment (Hose et al. 1989; Cross and Hose 1988; Thomas 1989). One form of reproductive impairment linked to pollution is atresia, the abnormal reabsorption of oocytes destined to be spawned. Atresia has been used as an indicator of pollutant-related reproductive impairment in fish living in the southern California Bight (Cross et al. 1984).

The objective of this investigation was to compare the spawning capability, as measured by pronounced atresia, of Pacific sanddab (*Citharichthys sordidus*) living around two oil platforms with fish inhabiting two natural areas. This species occurs from the western Gulf of Alaska to Bahia Magdalena, southern Baja California, and as an isolated population in the Gulf of California (Love et al. 2005). Pacific sanddab are very widespread throughout southern California waters and live both on soft sea floors at natural sites and around oil and gas platforms (Love et al. 1999; Allen et al. 2007). This flatfish spends much of its time lying on or partially covered in sediment, although we have also seen them well up in the water column. It is an opportunistic feeder, preying on such benthic infauna as polychaetes, as well as such benthic, epibenthic, and water column organisms including shrimps, gammarid amphipods, mysids, euphausiids, squids, and fishes (Allen 1982; Allen et al. 2002). Pacific sanddab spawn from late spring through late fall, probably peaking in the summer (Arora 1951; M. McCrea, pers. comm.). Regional surveys of chlorinated hydrocarbon contamination in the Southern California Bight imply that these fish do not make large-scale movements (Schiff and Allen 2000).

Based on its life history, it would be expected that the Pacific sanddab is an excellent species on which to model the reproductive effects of platform discharges. This fish is likely to come into contact with benthic pollutants, both through direct physical contact and absorption, and from its diet of both sea-floor infauna and epibenthic organisms. And, in fact, Pacific sanddab have been shown to bioaccumulate pollutants and have been used as a tool in assessments of tissue contamination (Schiff and Allen 2000; Allen et al. 2002). The lack of any evidence of substantial reproductive impairment in fish living around two oil and gas platforms implies that large-scale reproductive damage is unlikely to be occurring.

Methods

Pacific sanddab were collected at four sites in the Santa Barbara Channel, southern California, by hook and line in September 2005 (Figure 1, Table 1). The sampling sites were 1) Platform B, 2) Platform Gilda 3) a natural site located about 8 km offshore and southeast of Platform B ("Rincon"), and 4) a natural site off the northeast corner of Santa Cruz Island ("Santa Cruz Island"). Both platforms have produced oil and gas for many years, Platform B starting in 1968 and Platform Gilda in 1981 (Love et al. 2003). Platform fish were collected within 30-60 m of each structure.

After capture, fish were placed on ice and several hours later were measured (total length, cm), the ovaries fixed in 10% formalin, and later shipped to SRG. A section of each ovary was removed, dehydrated in ethanol of ascending concentrations, embedded in paraffin and serial sections were cut at 5μm using a rotary microtome. Sections were mounted on glass slides, stained with hematoxylin followed by eosin counterstain. Slides from each ovary were examined under a compound microscope. The presence of the following was noted: (1) hydrated eggs for upcoming spawning; (2) vitellogenesis (yolk deposition) in mode of smaller eggs for subsequent spawning; (3) postovulatory follicles (evidence of recent spawning); (4) follicular atresia (degenerating oocytes). Atresia was characterized as minor (<10% of eggs atretic) or pronounced (≥10% of eggs atretic) (Cross et al. 1984; McDermott et al. 2007).

Table 1. Details of Pacific sanddab, *Citharichthys sordidus*, collections made between 11 and 20 September 2005 in the Santa Barbara Channel.

Site	Date	Depth (m)	Location	n	TL (cm) ±SD
Platform B	11 Sept.	61 m	34°19’56” 119°37’18	18	16.1±1.6
Rincon	16 Sept.	87 m	34°15’21” 119·33’14”	19	19.3±4.8
Platform Gilda	20 Sept.	64 m	34°10’56” 119°25’09”	20	20.8±2.6
Santa Cruz I.	20 Sept.	64 m	34°01’48” 119°30’22”	21	20.7±3.4

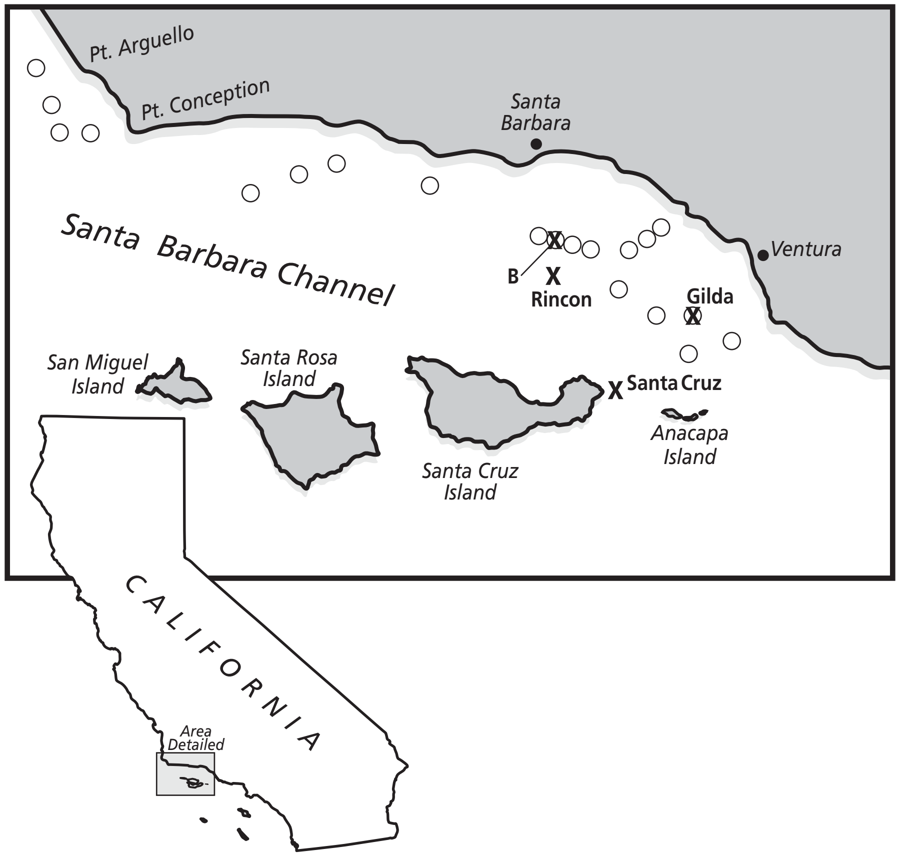

Figure 1. The location of the four Pacific sanddab sampling sites (latitudes and longitudes listed in Table 1).

Results

Within Pacific sanddab ovaries, we observed (1) hydrated eggs, (2) smaller eggs with yolks that would later grow and hydrate prior to spawning, (3) postovulatory follicles, (4) minor atresia, and (5) pronounced atresia (Figures. 2A-C, Table 2).

Egg hydration (Figure 2A) occurs just prior to spawning, when a mature oocyte expands to as much as four times its original volume (Wallace and Selman, 1981). Between 100% (Platform Gilda) and 50% (Rincon) of fish at each site contained hydrated eggs (Table 2). Smaller yolked eggs were found in between 100% (Platform Gilda) and 55% (Rincon) of fish (Table 2). Postovulatory follicles (Figure 2B) are remnants of the granulosa layer of the previously spawned egg that hypertrophies after the oocyte is released and thus their presence demonstrates that a female has spawned earlier in the season. Older postovulatory follicles (> 24 hours) can be distinguished from "fresh" ones by deterioration of original shape and loss of organization. The presence of postovulatory follicles from a recent spawning alongside maturing follicles destined for a subsequent spawning (Figure 2B) indicates Pacific sanddab spawn more than once during a reproductive season. Postovulatory follicles were found in between 65% (Santa Cruz Island) and 5% (Rincon) of fish (Table 2). At least some fish with minor atresia (Figure 2C) were noted at all of the sites, with levels ranging from 60% (Platform Gilda) to 15% at Santa Cruz Island. Pronounced atresia was relatively rare. No fish at Platform Gilda and Santa Cruz Island were identified as having pronounced atresia, while 6% of the sanddabs at Platform B and 16% at Rincon exhibited this condition (Table 2).

Table 2. Histological analyses of Pacific sanddab ovaries from four sites in the Santa Barbara. Values in each column are in percentages.

Site	n	Hydrated Eggs	Yolk in Smaller Modes	Post-ovulatory Follicles	Minor Atresia	Pronounced Atresia
B	18	95	95	61	22	6
Rincon	19	50	55	5	35	16
Gilda	20	100	100	35	60	0
SCI	21	85	85	65	15	0

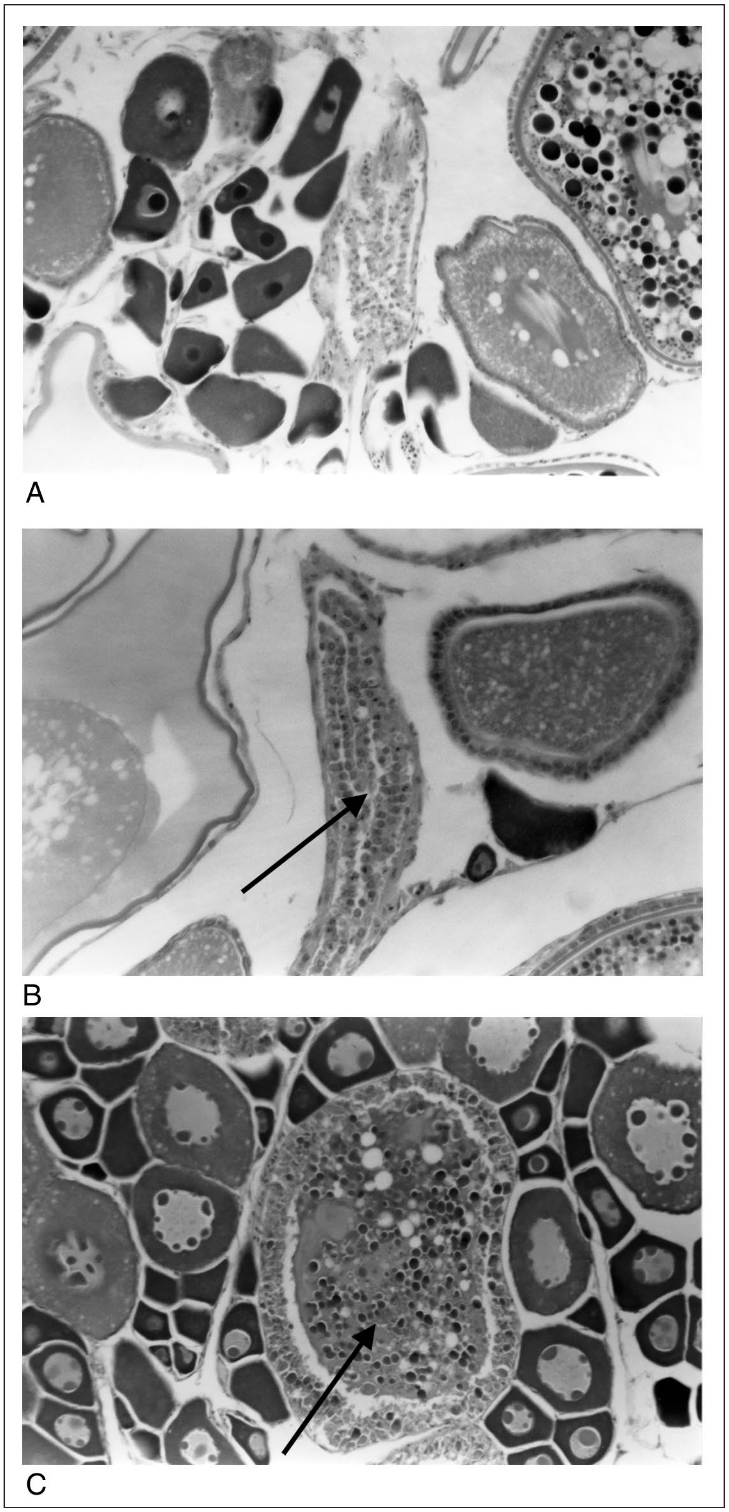

Figure 2. A) Ovary of Pacific sanddab showing hydrated oocyte (left) and yolk deposition in follicle (lower right); B) ovary of Pacific sanddab showing older postovulatory follicle (center); C) ovary of Pacific sanddab showing atretic follicle (center). Note enlarged height of granulosa cell layer and ingested yolk granules. All images at 160 X.

Discussion

In general, we observed relatively little evidence of conspicuous reproductive impairment in Pacific sanddab from both platforms and natural sites. Overall, most fish contained hydrated oocytes and were about to spawn and most contained oocytes with smaller yolks and thus were likely to spawn again. Many fish exhibited minor atresia. Atresia is the spontaneous degeneration of an oocyte at any stage in its development and it occurs at low frequency throughout the ovarian cycle. For instance, background (i.e., non-test) levels of minor atresia in laboratory-raised zebrafish, *Danio rerio*, were 58% (Rossteuscher et al. 2008). The frequency of minor atresia typically increases toward the end of the spawning cycle when follicles that initiated, but did not complete, yolk deposition degenerate (Goldberg, 1981).

We observed relatively few instances of pronounced atresia, a condition that is only occasionally observed in unstressed populations. As an example, pronounced atresia was only found in 2% of female Atka mackerel, *Pleurogrammus monopterygius*, taken from the relatively pristine Aleutian Islands (McDermott et al. 2007). A wide range of environmental stressors, including heavy metals (Pierron et al. 2008), endocrine disrupters (Pollino et al. 2007), and starvation and lipid-poor diets (Hunter and Macewicz 1985; Sherwood et al. 2007) can cause pronounced atresia. The level of pronounced atresia we documented was much less than that found in a study of longspine combfish, *Zaniolepis latipinnis*, and yellowchin sculpin, *Icelinus quadriseriatus*, from the vicinity of sewage outfalls in Santa Monica Bay and off Palos Verdes, southern California, or from a control site in Santa Monica Bay (Cross et al. 1984). That study documented high levels of pronounced atresia in 28-49% of females from around sewage outfalls and 42-44% from a control site (that was apparently also heavily polluted).

It might be argued that there was some reduction in the reproductive capacity of fish from Rincon as fewer fish from that site harbored 1) hydrated oocytes, 2) those smaller oocytes destined for later spawning, and 3) post-ovulatory oocytes. Furthermore, 16% of female Pacific sanddab from Rincon contained ovaries with pronounced atresia. We note that our sample size from Rincon was relatively small, encompassed only one year, and the factors responsible for the reduction in ovarian output in these females remain to be determined.

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