Trace elements in otoliths indicate the use of open-coast versus bay nursery habitats by juvenile California halibut

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ABSTRACT: Many coastal fishes use inshore nursery habitats as juveniles, but it is often difficult to define which nursery areas supply most recruits to adult populations. We tested whether trace element concentrations in otoliths can be used to identify which of 2 nursery habitats (bays or shallow open coast) were occupied by juvenile California halibut. Juveniles from bays in 1998 had concentrations of Cu and Pb in their otoliths that were higher than those in open coast juveniles of the same year. This broad-scale difference between bay and open coast juveniles remained intact when bay juveniles from 1994 to 1997 were added to the comparison, and juvenile halibut could be assigned to their nursery habitat of origin quite accurately (83%) using otolith concentrations of Cu and Pb. At a finer spatial scale, otolith concentrations of Cu and Pb differed among individual bays, and fish from the same bay could differ among years, precluding their use as markers of nursery habitat use at these scales. Like halibut otoliths, sediments from bays had higher concentrations of Cu and Pb than open coast nursery sites, and this difference was consistent over 11 yr. Otoliths and sediments from individual bays, however, showed no correlation in Cu and Pb concentrations. The concentration of Cu and Pb in sediments and their deposition in otoliths were thus loosely matched at a broad scale, though the underlying cause of this link is not known. A discriminant model, parameterized using Cu and Pb levels in juvenile otoliths, was used to classify prior nursery habitat use by 19 larger halibut (of unknown origin). Eleven of these halibut had high levels of Cu and Pb in the part of the otolith deposited as a juvenile, and were classified as of bay origin. The other 8 halibut had low otolith Cu and Pb levels in the juvenile portion of their otoliths and were classified as having used open coast nurseries. Overall, our results suggest that this approach has the potential to allow identification of nursery habitat use by California halibut at a broad scale (bay vs open coast) but not at a fine scale (individual bays).

KEY WORDS: Estuaries · Nursery habitat · Otoliths · Trace elements · Halibut

INTRODUCTION

Background

Most coastal fish have populations that occupy spatially distinct habitats at different stages in their life history. Many species, for example, use protected inshore habitats such as estuaries, bays and salt marshes as juvenile nursery grounds, but migrate offshore to deeper habitats as adults (Able & Fahay 1998). Many fish using inshore nurseries are the subject of commercially important fisheries (Houde & Rutherford 1993). The ability to quantify the degree to which fishes are exchanged among habitats is critical to understanding the ecology of these populations and to successfully manage their harvesting. For species using bays as nurseries, approaches used to assess the

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importance of the nursery habitat have often been qualitative, and quantitative approaches have usually been indirect. Some authors have assessed importance as the proportion of the life history (Cyrus & Martin 1991) or amount of time spent in a nursery (Szedlmayer & Able 1993). Many authors have determined the importance of nursery habitats based on the abundance of juvenile fishes in a specific nursery (Reichert & van der Veer 1991) or by comparing abundances among several nursery areas (Kerstan 1991). A limitation of these measures is that they do not identify natal origin or allow estimates of how many juveniles successfully recruit to the adult population from different nurseries.

A new approach that has the potential to provide more direct measures of the exchange of individuals among sites employs the analysis of trace elements in otoliths, balance organs found in the ear canals of all teleost fishes. Otoliths grow continually by the deposition of calcium carbonate crystals within a protein matrix. As the otolith material accretes, it incorporates trace elements from the environment that have been absorbed across the gills or gut and entered the fishes' bloodstream (Campana 1999). Fish collected from different locations often vary in the concentrations of various trace elements in their otoliths. Examples include comparisons of fish occupying different inshore sites (Gillanders & Kingsford 1996, 2000, Milton et al. 1997, Thorrold et al. 1997, 1998a,b, 2001), different areas on a continental shelf (Campana & Gagne 1994, Campana et al. 1994, 1995, 1999), different deep-water oceanic sites (Edmunds et al. 1991), and different coral reefs (Patterson et al. 1999, Swearer et al. 1999).

The causes of these site-specific differences in otolith chemistry are still poorly understood (Campana 1999). Some trace elements are deposited in otoliths in rough correspondence to their bulk concentration in the water column (Mugiya et al. 1991, Geffen et al. 1998, Bath et al. 2000, Milton & Chenery 2001), and water temperature and salinity also influence how some elements are incorporated into otoliths (Fowler et al. 1995a,b, Bath et al. 2000). Although not empirically documented, it is also possible that other environmental variables, including the uptake and incorporation of elements from the tissues of ingested prey, may influence variation in otolith elemental composition. Spatial gradients in any of these environmental conditions might produce reliable markers of site occupancy (elemental 'tags') if variation in otolith chemistry can be matched to specific environmental conditions. Site-specific differences in otolith chemistry might also occur, even in the absence of environmental gradients, if there are genetic differences among populations in the physiological regulation of elemental deposition (Campana & Gagne 1994).

Whatever their underlying cause, there is still a pressing need to test the consistency of observed site-specific differences in otolith chemistry. Most studies to date have compared relatively few sites, and have compared fish collected in only 1 year. Our primary aim in this study was to test for elemental tags indicating nursery habitat use at 2 spatial scales, and to assess whether patterns of trace-element deposition were consistent among years. We tested for large-scale elemental tags by comparing juvenile fishes from the 2 general nursery habitats occupied by our study species: bays and shallow open coast sites. We tested for small-scale elemental tags by comparing juveniles from individual bays. At both large and small spatial scales, we tested for temporal consistency in elemental tags by comparing juvenile fish collected over several years.

We also assessed whether variation in the concentration of trace elements in sediments varied in space and time in a way that matched the otolith chemistry of juvenile fishes resident in those locations. The lack of an association would allow us to exclude sediment trace elements as a cause of elemental deposition. A positive correlation, while not indicating causation, would suggest that a gradient in trace element concentrations across habitats caused any elemental tag in the otoliths. Lastly, we test whether it might be possible to identify the nursery habitat used previously by adults. To do this, we analysed otoliths from a small sample of larger fishes that had migrated to the adult habitat offshore and whose prior nursery habitat was unknown. Chemical analyses targeted both the central part of the otolith, deposited during residence in the nursery habitat, and the outer portion that was deposited after the fish had migrated to adult habitat. To test for natural tags indicating past nursery habitat use, the chemical composition of their otolith centre was compared to the composition of juvenile otoliths of known origin.

The study system

Our study species, the California halibut *Paralichthys californicus*, is the subject of a valuable fishery in California (Frey 1971) and has a life history typical of many coastal species. Adults occur on the continental shelf adjoining the West Coast of the United States and northern Baja California Sur, Mexico, but are concentrated primarily in the Southern California Bight (Frey 1971). Juvenile halibut (9 to 10 mm standard length [SL]) settle in shallow coastal habitats after a 20 to 30 d pelagic larval phase (Moser & Watson 1990). Juveniles then migrate into 2 distinct nursery habitats: (1) protected semi-enclosed bays and estuaries (which we will refer to as 'bays' for convenience), and (2) shal-

low (<30 m) inshore areas on the open coast (Kramer 1991). In both habitats, they are found in unvegetated areas on sandy sediments (Allen & Herbinson 1990, Valle et al. 1999). Juveniles on the open coast are at extremely low densities compared to those in bays, but since this habitat makes up virtually the entire coastline, the absolute number of juveniles occupying open coast habitat may be high (Kramer 1990). After about 1 yr, juveniles (140 to 220 mm SL) from both nursery habitats move offshore to join adult populations in deeper open coast areas. Surveys of the distribution and abundance of age-0 halibut can be used to estimate the total abundance of juveniles in different habitats and, by extrapolation, what fraction of the adult population originates from each (Allen et al. 1990). These extrapolations are difficult, however, because the scarcity of open coast juveniles makes it difficult to accurately estimate their abundance.

Chemical tags recorded in otoliths might provide a more direct estimate of prior residence in specific nurseries if the central portion of adult otoliths, deposited during the first year of life, retained a chemical tag indicating juvenile habitat. The importance of bay nurseries could then be measured as the fraction of successful recruits to the adult population that originated from bays. There are several trace elements that are potential markers for differentiating bay from open coast sites (Al, Mn, Co, Ni, Cu, Zn, Sr, Ag, Cd, Sn, Ba, and Pb). Most promising are elements (Al, Ni, Cu, Zn, Ag, Cd, Pb) whose concentrations in the water column and sediments are generally higher in bays than at inshore open coast sites in southern and central California (Johnson et al. 1988, Flegal & Sañudo-Wilhelmy 1993, van Geen & Luoma 1993, Sañudo-Wilhelmy & Flegal 1996, Zirino et al. 1998). In the deeper offshore coastal areas occupied by adult halibut, trace element concentrations are similar to, or slightly lower than, concentrations inshore on the open coast (Sañudo-Wilhelmy & Flegal 1996).

MATERIALS AND METHODS

Differences in otolith chemistry of juvenile halibut occupying bay and open coast nurseries. We first collected juvenile California halibut residing in bay and open coast nursery areas to test for differences in the trace element chemistry of their otoliths. Juvenile halibut from bays were collected using beach seines, and juveniles from the open coast were collected using trawls towed behind small boats. Open coast nursery sites are difficult to sample and contain very low densities of juvenile halibut. As a result, many collecting trips yielded no halibut, and we successfully sampled open coast sites only in 1998. In this year, we collected
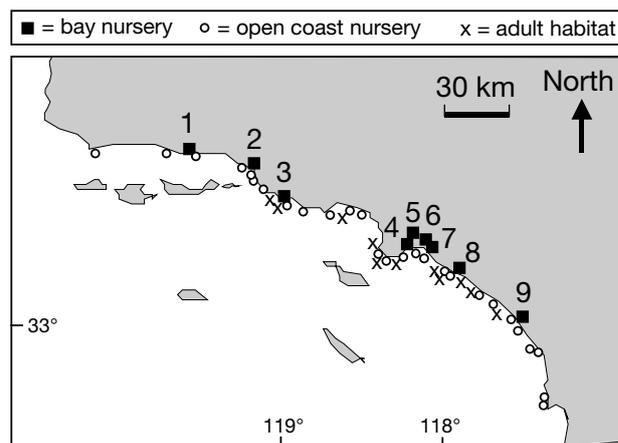

Fig. 1. Map of southern California showing bay and shallow open coast nursery sites sampled for juvenile California halibut, and deeper adult habitat sampled for larger fish. Bay sites are numbered as follows: 1 = Carpinteria Marsh, 2 = Ventura Harbor, 3 = Mugu Lagoon, 4 = Los Alamitos Bay, 5 = Los Angeles Harbor, 6 = Long Beach Harbor, 7 = Anaheim Bay, 8 = Newport Bay, 9 = Batiquitos Lagoon

69 juveniles from 27 inshore locations (depth <28 m) from San Diego to Pt. Conception (Fig. 1). In 1998, we also collected 65 juveniles from 7 bays in southern California spread along the same region of coastline (Fig. 1). The bays sampled were a reasonable cross section of those in the region in terms of size, fish habitat, and human impact (Mearns et al. 1991, Ferren et al. 1996). Comparison of the 1998 juveniles thus allowed us to test for an overall difference between bay and open coast halibut using fish collected in the same year. We collected 152 additional juveniles in bays from 1994 to 1997. Most of these juveniles were collected from the 7 original bays, but 2 additional bays were also sampled (Fig. 1, Table 1). We used these samples to assess the reliability of the bay natural tag. To do this, we tested whether any differences between bay and open coast juveniles that were detected in 1998 remained intact when bay juveniles from other years and sites were added. We also made more selective comparisons to test for spatial and temporal variation in the otolith chemistry of juveniles from specific bays. We tested for differences among years in a single bay, as well as differences among bays in a given year.

All juvenile halibut were frozen before preparation for chemical analyses to avoid effects of varied storage procedures on trace-element analysis (Milton & Chenery 1998). The juvenile halibut collected ranged in size from 33 to 206 mm SL (mean = 103 mm) and so had spent most of their lives in the nursery habitat from which they were collected (Kramer 1991). We therefore analysed entire otoliths (1 sagitta per fish) to get an integrated measure of their chemistry. Concentra-

Table 1. *Paralichthys californicus.* Mean concentrations (µg g^{-1} Ca) of Cu and Pb in the otoliths of juvenile halibut. The location of each bay is indicated in Fig. 1

Bay	n	Year sampled	Cu Mean	Cu SE	Pb Mean	Pb SE
Carpinteria Marsh	19	1994	1.554	0.062	0.030	0.004
Carpinteria Marsh	9	1995	5.141	0.977	0.065	0.011
Carpinteria Marsh	19	1996	4.832	0.861	0.061	0.021
Ventura Harbor	2	1998	0.749	0.531	0.282	0
Mugu Lagoon	7	1997	0.342	0.09	0.15	0.035
Mugu Lagoon	7	1998	0.414	0.103	0.172	0.073
Los Alamitos Bay	21	1994	1.670	0.09	0.014	0.002
Los Alamitos Bay	22	1996	0.898	0.158	0.106	0.027
Los Alamitos Bay	5	1998	0.050	0.034	0.030	0.021
Los Angeles Harbor	10	1996	0.336	0.04	0.021	0.002
Long Beach Harbor	8	1998	0.159	0.015	0.004	0.004
Anaheim Bay	20	1994	1.530	0.044	0.039	0.011
Anaheim Bay	25	1996	2.731	0.229	0.028	0.003
Anaheim Bay	3	1998	0.552	0.396	0.087	0.079
Newport Bay	7	1998	1.245	0.091	0.863	0.595
Batiquitos Lagoon	33	1998	0.754	0.166	0.057	0.018

tions of trace elements in otoliths were determined using inductively coupled plasma mass spectrometry (ICP-MS). The otoliths collected from both nursery habitats in 1998, and 103 additional otoliths collected from bays in earlier years, were analysed using a Finnegan MAT high-resolution ICP-MS. A further 113 otoliths, all collected in bays prior to 1997, were analysed using a different ICP-MS instrument (a VG Elemental PlasmaQuad 2+) because the Finnegan MAT was unavailable when these otoliths were analysed.

Otoliths analysed using the PlasmaQuad 2+ ICP-MS were first dissected and weighed to the nearest 10 µg. They were then crushed between 2 borosilicate plates and transferred to a 1.5 ml microcentrifuge vial. Each sample was then soaked in 500 µl of 5% NaOCl for a total of 72 h to remove organic contaminants. The NaOCl solution was changed 6 times (every 12 h) during the soaking process. The otoliths were then rinsed 3 times with de-ionized water (18 MOhm) and heat-rinsed for 1 h to remove any remaining bleach. After 3 more rinses with de-ionized water, the otoliths were transferred to acid-leached polypropylene 1.5 ml microcentrifuge vials. The otoliths were next acid-leached 3 times with 0.001 N HNO$_3$ (Fisher Optima grade) to remove adsorbed elements, and allowed to air-dry in a laminar flow bench. Each sample was then dissolved in 0.14 N HNO$_3$ (Fisher Optima grade). The volume of acid used

was varied, depending on the otolith weight, to yield a solution with a calcium concentration of approximately 0.06 M. Next, 25 µl of this primary sample solution was transferred to another acid-leached polypropylene 1.5 ml microcentrifuge vial. It was then diluted to a final volume of 1.5 ml with 1% HNO$_3$ (Fisher Optima grade) containing Sc, Y, and In as internal standards. These dilutions yielded a final sample calcium concentration of approximately 1 mM and were chosen to minimize the magnitude and between-sample variability of matrix effects.

Samples were introduced to the PlasmaQuad 2+ ICP-MS with a Cetac U5000 AT ultrasonic nebulizer modified to handle low sample flow rates. Samples were run in blocks of 13, with samples from each site and collection date placed randomly in each block, in order to avoid possible bias due to sequence effects. Also included in each block were 2 samples of a consistency standard (a matrix-matched solution containing all elements analyzed in concentrations typically found in an otolith). The concentrations of elements in the sample were calculated using calibrations derived from 3 external standards that were run before and after each block of samples. Standards were matrix-matched and optimized for each element to yield a range of concentrations that would bracket the range of expected sample concentrations.

The Finnegan MAT high-resolution ICP-MS was coupled with either a Cetac MCN-100 microconcentric nebulizer (pumped at 60 µl min^{-1}) or a glass expansion micromist nebulizer (pumped at 60 µl min^{-1}). Otoliths analysed using this instrument were prepared as just described, except that these otoliths were not crushed because paired tests revealed no differences in the chemistry of crushed and uncrushed otoliths. Other slight differences included the use of polyethylene rather than polypropylene vials and final dilution of the sample to a slightly different Ca concentration (5 to 7.5 mM). Trace element concentrations were also determined using a similar procedure to that described for the first 113 otoliths. Samples were run in blocks of 18, with samples from sites and collection date placed randomly in each block to avoid bias due to sequence effects. We also included matrix-matched external standards and a consistency standard in each block.

For both ICP-MS instruments, trace element concentrations were expressed relative to that of calcium

(µg element g Ca^{-1}). Some of the elements screened were discarded because of analytical problems with 1, or both, instruments (Al, Ni, Zn, Sn and Ag) or because their concentrations were often below detection limits (Co and Cd). For the remaining elements (Mn, Cu, Sr, Ba, Pb), we tested whether the results from the 2 instruments were comparable. To do this, we dissected the right and left sagitta from 10 halibut and analysed 1 otolith using each instrument. The levels of most elements in the paired otoliths were highly correlated (correlation for Mn, r = 0.91, p < 0.001; Cu, r = 0.97, p < 0.0001; Sr, r = 0.93, p < 0.0005; Pb, r = 0.75, p = 0.013), so we pooled the data from the 2 methods for these elements. For Ba the correlation was weaker (r = 0.65, p = 0.04) and measured concentrations were lower using the PlasmaQuad 2+ than the Element, so we analysed data from the 2 instruments separately. Detection limits (mean conc. of blank + 3 × SD) for the instrument with the higher limits were as follows (all units ng g^{-1}): Mn = 0.038, Cu = 0.098, Sr = 6.192, Ba = 0.014, Pb = 0.016. Precision estimates (% relative standard deviation) based on repeated analysis of the consistency sample were as follows: Mn = 1.9%, Cu = 3.4%, Sr = 1.5%, Ba = 1.8%, Pb = 2.3%.

We performed analyses of variance (ANOVA) to test for differences in the mean concentrations of trace elements among nursery habitats. Separate univariate tests were performed for each element, rather than a combined multivariate ANOVA, because sample sizes differed among the elements. Prior to this, and all other analyses, assumptions of normality and homoscedasticity were checked (following Johnson & Wichern 1998) and data always required log$_{10}$-transformation in order to meet these assumptions. We then conducted discriminant analyses to test how accurately we could classify juvenile halibut to their nursery habitat using the concentrations of trace elements (Mn, Cu, Sr, Ba and Pb) in their otoliths. Because Ba data from the 2

ICP-MS instruments were not directly comparable, models that included this element were constructed separately for each instrument. Neither raw nor transformed data met the additional assumption of equal covariance matrices required for discriminant analysis, so we used a quadratic model that is robust to departures from this assumption (Johnson & Wichern 1998). To evaluate the success of the discriminant model, we compared the numbers of samples actually taken from bays and open coast to a jackknifed estimate of the number predicted by the model to have been taken from each habitat (Lackenbruch & Mickey 1968).

Differences in sediment trace-element chemistry among nursery habitats. The second part of our study was to define patterns of variability in trace-element chemistry within and among the 2 nursery habitats. A general trend for elevated levels of trace elements in bays is well-established, so our goals were (1) to test whether the specific trace elements identified as good natural tags in otoliths differed between bay and open coast nursery sites, (2) to test whether differences among nursery habitats were consistent among years, and (3) to test whether concentrations in otoliths and sediments were correlated at the scale of individual bays.

We used data on trace element concentrations in sediments from 5 monitoring studies in the study area (Table 2). Only data from suitable halibut nursery sites were used (<30 m deep, close to seawater in salinity and over sandy/silty sediment). We compiled data on 3 elements measured in otoliths (Mn, Cu, and Pb). The remaining 2 elements analysed in otoliths (Sr and Ba) were not monitored in sediments. We used data from 1986 to 1998, collected in most of the bays within the study area (n = 23) and from open coast sites spread from San Diego to Pt. Conception (for details see Fig. 2). Sampling and analytical methods differed slightly among the programs, but are broadly comparable (see

Table 2. Details of environmental monitoring data used to compare levels of trace elements in sediment samples from within bays and on the open coast

| Agency	Bays			Open coast			Reference for methods
	n	Dates	Area sampled	n	Dates	Area sampled	
Bay Protection and Toxic Cleanup Program*	355	1992–1997	21 bays from Pt. Conception to San Diego	52	1992–1997	Pt. Conception to San Diego	Stephenson et al. (1994)
NOAA Status and Trends Program*	123	1986–1994	5 bays from Marina Del Rey to San Diego	106	1986–1994	Pt. Conception to San Diego	Lauenstein & Cantillo (1993)
Southern California Coastal Water Research Project	0	–	–	62	1994	Pt. Conception to San Diego	Schiff & Gossett (1998)
State Mussel Watch Program	18	1989–1993	6 bays from Los Angeles to San Diego	5	1990–1991	San Diego	Rasmussen (1996)
City of Los Angeles	47	1993–1996	Los Angeles/Long Beach Harbor area	75	1993–1996	Pt. Dume to Palos Verdes	City of Los Angeles (1997)
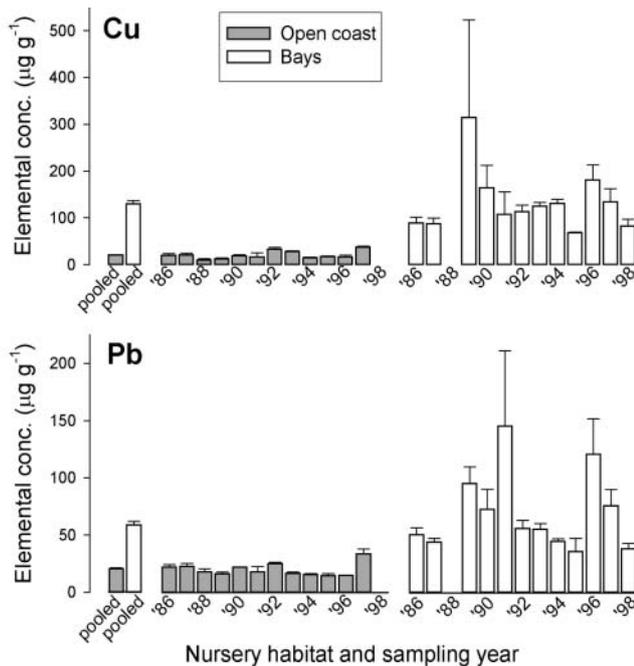
Fig. 2. Mean bulk concentrations (with SE) of Cu and Pb in sediments of open coast and bay nursery sites occupied by juvenile California halibut. Data are presented for all years pooled and for each year separately (1986–1998). A total of 300 samples were taken on open coast, and sample sizes each year were as follows: 1986 = 28, 1987 = 28, 1988 = 14, 1989 = 12, 1990 = 9, 1991 = 5, 1992 = 16, 1993 = 61, 1994 = 93, 1995 = 16, 1996 = 13, 1997 = 5, 1998 = 0. Samples were randomly located throughout the study region. Usually 1 sample was taken per location and a different set of locations within the study region was sampled each year. A total of 545 samples were taken in 23 bays, including the 9 sampled for halibut, but not all bays were sampled in all years. Number of bays sampled each year (n_{bay}) and number of samples each year (n) were as follows: 1986, n_{bay} = 3, n = 24; 1987, n_{bay} = 4, n = 33; 1988, n_{bay} = 2, n = 123; 1989, n_{bay} = 3, n = 12; 1990, n_{bay} = 5, n = 11; 1991, n_{bay} = 2, n = 3; 1992, n_{bay} = 5, n = 32; 1993, n_{bay} = 12, n = 135; 1994, n_{bay} = 16, n = 189; 1995, n_{bay} = 3, n = 3; 1996, n_{bay} = 7, n = 55; 1997, n_{bay} = 10, n = 52; 1998, n_{bay} = 4, n = 56

references in Table 2). Samples with elemental concentrations below detection limits were given the value of the appropriate detection limit. For each element, we tested for differences in concentrations among nursery habitats and years using a 2-factor ANOVA (both fixed effects). We also performed 1-factor ANOVAs to test for differences among nursery habitats using data from each monitoring program separately. These separate analyses served to check that any differences among nursery habitats were consistent among programs.

For some of the bays we sampled, we had data on Cu and Pb in both otoliths and sediments from the same year: these were Alamitos Bay (1994 and 1998), Ana-

heim Bay (1994), Carpinteria Marsh (1994), Los Angeles Harbor (1996), Mugu Lagoon (1997 and 1998), and Ventura Harbor (1998). These data were used to test whether the mean concentration of each element in halibut otoliths was correlated with its mean concentration in the sediment at the scale of individual bays.

Assessing the potential to define past nursery habitat use in older halibut. The last part of our study was a preliminary test of whether chemical tags indicating past nursery habitat use could be identified in larger halibut that had moved offshore to the adult habitat. For this test, we collected 19 larger halibut in 1998 using trawls towed at 11 haphazardly selected sites in the study region (Fig. 1). All sites were offshore and in deeper water (21 to 60 m) than those where juveniles were collected. The 19 halibut ranged in size from 240 to 345 mm SL (mean = 273 mm SL) and so were 1 to 2 yr old when collected (Haaker 1975, Kramer 1990).

All halibut were kept frozen after collection and 1 otolith per fish (the left sagitta) was dissected and ground to a thin section on a microscope slide (following Kramer 1990). The otolith section was then mounted on a glass cover slip using cyanoacrylate adhesive, sonicated in deionized water (18 MOhm) and given a brief acid wash with 0.001 N HNO_3 (Fisher Optima grade). Trace elements were analysed at 5 locations spaced roughly evenly from the otolith core to the outer edge using laser ablation ICP-MS. We estimated the size (mm SL) of the halibut at the location of each laser sample by measuring the otolith radius at the crater left by the laser. The size estimate was based on a relationship between otolith radius (*x*) and fish standard length (*y*) obtained by pooling our data with those from 2 previous studies ($y = -7.93 + 67.2x + 0.35x^2$; n = 169, $r^2 = 0.95$) (Jensen 1990, Kramer 1990). Halibut migrate out of nursery habitats when they are between 140 and 200 mm SL (Kramer 1990), so we conservatively assumed that halibut were in a nursery habitat when <140 mm SL and had moved offshore to adult habitat if >140 mm SL (Kramer 1990). Using the otolith radius as a guide to fish size, we designated each trace element measurement as targeting part of the otolith deposited during residence in a nursery area (usually the 3 inner measurements) or afterwards (usually the 2 outer measurements). We then calculated means of the measurements taken during and after nursery habitat occupancy, and these means were used for data analyses.

Trace elements were measured using a VG Elemental PlasmaQuad 2+ ICP-MS coupled with a custom laser ablation system (based on a LUMONICS YM-200 Nd-YAG laser). In preparation for analysis, the cover slip and otolith was placed in a sealed quartz chamber on a microscope stage. The laser beam was focused at the surface of the otolith and the site of each sample

was briefly pre-ablated to remove surface contaminants. The laser was run in Q-switched mode with a beam wavelength of 355 nm. The laser was pulsed at 2 Hz to ablate a sample, and each pulse was approximately 5 mJ. Each sample crater was 45 to 70 µm in diameter. Ablated material was transported from the quartz chamber to the ICP-MS torch by an argon gas stream. Data were acquired in multi-element scan mode using 20 channels per atomic mass unit, a dwell time of 320 µs per channel (for a total of 0.5 s per sweep of the mass range), and an total acquisition time of 45 s per location.

Rigorous calibration approaches for LA-ICP-MS (Gunther et al. 2000) had not been developed when our analyses were done, and matrix-matched standards were not available. We did, however, ablate a glass standard (NBS 612) and use this to calculate elemental concentrations. The glass standard and samples are not matrix-matched, and so the accuracy of the calculated concentrations is poor (Perkins et al. 1991). They were, however, sufficient for our purpose, which was simply to assess in rough terms whether the laser-ablation and solution-based measurements were comparable. Specifically, we wished to ensure that any high concentrations recorded in the laser samples were not an artefact of contamination. We present data on 4 elements previously evaluated as natural tags in the juvenile otoliths (Mn, Cu, Sr, and Pb). Six of the 95 samples were below detection limits (mean blank count rate + $3 \times$ SD) for Pb, but all samples were above limits for the other 3 elements.

Our test for natural tags in larger halibut is not dependent on quantitatively accurate laser ablation data, and is based on the following qualitative predictions about trace element profiles in the otoliths of large halibut. Since offshore adult habitat and inshore open coast nursery sites have quite similar physiochemical properties, a large halibut that had occupied an open coast nursery should display no marked change in trace element composition from the center to edge of the otolith. A halibut originating from a bay nursery should, in contrast, show distinctly elevated levels of Cu and Pb, but only in the central part of the otolith that was deposited in the nursery area. The outer region of the otolith should have lower levels of Cu and Pb comparable to fish originating on the open coast. Elements not elevated in bay juveniles (Mn and Sr) should not occur at high levels at the otolith center and should show little change in concentration from otolith center to edge.

We had previously tested discriminant models to classify juveniles of know origin as having come from bay or open coast habitat (the most accurate model was one using Cu and Pb, see 'Results'). To test whether the elemental tag derived from analysis of juvenile otoliths

yielded plausible predictions about prior use of nursery habitats by larger fish, we generated a discriminant model to classify the larger halibut to a nursery habitat. Since the solution-based and laser-based data were not comparable in strict quantitative terms, this new discriminant model was parameterized with normalized residuals (mean = 0, SD = 1) of Cu and Pb concentrations from the juvenile halibut of known origin. Use of residuals assumes that the 2 sets of data come from the same underlying distribution. The new model was then used to classify the 19 larger halibut of unknown origin using the normalized residuals of Cu and Pb measured in the central part of the otolith.

RESULTS

Differences in otolith chemistry of juvenile halibut occupying bay and open coast nurseries

We first used a 1-factor ANOVA to compare nursery habitats just in 1998, the year when we collected halibut from both habitats. Two trace elements (Sr and Ba) showed no significant difference in concentration in juveniles from bay and open coast habitats (Sr: $F_{1,132} = 1.73$, p = 0.190; Ba: $F_{1,132} = 0.12$, p = 0.726; Fig. 3). The remaining 3 elements (Mn, Cu and Pb) showed greater potential as markers of nursery habitat residence because they were at significantly higher concentrations in the otoliths of 1998 bay juveniles than in open-coast juveniles in the same year (Mn: $F_{1,132} = 19.2$, p < 0.0001; Cu: $F_{1,132} = 28.2$, p < 0.0001; Pb: $F_{1,132} = 38.4$, p < 0.0001; Fig. 3).

We next used a 1-factor ANOVA followed by a Tukey's multiple comparison to test whether elemental concentrations of the 1998 open coast juveniles differed from bay juveniles collected during 1994, 1995, 1996 and 1997 as well as in 1998. A different subset of the 1998 bays was sampled in each of these other years, and we sampled 2 additional bays not sampled in 1998 (Table 1). This was not a rigorous test for temporal change in bays, but simply assessed whether bay juveniles remained different from open coast juveniles when additional years (and sites) were added to the comparison. These analyses confirmed that Ba and Sr were poor markers of nursery habitat because only in some years did bay juveniles differ from the 1998 open coast fish (Fig. 3). In addition, Mn was revealed as an unreliable indicator of nursery occupancy because, despite the clear difference between habitats detected in 1998, the collections of bay juveniles from 1994 to 1997 did not differ from the open coast juveniles (Fig. 3). The 2 most promising elements were Cu and Pb because these elements were at lower concentrations in the 1998 open coast juveniles than in any of the
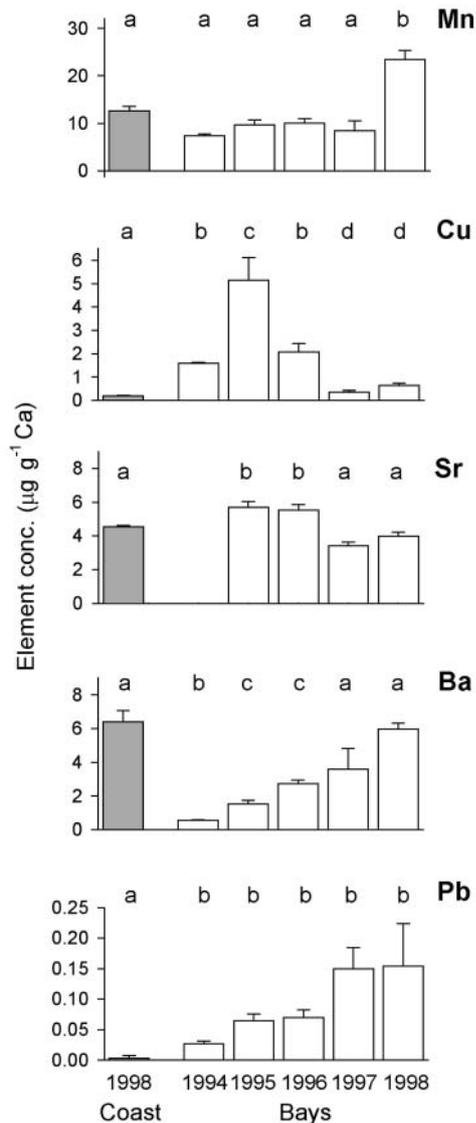
Fig. 3. *Paralichthys californicus.* Trace elements in the otoliths of juvenile halibut collected from open coast (gray bars) and bay (open bars) nursery sites. Plotted are means (with SE) for each year sampled in each habitat. Sample sizes and locations are given in 'Materials and methods' and in Table 2. Note that the number of bays sampled and sample sizes per bay differ among years. Letters above bars indicate results of a Tukey's test comparing means. Means not significantly different (p > 0.05) are indicated by the same letter

collections of bay juveniles (Fig. 3). Because of their greater promise as markers of habitat use, we concentrated our further analyses on Cu and Pb.

Since we collected juveniles from the open coast in 1998 only, we could not test for interannual consistency in trace element concentrations within this habitat. The samples were, however, scattered widely over the study area (Fig. 1) and this, coupled with the low within-sample variation (Fig. 3), indicates that there

was little spatial variation in Cu and Pb concentrations within the open coast nursery habitat. For bay juveniles, we were able to test for both spatial and interannual variation in otolith chemistry within this habitat. We used 1-factor ANOVAs to test for (1) differences among years in a single bay and (2) differences among bays in a given year (Table 1). We had large enough samples from 3 bays to meaningfully test for differences among years: Anaheim Bay (1994 and 1996), Carpinteria Marsh (1994, 1995 and 1996), and Los Alamitos Bay (1994 and 1996). For each of the 3 bays, there were differences among years in otolith Cu concentrations (Anaheim: $F_{1,26} = 21.5$, p < 0.0001; Carpinteria: $F_{2,44} = 8.82$, p = 0.001; Los Alamitos: $F_{1,41} = 17.6$, p < 0.0001). Otolith Pb concentrations were more temporally consistent, varying among years in Los Alamitos ($F_{1,41} = 11.1$, p = 0.002), but not in Carpinteria ($F_{2,44} = 1.68$, , p = 0.198) and Anaheim ($F_{1,26} = 0.36$, p = 0.553). To test for differences among bays within a single year, we used data from 1994 and 1996 (the 2 years for which we had the largest samples). In 1994, juveniles collected from different individual bays varied significantly in otolith Pb concentrations ($F_{2,57} = 3.21$, p = 0.048), but not in Cu concentrations (ANOVA, p = 0.31). In 1996, the pattern was reversed. There were significant among-bay differences in otolith Cu concentrations ($F_{3,55} = 14.9$, p < 0.0001) but not in levels of Pb ($F_{3,55} = 2.19$, p = 0.099). Overall, open coast juveniles had low and spatially consistent levels of Cu and Pb in their otoliths. In contrast, otoliths of bay juveniles had higher and more variable levels of Cu and Pb, which was due both to differences among individual bays and to change among years (Table 1).

When we pooled all samples, it was possible to assign the juveniles to their habitat of origin fairly accurately using trace element concentrations in their otoliths. A discriminant model using Cu alone correctly classified 76% of the bay juveniles and 86% of the open coast juveniles (78% overall). A model using Pb alone also had high classification accuracy (87% for bay juveniles, 68% for juveniles from the open coast and 83% overall). A model using data on both of these elements was similarly accurate (84% accuracy for bay juveniles, 81% for juveniles from the open coast and 83% overall). Other discriminant models that included data on Mn, Sr and Ba did not increase the accuracy of classification over that attained using Cu and Pb.

Differences in sediment trace-element chemistry among nursery habitats

A 2-factor ANOVA indicated that mean Mn concentrations (µg g^{-1}) in sediments did not differ significantly between bay and open coast nursery habitats overall

(coast mean ± SE = 404.1 ± 13.4, bay mean ± SE = 416.0 ± 12.771, $F_{1,546}$ = 0.75, p = 0.387), and there was no interaction ($F_{8,546}$ = 1.10, p = 0.210) that might indicate a difference between nursery habitats occurring in some, but not all years. Manganese concentrations did, however, differ significantly among years ($F_{8,546}$ = 2.30, p = 0.020). To check that the results were not biased by pooling data from the 5 monitoring programs, we used 1-factor ANOVAs to compare the 2 nursery habitats using data from each monitoring program separately. In all cases, the 1-factor ANOVAs produced qualitatively similar results to those just described for analyses on pooled data (detailed results not presented). At a finer spatial scale, Mn concentrations in sediments and otoliths from the same individual bay were not correlated (n = 6, r = 0.52, p = 0.287). Overall then, Mn in sediments showed a similar pattern to Mn in otoliths in that no consistent difference between bay and open coast habitats was observed.

In contrast, both Cu and Pb had significantly higher mean concentrations in bay sediments than in sediments at open coast nursery sites (Cu: $F_{1,817}$ = 42.9, p < 0.0001; Pb: $F_{1,817}$ = 30.6, p < 0.0001; Fig. 2). The lack of a significant interaction in the ANOVA (Cu: $F_{11,817}$ = 1.67, p = 0.076; Pb: $F_{11,817}$ = 1.01, p = 0.436) shows that this difference was maintained across all 11 yr sampled (Fig. 2) and for neither Cu nor Pb did mean concentrations differ significantly among years (Cu: $F_{11,817}$ = 1.51, p = 0.124; Pb: $F_{11,817}$ = 1.37, p = 0.327). At this broad spatial scale then, Cu and Pb in otoliths and sediments showed a similar pattern in that both elements occurred at higher and more variable concentrations in bays than on the open coast. As was the case for Mn, analyses of the data for each monitoring program separately produced results that were qualitatively similar to those on the pooled data (detailed results not presented). At a finer spatial scale, when we compared samples originating from the same bay in the same year, the match between sediments and otoliths was poorer. Juvenile halibut had concentrations of Cu and Pb in their otoliths that were not significantly correlated with the concentration of those elements in the sediment of their home bay (Cu: n = 9, r = 0.66, p = 0.054; Pb: n = 9, r = 0.38, p = 0.318).

Assessing the potential to define past nursery habitat use in older halibut

Trace element profiles in the otoliths of the 19 larger halibut provide tentative support for the hypothesis that otoliths retain chemical tags indicating prior nursery habitat use. First, Mn, Cu, Sr, and Pb concentrations estimated using the glass standard fell within the range of concentrations measured by solution-based

analysis of juvenile otoliths (Fig. 4), suggesting that relative differences among laser samples are probably indicative of habitat use and not contamination or other artifacts.

The discriminant model parameterized using normalized residuals of Cu and Pb concentrations in juvenile otoliths classified 11 of the large halibut as being of bay origin and 8 as having occupied open coast
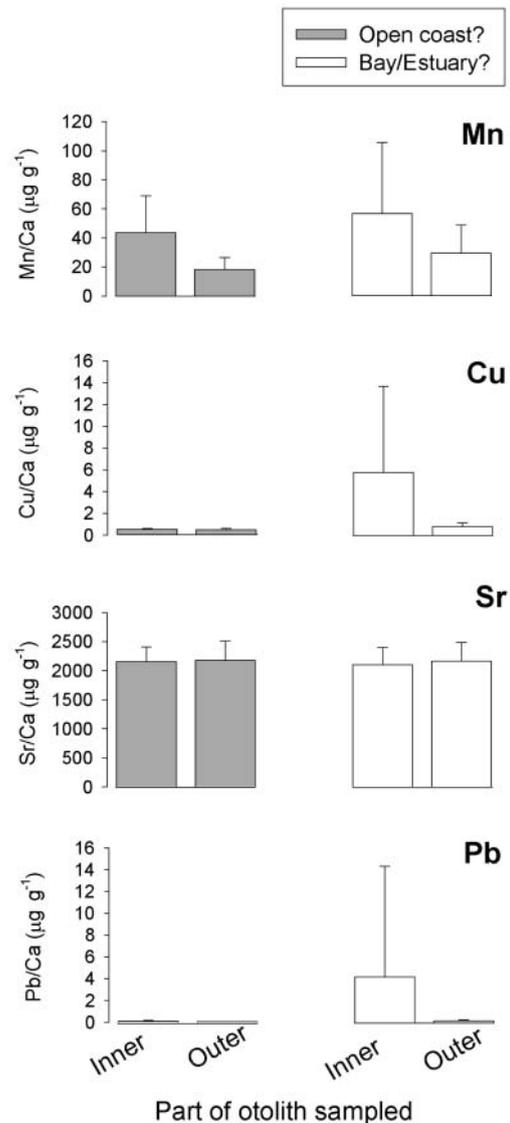

Fig. 4. *Paralichthys californicus.* Laser ablation ICP-MS analysis of trace elements in the otoliths of 19 large California halibut. Fish are grouped into those classified by a discriminant model as likely to have previously occupied open coast nurseries (left panel) and those that may have occupied bay nurseries (right panel). Each cross-sectioned otolith was sampled at 2 to 3 central locations deposited while a juvenile in a nursery habitat (during) and at 2 to 3 locations closer to the edge that were deposited after migration offshore to adult habitat (after). Plotted are mean concentrations (with SE) for each group

nurseries. Trace element profiles of these otoliths are consistent with our expectations about relative differences between parts of the otolith deposited during and after nursery habitat occupancy, and so suggest that these assignments are plausible (Fig. 4). Otoliths of the 8 halibut classified as originating from open coast nurseries showed minor changes in the concentration of all 4 trace elements from the otolith center to outer edge, a profile consistent with having spent their first yr in an open coast nursery and then migrated offshore to the adult habitat (Fig. 4). The 11 halibut classified as having occupied bay nurseries contained elevated levels of Cu and Pb, but only in the part of the otolith deposited in a nursery. The other elements, which were not significantly elevated in bay juveniles, showed little (Mn) or no (Sr) tendency to be elevated in the otolith centres of these 11 halibut. This trace element profile is thus suggestive of early residence in a bay nursery followed by movement to the offshore adult habitat (Fig. 4).

DISCUSSION

Reliable natural tags in juvenile California halibut otoliths?

The use of trace element concentrations in otoliths as a reliable natural tag requires firstly that otoliths of fish from the locations to be discriminated are chemically distinct. This criterion has been met in studies done in various habitats, including studies showing that juvenile fish collected from specific bays or estuaries have distinct trace element differences that can be used as natural tags (Milton et al. 1997, Thorrold et al. 1998a,b, 2001). A second important criterion for a reliable natural tag is that the marker be consistent over the time interval for which it is desired to characterize site usage. Some of the aforementioned estuarine studies were based on collections from a single year, and it is not known whether the site-specific differences in otolith chemistry would be consistent among years (Thorrold et al. 1998a,b, 2001). In fact, 1 study showed that differences among estuaries detected in 1 year were replaced by a new set of differences in fish collected 1 yr later (Milton et al. 1997). Our results for juvenile California halibut showed a similar lack of among-year consistency in trace element concentrations of otoliths at the level of individual bays. In our system, the lack of a consistent small-scale tag may be because most of the bays used as nursery sites by California halibut are quite small, and dynamic in their physiochemical features. Anthropogenic and terrigenous inputs of trace elements vary among bays depending on watershed features, and the hydrographic

regime is characterized by long summertime periods of low freshwater input, and restricted exchange with the ocean, interspersed with severe winter storms that 'reset' conditions in the bays (Nordby & Zedler 1991). It thus remains possible that there may be more reliable elemental tags for individual bays or estuaries in regions where bays and estuaries are larger and more hydrologically stable.

Despite the variability among individual bays, our results suggest that some trace elements may be reliable markers of nursery habitat use for California halibut at a broader spatial scale (all bays vs all open coast sites). A similar broad-scale difference was identified by Gillanders & Kingsford (1996, 2000), who were able to differentiate between the use of estuarine seagrass habitats and coastal reefs by juveniles of 2 species. These workers sampled juveniles in 1 year only. Two features of our results suggest that the difference between bay and open coast habitats in southern California is likely to be maintained over several years. Firstly, the difference between the 2 habitats that we detected in 1998 persisted when the bay sample was expanded to include additional sites and samples spanning 4 yr. Secondly, Cu and Pb in sediments were consistently lower in concentration on the open coast than in bays over 12 yr. If habitat-specific differences in otoliths and sediments are linked, this implies a temporally stable natural tag in juvenile halibut otoliths. Of course, establishing a causal link between elements in otoliths and sediments, or data on the otoliths of open coast juveniles over several years would be required to confirm that the natural tag shows long-term stability.

What causes habitat-specific differences in otolith trace element composition?

When we compared all bay samples to all open coast samples, Cu and Pb showed similar patterns of relative difference among habitats in otoliths and sediments. The fact that mean concentrations in the 2 habitats differed by similar amounts, and that variation around the means was of similar magnitude (compare Figs. 2 & 3), suggests some association between levels of Cu and Pb in otoliths and sediments. The fact that Cu and Pb concentrations in halibut otoliths did not match the subtler differences among sediments at a finer spatial scale (individual bays) indicates, however, that the association is not a tight one (see also Hanson & Zdanowicz 1999).

Our limited understanding of mechanisms regulating trace element deposition in otoliths makes it difficult to isolate the causal process underlying the correlation between Cu and Pb in sediments and otoliths. Two likely underlying causes are the uptake of dissolved Cu and Pb from the water column and/or

uptake across the gut of Cu and Pb from the tissues of ingested prey (Campana 1999). Both of these forms of Cu and Pb display a qualitatively similar concentration gradient to that observed in sediments: high levels in bays and lower levels on the open coast (Chapman et al. 1987, Johnson et al. 1988, Flegal & Sañudo-Wilhelmy 1993, van Geen & Luoma 1993, Sañudo-Wilhelmy & Flegal 1996, Fairey et al. 1998, Zirino et al. 1998). Laboratory experiments indicate that Cu and Pb are deposited in otoliths in proportion to their dissolved concentration in the surrounding water (Milton & Chenery 2001), as are Sr and Ba (Farrell & Campana 1996, Bath et al. 2000). As yet, however, there is no direct experimental evidence indicating that Cu and Pb deposition is a function of their concentration in the tissues of prey (Milton & Chenery 2001).

Applications of natural tags in California halibut

Our laser-ablation analysis of larger halibut suggests that the elemental tag of the 2 nursery habitats may be identifiable in older fishes. The classification algorithm we developed using the data on juveniles (of known origin) yielded a plausible prediction of prior nursery use by sub-adults (of unknown origin). We were forced to use residuals of elemental concentrations in this classification algorithm because the ICP-MS protocols used on adults and juveniles yielded raw data that were not quantitatively equivalent. This procedure is robust, as long as the 2 sets of raw data have similar underlying distributions; in other words, as long as the 2 methods are free of artifacts (see also Thorrold et al. 2001). A better approach would be to use the same ICP-MS protocol to analyse both juvenile otoliths and the core of adult otoliths (e.g. Gillanders & Kingsford 1996) so that a classification algorithm for juveniles (of known origin) could be used directly to predict prior nursery occupancy of adults (of unknown origin). By analysing a large representative sample of adults it may thus be possible to estimate the fraction of individuals recruiting to the adult population that originated from bay nurseries.

Such an estimate could prove useful in management of the California halibut population, which has declined steadily over the past 30 yr (Plummer et al. 1983, Kramer & Sunada 1992). One of the possible causes of this decline is the loss of bay nursery habitat (California Coastal Zones Commission 1975). If collections of otoliths made in the past were available, it might even be possible to assess whether the fraction of recruits coming from bay nurseries has declined over time and so test the habitat-loss hypothesis directly. The loss and deterioration of coastal wetlands and bays is a widespread phenomenon, and the use of trace ele-ments as natural tags to quantify the supply of recruits to fisheries from specific inshore habitats may be a useful technique for assessing 1 aspect of the functional status of those sites — as fish nurseries.

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