Defining the Extent of Larval Exchange among Kelp Rockfish (*Sebastes atrovirens*) Populations Using Otolith Microchemistry

Julie D. Standish
University of California, Santa Barbara
Department of Ecology, Evolution and Marine Biology
(Advisor: Robert Warner)

Introduction

Over 60 species of rockfish (*Sebastes*) inhabit the waters along the California coast. Rockfish are an important economical component of the state's commercial and recreational fisheries. Over the past two decades, rockfish populations along the coast of California have declined sharply leading to heightened concern over the management policies for rockfish and sustainability of existing stocks nearshore (Ralston 1998, Dorn 2002). Several measures, including a statewide network of marine protected areas (MPAs), are being considered to prevent overexploitation of nearshore stocks. In the last decade, the live-fish fishery that targets nearshore species, especially rockfishes, has grown dramatically yet little is known regarding the effect of the fishery on stock levels of the nearshore rockfish species. Critical to the effectiveness of fisheries management, including ecosystem-based fisheries methods and MPAs, is knowledge of the extent of larval movement and larval connectivity among populations. Information regarding the spatial scale of larval dispersal is required to determine the appropriate spacing between MPAs and the size of individual MPAs within a network in order to effectively sustain and replenish exploited stocks (Gaines et al. 2003). However, explicit information regarding the dispersal patterns of larvae remains unknown for any commercially important marine species.

Given the small size of fish and invertebrate larvae and their potential for long distance dispersal, tracking larvae as they disperse has been a very challenging task. Consequently, most of the information on larval dispersal has come from a variety of indirect methods, including correlations between settlement and oceanographic patterns (Meekan et al. 1993, Wing et al. 1995a, Wing et al. 1995b, Dixon et al. 1999, Morgan et al. 2000), the rate of range expansion following invasion of exotic species (Geller 1994, Grosholz & Ruiz 1995), and population genetics (Moberg & Burton 2000, Kinlan & Gaines 2003, Buonaccorsi et al. 2004, Sotka et al. 2004). Although these methods have provided valuable insights into the larval dispersal process and estimates of the degree of exchange among populations, they do not provide information on the specific origin of individuals. Alternative techniques that can explicitly determine the natal source and movement of larvae are needed (Jones et al. 1999).

Recently, environmental markers such as the isotopic and elemental composition in the otoliths of fishes have shown great promise as a means to track the history and quantify the movement among populations. Formed during the embryonic stage, otoliths grow by the daily accretion of calcium carbonate into a proteinaceous matrix, generating a pattern of concentric rings around the central nucleus. The central nucleus forms the core of the otolith as the fish grows. Trace elements from the environment are incorporated into the calcium carbonate matrix of the otolith. The elemental signatures within otoliths may be used as a natural tag of past environments experienced by an individual. Otolith microchemistry has been used to determine stock structure within marine species (Campana et al. 1994, Campana et al. 2000, Gillanders 2001, Rooker et al. 2003, Fowler et al. 2005, Ashford et al. 2006), to detect anadromy (Kalish 1990, Zimmerman & Reeves 2000,

Howland et al. 2001, Secor et al. 2001), and distinguish among individuals with different dispersal histories (Swearer et al. 1999, Sandin et al. 2005).

In this study, we examined environmental markers deposited in otoliths to determine the larval dispersal patterns for the kelp rockfish, (*Sebastes atrovirens*), which inhabits nearshore rocky reef communities along the California coast and is targeted by the nearshore recreational and live-fish fisheries. Like all species of the genus, females are primitively viviparous, internally brooding young before release to the pelagic phase (Wourms 1991). During incubation of the embryo within the mother, the natal portion of the otolith is formed. Females release small (~ 4 mm SL) larvae generally from February through June (Love et al. 2002) and after a 2- to 3-month pelagic stage (Gilbert 2000, Standish J., unpublished data), larvae settle to kelp canopies during the summer and early fall (Anderson 1983). Once settled, juveniles remain closely associated with kelp (Nelson 2001), and adults are relatively sedentary, generally moving only a few meters within their home reefs (Van Dykhuizen 1983).

Our first goal was to investigate larval connectivity within a limited geographic area in southern California. An earlier study (Warner et al. 2005) demonstrated that geochemical signatures in natal otoliths of larvae from late-term broods of *S. atrovirens* females varied significantly among three locations around the Santa Barbara Channel and Santa Cruz Island. Their data suggest that the chemistry of natal otoliths can be used to discriminate sites of origin, and that otolith chemistry may be used to measure the rate of larval exchange along the open coast. We assayed the natal portion of otoliths (the core) of recruits sampled from Santa Cruz Island and compared their elemental signatures to the natal data of Warner et al. (2005) from the three defined natal localities around the Santa Barbara Channel and Santa Cruz Island during one production-recruitment season. Our second goal was to examine natal elemental signatures in the otolith cores of recruit *S. atrovirens* to examine spatial patterns of larval dispersal along the open coast of California. We assayed otoliths from recruits sampled over a large spatial scale, from locations along the coast in 2001 and 2002. The otolith core elemental concentrations were grouped to classify distinct natal elemental signature types within each year. Natal signature types are assumed to represent chemically unique elemental signatures from source population(s), and we analyzed the spatial pattern of the occurrence of natal signature types among the collection locations for both 2001 and 2002

Methods

Sampling:

To examine larval connectivity within a limited spatial area in southern California, we sampled recruits from Santa Cruz Island and compared their otolith core signatures to the natal chemical signatures data from Warner et al. (2005). Newly settled recruits were collected biweekly from artificial collectors placed approximately 2 m below the surface near rocky subtidal reefs (see Ammann 2004 for artificial collector specifics). Collections were made between June and October 2002 from several sites on the north and south shores of Santa Cruz Island (Fig. 1). In 2002, no recruitment was observed on the mainland where pre-hatched larvae were collected.

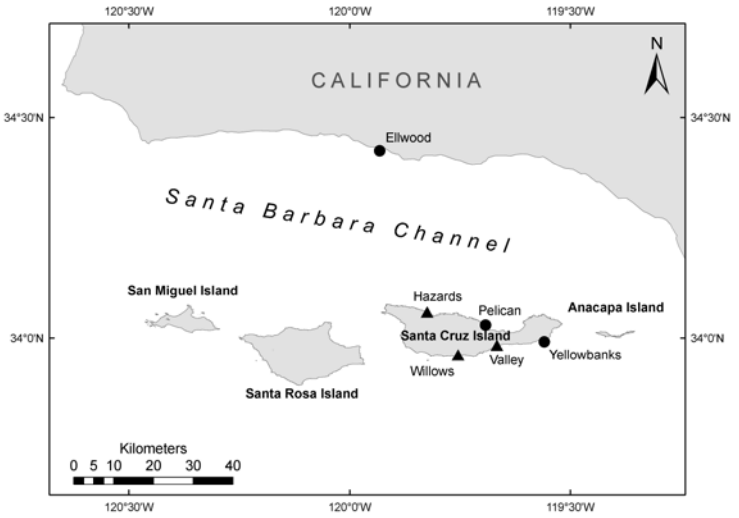

Figure 1. Map of collection sites of pre-hatch larvae and recruits in 2002. Black circles represent sites where pre-hatch larvae from brooding females were collected, and gray triangles represent sites where recruits were collected.

For the analysis of the spatial distribution of recruit natal signatures over two recruitment seasons, recruits were collected from near-surface kelp canopies using nets and near-surface artificial collectors. In 2001, recruits were collected between 8/24/01 – 8/27/01 at three sites (Hopkins, Stillwater and Monastery Beach) near the southern end of Monterey Bay (hereafter referred to as Monterey) and Big Creek (Fig, 2). Recruits collected at Purisima were sampled on 7/24/01 from artificial collectors (Fig, 2). In 2002, recruits were collected by net from 9/17/02 – 9/26/02 at mainland locations along the California coast (Fig. 2). Samples from Santa Cruz Island were collected biweekly between July and October 2002 from artificial collectors (Fig 2).

All samples were frozen immediately after capture. Natal otoliths were extracted, mounted onto slides, and cleaned for chemical analysis. Recruit otoliths were extracted, ground, aged, and cleaned for chemical analysis.

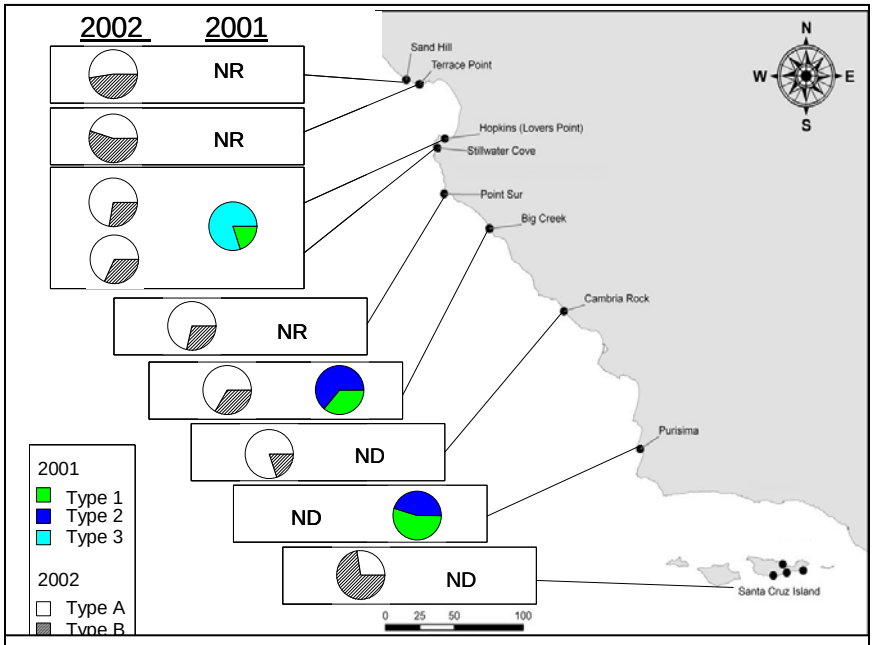

Figure 2. Map of south-central California, USA, with collection locations. Pie diagrams represent the proportion of natal signature types at each collection location in 2001 and 2002. NR, no recruitment of *Sebastes atrovirens* for the given collection year; ND, no data.

Analytical method:

All samples were analyzed using a precision laser and inductively coupled plasma mass spectrometry (LA-ICP-MS) to determine elemental concentrations. Natal otoliths were sampled from a brood (n = 10) and concentrations of the following elements were determined: Mg/Ca, Mn/Ca, Sr/Ca, Ba/Ca. Recruit otoliths were analyzed for the chemical composition of the core. We isolated the material associated with the core using a series of small, discrete vertical pits from the surface of the otolith through the visible core. This approach of discrete, successive pits approximates the amount of material analyzed when ablating natal otoliths and likely minimizes the material not associated with the pre-hatch core. Previous work has shown that the cores of otoliths in general contain elevated Mn (Brophy et al. 2004, Ruttenberg et al. 2005), and Mn has been shown to be an accurate

indicator of the location of the natal core of the otolith for *Sebastes atrovirens* recruits (Ruttenberg et al. 2005). We identified the specific pit containing the core material using elevated concentrations of Mn (at least 3x higher than surrounding material) as a proxy for the precise location of the core.

Results and Discussion

Analysis of connectivity within a limited geographic region

Earlier work by Warner et al. (2005) showed distinct geographical differences in natal otolith elemental concentrations from larval *Sebastes atrovirens* collected among several locations along the open coast in southern California in 2002, suggesting that the natal signatures could be used to identify dispersal patterns within this geographic area by assaying otoliths cores of recruits. We chemically analyzed the otolith core of recruits from Santa Cruz Island and compared them to the natal elemental signatures of the defined locations. Since the natal elemental signatures were characterized only within a restricted geographic area, the variation of natal elemental signatures among all potential contributing source populations is unknown. As a result, the correspondence between natal location and recruit cores could not be used to assign recruits unequivocally to site of origin because uncharacterized sources outside of our natal sampling area may have had a similar source signature to the defined natal locations. Instead, we were most interested in areas of non-overlap between the natal otolith and recruit core signatures. From the discriminant function plot, there was very little overlap between the otolith core signatures of Santa Cruz Island recruits and Ellwood natal signatures, suggesting that the mainland natal location was not an important contributor to Santa Cruz Island (Fig. 3). From the DFA assignment, only one recruit was identified to have potentially originated from Ellwood. This finding implies little connectivity between this site and the island in this recruitment year. In addition, although most of the island recruits had a chemical signature similar to the natal signatures of the island sites, a portion of the recruits appeared to have originated from a source with markedly different elemental concentrations than the natal locations we sampled. 5% of the recruit cores at Santa Cruz Island in 2002 had a greater than 95% probability of being sourced from outside the natal sampling area, suggesting that the island populations may not be completely self-recruiting. However, it remains possible that unsampled island larval sources may have had these distinctive natal otolith signatures. More comprehensive

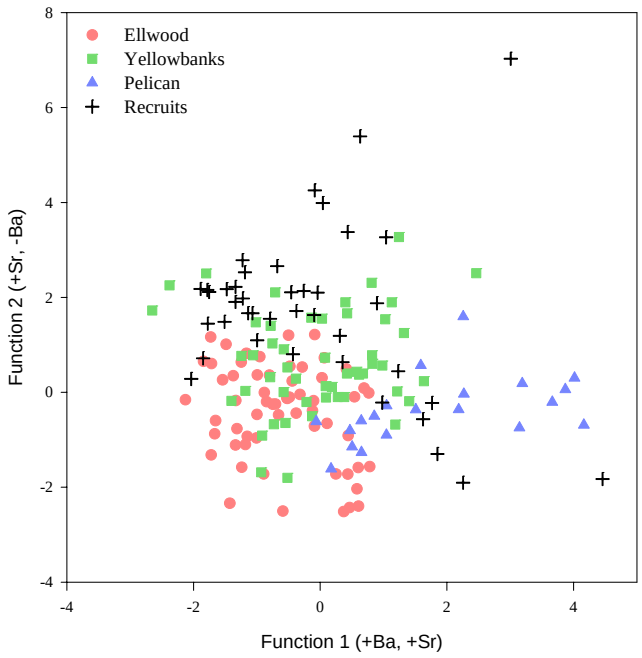

Figure 3. Discriminant function plot of natal otoliths and classification of Santa Cruz Island recruits. The discriminant functions were generated using concentrations of Ba and Sr in natal otoliths. Recruit cores were classified using these discriminant functions.

sampling of natal sources is necessary to resolve the spatial variation of natal elemental signatures around the island and determine the extent to which the island populations are self-recruiting. Nonetheless, the approach taken here, with a spatially restricted natal model, remains a useful method for identifying larval dispersal patterns with particular focus on sources that do *not* contribute to defined locations, and quantifying the number of recruits with distinct core signatures that were contributed by undefined sources and may have originated from outside the natal sampling area.

The assignment technique used here does place limitations on the interpretations that can be made from the multivariate comparisons between natal otoliths and recruit cores. First, recruits cannot be unequivocally attributed to a putative source whose otolith core signature is similar to the defined natal locations, because not all possible source natal signatures were characterized. Recruit core signatures that do correspond to the defined natal locations may have either originated from that source location or from an uncharacterized location with a similar source signature. Second, the assignment technique is that recruits that have different core multi-elemental profiles from the defined natal locations cannot be identified to source.

Spatial distribution of recruit natal signatures

We found sufficient variation in the elemental concentrations at the otolith cores of recently recruited individuals collected from locations along the coast of California resulting in unique elemental signature types. Three distinct natal signature types were classified in 2001 and two types were identified in 2002 among individuals (Fig 2). Recent studies analyzing the variation in chemical signatures at the early larval and juvenile portions of the otoliths of young fish have found significant differences among locations that imply separate origins and dispersal trajectories during the early larval and pelagic juvenile period (FitzGerald et al. 2004, Miller & Shanks 2004). For this study, we were most interested in differences of natal types between locations, such as the restriction of a natal type(s) to a particular region along the coast, to evaluate potential barriers to dispersal. In the 2001 recruitment season, there were strong differences in the presence of specific natal types observed at Monterey, Big Creek and Purisima, suggesting differences in the contribution of chemically distinct natal sources to the juvenile cohort at each location (Fig 2). From the cluster assignment, Type 2 natal signature was only present in recruits that recruited to Big Creek and Purisima while Type 3 was exclusive to Monterey recruits. Larval dispersal patterns are likely a result of many factors, including ocean currents and larval behavior. Possible barriers to dispersal, such as recirculating oceanographic currents and mesoscale eddies, are common along the coast of California and entrain planktonic fish larvae, possibly limiting alongshore transport (Wing et al. 1998, Nishimoto & Washburn 2002). In addition, larval behavior may retard passive dispersal within currents and assist in retaining individuals nearshore (Larson et al. 1994, Largier 2003, Shanks et al. 2003). The approach taken here of analyzing the spatial pattern of natal elemental signatures in the otoliths of recently settled individuals provides a useful method to quantify the degree to which natal signature types are *not* mixed among locations, and thus to examine larval dispersal patterns over a large geographic area.

Significant to California's coastal environmental policy

The California Fish and Game Commission adopted the Nearshore Fishery Management Plan that provides a management strategy for the targeted nearshore species (Sweetnam 2003). The plan offers several integrated management measures, including marine protected areas and regional management, as a means to provide for sustainable nearshore stocks and fisheries. Information regarding the level of connectivity among populations and the spatial dispersal patterns will be important to the management policy on this issue. The successful management of nearshore populations depends on certain assumptions about connectivity among populations. Our study provides valuable information of the potential source areas in southern California that are *not* contributors to recruits to Santa Cruz Island and quantifies potential larval input from sources outside the sampling area. In addition, the results of this study identify the regional scale at which natal signature types are mixed among locations along the coast for a nearshore rockfish species. Although most marine species have the potential for long distance dispersal, regions along the coast of California may not be as well mixed as expected.

Literature cited

Ammann AJ (2004) SMURFs: standard monitoring units for the recruitment of temperate reef fishes. J Exp Mar Biol Ecol 299:135-154

Anderson TW (1983) Identification and development of nearshore juvenile rockfishes (genus *Sebastes*) in central California kelp forests. MA Thesis, California State University, Fresno, California

Ashford JR, Arkhipkin AI, Jones CM (2006) Can the chemistry of otolith nuclei determine population structure of Patagonian toothfish Dissostichus eleginoides? Journal of Fish Biology 69:708-721

Brophy D, Jeffries TE, Danilowicz BS (2004) Elevated manganese concentrations at the cores of clupeid otoliths: possible environmental, physiological, or structural origins. Mar Biol 144:779-786

Buonaccorsi VP, Westerman M, Stannard J, Kimbrell C, Lynn E, Vetter RD (2004) Molecular genetic structure suggests limited larval dispersal in grass rockfish, *Sebastes rastrelliger*. Mar Biol 145:779-788

Campana SE, Chouinard GA, Hanson JM, Frechet A, Brattey J (2000) Otolith elemental fingerprints as biological tracers of fish stocks. Fish Res (Amst) 46:343-357

Campana SE, Fowler AJ, Jones CM (1994) Otolith elemental fingerprinting for stock identification of atlantic cod (*Gadus morhua*) using laser-ablation ICPMS. Can J Fish Aquat Sci 51:1942-1950

Dixon PA, Milicich MJ, Sugihara G (1999) Episodic fluctuations in larval supply. Science 283:1528-1530

Dorn MW (2002) Advice on west coast rockfish harvest rates from Bayesian meta-analysis of stock-recruit relationships. North American Journal of Fisheries Management 22:280-300

FitzGerald JL, Thorrold SR, Bailey KM, Brown AL, Severin KP (2004) Elemental signatures in otoliths of larval walleye pollock (*Theragra chalcogramma*) from the northeast Pacific Ocean. Fishery Bulletin 102:604-616

Fowler AJ, Gillanders BM, Hall KC (2005) Relationship between elemental concentration and age from otoliths of adult snapper (*Pagrus auratus*, Sparidae): implications for movement and stock structure. Mar Freshw Res 56:661-676

Gaines SD, Gaylord B, Largier JL (2003) Avoiding current oversights in marine reserve design. Ecol Appl 13:S32-S46

Geller JB (1994) Marine biological invasions as models of dispersal: tracking secondary spread and introgressive gene flow. CalCOFI Report 35:68-72

Gilbert EA (2000) Molecular genetic analysis of temporal recruitment pulses in juvenile kelp rockfish. MA Thesis, San Francisco State Univiversity, San Francisco, California.

Gillanders BM (2001) Trace metals in four structures of fish and their use for estimates of stock structure. Fishery Bulletin 99:410-419

Grosholz ED, Ruiz GM (1995) Spread and Potential Impact of the Recently Introduced European Green Crab, Carcinus-Maenas, in Central California. Marine Biology 122:239-247

Howland KL, Tonn WM, Babaluk JA, Tallman RF (2001) Identification of freshwater and anadromous inconnu in the Mackenzie river system by analysis of otolith strontium. Transactions of the American Fisheries Society 130:725-741

Jones GP, Milicich MJ, Emslie MJ, Lunow C (1999) Self-recruitment in a coral reef fish population. Nature 402:802-804

Kalish JM (1990) Use of otolith microchemistry to distinguish the progeny of sympatric anadromous and non-anadromous salmonids. Fishery Bulletin 88:657-666

Kinlan BP, Gaines SD (2003) Propagule dispersal in marine and terrestrial environments: A community perspective. Ecology 84:2007-2020

Largier JL (2003) Considerations in estimating larval dispersal distances from oceanographic data. Ecol Appl 13:S71-S89

Larson JF, Lenarz WH, Ralston S (1994) The distribution of pelagic juvenile rockfish of the genus *Sebastes* in the upwelling region off central California. CalCOFI Report 35:175-221

Love MS, Yoklavich MM, Thorsteinson L (2002) The rockfishes of the northeast Pacific, Vol. Univ. of California Press

Meekan MG, Milicich MJ, Doherty PJ (1993) Larval production drives temporal patterns of larval supply and recruitment of a coral-reef damselfish. Mar Ecol Prog Ser 93:217-225

Miller JA, Shanks AL (2004) Evidence for limited larval dispersal in black rockfish (*Sebastes melanops*): implications for population structure and marine-reserve design. Can J Fish Aquat Sci 61:1723-1735

Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. Mar Biol 136:773-784

Morgan LE, Wing SR, Botsford LW, Lundquist CJ, Diehl JM (2000) Spatial variability in red sea urchin (Strongylocentrotus franciscanus) recruitment in northern California. Fish Oceanogr 9:83-98

Nelson PA (2001) Behavioral ecology of young-of-the-year kelp rockfish, *Sebastes atrovirens* Jordan and Gilbert (Pisces: Scorpaenidae). J Exp Mar Biol Ecol 256:33-50

Nishimoto MM, Washburn L (2002) Patterns of coastal eddy circulation and abundance of pelagic juvenile fish in the Santa Barbara Channel, California, USA. Mar Ecol Prog Ser 241:183-199

Ralston S (1998) The Status of Federally Managed Rockfish on the U.S. West Coast. U.S. Department of Commerce NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-255:6-15

Rooker JR, Secor DH, Zdanowicz VS, De Metrio G, Relini LO (2003) Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. Fish Oceanogr 12:75-84

Ruttenberg BI, Hamilton SL, Hickford MJH, Paradis GL, Sheehy MS, Standish JD, Ben-Tzvi O, Warner RR (2005) Elevated levels of trace elements in cores of otoliths and their potential for use as natural tags. Mar Ecol Prog Ser 297:273-281

Sandin SA, Regetz J, Hamilton SL (2005) Testing larval fish dispersal hypotheses using maximum likelihood analysis of otolith chemistry data. Mar Freshw Res 56:725-734

Secor DH, Rooker JR, Zlokovitz E, Zdanowicz VS (2001) Identification of riverine, estuarine, and coastal contingents of Hudson River striped bass based upon otolith elemental fingerprints. Mar Ecol Prog Ser 211:245-253

Shanks AL, Grantham BA, Carr MH (2003) Propagule dispersal distance and the size and spacing of marine reserves. Ecol Appl 13:S159-S169

Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR (2004) Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. Mol Ecol 13:2143-2156

Swearer SE, Caselle JE, Lea DW, Warner RR (1999) Larval retention and recruitment in an island population of a coral-reef fish. Nature 402:799-802

Van Dykhuizen GS (1983) Activity patterns and feeding chronology of the kelp rockfish, *Sebastes atrovirens*, in a central California kelp forest. MA Thesis, San Francisco State University, San Francisco, California

Warner RR, Swearer SE, Caselle JE, Sheehy M, Paradis G (2005) Natal trace-elemental signatures in the otoliths of an open-coast fish. Limnol Oceanogr 50:1529-1542

Wing SR, Botsford LW, Largier JL, Morgan LE (1995a) Spatial structure of relaxation events and crab settlement in the northern California upwelling system. Mar Ecol Prog Ser 128:199-211

Wing SR, Botsford LW, Ralston SV, Largier JL (1998) Meroplanktonic distribution and circulation in a coastal retention zone of the northern California upwelling system. Limnol Oceanogr 43:1710-1721

Wing SR, Largier JL, Botsford LW, Quinn JF (1995b) Settlement and transport of benthic invertebrates in an intermittent upwelling region. Limnol Oceanogr 40:316-329

Wourms JP (1991) Reproduction and development of *Sebastes* in the context of the evolution of piscine viviparity. Environ Biol Fish 30:111-126

Zimmerman CE, Reeves GH (2000) Population structure of sympatric anadromous and nonanadromous *Oncorhynchus mykiss*: evidence from spawning surveys and otolith microchemistry. Can J Fish Aquat Sci 57:2152-2162

Name of Participants

Graduate support

Julie D. Standish, UCSB, (805) 893-7397

PI: Robert R. Warner, UCSB

Publications and manuscripts in preparation

Standish, J.D., M. Sheehy, R.R. Warner. The use of otolith natal element signatures as natural tags to evaluate connectivity among open-coast fish populations. To be submitted to Marine Ecology Progress Series.

Standish, J.D. and R.R. Warner. The spatial pattern of natal signatures in the otoliths of juvenile *Sebastes atrovirens* along the California coast: can differences in otolith

core chemistry among locations identify larval dispersal patterns for open-coast fish species? To be submitted to Marine Ecology Progress Series.

Professional society presentations

Standish, J.D. 2006. The use of otolith natal element signatures as natural tags to evaluate connectivity among open-coast fish populations. Western Groundfish Conference, Oregon, January