AN ABSTRACT OF THE DISSERTATION OF

Rebecca E. B. Baldwin for the degree of Doctor of Philosophy in Fisheries Science
Presented on September 21, 2010.

Title: Using Parasite Community Data and Population Genetics for Assessing Pacific Sardine (*Sardinops sagax*) Population Structure Along the West Coast of North America

Abstract approved:

Kym C. Jacobson Michael A. Banks

Pacific sardines (*Sardinops sagax*) are an economically and ecologically important forage fish which transfer energy from planktonic primary producers and secondary consumers to upper trophic predators. Previous genetics studies of Pacific sardine suggested a panmictic population with a shallow genetic structure. However, more than one subpopulation within the Central California Offshore management unit may exist based on recovering larger individuals at higher latitudes and a temporal difference in sardine spawning off Southern California and the Pacific Northwest. Potential for separate sardine subpopulations questions the long-standing paradigm of an annual migration of individuals to feeding grounds off the Pacific Northwest in the summer with migrants returning to Southern California in the fall to spawn the following spring. This study applied parasite community analyses and population

genetics techniques to assess migration patterns and stock structure of Pacific sardine in the California Current from Vancouver Island, British Columbia, Canada to San Diego, California, USA. A coastwide sardine migration is supported by the geographical distribution of *Myosaccium ecaude* (Trematoda), but a second migration pattern was identified within the Pacific Northwest from the geographic distribution of *Lecithaster gibbosus* (Trematoda). Population genetics studies identified a panmictic distribution for: 1) the trematode *M. ecaude* using a 283bp portion of the NADH-dehydrogenase subunit 1 (ND1) mitochondrial DNA (mtDNA) gene; and 2) three species of *Anisakis* nematodes (*A. simplex s.s.*, *A. pegreffii*, and *A. simplex* 'C') using a 524bp portion of the cytochrome c oxidase 2 (*cox2*) mtDNA gene. These results suggest that the extensive movement of all of the potential hosts utilized by these parasites, the limited oceanographic barriers, and complexity in the California Current are not preventing the mixing of *M. ecaude* or *Anisakis* species populations. The diversity and availability of fish and cetacean species that undergo extensive migrations along the full length of the California Current system may enable large geographically distributed population sizes of these parasite species. We thus cannot confirm the existence of separate Pacific sardine subpopulations within the California Current by the occurrence of parasite communities or the population genetics analyses of *M. ecaude* or the three *Anisakis* species.

Using Parasite Community Data and Population Genetics for Assessing Pacific Sardine (*Sardinops sagax*) Population Structure Along the West Coast of North America

by

Rebecca E. B. Baldwin

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 21, 2010
Commencement June 2011

Doctor of Philosophy dissertation of Rebecca E. B. Baldwin presented on September 21, 2010.

APPROVED:

Co-Major Professor, representing Fisheries Science

Co-Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Rebecca E. B. Baldwin, Author

ACKNOWLEDGEMENTS

My time living in Newport, Oregon and working at the Hatfield Marine Science Center is continually being filled with adventure, friendships with so many wonderful people, and learning how to be a better scientist. Many thanks are given to my co-major professors Dr. Kym Jacobson and Dr. Michael Banks. Kym: thank-you for hiring a recently-minted parasitologist from the Canadian prairies and introducing me to marine parasite ecology. You continue to kindle my passion for parasitology and your encouragement is unparalleled. Michael: thank-you for guiding me through the concepts of population genetics, and your patience with a student unfamiliar with genetics. Many thanks are given to my committee members Dr. Robert Emmett, Dr. Michael Blouin, Dr. Virginia Lesser and Dr. John Bolte for their helpful suggestions and guidance throughout my time as a Ph.D. student.

Thank-you to all past and present members of the Jacobson Parasitology Lab. Mary Beth Rew, Andrew Claxton, and James Losee you have gone above and beyond to help me with my seemingly endless questions, insightful discussions on interpreting data and editing all the draft copies of chapters. Thanks to Justin Atkins and Nick Hempfer for assisting me with examining fish for parasites. Thanks also go out to the past and present members of the Banks Genetics Lab. Dave Jacobson, thanks for getting me started on proper techniques for processing DNA samples. Dr. Mattias Johansson, thanks for guiding me through analyses and writing of genetic data.

I extend my sincerest thanks to all the scientists and fishermen I had the opportunity to discuss my research with, especially at the Trinational Sardine Forums. Further I thank all the scientists, fisherman and crew that kindly donated their time to collect sardines for this project. Without you my research would not have been possible.

I wish to thank all my family and friends that have supported me during my time as a Ph.D. graduate student. In particular: my mother Margaret Baldwin, Nikki and Justin Atkins, Renee Albertson, Amanda Claxton, Dr. Danna Schock, Dawn Hall, my sailing buddies from the YBYC (John and Barbara Dougherty, Ken and Stephanie Brown, Nairne Dickey, Dr. Laurie Weitkamp, Terry Lettenmaier, Jeff Breazile), all the members of the Hatfield Student Organization, the Newport Running Club (Tom and Sheila Swinford, Dennis Pingree and Rebecca Chuck), and all the local ultimate frisbee players.

CONTRIBUTION OF AUTHORS

Drs. Kym C. Jacobson and Michael A. Banks, my co-advisors, closely advised the planning and execution of this research. Both were involved in editing all the writing, and are therefore coauthors on all four chapters of this dissertation. Mary Beth Rew was involved in data gathering, data analysis and writing of Chapters 3 and 4. Dr. Mattias Johansson assisted with data analysis and writing of Chapter 3.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF TABLES

Tables Page

Using Parasite Community Data and Population Genetics for Assessing Pacific Sardine (*Sardinops sagax*) Population Structure Along the West Coast of North America

GENERAL INTRODUCTION

An estimated 81.9 million tons (FAO 2008) of marine fish were captured in 2006 by the world's fisheries. This demand for fish is predicted to continue increasing along with the human population, which is expected to reach over nine billion by 2050 (Ryman et al. 1995; Waples 1998; WGBH Educational Foundation 2004). With approximately 50% of the world's marine stocks currently considered over-exploited (FAO 2008), the sustainability of harvesting marine fish populations is in doubt.

The 'stock' concept was introduced to better understand the vulnerability of subpopulations within specific species to over-fishing, and to help delineate which fishing areas and components were most at risk of being over-fished. A stock is a group of individuals of a particular species or subpopulation whose genetic characteristics, and usually life history characteristics, are more similar to each other than other stocks (Waldman 2005). Stock identification of economically important fish species is needed to determine what regulates subpopulation abundance, reproduction, growth, and survival (Grant and Bowen 1998). If stocks can be identified, then it is possible to understand how or why a fishery might exploit specific components of a population (Waples 1998). Fish stock identification in the marine environment has been challenging since relatively few barriers exist that might delineate dispersal and

migration compared to those in terrestrial or freshwater environments (Waples 1998). Knowing stock structure is especially important for highly migratory fish species that cross state or country boundaries because changes in their distributions can result in changes to harvest availability in various state, national or international political jurisdictions. Most information on stock identification has been collected by applying morphological characteristics, artificial tags, biological tags and genetic techniques (Cadrin et al. 2005).

Pacific Sardines

The Pacific sardine (*Sardinops sagax*) is an ecologically important pelagic fish species, transferring energy from lower trophic prey species (algae and zooplankton) to upper trophic predators (fish, marine mammals and birds; Cury et al. 2000; Field et al. 2006). Pacific sardines are also economically important, harvested for bait, used in aquaculture and for human consumption (see review by Herrick et al. 2009, Hill et al. 2009). During the 1930s the Pacific sardine (*Sardinops sagax*) fishery was considered the largest in the Western Hemisphere (Wolf 1992), contributing almost 25% of the total fish landed in the United States (McEvoy and Scheiber 1984). Tagging studies in the 1930s and 1940s suggested that Pacific sardines in the California Current caught from British Columbia, Canada to southern California were one stock (Janssen 1938; Clark and Janssen 1945). Evidence indicated that after spawning off southern California in spring, the larger sardines migrated north to feeding grounds off of the Columbia River and into Canadian waters. At the onset of winter storms, sardines then

migrated back to Southern California to overwinter (Marr 1957; 1960). The coastwide fishery began to collapse in the 1940s, with closures off British Columbia in 1947, and off of Central California in 1967 (Radovich 1982).

By 1992 sardines re-appeared in large numbers in the Pacific Northwest (Vancouver Island, British Columbia, Canada, Washington and Oregon, USA) (Hargreaves et al. 1994), but it remains uncertain whether the coastwide migration pattern described prior to the fisheries collapse of the 1940s has resumed, or if there are sub-populations of sardine along the coast with limited migration among these smaller groups. Successful sardine recruitment has been observed off the Columbia River (Bentley et al. 1996, Emmett et al. 2005) lending support for a separate stock in the Pacific Northwest, but sardine genetic data have been inconclusive in identifying more than one stock between California and British Columbia (Hedgecock et al. 1989; Lecomte et al. 2004; J. Hyde pers. comm.). If more than one stock exists in this part of the California Current, understanding the connections between potential subpopulations has important management implications since the fisheries in the United States and Canada are currently managed as one stock (latest stock assessment by Hill et al. 2009).

Trophically-transmitted parasites

Using parasite species as biological tags is another approach applied to identify fish population stock structure (see review by MacKenzie 2002). Parasites are naturally

acquired by their fish hosts, and can provide ecological information on nursery grounds, migration and foraging history (Thomas et al. 1996). The total variation in the number of parasite species recovered (community diversity), the percentage of fish infected (prevalence) and the number of parasites per fish host (abundance) (Bush et al. 1997) have been used to discriminate between fish stocks (Lester 1990; Thomas et al. 1996; MacKenzie and Abaunza 1998). Parasites infect fish by direct attachment or penetration, or indirectly through consumption of infected prey (Marcogliese 1995; Rhode 1984). Once a fish is infected, parasites can remain in or on the host from months to years depending on the longevity of the parasite species, and other aspects of the host-parasite association (Rohde 1984).

There is a long history for applying parasites to delineate stocks of marine fish (Table 1.1). An early study by Herrington et al. (1939) used the presence of the parasitic copepod (*Sphyrion lumpi*) to separate two stocks of redfish (*Sebastes marinus*) harvested off the Atlantic coast of the United States. Prevalence of the parasitic copepod in redfish from the coast of Maine and New Hampshire averaged 11% compared to Browns Bank and eastward to Sable Island where 0% of the redfish were infected. Since that time, parasites (either single or multiple species) have been used to identify stocks of marine fish worldwide (Table 1.1) (MacKenzie and Abaunza 1998; Mattiucci 2006).

More recently, the combination of parasite community analyses and parasite population genetics has become a successful technique to assess fish population (see review by Abaunza et al. 2008a; b). Parasite genetic data can be especially useful when the genetic structure of the fish species themselves has been inconclusive in defining stock structure. Recently the HOMSIR (Horse Mackerel Stock Identification Research) project assessed the stock structure of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea in 2000 and 2001 (Abaunza et al. 2008a). A combination of fish genetics, morphometrics (e.g., body shape), life history traits (e.g., distribution) and parasites (Abaunza et al. 2008a; b; MacKenzie et al. 2008; Mattiucci et al. 2008) were examined from the same individual fish. The parasite community and fish morphometrics provided the strongest evidence for the presence of separate stocks within the Atlantic Ocean (Western and Southern) and the North Sea. Additionally, the parasite allozyme data further separated fish from the Mediterranean Sea into three different stocks, and provided evidence that horse mackerel may move between the southern Atlantic and the western Mediterranean (Abaunza et al. 2008b; Mattiucci et al. 2008). Thus, the integration of multiple techniques can enable the discovery of distinct fish stocks.

Review of methods to assess fish stock structure

Trophically-transmitted parasites have yet to be included in an assessment for Pacific sardine to assess migration patterns or stock structure. For Chapter one entitled "Integrating Fish and Parasite Data as a Holistic Solution for Identifying the Elusive

Stock Structure of Pacific sardines (*Sardinops sagax*)", the objectives were to 1) review different methods used to assess fish stock structure of Pacific sardines in comparison to other marine or anadromous fishes; 2) review the success of using parasites to examine fish stock structure; and 3) affirm the merits of complimenting fish data with parasite data (both community and genetic) to resolve more challenging stock identification scenarios.

Identifying migration patterns of Pacific sardines using biological tags

Pacific sardines have been described as migrating between their feeding and spawning grounds from Vancouver Island, British Columbia and San Diego, California (Dahlgren, 1936; Hart, 1943**;** Ahlstrom, 1957; Smith, 2005; Hill et al., 2009). Tagging studies in the 1930s and 40s suggested that Pacific sardine migrated back and forth between Southern California and Washington (Marr 1957), with evidence of a northward migration extending to Vancouver Island, British Columbia Marr (1960). Attempts to repeat these early tagging studies conducted between 1936 and 1942 for Pacific sardine have been difficult since mortalities before or shortly after release were up to 80% for fish $\leq$ 185 mm in total length and 70% for large fish (> 185 mm in total length) (Clark and Janssen 1945). Financial costs have also been prohibitive, with thousands of tagged fish needed to increase the probability of future recovery (Jacobson and Hansen 2005). Thus, cost restrictions and expectation of high mortality rates reduce the likelihood that a large-scale mark-recapture program for Pacific

sardine will be initiated soon to determine if the current migration patterns are similar to those observed more than 60 years ago.

For Chapter two entitled "Two Migration Patterns Identified for Northeastern Pacific Sardine (*Sardinops* sagax) using Trophically-transmitted Parasites as Biological Tags", I assessed if the parasite species infecting Pacific sardines could clarify migration patterns of sardines within the California Current System by: 1) comparing parasite communities of sardine collected from five regions along the west coast of North America; 2) identifying a single parasite or a suite of parasite species that may be used as biological tags for sardines; and 3) examining the parasite communities of northern anchovy (*Engraulis mordax*), another clupeid fish infected with limited migration capabilities (Bakun 1996) known to be infected with similar parasite species (Love and Moser 1983) that could provide information on the geographical restrictions of specific parasite species.

Parasite Population Genetics

Anisakis species

Anisakid nematodes are known to infect more than 200 species of pelagic fish (Sabater and Sabater 2000), and have been used as biological tags for fish population structure studies (see review by MacKenzie 2002). *Anisakis* species use euphausiids as their obligate first intermediate host, fish or squid as second intermediate or paratenic (transport hosts where no development occurs), and cetaceans as definitive hosts

(Oshima 1972; Smith and Wooten 1978). With the availability of molecular markers, morphologically similar larvae of *Anisakis* species recovered globally have been separated into nine genetically distinct species comprising two clades (Mattiucci et al. 2009). Despite the growing information on the global distribution of *Anisakis* species (see review by Mattiucci and Nascetti 2008; Klimpel et al. 2010) there is little information on the genetic diversity and population structure of these nematodes, which could be useful in assessing the stock structure of their fish hosts.

In the marine environment there are few obvious physical barriers limiting gene flow between fish populations. Nevertheless, some fish species have been designated as distinctively and geographically separated stocks or subpopulations based on genetically identifying *Anisakis* species. For example, separate stocks of the European hake (*Merluccius merluccius*) were identified within the Mediterranean Sea and the Atlantic Ocean based on the distribution of seven species of *Anisakis* identified using allozymes (Mattiucci et al. 2004). More recently, parasite community analysis (MacKenzie et al. 2008) and allozyme data from five *Anisakis* species (Mattiucci et al. 2008) identified separate stocks of the Atlantic horse mackerel (*Trachurus trachurus*) within the Atlantic Ocean (western and southern) and North Sea. Atlantic horse mackerel were further separated into three different stocks within the Mediterranean Sea with parasite data indicating the potential of fish exchange between the Atlantic southern stock and the west Mediterranean stock (Abaunza et al. 2008). In addition, Cross et al. (2007) suggested that *Anisakis simplex s.s.* may be a suitable biological tag

for Atlantic herring (*Clupea harengus*) since this nematode species can be recovered throughout the year.

For Chapter three entitled "Panmixia Rather Than Geographically Segregated Haplotypes Observed in Three Species of *Anisakis* Nematodes Recovered from Pacific Sardine (*Sardinops sagax*) Distributed Throughout the California Current System", The main goals of this study were to: 1) recover larval nematodes from Pacific sardine sampled off of Vancouver Island, British Columbia to San Diego, California the California Current; 2) genetically identify which *Anisakis* species infect Pacific sardine using sequences from the internal transcribed spacers (ITS1 and ITS2) and the 5.8s subunit of the nuclear ribosomal DNA.; 2) examine the genetic diversity and population structure of nematodes using the cytochrome c oxidase 2 (*cox2*) mitochondrial DNA gene; and 3) assess the potential of using *Anisakis* species as a biological tag to help discriminate stocks of Pacific sardine.

Myosaccium ecaude

The trematode *Myosaccium ecaude* was described by Montgomery (1957) from Pacific sardine (*Sardinops sagax*) caught in 1953 off La Jolla, California. Williams and Bunkley-Williams (1996) suggest this genus is commonly found in clupeid fish species. To date this species has only been reported from Pacific sardine (Montgomery 1957; Kunnenkeri 1962). Little information is available on the life history of this trematode in the California Current. However, based on other members of the family

Hemiuridae, the first intermediate host is likely a snail, the second intermediate host is either a calanoid or cyclopoid copepod (Margolis and Boyce 1969; Køie 1983) and the final host is a sardine. Previous surveys of marine fish parasites in the California Current either did not include Pacific sardine or were conducted after sardines disappeared from the northern end of this upwelling region (Love and 1983; McDonald and Margolis 1983). Baldwin et al. (CHAPTER 2, this volume) described an expanded distribution of *M. ecaude* from San Diego, California to Vancouver Island, British Columbia, Canada. It is uncertain if the presence of *M. ecaude* as far north as British Columbia is correlated with the return of Pacific sardine to the Pacific Northwest in the 1990s. Approximately 70% of *M. ecaude* individuals were recovered in sardines $\leq$ 200 mm in standard length (SL) (Baldwin et al. CHAPTER 2, this volume), which are considered non-migratory (Lo et al. unpublished).

For Chapter four entitled "Has the Population of the Marine Trematode *Myosaccium ecaude* Expanded in Association with the Return of Pacific Sardine (*Sardinops sagax*) to the Northern California Current?", the main goals of the study were to: 1) recover *M. ecaude* trematodes from Pacific sardine collected off of Vancouver Island, British Columbia to San Diego, California the California Current; 2) to examine the genetic diversity and population structure of *M. ecaude* using NADH-dehydrogenase subunit 1 (ND1) mitochondrial DNA gene; and 3) assess the potential of using the distribution of *M. ecaude* haplotypes as a biological tag to help discriminate stocks of Pacific sardine.

Significance of Research

The overall goals of this research were to use parasite community data and parasite population genetics data to describe the migration and stock structure of Pacific sardines along the west coast of North America by: 1) traditional morphological identification of the macroparasite species recovered from fish caught from 2005 through 2008 to assess differences in (a) parasite species populations, (b) species composition of macroparasite communities; and (c) identify candidate parasite species, or communities, as biological tags to distinguishing sardine populations from southern California to Vancouver Island, British Columbia; and 2) to investigate whether genetic differences from one nematode (*Anisakis* species) and one trematode (*M. ecaude*) can be used to assess and delineate the stock structure of Pacific sardines.

This research adds to and complements our current understanding of the migration patterns of Pacific sardine in the California Current System. Two trematodes, *Lecithaster gibbosus* and *M. ecaude*, have emerged as potential biological tags to identify two migration patterns of Pacific sardines that begin at the edges of our study area and spanning more than 2000 km of latitude. *Lecithaster gibbosus* were recovered in non-migrant ($\leq$ 200 mm standard length) and migrant (> 200 mm standard length) sardines primarily off Vancouver Island, British Columbia, Canada. *Myosaccium ecaude* were recovered throughout the study area, but were most common off central and Southern California in non-migrant fish. The distributional pattern of *M. ecaude* supported artificial tagging studies from 60 years ago that described a coastwide

migration pattern from the spawning grounds off southern California to the feeding grounds off the Pacific Northwest and Canada. We observed a secondary migration pattern with the geographic distribution of *L. gibbosus* from Vancouver Island, British Columbia to northern California, perhaps evidence for a migration pattern described from Regions 1 to 3 prior to the sardine fishery collapsing in the 1940s (Felin 1954; Radovich 1962; Radovich 1982).

We recovered the adult trematode *M. ecaude* and larval nematodes *Anisakis pegreffii*, *A. simplex s.s.*, and *A. simplex* 'C' throughout the California Current in Pacific sardines. To the best of our knowledge this is the first population genetics study for *M. ecaude*. Analyses of the mitochondrial DNA ND1 haplotypes (for *M. ecaude*) and *cox2* haplotypes (for each *Anisakis* species) supported a panmictic distribution of for both *M. ecaude* and all three *Anisakis* species in the California Current. Our results suggest that the extensive movement of all of the potential hosts utilized by these parasites, the limited oceanographic barriers, and complexity in the California Current are not preventing the mixing of *M. ecaude* populations or *Anisakis* species or populations.

References

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, E. Gallo, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Ramos, A. Ruggi, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008a. Considerations on sampling strategies for an holistic approach to stock identification: The example of the HOMSIR project. Fisheries Research **89**:104-113.

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Rarnos, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008b. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fisheries Research **89**:196-209.

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Bakun, A. 1996. Patterns in the ocean. Ocean processes and marine population dynamics. California Sea Grant College System, National Oceanic and Atmospheric Administration in cooperation with Centro de Investigaciones Biológicas del Noroeste, La Paz, BCS, Mexico.

Bentley, P. J., R. L. Emmett, N. C. H. Lo, and H. G. Moser. 1996. Egg production of Pacific sardine (*Sardinops sagax*) off Oregon in 1994. California Cooperative Oceanic Fisheries Investigations Reports **37**:193-200.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Cadrin, S. X., K. D. Friedland, and J. R. Waldman. 2005. Stock identification methods: an overview. Pages 3-6 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Applications in Fishery Science. Elsevier Academic Press, San Francisco.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Cross, M. A., C. Collins, N. Campbell, P. C. Watts, J. C. Chubb, C. O. Cunningham, E. M. C. Hatfield, and K. MacKenzie. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda: Anisakidae): implications for fisheries management. Marine Biology **151**:695-702.

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quinones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science **57**:603-618.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Felin, F. E. 1954. Population heterogeneity in the Pacific pilchard. Fisheries Bulletin **54**:201-225.

Field, J. C., R. C. Francis, and K. Aydin. 2006. Top-down modeling and bottom-up dynamics: Linking a fisheries-based ecosystem model with climate. Progress in Oceanography **68**:238-270.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity **89**:415-426.

Hargreaves, N. B., D. M. Ware, and G. A. McFarlane. 1994. Return of Pacific sardine (*Sardinops sagax*) to the British Columbia coast in 1992. Canadian Journal of Fisheries and Aquatic Science **51**:460-463.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Herrick, S. F., J. G. Norton, R. Hannesson, U. R. Sumaila, M. Ahmed, and J. Pena-Torres. 2009. Global production and economies. Pages 256-274 *in* D. Checkley, J. Alheit, Y. Oozeki, and C. Roy, editors. Climate Change and Small Pelagic Fish. Cambridge University Press, New York.

Herrington, W. C., H. M. Bearsem, and F. E. Firth. 1939. Observations on the life history, occurrence and distribution of the redfish parasite *Sphyrion lumpi*. US Bureau of Fisheries Special Report **5**:1-18.

Hill, K. T., N. C. H. Lo, B. J. Macewicz, P. R. Cronel, and R. Félix-Uraga. 2009. Assessment of the Pacific sardine resource in 2009 for U.S. management in 2010. NOAA-TM-NMFS-SWFSC-452:221.

Jacobsen, J. A., and L. P. Hansen. 2005. Internal and external tags. Pages 415-433 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in fishery science. Elsevier Academic Press, San Francisco.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Klimpel, S., M. W. Busch, T. Kuhn, A. Rohde, and H. W. Palm. 2010. The *Anisakis simplex* complex off the South Shetland Islands (Antarctica): endemic populations versus introduction through migratory hosts. Marine Ecology Progress Series **403**:1-11.

Køie, M. 1983. Digenetic trematodes from *Limanda limanda* (L.) (Osteichthyes, Pleuronectidae) from Danish and adjacent waters, with special reference to their life-histories. Ophelia **22**:201-228.

Kunnenkeri, J. K. 1962. Preliminary report on the parasites of the California sardine and the parasitic distribution in Clupeidae. Journal of Parasitology **48**:149.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Lester, R. J. G. 1990. Reappraisal of the use of parasites for fish stock identification. Australian Journal of Marine and Freshwater Research **41**:855-864.

Lo, N. C. H., B. J. Macewicz, and D. A. Griffith. unpublished. Biomass and reproduction of Pacific sardines off the northwest US in 2003-2005. 43pp.

Love, M. S., and M. Moser. 1983. A checklist of parasites of California, Oregon, and Washington Marine and Estuarine Fishes. NOAA Technical Report NMFS SSRF-777. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**:S153-S163.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research **38**:45-56.

MacKenzie, K., N. Campbell, S. Mattiucci, P. Ramos, A. L. Pinto, and P. Abaunza. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fisheries Research **89**:136-145.

Marcogliese, D. J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. Reviews of Fish Biology and Fisheries **5**:336-371.

Margolis, L., and N. P. Boyce. 1969. Life span, maturation, and growth of two Hemiurid trematodes, *Tubulovesicula lindbergi* and *Lecithaster gibbosus*, in Pacific salmon (Genus *Oncorhynchus*). Journal of the Fisheries Research Board of Canada **26**:893-907.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

Marr, J. C. 1960. The causes of major variations in the catch of the Pacific sardine, *Sardinops caerulea* (Girard). Pages 667-791 *in* H. Rosa and G. I. Murphy, editors. Proceedings of the World Scientific Meeting on the Biology of Sardines and Related Species. Food and Agriculture Organization of the United Nations.

Mattiucci, S. 2006. Parasites as biological tags in population studies of demersal and pelagic fish species. Parassitologia **48**:23-25.

Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008. *Anisakis* spp. larvae (Nematoda : Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Mattiucci, S., M. Paoletti, and S. C. Webb. 2009. *Anisakis nascettii* n. sp (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. Systematic Parasitology **74**:199-217.

McDonald, T. E., and L. Margolis. 1995. Synopsis of the parasites of fishes of Canada: Supplement (1978-1993). Canadian Special Publication of Fisheries and Aquatic Sciences.

McEvoy, A. F., and H. N. Scheiber. 1984. Scientists, entrepreneurs, and the policy process: a study of the post-1945 California sardine depletion. Journal of Economic History **44**:393-406.

Montgomery, W. R. 1957. Studies on digenetic trematodes from marine fishes of La Jolla, California. Transactions of the American Microscopy Society **76**.

Oshima, M. 1972. *Anisakis* and Anisakidiasis in Japan and adjacent area. Progress of Medical Parasitology in Japan **4**:305-393.

Radovich, J. 1962. Effects of sardine spawning stock size and environment on year-class production. California Fish and Game **48**:123-140.

Radovich, J. 1982. The collapse of the California sardine fishery. Pages 107-136 *in* M. H. Glantz and J. D. Thompson, editors. Resource Management and Environmental Uncertainty. Lessons from Coastal Upwelling Fisheries. John Wiley & Sons, New York.

Rohde, K. 1984. Ecology of marine parasites. Helgoländer Meeresunters **37**:5-33.

Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries **5**:417-446.

Sabater, E. I. L., and C. J. L. Sabater. 2000. Health hazards related to occurrence of parasites of the genera *Anisakis* and *Pseudoterranova* in fish. Food Science and Technology International **6**:183-195.

Smith, J. W., and R. Wooten. 1978. *Anisakis* and Anisakiasis. Advances in Parasitology **16**:93-163.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Thomas, F., O. Verneau, T. de Meeûs, and F. Renaud. 1996. Parasites as host evolutionary prints: Insights into host evolution from parasitological data. International Journal of Parasitology **26**:1137-1137.

Waldman, J. R. 2005. Definition of stocks: an evolving concept. Pages 7-16 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in Fisheries Science, San Francisco.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity **89**:438-450.

Williams Jr., E. H., and L. Bunkley-Williams. 1996. Platyhelminthes (Flatworms). Pages p.59 *in* Parasites of Offshore Big Game Fishes of Puerto Rico and the Western Atlantic. Antillean College Press, Mayaguez, Puerto Rico.

Wolf, P. 1992. Recovery of the Pacific sardine and the California sardine fishery. California Cooperative Oceanic Fisheries Investigations Reports **33**:76-86.

Electronic References:

FAO. 2008. The State of World Fisheries and Aquaculture (updated 2008). http://fao.org. Accessed: 29 January 2010

WGBH Educational Foundation. 2004. World in the Balance (updated 2004)

INTEGRATING FISH AND PARASITE DATA AS A HOLISTIC SOLUTION FOR IDENTIFYING THE ELLUSIVE STOCK STRUCTURE OF PACIFIC SARDINES (*SARDINOPS SAGAX*)

Rebecca E. Baldwin, Michael A. Banks, Kym C. Jacobson

Reviews in Fish Biology and Fisheries

Springer

233 Spring Street

New York, NY 10013

(In Prep)

Abstract

There is an urgency to clarify how different stocks or subpopulations of fish species are vulnerable to fishing pressure and unfavorable ocean conditions because of the increasing demand of fish for human consumption. For marine fishes the potential for high gene flow increases the difficulty in determining the number of subpopulations managed in a specific fishery. Although the use of fish-molecular data has become a common method in the past 15 years to identify fish subpopulations, no single technique or suite of techniques has been established for fish stock structure studies. We review the use of fish morphometrics, artificial tags, fish genetics, parasite genetics and parasites as biological tags to identify subpopulations of marine fishes with a focus on the Pacific sardine (*Sardinops sagax*) fishery off the west coast of North America. We suggest an integration of fish- and parasite-based techniques for future stock structure studies, particularly for pelagic fish species whose stock structure can be elusive. An integration of techniques may also resolve fish stock structure over small geographic areas by increasing the number of spatial and temporal scales studied simultaneously leading to methods for successful management of marine fish species.

Introduction

An estimated 81.9 million tons (FAO 2008) of marine fish were captured in 2006 by the world's fisheries. This demand for fish is predicted to continue increasing along with the human population, which is expected to reach over nine billion by 2050 (Ryman et al. 1995; Waples 1998; WGBH Educational Foundation 2004). With approximately 50% of the world's marine stocks currently considered over-exploited (FAO 2008), the sustainability of harvesting marine fish populations is in doubt. Early in the 20th century, fish stock models were developed to calculate marine fish stock biomass available to harvest each year while still maintaining viably productive populations (Beverton 2002). Some of the earliest mathematical models used to estimate fish population biomass yields included the length-based yield biomass equation (Baranov 1918), a simple logistic production curve to estimate maximum yield (Graham 1935), a physiological growth curve (von Bertalanffy 1938), a maximum yield versus recruitment curve (Ricker 1954), and a constant population recruitment model (Beverton and Holt 1957). Most fish harvest guidelines have been established based on these and more recent models; however, evidence continues to indicate that many fish populations are over-exploited (Pauly et al. 2000).

Models are only as good as the data used to create them (Beverton 1998), in part due to the difficulty identifying and assessing interactions among all the components of each fishery (Smith 1998; Schnute and Richards 2001). For instance, earlier models did not necessarily account for fish recruitment failures (Beverton 1998), the boom

and bust nature of forage fish species (Alder et al. 2008), or the accidental mixing of fish populations in fishery data making it difficult to later separate fish into distinct groups (Sinclair and Solemdal 1988). In addition, fisheries models published from 1948 to 1977 were created during a period with relatively stable ocean conditions compared to today (Mantua et al. 1997; Peterson and Schwing 2003). Important theories of fisheries management may have differed if these early modelers had been able to incorporate oceanographic conditions into their models, since the same model relationships may not be appropriate for use in different regimes or states of the ocean (Beamish et al. 2000).

Fish react to changes in their environment (physical and biological); however, single-species models may be too simple to capture the vicissitudes of complex ecosystems (Smith 1998). Changes in a fish population size via birth, individual movement, and death are often not well captured by abstract representations in a fisheries model, thus mathematical values become disconnected from the fish life-history characteristics they represent (Rose 1997; Beverton 1998; Schnute and Richards 2001). Further, one fishery model may not apply to different populations of the same fish species (Schnute and Richards 2001; Longhurst 2006) since it is unclear how fishing affects the ability of each population to overcome recruitment failures due to adverse environmental conditions (Mann 2000). It is also unclear if fish that are commercially exploited sustain sufficient rigor to withstand fluctuating ocean conditions as they would have done prior to exploitation (Mann 2000). Thus, model shortcomings and data

uncertainty in combination with inevitable fluxes in environmental conditions limits the value of using past data to define the future status of a specific fish population or species (Schnute and Richards 2001).

The ‘stock’ concept was introduced to better understand the vulnerability of subpopulations within specific species to over-fishing, and to help delineate which fishing areas and components were most at risk of being over-fished. A stock is a group of individuals of a particular species or subpopulation whose genetic characteristics, and usually life history characteristics, are more similar to each other than other stocks (Waldman 2005). Stock identification of economically important fish species is needed to determine what regulates subpopulation abundance, reproduction, growth, and survival (Grant and Bowen 1998). If stocks can be identified, then it is possible to understand how or why a fishery might exploit specific components of a population (Waples 1998). Fish stock identification in the marine environment has been challenging since relatively few barriers exist that might delineate dispersal and migration compared to those in terrestrial or freshwater environments (Waples 1998). Knowing stock structure is especially important for highly migratory fish species that cross state or country boundaries because changes in their distributions can result in changes to harvest availability in various state, national or international political jurisdictions. Most information on stock identification has been collected by applying morphological characteristics, artificial tags, biological tags and genetic techniques (Cadrin et al. 2005).

During the 1930s the Pacific sardine (*Sardinops sagax*) fishery was considered the largest in the Western Hemisphere (Wolf 1992), contributing almost 25% of the total fish landed in the United States (McEvoy and Scheiber 1984). Tagging studies in the 1930s and 1940s suggested that Pacific sardines in the California Current caught from British Columbia, Canada to Southern California were one stock (Janssen 1938; Clark and Janssen 1945). Evidence indicated that after spawning off Southern California in spring, the larger sardines migrated north to feeding grounds off of the Columbia River and into Canadian waters. At the onset of winter storms, sardines then migrated back to southern California to overwinter (Marr 1957; 1960). The coastwide fishery began to collapse in the 1940s, with closures off British Columbia in 1947, and off of Central California in 1967 (Radovich 1982).

By 1992 sardines re-appeared in large numbers in the Pacific Northwest (PNW) (Hargreaves et al. 1994), but it remains uncertain whether the coastwide migration pattern described prior to the fisheries collapse of the 1940s has resumed, or if there are sub-populations of sardine along the coast with limited migration among these smaller groups. Successful sardine recruitment has been observed off the Columbia River (Bentley et al. 1996, Emmett et al. 2005) lending support for a separate stock in the PNW, but sardine genetic data have been inconclusive in identifying more than one stock between California and British Columbia (Hedgecock et al. 1989; Lecomte et al. 2004; J. Hyde pers. comm.). If more than one stock exists in this part of the California Current, understanding the connections between potential subpopulations

has important management implications since the fisheries in the United States and Canada are currently managed as one stock (latest stock assessment by Hill et al. 2009).

Biological tags such as trophically-transmitted parasites have been used to assess migration patterns and stock structure of their fish hosts (see review by MacKenzie 2002), but parasites have yet to be included in an assessment for Pacific sardine. Our objectives for this review are to describe different methods used to assess fish stock structure of Pacific sardines, review the success of using parasites to examine fish stock structure, and affirm the merits of complimenting fish data with parasite data (both community and genetic) to resolve more challenging stock identification scenarios.

Fish stock identifications: morphology and artificial tags

The California Current Pacific sardine is an economically and ecologically important fish whose stock structure has been assessed using several different approaches (Smith 2005). Early sardine studies compared the variation in morphology (or meristics) between individual fish. Clark (1947) and Wisner (1960) suggested that sardine populations could be separated based on vertebrae number. Sardines from more northern latitudes (such as the PNW) had more vertebrae on average than sardines from southern latitudes (such as Southern California and the Gulf of California). Clark (1947) suggested that the development of more vertebrae resulted from colder ocean

temperatures during development, and that individuals could be traced to their source population by assessing vertebrae number. Other sardine meristic studies have included size at age determined by scales and otoliths (Felin 1954), blood groups (Sprague and Vrooman 1962), otolith morphometry (Félix-Uraga et al. 2005), and otolith microchemistry (Jones 2006).

Although fish are often opportunistically collected within the geographical range of a species (Jones 2006), there has been some success in identifying different stocks of Pacific sardine on a large geographic scale (Smith 2005). Recently, Félix-Uraga et al. (2004) developed a temperature distribution model that identified Pacific sardine groups from Southern California to the Gulf of California based on time of year and specific ranges in sea surface temperatures: 1) 13^{o} to 17^{o}C (cold water group) from San Pedro, California, to Ensenada, Baja California; 2) 17^{o} to 22^{o}C (temperate water group) from San Pedro, California to Magdalena Bay, Baja California; and 3) 22^{o} to 27^{o}C (warm water group) from Magdalena Bay, Baja California, to the Gulf of California. Although Radovich (1962; 1982) suggested a far northern stock existed in the California Current from Point Conception, California, to British Columbia, Canada, prior to the westcoast fishery collapse, only three Pacific sardine stocks are currently recognized: 1) Central California offshore; 2) Baja California Sur inshore; and 3) Gulf of California (Smith 2005).

Mark-recapture studies provide insight into fish migration patterns (Jakobsson 1970; Hansen and Jacobsen 2003; Orbesen et al. 2008), spatial separation of stocks (McFarlane et al. 1990; Neilson et al. 2006; Armannsson et al. 2007), and estimating the proportion of individuals intermingling among stocks (Teel et al. 2003; Hoenig et al. 2008). Early sardine migration studies used metal tags attached to the operculum or caudal fin, or inserted into the abdominal cavity (commonly known as "belly tags") (Rounsefell and Dahlgren 1933). When the electromagnetic tag-detection technology enabled an individual tag to be associated with a specific fish, belly tags became the preferred tagging method for pelagic fishes (Dahlgren 1936; Hart 1943**;** Ahlstrom 1957). Based on meristic and tagging studies, Marr (1957) concluded that Pacific sardine migrated back and forth between Southern California and the PNW. Further tagging research by Marr (1960) showed that the northward migration of Pacific sardine extended to Vancouver Island, British Columbia, and supported Clark's (1935) hypothesis that northern California Current Pacific sardine were one continuous population.

Attempts to repeat these early tagging studies for Pacific sardine have been difficult. High rates of tagging mortality are expected since up to 80% of small fish (< 185 mm in total length), and 70% of large fish (> 185 mm in total length) died before or shortly after release in tagging studies conducted between 1936 and 1942 (Clark and Janssen 1945). In general smaller fish were more prone to complications related to scale loss (Harrt 1963; Hansen and Jacobson 2003), which may explain the 'excessive mortality'

observed using belly tags in Pacific sardines smaller than 140mm in total length (Janssen and Alpen 1945). Clark and Janssen (1945) described mortality rates increasing with prolonged fish handling and holding fish in captivity, and currently there are limited facilities available to hold sardines with minimal mortality.

Thousands of tagged fish are needed to increase the probability of future recovery (Jacobson and Hansen 2005), hence financial costs also need to be considered as coded wire tags cost $0.15 each (Johnson 2004) compared to $20 for each electronic tag with temperature recording capabilities (Welch et al. 2003). Electronic tags are larger than coded wire tags; thereby their use is restricted to larger-sized fish. Thus, with cost restrictions and expectation of high mortality rates it is unlikely a large-scale mark-recapture program will be initiated soon for Pacific sardine to assess if migration patterns observed more than 60 years ago have resumed under current ocean conditions.

Fish stock identification: molecular markers

Technological advances over the past 25 years have enabled detailed examination of genetic material, and for the past 15 years molecular markers have become the most favored method to assess fish population stock structure. Fishery Scientists have used many different types of genetic markers including allozymes (Waples et al. 2008), mitochondrial DNA (mtDNA) (Waples et al. 2008), microsatellites (Hedrick 1999), and single nucleotide polymorphisms (SNPs) (Morin et al. 2004). All four types of

genetic markers are still in use today, providing opportunities to analyze fish stock structure over various time and spatial scales.

Allozymes, allelic forms of a protein (King and Stansfield 1985), were among the first genetic markers applied to the study of animals (Harris 1966; Hubby and Lewontin 1966; Lewontin and Hubby 1966). These markers have a low mutation rate (10^{-7} mutations per generation; Nei 1987), and provide a qualitative description for specific Mendelian loci (Avise 1998). Hedgecock et al. (1989) used 32 allozyme loci to examine Pacific sardine collected from Central California to the Gulf of California. Genetic variation was low with rare alleles shared among distant locations, thus sardines were considered one population (Hedgecock et al. 1989). In contrast, semi-isolated groups of European sardine (*Sardina pilchardus*) were described from the western Mediterranean Sea using 15 loci, with fish collected off Alboran considered distinct from the remaining geographic locations (Ramon and Castro 1997). Recently, allozyme data indicated a weak population structure for the European sardine in the Atlantic Ocean where samples from the Azores, Mauritania and Maderia were distinct from coastal Europe and northwest Africa (Laurent et al. 2007). In particular the super-oxidase dismutase (SOD*) locus was associated with genetic differences between geographic locations (Laurent et al. 2007), a pattern previously observed by Chlaida et al. (2006) whose study focused on sardine populations off Morocco. Although sardine populations can be identified using allozymes, they comprise less than 1% of the total genome (Nei 1987) and require reproductive isolation among

contributing subpopulations for several generations before genetic differences can accumulate (Koljonen and Wilmot 2005).

Mitochondrial DNA (mtDNA) is a haploid genetic marker that characterizes matrilineages within and among species (Avise 1998). Mitochondrial DNA markers are well represented in most genomes, have a high copy number (Allendorf and Luikart 2007), and have regions with varying mutation rates (10^{-1} to 10^{-3} mutations per generation) that are five to ten times faster than mutation rates estimated for some nuclear DNA (Brown 1983). Although discrete stocks of marine fish have been defined using mtDNA such as thornyheaded rockfish (*Sebastolobus alascanus*) (Stepien 1995) and blue rockfish (*Sebastes mystinus*) (Cope 2004), geographically separated populations have not been identified for either Pacific sardine in the California Current (Lecomte et al. 2004), or European sardine off Turkey (Sarmaşik et al. 2008). Evidence of a genetic bottleneck was described using 387 base pair (bp) portion of the mtDNA control region for European sardines caught off Safi, Morocco, but no other populations were identified for the Atlantic Ocean and Mediterranean Sea (Atarhouch et al. 2006). Historical divergences (over the last few million years) between populations or stocks can be detected using mtDNA, but these markers may fail to detect more recent divergences (within the last ten thousand years) (Hewitt 2004). Additionally it is impossible to resolve hybrid matings with a maternally inherited marker. Thus, mtDNA may be inappropriate for stock identification among

recently diverged subpopulations, or fish species like sardines that have expanding and contracting population dynamics.

Microsatellites are repetitive sequences of DNA, usually two to four nucleotides long (e.g., AC or GATA) often in assemblages ranging from five to 50 repetitions (Dewoody and Avise 2000). Microsatellites are co-dominant markers widely dispersed along and among chromosomes, with each locus defined by a specific DNA sequence. With 10^{-4} to 10^{-5} mutations per generation (Bruford and Wayne 1993), the mutation rate of microsatellites is high compared to allozymes. Sometimes unique alleles are observed for localized populations, but more often differences in allele frequencies are used to separate populations. For instance, Beacham et al. (2008) used 14 microsatellite loci to confirm that four stocks of Pacific herring (*Clupea pallasi*) in British Columbia, Canada, were distinct from each other as well as different from herring from Southeast Alaska, Washington, and California. Although eight microsatellite loci identified a weak genetic structure for European sardines among pairwise comparisons between Central Atlantic Ocean and the Mediterranean Sea, no geographically distinct populations were detected (Gonzalez and Zardoya 2007). So far eleven microsatellites have been published for Pacific sardine (Pereyra et al. 2004) with generally weak support for separate regional stocks; however, some cases of adjacent samples have appeared more distinct than samples collected at opposite ends of the California Current (J. Hyde pers. comm.).

The newest molecular markers assessed for stock identification are single nucleotide polymorphisms (SNPs) (Vignal et al. 2001; Morin et al. 2004), which are nucleotide sites with single nucleotide differences between alleles (Smith and Seeb 2008). Typically SNPs only have two alleles per marker (bi-allelic), and have been found throughout the genome in coding and non-coding regions roughly every 200 to 500 base pairs (Morin et al. 2004). Smith and Seeb (2008) suggested that SNPs have some concordance with microsatellites and allozymes in separating eleven chum salmon (*Oncorhynchus keta*) populations into seven reporting groups. Morin et al. (2004) suggest that several SNP loci exist in a more representative sample of the entire genome and have less sampling variance than microsatellite loci. More SNP loci are required to have a similar statistical power as microsatellite loci, and currently no information is available on the SNPs in sardines. Without *a priori* knowledge of the expected genetic variability in a fish species, SNPs may not be the best choice for a population genetics study.

With the ongoing development of new molecular markers, and increasing availability of genetic sequences, identifying individual fish to specific populations or stocks has become possible with different assignment methods (Hansen et al. 2001; Manel et al. 2005). Assignment tests categorize individuals of unknown origin to a specific population or stock that has been determined *a priori* (Manel et al. 2005). For instance, Narum et al. (2008) observed that both microsatellites and SNPs could differentiate 29 populations (> 99% of pairwise tests) of Chinook salmon (*O.*

tshawytscha) from the Pacific coast of North America, but 13 microsatellites had better resolution for separating closely related populations than the 37 SNPs applied in that study. Using data from eight microsatellites Gonzalez and Zardoya (2007) correctly assigned only 20.1% of individual European sardines to their source population, in part due to the difficulty in clarifying the number of source populations in the central Atlantic Ocean and the Mediterranean Sea. Instead of assigning individual fish, Population Differentiation assigns a proportion of individuals to a specific population of origin. Using nine microsatellite loci, Ruzzante et al. (2010) documented three spawning groups of Atlantic herring (*C. harengus harengus* L.) located in the North Sea, the Skagerrak and from the Kattegat and Western Baltic despite evidence for mixing in nursery, feeding and overwintering aggregations. To date, Population Differentiation has not been attempted for sardines. As for individual molecular markers, the power of assignment methods depend on the markers used, the number of loci and fish sampled, and the level of differentiation that exists between pre-determined populations (Manel et al. 2005).

Recommendations on fish molecular markers

Hedgecock et al. (1989) speculated that Pacific sardine have less genetic diversity than other clupeid fish species. Genetic information may be contributed by few founder individuals since Pacific sardine are frequently absent from the California Current as measured by scale deposits in anaerobic sediments (Soutar and Isaacs 1969; Baumgartner et. al 1992). Also, Pacific sardine may be a recent colonizer of the

California Current compared to other pelagic fish. Sardine fossil remains are absent in sediments from the Pliocene and Pleistocene when other pelagic fish species were present (Fitch 1969). When molecular markers are included in future stock assessments of Pacific sardine, the interpretation of fish population structure may differ depending on the type of marker used, the number of loci examined, and the geographical scope of populations included in the analysis. For example, Buonaccorsi et al. (2001) observed interspecific genetic differences for blue marlin (*Makaira nigricans*) caught in either the Atlantic or Pacific Ocean, using allozymes, mtDNA or five microsatellite loci. Even though almost 43% of all Atlantic samples comprised one of two clades, intraspecific population structure was difficult to detect. Interannual variability among locations (particularly for blue marlin caught off of Hawaii) was observed only using mtDNA, and a significant divergence among individual fish was suggested only by microsatellite loci. Microsatellite and mtDNA were more divergent and variable than allozymes, but there was no consistent support among the molecular markers for either higher or lower diversity in either the Atlantic or Pacific Ocean (Buonaccorsi et al. 2001). Grant and Utter (1984) suggested that allozymes may be insensitive to recently genetically separated populations of Pacific herring (*C. pallasi*), and further cautioned the use of any molecular markers for a forage fish species whose population size can expand and contract over decades. Furthermore, difficulty remains in resolving stock structure over relatively small geographic distances in the marine environment, where evidence for high rates of gene flow remains common irregardless of the type of molecular marker applied (Hedgecock 1986; Lessios et al. 1998; Smith

and Seeb 2008; Nielsen et al. 2009a; 2009b). We thus consider herein the potential for complimenting fish genetic data with other methods.

Fish stock identification: parasites

Using parasite species as biological tags is another approach applied to identify fish population stock structure (see review by MacKenzie 2002). Parasites are naturally acquired by their fish hosts, and can provide ecological information on nursery grounds, migration and foraging history (Thomas et al. 1996). Parasites infect fish by direct attachment or penetration, or indirectly through consumption of infected prey (Marcogliese 1995; Rhode 1984). Once a fish is infected, parasites can remain in or on the host from months to years depending on the longevity of the parasite species, and other aspects of the host-parasite association (Rohde 1984). The total variation in the number of parasite species recovered (community diversity), the percentage of fish infected (prevalence) and the number of parasites per fish host (abundance) (Bush et al. 1997) have been used to discriminate between fish stocks (Lester 1990; Thomas et al. 1996; MacKenzie and Abaunza 1998).

There is a long history for applying parasites to delineate stocks of marine fish (Table 1.1). An early study by Herrington et al. (1939) used the presence of the parasitic copepod (*Sphyrion lumpi*) to separate two stocks of redfish (*Sebastes marinus*) harvested off the Atlantic coast of the United States. Prevalence of the parasitic copepod in redfish from the coast of Maine and New Hampshire averaged 11%

compared to Browns Bank and eastward to Sable Island where 0% of the redfish were infected. Since that time, parasites (either single or multiple species) have been used to identify stocks of marine fish worldwide (Table 1.1; MacKenzie and Abaunza 1998; Mattiucci 2006).

The following characteristics are important in choosing a parasite species as a biological tag: 1) infection should not result in selective mortality; 2) the parasite is easy to recover and identify; 3) there is variation in parasite abundance between geographic locations; 4) individual parasites remain in or on a host for several months to years; and 5) the parasite is host specific (MacKenzie and Abaunza 1998; Bush et al. 2001; MacKenzie 2002). Host specificity may not be as stringent a requirement for marine parasite species compared to the other characteristics, since parasite generalists are more common among marine hosts (Marcogliese 2002). Generalist parasites have the ability to infect several different host species, are often transmitted by several different pathways in marine food webs, and are frequently observed when host distribution is patchy (Marcogliese 1995, 2002). For example, approximately 280 host species (both invertebrates and vertebrates) are known to exist in the northern latitudes for the nematode *Anisakis simplex sensu stricto* (Cross et al. 2007). Cross et al. (2007) advocated that *A. simplex s.s.* is a valuable biological tag since it had temporal stability throughout the year, and thus could be maintained within a specific geographical area over time. Although several related host species or taxa increase the complexity of a parasite life cycle, this may in fact increase the probability for transmission when large

geographic scales reduce encounter rates between first and final host (Marcogliese 1995, Nadler 1995).

While one parasite species can be used to discriminate between different host populations, in reality individual hosts are often infected with more than one parasite species at any given time. By examining parasite communities host populations could be further subdivided over several spatial and time scales (Nadler 1995; Marcogliese et al. 2003; Mattiucci et al. 2004; McClelland et al. 2005; Criscione et al. 2006). For instance, fish stocks of the western group of deep-water redfish (*Sebastes marinus*) within the Gulf of St. Lawrence and Newfoundland initially identified using fish molecular markers (Roques et al. 2002) were further subdivided into four smaller groups using three parasite species (Marcogliese et al. 2003). Also, the fish groups defined by the parasite community analysis supported the current stock boundaries previously defined by fisheries managers (Marcogliese et al. 2003).

Parasite identification and discrimination: morphological and molecular characteristics

The ease of correctly identifying parasites taxonomically is an important consideration when choosing parasites as biological tags. The ability to distinguish between parasite species has traditionally relied upon taxonomic keys based upon the morphology of adult worms. These keys compare the variation in characteristics that define a particular family, genus or species, but are difficult to use for identifying larval stages of parasites. However, with the development of molecular markers for parasites, it is

now possible to identify all developmental stages of complex life-cycles to species (Criscione et al. 2005, Locke et al. 2010). Also, individual parasite species can be morphologically similar but genetically different (Criscione et al. 2005; Mattiucci 2006). Cryptic species arise when genetic variation between closely related species is less conserved than morphological characteristics (Jousson et al. 2000). With the increasing availability of parasite DNA sequences, cryptic species have been discovered among several parasite taxa (Criscione and Blouin 2004; Vilas et al. 2005), and have altered our understanding of the geographic ranges of some platyhelminthes (Jousson et al. 2000). Mattiucci et al. (2004) used allozyme differences to demonstrate that seven genetically distinct species existed among morphologically similar individuals of *Anisakis* species collected from European hake (*Merluccius merluccius*). *Anisakis simplex,* originally considered a cosmopolitan species found globally, is now recognized as a mixture of nine genetically distinct species distributed across a large geographical area (Mattiucci et al. 2009). Vilas et al. (2005) suggested that parasite populations should be examined for cryptic species when parasite individuals are found in several habitats or host species.

The relative mutation rates of homologous loci between parasite DNA and that of their hosts suggests that some parasites have a faster generation time than hosts (Whiteman and Parker 2005), thus accumulating genetic variation faster and enabling host population structure to be inferred from parasite population structure. For example, Hafner et al. (1994) concluded that the mutation rates of chewing lice (*Geomydoecus*

spp. and *Thomomydoecus* spp.) were approximately three times faster than their pocket gopher hosts (*Orthogeomys* spp., *Zygogeomys* sp., *Pappogeomys* sp., *Cratogeomys* sp., *Geomys* sp., and *Thomomy* sp.). This implies that divergence times separating parasite populations would be shorter than divergence times separating host populations. Criscione et al. (2006) indicated that parasite genotypes of the trematode *Plagioporus shawi* were more accurate in assigning an individual steelhead trout (*O. mykiss*) to their natal stream than genotypic data of the fish. F_{st} values were ten times greater for *P. shawi* than for steelhead, suggesting less gene flow between parasite populations than steelhead populations. Additional research findings by Marcogliese et al. (2003) and Criscione and Blouin (2006) indicated that parasite genotypes supported fish management boundaries defined by other methods.

Similar to fish genetic data, results using parasite genetic data may differ depending on the type of molecular marker used and lead to different interpretations of stock structure. For example, Vilas et al. (2005) compared mitochondrial and ribosomal DNA sequences from the freshwater trematode *P. shawi*. They determined that NADH dehydrogenase1 (ND1) and cytochrome oxidase subunit 1 (CO1) mtDNA markers accumulated base pair substitutions at a faster rate than internal transcribe spacer ribosomal markers (ITS1 and ITS2). Cross et al. (2007) also reported high substitutions rates in mtDNA CO1 sequences from *A. simplex s.s.* infecting Atlantic herring (*C. harengus*). If the molecular marker chosen has a slow mutation rate then

parasite subpopulations that have recently been separated may still be noted as undifferentiated and mistakenly considered one population.

The gene flow between parasite populations is influenced by the mobility of host species (Prugnolle et al. 2005; Criscione et al. 2006). For instance, Criscione and Blouin (2004) suggested that parasites with allogenic life cycles (definitive host a mammal or bird) have more gene flow between distant parasite populations than parasites with autogenic life cycles (definitive host a fish) since geographic barriers are less likely to influence the movement of birds or mammals compared to fish. Thus more than one type of molecular marker and parasite should be examined from a given host under study to improve the understanding the parasite genetic structure and to determine which parasites are best suited as biological tags.

Recommendations on parasite molecular markers

In addition to the ecological characteristics important to identify candidate parasite biological tags, the characteristics and approaches necessary for parasite molecular markers include: 1) ease of recovery and identification (Aiken et al. 2007); 2) use of more than one genetic marker to verify that a parasite species is cryptic (Vilas et al. 2005); 3) assess mutation rates when choosing a specific genetic marker type (Vilas et al. 2005); 4) temporal and geographic stability in a parasite population to enable long-term monitoring of parasite populations (Cross et al. 2007); and 5) use of parasite phylogeography to infer host population structure (Mattiucci and Nascetti 2008).

Integrating techniques for fish stock identification

The techniques chosen to identify subpopulations or stocks of fish species, and the boundaries separating these stocks are critical for understanding and managing fish species. For instance, if small and locally important stocks of Pacific sardine exist in California and the Pacific Northwest (Oregon, Washington, and British Columbia, Canada), their identity could be masked by managing fish over this large geographical scale as one fishery. Recently the HOMSIR (Horse Mackerel Stock Identification Research) project assessed the stock structure of horse mackerel (*Trachurus trachurus*) in the northeast Atlantic and Mediterranean Sea in 2000 and 2001 (Abaunza et al. 2008a). A combination of fish genetics, morphometrics (e.g., body shape), life history traits (e.g., distribution) and parasite community and genetics data (Abaunza et al. 2008a, b; MacKenzie et al. 2008; Mattiucci et al. 2008) were examined from the same individual fish. The parasite community and fish morphometrics provided the strongest evidence for the presence of separate stocks within the Atlantic Ocean (Western and Southern) and the North Sea. Additionally, the parasite allozyme data further separated fish from the Mediterranean Sea into three different stocks, and provided evidence that horse mackerel may move between the Southern Atlantic and the Western Mediterranean (Abaunza et al. 2008b; Mattiucci et al. 2008). Thus, the integration of multiple techniques can enable the discovery of distinct fish stocks.

Conclusion

Fishing pressure can affect subpopulations differently, which can result in a loss in genetic diversity, and the over-harvest of small subpopulations compared to larger subpopulations (Stephensen 1999). High fishing pressure along with unfavorable ocean conditions can result in a dramatic decline of species diversity and fish biomass (Beamish et al. 2004; Alder et al. 2008), and fish may be less resilient at the edges of their distribution (Beverton 1998). However, it remains uncertain how the synergistic effects between these factors threaten the population genetic structure of exploited fish species (Alder et al. 2008; Casini et al. 2009), and the sustainability of marine ecosystems (Harley and Rogers-Bennett 2004; Crowder et al. 2008). When it is difficult to resolve the population structure of marine fish (such as the current situation with Pacific sardine), an integrated approach that includes both fish and parasite data could clarify ecological and evolutionary events that structure fish populations. The HOMSIR project which employed this integrated approach should serve as an important model for future stock structure studies, especially for pelagic fish species whose populations fluctuate in response to changing environmental conditions.

Acknowledgements

We would like to thank R. Emmett, M. Blouin, V. Lesser, J. Bolte, E. Casillas, and J. Scheurer for providing comments on earlier versions of this manuscript.

Table

Table 1.1. Examples of macroparasite biological tag studies that have been conducted for a variety of marine and anadromous fish species since 1939. References for this table are indicated by an asterisk (*) in the Literature Cited.

Year	Fish Species	Parasite Species	Study Location	Author(s)
1939	Redfish (*Sebastes marinus*)	Copepoda (*Sphyrion lumpi*)	western Atlantic Ocean (United States)	W.C. Herrington et al.
1963	Sockeye Salmon (*Oncorhynchus nerka*)	Cestoda (*Triaenophorus crassus*) & Nematoda (*Dacnitis truttae*)	north Pacific Ocean	L. Margolis
1984	Steelhead (*Salmo gairdneri*)	Trematoda (*Plagioporus shawi* & *Nanophyetus salmonicola*)	north Pacific Ocean	L. Margolis
1985	Atlantic Herring (*Clupea harengus*)	Larval Nematoda (*Anisakis simplex* & *Hysterothylacium aduncum*), & Trematoda Metacercariae (*Cryptocotyle lingua*)	northwestern Atlantic Ocean	S.E. McGladdery & M.D.B. Burt
1985	Herring (*Clupea harengus*)	metacercariae Trematoda (*Cercaria pythionike* & *C. doricha*), & plerocercus Cestoda (*Lacistorhynchus* sp.)	North Sea, north & west of Scotland	K. MacKenzie
1985	Skipjack Tuna (*Katsuwonus pelamis*)	Didymozoid Trematoda (*Didymocylindrus filiformis*, *D. simplex*, *Didymoproblema fusiforme*, *Lobatozoum multisacculatum*, *Coeliodidmyocystis* sp., *Oesophagocystis dissimilis*, *Kollikeria*/*Didymocystis* spp., *Didymocystoides intestinomuscularis*, & *Lagenocystis*/*Univitellannulocystis* spp.)	New Zealand	R.J.G. Lester et al.
1988	Orange Roughy (*Hoplostethus atlanticus*)	Larval Cestoda (*Callitetrarhynchus* sp. & *Hepatoxylon trichiuri*), Larval Nematoda (unidentified Spirurid, *Anisakis* sp. type 1, 2, & 3, *Terranova* sp.)	Australia, New Zealand & Tasmania	R.J.G. Lester et al.

Table 1.1 Continued.

1992	Yellowtail Rockfish (*Sebastes flavidus)*	Monogenea (*Microcotyle sebastis*)	Pacific Coast off North America	R.D. Stanley et al.
1993	Greenland Halibut (*Reinhardtius hippoglossoides*)	Acanthocephala (*Corynosoma strumosum*), Larval Nematoda (*Anisakis simplex , Psuedoterranova decipiens* , Contracaecienea), Trematoda Metacercariae (*Otodistomum* sp.)	Canadian NW Atlantic	J.R. Arthur & E. Albert
1993	Hake (*Merluccius capensis* & *M. paradoxus*)	Monogenea (*Anthocotyle merluccii*), Larval Cestoda (*Clestobothrium crassic* iceps*, Brachiella merluccii, Scolex pleuronectis, Leptotheca spec., Anisakis spec. 1)*	Namibia Coast, southwest Africa	L.W. Reimer
1995	Gemfish (*Rexea solandri*)	Nematoda (*Anisakis* sp. type 1), plerocercoid Cestoda (*Hepatoxylon trichiuri* & *Nybelinia* sp.)	southern Australia	K.B. Sewell & R.J.G. Lester
1995	Hake (*Merluccius australis* & *M. hubbsi*)	plerocercoid Cestoda (*Grillotia* sp. & *Hepatoxylon trichiuri*), Copepoda (*Trifur tortuosus*) & Digenea (*Elytrophalloides oatesi*)	southern Chile, Falkland Islands & Argentine shelf	K. MacKenzie & M. Longshaw
1995	Sailfish (*Istiophorus platypterus*)	Cestoda (*Callitetrarhynchus gracilis* & *Otobothrium dipsacum*), Copepoda (*Pennella instructa*) & Trematoda (*Cardicola grandis*)	east Coast Australia	P. Speare
1997	Sablefish (*Anoplopoma fimbria*)	Trematoda (*Derogenes varicus* , *Genolinea laticauda* , & *Lecithaster gibbosus*)	seamounts off Vancouver Island, British Columbia	D.J. Whitaker & G.A. McFarlane

Table 1.1 Continued.

1997	Atlantic Cod (*Gadus morhua*)	Copepoda (*Lemaeoceru brunchialis*), Trematoda (*Hemiurus levinseni*)	northern Norway	G. Larsen et al.
2002	Chilean Hake (*Merluccius gayi gayi*)	Larval Cestoda (*Clestobothrium crassiceps* & *Hepatoxylon trichiuri*), Monogenea (*Anthocotyle merlucci*) & Copepoda (*Neobrachiella insidosa f. pacifica*)	Chile & Peru	M.E. Oliva & I. Ballón
2003	Argentine Anchovy (*Engraulis anchoita*)	Monogenea (*Pseudanthocotyloides heterocotyle*), Digenea (Cardiocephaloides sp., *Lecithochirium microstomum* , & *Parahemiurus merus*), Larval Cestoda (*Scolex polymorphus*), Acanthocephala Cystacanth (*Corynosoma australe)* , Larval Nematoda (*Anisakis simplex* , *Contracaecum* sp.& *Hysterothylacium aduncum)*	south-west Atlantic (South America)	J.T. Timi
2003	Deepwater Redfish (*Sebastes mentella*)	Copepoda (Sphyrion lumpi), Nematoda (Anisakis simplex & Hysterothylacium aduncum)	northwest Atlantic coast (Canada)	D.J. Marcogliese et al.
2004	European Hake (*Merluccius merluccius*)	Larval Nematoda (*Anisakis* spp.)	east Atlantic Ocean & Mediterranean Sea	S. Mattiucci et al.
2006	Baltic Herring (*Clupea harengus*)	Larval Nematoda (*Anisakis simplex*)	Baltic Sea	M. Podolska et al.

Table 1.1 Continued.

2007	Anchoveta (*Engraulis ringens*)	Monogenea (*Pseudoanthocotyloides hetercotyle*), Copepoda (*Caligus* sp.), Isopoda (*Livoneca* sp.), adult Cestoda (*Bothriocephalus* sp.), larval Nematoda (*Anisakis* sp. & unidentified anisakid)	Chile & Peru	I.M. Valdivia et al.
2007	Atlantic Croaker (*Micropogonias undulatus*)	Monogenea (*Diplectanotrema* sp., *Encotyllabe* sp., & *Macrovalvitrematoides micropogoni*), Digenea (*Diplomonorchirs leistomi*, *Opecoeloides fimbriatus*, &	western north Atlantic Ocean	T.G. Baker et al.
2007	Atlantic Horse Mackerel (*Trachurus trachurus*)	Trematoda (*Derogenes varicus*, *Ectenurus lepidus*, & *Tergestia latticolis*), Monogenea (*Gastrocotyle trachuri*, *Heteraxinoides atlanticus*, *Pseudaxine trachuri*)	northeast Atlantic	N. Campbell et al.
unpublished	Pacific Sardines (*Sardinops sagax*)	Trematoda (*Parahemiurus* sp.)	Ecuador & Peru	K. MacKenzie et al.

References

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, E. Gallo, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Ramos, A. Ruggi, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008a. Considerations on sampling strategies for an holistic approach to stock identification: The example of the HOMSIR project. Fisheries Research 89:104-113.

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Rarnos, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008b. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fisheries Research **89**:196-209.

Abe, N. 2008. Application of the PCR-sequence-specific primers for the discrimination among larval *Anisakis simplex* complex. Parasitology Research **102**:1073-1075.

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Aiken, H. M., N. J. Bott, I. Mladineo, F. E. Montero, B. F. Nowak, and C. J. Hayward. 2007. Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tunas (*Thunnus* spp.). Fish and Fisheries **8**:167-180.

Alder, J., B. Campbell, V. Karpouzi, K. Kaschner, and D. Pauly. 2008. Forage Fish: From Ecosystems to Markets. Annual Review of Environment and Resources **33**:153-166.

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Australia.

Armannsson, H., S. T. Jonsson, J. D. Neilson, and G. Marteinsdottir. 2007. Distribution and migration of saithe (*Pollachius virens*) around Iceland inferred from mark-recapture studies. ICES Journal of Marine Science **64**:1006-1016.

*Arthur, J. R., and E. Albert. 1994. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **50**:2175-2181.

Atarhouch, T., L. Rüber, E. G. Gonzalez, E. M. Albert, M. Rami, A. Dakkak, and R. Zardoya. 2006. Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). Molecular Phylogenetics and Evolution **39**:373-383.

Avise, J. C. 1998. Conservation genetics in the marine realm. Journal of Heredity **89**:377-382.

*Baker, T. G., S. Morand, C. A. Wenner, W. A. Roumillat, and I. de Buron. 2007. Stock identification of the sciaenid fish *Micropogonias undulatus* in the western North Atlantic Ocean using parasites as biological tags. Journal of Helminthology **81**:155-167.

Baranov, F. I. 1918. On the question of the biological basis of fisheries. Nauchn. Issled. Iktiol. Inst. Izv. **1**:81-128.

Baumgartner, T. R., A. Soutar, and V. Ferreirabartrina. 1992. Reconstruction of the history of Pacific sardine and northern anchovy populations over the past two millennia from sediments of the Santa Barbara Basin, California. California Cooperative Oceanic Fisheries Investigations Reports **33**:24-40.

Beacham, T. D., J. F. Schweigert, C. MacConnachie, K. D. Le, and L. Flostrand. 2008. Use of Microsatellites to Determine Population Structure and Migration of Pacific Herring in British Columbia and Adjacent Regions. Transactions of the American Fisheries Society **137**:1795-1811.

Beamish, R. J., A. J. Benson, R. M. Sweeting, and C. M. Neville. 2004. Regimes and the history of the major fisheries off Canada's west coast. Progress in Oceanography **60**:355-385.

Beamish, R. J., G. A. McFarlane, and J. R. King. 2000. Fisheries climatology: understanding decadal scale processes that naturally regulate British Columbia fish populations. Pages 94-139 *in* P. J. Harrison and T. R. Parsons, editors. Fisheries Oceanography. An Integrative Approach to Fisheries Ecology and Management. Blackwell Science, Maldan, MA.

Bentley, P. J., R. L. Emmett, N. C. H. Lo, and H. G. Moser. 1996. Egg production of Pacific sardine (*Sardinops sagax*) off Oregon in 1994. California Cooperative Oceanic Fisheries Investigations Reports **37**:193-200.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Beverton, R. 1998. Fish, fact and fantasy: A long view. Reviews in Fish Biology and Fisheries **8**:229-249.

Beverton, R. 2002. Reflections on 100 Years of Fisheries Research. In: The Raymond JH Beverton Lectures at Woods Hole, Massachusetts. Three lectures on fisheries science given May 2-3, 1994. Pages 107-158 *in* NOAA Tech. Memo. NMFS-F/SPO-54. U.S. Department of Commerce.

Beverton, R., and S. J. Holt. 1957. On the Dynamics of Exploited Fish Populations. Ministry of Agriculture, Fisheries and Food.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pages 271-277 *in* K. R. Kerry and G. Hempel, editors. Evolution of Genes and Proteins. Sinauer, Sunderland, MA.

Buonaccorsi, V. P., J. R. McDowell, and J. E. Graves. 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Molecular Ecology **10**:1179-1196.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Cadrin, S. X., K. D. Friedland, and J. R. Waldman. 2005. Stock identification methods: an overview. Pages 3-6 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Applications in Fishery Science. Elsevier Academic Press, San Francisco.

*Campbell, N., K. MacKenzie, A. F. Zuur, E. N. Ieno, and G. M. Smith. 2007. Fish stock identification through neural network analysis of parasite fauna. Pages 449-462 *in* A. F. Zuur, E. N. Ieno, and G. M. Smith, editors. Analyzing Ecological Data. Springer, New York.

Casini, M., J. Hjelm, J. C. Molinero, J. Lovgren, M. Cardinale, V. Bartolino, A. Belgrano, and G. Kornilovs. 2009. Trophic cascades promote threshold-like shifts in pelagic marine ecosystems. Proceedings of the National Academy of Sciences of the United States of America **106**:197-202.

Chlaida, M., S. Kifani, P. Lenfant, and L. Ouragh. 2006. First approach for the identification of sardine populations *Sardina pilchardus* (Walbaum 1792) in the Moroccan Atlantic by allozymes. Marine Biology **149**:169-175.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Clark, F. N. 1935. A summary of the life-history of the California sardine and its influence on the fishery. California Fish and Game **21**:1-9.

Clark, F. N. 1947. Analysis of populations of the Pacific sardine on the basis of vertebral counts. California Department of Fish and Game Fisheries Bulletin **65**. 26pp.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Cope, J. M. 2004. Population genetics and phylogeography of the blue rockfish (*Sebastes mystinus*) from Washington to California. Canadian Journal of Fisheries and Aquatic Sciences **61**:332-342.

Criscione, C. D., and M. S. Blouin. 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution **58**:198-202.

Criscione, C. D., and M. S. Blouin. 2006. Minimal selfing, few clones, and no among-host genetic structure in a hermaphroditic parasite with asexual larval propagation. Evolution **60**: 553-562

Criscione, C. D., B. Cooper, and M. S. Blouin. 2006. Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. Ecology 87:823-828.

Criscione, C. D., R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Molecular Ecology **14**:2247-2257.

Cross, M. A., C. Collins, N. Campbell, P. C. Watts, J. C. Chubb, C. O. Cunningham, E. M. C. Hatfield, and K. MacKenzie. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda: Anisakidae): implications for fisheries management. Marine Biology **151**:695-702.

Crowder, L. B., E. L. Hazen, N. Avissar, R. Bjorkland, C. Latanich, and M. B. Ogburn. 2008. The impacts of fisheries on marine ecosystems and the transition to ecosystem-based management. Annual Review of Ecology Evolution and Systematics **39**:259-278.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

DeWoody, J. A., and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology **56**:461-473.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Felin, F. E. 1954. Population heterogeneity in the Pacific pilchard. Fisheries Bulletin **54**:201-225.

Félix-Uraga, R., V.M. Gómez-Muñoz, C. Quiñónez-Velázquez, F.N. Melo-Barrera, Hill, K.T., and W. García -Franco. 2005. Pacific sardine (*Sardinops sagax*) stock discrimination off the west coast of Baja California and southern California using otolith morphometry. California Cooperative Oceanic Fisheries Investigations Reports **46**:113-121.

Félix-Uraga, R., C. Quiñónez-Velázquea, K.T. Hill, V.M. Gómez-Muñoz, F.N. Melo-Barrera, and W. García-Franco. 2004. On the existence of Pacific sardine groups off the west coast of Baja California and southern California. California Cooperative Oceanic Fisheries Investigations Reports 25:146-151.

Fitch, J. E. 1969. Fossil records of certain schooling fishes of the California Current System. California Marine Research Communication. California Cooperative Oceanic Fisheries Investigations Reports **13**:71-80.

Gonzalez, E. G., and R. Zardoya. 2007. Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evolutionary Biology **7**.

Graham, M. 1935. Modern theory of exploiting a fishery, and application to North Sea trawling. Journal du Conseil International Exploration du Mer. **10**:264-274.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity **89**:415-426.

Grant, W. S., and F. M. Utter. 1984. Biochemical population genetics of Pacific herring (*Clupea pallasi*). Canadian Journal of Fisheries and Aquatic Sciences **41**:856-864.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Hafner, M. S., P. D. Sudman, F. X. Villablanca, T. A. Spradling, J. W. Demastes, and S. A. Nadler. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science **265**:1087-1090.

Hansen, L. P., and J. A. Jacobsen. 2003. Origin and migration of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in oceanic areas north of the Faroe Islands. ICES Journal of Marine Science **60**:110-119.

Hansen, M. M., E. Kenchington, and E. E. Nielsen. 2001. Assigning individual fish to populations using microsatellite DNA markers. Fish and Fisheries **2**:93-112.

Hargreaves, N. B., D. M. Ware, and G. A. McFarlane. 1994. Return of Pacific sardine (*Sardinops sagax*) to the British Columbia coast in 1992. Canadian Journal of Fisheries and Aquatic Science **51**:460-463.

Harley, C. D. G., and L. Rogers-Bennett. 2004. The potential synergistic effects of climate change and fishing pressure on exploited invertebrates on rocky intertidal shores. California Cooperative Oceanic Fisheries Investigations Reports **45**:98-110.

Harris, H. 1966. Enzyme polymorphism in man. Proceedings of the Royal Society Series B **164**:298-310.

Harrt, A. C. 1963. Problems in tagging salmon at sea. ICNAF Special Publication **4**:144-155.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science **39**:550-564.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Hedrick, P. W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution **53**:313-318.

*Herrington, W. C., H. M. Bearsem, and F. E. Firth. 1939. Observations on the life history, occurrence and distribution of the redfish parasite *Sphyrion lumpi*. US Bureau of Fisheries Special Report **5**:1-18.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Hewitt, G. M. 2004. The structure of biodiversity - insights from molecular phylogeography. Frontiers in Zoology **1**:4.

Hill, K. T., N. C. H. Lo, B. J. Macewicz, P. R. Cronel, and R. Félix-Uraga. 2009. Assessment of the Pacific sardine resource in 2009 for U.S. management in 2010. NOAA-TM-NMFS-SWFSC-452:221.

Hoenig, J. M., R. J. Latour, and J. E. Olney. 2008. Estimating stock composition of anadromous fishes from mark-recovery data: Possible application to American shad. North American Journal of Fisheries Management **28**:507-515.

Hubby, J. L., and R. C. Lewontin. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics **54**:577-594.

Jacobsen, J. A., and L. P. Hansen. 2005. Internal and external tags. Pages 415-433 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in Fishery Science. Elsevier Academic Press, San Francisco.

Jakobsson, J. 1970. On fish tags and tagging. Oceanography and Marine Biology **8**:457-499.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Janssen, J. F., and J. F. Alpen. 1945. The effect of internal tags upon sardines. Fisheries Bulletin **61**:43-62.

Jones, C. M. 2006. Estuarine and diadromous fish metapopulations. Pages 119-154 *in* J. P. Kritzer and P. F. Sale, editors. Marine Metapopulations. Elsevier Academic Press, San Francisco.

Jousson, O., P. Bartoli, and J. Pawlowski. 2000. Cryptic speciation among intestinal parasites (Trematoda : Digenea) infecting sympatric host fishes (Sparidae). Journal of Evolutionary Biology **13**:778-785.

King, R. C., and W. D. Stansfield. 1985. A Dictionary of Genetics, Third edition. Oxford University Press, New York.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Koljonen, M.-L., and R. Wilmot. 2005. Genetic analysis: allozymes. Pages 295-309 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in Fisheries Science. Elsevier Academic Press, San Francisco.

*Larsen, G., W. Hemmingsen, K. MacKenzie, and D. A. Lysne. 1997. A population study of cod, *Gadus morhua* L., in northern Norway using otolith structure and parasite tags. Fisheries Research **32**:13-20.

Laurent, V., B. Caneco, A. Magoulas, and S. Planes. 2007. Isolation by distance and selection effects on genetic structure of sardines *Sardina pilchardus* Walbaum. Journal of Fish Biology **71**:1-17.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Lessios, H. A., B. D. Kessing, and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proceedings of the Royal Society of London Series B-Biological Sciences **265**:583-588.

Lester, R. J. G. 1990. Reappraisal of the use of parasites for fish stock identification. Australian Journal of Marine and Freshwater Research **41**:855-864.

*Lester, R. J. G., A. Barnes, and G. Habib. 1985. Parasites of skipjack tuna, *Katsuwonus pelamis*: fishery implications. Fishery Bulletin **83**:343-356.

*Lester, R. J. G., K. B. Sewell, A. Barnes, and K. Evans. 1988. Stock discrimination of orange roughy *Hoplostethus atlanticus*, by parasite analysis. Marine Biology **99**:137-143.

Lewontin, R. C., and J. L. Hubby. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**:595-609.

Locke, S. A., J. D. McLaughlin, S. Dayanandan, and D. J. Marcogliese. 2010. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. International Journal of Parasitology **40**:333-343.

Longhurst, A. 2006. The sustainability myth. Fisheries Research **81**:107-112.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

*Mackenzie, K. 1985. The use of parasites as biological tags in population studies of herring (*Clupea harengus* L.) in the North Sea and to the north and west of Scotland. Journal du Conseil International Exploration du Mer **42**:33-64.

*MacKenzie, K. 1989. Parasites as biological tags for pacific sardines, *Sardinops sagax* (Jenyns) in coastal waters of Ecuador and Peru. DAFS Marine Laboratory, Aberdeen, Scotland.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**:S153-S163.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research **38**:45-56.

MacKenzie, K., N. Campbell, S. Mattiucci, P. Ramos, A. L. Pinto, and P. Abaunza. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fisheries Research **89**:136-145.

*Mackenzie, K., and M. Longshaw. 1995. Parasites of the hakes *Merluccius australis* and *M. hubbsi* in the waters around the Falkland Islands, southern Chile, and Argentina, with an assessment of their potential value as biological tags. Canadian Journal of Fisheries and Aquatic Sciences **52**:213-224.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2004. Assignment methods: matching biological questions with appropriate techniques. TRENDS in Ecology and Evolution **20**:1-7.

Mann, K. 2000. Commentary: environmental effects on fish stocks. Pages 140-145 *in* P. J. Harrison and T. R. Parsons, editors. Fisheries Oceanography. An Integrative Approach to Fisheries Ecology and Management. Blackwell Science, Maldan, MA.

Mantua, N. J., S. R. Hare, Y. Zhang, J. M. Wallace, and R. C. Francis. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bulletin of the American Meteorological Society **78**:1069-1079.

Marcogliese, D. J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. Reviews of Fish Biology and Fisheries 5:336-371.

Marcogliese, D. J. 2002. Food webs and the transmission of parasites to marine fish. Parasitology 124:S83-S99.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

*Marcogliese, D. J., E. Albert, P. Gagnon, and J.-M. Sevigny. 2003. Use of parasites in stock identification of the deepwater redfish (*Sebastes mentella*) in the Northwest Atlantic. Fisheries Bulletin **101**:183-188.

*Margolis, L. 1963. Parasites as indicators of the geographical origins of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the North Pacific Ocean and adjacent seas. International North Pacific Fisheries Commission Bulletin. **11**:101-156.

*Margolis, L. 1984. Preliminary report on identification of continent of origin of ocean-caught steelhead trout, *Salmo gairdneri*, using naturally occurring parasite "tags". unpublished report Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, BC.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

Marr, J. C. 1960. The causes of major variations in the catch of the Pacific sardine, *Sardinops caerulea* (Girard). Pages 667-791 *in* H. Rosa and G. I. Murphy, editors. Proceedings of the World Scientific Meeting on the Biology of Sardines and Related Species. Food and Agriculture Organization of the United Nations.

Mattiucci, S. 2006. Parasites as biological tags in population studies of demersal and pelagic fish species. Parassitologia **48**:23-25.

*Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Pages 47-148 *in* Advances in Parasitology.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Mattiucci, S., M. Paoletti, and S. C. Webb. 2009. *Anisakis nascettii* n. sp (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. Systematic Parasitology 74:199-217.

Mattson, M. T., J. R. Waldman, D. J. Dunning, and Q. E. Rose. 1990. Abrasion and protrusion of internal anchor tags in Hudson River striped bass. American Fisheries Society Symposium **7**:121-126.

McClelland, G., J. Melendy, J. Osborne, D. Reid, and S. Douglas. 2005. Use of parasite and genetic markers in delineating populations of winter flounder from the central and south-west Scotian Shelf and north-east Gulf of Maine. Journal of Fish Biology **66**:1082-1100.

McEvoy, A. F., and H. N. Scheiber. 1984. Scientists, entrepreneurs, and the policy process: a study of the post-1945 California sardine depletion. Journal of Economic History **44**:393-406.

McFarlane, G. A., R. S. Wydoski, and E. D. Prince. 1990. Historical review of the development of external tags and marks. American Fisheries Society Symposium **7**:9-29.

*McGladdery, S. E., and M. D. B. Burt. 1985. Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences **42**:1957-1968.

Morin, P. A., G. Luikart, R. K. Wayne, and S. N. P. Working Group. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution **19**:208-216.

*Moser, M. and J. Hsieh. 1992. Biological tags for stock discrimination in Pacific herring *Clupea harengus pallasi* in California. Journal of Parasitology **78**:1957-1968.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. Journal of Parasitology **81**:395-403.

Narum, S. R., M. Banks, T. D. Beacham, M. R. Bellinger, M. R. Campbell, J. Dekoning, A. Elz, C. M. Guthrie, C. Kozfkay, K. M. Miller, P. Moran, R. Phillips, L. W. Seeb, C. T. Smith, K. Warheit, S. F. Young, and J. C. Garza. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. Molecular Ecology **17**:3464-3477.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Neilson, J. D., W. T. Strobo, and P. Perley. 2006. Pollock (*Pollachius virens*) stock structure in the Canadian Maritimes inferred from mark-recapture studies. ICES Journal of Marine Science **63**:749-765.

Nielsen, E. E., J. Hemmer-Hansen, N. A. Poulsen, V. Loeschcke, T. Moen, T. Johansen, C. Mittelholzer, G. L. Taranger, R. Ogden, and G. R. Carvalho. 2009a. Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). BMC Evolutionary Biology **9**.

Nielsen, E. E., P. J. Wright, J. Hemmer-Hansen, N. A. Poulsen, L. M. Gibb, and D. Meldrup. 2009b. Microgeographical population structure of cod I in the North Sea and west of Scotland: the role of sampling loci and individuals. Marine Ecology-Progress Series **376**:213-225.

*Oliva, M. E., and I. Ballon. 2002. Metazoan parasites of the Chilean hake *Merluccius gayi gayi* as a tool for stock discrimination. Fisheries Research **56**:313-320.

Orbesen, E. S., J. P. Hoolihan, J. E. Serafy, D. Snodgrass, E. M. Peel, and E. D. Prince. 2008. Transboundary movement of Atlantic istiophoris billfishes among international and U.S. domestic management areas inferred from mark-recapture studies. Marine Fisheries Review **70**:14-23.

Pauly, D., V. Christensen, R. Froese, and M. L. Palomares. 2000. Fishing down aquatic food webs. American Scientist **88**:46-51.

Pereyra, R. T., E. Saillant, C. L. Pruett, C. E. Rexroad III, and A. Rocha-Olivares. 2004. Characterization of polymorphic microsatellites in the Pacific sardine *Sardinops sagax sagax* (Clupeidae). Molecular Ecology Notes **4**:739-741.

Peterson, W. T., and F. B. Schwing. 2003. A new climate regime in northeast pacific ecosystems. Geophysical Research Letters **30**:1-6.

*Podolska, M., J. Horbowy, and M. Wyszynski. 2006. Discrimination of Baltic herring populations with respect to *Anisakis simplex* larvae infection. Journal of Fish Biology **68**:1241-2156.

Prugnolle, F., A. Theron, J. P. Pointier, R. Jabbour-Zahab, P. Jarne, P. Durand, and T. de Meeûs. 2005. Dispersal in a parasitic worm and its two hosts: Consequence for local adaptation. Evolution **59**:296-303.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Radovich, J. 1962. Effects of sardine spawning stock size and environment on year-class production. California Fish and Game **48**:123-140.

Radovich, J. 1982. The collapse of the California sardine fishery. Pages 107-136 *in* M. H. Glantz and J. D. Thompson, editors. Resource management and environmental uncertainty. Lessons from coastal upwelling fisheries. John Wiley & Sons, New York.

Ramon, M. M., and J. A. Castro. 1997. Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. Heredity **78**:520-528.

*Reimer, L. W. 1993. Parasites of *Merluccius capensis*, and *M. paradoxus* from the coast of Namibia. Applied Parasitology **78**:520-528.

Ricker, W. E. 1954. Stock and recruitment. Journal of the Fisheries Research Board of Canada **11**:559-623.

Rohde, K. 1984. Ecology of marine parasites. Helgoländer Meeresunters **37**:5-33.

Roques, S., J.-M. Sevigny, and L. Bérnatchez. 2002. Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. Marine Biology **140**:297-307.

Rose, G. A. 1997. The trouble with fisheries science! Reviews in Fish Biology and Fisheries **7**:365-370.

Rounsefell, G. A., and E. H. Dahlgren. 1933. Tagging experiments on the Pacific herring, *Clupea pallasii*. Journal du Conseil **8**:371-384.

Ruzzante, D. E., S. Mariani, D. Bekkevold, C. Andre, H. Mosegaard, L. A. W. Clausen, T. G. Dahlgren, W. F. Hutchinson, E. M. C. Hatfield, E. Torstensen, J. Brigham, E. J. Simmonds, L. Laikre, L. C. Larsson, R. J. M. Stet, N. Ryman, and G. R. Carvalho. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proceedings of the Royal Society B-Biological Sciences **273**:1459-1464.

Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries **5**:417-446.

Sarmaşik, A., A. Çolakoğlu, and F., Altun. 2008. Mitochondrial DNA sequence and body size variations in Turkish sardine (*Sardina pilchardus*) stocks. Turkish Journal of Zoology **32**:229-237.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Schnute, J. T., and L. J. Richards. 2001. Use and abuse of fishery models. Canadian Journal of Fisheries and Aquatic Sciences **58**:10-17.

*Sewell, K. B., and R. J. G. Lester. 1995. Stock composition and movement of gemfish, *Rexea solandri*, as indicated by parasites. Canadian Journal of Fisheries and Aquatic Sciences **52**:225-232.

Sinclair, M., and P. Solemdal. 1988. The development of "population thinking" in fisheries biology between 1878 and 1930. Aquatic Living Resources **1**:189-213.

Smith, C. T., and L. W. Seeb. 2008. Number of alleles as a predictor of the relative assignment accuracy of short tandem repeat (STR) and single-nucleotide-polymorphism (SNP) baselines for chum salmon. Transactions of the American Fisheries Society **137**:751-762.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Smith, T. D. 1998. "Simultaneous and complementary advances": mid-century expectations of the interaction of fisheries science and management. Reviews in Fish Biology and Fisheries **8**:335-348.

Soutar, A., and J. D. Isaacs. 1969. History of fish populations inferred from fish scales in anaerobic sediments off California. California Marine Research Communication Cooperative Oceanic Fisheries Investigations Reports **13**:63-70.

*Speare, P. 1995. Parasites as biological tags for sailfish *Istiophorus platypterus*, from east coast Australian waters. Marine Ecology Progress Series **118**:43-50.

Sprague, L. M., and A. M. Vrooman. 1962. A racial analysis of the Pacific sardine *Sardinops caeruleus* based on studies of erythrocyte antigens. Annals of the New York Academy of Sciences **97**:131-138.

*Stanley, R. D., D. L. Lee, and D. J. Whitaker. 1992. Parasites of yellowtail rockfish, *Sebastes flavidus*, (Ayres, 1862) (Pisces: Teleostei), from the Pacific coast of North America as potential biological tags for stock identification. Canadian Journal of Zoology **70**:1086-1096.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Stephenson, R. L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fisheries Research **43**:247-249.

Stepien, C. A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. American Fisheries Society Symposium **17**:263-287.

Teel, D. J., D. M. Van Doornik, D. R. Kuligowski, and W. S. Grant. 2003. Genetic analysis of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington reveals few Columbia River wild fish. Fishery Bulletin **101**:640-652.

Thomas, F., O. Verneau, T. de Meeûs, and F. Renaud. 1996. Parasites as host evolutionary prints: Insights into host evolution from parasitological data. International Journal of Parasitology **26**:1137-1137.

*Timi, J. T. 2003. Parasites of Argentine anchovy in the south-west Atlantic: latitudinal patterns and their use for discrimination of host populations. Journal of Fish Biology **63**:90-107.

*Valdivia, I. M., R. A. Chavez, and M. E. Oliva. 2007. Metazoan parasites of *Engraulis ringens* as tools for stock discrimination along the Chilean coast. Journal of Fish Biology **70**:1504-1511.

Vignal, A., D. Milan, M. san Cristobal, and A. Eggen. 2001. A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution **34**:275-305.

Vilas, R., C. D. Criscione, and M. S. Blouin. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology **131**:839-846.

von Bertalanffy, L. 1938. A quantitative theory of organic growth**.** Human Biology **10**:181-213.

Waldman, J. R. 2005. Definition of stocks: an evolving concept. Pages 7-16 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods. Applications in fisheries science, San Francisco.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity **89**:438-450.

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

Waples, R. S., A. E. Punt, and J. M. Cope. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish and Fisheries **9**:423-449.

Welch, D. W., G. W. Boehlert, and G. P. Ward. 2003. POST-the Pacific Ocean salmon tracking project. Oceanologica Acta **25**:243-253.

*Whitaker, D. J., and G. A. McFarlane. 1997. Identification of Sablefish, *Anoplopoma fimbria* (Pallas, 1811), stocks from seamounts off the Canadian Pacific coast using parasites as biological tags. NMFS Technical Report 130, NOAA.

Whiteman, N. K., and P. G. Parker. 2005. Using parasites to infer host population history: a new rationale for parasite conservation. Animal Conservation **8**:175-181.

Wisner, R. L. 1960. Evidence of a northward movement of stocks of the Pacific sardine based on the number of vertebrae. California Cooperative Oceanic Fisheries Investigations Reports **8**:75-82.

Wolf, P. 1992. Recovery of the Pacific sardine and the California sardine fishery. California Cooperative Oceanic Fisheries Investigations Reports **33**:76-86.

Electronic Sources:

FAO. 2008. The State of World Fisheries and Aquaculture (updated 2008). http://fao.org. Accessed: 29 January 2010

Johnson, J.K. 2004. Regional Overview of Coded Wire Tagging of Anadromous Salmon and Steelhead in Northwest America (updated 2004). http://rmpc.org. Accessed: 20 September 2009

WGBH Educational Foundation. 2004. World in the Balance (updated 2004)

Footnote: Reference indicated by an asterisk (*) are noted in Table 1.1.

TWO MIGRATION PATTERNS IDENTIFIED FOR NORTHEASTERN PACIFIC SARDINE (*SARDINOPS* SAGAX) USING TROPHICALLY-TRANSMITTED PARASITES AS BIOLOGICAL TAGS

Rebecca E. Baldwin, Michael A. Banks, Kym C. Jacobson

Fisheries Research

Springer

233 Spring Street

New York, NY 10013

(In Prep)

Abstract

The Pacific sardine (*Sardinops sagax*) fishery crashed in the 1940s off the coasts of British Columbia (Canada), Oregon and Washington (USA). The sardine fishery resumed in 1999, followed by an interest in reassessing the management of sardines in the California Current off of western North America. Identifying Pacific sardine populations using trophically-transmitted parasites is a new approach to an ongoing multidisciplinary study between fisheries scientists from Canada, the United States and Mexico to assess migration patterns, and potential stock structure. A total of 1389 sardines were collected from 2005 through 2008 between 50^{o} to 32^{o} North latitude, and 120^{o} to 125^{o} West longitude. Two trematodes, *Lecithaster gibbosus* and *Myosaccium ecaude*, have emerged as potential biological tags that identify two migration patterns of Pacific sardines. *Lecithaster gibbosus* were recovered in non-migrant ($\leq$ 200 mm standard length) and migrant (> 200 mm standard length) sardines primarily to the north off Vancouver Island, British Columbia, Canada. *Myosaccium ecaude* were recovered throughout the study area, but were most common off central and southern California in sardines categorized as non-migratory. The distributional pattern of *M. ecaude* supported artificial tagging studies from 60 years ago that described a coastwide migration pattern from the spawning grounds in the south off southern California to the feeding grounds off the Pacific Northwest and Canada. Although we observed a secondary migration pattern with the distributional pattern of *L. gibbosus* from Vancouver Island, British Columbia to only Northern California, reproductively isolated stocks of sardines could not be unequivocally determined

using parasite community data. We thus suggest a conservative approach in management that recognizes two possible migration patterns of Pacific sardines.

Introduction

The Pacific sardine (*Sardinops sagax*) is an ecologically important pelagic fish species, transferring energy from lower trophic prey species (algae and zooplankton) to upper trophic predators (fish, marine mammals and birds; Cury et al. 2000; Field et al. 2006). Pacific sardines are also economically important, harvested for bait, used in aquaculture and for human consumption (see review by Herrick et al. 2009; Hill et al. 2009). In the 1930s and 40s, the sardine fishery was considered the largest fishery in the Western Hemisphere (Marr 1960; Wolf 1992), with more than 400,000 metric tons harvested each year off of California alone between 1934 and 1944 (Radovich 1982). In conjunction with heavy fishing pressure and cooling ocean temperatures, landings began to decline in the 1940s throughout the California Current System (CCS), with the fishery officially closing off the Pacific Northwest (PNW) by 1947, and off Central California by 1967 (Radovich 1982).

After almost 50 years of being absent from the PNW, the biomass of Pacific sardine increased dramatically in the northern CCS (Hargreaves et al. 1994). A purse seine fishery was re-established in the mid-1990s in the PNW off Oregon and Washington (off the Columbia River), and around Vancouver Island, British Columbia with all PNW landings ranging from 17,923 metric tons (mt) in 2000 to 54,254 mt in 2005

(Hill et al. 2009). Hedgecock et al. (1989) and Lecomte et al. (2004) identified no genetic differences between sardines captured in the PNW and those in California. Sardines caught from Oregon to British Columbia considered part of the Central California Offshore subpopulation are currently managed as migrating adults of the California stock. The age-class structure of commercially caught sardines, and successful spawning events observed between 1998 and 2003 suggest a separate spawning population in the PNW (Emmett et al. 2005), however the origin of sardines caught in the PNW fishery is uncertain.

Pacific sardines have been described as migrating between their spawning grounds off San Diego, California and feeding grounds off Vancouver Island, British Columbia (Dahlgren 1936; Hart 1943**;** Ahlstrom 1957; Smith 2005; Hill et al. 2009). Tagging studies in the 1930s and 40s suggested that Pacific sardine migrated back and forth between Southern California and the PNW (Marr 1957), and provided evidence for a northward migration extending to Vancouver Island, British Columbia (Marr 1960). Attempts to repeat these early tagging studies conducted between 1936 and 1942 for Pacific sardine have been difficult since mortalities before or shortly after release were up to 80% for fish $\leq$ 185mm in total length and 70% for large fish (>185mm in total length) (Clark and Janssen 1945). Financial costs have also been prohibitive, with thousands of tagged fish needed to increase the probability of future recovery (Jacobson and Hansen 2005). Thus, cost restrictions and expectation of high mortality rates reduce the likelihood that a large-scale mark-recapture program for Pacific

sardine will be initiated soon to determine if the current migration patterns are similar to those observed in the 1930s and 1940s.

Another approach applied to identify migration patterns and stock structure of fishes has been the use of parasite species as biological tags (Thomas et al. 1996; review by MacKenzie and Abaunza 1998; review by MacKenzie 2002). Many parasites are acquired by fishes through the consumption of infected prey and can remain in or on the host from months to years depending on the longevity of the parasite species (Rohde 1984). The total variation in the percentage of fish infected (prevalence), the number of parasites per fish host (abundance), and the number of parasite species recovered (community diversity) (Bush et al. 1997) have provided information on the migration and stock structure of fish species worldwide (see Baldwin et al. CHAPTER 1, this volume), but have yet to be included in an assessment for Pacific sardine. Our objective was to assess if the parasite species infecting Pacific sardines could clarify migration patterns of sardines within the CCS by: 1) comparing parasite communities of sardine collected from five regions along the west coast of North America; 2) identifying parasite species that may be used as biological tags for sardines; and 3) examining the parasite communities of northern anchovy (*Engraulis mordax*), another clupeid fish with limited migration (Bakun 1996) known to be infected with similar parasite species (Love and 1983) that could thus provide information on the geographical restrictions of specific parasite species.

Materials and methods

Study Area and Fish Collections

A total of 1389 Pacific sardine were opportunistically collected from the California Current from 2005 through 2008 between 32^{o} to 51^{o} N latitude, and 119^{o} to 128^{o} W longitude (Figure 2.1). We divided the study area into five geographic regions: 1) Vancouver Island, British Columbia (part of PNW); 2) Washington and Oregon (part of PNW); 3) Northern California; 4) Central California; and 5) Southern California (Figure 2.1). The number of fish examined varied per year: 2005 (n=272), 2006 (n=458), 2007 (n=560), and 2008 (n=99) (Table 2.1). Fewer fish were examined from 2008 to focus on the parasite communities of fish from Oregon and California classified as migratory (> 200 mm Standard Length) (Lo et al. unpublished). To help clarify if any parasite species were associated with specific geographical areas, a total of 168 northern anchovy (*Engraulis mordax*) were opportunistically collected off Washington in 2007 (Gray's Harbor, n = 20 and Willapa Bay, n = 97) and off California in 2008 (Point Hueneme, n = 51) (Table 2.1).

Sardines from Region 1 were caught between March and November using a modified Cantrawl 240 rope trawl (Cantrawl Nets Ltd., Richmond, British Columbia; see Morris et al. 2009 for details). Except for anchovies collected off Point Hueneme, California in Region 5, sardines and anchovies from Regions 3 to 5 were caught between April and September using a 30 m wide by 20 m deep mouth-opening 264 rope trawl, (Nor'Eastern Trawl Systems, Inc. Bainbridge Island, Washington, U.S.A.;

see Baldwin et al. 2008 for details). After each trawling event, consisting of one tow of the net, Pacific sardines were immediately frozen onboard and then stored in the lab at -80^{o}C until processed for parasites.

Parasite Analysis

Once thawed, each fish was weighed to the nearest 0.1 g, and standard length (SL) measured to the nearest mm. Fresh SL of individual sardine was estimated using the following regression: Fresh SL = 2.89 + 1.0286 (Frozen and then Thawed SL) (Lo et al., 2007). No regression formula was available for northern anchovy, so SL values are reported from fish frozen and then thawed. Parasite recovery followed standard necropsy procedures (Arthur and Albert, 1994; Baldwin and Goater 2003). The body cavity and external surface of the viscera were examined for macroparasites during removal of the stomach and intestine. No gall bladders were examined for parasites due to the fish being frozen and thawed prior to parasite examination. Gills were not examined after 2005 due to the recovery of only one monogenean trematode. Individuals of the parasitic copepod *Lepeophtheirus* sp. found in the stomach of three sardines may have been consumed along with infected prey, but were not included in any analyses. All macroparasites recovered were identified morphologically to species if possible, and then saved in 95% ethanol. Five anisakid species (*Contracaecum margolisi*, *C. rudolphi*, *Anisakis pegreffii*, *A. simplex s.s.* and *A. simplex* 'C') were genetically identified from 156 anisakid nematode larvae recovered from Pacific sardines in a related study by amplifying a molecular region that included the internal

transcribed spacers (ITS-1, ITS-2) and 5.8S subunit of the nuclear ribosomal DNA (methods described by Baldwin et al. CHAPTER 3, this volume).

Statistical Analysis

Parasite communities were described using parasite prevalence, mean abundance, and mean intensity as defined by Bush et al. (1997). We categorized sardines from each collection location within a year as either non-migratory (≤ 200 mm SL) or migratory (> 200 mm SL) following Lo et al. (unpublished) to clarify if specific parasite species are associated with fish that are capable of migration. To identify any spatial and temporal patterns in the parasite community data for each year, non-metric multidimensional scaling ordinations (MDS) were constructed (Clark and Gorley 2006) based on the Bray-Curtis Similarity Coefficient (Bray and Curtis 1957) with each fish considered an individual sampling unit. Parasite abundance data from all fish examined for parasites were square-root transformed to reduce skewness and stabilize the variance (Clark and Warwick 2001). A dummy parasite variable was included in the parasite abundance dataset to enable all fish to be included into ordinations analyses. One-way multivariate analysis of similarities (ANOSIM) permutation tests identified differences of parasite mean abundances among years or collection locations. A Global R value close to or equal to 1 suggested parasite communities were different (Clark and Warwick 2001). For parasite communities detected by ANOSIM as significantly different, we identified which parasite species contributed > 10% of these differences using the procedure SIMPER (similarity percentages). All

community analyses were performed using PRIMER (version 6.0) (Clark and Warwick 2001). Fishers Exact tests were calculated using Statview® (SAS, 1998) to assess regional differences in the recovery of the trematodes *Lecithaster gibbosus* and *Myosaccium ecaude* within the study area.

Results

General Pacific Sardine and Northern Anchovy Information

A total of 1389 sardines were recovered from 2005 through 2008, with one to eighty-six individual fish collected per sampling location (Table 2.1). The overall estimated fresh standard length (SL) ranged from 100.61 to 285.76 mm, (Figure 2.2A) of which 437 were classified as non-migrant fish ($\leq$ 200 m SL) and 952 were classified as migrant fish (> 200 mm SL) (Table 2.1). Although our sardines were not aged, we estimated an age range from 0 to 10 years old using a relationship between age and standard length for sardines caught in the California Current from 2003 to 2008 (Barbara Javor pers. comm.; Emmett and Lo 2009). Except for one sardine caught off Newport, Oregon (Region 2; estimated SL = 100.61 mm), the smallest sardines were recovered off Central California (Region 4; minimum estimated SL = 106.78 mm) and the largest were caught off British Columbia (Region 1) in 2006 (maximum estimated SL = 285.76 mm). In 2005, the range in SL was similar between non-migrant fish caught from Region 1 in March (148.08 to 193.22 mm) and Region 4 in April (147.12 to 196.10 mm). Also, non-migrant fish caught in March 2005 from Region 1 were smaller (mean SL = 163.26 mm) than non-migrant fish caught in July 2005 (mean SL

= 194.76). In 2008, similar mean SL (from 224.74 to 226.23 mm) and ranges (minimum SL = 208.61 to maximum SL = 257.98) in fish lengths were observed among the migrant fish caught in Regions 2 and 5.

Northern anchovies were collected in 2007 and 2008 with 20 to 97 individuals collected per sampling location (Table 2.1). The overall frozen then thawed SL ranged from 98.0 to 142.0 mm. The smallest anchovy was caught in Region 5 (98.00 mm SL), and the largest anchovy was caught in Region 2 (143.00 mm SL).

Parasite Species Recovery and Distribution Patterns in Pacific Sardine

Parasites recovered from Pacific sardines included trematodes, nematodes, acanthocephalans, larval cestodes, and a monogenean (Tables 2.2 – 2.8). To assess if any parasite species were associated with specific geographic locations, Pacific sardines were categorized as either non-migratory ($\leq$ 200 mm SL) or migratory (> 200 mm SL), with the assumption that parasites recovered in non-migratory fish were acquired within the geographic location where the fish was caught. Most individual parasites were identified to species, but some in poor condition remained as unknown. For *A. pegreffii* (n = 76), *A. simplex s.s.* (n = 51), *A. simplex* 'C' (N = 21), *C. margolisi*, (n = 3) and *C. rudolphi* (n = 3), individual worms were genetically identified as part of a population genetics study. The remaining *Anisakis* nematodes were likely a combination of *A. pegreffii*, *A. simplex s.s.*, and *A. simplex* 'C', but were

either not successfully sequenced (n = 37) or not yet recovered to sequence for the previous study (n = 136).

Parasite prevalence and mean intensity of infected fish varied by year, location and size of fish (Tables 2.2-2.8). In 2005, parasite prevalences ranged from 2.0 to 53.3% for non-migrant sardines (Table 2.2), and 3.3 to 66.7% for migrants (Table 2.3). Overall parasite mean intensities ranged from one to two worms per species, although off Region 1 in July the trematode *L. gibbosus* had a mean intensity of 14 (range one to 51 worms) in non-migrant fish, and 4.1 (range one to nine worms) in migrant fish. *Myosaccium ecaude*, another trematode, was found in all locations but was most prevalent in Region 3. The trematode *Parahemiurus merus* and the nematodes *Hysterothylacium* sp. and *Anisakis* species were recovered from all regions, but nematodes were more prevalent in migrant fish (Table 2.3). The acanthocephalan *Rhadinorhychus trachuri*, and an unknown monogenean were recovered in Region 4 in April 2005. Only one individual of the trematode *Pronoprynma petrowi* was recovered in 2005 from Region 1 in March.

In 2006 parasite prevalences ranged from 2.1 to 63.9% for non-migrant sardines (Table 2.4), with one non-migrant fish caught in November from Region 1 infected with three *Hysterothylacium* sp. nematodes. Prevalence ranged from 2.3 to 60.0% for migrants (Table 2.5). One migrant fish infected with a single *M. ecaude* was caught off Montana de Oro State Park (Region 4), and one migrant fish infected with a single

P. merus was caught off San Nicolas (Region 5). Most parasite mean intensities ranged from one to three worms per species except for 1) a mean intensity of 3.3 (range one to 29 worms) for *M. ecaude* in non-migrant sardines caught in May from Region 1, and 2) a mean intensity of 7.7 (range one to 25 worms) for *P. petrowi* in migrant sardines caught in November in Region 1. As seen in 2005, *M. ecaude* was most prevalent in non-migrant fish off California. *Lecithaster gibbosus* was again most prevalent from Region 1, with few worms recovered from Region 2 in May, and Region 3 in April. *Parahemiurus merus, Hysterothylacium* sp. and *Anisakis* species were recovered throughout the study area with no definitive spatial pattern observed.

In 2007 parasite prevalences ranged from 4.8 to 54.8% for non-migrant sardines (Table 2.6), and 2.0 to 97.2% for migrant fish (Table 2.7). One migrant fish caught off San Nicolas (Region 5) was infected with one *Hysterothylacium* sp. and one *P. merus*. Most parasite mean intensities ranged from one to three worms per species (Tables 2.6 and 2.7). *Lecithaster gibbosus* was recovered from Region 1 and 2, but were again most prevalent in migrant sardines from Region 1, with mean intensities from 4.0 to 5.7 (range one to 15 worms). Single worm infections of *P. merus* were observed in individual non-migrant fish caught from Region 1 in July, and Region 2 in June and July. In addition, one *M. ecaude* was recovered from a non-migrant fish caught off Golden Gate Inner, (Region 4). *Myosaccium ecaude* was found throughout the study area in 2007, but a group of non-migrant sardines caught off Point Arena/Point Reyes (Region 3) had the highest prevalence (54.8%), mean intensity (3.1) and range (one to

32 worms) of this trematode species. *Parahemiurus merus* was found throughout the study area but was most prevalent from Region 1. *Hysterothylacium* sp. and *Anisakis* species were also found throughout the study area, however, they did not have a specific geographic distribution. It is likely *A. pegreffii*, *A. simplex s.s.* and *A. simplex* 'C' were recovered Region 1, but these larval worms were not identified genetically. Only one individual *P. petrowi* was recovered in a migrant sardine from Region 1. Although still rare, the highest recoveries of *R. trachuri* were observed for the entire study during 2007.

In 2008 we examined only migrant sized fish to compare parasite communities among fish of similar size (Table 2.1). Parasite prevalences for these sardines ranged from 3.3 to 84.2%, and mean intensities ranged from one to 2.8 worms per species (Table 2.8). Except for the five *M. ecaude* recovered off Southern California (Region 5), the parasite communities were similar between migrant fish caught in Regions 2 and 5. Parasite communities were dominated by the nematodes *Hysterothylacium* sp. and *Anisakis* species, but *P. merus*, *R. trachuri*, and a single larval trypanorhynch cestode were also recovered. No *L. gibbosus* were recovered from sardines caught in 2008.

Parasite Species Recovery and Distribution Patterns in Northern Anchovy

For northern anchovy, parasite prevalences ranged from 5.0 to 45.0% in 2007 and 5.9 to 41.2% in 2008 (Table 2.9). *Lecithaster gibbosus* was only recovered off Gray's Harbor, Washington (Region 2), and a didymozoid trematode was only recovered off

Point Hueneme, Southern California (Region 5). *Anisakis* species, *Hysterothylacium* sp., and *P. merus* were observed in both Washington and Southern California anchovies, but *P. merus* was more prevalent off California. Overall parasite mean intensities ranged from one to two worms per infected fish, however up to six *L. gibbosus* were recovered from a single anchovy off Gray's Harbor, Washington. *Myosaccium ecaude* was not recovered in northern anchovy, and overall fewer parasites were recovered from northern anchovy than Pacific sardine.

MDS Ordinations and Fisher's Exact Tests for Pacific Sardine Parasites

Generally, the ordinations of parasite communities for the years 2005 (Figure 2.3A), 2006 (Figure 2.3B) and 2007 (Figure 2.3C) were defined by the abundance of four parasite species: *L. gibbosus*, *M. ecaude*, *Hysterothylacium* species and *P. merus*. Year had no effect on the geographic distribution of the parasite communities from 2005 through 2007 when all fish were included in a single MDS ordination (ANOSIM Global R = -0.013, p-value = 0.99). In contrast, the migrant fish examined in 2008 were dominated by *Hysterothylacium* species and *Anisakis* species (Figure 2.3D). When parasite communities were examined in a separate ordination for each year, there was a difference among collection locations (2005: ANOSIM Global R = 0.114, p-value = 0.001; 2006: ANOSIM Global R = 0.161, p-value = 0.001; 2007: ANOSIM Global R = 0.143, p-value = 0.001; 2008: ANOSIM Global R = 0.093, p-value = 0.006).

SIMPER analysis revealed that specific parasites species were associated with the northern and southern extreme regions of our study area from 2005 to 2007. From Region 1, parasite communities were defined by 1) *L. gibbosus* (contributing percentages ranging from 85.0% in 2005 to 30.5% in 2006), 2) *P. petrowi* in 2006 (41.0%), and 3) *P. merus* in 2007 (14.5%). In contrast, *M. ecaude* defined several parasite communities from 2005 to 2007 in Regions 3 - 5 (contributing percentages ranging from 24.4% in 2005 to 94.7% in 2006). With no migrant fish examined from Region 1 in 2008, only *Anisakis* species defined parasite communities in Region 2 (ranged from 91.0% to 99.4%). Both *Anisakis* species (43.3%) and *Hysterothylacium* sp. (49.7%) defined the parasite communities from Region 5 of migrant fish in 2008.

Fisher's Exact tests determined there that there was a spatial difference in the recovery of *L. gibbosus* and *M. ecaude* by region among non-migrant and migrant sardines (Table 2.10, Figure 2.3B and 2.3C). For non-migrants, *L. gibbosus* was recovered from only Region 1 and Region 2 (Figure 2.2B; Fisher's Exact test p-values ranged from < 0.0001 to 0.06). *Lecithaster gibbosus* was more common in migrant fish than non-migrant fish in Regions 1 and 2, with an additional three migrant fish infected in Region 3 (Fisher's Exact test p-values ranged from < 0.0001 to > 0.99). *Myosaccium ecaude* was observed in all 5 regions of the study area, but were most common in non-migrant fish from Region 5 (Fisher's Exact test p-values ranged from < 0.0001 to > 0.99) and migrant fish from Regions 3 and 4 (Fisher's Exact test p-values ranged from 0.0003 to 0.62). Out of 1389 sardines examined for parasites, only seven fish were

infected with both *L. gibbosus* and *M. ecaude*. Except for one migrant fish caught off Willapa Bay (Region 2) in 2006, the other six fish with this co-infection were caught in Region 1 (two non-migrants in 2005, one migrant in 2006 and three migrants in 2007).

Discussion

Pacific sardine were not infected with the same trophically-transmitted parasite species throughout the California Current. Five trematodes (*Lecithaster gibbosus*, *M. ecaude*, *P. petrowi*, and *P. merus*), and the nematodes *Hysterothylacium* sp. and *Anisakis* species contributed to differences in parasite communities between years. However, two trematode species were identified as biological tags for Pacific sardine in our study. *Lecithaster gibbosus* were recovered in non-migrant and migrant sardines primarily from Region 1 (Vancouver Island, British Columbia, Canada). *Myosaccium ecaude* were recovered throughout the study area, but were most common in non-migrant fish caught from Regions 4 and 5 (Central and Southern California).

Although *L. gibbosus* was not previously reported from Pacific sardine or northern anchovy, it is known to infect 82 fish species from Oregon to Alaska, the Bering Sea, the Sea of Japan and waters off the Soviet Union (Pratt and McCauley 1961; Love and Moser 1983; McDonald and Margolis 1995; Moles 2007). Boyce and Margolis (1969) suggested that *L. gibbosus* has an estimated longevity of two to nine months in juvenile salmon, but the recovery of infected sardines from March to November

suggests that fish can continually become infected while they remain in Region 1 and potentially Region 2 (Washington and Oregon). Additionally, only northern anchovies examined from Region 2 were infected with *L. gibbosus.* Northern anchovies are infected with similar parasite species as Pacific sardine (Love and Moser 1983), but more importantly are considered a less migratory clupeid species compared to Pacific sardine (Bakun 1996). The parasite communities of anchovies likely reflect what parasite species are present in a local geographical area where anchovies were caught, thus providing more support for a northern distribution of *L. gibbosus* in the California Current. Thus *L. gibbosus* has the potential to identify sardines that are either resident or that have migrated from the northern end of the California Current.

Montgomery (1957) described *M. ecaude* from Pacific sardine caught in 1953 off La Jolla, California. Williams and Bunkley-Williams (1996) suggested this genus is commonly found in clupeid fish species, yet to date this species has only been reported from Pacific sardine (Montgomery 1957; Kunnenkeri 1962). Little information is available on the distribution or life history of this trematode in the California Current. Previous surveys of marine fish either did not include Pacific sardine or were conducted after sardines disappeared after 1947 from the northern end of the California Current (Love and Moser 1983; McDonald and Margolis 1995; Radovich 1982). Additionally, *M. ecaude* was not recovered in either northern anchovy (this study) or Pacific herring (Pratt and McCauley 1961; Love and Moser 1983; McDonald and Margolis 1995; Moser and Hseih 1992; Moles 2007); lending further evidence

that Pacific sardine may be the only final host for *M. ecaude*. It is uncertain if the presence of *M. ecaude* in the Pacific Northwest and British Columbia is correlated with the return of Pacific sardine in the 1990s, but a population genetics study for *M. ecaude* could determine if more than one population of *M. ecaude* exists in the California Current (see Baldwin et al. CHAPTER 4, this volume).

Similar-sized non-migrant fish caught in Region 1 in March and Region 3 (central California) in April 2005 that differed in their infection with *L. gibbosus*, suggested a required intermediate host species for this trematode species may not be available throughout the study area. Sardines are non-selective omnivores consuming both phytoplankton and zooplankton in the pelagic food web, (van der Lingen 2002; van der Lingen et al. 2006). Either copepods (Hand and Berner Jr. 1959) or phytoplankton (Hart and Wailes 1931) have been identified as the dominant food source for Pacific sardines, however, amphipods, decapod larvae, ostracods, chateognaths, larvaceans, fish eggs (anchovy eggs in particular), and all stages of euphausiids from egg to adult have been identified in sardine diet (Hart and Wailes 1931; Hand and Berner Jr. 1959; van der Lingen 2002; Emmett et al. 2005; McFarlane et al. 2005; van der Lingen et al. 2006; Brodeur et al. 2008; Espinoza et al. 2008). Previous studies have suggested an ontogenetic shift in the diet from smaller sized zooplankton to more phytoplankton as sardines grow from juveniles to adults (van der Lingen 2002; van der Lingen et al. 2006). If the same prey species are available throughout the California Current, the reduction in prevalence of *M. ecaude* and increase in prevalence of *L. gibbosus* in

migrant fish may reflect a change in diet as sardines grow into adults. Alternatively, there may be a restriction in the geographical distribution of an invertebrate intermediate host limiting *L. gibbosus* from extending into California. By examining both stomach contents and parasite communities from the same individual fish host (Baldwin et al. 2008), one could identify potential host species to improve our understanding of the distribution of *L. gibbosus* and *M. ecaude*, and better understand the foraging history of Pacific sardine throughout its range.

The geographic distributions of *L. gibbosus* and *M. ecaude* observed from 2005 through 2007 suggest that Pacific sardines may have two overlapping migration patterns in the California Current. The recovery of *M. ecaude* in all five regions of the study area supported conclusions of previous artificial tagging studies conducted more than 60 years ago that sardines are migrating between Southern California and the Pacific Northwest (Janssen 1938; Clark and Janssen 1945; Marr 1957). In addition, our analyses identified another migration pattern that may be limited to the Pacific Northwest based on the geographic distribution of *L. gibbosus*. The recovery of only seven out of 1389 sardines infected with both *L. gibbosus* and *M. ecaude* (six off Vancouver Island, British Columbia, and one off of Willapa Bay, Washington) suggests that sardines migrating from Vancouver Island, British Columbia are not all returning to Southern California to overwinter in the same year they migrated north to feeding grounds. If the extensive migration pattern from British Columbia to Southern California was common, we would expect to recover more sardines infected with both

trematode species south off Washington and Oregon. Our recovery of only three migrant sardines from Northern California infected with *L. gibbosus* suggests very few sardines overwinter off Vancouver Island, British Columbia and return the following spring to spawn off Southern California. Alternatively, individual sardines may either clear infections of *L. gibbosus* before migrating back to Southern California to spawn, or grow to a specific standard length and no longer migrate back to Southern California.

Other potential candidates for biological tags were the trematodes *P. petrowi* and *Anisakis* species nematodes. Although *P. petrowi* was also restricted to the northern end of our study area it was only abundant in 2006. It was previously reported off British Columbia from Pacific herring (Arthur and Arai 1980), surf smelt (*Hyposmesus pretiosus*), and longfin smelt (*Spirinchus thaleichthys*) (Margolis and Ching 1965). In addition, surf smelt infected with *P. petrowi* were observed off Oregon and northern California (Jacobson et al. unpublished), but the presence of this parasite in Pacific sardine may be too variable to be used as a biological tag without also including *L. gibbosus*. *Anisakis* species were found throughout the study area and have been successfully used as biological tags in other fish population studies (e.g. MacKenzie 2002; Mattiucci and Nascetti 2008, Mattiucci et al. 2008). Three *Anisakis* species that infect Pacific sardine in the California Current were genetically identified, but there was no geographic separation among different haplotypes for *A. pegreffii*, *A. simplex* ‘C’ and *A. simplex s.s.* (Baldwin et al. CHAPTER 3, this volume).

Separate yet overlapping migration patterns based on the observed distributional patterns for *L. gibbosus* and *M. ecaude*, questions the proposed single coastwide migration pattern for Pacific sardines in the California Current. Larger sardines may have a restricted migration pattern in the Pacific Northwest from Vancouver Island, British Columbia to Oregon and may not all return to Southern California to spawn. Overlapping migration patterns were also described by Blaylock et al. (2002) for Pacific halibut (*Hippoglossus stenolepis*) from northern California to the Aleutian Islands, Alaska. Although reproductively isolated stocks among sardines could not be unequivocally determined using parasite community data, we suggest a conservative approach of management that recognizes two possible migration patterns of Pacific sardines.

Acknowledgements

We would like to thank the following people for their help with this project: 1) Paul Bentley, Joe Fisher & Laurie Weitkamp from National Oceanic and Atmospheric Administration (NOAA), Northwest Fisheries Science Center**;** 2) Bev Macewicz, Dave Griffith, Dimitry Abramenkoff, Ron Dotson, Barbara Javor, & David Field from NOAA, Southwest Fisheries Science Center; 3) Andrew Claxton, Mary Beth Rew, James Losee, Todd Sandell, Carrie Johnson, Paul Peterson, Marisa Litz, & Hongsheng Bi from Oregon State University, Cooperative Institute for Marine Resources Studies; 4) Motomitsu Takahashi & Rafael Gonzalez-Quiiros from University of California, San Diego/Scripps Institution of Oceanography; 5) Marc Trudel, Vanessa Hodes,

Linnea Flostrad, & Tyler Zubkowski from Fisheries and Oceans Canada; and Fishing Industry: Mike Okoniewski from Pacific Seafood, Rod Moore from West Coast Seafood Processors Association, Jerry Thon, Stan Schones, John Gingerich, Diane Pleshner-Steele, and all the fishermen who donated fish to this project, and 6) Justin Atkins, and Nick Hempfer. We would like to thank Michael Blouin, Virginia Lesser, John Bolte, and Edmundo Casillas for comments on previous versions of this manuscript. Funding was provided by NOAA Fisheries, the Mamie Markham Research Award (received by REB), and the Walter G. Jones Fisheries Development Award (received by REB).

References

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Arthur, J. R., and E. Albert. 1994. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **50**:2175-2181.

Arthur, J. R., and H. P. Arai. 1980. Studies on the parasites of Pacific herring (*Clupea harengus pallasi* Valenciennes): a preliminary evaluation of parasites as indicators of geographical origin for spawning herring. Canadian Journal of Zoology **58**:521-527.

Bakun, A. 1996. Patterns in the ocean. Ocean processes and marine population dynamics. California Sea Grant College System, National Oceanic and Atmospheric Administration in cooperation with Centro de Investigaciones Biológicas del Noroeste, La Paz, BCS, Mexico.

Baldwin, R. E., and C. P. Goater. 2003. Circulation of parasites among fishes from lakes in the Caribou Mountains, Alberta, Canada. Journal of Parasitology **89**:215-225.

Baldwin, R. E., T. W. Miller, R. D. Brodeur, and K. C. Jacobson. 2008. Expanding the foraging history of juvenile Pacific salmon: combining stomach-content and macroparasite-community analyses for studying marine diets. Journal of Fish Biology **72**:1268-1294.

Blaylock, R. B., L. Margolis, and J. C. Holmes. 2002. The use of parasites in discriminating stocks of Pacific halibut (*Hippoglossus stenolepis*) in the Northeast Pacific. Fishery Bulletin **101**:1-9.

Boyce, N. P. 1969. Parasite fauna of pink salmon (*Oncorhynchus gorbuscha*) of the Bella Coola River, central British Columbia, during their early sea life. Journal of the Fisheries Research Board of Canada **26**:813-820.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs **27**:325-349.

Brodeur, R. D., J. P. Fisher, D. J. Teel, R. L. Emmett, E. Casillas, and T. W. Miller. 2004. Juvenile salmonid distribution, growth, condition, origin, and environmental and species associations in the northern California Current. Fisheries Bulletin 102:25-46.

Brodeur, R. D., C. L. Suchman, D. C. Reese, T. W. Miller, and E. A. Daly. 2008. Spatial overlap and trophic interactions between pelagic fish and large jellyfish in the northern California Current. Marine Biology **154**:649-659.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Clark, K. R., and R. N. Gorley. 2006. Primer v6: user manual / tutorial. Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom.

Clark, K. R., and R. M. Warwick. 2001. Change in marine communities: An approach to statistical analysis and interpretation, Second edition. Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**:117-143.

Cross, M. A., C. Collins, N. Campbell, P. C. Watts, J. C. Chubb, C. O. Cunningham, E. M. C. Hatfield, and K. MacKenzie. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda : Anisakidae): implications for fisheries management. Marine Biology **151**:695-702.

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quinones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science **57**:603-618.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Emmett, R. L., and N. C. H. Lo. 2009. Proceedings of the 2008 Tri-national Sardine Forum. Northwest Fisheries Science Center. 89pp.

Espinoza, P., A. Bertrand, C. D. van der Lingen, S. Garrido, and B. R. de Mendiola. 2009. Diet of sardine (*Sardinops sagax*) in the northern Humboldt Current system and comparison with the diets of clupeids in this and other eastern boundary upwelling systems. Progress in Oceanography **83**:242-250.

Felin, F. E. 1954. Population heterogeneity in the Pacific pilchard. Fisheries Bulletin **54**:201-225.

Field, J. C., R. C. Francis, and K. Aydin. 2006. Top-down modeling and bottom-up dynamics: Linking a fisheries-based ecosystem model with climate. Progress in Oceanography **68**:238-270.

Hand, C. H., and L. Berner Jr. 1959. Food of the Pacific sardine (*Sardinops caerulea*). Fishery Bulletin **60**:175-184.

Hargreaves, N. B., D. M. Ware, and G. A. McFarlane. 1994. Return of Pacific sardine (*Sardinops sagax*) to the British Columbia coast in 1992. Canadian Journal of Fisheries and Aquatic Science **51**:460-463.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hart, J. L., and G. H. Wailes. 1931. The food of the pilchard, *Sardinops caerulea* (Girard), off the coast of British Columbia. Biological Board of Canada, Contribution to Canadian Biology and Fisheries **7**:247-254.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Herrick, S. F., J. G. Norton, R. Hannesson, U. R. Sumaila, M. Ahmed, and J. Pena-Torres. 2009. Global production and economies. Pages 256-274 *in* D. Checkley, J. Alheit, Y. Oozeki, and C. Roy, editors. Climate change and small pelagic fish. Cambridge University Press, New York.

Hill, K. T., N. C. H. Lo, B. J. Macewicz, P. R. Cronel, and R. Felix-Uraga. 2009. Assessment of the Pacific sardine resource in 2009 for U.S. management in 2010. NOAA-TM-NMFS-SWFSC-452:221.

Jacobsen, J. A., and L. P. Hansen. 2005. Internal and external tags. Pages 415-433 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods. Applications in fishery science. Elsevier Academic Press, San Francisco.

James, A. G. 1988. Are clupeid microphagists herbivorous or omnivorous? A review of the diets of some commercially important clupeids. South African Journal of Marine Science **7**:161-177.

Køie, M. 1983. Digenetic trematodes from *Limanda limanda* (L.) (Osteichthyes, Pleuronectidae) from Danish and adjacent waters, with special reference to their life-histories. Ophelia **22**:201-228.

Kunnenkeri, J. K. 1962. Preliminary report on the parasites of the California sardine and the parasitic distribution in Clupeidae. Journal of Parasitology **48**:149.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Lo, N. C. H., B. J. Macewicz, and D. A. Griffith. unpublished. Biomass and reproduction of Pacific sardines off the northwest US in 2003-2005. 43pp.

Lo, N. C. H., B. J. Macewicz, and R. L. Charter. 2007. Spawning biomass of Pacific sardine (*Sardinops sagax*) off California in 2007. NOAA-TM-NMFS-SWFSC-411 Technical Memo, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center:38pp.

Love, M. S., and M. Moser. 1983. A checklist of parasites of California, Oregon, and Washington Marine and Estuarine Fishes. NOAA Technical Report NMFS SSRF-777. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services. 574pp.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**:S153-S163.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research **38**:45-56.

Marcogliese, D. J. 2002. Food webs and the transmission of parasites to marine fish. Parasitology **124**:S83-S99.

Margolis, L., and N. P. Boyce. 1969. Life span, maturation, and growth of two Hemiurid trematodes, *Tubulovesicula lindbergi* and *Lecithaster gibbosus*, in Pacific salmon (Genus *Oncorhynchus*). Journal of the Fisheries Research Board of Canada **26**:893-907.

Margolis, L., and H. L. Ching. 1965. Review of the Trematode genera *Bacciger* and *Pentagramma* (Fellodistomatidae) and description of *P. petrowi* (Layman, 1930) n. comb. from marine fishes from the Pacific coast of Canada. Canadian Journal of Zoology **43**:381-405.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

Marr, J. C. 1960. The causes of major variations in the catch of the Pacific sardine, *Sardinops caerulea* (Girard). Pages 667-791 *in* H. Rosa and G. I. Murphy, editors. Proceedings of the World Scientific Meeting on the Biology of Sardines and Related Species. Food and Agriculture Organization of the United Nations.

Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008. *Anisakis* spp. larvae (Nematoda : Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Pages 47-148 *in* Advances in Parasitology.

McDonald, T. E., and L. Margolis. 1995. Synopsis of the parasites of fishes of Canada: Supplement (1978-1993). Canadian Special Publication of Fisheries and Aquatic Sciences.

McFarlane, G. A., L. MacDougall, J. Schweigert, and C. Hrabok. 2005. Distribution and biology of Pacific sardines (*Sardinops sagax*) off British Columbia, Canada. California Cooperative Oceanic Fisheries Investigations Reports **46**:144-160.

McGladdery, S. E., and M. D. B. Burt. 1985. Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences **42**:1957-1968.

Miller, T. W., and R. D. Brodeur. 2007. Diets of and trophic relationships among dominant marine nekton within the northern California Current ecosystem. Fishery Bulletin **105**:548-559.

Moles, A. 2007. Parasites of the fishes of Alaska and surrounding waters. Alaska Fishery Research Bulletin **12**:197-226.

Montgomery, W. R. 1957. Studies on digenetic trematodes from marine fishes of La Jolla, California. Transactions of the American Microscopy Society **76**.

Morris, J., M. Trudel, T. B. Zubowski, H. R. MacLean, J. M. R. Curtis, and L. Felli. 2009. CCGS W.E. Ricker Gulf of Alaska salmon survey, June 20-July 5, 2007. Canadian Data Report Fisheries and Aquatic Science 1221. Department of Fisheries and Oceans, Nanaimo, British Columbia.

Moser, M. 1991. Parasites as biological tags. Parasitology **7**:182-185.

Moser, M., and J. Hsieh. 1992. Biological tags for stock separation in pacific herring *Clupea harengus pallasi* in California. Journal of Parasitology **78**:54-60.

Nadler, S. A., and D. S. S. Hudspeth. 2000. Phylogeny of the ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: Hypotheses of structural and sequence evolution. Journal of Parasitology **86**:380-393.

Pauly, D., V. Christensen, R. Froese, and M. L. Palomares. 2000. Fishing down aquatic food webs. American Scientist **88**:46-51.

Pratt, I., and J. E. McCauley. 1961. Trematodes of the Pacific Northwest. An annotated catalog. Oregon University Press, Corvallis, OR.

Radovich, J. 1962. Effects of sardine spawning stock size and environment on year-class production. California Fish and Game **48**:123-140.

Radovich, J. 1982. The collapse of the California sardine fishery. Pages 107-136 *in* M. H. Glantz and J. D. Thompson, editors. Resource management and environmental uncertainty. Lessons from coastal upwelling fisheries. John Wiley & Sons, New York.

Rohde, K. 1984. Ecology of marine parasites. Helgoländer Meeresunters **37**:5-33.

SAS. 1998. Statview®. Using Statview. Version 5.0.1. *in*. SAS Institute Inc., Cary, NC.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Thomas, F., O. Verneau, T. de Meeûs, and F. Renaud. 1996. Parasites as host evolutionary prints: Insights into host evolution from parasitological data. International Journal of Parasitology **26**:1137-1137.

Valentini, A., S. Mattiucci, P. Bondanelli, S. C. Webb, A. A. Mignucci-Giannone, M. M. Colom-Llavina, and G. Nascetti. 2006. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *COX2* sequences, and comparison with allozyme data. Journal of Parasitology 92:156-166.

van der Lingen, C. D. 2002. Diet of sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. South African Journal of Marine Science **24**:301-316.

van der Lingen, C. D., L. Hutchings, and J. G. Field. 2006. Comparative trophodynamics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela: are species alternations between small pelagic fish trophodynamically mediated? African Journal of Marine Science **28**:465-477.

Williams Jr., E. H., and L. Bunkley-Williams. 1996. Platyhelminthes (Flatworms). Page 59 *in* Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Antillean College Press, Mayaguez, Puerto Rico.

Wolf, P. 1992. Recovery of the Pacific sardine and the California sardine fishery. California Cooperative Oceanic Fisheries Investigations Reports **33**:76-86.

Tables

Table 2.1. General information on 1389 Pacific sardine (*Sardinops sagax*) 167 northern anchovy (*Engraulis mordax)* and by year, location, region, date caught, and latitude. The mean (range) estimated mean standard length (SL) are provided for each fish collection. Sardines are separated into two size classes: and ≤ 200 mm SL (non-migrants) and > 200 mm SL (migrants).

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
Pacific sardine							
2005	Vancouver Island, British Columbia, Canada	1	Mar 3	48.72	> 200mm	26	163.26 (148.08-193.22)
			Jul 30	48.70	≤ 200mm	15	194.76 (181.69-199.94)
			Jul 30	48.70	> 200mm	15	215.50 (200.90-256.61)
	Willapa Bay, Washington, USA	2	Jun 4	46.67	≤ 200mm	9	186.71 (143.28-198.98)
			Jun 4		> 200mm	21	218.55 (200.90-258.53)
			Jun 26		≤ 200mm	26	188.53 (152.88-198.98)
			Jun 26		> 200mm	4	203.06 (200.90-204.74)
			Jul 7		≤ 200mm	23	190.34 (175.93-198.02)
			Jul 7		> 200mm	3	205.38 (200.92-214.35)
	Columbia River, Oregon, USA	2	Jun 6	46.17	> 200mm	30	254.88 (241.24-279.66)
	Santa Cruz, California, USA	4	Apr 11	36.98	≤ 200mm	50	171.63 (106.78-198.98)
	Point Arguello, California, USA	4	Apr 11	34.54	≤ 200mm	50	172.63 (147.12-196.10)

*Region 1 = Vancouver Island, British Columbia, Canada, Region 2 = Washington and Oregon, USA, Region 4 = Central California, USA

Table 2.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
Pacific Sardine							
2006	Vancouver Island, British Columbia, Canada	1	Nov 16	50.58	≤ 200mm	1	194.21
					> 200mm	44	224.55 (203.47-285.76)
	Willapa Bay, Washington, USA	2	May 12	46.67	≤ 200mm	10	168.29 (156.15-185.98)
			May 12		> 200mm	2	217.35 (200.38-234.33)
			Jun 13		≤ 200mm	5	196.68 (193.22-198.98)
			Jun 13		> 200mm	17	207.06 (200.90-212.43)
			Jul 18		≤ 200mm	5	186.60 (163.35-199.35)
			Jul 18		> 200mm	25	207.29 (200.38-232.27)
			Aug 15		≤ 200mm	1	199.35
			Aug 15		> 200mm	19	210.99 (200.38-265.18)
	Columbia River, Oregon, USA	2	May 27	46.17	>200mm	30	217.87 (201.41-255.93)
			Sep 25		≤ 200mm	5	196.27 (192.15-199.35)
			Sep 25		> 200mm	11	208.52 (203.47-214.78)
	Newport, Oregon, USA	2	Sep 18	44.67	≤ 200mm	24	172.27 (100.61-198.32)
			Sep 18		> 200mm	15	206.00 (200.38-213.75)
	Point Delgada, California, USA	3	Apr 24	40.24	≤ 200mm	13	195.58 (184.58-199.94)
			Apr 24		> 200mm	37	208.46 (200.90-247.00)

*Region 1 = Vancouver Island, British Columbia, Canada, Region 2 = Washington and Oregon, USA, Region 3 = Northern California, USA

Table 2.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
Pacific sardine							
2006	Manchester, California, USA	3	Apr 25	39.12	≤ 200mm	28	187.32 (120.23-199.94)
			Apr 25		> 200mm	22	211.73 (200.90-260.45)
			Apr 26		≤ 200mm	10	187.65 (168.25-198.98)
			Apr 26		> 200mm	7	204.20 (200.90-210.51)
	Montana de Oro State Park, California, USA	4	May 5	35.29	≤ 200mm	47	180.99 (163.35-196.27)
			May 5		> 200mm	1	200.38
	San Nicolas Island, California, USA	5	May 10	33.20	≤ 200mm	47	173.81 (157.18-190.10)
			May 10		> 200mm	1	204.50
	east of San Nicolas Island, California, USA	5	May 2	32.97	≤ 200mm	21	188.72 (158.21-198.32)
			May 2		> 200mm	5	203.26 (200.38-206.55)
2007	Vancouver Island, British Columbia, Canada	1	Jul 20	48.08	≤ 200mm	1	198.32
			Jul 20	48.08	> 200mm	51	222.08 (201.41-260.04)
			Sep 18	48.02	> 200mm	36	224.18 (202.44-255.93)
			Aug 26/Sep 2	50.69	> 200mm	30	225.65 (208.61-265.18)

*Region 1 = Vancouver Island, British Columbia, Canada, Region 3 = Northern California, USA, Region 4 = Central California, USA, Region 5 = Southern California, USA.

Table 2.1 Continued.

Year	Location	Region*	Date	Latitude (°N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
Pacific sardine							
2007	Willapa Bay, Washington, USA	2	Jun 25	46.67	≤ 200mm	1	179.81
			Jun 25		> 200mm	49	228.47 (206.55-276.50)
			Jul 23		≤ 200mm	2	196.78 (195.24-198.32)
			Jul 23		> 200mm	40	212.16 (201.41-255.93)
	Astoria, Oregon, USA	2	Jun 13	46.04	> 200mm	51	217.26 (201.41-255.93)
			Jul 17		≤ 200mm	2	199.35
			Jul 17		> 200mm	48	215.49 (200.38-227.13)
	Chetco River, California, USA	3	Aug 17	42.00	> 200mm	10	217.87 (209.64-229.18)
	Patrick's Point/Klamath River, California, USA	3	Aug 16	41.21	≤ 200mm	8	195.88 (190.10-199.35)
			Aug 16		> 200mm	24	208.61 (200.38-219.93)
	Point Delgada, California, USA	3	Aug	40.00	≤ 200mm	11	195.58 (184.58-199.94)
			Aug		> 200mm	12	204.07 (200.38-207.58)
	Point Arena/Point Reyes, California, USA	3	Aug 12/13	38.29	≤ 200mm	10	189.99 (182.90-199.35)
			Aug 12/13	38.29	> 200mm	3	202.78 (200.38-204.50)
			Aug 17	38.52	≤ 200mm	62	192.87 (170.55-199.35)
			Aug 17	38.52	> 200mm	24	203.90 (200.38-227.13)

*Region 2 = Washington and Oregon, USA, Region 3 = Northern California, USA

Table 2.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
Pacific sardine							
2007	Golden Gate Inner, California, USA	4	Aug 11	37.48	≤ 200mm	1	183.92
	Salmon Cone, California, USA	4	Apr 28	35.80	≤ 200mm	4	193.70 (185.98-199.35)
			Apr 28		> 200mm	35	211.58 (201.41-267.24)
	San Nicolas Island, California, USA	5	Apr 21	33.28	≤ 200mm	44	178.20 (161.29-194.21)
			Apr 21		> 200mm	1	205.52
Pacific sardine							
2008	Columbia River, Oregon, USA	2	May 25	46.17	> 200mm	19	224.74 (210.67-242.55)
	Cape Meares, Oregon, USA	2	May 23	45.48	≤ 200mm	1	195.24
			May 23	45.48	> 200mm	30	226.23 (208.61-251.81)
	Ventura, California, USA	5		34.28	> 200mm	49	223.30 (210.00-257.98)
Total						**1389**	

*Region 2 = Washington and Oregon, USA, Region 4 = Central California, USA, Region 5 = Southern California, USA.

Table 2.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	mean (range) frozen/thawed SL
Northern anchovy							
2007	Gray's Harbor, Washinton, USA	2	May 27	47.00	na	20	115.10 (104.00-125.00)
	Willapa Bay, Washington, USA	2	July 23	46.67	na	51	121.22 (100.00-143.00)
Northern anchovy							
2008	Point Hueneme, California, USA	5	Sept 29	34.15	na	97	111.40 (98.00-142.00)
Total						**167**	

*Region 2 = Washington and Oregon, USA, Region 5 = Southern California, USA.

Table 2.2. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 199 non-migrants (< 200 mm SL) of Pacific sardine (*Sardinops sagax*) in 2005. Single infected fish indicated by an asterisk (*).

	VI March 04 (n = 26)	VI July 06 (n=15)	WB June 04 (n=9)	WB June 26 (n=26)	WB July 07 (n=23)	SC April 11 (n=50)
Parasite Species	%, I(±SD)	%, I(±SD)	%, I(±SD)	%, I(±SD)	%, I(±SD)	%, I(±SD)
Anisakis pegreffii						
A. simplex 'C'						
A. simplex ss		6.7, 1.0*			4.3, 1.0*	
Anisakis species						
Total *Anisakis* species[A]		6.7, 1.0*			4.3, 1.0*	
anisakid nematode					4.3, 1.0*	
Hysterothylacium species	15.4, 1.3(±.5)	13.3, 1.5(±.7)	22.2, 1.0(±0)	15.4, 1.3(±.5)	8.7, 1.0(±0)	6.0, 1.0(±0)
Lecithaster gibbosus	30.8, 3.0(±1.3)	53.3, 14.0(±17.0)		3.8, 1.0*	8.7, 1.0(±0)	
Myosaccium ecaude	3.9, 5.0*	20.0, 1.0(±0)		15.4, 1.0(±0)	13.0 1.0(±0)	12.0, 1.5(±1.2)
Parahemiurus merus	3.9, 1.0*		11.1, 1.0*		4.3, 1.0*	30.0, 1.1(±.4)
Pronoprymna petrowi	3.9, 1.0*					
Rhadinorhynchus trachuri						2.0, 1.0*
unknown cestode	3.9, 5.0*					
unknown monogenean						2.0, 1.0*
unknown nematode	3.9, 1.0*			3.8, 1.0*		
unknown trematode	3.9, 1.0*					

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 1: VI = Vancouver Island, British Columbia, Region 2: WB = Willapa Bay, Washington, CR = Columbia River, Oregon, and Region 4: SC = Santa Cruz, Central California,

Table 2.2 Continued.

Parasite Species	PA April 24 (n=50) %, I(±SD)
Anisakis pegreffii	
A. simplex 'C'	
A. simplex ss	
Anisakis species	
Total *Anisakis* species[A]	
anisakid nematode	
Hysterothylacium species	6.0, 1.0(±0)
Lecithaster gibbosus	
Myosaccium ecaude	22.0, 1.6(±1.2)
Parahemiurus merus	18.0, 1.0(±0)
Pronoprymna petrowi	
Rhadinorhynchus trachuri	
unknown cestode	
unknown monogenean	
unknown nematode	
unknown trematode	

Region 4: PA = Port Arguello, Central California

Table 2.3. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 73 migrants (> 200 mm SL) of Pacific sardine (*Sardinops sagax*) in 2005. Single infected fish indicated by an asterisk (*).

	VI July 06 (n=15)	WB June 04 (n=21)	WB June 26 (n=4)	WB July 07 (n=3)	CR June 06 (n=30)
Parasite Species	%, I(±SD)	%, I(±SD)	%, I(±SD)	%, I(±SD)	%, I(±SD)
Anisakis pegreffii	6.7, 1.0*	4.8, 2.0*			16.7, 1.0(±0)
A. simplex C		9.5, 1.0(±0)			3.3, 1.0*
A. simplex ss		4.8, 1.0*			13.3, 1.0(±0)
Anisakis species		9.5, 1.5(±.7)			6.7, 1.5(±.7)
Total *Anisakis* species[A]	6.7, 1.0*	28.6, 1.3(.5)			33.3, 1.3(.7)
Hysterothylacium species	20.0, 1.0(±0)	14.3, 1.0(±0)		66.7, 1.0(±0)	36.7, 1.7(±1.6)
Lecithaster gibbosus	53.3, 4.1(±12.8)				
Myosaccium ecaude		9.5, 1.0(±0)		66.7, 1.0*	6.7, 1.0(±0)
Parahemiurus merus	26.7, 1.0(±0)	14.3, 1.3(±.6)			10.0, 1.0(±0)
Pronoprymna petrowi					
unknown nematode		4.8, 1.0*			16.7, 1.2(±.4)

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals,
Region 1: VI = Vancouver Island, British Columbia, and Region 2: WB = Willapa Bay, Washington,
CR = Columbia River, Oregon,

Table 2.4. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 217 non-migrants (≤ 200 mm SL) of Pacific sardine (*Sardinops sagax*) in 2006. Single infected fish indicated by an asterisk (*). Parasites from individual fish caught off Vancouver Island, British Columbia and Willapa Bay, Washington described in text.

Parasite Species	WB May 12 (n=10) %, I(±SD)	WB June 13 (n=5) %, I(±SD)	WB July 18 (n=5) %, I(±SD)	CR September 25 (n=5) %, I(±SD)	NH September 18 (n=24) %, I(±SD)	PD April 24 (n=13) %, I(±SD)
Anisakis pegreffii	10.0, 1.0*	20.0, 1.0*				7.7, 1.0*
A. simplex ss	20.0, 1.5(±.6)					
Anisakis species					8.3, 1.0(±0)	
Total *Anisakis* species[A]	30.0, 1.5(.7)				8.3, 1.0(±0)	
anisakid nematode					4.2, 1.0*	7.7, 2.0*
Tetraphyllidean sp. 1	10.0, 1.0*					
Contracaecum margolisi						
C. rudolphi						
Hysterothylacium species	40.0, 2.5(±2.4)				8.3, 1.5(±.7)	30.8, 1.5(±.6)
Lecithaster gibbosus	20.0, 1.0(±0)					
Myosaccium ecaude		20.0, 2.0*		40.0, 1.5(±.7)	8.3, 5.5(±6.4)	15.4, 1.5(±.7)
Parahemiurus merus	20.0, 1.0(±0)					7.7, 1.0*
Pronoprymna petrowi	10.0, 2.0*					
unknown nematode						7.7, 1.0*
unknown trematode						

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 1: VI = Vancouver Island, British Columbia, and Region 2: WB = Willapa Bay, Washington, CR = Columbia River, Oregon, NH = Newport, Oregon, and Region 3: PD = Point Arguello, Northern California

Table 2.4 Continued.

Parasite Species	MN April 25/26 (n=38) %, I(±SD)	MO May 5 (n=47) %, I(±SD)	SN May 10 (n=47) %, I(±SD)	ESN May 2 (n=21) %, I(±SD)
Anisakis pegreffii	5.3, 1.0(±0)	2.1, 1.0*		
A. simplex ss	2.6, 1.0*			
Anisakis species		2.1, 1.0*		9.5, 1.0(±0)
Total *Anisakis* species[A]	7.9, 1.0(±0)	4.3, 1.0(±0)		9.5, 1.0(±0)
anisakid nematode	2.6, 2.0*		6.4, 1.0(±0)	
Tetraphyllidean sp. 1				
Contracaecum margolisi			2.1, 1.0*	
C. rudolphi				4.8 1.0*
Hysterothylacium species	21.1, 1.0(±0)	12.8, 1.3(±.5)	29.8, 1.2(±.6)	57.1, 1.5(±.7)
Lecithaster gibbosus				
Myosaccium ecaude	28.9, 1.0(±1.0)	61.7, 2.3(±1.7)	63.9, 3.2(±5.2)	47.6, 2.2(±1.3)
Parahemiurus merus	5.3, 1.0(±0)	10.6, 1.4(±.9)	27.7, 1.5(±.8)	14.3, 1.0(±0)
Pronoprymna petrowi				
unknown nematode	2.6, 1.0*	8.5, 1.0(±0)		
unknown trematode	2.6, 1.0*		2.1, 1.0*	

Region 3: MN = Manchester, Northern California, PD = Point Delgada, Northern California, and Region 5: ESN = east of San Nicolas Island, Southern California

Table 2.5. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 241 migrant (> 200 mm SL) Pacific sardine (*Sardinops sagax*) in 2006. Single infected fish indicated by an asterisk (*). Parasites of individual fish caught off Montana de Oro State Park, California and San Nicolas Island, California are described in the text.

Parasite Species	VI November 16 (n=44) %, I(±SD)	WB May 12 (n=2) %, I(±SD)	WB June 13 (n=17) %, I(±SD)	WB July 18 (n=25) %, I(±SD)	WB August 15 (n=19) %, I(±SD)	WB August 29 (n=5) %, I(±SD)
Anisakis pegreffii	6.8, 1.0(±0)		11.8, 1.0(±0)	12.0, 1.0(±0)		
A. simplex C	6.8, 1.0(±0)			4.0, 1.0*		
A. simplex ss	18.2, 1.4(±.5)	100, 1.5(±.7)			5.3, 1.0*	
Anisakis species	6.8, 1.0(±0)			4.0, 1.0*		
Total *Anisakis* species[A]	34.1, 1.3(±.6)	100, 1.5(±.7)	11.8, 1.0(±0)	20.0, 1.0(±0)	5.3, 1.0*	
anisakid nematode	2.3, 1.0*		5.9, 3.0*			
Tetraphyllidean sp. 1		50.0, 1.0*				
Contracaecum margolisi						
C. rudolphi						
Hysterothylacium species	25.0, 1.3(±.6)	100, 2.0(±0)	23.5, 1.0(±0)	4.0, 1.0*	5.3, 1.0*	20.0, 1.0*
Lecithaster gibbosus	34.1, 2.9(±1.8)	50.0, 10.0*		4.0, 1.0*	5.3, 1.0*	
Myosaccium ecaude	4.5, 1.0(±0)		23.5, 1.0(±0)	4.0, 1.0*	31.6, 1.2(±.4)	20.0, 1.0*
Parahemiurus merus	18.2, 1.6(±.9)	50.0, 1.0*		5.9, 1.0*	5.3, 1.0*	
Pronoprymna petrowi	38.6, 7.7(±8.9)	50.0, 1.0*				
Rhadinorhynchus trachuri						
unknown nematode						

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 1: VI = Vancouver Island, British Columbia, and Region 2: WB = Willapa Bay, Washington

Table 2.5 Continued.

Parasite Species	CR May 27 (n=30) %, I(±SD)	CR September 25 (n=11) %, I(±SD)	NH September 18 (n=15) %, I(±SD)	PD April 24 (n=37) %, I(±SD)
Anisakis pegreffii	6.7, 1.5(±.7)	18.8, 1.0(±0)	6.7, 1.0*	8.1, 1.0(±0)
A. simplex 'C'				5.4, 1.0(±0)
A. simplex ss	6.7, 1.0(±0)			5.4, 1.0(±0)
Anisakis species	10.0, 1.0(±0)	18.2, 1.0(±0)		2.7, 1.0*
Total *Anisakis* species[A]	23.3, 1.1(±.4)	18.2, 2.0(±0)		21.6, 1.0(±0)
anisakid nematode				2.7, 1.0*
Tetraphyllidean sp. 1				
Contracaecum margolisi				
C. rudolphi				
Hysterothylacium species	16.7, 1.4(±.6)		6.7, 1.0*	45.9, 1.4(±.6)
Lecithaster gibbosus				8.1, 1.0(±0)
Myosaccium ecaude	3.3, 1.0*	9.1, 1.0*		5.4, 1.0(±0)
Parahemiurus merus	3.3, 1.0*		6.7, 1.0*	2.7, 1.0*
Pronoprymna petrowi				
Rhadinorhynchus trachuri	3.3, 1.0*			5.4, 1.0(±0)
unknown nematode				5.4, 1.0(±.0)

Region 2: CR = Columbia River, Oregon, NH = Newport, Oregon, and Region 3: PD = Point Delgada, Northern California

Table 2.5 Continued.

Parasite Species	MN April 25/26 (n=29) %, I(±SD)	ESN May 2 (n=5) %, I(±SD)
Anisakis pegreffii	3.4, 1.0*	20.0, 1.0*
A. simplex 'C'		
A. simplex ss	3.4, 1.0(±0)	
Anisakis species		
Total *Anisakis* species[A]	6.9, 1.0(±0)	20.0, 1.0*
anisakid nematode		20.0, 1.0*
Tetraphyllidean sp. 1		
Contracaecum margolisi		20.0, 2.0*
C. rudolphi		20.0, 1.0*
Hysterothylacium species	27.6, 1.0(±0)	60.0, 1.7(±.6)
Lecithaster gibbosus		
Myosaccium ecaude	17.2, 1.2(±.4)	20.0, 2.0*
Parahemiurus merus	3.4, 1.0*	
Pronoprymna petrowi		
Rhadinorhynchus trachuri		
unknown nematode		

Region 3: MN = Manchester, Northern California, and Region 5: ESN = east of San Nicolas Island, Southern California

Table 2.6. Prevalence (%) and mean intensity (I) ((±standard deviation, SD) of parasites recovered from 146 non-migrant (< 200 mm SL) Pacific sardine (*Sardinops sagax*) in 2007. Single fish indicated by an asterisk (*). Parasites of individual fish caught off Vancouver Island, British Columbia, Willapa Bay, Washington, and Golden Gate inner, California are described in the text.

Parasite Species	WB July 23 (n=2) %, I(±SD)	AS July 17 (n=2) %, I(±SD)	PP August 16 (n=8) %, I(±SD)	PD August (n=11) %, I(±SD)	PAR August 12/13 (n=10) %, I(±SD)	PAR August 17 (n=62) %, I(±SD)
Anisakis pegreffii			12.5, 1.0(±0)	9.1, 1.0*		4.8, 1.0(±0)
A. simplex 'C'			12.5, 1.0*	9.1, 1.0*		
A. simplex ss						1.6, 1.0*
Total *Anisakis* species[A]			25.0, 1.0(±.0)	18.2, 1.0(±0)		6.5, 1.0(±0)
anisakid nematode			12.5, 1.0*			
Hysterothylacium species			37.5, 1.3(±.6)	36.4, 2.0(±1.2)	30.0, 1.0(±0)	4.8, 1.0(±0)
Lecithaster gibbosus						
Myosaccium ecaude		50.0, 1.0*	50.0, 2.3(±2.5)	36.4, 1.3(±.5)	40.0, 1.8(±1.0)	54.8, 3.1(±5.7)
Parahemiurus merus	50.0, 1.0*	50.0, 1.0*			10.0, 1.0*	6.5, 1.4(±.7)
unidentifiable nematode				18.2, 1.0(±0)		
unknown nematode			12.5, 1.0*			

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 2: WB = Willapa Bay, Washington, AS = Astoria, Oregon, and Region 3: PP = Patrick's Point/Klamath River, Northern California, PD = Point Delgada, Northern California, PAR = Point Arena/Point Reyes, Northern California

Table 2.6 Continued.

Parasite Species	SL April 28 (n=4) %, I(±SD)	SN April 21 (n=44) %, I(±SD)
Anisakis pegreffii		2.3, 1.0*
A. simplex 'C'		
A. simplex ss		
Total *Anisakis* species[A]		2.3, 1.0*
anisakid nematode		
Hysterothylacium species	25.0, 1.0*	11.4, 1.0(±0)
Lecithaster gibbosus		
Myosaccium ecaude	50.0, 1.0(±0)	54.5, 1.6(±.7)
Parahemiurus merus	25.0, 1.0*	27.3, 1.1(±.3)
unidentifiable nematode		
unknown nematode		

Region 4: SL = Salmon Cone, Central California, and
Region 5: SN = San Nicolas Island, Southern California

Table 2.7. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 414 migrant (> 200 mm SL) Pacific sardine (*Sardinops sagax*) in 2007. Single fish indicated by an asterisk (*). Parasites from three fish caught off Point Arenas/Point Reyes, California and an individual fish caught off San Nicolas Island, California are described in the text.

Parasite Species	VI July 20 (n=51) %, I(±SD)	VI Aug 26/Sep 2 (n=30) %, I(±SD)	VI September 18 (n=36) %, I(±SD)	WB June 25 (n=49) %, I(±SD)	WB July 23 (n=40) %, I(±SD)	AS June 13 (n=51) %, I(±SD)
Anisakis pegreffii				2.0, 2.0*		2.0, 1.0*
A. simplex 'C'						
A. simplex ss						
Anisakis species	29.4, 1.4(±.7)	30.0, 1.7(±.9)	38.9, 1.5(±1.1)	30.6, 2.6(±1.9)	22.5, 1.6(±.9)	21.6, 1.5(±.9)
Total *Anisakis* species[A]	29.4, 14(±.7)	30.0, 1.7(±.9)	38.9, 1.5(±1.1)	30.6, 2.7(±1.9)	22.5, 1.6(±.9)	21.6, 1.5(±.9)
anisakid nematode						3.9, 1.0(±0)
Tetraphyllidean sp. 1				2.0, 1.0*		
Hysterothylacium species	15.7, 1.4(±.7)	10.0, 1.0(±0)	25.0, 1.1(±.3)	36.7, 1.9(±1.1)	32.5, 1.4(±.5)	7.8, 2.0(±1.2)
Lampitrema sp.						
Lecithaster gibbosus	17.6, 1.0(±0)	76.7, 4.0(±3.1)	97.2, 5.7(±3.7)	42.9, 4.3(±4.3)	2.5, 1.0*	19.6, 1.4(±.5)
Myosaccium ecaude	3.9, 1.5(±.7)		8.3, 1.3(±.6)		5.0, 1.0(±0)	7.8, 1.0(±0)
Parahemiurus merus	15.7, 1.4(±.5)	33.3, 1.3(±.7)	63.9, 2.5(±1.8)	2.0, 1.0*	10.0, 1.0(±0)	2.0, 1.0*
Pronoprymna petrowi			2.8, 1.0*			
Rhadinorhynchus trachuri	2.0, 2.0*			4.1, 1.5(±.7)		9.8, 1.2(±.5)
unidentifiable nematode			2.8, 1.0*			
unknown nematode				2.0, 2.0*		
unknown trematode					2.5, 1.0*	

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 1: VI = Vancouver Island, British Columbia, and Region 2: WB = Willapa Bay Washington, AS = Astoria, Oregon

Table 2.7 Continued.

Parasite Species	AS July 17 (n=48) %, I(±SD)	CH August 17 (n=10) %, I(±SD)	PP August 16 (n=24) %, I(±SD)	PD August (n=12) %, I(±SD)	PAR August 17 (n=24) %, I(±SD)	SL April 28 (n=35) %, I(±SD)
Anisakis pegreffii	2.1, 1.0*	40.0, 1.0(±0)	16.7, 1.3(±.5)		8.3, 1.0(±0)	17.1, 1.0(±0)
A. simplex 'C'	2.1, 1.0*					5.7, 1.0(±0)
A. simplex ss	2.1, 1.0*					11.4, 1.0(±0)
Anisakis species	25.0, 1.3(±.2)					2.9, 1.0*
Total *Anisakis* species[A]	29.2, 1.4(±.6)	40.0, 1.0(±.0)	16.7, 1.3(±.5)		8.3, 1.0(±0)	28.6, 1.3(+.9)
anisakid nematode						
Tetraphyllidean sp. 1						
Hysterothylacium species	25.0, 1.3(±.5)	30.0, 1.0(±0)	29.2, 1.7(±.8)	58.3, 1.1(±.4)	8.3, 2.0(±1.4)	14.3, 1.4(±.9)
Lampitrema sp.						2.9, 1.0*
Lecithaster gibbosus	6.3, 1.3(±.6)					
Myosaccium ecaude	8.3, 1.0(±0)	10.0, 1.0*	25.0, 1.7(±.9)	25.0, 1.3(±.6)	20.8, 1.8(±.8)	17.1, 3.0(±3.0)
Parahemiurus merus	8.3, 1.0(±0)	10.0, 1.0*	8.3, 1.0(±0)		8.3, 1.0(±0)	
Pronoprymna petrowi						
Rhadinorhynchus trachuri						5.7, 1.0(±0)
unidentifiable nematode			4.2, 1.0*			
unknown nematode	6.3, 1.3(±.6)					
unknown trematode						

Region 3: CH = Chetco River Northern California, PP = Patrick's Point/Klamath River, Northern California, PD = Point Delgada, Northern California, PAR = Point Arena/Point Reyes, Northern California, and Region 4: SL = Salmon Cone, Central California

Table 2.8. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 99 migrant (> 200 mm SL) Pacific sardine (*Sardinops sagax*) in 2008. Single fish indicated by an asterisk (*). Parasites from an individual non-migrant (< 200 mm SL) caught off Cape Meares, Oregon are described in the text.

Parasite Species	CR May 25 (n = 19) %, I(±SD)	CM May 23 (n=30) %, I(±SD)	VN April 25 (n=49) %, I(±SD)
Anisakis pegreffii			16.3, 1.1(±.4)
A. simplex 'C'			8.2, 1.5(±.6)
A. simplex ss B			10.2, 2.0(±1.0)
Anisakis species	84.2, 2.8(±3.2)	63.3, 1.8(±1.3)	18.4, 1.8(±1.6)
Total *Anisakis* species[A]	84.2, 2.8(±3.2)	63.3, 1.8(±1.3)	32.7, 2.6(±2.2)
C. rudolphi			2.0, 1.0*
Hysterothylacium species	10.5, 1.0(±0)	23.3, 1.1(±.4)	36.7, 1.8(±1.1)
Lampitrema sp.			
Myosaccium ecaude			10.2, 1.0(±0)
Parahemiurus merus	5.3, 1.0*	6.7, 1.0(±0)	4.1, 2.0(±1.4)
Rhadinorhynchus trachuri	5.3, 1.0*		6.1, 1.0(±0)
Trypanorhynch cestode		3.3, 1.0*	
unknown nematode	5.3, 1.0*		6.1, 1.0(±0)

A = all *Anisakis* species recovered includes taxonomically and genetically identified individuals, Region 2: CR = Columbia River, Oregon, CM = Cape Meares, Oregon, and Region 5: VN = Ventura, Southern California

Table 2.9. Prevalence (%) and mean intensity (I) (±standard deviation, SD) of parasites recovered from 168 northern anchovy (*Engraulis mordax*) in 2007 and 2008. Single infected fish indicated by an asterisk (*).

	2007		2008
	GH	WB	PH
	July 06	June 04	June 26
	(n=20)	(n=97)	(n=51)
Parasite Species	%, I(±SD)	%, I(±SD)	%, I(±SD)
Total *Anisakis* species[A]	10.0, 1.5(±.7)	5.2, 1.0(±0)	5.9, 1.0(±0)
Didymozoid trematode			41.2, 1.8(±.9)
Hysterothylacium species	5.0, 1.0*	7.2, 1.3(±.5)	17.6, 1.3(±.7)
Lecithaster gibbosus	45.0, 2.0(±1.6)		
Parahemiurus merus	5.0, 1.0*	2.0, 1.0(±0)	33.3, 1.3(±.5)
Tetraphyllidean sp. 1	5.0, 1.0*		
unknown nematode		11.3, 1.3(±.6)	9.8, 1.2(±.4)

A = all *Anisakis* species recovered only identified taxonomically, Region 2: GH = Gray's Harbor, Washington, WB = Willapa Bay, Washington, and Region 5: PH = Port Hueneme, Southern California

Table 2.10. Fisher's Exact tests comparing geographic recovery of the trematodes *Lecithaster gibbosus* and *Myosaccium ecaude* in non-migrant (<200mm SL) and migrant (>200mm SL) Pacific sardines (*Sardinops sagax*) caught in the California Current from 2005 through 2008. Comparisons among non-migrants are below the Xs, comparisons among migrants are above the Xs, and a dash means all fish were uninfected and was unable to compute a p-value.

A) *Lecithaster gibbosus*

		non-migrant sardines		migrant sardines	
		No. fish	% fish infected	No. fish	% fish infected
Region	Collection Location				
1	Vancouver Island, BC	43	32.09	176	51.14
2	Washington-Oregon	114	4.39	419	9.07
3	northern California	142	0	139	2.16
4	central California	152	0	36	0
5	southern California	112	0	56	0
Total No.		563		826	

Fisher's Exact test

Region	1	2	3	4	5
1	X	<0.0001	<0.0001	<0.0001	<0.0001
2	<0.0001	X	0.005	0.06	0.01
3	<0.0001	0.02	X	>0.99	0.56
4	<0.0001	0.01	-	X	-
5	<0.0001	0.06	-	-	X

Table 2.10 Continued.

B) *Myosaccium ecaude*

Region	Collection Location	non-migrant sardines No. fish	non-migrant sardines % fish infected	migrant sardines No. fish	migrant sardines % fish infected
1	Vancouver Island, BC	43	9.30	176	3.98
2	Washington-Oregon	114	11.40	419	4.16
3	northern California	142	41.55	139	15.83
4	central California	152	32.24	36	19.44
5	southern California	112	57.14	56	10.71
Total No.		563		826	

Fisher's Exact test

Region	1	2	3	4	5
1	X	0.19	0.0003	0.003	0.09
2	>0.99	X	0.004	0.02	0.42
3	<0.0001	<0.0001	X	0.62	0.50
4	0.003	<0.0001	0.12	X	0.36
5	<0.0001	<0.0001	0.02	<0.0001	X

Figure Titles

Figure 2.1. Geographic location of stations (identified by solid circles or gray arrows) where a total of 1389 Pacific sardines (*Sardinops sagax*) were caught in the California Current during: (A) 2005, (B) 2006, (C) 2007, and (D) 2008. The 200 m isobath is depicted by the solid black line oriented approximately north to south, west of the provincial and state coastlines.

Figure 2.2. Standard Length (SL in mm) size distribution Pacific sardines (*Sardinops sagax*) caught in five regions of the California Current from 2005 through 2008. (A) All fish whether, (B) Only those fish infected with *L. gibbosus*, and (C) Only those fish infected with *M. ecaude*. Region 1: off Vancouver Island, British Columbia (BC); Region 2: Washington (WA) and Oregon (OR); Region 3: Northern California (CA); Region 4: Central California (CA), and Region 5: Southern California (CA).

Figure 2.3. Non-metric multi-dimensional scaling (MDS) plots using parasite mean abundance data from non-migrant (≤200 mm SL) and migrant (>200 mm SL) Pacific sardine (*Sardinops sagax*). Individual fish were categorized by their collection locations from (A) 2005, (B) 2006, (C) 2007, and (D) 2008. Collection locations were from the following regions: Region 1: Vancouver Island, BC; Region 2: Willapa Bay, WA, Columbia River, OR, Astoria, OR, Cape Meares, OR, and Newport, OR; Region 3: Chetco River, CA, Patrick's Point/Klamath River, CA, Point Delgada, CA, Manchester, CA, and Point Arena/Point Reyes, CA; Region 4: Golden Gate, CA, Santa Cruz, CA, Salmon Cone, CA, and Montana de Oro State Park, CA; and Region 5: Ventura, CA, San Nicolas Island, CA, and east of San Nicolas Island, CA. The names of parasites outside the ordination graphs indicate species associated with collection locations. For clarity, only parasite genus names are included on the figures.

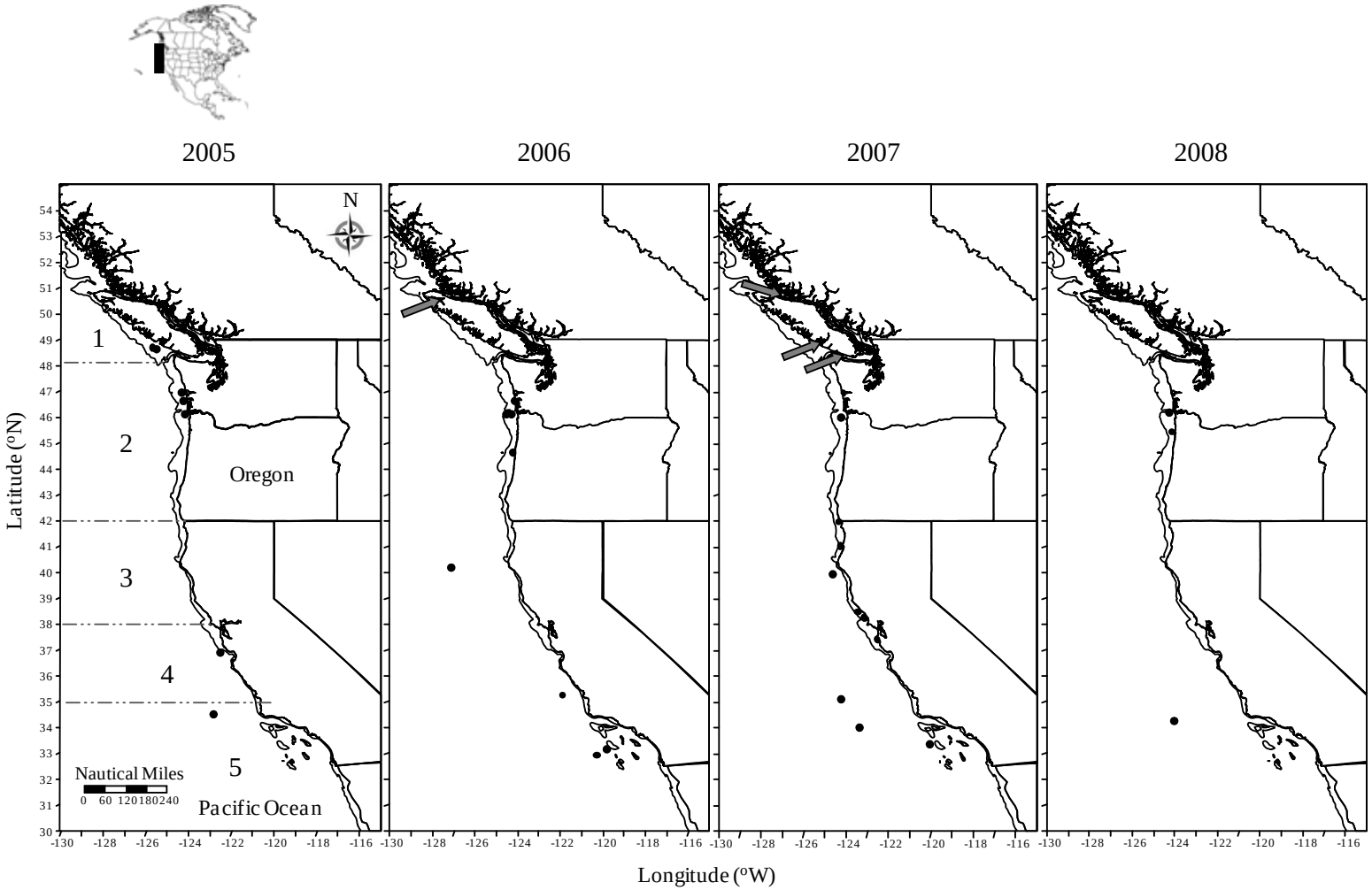
2005
2006
2007
2008
N
1
2
3
4
5
Oregon
Pacific Ocean
Nautical Miles
0 60 120180240
Latitude (°N)
Longitude (°W)

Figure 2.1.

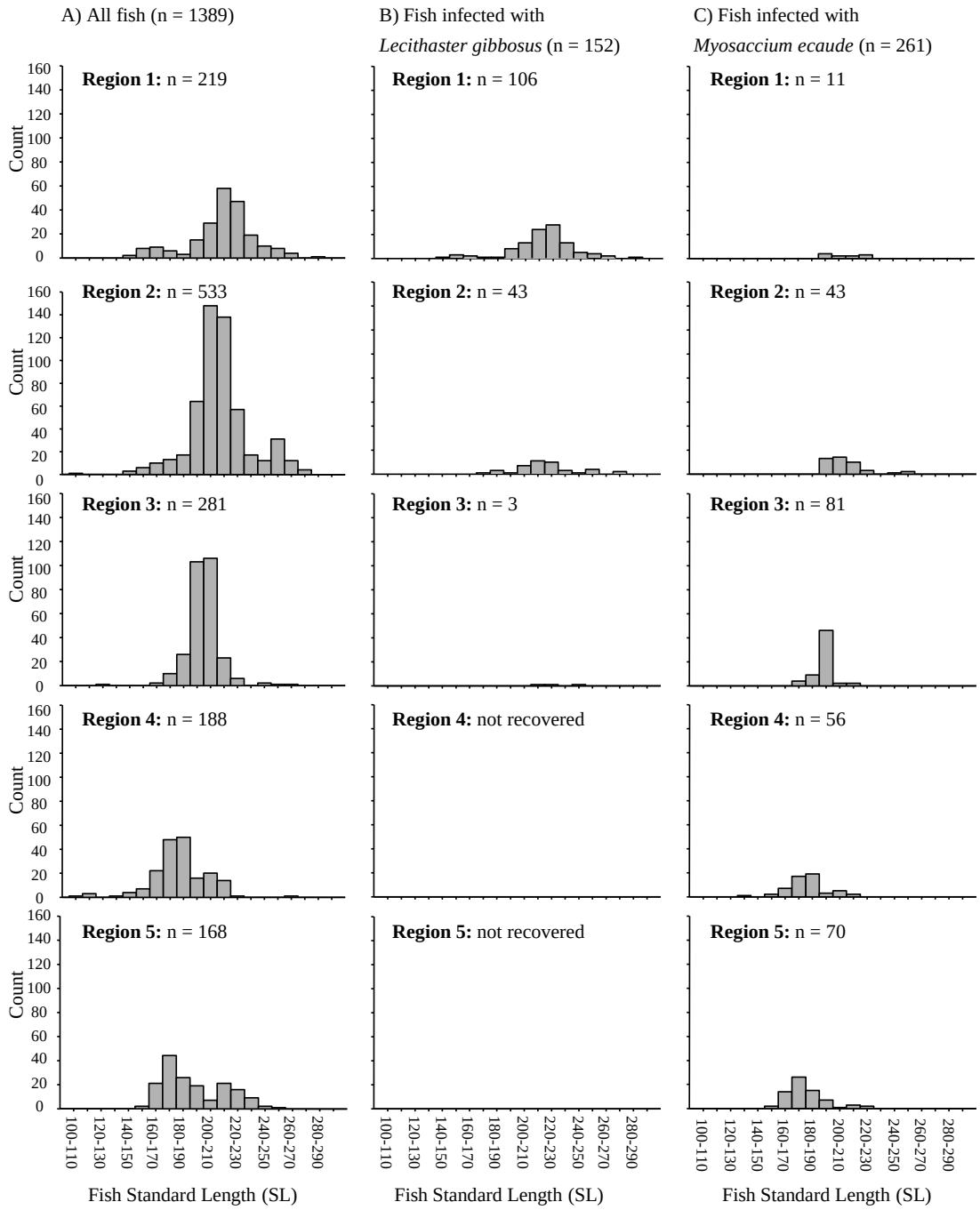
A) All fish (n = 1389)
B) Fish infected with *Lecithaster gibbosus* (n = 152)
C) Fish infected with *Myosaccium ecaude* (n = 261)
Region 1: n = 219
Region 1: n = 106
Region 1: n = 11
Region 2: n = 533
Region 2: n = 43
Region 2: n = 43
Region 3: n = 281
Region 3: n = 3
Region 3: n = 81
Region 4: n = 188
Region 4: not recovered
Region 4: n = 56
Region 5: n = 168
Region 5: not recovered
Region 5: n = 70
Count
0 20 40 60 80 100 120 140 160
100-110 120-130 140-150 160-170 180-190 200-210 220-230 240-250 260-270 280-290
Fish Standard Length (SL)

Figure 2.2

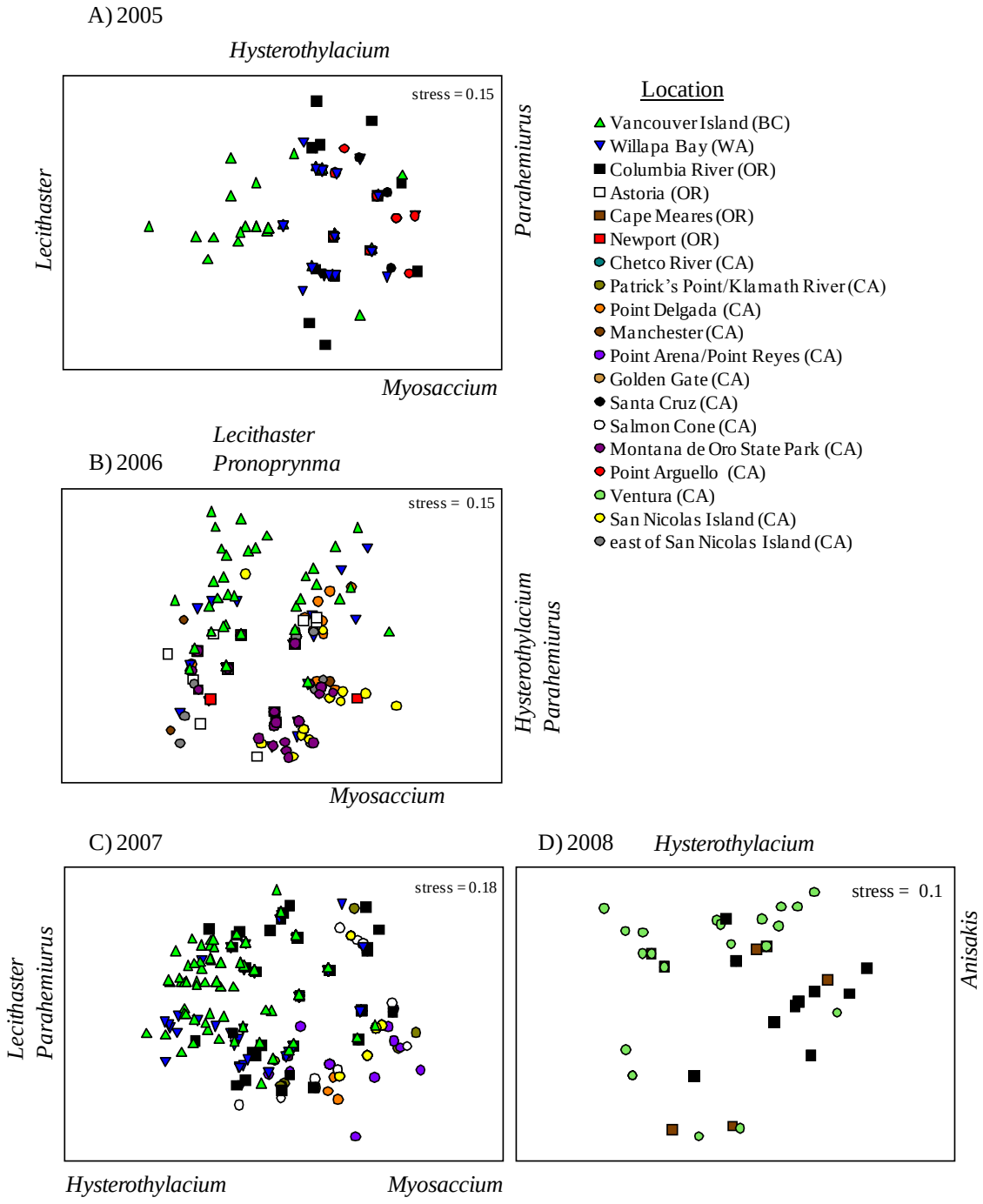
A) 2005
Hysterothylacium
stress = 0.15
Lecithaster
Parahemiurus
Myosaccium
Location
Vancouver Island (BC)
Willapa Bay (WA)
Columbia River (OR)
Astoria (OR)
Cape Meares (OR)
Newport (OR)
Chetco River (CA)
Patrick's Point/Klamath River (CA)
Point Delgada (CA)
Manchester (CA)
Point Arena/Point Reyes (CA)
Golden Gate (CA)
Santa Cruz (CA)
Salmon Cone (CA)
Montana de Oro State Park (CA)
Point Arguello (CA)
Ventura (CA)
San Nicolas Island (CA)
east of San Nicolas Island (CA)
B) 2006
Lecithaster
Pronoprynma
stress = 0.15
Hysterothylacium
Parahemiurus
Myosaccium
C) 2007
stress = 0.18
Lecithaster
Parahemiurus
Hysterothylacium
Myosaccium
D) 2008
Hysterothylacium
stress = 0.1
Anisakis

Figure 2.3.

PANMIXIA RATHER THAN GEOGRAPHICALLY SEGREGATED HAPLOTYPES OBSERVED IN THREE SPECIES OF *ANISAKIS* NEMATODES RECOVERED FROM PACIFIC SARDINE (*SARDINOPS SAGAX*) DISTRIBUTED THROUGHOUT THE CALIFORNIA CURRENT SYSTEM

Rebecca E. Baldwin, Mary Beth Rew, Mattias L. Johansson, Michael A. Banks, Kym C. Jacobson

Journal of Parasitology

Department of Biology

Wake Forest University

P.O. Box 7629

Winston-Salem, North Carolina, 27109

(In Prep)

Abstract

Members of the nematode family Anisakidae are known to infect over 200 pelagic fish species and have been frequently used as biological tags to identify fish populations. Despite information on the global distribution of *Anisakis* species, there is little information on the genetic diversity and population structure of this genus, which could be useful in assessing the stock structure of their fish hosts. From 2005 through 2008, 148 larval anisakids were recovered from Pacific sardine (*Sardinops sagax*) in the California Current upwelling zone and were genetically sequenced. Sardines were captured off Vancouver Island, British Columbia in the north to San Diego, California in the south. Three species, *Anisakis pegreffii*, *A. simplex* 'C' and *A. simplex s.s.* were identified using sequences from the internal transcribed spacers (ITS1 and ITS2) and the 5.8s subunit of the nuclear ribosomal DNA. The degree of nematode population structure was assessed using the cytochrome c oxidase 2 (*cox2*) mitochondrial DNA gene. All three *Anisakis* species were distributed throughout the study region from 32^{o}N - 50^{o}N latitude. There was no association between sardine length and either nematode infection intensity or *Anisakis* species recovered. Larval *Anisakis* species and mitochondrial haplotype distributions from both parsimony networks and AMOVA analyses revealed a panmictic distribution of these parasites which infect sardines throughout the California Current ecosystem using. Panmictic distribution of the larval *Anisakis* populations may be a result of the presumed migratory pathways of the intermediate host (the Pacific sardine), moving into the northern portion of the California Current in summer and returning to the southern portion to overwinter and

spawn in spring. However, the wider geographic range of paratenic (large piscine predators), and final hosts (cetaceans) can also explain the observed distribution pattern. As a result, the recovery of three *Anisakis* species and a panmictic distribution of their haplotypes could not be used to confirm or deny the presence of population subdivision of Pacific sardines in the California Current system.

Introduction

Anisakid nematodes are known to infect more than 200 species of pelagic fish (Sabater and Sabater 2000), and have been used as biological tags for fish population structure studies (see review by MacKenzie 2002). *Anisakis* species use euphausiids as their obligate first intermediate host, fish or squid as second intermediate or paratenic (transport hosts where no development occurs), and cetaceans as definitive hosts (Oshima 1972; Smith and Wooten 1978). With the availability of molecular markers, morphologically similar larvae of *Anisakis* species recovered globally have been separated into nine genetically distinct species comprising two clades (Mattiucci et al. 2009). Despite the growing information on the global distribution of *Anisakis* species (see review by Mattiucci and Nascetti 2008; Klimpel et al. 2010) there is little information on the genetic diversity and population structure of these nematodes, which could be useful in assessing the stock structure of their fish hosts.

In the marine environment there are few obvious physical barriers limiting gene flow between fish populations. Nevertheless, some fish species have been designated as

distinctively and geographically separated stocks or subpopulations based on genetic identification of *Anisakis* species. For example, separate stocks of the European hake (*Merluccius merluccius*) were identified within the Mediterranean Sea and Atlantic Ocean based on the distribution of seven species of *Anisakis* identified using allozymes (Mattiucci et al. 2004). More recently, parasite community analysis (MacKenzie et al. 2008) and allozyme data from five *Anisakis* species (Mattiucci et al. 2008) were used to identify separate stocks of the Atlantic horse mackerel (*Trachurus trachurus*) within the Atlantic Ocean (Western and Southern) and North Sea. Atlantic horse mackerel were further separated into three different stocks within the Mediterranean Sea with parasite data indicating the potential of fish exchange between the Atlantic southern stock and the west Mediterranean stock (Abaunza et al. 2008). In addition, Cross et al. (2007) suggested that *Anisakis simplex s.s.* may be a suitable biological tag for Atlantic herring (*Clupea harengus*), since this nematode species can be recovered throughout the year.

The Pacific sardine (*Sardinops sagax*) is an economically and ecologically important forage fish that transfers energy resources from planktonic primary producers and secondary consumers to upper trophic predators (Cury et al. 2000). Pacific sardine allozyme (Hedgecock et al. 1989) and mitochondrial DNA (mtDNA) data (Grant et al. 1998; Lecomte et al. 2004) suggest a panmictic population with a shallow genetic structure. However, there is some evidence of more than one subpopulation within the California stock management unit based on the recovery of larger individuals at higher

latitudes (Clark and Janssen 1945; Hill 1999; Emmett et al. 2005; McFarlane et al. 2005) and a temporal difference in sardine spawning off the Pacific Northwest versus Southern California (Emmett et al. 2005; Smith 2005). Furthermore, the potential for subpopulations with connectivity poses questions to the long-standing paradigm of an annual migration of individuals to feeding grounds off the Pacific Northwest in the summer with migrants returning to Southern California in the fall to spawn the following spring (Clark 1935). This accepted coastwide migration pattern was described with mark-recapture tagging studies prior to the fishery collapse in the 1940s (Janssen 1938; Clark and Janssen 1945). It is unclear if this exact pattern of migration has been reestablished since the return of Pacific sardines to Pacific Northwest and Canadian waters in the 1990s, or if there are some sardine subpopulations along the west coast with limited latitudinal migrations. The main goals of our study were to identify which *Anisakis* species infect Pacific sardine in the California Current, examine the genetic diversity and population structure of nematodes collected from sardines sampled off of Vancouver Island, British Columbia to San Diego, California, and assess the potential of using *Anisakis* species as a biological tag to help discriminate stocks of Pacific sardine.

Materials and Methods

Study area and parasite collection

From 2005 through 2008 a total of 1339 Pacific sardine were opportunistically recovered in the California Current (Table 3.1) between 32^{o} to 50^{o}N latitude, and 119^{o}

to 128^{o}W longitude (Figure 3.1). We divided the study area into five geographic regions: 1) Vancouver Island, British Columbia (part of PNW); 2) Washington and Oregon (part of PNW); 3) Northern California; 4) Central California; and 5) Southern California. Sardines from Canadian waters were caught using a modified Cantrawl 240 rope trawl (Cantrawl Nets Ltd., Richmond, BC; see Morris et al. 2007 for details), and sardines from Washington to California were caught using a 30 m wide by 20 m deep mouth-opening 264 rope trawl (Nor'Eastern Trawl Systems, Inc. Bainbridge Island, WA, U.S.A.; see Baldwin et al. 2008 for details). One tow of the net equaled one trawling event. Captured Pacific sardine were immediately frozen onboard and stored in the lab at -80^{o}C until processed for parasites. After being thawed, each fish was weighed to the nearest 0.1 g, and standard length (SL) measured to the nearest mm. Fresh SL of individual frozen sardine was estimated using the following regression: Fresh SL = 2.89 + 1.0286 (Frozen and then Thawed SL) (Lo et al. 2007). *Anisakis* nematodes were recovered from stomachs, intestines and body cavities according to standard necropsy procedures (Arthur and Albert 1994). A total of 191 nematodes belonging to the family Anisakidae were collected from these sardines and preserved in 95% ethanol.

Extraction, DNA amplification and sequencing

DNA was extracted from nematode tissue using a glass fiber plate DNA extraction protocol (Ivanova et al. 2006). Molecular markers were required to genetically identify larval *Anisakis* nematodes to species at two diagnostic nucleotide sites (Abollo et al.

2003; Nadler et al. 2005; Abe 2008), we used the polymerase chain reaction (PCR) to amplify a region including the internal transcribed spacers (ITS-1, ITS-2) and 5.8S subunit of the nuclear ribosomal DNA (rDNA) (hereafter referred to as ITS markers) using the forward primer #93 (5'-TTGAACCGGGTAAAAGTCG) and the reverse primer #94 (5'-TTACTTTCTTTTCCTCCGCT) (Nadler et al. 2005). All PCR reactions had a final volume of 20 μL comprised of 2 μL genomic DNA, 0.25 μM each forward and reverse primer, 0.25 mM deoxynucleoside triphosphates (dNTPs), 1.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit *Taq* DNA polymerase (Promega, Madison, Wisconsin). The temperature and cycling parameters included denaturation at 94^{o}C for 2 min, followed by 30 cycles at 94^{o}C for 30 sec, 53^{o}C for 30 sec, 72^{o}C for 45 sec, followed by postamplification extension at 72^{o}C for 10 min. To examine the population structure of anisakid nematodes, we amplified the mitochondrial DNA (mtDNA) *cox2* gene using the forward primer 210 (5'-CACCAACTCTTAAAATTATC) and the reverse primer 211 (5'-TTTTCTAGTTATATAGATTGRTTYAT) (Nadler and Hudspeth 2000). Modified from Valentini et al. (2006), all PCR reactions had a final volume of 20 μL comprised of 2 μL genomic DNA, 0.3 μM of each forward and reverse primer, 0.4 mM dNTPs, 2.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit *Taq* DNA polymerase (Promega, Madison, Wisconsin). The PCR temperature and cycling parameters included denaturation at 94^{o}C for 3 min, followed by 34 cycles at 94^{o}C for 30 sec, 46^{o}C for 1 min, 72^{o}C for 1 min and 30 sec, and postamplification extension at 72^{o}C for 10 min. All ITS and *cox2* PCR products were cleaned for direct nucleotide sequencing using an ExoSap-IT

clean-up protocol (GE Healthcare, Piscataway, New Jersey). Cycle sequencing was conducted using ABI-PRISM Big Dye terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, California), and DNA sequences were cleaned using a Sephadex protocol (GE Healthcare, Piscataway, New Jersey). Sequences were analyzed with an ABI 3730xl DNA automated sequencer (Applied Biosystems). DNA sequences were edited using BioEdit 7.0.1 (Hall 1999) and aligned with ClustalW (Thompson et al. 1994) following the default parameters. For each nematode species, all unique sequences were deposited into GenBank under the following accession numbers: *A. pegreffii* (XX), *A. simplex s.s.* (XX), and *A. simplex* 'C' (XX).

Data analysis

Estimated sardine standard lengths (SL) mm among regions were not normally distributed (Kolmogorov-Smirnov Test p-value 0.001), and variances were uneven among regions (Levene's Test p-value = 0.002; SPSS PASW Statistics 18). Thus for each year, Mann-Whitney U tests were used to compare estimated sardine SL of uninfected and infected sardine between regions and between nearshore and offshore samples. Fish collected east of the 200 m isobath (approximating the continental shelf break) were classified as inshore samples, and fish collected west of this line were considered offshore samples (Figure 3.1). A Mann-Whitney U test was also used to examine parasite species abundance between regions for fish caught nearshore versus offshore. We tested whether there was an effect of host size on parasite accumulation for each nematode species using Spearman's correlations (Ambrose and Ambrose

1987). All Mann-Whitney U tests and Spearman's correlations were calculated in Statview® (SAS, 1998). For each geographic region designated in the California Current, we calculated prevalence and intensity for each *Anisakis* species according to Bush et al. (1997).

For each nematode species we used DNAsp (v.5.00.07) (Librado and Rozas 2009) to calculate standard statistics: 1) haplotype diversity (h), the proportion of unique haplotypes recovered, 2) the number of polymorphic sites, 3) nucleotide diversity (π), the species-wide average number of nucleotide differences per site between two sequences, and 4) Tajima's D (Tajima 1989) to test for neutral selection among all molecular mutations, and to determine if the population size is constant (value near zero), shrinking (positive value) or growing (negative value). The number of unique haplotypes and mean pairwise differences among *cox2* sequences within and among species were determined using Arlequin 3.1 (Excoffier et al. 1992). Using an analysis of molecular variance (AMOVA) for each *Anisakis* species, genetic variation was attributed to its variance components (within and among region variation) where a negative value suggests the % variation is close to zero, and Phi_{ST} (Φ_{ST}) values (analogous to Fst values) were calculated using permutational estimates of significance in Arlequin 3.1 (Excoffier et al. 1992). Phi_{ST} (Φ_{ST}) values have a maximum value of 1, where 0 indicates no differentiation among sequences and 1 indicates complete differentiation among sequences from pre-defined regions. The

program TCS 1.13 (Clement et al. 2000) was used to create statistical parsimony networks of *cox2* haplotypes for each *Anisakis* species.

Parsimony networks were recalculated to assess the similarity among *cox2* sequences from our study and *cox2* sequences previously deposited in GenBank for *A. simplex s.s.*, *A. simplex* 'C' and *A. pegreffii* (Table 3.2). For sequences that were not included in a network at a 95% confidence level, we used a maximum of 100 steps to force sequences into the network to determine the number of steps from which these sequences differed from the main network. An AMOVA was used to compare the genetic subdivision between *A. simplex s.s.* sequences from Pacific sardine from the California Current (this study) to *A. simplex s.s.* recovered off Japan from walleye pollock (*Theragra chalcogramma*) (Quiazon et al. 2009) and chub mackerel (*Scomber japonicus*) (Suzuki et al. 2009).

Results

General Pacific Sardine Information

A total of 1339 sardines were processed during this study. These sardines had an estimated fresh standard length (SL) ranging from 100.61 to 285.76 mm (Table 3.1). The 809 fish caught nearshore were larger (mean 205.31 $\pm$ 21.76 mm with a mean rank of 747.84) than the 530 fish caught offshore (mean 193.23 $\pm$ 21.54 mm with a mean rank of 551.18; z-value = -9.10, p-value < 0.0001). Except for one sardine off of Newport, Oregon (estimated SL = 100.61 mm), the smallest sardines were recovered

in 2005 from region 4 (Central California; minimum estimated SL = 106.78 mm), and the largest were caught in 2006 from region 1 (Vancouver Island, British Columbia; maximum estimated SL = 285.76 mm).

A total of 9.1% of sampled sardines were infected with Anisakid nematodes. There was no difference in estimated SL for infected fish caught nearshore versus offshore when all years were combined (z-value = -0.92, p-value = 0.36) or for each year (2006: z-value = -0.84, p-value = 0.40; 2007: z-value = -0.82, p-value = 0.41). No nearshore versus offshore comparison could be made for 2005 (sampled only nearshore) or 2008 (sampled only offshore). Regionally, infected fish were smaller in region 1 (Vancouver Island, British Columbia; 198.02 $\pm$ 9.50mm, mean rank = 2.25) than in region 2 (Washington and Oregon, 248.60 $\pm$ 20.77mm; z-value = -2.01, p-value = 0.04; mean rank = 10.41) in 2005. However, in 2006 infected fish were larger in region 1 than region 2 (z-value = -3.63, p-value = 0.0003; region 1 mean rank = 28.29; region 2 mean rank = 14.74), and region 3 (Northern California) (z-value = -3.29, p-value = 0.001, region 1 mean rank = 20.12, region 3 mean rank = 9.46). In 2007 infected fish from region 3 were smaller than infected fish in region 2 (z-value = -1.93, p-value = 0.05; region 2 mean rank = 14.58; region 3 mean rank = 21.20). No comparison between regions was possible for 2008 since all fish used in this study from that year were caught in region 5 (Southern California). There were no correlations between the estimated fresh SL of infected fish and intensity of any

Anisakis species for any region (Rho= -0.59–0.50, p-value = 0.10–0.90) or by year (Rho = -0.011–0.51, p-value = 0.06–0.96).

Anisakis Species Recovery and Genetics Summary

Nematode intensity ranged from 1 to 4 worms per host, with most infected fish harboring a single worm (94 out of 122 fish). Of the 191 nematodes collected from five geographic regions, DNA was obtained from 148 nematodes. To identify to species, an 848 bp portion of ITS rDNA, which spanned two diagnostic sites, was used. Three genetically distinct species from the *Anisakis simplex* complex were recovered throughout the study area: *A. pegreffii* (n = 76), *A. simplex s.s.* (n = 51), and *A. simplex* 'C' (n = 21, Table 3.3 and Figure 3.1). Nine fish (7.3%) were infected with more than one species of *Anisakis*. Six of these fish were caught in regions 4 and 5 (California), two in Region 2 (Columbia River, Oregon) and one in Region 1 (Vancouver Island, British Columbia).

The population structure of each species of the *Anisakis simplex* complex was assessed using a 524 bp portion of the *cox2* mtDNA gene (Table 3.3). Shared haplotypes were highest for *A. pegreffii* (n = 10) followed by *A. simplex s.s.* (n = 3) and *A. simplex* 'C' (n = 2). *Anisakis simplex s.s.* had the most unique haplotypes (n = 48) followed by *A. pegreffii* (n = 33) and *A. simplex* 'C' (n = 17) (Table 3.3, Figure 3.2). Among the three *Anisakis* species, haplotype diversity ranged from 0.942 to 0.998 with a total of 98 unique sequences recovered from 148 individual worms. Nucleotide diversity ranged

from 0.007 to 0.018, and the number of polymorphic sites ranged from 23 to 79. All three *Anisakis* species had negative Tajima's D values, suggesting each *Anisakis* species may be increasing in population size, but these values were significant only for *A. simplex s.s.* and *A. simplex* 'C' (Table 3.3). The mean pairwise sequence differences within species were: 0.70% (*A. simplex* 'C.), 1.4% (*A. simplex s.s.*), and 1.8% (*A. pegreffii*). Corrected mean pairwise differences among the three species were higher than within each species: 3.1% (*A. pegreffii* versus *A. simplex s.s.*), 4.7% (*A. pegreffii* versus *A. simplex* 'C') and 5.4%. (*A. simplex s.s.* versus *A. simplex* 'C'), providing further evidence that three separate *Anisakis* species were recovered.

Geographic separation among *cox2* sequences was minimal for each *Anisakis* species when the individual worms were compared from the northern and southern ends of the study region (Table 3.4). Genetic variation was associated with differences within regions, as opposed to differences among populations resulting in non-significant AMOVA Φ_{ST} values ranging from -0.06 to 0.07. The remaining regional comparisons that were geographically closer were also non-significant (data not presented). Additionally, Φ_{ST} values (-5.88 to 2.46) were non-significant (p-values ranged from 0.10 to 0.52) for nearshore versus offshore nematode sequences compared throughout the study region. In contrast, a $\Phi_{ST} = 0.46$ (Table 3.4) was significantly different (p-value < 0.0001) with 46.28% of the variance explained by differences among regions of *A. simplex s.s.* from walleye pollock (data from Quiazon et al. 2009) and chub

mackerel (data from Suzuki et al. 2009) collected off Japan, and Pacific sardine caught in the California Current (this study).

For *Anisakis* species recovered in the California Current, the lack of distinct population structure associated with defined geographic regions was also evident in the *cox2* parsimony networks based on 524 bp of the sequence data (Figure 3.2). There was no separation by region within the network for individual *cox2* haplotypes, and shared *cox2* haplotypes were observed from multiple regions with 95% confidence. An additional sequence *A. simplex s.s.* from Region 2 was forced into the main network by 23 steps (Figure 3.2C). A similar pattern was observed in the parsimony networks based on 507 bp when 29 GenBank *cox2* sequences of the three *Anisakis* species were compared to our sequences (Figure 3, Table 3.2). Seventeen base pairs were removed from our sequences to enable an alignment with the GenBank sequences. Eight *A. pegreffii* sequences from GenBank fit into our network with 95% confidence, five of which were identical to sequences in our study (Figure 3.3A). Only four of the five *A. simplex* 'C' sequences fit into our network with 95% confidence (Figure 3.3B). When forced, the remaining *A. simplex* 'C' sequence was seven steps away from the main network. Ten *A. simplex s.s.* GenBank sequences fit into the network with 95% confidence, five of which were identical to our sequences. An additional sequence *A. simplex s.s.* from our data and six *A. simplex s.s.* GenBank sequences were forced into the main network by either 21 or 23 steps (Figure 3.3C).

Discussion

We recovered larval *A. pegreffii*, *A. simplex s.s.*, and *A. simplex* 'C' throughout the California Current in Pacific sardines. Analyses of the *cox2* haplotypes supported a panmictic distribution of larval *Anisakis* species in the California Current for all three *Anisakis* species. For each, haplotype diversity was high, nucleotide diversity was low, and related haplotypes among each species were distributed throughout our study area. This overall pattern of molecular variation across a large geographical region was similar to previous studies examining mtDNA of *A. simplex s.s.* infecting Atlantic herring (Cross et al. 2007), and parasitic nematodes infecting livestock (Blouin et al. 1995).

The ability of AMOVA to detect regional differences in haplotype diversity is well founded. Population subdivision was described among different river basins using AMOVA analyses for parasitic nematodes infecting freshwater fish. For example, Mejía-Madrid et al. (2007) observed 19.3% variation among seven river basins in Central Mexico for cytochrome c oxidase subunit 1 (*COI*) sequences of *Rhabdochona lichtenfelsi*. In addition, Wu et al. (2009) observed 46.6% variation among *COI* sequences of *Camallanus cottis* among three river basins in China, identifying haplotypes unique to the Pearl River compared to the Yangtze and Minjiang Rivers. Regional differences in our study were only detected by AMOVA when sequences of *A. simplex s.s.* from the California Current were compared to *A. simplex s.s.* sequences collected off Japan (Quiazon et al. 2009; Suzuki et al. 2009). If different anisakid

populations are observable in the Pacific Ocean only at the basin scale, then it is unlikely the population structure of Pacific sardines in the California Current can be determined using the recovery or population genetics of *Anisakis* nematodes.

Our *cox2* data support the hypothesis that host movement strongly influences the population structure of parasites (Jarne and Theron 2001). The Pacific sardines in this study are considered part of the Central California Offshore subpopulation and thought to be capable of migrating between Vancouver Island, British Columbia and San Diego, California (Dahlgren 1936; Hart 1943; Ahlstrom 1957; Smith 2005). In the *Anisakis* nematode life cycle, cetacean definitive hosts likely comprise the most mobile hosts, traveling thousands of kilometers during their annual migrations while dispersing nematode eggs. For example, humpback whales (*Megaptera novaeangliae*) are capable of traveling latitudinally between Mexico and Alaska (Lagerquist et al. 2008), and longitudinally between Japan and British Columbia (Perrin et al. 2009). With approximately 200 pelagic fish species known to be paratenic hosts for *Anisakis* species (Sabater and Sabeter 2000), opportunities for gene flow between geographically distant and potentially distinct populations of *Anisakis* species likely result from both migrating fish species and cetacean hosts (Nadler 1995; Cross et al. 2007; Mattiucci and Nascetti 2008). Thus, the panmixia of haplotypes found in our study could be a reflection of the extensive movement of all of the potential hosts utilized by these nematodes.

Our results suggest that the limited oceanographic barriers and complexity in the California Current are not preventing the mixing of anisakid species or populations. The major biogeographic break in the California Current at Point Conception, California, does not appear to limit the distribution of highly migratory fish or cetacean taxa (Checkley and Barth 2009) that propagate *Anisakis* species. Further, the hydrography of the north-south oriented California Current is considered less complex than the hydrography of the Atlantic Ocean from Europe to northwest Africa where five major currents interact along a European coastline that alternates between an east-west and north-south orientation (Checkley et al. 2009). The phylogeographic breaks located in the Mediterranean Sea (Peloponnesian) and the Atlantic-Mediterranean transition zone (Gibraltar and Oran-Almería) (Patarnello et al., 2007; Sala-Bozano et al. 2009) limit the movement of European sardines (*Sardina pilchardus*), as well as other fishes and cetaceans resulting in the recovery of different *Anisakis* species in the Mediterranean Sea and Atlantic Ocean. For example *A. pegreffii* was most common in the Mediterranean Sea, and *A. simplex s.s.* was most prevalent in the northeast Atlantic Ocean for both European hake (Mattiucci et al. 2004) and Atlantic horse mackerel (Mattiucci et al. 2008).

Our observations differ from the disjointed geographical recovery of *Anisakis* nematodes in European hake (Mattiucci et al. 2004), Atlantic horse mackerel (Mattiucci et al. 2008), and European sardines. To date European sardines infected with *Anisakis* spp. have been observed only along the Adriatic coast off Italy

(Fioravanti et al. 2006), and off western Portugal (Silva and Eiras 2003). No *Anisakis* sp. were observed in sardines off Northwest Spain in Galician waters (Abollo et al. 2001), Southern and Eastern Spain (Rello et al. 2008), or off Western Africa by Morocco and Mauritania (Kijewska et al. 2009). Larval *Anisakis* species in European sardines have not yet been genetically identified to species. However, the geographic distribution of genetically identified *Anisakis* in European hake (Mattiucci et al. 2004) and horse mackerel (Mattiucci et al. 2008) suggest that *A. simplex s.s.* and *A. pegreffii* could infect sardines off Portugual and *A. pegreffii*, *A. typica* and *A. physeteris* could infect sardines off Italy. Once *Anisakis* nematodes from European sardines are genetically identified, and a population genetic study is conducted for each *Anisakis* species, it can be determined if the panmictic distribution of the three *Anisakis* species in Pacific sardine is unique because of the hydrography of the California Current.

In summary, the distribution and population structure of *Anisakis* species throughout the California Current could suggest a single population of Pacific sardine. Just as elevated gene flow in several marine fish species obscures geographic structuring of genetic variation (Waples 1998), gene flow among marine parasites would also connect subpopulations from distant geographic locations. However, the diversity and availability of fish and cetacean species that undergo extensive migrations along the full length of the California Current system may enable large geographically distributed population sizes of anisakids. Thus we cannot confirm or deny the existence of Pacific sardine subpopulations within the California Current by the

distributional patterns of *Anisakis* species. Complex oceanographic conditions and host migrations may influence the genetic diversity and population structure of *Anisakis* species along other coastlines. Investigation of these influences could clarify whether high genetic diversity and connectivity among anisakid populations over large geographical distances is a common pattern, or whether hydrography can restrict gene flow of a widely dispersed generalist marine parasite.

Acknowledgements

We would like to thank R. Emmett, M. Blouin, V. Lesser, J. Bolte, E. Casillas, and JoAnne Butzerin for providing comments on earlier versions of this manuscript.

References

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Rarnos, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fisheries Research **89**:196-209.

Abe, N. 2008. Application of the PCR-sequence-specific primers for the discrimination among larval *Anisakis simplex* complex. Parasitology Research **102**:1073-1075.

Abollo, E., C. Gestal, and S. Pascual. 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitology Research **87**:492-499.

Abollo, E., L. Paggi, S. Pascual, and S. D'Amelio. 2003. Occurrence of recombinant genotypes of *Anisakis simplex s.s.* and Anisakis pegreffii (Nematoda: Anisakidae) in an area of sympatry. Infection, Genetics and Evolution **3**:175-181.

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Ambrose III, H. W., and K. P. Ambrose. 1987. A Handbook of Biological Investigation, Fourth edition. Hunter Textbooks Inc, Winston-Salem, NC.

Arthur, J. R., and E. Albert. 1994. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **50**:2175-2181.

Baldwin, R. E., T. W. Miller, R. D. Brodeur, and K. C. Jacobson. 2008. Expanding the foraging history of juvenile Pacific salmon: combining stomach-content and macroparasite-community analyses for studying marine diets. Journal of Fish Biology **72**:1268-1294.

Blouin, M. S., C. A. Yowell, C. H. Courtney, and J. B. Dame. 1995. Host movement and the genetic structure of populations of parasitic nematodes. Genetics **141**:1007-1014.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Checkley, D. M., P. Ayon, T. R. Baumgartner, M. Bernal, J. C. Coetzee, R. Emmett, R. Guevara-Carrasco, L. Hutchings, L. Ibaibarriaga, H. Nakata, Y. Oozeki, B. Planque, J. Schwiegert, Y. Stratoudakis, and C. van der Lingen. 2009. Habitats. Pages 12-44 *in* D. Checkley Jr., J. Alheit, Y. Oozeki, and C. Roy, editors. Climate Change and Small Pelagic Fish. Cambridge University Press, New York.

Checkley, D. M., and J. A. Barth. 2009. Patterns and processes in the California Current System. Progress in Oceanography **83**:49-64.

Clark, F. N. 1935. A summary of the life-history of the California sardine and its influence on the fishery. California Fish and Game **21**:1-9.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology **9**:1657-1659.

Cross, M. A., C. Collins, N. Campbell, P. C. Watts, J. C. Chubb, C. O. Cunningham, E. M. C. Hatfield, and K. MacKenzie. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda: Anisakidae): implications for fisheries management. Marine Biology **151**:695-702.

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quiñones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science **57**:603-618.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Excoffier, L., G. Laval, and S. Schneider. 1992. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics **1**:47-50.

Fioravanti, M. L., M. Caffara, D. Florio, A. Gustinelli, F. Marcer, M. Gradassi, S. Gavaudan, A. Paolini, A. Alessi, and D. Bisceglia. 2006. Anisakiasis in anchovies (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) caught along the Adriatic coast. Parassitologia **48**:285.

Grant, W. S., A. M. Clark, and B. W. Bowen. 1998. Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome b sequences. Canadian Journal of Fisheries and Aquatic Sciences **55**:2539-2547.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**:95-98.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Hill, K. T. 1999. Determining age composition of coastal pelagic species in northern California, Oregon, and Washington coastal waters. Project #1-IJ-9 Final Report. Pacific States Marine Fisheries Commission, Gladstone, Oregon.

Ivanova, N. V., J. R. Dewaard, and P. D. N. Herber. 2006. Glass fiber plate DNA extraction protocol: manual protocol employing centrifugation method.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Jarne, P., and A. Theron. 2001. Genetic structure in natural populations of flukes and snails: a practical approach and review. Parasitology **123**:S27-S40.

Kijewska, A., J. Dzido, O. Shukhgalter, and J. Rokicki. 2009. Anisakid parasites of fishes caught on the African Shelf. Journal of Parasitology **95**:639-645.

Klimpel, S., M. W. Busch, T. Kuhn, A. Rohde, and H. W. Palm. 2010. The Anisakis simplex complex off the South Shetland Islands (Antarctica): endemic populations versus introduction through migratory hosts. Marine Ecology Progress Series **403**:1-11.

Lagerquist, B. A., B. R. Mate, J. G. Ortega-Ortiz, M. Winsor, and J. Urban-Ramirez. 2008. Migratory movements and surfacing rates of humpback whales (*Megaptera novaeangliae*) satellite tagged at Socorro Island, Mexico. Marine Mammal Science **24**:815-830.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Librado, P., and J. Rozas. 2009. DNAsp v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**:1451-1452.

Lo, N. C. H., B. J. Macewicz, and R. L. Charter. 2007. Spawning biomass of Pacific sardine (*Sardinops sagax*) off California in 2007. NOAA-TM-NMFS-SWFSC-411 Technical Memo, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center:38pp.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**:S153-S163.

MacKenzie, K., N. Campbell, S. Mattiucci, P. Ramos, A. L. Pinto, and P. Abaunza. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fisheries Research **89**:136-145.

Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008a. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Pages 47-148 *in* Advances in Parasitology.

Mattiucci, S., M. Paoletti, and S. C. Webb. 2009. *Anisakis nascettii* n. sp (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. Systematic Parasitology **74**:199-217.

McFarlane, G. A., L. MacDougall, J. Schweigert, and C. Hrabok. 2005. Distribution and biology of Pacific sardines (*Sardinops sagax*) off British Columbia, Canada. California Cooperative Oceanic Fisheries Investigations Reports **46**:144-160.

Mejía-Madrid, H. H., E. Vázquez-Domínguez, and G. P. P. de León. 2007. Phylogeography and freshwater basins in central Mexico: recent history as revealed by the fish parasite *Rhabdochona lichtenfelsi* (Nematoda). Journal of Biogeography **34**:787-801.

Moles, A. 2007. Parasites of the fishes of Alaska and surrounding waters. Alaska Fishery Research Bulletin **12**:197-226.

Morris, J., M. Trudel, T. B. Zubowski, H. R. MacLean, J. M. R. Curtis, and L. Felli. 2009. CCGS W.E. Ricker Gulf of Alaska salmon survey, June 20-July 5, 2007. Canadian Data Report Fisheries and Aquatic Science 1221. Department of Fisheries and Oceans, Nanaimo, British Columbia.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. Journal of Parasitology **81**:395-403.

Nadler, S. A., S. D'Amelio, M. D. Dailey, L. Paggi, S. Siu, and J. A. Sakanari. 2005. Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from Northern Pacific marine mammals. Journal of Parasitology **91**:1413-1429.

Nadler, S. A., and D. S. S. Hudspeth. 2000. Phylogeny of the ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: Hypotheses of structural and sequence evolution. Journal of Parasitology **86**:380-393.

Oshima, M. 1972. *Anisakis* and Anisakidiasis in Japan and adjacent area. Progress of Medical Parasitology in Japan **4**:305-393.

Patarnello, T., A. M. Filip, F. Volckaert, and R. Castilho. 2007. Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? Molecular Ecology **16**:4426-4444.

Perrin, W. F., B. Würsig, and J. G. M. Thewissen 2009. Encyclopedia of marine mammals. Academic Press, London.

Quiazon, K. M. A., T. Yoshinaga, M. D. Santos, and K. Ogawa. 2009. Identification of Larval *Anisakis* spp. (Nematoda: Anisakidae) in Alaska Pollock (T*heragra chalcogramma*) in Northern Japan Using Morphological and Molecular Markers. Journal of Parasitology **95**:1227-1232.

Rello, F. J., F. J. Adroher, and A. Valero. 2008. *Hysterothylacium aduncum*, the only anisakid parasite of sardines (*Sardina pilchardus*) from the southern and eastern coasts of Spain. Parasitology Research **104**:117-121.

Sabater, E. I. L., and C. J. L. Sabater. 2000. Health hazards related to occurrence of parasites of the genera *Anisakis* and *Pseudoterranova* in fish. Food Science and Technology International **6**:183-195.

Sala-Bozano, M., V. Ketmaier, and S. Mariani. 2009. Contrasting signals from multiple markers illuminate population connectivity in a marine fish. Molecular Ecology **18**:4811-4826.

SAS. 1998. Statview®. Using Statview. Version 5.0.1. *in*. SAS Institute Inc., Cary, NC.

Silva, M. E. R., and J. C. Eiras. 2003. Occurrence of *Anisakis* sp. in fishes off the Portuguese west coast and evaluation of its zoonotic potential. Bulletin of the European Association of Fish Pathologists **23**:13-17.

Smith, J. W., and R. Wooten. 1978. *Anisakis* and Anisakiasis. Advances in Parasitology **16**:93-163.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Suzuki, J., R. Murata, M. Hosaka, and J. J Araki. 2009. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. International Journal of Food Microbiology **137**:88-93.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585-595.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weigh matrix choice. Nucleic Acids Research **22**:4673-4680.

Valentini, A., S. Mattiucci, P. Bondanelli, S. C. Webb, A. A. Mignucci-Giannone, M. M. Colom-Llavina, and G. Nascetti. 2006. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *COX2* sequences, and comparison with allozyme data. Journal of Parasitology **92**:156-166.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity **89**:438-450.

Wu, S. G., G. T. Wang, B. W. Xi, F. Xiong, T. Liu, and P. Nie. 2009. Population genetic structure of the parasitic nematode *Camallanus cotti* inferred from DNA sequences of ITS1 rDNA and the mitochondrial COI gene. Veterinary Parasitology **164**:248-256.

Tables

Table 3.1. Samples of Pacific sardine (*Sardinops sagax*) collected by year, region and latitude. The estimated mean standard length and range in millimeters (mm) are provided for each fish collection.

Year	Location	Region	Latitude (^{o}N)	No. Fish	estimated mean fresh SL(range SL)
2005	Vancouver Island, British Columbia, Canada	1	48.7	56	185.69 (148.08-256.61)
	Willapa Bay, Washington, USA	2	46.67	86	197.42 (143.28-258.53)
	Columbia River, Oregon, USA	2	46.17	30	254.88 (241.24-279.66
	Santa Cruz, California, USA	4	36.98	50	171.63 (106.78-198.98)
	Point Arguello, California, USA	4	34.54	50	172.63 (147.12-196.10)
2006	Vancouver Island, British Columbia, Canada	1	50.58	45	223.88 (194.21-285.76)
	Willapa Bay, Washington, USA	2	46.67	89	202.16 (156.15-265.18)
	Columbia River, Oregon, USA	2	46.17	46	213.28 (192.15-268.27)
	Newport, Oregon, USA	2	44.67	39	185.24 (100.61-213.75)
	Point Delgada, California, USA	3	40.24	50	205.11 (184.58-247.00)
	Manchester, California, USA	3	39.12	68	196.95 (120.23-260.45)
	Point Arguello, California, USA	4	35.29	48	181.40 (163.35-200.38)
	San Nicolas Island, California, USA	5	33.2	48	174.45 (157.18-204.50)
	east of San Nicolas Island, California, USA	5	32.97	26	191.52 (158.21-206.55)

Table 3.1. Continued.

2007	Willapa Bay, Washington, USA	2	46.67	92	220.16 (179.81-276.50)
	Astoria, Oregon, USA	2	46.04	21	220.56 (207.58-255.93)
	Columbia River, Oregon, USA	2	46.17	102	203.66 (189.07-217.87)
	Cape Blanco, Oregon, USA	2	43	3	211.70 (142.78-247.70)
	Chetco River, California, USA	3	42	10	217.87 (209.64-229.18)
	Patrick's Point/Klamath River, California, USA	3	41.21	32	205.43 (190.10-219.93)
	Point Delgada, California, USA	3	40	23	199.89 (189.07-207.58)
	Point Arena/Point Reyes, California, USA	3	38.29	95	195.57 (170.55-212.72)
	Golden Gate Inner, California, USA	4	37.48	1	183.92
	Salmon Cone, California, USA	4	35.8	39	209.74 (185.98-267.24)
	Point Arguello, California, USA	4	35.37	45	207.95 (120.15-235.35)
	San Nicolas Island, California, USA	5	33.28	45	178.80 (161.29-205.52)
2008	Ventura, California, USA	5	34.28	50	208.30 (193.18-264.15)
	San Diego, California, USA	5	32.48	50	222.26 (207.00-257.00)
Total				1339	

Table 3.2. GenBank *cox2* sequences available for three species of *Anisakis* nematodes.

GenBank Accession #	Host Species	Common Name	Geographic Location	Reference*
A. pegreffii				
EU933996	unknown	unknown	Mediterranean Sea	5**
EU993995	unknown	unknown	Mediterranean Sea	5**
EU933994	*Seriola dumerili*	Greater Amberjack	China	5**
AB517565	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517564	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517563	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517562	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517561	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
A. simplex s.s.				
EU560911	*Theragra chalcogramma*	Alaska Pollock	Iwate Prefecture, Japan	4
EU560907	*Theragra chalcogramma*	Alaska Pollock	Iwate Prefecture, Japan	4
AJ132189	unknown	unknown	unknown	2**
AY994157 or NC_007934	*Conger myriaster*	Conger Eel	Korea	1
AB517570	*Scomber japonicus*	Chub Mackerel		6
AB517569	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517568	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517567	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517566	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AB517560	*Scomber japonicus*	Chub Mackerel	Tokyo, Japan	6
AS09	*Phocoena phocoena*	Harbor Porpoise	Vancouver Island, BC	7
AS10	*Phocoena phocoena*	Harbor Porpoise	Vancouver Island, BC	7
AS11	*Pseudorca crassidens*	False Killer Whale	Canadian coast	7
AS12	*Pseudorca crassidens*	False Killer Whale	Canadian coast	7
AS13	*Pseudorca crassidens*	False Killer Whale	Canadian coast	7
AS14	*Pseudorca crassidens*	False Killer Whale	Canadian coast	7
***A. simplex* 'C'**				
AF179905 or AC01	unknown	Pacific Coast Rockfish	Californian coast	3 & 7
AF179906 or AC04	*Pseudorca crassidens*	False Killer Whale	Vancouver Island, BC	3 & 7
AC02	*Pseudorca crassidens*	False Killer Whale	Vancouver Island, BC	7
AC07	*Pseudorca crassidens*	False Killer Whale	Vancouver Island, BC	7
AC10	*Lissodelphis borealis*	Northern Right Whale Dolphin	Californian coast	7

*1 = Kim et al. (2006); 2 = Lahoz et al. submitted (1999); 3 = Nadler & Hudspeth (2000); 4 = Quiazon et al. (2009); 5 = Quiazon et al. submitted (2008); 6 = Suzuki et al. (2009); 7 = Valentini et al. (2006); **genetic sequence not associated with a specific citation.

Table 3.3. Summary information of the genetic variability among 524 nucleotide sites of *cox2* mitochondrial DNA for three *Anisakis* species.

	No. of sequences	No. shared haplotypes	No. unique haplotypes	h diversity	π	No. polymorphic sites	Tajima's D	p-value Tajima's D
Anisakis pegreffii	76	10	33	0.942	0.014	40	-0.321	0.47
A. simplex s.s.	51	3	48	0.998	0.018	79	-1.61	0.03
A. simplex 'C'	21	2	17	0.967	0.007	23	-1.718	0.03

h = haplotype diversity, π = nucleotide diversity

Table 3.4. AMOVA results for *cox2* mitochondrial DNA sequenced from three *Anisakis* species grouped by geographic region: Vancouver Island and southern California (VI vs SC); nearshore versus offshore (N vs O), where offshore includes stations west of the 200m isobath lines indicated in Figure 3.1; Japan versus California Current (J vs CC) only for *A. simplex s.s.* The number of worms compared per species and region are indicated for each AMOVA test.

	AMOVA test	Source	df	Sum of squares	Variance components	Percentage of variation	Φ_{ST}	p-value
Anisakis pegreffii	**VI vs SC**							
	VI = 4	Among regions	1	4.44	0.23	6.97	0.07	0.24
	SC = 14	Within regions	16	48.5	3.03	93.03		
		Total	17	52.94	3.26			
	N vs O	Among regions	1	4.99	0.04	0.98	0.01	0.21
	N = 45	Within regions	74	270.59	3.66	99.02		
	O = 31	Total	75					
A. simplex 'C'	**VI vs SC**							
	VI = 3	Among regions	1	1.44	-0.1	-5.88	-0.06	0.72
	SC = 6	Within regions	7	13	1.86	105.88		
		Total	8	14.44	1.75			
	N vs O							
	N = 10	Among regions	1	1.59	-0.02	-0.92	-0.01	0.52
	O = 11	Within regions	19	33.46	1.76	100.92		
		Total	20	35.05	1.74			

Table 3.4 Continued.

A. simplex s.s.	**VI vs SC**							
	VI = 12	Among regions	1	5.49	0.13	3.14	0.03	0.2
	SC = 10	Within regions	20	81.1	4.06	96.86		
		Total	21	86.59	4.19			
	N vs O							
	N = 33	Among regions	1	7.47	0.12	2.46	0.02	0.1
	O = 18	Within regions	49	230.61	4.71	97.54		
		Total	50	238.08	4.82			
	J vs CC							
	J = 8	Among regions	1	67.11	4.48	46.28	0.46	<0.0001
	CC = 51	Within regions	57	296.14	5.2	53.72		
		Total	58	363.25	9.67			

Figure Titles

Figure 3.1. Geographic location of stations (solid circles) where Pacific sardine (*Sardinops sagax*) were caught in five regions of the California Current, and the relative proportions of the *Anisakis* species are indicated for each region (pie charts: solid white - *A. pegreffii*; solid black - *A. simplex* 'C'; grey dotted - *A. simplex sensu stricto*). For each region, the number of *Anisakis* nematodes were included. Region 1: off Vancouver Island, British Columbia (n = 19); Region 2: Washington and Oregon (n = 55); Region 3: Northern California (n = 32); Region 4 Central California (n = 13), and Region 5: Southern California (n = 29). The 200m isobath is depicted by the solid black line oriented approximately north to south, west of the provincial and state coastlines.

Figure 3.2. (A) Regions in the California Current System where three species of *Anisakis* nematodes were recovered from Pacific sardine (*Sardinops sagax*): off Vancouver Island, British Columbia (blue), Washington and Oregon (green), Northern California (maroon), Central California (purple), and Southern California (yellow). (B-D) Statistical parsimony networks of *cox2* mitochondrial DNA sequences for each *Anisakis* species. (B) *A. pegreffii*; (C) *A. simplex sensu stricto*; and (D) *A. simplex* 'C'. Each connection is a single base difference, solid black circles are inferred haplotypes, and colored circles are observed haplotypes. The colored circles indicate the geographic region (as in A) where each haplotype was collected. The number of worms with identical sequences is represented by the size of the colored circles. Unless indicated by a number inside or next to the circle, each haplotype represents an individual nematode. Homoplasies among the sequences are indicated by reticulations within the networks.

Figure 3.3 (A-C) Statistical parsimony networks comparing 29 GenBank *cox2* mitochondrial DNA sequences to our *cox2* data for each *Anisakis* species. (A) *A. pegreffii*; (B) *A. simplex sensu stricto*; and (C) *A. simplex* 'C'. Each connection is a single base difference, solid black circles are inferred haplotypes, and colored circles are observed haplotypes. Each color indicates the geographic region where each haplotype was collected in the present study (see map in Figure 2), and all GenBank sequences are colored white. The number of worms with identical sequences is represented by the size of the colored circles. Unless indicated by a number inside or next to the circle, each haplotype represents an individual worm. Homoplasies among the sequences are indicated by reticulations within the networks. Connections forced into the network with a maximum of 100 steps are indicated by a broken line.

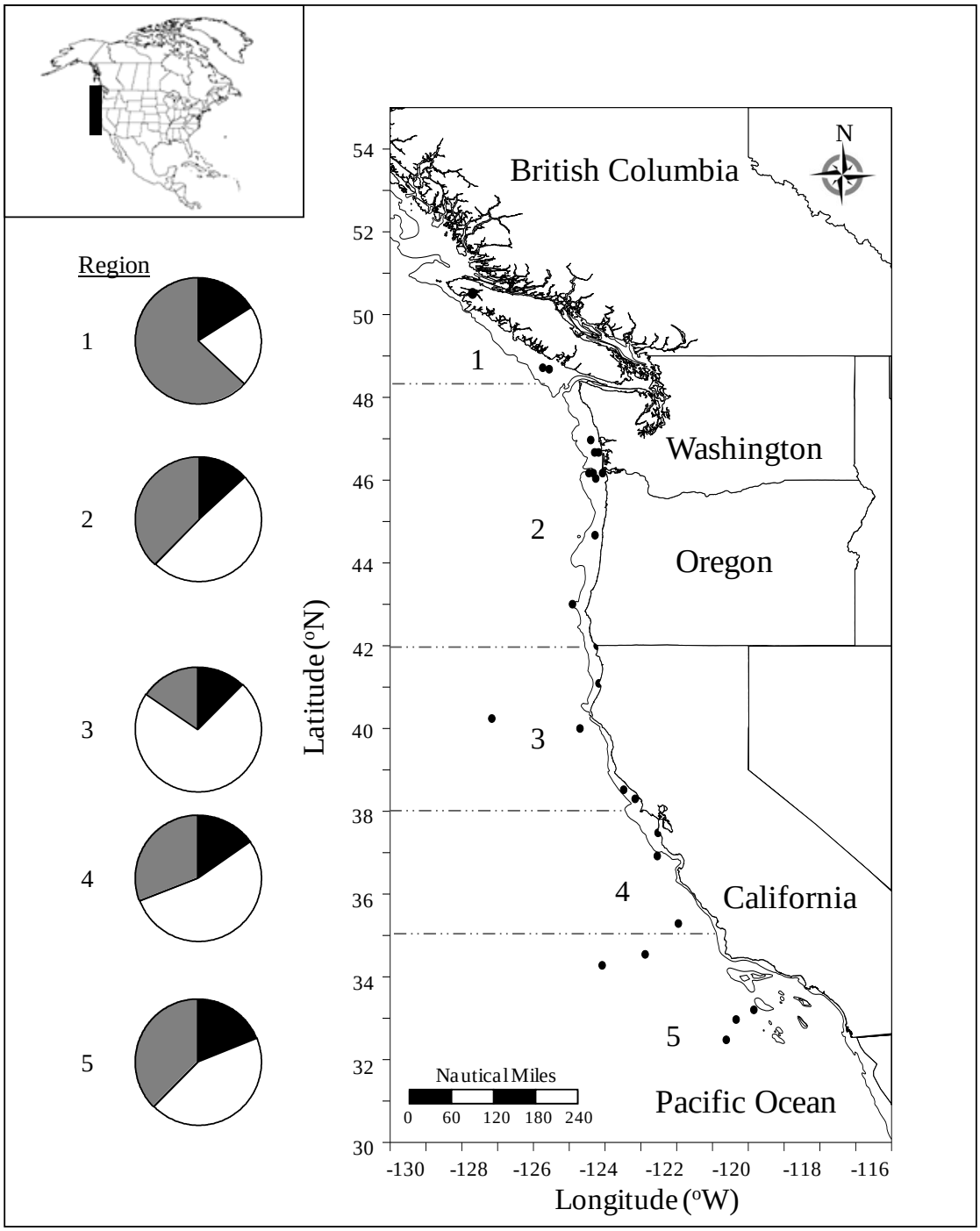
Region
1
2
3
4
5
British Columbia
Washington
Oregon
California
Pacific Ocean
N
Nautical Miles
0 60 120 180 240
Latitude (°N)
Longitude (°W)
54
52
50
48
46
44
42
40
38
36
34
32
30
-130
-128
-126
-124
-122
-120
-118
-116

Figure 3.1.

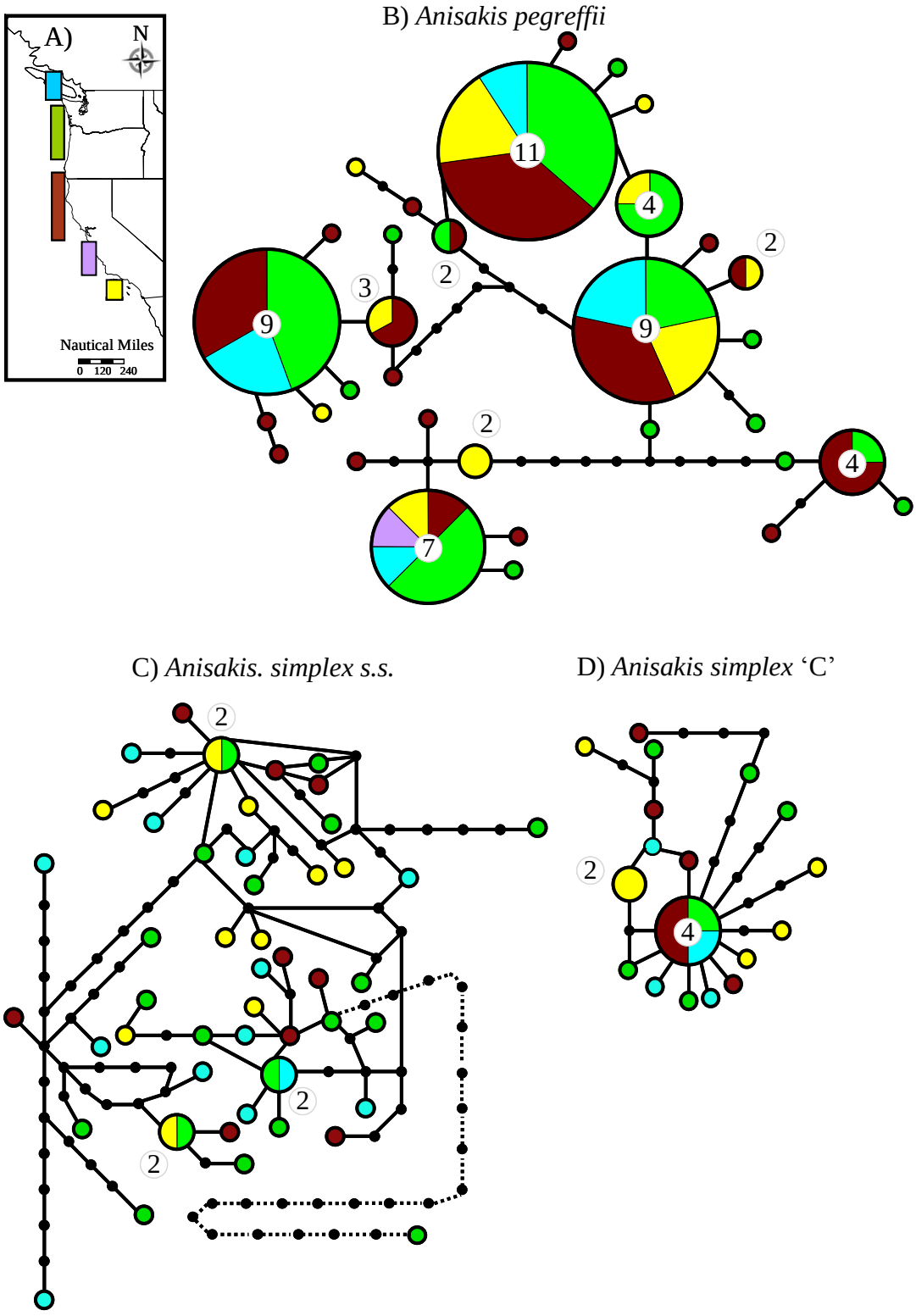
A)
N
Nautical Miles
0 120 240
B) *Anisakis pegreffii*
11
4
2
3
2
9
9
2
4
7
C) *Anisakis. simplex s.s.*
2
2
2
D) *Anisakis simplex* 'C'
2
4

Figure 3.2.

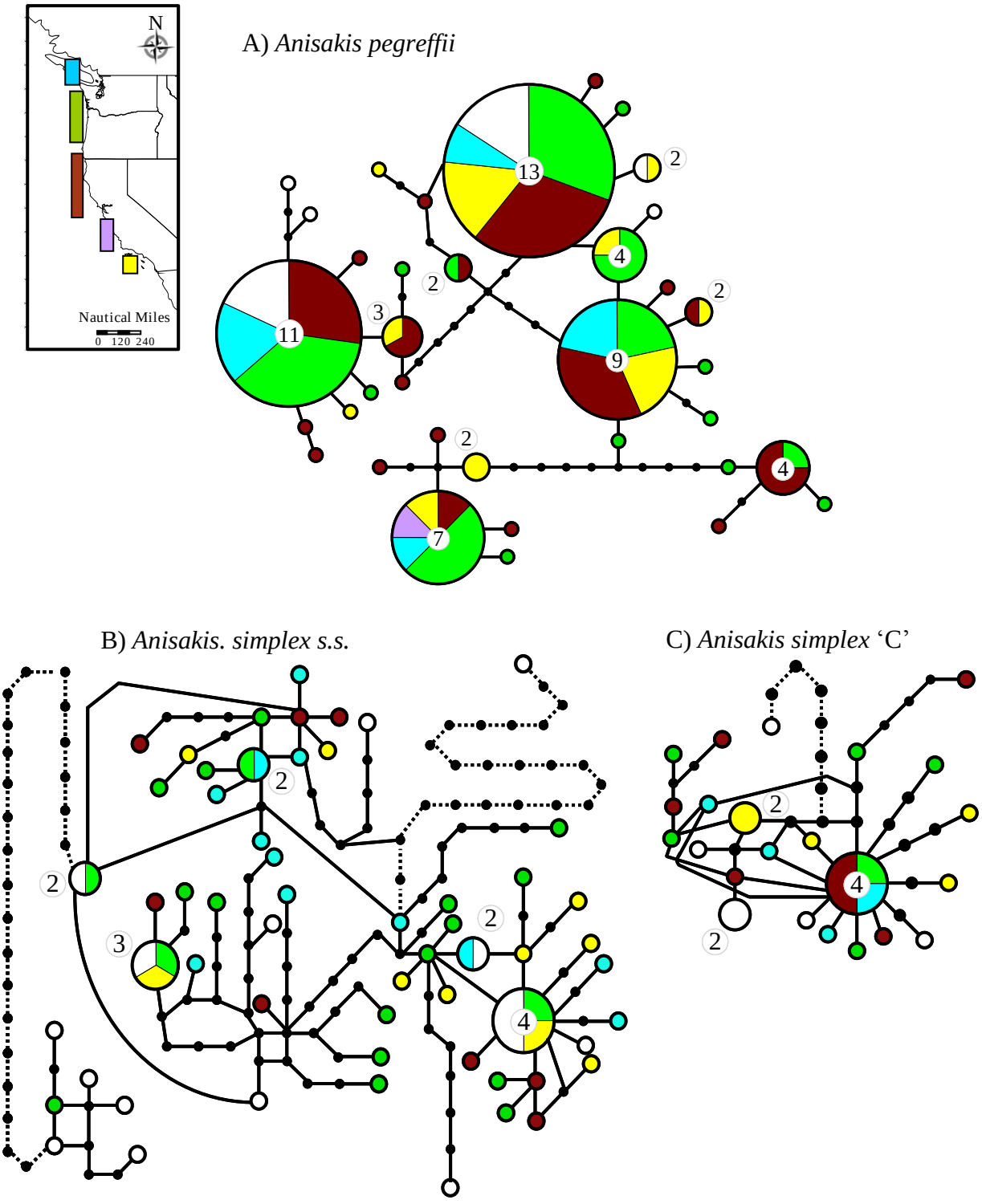
N
Nautical Miles
0 120 240
A) Anisakis pegreffii
13
2
4
2
2
3
11
9
2
4
2
7
B) Anisakis. simplex s.s.
C) Anisakis simplex 'C'
2
2
3
2
4
2
2
4

Figure 3.3.

HAS THE POPULATION OF THE MARINE TREMATODE *MYOSACCIUM ECAUDE* EXPANDED IN ASSOCIATION WITH THE RETURN OF PACIFIC SARDINE (*SARDINOPS SAGAX*) TO THE NORTHERN CALIFORNIA CURRENT?

Rebecca E. Baldwin, Mary Beth Rew, Michael A. Banks, Kym C. Jacobson

Abstract

Hemiuridae is one of ten trematode families that account for about two-thirds of the trematode species records in fishes. To date, *Myosaccium ecaude* has only been reported from Pacific sardine (*Sardinops sagax*) off La Jolla, California. From 2005 through 2008, 405 *Myosaccium ecaude* were recovered from Pacific sardine captured throughout the California Current from off Vancouver Island, British Columbia to San Diego, California. *Myosaccium ecaude* was distributed throughout the study region from 32^{o}N - 50^{o}N latitude, but were most common off Central and Southern California. The degree of trematode population structure was assessed using the NADH-dehydrogenase subunit 1 (ND1) mitochondrial DNA gene. Our study is the first to successfully sequence ND1 mtDNA from the marine trematode *M. ecaude*. Cryptic speciation was not observed among the 74 haplotypes identified, suggesting that only one species of *M. ecaude* was recovered. The two dominant ND1 haplotypes comprised 67.6% of the trematodes sequenced. Minimal base pair differences among related haplotypes and the dominance of two shared haplotypes in the parsimony network suggests that the population of *M. ecaude* may have recently expanded in the California Current. An expanding *M. ecaude* population may be in conjunction with a resurgence of Pacific sardine numbers in the California Current System that also enabled Pacific sardines to return to the Pacific Northwest in the 1990s.

Introduction

The Pacific sardine (*Sardinops sagax*) is a valuable economical and ecological forage fish that transfers energy resources from planktonic primary producers and secondary consumers to upper trophic predators (Cury et al. 2000). Sardine tagging studies in the 1930s and 40s suggested they made an annual migration to feeding grounds off the Pacific Northwest in the summer and returned to Southern California in the fall/winter to spawn the following spring (Clark 1935; Clark and Janssen 1945; Marr 1957).

This accepted coastwide sardine migration pattern was described prior to the fishery/population collapse in the 1940s (Janssen 1938; 1945), and it is unclear whether this same pattern of migration has been re-established since the return of Pacific sardines to the Pacific Northwest in the 1990s (Hargreaves et al. 1994). Pacific sardine allozyme (Hedgecock et al. 1989) and mitochondrial DNA (mtDNA) data (Grant et al. 1998; Lecomte et al. 2004) suggest a panmictic population with a shallow genetic structure. However, there is some evidence of more than one subpopulation within the California stock management unit based on the recovery of larger individuals at higher latitudes (Clark and Janssen 1945; Hill 1999; Emmett et al. 2005; McFarlane et al. 2005) and a temporal difference in sardine spawning off the Pacific Northwest versus southern California (Emmett et al. 2005; Smith 2005).

Williams and Bunkley-Williams (1996) suggested the trematode genus *Myosaccium* is commonly found in clupeid fish species: to date *M. ecaude* has only been reported

from Pacific sardine (Montgomery 1957; Kunnenkeri 1962). This trematode was first described by Montgomery (1957) from Pacific sardine caught in 1953 off La Jolla, California. Little information is available on the life history of this trematode in the California Current. However, based on the life history of other members of the family Hemiuridae, such as *Lecithaster gibbosus* and *Tubulovesicula lindbergi*, the first intermediate host is likely a snail, the second intermediate host could be either a calanoid or cyclopoid copepod (Margolis and Boyce 1969; Køie 1983) and the final host is a fish.

Previous parasite surveys of marine fish in the California Current either did not include Pacific sardine or were conducted after sardines disappeared from off the Pacific Northwest (British Columbia, Washington and Oregon) (Love and Moser 1983; McDonald and Margolis 1983). Recently, Baldwin et al. (CHAPTER 2, this volume) described the distribution of *M. ecaude* that ranged from San Diego, California to Vancouver Island, British Columbia, Canada. However, it is uncertain if the presence of *M. ecaude* as far north as British Columbia is correlated with the return of Pacific sardine to the Pacific Northwest in the 1990s since approximately 80% of *M. ecaude* individuals were recovered in sardines $\leq$ 200 mm in standard length (SL) (Baldwin et al. CHAPTER 2, this volume), which are considered non-migratory (Lo et al. unpublished). The main goals of our study were: 1) to examine the genetic diversity and population structure of *M. ecaude* collected from sardines sampled off of Vancouver Island, British Columbia to San Diego, California; and 2) assess the

potential of using the population genetics of *M. ecaude* as a biological tag to help discriminate stocks of Pacific sardine.

Materials and Methods

Study area and parasite collection

From 2005 through 2008 a total of 1491 Pacific sardine were opportunistically collected in the California Current (Table 4.1) between 32^{o} to 50^{o} N latitude, and 119^{o} to 128^{o} W longitude (Figure 4.1). We divided this study area into five geographic regions: 1) Vancouver Island, British Columbia; 2) Washington and Oregon; 3) Northern California; 4) Central California; and 5) Southern California. Sardines from Canadian waters were caught using a modified Cantrawl 240 rope trawl (Cantrawl Nets Ltd., Richmond, BC; see Morris et al. 2009 for details), and sardines from Washington to California were caught using a 30 m wide by 20 m deep mouth-opening 264 rope trawl, (Nor'Eastern Trawl Systems, Inc. Bainbridge Island, WA, U.S.A.; see Baldwin et al. 2008 for details).

One net tow equaled one trawling event. Captured Pacific sardine were immediately frozen on board and stored in the lab at -80^{o}C until processed for parasites. After being thawed, each fish was weighed to the nearest 0.1 g and standard length (SL) measured to the nearest mm. Fresh SL of individual frozen sardine was estimated using the following regression: Fresh SL = 2.89 + 1.0286 (Frozen and then Thawed SL) (Lo et al. 2007). *Myosaccium ecaude* trematodes were recovered from stomachs according to

standard necropsy procedures (Albert and Albert 1994). A total of 542 *M. ecaude* trematodes were collected from these sardines and preserved in 95% ethanol.

Extraction, DNA amplification and sequencing

DNA was extracted from trematode tissue using an extraction protocol solution of 100uL of 5% chelex and 5 µL of Proteinase k (Criscione and Blouin 2004). To genetically identify *M. ecaude* to species and assess the population structure of this trematode, we used the polymerase chain reaction (PCR) to amplify a region including the NADH-dehydrogenase subunit 1 (ND1) using the forward primer MB352 (5'-CGT AAG GGK CCT AAY AAG-3') (Criscione and Blouin 2004) and the reverse primer TremND1R1 (5'-CGT ACC TAC CCT AAA CAA CAA C-3') designed by Mary Beth Rew. All PCR reactions had a final volume of 20 µL comprised of 2 µL of genomic DNA, 0.40 µM each primer, 0.4 mM of each deoxynucleoside triphosphate (dNTP), 2.5 mM $MgCl_2$, 1X PCR buffer, and 1 unit *Taq* DNA polymerase (Promega, Madison, Wisconsin). The temperature and cycling parameters included denaturation at 95^{o}C for 3 min, followed by 35 cycles at 94^{o}C for 45 sec, 52^{o}C for 30 sec, 72^{o}C for 1 min, followed by postamplification extension at 72^{o}C for 7 min. All ND1 PCR products were cleaned for direct nucleotide sequencing using an ExoSap-IT clean-up protocol (GE Healthcare, Piscataway, New Jersey). Cycle sequencing was conducted using ABI-PRISM Big Dye terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, California), and DNA sequences were cleaned using a Sephadex protocol (GE Healthcare). Sequences were analyzed with an ABI 3730xl

DNA automated sequencer (Applied Biosystems). DNA sequences were edited using BioEdit 7.0.1 (Hall 1999) and aligned with ClustalW (Thompson et al. 1994) following the default parameters. All unique sequences of *M. ecaude* were deposited into GenBank under the following accession numbers: XX to XX.

Data analysis

Although the estimated sardine SL of uninfected and infected fish were normally distributed (Kolmogorov-Smirnov Test p-value 0.07), variances were uneven among regions (Levene's Test p-value = 0.002; SPSS PASW Statistics 18). Thus for each year, Mann-Whitney U tests were used to compare estimated sardine SL of uninfected and infected fish between 1) regions and 2) nearshore versus offshore. Fish collected east of the 200 m isobath (approximating the continental shelf break) were classified as inshore samples, and fish collected west of this line were considered offshore samples (Figure 4.1). All Mann-Whitney U tests were calculated in Statview® (SAS 1998). Parasite prevalence and intensity for *M. ecaude* were calculated according to Bush et al. (1997).

We used DNAsp (v.5.00.07) (Librado and Rozas 2009) to calculate standard statistics: 1) haplotype diversity (h), the proportion of unique haplotypes recovered, 2) the number of polymorphic sites, 3) nucleotide diversity (π), the species-wide average number of nucleotide differences per site between two sequences, and 4) Tajima's D (Tajima 1989) to test for neutral selection among all molecular mutations, and to

determine if the population size is constant (value near zero), shrinking (positive value) or growing (negative value). The number of unique haplotypes and mean pairwise difference among ND1 sequences was determined using Arlequin 3.1 (Excoffier et al. 1992). Using an analysis of molecular variance (AMOVA), genetic variation was attributed to its variance components (within and among region variation) where a negative value suggests the % variation is close to zero, and Phi_{ST} (Φ_{ST}) values (analogous to Fst values) were calculated using permutational estimates of significance in Arlequin 3.1 (Excoffier et al. 1992). Phi_{ST} (Φ_{ST}) values have a maximum value of 1, where 0 indicates no differentiation among sequences and 1 indicates complete differentiation among sequences from pre-defined regions. The program TCS 1.13 (Clement et al. 2000) was used to create statistical parsimony networks of ND1 haplotypes.

Results

General Pacific Sardine Information

A total of 1491 sardines were examined during this study. These sardines had an estimated fresh standard length (SL) ranging from 100.61 to 285.76 mm (Table 4.1). The 1060 fish caught nearshore were significantly larger (208.96 $\pm$ 21.03 mm with a mean rank of 852.91) than the 431 fish caught offshore (mean 189.95 $\pm$ 21.25 mm with a mean rank of 483.06; z-value -15.038, p-value < 0.0001). Except for one sardine collected off of Newport, Oregon (estimated SL = 100.61 mm), the smallest sardines were recovered in 2005 from region 4 (Central California; minimum

estimated SL = 106.78 mm), and the largest were caught in 2006 in region 1 (Vancouver Island, British Columbia; maximum estimated SL = 285.76 mm).

A total of 18.4% of the collected sardines were infected with *M. ecaude*. The 137 infected fish caught nearshore were larger (mean 201.38 $\pm$ 11.88 mm with a mean rank of 186.21) than the 138 infected fish caught offshore (mean 182.04 $\pm$ 15.11 mm with a mean rank of 90.14) when all years where combined (z-value = -10.02, p-value < 0.0001) and for each year (2005: z-value = -4.98, p-value = < 0.0001 with a nearshore mean rank of 26.00 and an offshore mean rank of 9.00; 2006: z-value = -6.98, p-value = < 0.0001 with a nearshore mean rank of 87.58 and an offshore mean rank of 41.78; 2007: z-value = -4.81, p-value = < 0.0001 with a nearshore mean rank of 73.21 and an offshore mean rank of 40.00). No nearshore versus offshore comparison could be made for 2008 (only offshore was sampled). Overall, infected fish were larger in Regions 1 and 2 than the other regions (Table 4.2). Additionally, infected fish were smaller in Regions 4 and 5 than the other regions (Table 4.2).

Myosaccium ecaude Recovery and Genetics Summary

Trematode intensity ranged from one to 32 worms per host with most infected fish harboring a single worm (174 out of 275 fish). Of the 524 trematodes collected from the five geographic regions, DNA was obtained from 405 individuals: Region 1 (n = 11), Region 2 (n = 46), Region 3 (n = 136), Region 4 (n = 96) and Region 5 (n = 116). We identified only one species of *Myosaccium* in our samples based on a 0.4%

pairwise difference among the ND1 sequences analyzed from all 405 trematodes recovered. Of the 75 haplotypes identified, 50 were unique and 25 were shared among multiple individual worms. We found two dominant trematode haplotypes that consisted of 67.6% of the trematodes sequenced (Figure 4.2). Seventy-six sardines (5.1%) were infected with more than one individual of *M. ecaude*, of which 79% of these sardines were infected with multiple haplotypes (up to 11 haplotypes). The population structure of *M. ecaude* was assessed using a 283 bp portion of ND1 mtDNA where: 1) haplotype diversity was 0.749; 2) nucleotide diversity was 0.004; and 3) there were 61 polymorphic sites. A significant Tajima's D value of -2.52 (p-value < 0.001) was obtained, suggesting a possible population size expansion in *M. ecaude*.

We found no geographic separation among ND1 sequences whereby all analyses by region resulted in negative among region variance components (Table 4.3). Genetic variation was associated with differences within regions, as opposed to differences among regions resulting in non-significant AMOVA Φ_{ST} values ranging from -0.005 to -0.01 (p-values ranged from 0.66 to 0.97). The lack of distinct population structure associated with defined geographic regions was also evident in the ND1 parsimony networks based on 283 bp of the sequence data (Figure 4.2). There was no separation by region within the network for individual ND1 haplotypes, and shared ND1 haplotypes were observed from multiple regions. Finally, when treating individual

multiple-infected fish as putative populations, AMOVA results still showed a lack of population differentiation (data not shown).

Discussion

Our study is the first to successfully sequence ND1 mtDNA from the marine trematode *M. ecaude*. Cryptic speciation was not observed among the 74 haplotypes identified, indicating only one species of *M. ecaude* was recovered. The population genetics data showed that haplotype diversity was high, nucleotide diversity was low, and the overall pattern of molecular variation was similar to *Nanophyetus salmonicola* (a freshwater trematode species) known to infect juvenile salmonids (Criscione and Blouin 2004).

A panmictic distribution was described previously for three *Anisakis* species recovered along with *M. ecaude* during a parasite community study of Pacific sardine in the California Current (Baldwin et al. CHAPTERS 2 and 3, this volume). As with *Anisakis*, the two dominant ND1 haplotypes for *M ecaude* were recovered in similar proportions throughout the study region. Further, related haplotypes were as likely to be found among different regions as within the same individual fish. Minimal base pair differences among related haplotypes and the dominance of two shared haplotypes in the parsimony network suggests that the population of *M. ecaude* may have recently expanded in the California Current. An expanding *M. ecaude* population may be in conjunction with a resurgence of Pacific sardine numbers in the California

Current System that also enabled Pacific sardines to return to the Pacific Northwest in the 1990s (Hargreaves et al. 1994).

Host movement appears to influence gene flow among distant parasite populations (Jarne and Theron 2001). Sardines migrating between Vancouver Island, British Columbia and San Diego, California (Dahlgren 1936; Hart 1943**;** Ahlstrom 1957; Smith 2005; Baldwin et al. CHAPER 2, this volume) are likely dispersing trematode eggs while they migrate. Similar to the dispersal capabilities of *Anisakis* nematodes described using cytochrome oxidase 2 (*cox2*) mtDNA (Baldwin et al. CHAPTER 3, this volume), our ND1 data suggest that *M. ecaude* eggs are also well dispersed in the California Current. Individual parasite haplotypes were just as likely to exist at the far ends of the study area as within the same sardine host, suggesting transmission dynamics of parasites can maintain genetic diversity through the wide dispersal of parasite eggs in the marine environment.

In addition to host movement, the complexity of a parasite life-cycle can also affect the population structure of a parasite. For example Criscione and Blouin (2004) described two different population structures among four freshwater trematode species with similar nucleotide diversities (π ranged from 0.008 to 0.01). Geographical separation of ND1 haplotypes was observed for *Deropegus aspina* A, *D. aspina* B, and *Plagioporus shawi* (freshwater trematodes that mature in fish), but a panmictic population structure was observed for *N. salmonicola* (a freshwater trematode that

matures in a fish-eating bird or mammal; Criscione and Blouin 2004). Although the *M. ecaude* life cycle is presently unknown, adult trematodes are known to mature in fish and have only been recovered from Pacific sardine (Montgomery 1957; Kunnenkeri 1962; Baldwin et al. CHAPTER 2, this volume). Even though *M. ecaude* appears to only infect Pacific sardines, our results suggest that the limited oceanographic barriers and complexity in the California Current are not preventing gene flow among *M. ecaude* populations.

In summary, host movement and oceanographic conditions may affect the gene flow of trophically-transmitted parasites in the marine environment. The distribution and population structure of *M. ecaude* suggests the range of this trematode has recently expanded throughout the California Current. Investigating how oceanographic conditions and host migration influence the genetic diversity and population structure of marine parasites that infect non-migratory fish species could clarify if high genetic diversity and connectivity for *M. ecuade* over large geographical distances is a common pattern, or if hydrography can restrict gene flow of other widely dispersed marine parasites.

Acknowledgements

We would like to thank R. Emmett, M. Blouin, V. Lesser, J. Bolte, and E. Casillas for providing comments on earlier versions of this manuscript.

References

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Ambrose III, H. W., and K. P. Ambrose. 1987. A Handbook of Biological Investigation, Fourth edition. Hunter Textbooks Inc, Winston-Salem, NC.

Arthur, J. R., and E. Albert. 1994. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **50**:2175-2181.

Baldwin, R. E., T. W. Miller, R. D. Brodeur, and K. C. Jacobson. 2008. Expanding the foraging history of juvenile Pacific salmon: combining stomach-content and macroparasite-community analyses for studying marine diets. Journal of Fish Biology **72**:1268-1294.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Clark, F. N. 1935. A summary of the life-history of the California sardine and its influence on the fishery. California Fish and Game **21**:1-9.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology **9**:1657-1659.

Criscione, C. D., and M. S. Blouin. 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution **58**:198-202.

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quinones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science **57**:603-618.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Excoffier, L., G. Laval, and S. Schneider. 1992. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics **1**:47-50.

Grant, W. S., A. M. Clark, and B. W. Bowen. 1998. Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome b sequences. Canadian Journal of Fisheries and Aquatic Sciences **55**:2539-2547.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**:95-98.

Hargreaves, N. B., D. M. Ware, and G. A. McFarlane. 1994. Return of Pacific sardine (*Sardinops sagax*) to the British Columbia coast in 1992. Canadian Journal of Fisheries and Aquatic Sciences **51**:460-463.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Hill, K. T. 1999. Determining age composition of coastal pelagic species in northern California, Oregon, and Washington coastal waters. Project #1-IJ-9 Final Report. Pacific States Marine Fisheries Commission, Gladstone, Oregon.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Janssen, J. F., and J. F. Alpen. 1945. The effect of internal tags upon sardines. Fisheries Bulletin **61**:43-62.

Jarne, P., and A. Theron. 2001. Genetic structure in natural populations of flukes and snails: a practical approach and review. Parasitology **123**:S27-S40.

Køie, M. 1983. Digenetic trematodes from *Limanda limanda* (L.) (Osteichthyes, Pleuronectidae) from Danish and adjacent waters, with special reference to their life-histories. Ophelia **22**:201-228.

Kunnenkeri, J. K. 1962. Preliminary report on the parasites of the California sardine and the parasitic distribution in Clupeidae. Journal of Parasitology **48**:149.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**:1451-1452.

Lo, N. C. H., B. J. Macewicz, and D. A. Griffith. unpublished. Biomass and reproduction of Pacific sardines off the northwest US in 2003-2005. 43pp.

Lo, N. C. H., B. J. Macewicz, and R. L. Charter. 2007. Spawning biomass of Pacific sardine (*Sardinops sagax*) off California in 2007. NOAA-TM-NMFS-SWFSC-411 Technical Memo, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center:38pp.

Love, M. S., and M. Moser. 1983. A checklist of parasites of California, Oregon, and Washington Marine and Estuarine Fishes. NOAA Technical Report NMFS SSRF-777. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services. 254pp.

Margolis, L., and N. P. Boyce. 1969. Life span, maturation, and growth of two Hemiurid trematodes, *Tubulovesicula lindbergi* and *Lecithaster gibbosus*, in Pacific salmon (Genus *Oncorhynchus*). Journal of the Fisheries Research Board of Canada **26**:893-907.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

McDonald, T. E., and L. Margolis. 1995. Synopsis of the parasites of fishes of Canada: Supplement (1978-1993). Canadian Special Publication of Fisheries and Aquatic Sciences.

McFarlane, G. A., L. MacDougall, J. Schweigert, and C. Hrabok. 2005. Distribution and biology of Pacific sardines (*Sardinops sagax*) off British Columbia, Canada. California Cooperative Oceanic Fisheries Investigations Reports **46**:144-160.

Montgomery, W. R. 1957. Studies on digenetic trematodes from marine fishes of La Jolla, California. Transactions of the American Microscopy Society **76**:13-35.

Morris, J., M. Trudel, T. B. Zubowski, H. R. MacLean, J. M. R. Curtis, and L. Felli. 2009. CCGS W.E. Ricker Gulf of Alaska salmon survey, June 20-July 5, 2007. Canadian Data Report Fisheries and Aquatic Science 1221. Department of Fisheries and Oceans, Nanaimo, British Columbia.

SAS. 1998. Statview®. Using Statview. Version 5.0.1. *in*. SAS Institute Inc., Cary, NC.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585-595.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weigh matrix choice. Nucleic Acids Research **22**:4673-4680.

Williams Jr., E. H., and L. Bunkley-Williams. 1996. Platyhelminthes (Flatworms). Pages p59 *in* Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Antillean College Press, Mayaguez, Puerto Rico.

Tables

Table 4.1. General information on 1491 Pacific sardine (*Sardinops sagax*) by year, location, region, date caught, and latitude. The mean (range) estimated mean standard length (SL) are provided for each fish collection. Sardines are separated into two size classes: and ≤ 200 mm SL (non-migrants) and > 200 mm SL (migrants).

Year	Location	Region*	Date	Latitude (°N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
2005	Vancouver Island, British Columbia, Canada	1	Mar 3	48.72	>200 mm	26	163.26 (148.08-193.22)
			Jul 30	48.70	≤ 200 mm	15	194.76 (181.69-199.94)
			Jul 30	48.70	>200 mm	15	215.50 (200.90-256.61)
	Willapa Bay, Washington, USA	2	Jun 4	46.67	≤ 200 mm	9	186.71 (143.28-198.98)
			Jun 4		>200 mm	21	218.55 (200.90-258.53)
			Jun 26		≤ 200 mm	26	188.53 (152.88-198.98)
			Jun 26		>200 mm	4	203.06 (200.90-204.74)
			Jul 7		≤ 200 mm	23	190.34 (175.93-198.02)
			Jul 7		>200 mm	3	205.38 (200.92-214.35)
	Columbia River, Oregon, USA	2	Jun 6	46.17	≤ 200 mm	30	254.88 (241.24-279.66)
	Santa Cruz, California, USA	4	Apr 11	36.98	≤ 200 mm	50	171.63 (106.78-198.98)
	Point Arguello, California, USA	4	Apr 11	34.54	≤ 200 mm	50	172.63 (147.12-196.10)

*Region 1 = Vancouver Island, British Columbia, Region 2 = Washington and Oregon, Region 4 = Central California

Table 4.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
2006	Vancouver Island, British Columbia, Canada	1	Nov 16	50.58	≤ 200 mm	1	194.21
					>200 mm	44	224.55 (203.47-285.76)
	Willapa Bay, Washington, USA	2	May 12	46.67	≤ 200 mm	10	168.29 (156.15-185.98)
			May 12		>200 mm	2	217.35 (200.38-234.33)
			Jun 13		≤ 200 mm	5	196.68 (193.22-198.98)
			Jun 13		>200 mm	17	207.06 (200.90-212.43)
			Jul 18		≤ 200 mm	5	186.60 (163.35-199.35)
			Jul 18		>200 mm	25	207.29 (200.38-232.27)
			Aug 15		≤ 200 mm	1	199.35
			Aug 15		>200 mm	19	210.99 (200.38-265.18)
	Columbia River, Oregon, USA	2	May 27	46.17	> 200 mm	30	217.87 (201.41-255.93)
			Sep 25		≤ 200 mm	5	196.27 (192.15-199.35)
			Sep 25		> 200 mm	11	208.52 (203.47-214.78)
	Newport, Oregon, USA	2	Sep 18	44.67	≤ 200 mm	24	172.27 (100.61-198.32)
			Sep 18		> 200 mm	15	206.00 (200.38-213.75)
	Point Delgada, California, USA	3	Apr 24	40.24	≤ 200 mm	13	195.58 (184.58-199.94)
			Apr 24		> 200 mm	37	208.46 (200.90-247.00)
	Manchester, California, USA	3	Apr 25	39.12	≤ 200 mm	28	187.32 (120.23-199.94)
			Apr 25		> 200 mm	22	211.73 (200.90-260.45)
			Apr 26		≤ 200 mm	10	187.65 (168.25-198.98)
			Apr 26		> 200 mm	7	204.20 (200.90-210.51)

*Region 1 = Vancouver Island, British Columbia, Region 2 = Washington and Oregon, Region 3 = Northern California

Table 4.1 Continued.

Year	Location	Region*	Date	Latitude (°N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
2006	Montana de Oro State Park, California, USA	4	May 5	35.29	≤ 200 mm	47	180.99 (163.35-196.27)
			May 5		> 200 mm	1	200.38
	San Nicolas Island, California, USA	5	May 10	33.20	≤ 200 mm	47	173.81 (157.18-190.10)
			May 10		> 200 mm	1	204.50
	east of San Nicolas Island, California, USA	5	May 2	32.97	≤ 200 mm	21	188.72 (158.21-198.32)
			May 2		> 200 mm	5	203.26 (200.38-206.55)
2007	Vancouver Island, British Columbia, Canada	1	Jul 20	48.08	≤ 200 mm	1	198.32
			Jul 20	48.08	> 200 mm	51	222.08 (201.41-260.04)
			Sep 18	48.02	> 200 mm	36	224.18 (202.44-255.93)
			Aug 26/Sep 2	50.69	> 200 mm	30	225.65 (208.61-265.18)
	Willapa Bay, Washington, USA	2	Jun 25	46.67	≤ 200 mm	1	179.81
			Jun 25		> 200 mm	49	228.47 (206.55-276.50)
			Jul 23		≤ 200 mm	2	196.78 (195.24-198.32)
			Jul 23		> 200 mm	40	212.16 (201.41-255.93)
	Astoria, Oregon, USA	2	Jun 13	46.04	> 200 mm	51	217.26 (201.41-255.93)
			Jul 17		≤ 200 mm	2	199.35
			Jul 17		> 200 mm	48	215.49 (200.38-227.13)
	Columbia River, Oregon, USA	2	8-Aug	46.17	≤ 200 mm	28	195.72 (189.07-199.35)
					> 200 mm	74	206.66 (200.38-217.87)
	Chetco River, California, USA	3	Aug 17	42.00	> 200 mm	10	217.87 (209.64-229.18)

*Region 1 = Vancouver Island, British Columbia, Region 2 = Washington and Oregon, Region 3 = Northern California, Region 4 = Central California, Region 5 = Southern California

Table 4.1 Continued.

Year	Location	Region*	Date	Latitude (^{o}N)	Fish size class (SL)	No. Fish	Estimated mean (range) fresh SL
2007	Patrick's Point/Klamath River, California, USA	3	Aug 16	41.21	≤ 200 mm	8	195.88 (190.10-199.35)
			Aug 16		> 200 mm	24	208.61 (200.38-219.93)
	Point Delgada, California, USA	3	Aug	40.00	≤ 200 mm	11	195.58 (184.58-199.94)
			Aug		> 200 mm	12	204.07 (200.38-207.58)
	Point Arena/Point Reyes, California, USA	3	Aug 12/13	38.29	≤ 200 mm	10	189.99 (182.90-199.35)
			Aug 12/13	38.29	> 200 mm	3	202.78 (200.38-204.50)
			Aug 17	38.52	≤ 200 mm	62	192.87 (170.55-199.35)
			Aug 17	38.52	> 200 mm	24	203.90 (200.38-227.13)
	Golden Gate Inner, California, USA	4	Aug 11	37.48	≤ 200 mm	1	183.92
	Salmon Cone, California, USA	4	Apr 28	35.80	≤ 200 mm	4	193.70 (185.98-199.35)
			Apr 28		> 200 mm	35	211.58 (201.41-267.24)
	San Nicolas Island, California, USA	5	Apr 21	33.28	≤ 200 mm	44	178.20 (161.29-194.21)
			Apr 21		> 200 mm	1	205.52
2008	Columbia River, Oregon, USA	2	May 25	46.17	> 200 mm	19	224.74 (210.67-242.55)
	Cape Meares, Oregon, USA	2	May 23	45.48	≤ 200 mm	1	195.24
			May 23	45.48	> 200 mm	30	226.23 (208.61-251.81)
	Ventura, California, USA	5		34.28	> 200 mm	49	223.30 (210.00-257.98)
Total						**1491**	

*Region 2 = Washington and Oregon, Region 3 = Northern California, Region 4 = Central California, Region 5 = Southern California

Table 4.2. a) Summary of regional recovery of Pacific sardines (*Sardinops sagax*) infected with the trematode *Myosaccium ecaude*, and b) Mann-Whitney U tests comparing the estimated fresh standard length (SL) of infected sardines caught in five regions of the California Current. Regions are defined as the following: Region 1 = Vancouver Island, British Columbia; Region 2 = Washington and Oregon; Region 3 = Northern California; Region 4 = Central California; and Region 5 = Southern California.

a) Regional summary

Region	No. fish	mean SL	SL sd*
1	11	208.34	(± 11.91)
2	57	206.85	(± 12.78)
3	81	196.38	(± 7.93)
4	56	180.73	(± 14.61)
5	70	180.01	(± 14.58)

b) Regional Mann-Whitney U tests

Region Comparsion	Mean Ranks		z-value	p-value
1 vs 2	1 = 37.18	2 = 33.98	-0.49	0.62
1 vs 3	1 = 69.32	3 = 43.40	-3.02	0.003
1 vs 4	1 = 58.05	4 = 29.28	-4.48	< 0.0001
1 vs 5	1 = 71.09	5 = 36.27	-4.57	< 0.0001
2 vs 3	2 = 91.11	3 = 54.29	-5.33	< 0.0001
2 vs 4	2 = 80.74	4 = 32.84	-7.77	< 0.0001
2 vs 5	2 = 93.54	5 = 39.95	-8.16	< 0.0001
3 vs 4	3 = 87.73	4 = 41.91	-6.64	< 0.0001
3 vs 5	3 = 101.09	5 = 46.97	-7.59	< 0.0001
4 vs 5	4 = 66.79	5 = 60.87	-0.90	0.37

*SL sd = standard length standard deviation in mm

Table 4.3. AMOVA analyses for ND1 mitochondrial DNA recovered from *Myosaccium ecaude* grouped by geographic region a) divided into 5 regions, b) divided into 2 regions (region 1 versus regions 2,3,4,5), c) divided into 2 regions (region 5 versus 1,2,3,4), d) divided into 2 regions (1,2,3 versus 4,5), and e) non-migrants versus migrants by region. The number of trematodes compared per region are indicated for each AMOVA test.

AMOVA test*	Source	df	Sum of squares	Variance components	Percentage of variation	Φ_{ST}	p-value
a) divided into 5 regions							
1 (n = 11)	Among regions	4	1.46	-0.003	-0.51	-0.005	0.97
2 (n = 43)	Within regions	400	235.55	0.59	100.51		
3 (n = 136)	Total	404	237.01	0.59			
4 (n = 96)							
5 (n = 119)							
b) divided into 2 regions							
1 (n = 11)	Among regions	1	0.41	-0.01	-1.46	-0.01	0.66
2,3,4,5 (n = 362)	Within regions	403	236.61	0.59	101.46		
	Total	404	237.01	0.58			
c) divided into 2 regions							
5 (n = 119)	Among regions	1	0.32	-0.002	-0.27	-0.003	0.90
1,2,3,4 (n = 286)	Within regions	403	236.67	0.59	100.27		
	Total	404	237.01				
d) divided into 2 regions							
1,2,3 (n = 190)	Among regions	1	0.37	-0.002	-5.88	-0.18	0.78
4,5 (n = 215)	Within regions	403	236.64	0.59	105.88		
	Total	404	237.01	0.59			

*1 = Vancouver Island, British Columbia, Canada, 2 = Washington and Oregon, 3 = Northern California, 4 = Central California, and 5 = Southern California

Table 4.3 Continued.

AMOVA test*	Source	df	Sum of squares	Variance components	Percentage of variation	Φ_{ST}	p-value
e) non-migrants vs migrants by region							
1: N (n = 5), M (n = 6)	Among regions	9	4.77	-0.002	-0.28	-0.003	0.66
2: N (n = 12), M (n = 31)	Within regions	396	233.30	0.59	100.28		
3: N (n = 110), M (n = 26)	Total	405	238.07	0.58			
4: N (n = 77), M (n = 19)							
5: N(112), m (n = 7)							

*1 = Vancouver Island, British Columbia, Canada, 2 = Washington and Oregon, 3 = Northern California, 4 = Central California, 5 = Southern California, N = non-migrant sardine; M = migrant sardine

Figure Titles

Figure 4.1. Geographic location of stations (solid circles or gray arrows) where Pacific sardine (*Sardinops sagax*) were caught in five regions of the California Current from 2005 through 2008, and the number of *Myosaccium ecaude* trematodes recovered per region: Region 1 Vancouver Island, British Columbia (n = 11); Region 2: Washington and Oregon (n = 43); Region 3: Northern California (n = 136); Region 4: Central California (n = 96); and Region 5: Southern California (n = 119). The 200m isobath is depicted by the solid black line oriented approximately north to south, west of the provincial and state coastlines.

Figure 4.2. Statistical parsimony network of ND1 mitochondrial DNA sequences for *Myosaccium ecaude*. Each connection is a single base difference, solid black circles are inferred haplotypes, and colored circles are observed haplotypes. The colored circles indicate the geographic region where each haplotype was recovered in the California Current from Pacific sardine (*Sardinops sagax*): Region 1: Vancouver Island, British Columbia (blue); Region 2: Washington and Oregon (green); Region 3: Northern California (maroon); Region 4: Central California (purple); and Region 5: Southern California (yellow). The number of worms with identical sequences is represented by the size of the colored circles, except the two haplotypes which are drawn at a quarter of their size. Unless indicated by a number inside or next to the circle, each haplotype represents an individual trematode. Homoplasies among the sequences are indicated by reticulations within the networks.

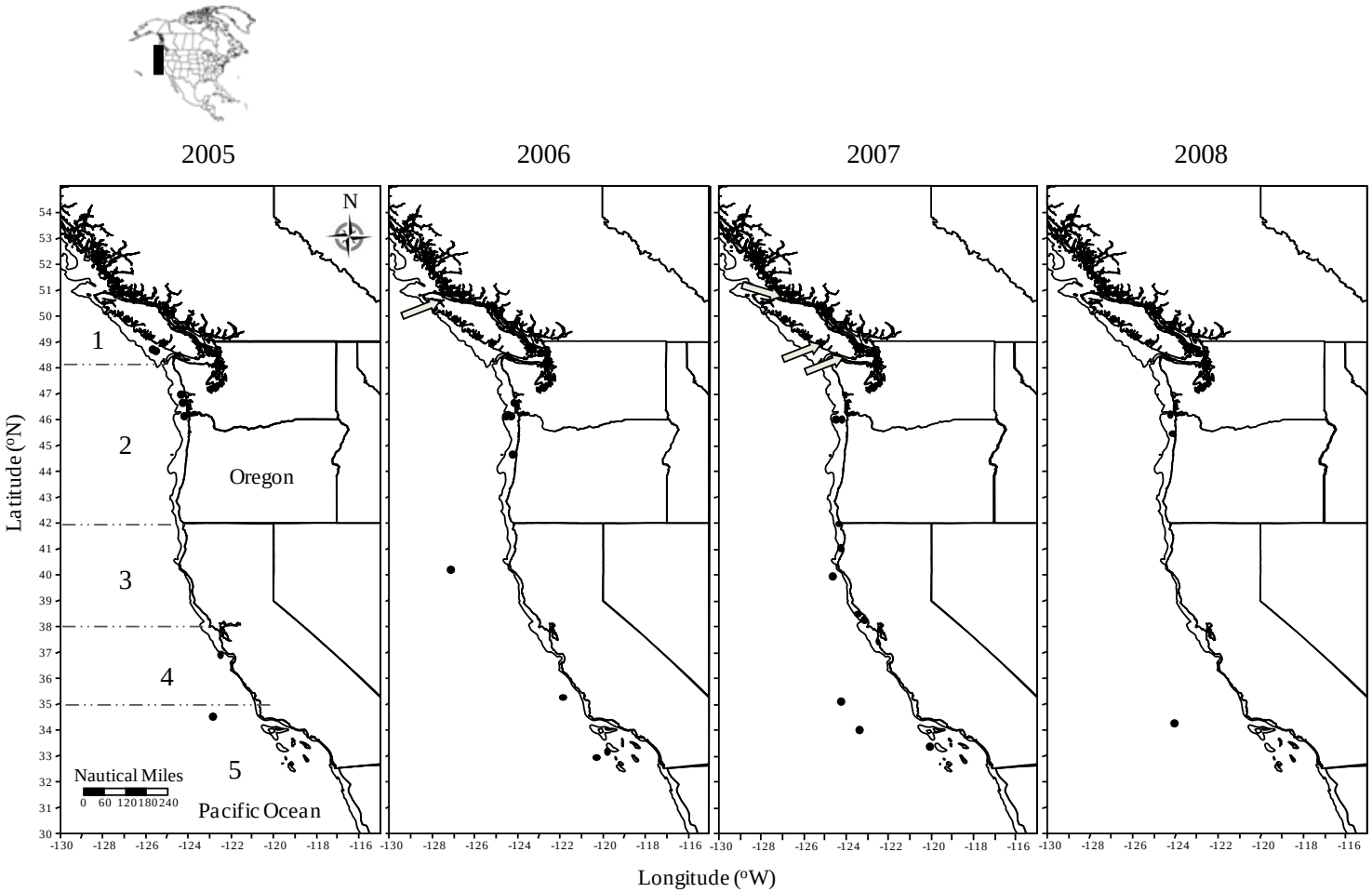
2005
2006
2007
2008
N
1
2
3
4
5
Oregon
Pacific Ocean
Nautical Miles
0 60 120180240
Latitude (°N)
Longitude (°W)

Figure 4.1.

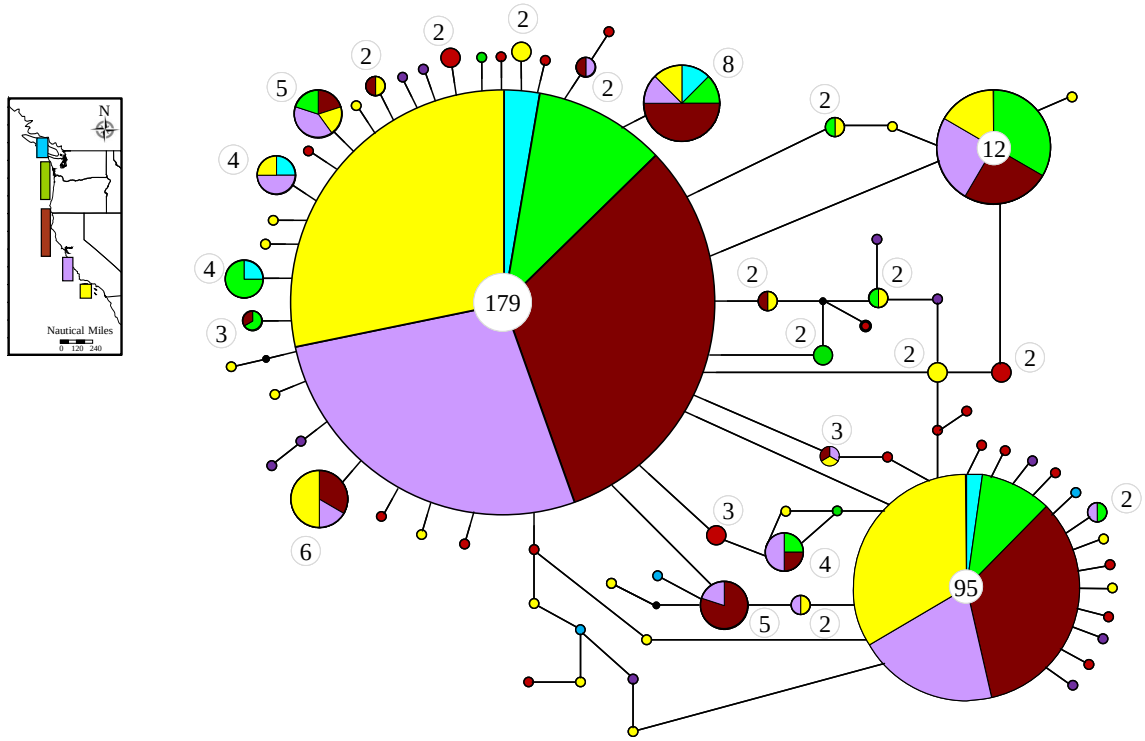

Figure 4.2.

GENERAL CONCLUSION

The overall objective of this research was to use parasite community data and parasite population genetics data to describe the migration and potential stock structure of Pacific sardines along the west coast of North America. In chapter one, I reviewed the history of different methods that have or could be used to assess the stock structure of Pacific sardines. In chapter two, I identified two trematodes, *L. gibbosus* and *M. ecaude* as promising biological tags in assessing the migration patterns of Pacific sardines in the California Current System. In chapter three, I genetically identified three species of larval nematodes (*A. simplex s.s.*, *A. pegreffii*, and *A. simplex* 'C') using the internal transcribed spacers (ITS1 and ITS2) and the 5.8s subunit of the nuclear ribosomal DNA. In addition, a panmictic distribution was identified for each *Anisakis* species using the cytochrome c oxidase 2 (*cox2*) mitochondrial DNA gene. In chapter four, a panmictic distribution from Vancouver Island, British Columbia, Canada, to San Diego, California, USA was also described for the trematode *M. ecaude* using the (ND1) mitochondrial DNA gene.

When it is difficult to resolve the population structure of marine fish (such as the current situation with Pacific sardine), an integrated approach that includes both fish and parasite data can be used to clarify ecological and evolutionary events that structure fish populations. The HOMSIR project which employed this integrated approach for the Atlantic horse mackerel (Abaunza et al. 2008a;b; MacKenzie et al. 2008; Mattiucci et al. 2008) should serve as an important model for future stock

structure studies, especially for pelagic fish species whose populations fluctuate in response to changing environmental conditions.

Pacific sardine are not infected with the same trophically-transmitted parasite species throughout the California Current. Five trematodes (*L. gibbosus*, *M. ecaude*, *P. petrowi*, and *P. merus*), and the nematodes *Hysterothylacium* species and *Anisakis* species contributed to differences in parasite communities between years. However, two trematode species were the most promising biological tags for Pacific sardine in our study. *Lecithaster gibbosus* were recovered in non-migrant and migrant sardines primarily off Vancouver Island, British Columbia. *Myosaccium ecaude* were recovered throughout the study area, but were most common off Central and Southern California in non-migrant fish.

The geographic distributions of *L. gibbosus* and *M. ecaude* observed from 2005 through 2007 suggested Pacific sardines may have two separate but overlapping migration patterns in the California Current. The recovery of *M. ecaude* in all five regions of the study area supported conclusions of previous artificial tagging studies conducted more than 60 years ago that sardines migrate between Southern California and the Pacific Northwest (Oregon, Washington and British Columbia; Janssen 1938; Clark and Janssen 1945; Marr 1957). However, our analyses identified another migration pattern within the Pacific Northwest comprising Regions 1 (Vancouver Island, British Columbia, Canada) and Region 2 (Washington and Oregon, USA)

based on the geographic distribution of *L. gibbosus*. The recovery of only seven out of 1389 sardines infected with both *L. gibbosus* and *M. ecaude* suggests that sardines migrating from Vancouver Island, British Columbia are not all returning to Southern California to overwinter in the same year they migrated to north to the feeding grounds. If this extensive migration was common, we would expect to recover more sardines infected with both trematode species south of Washington. Recovering only three migrant sardines off Central California in April infected with *L. gibbosus* suggests a few sardines overwinter off Vancouver Island and return the following spring to spawn of Southern California. Alternatively, individual sardines may either clear infections of *L. gibbosus* before migrating back to Southern California to spawn, or grow to a specific standard length and no longer migrate back to southern California.

Overlapping migration patterns based on the distributional patterns of *L. gibbosus* and *M. ecaude*, questions the proposed single coastwide migration pattern for Pacific sardines in the California Current. Some larger sardines may have a restricted migration pattern in the Pacific Northwest from Vancouver Island, British Columbia to Oregon and all may not return to Southern California to spawn. Overlapping migration patterns have been described by Blaylock et al. (2002) for Pacific halibut (*Hippoglossus stenolepis*) from Northern California to the Aleutian Islands, Alaska. While reproductively isolated stocks of sardines could not be determined using parasite community data, our data suggests a conservative approach to sardine

management that recognizes two possible migration patterns of Pacific sardines is warranted.

Our observations differ from the disjointed geographical recovery of *Anisakis* nematode in European hake (Mattiucci et al. 2004), Atlantic horse mackerel (Mattiucci et al. 2008), and European sardines. To date European sardines infected with *Anisakis* sp. have only been observed along the Adriatic coast off Italy (Fioravanti et al. 2006), and off western Portugal (Silva and Eiras 2003). No *Anisakis* sp. were observed in sardines off northwest Spain in Galician waters (Abollo et al. 2001), Southern and Eastern Spain (Rello et al. 2008), or off Western Africa by Morocco and Mauritania (Kijewska et al. 2009). Larval *Anisakis* species in European sardines have not yet been genetically identified to species, but the geographic distribution of genetically identified *Anisakis* species in European hake (Mattiucci et al. 2004) and horse mackerel (Mattiucci et al. 2008), suggest *A. simplex s.s.* and *A. pegreffii* could infect sardines off Portugual and *A. pegreffii*, *A. typica* and *A. physeteris* could infect sardines off Italy. Once *Anisakis* nematodes from European sardines are genetically identified, and a population genetic study is conducted for each *Anisakis* species, it can be determined if the panmictic distribution of the three *Anisakis* species in Pacific sardine is unique because of the hydrography of the California Current.

A panmictic distribution described for *M. ecaude* was also described previously for three *Anisakis* species in this study of Pacific sardine in the California Current

(Baldwin et al. CHAPTERS 2 and 3, this volume). The two dominant ND1 haplotypes of *M. ecaude* were recovered in similar proportions throughout the study region. Our results from the population genetics studies of *Anisakis* species and *M. ecaude* suggest that limited oceanographic barriers and complexity in the California Current are not preventing the mixing of the populations of either *M. ecaude* or each *Anisakis* species. The diversity and availability of fish and cetacean species that migrate along the California Current system may enable large geographically distributed populations of parasites, thus we cannot confirm or deny the existence of Pacific sardine subpopulations within the California Current by the distributional patterns of *M. ecaude* or the three *Anisakis* species.

References

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Rarnos, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fisheries Research **89**:196-209.

Abollo, E., C. Gestal, and S. Pascual. 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitology Research **87**:492-499.

Blaylock, R. B., L. Margolis, and J. C. Holmes. 2002. The use of parasites in discriminating stocks of Pacific halibut (*Hippoglossus stenolepis*) in the northeast Pacific. Fishery Bulletin **101**:1-9.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Fioravanti, M. L., M. Caffara, D. Florio, A. Gustinelli, F. Marcer, M. Gradassi, S. Gavaudan, A. Paolini, A. Alessi, and D. Bisceglia. 2006. Anisakiasis in anchovies (*Engraulis encrasicolus*) and sardines (*Sardina pilchardus*) caught along the Adriatic coast. Parassitologia **48**:285.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Kijewska, A., J. Dzido, O. Shukhgalter, and J. Rokicki. 2009. Anisakid parasites of fishes caught on the African Shelf. Journal of Parasitology **95**:639-645.

MacKenzie, K., N. Campbell, S. Mattiucci, P. Ramos, A. L. Pinto, and P. Abaunza. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fisheries Research **89**:136-145.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008. *Anisakis* spp. larvae (Nematoda : Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Rello, F. J., F. J. Adroher, and A. Valero. 2008. *Hysterothylacium aduncum*, the only anisakid parasite of sardines (*Sardina pilchardus*) from the southern and eastern coasts of Spain. Parasitology Research **104**:117-121.

Silva, M. E. R., and J. C. Eiras. 2003. Occurrence of *Anisakis* sp. in fishes off the Portuguese west coast and evaluation of its zoonotic potential. Bulletin of the European Association of Fish Pathologists **23**:13-17.

Bibliography

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, E. Gallo, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Ramos, A. Ruggi, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008a. Considerations on sampling strategies for an holistic approach to stock identification: The example of the HOMSIR project. Fisheries Research **89**:104-113.

Abaunza, P., A. G. Murta, N. Campbell, R. Cimmaruta, A. S. Comesana, G. Dahle, M. T. G. Santamaria, L. S. Gordo, S. A. Iversen, K. MacKenzie, A. Magoulas, S. Mattiucci, J. Molloy, G. Nascetti, A. L. Pinto, R. Quinta, P. Rarnos, A. Sanjuan, A. T. Santos, C. Stransky, and C. Zimmermann. 2008b. Stock identity of horse mackerel (*Trachurus trachurus*) in the Northeast Atlantic and Mediterranean Sea: Integrating the results from different stock identification approaches. Fisheries Research **89**:196-209.

Abe, N. 2008. Application of the PCR-sequence-specific primers for the discrimination among larval *Anisakis simplex* complex. Parasitology Research **102**:1073-1075.

Abollo, E., C. Gestal, and S. Pascual. 2001. *Anisakis* infestation in marine fish and cephalopods from Galician waters: an updated perspective. Parasitology Research **87**:492-499.

Abollo, E., L. Paggi, S. Pascual, and S. D'Amelio. 2003. Occurrence of recombinant genotypes of *Anisakis simplex s.s.* and *Anisakis pegreffii* (Nematoda: Anisakidae) in an area of sympatry. Infection, Genetics and Evolution **3**:175-181.

Ahlstrom, E. H. 1957. A review of recent studies of subpopulations of Pacific fishes. Pages 44-73 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208., Washington DC.

Aiken, H. M., N. J. Bott, I. Mladineo, F. E. Montero, B. F. Nowak, and C. J. Hayward. 2007. Molecular evidence for cosmopolitan distribution of platyhelminth parasites of tunas (*Thunnus* spp.). Fish and Fisheries **8**:167-180.

Alder, J., B. Campbell, V. Karpouzi, K. Kaschner, and D. Pauly. 2008. Forage Fish: From Ecosystems to Markets. Annual Review of Environment and Resources **33**:153-166.

Allendorf, F. W., and G. Luikart. 2007. Conservation and the genetics of populations. Blackwell Publishing, Australia.

Ambrose III, H. W., and K. P. Ambrose. 1987. A Handbook of Biological Investigation, Fourth edition. Hunter Textbooks Inc, Winston-Salem, NC.

Armannsson, H., S. T. Jonsson, J. D. Neilson, and G. Marteinsdottir. 2007. Distribution and migration of saithe (*Pollachius virens*) around Iceland inferred from mark-recapture studies. ICES Journal of Marine Science **64**:1006-1016.

Arthur, J. R., and E. Albert. 1994. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic. Canadian Journal of Fisheries and Aquatic Sciences **50**:2175-2181.

Arthur, J. R., and H. P. Arai. 1980. Studies on the parasites of Pacific herring (*Clupea harengus pallasi* Valenciennes): a preliminary evaluation of parasites as indicators of geographical origin for spawning herring. Canadian Journal of Zoology **58**:521-527.

Atarhouch, T., L. Rüber, E. G. Gonzalez, E. M. Albert, M. Rami, A. Dakkak, and R. Zardoya. 2006. Signature of an early genetic bottleneck in a population of Moroccan sardines (*Sardina pilchardus*). Molecular Phylogenetics and Evolution **39**:373-383.

Avise, J. C. 1998. Conservation genetics in the marine realm. Journal of Heredity **89**:377-382.

Baker, T. G., S. Morand, C. A. Wenner, W. A. Roumillat, and I. de Buron. 2007. Stock identification of the sciaenid fish *Micropogonias undulatus* in the western North Atlantic Ocean using parasites as biological tags. Journal of Helminthology **81**:155-167.

Bakun, A. 1996. Patterns in the ocean. Ocean processes and marine population dynamics. California Sea Grant College System, National Oceanic and Atmospheric Administration in cooperation with Centro de Investigaciones Biológicas del Noroeste, La Paz, BCS, Mexico.

Baldwin, R. E., and C. P. Goater. 2003. Circulation of parasites among fishes from lakes in the Caribou Mountains, Alberta, Canada. Journal of Parasitology **89**:215-225.

Baldwin, R. E., T. W. Miller, R. D. Brodeur, and K. C. Jacobson. 2008. Expanding the foraging history of juvenile Pacific salmon: combining stomach-content and macroparasite-community analyses for studying marine diets. Journal of Fish Biology **72**:1268-1294.

Baranov, F. I. 1918. On the question of the biological basis of fisheries. Nauchn. Issled. Iktiol. Inst. Izv. **1**:81-128.

Baumgartner, T. R., A. Soutar, and V. Ferreirabartrina. 1992. Reconstruction of the history of Pacific sardine and northern anchovy populations over the past two millennia from sediments of the Santa Barbara Basin, California. California Cooperative Oceanic Fisheries Investigations Reports **33**:24-40.

Beacham, T. D., J. F. Schweigert, C. MacConnachie, K. D. Le, and L. Flostrand. 2008. Use of Microsatellites to Determine Population Structure and Migration of Pacific Herring in British Columbia and Adjacent Regions. Transactions of the American Fisheries Society **137**:1795-1811.

Beamish, R. J., A. J. Benson, R. M. Sweeting, and C. M. Neville. 2004. Regimes and the history of the major fisheries off Canada's west coast. Progress in Oceanography **60**:355-385.

Beamish, R. J., G. A. McFarlane, and J. R. King. 2000. Fisheries climatology: understanding decadal scale processes that naturally regulate British Columbia fish populations. Pages 94-139 *in* P. J. Harrison and T. R. Parsons, editors. Fisheries Oceanography. An Integrative Approach to Fisheries Ecology and Management. Blackwell Science, Maldan, MA.

Bentley, P. J., R. L. Emmett, N. C. H. Lo, and H. G. Moser. 1996. Egg production of Pacific sardine (*Sardinops sagax*) off Oregon in 1994. California Cooperative Oceanic Fisheries Investigations Reports **37**:193-200.

Beverton, R. 1998. Fish, fact and fantasy: A long view. Reviews in Fish Biology and Fisheries **8**:229-249.

Beverton, R. 2002. Reflections on 100 Years of Fisheries Research. *In:* The Raymond JH Beverton Lectures at Woods Hole, Massachusetts. Three lectures on fisheries science given May 2-3, 1994. Pages 107-158 *in* NOAA Tech. Memo. NMFS-F/SPO-54. U.S. Department of Commerce.

Beverton, R., and S. J. Holt. 1957. On the Dynamics of Exploited Fish Populations. Ministry of Agriculture, Fisheries and Food.

Blaylock, R. B., L. Margolis, and J. C. Holmes. 2002. The use of parasites in discriminating stocks of Pacific halibut (*Hippoglossus stenolepis*) in the Northeast Pacific. Fishery Bulletin **101**:1-9.

Blouin, M. S., C. A. Yowell, C. H. Courtney, and J. B. Dame. 1995. Host movement and the genetic structure of populations of parasitic nematodes. Genetics **141**:1007-1014.

Boyce, N. P. 1969. Parasite fauna of pink salmon (*Oncorhynchus gorbuscha*) of the Bella Coola River, central British Columbia, during their early sea life. Journal of the Fisheries Research Board of Canada **26**:813-820.

Bray, J. R., and J. T. Curtis. 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs **27**:325-349.

Brodeur, R. D., J. P. Fisher, D. J. Teel, R. L. Emmett, E. Casillas, and T. W. Miller. 2004. Juvenile salmonid distribution, growth, condition, origin, and environmental and species associations in the northern California Current. Fisheries Bulletin **102**:25-46.

Brodeur, R. D., C. L. Suchman, D. C. Reese, T. W. Miller, and E. A. Daly. 2008. Spatial overlap and trophic interactions between pelagic fish and large jellyfish in the northern California Current. Marine Biology **154**:649-659.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pages 271-277 *in* K. R. Kerry and G. Hempel, editors. Evolution of Genes and Proteins. Sinauer, Sunderland, MA.

Buonaccorsi, V. P., J. R. McDowell, and J. E. Graves. 2001. Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). Molecular Ecology **10**:1179-1196.

Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83**:575-583.

Cadrin, S. X., K. D. Friedland, and J. R. Waldman. 2005. Stock identification methods: an overview. Pages 3-6 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Applications in Fishery Science. Elsevier Academic Press, San Francisco.

Campbell, N., K. MacKenzie, A. F. Zuur, E. N. Ieno, and G. M. Smith. 2007. Fish stock identification through neural network analysis of parasite fauna. Pages 449-462 *in* A. F. Zuur, E. N. Ieno, and G. M. Smith, editors. Analyzing Ecological Data. Springer, New York.

Casini, M., J. Hjelm, J. C. Molinero, J. Lovgren, M. Cardinale, V. Bartolino, A. Belgrano, and G. Kornilovs. 2009. Trophic cascades promote threshold-like shifts in pelagic marine ecosystems. Proceedings of the National Academy of Sciences of the United States of America **106**:197-202.

Checkley, D. M., and J. A. Barth. 2009. Patterns and processes in the California Current System. Progress in Oceanography **83**:49-64.

Chlaida, M., S. Kifani, P. Lenfant, and L. Ouragh. 2006. First approach for the identification of sardine populations *Sardina pilchardus* (Walbaum 1792) in the Moroccan Atlantic by allozymes. Marine Biology **149**:169-175.

Clark, F. N. 1935. A summary of the life-history of the California sardine and its influence on the fishery. California Fish and Game **21**:1-9.

Clark, F. N. 1947. Analysis of populations of the Pacific sardine on the basis of vertebral counts. Fisheries Bulletin for the California Department of Fish and Game **65**. 26pp.

Clark, F. N., and J. F. Janssen. 1945. Movements and abundance of the sardine as measured by tag returns. California Fish and Game **61**:1-42.

Clark, K. R., and R. N. Gorley. 2006. Primer v6: user manual / tutorial. Primer-E Ltd., Plymouth Marine Laboratory, United Kingdom.

Clarke, K. R. 1993. Non-parametric multivariate analyses of changes in community structure. Australian Journal of Ecology **18**:117-143.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology **9**:1657-1659.

Cope, J. M. 2004. Population genetics and phylogeography of the blue rockfish (*Sebastes mystinus*) from Washington to California. Canadian Journal of Fisheries and Aquatic Sciences **61**:332-342.

Criscione, C. D., and M. S. Blouin. 2004. Life cycles shape parasite evolution: comparative population genetics of salmon trematodes. Evolution **58**:198-202.

Criscione, C. D., and M. S. Blouin. 2006. Minimal selfing, few clones, and no among-host genetic structure in a hermaphrodictic parasite with asexual larval propagation. Evolution **60**:553-562.

Criscione, C. D., B. Cooper, and M. S. Blouin. 2006. Parasite genotypes identify source populations of migratory fish more accurately than fish genotypes. Ecology **87**:823-828.

Criscione, C. D., R. Poulin, and M. S. Blouin. 2005. Molecular ecology of parasites: elucidating ecological and microevolutionary processes. Molecular Ecology **14**:2247-2257.

Cross, M. A., C. Collins, N. Campbell, P. C. Watts, J. C. Chubb, C. O. Cunningham, E. M. C. Hatfield, and K. MacKenzie. 2007. Levels of intra-host and temporal sequence variation in a large CO1 sub-units from *Anisakis simplex sensu stricto* (Rudolphi 1809) (Nematoda: Anisakidae): implications for fisheries management. Marine Biology **151**:695-702.

Crowder, L. B., E. L. Hazen, N. Avissar, R. Bjorkland, C. Latanich, and M. B. Ogburn. 2008. The impacts of fisheries on marine ecosystems and the transition to ecosystem-based management. Annual Review of Ecology Evolution and Systematics **39**:259-278.

Cury, P., A. Bakun, R. J. M. Crawford, A. Jarre, R. A. Quinones, L. J. Shannon, and H. M. Verheye. 2000. Small pelagics in upwelling systems: patterns of interaction and structural changes in "wasp-waist" ecosystems. ICES Journal of Marine Science **57**:603-618.

Dahlgren, E. H. 1936. Further developments in the tagging of the Pacific herring, (*Clupea pallasii*). Journal du Conseil **11**:229-247.

DeWoody, J. A., and J. C. Avise. 2000. Microsatellite variation in marine, freshwater and anadromous fishes compared with other animals. Journal of Fish Biology **56**:461-473.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bentley, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific Northwest. California Cooperative Oceanic Fisheries Investigations Reports **46**:122-143.

Emmett, R. L., and N. C. H. Lo. 2009. Proceedings of the 2008 Tri-national Sardine Forum. Northwest Fisheries Science Center. 89pp.

Espinoza, P., A. Bertrand, C. D. van der Lingen, S. Garrido, and B. R. de Mendiola. 2009. Diet of sardine (*Sardinops sagax*) in the northern Humboldt Current system and comparison with the diets of clupeids in this and other eastern boundary upwelling systems. Progress in Oceanography **83**:242-250.

Excoffier, L., G. Laval, and S. Schneider. 1992. Arlequin (version 3.0): An integrated software package for population genetics data analysis. Evolutionary Bioinformatics **1**:47-50.

Felin, F. E. 1954. Population heterogeneity in the Pacific pilchard. Fisheries Bulletin **54**:201-225.

Félix-Uraga, R., V. M. Gómez-Muñoz, C. Quiñónez-Velázquez, F. N. Melo-Barrera, K. T. Hill, and W. García -Franco. 2005. Pacific sardine (*Sardinops sagax*) stock discrimination off the west coast of Baja California and southern California using otolith morphometry. California Cooperative Oceanic Fisheries Investigations Reports **46**:113-121.

Félix-Uraga, R., V. M. Gómez-Muñoz, C. Quiñónez-Velázquea, F. N. Melo-Barrera, and W. García-Franco. 2004. On the existence of Pacific sardine groups off the west coast of Baja California and southern California. California Cooperative Oceanic Fisheries Investigations Reports **25**:146-151.

Field, J. C., R. C. Francis, and K. Aydin. 2006. Top-down modeling and bottom-up dynamics: Linking a fisheries-based ecosystem model with climate. Progress in Oceanography **68**:238-270.

Fioravanti, M. L., M. Caffara, D. Florio, A. Gustinelli, F. Marcer, M. Gradassi, S. Gavaudan, A. Paolini, A. Alessi, and D. Bisceglia. 2006. Anisakiasis in anchovies (*Engraulis encrasicholus*) and sardines (*Sardina pilchardus*) caught along the Adriatic coast. Parassitologia **48**:285.

Fitch, J. E. 1969. Fossil records of certain schooling fishes of the California Current System. California Marine Research Communication. California Cooperative Oceanic Fisheries Investigations Reports **13**:71-80.

Gonzalez, E. G., and R. Zardoya. 2007. Relative role of life-history traits and historical factors in shaping genetic population structure of sardines (*Sardina pilchardus*). BMC Evolutionary Biology **7**.

Graham, M. 1935. Modern theory of exploiting a fishery, and application to North Sea trawling. Journal du Conseil International Exploration de Mer. **10**:264-274.

Grant, W. S., and B. W. Bowen. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: Insights from sardines and anchovies and lessons for conservation. Journal of Heredity **89**:415-426.

Grant, W. S., A. M. Clark, and B. W. Bowen. 1998. Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome b sequences. Canadian Journal of Fisheries and Aquatic Sciences **55**:2539-2547.

Grant, W. S., and F. M. Utter. 1984. Biochemical population genetics of Pacific herring (*Clupea pallasi*). Canadian Journal of Fisheries and Aquatic Sciences **41**:856-864.

Hafner, M. S., P. D. Sudman, F. X. Villablanca, T. A. Spradling, J. W. Demastes, and S. A. Nadler. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. Science **265**:1087-1090.

Hall, T. A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series **41**:95-98.

Hand, C. H., and L. Berner Jr. 1959. Food of the Pacific sardine (*Sardinops caerulea*). Fishery Bulletin **60**:175-184.

Hansen, L. P., and J. A. Jacobsen. 2003. Origin and migration of wild and escaped farmed Atlantic salmon, *Salmo salar* L., in oceanic areas north of the Faroe Islands. ICES Journal of Marine Science **60**:110-119.

Hansen, M. M., E. Kenchington, and E. E. Nielsen. 2001. Assigning individual fish to populations using microsatellite DNA markers. Fish and Fisheries **2**:93-112.

Hargreaves, N. B., D. M. Ware, and G. A. McFarlane. 1994. Return of Pacific sardine (*Sardinops sagax*) to the British Columbia coast in 1992. Canadian Journal of Fisheries and Aquatic Sciences **51**:460-463.

Harley, C. D. G., and L. Rogers-Bennett. 2004. The potential synergystic effects of climate change and fishing pressure on exploited invertebrates on rocky intertidal shores. California Cooperative Oceanic Fisheries Investigations Reports **45**:98-110.

Harris, H. 1966. Enzyme polymorphism in man. Proceedings of the Royal Society Series B **164**:298-310.

Harrt, A. C. 1963. Problems in tagging salmon at sea. ICNAF Special Publication **4**:144-155.

Hart, J. L. 1943. Tagging experiments on British Columbia pilchards. Journal of the Fisheries Research Board of Canada **6**:164-182.

Hart, J. L., and G. H. Wailes. 1931. The food of the pilchard, *Sardinops caerulea* (Girard), off the coast of British Columbia. Biological Board of Canada, Contribution to Canadian Biology and Fisheries **7**:247-254.

Hedgecock, D. 1986. Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bulletin of Marine Science **39**:550-564.

Hedgecock, D., E. S. Hutchinson, G. Li, F. L. Sly, and K. Nelson. 1989. Genetic and morphometric variation in the Pacific sardine, *Sardinops sagax caerulea*: Comparisons and contrasts with historical data and with variability in the northern anchovy, *Engraulis mordax*. Fishery Bulletin **87**:653-671.

Hedrick, P. W. 1999. Perspective: Highly variable loci and their interpretation in evolution and conservation. Evolution **53**:313-318.

Herrick, S. F., J. G. Norton, R. Hannesson, U. R. Sumaila, M. Ahmed, and J. Pena-Torres. 2009. Global production and economies. Pages 256-274 *in* D. Checkley, J. Alheit, Y. Oozeki, and C. Roy, editors. Climate Change and Small Pelagic Fish. Cambridge University Press, New York.

Herrington, W. C., H. M. Bearsem, and F. E. Firth. 1939. Observations on the life history, occurrence and distribution of the redfish parasite *Sphyrion lumpi*. US Bureau of Fisheries Special Report **5**:1-18.

Hewitt, G. M. 2004. The structure of biodiversity - insights from molecular phylogeography. Frontiers in Zoology **1**:4.

Hill, K. T. 1999. Determining age composition of coastal pelagic species in northern California, Oregon, and Washington coastal waters. Project #1-IJ-9 Final Report. Pacific States Marine Fisheries Commission, Gladstone, Oregon.

Hill, K. T., N. C. H. Lo, B. J. Macewicz, P. R. Cronel, and R. Felix-Uraga. 2009. Assessment of the Pacific sardine resource in 2009 for U.S. management in 2010. NOAA-TM-NMFS-SWFSC-452:221.

Hoenig, J. M., R. J. Latour, and J. E. Olney. 2008. Estimating stock composition of anadromous fishes from mark-recovery data: Possible application to American shad. North American Journal of Fisheries Management **28**:507-515.

Hubby, J. L., and R. C. Lewontin. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. I. The number of alleles at different loci in *Drosophila pseudoobscura*. Genetics **54**:577-594.

Ivanova, N. V., J. R. Dewaard, and P. D. N. Herber. 2006. Glass fiber plate DNA extraction protocol: manual protocol employing centrifugation method. *in*.

Jacobsen, J. A., and L. P. Hansen. 2005. Internal and external tags. Pages 415-433 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock identification methods. Applications in Fishery Science. Elsevier Academic Press, San Francisco.

Jakobsson, J. 1970. On fish tags and tagging. Oceanography and Marine Biology **8**:457-499.

James, A. G. 1988. Are clupeid microphagists herbivorous or omnivorous? A review of the diets of some commercially important clupeids. South African Journal of Marine Science **7**:161-177.

Janssen, J. F. 1938. Second report of sardine tagging in California. Fisheries Bulletin California Department of Fish and Game **24**:376-389.

Janssen, J. F., and J. F. Alpen. 1945. The effect of internal tags upon sardines. Fisheries Bulletin **61**:43-62.

Jarne, P., and A. Theron. 2001. Genetic structure in natural populations of flukes and snails: a practical approach and review. Parasitology **123**:S27-S40.

Jones, C. M. 2006. Estuarine and diadromous fish metapopulations. Pages 119-154 *in* J. P. Kritzer and P. F. Sale, editors. Marine Metapopulations. Elsevier Academic Press, San Francisco.

Jousson, O., P. Bartoli, and J. Pawlowski. 2000. Cryptic speciation among intestinal parasites (Trematoda: Digenea) infecting sympatric host fishes (Sparidae). Journal of Evolutionary Biology **13**:778-785.

Kijewska, A., J. Dzido, O. Shukhgalter, and J. Rokicki. 2009. Anisakid parasites of fishes caught on the African Shelf. Journal of Parasitology **95**:639-645.

King, R. C., and W. D. Stansfield. 1985. A Dictionary of Genetics, Third edition. Oxford University Press, New York.

Klimpel, S., M. W. Busch, T. Kuhn, A. Rohde, and H. W. Palm. 2010. The *Anisakis simplex* complex off the South Shetland Islands (Antarctica): endemic populations versus introduction through migratory hosts. Marine Ecology Progress Series **403**:1-11.

Køie, M. 1983. Digenetic trematodes from *Limanda limanda* (L.) (Osteichthyes, Pleuronectidae) from Danish and adjacent waters, with special reference to their life-histories. Ophelia **22**:201-228.

Koljonen, M.-L., and R. Wilmot. 2005. Genetic analysis: allozymes. Pages 295-309 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in Fisheries Science. Elsevier Academic Press, San Francisco.

Kunnenkeri, J. K. 1962. Preliminary report on the parasites of the California sardine and the parasitic distribution in Clupeidae. Journal of Parasitology **48**:149.

Lagerquist, B. A., B. R. Mate, J. G. Ortega-Ortiz, M. Winsor, and J. Urban-Ramirez. 2008. Migratory movements and surfacing rates of humpback whales (*Megaptera novaeangliae*) satellite tagged at Socorro Island, Mexico. Marine Mammal Science **24**:815-830.

Larsen, G., W. Hemmingsen, K. MacKenzie, and D. A. Lysne. 1997. A population study of cod, *Gadus morhua* L., in northern Norway using otolith structure and parasite tags. Fisheries Research **32**:13-20.

Laurent, V., B. Caneco, A. Magoulas, and S. Planes. 2007. Isolation by distance and selection effects on genetic structure of sardines *Sardina pilchardus* Walbaum. Journal of Fish Biology **71**:1-17.

Lecomte, F., W. S. Grant, J. J. Dodson, R. Rodriguez-Sanchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Molecular Ecology **13**:2169-2182.

Lessios, H. A., B. D. Kessing, and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. Proceedings of the Royal Society of London Series B-Biological Sciences **265**:583-588.

Lester, R. J. G. 1990. Reappraisal of the use of parasites for fish stock identification. Australian Journal of Marine and Freshwater Research **41**:855-864.

Lester, R. J. G., A. Barnes, and G. Habib. 1985. Parasites of skipjack tuna, *Katsuwonus pelamis*: fishery implications. Fishery Bulletin **83**:343-356.

Lester, R. J. G., K. B. Sewell, A. Barnes, and K. Evans. 1988. Stock discrimination of orange roughy *Hoplostethus atlanticus*, by parasite analysis. Marine Biology **99**:137-143.

Lewontin, R. C., and J. L. Hubby. 1966. A molecular approach to the study of genetic heterozygosity in natural populations. II. Amount of variation and degree of heterozygosity in natural populations of *Drosophila pseudoobscura*. Genetics **54**:595-609.

Librado, P., and J. Rozas. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics **25**:1451-1452.

Lo, N. C. H., B. J. Macewicz, and D. A. Griffith. unpublished. Biomass and reproduction of Pacific sardines off the northwest US in 2003-2005. 43pp.

Lo, N. C. H., B. J. Macewicz, and R. L. Charter. 2007. Spawning biomass of Pacific sardine (*Sardinops sagax*) off California in 2007. NOAA-TM-NMFS-SWFSC-411 Technical Memo, U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southwest Fisheries Science Center:38pp.

Locke, S. A., J. D. McLaughlin, S. Dayanandan, and D. J. Marcogliese. 2010. Diversity and specificity in *Diplostomum* spp. metacercariae in freshwater fishes revealed by cytochrome c oxidase I and internal transcribed spacer sequences. International Journal of Parasitology **40**:333-343.

Longhurst, A. 2006. The sustainability myth. Fisheries Research **81**:107-112.

Love, M. S., and M. Moser. 1983. A checklist of parasites of California, Oregon, and Washington Marine and Estuarine Fishes. NOAA Technical Report NMFS SSRF-777. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Services. 254pp

Mackenzie, K. 1985. The use of parasites as biological tags in population studies of herring (*Clupea harengus* L.) in the North Sea and to the north and west of Scotland. Journal du Conseil International Exploration de Mer **42**:33-64.

MacKenzie, K. 1989. Parasites as biological tags for pacific sardines, *Sardinops sagax* (Jenyns) in coastal waters of Ecuador and Peru. DAFS Marine Laboratory, Aberdeen, Scotland.

MacKenzie, K. 2002. Parasites as biological tags in population studies of marine organisms: an update. Parasitology **124**:S153-S163.

MacKenzie, K., and P. Abaunza. 1998. Parasites as biological tags for stock discrimination of marine fish: a guide to procedures and methods. Fisheries Research **38**:45-56.

MacKenzie, K., N. Campbell, S. Mattiucci, P. Ramos, A. L. Pinto, and P. Abaunza. 2008. Parasites as biological tags for stock identification of Atlantic horse mackerel *Trachurus trachurus* L. Fisheries Research **89**:136-145.

Mackenzie, K., and M. Longshaw. 1995. Parasites of the hakes *Merluccius australis* and *M. hubbsi* in the waters around the Falkland Islands, southern Chile, and Argentina, with an assessment of their potential value as biological tags. Canadian Journal of Fisheries and Aquatic Sciences **52**:213-224.

Manel, S., O. E. Gaggiotti, and R. S. Waples. 2004. Assignment methods: matching biological questions with appropriate techniques. TRENDS in Ecology and Evolution **20**:1-7.

Mann, K. 2000. Commentary: environmental effects on fish stocks. Pages 140-145 *in* P. J. Harrison and T. R. Parsons, editors. Fisheries oceanography. An integrative approach to fisheries ecology and management. Blackwell Science, Maldan, MA.

Mantua, N. J., S. R. Hare, Y. Zhang, J. M. Wallace, and R. C. Francis. 1997. A Pacific interdecadal climate oscillation with impacts on salmon production. Bulletin of the American Meteorological Society **78**:1069-1079.

Marcogliese, D. J. 1995. The role of zooplankton in the transmission of helminth parasites to fish. Reviews of Fish Biology and Fisheries **5**:336-371.

Marcogliese, D. J. 2002. Food webs and the transmission of parasites to marine fish. Parasitology **124**:S83-S99.

Marcogliese, D. J., E. Albert, P. Gagnon, and J.-M. Sevigny. 2003. Use of parasites in stock identification of the deepwater redfish (*Sebastes mentella*) in the Northwest Atlantic. Fisheries Bulletin **101**:183-188.

Margolis, L. 1963. Parasites as indicators of the geographical origins of sockeye salmon, *Oncorhynchus nerka* (Walbaum), occurring in the North Pacific Ocean and adjacent seas. International North Pacific Fisheries Commission Bulletin **11**:101-156.

Margolis, L. 1984. Preliminary report on identification of continent of origin of ocean-caught steelhead trout, *Salmo gairdneri*, using naturally occurring parasite "tags". unpublished report Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, BC.

Margolis, L., and N. P. Boyce. 1969. Life span, maturation, and growth of two Hemiurid trematodes, *Tubulovesicula lindbergi* and *Lecithaster gibbosus*, in Pacific salmon (Genus *Oncorhynchus*). Journal of the Fisheries Research Board of Canada **26**:893-907.

Margolis, L., and H. L. Ching. 1965. Review of the Trematode genera *Bacciger* and *Pentagramma* (Fellodistomatidae) and description of *P. petrowi* (Layman, 1930) n. comb. from marine fishes from the Pacific coast of Canada. Canadian Journal of Zoology **43**:381-405.

Marr, J. C. 1957. The subpopulation problem in the Pacific sardine, *Sardinops caerulea*. Pages 108-125 *in* J. C. Marr, editor. Contributions to the Study of Subpopulations of Fishes. Special Scientific Report, Fisheries No. 208, Washington, DC.

Marr, J. C. 1960. The causes of major variations in the catch of the Pacific sardine, *Sardinops caerulea* (Girard). Pages 667-791 *in* H. Rosa and G. I. Murphy, editors. Proceedings of the World Scientific Meeting on the Biology of Sardines and Related Species. Food and Agriculture Organization of the United Nations.

Mattiucci, S. 2006. Parasites as biological tags in population studies of demersal and pelagic fish species. Parassitologia **48**:23-25.

Mattiucci, S., P. Abaunza, L. Ramadori, and G. Nascetti. 2004. Genetic identification of *Anisakis* larvae in European hake from Atlantic and Mediterranean waters for stock recognition. Journal of Fish Biology **65**:495-510.

Mattiucci, S., V. Farina, N. Campbell, K. MacKenzie, P. Ramos, A. L. Pinto, P. Abaunza, and G. Nascetti. 2008a. *Anisakis* spp. larvae (Nematoda: Anisakidae) from Atlantic horse mackerel: Their genetic identification and use as biological tags for host stock characterization. Fisheries Research **89**:146-151.

Mattiucci, S., and G. Nascetti. 2008. Advances and trends in the molecular systematics of anisakid nematodes, with implications for their evolutionary ecology and host-parasite co-evolutionary processes. Pages 47-148 *in* Advances in Parasitology.

Mattiucci, S., M. Paoletti, J. Olivero-Verbel, R. Baldiris, B. Arroyo-Salgado, L. Garbin, G. Navone, and G. Nascetti. 2008b. *Contracaecum bioccai* n. sp from the brown pelican *Pelecanus occidentalis* (L.) in Colombia (Nematoda : Anisakidae): morphology, molecular evidence and its genetic relationship with congeners from fish-eating birds. Systematic Parasitology **69**:101-121.

Mattiucci, S., M. Paoletti, and S. C. Webb. 2009. *Anisakis nascettii* n. sp (Nematoda: Anisakidae) from beaked whales of the southern hemisphere: morphological description, genetic relationships between congeners and ecological data. Systematic Parasitology **74**:199-217.

Mattson, M. T., J. R. Waldman, D. J. Dunning, and Q. E. Rose. 1990. Abrasion and protrusion of internal anchor tags in Hudson River striped bass. American Fisheries Society Symposium **7**:121-126.

McClelland, G., J. Melendy, J. Osborne, D. Reid, and S. Douglas. 2005. Use of parasite and genetic markers in delineating populations of winter flounder from the central and south-west Scotian Shelf and north-east Gulf of Maine. Journal of Fish Biology **66**:1082-1100.

McDonald, T. E., and L. Margolis. 1995. Synopsis of the parasites of fishes of Canada: Supplement (1978-1993). Canadian Special Publication of Fisheries and Aquatic Sciences.

McEvoy, A. F., and H. N. Scheiber. 1984. Scientists, entrepreneurs, and the policy process: a study of the post-1945 California sardine depletion. Journal of Economic History **44**:393-406.

McFarlane, G. A., L. MacDougall, J. Schweigert, and C. Hrabok. 2005. Distribution and biology of Pacific sardines (*Sardinops sagax*) off British Columbia, Canada. California Cooperative Oceanic Fisheries Investigations Reports **46**:144-160.

McFarlane, G. A., R. S. Wydoski, and E. D. Prince. 1990. Historical review of the development of external tags and marks. American Fisheries Society Symposium **7**:9-29.

McGladdery, S. E., and M. D. B. Burt. 1985. Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*). Canadian Journal of Fisheries and Aquatic Sciences **42**:1957-1968.

Mejía-Madrid, H. H., E. Vázquez-Domínguez, and G. P. P. de León. 2007. Phylogeography and freshwater basins in central Mexico: recent history as revealed by the fish parasite *Rhabdochona lichtenfelsi* (Nematoda). Journal of Biogeography **34**:787-801.

Miller, T. W., and R. D. Brodeur. 2007. Diets of and trophic relationships among dominant marine nekton within the northern California Current ecosystem. Fishery Bulletin **105**:548-559.

Moles, A. 2007. Parasites of the fishes of Alaska and surrounding waters. Alaska Fishery Research Bulletin **12**:197-226.

Montgomery, W. R. 1957. Studies on digenetic trematodes from marine fishes of La Jolla, California. Transactions of the American Microscopy Society **76**:13-35.

Morin, P. A., G. Luikart, R. K. Wayne, and S. N. P. Working Group. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology and Evolution **19**:208-216.

Morris, J., M. Trudel, T. B. Zubowski, H. R. MacLean, J. M. R. Curtis, and L. Felli. 2009. CCGS W.E. Ricker Gulf of Alaska salmon survey, June 20-July 5, 2007. Canadian Data Report Fisheries and Aquatic Science 1221. Department of Fisheries and Oceans, Nanaimo, British Columbia.

Moser, M. 1991. Parasites as biological tags. Parasitology **7**:182-185.

Moser, M., and J. Hsieh. 1992. Biological tags for stock separation in pacific herring *Clupea harengus pallasi* in California. Journal of Parasitology **78**:54-60.

Nadler, S. A. 1995. Microevolution and the genetic structure of parasite populations. Journal of Parasitology **81**:395-403.

Nadler, S. A., S. D'Amelio, M. D. Dailey, L. Paggi, S. Siu, and J. A. Sakanari. 2005. Molecular phylogenetics and diagnosis of *Anisakis*, *Pseudoterranova*, and *Contracaecum* from Northern Pacific marine mammals. Journal of Parasitology **91**:1413-1429.

Nadler, S. A., and D. S. S. Hudspeth. 2000. Phylogeny of the ascaridoidea (Nematoda: Ascaridida) based on three genes and morphology: Hypotheses of structural and sequence evolution. Journal of Parasitology **86**:380-393.

Narum, S. R., M. Banks, T. D. Beacham, M. R. Bellinger, M. R. Campbell, J. Dekoning, A. Elz, C. M. Guthrie, C. Kozfkay, K. M. Miller, P. Moran, R. Phillips, L. W. Seeb, C. T. Smith, K. Warheit, S. F. Young, and J. C. Garza. 2008. Differentiating salmon populations at broad and fine geographical scales with microsatellites and single nucleotide polymorphisms. Molecular Ecology **17**:3464-3477.

Nei, M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Neilson, J. D., W. T. Strobo, and P. Perley. 2006. Pollock (*Pollachius virens*) stock structure in the Canadian Maritimes inferred from mark-recapture studies. ICES Journal of Marine Science **63**:749-765.

Nielsen, E. E., J. Hemmer-Hansen, N. A. Poulsen, V. Loeschcke, T. Moen, T. Johansen, C. Mittelholzer, G. L. Taranger, R. Ogden, and G. R. Carvalho. 2009a. Genomic signatures of local directional selection in a high gene flow marine organism; the Atlantic cod (*Gadus morhua*). BMC Evolutionary Biology **9**.

Nielsen, E. E., P. J. Wright, J. Hemmer-Hansen, N. A. Poulsen, L. M. Gibb, and D. Meldrup. 2009b. Microgeographical population structure of cod I in the North Sea and west of Scotland: the role of sampling loci and individuals. Marine Ecology Progress Series **376**:213-225.

Oliva, M. E., and I. Ballon. 2002. Metazoan parasites of the Chilean hake *Merluccius gayi gayi* as a tool for stock discrimination. Fisheries Research **56**:313-320.

Orbesen, E. S., J. P. Hoolihan, J. E. Serafy, D. Snodgrass, E. M. Peel, and E. D. Prince. 2008. Transboundary movement of Atlantic istiophoris billfishes among international and U.S. domestic management areas inferred from mark-recapture studies. Marine Fisheries Review **70**:14-23.

Oshima, M. 1972. *Anisakis* and Anisakidiasis in Japan and adjacent area. Progress of Medical Parasitology in Japan **4**:305-393.

Patarnello, T., A. M. Filip, F. Volckaert, and R. Castilho. 2007. Pillars of Hercules: is the Atlantic-Mediterranean transition a phylogeographical break? Molecular Ecology **16**:4426-4444.

Pauly, D., V. Christensen, R. Froese, and M. L. Palomares. 2000. Fishing down aquatic food webs. American Scientist **88**:46-51.

Pereyra, R. T., E. Saillant, C. L. Pruett, C. E. Rexroad III, and A. Rocha-Olivares. 2004. Characterization of polymorphic microsatellites in the Pacific sardine *Sardinops sagax sagax* (Clupeidae). Molecular Ecology Notes **4**:739-741.

Perrin, W. F., B. Würsig, and J. G. M. Thewissen 2009. Encyclopedia of marine mammals. Academic Press, London.

Peterson, W. T., and F. B. Schwing. 2003. A new climate regime in northeast pacific ecosystems. Geophysical Research Letters **30**:1-6.

Podolska, M., J. Horbowy, and M. Wyszynski. 2006. Discrimination of Baltic herring populations with respect to *Anisakis simplex* larvae infection. Journal of Fish Biology **68**:1241-2156.

Pratt, I., and J. E. McCauley. 1961. Trematodes of the Pacific Northwest. An annotated catalog. Oregon University Press, Corvallis, OR.

Prugnolle, F., A. Theron, J. P. Pointier, R. Jabbour-Zahab, P. Jarne, P. Durand, and T. de Meeûs. 2005. Dispersal in a parasitic worm and its two hosts: Consequence for local adaptation. Evolution **59**:296-303.

Quiazon, K. M. A., T. Yoshinaga, M. D. Santos, and K. Ogawa. 2009. Identification of Larval *Anisakis* spp. (Nematoda: Anisakidae) in Alaska Pollock (T*heragra chalcogramma*) in Northern Japan Using Morphological and Molecular Markers. Journal of Parasitology **95**:1227-1232.

Radovich, J. 1962. Effects of sardine spawning stock size and environment on year-class production. California Fish and Game **48**:123-140.

Radovich, J. 1982. The collapse of the California sardine fishery. Pages 107-136 *in* M. H. Glantz and J. D. Thompson, editors. Resource Management and Environmental Uncertainty. Lessons from Coastal Upwelling Fisheries. John Wiley & Sons, New York.

Ramon, M. M., and J. A. Castro. 1997. Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. Heredity **78**:520-528.

Reimer, L. W. 1993. Parasites of *Merluccius capensis*, and *M. paradoxus* from the coast of Namibia. Applied Parasitology **78**:520-528.

Rello, F. J., F. J. Adroher, and A. Valero. 2008. *Hysterothylacium aduncum*, the only anisakid parasite of sardines (*Sardina pilchardus*) from the southern and eastern coasts of Spain. Parasitology Research **104**:117-121.

Ricker, W. E. 1954. Stock and recruitment. Journal of the Fisheries Research Board of Canada **11**:559-623.

Rohde, K. 1984. Ecology of marine parasites. Helgoländer Meeresunters **37**:5-33.

Roques, S., J.-M. Sevigny, and L. Bérnatchez. 2002. Genetic structure of deep-water redfish, *Sebastes mentella*, populations across the North Atlantic. Marine Biology **140**:297-307.

Rose, G. A. 1997. The trouble with fisheries science! Reviews in Fish Biology and Fisheries **7**:365-370.

Rounsefell, G. A., and E. H. Dahlgren. 1933. Tagging experiments on the Pacific herring, *Clupea pallasii*. Journal du Conseil **8**:371-384.

Ruzzante, D. E., S. Mariani, D. Bekkevold, C. Andre, H. Mosegaard, L. A. W. Clausen, T. G. Dahlgren, W. F. Hutchinson, E. M. C. Hatfield, E. Torstensen, J. Brigham, E. J. Simmonds, L. Laikre, L. C. Larsson, R. J. M. Stet, N. Ryman, and G. R. Carvalho. 2006. Biocomplexity in a highly migratory pelagic marine fish, Atlantic herring. Proceedings of the Royal Society B-Biological Sciences **273**:1459-1464.

Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. Reviews in Fish Biology and Fisheries **5**:417-446.

Sabater, E. I. L., and C. J. L. Sabater. 2000. Health hazards related to occurrence of parasites of the genera *Anisakis* and *Pseudoterranova* in fish. Food Science and Technology International **6**:183-195.

Sala-Bozano, M., V. Ketmaier, and S. Mariani. 2009. Contrasting signals from multiple markers illuminate population connectivity in a marine fish. Molecular Ecology **18**:4811-4826.

SAS. 1998. Statview®. Using Statview. Version 5.0.1. *in*. SAS Institute Inc., Cary, NC.

Schnute, J. T., and L. J. Richards. 2001. Use and abuse of fishery models. Canadian Journal of Fisheries and Aquatic Sciences **58**:10-17.

Sewell, K. B., and R. J. G. Lester. 1995. Stock composition and movement of gemfish, *Rexea solandri*, as indicated by parasites. Canadian Journal of Fisheries and Aquatic Sciences **52**:225-232.

Silva, M. E. R., and J. C. Eiras. 2003. Occurrence of *Anisakis* sp. in fishes off the Portuguese west coast and evaluation of its zoonotic potential. Bulletin of the European Association of Fish Pathologists **23**:13-17.

Sinclair, M., and P. Solemdal. 1988. The development of "population thinking" in fisheries biology between 1878 and 1930. Aquatic Living Resources **1**:189-213.

Smith, C. T., and L. W. Seeb. 2008. Number of alleles as a predictor of the relative assignment accuracy of short tandem repeat (STR) and single-nucleotide-polymorphism (SNP) baselines for chum salmon. Transactions of the American Fisheries Society **137**:751-762.

Smith, J. W., and R. Wooten. 1978. *Anisakis* and Anisakiasis. Advances in Parasitology **16**:93-163.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) population off western North America. California Cooperative Oceanic Fisheries Investigations Reports **46**:75-82.

Smith, T. D. 1998. "Simultaneous and complementary advances": mid-century expectations of the interaction of fisheries science and management. Reviews in Fish Biology and Fisheries **8**:335-348.

Soutar, A., and J. D. Isaacs. 1969. History of fish populations inferred from fish scales in anaerobic sediments off California. California Marine Research Communication Cooperative Oceanic Fisheries Investigations Reports **13**:63-70.

Speare, P. 1995. Parasites as biological tags for sailfish *Istiophorus platypterus*, from east coast Australian waters. Marine Ecology Progress Series **118**:43-50.

Sprague, L. M., and A. M. Vrooman. 1962. A racial analysis of the Pacific sardine *Sardinops caeruleus* based on studies of erythrocyte antigens. Annals of the New York Academy of Sciences **97**:131-138.

Stanley, R. D., D. L. Lee, and D. J. Whitaker. 1992. Parasites of yellowtail rockfish, *Sebastes flavidus*, (Ayres, 1862) (Pisces: Teleostei), from the Pacific coast of North America as potential biological tags for stock identification. Canadian Journal of Zoology **70**:1086-1096.

Stephenson, R. L. 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fisheries Research **43**:247-249.

Stepien, C. A. 1995. Population genetic divergence and geographic patterns from DNA sequences: examples from marine and freshwater fishes. American Fisheries Society Symposium **17**:263-287.

Suzuki, J., R. Murata, M. Hosaka, and J. J Araki. 2009. Risk factors for human *Anisakis* infection and association between the geographic origins of *Scomber japonicus* and anisakid nematodes. International Journal of Food Microbiology **137**:88-93.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics **123**:585-595.

Teel, D. J., D. M. Van Doornik, D. R. Kuligowski, and W. S. Grant. 2003. Genetic analysis of juvenile coho salmon (*Oncorhynchus kisutch*) off Oregon and Washington reveals few Columbia River wild fish. Fishery Bulletin **101**:640-652.

Thomas, F., O. Verneau, T. de Meeûs, and F. Renaud. 1996. Parasites as host evolutionary prints: Insights into host evolution from parasitological data. International Journal of Parasitology **26**:1137-1137.

Thompson, J. D., D. G. Higgins, and T. J. Gibson. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weigh matrix choice. Nucleic Acids Research **22**:4673-4680.

Timi, J. T. 2003. Parasites of Argentine anchovy in the south-west Atlantic: latitudinal patterns and their use for discrimination of host populations. Journal of Fish Biology **63**:90-107.

Valdivia, I. M., R. A. Chavez, and M. E. Oliva. 2007. Metazoan parasites of *Engraulis ringens* as tools for stock discrimination along the Chilean coast. Journal of Fish Biology **70**:1504-1511.

Valentini, A., S. Mattiucci, P. Bondanelli, S. C. Webb, A. A. Mignucci-Giannone, M. M. Colom-Llavina, and G. Nascetti. 2006. Genetic relationships among *Anisakis* species (Nematoda: Anisakidae) inferred from mitochondrial *COX2* sequences, and comparison with allozyme data. Journal of Parasitology **92**:156-166.

van der Lingen, C. D. 2002. Diet of sardine *Sardinops sagax* in the southern Benguela upwelling ecosystem. South African Journal of Marine Science **24**:301-316.

van der Lingen, C. D., L. Hutchings, and J. G. Field. 2006. Comparative trophodynamics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela: are species alternations between small pelagic fish trophodynamically mediated? African Journal of Marine Science **28**:465-477.

Vignal, A., D. Milan, M. san Cristobal, and A. Eggen. 2001. A review on SNP and other types of molecular markers and their use in animal genetics. Genetics Selection Evolution **34**:275-305.

Vilas, R., C. D. Criscione, and M. S. Blouin. 2005. A comparison between mitochondrial DNA and the ribosomal internal transcribed regions in prospecting for cryptic species of platyhelminth parasites. Parasitology **131**:839-846.

von Bertalanffy, L. 1938. A quantitative theory of organic growth. Human Biology **10**:181-213.

Waldman, J. R. 2005. Definition of stocks: an evolving concept. Pages 7-16 *in* S. X. Cadrin, K. D. Friedland, and J. R. Waldman, editors. Stock Identification Methods. Applications in fisheries science, San Francisco.

Waples, R. S. 1998. Separating the wheat from the chaff: Patterns of genetic differentiation in high gene flow species. Journal of Heredity **89**:438-450.

Waples, R. S., A. E. Punt, and J. M. Cope. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish and Fisheries **9**:423-449.

Welch, D. W., G. W. Boehlert, and G. P. Ward. 2003. POST-the Pacific Ocean salmon tracking project. Oceanologica Acta **25**:243-253.

Whitaker, D. J., and G. A. McFarlane. 1997. Identification of Sablefish, *Anoplopoma fimbria* (Pallas, 1811), stocks from seamounts off the Canadian Pacific coast using parasites as biological tags. NMFS Technical Report 130, NOAA.

Whiteman, N. K., and P. G. Parker. 2005. Using parasites to infer host population history: a new rationale for parasite conservation. Animal Conservation **8**:175-181.

Williams Jr., E. H., and L. Bunkley-Williams. 1996. Platyhelminthes (Flatworms). Pages p59 *in* Parasites of offshore big game fishes of Puerto Rico and the western Atlantic. Antillean College Press, Mayaguez, Puerto Rico.

Wisner, R. L. 1960. Evidence of a northward movement of stocks of the Pacific sardine based on the number of vertebrae. California Cooperative Oceanic Fisheries Investigations Reports **8**:75-82.

Wolf, P. 1992. Recovery of the Pacific sardine and the California sardine fishery. California Cooperative Oceanic Fisheries Investigations Reports **33**:76-86.

Wu, S. G., G. T. Wang, B. W. Xi, F. Xiong, T. Liu, and P. Nie. 2009. Population genetic structure of the parasitic nematode *Camallanus cotti* inferred from DNA sequences of ITS1 rDNA and the mitochondrial COI gene. Veterinary Parasitology **164**:248-256.

Electronic Sources:

FAO. 2008. The State of World Fisheries and Aquaculture (updated 2008). http://fao.org. Accessed: 29 January 2010

Johnson, J.K. 2004. Regional Overview of Coded Wire Tagging of Anadromous Salmon and Steelhead in Northwest America (updated 2004). http://rmpc.org. Accessed: 20 September 2009

WGBH Educational Foundation. 2004. World in the Balance (updated 2004)