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Local Adaptation along a Latitudinal Gradient in Pacific versus Atlantic-Coast Fishes

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Understanding how species adapt to spatial climate gradients can provide clues to potential evolutionary responses to climate change. Species distributed across broad environmental gradients, such as those that occur along latitudes or altitudes, often exhibit adaptive genetic variation. However, little attention has been given to how the type of environmental gradient shapes adaptive responses. To provide insight into this, local adaptation is compared in related fish species across two very different environmental gradients: the Atlantic and Pacific coasts of North America. Local adaptation is first examined in the California grunion (*Leuresthes tenuis*) and then results are compared to previous work on the Atlantic silverside.

Common garden experiments and wild fish studies were used to test for local adaptation among several traits (growth capacity, sex determination, and vertebral number) of the California grunion across three latitudinal populations: Monterey, CA (36.6°N), Malibu, CA (34.0°N), and Ensenada, MX (31.9°N). Consistent genetic differences in growth capacity between latitudinal

populations were not observed. Wild southern grunion were slightly larger and grew faster than more northern grunion, likely due to environmental effects. Temperature ($p < 0.001$) and photoperiod ($p = 0.011$) were found to significantly affect sex ratios of laboratory reared fish, indicating that grunion have environmental sex determination (ESD); however the level of ESD did not differ among populations. Mean vertebral numbers in wild grunion were nearly identical for all populations.

The lack of latitudinal variation in these traits of the grunion is in direct contrast to the Atlantic silverside, which exhibits a high degree of genetic differentiation in all of the above traits. Results also differ from recent work on the topsmelt, another Pacific coast silverside species. Failure to observe latitudinal variation in the grunion unlike its other taxonomic relatives may be due to its oceanic rather than estuarine habitat, which provides a greater opportunity for broad-scale gene flow and results in a more homogenous environment. Implications for climate change are discussed.

Table of Contents

List of Tables	vi
List of Figures	vii
Acknowledgements	vix
Introduction	1
I. California grunion life history	8
II. Objectives	10
Methods	12
I. Field Collections	12
II. Laboratory Reared Fish	12
Laboratory Rearing	13
Growth Experiments	14
Sex Determination	15
Photoperiod Experiment	16
III. Wild Fish Analysis	17
Length Distributions	17
Vertebral Analysis	17
Age and Growth Analysis	18
Results	20
I. Wild Fish Length Distributions	20
II. Laboratory Reared Fish	20
Hatching	20
Growth Experiments	21
Mortalities	22
Sex Ratios	23
Photoperiod Experiment	24
III. Wild Fish Analysis	25
Ages	25
Growth	26
Sex Ratios	27
Vertebral Numbers	27
Discussion	28
I. Larval Thermal Range	28
II. Growth in Young Grunion	29
III. Growth in Wild Grunion	30
IV. Sex Determination	32
V. Sex Ratios at Spawning	36
VI. Vertebral Numbers	36
VII. Local Adaptation in Pacific versus Atlantic-Coast Fishes	37
VIII. Implications for a Changing Climate	43
IX. Further Studies	44
References	73

List of Tables

Table 1: 2009 hatch lengths for each treatment and population	46
Table 2: 2010 hatch lengths for each population	46
Table 3: 2009 percent mortality by population and temperature	47
Table 4: 2010 percent mortality by population and temperature	47
Table 5: 2009 and 2010 average lengths of lab reared fish by sex	48
Table 6: 2010 sex ratios by container for the 21°C long photoperiod treatment (15 light hours: 9 dark hours)	49
Table 7: Sex ratios by container for the short photoperiod treatment (12 light hours: 12 dark hours)	49
Table 8: 2009 scale data for wild grunion	50
Table 9: 2010 scale data for wild grunion	50
Table 10: 2009&2010 combined scale data	51
Table 11: Sex ratios of wild grunion on the spawning grounds	52
Table 12: Frequency of vertebral counts in wild grunion for each population	53
Table 13: Hypothetical interaction of temperature and photoperiod on sex determination	54

List of Figures

Figure 1: Contour Representation of average weekly sea surface temperatures by latitude for the Atlantic (A) and Pacific (B) coasts of North America	55
Figure 2: 2009 Length frequency distributions for wild grunion from Ensenada (A), Malibu (B), and Monterey (C)	56
Figure 3: Box plot of 2009 wild fish lengths by population and sex	57
Figure 4: 2010 length frequency distributions for wild grunion from Ensenada (A) Malibu (B)	58
Figure 5: Growth in length (A) and weight (B) for 2009 growth experiments	59
Figure 6: Growth in length during 2009 growth experiments from the initial to mid sample (A) and from the mid to final sample (B)	60
Figure 7: Growth in length (A) and weight (B) for 2010 growth experiments	61
Figure 8: Growth in length during 2010 growth experiments from the initial to mid sample (A) and from the mid to final sample (B)	62
Figure 9: Comparison of growth in length between 2009 and 2010 from the initial to final sample (A), initial to mid sample (B), and mid to final sample (C)	63
Figure 10: 2009 sex ratios by population and temperature	64
Figure 11: 2010 sex ratios by population and temperature	65
Figure 12: Sex ratios for the two photoperiod treatments	66
Figure 13: 2009 length frequency distributions by age for wild grunion from Ensenada (A) and Malibu (B)	67
Figure 14: 2010 length frequency distributions by age for wild grunion from Ensenada (A) and Malibu (B)	68
Figure 15: 2009&2010 combined length frequency distribution for wild Ensenada males (A) and females (B)	69
Figure 16: 2009&2010 combined length frequency distribution for wild Malibu males (A) and females (B)	70
Figure 17: Scale to fish length relationship for wild grunion from Ensenada (A) and Malibu (B)	71

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Introduction

Understanding how species adapt to climate variation is becoming increasingly important as we consider the effects that global climate change will have on species' distributions and life histories. One way to shed light on this issue is to examine how species adapt to spatial environmental gradients, such as those that occur along latitudes or altitudes (Conover et al. 2009). Species distributed across broad environmental gradients often display phenotypic variation (V_P). Some of this phenotypic variation may be attributed to genetic variation (V_G) which may represent genotypic differences that are adaptive and maximize fitness in their given environment, while some may be due to environmental variation (V_E), where species simply adjust their phenotypic expression as the environmental conditions change (i.e. phenotypic plasticity). By determining both the genetic and environmental variation within species along spatial climate gradients, not only can we discern how species have locally adapted, but we can then use this knowledge to make predictions about potential adaptation mechanisms to future environmental change.

The phenotypic variance in a trait can be described as $V_P = V_G + V_E + V_{G \times E} + \text{Cov}(G,E)$. $V_{G \times E}$ is the genotype by environment interaction and represents variation by different genotypes in their plastic response to the environment. The covariance term, $\text{Cov}(G,E)$, is the degree to which genotypes are distributed in a predictable pattern across environments that also influence the same phenotypic traits (Conover and Schultz 1995). If the genotypes are distributed across environments in the same direction as the environmental influence this is cogradient (CoGV) variation and the $\text{Cov}(G,E)$ term is positive (Conover and Schultz 1995). In this case the genetic

and environmental variation add together to enhance the phenotypic expression. If however the genotypes are distributed across the environment in a manner opposite to the environmental pattern, this is countergradient variation (CnGV) and the $Cov(G,E)$ term is negative (Conover and Schultz 1995). In the case of countergradient variation, the phenotypic expression is diminished and the direction of the phenotypic pattern depends on whether the environmental or genetic influence is stronger; if the two exactly oppose each other then there will be no phenotypic variation (Conover and Schultz 1995).

To examine species' adaptations across climatic gradients, both the phenotypic pattern in nature should be measured as well as the genetic and environmental contributions from laboratory experiments (Conover et al. 2009). Separating the environmental influence from the genetic influence on a trait can be accomplished by using common-garden experiments, in which individuals from different habitats are raised in the same environment. Individuals should be sampled from multiple locations and multiple environmental conditions should be used, preferably those that span the range of conditions that the species encounters in nature (Conover et al. 2009). The trait reaction norms can then be examined for the populations across the varying environmental conditions. Variation in the trait between environmental conditions gives the environmental pattern, while variation in the trait between populations can be attributed to genetics. If the trait varies between populations across all environmental conditions, such that the reaction norms are above or below each other this indicates either cogradient or countergradient variation depending on the direction of the environmental pattern (Conover et al. 2009). If the reaction norms have different slopes this indicates a genotype by environment interaction.

Examples of counter and cogradient variation are fairly prevalent in the literature. Evidence of countergradient variation has been found for 60 species, primarily for physiological traits, such as growth and development time (Conover et al. 2009). The first example of countergradient variation was discovered in fruit flies along an altitudinal gradient in the mountains of Puerto Rico. In nature, flies from cooler, high altitudes are larger than those from the dry, coastal regions; however, when flies from both localities were raised in common environments the lower coastland flies grew larger (Levins 1969). It was postulated that there was selection for large size in the coastland populations because it is an adaptation to desiccation stress in these dry habitats (Levins 1969). In this case the phenotypic pattern in nature masks the underlying genetic variation between the fruit fly populations.

In ectotherms the observation of Bergmann's rule, which states that body size increases with latitude, may also be the result of countergradient variation (Blanckenhorn and Demont 2004). For example, in the ant lion body size increases with latitude despite the fact that in the north growth should be limited due to the reduced growing season and availability of food (Arnett and Gotelli 1999). Common garden experiments revealed that northern populations display faster growth and shorter development times across all temperatures (Arnett and Gotelli 1999). Thus the overcompensation in growth rate by northern populations is responsible for the Bergmann's body size cline found in this species (Arnett and Gotelli 1999).

Evidence of cogradient variation has only been found in 11 species thus far. In contrast to countergradient variation, cogradient variation typically involves morphological traits (Conover et al. 2009). For example, in *Drosophila* species body size and wing size show cogradient variation; the genetic variation results in greater sizes at higher latitudes where low temperatures also confer large size (James et al. 1995). When *Drosophila subobscura* was

introduced into North and South America, similar latitudinal clines in wing size evolved in just two decades (Gilchrist et al. 2004).

While many cases of countergradient variation and cogradient variation have been observed within species distributed along environmental gradients, how different types of environmental gradients shape local adaptation has been given little attention. Selection pressures will vary in both type and strength across climatic gradients; therefore, species will likely exhibit varying degrees of adaptation and different strategies depending on the type of environment they are distributed along. To provide insight into how the environmental gradient structures adaptive responses, patterns of variation can be compared across differing climatic gradients.

For example, the environmental gradients along the Atlantic and Pacific coasts of North America provide an excellent contrast. The Atlantic coast of North America is one of the steepest temperature gradients in the world. In the summer temperatures are fairly similar, but in winter water temperatures decline by $\sim 1-5^{\circ}\text{C}$ for every degree of latitude, causing a large range in annual temperatures of up to 20°C in the north (Fig 1A) (Schroeder 1966). This means that winters are not only harsher in the north, but that winter begins earlier resulting in a growing season that is 2.5 times shorter (Conover 1992). On the other hand, the temperature gradient on the Pacific coast is much weaker, with a mean latitudinal temperature decrease that is three times slower ($\sim 0.4^{\circ}\text{C}/^{\circ}\text{N}$) than that observed on the Atlantic coast ($\sim 1.1^{\circ}\text{C}/^{\circ}\text{N}$) (Baumann and Conover *in press*). Summer sea surface temperatures along the Pacific coast (26°N - 47°N) range from 14° - 26°C in the summer and from 9° - 19°C in the winter (UCAR 2010). Seasonal temperature fluctuations are also much smaller, with summer and winter temperatures differing by only 2- 6°C (UCAR 2010) (Fig 1B).

Given the large climatic gradient of the Atlantic coast of North America, we would expect several adaptations would be needed for species to proliferate at both northern and southern latitudes, and this has indeed been shown to be the case; numerous cases of countergradient and cogradient variation have been cited for Atlantic coast species. A prime example is the Atlantic silverside, *Menidia menidia*, an estuarine fish found from northern Florida (30°N) to the Gulf of St. Lawrence (46°N) (Conover and Present 1990). Genetic variation has been found in several traits along this species latitudinal gradient, and trait differences between populations appear to be adaptations to the local environment

For example, the Atlantic silverside displays countergradient variation in growth capacity, an adaptation which allows northern populations to make up for the reduced growing season they experience (Conover and Present 1990). Common garden experiments showed that higher latitude populations grow faster than southern populations when raised under similar conditions (Conover and Present 1990). This faster growth is achieved through increased food consumption, growth efficiency, and foraging activity (Present and Conover 1992, Chiba et al. 2007). In addition, northern fish have evolved increased phenotypic plasticity, allowing them to further accelerate their growth at higher temperatures (Conover and Present 1990). The increased growth rate by northern fish almost exactly compensates for the reduced growing season they experience so that populations from all latitudes are similar in size by their first winter (Conover and Present 1990).

Selection for increased growth and large size in the north was shown to be a result of size-selective winter mortality; only large fish are capable of surviving the winter because they can store more energy reserves than smaller fish and thus avoid starvation (Schultz and Conover 1997, Schultz et al. 1998, Munch et al. 2003). However, the increased growth by northern fish

comes at a cost, which explains why southern fish would evolve sub-maximal growth rates. Northern fish show reduced swimming endurance and burst swimming speeds (Billerbeck et al. 2001), as well as increased predator mortality compared to southern fish (Lankford et al. 2001, Munch and Conover 2003). These trade-offs are the result of the increased metabolic cost of increased growth and consumption; northern fish have a higher standard metabolic rate and a lower scope for activity than southern fish (Arnott et al. 2006). In the north, *Menidia* maximize their growth despite the costs because of the necessity to be large for winter survival; however in the south, where winters are less severe, these fish choose to balance growth with swimming performance.

In contrast to growth, vertebral number in the Atlantic silverside shows a pattern of cogradient variation. Vertebral number increases with latitude in accordance with Jordan's rule (Billerbeck et al 1997). This variation is a result of both genetic and environmental factors. Lower temperatures result in higher numbers of vertebrae and common garden experiments showed that northern populations have a higher vertebral number across all temperatures (Billerbeck et al. 1997). Genetic variance is responsible for most of the phenotypic variance in vertebral number observed between northern and southern populations, while the temperature effect is small (Billerbeck et al. 1997). Therefore, it would appear that Jordan's rule is adaptive, but its significance is still unclear (Billerbeck et al. 1997).

In addition to variation in growth capacity and vertebral number, Atlantic silversides have also adjusted their sex-determination strategy to account for differences in growing and breeding season lengths along their latitudinal gradient. Silversides display both environmental sex determination (ESD) and genetic sex determination (GSD). For populations that display ESD, females are produced early in the breeding season when temperatures are cold while males

are produced later in the breeding season when temperatures are warm (Conover and Kynard 1981). This sex determination strategy allows females more time to grow before spawning, which thus increases their relative fecundity (Conover 1984). As latitude increases though, the level of ESD tends to decline and some of the northern populations only display GSD (Conover and Heins 1987a). This occurs because ESD is no longer beneficial at northern latitudes since the reduced growing and breeding seasons do not allow time for females produced early on to gain a size advantage (Conover and Heins 1987a).

These experiments on the Atlantic silverside provided significant insights into the kinds of adaptive strategies that species use. In addition, they revealed that the primary selection agents acting on the silverside, and likely many other Atlantic species, are growing season length and size-selective winter mortality.

On the Pacific coast, adaptation pattern patterns may differ from those seen on the Atlantic coast given the different environmental gradient. Environmental selection pressures on the Pacific coast are likely weaker; consequently, the genetic variation in traits between latitudinal populations should be smaller. Phenotypic plasticity is also predicted to be diminished given the small yearly fluctuation in temperature. Furthermore, growing season length and size-selective winter mortality are unlikely to be the principal selection agents on the Pacific coast, like they are on the Atlantic coast, and thus alternate mechanisms of adaptation may be observed.

For example, Pacific coast organisms may exhibit latitudinal compensation in growth just like Atlantic coast organisms, but they may do so by utilizing a different growth strategy. Unlike the Atlantic coast where growth is constrained by season, Pacific coast organisms are likely able to grow throughout most of the year. Therefore, countergradient variation in growth

would not be expected since it is thought to be an adaptation strategy that evolves in response to variation in growing season length (Conover and Present 1990, Yamahira and Conover 2002). Instead, Pacific coast organisms may exhibit thermal adaptation in growth. In this case, all individuals have the same maximal growth rate, but growth is maximized at the temperature that is most commonly experienced in their environment and then reduced in other environments (Levinton 1983, Yamahira and Conover 2002). This scenario is consistent with a genotype by environmental interaction and is expected when local mean temperatures vary between populations (Yamahira and Conover 2002).

The latitudinal pattern and type of environmental sex determination (ESD) may also vary between Atlantic and Pacific organisms. In Pacific species with ESD, similar levels would be expected for all latitudinal populations, since there is little variation in the length of the growing and breeding seasons along most Pacific coast species' distributions. In addition, it is also possible that an environmental cue other than temperature may be responsible for sex determination in Pacific species. Since temperature changes throughout the year are relatively small on the Pacific coast, temperature may not be the most reliable predictor of the environment that offspring are entering; other cues, such as photoperiod length, may be better environmental indicators. In the Atlantic silverside, a photoperiod influence on sex-determination was tested for, but no effect was found (Conover and Heins 1987b).

To contrast local adaptation in Atlantic and Pacific species, patterns of latitudinal variation were compared in two ecologically equivalent fish species, the Atlantic silverside (*Menidia menidia*) and the California grunion (*Leuresthes tenuis*). The California grunion was chosen as the Pacific counterpart to the Atlantic silverside because it is the same family as the

silverside (Atherinopsidae), and has a similar life cycle and reproductive ecology. In addition, the California grunion, like the Atlantic silverside, has a broad geographic range (24°-36.6°N).

California grunion life history

The California grunion (hereafter referred to as grunion) is found along the west coast of North America from Bahia Magdalena, Baja California, Mexico (24°N) to Monterey Bay, California (36.6°N) (Reynolds et al. 1977). The principal spawning range occurs between Punta Abreojos, Baja California (26.7°N) and Point Conception, California (34.5°N) (Eschmeyer et al. 1983), but a small spawning population also exists in Monterey Bay (Phillips 1943). Along its distribution, monthly mean temperatures range from 12-27.5°C annually (Reynolds et al. 1977). The grunion is thought to remain in shallow waters for the majority of its life, but little is known about where this species spends its time when it is not breeding. Allozymic variation analysis showed that there is enough gene flow between populations to limit the formation of distinct subpopulations, but that there may be some degree of natal homing (Gaida et al. 2003).

Spawning in grunion takes place from February to August on the 3-4 nights surrounding the new and full moon (Smyder and Martin 2002), with the exception of the Monterey population in which spawning occurs from June-August on the full moon only. The largest females start spawning early in the season and then spawning peaks in April and May when the smaller fish join in (Clark 1925). Grunion are batch spawners, meaning that females lay their eggs every two weeks throughout the season until July and August when spawning slows. Fecundity in the grunion ranges from 1,000-3,000 eggs per fish and is size dependent (Thompson 1919, Clark 1925).

The grunion's unique spawning behavior has generated a lot of public interest in this species; during spawning grunion fully emerge from the water and come onto the beach to lay

their eggs during the 1-2 hours following high tide. They come ashore with the waves and the females bury themselves in the sand tail first to lay their eggs while multiple males (2-9) surround them to release milt (Walker 1952, Byrne and Avise 2009). The fish then return to the ocean on the next set of the waves. These spawning occurrences are referred to as grunion runs and anywhere from a few to thousands of fish may come onto the beach during these runs. Over the next several days, the eggs are buried 15-20 centimeters below the surface (Moffatt and Thompson 1978). They incubate in the sand for about 14 days until the next set of semilunar high tides comes to wash them out to sea (Walker 1952). Eggs are ready to hatch in about 10 days, but wave-action or mechanical agitation is needed to trigger actual hatching (Greim and Martin 2000).

Newly hatched grunion larvae are thought feed on plankton in the surface waters (Fritzche et al. 1985) and then may enter bays and harbors (Ehrlich and Muszynski 1982, Reynolds et al. 1977). During the grunion's first year of life they grow rapidly, reaching sizes of 11 cm and greater, and they are ready to spawn by the end of their first year (Clark 1925). Growth is rapid in late summer/early fall, slows in the winter, and accelerates again right before spawning (Clark 1925). During the breeding season, growth comes to a halt and when it resumes in late July or August, a breeding annulus is laid down on the scales (Clark 1925). A winter annulus has also been found in a few fish, but this is rare, and the winter annulus has different characteristics than the breeding annulus (Clark 1925). In the following years, growth in the grunion slows considerably; in years two and three grunion only grow another 2cm and 1.5cm, respectively. (Clark 1925). Grunion can live between 2-4 years, but there are few fish that live to the end of their third year, and four year olds are extremely rare (Clark 1925).

Objectives

The objective of this study is to contrast local adaptation in fishes along the Atlantic and Pacific coasts of North America. Common garden experiments and wild fish studies were used to examine local adaptation in multiple traits (growth rate, sex ratios, and vertebral number) of the California grunion *Leuresthes tenuis* across its latitudinal gradient. Results were then compared with previous work on latitudinal variation in the Atlantic silverside *Menidia menidia*. The questions being asked are, do Pacific coast-fishes locally adapt to their environment as is seen in Atlantic coast fishes; and if so how do the different environmental gradients between coasts impact the type of adaptations, they degree to which species adapt, and the trait reaction norms.

Methods

Field Collections

In the spring of 2009 and 2010, grunion eggs and mature adults were collected from three latitudinal populations: Monterey Bay, CA (36.6°N), Malibu, CA (34.0°N), and Ensenada, MX (31.9°N). In 2009, field collections occurred on April 25th and 26th in Ensenada, May 11th in Malibu, and June 7th in Monterey. In 2010, fish were taken on April 30th and May 1st from Ensenada and May 29th and 30th from Malibu. Eggs could not be obtained from Monterey in 2010 due to a lack of spawning fish at this location. In both years an attempt was made to collect eggs south of Ensenada, but a spawning population could not be found.

To collect grunion eggs, mature grunion were captured by hand during their beach spawning runs. Fish were strip-spawned, and eggs and milt were mixed in plastic containers to allow fertilization. To ensure genetic diversity at least 10-20 fish of each sex were used for strip-spawning, except for Monterey, where due to the small size of this population only 5 females and 8 males were able to be obtained. Eggs were transported back to Flax Pond Marine Lab within 60 hours after fertilization (Stony Brook University, Long Island, NY) by wrapping embryos in damp paper towels and storing them in thermos cans. Once at Flax Pond Lab, eggs were incubated in temperature controlled baths. Mature fish used for strip-spawning and any extra fish taken were frozen for later analysis. In 2010, the mature fish used for strip-spawning in Malibu could not be kept, but 35 mature fish were sacrificed from Malibu on June 29th to be used for this study.

Laboratory Reared Fish

Laboratory Rearing

Grunion were reared in common environments (same light, temperature, food, salinity, etc.) at three rearing temperatures spanning the range of temperatures that grunion experience in their natural environment. In 2009, grunion eggs from Ensenada were hatched out at 27°C, 21°C, and 15°C. However, due to poor hatching success at 27°C and 15°C, eggs from the remaining populations in 2009 and eggs in 2010 were hatched out at 21°C. Once larva reached 6-9 mm they were separated into the different temperature treatments. While this differs slightly from the protocol used in the Atlantic silverside experiments (Conover and Present 1990), where hatching took place at the same temperature as larval/juvenile growth, it was necessary given the narrow thermal hatching tolerance of grunion (Smyder and Martin 2002, Ehrlich and Farris 1971), and likely had little impact on detection of trait differences between populations. In addition, due to poor survival of Ensenada fish at 27°C, the bath was turned down to 25°C. Ensenada larvae of 7-9 mm were taken from the 21° bath and acclimated to 25°C degrees to be used for the experiments. Thus rearing temperatures for 2009 were 25°C, 21°C, and 15°C. In 2010, rearing temperatures were 25°C, 21°C, and 17°C. In 2010, 17°C was used instead of 15°C because of the low survival and poor growth at 15°C in 2009.

Grunion were reared in aerated 20L containers sitting in large temperature controlled baths (700L). Each container had three 5cm holes with 400µm mesh screen to allow for water exchange within the bath. Water for the baths was drawn from saline ground wells. Bath temperatures were controlled using immersion heaters and chillers. Salinity was 30‰ (±2‰). A photoperiod of 15 light hours to 9 dark hours was used.

Hatching was initiated by turning up the air within the buckets. A hatch sample of 8-12 fish was taken each year from all populations and total length (TL) was measured to the nearest

0.1 mm using calibrated digital pictures and ImagePro in 2009 and ImageJ in 2010. Grunion larvae were fed both rotifers (*Brachionus plicatilis*, Atlantis Marine World, Riverhead, NY) and newly hatched brine shrimp nauplii (*Artemia salina*, San Francisco strain, Brine Shrimp Direct, Inc.) for the first 5-7 days. After which, they were only fed nauplii.

Growth Experiments

Growth experiments were initiated 7-12 days after hatching, once fish reached 6-9 mm. Larvae from each population were randomly separated into three replicates of 40 fish each per temperature treatment and acclimated to their assigned temperature. In addition, a sample of 15-20 fish were sacrificed at the start of the growth experiments to obtain initial total length measurements (nearest 0.1mm using calibrated digital photos and ImagePro or Image J software). In 2010, an initial pooled wet mass (nearest 0.1mg, Mettler AE163) was also obtained. Leftover fish were divided into replacement buckets for each temperature treatment. Any fish that died within the first 3 days of the experiment was replaced with fish from the replacement bucket to compensate for mortality due to handling.

There were some exceptions to the above protocol. Due to a lack of eggs from the Monterey population there were only two replicates of 40 fish each for the 25°C and 21°C temperature treatments and no replicates for the 15°C treatment. Due to a shortage of surviving Ensenada larvae at 25°C in 2009, replicates contained 33, 33, and 37 fish, respectively. For Ensenada fish grown at 15°C there were only enough fish for one replicate. There were no replacement fish for the Monterey or Ensenada growth experiments in 2009.

During growth experiments fish were fed an unlimited amount of 24 hour old brine shrimp nauplii. Halfway through the experiment, when larvae reached 20-22 mm, a mid-sample was taken from each replicate. For the mid-sample in 2009, a sample of 8-15 fish from each

replicate was euthanized using MS-222 (Tricaine Methanensulfonate) and total length (0.1 mm, using calipers), standard length, and wet weight (same scale) were measured for each fish. In 2010, the mid sample was obtained by taking live total length measurements (nearest 1 mm) on 13-15 fish from each replicate. At the end of the experiment all remaining fish were sacrificed using MS-222 to obtain final length and weight measurements and all fish were preserved in 10% borax buffered formalin. Experimental durations varied depending on treatment: the 25°, 21°, and 17° treatments lasted 49-55, 71-74, and 93-94 days, respectively. This was done so that all fish were 35-40 mm at the end of the experiment.

The growth rate for each replicate was calculated by taking the difference between the average final length/weight measurements and the average initial length/weight measurements and then dividing by the experimental duration; thus for each replicate there is one mean daily growth rate (Conover and Present 1990). In the same manner growth rates were also calculated from the initial to mid sample and from the mid sample to the final sample to determine whether growth stays the same throughout the experiment (Conover and Present 1990). General linear models (LM) were used to test for significant population effects, temperature effects, population by temperature interactions, and year effects for all experimental intervals. LMs were followed by Tukey and Holm-Sidak multiple comparison tests. Statistical tests were performed using Sigma Stat 3.11 (2004 Systat Software, Inc) and Minitab 16.1.0 (2010 Minitab Inc)

Percent mortality for each replicate was calculated by taking the difference between the initial number of fish and the number of fish left at the end of the experiment divided by the initial number of fish. Two-way chi square tests of independence were used to test for significant differences in percent mortality among populations and temperatures.

Sex Determination

Fish used in the growth experiments and extra fish, when available, were used for sex ratios. Fish were reared at their experimental temperatures until they reached a length of 35-40 mm where sex can easily be determined. Fish were dissected and their gonads removed for examination under the microscope (100x). A female was defined as any fish that had oocytes and male was defined as any fish that did not have oocytes. The sex of the fish was considered determinable down to the smallest size at which a female could easily be recognized. For each population at each temperature treatment the sex ratio was calculated as the proportion of females to the total number of fish sexed ($F/(F+M)$). Two-way chi square tests of independence were performed to test for a temperature effect and a population effect within each temperature treatment. One way goodness of fit tests were also used in 2009 to test for departures from 1:1 ratios. The level of environmental sex determination (ESD) was calculated for each population by subtracting the proportion of females ($F/(F+M)$) at the 25° treatment from the proportion of females ($F/(F+M)$) at the 17° treatment (Conover and Heins 1987a). Additionally length was recorded with sex, and ANOVAs and t-tests were used to test for sexual dimorphism.

Photoperiod Experiment

In 2010, in addition to grunion being reared at a photoperiod of 15 light hours to 9 dark hours, a subsample of grunion from each population were also reared under a short photoperiod of 12 light hours to 12 dark hours at 21°C. Hatching took place at the longer photoperiod at 21°C, and then larvae were transferred to the short photoperiod treatment once they reached sizes of 6-8 mm. For the Ensenada population three replicates of 25 fish each were created and for the Malibu population three replicates of 55 fish each were created. Fish were raised until they reached a size in which sex could easily be determined (40-45mm) and were sexed in the manner described above. Sex ratios were determined and compared with the sex ratios at the 21°C long

photoperiod treatment (15 light hours:9 dark hours). Due to high amounts of variation between replicated containers, sex ratios were analyzed as proportion data. Female proportions were arcsine transformed and then a two-way ANOVA with replication was performed to test for a photoperiod effect and a population effect. This was followed by Tukey multiple comparison tests.

Wild Fish Analysis

Length Distributions

In 2009 and 2010 mature grunion were separated into males and females and length was recorded for each fish to the nearest 0.5 cm. In 2010, a random sample was obtained to determine to determine the sex ratio at spawning. Since the data either failed normality or equal variance tests, Krustal-Wallis one-way ANOVAs and Mann-Whitney 2 sample tests were performed on length to test for population and sex effects. This was followed by Dunn multiple comparisons.

Vertebral Analysis

Radiographs of approximately 50 mature fish from each population, except for the Monterey population in which only 13 fish were available, were taken using an analog Kramex PX- 20N x-ray system (10mA/80kVp, exposure time 2 seconds) and Kodak Industrex type M film. Radiographs were developed in a dark room and then viewed against a light board to count vertebrae directly. Vertebral counts included the centra between the basioccipital and urostyle (Billerbeck 1997). Sex and total fish length were also recorded. Since the data did not conform to normality, Krustal-Wallis one-way ANOVAs and Mann-Whitney 2 sample tests were used to test for significant population and sex effects. In addition, a correlation between vertebral number and fish length was also tested for.

Age and Growth Analysis

To determine age and calculate growth of mature grunion, scales were removed from a subset of Malibu and Ensenada fish that were collected in 2009 and 2010. Fish were divided into length classes (1/2 cm) and fish from each length class were used in the analysis. In 2009, approximately 50 fish were used from each population. In 2010, 60 fish were used from Ensenada and 35 fish from Malibu.

Fifteen to twenty scales from each fish were removed from just below the silvery lateral band between the origin of the first dorsal fin and the posterior margin of the second dorsal fin, since this was found to be the best region of the fish to analyze scales from (Clark 1925). For each fish, total length was measured and sex was recorded. A Carver Scale Press was then used to make imprints of the scales onto slides. The scale imprints were examined and the number of annuli counted to age each fish. Annuli were determined by looking for ridges running obliquely to the preceding ridges and a narrow zone of broken ridges in the anterior region that occurs just before the annulus (Clark 1925). Since grunion lay down breeding annuli, a fish with zero annuli was labeled as age one, a fish with one annulus was labeled as age two, and so on (Clark 1925). Average lengths at age were calculated for males and females. General linear models (LM) were used to test for population effects, sex effects, population by sex interaction, and year effects on length at ages 1 and 2.

The three clearest scales were chosen for each fish to use for scale length measurements. Measurements were made from the core to the center anterior tip of the scale. For fish in which annuli were present, measurements were also made from the core to each annulus. The average scale length was calculated for each fish and used to construct a scale to fish length relationship

for each population. An analysis of covariance was conducted to determine if the relationship differed between populations.

The scale to fish length relationship was then used to back-calculate the length at age one for fish that were two years and older. Back-calculations were performed using the Fraser-Lee formula: $L_i = c + (L_c - c)(S_i/S_c)$ where L_i = back-calculated length at age 1, c = intercept of the scale to fish length regression, L_c = length of fish at capture, S_i = mean length of scale to the first annulus, and S_c = mean total scale length. Growth during year one (mm/day) was then calculated by dividing the average back-calculated length at age one by 365 days. General liner models were used to test for a population effect, a sex effect, a population by sex interaction, and year effects.

Results

Wild Fish Length Frequency Distributions

In 2009, wild fish lengths ranged from 12 to 20 cm. Fish lengths declined with increasing latitude ($p < 0.001$) (Fig 2 and Fig 3). The average (\pm SE) lengths for Ensenada, Malibu, and Monterey fish were 17.0 ± 0.15 cm, 15.6 ± 0.19 cm, and 14.8 ± 0.49 cm, respectively. In addition wild female grunion (17.0 ± 0.25 cm) were significantly larger than wild males (15.8 ± 0.15 cm) ($p < 0.001$) (Fig 3). Dunn pairwise comparisons indicated that the Ensenada fish were significantly larger than the Malibu and Monterey fish ($p < 0.05$), but that Malibu and Monterey wild fish did not differ in length.

In 2010, wild fish lengths ranged from 11 to 19 cm. Differences in length were not observed between populations (Fig 4). Ensenada grunion had an average length of 14.6 ± 0.13 cm and Malibu grunion had an average length of 15.1 ± 0.30 cm. Females were again significantly larger (15.5 ± 0.22 cm) than males (14.4 ± 0.17 cm) ($p < 0.001$) (Fig 4). The Ensenada 2010 wild fish were significantly smaller than Ensenada 2009 fish ($p < 0.001$), but there was no difference in lengths between years for Malibu grunion.

Laboratory Reared Fish

Hatching

In 2009, grunion larvae were between 5 and 7 mm at hatching. Differences in hatch lengths between temperature treatments or populations were not observed (Table 1). Hatching occurred sooner at higher temperatures (Table 1). For Ensenada grunion, hatching at 27°C, 21°C, and 15°C occurred at 7 days, 10 days, and 15 days after fertilization, respectively. Once

hatching was initiated, larvae continued to emerge from the eggs over the next several days. In 2010, Ensenada larvae hatched out at considerably smaller sizes (4.42 ± 0.16 mm) than Malibu larvae (6.03 ± 0.19 mm) (Table 2)

In 2009 when the Ensenada eggs were hatched at different temperatures, survival after hatching at the two extreme temperatures, 27°C and 15°C, was very low. The majority of larvae died within the first few weeks. Due to this low survival, fish from 21°C were acclimated to 25°C for the growth experiments and only one growth replicate was able to be created at 15°C. Only 13 fish hatched out at 27°C survived until the termination of the experiment, and only 1 fish survived from those hatched out at 15°C.

Growth Experiments

In 2009, due to poor survival at 15°C growth rates were only estimated for the 21°C and 25°C treatments. Growth rates were significantly faster at 25°C than 21°C in both length ($p < 0.001$) and weight ($p < 0.001$) (Fig 5A-B). Over the entire experiment growth in length averaged 0.59 ± 0.012 mm/day at 25°C and 0.43 ± 0.006 mm/day at 21°C. Growth in weight from the mid to final sample averaged 9.0 ± 0.27 mg/day at 25°C and 5.8 ± 0.20 mg/day at 21°C. In all cases growth in length from the initial to the mid sample was faster than growth from the mid to final sample (Fig 6A-B). From the initial to the final sample (8-40 mm), growth rates differed among populations at 21°C ($p = 0.021$), but not at 25°C. Tukey comparisons indicated that significant population differences in growth occurred only between the Ensenada and Monterey fish (Fig 5A). Malibu and Monterey grunion grew faster than Ensenada grunion at both the 25°C and 21°C temperature treatments. Monterey grunion grew faster than Malibu grunion at 21°C, but not at 25°C. The majority of the growth differences occurred from the initial to mid sample (8-20mm), even though a significant population effect was not found at the

0.05 level ($p=0.08$) (Fig 6A). From the mid sample to the final sample growth rates were nearly the same for all populations (Fig 6B). There was no significant population effect for growth in weight (Fig 5B).

In 2010, growth rates increased with increasing temperature for length and weight ($p<0.001$). Average growth rates in length from the initial to final sample for the 17°C, 21°C, and 25°C treatment were 0.34 ± 0.003 mm/day, 0.45 ± 0.005 mm/day, and 0.59 ± 0.011 mm/day, respectively (Fig 7A). Growth in weights from the initial to the final sample were 3.3 ± 0.05 mg/day for 17°C, 4.5 ± 0.06 mg/day for 21°C, and 5.6 ± 0.12 mg/day for 25°C (Fig 7B). Again, growth rates were faster during the first half of the experiment than the second half (Fig 8A-B). Overall, Ensenada grunion grew faster than Malibu grunion at all three temperatures ($p<0.001$) (Fig 7A). There was also a significant population by temperature interaction ($p=0.003$). Tukey pairwise comparison tests between populations were only significant at 25°C ($p<0.001$) and 21°C ($p=0.024$). The variation in growth between populations was only evident from the initial to the mid sample ($p<0.001$) (Fig 8A). From the mid sample to the final sample population growth rates were nearly equal (Fig 8B). There was no difference in growth in weight between populations (Fig 7B).

Ensenada grunion grew significantly faster in length in 2010 compared to 2009 ($p<0.001$) (Fig 9A). The majority of this difference in growth between years occurred between the initial and mid samples ($p=0.019$) (Fig 9B). There was no year effect for growth rates from the mid to final sample (Fig 9C). Growth rates for Malibu grunion did not differ between years (Fig 9A-C).

Mortalities

In 2009, temperature significantly influenced percent mortality for Ensenada and Malibu grunion ($p<0.001$). Mortality for grunion larvae reared at the 15°C treatment was nearly 100%

(Table 3). Towards the end of the experiments, day 56 of the Ensenada experiment and day 58 of the Malibu experiment, the 15°C bath was turned up to 17°C to try and keep the remaining fish alive to be sexed. At this point only 3 Ensenada and 10 Malibu larvae remained, and none had grown beyond 20mm. Once the bath was turned up to 17°C larval growth and swimming increased, and no Malibu larvae died after this. Average percent mortality for the 25°C and 21°C treatment ranged from 0.06 to 0.5 (Table 3), differing among populations ($p < 0.001$). Percent mortalities for the Monterey population were considerably lower than those for the Ensenada and Malibu populations (Table 3).

In 2010, average percent mortalities were lower than 2009, ranging from 0.1 to 0.37. Percent mortalities for Malibu larvae were lower than Ensenada larvae at all temperature treatments, and differed significantly at 25°C ($p < 0.001$) and 21°C ($p = 0.02$) (Table 4). In both years, the majority of the mortalities occurred before the mid sample was taken.

Sex Ratios

Sex was able to be identified in fish as small as 30 mm. In 2009, sex ratios for all temperature treatments and populations were male biased and were found to deviate significantly from a 1:1 ratio (p values ranged from < 0.001 to 0.013) (Fig 10). For the 25°C treatment, the proportions of females ($F/(F+M)$) for Ensenada, Malibu, and Monterey grunion were 0.17, 0.30, and 0.30, respectively. For the 21°C treatment the proportions of females were 0.17 for Ensenada, 0.21 for Malibu, and 0.32 for Monterey. Due to poor survival of fish at the 15°C treatment, sex ratios were unable to be determined. Sex ratios did not differ significantly between temperatures or populations.

For both Ensenada and Malibu grunion in 2010, temperature significantly impacted sex ratios ($p < 0.001$); the proportion of females increased as temperatures decreased (Fig 11). For

Ensenada grunion the proportions of females ($F/(F+M)$) for the 25°C, 21°C, and 17°C treatments were 0.29, 0.44, and 0.77, respectively. For all temperature treatments the proportion of females for Malibu grunion was higher than Ensenada grunion (Fig 11). Proportions of females for Malibu grunion were 0.43, 0.69, and 0.93 for the 25°C, 21°C, and 17°C treatments, respectively. Sex ratios between populations were significantly different at 21°C ($p < 0.001$), but not at the other temperature treatments. The levels of environmental sex determination (ESD) [$(F/(F+M))$ at 17°C - $(F/(F+M))$ at 25°C] was 0.477 for Ensenada and 0.504 for Malibu

It should be noted that sex ratios for the 21°C treatment were significantly different between containers for both Ensenada ($p = 0.034$) and Malibu ($p < 0.001$) populations, with some containers being male biased and others female biased (Table 6). In addition, it was noted that some of the female oocytes from the 17°C treatment were lighter in color and less developed than females in the other two treatments; however females were still easily recognizable. Several lighter and less developed female oocytes were also observed in the 21°C containers that were female biased.

In 2009, female grunion were found to be significantly larger than male grunion ($p < 0.002$); however differences were small (~1mm) (Table 5). In 2010, females and males were similar in size overall (Table 5), but t-tests revealed that females were slightly larger than males ($p < 0.05$) in 3 out of the 6 population/temperature treatments.

Photoperiod Experiment

For both the Ensenada and Malibu populations female proportions were lower at the short photoperiod (12 light hours:12 dark hours) compared to the long photoperiod (15 light hours:9 dark hours) (Fig 12). Proportions of females at the short photoperiod for Ensenada and Malibu populations were 0.16 and 0.44, respectively. Sex ratios between containers did not differ for the

short photoperiod treatment (Table 7), but they were heterogeneous for the long photoperiod treatment and thus were not pooled (Table 6). The data were analyzed using proportions of females rather than counts. A two-way ANOVA with replicates indicated a significant overall photoperiod effect ($p=0.011$) and a significant population effect ($p=0.027$). Tukey comparisons indicated a significant difference in the proportion of females between photoperiods for the Malibu population ($p=0.038$), but not for the Ensenada population ($p=0.063$). Female oocytes were lighter in color and less developed at the short photoperiod compared to the long photoperiod, and in some instances only a few oocytes were present. The Malibu fish were raised for nine days longer than the Ensenada fish in an attempt to make sure that all females could be recognized.

Wild Fish Analysis

Ages

In 2009, fish were primarily one and two years old. Only a few fish were found to be age 3. For Ensenada grunion, lengths at age 1 ranged from 14 to 18 cm and at age 2 from 15 to 20 cm (Fig 13A). Malibu lengths ranged from 12 to 16 cm for age 1 fish and 15 to 19 cm for age 2 fish (Fig 13B). Average lengths at age for both 1 and 2 year olds were significantly larger for Ensenada fish compared to Malibu fish ($p<0.001$), and females were significantly larger than males ($p<0.001$) (Tables 8).

In 2010, fish were primarily ages 1 and 2, with a few 3 year olds; however, in 2010 there were many more age 1 fish than age 2 fish, particularly for the Ensenada population (Fig 14A-B). For Ensenada grunion, lengths at age 1 ranged from 12 to 17 cm and lengths at age 2 ranged from 14 to 18 cm (Fig 14A). Malibu age 1 lengths ranged from 11-17 cm and age 2 lengths ranged from 16-18 cm (Fig 14B). Females were significantly larger than males ($p<0.001$), but

there was no difference in lengths at age 1 between populations (Table 9). Due to the small numbers of age 2 fish, they were not included in the analysis. The 2010 Ensenada grunion were significantly smaller than 2009 fish at ages 1 ($p < 0.001$) and 2 ($p = 0.003$) (Table 8&9).

Overall, age 2 fish were 2-3 cm larger than age 1 fish and females were about 1 cm larger than males (Table 10, Fig 15&16). Average lengths at age tended to be slightly larger for Ensenada grunion compared to Malibu grunion (Table 10).

Growth

The intercepts of the scale to fish length relationships were significantly different between populations ($p = 0.004$), therefore separate back-calculation equations were used. Both scale to fish linear regressions were significant ($p < 0.001$) and yielded coefficients of determination, R^2 , of about 0.80 (Fig 17). Average back-calculated lengths at age 1 using these relationships tended to produce lengths about 1 cm larger than average lengths of age 1 wild grunion (Tables 8, 9&10).

In 2009, growth rates for Ensenada grunion ranged from 0.35 to 0.46 mm/day for males and 0.43 to 0.50 mm/day for females. Malibu grunion growth rates ranged from 0.38 to 0.44 mm/day for males and 0.41 to 0.45 mm/day for females. Growth rates were significantly greater for Ensenada grunion compared to Malibu grunion ($p < 0.001$) and females grew significantly faster than males ($p < 0.001$) (Tables 8).

In 2010, growth rates for male and female Ensenada grunion ranged from 0.35 to 0.46 mm/day and 0.40 to 0.47 mm/day, respectively. Malibu grunion growth rates were between 0.38 and 0.44 mm/day for males. There was only one age 2 Malibu female, and thus an average growth rate could not be calculated. In 2010, a population difference in growth rates was not observed (Table 9). Females grew significantly faster than males ($p = 0.046$) (Table 9).

Ensenada grunion in 2010 grew significantly slower than in 2009 ($p=0.005$), but there was no difference in growth between years for Malibu grunion (Tables 8&9).

Combined scale data indicates that Ensenada grunion grew faster than Malibu grunion ($p=0.02$) and that females grew faster than males ($p<0.001$) (Table 10).

Sex Ratios

On the spawning grounds male grunion were found to be 2-3 times more abundant than female grunion (Table 11). In Ensenada the random sample contained 70 males and 30 females. In Malibu the random sample consisted of 26 males and 9 females.

Vertebral Numbers

Mean vertebral numbers for all populations were nearly identical and did not vary between males or females (Fig 18). Mean vertebral numbers for Ensenada, Malibu, and Monterey were 47.6 ± 0.09 , 47.5 ± 0.13 , and 47.3 ± 0.29 , respectively. While vertebral numbers ranged from 43 to 49, almost all grunion had either 47 or 48 vertebrae (Table 12). No correlation was found between vertebral number and fish length.

Discussion

In this study, common garden experiments and wild fish studies were used to test for local adaptation in several traits (growth capacity, sex determination, and vertebral number) of the grunion across latitudinal populations. Common garden growth experiments did not reveal consistent differences in growth capacity among latitudinal populations and growth differences in wild fish appear to be the result of phenotypic plasticity. Experiments suggest that grunion have environmental-dependent sex determination (ESD), but the level of ESD did not vary between populations. Mean vertebral numbers were nearly equal in wild grunion from all populations. Overall, little variation in these traits was found between latitudinal populations.

Larval Thermal Range

Laboratory experiments on the grunion indicate that both hatching and larval survival are limited to a narrow thermal range of 17°-25°C. Hatching and subsequent survival was very low at 15°C and 27°C. In addition, larval mortalities at 15°C were nearly 100%, and both growth and swimming were extremely poor. There was no indication that temperature tolerances of larvae differed between populations, although Monterey larvae were never exposed to cold temperatures. However, there is some indication that overall survival is enhanced at increasing latitudes (Table 3&4).

Other studies have also indicated narrow temperature tolerances for grunion hatching and larval survival, noting that hatching is maximized from 16-25.5°C and viability of yolk-sac larvae is reduced above 25°C (Ehrlich and Muszynski 1982, Smyder and Martin 2002). It has also been shown that newly hatched grunion larvae tend to avoid waters colder than 20°C and

that both larvae and juveniles up to 4 months old have a modal temperature preference of 25°C (Ehrlich and Muszynski 1982, Reynolds et al. 1977). Given that ocean temperatures during larval development are often below 20°C and even below the lower thermal limit of 17°C (UCAR 2010), this would suggest that grunion larvae are likely seeking out warm environments. Grunion larvae have been observed in warm harbor basins and bays and may also be associated with habitat formers such as bryozoans and kelp (Ehrlich and Muszynski 1982, Reynolds et al. 1977).

Growth in young grunion

In 2009, common garden experiments indicated that higher latitudinal populations may grow slightly faster than lower latitudinal populations, providing some evidence for countergradient variation in growth rate (Fig 5&6). However, in 2010, the opposite was found; the southern population (Ensenada) grew faster than the more northerly population (Malibu) (Fig 7&8). This reversed pattern was due to higher growth rates of Ensenada grunion in 2010 compared to 2009. In both years though growth rates from the mid sample to the final sample were virtually the same for all populations, indicating that even if growth differences do exist between populations they do not persist for long (Fig 6&8).

Differences in growth rate patterns between years may be a result of differences in the parental environments. In this study experiments were conducted on first generation fish; therefore, maternal effects and environmental influences on adult grunion used in strip-spawning may have confounded results. 2010 was an El Niño year and water temperatures increased faster compared to 2009 (NOAA buoy data). This may have influenced the size structure of fish at spawning and caused younger fish to spawn earlier in 2010 than 2009, explaining the much smaller fish sizes for the Ensenada population in 2010 (Fig 2&4). Alternatively differences in

food levels between years could have also impacted the size structure of the population.

Differences in the adult Ensenada population between years may have resulted in the observed differences in laboratory growth rates. Another potential explanation is that results in 2009 may have been hampered by rearing difficulties and high mortalities.

Given the inconsistent population differences in growth rates between years and the fact that growth rate differences did not continue over the entire experimental duration, these results do not provide evidence for countergradient variation in growth rate. There was also no evidence for latitudinal compensation in growth via thermal adaptation (Levinton 1983). Under the thermal adaptation scenario growth reaction norms differ in their temperature response among populations (Levinton 1983, Yamahira and Conover 2002). This was not found for the grunion. For all populations growth rates similarly increased with increasing temperature. While it is possible that growth rate reaction norms could cross at higher experimental temperatures, this is unlikely since results from this study suggest that grunion are unable to survive at temperatures much beyond 25° C . Therefore, all grunion populations likely experience maximal growth at the same temperature.

Growth in wild grunion

Most wild grunion were found to be one and two years old. Three year olds were rare and four year olds were absent. Similarly, Clark (1925) found age one fish to be the dominant age group, and found low numbers of three and four year olds. Average lengths at age in this study tended to be about 3 cm larger than average length at age observed by Clark (1925). However, analogous to this study, Clark (1925) found that females tended to be about 1 cm larger than males and that age two fish were 2-3 cm larger than age one fish.

Scale to fish length linear regressions differed significantly in their intercepts between populations, thus separate equations were used to perform back-calculations at age for each population. Average back-calculated lengths tended to be about 1 cm larger than the average lengths of age 1 wild fish. This may be due to an incorrect intercept estimate in the scale to fish length linear regression, resulting from a lack of data on fish younger than age 1 or differential mortality on the smallest fish in the population. Growth rates of wild grunion during their first year of life (0.41-0.46mm/day) were consistent with those calculated for laboratory reared young grunion (0.3-0.7mm/day) over the temperatures 17-25°C. They were also within the range of growth estimates calculated by Clark (1925), who estimated that wild grunion during their first year grow 0.7 mm/day during summer and 0.18 mm/day during fall and winter.

Similar to common garden experiments, wild fish growth patterns were inconsistent between years. In 2009, there appeared to be a latitudinal trend to fish sizes, with larger sizes occurring in the south (Fig 2&3). In addition, scale analysis indicated larger mean lengths at age and faster growth for Ensenada grunion compared to Malibu grunion (Table 8). In 2010 neither size nor growth differences were observed between Malibu and Ensenada grunion. The small number of grunion available in the 2010 growth analyses (those ages two and older) may have hampered the detection of population differences. Combined 2009 and 2010 scale data suggest that Ensenada grunion tend to grow faster and be slightly larger at age than Malibu grunion (Table 10). Size differences between north and south grunion were also observed by Johnson et al. (2009); grunion from Los Angeles and Orange County were found to be significantly larger (16.5cm) than those from San Francisco (12cm).

Phenotypic plasticity is likely responsible for the size differences observed between latitudinal populations of the grunion. Temperatures are slightly warmer in the south, which

enables faster growth and larger sizes. Data on growth in wild grunion thus suggests that latitudinal compensation in growth either does not occur, or that it occurs, but is unable to fully counteract the environmental influence. Given the lack of evidence from the laboratory experiments for either countergradient variation in growth or thermal adaptation, latitudinal growth compensation likely does not occur in the grunion.

Sex Determination

Sex ratios strongly suggested that grunion have temperature-dependent sex determination (TSD). In 2009, sex ratios for all populations were male biased at warm temperatures, suggesting an environmental influence. In 2010, temperature significantly influenced sex ratios of Ensenada and Malibu grunion over the range 17-25°C, confirming TSD; the proportion of females increased as temperatures decreased (Fig 11). Since Monterey grunion eggs were unable to be obtained in 2010, TSD could not be conclusively shown in this population. However, given that sex ratios of Monterey grunion were male biased at warm temperatures in 2009 this population likely exhibits TSD as well.

Sex ratios results at the 21°C rearing temperature in 2010 were intriguing. While overall the proportion of females fell with the middle of females proportions at the two extreme temperatures, ratios between containers varied greatly; some containers were male biased while others were female biased. Reasons for this variation are unknown. All containers were in the same bath and visible differences in environmental conditions among containers were not evident. Fish were randomly divided into containers, so genetic differences are unlikely to be responsible. It seems that 21°C may be close to the threshold temperature separating male biased ratios from female biased ratios, but in this case intermediate sex ratios would be expected for all containers. In 2009, variability was much smaller and all containers were male biased. The

differences in sex ratio results at 21° between the two years may simply be an artifact of the smaller samples and higher mortality rates in 2009. Alternatively it may be due to differences in the paternal environment between years.

In addition to a temperature effect on sex determination, some evidence for a photoperiod effect was found. Proportions of females were lower at the short photoperiod (12 light hours: 12 dark hours) compared to the long photoperiod (15 light hours: 9 dark hours) for both populations (Fig 12). Unfortunately, due to the large variation in sex ratios of replicated containers at the 21°C long photoperiod treatment, results are hard to interpret. Despite this variation, a significant photoperiod effect was found for the Malibu sex ratios. However, this effect was non-significant for Ensenada sex ratios, although just barely. While other environmental factors besides temperature, such as pH and social factors, are known to affect sex determination (reviewed by Sandra and Norma 2010), a photoperiod effect on sex determination has not been found for any other species to date. On the Pacific coast, where temperatures variations during the spawning season are slight, photoperiod may be a better environmental cue than temperature.

It is unclear though why a species would evolve two different environmental sex determining mechanisms, and how these two environmental cues would interact. Since photoperiods were only studied at one temperature, further insights about this potential interaction require additional experiments. However, by utilizing current knowledge on sex determination in other fish and grunion growth rates we can infer when sex determination in the grunion is likely to occur.

In the Atlantic silverside, and most fish species, sex is determined during a critical period just prior to differentiation (Conover and Kynard 1981). Thus, based on observations of female oocytes in grunion of varying sizes, sex determination in the grunion likely occurs between 20-

30mm. This means that for fish produced at the beginning of the breeding season (early to mid March) the critical sex determining stage (20mm) will likely not be reached until early May. At this time the photoperiod is lengthening from 13.5-14 light hours and water temperatures would be relatively cold; therefore, highly female biased sex ratios would be expected (Table 13). For fish produced at the end of the spawning season (early August), they would likely reach the 20mm sex determination period in early to mid September. During this time, the photoperiod would be relatively short (13-12.5 light hours) and temperatures warm, thus resulting in highly male biased sex ratios (Table 13). Therefore a photoperiod effect may have evolved to further enhance the temperature effect, or to provide a more stable environmental cue.

Regardless of whether there is only a temperature effect on sex determination, or a temperature and photoperiod effect, the result should be the production of females early in the breeding season and the production of males later in the breeding season. This is the same effect seen in the Atlantic silverside, and thus environmental sex determination (ESD) in the grunion may have a similar adaptive value. In the Atlantic silverside, ESD evolved as a means to enhance female fitness. By ensuring that females are produced early in the breeding season they are afforded the longest growing season possible, which allows them to attain larger sizes and increase their relative fecundity (Conover 1984). Like the Atlantic silverside, female grunion are larger than males by their first spawning and there is evidence that fecundity is positively correlated with female size (Clark 1925, Thompson 1919). This suggests that fitness in female grunion is improved by the attainment of large sizes and thus production of females early in the breeding season should be adaptive in the grunion as well.

Alternatively though, females could achieve larger sizes than males by growing faster. Sizes of male and female grunion from the laboratory suggest that growth rates may vary slightly

between the sexes (Table 5); however this difference was only evident for 2009 and size differences were an order of magnitude lower in the lab (~1mm) compared to those observed in the field (1-2cm). This therefore supports the notion that the size difference between the sexes is a result of different birth dates rather than differences in growth. Further evidence is provided by the fact that this size difference between males and females does not increase after year one. It should be noted though that previous work on growth in grunion by Clark (1925) suggests that females grow faster than males just prior to the onset of spawning (February-March). However, in this study growth rates are only estimates since they are based on length data at different stages during the grunion's first year of life and all fish were assumed to be born on the same date. Further analysis is needed to determine whether growth rates differ in males and females.

Since sex ratios were not 100% male or female at any environmental rearing treatment, genetic factors likely influence sex determination in the grunion as well. In 2010, there was not an extremely male biased sex ratio for Malibu grunion at any temperature treatment; the lowest proportion of females observed was about 0.4. Female proportions for Malibu grunion in 2010 were higher than those observed in 2009. This may be due to differential genetic by temperature responses in wild Malibu grunion among years. In 2010, Malibu fish used in strip-spawning may have had more 'female producing' genes compared to fish used in strip-spawning in 2009. In the Atlantic silverside, females from populations with environmental sex determination were found to vary greatly in their response to temperature, indicating that both genetics and temperature play a role in sex determination (Lagomarsino and Conover 1993).

In the Atlantic silverside the influence of the environmental versus genetic component of sex determination was found to vary with latitude, with the level of ESD decreasing at higher latitudes (Conover and Heins 1987a, Lagomarsino and Conover 1993). This pattern is observed

because as latitude increases on the east coast, the growing and breeding seasons decrease, making ESD less beneficial (Conover and Heins 1987a). For grunion, there does not appear to be a difference in the level of ESD among populations, although information is not available for the Monterey population. Levels of ESD in the grunion for Malibu and Ensenada populations were 0.48 and 0.50, respectively, which is similar to the level of ESD observed in mid latitude Atlantic silverside populations (Conover and Heins 1987a). Similar levels of ESD for would be expected for most grunion populations since the growing and spawning seasons do not vary over the grunion's primary breeding range. However, in Monterey the spawning season is much shorter (June-August) and spawning occurrences are very infrequent, thus the level of ESD would be expected to be lower or non-existent in this population. In 2009, Monterey sex ratios were male biased and similar to Ensenada and Malibu sex ratios at 25°C and 21°C, suggesting that they have a similar level of ESD to the other populations.

Sex Ratios at Spawning

In the wild, males are about 2-3 more times abundant on the spawning grounds than females. This is unlikely to be the true sex ratio in the wild and is likely due to the fact that females only spawn their eggs once during the four night spawning period that occurs around the full and new moons, whereas males spawn every night. Several males surround one female during spawning and multiple paternity has been shown to be common in the grunion (Brine and Avise 2009). Further analysis on the true sex ratio in the wild is needed.

Vertebral Number

The common phenomenon in fish of increased vertebral number at higher latitudes, known as Jordan's rule (Jordan 1891) was not found in the grunion. For all populations mean vertebral numbers were very similar. Vertebral counts ranged from 43-49, but the majority of

fish had either 47 or 48 vertebrae (Fig 18&19). Similar vertebral counts in grunion were found in a previous study (McHugh 1954).

Jordan's Rule is commonly attributed to temperature differences along latitudes, but genetic factors have also been shown to be responsible for the observed vertebral number variation (Billerbeck et al. 1997, Yamahira et al. 2006). The adaptive significance of this variation though still remains unclear (McDowall 2008). In the Atlantic silverside, selection on vertebral number has been hypothesized to be a correlated response to selection on body size, resulting from size-selective winter mortality (Billerbeck et al 1997, Yamahira et al. 2006). Another explanation is that the number of vertebrae affects swimming performance and thus predation vulnerability in waters of different temperatures and viscosities (Swain 1992a&b). Regardless of the exact mechanism, recent work indicates that the adaptive response is strongly correlated to the temperature gradient (Baumann et al. *under review*). Therefore, the lack of variation in vertebral number in the grunion may indicate that temperature variations across the grunion's range are not great enough to necessitate differences.

Local adaptation in east verses west-coast fishes

The apparent lack of adaptive latitudinal variation in traits of the grunion, like growth rate, sex determination, and vertebral number contrasts to previous work on the related Atlantic silverside, which exhibits a high degree of genetic differentiation in all of the above traits along its latitudinal gradient. Maximal growth rates for northern silversides are twice as great as those from southern populations (Conover and Present 1990). In addition, a difference of approximately 7 vertebrae is observed between the southernmost and northernmost populations (Yamahira et al. 2006). The level of ESD also varies from 0 to 0.8 along its latitudinal gradient (Conover and Heins 1987a). Given the differences in latitudinal variation between the Pacific

grunion and the Atlantic silverside, one might conclude that the environmental gradient on the Pacific coast is not strong enough to induce adaptive responses in Pacific coast species.

However, recent work on topsmelt (*Atherinops affinis*), another Pacific coast fish in the silverside family, indicates that this species displays similar patterns of genetic variation in both growth and vertebral number to that observed in the Atlantic silverside, although to a lesser degree. Over the latitudinal range of 28°-43°N, the northernmost topsmelt fish grew approximately 0.3 mm/day faster than the southernmost fish (Baumann and Conover *in press*). Vertebral number was also found to increase with latitude, with a maximum difference of about 4 vertebrae (Baumann et al. *under review*). This trait variation in the topsmelt is less than half of the variation observed in the Atlantic silverside, reflecting the much weaker temperature gradient on the Pacific coast compared to the Atlantic coast (Baumann and Conover *in press*, Baumann et al. *under review*). These results thus also contrast to results from this study, and indicate that Pacific coast species are capable of locally adapting like Atlantic coast species. Therefore, another explanation is needed to explain the lack of variation in the grunion compared to its other taxonomic relatives.

It is possible that experimental limitations prevented detection of trait variation in the grunion. Since a southern spawning population of grunion could not be found, the latitudinal range was not as large as desired, spanning only about five degrees of latitude. In addition, only one year of data could be obtained for Monterey grunion, further preventing our chances of observing trait variation. Given that variation in west coast organisms is much smaller than variation found in east coast species (Baumann and Conover *in press*), the latitudinal range in this study may not have been large enough to allow for population differences to be observed. In the topsmelt study, in which evidence of local adaptation was found, the latitudinal range was

much broader, spanning 15 degrees. However, trait differences were still detectable between populations separated by only 4-5° of latitude (Baumann and Conover *in press*). Therefore if similar latitudinal patterns occurred in the grunion, at least some evidence of trait differentiation would be expected.

Alternatively, the lack of genetic differentiation between latitudinal populations of the grunion may be a result of its oceanic life history. In contrast to the more estuarine Atlantic silverside and topsmelt, the oceanic habitat of the California grunion provides a much greater opportunity for gene flow; and high levels of gene flow can constrain adaptation (Felsenstein 1976, Slatkin 1987). Several empirical studies have supported this notion by showing that adaptation occurs within species when gene flow is limited, but not when populations are highly connected (Storfer 1999, Postma and van Noordwijk 2005, Moore et al. 2007).

The Atlantic silverside and topsmelt lay their eggs in intertidal estuaries where their eggs attach to submerged vegetation (Conover and Kynard 1984), so most of their larvae are likely retained within the estuaries in which they hatch. In addition, juvenile Atlantic silversides are known to stay within their natal estuaries throughout their first growing season (Griffin and Valiela 2002). This life history strategy would therefore promote local adaptation (Conover et al. 2006). In contrast, grunion eggs hatch in the near-shore open ocean where there is a high potential for larvae to be dispersed great distances by ocean currents. This would homogenize genetic variation and work against local adaptation (Conover et al. 2006).

Unfortunately, little information is available on the distribution of grunion larvae since they are rarely captured in nets (Johnson et al. 2009). It is thought that grunion larvae seek out warm environments such as bays and harbors and that they may be associated with habitat formers such as bryozoans and kelp (Ehrlich and Muszynski 1982, Reynolds et al. 1977).

Grunion larvae have been captured at night in ocean surface waters (Kauffman et al. 1981), and in Los Angeles harbors (Allen et al. 1983). Young grunion have also been found in San Diego Bay (Allen et al. 2002) and San Pedro Harbor (Clark 1925). Whether larvae and juveniles seek out the closest bays and harbors or whether they are first dispersed throughout the coast is unknown.

In addition to larval dispersal, movements among adults may also lead to high gene flow. In the Atlantic silverside, mixing occurs among adults from different populations likely during their offshore winter migrations, but there is also evidence that some silversides exhibit natal homing (Clarke et al. *in press*). Recent work also indicates that while mixing does occur that gene flow is at least limited regionally, which may facilitate adaptation (Mach et al. 2010). For the grunion, little is known about where adults spend their time when they are not spawning. In a tagging study by Walker (1949), he suggests that grunion move little between populations and that they may show some degree of site fidelity. However, tag recovery rates in this study were low and site fidelity is considered unlikely since grunion are known to spawn on man-made or replenished beaches (Johnson et al. 2009).

Since grunion are elusive from nets, the population structure has attempted to be assessed by examining genetic variation in neutral markers. In an allozyme study by Gaida et al. (2003), the majority of results provided evidence for a panmixia population structure of free genetic interchange and mixing with no divergence; allelic frequencies were found to be similar among four southern California populations and few private alleles were detected (Gaida et al. 2003). However, a significant correlation was found between allozyme divergence and geographic distance, suggesting that all larvae are not dispersing or that there may be some degree of natal homing (Gaida et al. 2003).

In a recent study by Johnson et al. (2009) based on microsatellite and mitochondrial analysis little evidence of genetic differentiation was found between central California grunion (San Francisco) and southern California grunion (Orange County and Malibu). Microsatellite data indicated no significant genetic differences between populations, showing a high number of migrants between populations and little subdivision (Johnson et al. 2009). For the mitochondrial data, results varied depending how the data were analyzed; haplotype frequency data indicated significant population differences, as several unique haplotypes were found, whereas genetic distances among haplotypes were not significant (Johnson et al. 2009). Since no correlation was found between genetic divergence and geographic population though, the authors conclude that the high amount of unique haplotypes is a result of a large effective population size and that most evidence points to little genetic differentiation between north and south grunion (Johnson et al. 2009).

While studies that measure direct movements of larvae and adults movements are still needed, the majority of results from the above studies suggest that gene flow is sufficient enough to prevent genetic divergence between populations. These studies are therefore consistent with conclusions from this study and together they indicate that gene flow is likely high enough to overcome natural selection pressures imposed on the California grunion.

Not only does the potential for gene flow differ between oceanic and estuarine habitats, but the environmental gradients may vary as well. On the west-coast, the California current combined with upwelling waters creates similar oceanic temperature conditions along the majority of the coast; therefore, latitudinal and annual temperature variations are small. Estuarine environments, however, are more likely to be impacted by atmospheric conditions, since their higher surface area to volume ratios allows for more rapid heating and cooling

compared to oceanic waters. Temperatures in Tijuana estuary (San Diego, CA) and Elkhorn Slough (Monterey, CA) estuary may vary throughout the year from 10-27°C and 8-22°C, respectively (NOAA NERR). Comparable coastal ocean temperatures for these latitudes are 15-21°C (San Diego, CA), and 12-15°C (Monterey, CA). This suggests that temperature fluctuations in estuarine environments are greater than those in oceanic environments. Therefore, not only do environmental selection pressures differ between the east and west-coast, but they may also differ between oceanic and estuarine habitats.

Since for most marine species the degree of local adaptation will be a function of gene flow and environmental selection differentials (Felsenstein 1976, Lenormand 2002, Conover et al. 2006), we can explain the varying degrees of adaptation in the three silverside species by considering how gene flow and selection levels differs among them. In the case of the Atlantic silverside, while gene flow may be moderate, environmental selection pressures are very high thus resulting in a high degree of local adaptation in this species. For the topsmelt, selection pressures are much lower than those on the Atlantic silverside, but given its estuarine life history gene flow is likely low enough to still permit small amounts of adaptation. In the grunion though, where selection may be even weaker and the opportunity for gene flow is higher, latitudinal variation is not observed.

This study therefore highlights the importance of considering how both gene flow and environmental selection impact adaptation. For species living along steep climatic gradients, like the Atlantic coast of North America, where environmental selection pressures are strong, even high amounts of gene flow will be unlikely to completely limit adaptation from occurring (Schneider et al. 1999, Saint-Laurent et al. 2003). Local adaptation has been found in many species with varying life histories along the Atlantic coast of North America (reviewed by

Conover et al. 2009). However, for species living along gradients where environmental selection is weak (i.e. Pacific coast of North America), only low amounts of gene flow may confer local adaptation. Local adaptation on the Pacific coast has been observed in several intertidal species that are considered to have low amounts of gene flow (Bernadi and Talley 2000, Willet 2010, Sanford and Worth 2010). Pacific Salmon also often show local adaptation, but these are anadromous species that exhibit natal homing (Taylor 1991, Quinn and Dittman 1990). Adaptations in oceanic species along the Pacific coast may be less common compared to estuarine or intertidal species, given the greater potential for higher gene flow.

Implications for a Changing Climate

Examining how species adapt to spatial climatic gradients may be useful for predicting future evolutionary adaptations to global climate change, since traits may evolve similarly. (Conover et al. 2009). While studies on adaptation to temporal climatic variation are rare, similar patterns of adaptive variation have been found across time-scales over which the environment has fluctuated (Garant et al. 2004, Merila et al. 2001). The response to future environmental change is expected to be a poleward shift in genotypes, either through distribution shifts or natural selection, to counter the plastic effects of the changing environment (Baumann and Conover 2010 *in press*). However, for species like the grunion, with homogenous genotypes restricted to a narrow range of stable environmental conditions, they may be more limited in their ability to adapt and expand their range compared to species with divergent genotypes adapted to high amounts of environmental variation (i.e. Atlantic silverside and topsmelt). This may mean that oceanic species may be less suited to adapt to climate change than estuarine or intertidal species.

Potential impacts of global climate change on species with environmental sex determination deserve considerable attention. For most fishes with TSD, the proportion of males increases with increasing temperatures; therefore will climate change cause a disproportional increase in males within populations (Ospina-Alvarez and Piferrer 2008)? An increase of 1.5°C and 4°C has been predicted to increase the male proportion in populations with TSD to 62% and 78%, respectively (Ospina-Alvarez and Piferrer 2008). However, distribution shifts may mitigate these effects. It is also possible that species may evolve increased genetic sex determination mechanisms (GSD) or adjust their sex ratio-temperature response (Ospina-Alvarez and Piferrer 2008). Both of these responses were seen in laboratory reared Atlantic silverside that were exposed to extreme temperature changes; sex ratios responses evolved rapidly until 1:1 sex ratios were again achieved (Conover et al. 1992). For the grunion, if both photoperiod and temperature influence sex ratios, the photoperiod effect may help prevent future changes in population sex ratios.

Further studies on the California grunion

With the exception of spawning and hatching information, very little is known about the California grunion, particularly with regard to its population structure. Studies on larval dispersal and adult movements in the California grunion are needed to assess how much gene flow may occur between populations. Currently, grunion movements are attempting to be assessed using otolith microchemistry (Dr. Danielle Zacherl, California State University Fullerton).

Environmental sex determination (ESD) in the grunion should be further investigated. Experiments are needed to assess how temperature and photoperiod interact in the sex determination process; several combinations of temperature and photoperiods should be used.

In addition, ESD should be confirmed in the Monterey population and exactly when sex determination occurs should be established.

Further studies on local adaptation among species

While local adaptation has been well established in a variety of species, more studies on contrasting patterns of local adaptation across varying environmental gradients are needed.

Studies that contrast adaptation in species with different patterns of connectivity should also be conducted to assess how gene flow affects the degree of adaptation, and how much gene flow is needed to override different magnitudes of selection differentials. Understanding how varying spatial climatic gradients and differing life histories influence adaptive genetic variation within species will shed further light on the types of evolutionary responses that will be seen in the coming years as global climate change continues, as well as who the winners and losers may be in a changing world.

Table 1: 2009 hatch lengths for each treatment and population.
 Numbers inside parentheses indicate sample size.

Treatment	Days from Fertilization to Hatch	Average Hatch Length (mm)
Ensenada 27°C	7	6.14 ± 0.27 (9)
Ensenada 21°C	10	5.97 ± 0.13 (8)
Ensenada 15°C	15	6.25 ± 0.27 (6)
Malibu 21°C	9	5.77 ± 0.18 (13)
Monterey 21°C	8	5.86 ± 0.14 (9)

Table 2: 2010 hatch lengths at 21°C for each population

Population	Days from Fertilization to Hatch	Average Hatch Length (mm)
Ensenada	9	4.42 ± 0.16 (9)
Malibu	10	6.03 ± 0.19 (11)

Table 3: 2009 percent mortality by population and temperature
(ND = No data)

Temperature	Population		
	Ensenada	Malibu	Monterey
25°	0.338	0.208	0.075
21°	0.442	0.517	0.063
15°	0.973	0.917	ND

Table 4: 2010 percent mortality rates by population and temperature

Temperature	Population	
	Ensenada	Malibu
25°	0.367	0.100
21°	0.317	0.180
17°	0.25	0.170

Table 5: 2009 and 2010 average lengths of lab reared fish by sex

Year	2009			2010		
Sex	n	Average Length (cm)	SE	n	Average Length (cm)	SE
Males	80	39.5	0.22	322	39.5	0.33
Females	24	40.8	0.38	374	39.6	0.22

Table 6: 2010 sex ratios by container for the 21° long photoperiod treatment (15 light hours: 9 dark hours).

Replicate	Ensenada			Malibu		
	F	M	(F/(F+M))	F	M	(F/(F+M))
1	8	18	0.308	24	6	0.800
2	10	16	0.385	15	19	0.441
3	18	10	0.643	25	4	0.862
Average	0.444			0.688		

Table 7: Sex ratios by container for the short photoperiod treatment (12 light hours: 12 dark hours).

Replicate	Ensenada			Malibu		
	F	M	(F/(F+M))	F	M	(F/(F+M))
1	1	15	0.063	17	32	0.436
2	3	8	0.273	16	26	0.381
3	4	18	0.182	15	28	0.349
Average	0.16			0.387		

Table 8: 2009 scale data for wild grunion

Population	Ensenada						Malibu					
	Males			Females			Males			Females		
Sex	n	Average	SE	n	Average	SE	n	Average	SE	n	Average	SE
Length age 1 (cm)	11	14.94	0.24	5	18.30	0.21	20	14.21	0.18	10	15.36	0.26
Length age 2 (cm)	27	17.01	0.14	8	18.83	0.37	9	16.37	0.26	9	17.47	0.26
Back-calculated Length age 1 (cm)	28	15.66	0.17	8	17.15	0.30	13	15.00	0.25	11	15.74	0.16
Growth Year 1 (mm/day)	28	0.430	.005	8	0.470	.008	13	0.411	.007	11	0.431	.004

Table 9: 2010 scale data for wild grunion (ND = No data)

Population	Ensenada						Malibu					
	Males			Females			Males			Females		
Sex	n	Average	SE	n	Average	SE	n	Average	SE	n	Average	SE
Length age 1 (cm)	24	14.20	0.23	26	15.05	0.19	16	13.36	0.44	8	15.39	0.57
Length age 2 (cm)	6	16.43	0.47	4	17.0	0.48	9	17.03	0.20	1	ND	ND
Back-calculated Length age 1 (cm)	7	15.16	0.52	4	15.43	0.60	10	15.19	0.24	1	ND	ND
Growth Year 1 (mm/day)	7	0.415	.014	4	0.423	.017	10	0.416	.007	1	ND	ND

Table 10: 2009&2010 combined scale data

Population	Ensenada				Malibu			
Sex	Males		Females		Males		Females	
	Average	SE	Average	SE	Average	SE	Average	SE
Length at Age 1 (cm)	14.45	0.22	15.59	0.23	13.83	0.22	15.37	0.31
Length at Age 2 (cm)	16.96	0.16	18.22	0.27	16.68	0.22	17.50	0.27
Back-calculated length at age 1 (cm)	15.59	0.17	16.61	0.36	15.13	0.17	15.96	0.22
Growth during year 1 (mm/day)	0.426	0.005	0.454	0.010	0.413	0.005	0.436	0.006

Table 11: Sex ratios of wild grunion on the spawning grounds

Population	Males		Females	
	#	Average Length (cm)	#	Average Length (cm)
Ensenada	70	14.3	30	15.3
Malibu	26	14.8	9	15.8

Table 12: Frequency of vertebral number counts in wild grunion for each population

Population	Vertebral Number						
	43	44	45	46	47	48	49
Ensenada	0	0	0	1	18	25	1
Malibu	1	0	0	0	23	22	4
Monterey	0	0	1	1	5	5	1

Table 13 : Hypothetical interaction of temperature and photoperiod on sex determination for grunion born at different times during the spawning season

Birth month	Growth rate (mm/day) during larval stage	# of days to reach critical sex det. period (20 mm)	Month of sex det.	Temp. during sex det.	Photo-period during sex det.	Sex ratio response
Early to mid march	~0.34mm/day at 17°C	52 days	Early May	Cold	long (13.5-14 light hours)	Highly female biased
Mid to end of May	Between 0.34mm/day (17°C) and 0.5mm/day (21°C)	40-50 days	Early to mid July	Fairly warm	Long (14 light hours)	Equal males and females
Late July to Early August	Between 0.5 mm/day (21°C) and 0.65mm/day (25°C)	30-40 days	Early Sept.	Warm	Short (13-12.5 hours)	Highly male biased

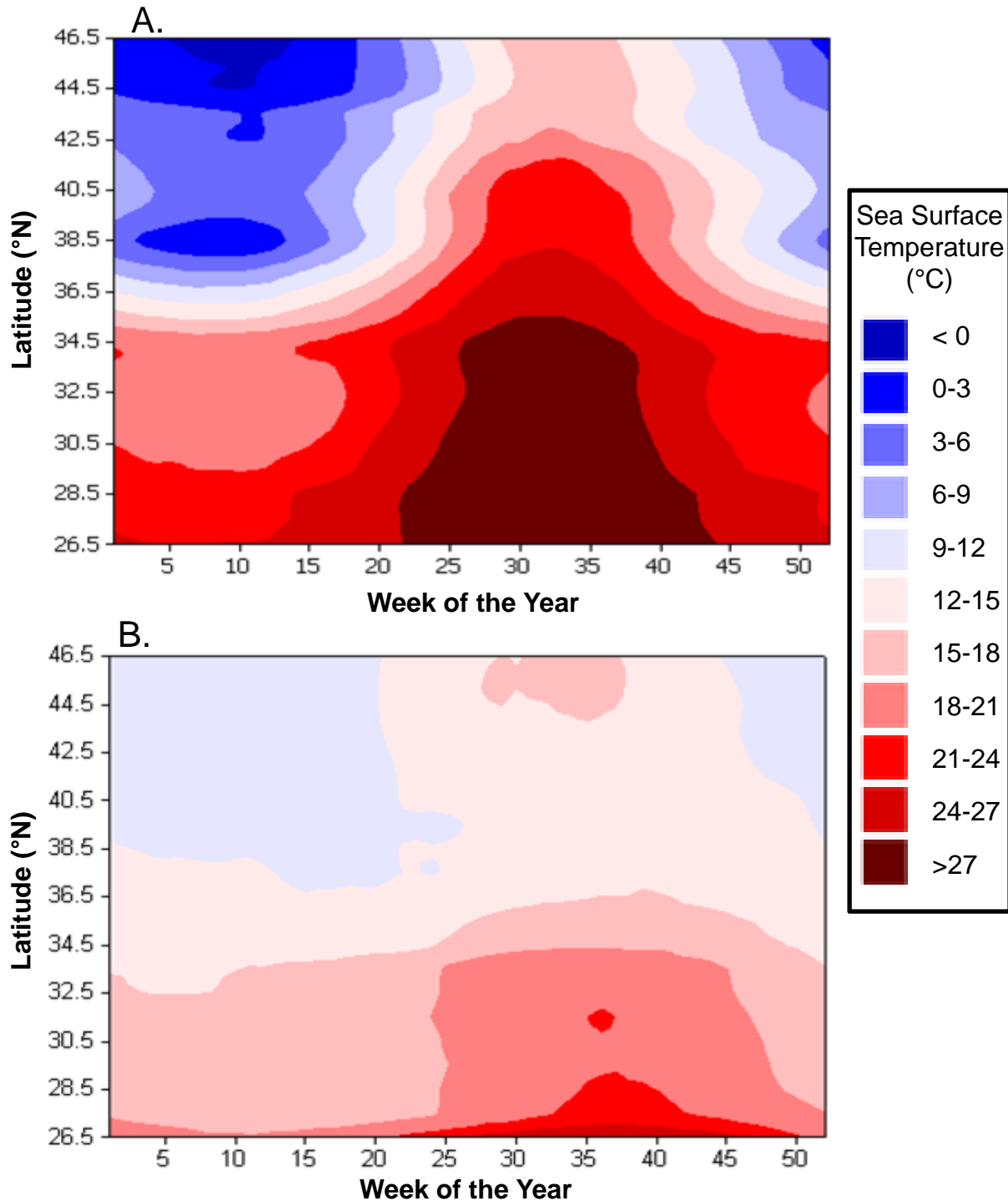


Figure 1: A-B, Contour representation of average weekly coastal sea surface temperatures by latitude for the Atlantic (A) and Pacific (B) coasts of North America. Temperatures are derived from NOAA SST data from 1989-2009, based on optimum interpolations over 1° grids. Temperatures are from the grid cell immediately next to land (UCAR 2010).

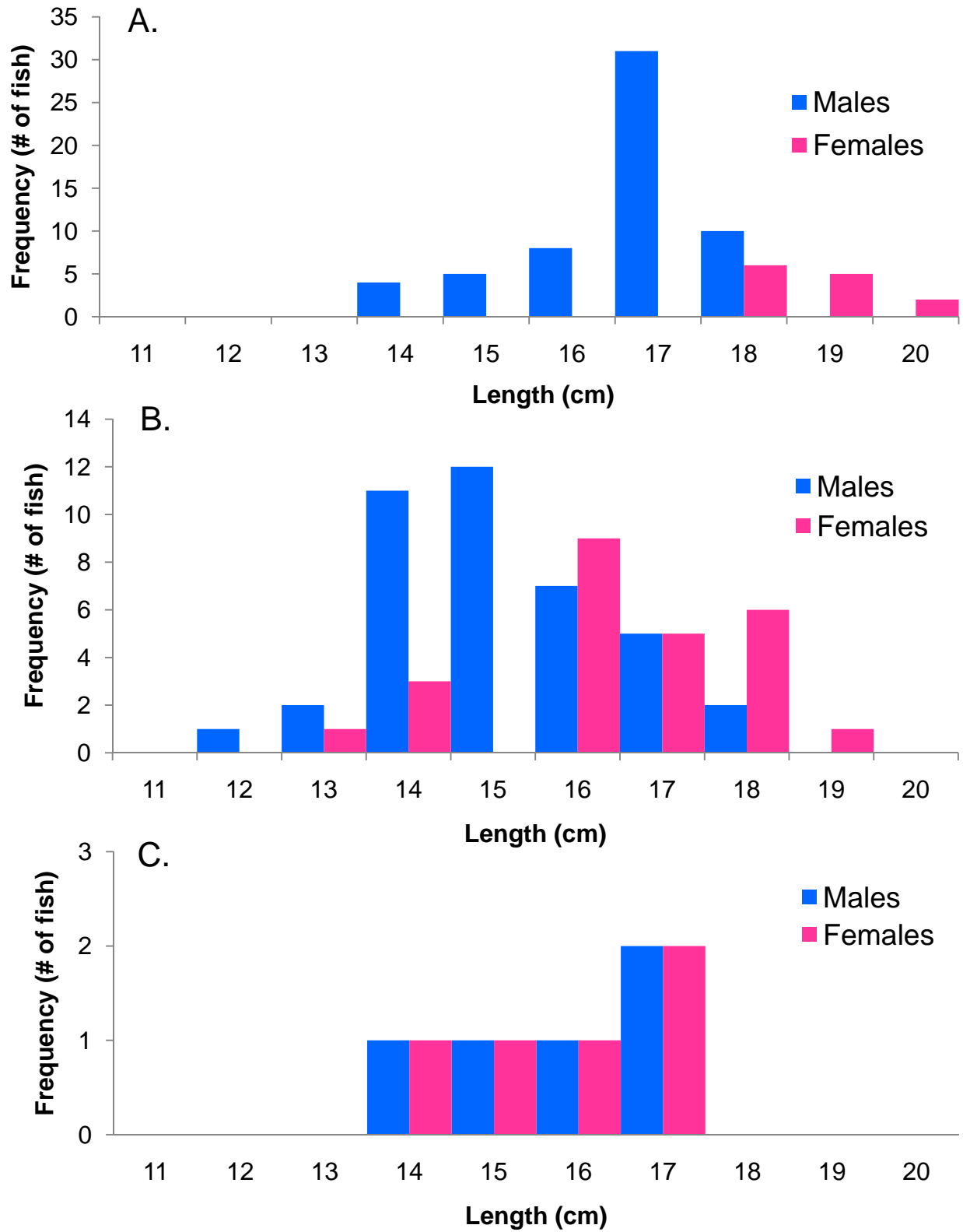


Figure 2: A-C, 2009 length frequency distributions for wild grunion from Ensenada (A), Malibu (B), and Monterey (C).

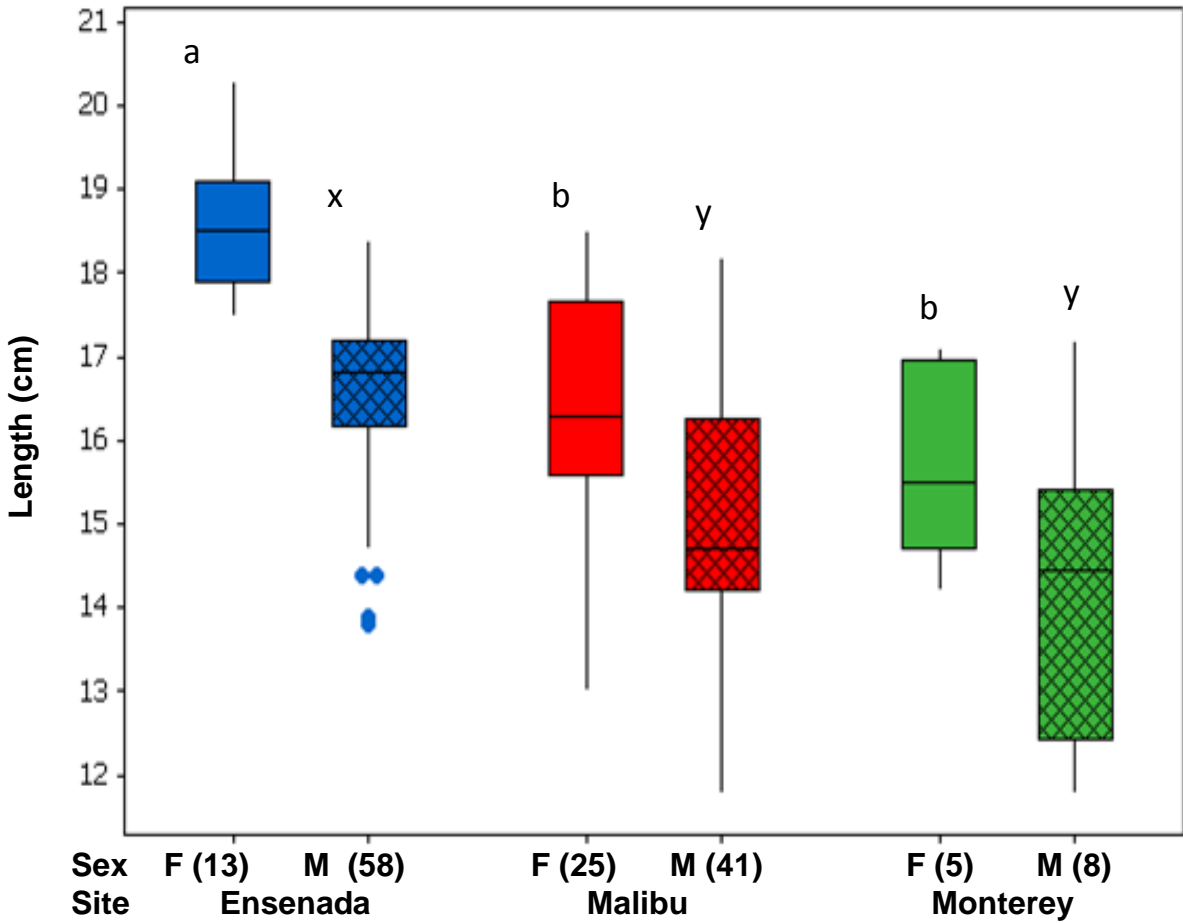


Figure 3: Box plot of 2009 wild fish lengths by population and sex. Boxes shows the 25th to 75th percentiles for length and the horizontal bar within the box represents the median. Vertical bars extend to lower and upper limits of the data within the 1.5 interquartile range. Individual points are those outside the limits. Boxes with different letters above them are significantly different from each other..

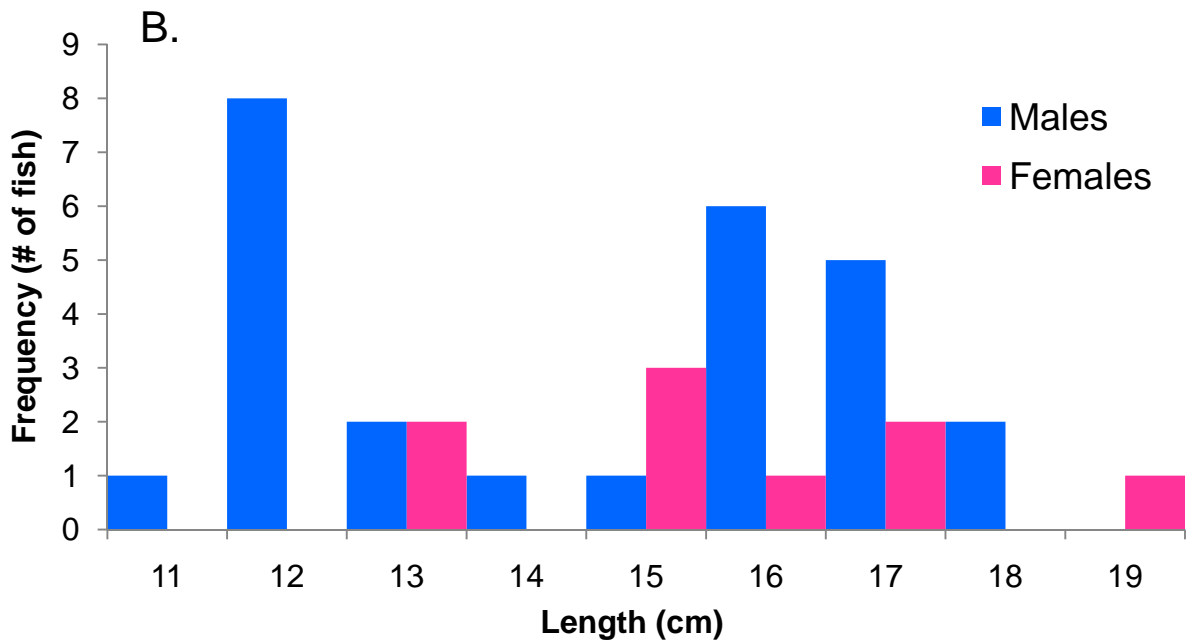
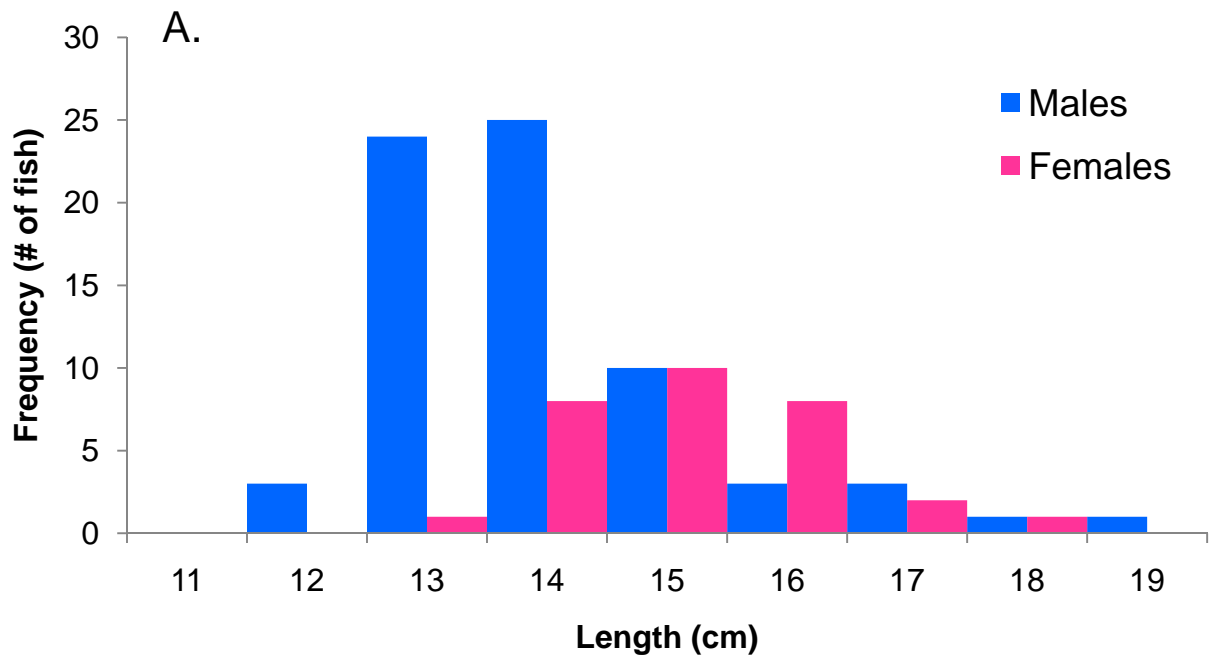


Figure 4: A-B, 2010 length frequency distributions for wild grunion from Ensenada (A) and (B).

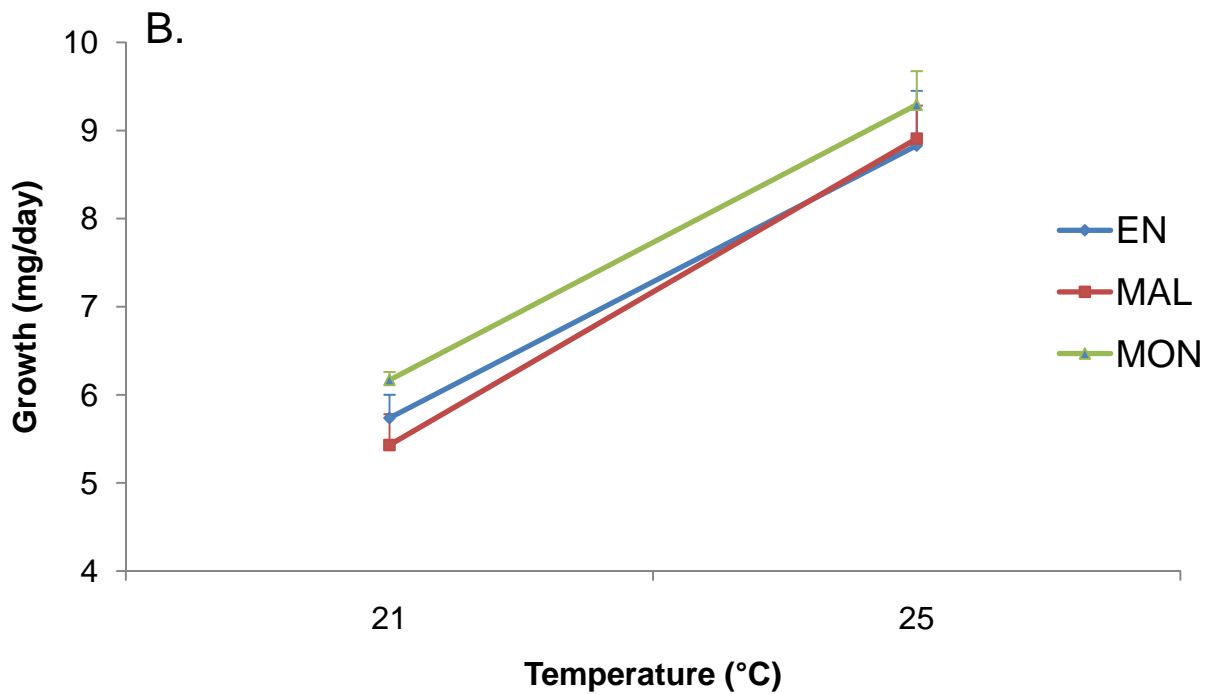
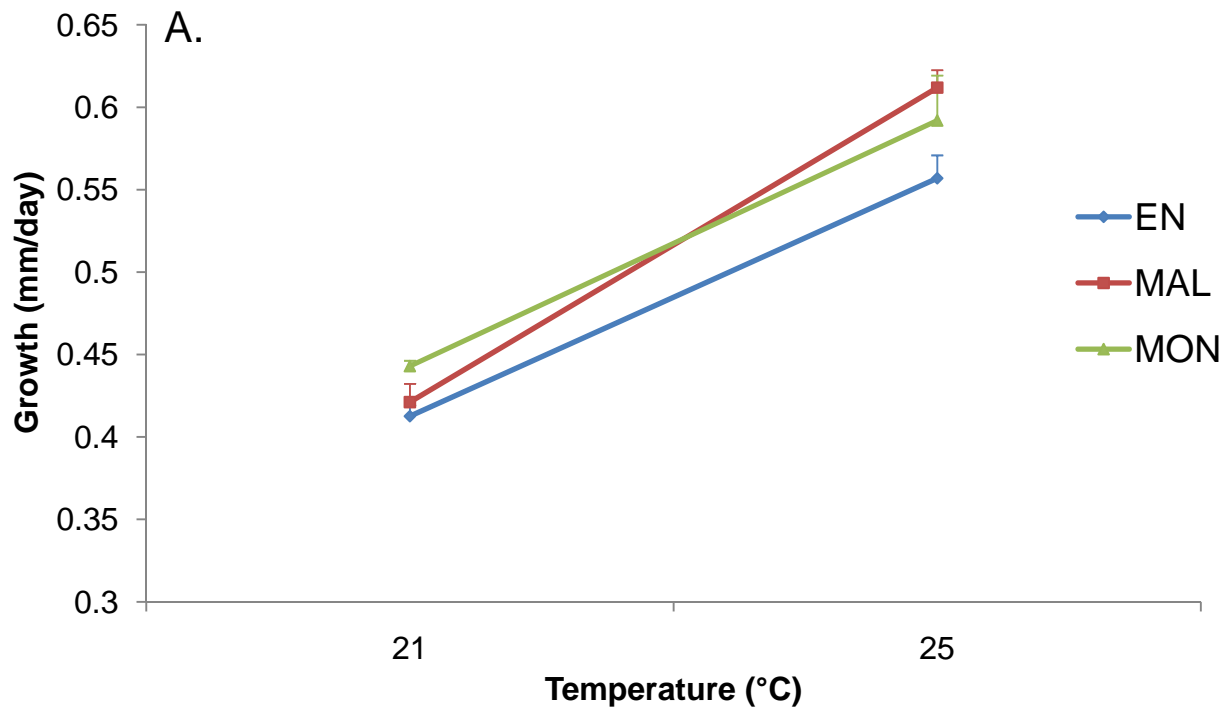


Figure 5: A-B, Growth in length from the initial to final sample (A) and in weight from the mid to final sample (B) during the 2009 growth experiments. Vertical bars represent standard errors. Legend: EN = Ensenada, MAL = Malibu, and MON = Monterey.

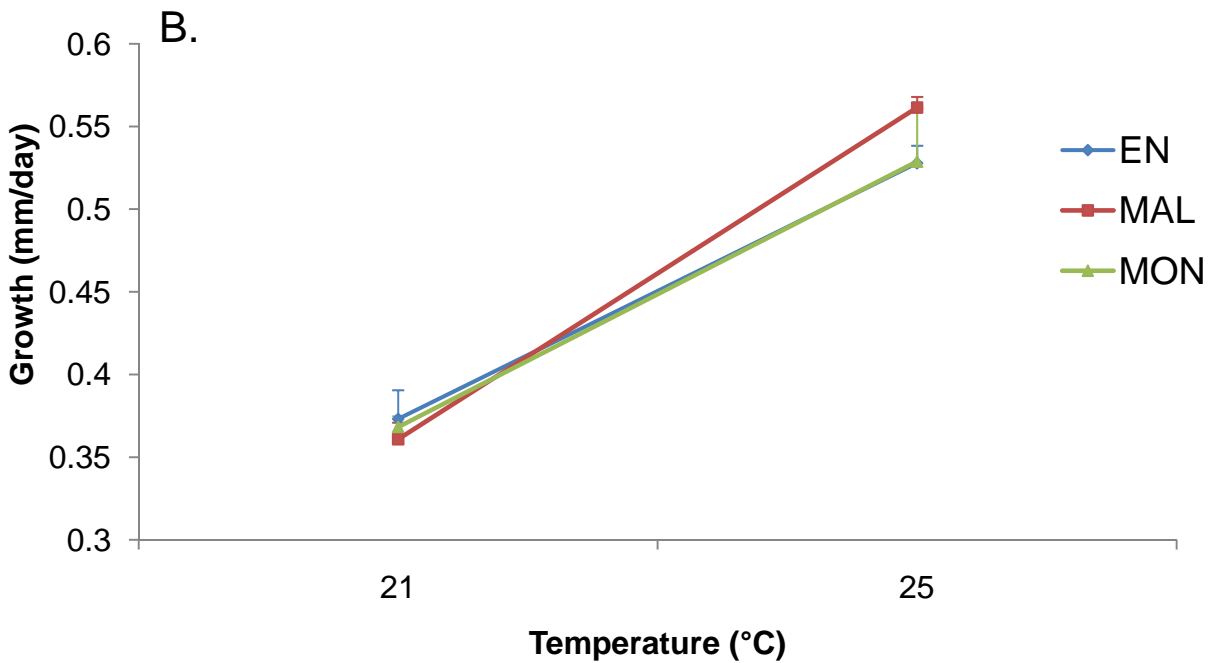
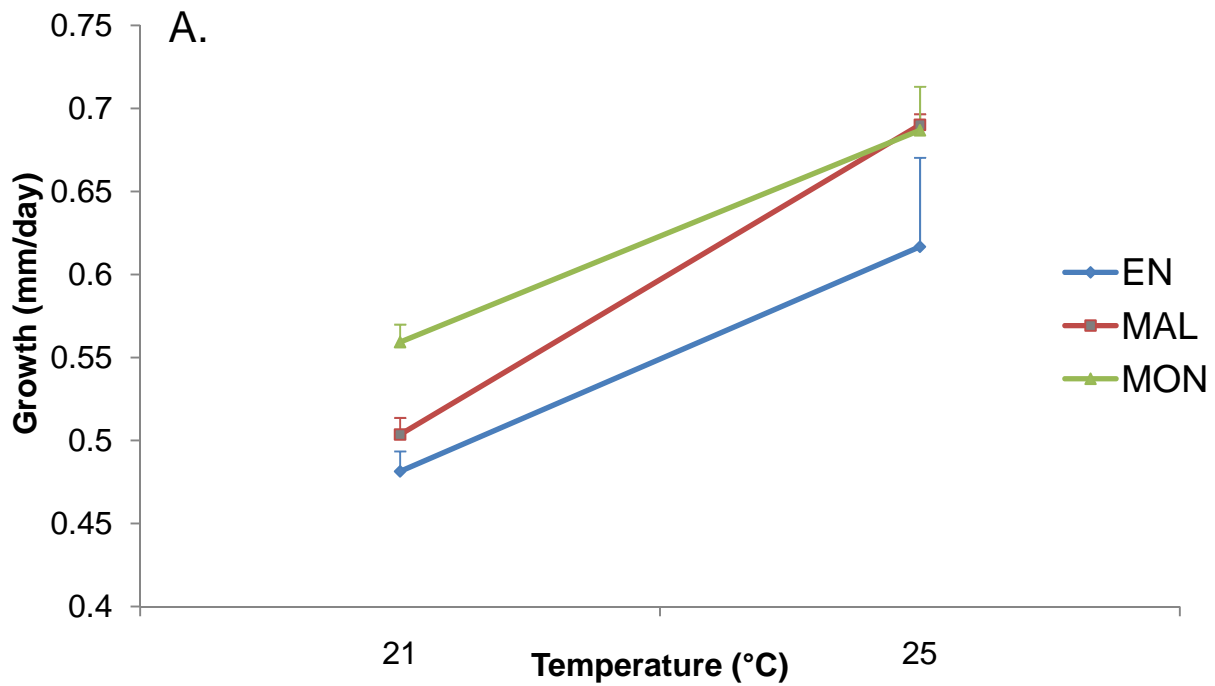


Figure 6: A-B, Growth in length during the 2009 growth experiments from the initial to the mid sample (8-20mm) (A) and from the mid to final sample (20-40mm) (B). Vertical bars represent standard errors. Legend: EN = Ensenada, MAL = Malibu, and MON = Monterey.

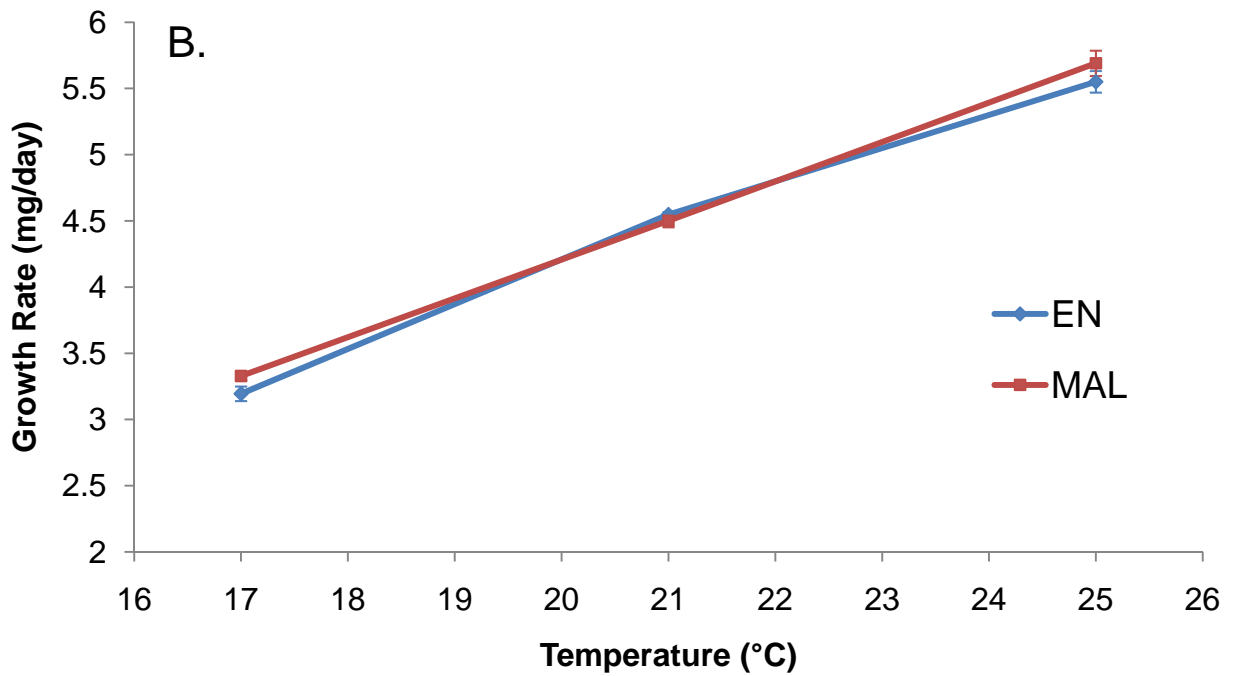
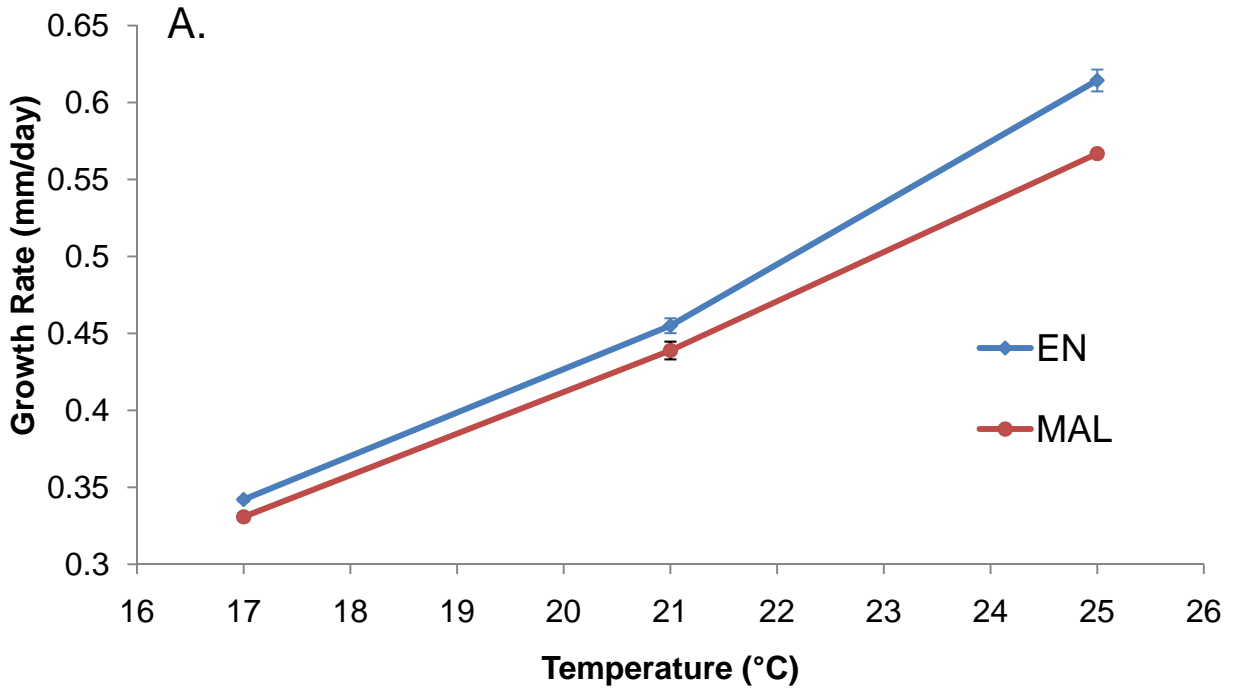


Figure7: Growth in length (A) and weight (B) during the 2010 growth experiments from the initial to final sample (6-40mm). Vertical bars represent standard errors. Legend: EN = Ensenada and MAL = Malibu.

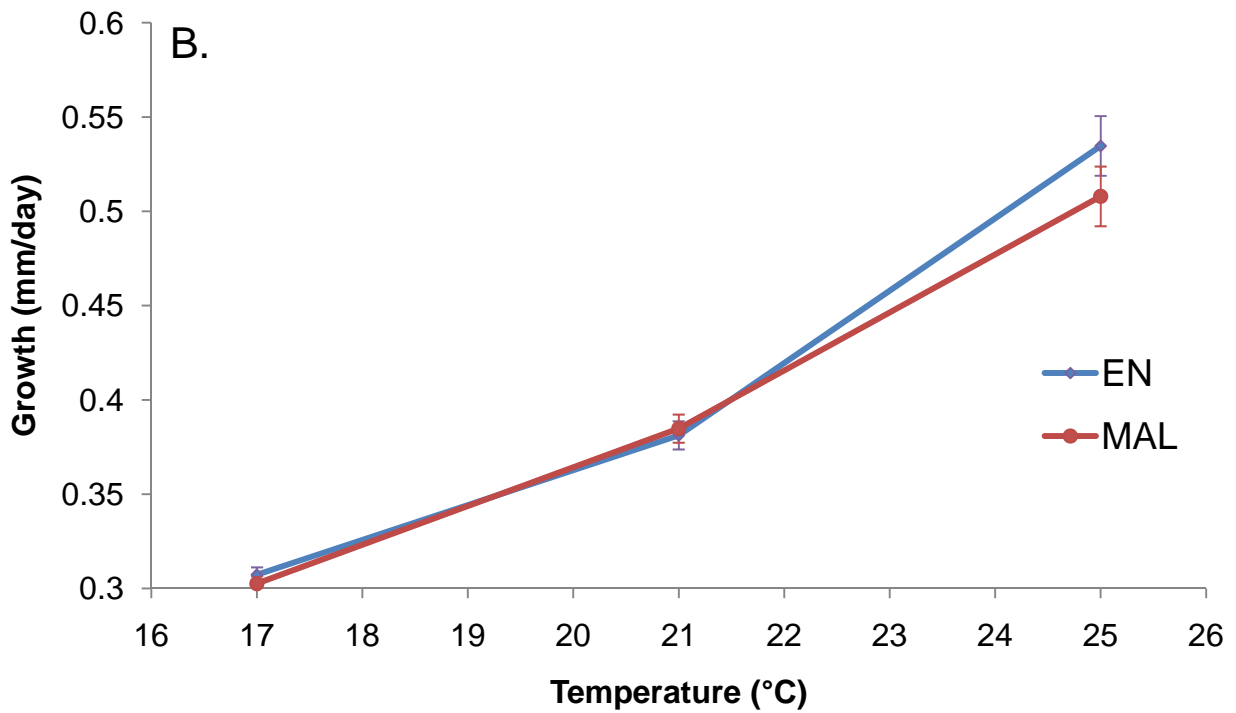
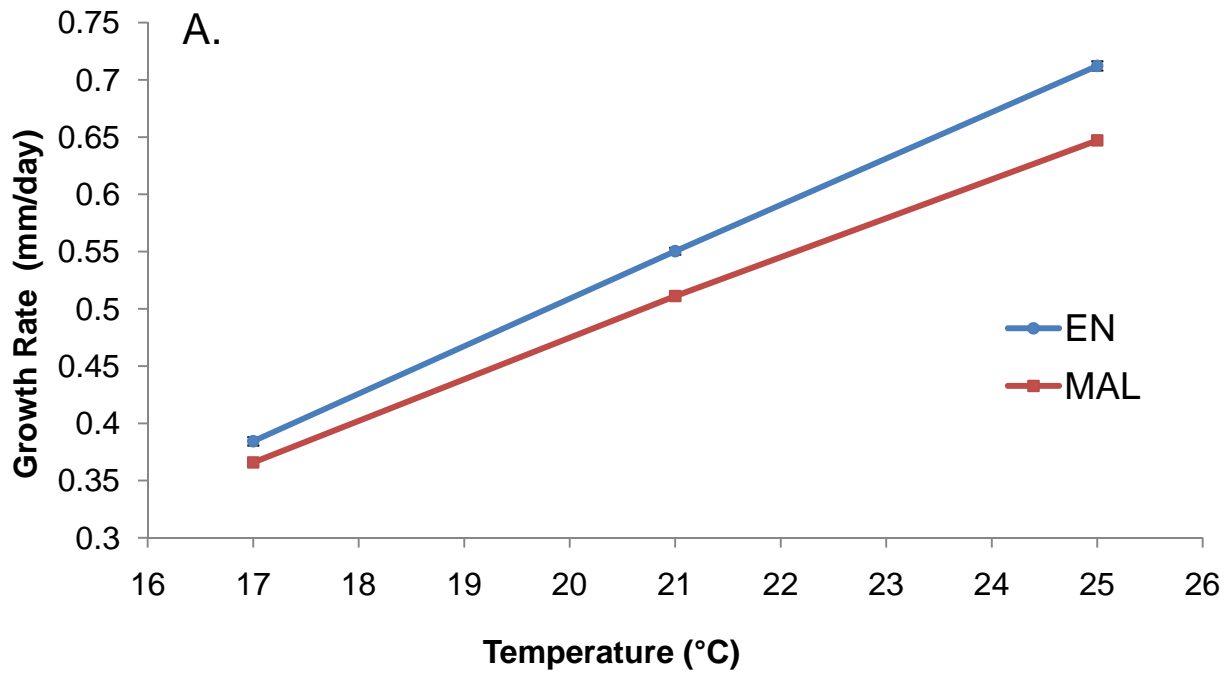


Figure 8: A-B, Growth in length from the initial to mid sample (6-22mm) (A) and from the mid to final sample (22-40mm) (B) during the 2010 growth experiments. Vertical bars represent standard error. Legend: EN = Ensenada and MAL = Malibu.

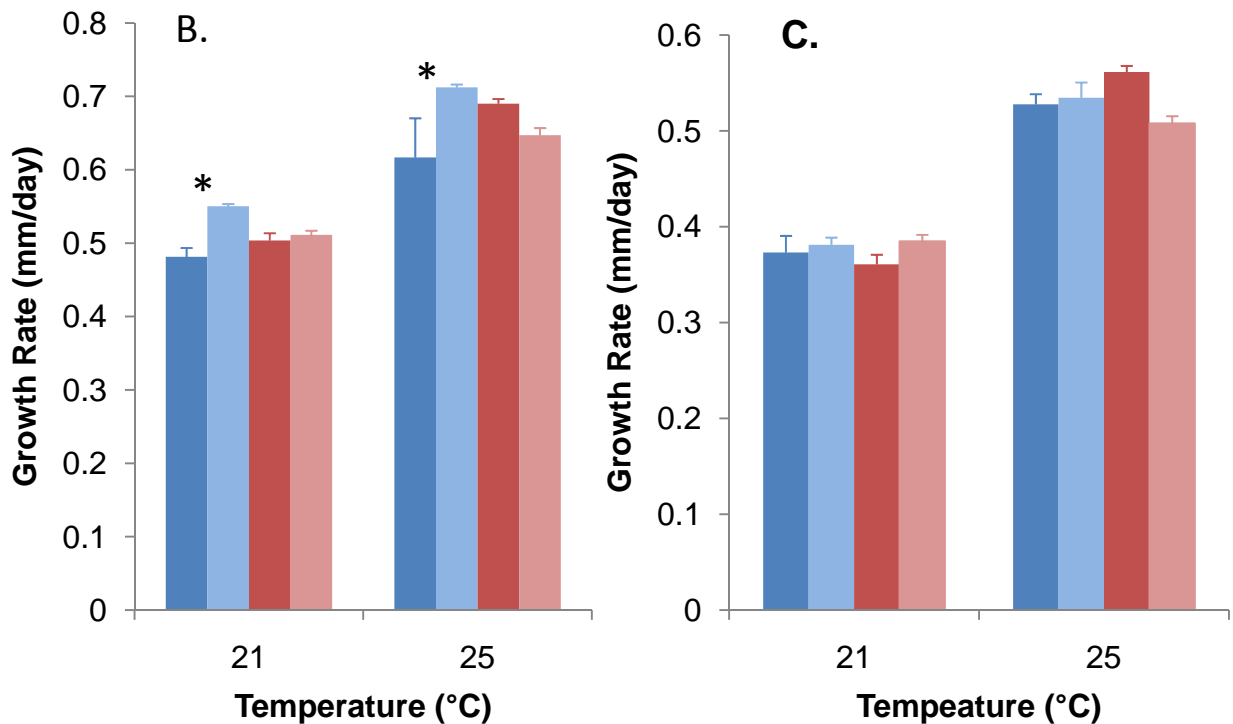
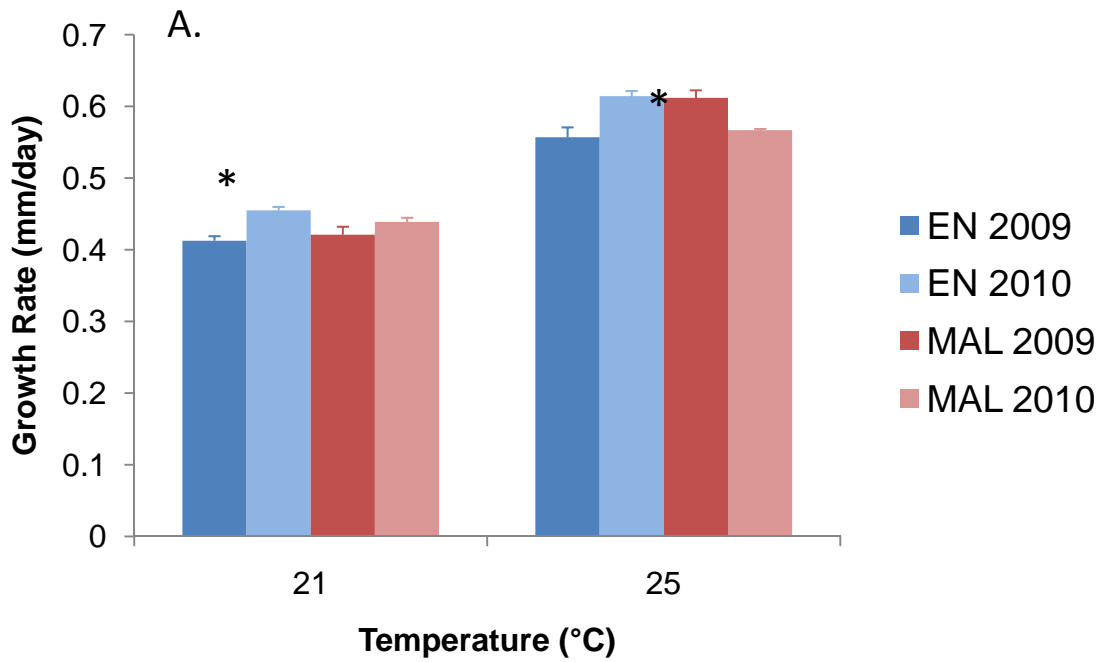


Fig 9: A-C, Comparison of growth in length between 2009 and 2010, from initial to final sample (6-40mm) (A), initial to mid sample (6-22mm) (B), and mid to final sample (22-40mm) (C). Vertical bars represent standard error. Asterisks indicate significant difference between years. Legend: EN = Ensenada and MAL = Malibu.

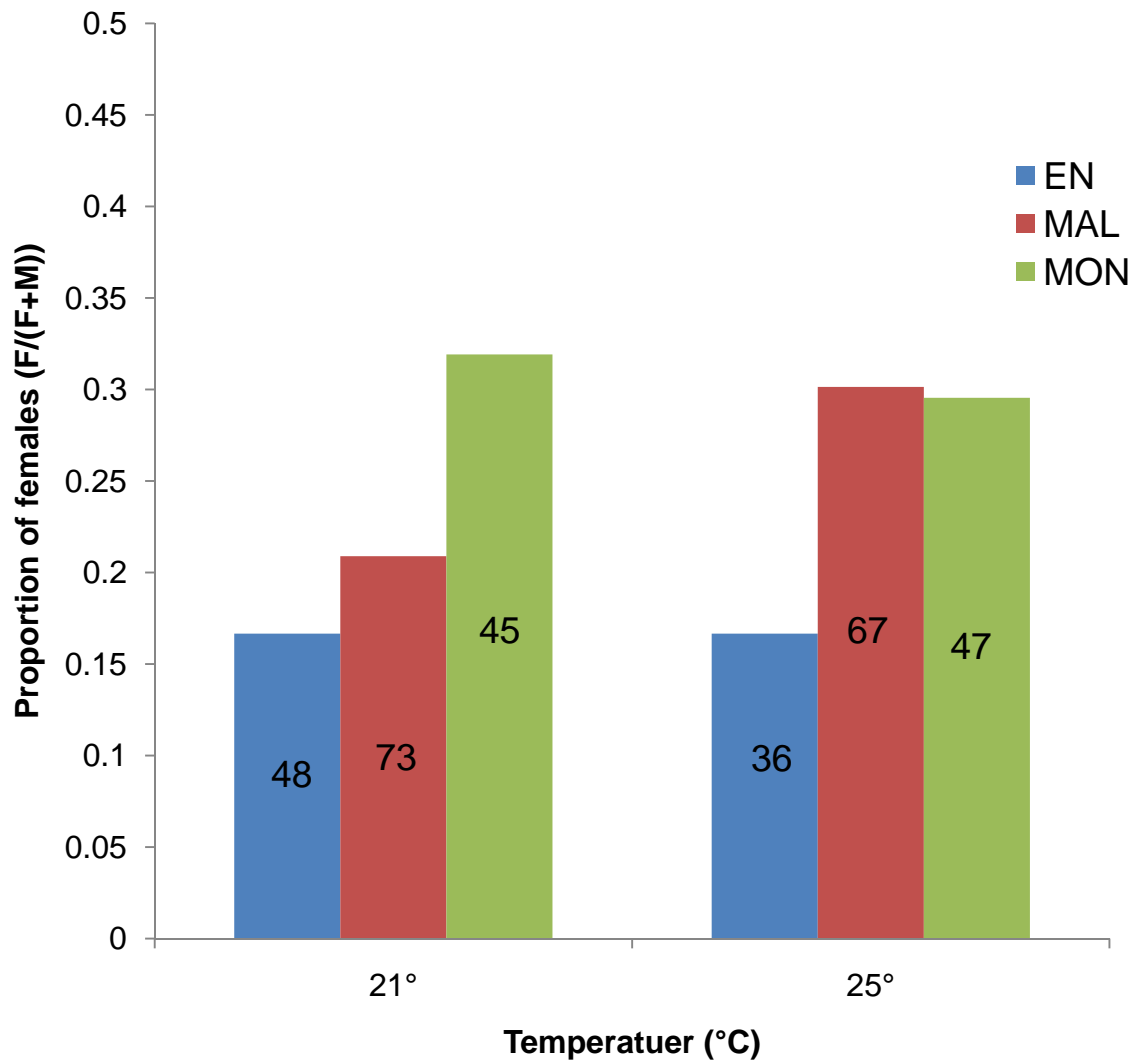


Figure 10: 2009 Sex Ratios by population and temperature. Numbers inside the bars indicate sample size. Legend: EN = Ensenada , MAL = Malibu, and MON = Monterey.

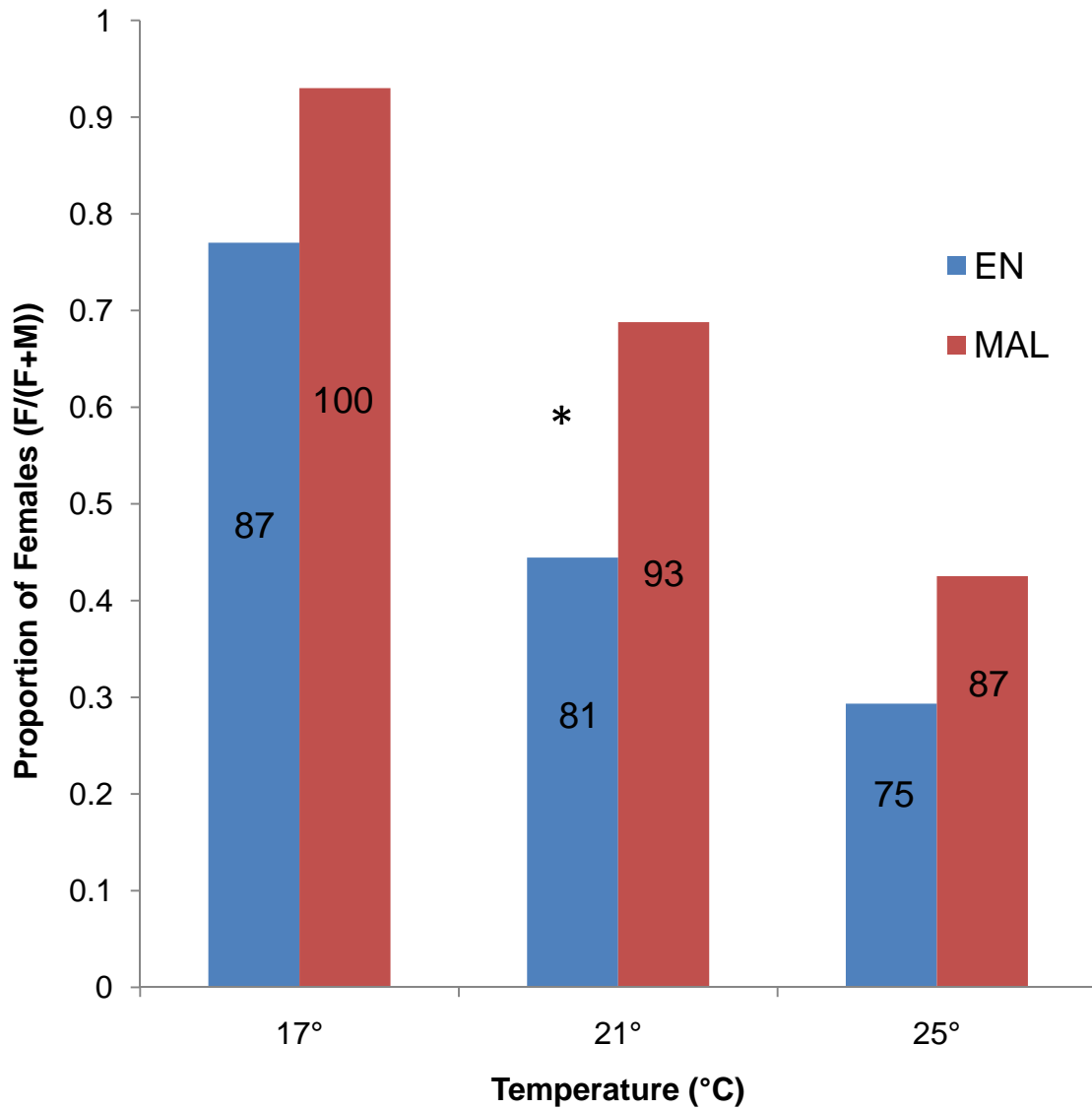


Figure 11: 2010 sex ratios by population and temperature. Numbers inside the bars indicate sample sizes. Asterisk indicates significant difference between populations within a treatment. Legend: EN = Ensenada and MAL = Malibu.

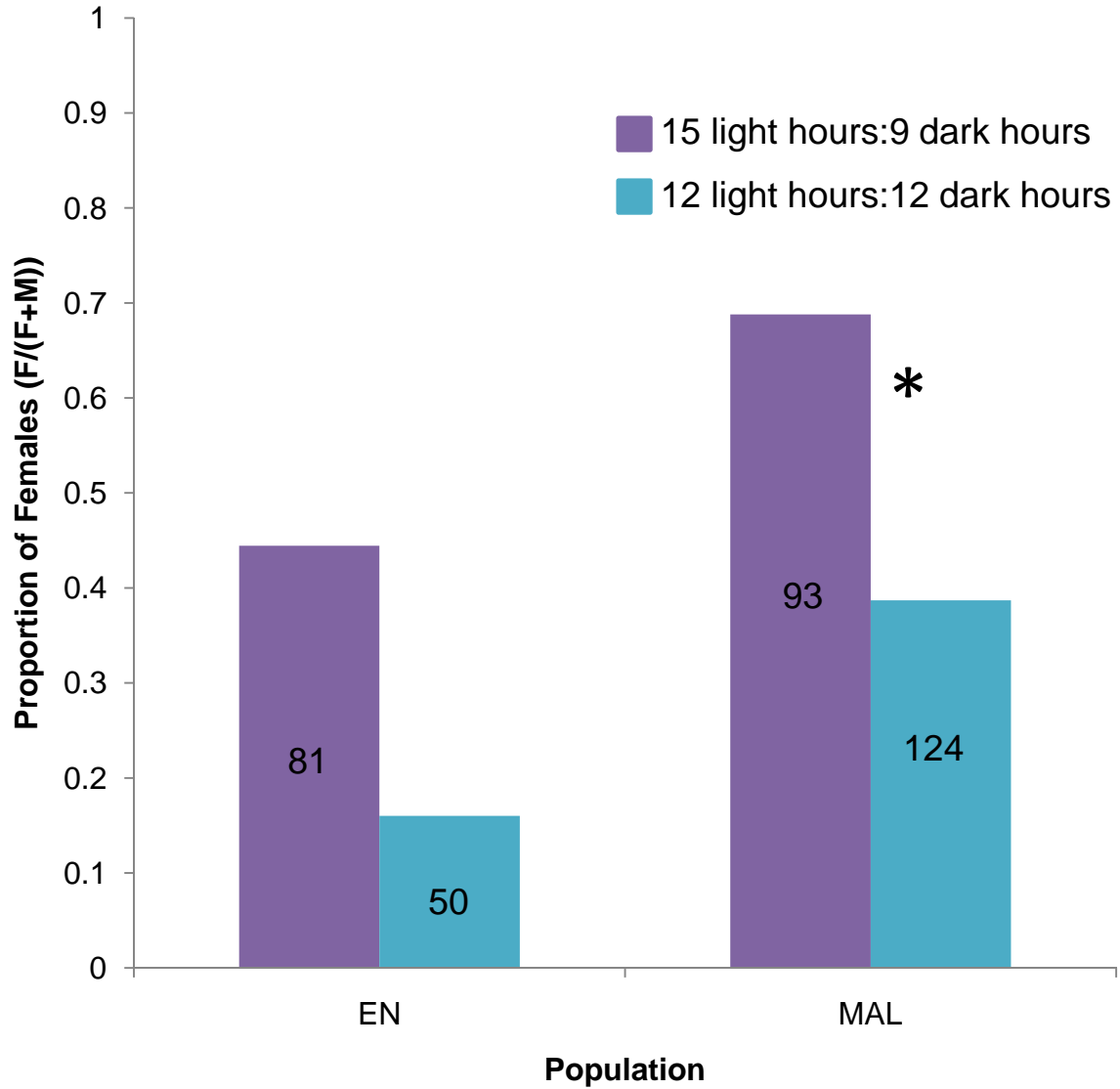


Figure 12: Sex ratios for the two photoperiod treatments for the Ensenada (EN) and Malibu (MAL) populations. Numbers inside the bars indicate sample sizes. Asterisk indicates significant photoperiod effect.

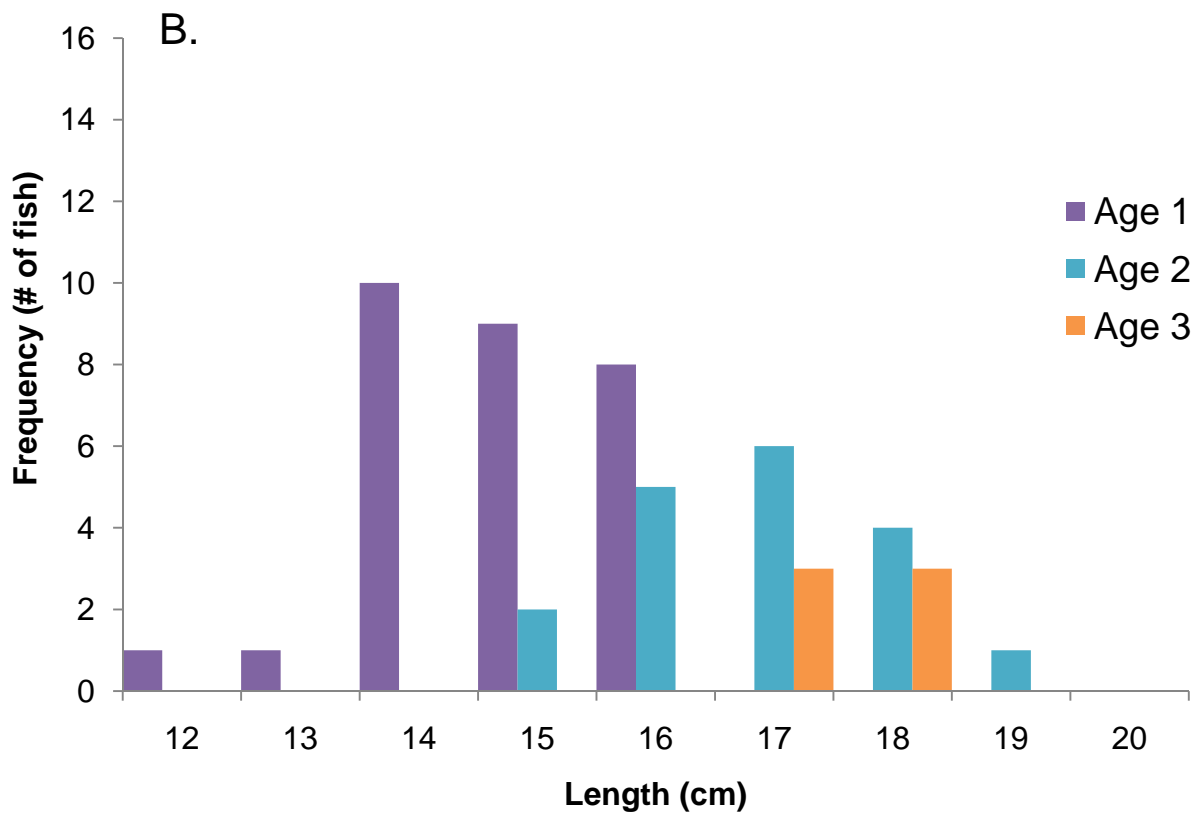
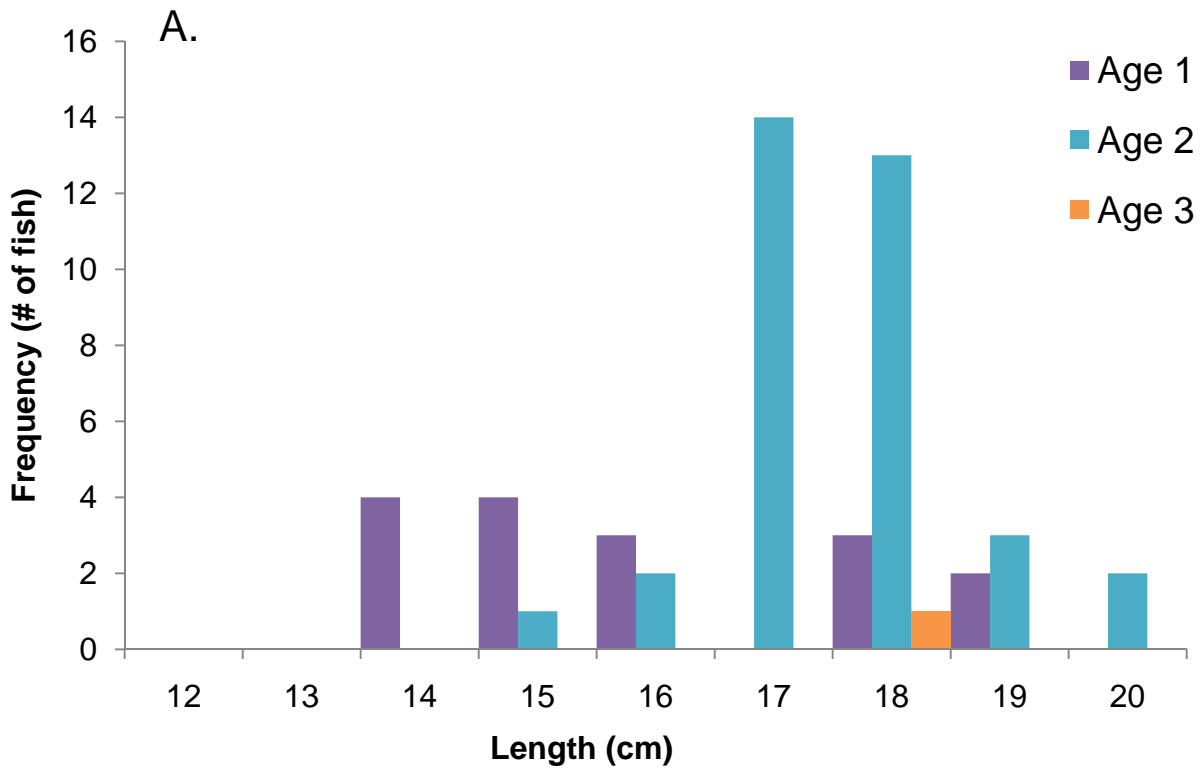


Figure 13: 2009 length frequency distributions by age for wild grunion from Ensenada A) and Malibu (B).

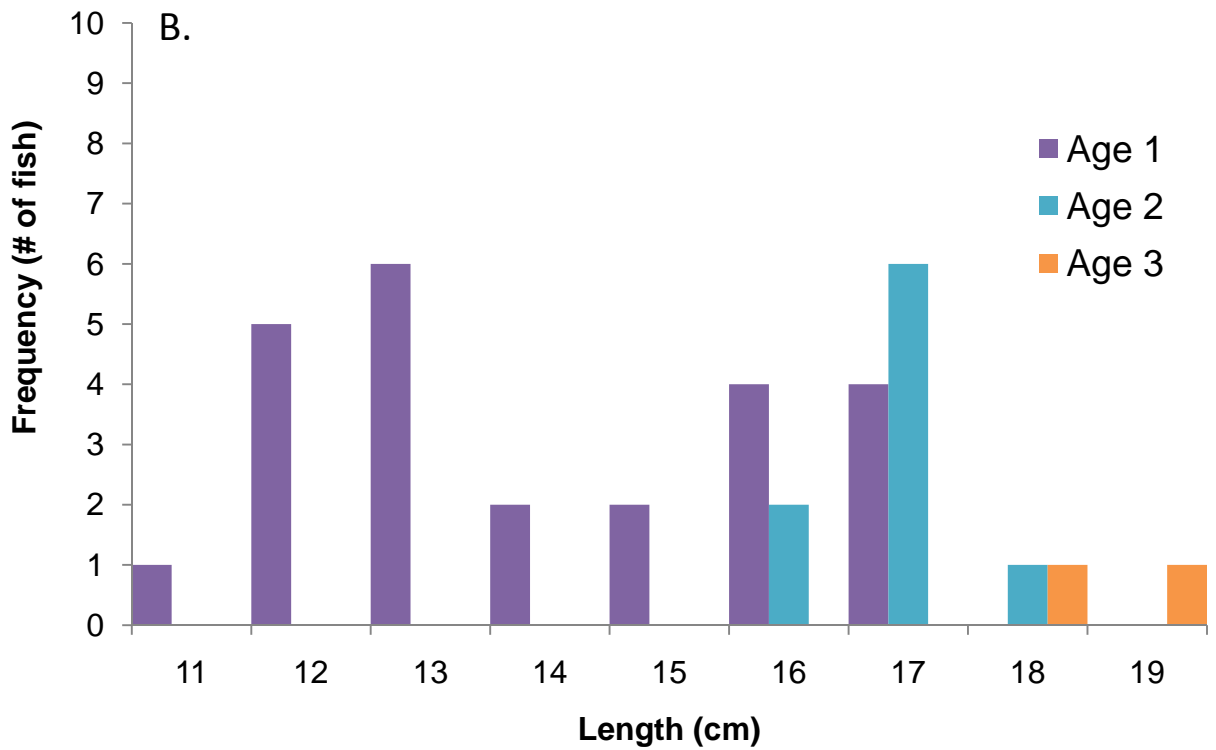
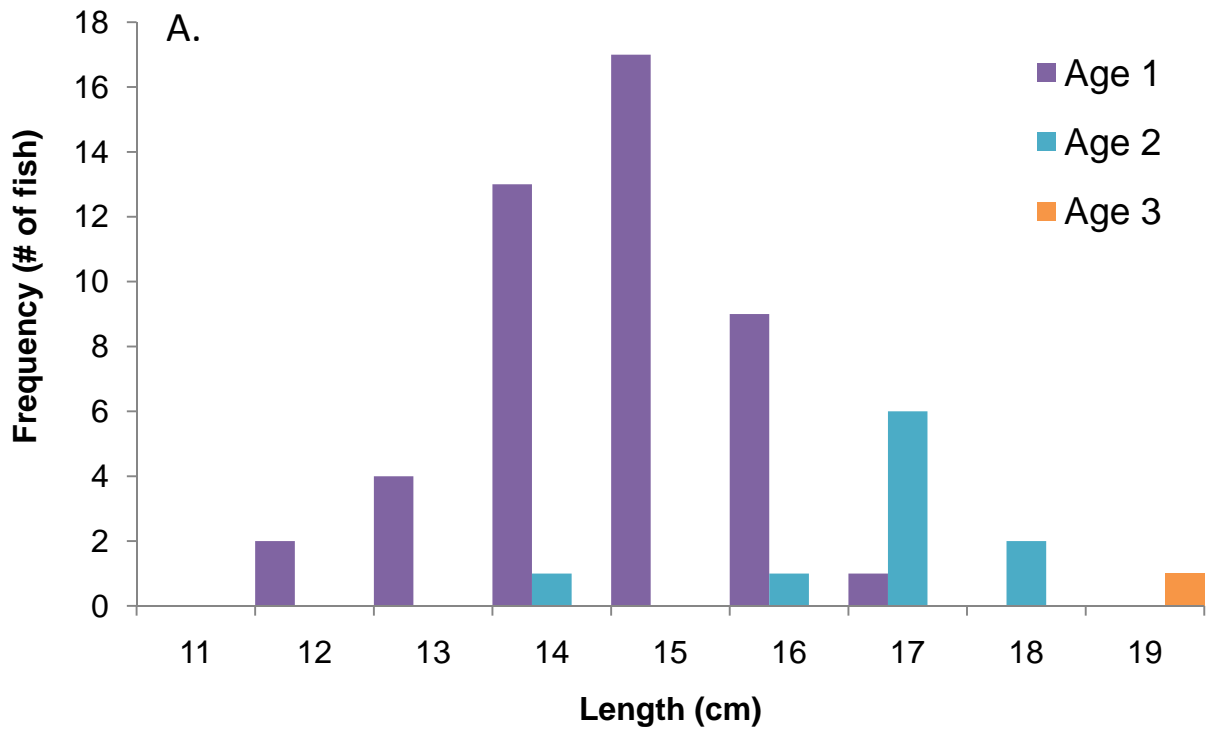


Figure 14: A-B, 2010 length frequency distribution by age for wild grunion from Ensenada (A) and Malibu (B).

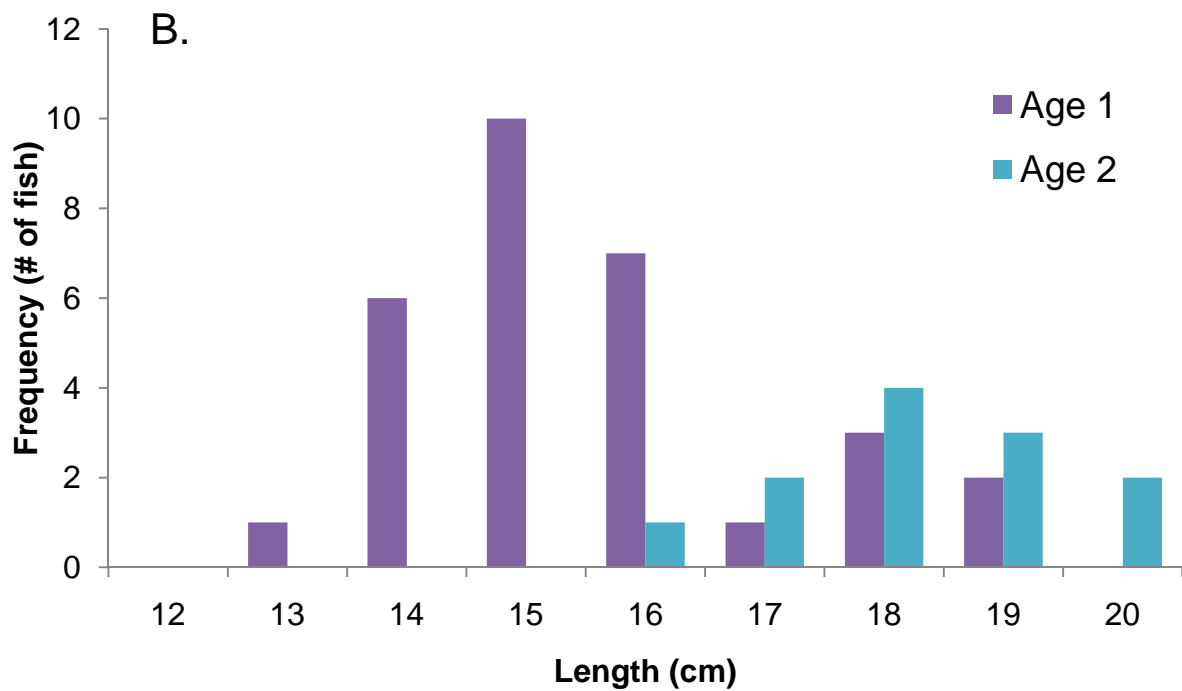
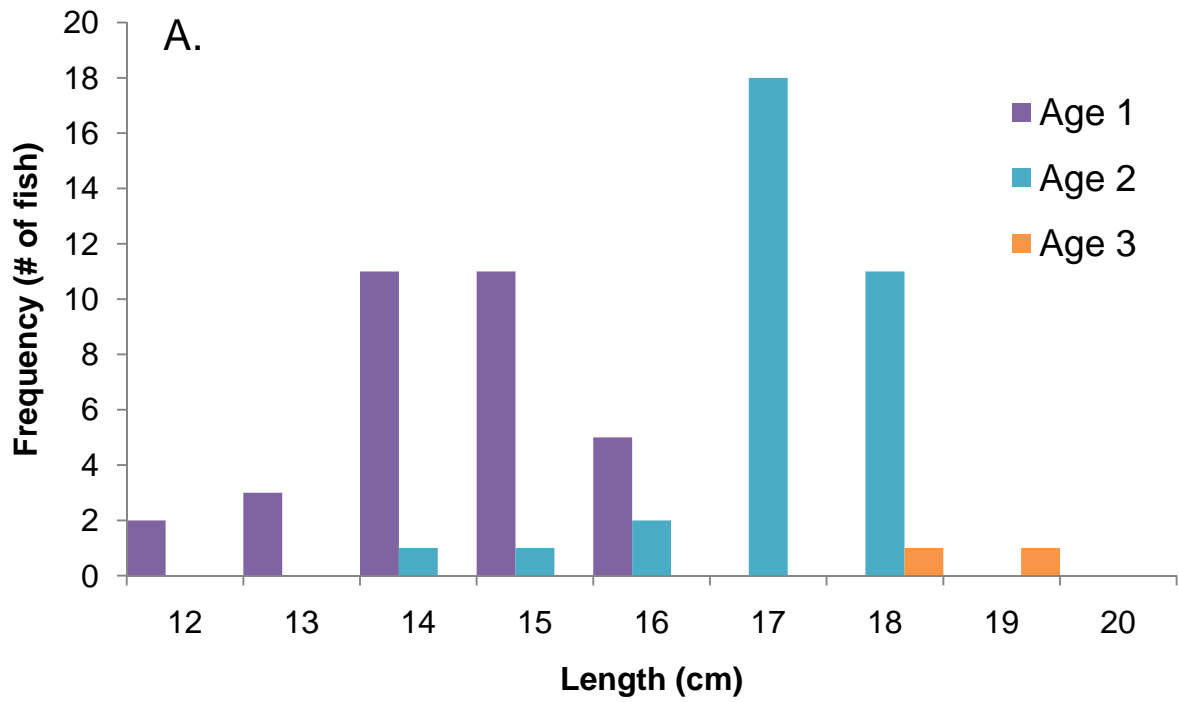


Figure 15: A-B, 2009&2010 combined length frequency distributions by age for Ensenada males (A) and females(B)

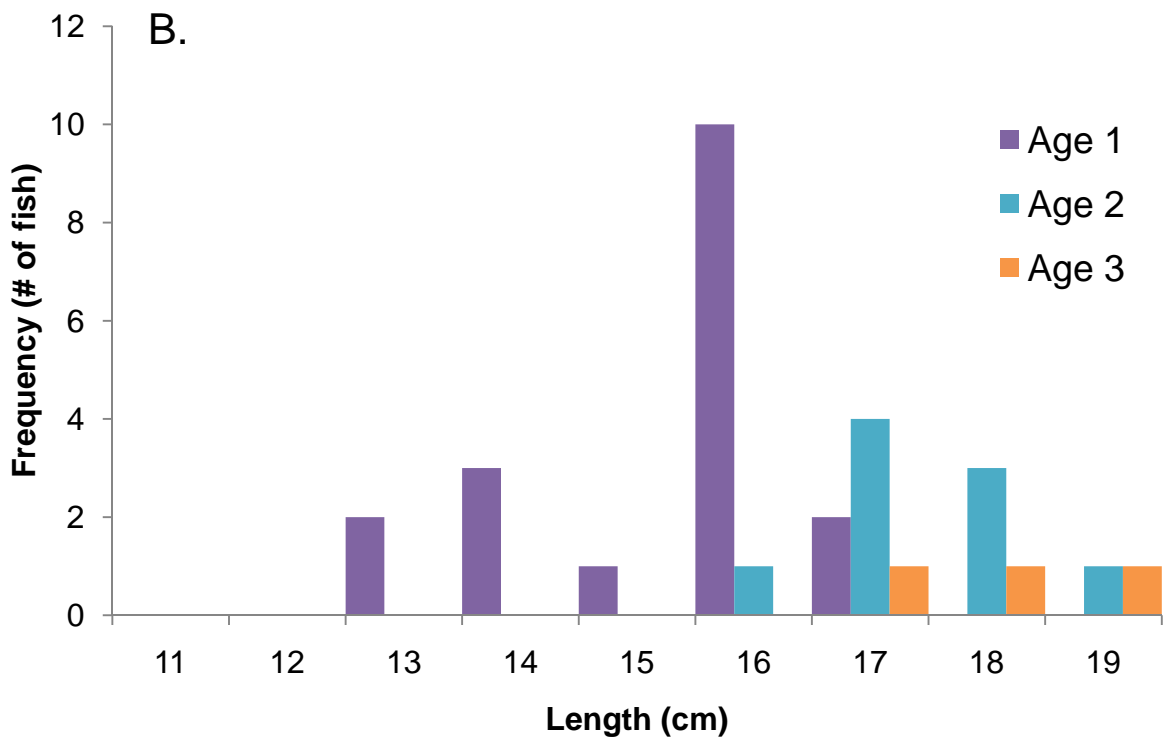
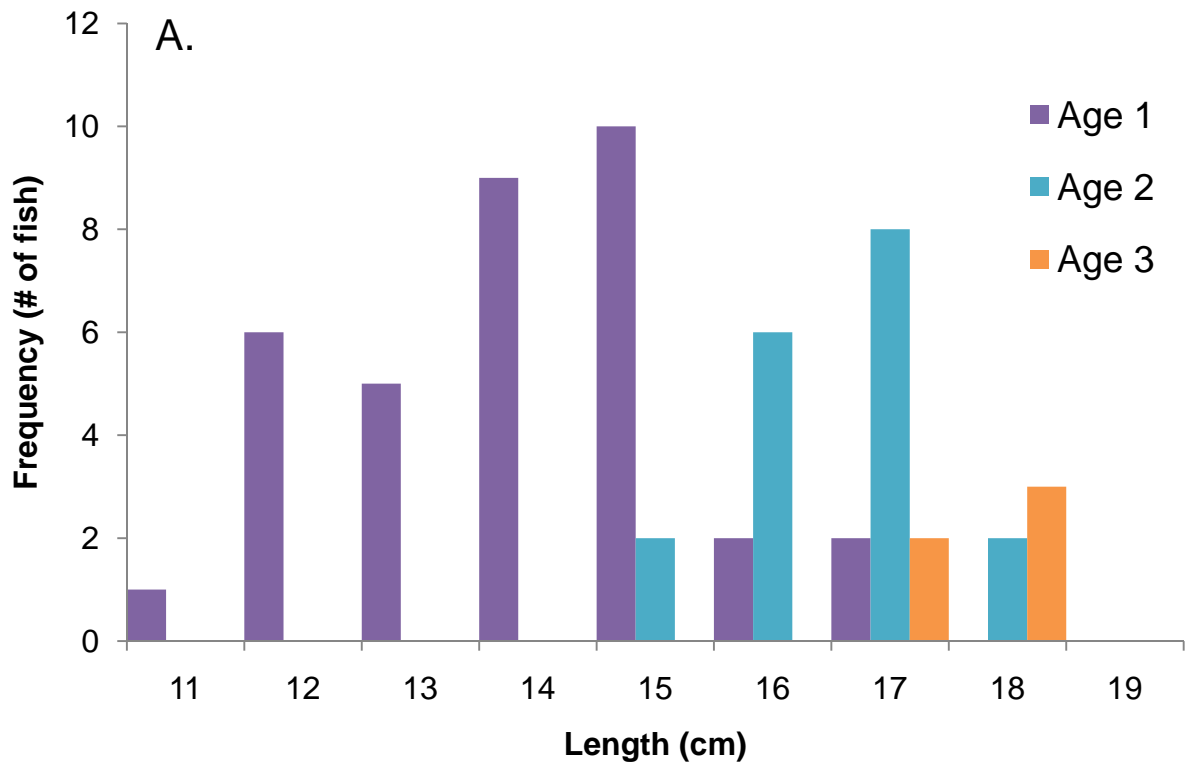


Figure 16: A-B, 2009&2010 combined length frequency distributions by age for Malibu males (A) and females (B).

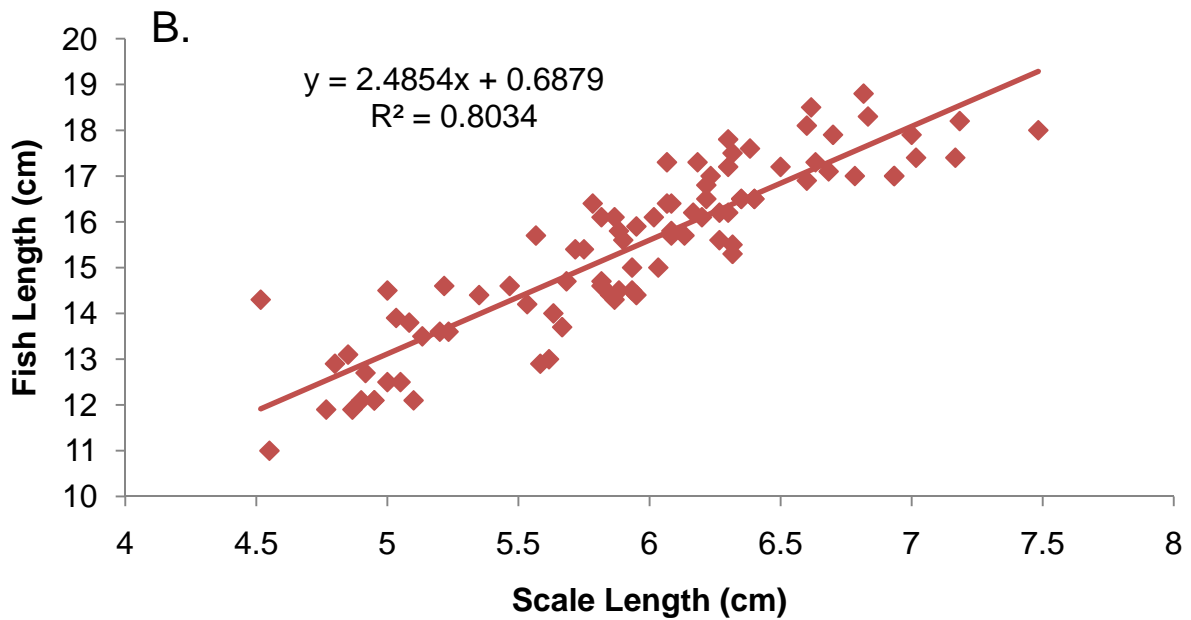
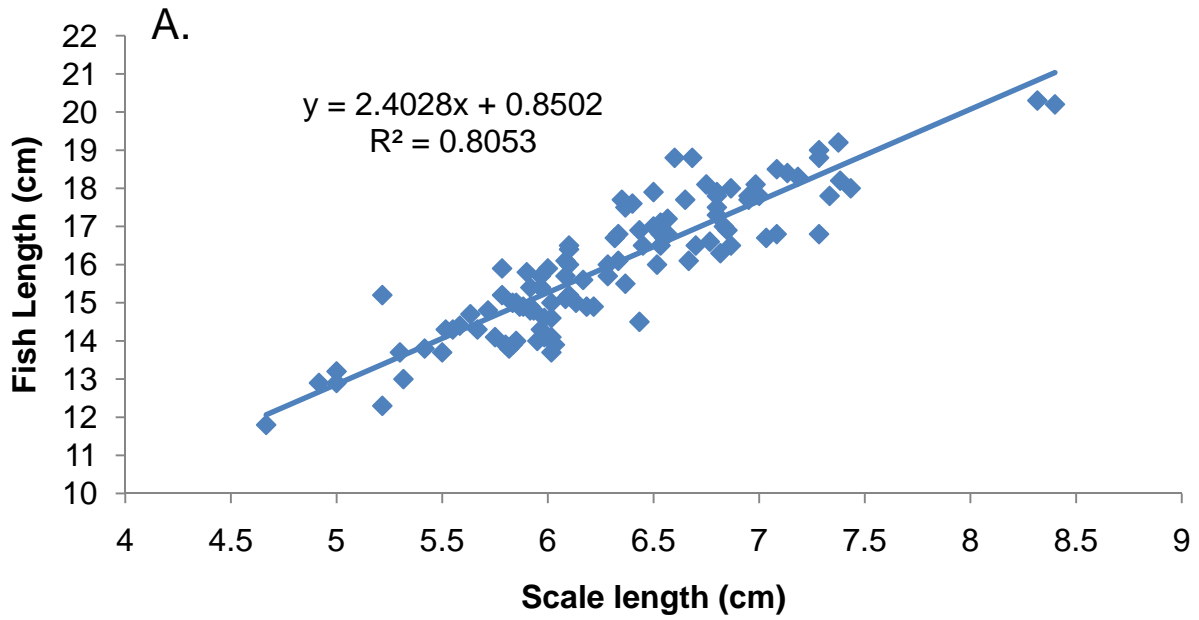


Figure 17: Scale to fish length relationship for Ensenada (A) and Malibu (B) wild grunion. Scale lengths are not actual, but are lengths measured under magnification.

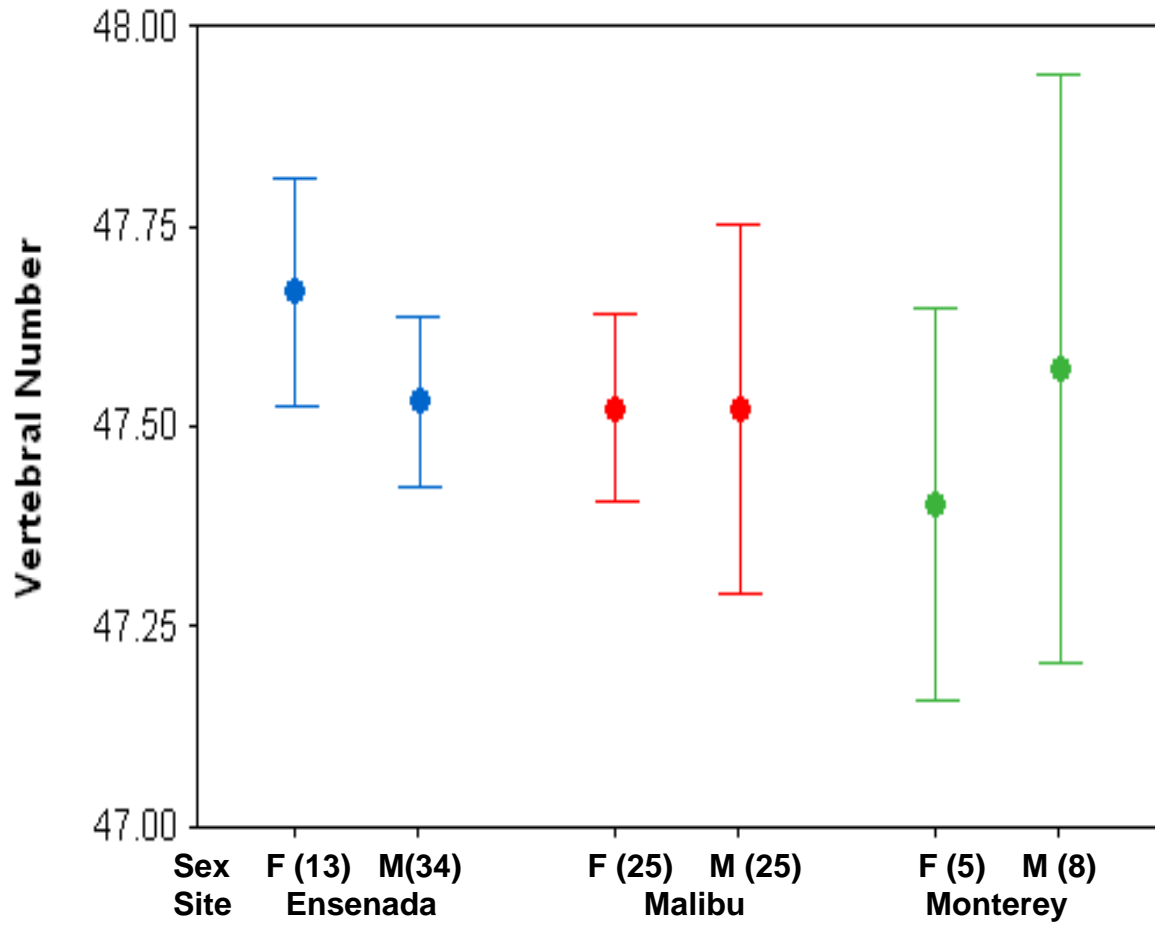


Figure 18: Mean vertebral numbers by population and sex. Vertical bars indicate standard errors.

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