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AN EVALUATION OF THE NURSERY ROLE OF ESTUARIES FOR FLATFISH POPULATIONS IN CENTRAL CALIFORNIA

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AN EVALUATION OF THE NURSERY ROLE OF ESTUARIES FOR FLATFISH POPULATIONS IN CENTRAL CALIFORNIA

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ABSTRACT

The purpose of this research was to determine if estuaries in central California are higher quality juvenile habitats than coastal sandy habitats and, thus, function as nurseries by contributing more individuals to the adult populations than an equivalent area of coastal habitat. I evaluated the nursery role of estuaries for two species of flatfish - the English sole (*Pleuronectes vetulus*) and the speckled sanddab (*Citharichthys stigmaeus*).

I assessed relative habitat quality by comparing growth rates of juveniles in estuarine and coastal habitats using two methods: 1) a caging experiment in which juveniles of each species were held for 28 days in August 2000 Elkhorn Slough and Monterey Bay; and 2) a comparison of the width of daily increments in otoliths from juvenile speckled sanddab collected from estuaries and coastal areas in four regions in 1999 and 2000. Results from both the caging experiment and the comparison of daily increments indicated that juvenile flatfish grow faster in estuaries. Thus, based on comparisons of growth rates, estuaries were determined to be the higher quality juvenile habitat.

I directly assessed the nursery role of estuaries by determining the proportion of the adult population that recruited from estuaries. The chemical composition of otoliths (Sr/Ca and Li/Ca) was used to differentiate between fish that had resided as juveniles in either estuaries or sandy coastal habitats. Classification models based on juveniles collected in both habitat types were used to assign juvenile and adult fish to either the estuarine or coastal habitat group. Juveniles were assigned to the habitat type where they were captured with approximately 80% accuracy. The proportion of adult fish that were assigned to the estuarine habitat group was estimated to range between 45% and 57%. This is a much higher level of contribution than would be expected based on the relative area of estuarine and sandy coastal habitats in central California. These results indicate that estuarine habitats are an important source of new individuals for adult flatfish populations and conservation of estuaries may help maintain high levels of recruitment to harvested populations in central California.

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INTRODUCTION

Many coastal fish species have juvenile and adult life stages that occupy spatially separated habitats. The juveniles often recruit to nearshore habitats where they reside for months to years before migrating to offshore habitats to join the adult population. In addition, juveniles of many species with this life history pattern recruit to more than one type of nearshore habitat, for example estuaries and shallow sandflats, and those different habitats are likely to vary in quality. The highest quality juvenile habitats are often referred to as 'nursery' habitats. Recently, the definition of a nursery habitat was clarified by Beck et al. (2001): "A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur." Determining which juvenile habitats are functioning as nursery habitats is important to both understanding the ecological roles of the different juveniles habitats and managing harvested fish populations and coastal resources. Identification of nursery habitats is particularly important when some of the habitats used by juvenile fish are vulnerable to degradation or loss.

Along the Pacific coast of the United States estuaries are few in number, small in size and vulnerable to degradation from surrounding human activities and industries. Though many species of fish and invertebrates have juvenile stages that occur in these estuaries, very few species are estuarine-dependant. Most species that use estuaries also occur in other, more abundant, habitats such as sandy bottom or rocky reef. The purpose of this dissertation research was to evaluate the nursery role of the estuarine habitats for two species of flatfish – the English sole (*Pleuronectes vetulus*) and the speckled sanddab (*Citharichthys stigmaeus*). This evaluation was composed of two parts: 1) a comparison of the quality of estuaries and subtidal sandy coast as juvenile habitats; and 2) an estimate of the proportion of the adult population that recruited from estuarine and coastal habitats.

The relative quality of juvenile habitats is often assessed by comparing the density, survivorship rates, or growth rates of individuals residing in alternative habitat types. I focused on growth rates because higher growth rates during the juvenile phase can have a marked influence on an individual's success in both the juvenile and subsequent adult phases. For example, rapidly growing juveniles will be less vulnerable to size-selective mortality and will attain a larger size at the end of the juvenile period, which may improve recruitment success to the adult habitat.

Therefore, a habitat that promotes higher growth rates may act as a nursery habitats by contributing more and larger juveniles to the adult population.

In the first chapter of this dissertation, I employed two methods for measuring habitat-specific growth rates: a caging experiment and a comparison of the widths of daily increments in otoliths (bones in the inner ear of fish that can record age and growth rate). In the caging experiment, juvenile English sole and speckled sanddab were held in cages for 28 days in Elkhorn Slough estuary and in the surrounding coastal habitats of Monterey Bay. I found that both species experienced faster growth rates in the estuary, but for one species – the speckled sanddab – the growth advantage of the estuary diminished as the size of the fish increased. In the second method, I compared the width of daily increments in the otoliths of juvenile speckled sanddab that were collected in 1999 and 2000 from estuarine and coastal habitats in four regions along the central coast of California. I found that daily increments were wider in fish collected from estuarine habitats, and that this pattern occurred in all four regions and in both years of the study. Comparison of the results from both methods indicates that estuaries support faster growth rates than coastal habitats. Thus, estuaries are higher quality habitats for juvenile flatfish and may be functioning as nursery habitats for these two species.

The second objective my research was to directly evaluate if estuaries were nursery habitats by measuring the proportion of the adult population that recruited from estuarine and coastal habitats. Determining which juvenile habitats are contributing more individuals to adult populations requires identifying the juvenile habitats in which the adults once lived. One way to determine prior residence of adult fish is to manually tag fish in all the alternative juvenile habitats and, subsequently, recover those tagged individuals as adults. An alternative method to manual tagging requires that juveniles incorporate markers (e.g., elements, isotopes) characteristic of and specific to the habitat in which they reside and that these natural “habitat tags” that can be used to identify individuals from different juvenile habitats. Such natural habitat tags have been found in the otoliths of fish. The specific purpose of this portion of my research was to: 1) determine if a chemical habitat tag exists in the otoliths of juvenile English sole and speckled sanddab that could be used to differentiate fish collected from estuarine and coastal habitats in central California ; 2) determine if this chemical habitat tag was present in adult English sole collected in Monterey Bay by analyzing the portion of the adult otolith that was laid down when the fish was a juvenile; and 3) determine the proportion of the adults that originated in estuarine habitats. Determining the proportion of the adults that recruited from estuarine habitats will give insight into whether estuarine habitats are functioning as nurseries for the English sole population in central California.

In my second chapter, I compared the chemical composition of otoliths from juvenile English sole and speckled sanddab collected from estuarine and coastal habitats located along a 500 km section of the California coastline. Multiple estuaries and coastal sites were sampled in each of three years – 1998, 1999, and 2000. I used discriminant function analysis on the elemental composition of the otoliths (Li, Sr, Ba and Mn) to classify fish to groups based on the habitat type in which they were captured.

For each species, the global model, which pooled juveniles collected from all sites over three years, was able to classify fish into estuarine and coastal groups with close to 80% accuracy. Classification success of juveniles was modestly improved in some cases by generating separate discriminant functions for each year. These improvements were due to two elements, Ba and Mn, that differed between habitats in only some years. However, the two main elements in the discriminant models, Sr and Li, differed consistently between habitats over all three years. Given that the years examined in this study differed markedly in oceanographic conditions (e.g., El Niño and La Niña), this chemical habitat tag appears to be robust to temporal changes in environmental conditions. Thus, the chemical habitat tag found in this portion of the study appeared to be promising tool for determining contribution of estuarine and coastal habitats to the central California populations of English sole and speckled sanddab.

In addition, I found that English sole and speckled sanddab had striking similarities in their chemical habitats tags and that, in some cases, one species could be used as a proxy to classify juveniles of the other species without compromising the accuracy of the habitat tag. The ability to use a ‘proxy classification model’ would significantly reduce the number of juvenile fish that would need to be collected and analyzed in order to classify adults of ecologically similar species.

In my third chapter, I determined whether the habitat tag found in juvenile English sole could be used to classify adult of this species. Adult fish were collected from the Monterey Bay Region in 2001 and 2002 and individuals that were born in 1998-2000 (the years that juvenile fish were collected) were selected for the analysis. The ‘juvenile core’ of each adult otolith was extracted and its chemical composition determined. The range of Sr/Ca and Li/Ca values in the juvenile cores were similar to the range of Sr/Ca and Li/Ca values found in juvenile otoliths. Therefore, the discriminant functions based on the chemical composition of otoliths from estuarine and coastal juveniles could be used to classify adult fish as having recruited from either estuarine or coastal habitats.

The percentage of the adults that were identified as having resided as juvenile in estuarine habitats was estimated to range between 46% and 57% for the entire study area and 45% and 53% for the Monterey Bay region. That is, estuarine contribution to the central California English sole population was estimated to be approximately 50% even though much less than 50% of the habitat available for use by juvenile English sole is estuarine habitat. For example, in the Monterey Bay region, it was estimated that estuaries comprise approximately 6% of the available juvenile habitat. This result strongly suggests that estuarine habitats in this region are acting as ‘nursery habitats’ by contributing more individuals per unit area to the adult English sole population than the adjacent coastal habitats. The disproportionate contribution of estuarine habitats to the adult populations may be due to fish in that habitat having higher densities, higher growth rates (as found in Chapter 1), lower mortality, or more successful recruitment to the adult population.

Many estuarine habitats in California, and around the globe, are vulnerable to loss or deterioration from a variety of processes, including erosion, pollution, and urbanization. Conservation of these estuarine ‘nursery’ habitats would protect an important source of new individuals to offshore adult populations and appears to be a useful strategy for maintaining high levels of recruitment to harvested flatfish populations in central California.

CHAPTER 1:

Variability in growth rates of juvenile flatfish in estuarine and open coast habitats: Evidence for estuaries as fish nurseries in central California

Introduction

Many marine fish and invertebrate species have a life history in which juveniles occur in habitats that are spatially distinct from those in which adults are found (reviewed in Gillanders et al. 2003). In addition, the juvenile phase often occurs in more than one habitat type (e.g., estuary, rocky reef, sandflat). When juveniles occur in multiple habitats, it is likely that the different areas will vary in their relative quality as juvenile habitat. Relative quality is frequently assessed by comparing density, growth, and survivorship of juveniles in two or more habitat types (e.g., Wilson et al. 1990, Irlandi and Crawford 1997, Rozas and Minello 1997, Meng et al. 2000).

Habitats that promote rapid growth are generally assumed to be high quality areas for juvenile fish and crustaceans because rapid growth implies that sufficient food is available and that juveniles will be less vulnerable to size-selective mortality. Size-selective mortality is common in juveniles and the most common sources of this type of mortality are predation and environmental stress (reviewed by Sogard 1997). Predators tend to take smaller individuals due to the gape limitation of fish predators, handling limitations of crustacean and bird predators, and higher avoidance and escape abilities of larger prey (e.g., van der Veer and Bergman 1987, Witting and Able 1993, Ellis and Gibson 1995). Smaller juveniles are also more vulnerable to over-winter mortality due to starvation or intolerance to the harsh conditions experienced during winters (Oliver et al. 1979, Hales and Able 2001). Higher growth rates not only decrease exposure to size-selective mortality, but because swimming speed and endurance tend to increase with fish size (Beamish 1978, Domenici 2001, Plaut 2001), individuals attaining a larger size at the end of the juvenile period may be more successful in completing the process of migration to the adult habitat. Therefore, a habitat that promotes higher growth rates may contribute more and larger juveniles to the adult population.

Growth rates have been shown to vary substantially between juvenile habitat types and locations, especially in estuarine environments. For example, many studies comparing growth rates in vegetated (seagrass, saltmarsh) and unvegetated (mudflat, open water) habitats have found that most species of fish and crustaceans grow faster in vegetated habitats (Minello et al. 2003, Heck et al. in press). However, unvegetated habitats have been shown to support equal or higher growth rates in certain species of benthic fish, such as flatfishes (Sogard 1992, Meng et al. 2000). The vast majority of studies comparing estuarine habitat types occurred in geographic regions where estuaries are large and numerous and contain many species that are estuarine-dependant (such as the northern Gulf of Mexico and the Atlantic coast of the United States; Houde and Rutherford 1993, Minello et al. 2003, Heck et al. in press).

Along the Pacific coast of the United States estuaries are few in number, small in size and isolated from one another by vast stretches of open coast (Nybakken et al. 1982). Though many species of fish and invertebrates have juvenile stages that occur in these estuaries (Horn and Allen 1976, Yoklavich et al. 1991, Valle et al. 1999), very few species are estuarine-dependant (Onuf et al. 1978, Monaco et al. 1992, Nybakken et al. 1982). Most species that use estuaries also occur in other, more abundant, habitats such as sandy bottom or rocky reef (Allen 1985). Therefore, studies attempting to determine the quality of juvenile habitats along the Pacific coast have focused primarily on comparing the relative quality of estuarine habitats as a whole to open coast habitats. One common method for making this comparison has been to measure growth rates in estuaries and the adjacent coastal habitats.

Juvenile flatfish are abundant in both estuaries and open coast sandy bottom habitats (hereafter referred to as “coastal habitats”) along the coast of California, Oregon and Washington (Olson and Pratt 1973, Krygier and Pearcy 1986, Allen and Herbinson 1990, Rogers et al. 1988, Kramer 1991). Determining the relative importance of estuarine and coastal habitats in supplying fish to the adult population is of great interest because of the long history of anthropogenic degradation of estuarine habitats, and because many of these species support commercial fisheries. However, studies that have compared the quality of estuarine and coastal habitats using growth rates of juvenile flatfish have found mixed results. Size-at-age comparisons of juvenile speckled sanddab (*Citharichthys stigmaeus*) and modal length progression comparisons of English sole (*Pleuronectes vetulus*) suggest that individuals living in estuaries have faster growth rates (Krygier and Pearcy 1986, Kendall 1993). However, Rosenberg (1982) and Kramer (1991) found, using similar methods, that the growth rates of English sole and California halibut (*Paralichthys californicus*) in estuarine and coastal habitats were indistinguishable. The purpose of this study was to evaluate the relative quality of estuaries and coastal habitats for flatfish populations along the central and northern California coast by comparing growth rates in the two habitat types.

Two methods for measuring habitat-specific growth rates were employed: caging experiments and analysis of the microstructure of otoliths (bones in the inner ear of fishes that can record the age and growth rate of fishes; reviewed in Campana and Neilson 1985). Cage experiments have the advantage of allowing the researcher to directly measure somatic growth in each habitat of interest. The disadvantage of this method are potential caging artifacts; altering growth rates by restricting foraging patterns, altering prey abundance, and releasing fish from predation and competition that would otherwise affect foraging rate or efficiency (Hulberg and Oliver 1980, Peterson and Black 1994). Analysis of otolith microstructure allows a comparison of growth rates of fish that were able to forage under natural conditions, but it assumes that fish spent a significant amount of time in the habitat of interest prior to capture and it requires that there is a positive linear relationship between otolith growth and somatic growth (Campana and Neilson 1985). By using both methods, I was able to determine if a pattern detected in habitat-specific growth rates using one method was consistent with that found using the other method.

The caging portion of this study was used to address an additional question: Are patterns in habitat-specific growth rates consistent in two species with similar life histories? English sole, *Pleuronectes vetulus,* and speckled sanddab, *Citharichthys stigameus,* were selected for the caging experiment because juveniles of both species: 1) occur in estuaries and coastal habitats in the geographic area of interest; 2) are small in size, demersal, and relatively sedentary; and 3) consume small, mobile crustaceans and worms that can move freely through cages.

The otolith microstructure portion of the study also was used to address an additional question: Are patterns in habitat-specific growth rates consistent over space (within habitats) and time (among years)? Otoliths from the speckled sanddab were used in this comparison because: 1) there is compelling evidence that increments in juvenile speckled sanddab are formed daily and can be used to detect variability in somatic growth rates (Kendall 1993); and 2) juveniles were sufficiently and consistently abundant in estuarine and coastal habitat along the central California coast to allow such comparisons.

Materials and Methods

This study focused on estuarine and coastal habitats along the approximately 500 km section of California between Bodega Harbor in the north and Pismo Beach in the south (Figure 1). Seven estuaries occur along this region of coastline. With the exception of San Francisco Bay, all are fairly small (~850 - 2,500 acres) with deep main channels, extensive mud flats, and varying amounts of tidal creeks, seagrass beds and salt marsh in the inland portions. Subtidal sandy bottom constitute the majority of the coastal habitats adjacent to the estuaries. Coastal habitats and estuaries tend to differ in their physical and biological attributes (temperature, prey availability) which may lead to differential growth rates in resident juvenile fish.

Caging Experiment

Monterey Bay is a half-moon shaped embayment (37 km wide) located on the central California coast (Figure 2). The majority of nearshore benthic habitat is composed of gently sloping sand bottom. Elkhorn Slough is a narrow, seasonal estuary that is located in the middle of the curve of Monterey Bay (Figure 2). It reaches 11 km inland and covers approximately 2,500 acres. English sole and speckled sanddab were held in cages to compare growth rates in the estuary to those in the adjacent coastal habitats of Monterey Bay. Cages were installed at four sites on the south bank of the main channel in Elkhorn Slough and four sites in Monterey Bay (Figure 2; Table 1). Substrate at the estuarine sites ranged from sandy mud at the bridge site to silty mud above the dairy. On the coast, cages were located on sand bottom at sites and depths (12-18 meters) where previously both species had been collected by otter trawl.

Cages consisted of a 0.75 x 0.75 x 0.25 m frame made of 3/4" diameter PVC pipe and elbows. Holes were drilled in the PVC frame to allow submergence and additional weight was added by enclosing pieces of rebar in the bottom sides of the frame. Each frame was fully enclosed with a stiff plastic mesh with holes (15 x 10 mm) that were small enough to prevent fish from escaping, but large enough to allow prey items move through the cages. During installation, sand anchors and polypropylene rope were used to cinch down the cages until the entire bottom of the cage was below the surface of the substrate. Sediment was gathered from surrounding areas and sifted through the mesh top of the cage until the bottom was covered with approximately 2 cm of sediment. Fish were released into each cage through a small re-sealable opening in the top.

Fish were collected two days prior to the beginning of the experiment by otter trawl from the subtidal sand habitats of northern Monterey Bay (Figure 2). The fish with the healthiest appearance were selected for the growth experiment. Fish of each species were sorted into four size classes (English sole: 83-89, 90-95, 96-99, 100-102 mm TL; speckled sanddab: 64-69, 70-75, 76-81, 82-94 mm TL) and each size class received a unique mark using a subdermal injection of colored elastomer (Visible Implant Fluorescent Elastomer). One fish from each size class was randomly assigned to a cage to ensure similar initial size ranges among cages. Each site had one cage containing four English sole and one cage containing four speckled sanddab. There was no significant difference in initial size of fish among habitats or sites (nested within habitats) for either species (two-way nested ANOVA, all $p>0.05$; Table 1).

Fish were re-measured and total length recorded (mm) immediately prior to release into cages. Fish were held in the cages for approximately 27 days in August of 2000 (Table 1). This time period was chosen to coincide with the lowest low tides of the monthly series (July 31 - August 30, 2000) so that cages in the estuary were slightly exposed and accessible (by wading from shore) during installation and removal, but fully submerged for the duration of the experiment. In addition, this was a time period in which both species were abundant in estuarine and coastal habitats and corresponded to the middle of the juvenile growth season.

All estuarine fish and all but two coastal fish were recovered at the end of the experiment (Table 1). Fish were measured while alive and growth rates were calculated as change in total length over the duration of the experiment (mm/d). One Elkhorn Slough site, ‘bend’, was removed from the analysis because it was the only estuarine site where cages were separated from the main channel by a sand/mud bar and appeared to have been an atypical growth environment. The effect of habitat (estuary and coast) and site (4 sites nested within each habitat) was estimated using a 2-way nested analysis of covariance using initial size as the covariate. The assumption of homogeneity of slopes was tested for all sites. Sites nested within habitat and habitat were random and fixed factors, respectively, in the analysis.

Otolith Microstructure

Speckled sanddabs between 46 and 95 mm (TL) were collected from estuarine and coastal habitats in four regions (Bodega, Tomales, Monterey, and Morro) along the coast of California in the summer of 1999 and 2000 (Figure 1). These regions were chosen to determine if there were spatial differences in growth rates over the geographic area of interest. Total length (mm) was recorded prior to dissection. Both sagittal otoliths were removed, cleaned of adhering tissue and stored dry in plastic vials.

The left sagittal otolith was imbedded in thermoplastic cement (Crystal Bond®) on a glass slide. Material was removed from the exposed surface (sagittal plane) using an Ingram Thin Sectioning Grinder Model 400U and a slurry with 20 µm grit until the primordium became visible under transmitted light. The thermoplastic cement was heated, the otolith turned over and then ground from the opposite side using the grit slurry and 6 µm diamond polishing compound until the microstructure was visible on the edges of the otolith surrounding the sulcus (at the rostrum). This region of the otoliths was targeted for analysis for two reasons. First, Sogard (1991) found that the rostrum of the left sagittal otolith provided the strongest relationship between somatic growth and otolith growth in winter flounder (*Pseudopleuronectes americanus*). Second, a visual survey of speckled sanddab otoliths revealed that the rostrum consistently had clearer microstructure than other regions of the otolith and that the sulcus was an easily identified landmark in this region.

However, choosing a transect along which to measure the width of daily increments that would be uniform across individuals was not possible for this species because of the structure of the otoliths. Speckled sanddab otoliths contained a variable number of accessory primordia which form during metamorphosis. Multiple accessory primordia caused the edges of juvenile otoliths to appear scalloped (Figure 3) with varying amounts of curvature in different individuals. To account for this variability in otolith structure among individuals, I chose to measure the width of randomly selected increments rather than a series of consecutive increments along a fixed transect. Increments were randomly selected within a wedge-shaped portion of the rostrum spanning 40 degrees and within 50 µm of the otolith edge. To select an increment for measurement, a transect line (-20 to +20 degrees from sulcus) and a distance (0-50 µm) were assigned randomly and the width of the increment closest to this point was measured. This procedure was repeated eight times for each otolith and the average width of the eight increments was used as an estimate of recent growth (Figure 3).

Five fish were selected for analysis to span the size range (TL) available for each habitat by region by year combination (Table 2). There was no difference in size of fish among habitats, regions or years (3-way ANOVA, all $p>0.10$). The width of increments was measured using Optimas image analysis software (V.6.5, Media Cybernetics). Images were captured using a SV Micro (Sound Vision) digital camera fitted to a Leica DMLB compound microscope (using 400X magnification).

Differences in average increment widths among the 2 years (1999 and 2000), 4 regions (Bodega, Tomales, Monterey and Morro) and 2 habitats (estuary and coast) were assessed with a 3-way ANCOVA using fish size (TL) as the covariate. The assumption of homogeneity of slopes was tested as described in Quinn and Keough (2002). Year and region were considered fixed factors in the analysis. Year was fixed because the two consecutive years were not randomly chosen from all possible years and inference should not be made to other types of years (e.g., an El Niño year).

Relationship Between Fish Size and Otolith Size

Daily increments are found in the otoliths of juveniles of many species and the width of these increments have been shown to correlate with somatic growth rates when a positive linear relationship exists between otolith size and fish size (Campana and Neilson 1985, Suthers 1998). Though Kendall (1993) found a strong linear relationship between otolith radius and total length for juvenile speckled sanddab collected from the San Francisco area, I tested for this relationship because the fish in this study were from a larger size class and expanded geographic area. The radius of otoliths was measured using the same image analysis system used to measure increment widths. Two measurements were made between the central primodium and the edge of the otolith; one to the point of maximum curvature in the scallop immediately to the left and one to the point of maximum curvature in the scallop immediately to the right of the sulcus (Figure 3). Left and right otolith radius were regressed on fish size (TL) to determine if there was a linear relationship between fish size and otolith size. All speckled sanddab used in the microstructure study (n=80) were used to determine this relationship.

Water Temperature in Monterey Bay and Elkhorn Slough

Because growth rates of fish are often positively related to water temperature, I compared water temperatures at estuary and coastal sites in Monterey Bay to determine if habitat-specific patterns in growth rates corresponded with differences in water temperature among habitats. The range of water temperatures experienced by juvenile flatfish in Elkhorn Slough was determined using data collected by the Elkhorn Slough National Estuarine Research Reserve water quality monitoring program. Bottom temperature was collected every 30 minutes by electronic temperature recorders. Data were available from January 1999 to June 2002 from the South Marsh site (a shallow marsh located above the caging sites) and March 2001 to June 2002 for the Vierra's site (located between caging sites along the south shore of the main channel) (Figure 2). Water temperatures experienced by fish living on the shallow sandy coastal habitats of Monterey Bay were determined from data collected November 1999 to June 2002 by the Partnership for the Interdisciplinary Study of Coastal Oceans (PISCO). Bottom temperature was collected hourly at Terrace Point (located in the northern portion of the bay) and Hopkins Marine Station (located in the southern portion of the bay) (Figure 2). A daily maximum, mean and minimum temperature was calculated for each site. These daily values were used to calculate monthly maximum, mean and minimum temperatures which then were used to compare the range of temperatures experienced by fish in the estuarine and coastal habitats of Monterey Bay.

Results

Caging Experiment

Speckled sanddab. For individual fish, the increase in total length over the duration of the experiment varied from 1 -12 mm in the estuary and 0 – 4 mm on the coast. ANCOVA results revealed that the relationship between growth rate and initial fish size differed between habitats (Table 3; Figure 4). Subsequent regression analysis determined that growth rates in Elkhorn Slough declined with increasing fish size (slope = -0.011, $F_{1,10}$=8.52, p=0.004) and that this relationship explained 58% of the variance in growth rates among estuarine fish. However, the relationship between growth rate and initial size was not significant in coastal fish (slope = -0.002, $F_{1,10}$=8.52, p=0.145). Plotting the 95% confidence intervals around the regression lines reveal that there is very little overlap in growth rates between habitats and that, over most of the size range examined, estuarine fish had higher growth rates than similarly sized coastal fish (Figure 4).

Comparison of growth rates of speckled sanddabs caged in a preliminary experiment I conducted in Elkhorn Slough in 1999 revealed a similar declining trend of growth rate on initial size (slope=0.010, $F_{1,29}$=11.436, p=0.002). However, the slope of this line is elevated compared to the data from 2000 suggesting faster growth rates in Elkhorn Slough in 1999. Growth rates ranged from 0.04-0.46 and 0.00-0.15 mm/day in Elkhorn Slough and Monterey Bay in 2000 and 0.00-0.55 mm/day in Elkhorn Slough in 1999.

English sole. For individual fish, the increase in total length over the duration of the experiment varied from 2 -11 mm in the estuary and 0 – 3 mm on the coast. Growth varied significantly between estuarine and coastal habitats, but not among sites nested within habitats or as a function of initial size (Table 3). Fish grew faster in Elkhorn Slough than in Monterey Bay with mean growth rates of 0.18 and 0.04 mm/day, respectively (Figure 4).

Otolith Microstructure

The average width of daily increments in juvenile speckled sanddab otoliths ranged from 2.05–3.74 μm. The three-way ANCOVA revealed that the assumption of homogeneity of slopes was not rejected and total length covaried with otoliths width (Table 4). However, this relationship with fish size (increment = 3.41-0.011*TL; $F_{1,78}$=0.882, p=0.008) only accounted for 9% of the variability in increment widths. The ANCOVA also detected a significant habitat by year interaction (Table 4). In 1999 increment widths were slightly smaller on the coast and larger in the estuary compared to those in 2000 (Figure 5). This result is consistent with the pattern in the cage experiment in which speckled sanddab had higher growth rates in the estuary in 1999. Though the interaction between habitat and year was significant, it did not overwhelm the general effect of habitat - in both years estuary fish had significantly larger increments than coastal fish (Figure 5). Mean increment widths (in μm) for 1999 and 2000 were 2.99 and 2.91 in estuarine and 2.30 and 2.55 in coastal habitats. Spatial variability, at the scale of regions, did not explain a significant portion of the variability in increment widths (Table 4).

Relationship Between Fish Size and Otolith Size

The radius of speckled sanddab otoliths increased with increasing fish size (TL) with TL explaining 77% of the variance in otolith radius (Figure 6). This relationship was similar for measurements taken on the left (OR = 10.50*TL + 188; $r^2 = 0.77$, $p < 0.001$) and right side of the sulcus (OR = 11.23*TL + 160; $r^2 = 0.76$, $p < 0.001$).Using the mean width of daily increments from all fish in the otolith microstructure study (2.69 μm), I calculated a daily increase in total length of 0.26 and 0.24 mm/d using the slope for the left and right radius, respectively. This range falls within the range of growth rates observed for speckled sanddabs in the cage experiment.

Water Temperature in Monterey Bay and Elkhorn Slough

Coastal bottom water temperatures from fall 1999 to summer 2002 ranged between 10-14˚C (Figure 7). Mean temperature tended to peak in the fall (September – November) and this temporal pattern was consistent across the two sites located at opposite ends of the Monterey Bay. A greater range of water temperatures was recorded in Elkhorn Slough (Figure 7). Temperatures ranged between 11-18˚C in the lower channel (Vierra's) and 10-21˚C in a shallow marsh (South Marsh). Despite this difference in the range of temperatures, both sites showed similar temporal patterns in water temperature, with the lowest temperatures occurring in the winter (Dec-Feb) and the highest temperatures occurring in the summer (May-September).

Discussion

Caging Experiment

The caging experiment demonstrated that juvenile English sole and speckled sanddab had higher growth rates in Elkhorn Slough as compared to Monterey Bay. Caging experiments are a valuable tool for comparing the relative growth of small juvenile fish in different habitats, but are known to have certain limitations (Peterson and Black 1994). Cages confine fish to a small space preventing them from moving to surrounding areas in search of food or to avoid sub-optimal environmental conditions. Cages may remove the negative impact that predator avoidance and competition for resources can have on growth rates. Cages can also alter local hydrodynamic patterns that influence sediment structure and both benthic and planktonic prey abundance. In addition, one must assume that any treatment effects of caging are constant across all habitats being compared.

There was little evidence that caging significantly altered the natural growth rates of speckled sanddab. Estimates of daily growth (0.24-0.26 mm/d) based on the slope of the relationship between fish length and otolith radius for wild fish (Figure 6) were within the range of growth rates experienced by caged fish (0-0.55 mm/d). In addition, growth rates in cages were similar to somatic growth rates estimated for speckled sanddab (0.15 mm/d) and Pacific sanddab, *Citharichthys sordidus* (0.13 mm/d) based on length-at-age and for Pacific sanddab (0.11-0.15 mm/day) based on modal length progression (Kendall 1983, Donohoe 2000).

Speckled sanddabs held in estuarine cages showed a decline in growth rate with initial body size. If this trend reflects a natural decline in growth rates as estuarine fish reach a larger size, it may explain the emigration of large juvenile speckled sanddab from the estuary in the fall. Perhaps the estuary is a particularly good growth environment for smaller juveniles, but this advantage diminishes with fish size until growth is similar to, or less than, that of fish living in coastal subtidal sand habitats. However, this decline in growth rates was not detected over the broader size range of fish used in the otolith microstructure analysis. Alternatively, cages may be more restrictive for larger fish resulting in lower growth rates for these individuals. Larger fish may require more food items or a different type of food item (size or species) than was available in the estuarine cages.

The growth rates of English sole in cages (0-0.39 mm/d) were lower than those (0.26-0.49 mm/d) reported in a number of studies (summarized by Shi et al.1997). Some of the discrepancy in these estimates may be due to the different methods that were used to estimate natural growth rates in English sole: this study directly measured the change in body size of individual fish; Rosenberg (1982) found a mean growth rate of 0.33 mm/d using length-at-age from fortnightly banding in otoliths; and three studies used modal length progression to estimate growth rates of 0.26-0.49 mm/d (Westrheim 1955, Krygier and Pearcy 1986, Shi et al. 1997). Each of these methods have associated problems that can result in under or over-estimation of natural growth rates. In addition, all the previous studies estimating the growth of juvenile English sole have focused on juveniles living in the estuarine and coastal habitats of Oregon and Washington. The lower growth rates measured in this study may be caused by lower habitat quality in California. English sole are not known to use estuarine habitats south of Morro Bay. Yoklavich (1982) proposed that estuaries in southern California were poor habitats for juveniles due to negative environmental conditions, particularly warm temperatures. Perhaps estuarine and coastal habitats in central California, though better then those in southern California, cannot support the high growth rates achieved by English sole farther to the north.

Using cages to compare growth rates requires the assumption that any effect of the treatment is consistent across habitats (Peterson and Black 1994). If this assumption holds, then the magnitude of the difference in growth rates is real even if the estimated growth rates do not reflect those attained under natural conditions in each habitat type. Hulberg and Oliver (1980) measured changes in sedimentation and invertebrate abundance inside cages placed on shallow subtidal sandflats in Monterey Bay and in the main channel of Elkhorn Slough. They found increased deposition of sediment and increased abundance of polychaetes inside cages in the estuary and on the coast. The presence of fish predators did not affect the abundance of invertebrates inside or near cages. Similarly, Meng et al. (2000) found the presence of cages and fish predators did not alter the density of invertebrates from ambient levels. These studies suggest that even though cages may alter the natural sedimentary processes or prey communities in and around cages, these changes probably occur in a similar fashion in all habitats. The growth rates measured in this caging experiment may not be natural growth rates, but the difference in growth rates probably reflects a real difference the quality of Elkhorn Slough and Monterey Bay as juvenile habitat for flatfish.

Otolith Microstructure

Daily increments in the otoliths of juvenile fish offer a valuable means for obtaining growth rates of individuals under natural conditions given two assumptions: 1) increment formation is daily in periodicity; and 2) otolith size positively correlated with fish size (Campana and Neilson 1985). Multiple attempts to experimentally verify the daily periodicity of increments in speckled sanddab failed because otolith material laid down while fish were held in captivity (either in the field or lab) contained very faint or completely invisible increments. Faint or invisible increments in captive fish have been found in other studies attempting to verify daily increments in the otoliths of speckled sanddab and Pacific sanddab (C. Donohoe pers. com.), English sole (Laroche et al. 1982) and winter flounder *Pseudopleuronectes americanus* (Sogard 1991). Despite this problem, direct validation was possible in some cases (Laroche et al. 1982, Sogard 1991) and in the others, there was substantial corroborating evidence that increments formed with daily periodicity (Kendall 1993, Donohoe 2000). The width of 'daily' increments was used to measure growth rates in all these studies because the second assumption - a positive linear relationships between fish size and otolith size – was met in all cases (Laroche et al. 1982, Sogard 1992, Kendall 1993, Donohoe 2000). In this study, increment widths were used to compare habitat-specific growth rates because I also found a strong relationship between otolith radius and fish length relationship for the size range of speckled sanddab examined (Figure 6).

Increment widths ranged from 2.05–3.74 µm in this study, which was similar to those measured previously in speckled sanddab (2.05 µm; Kendall 1983) and Pacific sanddab (1.7-2.2 µm; Donohoe 2000). I found that estuarine fish had consistently wider increments than coastal fish in four different regions and two consecutive years. This result is consistent with a study of speckled sanddab from the San Francisco Bay area in which speckled sanddab collected inside the estuary had, on average, otoliths with a larger radius (wider increments) than coastal individuals of the same age (see Figure 7 in Kendall 1983). Based on otolith microstructure, it appears that estuarine environments promote faster growth of juvenile speckled sanddab along the central California coast.

Factors Affecting Fish Growth

Food quality and quantity is a critical factor affecting fish growth. A number of studies that have found that juvenile flatfish living in areas with more or better quality food experience elevated growth rates (Jenkins et al. 1993, Berghahn et al.1995). In addition, growth rates of juvenile

English sole reared in captivity have been shown to be positively related to food ration over a wide range of temperatures (Williams and Caldwell 1978, Yoklavich 1982).

Juvenile speckled sanddab and English sole eat primarily infaunal and epifaunal polychaetes, crustaceans and mollusks (Hogue and Carey 1982, Gunderson et al. 1990). Suitable prey items have been found in subtidal sandflats and estuaries along the Pacific coast (Oliver et al. 1980, Nybakken et al. 1982, Gunderson et al. 1990), but there is some evidence that more prey items are available in estuaries, particularly in the summer months. In the Monterey Bay area, densities of infaunal and epifaunal invertebrates can reach 10,000/m^2 on the coast (Oliver et al. 1980) and 50,000/m^2 in Elkhorn Slough (Nybakken et al. 1977). Gunderson et al. (1990) reported similar differences in prey densities between Grays Harbor estuary and the coastal habitats in Washington. These higher densities of prey items in estuaries may support higher growth rates in juvenile flatfish living in estuaries compared to those living on the coast.

Water temperature can also have a strong influence on growth rates of juvenile flatfish. Reichert and van der Veer (1991) and Baltz et al. (1998) found that warmer water temperatures were responsible for faster growth rates in a variety of juvenile fish species. However, other studies found growth rates of juvenile flatfish to be negatively effected by rising water temperature (Sogard 1992, Meng et al. 2000, Manderson et al. 2002). This discrepancy is probably due to the fact that the relationship between growth rate and temperature is parabolic; growth rates tend to increase with temperature to an optimum beyond which growth rates decline rapidly (Williams and Caldwell 1978, Reichert and van der Veer 1991). In some cases, fish may be living in habitats with water temperatures that span their optimal range. Researches may inadvertently place cages in some locations where temperatures exceed the optimum resulting in lowered growth rates.

Laboratory experiments measuring growth rates of juvenile English sole under a range of water temperatures suggest an optimum temperature of about 15ºC; growth rates were unaffected by temperature changes between 9-15ºC, but were negatively effected as temperatures approached 18ºC (Williams and Caldwell 1978, Yoklavich 1982). Field surveys of speckled sanddab found that most individuals were located in areas with water temperatures between 11-17 ºC and the mean occurrence was 14 ºC (Ehrlich et al. 1979). Water temperatures in Elkhorn Slough during the summer months range between 13-18˚C in the main channel and 15-21ºC in the shallow marsh habitats. On the coast during the same period, temperatures vary between

10-13˚C. Increased growth rates in Elkhorn Slough may be promoted by the higher water temperatures found in the estuary. However, given that the temperature optimum of both species appear to be near 15 ºC, juvenile speckled sanddab and English sole probably avoid the shallower areas at times when temperatures far exceed their optimum. Surveys of flatfish distribution in Elkhorn Slough confirm this expectation – fish are much more abundant in the main channel than in shallow habitats such as tidal creeks and salt marshes (Yoklavich et al. 1991).

Conclusions

Because smaller fish are more vulnerable to predation and other sources of size-selective mortality, rapid growth is considered to be advantageous for juvenile (Sogard 1997). The results from my experiments suggest that estuarine habitats along the coast of central California support higher growth rates in juvenile flatfish than the adjacent sandy coastal habitats. Higher growth rates were found for both English sole and speckled sanddab held in cages in the Elkhorn Slough in one year (2000). In addition, otolith microstructure analysis using speckled sanddab indicated that these elevated growth rates are found in multiple estuarine habitats along the central coast of California over the two years studied. Though finer scale temporal differences in growth rate were detected - faster growth in estuaries in 1999 compare to 2000 – these differences did not obscure the overall effect of higher growth rates in estuarine habitats (Figure 5).

The definition of a nursery habitat was clarified recently by Beck et al. (2001): "A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur." Based on the potential advantages of faster growth rates- decreased vulnerability to size-selective mortality and increased migration success - estuarine habitats (especially Elkhorn Slough) may be functioning as nursery habitats for some flatfish populations in central California. To more fully determine the nursery value of estuaries in this (and other) regions, future studies are needed to quantify the actual biomass being produced per unit area of each habitat type.

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Table 1-1. Summary information for the experiment comparing growth rates of juvenile speckled sanddab and English sole in Monterey Bay and Elkhorn Slough in summer 2000. Shown are the location of sites where cages were installed, the start, end date and duration of the experiment, the number of fish recovered at the end of the experiment, and the initial size of fish (mean ± S.E., mm total length). There was no significant difference in initial size of fish among habitats or sites nested within habitats for either species (two-way nested ANOVA, all p>0.05).

Sites	Latitude	Longitude	------------ Date ------------		Duration	# Fish Recovered		--------- Initial Size ---------	
			Start	End	(days)	sanddab	sole	sanddab	sole
Elkhorn Slough									
Bridge	N36º48.63'	W121º46.94’	7/31/2000	8/28/2000	28	4	4	74.8 ± 4.0	94.3 ± 2.4
Bend	N36º48.74'	W121º46.22’	7/31/2000	8/27/2000	27	4	4	73.0 ± 5.3	95.3 ± 1.9
Below Dairy	N36º48.78'	W121º45.91’	8/1/2000	8/27/2000	26	4	4	73.3 ± 4.7	94.3 ± 3.0
Above Dairy	N36º48.85’	W121º45.20’	8/1/2000	8/27/2000	26	4	4	76.5 ± 3.5	93.3 ± 2.9
Monterey Bay									
Pacific Grove	N36º37.51'	W121º54.57'	8/1/2000	8/30/2000	29	3	4	82.0 ± 6.7	94.0 ± 3.9
Del Monte	N36º36.81'	W121º51.95'	8/3/2000	8/30/2000	27	4	4	78.8 ± 6.5	96.3 ± 3.1
Rio Del Mar	N36º57.48'	W121º54.56'	8/2/2000	8/29/2000	27	4	4	75.5 ± 3.9	95.0 ± 2.9
New Brighton	N36º57.98'	W121º55.97'	8/2/2000	8/29/2000	27	4	3	79.8 ± 5.9	94.7 ± 4.1

Table 1-2. Summary information showing mean size and size range of fish (mm TL) by habitat type (estuary or coast) in four regions and two years. There was no significant difference in size of fish among habitats, regions or years (three-way ANOVA, all p>0.10).

Region	-----------1999-----------		------------2000-----------	
	Mean TL	Range TL	Mean TL	Range TL
Bodega				
coast	63.3	55 - 71	67.4	61 - 77
estuary	59.4	52 - 67	62.8	52 - 76
Tomales				
coast	62.8	50 - 75	71.6	52 - 87
estuary	72.6	58 - 95	68.0	59 - 79
Monterey				
coast	67.8	58 - 73	76.0	60 - 89
estuary	71.6	64 - 79	66.4	51 - 90
Morro				
coast	69.0	46 - 85	78.0	67 - 87
estuary	65.6	56 - 85	70.6	52 - 90

Table 1-3. Effect of habitat (Elkhorn Slough and Monterey Bay), site, and total length (TL, covariate) on growth rates of speckled sanddab and English sole. The reduced ANCOVA model is shown for English sole because the slopes were homogeneous. df = degrees freedom; MS = mean squares.

Source of variation	df	MS	F	p
Speckled sanddab				
Habitat	1	0.051	18.09	0.008
TL	1	0.066	15.87	0.001
Site(Habitat)	5	0.003	0.67	0.650
Habitat x TL	1	0.036	8.52	0.009
Residual	18	0.004		
English sole				
Habitat	1	0.143	34.65	<0.001
TL	1	0.006	1.51	0.235
Site(Habitat)	5	0.004	0.93	0.485
Residual	19	0.004		

Table 1-4. Effect of habitat (estuary and coast), region, year and total length (TL, covariate) on increments widths in otoliths from juvenile speckled sanddab collected along the central California coast. The reduced ACOVA model is shown because slopes for all treatment combinations were homogeneous. df = degrees of freedom; MS = mean squares.

Source of variation		MS	F	p
Habitat	1	5.481	4.534	<0.001
Region	3	0.118	0.815	0.154
Year	1	0.134	0.068	0.155
TL (covariate)	1	0.945	14.581	<0.001
Habitat x Region	3	0.050	0.764	0.519
Region x Year	3	0.153	2.362	0.080
Habitat x Year	1	0.524	8.084	0.006
H x R x Y	3	0.148	2.286	0.087
Residual	63	0.065		

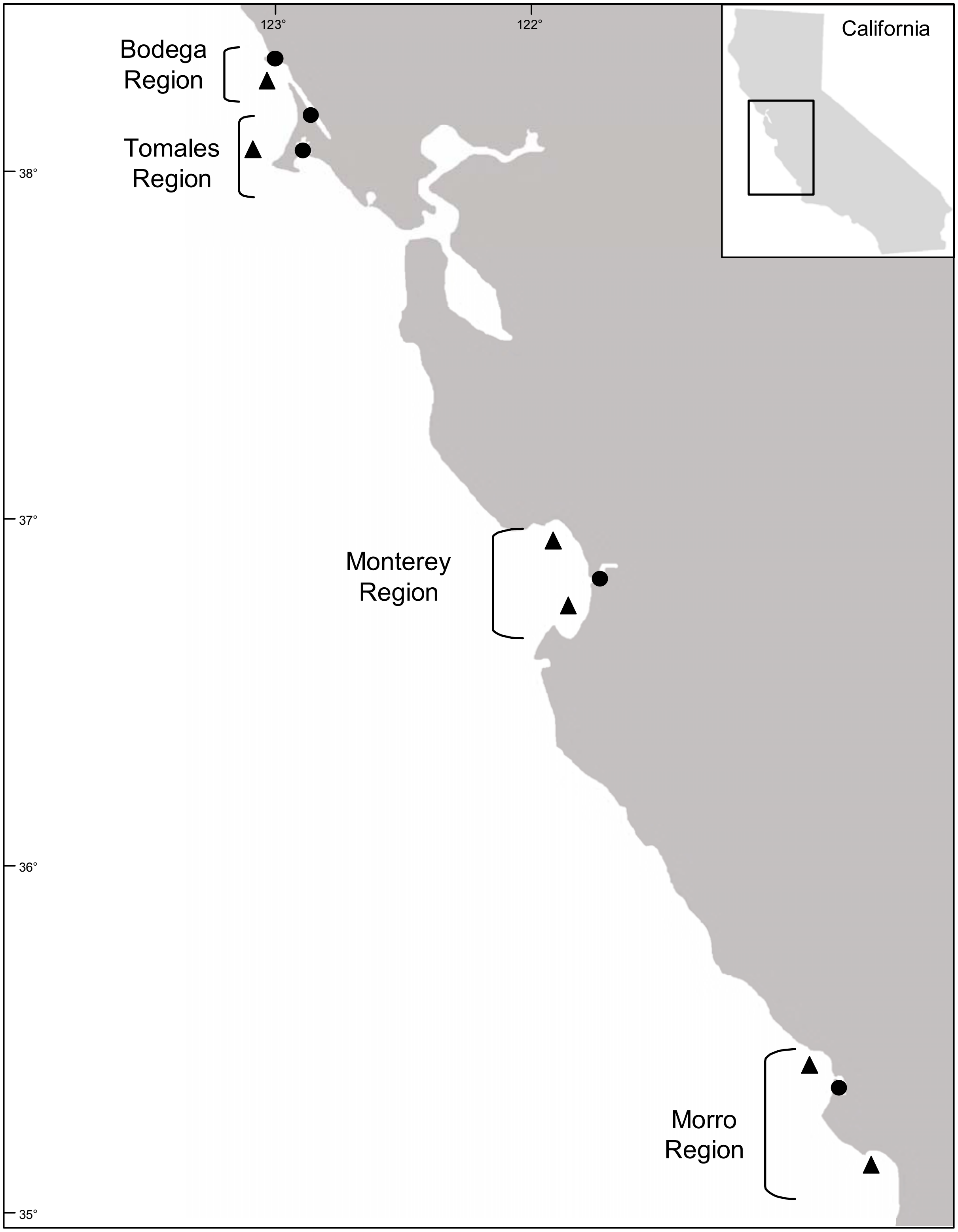

Figure 1-1. Map of California (inset) and the four regions along the coastline in which speckled sanddab were collected for comparison of growth rates using the width of daily increments in otoliths. In each region, otoliths from five fish in each of two habitats (open coast = triangle; estuary = circle) and two years (1999 and 2000) were examined.

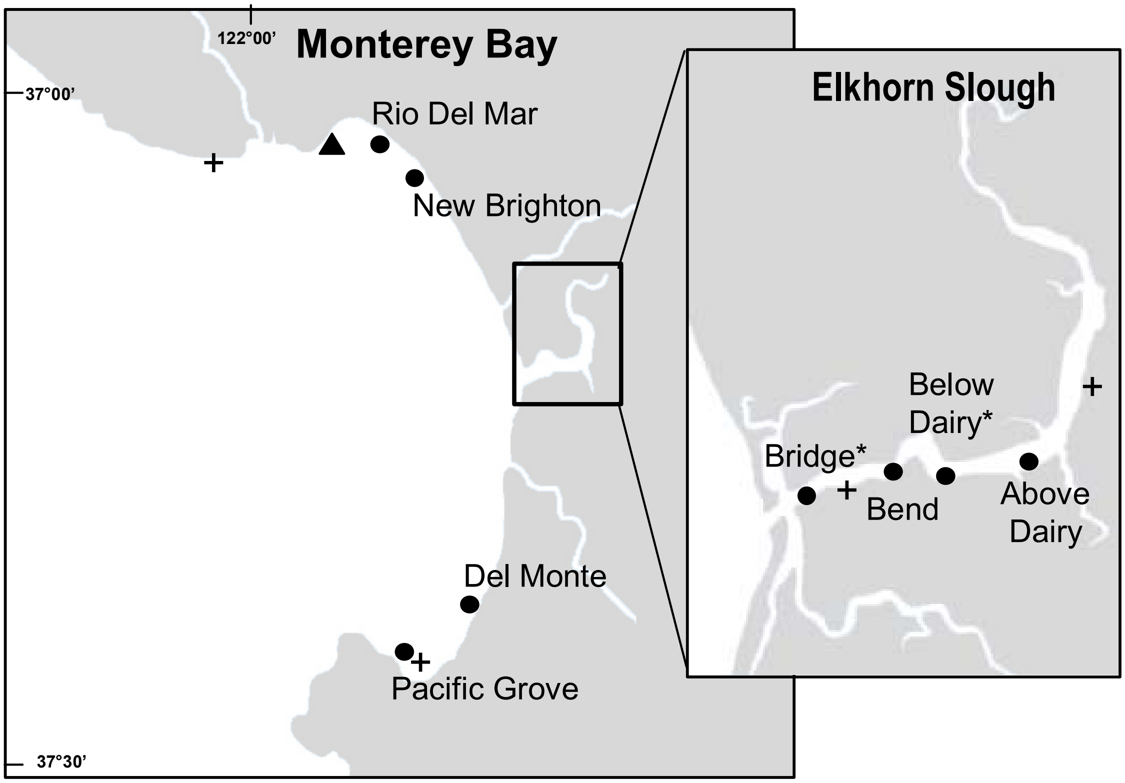

Figure 1-2. Map of Monterey Bay and Elkhorn Slough located on the coast of central California. Black circles denote sites where English sole and speckled sanddab were held in cages during the growth experiment in August 2000. The black triangle indicates the location where experimental fish were collected. Asterisks indicate sites in Elkhorn Slough where speckled sanddabs were recovered at the end of a growth experiment in October 1999. '**+**' indicates locations where bottom temperatures were recorded.

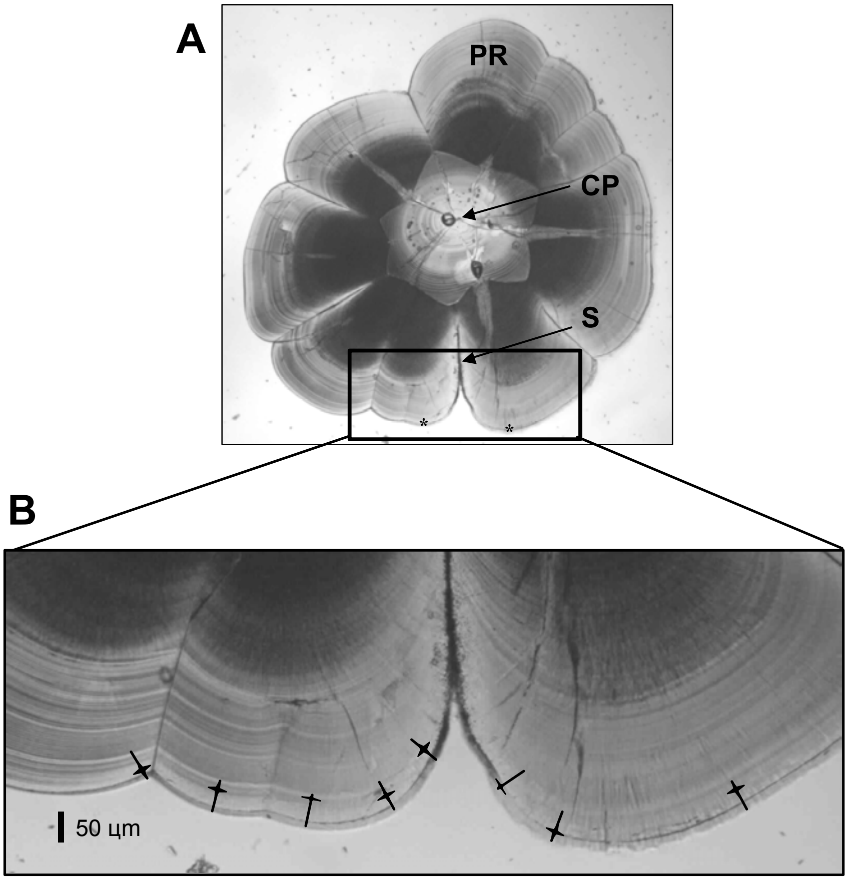

Figure 1-3. (A) Image of a left sagittal otolith from a speckled sanddab (50x magnification). Rectangle indicates the part of the rostrum surrounding the sulcus (S) where the widths of daily increments were measured (PR = post-rostrum; CP= central primordium). Radius was measured between central primordium and a point to the left and right of the sulcus (indicated by asterisks). (B) Enlargement (100x magnification) of the rostrum section demonstrating how eight increments were chosen for measurement using randomly assigned transects (± 20 degrees around sulcus) and distances (0-50 µm from edge).

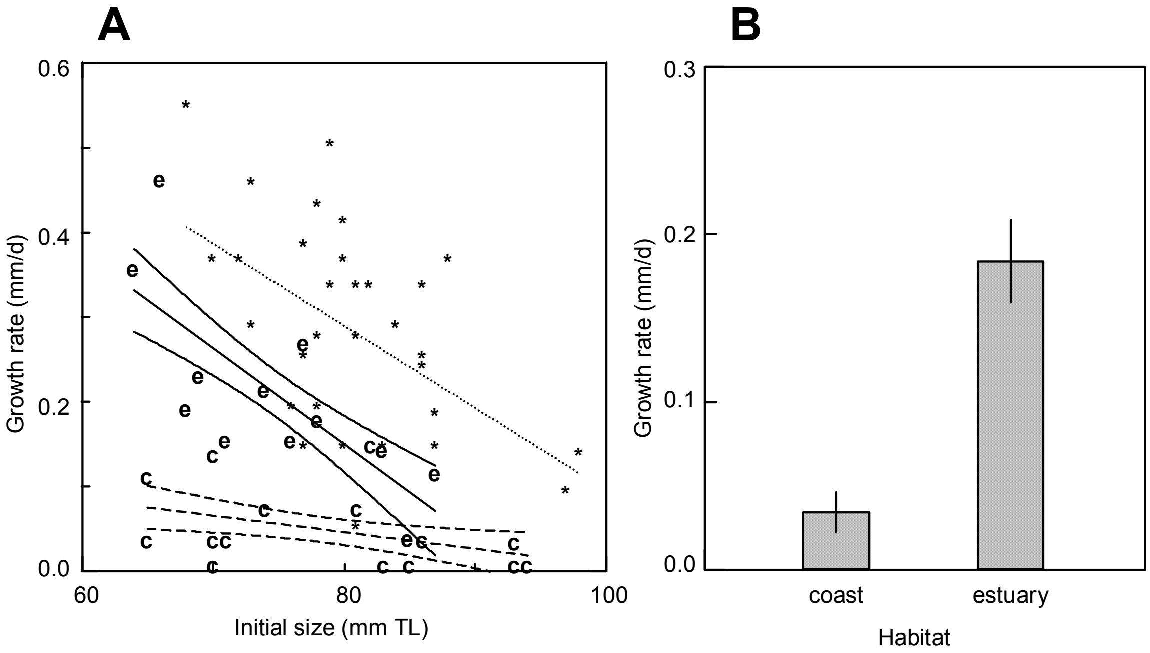

Figure 1-4. Growth rate of juvenile flatfish held in experimental cages located in an estuary (Elkhorn Slough) and on the coast (Monterey Bay). (A) Growth rates as a function of initial size for speckled sanddab caged in the estuary (e) and coast (c) in 2000. Slopes for estuary fish (-0.011, p=0.004; solid line ± 95% CI) and coast fish (-0.002, p=0.145; dashed line ± 95% CI) are heterogeneous (ANCOVA, $F_{1,18}$=8.52, p=0.009). Data from speckled sanddabs caged during a preliminary study conducted in the estuary in 1999 (*) are included for comparison (small dashed line; slope=0.010, p=0.002). (B) Least squares mean growth rate (± 1 SE) of English sole caged in coast and estuary habitats (initial size was not a significant covariate; $F_{1,19}$ = 1.51, p=0.24).

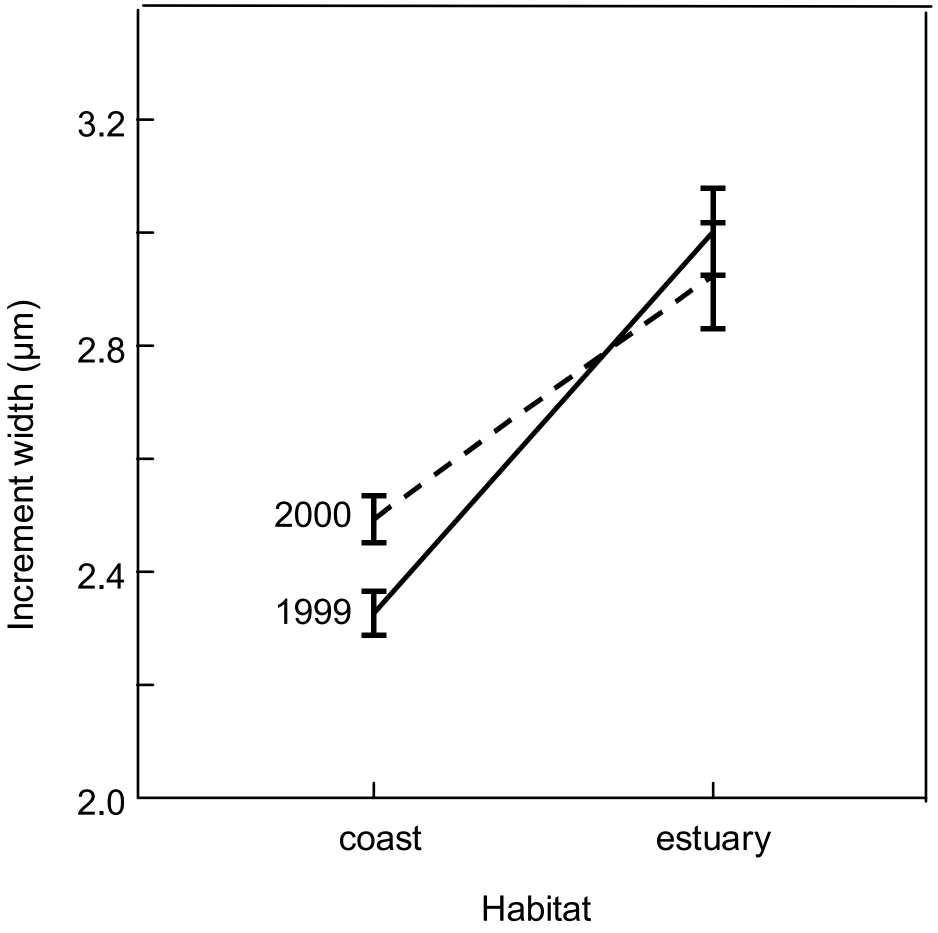

Figure 1-5. Interaction plot showing the width (LS mean ± SE) of daily increments in speckled sanddab otoliths for the two habitats and years examined. Though the interaction is significant, the plot illustrates that larger increments occurred in the otoliths of estuarine fish in both years.

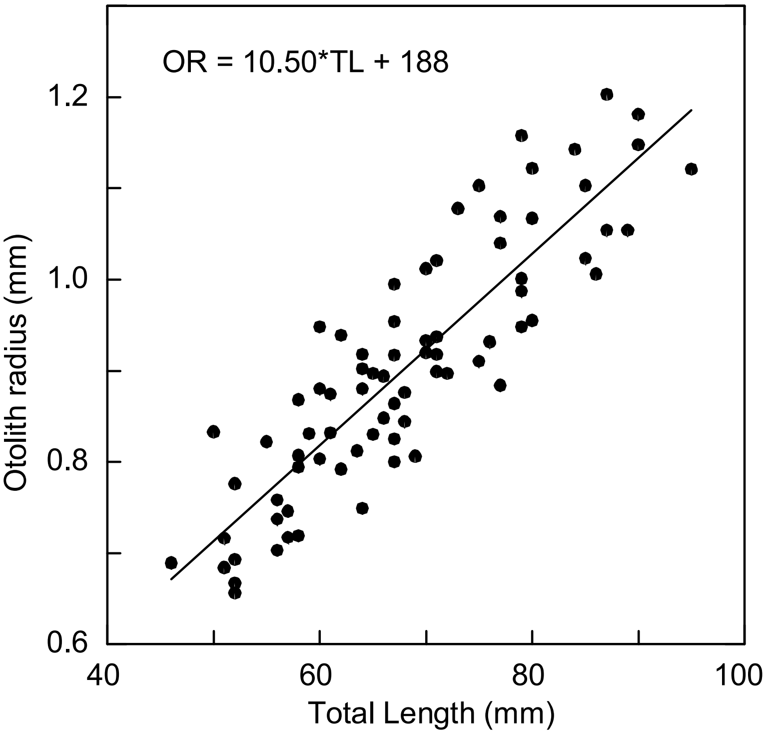

Figure 1-6. Relationship between otolith radius (µm) and total length of fish (mm) for juvenile speckled sanddab, *Citharichthys stigmaeus* (r^2 = 0.77, $p < 0.001$). Data shown are for the radius measured to the left of the sulcus. A similar relationship was found for the radius measured to the right of the sulcus (OR = 11.23*TL + 160; r^2 = 0.76, $p < 0.001$).

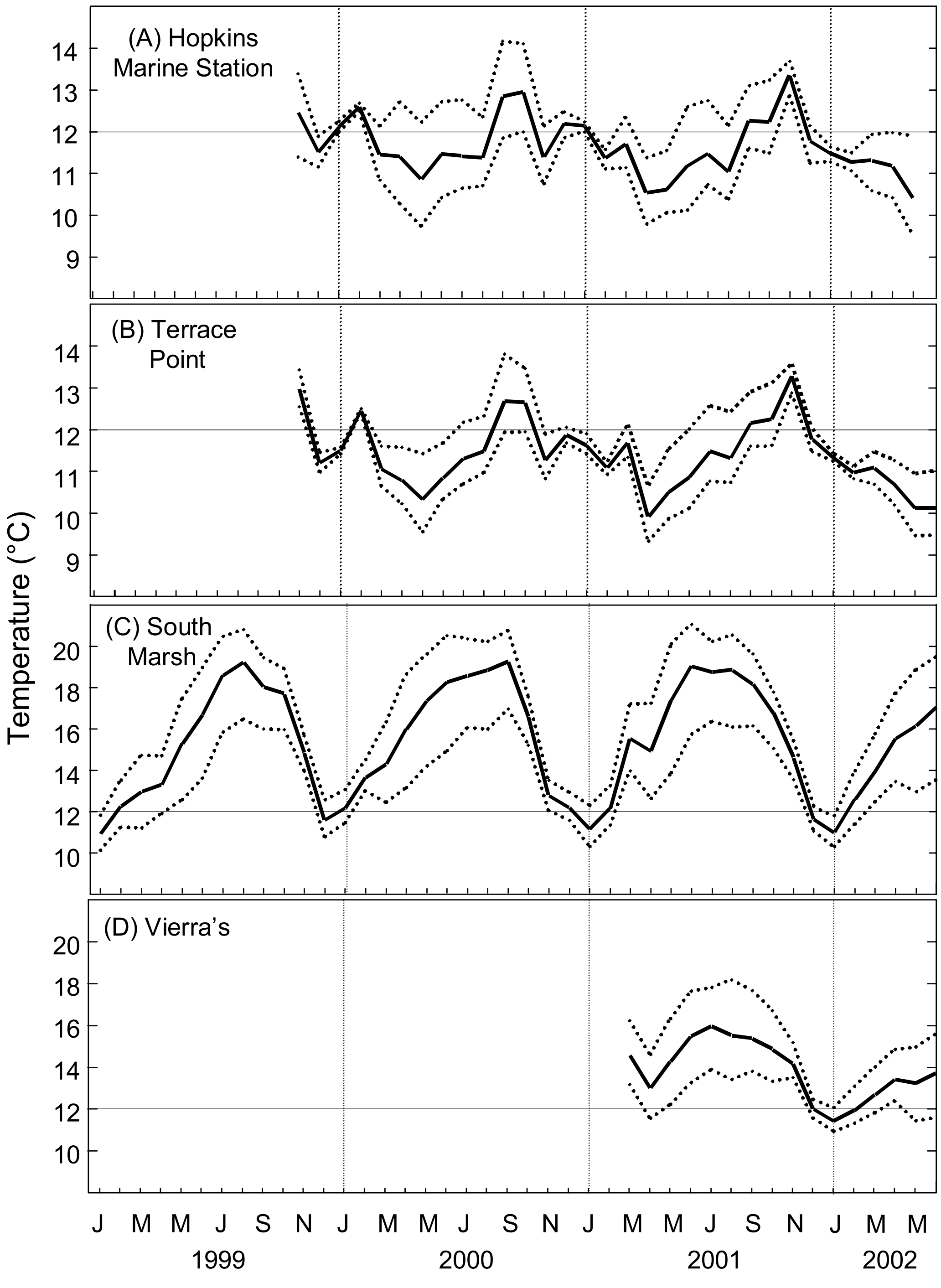

Figure 1-7. Monthly average of the daily maximum (dashed line), mean (solid line), and minimum (dashed line) bottom temperatures at nearshore sites in southern (A) and northern (B) Monterey Bay and two estuary sites in Elkhorn Slough; a shallow marsh site (C) and a main channel site (D). A line was drawn at 12°C as a reference due to the different temperature scales used for Monterey Bay and Elkhorn Slough sites.

CHAPTER 2:

Classification of juvenile flatfishes to estuarine and coastal habitats based on the elemental composition of otoliths

Introduction

Many species of marine fish have juvenile and adult phases that occupy separate habitat types. Often, the adults tend to occur in offshore habitats while the juveniles are found in one or more nearshore habitats such as estuaries, reefs, or shallow subtidal sandflats. For species with multiple juvenile habitat types, knowledge of the relative contribution of different habitat types to juvenile production and recruitment to offshore adult populations is fundamental to understanding population structure and dynamics. Such information is critical for both managing coastal fish populations and identifying ecologically important habitats and their resources, functions and services (Beck et al. 2001).

Most studies that have attempted to determine the relative contribution of different juvenile habitats have done so by comparing the ‘quality’ of those habitats. High quality habitats – habitats with higher density, growth rates or survivorship rates of resident juveniles – are assumed to contribute more juveniles to adult populations than lower quality habitats (reviewed in Minello et al. 2003 and Heck et al. in press). This method, however, does not directly measure contribution because the number of juveniles from each habitat type that successfully recruit to the adult population is not measured. The marking of juvenile fish in alternative habitats and subsequent recapture of those individuals as adults would allow a direct measure of the proportion of adult fish that recruited from different juvenile habitats. However, this method is logistically difficult to implement given the small size of juveniles, high rates of juvenile mortality, and large number of individuals that would have to be tagged to get meaningful results. A promising and more powerful alternative to manual tagging is available if fish residing in the juvenile habitats acquire naturally occurring ‘habitat tags’. Such natural habitat tags have been found in the chemical composition of fish otoliths.

The chemical composition of otoliths can be influenced by both physiological factors (growth rate, stress, reproductive activity) and environmental factors (salinity, temperature, elemental abundance in the water; reviewed in Campana 1999). If juvenile habitats differ in some of these factors, then those differences may be recorded in the otoliths of resident fish creating a chemical habitat tag (Fowler et al. 1995, Secor and Rooker 2000, Thorrold and Shuttleworth

2000). In addition, because otoliths are metabolically inert (Campana 1999), the habitat tag is permanently recorded in the otolith for the lifetime of the fish. Chemical habitat tags in the otoliths of juvenile fish have been used to differentiate individuals from different estuarine/riverine systems (Thorrold et al. 1998a, Thorrold et al. 1998b, Gillanders and Kingsford 2000, Gillanders 2002b) and alternative types of nearshore habitats, including estuary versus rocky reef (Gillanders and Kingsford 1996) and estuary versus subtidal sandflats (Yamashita et al. 2000, Forrester and Swearer 2002). In addition, through chemical analysis of the juvenile core of adult otoliths, the habitat tag has been used to determine the proportion of the adult population that resided in different juvenile habitats (Yamashita et al. 2000, Thorrold et al. 2001, Gillanders 2002a).

In order for chemical tags to be useful for differentiating adults that recruited from alternative juvenile habitats, detectable differences in the concentration of elements must be present in the otoliths of juveniles residing in those habitats and these differences should be spatially and temporally consistent (Campana et al. 2000). Spatial consistency in the habitat tag is needed over the geographic range of the adult population of interest. For example, if a chemical signature is to be used to distinguish adults that recruited from estuaries from those that recruited from coastal sandflats, then the chemical habitat tags must allow differentiation of juveniles collected from all estuarine and coastal sandflat habitats located within geographic range of the adult population. Temporal consistency in the habitat tag is required over the time period during which assignment of adults will be made. If the tag is found to be consistent over multiple years, particularly years with marked variation in environmental conditions (e.g., temperature, salinity, upwelling), then it can be used to classify adults of any age. However, if the tag changes from year to year, then adults should be classified using a habitat tag derived from the otoliths of juveniles collected in same year that the adults were residents in the juvenile habitats.

Numerous studies that have sampled multiple habitats in one year have found sufficient consistency in the chemical habitat tags to distinguish habitat types (e.g., different estuarine/riverine systems) over spatial scales ranging from 10s to 1000s of kilometers (Dove et al. 1996, Gillanders and Kingford 1996, Dove and Kingsford 1998, Secor and Zdanowicz 1998, Thorrold et al. 1998b) sometimes despite within year variability (Thorrold et al. 1998a). However, many of the studies that have compared chemical composition between habitats over two or more years have found significant interannual variability in the habitat tags (Milton et al.

1997, Dove and Kingsford 1998, Patterson et al. 1999, Gillanders and Kingsford 2000). For example, Gillanders and Kingsford (2000) found that juveniles collected from several estuaries could be differentiated within each year examined, but that the years could not be pooled because the chemical tag of some estuaries varied between years. Interannual variability in the habitat tag has limited comparisons between juveniles and adults in some studies because the adults had to be classified using habitat tags derived from juveniles collected in the same year(s) that the adults were born (Thorrold et al. 2001, Gillanders 2002a). However, interannual variability in the elemental composition of otoliths does not always obscure the habitat tag. For example, Yamashita et al. (2000) and Forrester and Swearer (2002) found that despite interannual variability in elemental concentrations in different estuaries, the estuaries as a group could still be differentiated from the open coast habitats over multiple years.

Purpose of this study was to determine if: 1) a chemical habitat tag existed that could be used to differentiate juvenile flatfish residing in estuaries and open coast habitats; 2) the habitat tag allowed differentiation of juveniles collected from estuarine and coastal habitats located throughout the geographic range of the adult population; and 3) the habitat tag was consistent over multiple years. Two additional aims of this study were to determine if: 1) two flatfish species shared a similar chemical habitat tag; and 2) by extension, one species could be used as a proxy to classify the other species. If elemental composition of otoliths differs between estuarine and coastal juveniles and those differences are due to environmental factors, then a similar habitat tag may be present in the otoliths of all resident species with a similar life history.

Study system

This study focused on two species of economically important flatfish with similar life histories – the English sole *Pleuronectes vetulus* and the speckled sanddab *Citharichthys stigmaeus*. Both species are found in abundance as juveniles and adults along the west coast of North America. Juveniles reside in either estuaries or shallow (<30m) coastal sandy habitats during the spring and summer of their first year. At the end of this first growth season, juveniles from both nearshore habitats move offshore to deeper coastal habitats to join the adult population (Laroche and Holton 1979, Starr et al. 1998). English sole and speckled sanddab larger than 140 and 105 mm (TL) are rarely encountered in estuarine habitats (Shi et al. 1997, Smith and Nitsos 1969, J. Brown unpublished data) so fish smaller than this size were considered juveniles for the purpose of this study.

Prior research on these two species, as well as other flatfish species along the west coast of North America, have found that densities tend to be higher and growth rates tend to be faster in estuaries compared to adjacent coastal habitats (Krygier and Pearcy 1986, Allen and Herbinson 1990, Rogers et al. 1988, Kramer 1991, Kendall 1993, Brown [Ch 1]). These results have been used to infer that estuaries are higher quality habitats that contribute more juvenile flatfish to the adult population than an equivalent area of coastal habitat (Olson and Pratt 1973, Rogers et al. 1988, Kramer 1991). However, these studies did not measure contribution directly. The existence of a reliable chemical habitat tag in the otoliths of flatfish would allow a more direct measure of the proportion of the adult populations that was reared in estuarine habitats and determine whether estuaries contribute disproportionately more individuals to the adult populations than do coastal habitats.

To effectively identify adult fish that recruited from either estuaries or sandy coastal habitats, the chemical habitat tags must allow differentiation of juveniles collected from all estuarine and coastal habitats located within geographic range of the adult population. The 500 km section of the California coast between Port San Luis and Bodega Bay (Figure 1) was selected for study for two reasons. First, this coastal section encompasses the geographic range of the ‘south-central California’ stock of the English sole population (as defined by Jow 1969). Hence, there was a low probability that adults collected along this section of the coast had resided as juveniles in estuarine and coastal habitats located outside of the study area. It was assumed that this area was sufficient in size to encompass the adult population of speckled sanddabs because they probably have less mobility than English sole given their smaller size and shorter life span. Second, this section of the coastline consisted of three distinct regions with coupled estuarine and coastal areas; the northern, central and southern regions (Figure 1). The existence of distinct regions allowed an assessment of the consistency of the habitat tag over two spatial scales; the global scale (the entire 500 km section) and the regional scale (100-200 km).

In addition, the temporal consistency of the habitat tag must be determined before it can be used to assign adult fish to juvenile habitats groups. I examined the temporal consistency of the juvenile habitat tag over three years with very different oceanographic conditions: 1) 1998 (El Niño year) was characterized by elevated water temperature, increased rainfall and decreased upwelling activity; 2) 1999 (La Niña year) was characterized by decreased water temperature and rainfall and increased upwelling activity; and 3) 2000 was characteristic of a ‘typical year’ with intermediate levels of all three factors.

Material and Methods

Sample Collection

Juvenile English sole and speckled sanddab were collected between May and September from up to 7 estuaries and 11 coastal sites along the 500 km portion of the California coast (Figure 1). The central and southern regions each contained one estuarine site surrounded by multiple coastal sites. The northern region contained multiple estuaries with coastal sites interspersed between them. All sites were sampled in 1999 and 2000. Due to logistical constraints, only coastal sites in the central region and a subset of all the estuarine sites were sampled in 1998 (Table 1). Coastal fish were collected by otter trawl and estuarine fish were collected using a combination of otter trawl and beach seine. Fish were kept on ice or frozen until dissection. Fish were measured (mm total length) and sagittal otoliths were removed, cleaned of adhering tissue, and stored dry in plastic vials.

Otolith Preparation and Analysis

Between 10-15 fish per species per site per year were selected for analysis when available (Table 1). Individuals were selected to cover the size range collected at each site. In some cases fewer than 10 fish had been collected so all individuals were analyzed. The right sagittal otolith from each fish was transferred to acid-cleaned Eppendorf microcentrifuge tubes, weighed (± 0.01 mg) and left to soak for 6 hours in 30% Suprapur (EM Scientific) H_2O_2 to remove organic material. Otoliths were then double-rinsed in Milli-Q water, triple-washed in dilute acid (0.01N HNO_3) to remove surface contaminants, and double-rinsed again in Milli-Q water. Otoliths were transferred to 4 ml Nalgene HDPE bottles and dissolved in 1% HNO_3 at a ratio of 2 ml of solution per 1 mg otolith (final concentration of ~200 ppm Ca). After weighing, all sample handling was performed in a HEPA class 100 laminar flow hood. Unless otherwise stated, all HNO_3 used was Trace Metal Grade (Fischer Scientific).

A procedural blank was prepared in the same manner as samples, but no otolith was present. The procedural blank was compared to the system blank to determine if contamination occurred during processing. System blanks were made from the same acid used for sample dissolution. System blanks were run every four samples and were used for blank corrections and to determine limits of detection. A spike standard was prepared by gravimetrically spiking a Ca standard solution with appropriate concentrations of Li, Mg, Mn, Ni, Cu, Zn, Sr, Ba, Pb to match the typical elemental composition of the otoliths. The spike standard was analyzed every four samples to track, and correct for, instrument drift. A consistency standard (a bulk digestion of English sole and speckled sanddab otoliths) was analyzed at the beginning of each run to assess consistency of measurements over multiple runs within a day and over multiple days. All measurements were carried out on a Finnigan MAT (Bremen, Germany) Element sector field inductively coupled plasma mass spectrometer (ICPMS).

Initially, all plasticware used for sample preparation and analysis was leached in 2N HNO_3 for 72 hours, rinsed thoroughly (five times) with Milli-Q water and dried in the clean hood prior to use. This procedure was changed mid-way through the experiment when continued leaching of Mg from the 4 ml Nalgene bottles was detected in procedural blanks that had a lag period between preparation and analysis. In the new cleaning procedure, bottles were leached for one week in heated 3 N HCl (ACS grade), rinsed in Milli-Q, leached again in heated 3 N HNO_3 (ACS grade), and rinsed thoroughly in Milli-Q. Bottles were either dried in the hood and used immediately or left soaking in a bath of 2 N HNO_3 (trace metal grade) until needed. Contamination from plasticware was not detected for any of the other elements of interest.

Ten elements (^{7}Li, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{62}Ni, ^{63}Cu, ^{66}Zn, ^{87}Sr, ^{138}Ba, ^{208}Pb) were initially chosen for study based on preliminary analysis of the composition of juvenile flatfish otoliths. The limits of detection of each element were calculated for each run as three times the standard deviation of the system blank and were applied to blank corrected intensities. Ni, Cu, Zn, and Pb intensities were frequently at or below detection limits and, therefore, were removed from the analysis. Mg was removed due to possible contamination from 4 ml bottles as noted above. Li, Mn, Sr, and Ba were consistently above detection limits and were used for statistical analysis. Element/Ca^{43} ratios were determined from blank-corrected intensities using the spiked standard to correct for instrumental mass discrimination (following Rosenthal et al. 1999). Estimates of precision (% relative standard deviation) based on repeated analysis of the otolith consistency standard were determined for each elemental ratio to be: ^{7}Li/^{43}Ca = 2.62%, ^{55}Mn/^{43}Ca = 1.17%, ^{87}Sr/^{43}Ca = 1.15%, ^{138}Ba/^{43}Ca = 1.49%.

Data analysis

Prior to statistical analyses of elemental composition of otoliths, data from each element was checked for univariate normality and homogeneity of variances (following Quinn and Keough

2002). For both species, Mn, Sr, and Ba were normally distributed after $\log_{10}$-transformation; Li was normally distributed without transformation. To ensure that differences in otolith size (≈ fish size) among samples did not confound any spatial or temporal patterns in elemental composition, I tested for a relationship between otolith weight and elemental ratio. Only Li showed a significant linear relationship between otolith weight and element concentration; this relationship was consistent among habitats, regions and years. Hence, the Li values were detrended using a two step process: 1) the slope of the regression line was used to estimate a Li value for a pre-determined otolith weight (4.5 mg for speckled sanddab and 7.0 mg for English sole); and 2) for each sample, the specific residual from the regression was added to this estimated Li value to produce a distribution of sample Li values that was unaffected by the effects of otolith weight. The pre-determined otolith weights were the average weight of otoliths in juveniles collected at the end of the first growth season (corresponding to 140 and 105 mm TL for English sole and speckled sanddab, respectively). By detrending Li values to an 'end of season' otolith weight, the resulting statistical models could be used to classify adult fish using the chemical composition of the juvenile core of the adult otolith. Juvenile cores contain otolith material that was accumulated over the entire juvenile growth season (see Brown [Ch 3]).

Discriminant function analysis (DFA) was used to determine whether a chemical habitat tag existed in the otoliths of speckled sanddab and English sole. Discriminant functions were calculated using SYSTAT and these functions were used to classify juveniles into two groups based on habitat type (estuary or coast) using a jack-knife (leave-one-out) approach. The classification accuracy was determined by comparing the jack-knife predicted group membership to the actual group membership and calculating the percentage of individuals that were correctly classified. For each species three classification models were examined; global, years and regions. In addition, DFA was used to the compare the habitat tags between the two species. Assumptions of DFA, including multivariate normality and homogeneity of variance-covariance matrices, were checked graphically using squared-Mahalanobis distance plots and by examining the spread of each group on the canonical variate axes (following Tebachnick and Fidell 2001).

Global Classification Model

A robust chemical habitat tag would allow fish to be classified into juvenile habitat type (estuary or coast) regardless of the region or year in which it was collected. The "global model" used here was intended to determine how accurately elemental composition could be used to classify juveniles of a given species over all regions and years combined. Given the large sample sizes (490 for speckled sanddab and 455 for English sole), quadratic DFA (QDFA) could be used. Even though the data did not violate the assumptions of linear DFA (LDFA) including homogeneity of variance-covariance matrices, slight difference in the variance structure of the groups can be accounted for in QDFA and the overall classification accuracy of the model can be improved.

Years Classification Model

To determine if annual changes in the elemental composition of otoliths deceased my ability to accurately classify juvenile fish, I ran four LDFAs: one which pooled data over all years and one for each of the three years in the study. The classification accuracy of each year was compared to that of all years combined to determine if classification could be improved by treating years separately. The four classification models only included sites from which individuals of a given species had been collected in all three years (Table 1). Five estuaries (BH, TB, SFB, ES, MORR) and three coastal sites (PR, SR, MY) were included in the models for speckled sanddab. Four estuaries (TB, SFB, ES, MORR) and three coastal sites were included (SC, PR, SR) in the models for English sole. I used LDFA because samples sizes were too small for QDFA in some of the groups and because evaluation of the assumptions of LDFA (multivariate normality and equality of variance-covariance matrices) revealed no violation of these assumptions.

Regional Classification Model

To determine if regional differences in elemental composition of otoliths decreased my ability to accurately classify juvenile fish, I compared a LDFA which pooled across all regions to LDFAs for each region separately. The classification accuracy of each region was compared to that of all regions combined to determine if classification could be improved by treating regions separately. The four classification models only included fish collected in 1999 and 2000 because coastal habitats were not sampled in the northern and southern regions in 1998 (Table 1). Again, I used LDFA to classify individuals in these analyses because of reduced sample sizes in some of the comparisons and because evaluation of the assumptions revealed no violations.

Species Comparison

English sole and speckled sanddab collected in this study should have experienced very similar environmental conditions during residence in the juvenile habitats. To determine if differences in estuarine and coastal habitats translated into similar habitat tags in the otoliths of the two species, I combined the two transformed and detrended datasets (see above). First, I used QDFA on the combined dataset to determine if estuarine and coastal fish could be classified using data from both species combined. Then, to determine if one species could be used as a proxy for the other, I used the discriminant function from the speckled sanddab global classification model to classify English sole. This was repeated using the English sole global model to classify speckled sanddab. To determine if classification accuracy was impacted by combining years, I repeated the analysis using only data from fish collected in either 1999 or 2000.

Patterns in Elemental Concentrations

In an attempt to explain the classification accuracy of the various models in this study, I investigated spatial and temporal patterns in elemental concentrations using ANOVA techniques. For the global classification model, I compared elemental concentration between estuarine and coastal habitats for each species using a one-way ANOVA procedure. For the years classification model, I compared elemental concentrations using a two-way ANOVA procedure with habitat and year as main effects. Only sites where fish were collected in all three years were used in the analysis. For the regions classification model, I compared elemental concentrations using a three-way ANOVA procedure with habitat, region and year as main effects. Habitats, years and regions were considered fixed factors because I was only evaluating patterns related to the specific regions and years examined in this study.

Results

English sole

Global Classification Model

The discriminant function was able to separate estuarine and coastal groups (Wilk's λ, $F_{4,450}=78.60$, $p<0.001$). Using the jack-knife classification procedure on the sample of 455 fish, 80% of coastal fish (n=238) and 76% of estuarine fish (n=217) were correctly classified for an overall classification accuracy of 78% (Table 2). The canonical correlations of predictor variables with the discriminant function suggested that the best single variables for distinguishing between estuarine and coastal juveniles were Sr and Li (Table 3). Estuarine fish had higher Sr (0.30 ± 0.003 mmol/mol) and lower Li (4.38 ± 0.042 µmol/mol) than coastal fish (0.24 ± 0.003 mmol/mol; 5.09 ± 0.040 µmol/mol) (Figures 2A & 3A). Figure 4 shows the percentage of fish that were correctly classified by the global model by site for all years and for each year separately. Most sites have a high classification accuracy; greater than 80% of fish were correctly classified for 12 of the 18 sites. The sites with a large percentage of misclassified fish tended to have levels of Sr or Li that were different from the other sites in the same habitat group. For example, Drake's Bay and Bodega Bay had higher Sr values that the other coastal sites (Figure 2A) and Bolinas Lagoon had higher Li values than the other estuarine sites (Figure 3A).

Years Classification Model

Across all years combined, the jack-knife classification procedure correctly classified 91% of coastal fish and 84% of estuarine fish for an overall classification accuracy of 87% (Table 2). By making separate discriminant functions for each year, I was able to increase the total classification accuracy to 93%, 89% and 92% in 1998, 1999 and 2000, respectively. The increases were due to increased classification success for coastal fish in 1999 (97%) and for estuarine fish in 1998 (97%) and 2000 (93%) (Table 2).

The canonical loadings suggested that the best predictors for distinguishing between estuarine and coastal juveniles in all years combined were Sr and Li (Table 3). However, increased classification accuracy in individual years was due to Mn becoming a useful predictor in 1998 and 1999 and Ba becoming a useful predictor in 2000 (Table 3). Significant habitat, year, and habitat by year interaction effects in the two-way ANOVAs indicated that concentrations of Mn and Ba in estuarine and coastal habitats differed among years (Table 4). Ba and Mn were higher in coastal fish in some years, especially at the sites used in this reduced classification model (coastal sites in the central region) (Figures 5C and 6C).

Regional Classification Model

Across all regions, the jack-knife classification procedure correctly classified 78% of coastal fish and 73% of estuarine fish for an overall classification accuracy of 76% (Table 2). By creating separate discriminant functions for each region, I was able to increase the total classification accuracy to 80%, 86% and 80% in northern, central and southern regions, respectively. The improvements were due to increased classification success for coastal fish in the central region

(87%) and for estuarine fish in the northern (80%), central (82%) and southern (81%) regions (Table 2).

The canonical loading suggested that the best predictors for distinguishing between estuarine and coastal juveniles across all regions combined and within separate regions were Sr and Li (Table 3). Li was higher in coastal fish and this difference was consistent across all regions (Figure 3). ANOVA revealed significant habitat and region effects on Sr concentrations (Table 4). Sr was higher in estuarine fish compared to coastal fish in all three regions, but Sr concentrations were elevated in all fish from the northern region as compared to the other two regions (Figure 2). Pooling across regions probably caused a decrease in classification success due to coastal sites from the north resembling estuarine sites from the central and southern regions.

Speckled sanddab

Global Classification Model

The discriminant function was able to separate the estuarine and coastal groups (Wilk's λ, $F_{4,485}$=52.75, p<0.001). Using the jack-knife classification procedure for the sample of 490 fish, 77% of coastal fish (n=281) and 81% of estuarine fish (n=209) were correctly classified for an overall classification accuracy of 79% (Table 2). Similar to the findings for English sole, the canonical correlations of predictor variables with the discriminant function suggested that the best single variables for distinguishing between estuarine and coastal fish were Sr and Li (Table 3). Estuarine fish had higher Sr (0.29±0.002 mmol/mol) and lower Li (4.66±0.033 µmol/mol) than coastal fish (0.26±0.002 mmol/mol; 4.97±0.028 µmol/mol) (Figures 2B & 3B). Figure 7 shows the percentage of fish that were correctly classified in the global model by site for all years and for each year separately. Most sites have a high classification accuracy; 12 of 18 sites have more than 75% of fish correctly classified. The sites with large numbers of misclassified fish tended to have levels of Sr or Li that were different from the other sites in the same habitat group. For example, Salinas River had higher mean Sr values that the other coastal sites (Figure 2B) and Bodega Harbor had higher mean Li values than the other estuarine sites (Figure 3B).

Years Classification Model

Across all years combined, the jack-knife classification procedure correctly classified 73% of coastal fish and 77% of estuarine fish for an overall classification accuracy of 76% (Table 2). By making separate discriminant functions for each year, I was able to increase the total classification accuracy to 85% and 80% in 1998 and 2000, respectively. The improvement was due to increased classification success for both coastal (85%) and estuarine fish (86%) in 1998 and for estuarine fish (83%) in 2000. In sharp contrast, classification rates were reduced to 66% and 68% for coastal and estuarine fish in 1999 for a total of only 67% correctly classified.

The canonical loadings suggested that the best predictors for distinguishing between estuarine and coastal juveniles across all years combined and within each year were Sr and Ba (Table 3). ANOVA revealed a strong effect of habitat on Sr concentrations (Table 4) with Sr being consistently higher in estuarine fish (Figure 2). Ba has strong habitat, year and habitat by year interaction effects, which probably caused classification success to differ among years (Table 4). Ba was higher in coastal fish in 1998 and 2000 allowing high classification success in those years, but there was no difference in Ba concentration between habitats in 1999 leading to decreased classification success in that year (Figure 5).

Regional Classification Model

Across all regions, the jack-knife classification procedure correctly classified 76% of coastal fish and 78% of estuarine fish for an overall classification accuracy of 77% (Table 2). By creating separate discriminant functions for each region, I was able to increase the total classification accuracy to 79% and 88% in northern and southern regions, respectively. The improvements were due to increased classification success for estuarine fish in the southern region (82%) and for coastal fish in the northern (83%) and southern (89%) regions. Total classification success decreased in the central region to 67% with only 58% of estuarine fish classified correctly (Table 2).

The canonical loadings suggested that the best predictor for distinguishing between estuarine and coastal juveniles across all regions combined and within each region separately was Sr (Table 3). Sr was consistently higher in estuarine fish (Figure 2), but ANOVA revealed significant region and region by habitat interaction effects on Sr concentrations (Table 4). Sr concentrations appear to be elevated in coastal fish from sites in the central region (Figure 2). Higher Sr in coastal fish causes less separation between estuarine and coastal groups from the central region and probably led to decrease classification accuracy relative to the other two regions.

Species comparison

The otoliths of juvenile English sole and speckled sanddab showed similar patterns in elemental concentrations in estuarine and coastal habitats. The discriminant function produced from the combined dataset was able to significantly separate the estuarine and coastal groups (Wilk's λ, $F_{4,940}$=116.3, p<0.001). The jack-knife classification procedure correctly classified 79% of coastal fish and 75% of estuarine fish for an overall classification accuracy of 77% (Table 5). The canonical correlations of predictor variables with the discriminant function again suggested that the best single variables for distinguishing between estuarine and coastal juveniles were Sr and Li (Table 3). Estuarine fish had higher Sr and lower Li than coastal fish, which is consistent with the patterns found for each species individually.

The total classification accuracy decreased to 71% and 69 % when the discriminant functions derived from the global model of one species was used to classify individuals of the other species (Table 5). This decrease was due to lower classification success for the estuarine fish; 64% of estuarine speckled sanddab and 54% of estuarine English sole were correctly classified. The decreased classification accuracy may have been caused by differences in the mean value of Sr and Li between estuarine speckled sanddab (0.286 mmol/mol Sr; 4.66 µmol/mol Li) and English sole (0.300 mmol/mol Sr; 4.384 µmol/mol Li). However, classification success was somewhat improved by limiting the analysis to fish collected in only one year (Table 5).

Discussion

Global Classification Model

The use of a chemical tag to differentiate fish from alternative juvenile habitats requires that there are measurable differences in the elemental composition of otoliths in fish residing in those habitats. In addition, to use this tag to determine prior residence of adult fish, the chemical differences must be sufficiently consistent to distinguish the habitats of interest over both the geographic range of the adult population and the time period during which adults are to be classified. The most important finding in this study, was that juveniles collected from the two habitats had otoliths that differed significantly in their elemental composition and that these differences could be used as a habitat tag to identify estuarine and coastal English sole and speckled sanddabs. This habitat tag was found to be stable over a 500 km section of the California coastline that encompasses the geographic range of the adult populations. In addition, this habitat tag was found to be stable over three years with very different environmental conditions: El Niño, La Niña and typical years. The close to 80% classification success of the global model was due to spatial and temporal consistency in the levels of Sr and Li in the otoliths of estuarine and coastal fish. For both species, Sr/Ca was consistently higher and Li/Ca was consistently lower in estuarine habitats.

Sr levels in otoliths have been shown to be influenced by a number of environmental and physiological factors, such as temperature, salinity, and growth rates. Generally, Sr/Ca ratios have been reported to be positively correlated with salinity (Kalish 1990, Secor 1992, Secor et al. 1998, Secor and Rooker 2000) and negatively correlated with temperature (Radtke 1989, Townsend et al. 1995, Secor and Rooker 2000) and growth rate (Toole et al. 1993, Sadovy and Severin 1992,1994). However, a few studies have found the opposite patterns; Sr/Ca ratios negatively correlated with salinity (Radtke et al. 1988, Yamashita et al. 2000) and positively correlated with temperature and growth rate (Kalish 1989, Yamashita et al. 2000). Yamashita et al. (2000) suggested that elevated Sr/Ca in the otoliths of estuarine juveniles may not be related directly to the temperature or salinity of the water *per se*, but instead may be caused by higher ‘stress’ in estuarine environments due to daily fluctuations in water temperature and salinity. The estuarine habitats examined in this study are characterized (during the summer/fall juvenile growth season) by higher salinities, faster growth rates and warmer and more variable water temperatures than the surrounding coastal habitats (Broenkow 1977, [Brown Ch 1]). The present study could not determine which of these factors may have been responsible for causing the pattern of elevated Sr/Ca values in estuarine fish, but the pattern appears to be due to one or more factors that differed consistently between estuarine and coastal habitat types over three years with different oceanographic conditions.

Though multiple studies have found Li to be a useful element for differentiating habitat groups (Milton et al. 1997, Campana et al. 2000, Gillanders 2002b), very little attention has been given to determining the environmental and physiological factors that effect Li incorporation into otoliths. Fluvial input from continents is one of the major sources of dissolved Li in coastal waters and the abundance of this element has been shown to be negatively correlated with salinity (Bruland 1983). There is some evidence that Li/Ca ratios in otoliths may be inversely related to salinity. Milton and Chenery (2001) found that juvenile barramundi held in freshwater had lower Li/Ca levels in their otoliths than fish held in seawater. A negative relationship between Li/Ca and salinity is consistent with the pattern found in this study of elevated Li/Ca values in the otoliths of coastal juveniles. However, the present study also found a significant relationship between otolith weight and Li/Ca indicating that Li incorporation into otoliths was also influenced by ontogenic processes. These results indicate that the processes influencing the uptake of Li into otoliths requires further study. Fortunately, it is not necessary to understand the mechanisms responsible for generating habitat related differences in elemental concentrations to be able to use those chemical differences as habitat tags (Thorrold et al. 1998a).

The global classification model had an accuracy of approximately 80% indicating that approximately 20% of the juvenile fish examined in the study were misclassified. Misclassified fish tended to have Sr/Ca or Li/Ca values that were not typical of the habitat type in which the fish were collected. Two possible causes of misclassifications are: 1) some coastal or estuarine sites have environmental conditions that are atypical compared to other sites in the same habitat group; and 2) fish collected at a site had recently moved into the collection habitat from the adjacent alternative habitat type.

Under the first scenario, sites with atypical environmental conditions would probably be characterized by a high proportion of misclassified fish. There were some sites that fit this scenario. Under the global model for English sole, Bodega Harbor, Bodega Bay, Bolinas Lagoon and Elkhorn Slough had less than 70% of fish correctly classified. For speckled sanddab, the sites with high misclassifications were Bodega Harbor, Santa Cruz, and Salinas River. Bodega Harbor was the only site in which both species experienced high misclassification rates under the global model. In addition, misclassification rates were high at this site in most years. Bodega Harbor is the northern most estuary in the study area. It is possible that atypical environmental conditions, such as cooler water temperatures, exist in this estuary and were responsible for increased misclassification rates at this site. As for the other sites, the high misclassification rates were found in only one of the two species. Given the general pattern of similarity in habitat tags between the two species, it does not seem likely that the misclassification rates were due to atypical environmental conditions.

Under the second scenario, movement of juvenile flatfish between estuarine and coastal habitats prior to capture would result in a chemical tag that was either typical of the alternative habitat type or an intermediate value depending on the timing of the movement. Speckled sanddab and English sole are known to move between estuarine and coastal habitats during at least two stages of the juvenile phase: 1) small juveniles move from coastal waters to estuarine habitats during or soon after metamorphosis and settlement; and 2) large juveniles leave the estuaries and move into progressively deeper coastal habitats prior to the onset of the first winter (Krygier and Pearcy 1986, Boehlert and Mundy 1987, Rogers et al. 1988, Kendall 1993). No tagging or tracking studies have been preformed to date to determine if these are the only time periods during which juveniles move between habitats. In the present study, every effort was made to minimize the chance of collecting juveniles that were in the process of moving between habitats; small individuals were not collected from estuaries in the spring and early summer and large individuals were not collected from coastal habitat in the fall. The overall consistency of the habitat tag implies that the majority of fish collected in this study had resided for a significant amount of time in the habitat in which they were captured. However, it is possible that some individuals, particularly those that were misclassified, had recently moved from the adjacent alternative habitat type.

One method for determining if misclassifications were due to the movement of fish between habitat types employs a laser, or other subsampling equipment, to measure the chemical composition of the otolith along a primorium-to-edge transect. Subsampling along a transect can be used both to detect environmentally and ontogenically induced shifts in the elemental composition of otoliths and to determine the timing of these shifts (Toole et al. 1993, Secor et al. 1998, Milton and Chenery 2001). Therefore, this method could be used not only to determine if misclassifications were due to individuals moving between juvenile habitats, but also to determine when those movements occurred. Fish that were identified as having recently moved between habitats could then be removed from the classification models, which would increase the discriminatory power of these models. In addition, an evaluation of the timing of movements between juvenile habitats for fish of different sizes would substantially increase knowledge of how juvenile flatfish utilize alternative habitats types.

Years Classification Model

Though the habitat tag based on Sr and Li allowed differentiation of estuarine and coastal groups over broad spatial and temporal scales, the classification accuracy of the model was improved, in most cases, by examining each year separately. The modest improvements in the classification accuracy of the yearly models were due to Ba and Mn. These two elements showed significant interannual variability and, in some years, Ba/Ca and Mn/Ca rations differed between the habitat groups. Specifically, Ba/Ca and Mn/Ca were found to be higher in coastal fish in some years. These results indicate that classification success can be improved by examining years separately if elements, that were not informative in the global model, become informative in one or more of the yearly models.

Spatial and temporal variability in Mn and Ba have been attributed to a variety of processes including: upwelling (Patterson et al. 1999), pollution/run off (Dove et al. 1996), and reducing conditions in the water column or sediments (Thorrold and Shuttleworth 2000). In this study Ba/Ca and Mn/Ca were found to be higher in coastal fish in 1998 and, to a lesser extent, in 2000. The incorporation of Mn and Ba into the otoliths appears to have been influenced by the prevailing oceanographic conditions because the magnitude of the increase of Ba/Ca and Mn/Ca in coastal fish tracked the magnitude of the changes in the oceanographic conditions. For example, coastal water temperatures and rainfall levels were highest in 1998 when Ba/Ca and Mn/Ca values were highest, and lowest in 1999 when Ba/Ca and Mn/Ca values were lowest. The opposite relationship occurred for upwelling activity; upwelling was lowest in 1998 and highest in 2000. Which, if any, of these environmental factors were responsible for generating these interannual patterns in Ba/Ca and Mn/Ca could not be identified using the data collected in the present study.

Regional Classification Model

Generating separate discriminant models for each region also improved classification success in many cases. This increase was mostly due to differences in Sr/Ca values across regions. For example, English sole collected from estuarine and coastal habitats in the northern region had elevated Sr/Ca compared to fish collected from the same habitats in the other two regions. In the global model, classification success was decreased due to coastal sites from the north resembling estuarine sites from the central and southern regions. By separating the fish into regions, the overlap in the distribution of Sr/Ca values in estuarine and coastal fish was reduced in each discriminant model and, thus, classification accuracy improved.

This finding suggests that the discriminatory power of a habitat tag may decrease as the spatial scale over which the tag is being applied increases. To generate the most robust chemical habitat tag, it might be advantageous to limit the collection of juvenile fish to habitats that are within the geographic range of, or are likely sources of recruits to, the adult population of interest. Therefore, determining the geographic range of the adult population appears to be a critical first step in the process of using otolith chemical composition to identify prior habitat residence of adult fish.

Species Comparison

This study is among the first to compare chemical habitat tags in two species with similar life histories. Differences in the elemental composition of otoliths from estuarine and coastal fish were similar for both species; estuarine fish had higher Sr/Ca and lower Li/Ca values than coastal fish. These similarities caused the classification accuracy of the discriminant model using data from both species combined (77%) to be very similar to those generated for English sole (78%) and speckled sanddab (79%) separately. The utility of the habitat tag was not significantly diminished by combining data from the two species. In addition, the overall similarities in the chemical composition of English sole and speckled sanddab otoliths suggested that the otoliths are recording true environmental differences between estuarine and coastal habitats.

Given the similarities in the English sole and speckled sanddab habitat tags, I used the habitat tag in one species as a proxy to classify coastal and estuarine individuals of the other species. The species did not perform as well as expected when they were used as proxies, especially for estuarine fish. This decreased classification success was probably due, at least in part, to slight differences in the mean values of Sr and Li in estuarine English sole and speckled sanddab. However, the classification success of the proxy models was improved by limiting the analysis to fish collected in only one year. The ability to use the habitat tag of one species as a proxy to classify individuals from another species is a very exciting development. It would allow the creation of a classification system for many species of adults based on juveniles of only one species. This would significantly decrease the number of juvenile fish that would need to be collected and analyzed to generate a classification model for adults from two or more species with similar life histories. The success of the proxy model concept requires further testing with other species of fish and in other types of juvenile habitats.

Conclusions

For each species, the global model, which pooled juveniles collected from three regions over multiple years, was able to classify fish into estuarine and coastal groups with close to 80% accuracy. Classification success of juveniles was modestly improved in some cases by generating separate discriminant functions for each year. These improvements were due to two elements, Ba and Mn, that differed between habitats in only some years. However, the two main elements in the discriminant models, Sr and Li, differed consistently between habitats over all three years. Given that the years examined in this study differed markedly in oceanographic conditions (e.g., El Niño and La Niña), this chemical habitat tag appears to be robust to temporal changes in environmental conditions. Consistent with my results are the recent findings by Yamashita et al. (2000) and Forrester and Swearer (2002) that chemical habitat tags were present in the otoliths of juvenile stone flounder and California halibut that allowed differentiation of coastal and estuarine individuals over multiple years. In both cases, the habitat tag could be used to classify adults into estuarine and coastal habitat groups. The chemical habitat tags found in this study appears to be promising tools for determining contribution of estuarine and coastal habitats to the central California populations of English sole and speckled sanddab.

In addition, I found that English sole and speckled sanddab had striking similarities in their chemical habitats tags and that, in some cases, one species could be used as a proxy to classify juveniles of the other species without compromising the accuracy of the habitat tag. The ability to use a proxy classification model would significantly reduce the number of juvenile fish that would need to be collected and analyzed in order to classify adults of ecologically similar species. The success of the proxy model concept needs to be explored further using other species of fish and in other types of juvenile habitats.

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Table 2-1. Summary information for English sole *Pleuronectes vetulus* and speckled sanddab *Citharichthys stigmaeus* collected from coastal (plain type) and estuarine (bold type) habitats for comparison of elemental composition of otoliths. Shown are locations (with abbreviation) of collection, mean fish size (TL ± S.E, mm) and sample size (in parenthesis). nd = no data; 0 = location sampled, but no fish collected

Location	English sole			Speckled sanddab		
	1998	1999	2000	1998	1999	2000
Bodega Harbor (BH)	nd	65.4 ± 2.7 (12)	78.5 ± 4.1 (11)	84.0 ± 8.5 (6)	57.2 ± 2.0 (12)	66.8 ± 3.6 (11)
Bodega Bay (BB)	nd	88.4 ± 2.7 (14)	74.6 ± 2.9 (13)	nd	69.2 ± 4.0 (10)	75.4 ± 3.7 (10)
Tomales Bay (TB)	71.6 ± 3.5 (10)	102.5 ± 4.2 (15)	78.6 ± 7.0 (11)	71.0 ± 3.3 (10)	73.6 ± 7.1 (8)	73.6 ± 5.2 (13)
10 Mile Beach (TMB)	nd	(0)	57.0 ± 21.0 (2)	nd	61.1 ± 2.4(11)	67.4 ± 3.3 (11)
Drake’s Bay (DB)	nd	83.3 ± 6.6 (10)	(0)	nd	66.6 ± 4.2 (11)	67.1 ± 3.7 (11)
Drake’s Estero (DE)	nd	74.7 ± 3.6 (13)	68.5 ± 3.2 (11)	nd	59.4 ± 3.2 (11)	58.4 ± 3.8 (12)
Bolinas Lagoon (BL)	nd	81.9 ± 3.8 (12)	68.9 ± 3.8 (10)	nd	53.6 ± 3.1 (10)	59.9 ± 2.3 (12)
San Francisco Bay (SFB)	94.3 ± 4.6 (12)	83.4 ± 4.5 (13)	71.6 ± 2.9 (15)	86.7 ± 2.6 (12)	73.7 ± 3.5 (12)	67.6 ± 3.7 (11)
Half Moon Bay (HMB)	nd	107.3 ± 3.0 (12)	88.7 ± 2.6 (11)	nd	88.2 ± 2.5 (12)	75.7 ± 4.7 (12)
Santa Cruz (SC)	113.4 ± 3.6 (5)	101.7 ± 8.7 (7)	82.7 ± 4.7 (11)	(0)	67.1 ± 3.9 (10)	66.6 ± 3.9 (10)
Pajaro River (PR)	80.3 ± 3.2 (10)	92.7 ± 4.2 (12)	78.5 ± 3.7 (11)	75.7 ± 4.9 (12)	64.8 ± 3.1 (10)	71.2 ± 4.0 (12)
Elkhorn Slough (ES)	77.0 ± 6.7 (3)	95.0 ± 3.0 (15)	84.9 ± 4.8 (19)	74.5 ± 4.9 (11)	82.4 ± 4.4 (13)	79.0 ± 4.0 (13)
Salinas River (SR)	84.6 ± 4.9 (11)	87.3 ± 4.5 (12)	76.3 ± 4.2 (12)	77.6 ± 4.7 (10)	64.3 ± 3.1 (11)	68.8 ± 3.3 (11)
Monterey (MY)	62.1 ± 2.4 (11)	(0)	59.2 ± 2.2 (12)	76.7 ± 4.3 (11)	64.5 ± 3.1 (11)	69.4 ± 4.2 (11)
Estero Bay – Cayucos (EBC)	nd	93.0 ± 4.8 (11)	78.1 ± 3.4 (15)	nd	74.0 ± 4.3 (12)	69.1 ± 3.7 (12)
Estero Bay – Strand (EBS)	nd	81.6 ± 4.8 (10)	71.0 ± 4.3 (10)	nd	67.9 ± 3.3 (13)	67.7 ± 4.3 (10)
Morro Bay (MB)	85.7 ± 6.1 (9)	86.2 ± 5.6 (13)	83.2 ± 5.0 (13)	68.3 ± 4.4 (10)	80.3 ± 5.3 (11)	78.5 ± 4.8 (11)
Port San Luis (PSL)	nd	86.7 ± 6.4 (3)	81.5 ± 5.7 (13)	nd	77.9 ± 4.0 (13)	72.5 ± 3.7 (14)

Table 2-2. The percentage of English sole and speckled sanddab correctly classified into habitat groups (coast or estuary) and the total correctly classified using discriminant function analysis and jack-knife cross-validation on the concentration of elements (Li, Mn, Sr, Ba) in otoliths. Three classification models were examined for each species. (1) The global model classified fish collected from all sites and all years. (2) The years model excluded sites where fish were not collected in all three years. Fish were classified in all years combined and each year separately. (3) The regions model excluded fish collected in 1998. Fish were classified in all regions combined and each region separately. Sample sizes are shown in parenthesis.

Classification Model	Coast	Estuary	Total
English sole			
(1) Global model	**80** (238)	**76** (217)	**78**
(2) Years (reduced model)			
All	**91** (91)	**84** (148)	**87**
1998	**88** (26)	**97** (34)	**93**
1999	**97** (31)	**84** (56)	**89**
2000	**91** (34)	**93** (58)	**92**
(3) Regions (reduced model)			
All	**78** (201)	**73** (183)	**76**
Northern	**79** (62)	**80** (123)	**80**
Central	**87** (77)	**82** (34)	**86**
Southern	**79** (62)	**81** (26)	**80**
Speckled sanddab			
(1) Global model	**77** (281)	**81** (209)	**79**
(2) Years (reduced model)			
All	**73** (99)	**77** (164)	**76**
1998	**85** (33)	**86** (49)	**85**
1999	**66** (32)	**68** (56)	**67**
2000	**74** (35)	**83** (60)	**80**
(3) Regions (reduced model)			
All	**76** (248)	**78** (160)	**77**
Northern	**83** (88)	**76** (112)	**79**
Central	**70** (86)	**58** (26)	**67**
Southern	**89** (74)	**82** (22)	**88**

Table 2-3. Correlations of predictor variables with discriminant functions (canonical loadings) used to classify juvenile English sole and speckled sanddab to habitat of capture (estuary or coast) based on the elemental composition of otoliths (using Li, Mn, Sr, Ba). Three classification models were examined for each species. (1) The global model classified fish collected from all sites and all years. (2) The years model excluded sites where fish were not collected in all three years. Fish were classified in all years combined and each year separately. (3) The regions model excluded fish collected in 1998. Fish were classified in all regions combined and each region separately. In addition, a global model was calculated for both species combined.

Classification Model	Canonical Loadings			
	Sr	Li	Ba	Mn
English sole				
(1) Global model	-0.82	0.69	0.16	0.26
(2) Years (reduced model)				
All	-0.67	0.64	0.37	0.51
1998	-0.62	0.32	0.36	0.79
1999	0.84	-0.58	0.01	-0.59
2000	0.38	-0.73	-0.56	0.17
(3) Regions (reduced model)				
All	0.80	-0.73	-0.03	-0.18
Northern	0.48	-0.67	0.43	0.19
Central	-0.00	0.70	0.32	0.25
Southern	-0.90	0.36	0.06	0.43
Speckled sanddab				
(1) Global model	-0.83	0.49	0.13	0.05
(2) Years (reduced model)				
All	-0.56	0.26	0.53	0.41
1998	-0.40	0.32	0.65	0.40
1999	-0.75	-0.09	0.52	-0.25
2000	0.29	0.34	0.66	0.10
(3) Regions (reduced model)				
All	-0.88	0.43	0.10	-0.01
Northern	-0.90	0.36	-0.46	-0.37
Central	-0.74	0.35	0.27	0.11
Southern	-0.77	0.34	0.34	0.65
Species combined				
(1) Global model	0.86	-0.64	-0.10	-0.17

Table 2-4. Differences in concentrations of individual elements in the otoliths of English sole and speckled sanddabs. (A) One-way ANOVA using habitat as the main effect (global model; all sites and all years); (B) 2-way ANOVA with habitat and years (1998, 1999 and 2000) as main effects; and C) 3-way ANOVA with regions, years (1999 and 2000) and habitat as main effects. All factors are considered fixed in the analyses. SS. Sum-of-squares; df, degrees freedom; * p < 0.05, ** p < 0.01, *** p < 0.001

	Source of variation	df	SS log(Sr/Ca)	SS Li/Ca	SS log(Mn/Ca)	SS log(Ba/Ca)
(A)	**English sole**					
	Habitat	1	0.3756***	56.925***	2.948***	0.681**
	Residual	453	0.8048	171.333	62.524	38.634
	Speckled sanddab					
	Habitat	1	0.1187***	11.158***	0.030	0.127
	Residual	488	0.394	107.433	27.931	18.076
(B)	**English sole**					
	Habitat	1	0.2504***	44.001***	7.692***	1.934***
	Year	2	0.0330***	6.582***	1.080**	4.411***
	Habitat x Year	2	0.0137*	3.222*	1.945***	1.060***
	Residual	233	0.3605	80.430	18.457	7.861
	Speckled sanddab					
	Habitat	1	0.0289***	2.090**	1.177***	0.973***
	Year	2	0.0000	6.137***	2.549***	2.541***
	Habitat x Year	2	0.0023	1.695*	0.541*	0.898***
	Residual	257	0.2167	56.383	15.037	6.378
(C)	**English sole**					
	Habitat	1	0.1515***	27.223***	0.436*	0.014
	Region	2	0.0737***	1.484	5.019***	0.743***
	Year	1	0.0077*	0.720	0.008	0.219*
	Habitat x Year	1	0.0002	3.269**	0.076	0.000
	Habitat x Region	2	0.0048	3.068*	2.000***	1.480***
	Region x Year	2	0.0048	1.449	0.438	2.334***
	H x R x Y	2	0.0040	0.721	0.583	0.794***
	Residual	372	0.5633	131.336	41.735	14.930
	Speckled sanddab					
	Habitat	1	0.0866***	3.598***	0.166*	0.018
	Region	2	0.0090**	0.074	1.175***	1.139***
	Year	1	0.0030*	1.921**	0.031	0.185**
	Habitat x Year	1	0.0032*	3.366***	0.058	0.056
	Habitat x Region	2	0.0098**	0.207	1.624***	0.665***
	Region x Year	2	0.0138***	2.669***	0.484***	0.164*
	H x R x Y	2	0.0026	2.187**	0.140	0.098
	Residual	396	0.2745	74.228	13.437	8.516

Table 2-5. The percentage of individuals correctly classified into habitat groups (coast or estuary) and the total correctly classified using discriminant function analysis and jack-knife cross-validation procedure on the concentration of elements (Li, Mn, Sr, Ba) in otoliths. Three classification models were examined for all years combined and 1999 and 2000 separately: (1) data from all English sole and speckled sanddab collected in the study were used to classified fish into habitat groups; (2) the English sole 'global model' was used to classify speckled sanddab; and (3) the speckled sanddab 'global model' was used to classify English sole. Sample sizes are shown in parenthesis

Classification Model	Coast	Estuary	Total
All years			
(1) Species Combined	**79** (519)	**75** (426)	**77**
(2) Using English sole to classify speckled sanddab	**77** (281)	**64** (209)	**71**
(3) Using speckled sanddab to classify English sole	**83** (238)	**54** (217)	**69**
1999 only			
(1) Species Combined	**80** (215)	**72** (170)	**76**
(2) Using English sole to classify speckled sanddab	**88** (91)	**64** (93)	**79**
(3) Using speckled sanddab to classify English sole	**74** (124)	**72** (77)	**73**
2000 only			
(1) Species Combined	**76** (234)	**76** (173)	**76**
(2) Using English sole to classify speckled sanddab	**70** (110)	**81** (90)	**74**
(3) Using speckled sanddab to classify English sole	**82** (124)	**59** (83)	**72**

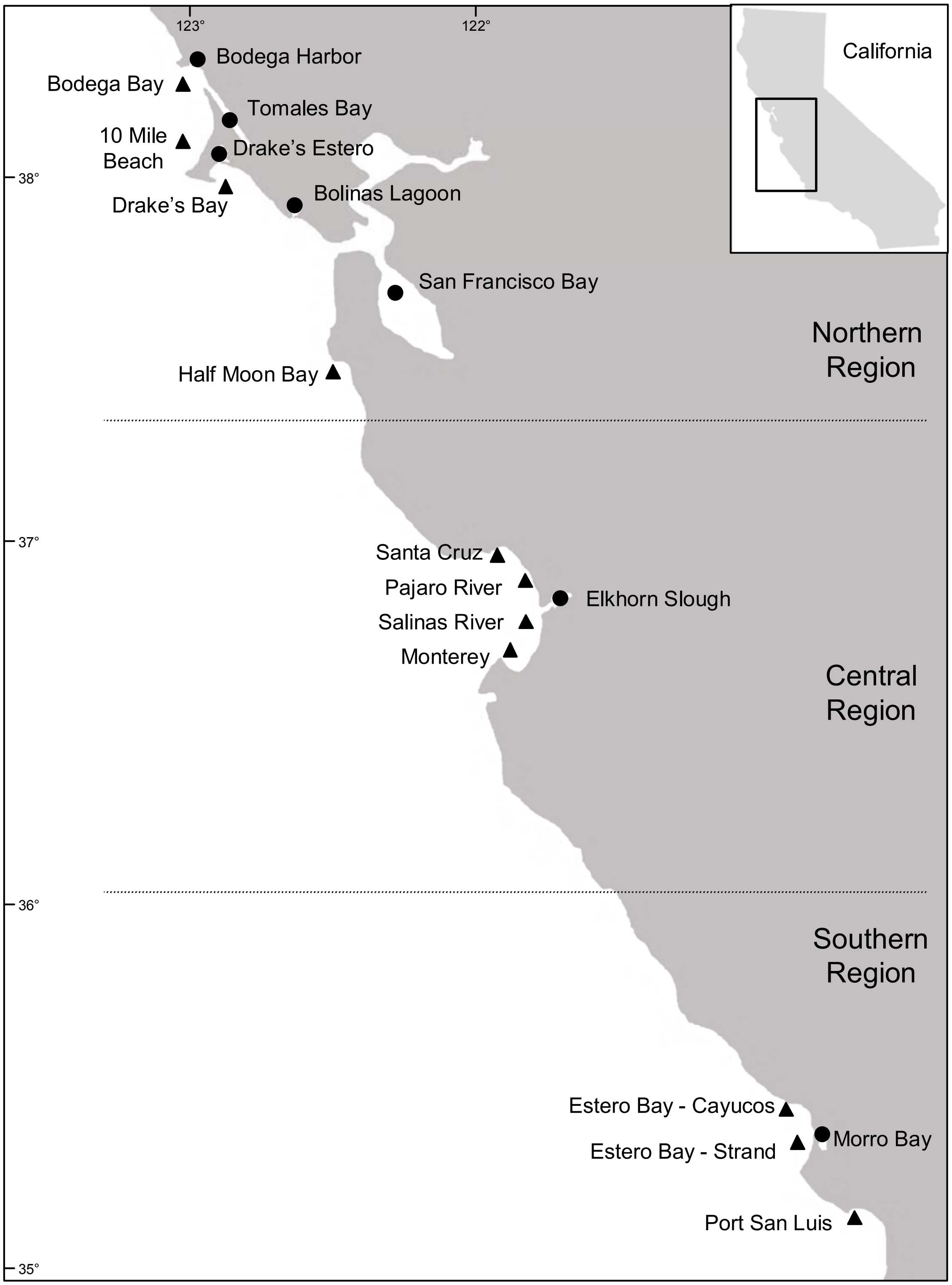


Figure 2-1. Map of California (inset) and the section of the coast examined in this study. Open coast (triangle) and estuary (circle) sites fell into three regions: Northern, Central and Southern. All sites were sampled in 1999 and 2000 and a subset was sampled in 1998.

Figure 2-2. Concentration of Sr in otoliths by collection site pooled over three years for (A) English sole and (B) speckled sanddab. Collection locations are grouped by habitat type (coast and estuary) and listed from north to south (left to right) within each group. Dashed lines denote group means. See Table 1 for an explanation of abbreviations for collection sites. Box plot: the center horizontal line = median; the box = central 50% of values; the whiskers = statistical range of values; asterisks = outliers; and empty circles = far outliers. Concentration of Sr in otoliths from fish collected in coastal and estuarine habitats by region and year of collection for (C) English sole and (D) speckled sanddab.

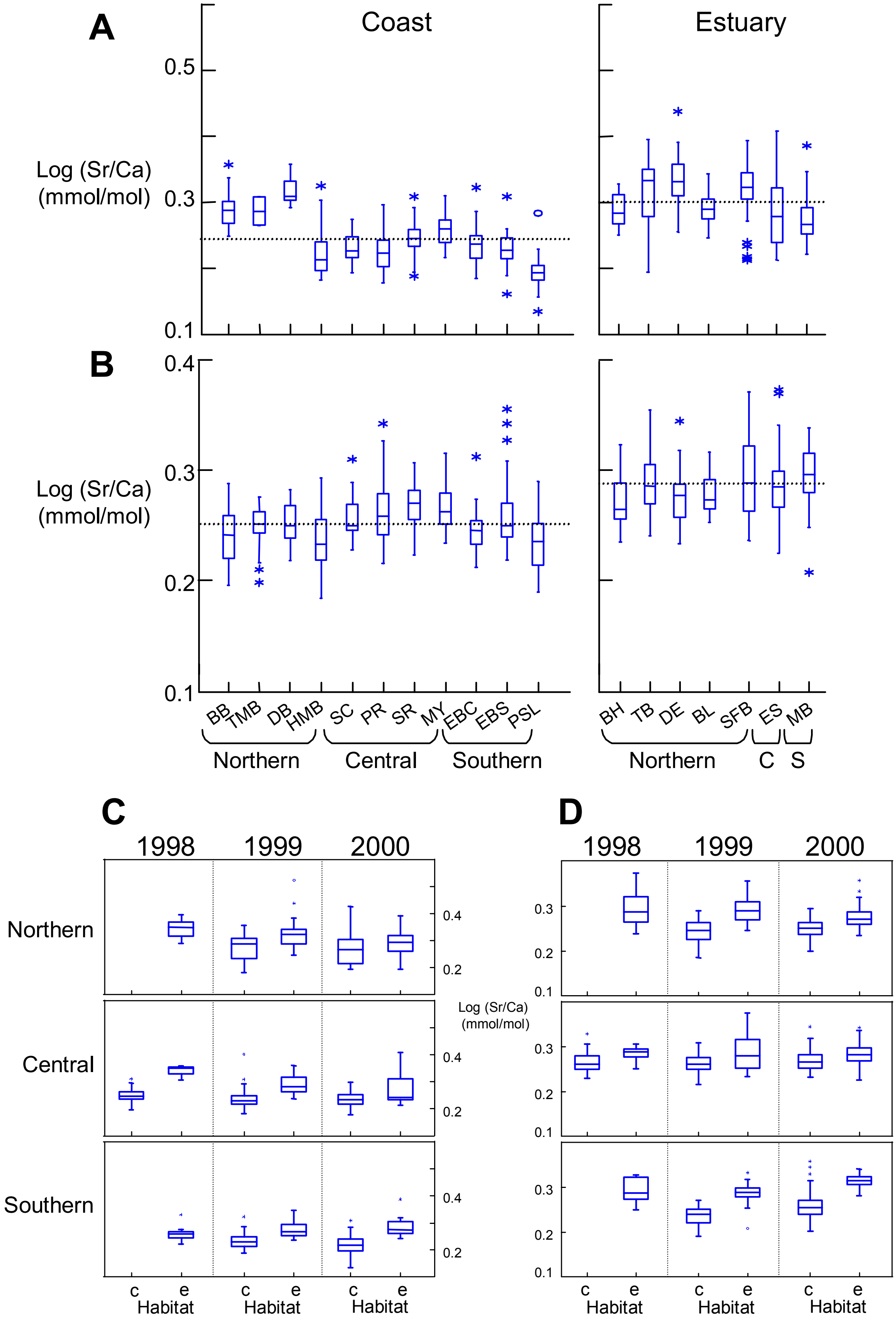
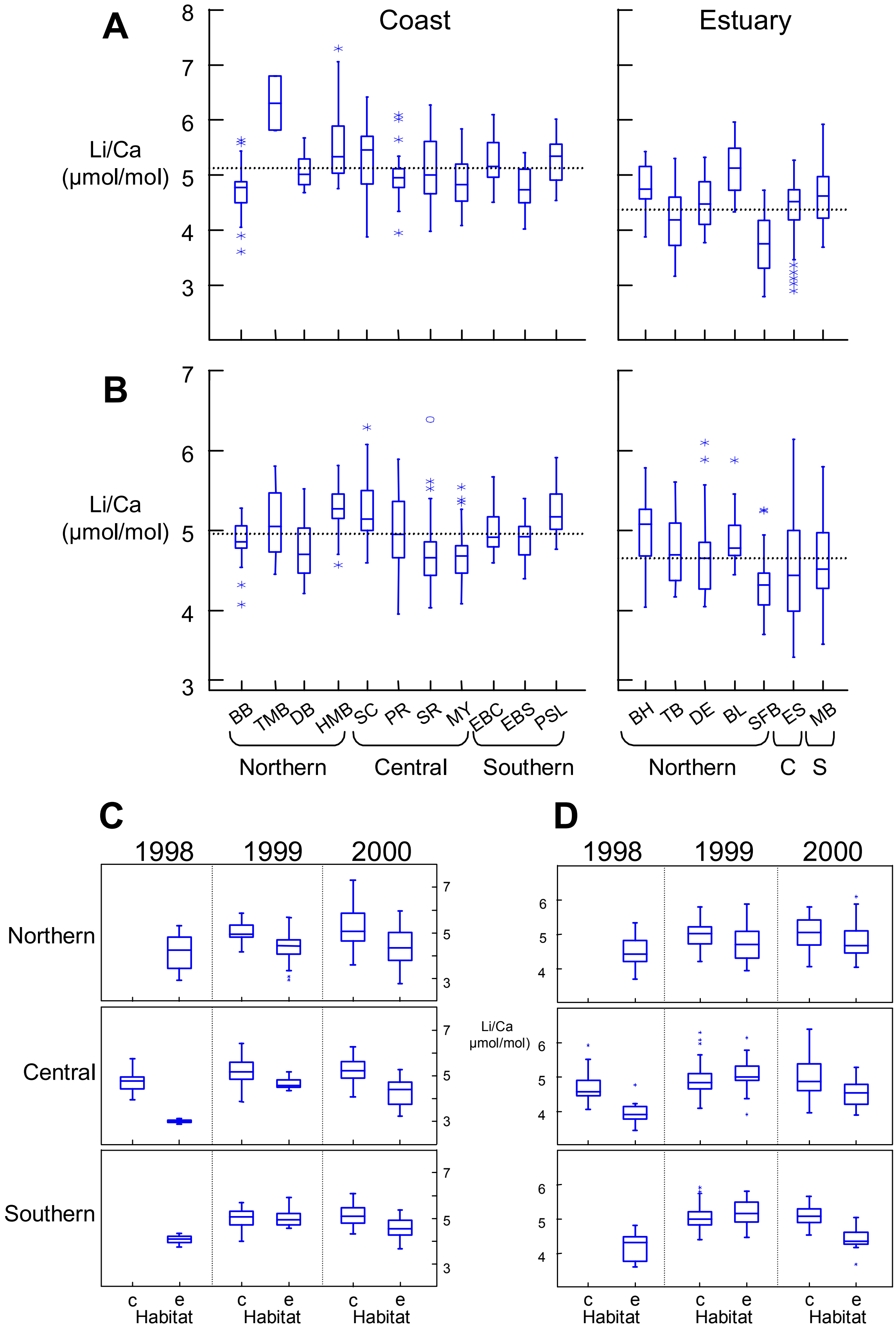

A
Coast
Estuary
Log (Sr/Ca)
(mmol/mol)
0.5
0.3
0.1
B
0.4
0.3
0.2
0.1
BB
TMB
DB
HMB
SC
PR
SR
MY
EBC
EBS
PSL
Northern
Central
Southern
BH
TB
DE
BL
SFB
ES
MB
Northern
C
S
C
1998
1999
2000
Northern
Central
Southern
0.4
0.2
c
e
Habitat
D
1998
1999
2000
0.3
0.2
0.1
Log (Sr/Ca)
(mmol/mol)
c
e
Habitat

Figure 2-3. Concentration of Li in otoliths by collection site polled over three years for (A) English sole and (B) speckled sanddab. Collection locations are grouped by habitat type (coast and estuary) and listed from north to south (left to right) within each group. Dashed lines denote group means. See Table 1 for an explanation of abbreviations for collection sites. Box plot: the center horizontal line = median; the box = central 50% of values; the whiskers = statistical range of values; asterisks = outliers; and empty circles = far outliers. Concentration of Li in otoliths from fish collected in coastal and estuarine habitats by region and year of collection for (C) English sole and (D) speckled sanddab.


A
B
C
D
Coast
Estuary
Li/Ca (µmol/mol)
8
7
6
5
4
3
BB
TMB
DB
HMB
SC
PR
SR
MY
EBC
EBS
PSL
BH
TB
DE
BL
SFB
ES
MB
Northern
Central
Southern
C
S
1998
1999
2000
Northern
Central
Southern
Li/Ca µmol/mol)
c
e
Habitat

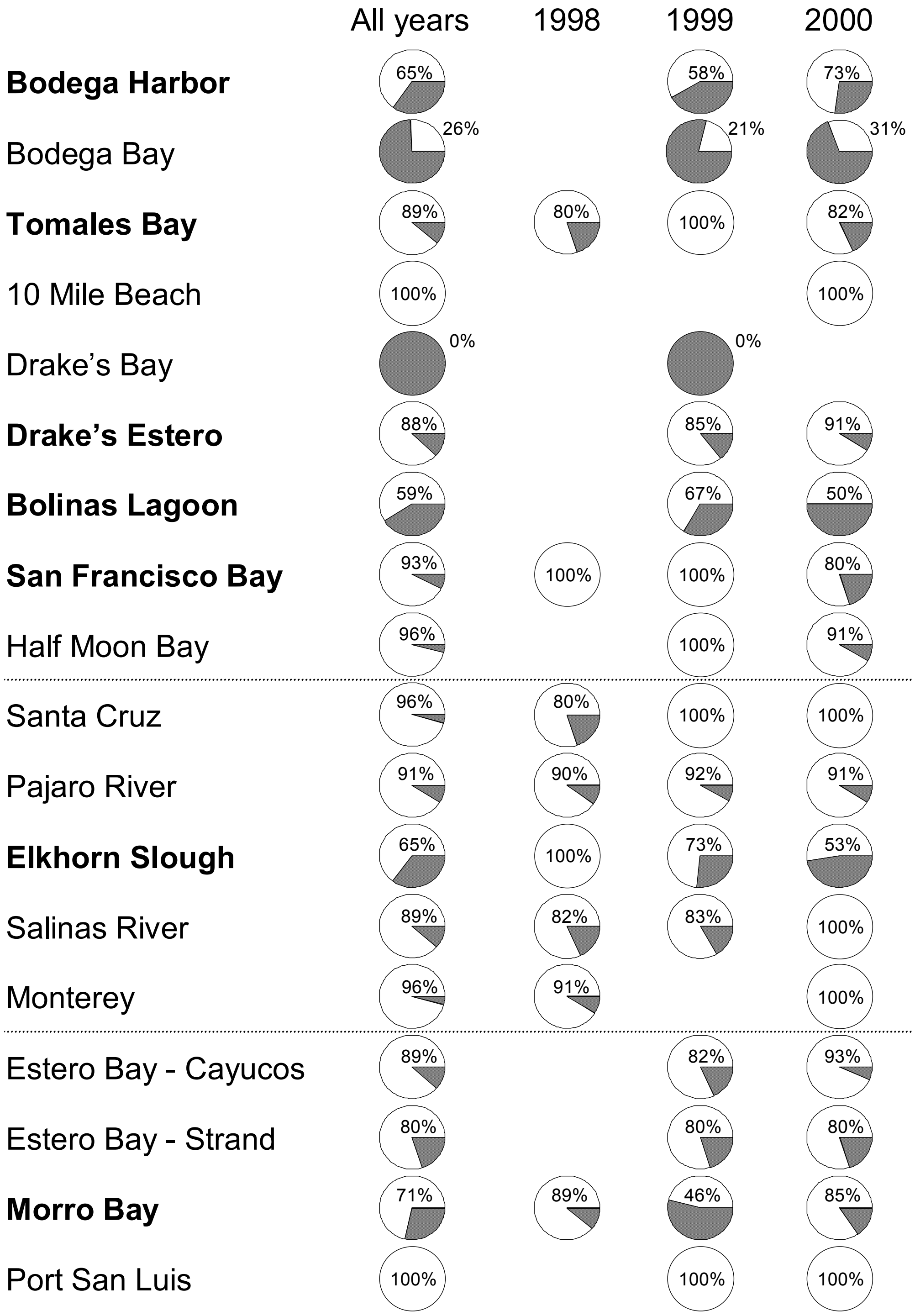


Figure 2-4. Classification accuracy of the global classification model using the chemical composition of otoliths to classify juvenile English sole into habitat of capture (estuary or coast). The pie diagrams show percent correctly (white, % shown) and incorrectly (grey) classified from each collection site. Sites are listed from north (top section) to south (bottom section) and designated as estuary or coast using type face (bold or plain type respectively). The global model uses fish collected over three years; results are shown for all years combined and each year individually. See Table 1 for sample sizes.

Figure 2-5. Concentration of Ba in otoliths by collection site pooled over three years for (A) English sole and (B) speckled sanddab. Collection locations are grouped by habitat type (coast and estuary) and listed from north to south (left to right) within each group. Dashed lines denote group means. See Table 1 for an explanation of abbreviations for collection sites. Box plot: the center horizontal line = median; the box = central 50% of values; the whiskers = statistical range of values; asterisks = outliers; and empty circles = far outliers. Concentration of Ba in otoliths from fish collected in coastal and estuarine habitats by region and year of collection for (C) English sole and (D) speckled sanddab.

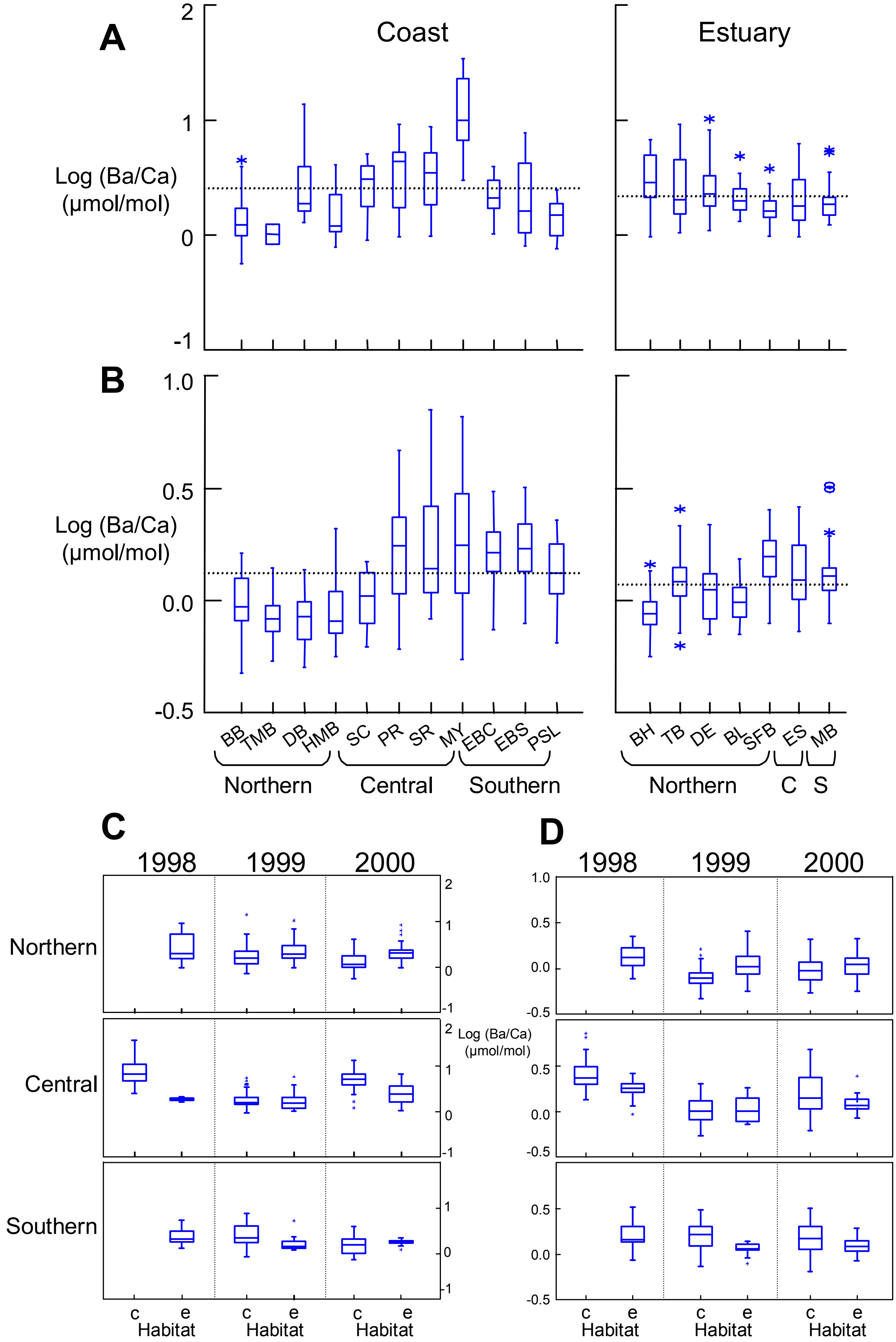
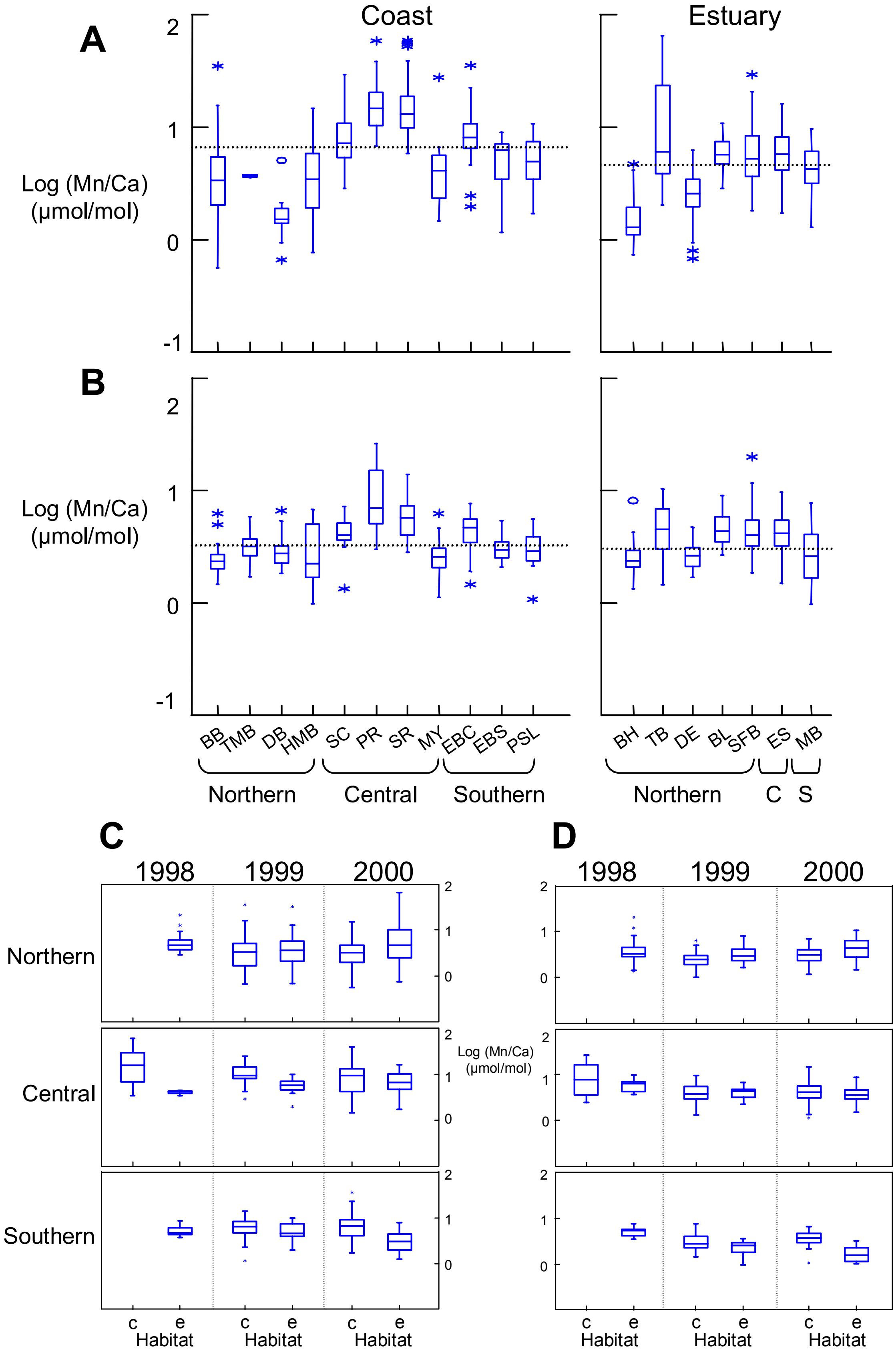

A
Coast
Estuary
Log (Ba/Ca)
(µmol/mol)
2
1
0
-1
B
1.0
0.5
0.0
-0.5
Log (Ba/Ca)
(µmol/mol)
BB
TMB
DB
HMB
SC
PR
SR
MY
EBC
EBS
PSL
BH
TB
DE
BL
SFB
ES
MB
Northern
Central
Southern
Northern
C
S
C
1998
1999
2000
Northern
Central
Southern
2
1
0
-1
c
e
Habitat
D
1998
1999
2000
1.0
0.5
0.0
-0.5
Log (Ba/Ca)
(µmol/mol)
c
e
Habitat

Figure 2-6. Concentration of Mn in otoliths by collection site pooled over three years for (A) English sole and (B) speckled sanddab. Collection locations are grouped by habitat type (coast and estuary) and listed from north to south (left to right) within each group. Dashed lines denote group means. See Table 1 for an explanation of abbreviations for collection sites. Box plot: the center horizontal line = median; the box = central 50% of values; the whiskers = statistical range of values; asterisks = outliers; and empty circles = far outliers. Concentration of Mn in otoliths from fish collected in coastal and estuarine habitats by region and year of collection for (C) English sole and (D) speckled sanddab.


A
B
C
D
Coast
Estuary
Log (Mn/Ca)
(µmol/mol)
2
1
0
-1
BB
TMB
DB
HMB
SC
PR
SR
MY
EBC
EBS
PSL
BH
TB
DE
BL
SFB
ES
MB
Northern
Central
Southern
Northern
C
S
1998
1999
2000
Northern
Central
Southern
c
e
Habitat
Log (Mn/Ca)
(µmol/mol)

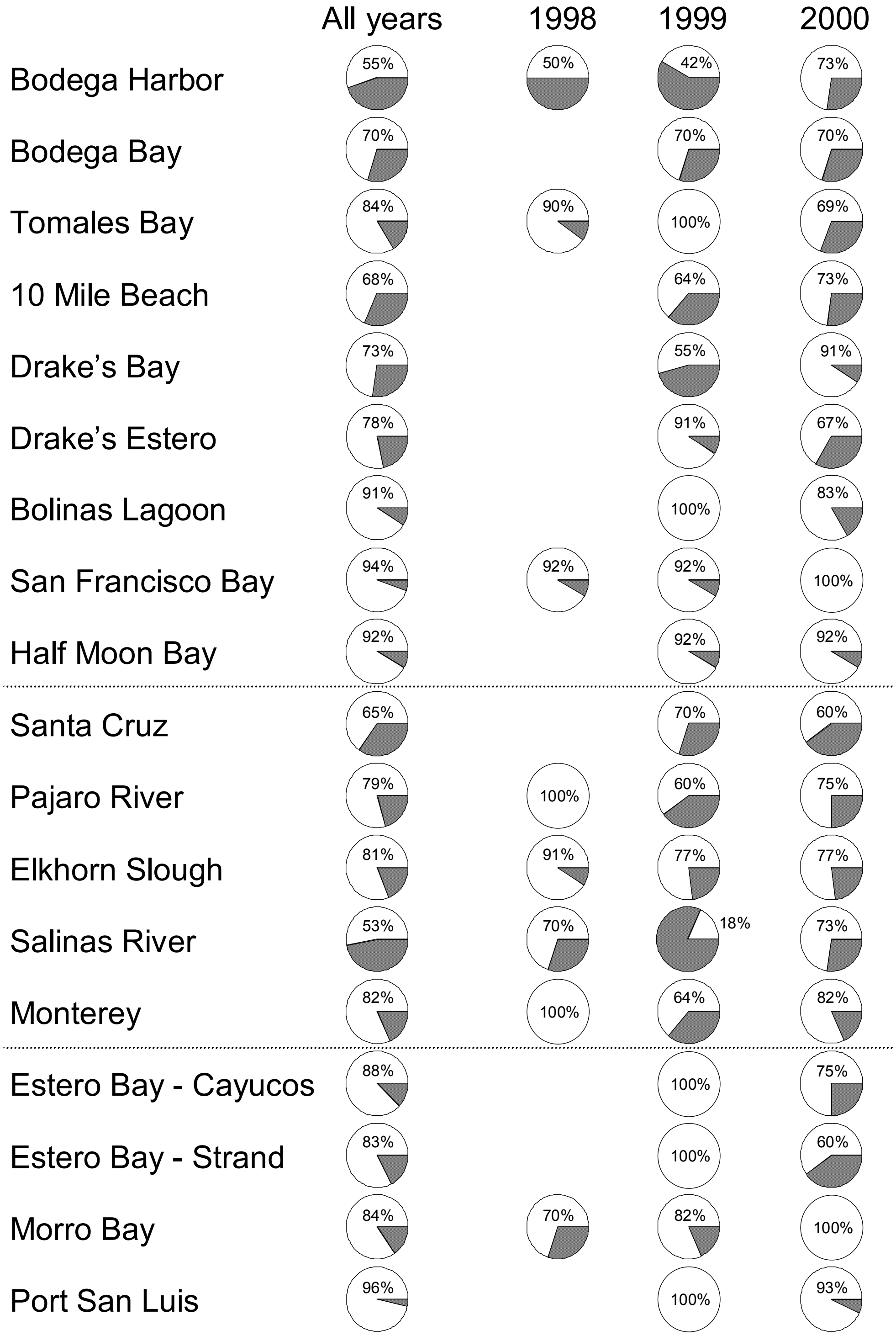


Figure 2-7. Classification accuracy of the global classification model using the chemical composition of otoliths to classify juvenile speckled sanddab into habitat of capture (estuary or coast). The pie diagrams show percent correctly (white, % shown) and incorrectly (grey) classified from each collection site. Sites are listed from north (top section) to south (bottom section) and designated as estuary or coast using type face (bold or plain type respectively). The global model uses fish collected over three years; results are shown for all years combined and each year individually. See Table 1 for sample sizes.

CHAPTER 3:

Using the chemical composition of otoliths to evaluate the nursery role of estuaries for English sole (*Pleuronectes vetulus*) populations in central California

Introduction

Many coastal fish species have juvenile and adult life stages that occupy spatially separated habitats. The juveniles often recruit to nearshore habitats where they reside for months to years before migrating to offshore habitats to join the adult population. In addition, juveniles of many species with this life history pattern recruit to more than one type of nearshore habitat, for example estuaries and shallow sandflats, and those different habitats are likely to vary in quality. The highest quality juvenile habitats are often referred to as ‘nursery’ habitats. Recently, the definition of a nursery habitat was clarified by Beck et al. (2001): “A habitat is a nursery for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur.” Determining which juvenile habitats are functioning as nursery habitats is important to both understanding the ecological roles of the different juveniles habitats and managing harvested fish populations and coastal resources. Identification of nursery habitats is particularly important when some of the nearshore habitats used by juvenile fish are vulnerable to degradation or loss.

Determining which juvenile habitats are contributing more individuals to adult populations requires identifying the juvenile habitats in which the adults once lived. One way to determine prior residence of adult fish is to manually tag fish in all the alternative juvenile habitats and, subsequently, recover those tagged individuals as adults. However, this method is logistically difficult to implement because the small size of juveniles and the high rate of juvenile mortality necessitate that a large number of individuals have to be tagged to get meaningful results. An alternative method to manual tagging requires that juveniles incorporate markers (e.g., elements, isotopes) characteristic of and specific to the habitat in which they reside. Such natural “habitat tags” that allow differentiation of individuals from different juvenile habitats have been found in the calcified structures of many different marine organisms, including coral skeletons, bivalve shells, marine mammal teeth, invertebrate statoliths, and fish otoliths (Campana and Thorrold 2001).

Chemically-based habitat tags incorporated within otoliths are particularly useful tools for determining prior residence of fish for three reasons. First, the deposition rate of trace elements into otolith material (aragonite) can be influenced by environmental factors (e.g., water temperature, salinity, trace metal abundance in the water) and physiological factors (e.g., growth rate, 'stress', ontogenic stage) that may differ between habitats (Campana 1999). Therefore, juvenile habitats with different environmental conditions may produce fish with otoliths that differ in their chemical composition. Second, otoliths are metabolically inert (Campana and Neilson 1985) which results in the permanent retention of the habitat tag. Third, because otoliths grow continuously and contain daily and annual banding patterns, they can be used as long-term chronological recorders of environmental conditions (Campana and Thorrold 2001). Hence, the environmental history of a fish at a specific time period in the past can be determined by analyzing the chemical composition of the portion of the otolith that corresponds to that specific time period.

Differences in the chemical composition of whole otoliths have been used to identify individuals with different dispersal histories (Swearer et al. 1999) or migratory patterns (Secor et al. 2001) and to differentiate individuals from different stocks (Campana et al. 1995, Campana et al. 2000), estuarine/riverine systems (Milton et al. 1997, Thorrold et al. 1998a, 1998b, Gillanders and Kingsford 2000, Gillanders 2002b), or nearshore habitats, including rocky reef and estuary (Gillanders and Kingsford 1996) and open coast and estuary (Yamashita et al. 2000, Forrester and Swearer 2002). Analysis of the chemical composition of the otolith along a transect from center to edge (i.e., over the ontogeny of an individual) has been used to determine timing of migrations, annulus (annual band) formation and other life history events (Toole et al. 1993, Halden et al. 2000, Secor and Rooker 2000). In addition, analysis of only the juvenile portion of adult otoliths has allowed researchers to hindcast the juvenile habitat type from which adult fish originated (Yamashita et al. 2000, Thorrold et al. 2001, Forrester and Swearer 2002, Gillanders 2002a).

This study investigates the utility of using naturally occurring habitat tags in the otoliths of English sole (*Pleuronectes vetulus*) to determine the proportion of the adult population that recruited from two alternative juvenile habitats – estuaries and shallow coastal sandy habitats. The English sole is a commercially harvested species abundant along the Pacific coast of North America (Starr et al. 1998, Sampson and Al-Jufaily 1999). Juveniles recruit to estuaries or shallow sandy habitats along the open coast in the spring and early summer. In the fall, juveniles leave the nearshore habitats and move into progressively deeper water where they join the adult population offshore (Starr et al. 1998). I showed [Ch 2] that a distinguishable chemical habitat tags exist in the otoliths of juvenile English sole and speckled sanddab that were collected from estuarine and coastal habitats along the central California coast. The purpose of this study was to: 1) characterize the habitat tag in juvenile English sole collected from estuarine and coastal habitats; 2) determine if this chemical habitat tag was present in adult English sole collected in Monterey Bay by analyzing the portion of the adult otolith that was laid down when the fish was a juvenile; and 3) determine the proportion of the adults that originated in estuarine habitats. Determining the proportion of the adults that recruited from estuarine habitats will give insight into whether estuarine habitats are functioning as nurseries for the English sole population in central California.

Material and Methods

Collection of Juveniles

The juvenile English sole used to characterize the juvenile habitat tag were collected between May and September from 7 estuaries and 11 coastal sites along a 500 km portion of the California coast (Figure 1). All sites were sampled in 1999 and 2000, but due to logistical constraints, only a subset of these sites were sampled in 1998 (Table 1). English sole collected from Elkhorn Slough in 1996 and 1997 (by Dave Lindquist, Moss Landing Marine Laboratory) were also included in the analysis to increase the sample size of estuarine fish collected in the Monterey Bay region. Coastal fish were collected by otter trawl and estuarine fish were collected using a combination of otter trawl and beach seine. Fish were kept on ice or frozen until dissection. Fish were measured (mm total length) and sagittal otoliths were removed, cleaned of adhering tissue, and stored dry in plastic vials.

Collection of Adults

Adults English sole were collected in the Monterey Bay region (Figure 1) at three different times; Mont1 and Mont2 fish were collected on research cruises in the summer of 2001 and Mont3 fish were obtained from a commercial processor in May 2002 (Table 1). Fish were kept on ice or frozen until dissection. The sagittal otoliths were removed, cleaned of adhering tissue, and stored dry in plastic vials.

Preparation of juvenile otoliths

Between 10-15 juveniles per site per year were selected for analysis when available (Table 1). Individuals were selected to span the size range collected at each site. In some cases fewer than 10 fish had been collected so all individuals were analyzed. The right sagittal otolith from each fish was transferred to acid-cleaned Eppendorf microcentrifuge tubes, weighed (± 0.01 mg) and left to soak for 6 hours in 30% Suprapur (EM Scientific) H_2O_2 to remove organic material. Otoliths were then double-rinsed in Milli-Q water, triple-washed in dilute acid (0.01N HNO_3) to remove surface contaminants, and double-rinsed again in Milli-Q water. Otoliths were transferred to 4 ml Nalgene HDPE bottles and dissolved in 1% HNO_3 at a ratio of 2 ml of solution per 1 mg otolith (final concentration of ~200 ppm Ca). Following weighing, all sample handling was performed in a HEPA class 100 laminar flow hood. Unless otherwise stated, all HNO_3 used was Trace Metal Grade (Fischer Scientific).

Preparation of adult otoliths

Otoliths from adult fish of many species contain an annual banding pattern; wide opaque summer bands alternating with narrow translucent winter bands when viewed under reflected light (Campana 2001). Annual banding has been found in the otoliths and interopercular bones of adult English sole, with the first annulus (translucent band) formation beginning at the end of the first summer/fall growth period (Smith and Nitsos 1969, Van Cleve and El-Sayed 1969). To determine the birth year of the adult English sole collected in this study, the right sagittal otolith of each adult was viewed under a dissecting microscope using reflected light and a black background. Age (years) was determined by counting translucent bands and using the date of collection to back-calculate birth year. The annual banding pattern was more easily discerned in some individuals that in others. Only adults that had a high probability of being born in one of the three years in which juveniles were collected (1998, 1999 and 2000) were selected for analysis (n=67).

For the purpose of chemical analysis, the right sagittal otolith was embedded in epoxy (Epoxicure, Beuhler Ltd) and the juvenile core extracted. Juvenile cores consisted of all material deposited during the larval and juvenile growth seasons before formation of the first annuals. Cores were extracted by cutting away the surrounding otolith material in a multi-step process (Figure 2). The first two steps used a low speed isomet saw fitted with diamond blades and run with MilliQ water as the lubricant. First, the rostrum and postrostrum were removed by cutting with a single blade through the first annulus. Double blades fitted with spacers were then used to cut a thin section (approximately 300 um thick) along the sagittal plane. The thin section was attached to a microscope slide using double-sided tape and then a microdrill was used to free the juvenile core from the surrounding otolith material by drilling along the first annulus. The thin sections were fragile and frequently cracked during the drilling process. All available pieces of each core were removed from the tape, placed in an acid-cleaned epindorf tube and rinsed twice with MilliQ water to remove dust contamination from the drilling process. The cores were left to dry over night in a laminar flow hood. Cores were then weighed and cleaned following the same procedure used for juvenile otoliths.

Sample Analysis

A procedural blank was prepared in the same manner as samples, but no otolith was present. The procedural blank was compared to the system blank to determine if contamination occurred during processing. System blanks were made from the same acid used for sample dissolution. System blanks were run every four samples and were used for blank corrections and to determine limits of detection. A spike standard was prepared by gravimetrically spiking a Ca standard solution with appropriate concentrations of Li, Mg, Mn, Ni, Cu, Zn, Sr, Ba, Pb to match the typical elemental composition of the otoliths. The spike standard was analyzed every four samples to track, and correct for, instrument drift. A consistency standard (a bulk digestion of flatfish otoliths) was analyzed at the beginning of each run to assess consistency of measurements over multiple runs within a day and over multiple days. All measurements were carried out on a Finnigan MAT (Bremen, Germany) Element sector field inductively coupled plasma mass spectrometer (ICPMS).

Initially, all plasticware used for sample preparation and analysis was leached in 2N HNO_3 for 72 hours, rinsed thoroughly (five times) with Milli-Q water and dried in the clean hood prior to use. This procedure was changed mid-way through analyzing the juvenile otoliths when continued leaching of Mg from the 4 ml Nalgene bottles was detected in procedural blanks that had a lag period between preparation and analysis. In the new cleaning procedure bottles were leached for one week in heated 3 N HCl (ACS grade), rinsed in Milli-Q, leached again in heated 3 N HNO_3 (ACS grade), and rinsed thoroughly in Milli-Q. Bottles were either dried in the hood and used immediately or left soaking in a bath of 2 N HNO_3 (trace metal grade) until needed. Contamination from plasticware was not detected for any of the other elements of interest. All bottles used for preparation of adult otoliths were cleaned using the new method.

Ten elements (^{7}Li, ^{25}Mg, ^{43}Ca, ^{55}Mn, ^{62}Ni, ^{63}Cu, ^{66}Zn, ^{87}Sr, ^{138}Ba, ^{208}Pb) were initially chosen for study based on preliminary analysis of the composition of juvenile flatfish otoliths. The limits of detection of each element were calculated for each run as three times the standard deviation of the system blank and were applied to blank corrected intensities. Ni, Cu, Zn, and Pb intensities were frequently at or below detection limits and, therefore, were removed from the analysis. Mg was removed due to possible contamination from 4 ml bottles (see above). Li, Mn, Sr, and Ba were consistently above detection limits and could be used for statistical analysis. Element/Ca^{43} ratios were determined from blank-corrected intensities using the spiked standard to correct for instrumental mass discrimination (following Rosenthal et al. 1999). Estimates of precision (% relative standard deviation) based on repeated analysis of the otolith consistency standard were determined for each elemental ratio to be: ^{7}Li/^{43}Ca = 2.62%, ^{55}Mn/^{43}Ca = 1.17%, ^{87}Sr/^{43}Ca = 1.15%, ^{138}Ba/^{43}Ca = 1.49%.

Prior investigation of spatial and temporal patterns in element concentrations in the otoliths of juvenile English sole (Brown [Ch 2]) showed that Sr and Li concentrations differed between estuarine and coastal habitats and that these differences were consistent over the three years studied (1998-2000). Though Ba and Mn also showed significant habitat differences, these differences were only present in some years. Because the adults used in this study were born over the three years in which juveniles were collected, only the elements (Sr and Li) that showed temporal stability in the chemical tag were included in statistical analysis.

Analysis of Data from Juveniles

Data from each element was checked for univariate normality and homogeneity of variances (following Quinn and Keough 2002). Sr was normally distributed after $\log_{10}$-transformation; Li was normally distributed without transformation. To ensure that differences in otolith size (≈ fish size) among samples did not confound any spatial patterns in elemental composition, the effect of otolith weight on element ratio was examined. A significant relationship was found for Li (Figure 3; $Li = -0.314OW+6.939$; $r^2 = 0.172$, $p < 0.001$), but not for Sr. Hence, Li values were detrended using a two step process: 1) the slope of the regression line was used to estimate a Li value corresponding to an otolith weight of 7.0 mg (an approximate ‘end-of-season’ otolith weight; see below); and 2) for each sample, the specific residual from the regression relationship was added to the estimated Li value. This procedure produced a distribution of sample Li values that were unaffected by otolith weight. By detrending Li values to an end-of-season otolith weight, the resulting statistical models could be used to classify adults using their otolith cores, which contained material deposited during the entire juvenile growth season. Using the relationship between otolith weight and total length for juvenile and sub-adult English sole (Figure 4; sqOW=0.021TL-0.249, r^2=0.88, p<0.001), I calculated that an otolith weight of 7.0 mg corresponded to a fish size of 140 mm (TL). This end-of-season size was selected because juveniles larger than 140 mm are rarely encounter in estuarine habitats (summarized in Shi et al. 1997), and a first annulus begins to form in the interopercular bones of English sole that are approximately 135 mm (Smith and Nitsos 1969).

Linear discriminant function analysis (LDFA) was used to classify juvenile fish as being either from estuarine or coastal habitats based on the Sr and Li concentrations in their otoliths. The classification accuracy of the discriminant functions was determined by comparing the jack-knife (leave-one-out) predicted group membership to the actual group membership and calculating the percentage of individuals that were correctly classified. Two different juvenile classification models were created. The ‘all sites’ model used juveniles collected from every site in the study region. This model was created under the assumption that the adults, although collected in the Monterey Bay region, could have recruited to that region from juvenile habitats to the north or south. The ‘Monterey Bay’ model only includes juveniles collected from sites in the Monterey Bay region under the assumption that adults collected in Monterey Bay most likely resided in that same region as juveniles. Assumptions of LDFA, including multivariate normality and homogeneity of variance-covariance matrices, were checked graphically using squared-Mahalanobis distance plots and by examining the spread of each group on the canonical variate axes (following Tabachnick and Fidell 2001).

Adult Classification

Juvenile cores isolated from adult otoliths were assigned to either estuarine or coastal habitat group using the ‘all sites’ and ‘Monterey Bay’ classification models. In each case, the default classification model used Li values that had been detrended to an ‘end-of-season’ size of 140 mm (TL) to classify the juvenile fish. To investigate the impact that selecting 140 mm as the ‘end-of-season’ size may have had on the classification of adults, I also created classification functions using Li values detrended to 135 and 145 mm (TL). These alternative classification models would not change the classification accuracy of the models for juveniles fish, but they could alter the assignment of adults to estuary and coastal habitat groups.

Edge Contamination

Despite intense efforts to remove all material surrounding the juvenile cores (hereafter referred to as ‘edge’ material), it is possible that edge material was not completely removed from some cores during the sectioning and drilling process. It is likely that edge material differed in chemical composition from core material. Thus, the presence of edge material in a core sample might cause inaccurate measurement of the chemical habitat tag in the cores. I analyzed the chemical composition of the edge material removed from 13 of the adult otoliths. A paired t-test was used to determine if the core and edge material differed in chemical composition and the magnitude of this difference. This latter estimate of the chemical composition of adult edges was then used to correct for potential contamination of the core.

To explore how contamination of cores with edge material might effect the classification of adults, two alternative scenarios were explored. The elemental concentrations measured in a core were adjusted assuming that either 10% or 25% of the mass of the core was actually edge material. To determine the ‘true’ chemical composition of the core material, the values of Sr and Li measured in the cores had to be adjusted to remove the effect of chemical contamination by edge material. The following equation was used to adjust the Li/Ca concentrations measured in each core under the assumption of 10% contamination:

$$\mathrm{Li}_{10} = [\mathrm{Li}_{\mathrm{measured}} - (0.10 * \mathrm{E}_{\mathrm{mean}})]/(1 - 0.10) \qquad (1)$$

where Li_{10} is the adjusted concentration of core Li, $\mathrm{Li}_{\mathrm{measured}}$ is the Li concentration measured in the core, and $\mathrm{E}_{\mathrm{mean}}$ is the mean Li concentration of edge material (n=13). Similar equations were used to adjust Li values assuming 25% contamination and to adjust Sr values assuming 10% and 25% contamination. The adjusted values represented the best estimate of the habitat tag in the juvenile core of each adult otolith. The core values, adjusted under both the 10% and 25% contamination scenarios, were classified as either estuarine or coastal using the default ‘all sites’ and ‘Monterey Bay’ discriminant models (juvenile Li values detrended to 140 mm TL).

Correction of Adult Classification

Adults were assigned to estuarine or coastal habitat groups using discriminant models based on the chemical composition of the otoliths of juvenile estuarine and coastal fish. Although these models showed high classification accuracies for juvenile fish, they were not perfect (i.e, the classification accuracy of estuarine and coastal juveniles was less than 100%). That is, the discriminant models misclassified some juvenile fish. It is likely that errors also occurred when the discriminant models were used to assign adults to estuarine and coastal habitat groups. Under the assumption that a similar error rate occurred during assignment of juveniles and adults, I used the following equation to correct the number of adults assigned to the estuarine group to reflect the probabilities that 1) estuarine fish had been correctly classified as estuarine and 2) estuarine fish had been incorrectly classified as coastal:

$$N[adjusted, e] = P[e]*N[e] + (1 - P[c])*N[c] \qquad (2)$$

where N[e] and N[c] are the number of adults classified as estuarine and coastal by the classification model and P[e] and P[c] are the probabilities that an estuary fish and a coastal fish are correctly assigned by the classification model (based on the % of juveniles correctly classified in the model).

Results

Juvenile Classification Model

The 'all sites' discriminant function was able to separate the juvenile fish into estuarine and coastal groups (Wilk's λ, $F_{2,469}=161.6$, $p<0.001$). Using the jack-knife classification procedure on the sample of 472 fish, 81% of coastal fish (n=238) and 73% of estuarine fish (n=234) were correctly classified for an overall classification accuracy of 77% (Table 2). Classification of fish into habitat groups was possible because estuarine fish had higher Sr (0.29±0.003 mmol/mol) and lower Li (4.34±0.044 µmol/mol) than coastal fish (0.23±0.003 mmol/mol; 5.09±0.037 µmol/mol) (Figure 5).

The 'Monterey Bay' discriminant function also was able to separate juvenile fish into habitat groups (Wilk's λ, $F_{2,165}=62.5$, $p<0.001$). Using the jack-knife classification procedure on this reduced sample of 168 fish, 85% of coastal fish (n=114) and 74% of estuarine fish (n=54) were correctly classified for an overall classification accuracy of 82% (Table 2). The mean concentration of Sr and Li was 0.27 (±0.007 mmol/mol) and 4.17 (±0.098 µmol/mol) in estuarine fish and 0.23 (±0.003 mmol/mol) and 5.06 (±0.049 µmol/mol) in coastal fish (Figure 5).

Adult Classification

The concentration of Sr and Li measured in juvenile cores of adult otoliths was very similar to the Sr and Li concentrations found in otoliths from estuarine and coastal juveniles (Figure 5). Under the default 'all sites' model, 47 adults were classified as estuarine and 20 were classified as coastal (Table 3). However, the default model had misclassified 19% of coastal juveniles and 27% of estuarine juveniles. Correcting for these errors in the classification model (using equation 2) resulted in an estimate of 38 estuarine and 29 coastal adults. That is, 57% of the adult fish were estimated to have spent a portion of their juvenile period in estuarine habitats. This result was unchanged using Li values detrended to 135 mm (TL). Using Li values detrended to 145 mm resulted in a slightly lower estimate of the percentage of the adult population that came from estuaries (52%).

The ‘Monterey Bay’ classification model provided slightly lower estimates of estuarine residence. Under the default model, 43 adults were classified as estuarine and 24 were classified as coastal (Table 3). Correcting these values for error in the default classification model resulted in an estimate of 35 estuarine and 32 coastal adults. 53% of the adults were estimated to have resided during a portion of their juvenile period in Elkhorn Slough, the only estuarine habitat located in the Monterey Bay region. Using Li values detrended to 135 and 145 mm resulted in estimates that 56% and 48% of the adults came from Elkhorn Slough (Table 3).

Edge Contamination

The core and edge portions of adult otoliths differed significantly in their Li content (paired t-test, $t_{12}=-5.621$, $p<0.001$). In general, Li was less abundant in edges (3.50 µmol/mol) than in cores (4.97 µmol/mol) (Figure 5). All but two adults showed similar decreases in Li content between the core and edge portions of their otoliths (Figure 6). The Li concentrations in edges was similar to the Li concentrations found in otoliths from estuarine juveniles (Figure 5). Therefore, contamination of cores with edge material would decrease the Li values measured in some cores (particularly those with higher Li concentrations) and cause them to more closely resemble estuarine juveniles. However, contamination with edge material would not significantly alter Li values in cores with low Li concentrations. By adjusting the core values under the assumption of 10% or 25% contamination of the core mass with edge material, the Li concentration of cores with already high Li was increased. These elevated core values were within the range of Li values observed in juvenile otoliths under the 10% contamination scenario, but not under the 25% contamination scenario (Figure 7). Therefore, a level of 25% contamination was considered unlikely based on adjusted Li values.

Cores and edges also differed significantly in their Sr content (paired t-test, $t_{12}=13.40$, $p<0.001$). Sr was more abundant in edges (0.522 mmol/mol) than in cores (0.298 mmol/mol) (Figure 5). All adults analyzed showed similar increases in Sr content between the core and edge portion of the otolith (Figure 6). Sr concentrations in edges were well above the Sr concentrations measured in otoliths of juveniles from either estuarine or coastal habitats. Therefore, contamination of cores with edge material would elevate the true Sr values of cores, and make the cores more closely resemble estuarine juveniles. By adjusting the core values under the assumption of 10% or 25% contamination, the Sr value for all cores was decreased. The decreased core values were within the range of Sr values observed in juvenile otoliths under the 10% contamination scenario, but not under the 25% contamination scenario (Figure 7). As with Li, a level of 25% contamination of core mass was considered unlikely based on adjusted Sr values.

Given that the 10% contamination scenario appeared plausible, cores were reclassified with the default ‘all sites’ and ‘Monterey Bay’ classification models (Li detrended to 140 mm TL) using Li and Sr values adjusted for 10% contamination. Under the default ‘all sites’ model, and after correction for assignment error in that model, 31 adults were classified as estuarine and 36 were classified as coastal (Table 3). That is, 46% of the adult fish were estimated to have spent time in the estuarine habitats. Similar estimates were found using the default ‘Monterey Bay’ model; for example, 45% of the adult fish were estimated to have spent a portion of their juvenile period in the Elkhorn Slough estuary (Table 3).

Discussion

Juvenile Classification Model

The use of a chemical tag to discriminate fish residing in different habitats requires that the otoliths of fish collected from those habitats differ in their chemical composition. In addition, the chemical habitat tag may be used to identify adult fish that once resided in the different juvenile habitats if the tag meets two criteria (summarized in Campana et al. 2000). First, the habitat tag should be characterized using individuals collected from all possible source habitats for the adult population under study. Second, the habitat tag should be stable over the time period during which habitat assignment is to be made. My results indicate that juvenile English sole living in estuarine and coastal habitats had otoliths that differed significantly in their chemical composition. These chemical habitat tags (estuarine and coastal) were present and distinguishable in juveniles collected over a large geographic area (500 km). This area encompassed most, if not all, of the juvenile habitats that contribute individuals to the central California English sole population (based on Jow 1969). In addition, the habitat tag was found to be present and consistently distinguishable in juveniles collected over a three-year time period (1998-2000) – the time period during which the adult fish used in this study would have been living in the juvenile habitats. Thus, this habitat tag was found to meet all criteria for use in identifying the juvenile habitats from which adult fish recruited.

The classification success of the discriminant models generated in this study was due to consistent differences in Sr/Ca and Li/Ca ratios in the otoliths of estuarine and coastal fish; Sr/Ca was higher and Li/Ca was lower in estuarine habitats. Deposition of trace elements in otoliths has been shown to be influenced by a number of environmental and physiological factors, such as temperature, salinity, and growth rates. The estuarine habitats examined in this study are characterized (during the summer/fall juvenile growth season) by higher salinities, faster growth rates and warmer and more variable water temperatures than the surrounding coastal habitats (Broenkow 1977, Brown [Ch 1]). Thus, the pattern found in this study of elevated Sr/Ca in estuarine fish may have been caused by Sr/Ca concentrations being positively correlated with any of these factors. Previous studies have found Sr/Ca to be positively correlated with temperature (Kalish 1989, Yamashita et al. 2000), salinity (Kalish 1990, Secor and Rooker 2000) and growth rates (Yamashita et al. 2000). Alternatively, increased deposition of Sr in otoliths of estuarine fish may be due to higher ‘stress’ levels caused by the frequent and extreme fluctuations in water temperature and salinity in estuarine environments (Kalish 1992, Yamashita et al. 2000). Controlled laboratory experiments are needed to determine which of these factors may be responsible for the elevated Sr/Ca values in estuarine English sole.

Though multiple studies have found Li to be a useful element for differentiating habitat groups (Milton et al. 1997, Campana et al. 2000, Rooker et al. 2001, Gillanders 2002b), very little attention has been given to determining the environmental and physiological factors that effect Li incorporation into otoliths. Milton and Chenery (2001) found that juvenile barramundi held in freshwater had lower Li/Ca levels in their otoliths than fish held in seawater. A negative relationship between Li/Ca and salinity is consistent with the pattern found in this study of elevated Li/Ca values in the otoliths of coastal juveniles (estuaries in central California are hypersaline in summer months). The present study also found a significant negative relationship between otolith weight and Li/Ca indicating that Li incorporation into otoliths was influenced by ontogenic processes. These results indicate that multiple factors may influence the deposition of Li in otoliths. This is an area that requires further study. Fortunately, it is not necessary to understand the mechanisms responsible for generating habitat related differences in elemental concentrations to be able to use those chemical differences as habitat tags (Thorrold et al. 1998a).

The discriminant models, based on the habitat related differences in Sr/Ca and Li/Ca, classified coastal juveniles with more accuracy than estuarine juveniles. One possible reason for increased classification error associated with estuarine juveniles is that environmental conditions are more variable in estuarine habitats resulting in a more variable estuarine habitat tag. Environmental variability in estuarine habitats exists at two spatial scales: within estuary and among estuaries. Within an estuary, individual fish can experience vastly different environmental conditions, such as water temperature and salinity, depending on their location within the estuary. For example, in Elkhorn Slough, fish that reside in the upper channels and mudflats will experience higher temperatures and salinities than fish living closer to the mouth (Broenkow 1977). The variability in environmental conditions among estuaries is also quite large. Some estuaries in this study are relatively small and shallow (Bolinas Lagoon) while others are large and deep (Drake’s Estero). Some receive large inputs of agricultural runoff (Elkhorn Slough) while others receive large amounts of industrial runoff (San Francisco Bay). Compared to estuaries, the coastal habitats have fairly constant environmental conditions (Broenkow 1977, Brown [Ch2]). Given the increased variability in environmental conditions in estuaries compared to the coast, the estuarine habitat tag is probably more variable among individual fish than the coastal habitat tag and, thus, may be more difficult to accurately classify.

An alternative explanation for error in the classification models is that misclassified fish had recently moved into the collection habitat from the adjacent alternative habitat type. Movement of individuals between estuarine and coastal habitats prior to capture would result in a chemical tag that was either typical of the alternative habitat type or of an intermediate value depending on the timing of the movement. Krygier and Pearcy (1986) found that juvenile English sole enter estuaries over a protracted period (several months) and large size range (up to 60 mm TL). It is possible that some individuals collected from estuarine habitats in this study had recently moved into those habitats from the surrounding coastal habitats. These estuarine individuals would have been misclassified by the discriminant model because their otoliths would contain a ‘coastal’ habitat tag. Error in classification of coastal fish may have been caused by the reverse phenomena – fish collected on the coast having recently left the estuary.

This potential cause of misclassification was explored by examining the probability with which a fish was assigned to each habitat group by the classification modal (i.e., posterior probabilities). A fish that had moved recently should have a low probability of classification in the habitat from which it was collected and, thus, a high probability of classification in the alternative habitat type. In the current study, a few fish were identified that fit the above profile and, thus, may have been misclassified because they recently moved into the collection habitat. However, the majority of the fish that were misclassified in this study had intermediate probabilities (0.40 - 0.60) of classification in either habitat group. This pattern of posterior probabilities could be caused by fish spending a significant amount of time in both juvenile habitats types; either by moving frequently between habitats or by moving into the habitat of capture weeks to months prior to capture. However, intermediate posterior probabilities may also have been caused by a fish residing an area that had environmental conditions that were either 'atypical' or 'intermediate' in comparison to those found in most estuarine and coastal habitats. The current study could not differentiate between these alternative causes of misclassifications.

Two discriminant models were created in this study: 1) the 'all sites' model used juveniles collected from all estuarine and coastal habitats in the study area; and 2) the 'Monterey Bay' model used only those fish that had been collected at sites in the Monterey Bay. The Monterey Bay model had a higher total classification accuracy (82%) than the all sites model (77%). This result may have been caused by latitudinal differences in the coastal habitat tag. Fish collected from both estuarine and coastal habitats located to the north of Monterey Bay had higher Sr values than fish collected from Monterey Bay habitats (Brown [Ch 2]). In addition, fish collected from coastal and estuarine habitats south of Monterey Bay did not differ substantially in their Li concentrations (Brown [Ch 2]). By restricting the classification model to sites located within Monterey Bay, this latitudinal variability in the habitat tag was removed and classification success was increased. The reduced Monterey Bay model could be used to classify adult fish collected in Monterey Bay under the assumption that those fish once resided in the juvenile habitats located in that region. This assumption is supported by a tagging study of adult English sole, which found that most individuals were recaptured near the release area (Jow 1969).

Adult Classification

To use chemical habitat tags to identify the juvenile habitats from which adult fish recruited, the chemical composition of the juvenile portion of the adult otoliths must be determined. In this study, the juvenile portion of adult otolith was extracted by cutting away the surrounding otolith material and analyzing the chemical composition of the juvenile core with the same methods used on otoliths from juvenile fish. This 'core extraction' method was successfully used by Gillanders and Kingsford (1996) to differentiate adult blue groper that had recruited from rocky reefs and estuaries. This method appears to have been successfully applied in this study as well. The range of Sr/Ca and Li/Ca values in the juvenile cores were similar to the range of Sr/Ca and Li/Ca values found in juvenile otoliths. Therefore, the discriminant functions based on the chemical composition of otoliths from estuarine and coastal juveniles could be used to classify adult fish as having recruited from either estuarine or coastal habitats.

Using the default 'All sites' and 'Monterey Bay' classification models, I estimated that 57% and 53% of the adult fish had lived as juveniles in estuarine habitats, respectively. These percentages were determined by correcting the initial estimates generated by the classification models to account for errors in habitat assignment. Errors in habitat assignment had to be corrected for because the error rates were not equivalent for the two habitat groups; the model had a higher probability of mis-assigning a fish to the estuarine group than to the coastal group. The corrected percentages represented better estimates of true estuarine contribution than the initial values produced by the models, as long as the error rates in assignment were equal for juvenile and adult fish. Because the adult fish used in this study were living in the same habitats during the same years as the juvenile fish used to create the classification models, there was no obvious reason to doubt the validity of this assumption.

The default 'All sites' and 'Monterey Bay' classification models used Li values for juvenile fish that were detrended to an 'end-of-season' fish size of 140 mm. The negative relationship between Li/Ca and otolith size (≈fish size) had to be removed for two reasons. First, the habitat-related difference in Li/Ca values between estuarine and coastal fish would have been confounded by ontogenetic trend and the classification accuracy of discriminant models would have been diminished. Second, the juvenile cores extracted from the adult otoliths contained material deposited over the entire juvenile growth season. Thus, Li/Ca values in cores are representative of Li/Ca values found in the otoliths of large juveniles. If the classification model had not used Li values detrended to an 'end-of-season' fish size, then the distribution of Li values in juvenile otoliths would not have overlapped the distribution of Li values in juvenile cores. Classification of adults would not have been possible if discriminant models had used 'raw' Li/Ca values.

A size of 140 mm TL was chosen for standardizing the analysis because juvenile fish larger than 140 mm TL are rarely encountered in estuarine habitats (summarized in Shi et al. 1997). In addition, Smith and Nitsos (1969) determined that the first annulus (winter band) began to form in the interopercular bones of English sole that were approximately 135 mm. Choosing an appropriate 'end-of-season' fish size is important because it determines the mean of the Li distribution for juvenile fish and, thus, can affect the assignment of adults to habitat groups. Changing the 'end-of-season' fish size by ± 5 mm (TL) did alter the percentage of the adult fish that were assigned to estuarine and coastal habitat groups. However, the change was not substantial (i.e., 5% or less in either direction).

In the core extraction method, which requires cutting away the surrounding otolith material, there was the potential for contamination of the core by edge material. Contamination can modify the habitat tag present in the cores if edge material has a different chemical composition than core material. Core material and edge material was found to have different Sr/Ca and Li/Ca concentrations and, because edge material had higher Sr/Ca and lower Li/Ca, contamination may have led to the misclassification of some coastal fish and, thus, an over estimate of estuarine contribution. When adult fish were re-classified using core values adjusted for 10% contamination, the estimates of estuarine contribution were reduced to 46% and 45% for the default 'All sites' and 'Monterey Bay' classification models. However, this is probably an extreme estimate of the impact of contamination on adult classification because it is unlikely that all cores were contaminated. In addition, the concentration of Li and Sr in the material that would have caused the contamination (material located immediately adjacent to the cores) may not have been as different in chemical composition from the cores as the edge material that was analyzed in this study (material located near the exterior of adult otoliths). Therefore, the presence of edge material in extracted cores may not have had as large an influence on the chemical composition of cores as was assumed in the contamination model.

Are Estuaries Nursery Habitats for English sole in Monterey Bay?

The percentage of the adults that were identified as having resided as juvenile in estuarine habitats was estimated to range between 46% and 57% for the entire study area and 45% and 53% for the Monterey Bay region. That is, estuarine contribution to the central California English sole population was estimated to be approximately 50% even though much less than 50% of the habitat available for use by juvenile English sole in this region is estuarine habitat. For example, in the Monterey Bay region, it was estimated that the intertidal and subtidal habitats of Elkhorn Slough (the only estuary in the region) cover an area of 11.5 km^2 compared to the approximately 177km^2 of suitable coastal habitats (sandy bottom between 10 and 30 m depth; Krygier and Pearcy 1986). Though estuarine habitat comprises only approximately 6% of the available juvenile habitat in the region, approximately half of the adult fish collected in that region may have resided in the estuary as juveniles. This result suggests that estuarine habitats in this regions may be acting as 'nursery habitats' by contributing more individuals per unit area to the adult English sole population than the adjacent coastal habitats (Beck et al. 2001).

The disproportionate contribution of one juvenile habitat to the adult populations may be due to fish in that habitat having higher densities, higher growth rates, lower mortality, or more successful recruitment to the adult population (Beck et al. 2001). Past research on English sole along the coast of North America has found evidence that estuaries can support higher densities (Kygier and Pearcy 1986, Rogers et al. 1988, Gunderson et al. 1990) and faster growth rates (Kygier and Pearcy 1986, Brown [Ch 2]) than coastal habitats. In addition, one study using parasites as a natural tag of estuarine residence, concluded that the majority of adult English sole had recruited to the adult population from estuarine habitats (Olson and Pratt 1973). These findings suggest that estuarine habitats may be producing more juvenile English sole because they support a higher density of faster growing fish that successfully recruit to the adult population.

Evidence for disproportionate contribution of estuarine habitats to adult flatfish populations have recently been found for two other flatfish species - rock sole in Sendai Bay, Japan (Yamashita et al. 2000) and California halibut in southern California (Forrester and Swearer 2002). These findings combined with those of the current study suggest that estuarine habitats may commonly function as nursery habitats for juvenile flatfish populations. Many estuarine habitats in California, and around the globe, are vulnerable to loss or deterioration from a variety of processes, including erosion, pollution, and urbanization. Conservation of these estuarine 'nursery' habitats may be an important step in maintaining high levels of recruitment to harvested flatfish populations. To more fully determine the importance of estuarine habitats to the maintenance of English sole populations in California, the contribution of estuaries should be determined over a larger spatial scale. Identifying the regions in which disproportionate contribution occurs would help to determine which estuarine habitats should be targeted for protection.

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Table 3-1. Summary information for juvenile and adult English sole *Pleuronectes vetulus* collected from coastal (italic type) and estuarine (bold type) habitats for comparison of elemental composition of otoliths. Shown are collection sites (with abbreviation), mean fish size (mm TL ± S.E) and sample size (in parenthesis). ns = no data; 0 = location sampled, but no fish collected

Site	1996	1997	1998	1999	2000	2001	2002
Juveniles							
Bodega Harbor (BH)	nd	nd	nd	65.4 ± 2.7 (12)	78.5 ± 4.1 (11)	nd	nd
Bodega Bay (BB)	nd	nd	nd	88.4 ± 2.7 (14)	74.6 ± 2.9 (13)	nd	nd
Tomales Bay (TB)	nd	nd	71.6 ± 3.5 (10)	102.5 ± 4.2 (15)	78.6 ± 7.0 (11)	nd	nd
10 Mile Beach (TMB)	nd	nd	nd	(0)	57.0 ± 21.0 (2)	nd	nd
Drake's Bay (DB)	nd	nd	nd	83.3 ± 6.6 (10)	(0)	nd	nd
Drake's Estero (DE)	nd	nd	nd	74.7 ± 3.6 (13)	68.5 ± 3.2 (11)	nd	nd
Bolinas Lagoon (BL)	nd	nd	nd	81.9 ± 3.8 (12)	68.9 ± 3.8 (10)	nd	nd
San Francisco Bay (SFB)	nd	nd	94.3 ± 4.6 (12)	83.4 ± 4.5 (13)	71.6 ± 2.9 (15)	nd	nd
Half Moon Bay (HMB)	nd	nd	nd	107.3 ± 3.0 (12)	88.7 ± 2.6 (11)	nd	nd
Santa Cruz (SC)	nd	nd	113.4 ± 3.6 (5)	101.7 ± 8.7 (7)	82.7 ± 4.7 (11)	nd	nd
Pajaro River (PR)	nd	nd	80.3 ± 3.2 (10)	92.7 ± 4.2 (12)	78.5 ± 3.7 (11)	nd	nd
Elkhorn Slough (ES)	90.5 ± 3.1 (8)	65.7 ± 6.2 (9)	77.0 ± 6.7 (3)	95.0 ± 3.0 (15)	84.9 ± 4.8 (19)	nd	nd
Salinas River (SR)	nd	nd	84.6 ± 4.9 (11)	87.3 ± 4.5 (12)	76.3 ± 4.2 (12)	nd	nd
Monterey (MY)	nd	nd	62.1 ± 2.4 (11)	(0)	59.2 ± 2.2 (12)	nd	nd
Estero Bay Cayucos (EBC)	nd	nd	nd	93.0 ± 4.8 (11)	78.1 ± 3.4 (15)	nd	nd
Estero Bay Strand (EBS)	nd	nd	nd	81.6 ± 4.8 (10)	71.0 ± 4.3 (10)	nd	nd
Morro Bay (MB)	nd	nd	85.7 ± 6.1 (9)	86.2 ± 5.6 (13)	83.2 ± 5.0 (13)	nd	nd
Port San Luis (PSL)	nd	nd	nd	86.7 ± 6.4 (3)	81.5 ± 5.7 (13)	nd	nd
Adults							
Monterey Bay (MONT 1)	nd	nd	nd	nd	nd	197.9 ± 4.7 (8)	nd
Monterey Bay (MONT 2)	nd	nd	nd	nd	nd	244.5 ± 7.3 (17)	nd
Monterey Bay (MONT 3)	nd	nd	nd	nd	nd	nd	277.5 ± 3.1 (42)

Table 3-2. The percentage of juvenile English sole correctly classified into coast and estuary habitat groups and the total percentage correctly classified using discriminant function analysis and jack-knife cross-validation on the concentration of elements (Li and Sr) in otoliths. Two classification models were examined: (A) the 'All Sites' model classified juveniles from all collection sites between Bodega Bay and Port San Luis; and (B) the 'Monterey Bay' model used only fish collected from sites in Monterey Bay. Sample sizes are shown in parenthesis.

Classification Model	% Coast	% Estuary	% Total
(A) All sites	**81** (238)	**73** (234)	**77**
(B) Monterey Bay	**85** (114)	**74** (54)	**82**

Table 3-3. The number of adult English sole classified into either coast or estuary habitat group, the number in the estuary group corrected (using equation 2) for error in the juvenile classification model, and the resulting percentage in the estuary group using discriminant functions derived from juvenile fish collected from (A) all sites between Bodega Bay and Port San Luis and (B) Monterey Bay only (Table 2). The default classification model used Li values detrended to a juvenile fish size of 140 mm (TL). Discriminant functions based on Li values detrended to 135 and 145 mm were also used to classify adults. The default model assumed no contamination of adult otolith cores with material from the otolith edge. Sr and Li (detrended to 140 mm) values in cores were adjusted (using equation 1) assuming 10% of core material was contaminated with edge material before classification.

Classification Model	# Coast	# Estuary	# Estuary (Corrected)	% Estuary (Corrected)
A) All Sites				
Default	**20**	**47**	**38**	**57**
Li/Ca (135 mm TL)	**20**	**47**	**38**	**57**
Li/Ca (145 mm TL)	**26**	**41**	**35**	**52**
10% Contamination	**33**	**34**	**31**	**46**
B) Monterey Bay				
Default	**24**	**43**	**35**	**53**
Li/Ca (135 mm TL)	**20**	**47**	**38**	**56**
Li/Ca (145 mm TL)	**29**	**38**	**32**	**48**
10% Contamination	**33**	**34**	**30**	**45**

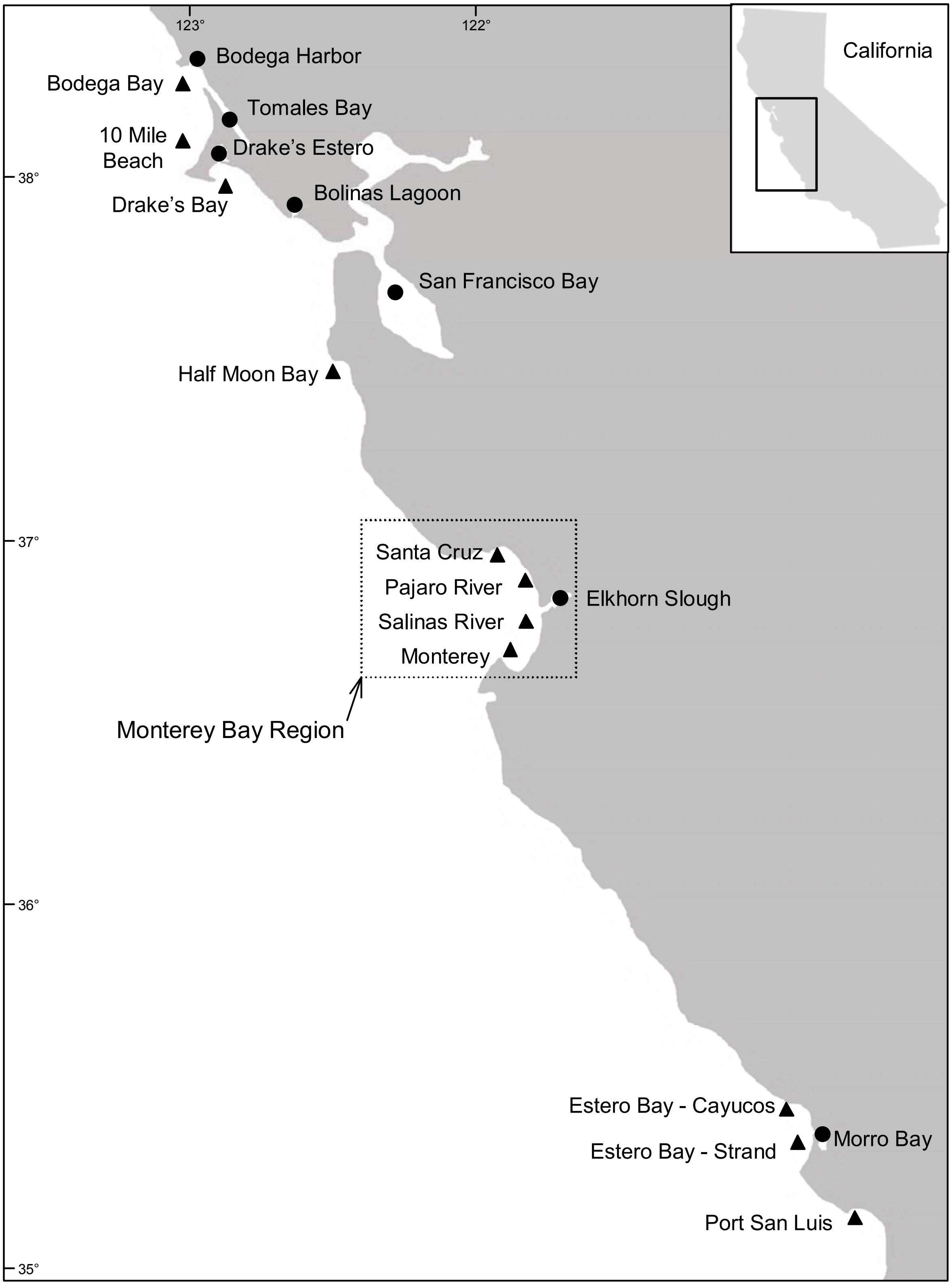

Figure 3-1. Map of California (inset) and the open coast (triangle) and estuary (circle) sites from which juvenile English sole were collected. All adult English sole examined in this study were collected in the Monterey Bay region (boxed area).

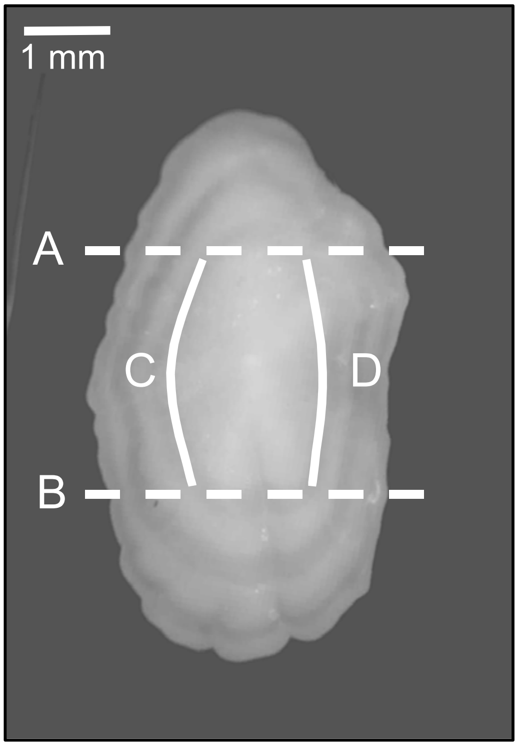

Figure 3-2. Juvenile cores were isolated from the otoliths of adult English sole in a three step process. An isomet saw was used to 1) remove edge material from the rostrom and post-rostrum along cuts A and B and 2) make a thin section approximately 300 µm thick along the sagittal plane (the plane of this sheet of paper). A microdrill was used to 3) remove the right and left sides from the thin section by drilling along the translucent band corresponding to the 1st winter (paths C and D).

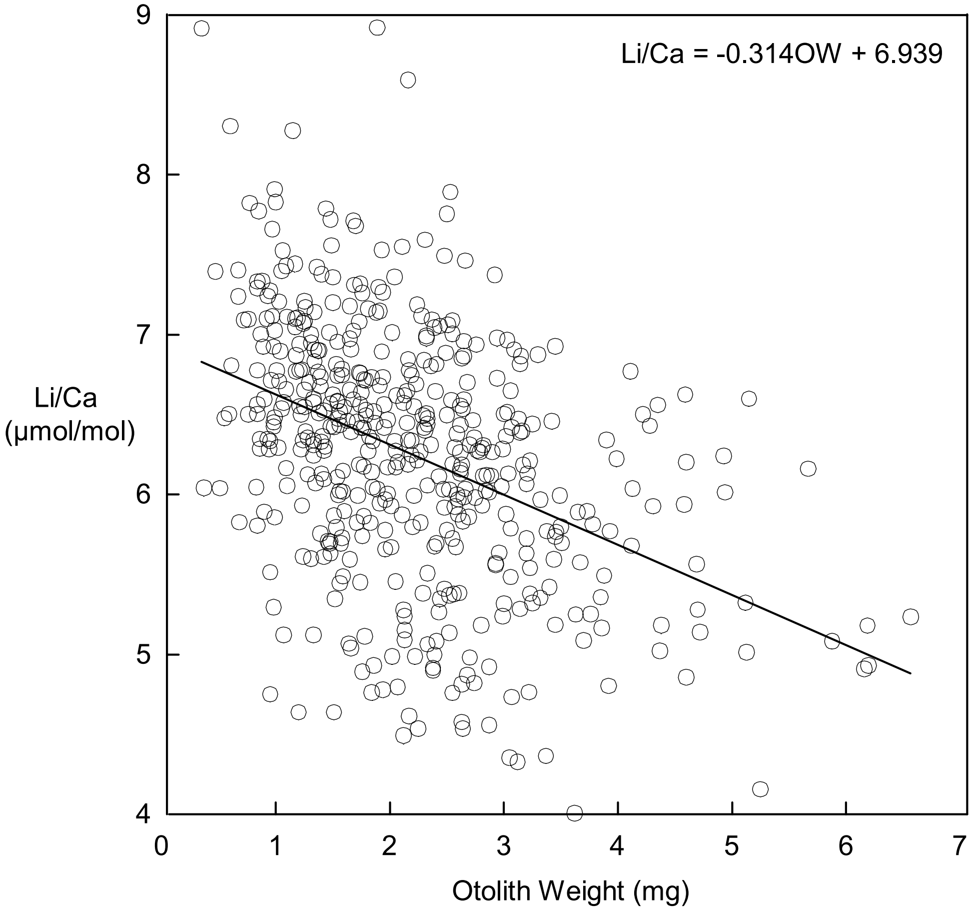

Figure 3-3. Relationship between otolith weight (mg) and Li/Ca (µmol/mol) in the right sagittal otoliths of juvenile English sole ($r^2 = 0.172$, $p < 0.001$).

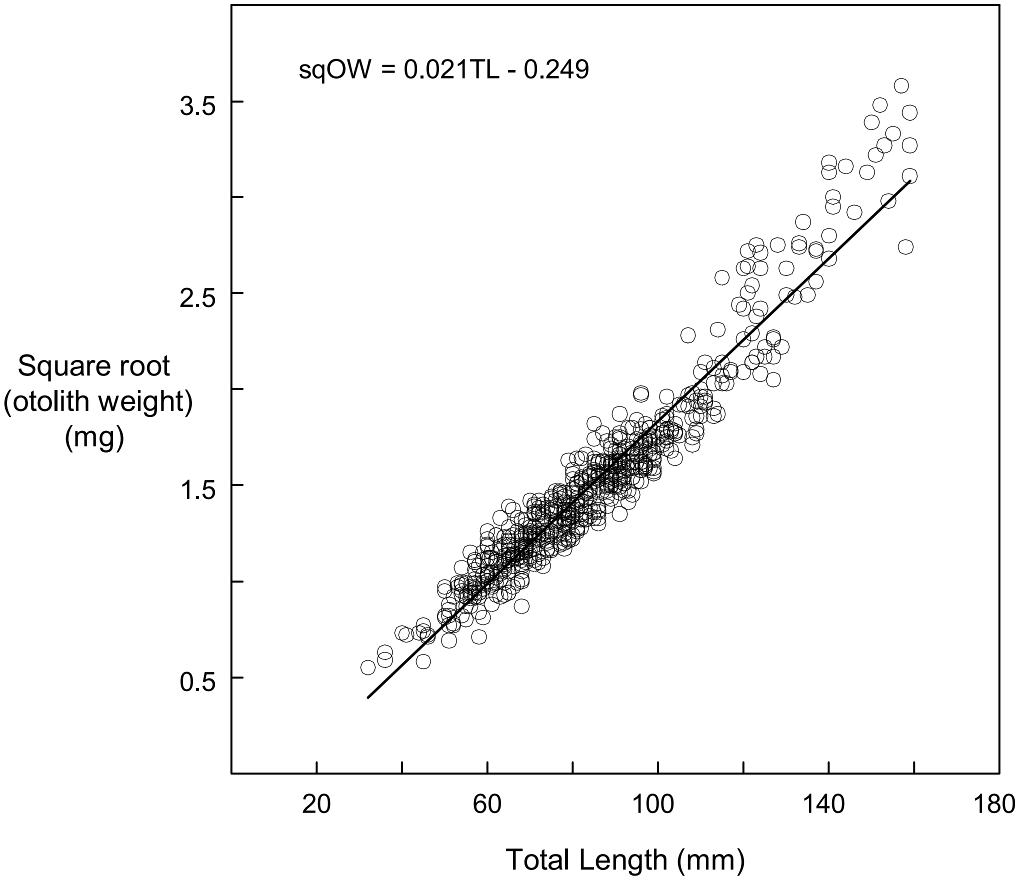

Figure 3-4. Relationship between total length (mm) and otolith weight (mm; right sagittal otolith) for English sole ($r^2 = 0.88$, $p < 0.001$).

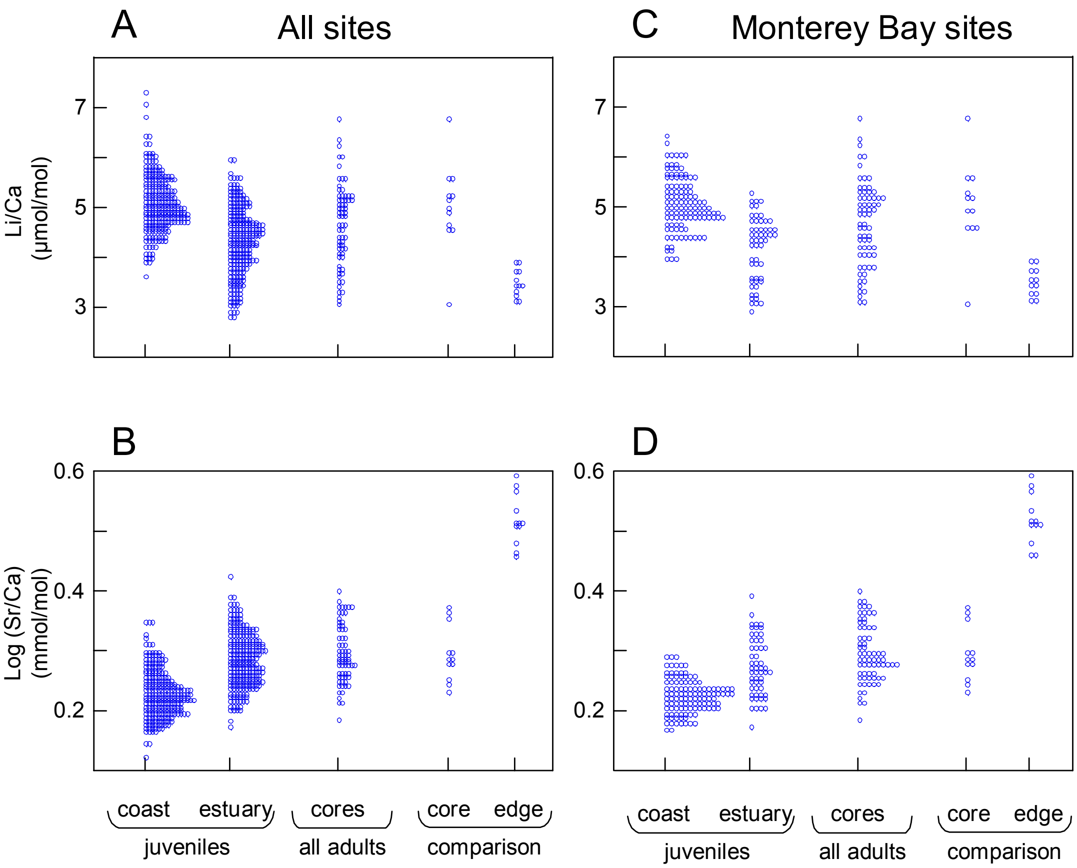

Figure 3-5. Comparison of the concentration of Li and Sr in the otoliths of juvenile and adult English sole. Sr and Li concentrations in otoliths of juvenile fish collected from open coast and estuary habitats are shown for all sites between Bodega and Port San Luis (panels A and B) and Monterey Bay sites only (panels C and D). Sr and Li concentration are shown for the cores of 'all adult' otoliths collected from Monterey Bay (n=67) and for a 'comparison' of the chemical composition of the cores and edges for a subset of these adults (n=13).

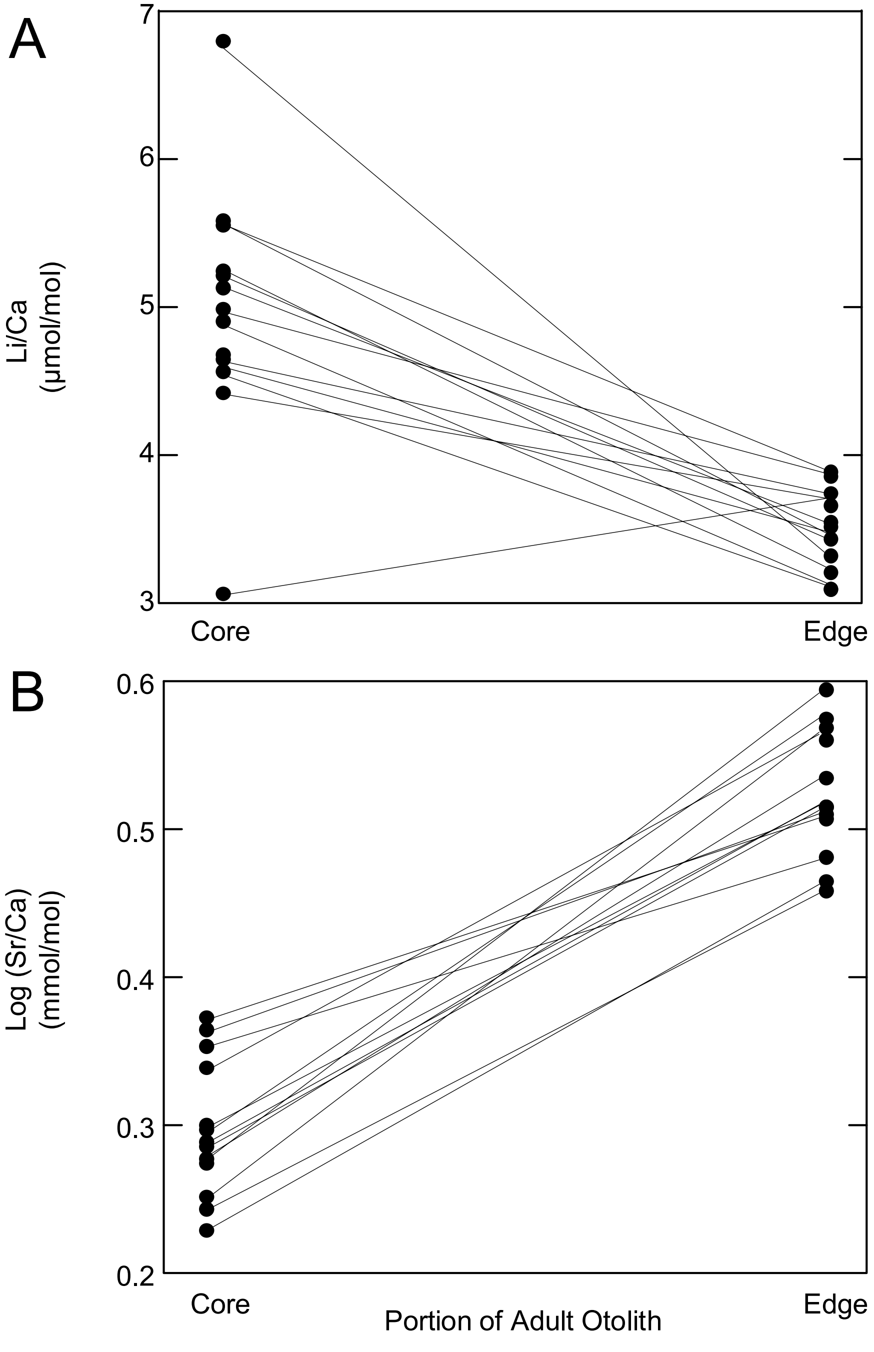

Figure 3-6. A comparison of (A) Li and (B) Sr concentration in two portions of otoliths from adult English sole. The ‘core’ corresponds to the portion of the otoliths formed during the first summer of growth and the ‘edge’ corresponds to the portion formed after the first annulus (winter). Each line connects the core and edge of a given adult (n=13).

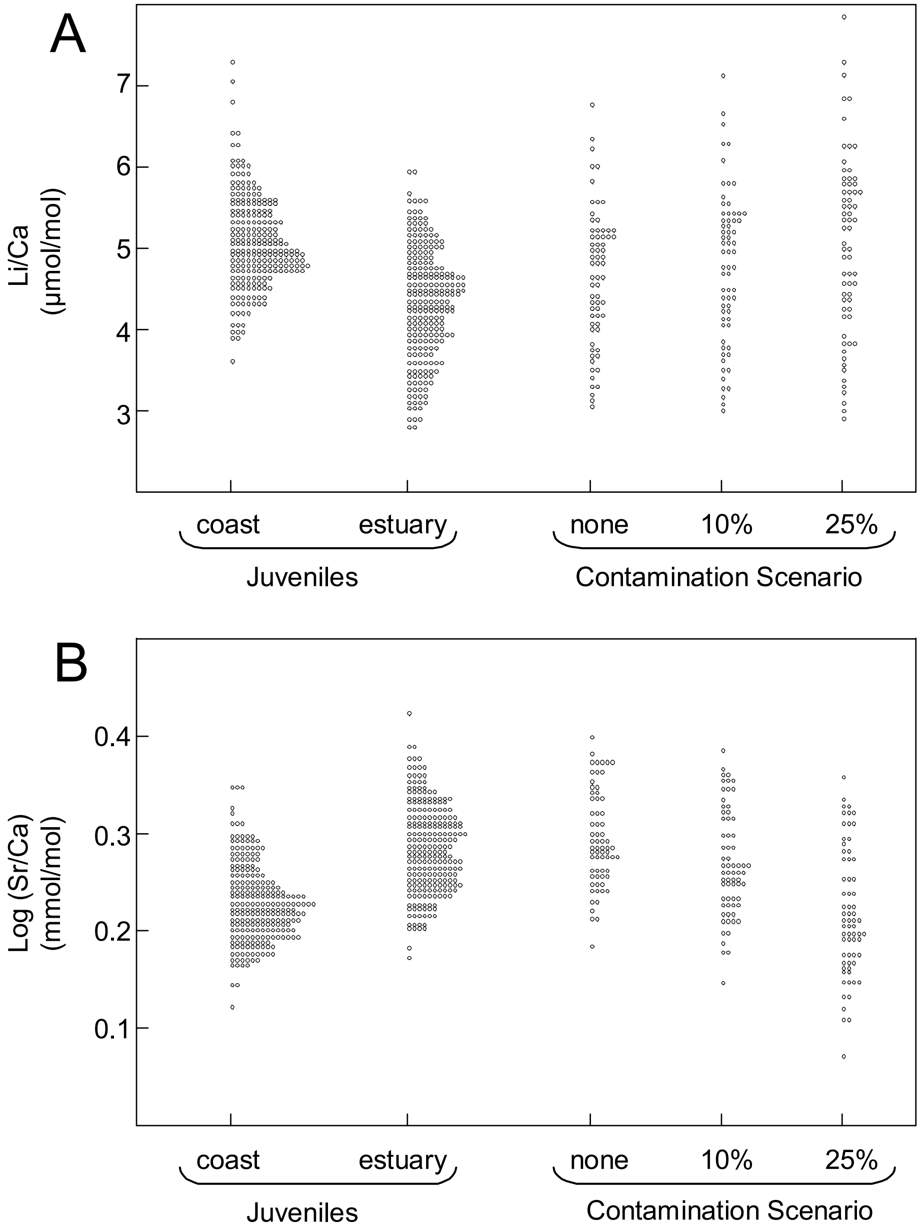

Figure 3-7. Concentration of (A) Li and (B) Sr in otoliths from juvenile and adult English sole. Elemental concentrations are shown for juveniles collected in coastal and estuarine habitats and for cores of otoliths from adults collected in Monterey Bay. Core values are shown under three contamination scenarios: no contamination; 10% of core contaminated with edge material; and 25% of core contaminated with edge material. Core values were adjusted to remove the effects of contamination using equation (1) .

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