https://doi.org/10.25923/zzvw-x557

NOAA Technical Memorandum NMFS

FEBRUARY 2025

THE SUBPOPULATION PROBLEM IN PACIFIC SARDINE, REVISITED

Matthew T. Craig[1], Brad E. Erisman[1], Ella S. Adams-Herrmann[2], Kelsey C. James[1], and Andrew R. Thompson[1]

[1] NOAA Fisheries, Southwest Fisheries Science Center
Fisheries Resources Division, La Jolla, California

[2] University of Central Florida
Department of Biology, Orlando, Florida

NOAA-TM-NMFS-SWFSC-713

U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
National Marine Fisheries Service
Southwest Fisheries Science Center

About the NOAA Technical Memorandum series

The National Oceanic and Atmospheric Administration (NOAA), organized in 1970, has evolved into an agency which establishes national policies and manages and conserves our oceanic, coastal, and atmospheric resources. An organizational element within NOAA, the Office of Fisheries is responsible for fisheries policy and the direction of the National Marine Fisheries Service (NMFS).

In addition to its formal publications, the NMFS uses the NOAA Technical Memorandum series to issue informal scientific and technical publications when complete formal review and editorial processing are not appropriate or feasible. Documents within this series, however, reflect sound professional work and may be referenced in the formal scientific and technical literature.

SWFSC Technical Memorandums are available online at the following websites:

SWFSC: https://swfsc-publications.fisheries.noaa.gov/

NOAA Repository: https://repository.library.noaa.gov/

Accessibility information

NOAA Fisheries Southwest Fisheries Science Center (SWFSC) is committed to making our publications and supporting electronic documents accessible to individuals of all abilities. The complexity of some of SWFSC's publications, information, data, and products may make access difficult for some. If you encounter material in this document that you cannot access or use, please contact us so that we may assist you.
Phone: 858-546-7000

Recommended citation

Craig, Matthew T., Brad E. Erisman, Ella S. Adams-Herrmann, Kelsey C. James, and Andrew R. Thompson. 2025. The subpopulation problem in Pacific sardine, revisited. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-713. https://doi.org/10.25923/zzvw-x557

The Subpopulation Problem in Pacific Sardine, Revisited

Matthew T. Craig[1], Brad E. Erisman[1], Ella S. Adams-Herrmann[2], Kelsey C. James[1], and Andrew R. Thompson[1]

[1]NOAA National Marine Fisheries Service, Southwest Fisheries Science Center, Fisheries Resources Division, 8901 La Jolla, CA, 92037, USA

[2]University of Central Florida, Department of Biology, 4110 Libra Dr, Orlando, FL, 32816, USA

Table of Contents

Author's Note 3
Abstract 4
Introduction 5
Comments on Terminology 9
Methods 10
Results and Discussion 10
  Vertebral Counts 10
  Serological Antigen Response 15
  Tagging Data 18
  Parasites as Natural Tags 22
  Otolith Morphology and Morphometry 22
  Spawning Location, Timing, and Temperature 28
  Genetic Data 36
  External Morphology 38
  Temperature and Landings Data 40
  Growth Patterns 44
  Demographics 48
  How did we get here? 49
Summary 50
Acknowledgments 53
Author's Contributions 53
Literature Cited 53

Author's Note

This manuscript was submitted, favorably reviewed, and provisionally accepted for publication in Fishery Bulletin. It was ultimately determined to be too long for that Journal's format and was withdrawn. References to this work as "accepted" in Fishery Bulletin may therefore exist in the literature.

Abstract

Pacific sardine (*Sardinops sagax*) is federally-managed in the U.S. under the assumption of three subpopulations. This hypothesis was formalized by John Marr in the late 1950s. In the decades since, studies of subpopulation structure in Pacific sardine were designed with the assumption that subpopulations exist. We conducted a critical review of studies in the literature in reference to subpopulation structure in the species across its North American range, including those used by Marr to formulate his hypothesis. Our review of these older works (1925-1970) revealed that, while based on the best available information and scientific practices at the time, many do not support the hypothesis of subpopulation structure in Pacific sardine. In literature from 1970 onwards, our review shows that nearly all studies either stated that the data did not support a multipopulation hypothesis or had methodological or other weaknesses that introduced uncertainty to their conclusions despite having been cited as supporting it by works in the past two decades. Based on this review of a century of literature, we conclude that there is little, if any, evidence supporting a hypothesis of multiple subpopulations of Pacific sardine throughout their North American range and no evidence that can be used to reject the null hypothesis of a single population.

Introduction

"Twenty years ago it was generally believed that there was only one major group of sardines, which was produced in the southern part of its range and, with increasing size (or age), performed successively longer feeding migrations to the north in the spring and summer and spawning migrations to the south in the fall and winter. (The sardines off the west coast of southern Lower California and in the Gulf of California were considered to be of uncertain relationship to the northern group.)" Marr, 1957, p. 108

Spatial structure plays an important role in population dynamics and the sustainable harvest of marine organisms (Fogarty and Botsford, 2007; Cadrin and Secor, 2009; Cadrin, 2020). For spatially explicit management strategies, the definition of the geographic boundaries of management units (i.e., stocks) is ideally based on biological population principals, and is a critical component to their success as most stock assessment models rely on the assumption of panmictic unit stocks (Cadrin, 2020; Cadrin, et al., 2023). Because of this, the spatial boundaries of management (i.e., "stock") and biological (i.e., "population") units should necessarily coincide (i.e., should have "coherent dimensionality" *sensu* Berger, et al., 2021), and inconsistencies between them can have adverse outcomes (e.g., Cope and Punt, 2011; Hintzen, et al., 2015; Kerr, et al., 2017). Often the theoretical aspects that define a biological population relative to spatially disjunct fishing efforts are dismissed in practice despite evidence that spatially defined units and/or structure are incorrectly specified (e.g., fishery stocks may be defined based on factors such as geopolitical boundaries; Cadrin, 2020; Cadrin, et a., 2023). This highlights the importance of approaching stock assessments in fisheries with a more holistic view that includes testing the performance of models and management strategies under alternative hypotheses of spatial structure in addition to the *status quo* (Cadrin, 2020).

The Pacific sardine (*Sardinops sagax*; hereafter "sardine") once supported the largest fishery in the western hemisphere (Norton and Mason, 2005). Its geographic range is both expansive and dynamic, extending in the California Current system across three coastal zoogeographic provinces, an entire coastal upwelling system, and three oceanic water masses (Moser et al., 1993; Herandez-Vazquez, 1994). Current management in the U.S. operates under the assumption that there are three subpopulations of sardine across their North American range: a northern subpopulation (NSP), a southern subpopulation (SSP), and a Gulf of California subpopulation (see below for comments on terminology). The distributional limits of each subpopulation have been variously defined in the literature but are generally consistent as follows: the NSP ranges from northern Baja California (MX) from approximately Punta Baja northwards to Alaska, the SSP ranges from the southern tip of the Baja California Peninsula to southern California (U.S.) at approximately Point Conception, and the Gulf of California subpopulation is restricted to that body of water (e.g., see Yau, 2022; Kuriyama et al., 2024; PFMC 2024) (Fig. 1). While the geographic limits of the NSP and SSP are hypothesized to overlap in northern Baja California and in the Southern California Bight, it is thought that the two groups do not fully occupy the overlapping area at the same time of the year (i.e., they undergo simultaneous yet spatially discrete migrations) and have segregated spawning areas (Demer and Zwolinski, 2014). Moreover, these separate subpopulations are hypothesized to possess different biological traits (e.g, growth patterns, demographics, vertebral counts, thermal tolerance ranges) that reflect a persistent isolation (e.g., Felin, 1954; Felix-Uraga et al., 2004; Zwolinski and Demer, 2023).

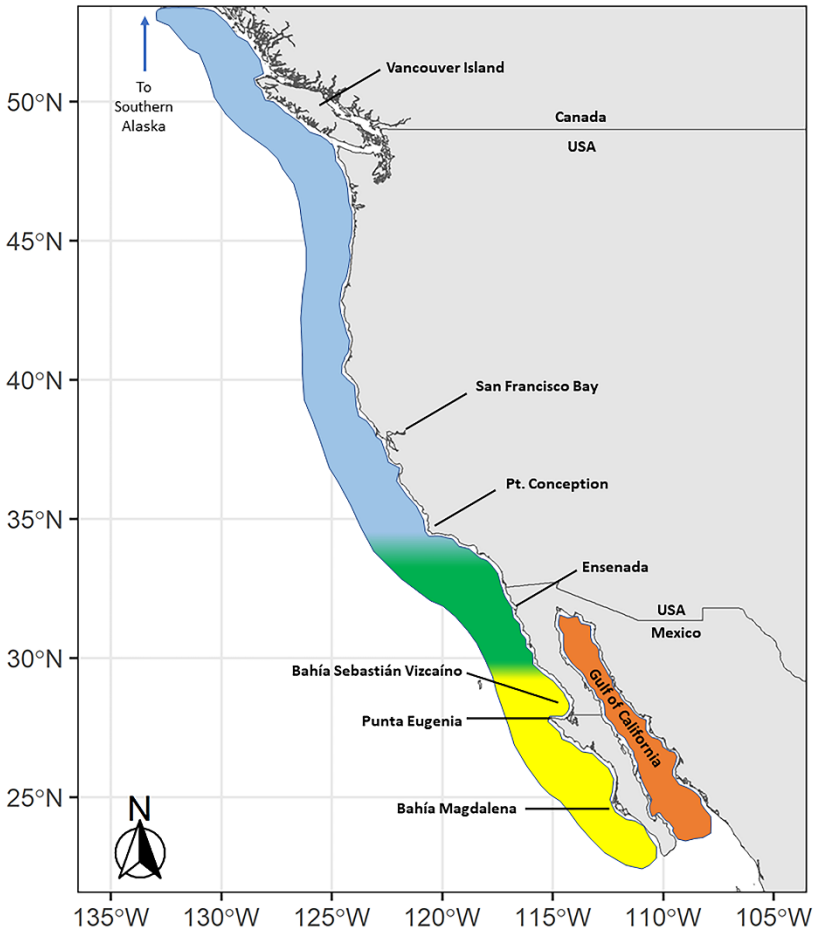

Figure 1. Generalized distributions of the hypothesized Northern Subpopulation (blue), Southern Subpopulation (yellow), and Gulf of California subpopulation (orange) of Pacific sardine (*Sardinops sagax*). While the Northern and Southern subpopulations are not thought to fully occupy the same region at the same time, their potential range of overlap is shown in green.

While it is difficult to pinpoint exactly when the first hypotheses arose, questions about putative subpopulation structure in sardine along the Pacific coast of North America have been posed for at least the last century and appear to have begun with Hubbs (1925). That multiple subpopulations of sardine may exist, and that their associated dynamics have an effect on local abundance and landings, became acutely important following precipitous declines in U.S. and Canadian sardine landings beginning in the 1940s which decimated this once productive fishery, particularly from central California northward (Murphy, 1966; Radovich, 1982). In the late 1950s, John C. Marr, then director of the U.S. Bureau of Commercial Fisheries Honolulu Biological Laboratory, published two seminal papers reviewing the ecological, biological, and fishery data for sardine (Marr, 1957; Marr, 1960). Marr's (1960) review, in particular, had a lasting impact on the study of subpopulation structure in sardine.

Prior to the 1950s, many empirical studies concluded that sardine comprised a single population across their North American range (e.g., Hubbs, 1925; Hart, 1933a, b; Clark, 1936; Clark, 1947; Clark and Janssen, 1945), with some speculation as to if the sardine in the Gulf of

California were a separate group (Clark, 1936; Clark, 1947; Clark and Janssen, 1945). This was synthesized in two papers published by Marr in 1957 entitled "The problem of defining and recognizing subpopulations of fishes" (Marr, 1957a), and "The subpopulation problem in the Pacific sardine, *Sardinops caerulea"* (Marr, 1957b). In the latter paper, Marr (1957b) concluded that much of the biological data gathered to date had shown high variability both within and among groups and were based on phenotypic characters (by this drawing a contrast with "genotypic", implying that they were not fully under genetic control but also influenced by environmental factors), thus largely irrelevant to questions about subpopulations:

> "The subpopulation problem in the Pacific sardine is concerned with the number and identity of genetically self-sustaining units within the population. Evidence of many kinds shows that there are between- and within-season, port and year-class differences. This evidence includes migration, growth, meristic characteristics, etc. It is not known whether these differences are phenotypic or genotypic; the weight of the evidence is that they are phenotypic." (p. 116)

In the late 1950s and early 1960s, three papers were published which solidified the scientific consensus relative to subpopulation structure in the sardine and which formed the foundation for all future studies on subpopulation structure (Marr, 1960; Sprague and Vrooman, 1962; Vrooman, 1964). Marr (1960) formulated a hypothesis of subpopulation structure in sardine in which he placed great emphasis on the ongoing serological antigen work of Lucian Sprague and Andrew Vrooman that provided a tantalizing glimpse of what researchers at the time considered to be genetic data supporting the existence of multiple subpopulations:

> "…[It] was postulated that the catch [in the U.S. and Canada] consisted of fish produced in the southern California and central Baja California spawning grounds. The question of whether or not the fish spawning in these two areas represent genetically distinct subpopulations cannot now be answered. Until very recently, it might have been concluded that the two spawning groups were not distinct…Furthermore, Dr. Lucian Sprague had observed no differences in two blood group systems between samples from several localities. However, he has recently informed me that additional samples have revealed the existence of two genetically distinct subpopulations. Whether these subpopulations represent in fact the fish spawning on the southern California and central Baja California spawning grounds is not yet known." (p. 767)

This new "genetic" information led Marr (1960) to formulate a hypothesis that provided a potential mechanism for the formation and persistence of subpopulation structure, a possible explanation as to the dynamics of sardine availability to the fishery, and a plausible reason for why the fishery collapsed:

> "[S]ardines which support the United States and Canadian catch are produced off southern California and central Baja California. Those produced off southern California migrate as far north as British Columbia, support the fishery there and contribute to the fisheries at San Francisco, Monterey and San Pedro. Those produced off central Baja California migrate as far north as central California and

> contribute to the California fisheries, especially at San Pedro. Lack of spawning success on the southern California spawning grounds since about 1943 could account for the observed changes in the fishery." (p. 783)

Following these publications, and with the formal publication of the "genetic" data Marr cited (Sprague and Vrooman, 1962; Vrooman, 1964), the issue seemed settled, and thus began what has, for the most part, been a dogmatic adherence to a subpopulation model for sardine. Subsequent studies were largely confirmational, seeking to show evidence of the subpopulation hypothesis through descriptive work in hypothesis-free study designs that began with the accepted existence of population structure. This culminated in what has been described as "general scientific consensus" that three subpopulations of sardine exist and that landings at U.S. ports represent a mixture of the NSP and SSP (Hill et al., 2018; Kuriyama et al., 2020).

The management of sardine has been shaped by this consensus and is most complex at the border of the U.S. and Mexico where the fisheries are considered to interact with more than one subpopulation. In Canada, all sardine are assumed to be a part of the NSP (see Fisheries and Oceans Canada, 2024). In Mexico, biomass estimates are separated into three groups (the cold, temperate, and warm stocks of Felix-Uraga et al., [2005]) based on a temperature threshold. The cold and temperate stock are roughly equivalent to the NSP and SSP, respectively, and the warm stock occurs at southern extreme of the Baja California Peninsula and in the GOC. Mexico manages the warm and temperate stock and excludes cold stock landings from the Ensenada fishery when assessing the temperate stock (Enciso-Enciso et al., 2023). In the U.S. only the NSP is managed. Landings and biomass estimates of sardine are apportioned to the NSP through the use of a model which estimates its potential habitat (see Zwolinski et al., 2011, Demer and Zwolinski, 2014, and Zwolinski and Demer, 2023, for detailed discussion of the model). All landings in the U.S., however, are counted towards annual catch limits of the NSP, even if determined to have been taken in habitat not aligned with the NSP.

Although a majority of the geographic range (and thus presumably a substantial part of the biomass) of the NSP occurs in U.S. waters, in 2021 the U.S. stock assessment for sardine showed that landings from the Mexican fishing fleet (which does not fish in U.S. waters) that were attributed to the NSP using the potential habitat model were on the same order as its entire estimated biomass (Kuriyama et al., 2021). It is clear from this that the model version used at that time to apportion fish to the NSP was operating sub optimally. One way to address this is to adjust the model itself or reevaluate how model outputs are interpreted to improve its perceived performance. For example, excluding some variables and lowering tolerance thresholds was used as way to reconcile the disparity noted above (see PFMC Scientific and Statistical Committee Report, April, 2024 and Zwolinski and Demer, 2023 for details). We have chosen to take a different tack to address this sub-optimal model performance by reevaluating one of its underlying hypotheses, that of the existence of subpopulations in sardine, by focusing on the strength of the biological evidence that supports it. What we found was surprising given the impression left after reading recent literature. Among the myriad problematic factors that we found, particularly with data used to form the foundation of the population structure hypothesis, was a pattern of within-group variation equaling or exceeding among-group variation that was acknowledged by the original authors but that was unevaluated or dismissed by later researchers. In many cases, this resulted in a pattern of somewhat inaccurate citations, a reliance on data derived from flawed or antiquated methods, and a rather dogmatic approach to the study of sardine subpopulation structure that is antithetical to the scientific method.

Comments on Terminology

"*Suffice it to say that I use the term 'subpopulation' in the sense that it is a self-sustaining unit; subpopulations segregate at spawning time and their characteristics are heritable*." Marr, 1957b, p. 108

While many definitions of the term "population" have been published, they typically share a similar, biological underpinning such as the following: "…[A] group of individuals of the same species or subspecies that are spatially, genetically, or demographically separated from other groups" (Wells and Richmond, 1995; Pope et al., 2010). In fisheries biology, and particularly for Pacific sardine, the term "stock" is often conflated with the term "population" or "subpopulation". This is because of what have been called the "unit stock assumptions" of stock assessment models which are based on biological criteria (see Cadrin, et al., 2023 for a review of this topic). For these assumptions to hold true, fisheries stocks must be defined based on biological populations (Cadrin, et al., 2023). Unfortunately, this is not always possible due to a variety of practical or operational limitations. What is thus defined as a "stock" is therefore variable, the term somewhat labile, and numerous, conceptually discordant definitions exist in the literature. At times, the definitions of "stock" and "population" border on the synonymous (e.g., "[A] stock is a population of organisms, ideally sharing a common gene pool, that is sufficiently discrete and nominally identifiable to warrant consideration as a self-perpetuating system that can be managed." [MacCall, 1986]). At others, these definitions could not be more antonymic (e.g., "The part of a fish population which is under consideration from the point of view of actual or potential utilization" [Ricker, 1975]). This lability in the definition of "stock" reflects the fact that it is an operational term. That is, fishery stocks have historically been defined based on criteria that are useful for implementing management strategies (but see Cadrin, 2020 and Cadrin et al., 2023 for cautionary notes on this practice). Populations, however, cannot be defined based on purely operational terms, but rather are a process-oriented, biological concept. What is clear, however, is that "stocks" are management units, whereas "populations" are biological units.

It is therefore important at the outset of this paper to provide the definition of "population" as used herein. Given that we are interested in coherent dimensionality in fisheries management (Berger, et al., 2021), and that that coherence is based on the alignment of biological units (populations) and management units (stocks; Cadrin, 2020; Cadrin, et al., 2023), we use the definition of "population" most often used in modern biology: "A group of individuals of the same species living in the same area at the same time sharing a common gene pool, with little or no immigration or emigration" (FAO Term Portal accessed 7/22/24 within "Fisheries" term collection https://www.fao.org/faoterm/collection/fisheries/en/). We intentionally avoid using the NOAA Fisheries Glossary (Blackheart, et al., 2006) definition of "population" ("The number of individuals of a particular species that live within a defined area") as it more accurately defines the term "census size". Notably, this reflects the nuanced difference between "a" population (biological) and "the" population (non-biological).

The taxonomic history of species in the genus *Sardinops* is complex and they have been variously treated as one or several species worldwide. Currently, Pacific sardine (*Sardinops sagax*) is recognized as a valid species inhabiting the shallow marine waters along the west coasts of both North and South America (Fricke, et al. 2023). Assuming little or no connectivity between groups of sardine in North and South America, this would render those living along the

North American coast a “population”, and biological divisions within that group would be “subpopulations”. In this context, the fundamental meaning of the terms “subpopulation” and “population” is the same and is based on biological processes (i.e., a subpopulation is a distinct subunit of a population sharing a common gene pool and with little immigration and emigration to other subpopulations), and we therefore use “subpopulation” to remain consistent with the literature being reviewed.

Methods

We began by reviewing studies commonly cited and used to support the hypothesis of the existence of subpopulations in sardine along the west coast of North America. We then performed a “literature trace” in which we reviewed citations within citation lists focusing on the topic of subpopulation structure. We also searched common, public databases (e.g., Web of Science) for original data papers concerning subpopulation structure in sardine. We excluded review papers that were derived from or summarized the results of those original data papers but did not provide new data, analyses, or results. However, review papers were used to identify papers commonly cited as evidence of subpopulation structure and papers that discussed the topic. We also excluded modeling papers that did not explicitly test a hypothesis but rather provided conceptual or quantitative models of subpopulation distributions based on empirical data from prior studies. Several data types emerged as the foundation for studies on sardine population biology. These included vertebral counts, serological antigen response in erythrocytes, tagging, variations in size-at-age, otolith morphometry and morphology, otolith microchemistry, landings and temperature data, gross overall morphology, demographics, growth, and spawning location and timing. Where multiple papers were written on a common data type, data were examined both on a case-by-case and combined manner. For each paper, we summarized the conclusions, interpreted the data, and critically evaluated the results and conclusions viewed through the lens of modern scientific best practices. Particular attention was paid to studies predating the work of Marr (1957, 1960) which emerged from our review as forming the foundation of the subpopulation hypothesis for sardine. Occasionally, where possible, data were transcribed and compiled to enable accurate depiction of data from multiple sources or from a single source that had not presented the data in a compiled manner. Place and region names mentioned in the text are depicted in Figure 2.

Results and Discussion

Vertebral Counts

> *“One has only to consider what is known of sardine spawning to realize how meaningless (from the standpoint of subpopulation characters) meristic characters may be.”* Marr, 1957b, p. 112.

Among the earliest and most frequently cited papers in support of subpopulation structure in sardine are those examining vertebral counts. While authors of the earliest studies on vertebral counts tended to conclude that evidence for separate populations was weak or absent, later

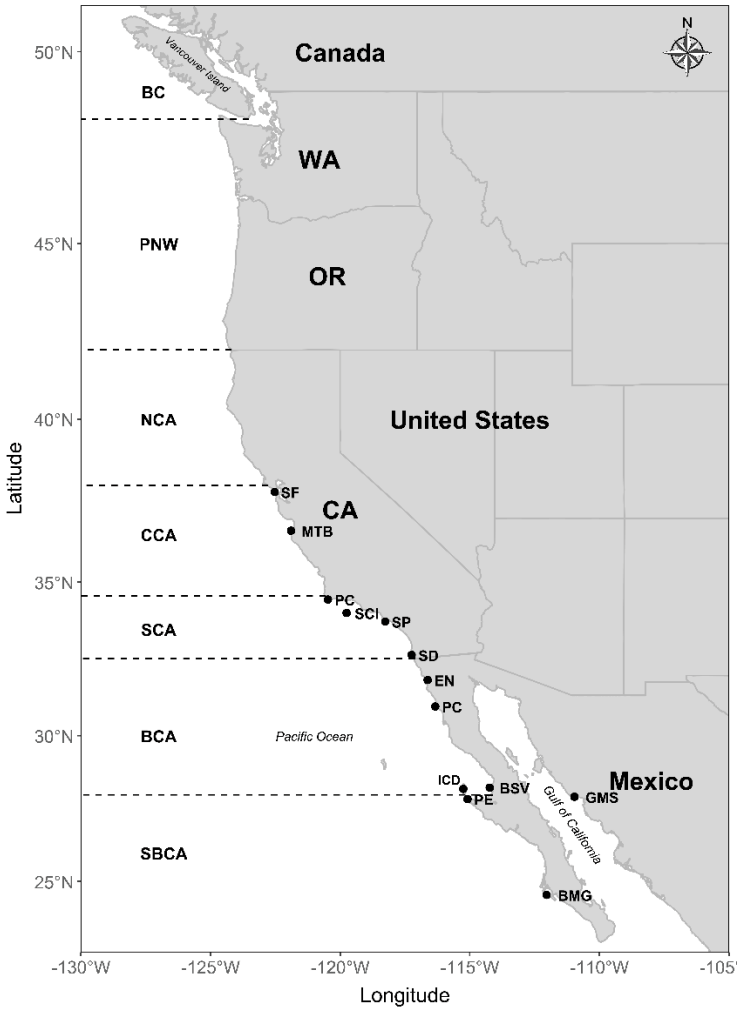

Figure 2. Locations and regions mentioned in this review. BC = British Columbia, BCA = Baja California, BMG = Bahía Magdalena, BSV = Bahía Sebastían Vizcaíno, CCA = Central California, EN = Ensenada, GMS = Guaymas, ICD = Isla de Cedros, MTB = Monterey Bay, NCA = Northern California, PC = Point Conception, PE = Punta Eugenia, PNW = Pacific Northwest, SBCA = Southern Baja California, SCA = Southern California, SCI = Santa Cruz Island, SD = San Diego, SF = San Francisco, SP = San Pedro.

studies asserted the existence of separate subpopulations based on these same studies. A century ago, Hubbs (1925) presented and evaluated data on vertebral counts in sardine from San Francisco Bay south to San Diego (California, U.S.). Based on counts from 1,910 individuals (800 from Central California and 1,110 from Southern California), Hubbs (1925) reported an average vertebral count of 51.78 and 51.69, respectively, with a range of 50-53 and a modal value of 52 for both regions. While Hubbs commented that the difference in the average value appeared to be statistically significant ("...being five times the probable error of the difference…"), he also noted that greater differences were apparent between averages of counts from samples taken at the same location at different times. He concluded that these differences were a result of annual variation and were of "…purely individual and not a racial significance"

(Hubbs and others at that time used the term "racial" to describe subpopulations within a species).

Thompson (1926) examined sardine from California and Europe, thus his larger conclusions are irrelevant to the present review. However, it is worth mentioning that Thompson concluded that vertebral counts were not indicative of subpopulations as "[i]t is entirely possible that the count supposed to be characteristic for a given locality varies considerably from time to time, or as between one school of fish and another, or that differences in method of counting give rise to error."

Hart (1933a) added to the literature 9,000 vertebral counts of sardine from British Columbia (Canada) and central California and concluded that "…definite racial distinctions in vertebra number between California and British Columbia pilchards or British Columbia pilchards from the two localities treated are not demonstrated by the data available" (p. 83). This conclusion was based in large part on the observation that the magnitude of differences in averages for different years at the same locality was as large as any average difference between localities as a whole. Hart thus agreed with Hubbs (1925) that annual variation in the number of vertebrae at a locality was common and that vertebral number did not show any pattern indicative of separate subpopulations. Hart (1933b) added 600 counts from an additional location in British Columbia (Barkley Sound, Vancouver Island) and concluded that these additional counts "…confirm the interpretation of the previous paper [Hart 1933a]".

Clark (1936) added counts and locations to those published by Hubbs (1925) and Hart (1933a/b), bringing the total number of published vertebral counts to 18,214. Among these, Clark (1936) included counts from 5,356 "young" fish (she described "adult" fish as "…sardines larger than 170 or 180 mm, standard length" and "young" as "sardines of smaller size", and commented that "very few young fish exceeding 150 mm in standard length were used."). Clark's sampling included 12 sites ranging from Alaska (U.S.) to Baja California Sur (MX), thus considerably expanding the geographic coverage of samples. Additionally, Clark (1936) adjusted all counts to include the hypural (previous studies did not explicitly state that they included or excluded the hypural; however, the counts are similar in range so we assume the counts were made to include the hypural in the earlier studies). Clark (1936) concluded that "[t]he present data, consisting of vertebral counts of 12,858 adult sardines, indicate, therefore, a complex adult population along the Pacific coast of North America." Differences in the mean values of "adult" and "young" sardine from California and southern Baja California, and the similarity between the mean value of Californian "young" and "adults" to the north, prompted Clark to surmise a lack of admixture between young fish in California and those from southern Baja California.

Clark (1947) compiled additional vertebral counts in the intervening years and provided a summary of all available counts, published and unpublished, for sardine from British Columbia to the Gulf of California, bringing the total number of published vertebral counts to 49,735. Clark (1947) continued to investigate differences in vertebral counts between age/size groups and defined "0 group" fish as those between ~30-120mm, "1 group" as those ~100-150mm, and "adult" as those fish greater than or equal to~150mm standard length. As mentioned in her previous study, Clark (1947) noted that the number of vertebrae varied between year classes. Clark's assessment was that "[t]he tentative conclusion seems justified, therefore, that sardines found in southern Lower California and the Gulf of California constitute a separate population which rarely intermingles with the northern population, but that a considerable, and perhaps variable, amount of interchange takes place throughout the range of the northern population from Alaska to Pt. San Eugenio in central Lower California [MX].".

Wisner (1960) evaluated a new set of vertebral counts in his study on northward movements of sardine stocks and compared them with the values from Clark (1947). Wisner added 18,733 vertebral counts from San Pedro (California, U.S.) to the Gulf of California, bringing the total number of published vertebral counts to 68,468. Wisner noted a lower average number of vertebrae from northern Baja California and southern California as compared to the data reported by Clark (1947; 51.50 and 51.73, respectively). He concluded that the sardine taken in northern Baja California during his study period (1950-1959) comprised a different lineage than those taken prior to 1941 (i.e., those reported in Clark, 1947). He based this on the notion that the reduction in the mean vertebral number was contrary to an expected phenotypic increase in vertebral number associated with cooler water (see below for context) and, as such, the change in average vertebral count in southern California was due to decreasing influx from a northern subpopulation that had recently declined in abundance. A summary of all vertebral counts from the studies mentioned above is shown in Fig. 3.

There is no expectation that individuals with a certain vertebral count will produce offspring with exactly that same count and thus the use of vertebral counts (and other meristic characters) in subpopulation studies has been criticized (e.g., Pawson and Jennings, 1996). Additionally, at the turn of the 20th century, there was a strong belief in "Jordan's Rule" for vertebral counts which stated that vertebral number should increase with latitude within a species (Jordan himself did not claim to have first described this rule and recognized Albert Günther as the first to make note of it). As Clark (1936) stated "Since decrease in temperature is associated with increase in latitude, sardines from northern waters…should have a higher average number of vertebrae than do those found in Californian or Mexican waters, provided the populations do not mix." As attractive as this hypothesis was at the time, we now understand that the number of factors influencing vertebral number (and other enumerable characters) in fishes is the function of a complex interaction of both environmental and genetic causes (see McDowall, 2008, for a discussion). As it is currently understood then, there is no expectation that sardine in northern latitudes should have a higher number of vertebral elements than those from more southerly areas.

Much of the focus in these early studies was on very small differences in the mean number of vertebral elements between groups collected at different locations. This presents a somewhat philosophical problem in that meristic characters are discrete and require a different set of statistical analyses (Winans, 1985). Comparing means is also difficult to interpret as an organism cannot have or inherit a fractional element, thus the information contained in a mean value is somewhat meaningless in terms of subpopulation studies. The simplest method to avoid this nuance is to use modal values (and shifts in modal frequencies between groups). An observed modal shift that is associated with geography may yield useful information on subpopulation structure, but only if all environmental factors are considered and eliminated. Thus, in evaluating the utility of these data, it is important to recognize these caveats.
Another important consideration in evaluating these early studies concerns the use of size at age data. Clark's (1936, 1947) papers presented vertebral counts sorted by age, with age determination being based on individual size. As noted by Wisner (1960) and validated since "…adult sardines (2 years or older) vary in length from 1.5 to 2.5 inches in each age group." More recent studies have demonstrated that individual variation in length at age in sardine is even higher than noted by Wisner (1960) and that the length ranges of fish of ages 0 through 4

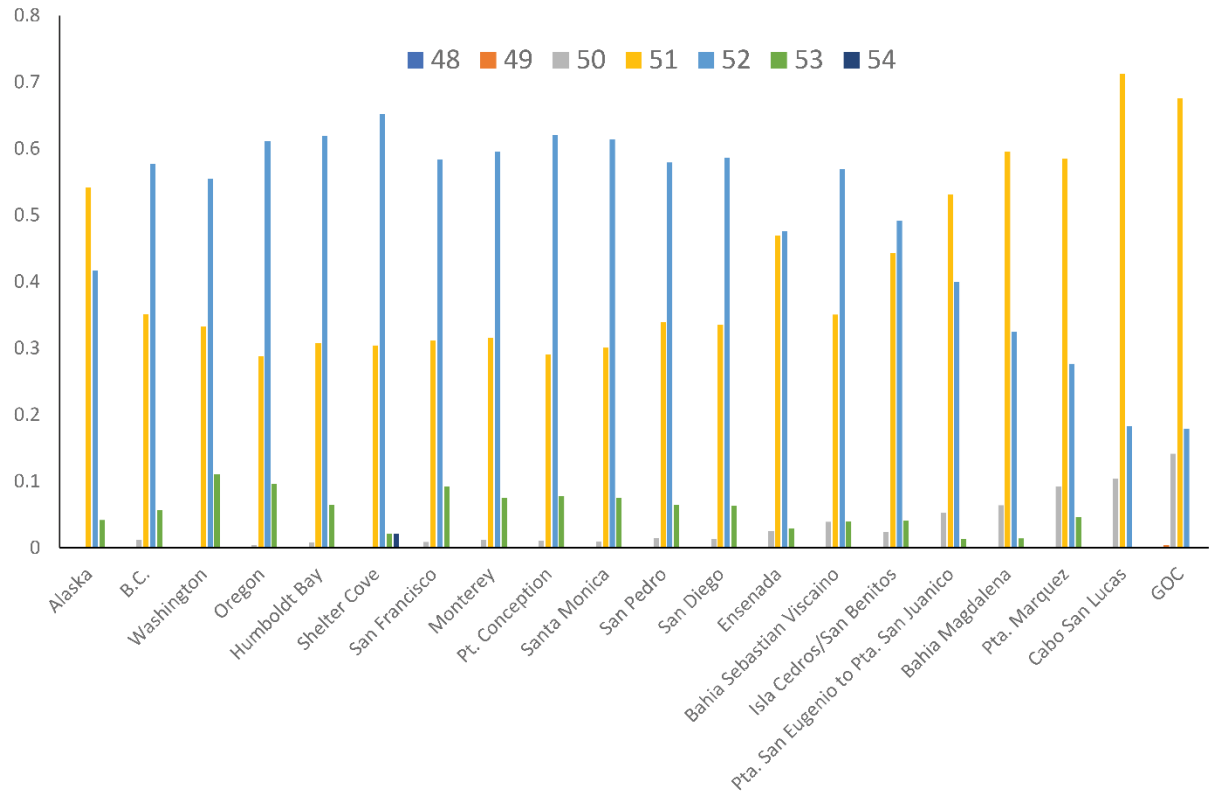

Figure 3. Vertebral counts for Pacific sardine (*Sardinops sagax*). Data were compiled from several primary sources (Hubbs, 1925; Hart, 1933a/b; Clark, 1936; Clark, 1947; and Wisner, 1960).

years completely overlap (e.g., Dorval et al., 2015). Therefore, any age-related data that are not derived empirically (e.g., from direct aging of an individual through such methods as counting annuli in otoliths) carry a high degree of uncertainty and thus are unsuitable for use in most applications seeking to understand age-related patterns in sardine.

With all this in mind, we can examine the nearly 70,000 published vertebral counts *en toto*. Figure 3 shows the frequency distributions for vertebral counts for all fish in the aforementioned studies arranged by locality from North to South. Ignoring the samples from Alaska (U.S.; vertebrae from only 29 fish have been counted from this extreme northern edge of the range), it is clear that there are two modal values, 51 and 52, with an apparent modal shift from 51 to 52 in samples collected south of Bahía Sebastián Vizcaíno (MX; latitude ~ 28°N). If this modal shift is relevant (and it can be argued that it is not based on the discussion above), it could imply that there is a difference between two groups: one extending from British Columbia to Bahía Sebastián Vizcaíno and one extending from Bahía Magdalena (MX) south and into the Gulf of California (Bahía Magdalena is considered as part of the Cortez biogeographic province and is aligned with the Gulf of California; Hastings, 2000). Division into two such groups reflects the well-studied biogeographic patterns of marine fishes in the eastern Pacific, and agrees with Clark's (1947) conclusion:

> "The tentative conclusion seems justified, therefore, that sardines found in southern Lower California and the Gulf of California constitute a separate

> population which rarely intermingles with the northern population, but that a considerable, and perhaps variable, amount of interchange takes place throughout the range of the northern population from Alaska to Pt. San Eugenio in central Lower California." (p. 25)

It is surprising that well into the 21st century, investigators have continued to cite papers using vertebral count (or other meristic) data as a means of investigating subpopulation structure in sardine without having first evaluated the utility of the method. That is, it is well known that differences in vertebral number among populations of fishes may be caused by myriad factors including environment, ecology, body size, life history, genetics, and body shape (McDowell, 2008; Tibblin et al., 2016). As early as the 1950s it was recognized that the use of meristic data for subpopulation studies was imperfect, at best. Marr (1957) eloquently stated that:

> "[o]nly under certain ideal conditions would such environment modified characters be of value…Under these ideal conditions such characters would serve as useful 'natural tags' with respect to geographic origin of fish, extent of dispersion, migrations and related problems. They would not, however, provide any information on the more fundamental problem of genetic difference. One has only to consider what is known of sardine spawning to realize how meaningless (from the standpoint of subpopulation characters) meristic data may be." (p. 112.)

Considering all existing data gathered to date, vertebral count data cannot be used to reject the null hypothesis of a single population along the west coast of North America. Given the observed modal shift in vertebral number in fish from southern Baja California and the Gulf of California an argument could be made that there are two, weakly defined groups of sardine in the north eastern Pacific, one spanning the region from Alaska to central or southern Baja California, and one from Bahía Magdalena south and into the Gulf of California. However, given that vertebral number is influenced by a complex set of environmental and biological factors (e.g., temperature during development, phylogeny, body shape; see McDowell, 2008), this modal difference may not reflect true subpopulation structure.

Serological Antigen Response

"While these different techniques [serological antigens and muscle amino acids] are rather exact and well known, they need extensive testing in order to determine whether or not differences observed through their use are a reflection of genetic differences." Marr 1957a, p. 2.

Among the earliest biochemical data types used in population studies was one based on the immunological erythrocyte antigen response (blood clotting). Two papers (Sprague and Vrooman, 1962; Vrooman, 1964) sought to use such data to "…[characterize] reproductively isolated groups of sardine." (p. 131). Sprague and Vrooman (1962) collected blood samples from >2,000 sardine at various locations from Monterey (U.S.) to Bahía Magdalena from 1958-1960. Sprague and Vrooman (1962) identified two groups in the data, one with a higher distribution of values for individuals with a positive antigen response termed "C+" (i.e., clotting; 11.03 - 19.51%, mean 13.2%; hereafter "Pacific high group") and one group with a lower distribution of

values (3.8 - 6.67%, mean 9.2%; hereafter “Pacific low group”). These groups roughly corresponded to northern and southern groupings of localities with some overlap, particularly towards the midpoint of the geographic range of samples.

Vrooman (1964) expanded upon the previous study, adding 2,844 samples from Santa Cruz Island (California, U.S.) to Isla Cresciente (southern end of Bahía Magdalena) on the Pacific coast and from eight sites in the Gulf of California taken from 1960 to 1962. Vrooman noted similar distributions of values as in the previous paper (Pacific low group 5.3 - 8.1% C+, mean 6.5%; Pacific high group 11.0 - 18.4% C+, mean 13.5%) and also noted that samples from the Gulf of California had a somewhat higher range and mean (12.2 - 21.2% C+, mean 16.8%). All data from these two studies are presented in Figures 4-5.

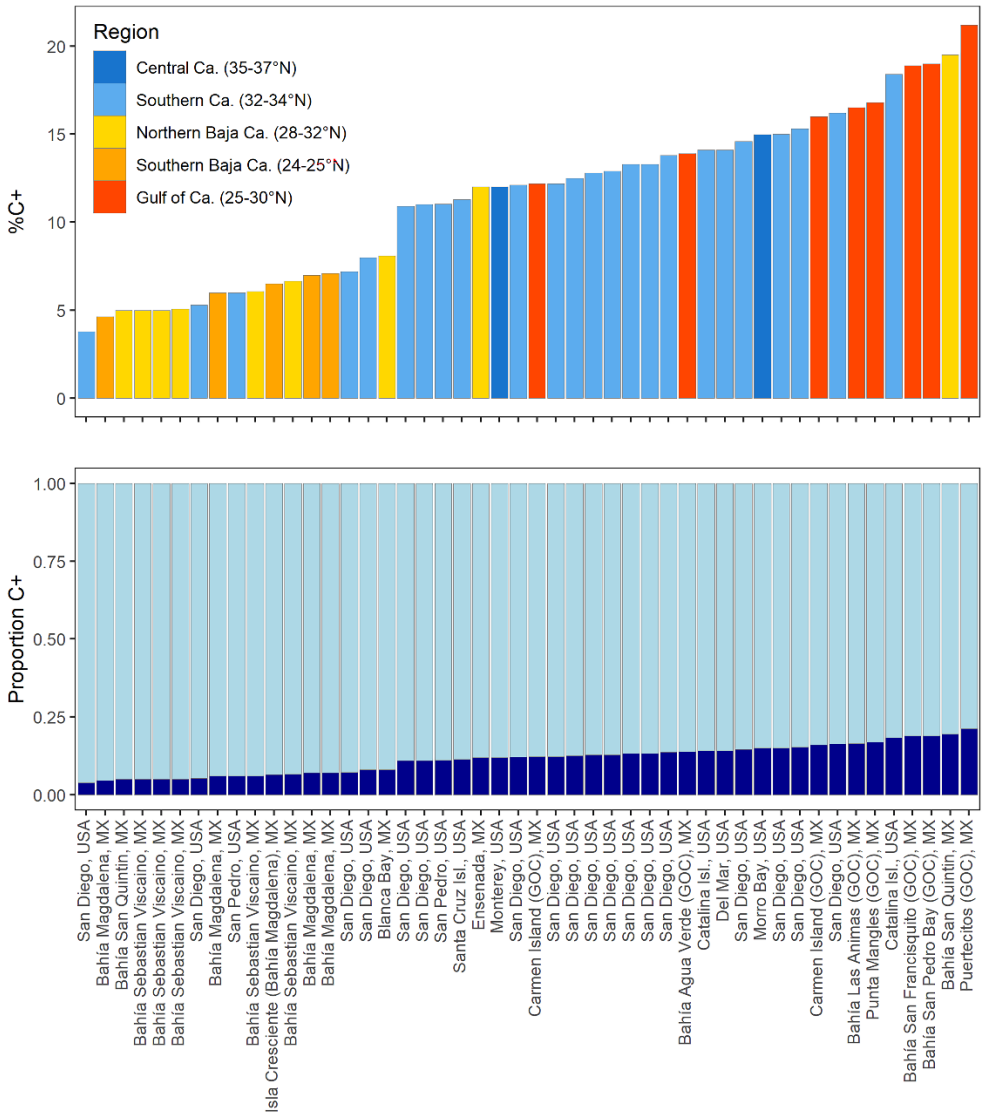

Figure 4. Percent (top) and proportion (bottom) of positive serological antigen response in Pacific sardine (*Sardinops sagax*) compiled from Sprague and Vrooman (1962) and Vrooman (1964) arranged from smallest to largest value with collection locality on the horizontal axis. Top panel is color coded by general geographic region. Note that vertical axis scales have differing maxima.

When grouped into North and South regions and presented in tabular format as in the original papers, there certainly appeared to be the suggestion of two groups along the Pacific coast that overlap near San Diego (U.S.), a scenario that would support a hypothesis of two subpopulations whose ranges overlap in southern California. It is important to note, however, that their grouping of locations into "north" and "south" was based on a *post hoc* categorization of the "high" and "low" groups as corresponding to a northern and southern group (this is particularly evident when reviewing the data presented for San Diego; see Figs. 4-5). When all data are evaluated together, this pattern is not apparent, especially when considering the samples from the Gulf of California. Samples within the Pacific low group are found from San Pedro (U.S.) to Bahía Magdalena and within the Gulf of California. Samples within the Pacific high group are found throughout the geographical range of samples (Fig. 4). Additionally, there was disproportionate sampling among locations, particularly for San Diego, which had 19 of the 48 total samples (~40%). When samples are combined and grouped by location, there is even less of a pattern of any discrete groups (Figure 5).

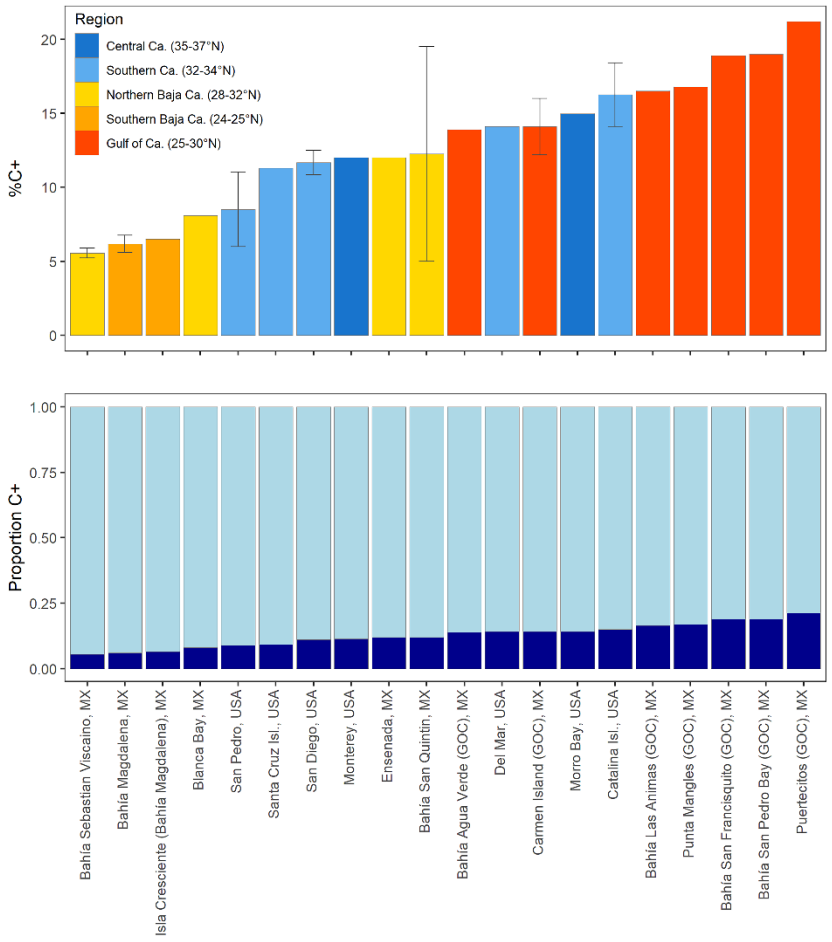

Figure 5. Percent (top) and proportion (bottom) of positive serological antigen response in Pacific sardine (*Sardinops sagax*) compiled from Sprague and Vrooman (1962) and Vrooman (1964). Samples from each locality are combined. Top panel depicts mean value =/- one standard error and color coded by general geographic region. Note that vertical axis scales have differing maxima.

It is difficult to interpret the proportional changes in C+ phenotypes as being meaningful given that among all samples only 10.8% are C+. That is, nearly 90% of all samples did not display a clotting response. If treated as a discrete character (i.e., clot or no clot) it is clear that there is little to suggest differences among geographic localities. Regardless, if we assume that the proportional differences of this low frequency character are biologically relevant as an indicator of subpopulation divisions, our interpretation is very different from that of Sprague and Vrooman (1962) and Vrooman (1964). Given the large geographic range of samples that contain the Pacific high group which exceeds the bounds of the geographic coverage of the Pacific low group, the most parsimonious explanation is that there is a single group with a range of proportional values for C+ fish that does not correspond to geography.

Perhaps more importantly, serological antigen response in erythrocytes is no longer used in studies of subpopulation structure. These immunological responses are notably unreliable and many times artifactual reactions that are elicited from other cellular components are difficult to control, thus providing misleading results (Utter, 1991). Additionally, it has been known since the 1930s that these responses are influenced by the temperature at which an individual has been acclimated (reviewed in Corbel, 1975). As one cautionary example, variation in erythrocyte agglutination in Atlantic herring, *Clupea harengus*, was presumed to be the result of direct genetic influence (Sinderman and Mairs, 1959) but was later shown to be an effect of dietary- or temperature-induced anemia, as noted by Ridgeway (1971). It is clear that the immune reactions elicited in these types of studies are a phenotypic trait and are therefore only crude proxies for an organism's underlying genotype, and that they are influenced by myriad non-heritable factors. Thus, the conclusions of earlier authors that these methods were able to unambiguously assign samples to a particular subpopulation (e.g., Murphy, 1966) can no longer be thought of as robust. Despite being the best available information at the time, this method is flawed and it would be imprudent to use Sprague and Vrooman's work as supporting data for subpopulation studies in sardine.

Tagging Data

> **"***Tagging or marking experiments have usually been carried out in order to learn about migrations or to make estimates of population size. They can also be used to learn something about subpopulations, provided that the tagging and recapturing are done on the spawning grounds.*" Marr, 1957a, p. 2.

From the mid-1930s through the early 1940s, an ambitious and remarkable tagging study took place along the west coast of the U.S. and Canada. Through a collaborative effort between researchers in British Columbia and the coastal western U.S., more than 140,000 sardines were fitted with small metal tags and released at various locations over an eight-year period. These metal tags were then recovered during the fish reduction process (i.e., creating fish meal and oil) on magnets within reduction plant machinery. Hart (1944) summarized tagging and recovery of sardine in British Columbia from 1936 to1942, while Clark and Janssen (1945) summarized tagging and recovery of sardine along the west coast of the U.S. from 1936 to1944.

These studies unequivocally demonstrated that at least some sardine along the west coast of North America move extensively, particularly among fish of larger size. Figures 6-7 show generalized movements of fish relative to their location of release. Fish released at Vancouver Island, British Columbia (Canada), were recovered at all participating reduction plants to the

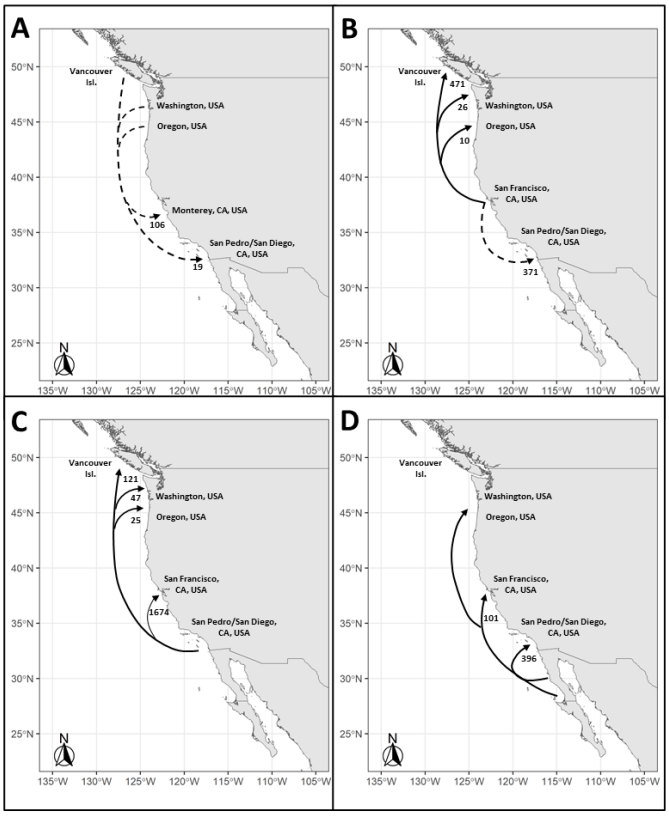

Figure 6. Release and recovery locations of tagged Pacific sardine (*Sardinops sagax*) redrawn from Clark and Janssen (1945). Northward paths are drawn in solid lines, while southward paths are drawn in dashed lines. Numbers indicate number of tag returns. Note that Clark and Janssen (1945) inadvertently omitted low frequency tag returns in Washington, U.S., release from central Baja California, MX, but were later reported by Clark and Marr (1955) and are included here. Numbers indicate tag recoveries.

south (Hart, 1944), as were fish released off Washington and Oregon (U.S.; Clark and Janssen, 1945). Fish released in San Francisco (U.S.) were recovered at all participating plants to the north and the south (Clark and Janssen, 1945). Fish released in San Diego were recovered at all participating plants to the north (Clark and Janssen, 1945). A limited number of fish were released in Baja California at Cabo (Punta) Colnett, Bahía Sebastián Vizcaíno, and Bahía Magdalena. Recoveries from Cabo (Punta) Colnett and Bahía Sebastián Vizcaíno occurred in San Pedro, San Diego, San Francisco (U.S.), and Washington. No tags were recovered from the small number of fish released in Bahía Magdalena.

In addition to the rather long-distance movement observed, perhaps more remarkable is the speed at which these movements took place. Fish tagged off southern California (U.S.) in February and March were recovered off Vancouver Island, British Columbia (Canada) the following July (Clark and Janssen, 1945). Similarly, fish released off Vancouver Island in July and August were recovered off California the following December and January (Clark and

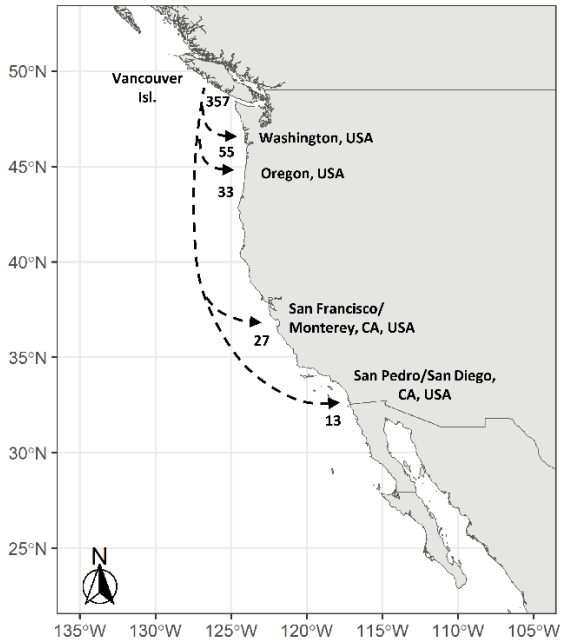

Figure 7. Release and recovery locations of tagged Pacific sardine (*Sardinops sagax*) drawn from data in Hart (1944).

Janssen, 1945). Hart (1944) estimated a daily movement rate of ~11 km per day for northward movements, and ~6 - 14 km per day for southward movements.

Longer distance movements were also clearly associated with larger fish. Smaller fish were more often recaptured near their location of release in the following year (Clark and Janssen, 1945). Clark and Janssen (1945) noted that there was a proportionate decrease in the recoveries of the larger fish in each succeeding season on the fishing grounds where they were released. Clark and Janssen (1945) noted that the observation that commercial landings in more northerly locations were dominated by larger size classes was consistent with this size-based movement.

In 1938, 963 tagged sardines were released in Bahía Magdalena, but no tagged fish were recovered. Clark and Janssen (1945) posited that this was an indication either that fish from southern Baja California and in the Gulf of California do not intermingle with fish to the north, or that an insufficient number of fish were released to reasonably expect tag returns. Nearly 16,000 tagged sardines (~11% of total tagged fish released) were released in Baja California from Cabo Colnett to Sebastián Vizcaíno Bay from 1939-1941 (~4,000 in 1939, ~6,800 in 1940, and ~5,000 in 1941; Clark and Janssen, 1945). While fish were recovered from all three years, the highest number of tag returns was from the 1941 group. In all three years, tagged fish were recovered as far north as San Francisco, (Clark and Janssen, 1945). Importantly, but often overlooked in citations of Clark and Janssen (1945; e.g., Marr, 1960), the authors noted that movements northward of San Francisco were possible but could not be determined as insufficient time had elapsed since release when their study ended, especially given that most returns originated from the last release (1941). Clark and Janssen (1945) also omitted from their summary the recovery of tagged fish released in central Baja California in the Pacific Northwest of the U.S. Clark and Marr (1955) report that "[f]ish tagged off central and northern Baja California were recovered in large numbers in the southern California catch, moderately in the

central California catch and negligibly in the catch of the Pacific Northwest" and reported a 1.1% tag return rate for the latter. Even more surprising is that Marr (1960) also omitted these tag returns in the formulation of his hypothesis of subpopulation structure. While only a few tags were recovered, they should not be discounted as they unequivocally demonstrate that at least a portion of sardine from Baja California make the journey to the Pacific Northwest. Also, being the furthest distance from the release site, it is not to be expected that a large number of fish released in Baja California would be recovered in the Pacific Northwest of the U.S. given the relatively short post release time as noted by the authors.

It is important to note that no reduction plants in Baja California participated in this tag recovery program. Any movements of tagged sardine south of the border between the U.S. and Mexico would therefore not be reflected in the data. Given the lack of any major oceanographic or physical barrier between southern California and Baja California it seems likely that southerly movements beyond the U.S./Mexico border were simply not recorded due to the lack of participating reduction plants and not due to any biological or ecological reasons.

Due to the absence of tagging data on the southward movements of fish beyond the U.S./Mexico border there remains some uncertainty as to the full extent of movements in sardine. However, the overlooked northward movement to the Pacific northwest of the U.S. by fish tagged off central Baja California provides data that do not support Marr's original hypothesis (or subsequent interpretations thereof). The results of these tagging studies confirmed that sardine, at a minimum, migrate between British Columbia and Southern California, and between central Baja California and the Pacific Northwest of the U.S. It cannot be discounted that large sardine found in the Pacific Northwest include individuals that migrated from central Baja. Likewise, the degree to which adults from the Pacific Northwest and Southern California migrate southward into Mexico is unknown as no study has been executed to determine the full migration distance of individual sardine along the Pacific coast. Moreover, the degree to which all individuals migrate seasonally or whether some adults do not migrate at all (i.e., partial migration behavior; Chapman et al., 2012) has not been investigated but could explain regional variations in growth patterns and other life history traits (Gillanders et al., 2015). In fact, Clark and Janssen's (1945) data contain several instances of tag recoveries in the same region where they were released but in the following sampling years, a result that could be explained by either migration and return, or no migration at all. However, with these important caveats in mind, we think it safe to infer that broad and extensive movements of sardine occur from central Baja California to British Columbia, and we agree with Clark and Janssen (1945) in their conclusion "… that all the fisheries along the Pacific Coast are drawing from the same sardine population…".

These tagging studies are the sole provider of direct evidence of movement/migration in sardine. While there are more recent papers purporting to provide information on sardine migration (e.g., Lo et al., 2011, Demer, et al., 2012), none provided any direct evidence. For example, Lo et al. (2011) inferred migration of large fish (>200mm) away from the Pacific northwest of the U.S. to the coast of California by calculating a change in biomass between July and March from 2003-2005. In a brief section of their discussion, Demer et al. (2012) reported on the distributions of sardine along the coast of the western U.S. and by comparing them to the results of a potential habitat model they concluded that they had shown evidence of migration. While interesting, these types of studies only measure proxies for movements, and many factors could introduce bias.

Parasites as Natural Tags

"*It is obvious that, regardless of the method used to attack this problem, the crucial test will be the determination of the amount of mixing at spawning time. Complete mixing, lack of mixing or any intermediate condition during the rest of the year are not pertinent to this problem*." Marr, 1957b, p. 115.

As biological tags, parasites have been examined in the sardine (Baldwin, 2010; Baldwin et al., 2012; Jacobson et al., 2019). Based on the presence of two parasite species, one widespread along the west coast of North America and one restricted to the Pacific Northwest of the U.S., Baldwin (2010) hypothesized two migratory pathways. One of these mirrored the long-distance north/south movements shown by conventional tagging, and one in which fish overwinter in the Pacific northwest of the U.S. and do not perform the north-south movements. Baldwin (2010) classified sardine as "non-migrant" and "migrant" based on size (<200 mm SL and >200mm SL, respectively) and assumed that parasites found in "non-migratory" fish infected the host individual within the geographic location where the fish was caught.
This work has gone largely overlooked but is important. These data suggest that not all sardine migrate in the same manner, a phenomenon that is not unusual in migratory species. In many migratory species, the entire population does not perform the typical migratory pattern, a pattern known as "conditional migration" (Secor, 2015). It is therefore possible that some portion of the sardine population does not engage in the migratory behavior as convention has described (although whether or not overwintering in the Pacific northwest of the U.S. varies annually at the individual level is unknown). The smaller size of the "non-migrant" fish described in Baldwin (2010) also implies some level of recruitment of sardine to the Pacific Northwest. The larger size of the "migrant" fish in that same study implies that southward migration of fish that recruit to the PNW follows a similar size/age-based pattern as that of fish that recruit in more southerly locations. Baldwin (2010), however, explicitly stated that they could not confirm the existence of subpopulations in sardine based on these parasite data.

Parasites have been successfully used to help answer questions about host diet and feeding behavior, movements and ranges, connectivity of host groups, and to a lesser degree recruitment patterns and phylogenies (reviewed in Catalano, et al., 2014). As with conventional tagging, parasite data are best used in concert with other proxies of subpopulation structure as it is only in exceptional circumstances that they can define the limits of fish subpopulations (see Lester, 1990, MacKenzie, 1999 and other papers reviewing this topic). Currently, the available data on parasites of sardine cannot be used to reject the null hypothesis of a single subpopulation or to support the hypothesis of the existence of subpopulations.

Otolith Morphology and Morphometry

"A second method is to pick [morphological or meristic] characteristics which are known to be genotypic…At present, the extent to which any particular characteristic is an expression of the genetic constitution of an individual is unknown. Therefore, if this method is used, it will first be necessary to establish experimentally the genotypic nature of as many characteristics as possible..." Marr, 1957b, p.116.

Otolith morphometry and microchemistry studies have been conducted to test the three-stock hypothesis in the sardine and to examine location of origin (birth). Felix-Uraga et al. (2005) examined sagittal otoliths taken from 1,849 one-year old fish from two locations (Ensenada and Bahía Magdalena) collected from 1994-2002, and measured four distances: distance from antirostrum to the posterior edge, distance from posterior edge to rostrum, distance of rostrum to antirostrum, and width at the focus. For comparisons of these data, fish were assigned to a putative stock based on month of landing which Felix-Uraga et al. (2004) determined to be the temporal bounds of stock presence at the two locations: August to October for the "warm stock" at Bahía Magdalena and the "temperate stock" in Ensenada, March to May for the "temperate stock" in Bahía Magdalena, and February to April for the "cold stock" in Ensenada (see "Temperature and Landings Data" section for definitions of these stocks). In order to account for uneven sample sizes, synoptic data sets were created by randomly subsampling 50 non-repeated measurements to create pseudo-replicates and this was repeated 50 times. Multidimensional analysis (MDA) was then performed on two balanced, non-replacement, random sub-samples from each group. Finally, the accuracy of the MDA was tested using 100 random samples from each group.

In every pairwise comparison of the four groups, the Wilks' Lambda tests were statistically significant, including between the "temperate" stock landed at Ensenada and Bahía Magdalena, meaning that the data in each group were unlikely to have been drawn from the same statistical population. The percentage of non-significant test values among iterative runs showed a progression from none to many that correlated with the most spatially and thermally different groups (e.g., "cold Ensenada" and "warm Bahía Magdalena" had zero non-significant test values whereas "temperate Bahía Magdalena" and "temperate Ensenada" had 68% non-significant test values). MDA error rates for stock assignment were 38% for the "warm" stock, 43% for the "cold" stock, and 58% for the "temperate" stock. Felix-Uraga et al. (2005) interpreted these results as confirmation of their three-stock hypothesis and stock assignments of Felix-Uraga et al. (2004).

There are a number of considerations which make it difficult to accept the conclusions of Felix-Uraga et al. (2005). First and foremost is that when the data were analyzed *en toto*, all pairwise comparisons were statistically significant, including the sole comparison of a putative stock from multiple locations (i.e., the "temperate" stock from Ensenada and Bahía Magdalena). This implies that the within-group variation (in this case within the "temperate" group) is comparable to the among-group variation for the otolith measurements analyzed.
Nearly as concerning are the high error rates in the MDA classification. Ranging from 33-58%, these rates signal a near inability to discriminate among putative stocks. An error rate of 58%, in fact, means that less than half of the samples were correctly classified.

Other factors make it difficult to interpret the conclusions of Felix-Uraga et al. (2005), including the absence of methodology for aging of fish (e.g., fish aged as one year old may actually be 6 months or 18 months old); if fish length was used as a proxy for age, variation in length at age confounds age estimates by up to several years (Erisman et al., 2025) the absence of samples from the "warm Bahía Magdalena" group from 1997-2000, and the lack of meaningful consideration for the effects of the environment on otolith size and shape (see discussion below). Without invoking some special consideration or relying on qualitative evaluation of the statistical test values (i.e., fewer or more non-significant individual pairwise comparisons), the data in this paper cannot be used to reject the null hypothesis of a single population.

Valle and Herzka (2008) calculated $\delta^{18}O$ compositions in otoliths from juvenile sardine (up to 18 months old) to test the hypothesis that young fish were spawned near their capture location. Samples were collected from four areas in Mexico: Ensenada, Isla de Cedros, Bahía Magdalena, and the Gulf of California. Valle and Herzka (2008) compared $\delta^{18}O$ values with isotopic ratios predicted from local temperature and salinity, as well as the integrated temperature to which an individual was exposed over its lifespan. The data showed that fish from a given area were born both locally and at locations from afar. Importantly, this was true for samples collected in the Pacific Ocean and the Gulf of California, indicating either that there is a high degree of connectivity among these locations, or that $\delta^{18}O$ compositions lack the power to detect an absence of mixing among groups.

Javor et al. (2011) conducted a large-scale study of otolith morphology with sampling from the Gulf of California north to Vancouver Island (Canada) from 1991-2010. Eight morphometric measurements were taken from >2,000 individuals across ages but with an emphasis on age one fish. Fish were grouped by seven geographic regions and Principal Component Analysis (PCA) and Multivariate Analysis of Variance (MANOVA) were used for comparisons. Javor et al. (2011) identified length, area, perimeter, and weight as the most informative dimensions based on PCA. These selected measurements (length, area, perimeter, and weight) were then standardized and a MANOVA was used to test for differences between regions or clusters of regions by using the Wilks's Lambda test of significance. Only age-1 individuals were used in these analyses as they were the only age group collected from all regions.

From this initial analysis, Javor et al. (2011) developed a novel calculation called Perimeter Weight Profiles (PWP). Regression equations for the four measurements identified in the PCA (above) were calculated for the following combinations: perimeter vs. area, perimeter vs. length, and weight vs. length. These regression equations were then used to calculate the expected average perimeter and weight from the otolith area or length. The differences between observed and calculated measurements (residuals) were used to describe regional characteristics of the otoliths. Based on the null hypothesis that, for a given region, half of the measurements for a region should fall above and half should fall below the regression line for the entire data set if there are no regional differences, Javor et al. (2011) calculated the PWP as the sum of a binary indicator of a measurement being greater or less than an expected value (e.g., 1 if greater, 0 if less) divided by the sample size.

In the initial comparisons of correlations of the four variables (length, perimeter, area, and weight) based on all ages and all regions, no regional patterns were observed. The MANOVA using these four variables for only age-one fish indicated that otolith sizes were not the same for all regions. In summary, Javor et al. (2011) concluded that regional differences existed in otolith morphology for age-1 sardine between regions or clusters of regions. PWP of otoliths of the same length or area showed both similarities and differences among some regions. The authors pointed to high inter-annual variation in PWPs as a complicating factor in the analyses. Despite this, they highlighted a geographical pattern of differences in weight and area as an indication of a potential separate group in the southernmost distribution of sardine that is distinct from U.S. and Canadian subpopulations (the former having a lighter and more rugose otolith than the latter). Finally, the authors concluded that PWPs could be applied as a tool for understanding migration and connectivity when used alongside otolith chemistry, aging, genetics, and other methods.

One factor that could help to explain the high degree of variation in Javor et al.'s (2011) data is potential aging error. Javor et al. (2011) used otolith weight as a proxy for age following Yaremko (1996). It is now understood that there is considerable individual variation of otolith weight-at-age (E. Dorval and K. James, SWFSC, unpublished data). Any age-specific signal may have thus been diluted by the inclusion of multiple age classes.

On the premise that otoliths from southern sardine were lighter and more rugose (termed Type I) and those from northern sardine were heavier and smoother (Type II; Javor *et al.* 2011), Javor (2013) examined otoliths from monthly collections of sardines from 2006-2012 caught off of San Diego and Monterey (U.S.) by the live bait fishery. PWPs (Javor et al, 2011; see above) were calculated to evaluate the efficacy of this morphological measure as a tool to discriminate among putative stocks of sardine. This ambitious study approached this question by: 1. Examining temporal trends in juvenile otolith morphology, 2. Calculating stable oxygen isotope composition to discern relationships between otolith morphology, temperature, and date of capture, 3. Determining the relative abundance of the Type I and Type II PWPs in adult sardine, 4. Searching for relationships to spawning condition, length, and growth rates, 5. Examining any correlation of otolith characteristics with spawning stock biomass, and 6. Comparing the frequencies of Type I and Type II otoliths between locations in the U.S. (Monterey and San Diego) and Mexico (Ensenada and Bahía Magdalena).

Javor (2013) observed an increase in Type I otoliths in young (age 0-2) sardine after 2008. In San Diego, from 2006-2008, 21% of the total otoliths were Type I and 24% were Type II, whereas from 2008-2012, Type I and Type II otoliths represented 49% and 9% of the total, respectively. Data from the juvenile otoliths examined from Monterey were not shown but reported as having similar temporal trends.

Temperature during otolith formation as measured by $\delta^{18}O$ composition in otoliths from San Diego did not show a significant difference between Type I and Type II otoliths for either time period examined (before or after July, 2008). However, there was a significant difference in calculated temperature during formation for all otoliths of about 2°C after July 2008. Interannual comparisons of temperature during otolith formation could not be calculated for Monterey due to annual variation in otolith weight. However, three collection periods were able to be compared: 1996-7, 2008, and 2009. No significant differences between temperature and otolith type were found.

Javor (2013) then examined PWPs in otoliths from adults collected from 2004-2012. There was no apparent shift in the frequencies of Type I and Type II otoliths. No differences in the frequency of Type I and Type II otoliths were found when adults were grouped by sex (assessed visually) from collections in 2012. Mature males had 20% Type I and 23% Type II, and females 24% and 28% Type I and II, respectively. Maturity index of adult sardine showed no pattern in the distribution of Type I and Type II otoliths.

There was a high correlation coefficient for the relationship between PWP-weight (a calculation based on one of the four most important characteristics identified in Javor et al. [2011]) and spawning stock biomass off California from 2006-12. The author concluded that this supported the hypothesis that young sardine with lighter weight otoliths (reported as Type I but not based on all characters measured) from the putative southern or temperate stock became proportionally more abundant as the putative northern stock diminished.

Comparisons of the frequency of Type I and Type II otoliths between locations in the U.S. and Mexico before and after July 2008 showed a frequency shift. In Monterey, Type II otoliths were more abundant before July 2008, whereas they were less abundant after this. In San Diego,

frequencies were nearly equal before July 2008, but Type I was more abundant in the later part of the study period. In Ensenada (1991-1992) and Bahía Magdalena (2004), the Type I otolith was always more abundant; however, the time periods did not overlap with those used for Monterey and San Diego (1991-1992 for Ensenada, 2004 for Bahía Magdalena, and 2006-2007 and 2009-2010 for Monterey and San Diego).

The conclusions of Javor (2013) are based on the assumed existence of subpopulations of sardine and that Type I and Type II otoliths are capable of diagnosing the subpopulations. Several aspects of the underlying data, however, indicate that any subpopulation signal is weak. $\delta^{18}O$ compositions showed that there was no direct effect of temperature on otolith morphology (relative to Type I and Type II) in sardine. Both Javor et al. (2011) and Javor (2013) relied upon subpopulation delineations as provided by Felix-Uraga et al. (2005) that were based on temperature. As we have discounted temperature as a useful way to separate putative subpopulations (see "Temperature and Landings Data" section), and that temperature did not appear to affect the formation of Type I and Type II otoliths, we conclude that using Type I and Type II otoliths to diagnose putative subpopulation identity to be equivocal.

Vergara-Solana et al. (2013) examined sardine body and otolith morphology using a geometric morphometric approach (here we only review their data on otoliths). Samples from Bahía Magdalena, were collected from September and December in 2007 and from April and July in 2008 (N = 260). Canonical Variation Analysis (CVA) on 43 otolith landmarks grouped by month of collection showed significant differences between all groups for the first coefficient of variation, but not for the second or third. The greatest differences among otolith shape at CV1 were between an April and December/September group. Correct assignments using CVA (PeCoAs) ranged from 29-80%. This range of discriminatory power is a direct reflection of the variability between the temporally assigned groups shown by the CVA and likely is an indication that the underlying data are not suitable for the purpose. These data are also not corrected for age/size biases which are known to be important factors when analyzing otoliths (see below).

Javor and Dorval (2017) used trace element analysis on whole sardine otoliths to compare cohorts of age-1 fish from central California (Monterey), the Southern California Bight (SCB; Los Angeles, San Diego), and Ensenada. These sites were chosen based on the assumption that fish from central California belong to the putative "cold" stock, while fish from the SCB consist of a mixture of the "cold" and "temperate" stocks of Felix-Uraga et al. (2004; see "Temperature and Landings Data" section for discussion of these stock delineations). Fish were sampled in spring and assumed to be one year of age. A standard trace element panel measured Mg, P, Ca, Mn, Sr, and Ba. The non-parametric k-nearest neighbor method with k = 3 was used to evaluate accuracy of classification to collection site and year based on five trace element ratios, but only for the SCB and Ensenada. Multidimensional scaling (MDS) analysis was used to evaluate the influence of temperature on the temporal variability of trace element composition within the SCB only. Temporal trends in element ratios and temperature were also compared using Spearman's correlation coefficient. A 2 x 2 ANOVA was used to compare element ratios between samples from 1996 and 1997 that were from fish presumed to be born during the same spring spawning event. Javor and Dorval neatly summarized their findings: "The overall picture of trace element profiles of age-1 otoliths of sardine captured in the SCB and Monterey is one of complex interactions between collection sites, year of capture, and seawater temperature."

Beginning in the early 1990s, intraspecific differences in otolith morphology began to be considered as indicators of subpopulation or stock origin for marine fishes with varying degrees

of success. Otolith morphology varies within a species and this variation is sometimes correlated with geography (see Javor et al. 2011 and references therein). However, because otolith morphology is dually affected by both genetics and the environment, a particular morphology may be more indicative of a fish's environment than its hereditary identity (see Javor et al., 2011 and references therein). For species with low dispersal potential, measurements of their environment may coincide with subpopulation boundaries. For species with large dispersal potential such as sardine that experience a patchwork of environmental conditions throughout their lifetime, such measures will carry far less predictive power. As Javor et al. (2011) noted, otolith morphology may help provide confirmation of subpopulation identity along with other indicators of subpopulation structure (ideally genetic) but should not be used on its own.

In addition to being affected by environmental conditions, otolith morphology has been shown to vary with growth rate, both of the individual and of the otolith itself. Campana and Casselman (1993) provided data on Atlantic cod (*Gadus morhua*), arguably one of the most well-studied species on the planet, and convincingly showed that both otolith size and shape varied among geographic regions where growth rate of cod also varies predictably. Campana and Casselman (1993) concisely summarized why great care must be taken when using otolith morphology for stock discrimination:

> "With the influence of the environment being paramount, the utility of otolith shape for stock identification would depend on the relative constancy of the environment in a given stock area, integrated over the lifetime of the fish. For all but short-lived species, this would be a reasonable assumption, since year-to-year differences in the environment would be smoothed out over the lifetime of the fish." (p. 1079)

Clearly, then, Pacific sardine is not a species for which otolith morphology would have a high predictive power for area of origin or putative stock identity. Fluctuating physical and biological oceanographic conditions experienced over the lifetime of an individual can result in highly variable individual growth rates in sardine. Given this, it is expected that otolith morphology in sardine would be particularly susceptible to individual variation. It would also be expected that otolith morphology would change throughout the lifetime of the individual with changing growth rate intervals. This may confound attempts to link frequencies of an otolith type from year to year (Javor, 2013).

Otolith microchemistry has also failed to demonstrate high predictive power for region/subpopulation origin. In many ways this is to be expected given the life history of sardine. In other ways, investigators may not have fully satisfied all assumptions of otolith microchemistry methods. Elsdon et al. (2008) provided a thorough review of the uses, assumptions, and limitations of otolith microchemistry studies and we summarize some of their salient points below. Whole otolith microelement compositions reflect conditions experienced over the lifetime of the individual. Given sardine's proclivity for movements, a putative subpopulation signal could be diluted or lost entirely, especially without accurate determination of age (i.e., age/size-biased movements are known to occur in sardine). Additionally, it is imperative that growth differences are quantified. Given the high variance of individual growth rates in sardine (Dorval et al. 2015), it would be difficult to discriminate between changes due to growth and changes due to a group-specific signal. Finally, prior to using otolith microchemistry for studies of connectivity between groups, it must be shown that there is a distinctly different

chemical signal between those groups and that there is segregation of those groups to incorporate the unique chemical signals. Thus far, no studies have met these assumptions for sardine.

Only one study (Felix-Uraga et al., 2005) made a strong claim that otoliths show high diagnostic success for putative subpopulation assignment (but see remarks above). However, several other studies using otoliths have been cited as showing evidence for the existence of subpopulations in sardine. As a whole, data on otolith morphology and microchemistry are unable to reject the null hypothesis of a single sardine subpopulation and, in general, do not support the alternative hypothesis of the existence of subpopulations.

Spawning Location, Timing, and Temperature

"*If all sardine spawning took place at a single time and in a single place, then there would be no subpopulation problem since there would be opportunity for gene flow throughout the population."* Marr 1957b, p. 113.

That spawning in sardine occurs over a large spatial expanse has been known since at least the 1930s. Upon finding sardine eggs and larvae across a wide swath of the California (U.S.) coast in what we believe to be the earliest published attempt to define the geographical limits of sardine spawning, Scofield (1934) remarked "This find was not only startling, but also rather depressing as it uncovered the fact that future work would have to be extended several hundred miles to sea as well as along the entire coast line of California and Baja California." The body of literature on the oceanographic conditions related to and spatial extent of sardine spawning is large, to say the least. Much of this literature is regionally focused and most is also restricted temporally (i.e., sampling occurred for only a single month or season). These spatiotemporally restricted studies provide only a glimpse of the totality of sardine spawning patterns.

It should be noted that in all studies that were reviewed, the presence of eggs was used as a proxy for spawning. Although spawning behavior has been casually observed (e.g., Wolf, 1964), to our knowledge direct observation of spawning in sardine in the wild has never been reported. Given that sardine eggs hatch, and that non-viable eggs would likely sink to a depth where standard collection methods would not catch them, within ~48 hours (W. Watson, pers. comm.), the presence of eggs appears to be a reasonable proxy for the location of spawning with a small margin of distance error. This distance, however, may vary depending on oceanographic conditions so comparisons among very fine-scale patterns of spawning in sardine may not able to be described with accuracy.

Scofield (1934) described a series of plankton surveys in waters off of California, and Baja California, from 1929-1932 by the Hydrobiological Survey of the Hopkins Marine Station. Over a four-year survey period, 358 stations were occupied over an area of ~250,000 square miles spanning the region between northern California to southern Baja California. Sampling sites varied between years but there were areas of overlap. Distance from shore varied annually from the nearshore to 400 miles offshore (Figure 8) and was inconsistent between years. Surveys were conducted from February to June over the four-year period, with February being absent from the 1929 survey and June being absent from later years.

Scofield (1934) concluded that while "general spawning" was found from Cabo San Lucas (MX), to San Francisco (U.S.), and offshore to 250 miles, spawning was concentrated in a 200 mile stretch of the waters off southern California between San Diego and Point Conception

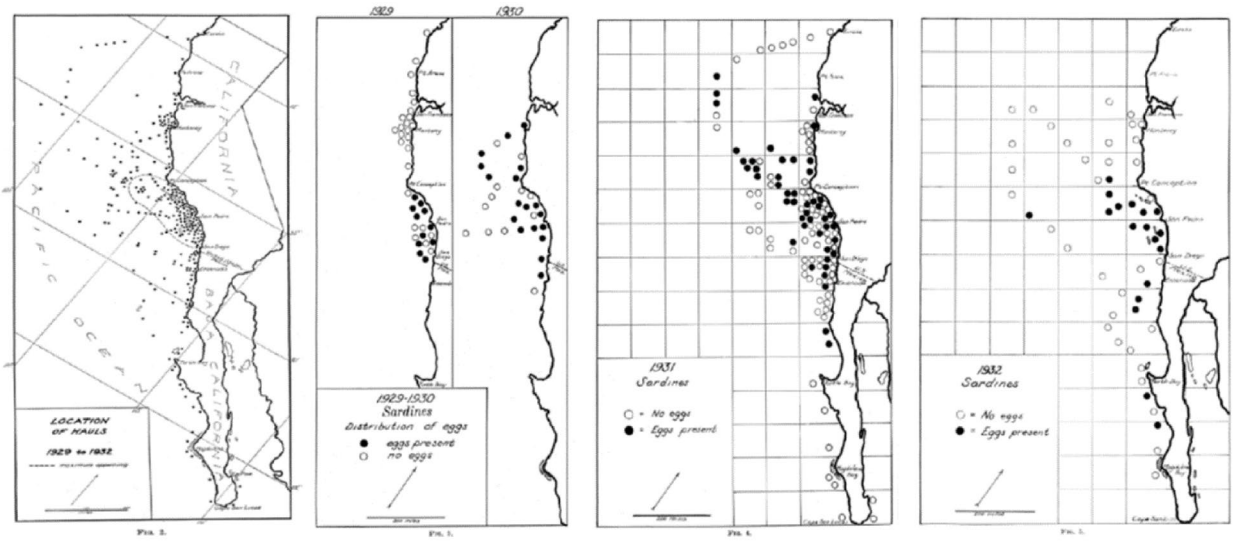

Figure 8. Figures 2-5 from Scofield (1934) depicting plankton sampling by the Hydrobiological Survey of the Hopkins Marine Station from 1929-1932 in an effort to describe the spawning area of Pacific sardine (*Sardinops sagax*). The left-most panel depicts the entire sampling effort over the four-year study, while the subsequent panels depict stations where sardine eggs were present or absent in individual years. Figures reproduced by permission of CADFW.

offshore to 100 miles. Scofield (1934) posited that there was a relationship between temperature and spawning in sardine although he provided only generalized information on SST during certain years. Of the variation in observations of sardine spawning intensity among the sampled years, Scofield (1934) wrote:

> "During 1930 and 1931, when the greatest variations occurred, water conditions varied also. The main peculiarity was that much warmer water than average prevailed along the entire California coast. During these two years the sardine spawn was found well scattered to the north and south and well offshore. During 1932, when colder water temperature was evident, the sardine spawn was in very restricted areas and only found south of Point Conception and comparatively close to the mainland. It might be assumed from this that 1929 also was a year in which the sardine spawn was restricted to a small territory, because that year, like 1932, experienced colder water temperatures." (p. 19)

Scofield (1934) also showed that while sardine eggs are present from the nearshore to hundreds of miles offshore, the bulk of late-stage larvae are found nearshore. This complemented Godsil's (1930) description of nursery habitat being in waters 10m or less. Thus, this work described the ontogenetic shifts in habitat from offshore egg to inshore settlement and recruitment in sardine.

While Scofield's (1934) was the first to recognize the extreme geographic area over which sardine spawn, there are some caveats to his conclusion that the main spawning area is off of southern California. Principally, these caveats lie with the relative dearth of sampling south of the U.S./Mexico border coupled with sampling that was restricted to the spring. As shown in

Figure 8, sampling for that study was concentrated in southern California across all four years. Sampling south of the international border between Mexico and the U.S. was not only restricted in time (largely only in 1931 and 1932), but also spatially in that a relatively few samples were collected and those mainly nearshore or sporadically offshore. Therefore, while Scofield (1934) provided the first thorough documentation of spawning in sardine and the first hypothesis of its association with water temperature, it provided less-than-optimal documentation of spawning patterns in Mexico.

Tibby (1937) published the first directed investigation to determine the relationship between ocean temperature and the occurrence of sardine spawning. Using data collected by Scofield (1934) from 1929-1932 from northern California to Cabo San Lucas, and additional data from 1936 along Baja California, Tibby (1937) concluded that spawning in sardine occurred at temperatures between 13˚C and 24˚C, with an estimated optimum temperature for spawning between 15˚C and 18˚C and a peak at 16˚C for the spawning areas off southern California and central Baja California (Bahía Sebastián Vizcaíno and Punta Eugenia). The data examined by Tibby (1937) were heavily weighted by those collected in years when SST off California was anomalously high, resulting in five records of spawning off southern California at temperatures above 20˚C. Tibby (1937) noted that because SST is generally warmer along the Baja California Peninsula where sardine spawning in concentrated, the heaviest spawning in that region likely occurs earlier than the heaviest spawning in the Southern California region but that it continues longer and thus overlaps temporally. Tibby (1937) also noted a "sizeable" spawning in the region of Bahía Sebastián Vizcaíno that was not noted by Scofield (1934) in the 1929-1932 surveys. This spawning area was undoubtedly unobserved by Scofield due to the limited sampling in Mexico (Scofield 1934).

In 1949, the California Cooperative Oceanic Fisheries Investigations (CalCOFI) began an ambitious physical and biological oceanographic survey within the California Current Ecosystem (McClatchie, 2014). The primary goal of this survey at the time of its inception was to investigate the distribution and abundance of sardine eggs and larvae (Ahlstrom, 1954). Prior to the CalCOFI program, it was known that sardine spawned over a large geographic area from the Gulf of California (Godsil, 1941) to at least Cascade Head, OR (U.S.; Ahlstrom, 1948). Walford and Mosher (1941) and Hart (1943) provided evidence for at least some spawning as far north as British Colombia. Within this range, it was known that southern California was a location where abundant spawning occurred (Scofield, 1934; Sette and Ahlstrom, 1948), but, aside from the region near Bahía Sebastian Vizcaíno reported by Tibby (1937), it was not known if there were other important areas. In 1948, a dedicated survey was made to document sardine spawning off of Baja California. Abundant spawning was found in the region between Punta Eugenia and Punta Abreojos (MX; as reported in Ahlstrom, 1954). Of this region, Ahlstrom (1954) stated "[t]his is the area that has proved, since 1949, to be the principal spawning ground of the sardine." These "knowns" (and "unknowns") of the geographic and temporal limits of sardine spawning provided the impetus for the subsequent CalCOFI sampling.

From 1951-1966, the CalCOFI surveys set the gold standard for sampling that has not been duplicated in other studies attempting to describe spatiotemporal patterns in Pacific sardine spawning. Using three or four vessels (depending on the year) working simultaneously, and following a set sampling grid (described in Ahlstrom, 1954) the region from northern California to Cabo San Lucas was surveyed on a monthly or near-monthly basis. In subsequent years, the sampling area and timing was variably less frequent and less expansive. The extensive and intensive sampling during the first decade of CalCOFI offers the most complete dataset with

which the spawning habitat of the sardine may be described with reasonable certainty (this is nicely illustrated in Figures 10 and 11 of Lluch-Belda, 2003). The data collected on sardine egg distributions and accompanying oceanographic data during that period -were reported by Ahlstrom (1954; 1959), Kramer (1970), and others. These data, particularly those summarized for the years 1950-1956 by Ahlstrom (1954; 1959) weighed heavily in Marr's (1960) subpopulation model and we begin our review with those papers.

Based on collections from 1950-1956, Ahlstrom (1954; 1959) recognized two spawning centers, a northern center off of southern California (U.S.) and northern Baja California, and a southern center off of central Baja California in the region of Bahía Sebastián Vizcaíno. Ahlstrom (1954) documented a northern progression of spawning during the spawning season (i.e., spawning began earlier in the year in more southerly regions). Ahlstrom (1949) identified two spawning seasons. In the first season, January through June/July, spawning occurs in both the northern and southern spawning areas. In the second, July through December, spawning was largely restricted to the southern area. Ahlstrom (1959) summarized these findings in his Figure 9 (reproduced here as Figure 9).

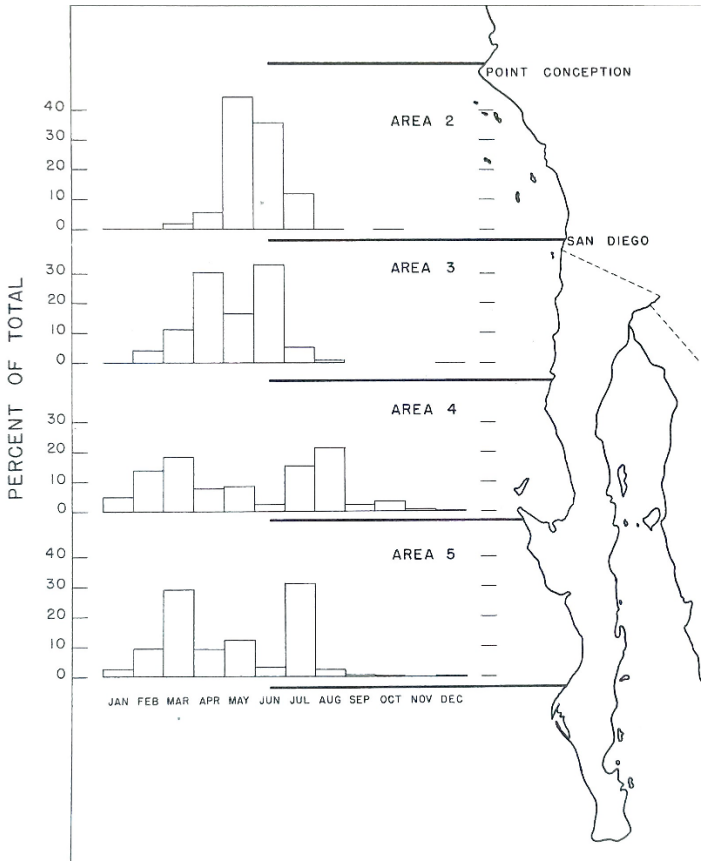

Figure 9. Figure 9 from Ahlstrom (1959) depicting spatiotemporal distribution of Pacific sardine eggs from 1951-1956. Figure reproduced by permission of CalCOFI.

Ahlstrom (1959) noted that these differences in timing of spawning led some to speculate that the late spawning fish represented a separate subpopulation, of which he noted "[t]he subpopulation problem in the sardine is discussed by Marr (1957). Although the question has been posed from a consideration of time and temperature differences at spawning, the problem will have to be solved by other techniques such as genetic studies of spawning fish of the two groups, growth studies, and a suitable tagging program."

Ahlstrom (1959) provided two alternatives for the supposition that subpopulations had evolved with discrete spawning areas and timing: 1. Spawning that occurred throughout the year off central Baja California is carried out by the same group of fish, or 2. Late season spawning is carried out by young fish spawning for the first time. Ahlstrom (1959) stressed the importance of knowing if late season spawners were a different subpopulation noting that if they were an intrinsically different entity, they may have different optimal environmental conditions and thus may be unavailable to fisheries in the U.S.

In 1964, Ahlstrom presented a paper at the International Commission for the Northwest Atlantic Fisheries Environmental Symposium (later published in 1965) in which he provided some updates on the CalCOFI program's sardine egg and larval distribution data. Ahlstrom (1965) noted that during the decade of the 1950s, sardine spawning occurred over a broad area between Point Conception (U.S.), and Punta San Juanico (MX), with interannual variation in the locations of the highest egg and larval densities (e.g., 1952-3 had low egg and larval densities off of southern California and northern Baja California but 1954 had high densities). Ahlstrom also noted that changes in egg and larval densities appeared to correlate with warmer water, particularly following a period of below average temperatures from 1950-1956. The warmer temperatures in 1957-58 not only influenced the location of spawning, but also the timing. Spawning off southern California that had been observed primarily in May and June from 1950-1956 occurred earlier and was more protracted over time in 1958. Ahlstrom (1965) reiterated the lower temperature limit for sardine spawning of 13˚C and the optimal range from 13˚C - 18˚C. Ahlstrom (1965) refined the geographical limits of the northern and southern spawning centers: the northern comprising all of California and northern Baja California to Punta San Quintin, the southern center comprising central and southern Baja California. In this paper, Ahlstrom (1965) mentioned the work of Sprague and Vrooman (1964) that used serological antigen response as a method to investigate genetic structure in sardine (but see "Serological Antigens" section for reasons why this is no longer considered a valid method). Based on the supposition that two genetically distinct stocks existed, Ahlstrom commented "Since the offseason spawning occurs at temperatures which average 2 to 3˚C higher than the spring spawning, we would like to believe that the 'southern' stock is a physiologically distinct group of fish."

Ahlstrom (1966) provided an update on sardine larval distributions based on CalCOFI data from 1951-1964. While focused on larval distributions which may be a less-than-optimal predictor of spawning area given their 30- to 40-day larval duration (Moser, 1996), Ahlstrom (1966) provided some comments on egg distributions. Among these comments he noted that the waters of central California were an unimportant spawning area during the 1950s (however he noted that spawning in this area was not absent). Ahlstrom noted that cruises north of San Francisco were infrequent during the period 1951-1964, but that no eggs or larvae were found on these cruises. Ahlstrom (1966) stated that the most drastic change in the distribution of sardine eggs was between the 1953 and 1954 cruises. In the former, less than 1% of eggs or larvae were collected off of southern California and northern Baja, while in 1954, 38% and 33% of eggs and

larvae, respectively, were collected in this area. No apparent reason, physical or biological, was identified to explain this change.

In summary, the first decade or so of the CalCOFI program yielded the most complete description of the geographic and temporal limits of sardine spawning that has been reported. These data show that spawning of sardine is a spatiotemporally dynamic process that, while having consistently concentrated spawning in the Punta Eugenia region, also has periods of concentrated spawning in the southern California region. This is coupled with additional spawning that may occur at any time of the year and at any place from northern California to southern Baja California. In some years, spawning from southern California to Punta Eugenia is nearly uniform in concentration resulting in a geographically broad and connected spawning event. In general, the data show that there is a northward progression of spawning during the season with spawning usually beginning earlier off central Baja California than off of southern California and elsewhere, presumably corresponding to the later timing of water temperature increase to the north. Spawning season is more protracted in the southern areas (January to July) than in the northern areas, where it is more concentrated in spring and early summer (May and June). While providing a nearly complete picture of where and when sardine spawn, one limitation to these studies is the absence of sampling in the Pacific Northwest.

From 1967 on, the CalCOFI program continued to sample at least quarterly and published reports were largely descriptive (e.g., egg and larval occurrence maps). In 1991, Lluch-Belda et al. analyzed spawning patterns in sardine across the California current from Cape Mendocino, California, to Cabo San Lucas, Baja California Sur, in relation to sea surface temperature (SST) and upwelling. Lluch-Belda et al. (1991) used CalCOFI sardine egg counts from 1951 through 1989 that comprised approximately 19,500 sample collections. Because the absolute number of stations having sardine eggs depends on the total number of stations sampled at a particular temperature, they chose to examine the proportion of positive stations at each temperature as the appropriate measure with which to make comparisons of SST and spawning intensity (i.e., the quotient between the smoothed temperature distribution of eggs and the smoothed SST frequency distribution). Sardine eggs were collected over a wide SST range from approximately 9˚C to 28˚C and they concluded that sardine spawn from 13.5˚C to 25°C. Lluch-Belda et al (1991) also observed two maxima in the frequency of occurrence of eggs, one at ~15˚C and another at ~23˚C. They concluded that the two observed maxima do not represent an intrinsic (biological) characteristic of sardine but rather are an environmentally caused feature (a conclusion often overlooked in subsequent reviews of this work). They further concluded that the "gap" between the two maxima was related to a combination of temperature and upwelling index (often used as a proxy for food availability) which is geographically variable along the Baja California Peninsula. This area generally has SSTs matching those in the "gap" during spawning periods. Thus, for sardine, Lluch-Belda et al. (1991) concluded that "[s]st is a good indicator of spawning only at the limits, particularly at the lower one; otherwise, it is a poor indicator, since there is a very wide range of appropriate temperatures. However, the combination of temperature and upwelling appears to determine time and space of sardine spawning." Overall, based on CalCOFI data from 1951-1989. Lluch-Belda et al. (1991) found a consistent, broad thermal range of spawning along the entire survey area of the Pacific coast which corroborated the findings of earlier studies (e.g., Tibby, 1937 and others).

Moser et al. (1993) summarized spatial and temporal patterns of sardine eggs and larvae from CalCOFI surveys conducted from 1951 to 1984. The results showed that spawning occurred off central Baja (Punta Eugenia and Bahia Sebastián Vizcaino) throughout the year,

with maximum spawning from January to September. Moser et al. (1993) also showed that while spawning off the southern California Bight area tended to peak during April to June, it was highly variable among years, peaked in late summer (July-August) in some years, and also occurred year-round in measurable amounts.

In 1994, Hernandez-Vazquez published one of the most overlooked papers describing the seasonal and geographical variation in sardine egg and larval distribution from 1951-1989 from the CalCOFI sardine egg dataset. The percent of stations in which sardine eggs and larvae occurred was analyzed "globally" (all samples combined), regionally over six geographically defined blocks from Cape Mendocino, to the southern tip of Baja California, by CalCOFI line, and by distance from the coast. Consistent with previous studies, Hernandez-Vazquez (1994) concluded that, overall, sardine eggs and larvae were present throughout the year along Baja California and California, however he noted that the spawning season was longer than previously reported and extended from February to September with the highest values recorded from August and September. The highest values for occurrence of sardine larvae were in the Punta Eugenia region. The occurrence of sardine eggs from the southern California bight to the Punta Eugenia region varied little but diminished both to the north and the south of this large swath of coastline. The concentration of sardine eggs was fairly even from just south of Point Conception to just north of Bahía Magdalena, but with a sharp peak in the Punta Eugenia region. Corroborating earlier findings, Hernandez-Vazquez (1994) showed that peak spawning seasons differed between the southern California bight and the Punta Eugenia region, the former beginning in February and lasting to July, and the latter beginning in March and extending through October (peaking from August to October). In the southern California bight region, sardine eggs and larvae were present out to 100 nm with a slight peak in the 20 nm closest to shore. From Punta Baja southwards, a majority of the eggs and larvae were found closer to shore. This pattern had a seasonal component. From August to February, offshore occurrence of eggs and larvae is nearly absent in the southern California and Punta Baja regions. These data firmly established that, during a calendar year, sardine eggs and larvae can be observed in any one month and at any one place in Southern California and Baja California.

Lluch-Belda et al. (2003) expanded their previous study and examined sardine egg distributions from CalCOFI cruises from 1951-1997 relative to highly productive, upwelling regions from Point Conception to the southern tip of Baja California. Their goal was to describe the spatiotemporal dynamics of oceanographic and biological features (SST, Sea Surface Height, and macrozooplankton volume) of these highly productive areas with an emphasis on the temperate-tropical transition zone in the region of Punta Eugenia, and to assess this region's role as a refuge for sardine during times of unfavorable environmental conditions. The authors evaluated the abundance of sardine eggs collected by the CalCOFI program in two temporally discrete "bins", 1951-1967 and 1951-1997. The reason for this, as the authors point out, is because until 1967 the CalCOFI program made regular incursions into Mexican waters and sampled on a monthly (until 1960) or nearly monthly (1960-1967) basis, whereas after 1967 sampling was largely restricted to U.S. waters and sampling frequency was variable but mostly on a quarterly basis. For the period 1951-1967, Lluch-Belda et al. (2003) showed widespread spawning of sardine from Point Conception to slightly north of Bahía Magdalena. The average latitude of spawning (i.e., the center of gravity) varied among years from Punta Eugenia to Southern California. When the center of gravity of sardine spawning shifted northward to Southern California, water temperatures were anomalously warm and associated with the 1957-58 El Niño event, after which, the center of gravity of spawning retreated south, returning to the

Punta Eugenia region. From 1967 to 1982, spawning was concentrated off Bahía Magdalena with additional spawning off Punta Eugenia during the latter years. This corresponded to a period of anomalously cool water temperatures. After 1984, the CalCOFI survey made few incursions into Mexican waters (only one after 1984) so there are no data available on sardine egg abundance during this time. However, as would be predicted by the anomalously warm waters precipitated by the 1982-83 El Niño, there was increased sardine egg abundance from the U.S./Mexico Border to San Francisco Bay (U.S.). In 1996, CalCOFI again returned to Mexican waters and, in addition to the spawning observed in the northern portion of the survey grid, sardine eggs were also found off of Punta Eugenia (it should be noted that this was a single sampling event, i.e., was not repeated). Lluch-Belda et al. (2003) concluded that the region off Punta Eugenia acted as a refuge for sardine as environmental conditions were favorable (i.e., there was high productivity) throughout the entire calendar year. This was contrasted with other regions of high productivity such as the southern California bight which is characterized by only seasonally high productivity. Thus Lluch-Belda et al. (2003) suggested that the Punta Eugenia region is expected to retain the highest abundance of sardine during periods of unfavorable environmental conditions and serves as the source for an expansion of the sardine biomass as conditions improve to the north and south. This hypothesis is supported not only by the authors' analyses, but also by studies of scale deposition in anoxic basins indicating that this is a long-term rather than an ephemeral process (e.g., Field et al., 2009).

Valencia-Gasti et al. (2018) presented data on sardine egg density using data collected with a Continuous Underway Fish Egg Sampler (CUFES) along the west coast of the U.S. and Baja California during spring (primarily April) from 2000-2013. The authors concluded that in the spring, most sardine spawning occurred in U.S. waters with only a small percentage of eggs (<11%) collected in Mexican waters. The purported absence of sardine eggs in Mexican waters, however, could be explained by sampling bias. Data from U.S. waters originated from multiple surveys including the CalCOFI, SWFSC DEPM, and CCEES surveys. These surveys only rarely ran concurrently aboard the same vessel, thus many stations were sampled more than once in a given April, whereas in Mexican waters, only one survey sampled each year. Given the patchy distribution of fish eggs in general this "double" sampling in the U.S. would increase the chances of encountering egg patches and would artificially inflate egg counts as compared to the less-well-sampled stations in Mexico. This study was also temporally restricted.

While there are few studies that have examined spawning in sardine over sufficient spatiotemporal scales to characterize its geographical and oceanographic limits, several regionally or temporally restricted studies provide finer-scale insights. For example, Watson (1992) reported on the distribution of sardine larvae from 1978-1986 in shallow waters between Oceanside and San Onofre, California (U.S.) and found larvae present year-round with peak abundance in summer and fall from which he surmised that the sardine resurgence in the 1980s began in 1981 from successful spawning off central California that produced the larvae collected in his study. Lynn (2003) documented sardine egg distributions from San Francisco to the U.S./Mexico border in the spring of 1996-1999 and described changes presumably associated with the strong El Niño event of 1997/98. Emmett et al. (2005) documented spawning off the Pacific Northwest of the U.S. from 1994-1998. Lo et al. (2010) further described sardine spawning off the Pacific Northwest of the U.S. from 2003-2005 and documented a southward and shoreward shift in spawning area as compared to observations in the 1990s. These (and other) regional studies have by and large demonstrated that spawning in sardine is a spatiotemporally dynamic process that occurs over a large geographic range. None, however,

have demonstrated that these spawning areas are composed of isolated groups of sardine. Rather, these studies have shown that spawning of sardine along the Pacific coast varies in magnitude and spatial distribution at monthly, seasonal, and annual scales. Annually, the spatial distribution of spawning can quickly shift from being highly patchy and concentrated in specific regions (e.g., southern California and central Baja California) to being continuously distributed from southern Baja California to central California (Kramer 1970; Ahlstrom 1959, 1965; Moser et al. 1993). These same patterns and variations have been observed at monthly scales within and among individual years (Kramer 1970; Ahlstrom 1954; Hernandez-Vasquez 1994).

In summary, the available data on spawning in sardine indicate that while there are regions and time periods where spawning intensity typically increases, it is best characterized as a geographically dynamic and temporally protracted process. Spawning temperature appears to have a lower limit around 13˚C and an upper limit of about 22˚C with a unimodal thermal range of spawning across a wide latitudinal range (Tibby 1937; Ahlstrom 1959; Lluch-Belda et al. 1991). However, temperature alone seems to be a limited predictor of spawning and is best used in combination with other oceanographic characteristics such as upwelling index (Lluch-Belda, et al., 2003). There is no indication that there are spatiotemporally discrete spawning areas that could maintain subpopulation separation. Importantly, since the early years of the CalCOFI surveys, the absence of surveys conducted at spatiotemporal scales appropriate to describe the full extent of spawning in sardine confounds the ability to assess if spawning patterns have changed since the early CalCOFI studies.

Genetic Data

"Only under certain ideal conditions would such environment modified characters be of value…They would not, however, provide any information on the more fundamental problem of genetic difference." Marr, 1957b, p. 112.

Given the longstanding belief that sardine are partitioned into subpopulations, it is somewhat surprising that only a few genetic studies have been published. The first genetic study on sardine relative to the subpopulation problem was by Hedgecock, et al. (1989) who examined heterozygosity in allozymes. Starch-gel electrophoresis was used to show variation in 30 enzymes and other proteins. Samples were collected from Guaymas (Gulf of California, MX) (; N = 48), Bahía Magdalena, the Southern California Bight, (N = 36), Monterey Bay, (N = 29), and Tomales Bay (San Francisco, U.S; N = 5). Hedgecock et al. (1989) found low levels of polymorphism and individual heterozygosity, and almost no variation among sample sites in the frequencies of the allozymes examined. The authors concluded that the five locations sampled represented genetically identical groups.

Bowen and Grant (1997) and Grant et al. (1997) used DNA sequence data from the mitochondrial control region to assess biogeographic relationships among putative species of *Sardinops*. While these studies did not explicitly examine subpopulation structure of the sardine, neither study presented data that suggested genetic differences among sardine from the west coast of the U.S.

Lecompte et al. (2004) examined DNA sequences of the mitochondrial cytochrome *b* gene in sardine from 107 individuals collected at Vancouver Island (Canada), San Diego (U.S.) Bahía de Todos Santos (MX), and Guaymas (MX). There was no geographic heterogeneity in the

distribution of haplotypes and AMOVA analysis showed no differences when samples were grouped by putative stocks (i.e., Northern and Southern subpopulations).

Gutierrez-Flores (2007) examined two mitochondrial genes (NADH-5 and NADH-6) and eight microsatellite loci in 475 sardines from Vancouver Island to Bahía Magdalena and the gulf of California. Range-wide analyses demonstrated high geneflow and spatial homogeneity (ΦST = 0.0098, p = 0.27 in NAD5; ΦST = 0.0005, p = 0.51 in NAD6; and RST = -0.012, p = 0.99 in microsatellites). Genetic homogeneity was also observed among the three groups of Felix-Uraga et al. (2004; see "Temperature and Landings Data" section for a discussion of these group: ΦCT = 0.0018, p = 0.26 in NAD5 and ΦCT = 0.008, p = 0.34 in NAD6; RST =-0.00487; p = 0.83 in microsatellites).

García-Rodríguez et al. (2011) examined DNA sequence data from the mitochondrial control region of sardine from Ensenada (N = 53) and Bahía Magdalena (N = 106). Samples were grouped into the three proposed groups of Felix-Uraga et al. based on SST (2004; see discussion in "Temperature and Landings Data"). AMOVA analysis indicated significant genetic structure overall (Φ_{ST} = 0.0293, p<0.001) as well as significant differences in pairwise comparisons of sampling sites.

García-Rodríguez et al. (2011) is the only study to have reported significant genetic structure among sardine. Previous studies (see above) using mitochondrial DNA and over a broader geographic range had contrary findings. Their finding is surprising given the high haplotype diversity reported (H = 0.999) indicating that almost every individual possessed a unique haplotype. It is difficult to mathematically reconcile how within- and among-group variation could be partitioned to yield significant structure with such high overall haplotype diversity. Unfortunately, the data from this paper were not deposited in a public database (e.g., GenBank) so we cannot comment further through reanalysis. Despite demonstrating genetic structure when partitioned into the three groups proposed by Felix-Uraga et al. (2004) based on SST, the authors state that "[A] phylogeographic pattern from total haplotypes was not apparent, as the associations between clades and particular groups were unclear (data not shown)." This statement is unclear to us and in the absence of a presentation of the data, we cannot adequately address it.

Adams and Craig (2024) examined DNA sequence data from the mitochondrial cytochrome *b* gene from 434 sardines from Vancouver Island, Canada, to the Gulf of California. The authors found low but significant genetic structure overall (ϕ_{ST}= 0.01136, p = 0.032) and low but significant structure when samples from Bahía Magdalena were grouped with the Gulf of California and compared to a group of Pacific Ocean sites (ϕ_{CT} = 0.00923, p = 0.021). The authors considered the statistical significance of the genetic structure to be artifactual and not biologically meaningful due to the high diversity of haplotypes and comparisons to other published studies with similar results.

As a whole, the published genetic data do not show a pattern of structure in sardine. However, a vast majority of the data are from the mitochondrial genome which evolves at a relatively slow pace. The genetic homogeneity in the distribution of mitochondrial gene sequences in sardine has been attributed to rapid population growth following the last glacial maximum (Grant and Bowen, 1998). This also results in a pattern of extremely high genetic diversity comprised largely of single nucleotide polymorphisms which could confound the ability to detect recent population separations with the data thus far published. Unpublished data from microsatellite loci which evolve at a relatively faster rate than mitochondrial genes also showed extremely high levels of genetic variation with most of the loci examined being out of

Hardy-Weinberg equilibrium and thus unsuitable for analysis (J. Hyde, unpublished data). Ongoing work using low coverage whole genome sequencing, which can detect changes on time scales of only a few generations, from the Pacific Northwest of the U.S. to the Gulf of California has also shown a pattern consistent with genetic homogeneity (G.C. Longo and M.T. Craig forthcoming).

External Morphology

"[I]t is well known from both empirical and experimental evidence that body form, numbers of vertebrae, etc., are influenced by environmental variables (such as food and temperature, for example). Obviously, then, in using such characteristics the risk exists of studying the effects of environmental conditions rather than the effects of genetic isolation." Marr, 1957a, p. 2.

Several papers have examined external morphological characters in the context of defining subpopulations of sardine in North America. Mais (1972) generated data for four external measurements (standard length, head length, pectoral-fin length, and postpelvic length) for 3,706 individuals from central California to southern Baja California and the Galápagos Islands (Ecuador) from 1958-1962. Mais (1972) devised a strategy for overcoming possible effects of allometric growth by dividing the samples into three groups: small (110-139 mm SL), medium (140-169 mm SL), and large (170-209 mm SL). Each of these groups was then compared to one another by region and subjected to ANOVA. Significant differences were found for each area and character. Mais (1972) then performed regression analysis adjusting mean values to a "standard fish size" for each group. Finally, Mais (1972) performed an "overlap" analysis using an early type of discriminant function analysis. Between small and medium fish across the geographic range studied Mais stated "[F]ish from these areas overlapped so greatly that no inference of separate stocks can be made." Mais speculated that this was due to the influence of migrants from Mexico and lack of native-born fish in the California samples. Of large fish, Mais also noted small differences in the overlap analysis. Among all size groups, the Gulf of California fish had the least similarity to other areas. Despite the large degree of overlap and similarities among regions, Mais concluded that three subpopulations existed. We do not agree with this conclusion given the data that was contrary to it. Additionally, rather than the accepted method of standardizing each measurement by the standard length of the individual, Mais (1972) devised a scheme not used before or after. This hinders comparisons of the data with other studies and does not allow for an evaluation of the method's utility.

Using discriminant function and principal component analysis of 12 external morphological characters, Hedgecock et al. (1989) found significant differences between five groups collected from Guaymas, Bahía Magdalena, Southern California, Monterey (U.S.), and Tomales Bay (San Francisco, U.S.). PCA of the traits that contributed to the between-group variance of the DFA resulted in a single factor explaining 97% of the variance. Hedgecock et al. (1989) reported that this was likely a reflection of differences in size of individuals among the collecting sites. Overall, the authors concluded that the minor differences observed in external morphology were the result of environmental factors and not genetic forcing and challenged the view of previous authors that they were evidence of subpopulation structure.

Aguero et al. (2004) used a geometric morphometric approach to evaluate its use in distinguishing among stocks of sardine. It is important to note that their operational definition of "stock" ("[W]e use the term stock as groups of individuals within a species population that have

sufficient spatial and temporal integrity to warrant consideration as self-perpetuating units…") has a biological underpinning and is similar to "subpopulation" as used here. Nine external landmarks were established and produced 19 truss elements (distance measurements) for fish collected from the fisheries at Isla de Cedros and Bahía Magdalena (MX). Analysis of covariance (ANCOVA) revealed sexual dimorphism for some characters so analyses were run both for same sex groups and mixed sex groups (i.e., all samples). CVA using the four groups (each sex and location) showed significant differences among the groups. Females from Bahía Magdalena were the most different (based on Mahalanobis distance) from all other groups but the classification accuracy was low. On average, 54% of fish were correctly classified: 41% for males from Bahía Magdalena, 54% for females from Isla de Cedros, 58% for females from Bahía Magdalena, and 68% for males from Isla de Cedros. The authors concluded that significant differences were found between groups, but highlighted environmental differences in spawning and larval development areas as influencing morphotypes, and thus they considered these differences to be unreliable indicators of stock identity.

García-Rodríguez et al. (2011) used a geometric morphometric approach to compare sardine from two landing ports on the west coast of the Baja California Peninsula (Ensenada and Bahía Magdalena). Sampling from Ensenada was conducted from January to April, while sampling from Bahía Magdalena was conducted both from February to June and August to January (the former representing the hypothesized "Temperate" stock and the latter representing the "warm" stock of Félix-Uraga et al. 2005; see "Temperature and Landings Data" section for discussion of these groupings). PCA and CVA were employed and a Chi-squared test was used to test for significant PCs. Significant PCs were used to compare groups using ANOVA. Mahalanobis distance from the test value to the nearest group was used to assign each specimen to a group. Finally, Procrustes distance means were calculated for paired comparisons, and distances obtained were visualized using a Neighbor-Joining tree. ANOVA of the PCA scores were significant along PC1 and the "warm" group was statistically different from the two "temperate" groups which were not statistically different from each other. Along PC2, the "temperate" Ensenada group was statistically different from the other two groups which were not statistically different from each other. CVA indicated significant differences among groups and the assignment to group using Mahalanobis distance ranged from 80-88% accurate. Procrustes distances revealed that the "warm" group represented the most divergent morphotype. The authors concluded that the three morphotypes characterized by their analysis were possibly a result of phenotypic plasticity rather than genetic variation and that this morphological divergence could be driven largely by environmental conditions.

Vergara-Solana et al. (2013) compared otolith shape and external morphology as uses for discriminating among stocks of sardine as determined by SST following Felix-Uraga et al. (2004; see "Temperature and Landings Data" section for discussion of these groupings) for 260 fish from Bahía Magdalena (we only discuss external morphology here, see "Otolith" section for discussion of otolith results). The authors used 18 external landmarks to derive their measurements (number of measurements not stated). CVA indicated significant differences between groups, and overall classification accuracy was 85.1%. CV1 separated two main groups: one containing fish collected in September and December and another containing fish collected in April and June. The authors considered their results to be concordant with stock discrimination based on SST as defined by Felix-Uraga et al. (2004). In that paper, the authors defined a warm stock for fish landed when SST was >22˚C and a temperate stock for fish landed when SST was between 17-22˚C. Vergara-Solana et al. (2013) obtained satellite-derived SST for

the lagoon complex of Bahía Magdalena, where landings occurred and which were presumably the fishing grounds (although this was not stated), and their groupings of fish by SST were discriminated by the CVA. The SST in September was ~26˚C, ~21˚C in December, ~ 18˚C in April, and ~21˚C in July in this region (temperature was only displayed graphically so exact temperatures are unclear). Based on these temperatures, only one group (September) would be assigned to the warm stock of Felix-Uraga et al. (2004) thus it is curious that Vergara-Solana et al. (2013) dismiss the lower temperature of the December sample. The authors justify this by hypothesizing delayed movement in response to sudden changes in water temperature or that the temperature ranges given by Felix-Uraga et al. (2004) are not exact. While it is certainly possible that the temperatures given by Felix-Uraga et al. (2004) are not absolute, we feel that discounting the lower temperature in December lends some uncertainty to the authors' conclusions.

Nearly all of the studies using external morphology in sardine as a means of discriminating among subpopulations note that phenotypic plasticity in response to environmental conditions is a dominant factor in shaping the characters examined. It is therefore apparent that they should not be used for that purpose. If sardine were not a highly mobile species, these environmentally driven differences may in fact be an indicator of a lack of connectivity and thus indicate subpopulation distinction. However, given that they are highly mobile, and an individual may experience extreme fluctuations in environmental conditions throughout its life which potentially influence a suite of characteristics including growth rate and external morphology, these characters are ill-suited to demonstrate population subdivision.

Temperature and Landings Data

"*Information arising from a long period of study, plus greatly intensified studies in recent years, now makes it possible to ask intelligent questions about the number and location of sardine subpopulations and to suggest critical methods of examining these questions*." Marr (1957b), p. 108.

One of the most widely cited papers as evidence of the existence of subpopulations in sardine is Felix-Uraga et al. (2004), which analyzed an impressive 20-year long data set of sardine landings. In fact, this study is so influential that it forms the basis for the management of sardine in Mexico. Given its importance in influencing the discussion of subpopulations of sardine, we devote time here to thoroughly discussing the data therein. Through the analysis of landings data and SST from four fishing ports (San Pedro, U.S., and Ensenada, Isla de Cedros, and Bahía Magdalena, MX), Felix-Uraga et al. (2004) proposed that there are three groups of sardine along the west coast of Baja California and Southern California: a "warm" group caught at SST >22˚C, a "temperate" group caught at SST between 17-22˚C, and a "cold" group caught at SST <17˚C. From the results, the authors provided a conceptual model describing the spatiotemporal distribution of sardine in the study region.

To reach their conclusions, Felix-Uraga et al. (2004) examined monthly catch data from the four major landings ports mentioned above from 1981-2002 and satellite-derived SST for 2˚ latitude x 2˚ longitude squares which presumably represented fishing grounds and which extended roughly 100 miles offshore. Landings data were grouped into 1˚C bins for each zone, summed, and plotted using contour graphs. The authors then looked ("eye-balling" as stated by the authors) for discrete peaks in the temperature/landings/month covariates. This qualitative approach revealed two SST/landings contour peaks in the data collected from Bahía Magdalena,

one peak at Isla de Cedros and two peaks at San Pedro (U.S.). The contour peak for Bahía Magdalena at SSTs above 22°C roughly corresponded to landings from July to December. The second contour peak for Bahía Magdalena, at SSTs of 17–22°C corresponded primarily to landings from February to June. It was presumed that this contour peak represented the same group of fish as that producing the contour peak from July to November off Ensenada and San Pedro. A third contour peak was apparent in Ensenada and San Pedro at SSTs of 13–17°C that corresponded to landings from December to May. Following the examination of the contour plots, Felix-Uraga et al. (2004) plotted aggregated (total) landings against SST both for individual ports and for all ports combined. The former plot had what the authors viewed as two "peaks" for each port at different temperature ranges. When all ports were plotted together, the authors interpreted that the distribution had three peaks. The authors assumed that each peak corresponded to a different group of sardine.

While providing useful visualizations of landings data over a roughly two-decade period, Felix-Uraga et al. (2004) provided no statistical analysis of their data to test their proposed grouping of sardine. In fact, the authors took great care to highlight that their data were not evidence of subpopulation structure, but rather were a possible way to discriminate among groups stating that "[a]ssuming that the observed patterns are indicative of sardine stock structure, this method also serves as a practical approach to partitioning and attributing the catch data of each fishing zone to each sardine group, thus improving estimates of population abundance from stock assessment models."

There are several factors that make it difficult to accept that the patterns observed by Félix-Uraga et al. (2004) indeed represent evidence of groups, stocks, or subpopulations and we address the most salient of these here. First and foremost is that, as pointed out by the authors, the use of landings data as a proxy for abundance (or any other metric) carries a high degree of uncertainty. Landings data may be highly variable over time due to any number of reasons that are unrelated to abundance (Pauley et al., 2013). The choice by Félix-Uraga et al. (2004) to examine aggregated (i.e., summed) landings over the ~20-year time period spanned by their data therefore seems ill conceived. In doing so, inter- and intra-annual variance is completely obscured and, if fishing effort data are available its variation is also obscured. A more informative way to examine these data, especially when looking for persistent patterns associated with a covariate (in this case trends in landings associated with SST), is to examine mean values with a measure of variance. When this is done using the same data as those in Félix-Uraga et al. (2004) it is readily apparent that the variation around the mean is large and, in many cases, is greater than absolute differences in mean values (Figure 10a). Additionally, when viewed as the mean value of landings +/- the 95% confidence interval (Figure 10b) it is apparent that many of these mean values cannot be considered different from one another. While there are peaks and valleys that roughly agree with the pattern observed in Figure 6 of Felix-Uraga et al. (2004), they are rather unremarkable given the degree of variation in the underlying data and are not significantly different from one another (Kruskal-Wallace rank sum text, $\chi^2 = 18.915$, df = 15, p = 0.2176).

Also masked by the use of aggregated landings data in Félix-Uraga et al. (2004) is the marked increase in landings beginning in about 1990. From 1981-1990, annual landings were never above 17,000 mt at any port (Figure 11). From 1990 onwards a steady increase in annual landings occurred for all ports with the exception of Bahía Magdalena and Isla de Cedros which showed a stark drop in annual landings from 1996-1999, a time period marked by one of the strongest El Niño events on record when water temperatures there exceeded 28˚C (28.4 ˚C in

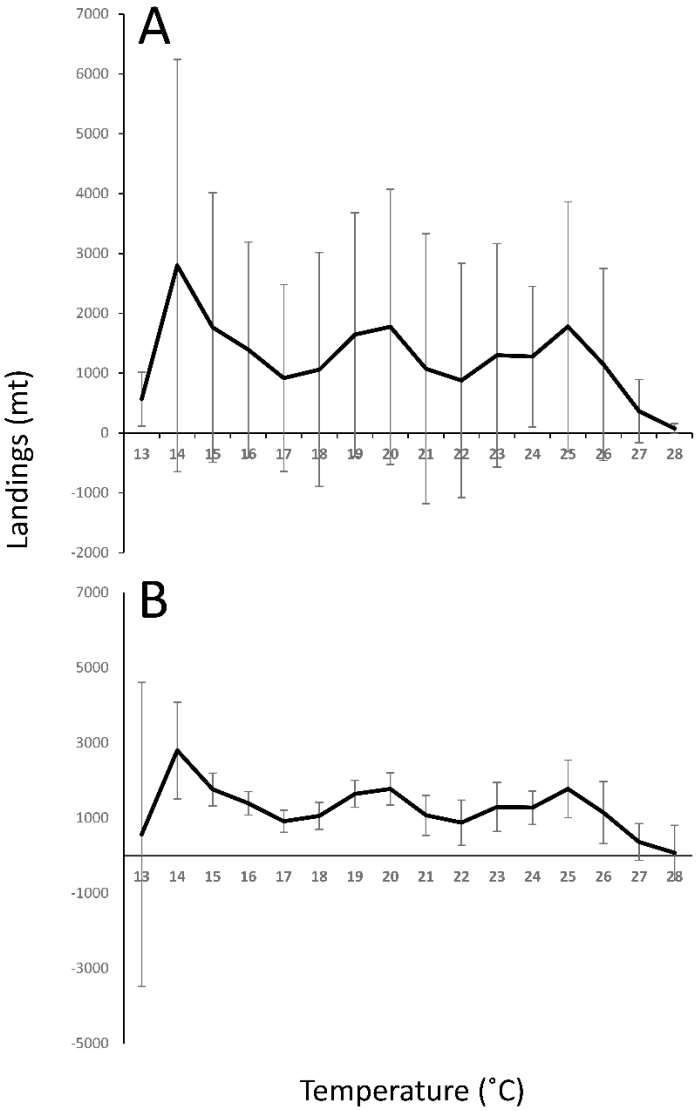

Fig. 10. (A) Mean (+/- standard deviation) and (B) mean +/- 95% confidence interval of Pacific sardine (*Sardinops sagax*) landings (mt) plotted against sea surface temperature from four sampling regions in southern California, US, and Baja California, MX. (Data from Felix-Uraga et al., 2004).

August of 1997; HadISST). This large change in annual mean landings indicates that any patterns in the data would largely be driven by data from 1990 onwards and by data from San Pedro and Ensenada. It should also be noted that landings from Isla de Cedros (MX), were consistently lower than any other region to such a degree that Félix-Uraga, et al. (2004) used a secondary axis with a much smaller scale in their visualizations that gives the impression that landings at Isla de Cedros were equivalent to the other ports. In doing so, the casual reader may have inferred artificially amplified trends in those landings data. Furthermore, the fishery at Isla de Cedros did not operate due to closure of the cannery there from 1995-2002, thus their inclusion in the graphical presentations is somewhat misleading.

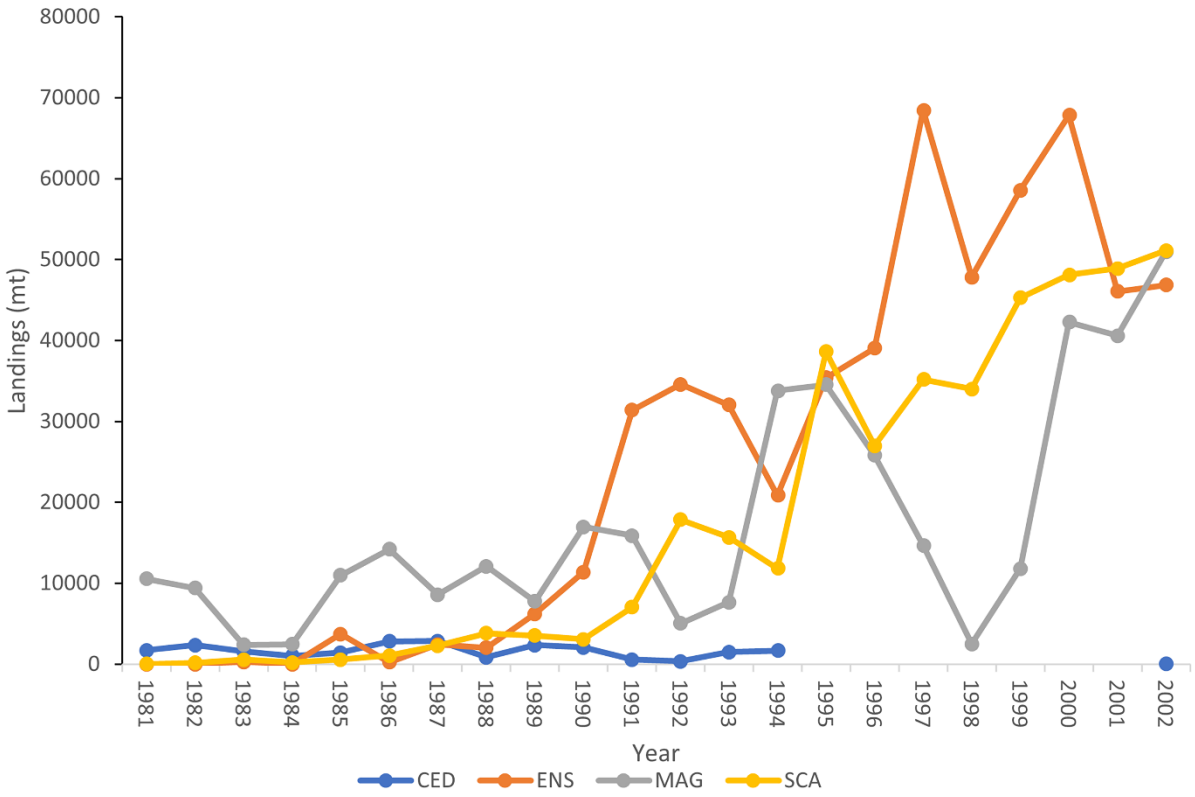

Figure 11. Annual landings of Pacific sardine (*Sardinops sagax*) by port from Felix-Uraga et al. (2004). CED = Isla de Cedros, ENS = Ensenada, MAG = Bahía Magdalena, SCA = Southern California.

Another potential source of uncertainty is in the assumption that sardine landed at each port were taken within a reasonable distance of that port. It has been demonstrated that for many species of fish, landings at Ensenada, for example, comprise catches from far distant fishing grounds that are reported at the local fishery office in Ensenada (MX) (Erisman, et al. 2011). Notably, fishing effort of the sardine fishery in Northwest Mexico is highest in the Gulf of California, and it is very common for fish captured inside the Gulf of California to officially be "landed" (i.e., reported) in Ensenada. Relative to sardine, the fishery from Isla de Cedros now lands its catches at Ensenada or Bahía Magdalena (K. Hill, SWFSC, pers. Comm.). While these landings are relatively small, it highlights the importance of accounting for distant catches being included at a particular port.

While Felix-Uraga et al. (2004) took steps to highlight that their data were not evidence of subpopulation structure, they made a bold assumption that the three peaks in landings represented groups that are adapted to different thermal ranges. This implies that the authors believe these groups to be so distinct as to have evolved to inhabit specific thermal ranges, a scenario that is expected to result in sufficient genetic differences so as to be readily detectable by modern methods. This assertion can only be regarded as speculative and is not supported by their data, the known biology of sardine, or existing genetic evidence. Intraspecific thermal preference/tolerance in fishes varies in concert with several factors including life stage, phenotypic plasticity, and heritable genetic differences (see McKenzie, et al., 2020 and

references therein), and disentangling which factors are most at play in a given study system is a daunting task. Unfortunately, the data presented in Felix-Uraga et al. (2004) fall short of those necessary to ascertain thermal adaptation by groups of sardine as they do not indicate any heritable genetic factors that are a requirement to support a hypothesis of adaptation.

Growth Patterns

"These studies have shown that there are between-season, between-port and between-year-class differences in 'size on age' curves. Similarly, there are within-season, within-port and within-year-class differences. Some of the differences appear to be associated with latitude. Insofar as the characteristics examined are concerned, the sardine population, as sampled by the fishery, is not homogeneous. The nature of the observed differences is not definitely known, but they are probably phenotypic." Marr, 1957b, p. 113.

Growth rates in sardine have been purported to show evidence for the existence of subpopulations of sardine (e.g., Smith, 2005; Zwolinski and Demer, 2023). Erisman et al. (2025) presented a detailed review of studies on growth rates relative to subpopulation structure in sardine and we present a summary here. Phillips (1948) provided one of the first comparative analyses of growth in sardine. That study presented data on annual and regional variation in growth among fishing ports in the Pacific Northwest (British Columbia, Washington, Oregon), Central California (San Francisco, Monterey), and Southern California (San Pedro) for 46,045 fish aged from scales. Phillips (1948) found that the average length of age 2 fish caught in Southern California (U.S.), was noticeably greater than fish caught in Central California and the Pacific Northwest of the U.S. At age 4 and older, a clinal pattern was observed wherein the average growth attained at each age was progressively greater from Southern California to Central California to the U.S. Pacific Northwest. Phillips (1948) also reported qualitative seasonal, annual, and sex-related differences in mean length at age within and among regions. Based on visual interpretation of differences in plots of growth curves for each region using mean values (all individuals and years) of length at age across all age classes from each region, Phillips (1948) concluded that there were variations in growth patterns among fish harvested in the three fishery regions.

Phillips (1948) provided several explanations for these observed patterns. Noting that Godsil (1931) had shown that older fish off San Diego, California, occurred more offshore, whereas smaller fish were more common inshore and thus interacted disproportionately with the fishery there, that tagging studies (e.g., Clark and Janssen, 1945) had shown size-biased migratory behavior, and that age 2 fish were rare in the Pacific Northwest because spawning was much less frequent there, Phillips (1948) determined that regional differences in length at age were expected. Phillips (1948) also highlighted regional differences in the seasonal timing of spawning (i.e., earlier in the south compared to the north) based on work by Tibby (1937) as an explanation for larger size at age of young fish from the southern California fishery compared to regions to the north. He explained the "flatter growth pattern" of fish from southern California after age 3 to the presence of "residual", slow-growing fish that did not migrate, with the possibility of an influx of slower-growing fish from southward locations (i.e., partial migration). In summary, Phillips (1948) considered the observed regional differences in growth patterns as reflections of sampling bias caused by biological, ecological, and behavioral characteristics of the species. Consequently, he concluded that somatic growth in sardine is best represented by a

single growth curve that considers the complex life history and the spatial and temporal variation in individual growth patterns within a single population that extends from at least the Pacific Northwest of the U.S. southern California (Phillips, 1948).

The most commonly cited study on spatiotemporal patterns in somatic growth in sardine is Felin (1954). Felin (1954) asked whether sardine taken by fisheries from southern California (U.S.), to Canada represented a single, homogeneous population or a complex of several, heterogenous populations. Using data on length-at-age and other vital statistics from fish landed in five major fishing regions (British Columbia, Canada, Pacific Northwest of the U.S., San Francisco, Monterey, and San Pedro) across 9 fishing seasons (1941-42 through 1949-50), Felin (1954) calculated the average observed lengths for each age class and calculated an unweighted mean of means length for each age class in each region across all years. These values were then plotted to represent a generalized growth curve for each region.

Using the direct-proportion approach (Walford, 1946), Felin (1954) back-calculated mean length-at-age for all age classes and plotted the mean of means lengths at age for all five regions against their back-calculated length at age in the successive year. Felin (1954) qualitatively (visually) examined these curves and concluded that they deviated from a linear pattern that would be expected if growth rates were the same across all regions.

Felin (1954) then compared growth patterns of sardine from the 1939 year-class taken by the fisheries in British Columbia and San Pedro. She again back-calculated lengths-at-age for each fish from each region using the direct-proportion method (Walford, 1946). She then compared growth curves derived from mean calculated lengths to those derived from mean observed lengths for each year class. She concluded that the two growth curves were the same. After a second transformation of the data, Felin (1954) concluded that the slope of the regressions (k) for each region was linear but different between the two regions.
Next, Felin (1954) constructed growth curves for the year classes 1937-1942 for British Columbia and San Pedro using the same methods described above to compare growth over a longer time period. Based on analysis of covariance, she found no differences in mean k values among year classes or between the two regions (i.e., the estimated mean growth rate was consistent among years for each port and between ports). There was, however, a significant difference ("at the 1-percent level") in the predicted y-intercept from the transformations of mean calculated and observed maximum length (L_∞) between the two ports across each year class.

Based on the relationship between the y-intercept and the maximum predicted L_∞, Felin (1954) concluded that L_∞ was different between these two regions. In her discussion of these results, Felin (1954) highlighted that differences in L_∞ could be phenotypic rather than genotypic, and that environmental factors may contribute to the plasticity of L_∞. Felin (1954) also recognized potential shortfalls in the use of back-calculated lengths as well as that comparisons of means and means of means of growth obscured the large variations in length at age among individual fish. However, she dismissed these concerns as only minor complications and concluded that there were in fact differences in growth rates between sardine landed at San Pedro and British Columbia and argued that the greater size of fish in northern waters could not entirely be explained by the migratory behavior of sardine. She speculated that the calculated differences were the result of "intraspecific populations" with limited exchange. She also speculated that sardine may have a series of overlapping coastal migrations of more than one group rather than a general, coastwide migration pattern.

With the contemporaneous documentation (see Ahlstrom 1954) of a large spawning area off central Baja California that Felin (and her contemporaries) assumed to be discrete from the

known spawning areas off southern California, Felin suggested that this area gave rise to catches off San Pedro (and to a lesser degree to those off central California), while the larger fish were hypothesized to originate off southern California and occasionally to the north (thus predating Marr's publication of the same idea).

Clark and Marr (1955) contributed a brief report discussing growth rates in sardine relative to a subpopulation hypothesis. No methods, data, or analyses were presented, but they presented a figure showing different growth curves. This appears to be a conceptual diagram only and we speculate that the figure is derived from Felin (1954). Clark and Marr (1955) concluded that there were differences in growth patterns in sardine taken in the fisheries off the Pacific Northwest of the U.S. and off San Pedro.

Marr (1960) compiled age and length data from previous studies that included fish taken in the fisheries from San Diego to British Columbia. These data included direct estimates of length and age, as well as back-calculated values for the same. In his comparison, Marr (1960) noted that the mean length of age 1 fish was highest in San Pedro and lowest in British Columbia. He also showed that the mean length of age 1 fish varied from year to year within individual ports. Marr concluded that this variation was due to a density-dependent relationship between growth and competition for resources as smaller fish are more restricted in their ability to move long distances. As with the studies mentioned above, Marr (1960) plotted the mean of means of length at age and recovered similar relationships to those reported by Felin (1954). Marr (1960) hypothesized that there were at least two groups of fish with different growth patterns and concluded that the observed patterns could not be explained solely by the migratory behavior of sardine. However, Marr (1960) stopped short of explaining these patterns as the product of genetically distinct subpopulations and argued that the different growth patterns were likely influenced by the environment.

Studies on sardine age and growth in the U.S. languished for several decades, likely in response to a near absence of sardine from U.S. waters. In 1996, Butler et al. presented age and growth data for 1,079 individuals collected from Punta Eugenia in central Baja California to Monterey (U.S.). A majority of the samples were collected off southern California (U.S.; n = 667). Samples were pooled into 3 regions: Monterey (north of 34˚N), southern California, and Baja California (south of 31˚N). Otolith annuli were counted by five readers with low (31%) agreement and the mean age among readers was used for final age estimates. Butler et al. (1996) fixed the theoretical age when size is 0 (t_0) to 0 because there were no small or young fish sampled. The oldest fish in the sample were aged seven (thus the estimate for L_∞ was artificially low as sardine are known to reach greater ages). Butler et al. (1996) concluded that it was uncertain whether observed regional differences in length-at-age were due to difference in growth patterns, small sample sizes, differences in spawning seasonality, or inaccurate age determination.

As noted by Erisman et al. (2025), the studies above failed to address the effects of individual variance in growth rates in sardine (e.g., the use of mean and means of mean lengths at age obscures individual variation). As early as the 1930s, data showing individual variation in length at age were available, thus it is surprising that this was virtually ignored by so many authors. The large degree of individual variation in length at age was shown by Dorval et al. (2015) who examined growth patterns in sardine from central and southern California collected from 1994-2010. That study showed, for example, that individuals 160 mm SL ranged in age from 0 to 4 years, and fish 220 mm SL ranged in age from 0 to 7 years in age. This variation was also shown by Enciso-Enciso et al. (2022) for fish collected from Baja California wherein fish of

a given length were represented by as many as 5 age classes. As reported by Erisman et al. (2025), when the mean of mean values presented by Felin (1954) for each age class and each region are plotted against the data presented in Dorval et al. (2015) it is readily apparent that Felin's reported growth differences are encompassed by the range of fish length for each age class presented in Dorval et al. (2015; Figure 12). We suspect that, despite this well-documented variation in length at age, modern reliance on suspected regional differences in growth rates to demonstrate subpopulation differences (e.g., Enciso-Enciso, et al., 2022; Zwolinski and Demer, 2023) has been heavily influenced by a review published in the mid-2000s (Smith, 2005). Through hyperbole, Smith (2005) gave the impression that regional differences in growth patterns were of a greater magnitude than those shown by the data being reviewed ("Once aging from scales and otoliths became available…two radically different growth patterns were detected [Felin 1954]").

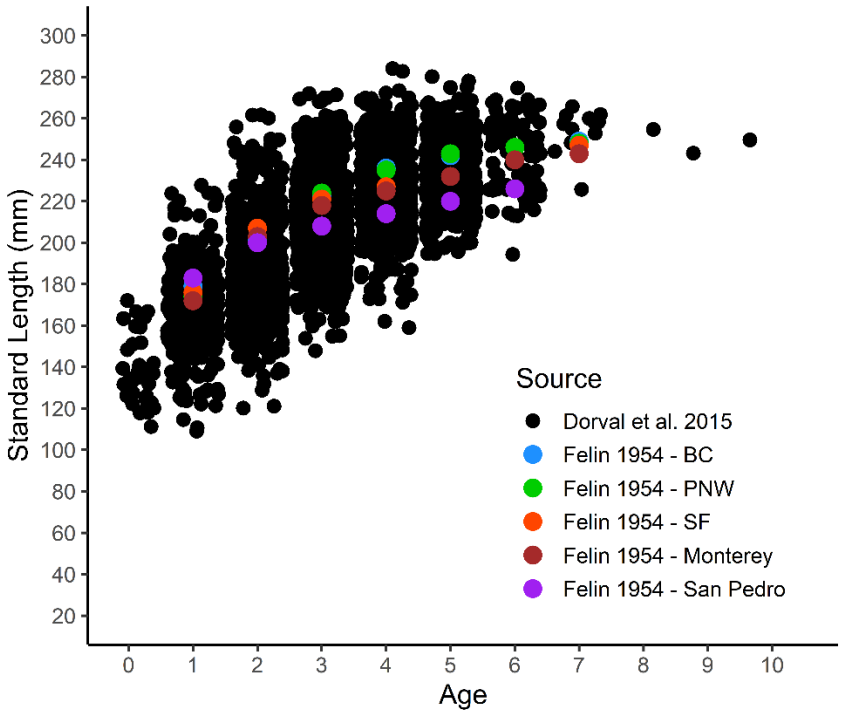

Figure 12. Figure 4 from Erisman et al. (2024). Length at age of Pacific sardine (*Sardinops sagax*). Black data points are from Dorval et al. (2015) while colored data points are from Felin (1954). Figure reproduced by permission of the authors.

Given that growth characteristics are heavily influenced by environmental factors, that migratory behavior may obscure regional patterns in growth rates, that individual variation in growth rates is the rule rather than the exception, and that no studies have demonstrated a persistent pattern of differential growth over time (e.g., have excluded interannual variation among regions as an explanation for observed differences), it seems unlikely that growth is a reliable characteristic for demonstrating the existence of subpopulations in sardine or for corroborating allocation of sardine to a particular subpopulation. This has also been demonstrated for other coastal pelagic species. For example, Silva et al. (2008) attributed geographical variation in growth patterns to age-related migration in European sardine (*Sardina*

pilchardus). Rogers and Ward (2007) noted differences in size and age distributions in offshore versus inshore habitats in Australian sardine (*Sardinops sagax*) indicating that data collected from one area will provide a skewed understanding of growth at the population level. In fact, the use of growth patterns in any fish species for the purposes of distinguishing among geographically delimited groups is inherently problematic due to its phenotypically plastic nature (Enberg, et al., 2012; Lorenzen, 2016).

Demographics

"*Data on the length frequency distributions of sardines have been collected almost from the inception of the fishery*." Marr, 1957b, p. 113.

Another characteristic of sardine that has been lauded as informative as to the existence of subpopulations is regional differences in the demographics of fish collected by a particular fishery (herein "demographics" refers to age/length composition of samples). As Marr (1957) pointed out, there is abundant data available from which to evaluate the age and length structure of sardine landed at various ports dating back to the 1930s. These data have consistently shown similar patterns of qualitatively different age/length compositions of fish landed in different regions and during different times of the year. What has varied over time, however, is how these differences have been interpreted and their contribution to the discussion of subpopulations in sardine. Given the number of data papers available that are purely descriptive, we highlight a few papers below that examined those data to demonstrate how their interpretation has changed over the years and to provide our own comments.

Phillips (1948), Felin (1954), and Marr (1960) all evaluated differences in the age/length structure of fishery samples collected from regionally-defined fisheries. Whereas Phillips (1948) and Marr (1960) explained these differences by invoking the biology of sardine and fishery behavior, Felin (1954) concluded that they were the result of population heterogeneity (many authors seem to have interpreted "heterogeneity" to mean "subpopulation structure"; Felin, however explicitly stated that the cause of the heterogeneity could not then be determined). Phillips (1948) alluded to two factors that influenced the observed regional demographic patterns: selectivity of the fishery in different regions and size/age-biased migratory behavior. These well-known pitfalls of using fishery dependent data can lead to a false impression of the true demographic structure of a species and Phillips (1948) seemed to have felt that sardine are no exception. Smith (2005) neatly described the effects of fishery behavior in discussing the various canning sizes of fish landed at the different ports (although he seems to have attributed that to availability of different size class rather than fishery behavior). Marr (1960) pointed out that when a dominant year class first enters a fishery, the mean age of fish landed will necessarily decrease. As this dominant age class moves through the fishery, the mean age will increase. In the case of sardine, the California fisheries receive more dominant age classes than those of the Pacific northwest of the U.S. due to the higher concentration of spawning and recruitment that occurs there annually but that does not occur in the Pacific northwest of the U.S. (see "Spawning" section for discussion of spawning patterns). Simultaneously, the effects of size- and age-based migration lead to further increases in mean size of fish landed in regions of the Pacific northwest of the U.S. Together these factors lead to a skewed age/size distribution of fishery landings towards older/larger fish in more northern localities. It is also important to remember that there is a logical inconsistency in considering fish from different regions to be

intrinsically different. That is, the migratory behavior of sardine means that fish from the same group (population/subpopulation) move from one region to another and thus the comparison above would be akin to comparing something to itself. It is thus evident that using demographic structure in sardine as a means to differentiate between putative biological groups is inherently difficult if not impossible. Nevertheless, current research and stock assessments assume that differences in age and length distributions from fisheries dependent and independent surveys are representative of the population and thus reflect biologically meaningful differences in demography (e.g., natural mortality) rather than as an effect of sampling bias (e.g., Zwolinski and Demer, 2023; Kuriyama, et al., 2024).

How did we get here?

After reviewing the primary literature and finding little support for the hypothesis of subpopulation structure in the sardine, we sought an explanation for how this notion became so deeply ingrained that it became dogma and thus impacted both science and management. When sardine landings plummeted to levels where the fishery could no longer operate, questions about subpopulations moved from a purely academic endeavor to a practical one. Marr's (1960) hypothesis was written almost as an obituary to the fishery, as by 1960 it was all but eliminated in the U.S. Few primary research papers on sardine appear in the literature in the 1970s and 1980s, a result we believe is simply due to the fact that there were few sardine available to study and that no management actions were necessary as the fishery was closed. In the late 1980s, sardine numbers began to rise in the northern portion of its range spawning a flurry of action by resource managers in the U.S. that was based on research that had all but ceased by the end of the 1960s including Marr's untested hypothesis. Concern arose that the northern portions of the range of sardine were being repopulated with Marr's southern stock and that that southern stock, thought to be genetically distinct based on the work of Sprague and Vrooman (1962) and Vrooman (1964) (but see "Blood Antigens" section above for explanation of why this is not a valid approach), could have biological characteristics that were sufficiently different from Marr's northern stock as to cause a change in productivity to the fishery. This was neatly encapsulated by Alec MacCall in 1984 in reference to the sardine:

> "For a person like me who is going to have to write a management plan for this fish, it is rather important to determine whether the northern stock still exists. Is the new sardine stock going to be the same as the old or is it going to be something different? If the genetic composition is different, can we expect the productivity to be the same as for the old stock? If it looks as if it is the same stock as it used to be, we would feel more confident that the productivity would be the same. On the other hand, we might want to be more conservative if we see that the genetic composition is actually different than it used to be." (p.12)

The idea that there may be two different entities within the larger sardine population thus became a central theme as managers prepared a strategy to potentially reopen a sardine fishery in the U.S. Rueben Lasker summed up the issue when he remarked during a workshop on identifying fish subpopulations "From a management point of view, I would like to know if there are two pots [of fish]; then perhaps I would change my strategy and fish only from the larger one" (in Hedgecock, 1984). In 1998, management of sardine in U.S. waters shifted from state

agencies to the Pacific Fishery Management Council under their Coastal Pelagic Species Fishery Management Plan. This initiated a flurry of federally sponsored research into sardine. In 2005, Paul Smith at NOAA's Southwest Fisheries Science Center wrote a paper discussing the history of subpopulation studies in sardine. In that paper, he provided a cursory summary of many of the papers reviewed here, and suggested that in order to be precautionary, management should operate under a subpopulation model as, at the time, there were no data to the contrary (but see Lluch-Belda et al. 2003). This last aspect (absence of data to the contrary) seems to have been largely ignored by subsequent researchers, which resulted in Smith's paper being cited as evidence for subpopulations in sardines through the present day when clearly it was not.

Following Smith's (2005) paper, a nearly circular pattern of citation began within the sardine literature. We observed that a somewhat "standard" citation string began to be used for papers purported to show evidence of subpopulation structure. This standard string often contained papers that did not show evidence of subpopulation structure (either by their author's analysis or through reconsideration of methods and data quality), were papers that summarized other papers, or were papers that themselves used a similar citation string. The end result was that readers interested in the topic were consistently given the impression that the issue was settled: sardine *are* divided into subpopulations *unequivocally*. However, when the path to this standard string was followed, all roads led to Marr (1960) and the three main data sets that he relied upon to construct his hypothesis: vertebral counts, serological antigen responses, and spawning, all of which our review has shown do not support a hypothesis of subpopulation distinction.

In light of our review, it is surprising that the subpopulation paradigm has persisted for so long. While taking a precautionary approach to management of sardine has certainly influenced this course of action, we also speculate that there may have been a reluctance to challenge the *status quo* in an attempt to avoid conflict among the community of sardine scientists. While we could not find any papers that directly challenged the subpopulation paradigm, there are some works which examined population dynamics and distributions of sardine that make no mention of subpopulations in their studies (e.g., Lluch-Belda et al., 1991; Rodriguez-Sánchez, et al., 2002; Lluch-Belda et al., 2003).

Summary

"*While my model does relate present knowledge of the Pacific sardine in what seems to me to be a logical manner, it is obvious that the evidence is not always as clear cut as one might desire. The judgements made in the course of constructing the model were, perforce, sometimes made on the basis of the most likely alternative among several, rather than on the basis of a simple choice between opposing alternatives. The model must, therefore, be regarded as only a first approximation. It is my hope that it is realistic enough to both warrant and inspire critical testing in the laboratory and at sea*." Marr, 1960, p. 780.

Our review of the sardine literature has shown that what was once considered as compelling evidence of subpopulation structure in sardine can no longer be considered as appropriate to use in studies of subpopulation divisions when modern best principles are applied. With the recognition that Jordan's Rule for meristic elements does not hold, there is no reason to expect changes in the mean number of vertebrae to be a reliable indicator of anything other than

phenotypic plasticity. The early serological antigen methods used by Vrooman (1964) and Sprague and Vrooman, (1962) are fatally flawed, being affected by, among other things, dietary-induced anemia and not having an underlying genetic cause. Growth rates in sardine are variable at the level of the individual to such a degree that they are incapable of demonstrating local adaptation and subpopulation structure and again reflect phenotypic plasticity under the effect of the environment that varies more within geographically partitioned groups than among them. It is abundantly clear that the notion of the existence of subpopulations in sardine is grounded in the serological antigen work of Sprague and Vrooman (1962) and Vrooman (1964). Marr's hypothesis is explicitly based on these works as highlighted above. Reliance on these data persisted through the mid-1980s as highlighted by McCall (1984) ("We had reason to believe from blood serological research that there was a northern and southern stock of sardines") and was (and still is) explicitly mentioned in the PFMC CPS FMP. Prior to Sprague and Vrooman's work, Marr's hypothesis lacked the key "genetic" data upon which he based his suppositions. Had Marr had the benefit of modern genetic and biological data, we suspect that he would have rejected or greatly altered his hypothesis The bottom line is that the singular, decisive piece of the puzzle that solidified the subpopulation hypothesis for sardine is based on data that are incapable of doing so. The proverbial house of cards that has been built on this unstable foundation is thus incapable of supporting itself.

While it may seem odd to provide such an in-depth review of this literature in the context of what many consider to be basic biological principles, it is nevertheless important for many reasons. The present state of sardine management is (and has been) based on a subpopulation hypothesis posed in the 1960s but that was by and large never adequately tested or refined despite the call to do so from its originator (see quote above). This is likely because at the advent of many techniques for aging and the development of the knowledge base of alosid life histories, the fishery for the sardine was non-existent and therefore comparatively little research was undertaken on the species using modern methods. It was not until the resurgence of sardine in the late 1990s and early 2000s that interest in sardine was reignited and research resumed. This resulted in an uncritical acceptance of these early hypotheses that was in part influenced by one of the most cited papers on the topic, Smith (2005), which left the reader with the impression that the subpopulation hypothesis was well supported. In reaching his conclusions, Smith (2005) deemphasized the only available direct information on sardine movements that found mixing of sardine tagged and recaptured at various locations (Clark and Jannson, 1945, see discussion above) and placed greater emphasis on the finding of growth differences by Felin (1954), the vertebral count work of the various authors cited here, and the serological antigen work of Sprague and Vrooman (1966) and Vrooman (1964), all of which we have shown to be nugatory. Smith also dismissed the absence of genetic evidence for subpopulation structure in sardine stating the familiar adage "the absence of evidence is not evidence of absence." While we cannot dispute the philosophical truth of this statement, this often dismissively employed red herring could also be used to argue for the existence of the fabled Loch Ness Monster.

There is a historical context within which Smith's (2005) conclusions are understandable. In the past, population studies, particularly those that were based on purely genetic data, often suffered from a common problem: lack of consideration for the ecology of the species being studied. The advent of molecular techniques that could directly measure gene flow, and thus exchange, among groups led to an explosion of studies by geneticists with little background in the biology or ecology of their study species (Choat, 2006). The results of this were that data were interpreted incorrectly based on known biological patterns, leaving many outside of the

genetics arena unsatisfied that the methods had merit. At the time of Smith's (2005) writing, genetic techniques were also less capable of detecting differences at time scales relevant to fisheries management than they are now. It is likely that these two factors conspired against the acceptance of genetic data showing panmixia in sardine.

Many of the studies attempting to confirm the existence of subpopulations have done so using characters that are by and large under strong influence of the environment and only to a lesser degree shaped by genetics. Measuring characters that are under environmental control may indeed provide data that are concordant with subpopulation structure in certain, constrained scenarios. For example, an organism that has limited dispersal potential (as adults or otherwise) may experience a consistent and constrained set of environmental conditions throughout its lifetime, thus paving the way for selection and local adaptation. This is hardly the case with sardine. With their roughly 40-day pelagic egg and larvae phase, capacity for long-distance migration, and geographically and temporally protracted spawning habits, there does not appear to be a mechanism whereby genetic and environmentally-driven phenotypic characters could simultaneously be reinforced. When considering the ecology and biology of the sardine, it is therefore not surprising that the bulk of studies reviewed herein were unable to find data that could be used to reject the null hypothesis of no population structure or that could be used to support the alternative hypothesis of population structure.

Additionally, many studies of subpopulation structure in sardine are based on data that were collected over inadequate spatiotemporal scales to support those claims. For example, current management of the sardine in the U.S. apportions landings and surveyed biomass to the NSP using a potential habitat model (see Demer and Zwolinski, 2014; Zwolinski and Demer, 2023). This model is based on spring egg collections from southern California, U.S., despite the fact that earlier studies have demonstrated that sardine spawning is temporally dynamic over a spatial range encompassing central Baja California to Oregon (U.S.). As a result of this method, sardine present in southern California but not within the modelled environmental envelope of the NSP are removed from biomass estimates.

This review also highlights the importance of citation accuracy (see Pavlovic, et al. 2021, Texeira, et al., 2013, and Hosseini, et al. 2020 for discussion on this topic). Long-term and repetitive mis-citation may result in the building of a false narrative, blurring the line between what we know (data) and what we think we know (speculation based on those data). In the case of sardine, our review revealed that the foundation of the subpopulation structure discussion was at least partially based on misinterpretations of the original studies. In other instances, recent citations to past works have provided a false sense of confidence in prior studies that utilized outdated and/or error prone methodology. This has resulted in a circularity of citation that misleads readers towards the inference that the matter is settled, when in fact there are no data that can be used to reject the null hypothesis of a single, well-mixed population of sardine along the west coast of North America.

Recognizing biological populations and the limits of their geographic distributions is critical for effective fisheries management (Reiss, et al., 2009; Cianelli, et al., 2013), and the accuracy with which we interpret historical data to form conclusions about population boundaries is of great importance. The alignment of geographically-based management measures with these boundaries can prevent undesired management outcomes (Berger, et al., 2021; Cadrin, et al., 2023). In the case of the sardine, current management is based on the hypothesized presence of three subpopulations that vary in their geographic limits which themselves vary seasonally and with environmental conditions. Our conclusion based on a critical review of

studies into population structure in sardine is also the most parsimonious: there is a single, well-mixed population of sardine along its northeastern Pacific range.

Our review has implications for the management of sardine. What those implications are, however, is unclear. While the alignment of management units with biological units is desirable for myriad reasons, our review shows that this is currently not the case for sardine. Undesirable outcomes of such scenarios are most obvious when a single management unit is assumed but multiple biological units exist, yet in this case, multiple management units have been assumed for a single biological unit. Whether or not this is problematic ultimately depends on the goals of a particular management framework (i.e., strict conservation, optimized harvest, or some combination of both). The impacts of this review for management of sardine will need to be evaluated by a broad array of stakeholders including managers, scientists, and the broader fishing community. Innovative modern genetic techniques, interdisciplinary approaches, or new numerical modelling may serve to help assess how this review may or may not impact the current management of sardine.

Acknowledgments

Several people provided input for this manuscript in various ways and we would like to acknowledge their roles in improving it: B. Muhling, A. MacCall, R. McBride, R. Parrish, K. Hill, S. Cadrin, G. Longo, E. Dorval, B. Schwartzkopf, B. Javor, and J. Hyde. D Losey provided invaluable assistance in locating obscure and difficult-to-find references. We thank W. Watson, J. Hyde, A. Yau, and two anonymous reviewers for reviews of this manuscript.

Author's Contributions

Order of authorship arranged in descending order of contribution. MTC and BE conceived of the paper, reviewed and extracted all relevant information and results from the literature. MTC led the writing of the manuscript with major contributions by BEE. ESA-H transcribed and digitized data from pre-electronic resources, assisted with literature searches, contributed to the intellectual development of this study, and assisted with the writing. KCJ and ART contributed to the intellectual development of this review and assisted with the writing. All authors reviewed and edited drafts of the manuscript.

Literature Cited

Adams, E. S., and M. T. Craig. 2024. Phylogeography of the Pacific Sardine, *Sardinops sagax*, across its Northeastern Pacific range. So. Cal. Acad. Sci. Bull. 123:10-24.

Ahlstrom, E. H. 1948. A record of pilchard eggs and larvae collected during surveys made in 1939 to 1941. U.S. Dept. Interior, Fish and Wildlife Service, Special Scientific Report No. 23, 26pp.

Ahlstrom, E. H. 1954. Distribution and abundance of egg and larval populations of the Pacific sardine. Fish. Bull., U.S. 93:83-140.

Ahlstrom, E. H. 1959. Distribution and abundance of the eggs of the Pacific sardine, 1952–1956. Fish. Bull., U.S. 60:185–213.

Ahlstrom, E. H. 1965. A review of the effects of the environment of the Pacific sardine. International Commission for the Northwest Atlantic Fisheries Special Publication No. 6:53-74.

Ahlstrom, E. H. 1966. Distribution and abundance of sardine and anchovy larvae in the California Current region off California and Baja California, 1951-64: A summary. U.S. Department of the Interior Fish and Wildlife Service Special Scientific Reports No. 534. Pp. 71.

Baldwin R. E. 2010. Using parasite community data and population genetics for assessing Pacific sardine (*Sardinops sagax*) population structure along the west coast of North America. Ph.D. diss., Oregon State University, Corvallis, Oregon, 207 pp.

Baldwin, R. E., M. A. Banks, and K. C. Jacobson. 2012. Integrating fish and parasite data as a holistic solution for identifying the elusive stock structure of Pacific sardines (*Sardinops sagax*). Rev. Fish. Biol. Fisheries 22:137-156.

Berger, A.M., J. J. Deroba, K. M. Bosley, D. R. Goethel, B J. Langseth, A. M. Schueller, and Hanselman, D. H. 2021. Incoherent dimensionality in fisheries management: consequences of misaligned stock assessment and population boundaries. ICES J. Mar.Sci. 78:155-171.

Blackheart, K., D. G. Stanton, and A. B. Shimada. 2006. NOAA fisheries glossary. NOAA Tech. Memo. NMFS-F/SPO 69.

Bowen, B. W., and S. W. Grant. 1997. Phylogeography of the sardines (*Sardinops* spp.): Assessing biogeographic models and population histories in temperate upwelling zones. Evolution 51:1601-1610.

Butler, J. L., G. M. L. Granados, J. T. Barnes, M. Yakemko, and B. J. Macewicz. 1996. Age composition, growth, and maturation of the Pacific sardine (*Sardinops sagax*) during 1994. California Cooperative Oceanic Fisheries Investigations Reports 37:152–159.

Cadrin, S. X. 2020. Defining spatial structure for fishery stock assessment. Fisheries Research 221:105397.

Cadrin, S. X., D. R. Goethel, A. Berger, and F. Jardim. 2023. Best practices for defining spatial boundaries and spatial structure in stock assessment. Fisheries Research 262:106650.

Cadrin, S. X., and D. H. Secor. 2009. Accounting for spatial population structure in stock assessment: past, present and future. *In*: The Future of Fisheries Science in North America (R. J. Beamish, B. J. Rothschild, eds), p. 405–425. Springer, New York.

Campana, S. E., and J. M. Casselman. 1993. Stock discrimination using otolith shape analysis. Can. J. Fish. Aquat. Sci. 50:1062-1083.

Catalano, S. R., I. D. Whittington, S. C. Donnellan, and B. M. Gillanders. 2014. Parasites as biological tags to assess host population structure: Guidelines, recent genetic advances and comments on a holistic approach. Int. J. Parasitol.: Parasites and Wildlife 3:220-226.

Chapman, B. B., C. Skov, K. Hulthén, J. Brodersen, P. A. Nilsson, L. A. Hansson, and C. Brönmark. 2012. Partial migration in fishes: definitions, methodologies and taxonomic distribution. J. Fish. Biol. 81:479-499.

Choat, J. H. 2006. Phylogeography and reef fishes: bringing ecology back into the argument. J. Biogeog. 33:967-968.

Cianella, L., J. A. D., Fisher, M. Skern-Mauritzen, M.E. Hunsicker, M. Hidalgo, K. T. Frank, and K. M. Bailey. 2013. Theory, consequences and evidence of eroding population spatial structure in harvested marine fishes: A review. Mar. Ecol. Prog. Ser. 480:227-243.

Clark, F. N. 1936. Variations in the Number of Vertebrae of the sardine, *Sardinops caerulea* (Girard). Copeia, 1936 (3), 147-150.

Clark, F. N. 1947. Analysis of populations of the Pacific sardine on the basis of vertebral counts. Fish. Bull. 65:1-29.

Clark, F. N., and J. F. Janssen, Jr. 1945. Movements and abundance of the sardine as measured by tag returns. Fish. Bull. 61:7-42.

Clark, F. N., and J. C. Marr. 1955. Population dynamics of the Pacific sardine. Prog. Rep. Calif. Oceanic Fish. Invest. 1 July 1953 to 31 March 1955:11-48.

Cope, J. M., and A. E. Punt. 2011. Reconciling stock assessment and management scales under conditions of spatially varying catch histories. Fish. Res. 107:22–38.

Corbel, M. J. 1975. The immune response in fish: a review. J. Fish. Biol. 7:539-563.

Demer, D. A., and J. P. Zwolinski. 2014. Corroboration and refinement of a method for differentiating landings from two stocks of Pacific sardine (*Sardinops sagax*) in the California Current. ICES J. Mar. Sci. 71:328-335.

Demer, D. A., J. P. Zwolinkski, K. A. Byers, G. R. Cutter, J. S. Renfree, T. S. Sessions, and B. J. Macewicz. 2012. Prediction and confirmation of seasonal migration of Pacific sardine (*Sardinops sagax*) in the California Current Ecosystem. Fish. Bull. 110:52-70.

Dorval, E., J. D. McDaniel, B. J. Macewicz, and D. L. Porzio. 2015. Changes in growth and maturation parameters of Pacific sardine *Sardinops sagax* collected off California during a period of stock recovery from 1994 to 2010. J. Fish Biol. 87:286-310.

Enberg, K., C. Jørgensen, E. S. Dunlop, Ø. Varpe, D. S. Boukal, L. Baulier, S. Eliassen, and M. Heino. 2012. Fishing-induced evolution of growth: concepts, mechanisms and the empirical evidence. Marine Ecology 33:1–25.

Enciso-Enciso, C., M. O. Nevárez-Martínez, R. Sánchez-Cárdenas, E. Marín- Enríquez, E., L. A. Salcido-Guevara, and C. Minte-Vera. 2022. Allometry and individual growth of the temperate Pacific sardine (*Sardinops sagax*) stock in the Southern California current system. Fishes 7:226.

Elsdon, T. S., B. K. Wells, S. E. Campana, B. M. Gillanders, C. M. Jones, K. E. Limburg, D. H. Secor, S. R. Thorrold, and B. D. Walther. 2008. Otolith chemistry to describe movements and life-history parameters of fishes: Hypotheses, assumptions, limitations and inferences. Oceanography and Marine Biology: An Annual Review 46:297-330.

Emmett, R. L., R. D. Brodeur, T. W. Miller, S. S. Pool, G. K. Krutzikowsky, P. J. Bently, and J. McCrae. 2005. Pacific sardine (*Sardinops sagax*) abundance, distribution, and ecological relationships in the Pacific northwest. CalCOFI Rep. 46:122-143.

Enciso-Enciso, C., M. O. Nevárez-Martínez, R. Sánchez-Cárdenas, L. A. Salcido-Guevara, C. Minte-Vera, E. Marín-Enríquez, and M. E. Hernández-Rivas. 2023. Assessment and management of the temperate stock of Pacific sardine (*Sardinops sagax*) in the south of California Current System. Reg. Stud. Mar. Sci. 62:102972.

Erisman, B. E., G. A. Paredes, T. Plomozo-Lugo, J. Cota-Nieto, P. A. Hastings, and O. Aburto-Oropeza. 2011. Spatial structure of commercial marine fisheries in northwest Mexico. ICES Jour. Mar. Sci. 68:546-571.

Erisman, B., M. Craig, K. James, B. Schwartzkopf, E. Dorval. 2025. Systematic review of somatic growth patterns in relation to population structure for Pacific Sardine (*Sardinops sagax*)

along the Pacific Coast of North America. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-708.

Felin, F. E. 1954. Population heterogeneity in the Pacific pilchard. Fish. Bull. U.S. 54:201–225.

Félix-Uraga, R., V. M. Gómez-Muñoz, C. Quiñonez-Velazquez, 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. CalCOFI Rep. 45:146-151.

Félix-Uraga, R., V. M. Gomez-Munoz, C. Quiñonez-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. CalCOFI Rep. 46:113-121.

Field, D. B., T. R. Baumgartner, V. Ferreira, D. Gutierrez, H. Lozano-Montes, R. Salvatteci, and A. Soutar. 2009. Variability from scales in marine sediments and other historical records. *In* Climate Change and Small Pelagic Fish (D. Checkley D., J. Alheit, Y. Oozeki, and C. Roy, eds.), p. 45-63. Cambridge University Press, Cambridge.

Fisheries and Oceans Canada. 2024. Pacific Region Integrated Fisheries Management Plan, Pacific Sardine. September 30, 2024 to May 31, 2029. 23-2365:15p.

Fogarty, M. J., and L. W. Botsford. 2007. Population connectivity and spatial management of marine fisheries. Oceanography. 20:112–123.

Fricke, R., W. N. Eschmeyer, and R. Van der Laan, R. 2023. Eschmeyer's Catalog of Fishes: Genera, Species, References. (http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed 17 October 2023.

García-Morales, R., B. R. Shirasago-Germán, R. Félix-Uraga, and L. Pérez-Lezama. 2012. Conceptual models of Pacific sardine distribution in the California Current System. Curr. Devel. Oceanog. 5:23-47.

García-Rodríguez, F. J., S. A. García-Gascab, J. De La Cruz-Agüeroa, and V. M. Cota-Gómeza. 2011. A study of the population structure of the Pacific sardine *Sardinops sagax* (Jenyns, 1842) in Mexico based on morphometric and genetic analyses. Fish. Res. 107:169-176.

Gillanders, B. M., C. Izzo, Z. A. Doubleday, and Q. Ye. 2015. Partial migration: growth varies between resident and migratory fish. Biol. Lett., 11:20140850.

Godsil, H. C. 1930. A discussion of the localities in which the California sardine (*Sardina caerulea*) was taken in the San Diego region, 1928–1929. Calif. Div. Fish Game Fish Bull. No. 25, pp. 40–44.

Godsil, H. C. 1931. The commercial catch of adult California sardines (*Sardina caerulea)* at San Diego. Calif. Div. of Fish and Game. Fish Bull. 31:41–53. 1941. "N. B. Scofield": Progress report for 1940. Cal. Fish Game, 27:39-43.

Grant, W. S., A. M. Clark, and B. W. Bowen. 1997. Why restriction fragment length polymorphism analysis of mitochondrial DNA failed to resolve sardine (*Sardinops*) biogeography: insights from mitochondrial DNA cytochrome *b* sequences. Can. J. Fish. Aquat. Sci. 55:2539-2547.

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. J. Hered. 89:415-426.

Gutiérrez Flores, C. 2007. Estructura genética poblacional de la sardina del Pacífico nororiental *Sardinops sagax caeruleus*. Masters Thesis. Centro de Investigación Científica y de Educación Superior de Ensenada.

Hart, J. L. 1933a. Statistical studies on the British Columbia pilchard: Vertebra counts. Trans. Roy. Soc. Canada 3:79-85.

Hart, J. L. 1933b. A report on the Investigation of the Life-History of the British Columbia Pilchard. Report of the Commissioner of Fisheries, 1933.

Hart, J. L. 1943. The pilchard *Sardinops caerulea* (Girard) on Canadian fishing grounds with special reference to an unusual abundance of young fish. Trans. Roy. Soc. Canada, section V, pp. 55-73.

Hart, J. L. 1944. Pilchard-tagging and Pilchard-tag recovery from 1936-1943. Report of the Provincial Fisheries Department 1943:43-52.

Hastings, P. A. 2000. Biogeographic of the Tropical Eastern Pacific: distribution and phylogeny of chaenopsid fishes. Zool. J. Lin. Soc. 128:319-335.

Hedgecock, D. 1984. Identifying fish subpopulations. California Seagrant College Program Report T-CSGCP-013.

Hedgecock, D., E. S. Hutchinson, G. Li, F. 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 Bull. 87:653-671.

Hernandez-Vazquez, S. 1994. Distribution of eggs and larvae from sardine and anchovy off California and Baja California, 1951-1989. CalCOFI Rep. 35:94-107.

Hill, K. T., P. R. Crone, and J. P. Zwolinski. 2018. Assessment of the Pacific sardine resource in 2018 for U.S. management in 2018-19. NOAA Technical Memorandum NOAA-TM-NMFS-SWFSC-600.

Hintzen, N. T., B. Roel, D. Benden, M. Clarke, A. Egan, R. D. M. Nash, N. Rohlf, and E. M. C. Hatfield. 2015. Managing a complex population structure: exploring the importance of information from fisheries-independent sources. ICES J. Mar. Sci. 72:528–542.

Hosseini, M., M. P. Eve, B. Gordign, and C. Neylon. 2020. MyCites: a proposal to mark and report inaccurate citations in scholarly publications. Res. Integr. Peer Rev. 5:13.

Hubbs, C. L. 1925. Racial and seasonal variation in the Pacific herring, California sardine and anchovy. Fish. Bull. 8:1-23.

Jacobson, K., R. Baldwin, M. Banks, and R. Emmett. 2019. Use of parasites to clarify residency and migration patterns of Pacific sardine (*Sardinops sagax*) in the California Current. Fish. Bull. 117:196–210.

Javor, B. J. 2013. Do shifts in otolith morphology of young Pacific sardine (*Sardinops sagax*) reflect changing recruitment contributions from northern and southern stocks. CalCOFI Rep. 54:1-12.

Javor, B. J., and E. Dorval. 2017. Composition and inter-annual variability in trace element profiles of Pacific sardine otoliths. CalCOFI Rep 58:95-104.

Javor, B., N. Lo, and R. Vetter. 2011. Otolith morphometrics and population structure of Pacific sardine (*Sardinops sagax*) along the west coast of North America. Fishery Bulletin, 109:402-415.

Kerr, L. A., N. T. Hintzen, S. X. Cadrin, C. Worsøe, M. Dickey-Collas, D. R. Goether, E. M. C. Hatfield, J. P. Kritzer, and R. D. M. Nash. 2017. Lessons learned from practical approaches to

reconcile mismatches between biological population structure and stock units of marine fish. ICES J. Mar. Sci. 74:1708–1722.

Kramer, D. 1970. Distributional atlas of fish eggs and larvae in the California Current region: Pacific sardine, *Sardinops caerulea* (Girard), 1951-1966. CalCOFI Atlas No. 12.

Kuriyama, P. T., J. P. Zwolinski, K. T. Hill, and P. R. Crone. 2020. Assessment of the Pacific sardine resource in 2020 for U.S. management in 2020-2021. U.S. Department of Commerce, NOAA Technical Memorandum NMFS-SWFSC-628.

Kuriyama, P. T., K. T. Hill, J. P. Zwolinski, and P. R. Crone. 2021. Catch-only projection of the Pacific sardine resource in 2021 for U.S. management in 2021-2022. Report to PFMC Agenda Item E4, April, 2021.

Lecompte, F., S. W. Grant, J. J. Dodson, R. Rodríguez-Sánchez, and B. W. Bowen. 2004. Living with uncertainty: genetic imprints of climate shifts in East Pacific anchovy (*Engraulis mordax*) and sardine (*Sardinops sagax*). Mol. Ecol. 13:2169-2182.

Lester, R. J. G. 1990. Reappraisal of the use of parasites for fish stock identification. Aust. J. Mar. Freshwater Res. 41:855-64.

Lluch-Belda, D., D. B. Lluch-Cota, S. Hernandez-Vazquez, C. A. Salinas-Zavala, and R. A. Schwartzlose. 1991. Sardine and anchovy spawning as related to temperature and upwelling in the California Current system. CalCOFI Rep. 32:105-111.

Lluch-Belda, D., D. Lluch-Cota, D., S. E. Lluch-Cota. 2003. Baja California's biological transition zones: Refuges for the California sardine. J. Oceanog. 59:503-513.

Lo, N. C. H., B. J. Macewicz, and D. A. Griffith. 2010. Biomass and reproduction of Pacific sardine (*Sardinops sagax*) off the Pacific northwestern United States, 2003-2005. Fish. Bull. 108:174-192.

Lorenzen, K. 2016. Toward a new paradigm for growth modeling in fisheries stock assessments; Embracing plasticity and its consequences. Fisheries Res. 180:4-22.

Lynn, R. J. 2003. Variability in the spawning habitat of Pacific sardine (*Sardinops sagax*) off southern and central California. Fish. Oceanogr. 12:541-553.

MacCall, A. D. 1984. Review of the biological rationale for identifying subpopulations in fisheries. California Seagrant College Program Report T-CSGCP-013.

Mais, K. F. 1972. A subpopulation study of the Pacific sardine. Calif. Fish and Game 58:296-314.

Marr, J. C. 1957a. The problem of defining and recognizing subpopulations of fishes. In: Contributions to the study of subpopulations of fishes. Special Scientific Report - Fisheries No. 208.

Marr, J. C. 1957b. The subpopulation problem in the Pacific sardine *Sardinops caerulea*. In: Contributions to the study of subpopulations of fishes. Special Scientific Report - Fisheries No. 208.

Marr, J. C. 1960. The causes of major variations in the catch of the Pacific sardine *Sardinops caerulea* (Girard). *In* Proceedings of the World Scientific Meeting on the Biology of sardines and Related Species (H. R. Rosa, G. Murphy eds.), Vol. 3, p. 667-791.

McClatchie, S. 2014. Regional fisheries oceanography of the California Current System and the CalCOFI program. Springer.

McDowall, R. M. 2008. Jordan's and other ecogeographical rules, and the vertebral number in fishes. J. Biogeog. 35:501-508.

McKenzie, D.J., Y. Zhang, E. J. Eliason, P. M. Schulte, G. Claireaux, F. R. Blasco, J. J. H. Nati, and A. P. Farrell. 2020. Intraspecific variation in tolerance of warming in fishes. J. Fish Biol. 98:1536-1555.

Moser, H. G., R. L. Charter, P. E. Smith, D. A. Ambrose, S. R. Charter, C. A. Myer, E. M. Sandknop, and W. Watson. 1993. Distributional atlas of fish larvae and eggs in the California Current region: taxa with 1000 or more total larvae, 1951 through 1984. CalCOFI Atlas 31, 233pp.

Moser, H. G. 1996. The early stages of fishes in the California Current region. CalCOFI Atlas no. 33.

Murphy, G. I. 1966. Population biology of the Pacific sardine (*Sardinops caerulea*). Proc. Calif. Acad. Sci. Fourth Ser. 34:1-84.

Norton, J. G., and J. E. Mason. 2005. Relationship of California sardine (*Sardinops sagax*) abundance to climate-scale ecological changes in the California Current system. CalCOFI Rep. 46:83-92.

Pauly, D., R. Hilborn, and T A. Branch. 2013. Fisheries: Does catch reflect abundance? Nature 497:303-306.

Pavlovic, V., T. Weissgerber, D. Stanisavljevic, T. Pekmezovic, O. Milicevic, J. M. Lazovic, A. Cirkovic, M. Savic, N. Rajovic, P. Piperac, N. Djuric, P. Madzarevic, A. Dimitrijevic, S. Randjelovic, E. Nestorovic, R. Akinyombo, A. Pavlovic, R. Ghamrawi, V. Garovic, and N. Milic. 2021. How accurate are citations of frequently cited papers in papers in biomedical literature? Clin. Sci. (Lond.) 135:671-681.

Pawson, M. G., and S. Jennings. 1996. A critique of methods for stock identification in marine capture fisheries. Fish. Res. 25:203-217.

PFMC. 2022. Terms of Reference for the Coastal Pelagic Species Stock Assessment Review Process for 2023-2024. Pacific Fishery Management Council, November 2022. 40 pp. 2024. Coastal Pelagic Species Fishery Management Plan as Amended through Amendment 21. Pacific Fishery Management Council, April 2024.

Phillips, J. B. 1948. Growth of the sardine, *Sardinops caerulea*, 1941-42 through 1946-47. Fish Bull. 71:5-37.

Pope, K. L., S. E. Lochmann, and M. K. Young. 2010. Methods for assessing fish populations. IN: Quist, M.S., and Hubert, W.A., EDS. Inland Fisheries Management in North America, 3rd Edition. American Fisheries Society, Bethesda, MD.

Radovich, J. 1982. The Collapse of the California Sardine Fishery: What have we learned? CalCOFI Rep. XXIII, 56-78.

Reiss, H., G. Hoarau, M. Dickey-Collas, and W. J. Wolff. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fish. 10:361-395.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada, 191.

Ridgway, G. J. 1971. Problems in the application of serological methods to population studies on fish. Special meeting on the biochemical and serological identification of fish stocks. Conseil International pour L'Exploration de la Mer. Rapports et Proces Verbaux 161:1c-14.

Rodriguez-Sánchez, R., D. Lluch-Belda, H. Villalobos, and S. Ortega-Gargica. 2002. Dynamic geography of small pelagic fish populations in the California Current System on the regime time scale (1931–1997). Can. J. Fish. Aquat. Sci. 59:1980-1988.

Rogers, P. J., and T. M. Ward. 2007. Application of a 'case building approach' to investigate the age distributions and growth dynamics of Australian sardine (*Sardinops sagax*) off South Australia. Mar. Freshw. Res. 58:461-474.

Schofield, E. C. 1934. Early life history of the California sardine (*Sardina caerulea*), with special reference to distribution of eggs and larvae. Fish. Bull. 41:1-48.

Secor, D. H. 2015. Migration Ecology of Marine Fishes. Johns Hopkins Univ. Press. 292pp.

Sette, O. E., and E. H. Ahlstrom. 1948. Estimations of abundance of the eggs of the Pacific pilchard (*Sardinops caerulea*) off southern California during 1940 and 1941. Jour. Mar. Res.7:511-542.

Silva, A., P. Carrera, J. Massé, A. Uriarte, M. B. Santos, P. B. Oliveira, E. Soares, C. Porterio, and Y. Stratoudakis. 2008. Geographic variability of sardine growth across the northeastern Atlantic and the Mediterranean Sea. Fish. Res. 90:56-69.

Sinderman, C. J. and D. F. Mairs. 1959. A major blood group system in Atlantic Sea herring. Copeia 1959:228-232.

Smith, P. E. 2005. A history of proposals for subpopulation structure in the Pacific sardine (*Sardinops sagax*) populations off western North America. CalCOFI Rep. 46:75-82.

Sprague, L. M., and A. M. Vrooman. 1962. A racial analysis of the Pacific sardine (*Sardinops caerulea*) based on studies of erythrocyte antigens. Annal. New York Acad. Sci. 97:131-138.

Teixeira M. C., S. M. Thomaz, T. S. Michelan, R. P. Mormul, T. Meurer, J. V. B. Fasolli, and M. J. Silveira. 2013. Incorrect Citations Give Unfair Credit to Review Authors in Ecology Journals. PLoS ONE 8:e81871. doi.org/10.1371/journal.pone.0081871.

Tibby, R. B. 1937. The relation between surface water temperature and the distribution of spawn of the California sardine. Cal. Fish Game 23:132-137.

Utter, F. M. 1991. Biochemical genetics and fishery management: an historical perspective. J. Fish Biol. 39 (Supplement A):1-20.

Valencia-Gasti, J.A., R. Durazo, E. D. Weber, S. McClatchie, T. Baumgartner, and C. E. Lennert-Cody. 2018. Spring spawning distribution of Pacific sardine in US and Mexican waters. CalCOFI Rep. 59:79-85.

Valle, S. R., and S. Z. Herzka. 2008. Natural variability in $\delta^{18}O$ values of otoliths of young Pacific sardine captured in Mexican waters indicates subpopulation mixing within the first year of life. ICES Journal of Marine Science, 65:174-190.

Vergara-Solana, F. J., F. J. García-Rodríguez, and J. De La Cruz-Agüero. 2013. Comparing body and otolith shape for stock discrimination of Pacific sardine, *Sardinops sagax* Jenyns, 1842. J. App. Ich. 29:1241-1246.

Vrooman, A. M. 1964. Serologically differentiated subpopulations of the Pacific sardine, *Sardinops caerulea*. J. Fish. Board Can. 21:691-701.

Waldman J. R. 2005. Definition of stocks: an evolving concept. *In* Stock identification methods. Applications in fisheries science (S. X. Cadrin, K. D. Friedland, R. Waldman, eds.). p. 7- 16. Elsevier Academic Press, San Francisco.

Walford, L. A., and K. Mosher. 1941. Extension of pilchard spawning to northern Pacific waters indicated. Pacific Fisherman, February, p. 47.

Walford, L. A. 1946. A new graphic method of describing the growth of animals. Biology Bulletin 90: 141–147.

Watson, W. 1992. Distribution of larval Pacific sardine, *Sardinops sagax*, in shallow coastal waters between Oceanside and San Onofre, California: 1978-1986. CalCOFI Rep. 33:89-99.

Wells, J. V., and M. E. Richmond. 1995. Populations, metapopulations, and species populations: what are they and who should care? Wildlife Society Bulletin 23:458–462.

Winans, G. A. 1985. Using morphometric and meristic characters for identifying stocks of fish. NOAA Tech. Memo. NMFS-SWFSC-199.

Wisner, R. L. 1961. Evidence of a northward movement of stocks of the Pacific sardine based on the number of vertebrae. CalCOFI Rep. 8:75-82.

Wolf, R. S. 1961. Age composition of the Pacific sardine 1932–1960. Fish and Wildlife Service, United States Department of Interior, Washington. Research Report 53.

Wolf, R. S. 1964. Observations on spawning Pacific sardines. Cal. Fish Game 50:53-57

Yau, A. 2022. Report from the Pacific Sardine Stock Structure Workshop, November, 2022. Southwest Fisheries Science Center.

Zwolinski, J. P., R. L. Emmett, and D. A. Demer. 2011. Predicting habitat to optimize sampling of Pacific sardine (*Sardinops sagax*). ICES J. Mar. Sci. 68:867-879.

Zwolinski, J. P., and D. A. Demer. 2023. An updated model of potential habitat for northern stock Pacific Sardine (*Sardinops sagax*) and its use for attributing survey observations and fishery landings. Fisheries Oceanography, 33:1-14.