**UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ**

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## HOJA DE APROBACIÓN DE TRABAJO DE TITULACIÓN

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# DEDICATORIA

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# RESUMEN

A nivel mundial las aguas ricas en nutrientes producidas por afloramientos son un factor ambiental clave que influye la naturaleza de las cadenas tróficas. La Reserva Marina de Galápagos es conocida por la gran variación espacial y temporal de los afloramientos, lo que permite la existencia de una biota única en aguas ecuatoriales. Se sabe que las diferencias en niveles de afloramiento produce una variación en la estructura de las comunidades bentónicas. Sin embargo, se desconoce cómo dichas diferencias afectan a las comunidades locales de peces y cómo las respuestas de éstos contribuyen al entendimiento de los patrones de afloramientos.

El objetivo general de este estudio fue el de entender los patrones de crecimiento de la damisela de cola amarilla (*Stegastes arcifrons*), un pez territorial, así como de las señales de química elemental en sus otolitos, bajo diferentes regímenes de afloramiento, utilizando métodos descriptivos y experimentales.

Se colectaron 10 peces adultos, con un diseño anidado, en ocho sitios cuatro con alto, cuatro con bajo afloramiento), se utilizó los otolitos sagitales para determinar la edad de los individuos, la tasa de crecimiento (relación entre talla y edad), crecimiento anual (incremento entre los anillos de crecimiento en el otolito). También se realizaron análisis de química elemental mediante el uso de Espectometría de Masas con Plasma Acoplado Inductivamente (ICP-MS), sola y mediante Ablación con Láser. Se obtuvieron datos de la temperatura superficial del mar de imágenes satelitales, para comparar con la carga elemental de los otolitos. Adicionalmente, se colectaron 36 peces juveniles con los que se llevó a cabo un experimento de mesocosmos para evaluar los efectos de temperatura y alimento en la química de los otolitos. Se utilizaron métodos estadísticos univariados y multivariados para el análisis de datos.

Se encontró que la edad máxima de un pez fue 23 años. Los peces por lo general fueron más grandes en áreas de alto afloramiento, aún así las curvas de Von Bertalanffy demostraron que la talla al infinito fue similar entre los tratamientos (~ 116 mm SL). Sin embargo, la tasa de crecimiento hacia $L\infty$ fue mayor en peces provenientes de áreas con alto afloramiento (K = 0.8, *cf* 0.5 en bajo afloramiento). El incrementos anual entre los anillos de crecimiento se estabilizó alrededor de los 8 años y la variación del incremento a partir de ese tiempo se consideró un aproximamiento para la variación anual de crecimiento. Los menores incrementos ocurrieron en sitios de bajo afloramiento.

Se encontraron patrones contrastantes en las proporciones elementales de los otolitos enteros de los peces provenientes de diferentes regímenes de afloramiento. Las proporciones Ba/Ca fueron significativamente menores en sitios con alto afloramiento comparados con sitios de bajo afloramiento. Además, la mayoría de la variación (46%) fue explicada por estas diferencias. Para las proporciones Mn/Ca también se encontraron diferencias significativas en diferentes regímenes de afloramiento, sin embargo, la mayoría de la varianza fue explicada por la variabilidad entre sitios. Por otro lado, para Mg/Ca y Sr/Ca no se encontraron diferencias significativas entre diferentes regímenes de afloramientos y la mayor parte de la variación fue entre los peces (a nivel de individuos), y entre sitios, respectivamente.

Los análisis multivariados (PCA) detectaron que existen diferencias en las firmas elementales entre sitios, que los distinguen dentro de los regímenes de afloramiento. Se encontró una fuerte y positiva correlación entre la temperatura y las proporciones Sr/Ca. Los resultados de los análisis hechos a los otolitos con el uso de ablación de láser y ICP- concuerdan con las conclusiones para los análisis de otolitos enteros, en el sentido de que se encontraron diferencias entre los tratamientos consistentes entre ambas técnicas. Inclusive se pudo detectar patrones temporales de incremento de la proporción Sr/Ca que puede corresponder al incremento de la temperatura que caracteriza eventos de El Niño. Por otro lado, los hallazgos del experimento concuerdan con la relación entre más altas proporciones de Sr/Ca y mayores temperaturas, como en el caso de los otolitos de los peces adultos provenientes de sitios con bajo afloramientos. También se evidenció una tendencia fuerte en el aumento de la proporción Ba/Ca con el incremento en la temperatura y la cantidad de alimento, pero ésta no fue significativa.

En conclusión, la variación espacial y temporal en los patrones de crecimiento, el incremento en los anillos de crecimiento dentro de los otolitos y la química elemental se pueden relacionar con los niveles de afloramiento. Los patrones detectados en este estudio proveen un mayor entendimiento de la ecología de peces territoriales de arrecife.

*Palabras clave*: Afloramiento, peces damisela, otolitos, patrones de crecimiento, química elemental.

# ABSTRACT

Worldwide the upwelling of nutrient rich waters is a key environmental factor that influences the nature of marine food chains. The Galapagos Marine Reserve (GMR) is known for great spatial and temporal variation in upwelling and this supports a unique fauna in equatorial waters. It is known that differences in levels of upwelling (ie. High vs. Low) translates in the variation in benthic community structure. There is, however, a paucity of knowledge on how such differences affect local fish communities and how the responses of fishes can be informative about patterns of upwelling. Otolith elemental chemistry can vary according to environmental conditions during the life of a fish.

The broad objective of this thesis was to study patterns of growth and elemental signals in the otoliths of a territorial damselfish (*Stegastes arcifrons*) to identify responses to different levels of upwelling; descriptive and experimental methods were used.

Adult fish (n=10) were collected at 8 sites, in areas of Low and High upwelling regimes, where four sites were nested in each treatment. Sagittal otoliths were used to determine the age of individuals, growth rate (size vs. age), yearly growth (increment of interannual spacing). Also elemental chemistry analysis were performed with inductively coupled plasma mass spectrometry (ICP-MS), in some cases coupled with a laser ablation system. Sea surface temperatures among sites were determined using satellite imagery, to relate it to elemental loads. Additionally, 36 juvenile fish were collected to perform a mesocosm experiment to evaluate the effects of temperature and food in otolith chemistry. Univariate and multivariate statistical methods were used for data analysis.

The maximum age of fish was 23 years. Fish were generally larger in areas of High upwelling, but Von Bertalanffy curves demonstrated that length at infinity was similar between upwelling treatments (~ 116 mm SL). However, the rate of growth to $L\infty$ was higher in fish from areas of High upwelling (K = 0.8, *cf* 0.5 in low upwelling). The spacing of increments plateaued after about 8 years and variation in spacing from that time was considered to be a proxy for annual variation in growth. Increments were more closely spaced at Low upwelling sites.

Contrasting patterns of elemental ratios were found in whole otoliths of fish among upwelling treatments. Ba/Ca ratios were lower in High upwelling sites compared to Low upwelling sites and significant differences were found among sites within upwelling regimes. For Mn/Ca and Sr/Ca ratios variance components indicated that most of the variation was found among sites. Most of the variation for Mg/Ca ratios was detected at the residual level and high levels of individual variation was recorded for all elements. Multivariate analyses (PCA) detected differences in elemental fingerprints among sites, within treatments. Ba/Ca and Sr/Ca ratios had greatest influence in the elemental fingerprints. A positive correlation was detected between temperature and Sr/Ca ratios. Laser ICP-MS allowed me to test for temporal variation within otoliths. Laser data concurred with the conclusions of whole otolith analyses in that the differences found between upwelling treatments were consistent for both methods. Furthermore, temporal patterns were detected within fish that apparently correlates with the timing of major El Niño events, with Sr/Ca increments.

The experiment concurred with the relationship found in adult fish in that significant differences in Sr/Ca rations were detected among temperature treatments. Sr/Ca ratio where highest in the highest temperature treatment. There was a strong trend for Ba/Ca ratios to increase with temperature and quantity of food, but this was not significant.

In conclusion, spatial and temporal variation in patterns of growth, increment spacing within otoliths and elemental chemistry could be related to level of upwelling. The patterns detected in this study provide greater insight to the ecology of a reef fish.

*Key words*: Upwelling, damselfish, Otoliths, growth patterns, elemental chemistry.

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# 1. INTRODUCTION

In marine ecosystems, oceanic conditions affect local biodiversity, community composition and important ecological processes. Ocean currents are particular significant because they provide continuous water mass movement that greatly influences biological productivity (Sigman & Hain, 2012). As currents meet and mix, reach continental landmasses or major rivers, or move over shallower depths, various types of vertical circulation patterns are generated that affect nutrient availability (Lalli & Parsons, 1997). There are two general patterns of nutrient supply in the ocean: (i) at high latitudes and during winter deep mixing, the thermocline decreases and nutrient mixing increases; (ii) at low latitudes where seasonality is often weak, as in the Eastern Tropical Pacific, wind-driven surface water flow produces upwelling (Pennington et al., 2006).

Generally speaking, high latitude oceans have nutrient-rich waters (Sigman & Hain, 2012). This feature contributes to high primary productivity that supports large fish populations and important commercial fisheries. In contrast, tropical nutrient-poor waters host a great diversity of fish species, however population sizes and biomass are smaller compared to the temperate or polar regions (Bone & Moore, 2008). Coastal upwelling is a wind-driven circulation process that results in high nutrient supply to the surface ocean, and when followed by stratification and an optimal light environment leads to greatly enhanced levels of primary production relative to other regions of the world ocean. While coastal upwelling can occur along any coastline it is most prevalent in the eastern boundaries of the Atlantic and Pacific Oceans (Messié & Chavez, 2015). These general patterns however are affected by regional climatic variability. For example, in the North Pacific there are well documented “regime shifts” with abrupt physical and biological changes that persist for over a decade (Lalli & Parsons, 1997). Such changes have significant consequences for productivity and species composition of fisheries resources in the region (Lalli & Parsons, 1997). In the Eastern Pacific, the inter-annual and decadal climatic variability affects annual catches of the Peruvian anchoveta, the largest single-species fishery in the world (Brander, 2007). During El Niño Southern Oscillation (ENSO) events, upwelling is reduced and warmer water spreads and persists in the region for several months, reducing significantly the primary productivity and therefore the population of the Peruvian anchoveta, with catastrophic consequences for the seabirds that feed on them, as well as for the fisheries that depend on them (Brander, 2007).

## 1.1. Oceanographic settings surrounding the Galápagos Marine Reserve (GMR)

The particular geographic and oceanographic settings that surround the Galápagos Archipelago make these oceanic islands unique in terms of diversity and endemism, since it possesses marine ecosystems with characteristics from both tropical and subtropical/temperate regions (Edgar, Banks, Fariña, Calvopiña, & Martínez, 2004). The Galápagos Islands are located at the equator at the confluence of several major oceanic currents in the Eastern Tropical Pacific Ocean. The warm Panama Current flows from the northeast and affects the northern and central islands. In addition, cool water flows from east to west carried by the South Equatorial Current. This current is also affected by the Panama current in the north and by the Humboldt Current, which is generated in the Peru upwelling zone, and affects the central and southern areas of the Galápagos. In addition, the eastward-flowing subsurface Equatorial Undercurrent (EUC, also known as the Cromwell Current), brings cold nutrient-rich waters to the surface by means of topographically generated upwelling, and mainly affects the western section of the archipelago (Palacios, 2004), with some influence in the central archipelago as well (Witman, Brandt, & Smith, 2010). These currents show seasonal variability and are also influenced by trade winds and other atmospheric processes that change the water mass properties in terms of salinity, pH and temperature (Sweet et al., 2007, 2007).

During an El Niño event, the Equatorial Undercurrent weakens, upwelling is suppressed, the ocean surface around the Galápagos warms substantially and the islands receive significantly more rainfall than normal (Cane, 2005; Sachs & Ladd, 2010; (Liu, Xie, Morrison, & Kamykowski, 2013). The warmer water is poorer in nutrients compared to the cool waters that normally surround the archipelago, primary production decreases, and fish numbers diminish (Liu et al., 2013). These changes had devastating impacts on the marine ecosystems, causing mass mortality of corals, seabirds, marine iguanas, marine mammals, fish and invertebrates during the most intense events, such as those of 1982–3 and 1997–8 (Victor, Wellington, Robertson, & Ruttenberg, 2001; Edgar, Banks, Brandt, Bustamante, Chiriboga, Earle & others, 2010; Sachs & Ladd 2010); both the strongest historically recorded (Cane, 2005; Liu et al., 2013).

## 1.2. Upwelling-related ecological processes in Galápagos

The topography on the seafloor on the Galápagos also contributes to the contrasting oceanographic conditions within the archipelago: in several sites closed to deep waters there is frequent upwelling, commonly reported by scuba divers (Witman & Smith, 2003). Gordon Rocks (northeast of Santa Cruz Island), a site with strong upwelling, experienced daily temperature fluctuations that corresponded to upwelling events (Witman & Smith, 2003). As a consequence, there was a rapid epifaunal community change over one year, doubling the species richness within that period. At the same time some macro invertebrates showed great changes in percentage cover, biomass and/or density. This study took place between 1999 and 2000, after the severe 1997-98 El Niño event, when an important portion of the shallow water communities experienced severe changes (Witman & Smith, 2003). In another study between 2006 and 2009, in the intertidal rocky shores in an upwelling gradient in the Galapagos, (Vinueza, Menge, Ruiz, & Palacios, 2014) demonstrated that in areas of Intermediate and High upwelling, the bottom up effects were stronger on algal biomass, whereas in sites of low upwelling the top down control of macro-herbivores was the prevailing mechanism regulating algal abundance.

On the other hand, Krutwa (2014) demonstrated experimentally, by comparing the sessile communities in two sites in Floreana, that the Low upwelling site experienced seasonal changes in community structure, while the High upwelling site showed a more stable community composition, because it received cold water pulses frequently, which masked the effects of seasonality. In another study that compared several sites with High, Mid and Low upwelling regimes in the central archipelago, High upwelling sites supported a greater recruitment and abundance of the barnacle *Megabalanus peninsularis*, an important suspension feeder of the archipelago, and their abundance was also correlated to greater abundances of one of its main predator, the whelk *Hexaplex princeps* (Witman et al., 2010). In addition, there were higher predation rates from whelks and fish on the barnacles in the High upwelling sites (Witman et al., 2010).

The findings of these studies suggested that High upwelling sites are very important as a frequent source of nutrients and food availability. Also High upwelling sites seem to have less environmental variation compared to Low upwelling sites, which has a direct impact on local community structure. Understanding how this productivity supports local diversity and ecological processes has been studied mostly on benthic organisms, however the importance of upwelling on the ecology of reef fish remains unstudied in the Galápagos. For this reason, the objective of my project was to determine the effects of upwelling on the patterns of growth and elemental loads in the otoliths of a wide spread herbivore fish, the yellow-tail damselfish, *Stegastes arcifrons*. It was thought possible that elemental and growth records in the otoliths of fish could provide an environmental record that would give better resolution of spatial and temporal patterns of upwelling.

## 1.3. Otoliths as environmental recorders

There are several tools to quantify marine environmental variables that can be related to primary productivity: water flow, temperature, chlorophyll-α and dissolved oxygen concentrations. A viable alternative is the utilization of satellite imagery that uses chlorophyll-α concentration as a proxy for primary productivity, however this tool has its limitations.

Different plankton species have different photosynthetic rates, which derives into dissimilar chlorophyll cellular concentrations that are not necessarily related to primary productivity. Also, small variations in grazing can have considerable proportional effects on plankton biomass, affecting the image taken (Sigman & Hain, 2012). In addition, both image quality and processing tools are continuously improving along with different levels of resolution, making a standardized method difficult to define (Thengade & Agale, 2014). Although satellite-derived ocean color observations have transformed our view of oceanic productivity, in order to elucidate environmental histories at the habitat level, it is perhaps a better option to work directly with living organisms, because satellite data are not easily available.

Many sessile organisms, such as scleractinian corals and bivalves possess carbonate skeletons that are useful for the reconstruction of temporal and spatial variation of environmental conditions in marine systems. The deposition of calcium carbonate in most skeletons provides a chronological record that is generally not deconstructed through metabolism. Not only is there sequential deposition, but discontinuities can represent years and even days. Furthermore, variation in microchemistry can provide geochemical signals that can be related to variation in the environment (Walther, Kingsford, & McCulloch, 2013).

In order to reconstruct in the Galápagos Archipelago the temporal and spatial dynamics of some elements as geochemical signals, the use of fish otoliths seems a good alternative because as opposed to other organisms that need specific locations to survive (e.g. scleractinian corals) fish are present all over the archipelago. In this regard, the yellow-tail damselfish (*Stegastes arcifrons*) is a perfect candidate species, because it has wide-spread distribution in the Archipelago (Allen & Woods, 1980; Allen et al., 2010). In addition, by being a territorial and sedentary fish, variation in patterns of growth and elemental chemistry could be attributed to variation in the environment rather the potentially confounding influence of fish movement.

Teleost fishes have three pairs of structures called otoliths. The sagittae, lapilli and the astericus are located in the inner ear and are used for balance and auditory senses. Otoliths are made of calcium carbonate (aragonite) deposited on a protein matrix. New material is incorporated on a daily basis to the exterior surface of this structure. In addition to the major constituents such as Ca, minor (e.g. Sr) and trace elements (e.g. Ba) are incorporated into the aragonite matrix. The incorporation of these elements and nightly increases in protein deposition is continuous and it leads to the formation of daily and annual growth rings that have been used for over a century to determine the age of fishes (Campana, 1999). Otoliths are acellular and metabolically inert, therefore once elements are incorporated into the calcareous material they are not subject to reabsorption and the chemical composition of a given layer will remain constant over time (Campana & Thorrold, 2001; Walther & Thorrold, 2006).

Environmental variables such as temperature, salinity and water chemistry influence how elemental incorporation takes place (Elsdon & Gillanders, 2004). Because of this, otoliths can provide a chemical chronology of the entire life of a fish (Elsdon et al., 2008). Reconstructions of life histories are possible with modern spectrometry tools that allow finer resolution analysis (Elsdon & Gillanders, 2004). The use of elemental signature in otoliths has helped to determine differences in chemical composition even at a small spatial scale (200 m), which may be useful for determining the movements of fishes (salmon), recruitment areas (Gillanders & Kingsford, 1996) and population connectivity studies (Lo-Yat et al., 2005). There is not a clear pattern on how environmental water chemistry and oceanographic physical conditions determine the element up take into the otolith (Bath et al., 2000), and apparently there are significant differences that are species or stock-specific (Barnes, Gillanders, & Rose, 2013). In a recent study it was found that Strontium (Sr) was considered one of the most informative and extensively used elements in otolith chemistry; it substitutes Ca within the aragonite crystal, which means that its presence in the otolith will depend on the availability on the environment (Doubleday, Harris, Izzo, & Gillanders, 2013). As a consequence of this and as reviewed by Campana (1999), elements in the otolith are likely to provide an accurate prediction of the chemical and physical properties of the water mass.

In addition, the use of otoliths for aging fishes began more than a century ago; over the years mostly important temperate fisheries-related species have received sufficient attention (Campana & Thorrold, 2001; Campana, 2005). Since the 1990s, more studies have focused on tropical species, that also experience seasonal variation, which in turn, due to physiochemical regimes produce different markings on the otoliths, translated into opaque and translucent bands, that together are accepted to be produced during one-year periods (Fowler, 2009).

## 1.4. *Stegastes arcifrons* (Heller & Snodgrass, 1903)

The species of focus in my study belongs to the Pomacentridae. It inhabits the Galápagos, Cocos and Malpelo Islands, where is common and abundant (Allen & Woods, 1980). In

Galápagos the yellow-tail damselfish suffered a minor population decline following the 1997-98 El Niño event, however it recovered completely after one year (Allen et al. 2010). It is an omnivorous species that feeds on algae and several invertebrates including tubeworms, small crustaceans and anemone’s tentacles (Allen et al. 2010). As is typical of many reef fish, the yellow-tail damselfish disperses during planktonic larval stage and once settled it spends its remaining life in a specific territory; such a behavior is common in several damselfish species (Munday & Jones, 1998; (Ceccarelli, Jones, & McCook, 2001). *Stegastes arcifrons* defends its ‘farmed' algal turf from other herbivorous fish and small invertebrates, and this has a positive effect on maintenance of algal composition and diversity (as well as associated fauna) of the habitat, since the fish will favor certain algal species inside their territory (Irving & Witman, 2009).

*Stegastes arcifrons* is particularly useful for this study because it is relatively long-lived; it has been reported to live 25+ years (Meekan, Wellington, & Axe, 1999). Although it has been reported to be present up to 20 m (Allen et al. 2010), currently it is difficult to find individuals deeper than 7 m (*pers. obs.*). This means that it spends its life exposed to the shallow water conditions historically documented to equate with Sea Surface Temperature (SST) conditions. Meekan et al. (1999) found bands on the otoliths of the yellow-tail damselfish that corresponded to the 1982-1983 El Niño event, suggesting that environmental conditions are indeed recorded in these structures; however, chemical load differences from ‘normal’ and El Niño years, or between sites are still unknown.

The objective of the study was to determine variation in growth and elemental chemistry of *Stegastes arcifrons* otoliths in areas of Low and High upwelling. The specific aims were:

1. To describe the patterns of growth of fish collected in areas of High and Low upwelling sites.

2. To use the spacing of annual increments in the otoliths as a proxy of growth, and to determine the temporal variation that could be related to upwelling.
3. To use a mensurative experimental design to determine any differences in the elemental loads in otoliths of fish from sites of High and Low upwelling and,
4. To experimentally determine the influence of temperature and the amount of food ingested on elemental fingerprinting.

# 2. PATTERNS OF GROWTH

## 2.1. Hypothesis

I hypothesized that the environmental conditions (i.e. water temperature, primary productivity) that are known to vary between sites with High or Low upwelling intensity would have an effect in the growth patterns of fish from different upwelling regimes, and this will be reflected in the annuli growth of the otoliths of the yellow-tail damselfish.

## 2.2. Materials and methods

### 2.2.1. Selection of study sites

Eight sites in the GMR were chosen according to the average environmental conditions described in previous studies that were related to upwelling, marine biogeography and satellite imagery analysis of sea surface temperatures and chlorophyll concentrations (Table 1; Figure 1). Witman et al. (2010) used *in-situ* measurements to classify upwelling regimes according to vertical flow speed, vicinity of sites to deep waters and persistence of the EUC. Schaeffer et al. (2008) collected *in-situ* water samples to estimate chlorophyll and nitrate concentrations, salinity, and temperature, which they compared to satellite-derived information for determining productive habitats in the GMR. Their findings support the idea of topographically induced upwelling of the EUC to surface waters, similar to Witman et al. (2010). Finally, Edgar et al. (2004) analyzed fish and macro invertebrate distribution and abundances, and recognized four major biogeographic regions that are determined by different environmental conditions. Based on these publications, three sites were treated as High Upwelling sites and three others as Low Upwelling sites. In addition, the classification of the sites in San Cristóbal were based on personal observations at the moment of fish collections. Cerro Brujo (classified as a High Upwelling Site) had a temperature 4°C lower than North Tijeretas, and the water column seem to have more particles in suspension. Those conditions were similar to High upwelling sites, such as La Botella in Floreana Island, were sample collection took place earlier (May of 2015). In comparison North Tijeretas had higher temperatures and a better visibility due to less suspended particles, and was classified as a Low Upwelling Site.

### 2.2.2. Sample design and collection of specimens

A mensurative approach was used to determine the influence of upwelling on patterns of growth. The sampling design was fully nested and hierarchical with the sources of variation and treatments as follows: Upwelling (Low and High); sites nested in upwelling (b = 4); n = 10 adults fish per site. Fish were collected in May and October 2015 using hand spears while SCUBA diving; all fish were in the adult size range (>100 mm total length). In May a field trip was conducted to the sites in San Cristóbal and Floreana Islands; in October fish were collected in Baltra, Fernandina, Pinta and Santiago (Table 1). In May fish were collected during daily field trips, stored on ice and transported to be processed at the Marine Ecology Laboratory of the Galápagos Science Center (GSC) facility on San Cristóbal Island. Fish collected in October were processed on board of the MV Queen Mabel immediately after the dive.

### 2.2.3. Otolith extraction

In order to extract the otoliths I followed the methods of studies of other pomacentrid species (Kingsford, Hughes, & Patterson, 2009); (Walther, Kingsford, O’Callaghan, & McCulloch, 2010) *Acanthochromis polyacanthus*; (Sih & Kingsford, 2015) *Pomacentrus amboinensis*. Independently of the time of collection, all fish were maintained in a flat posture before *rigor mortis* to minimize measurement errors. All fish were measured for standard length (SL), fork length (FL) and total length (TL) to the nearest millimeter before dissection. Afterwards, the largest pair of otoliths (sagittae) were removed, cleaned with Milli-Q water to remove of any tissue left, dried and placed into Eppendorf tubes. Then they were transported to the College of Science and Engineering at James Cook University (Townsville, Australia) where otolith preparation and chemical analysis took place.

### 2.2.4. Otolith preparation

One sagittal otolith was used for aging the fish, while the other for elemental load analysis (section 3.2). The former was sectioned transversally, placed in Crystalbond resin on glass microscope slides, grounded and polished with lapping film. Mounted otoliths were viewed under a Leica DMLB compound microscope using a cold light source. Images were taken with the attached Leica DC300 camera and IM50 software. The images were used for aging the fish and for width increment measurements, which are considered to be a proxy for annual variation in growth (Campana & Thorrold, 2001; Kingsford, O'Callaghan, Liggins, & Gerlach, 2017).

Sectioned otoliths were examined at 200x and opaque annuli (discontinuous zone) and translucent bands (continuous zones) identified and the opaque bands were counted from the nucleus to the edge of the sagittae. One reader made three different readings of every otolith, with an interval of five days between readings. The identification and size of each fish was unknown to avoid potential bias in estimates of age. The data of the different readings were recorded in different files. After the three readings were done, the information was put together in one file to compare. In the cases were readings did not match, a second reader, made at least two independent readings before an age was determined, and a consensus was reached between the two readers.

### 2.2.5. Growth rate

Growth rates were described using the von Bertalanffy growth function, a widely known method for fishes. This model consists of the measurement of length and the assignment of age to individual fish at the time it was collected (Choat & Robertson, 2002). The function has been used to describe with accuracy the growth of other pomacentrids (Kingsford & Hughes, 2005), *Acanthochromis polyacanthus;* (Kingsford et al., 2017), *Pomacentrus coelestis*; (Schwamborn & Ferreira, 2002), *Stegastes fuscus*, and it is described as follows:

$$L_t = L_\infty(1 - exp^{-k(t-t_0)})$$

where

$L_t$ = length at age t;

$L_\infty$ = the asymptote of the growth curve (average maximum length);

*k* = the rate at which the growth curve approaches the asymptote ($L\infty$);

*t* = age of fish in years;

$t_o$ = the theoretical origin of the growth curve (i.e., the hypothetical age of the fish when it has no length); and

*exp* = the base of the natural logarithm.

Values of K and $L\infty$ were attained through the Solver routine in Excel. In addition, the four oldest fish from all sites were chosen to analyze growth rates. All fish were seven years old or older (up to 23 yrs.) and in order to minimize the possible bias that during early years growth rates are higher, bandwidth data for the last seven yrs. was used (2009-2015).

Repeated Measures ANOVA (RM-ANOVA) was used to analyze annual increment since the same otoliths were being used, and therefore the data were not independent. This statistical approach has been used to analyze temporal variation in element loading during several stages of a fish's life (Sturrock, Trueman, Darnaude, & Hunter, 2012); (Sih & Kingsford, 2015).

## 2.3. Results

### 2.3.1. Age estimation

The collected fish included ages from 3 up to 23 yrs. The mean age of fish from High upwelling sites was 7.944 yrs. (SE=0.631) and was very similar to that of fish from the Low upwelling treatment (mean age = 7.881 yrs, SE=0.602); however the age distribution for High upwelling sites is normal, while in the Low upwelling regime there are several young individuals (Fig. 2).

The maximum age of fish was found at a High upwelling site (La Botella, Floreana Island) and it was 23 yrs., while the oldest fish found across all Low upwelling sites was 19 yrs. old and it was collected in Beagle Rocks (Figs. 2 & 3). On the other hand, there was a trend for fish to be larger in areas of High upwelling (SL Mean=119.2; SE=1.18) compared to lLow upwelling sites (SL Mean=113.6; SE=1.53, Figs. 4 & 5).

### 2.3.2. Growth of all individuals

Although von Bertalanffy growth curves demonstrated that length at infinity was similar between upwelling regimes (~ 119 mm SL, Fig. 6), the rate of growth to $L\infty$ was higher in fish from areas of High upwelling (K = 0.8, *cf* 0.5 in Low upwelling, Table 2). This parameter expresses the mean length the fish of a given stock would reach if they were to grow for an infinitely long period. Fish grew more rapidly, therefore, early in life at upwelled areas compared to areas of Low upwelling

The spacing of increments (space between annuli) also showed that for fish of similar age the increments were more widely spaced early in life and when compared to fish from Low upwelling sites.  Late in life, when growth had slowed, increment spacing was similar between upwelling regimes (Fig. 7). Accordingly, the ability to detect differences in growth between upwelling regimes was best when fish were young (ie < 6 yrs. old) (Fig. 7).

### 2.3.3. Annual growth rates

The growth rates through time were significantly different, however not across different upwelling regimes (RM ANOVA, Table 3a). Nevertheless, there is a strong trend for fish of High upwelling sites having wider average annual increments than fish from Low upwelling sites (Fig. 7), which suggest a faster growth in High upwelling sites, specifically early in life. On the other hand, growth rates were significantly different across all sites and years and their interaction (RM ANOVA, Table 3b).

# 3. Elemental Chemistry Analysis

## 3.1. Hypothesis

I hypothesized that the variation in environmental conditions (i.e. water temperature, primary productivity) between sites with High or Low upwelling regime should be reflected in the elemental load in the otoliths of the yellow-tail damselfish. In order to test this, two approaches were used: (1) a mensurative experiment where fish were collected from areas known to be characterized by Low and High upwelling; sites of collection in the nested design were the same as in Chapter two. (2) a manipulative experimental design was used to determine the effects of temperature and food ration on elemental signals.

## 3.2. Materials and Methods

### 3.2.1. Elemental chemistry analysis with all adult fish

One otolith from each pair (of samples collected according to section 2.2.2) was randomly selected and weighed using a Sartorius Genius microbalance to the nearest 0.00001 gr. Otoliths were then cleaned in 1% HNO3 (65% Merck Suprapure) for 5 to 10 s, rinsed three times in Milli-Q water and allowed to dry inside a positive flow Class-100 laminar flow cabinet (AS 1807). Samples were dissolved in 500 µl of HNO3 (20%); the solution was then made up to a final volume of 5 ml with Milli-Q water; otoliths were previously partitioned into groups by eight category for appropriate dilution factor. Samples were analysed on a Varian 820 inductively coupled plasma mass spectrometer (ICP-MS). Blank samples (without otoliths), were prepared in the same fashion and were used for ‘blank corrections’ and to calculate limits of detection (Kingsford et al., 2009).

### 3.2.2. LA-ICP-MS analysis of selected adult fish

Sectioned otoliths, corresponding to the two oldest fish from Champion, La Botella, Cabo Ibbetson and Punta Mangle, were analyzed with a GeoLas 200 Excimer Laser Ablation System (193 nm) coupled to a Varian 820 ICP-MS at the Advanced Analytical Centre in James Cook University. Certified reference material was used to calibrate the equipment. All samples were pre-ablated from the core through the margin with one spot path at 1 Hz with a 24 µm aperture to remove surface material. Ablation was performed on the previous path with a 16 µm aperture at 5 Hz with 10 pulses per spot (58 mJ). Ablated material was delivered to the ICP-MS via an Ar-He carrier gas.

These sites were chosen considering the evidence described in the literature that strongly suggest that La Botella (Floreana) and Punta Mangle (Fernandina) have a strong upwelling regime, whereas Champion (Floreana) and Cabo Ibbetson (Pinta) have weak upwelling regimes (Edgar et al., 2004; Sweet et al., 2007; Witman et al., 2010; Krutwa, 2014; Vinueza et al., 2014). The two oldest fish per site (≥ 9 yrs. old) were selected for this analysis in order to maximize the detection of temporal variation.

### 3.2.3. Elemental chemistry analysis with juvenile fish

As both of these factors are known to affect elemental ratios in other studies (e.g. in Walther et al. 2010), I hypothesized that water temperature and the food ration would alter damselfish otolith chemistry. To test this, I performed an experiment with an orthogonal design with temperature (three levels: High, Mid, Low) and food quantity (three levels: High, Mid, Low) as fixed factors and the ratio between the different chemical elements and Ca as the response variable. The amount of food was based on levels that had the potential to affect growth, and were also used by (Munday, Kingsford, O'callaghan, & Donelson, 2008) and Walther et al. (2010), where somatic differences by food ration were measured. On May 21st, 2015, 40 juvenile *S. arcifrons* (< 50 mm TL) were collected at Tijeretas, San Cristóbal Island, using hand nets and 1:10 clove oil-to-ethanol mixture to enhance survivorship prior the experiment. All fish were taken to the GSC Marine Ecology Lab and placed in a 200 L tank with constant airflow and filtered seawater. Fishes were allowed to acclimatize for 24 hrs at 22°C. During this period fish were *ad libatum* during the day every 6 hrs with a mixture of the following commercial fish pellets (INVE Aquaculture): NRD 3/5 (0.3-0.5 mm), NRD G8 (0.8 mm) and NRD G12 (1.2 mm).

For the experimental phase, 36 fish were randomly chosen and placed in pairs in one of the 18 50 L fish tanks that were used for the experiments. All tanks had a constant flow of filtered seawater and were internally divided in two with a plastic mesh, which allowed having two fish per tank sharing the same temperature treatment, but preventing the fish from affecting each other's food ration. Care was taken to drop food close to target fish to minimize the chances that they would miss the ration.

All Food treatments consisted of the same quantity of commercial fish pellets per fed, however the frequency of food provision varied. High Food Treatment fish were fed twice per day and every day during the experiment, Medium Food Treatment fish were fed once every day, and Low Food Treatment fish only once every other day. Each ration consisted of two NRD G8 (0.8 mm) and three NRD 3/5 (0.3-0.5 mm) INVE Aquaculture pellets, which were the sizes preferred by the fish during the acclimatization period.

I chose three different temperatures for the Temperature Treatment: 18°C, 22°C and 24°C (Low, Medium and High Temperature, respectively). These temperatures were chosen to reflect average SST in distant sites of the archipelago that are known to have variable oceanic conditions, including different upwelling regimes. For instance, in Punta Espinosa in Fernandina Island (located in the West, it is considered a High Upwelling Site), an average SST of 18°C was recorded before the 1997-1998 El Niño event, while in Wolf Island (located in the far North, where water temperature is the highest) the average SST was 24°C (Wellington, Strong, & Merlen, 2001). Similar average water temperatures were also reported in more recent studies; the northeast side of the archipelago having >25°C and the upwelling zone west to Isabela, <20°C (Sweet et al., 2007). The 22°C treatment was chosen as the midpoint between the other two.

At the end of the experiment (9 d), all fish were euthanized with Ethanol (95%), stored in individual 12 ml Falcon Vials and transported to the College of Science and Engineering of James Cook University, where otolith extraction, preparation and chemical analysis took place. Before dissection, all fish were measured to the nearest ml for standard (SL) and total length (TL) and weighted to the nearest 0.001 gr. Both sagittae were extracted and cleaned with Milli-Q water to get rid of any remaining tissue. Then they were let dry for 24 hrs before processed. One otolith per fish was randomly chosen, transverse- sectioned, placed in Crystalbond resin on glass microscope slides, grounded and polished with lapping film; similarly to the handling and preparation of adult otoliths.

The otoliths of the experimental fish were analyzed with La-ICP-MS the same way as for adult fish. However, only the data obtained from the laser ablation spots performed on the external edge of the otolith were used on the analysis since that section corresponded to the growth that took place during the experiment.

### 3.2.4. Data Analyses

*Otolith Elemental Chemistry of Adult Fish* (*Mensurative Design*) - In order to analyze individual element loads, a Nested ANOVA design was applied with upwelling regime (two levels, High or Low) and sites (b=4) nested in each upwelling regime as fixed and random factors, respectively. Some otoliths were lost; in these cases a degree of freedom was subtracted for each loss, so the balanced design (n=10 fish per site) was not affected (Underwood, 1996).

The elemental fingerprints were also analyzed using Principal Component Analysis (PCA). Raw elemental ratios contributed to PC1 and PC2. The PC scores were used as an aggregate score representing a multi-element signal. These scores were then analyzed with the same Nested ANOVA design as above. This approach was also used for the data analysis corresponding to the manipulative experiment.

Data obtained from the LA-ICP-MS phase was plotted after outliers were removed, comparing elemental loads with the literature (Ruttenberg & Warner, 2006; Kingsford et al., 2009). Considering that the use of LA-ICP-MS was to show how element incorporation took place through the life of the fish, exponential smoothing was performed in order to reduce noise in the visualization of the data (5-point moving average, 0.5 damping factor). The data input for the plots was done with an emphasis in overlapping the margin and going backwards to the core, considering that not all fish were born in the same year.

In order to determine any patterns related to temperature and elemental load, satellite derived SST monthly averages data near our collection sites was obtained for a 20 yr. period (1995-2015) (IRI/LDEO Climate Data Library: http://ingrid.ldgo.columbia.edu). Element ratios (Ba/Ca and Sr/Ca) were correlated to the average temperature for that 20 yr. period on every site.

*Otolith Elemental Chemistry of Juvenile Fish* (*Manipulative design*) - A Two-Way ANOVA design was applied with Food (three levels: High, Mid, Low) and Temperature (three levels: High, Mid, Low) as fixed factors. Similarly to the adult fish otolith analysis, a PCA was also applied for elemental fingerprint analyses, with PC1 and PC2 used in separate analyses. Minitab Statistical Software 17.0 was used for all statistical analysis and plots.

## 3.3. Results

### 3.3.1. Elemental chemistry analysis with all adult fish

Contrasting patterns of elemental ratios were found among upwelling regimes (Fig. 8). Some elements showed significant differences between High Upwelling and Low Upwelling sites (Ba/Ca and Mn/Ca), while others did not (Mg/Ca and Sr/Ca) (Fig. 8, Table 4). Significant differences were found among Sites within Upwelling Regimes for all elements (Table 4), and this variation explained 19.1 to 62.9 % of the total variation in the analyses. Variation among replicate fish, within sites, was 25.8 to 80.9 % of the total variation (Table 4).

In general, Ba/Ca ratios were lower in High upwelling sites (Mean 11.77 µmol/mol [1.00 SE]) compared to Low upwelling sites (Mean 33.39 µmol/mol [3.31 SE], Figure 8). There were also significant differences among sites within upwelling regimes, however the upwelling regime explained most of the variation (42.6%), while site within upwelling regime explained 24.6% and replicate fish 32.8% (Table 4). Similar to the Ba/Ca ratio, the Mn/Ca ratio was significantly lower in High upwelling sites compared to Low upwelling sites (Figure 8); with a High upwelling Mean of 1.33 µmol/mol [0.133 SE] and a Low upwelling Mean of 2.23 µmol/mol [0.114 SE]), however it only explained 25.7% of the variation (Table 4).

There were not significant differences for the Mg/Ca (High upwelling Mean 63.89 µmol/mol [5.51 SE]; Low upwelling Mean 71.94 µmol/mol [4.91 SE]) and the Sr/Ca (High upwelling Mean 5.48 mmol/mol [0.213 SE]; Low upwelling Mean 6.97 mmol/mol [0.392 SE]) ratios between Upwelling Regimes (Table 4, Fig. 8). Most of the variation for the Mg/Ca ratio was explained by the replicate fish (80.9%, Table 4). This contrasts to the greater percentage of the variation being explained by the Sites within Upwelling regime, for both the Mn/Ca and the Sr/Ca ratios (48.5 and 62.9%, respectively, Table 4).

The results from the PCA indicated great variation in individual elemental fingerprints (individual fish). The majority of fishes in Low upwelling areas had positive values in PC1 in contrast with those in High upwelling areas that had negative values (Fig. 9). PC1 explained 50.7% of the variance, and was characterized by high Sr and Ba values whereas PC2 (21.7% of the variance) presented high negative Mg values. Mn was the element that contributed the less in the two principal components and in the elemental fingerprint in general (Table 5; Fig. 9).

PC1 multi-element signatures had a strong trend for upwelling, however it was not significant (Nested ANOVA, Table 5). Most of the variation was explained at the site level (within upwelling treatments, 38.5%), with individual fish explaining 37.71% of the variation. As for PC2 the residual explained 86.64% of the variation according to the analysis (Table 5). PCA values for individual fish for PC1 were higher in Low upwelling sites compared to High upwelling sites; PC2 values were negative for High and Low upwelling regime sites (Fig. 10).

Sr/Ca ratios profiles from the LA-ICP-MS transects (Fig. 11) support the findings described above, with Low upwelling sites having higher Sr loads. Two peaks for the Low upwelling sites can be observed, one around spot 350, and another between spots 450 and 500. Ba/Ca ratio detection limit was two orders of magnitude lower compared to otolith analysis of adult fish (which was not the case for Sr/Ca), the profile does not show a very clear pattern, however there is a consistency in that in average metal-to-calcium ratio in Low upwelling sites have a higher Ba/Ca ratio.

The correlation between SST and elemental load was strong for Sr/Ca ratios ($r^2$=0.411, Fig. 12), with the fish collected in some Low upwelling sites (Cabo Ibbetson and Champion) having a higher Sr load than sites from High upwelling (La Botella and Punta Mangle). A similar pattern was observed for Ba/Ca ratios, although it was weaker relation ($r^2$=0.269, Fig. 12).

### 3.3.2. Elemental chemistry analysis with juvenile fish

In general elemental differences between the experimental treatments were rare, although there were strong trends for some elements. Sr/Ca ratio showed significant differences among temperature treatments, having consistently highest values for high temperature treatments and this was independent of levels of food rations that were given to fish (Table 6; Fig. 13). In addition, high residual differences for most element ratios suggest great differences between individual fish (Table 6, this was represented by SE of up to 50% of the mean). There appeared to be an interaction between temperature and food ration for Ba/Ca ratios, but this was not significant (Fig. 13). For Mg/Ca and Zn/Ca ratios, although there were no significant differences among treatments, a strong trend for high element/Ca values can be found in the high food and high temperature treatments (Fig. 13).  There were no significant differences for Mn/Ca and Cu/Ca ratios either, however there was a strong trend for low values where the food ration was low (Fig. 13).

No differences in elemental fingerprints were identified (Fig. 14) and the two-way ANOVA did not detect any significant differences among PC scores (Table 7). The use of multivariate analysis did not result as useful as for the adult fish. The results from the PCA indicated great variation in individual elemental fingerprints. The majority of fishes in medium temperature and medium food treatments had negative values both in PC1 and PC2, while high food and high temperature treatments had positive PC1 values (Fig. 14). PC1 explained 56.7% of the variance, and was characterized by high Zn/Ca and Cu/Ca values, Mg/Ca and Ba/Ca values were also important in PC1. PC2, explaining 19.6% of the variance, presented high Sr/Ca values and high Mn/Ca negative values. However multi-element signatures have a strong trend for high food and high temperature treatments for PC1 (Table 7, Fig. 15). As for PC2 no significant differences were found according to the analysis, although there was a trend for low and mid temperatures for higher PC value no matter the food treatment (Table 7, Fig. 15).

# 4. DISCUSSION

I have provided strong evidence that fishes collected in Low and High areas of upwelling have different patterns of growth and elemental chemistry of the otoliths. My conclusions were based on a broad scale mensurative design and was supported by *a-posteriori* analyses of temperature data from satellites and a manipulative experiment. It was clear that strong inference on the environmental regime that fish experience could be deduced from growth and elemental chemistry without site-specific historical environmental measurements. I also predict that interactions among individuals, particularly with respect to the availability of food is likely to be highest where upwelling is low and variance in elemental signatures was as Ba/Ca were highest.

## 4.1. Patterns of growth

The findings presented here (von Bertalanffy parameters) support the hypothesis that fish grow faster in areas of High upwelling, this was especially the case for young fish that are shorter for their age in Low upwelling regime compared to the fish growing in High upwelling regimes. In a previous study growth parameters for *S. arcifrons* were reported for the whole Archipelago without considering environmental variation, even though fish were collected in several locations with different conditions and the sample size was fairly large (n=301), the mean asymptotic standard length was smaller than in this thesis compared to either High or Low upwelling fish (103.8 mm SL), however the coefficient of growth was larger than my findings for Low upwelling sites (0.75) (Meekan, Ackerman, & Wellington, 2001). The results that I describe show a similar pattern to what was found by Kingsford & Hughes (2005) in *Acanthochromis polyacanthus* (Pomacentridae) (inner, mid-, and outer shelf comparison) growth correlated with an environmental gradient, however they found similar maximum age, suggesting that the differences in growth patterns correspond to quantity and quality of food.

This idea is supported by another study of the Rockfishes (*Sebastes* spp.) that showed higher individual growth (as well as recent settlement and juvenile recruitment) in a year of productivity bloom in the California upwelling system (Wheeler, Anderson, Bell, Morgan, & Hobbs, 2016). Similarly, the growth of the deep sea fish hapuku (*Polyprion oxygeneios* is favored by the Leeuwin Current, an east boundary current that influences the primary productivity in south-western Australia (Nguyen et al., 2015).

In another study of *A. polyacanthus*, it was experimentally demonstrated that temperature and food supply affected the growth of juveniles and adults. Individuals grew more on high food rations, but growth declined with increasing temperature, in fact at the highest temperature treatment (30 ºC) growth was very similar disregarding food ratio (Munday et al. 2008).

Contrastingly, (Neuheimer, Thresher, Lyle, & Semmens, 2011) reported that populations of banded morwong (*Cheilodactylus spectabilis*) in the Tasman Sea near the center of the species range showed acceleration of growth with increasing temperature, while those at the warm margin of the range showed decreased growth rates, indicating that temperatures may have already reached levels associated with increased metabolic costs. (Rountrey, Coulson, Meeuwig, & Meekan, 2014) observed that the growth of the western blue groper (*Achoerodus gouldii*), in Wetern Australia, tend to respond positively to increased temperature which may indicate a physiological effect, also it could be related to prey availability or other biotic interactions, or a combination of both. It is worth mentioning that the abundances of several other marine invertebrate and fish species found off southwestern Australia appear to be influenced by the strength of the Leeuwin Current, therefore food availability may be the limiting factor for growth in this case.

The results I am presenting agree with what was reported by (Schwamborn & Ferreira, 2002) for *Stegastes fuscus* in Brazilian waters: high individual variability in growth therefore suggesting that length frequencies are not ideal to determine growth rate, nonetheless otoliths proof to be reliable for age determination. Greater variance on the SL of fish of a given age for those individuals collected at Low upwelling sites probably contributed to this pattern.

Estimations of age, growth and mortality in wild fish generally required a large sample size of different age classes. For this reason my comparisons of age and size among sites within upwelling regimes were not considered robust. However, at the level of upwelling the evidence was strong. Only the largest fish were collected (ie close to $L\infty$) and it was not possible that we could have selected ages and sizes by treatment. Potential differences among sites could be explored with large sample sizes.

## 4.2. Elemental chemistry

My findings demonstrate that there are differences in element incorporation into the otolith of fish between upwelling regimes, and furthermore within treatments there was significant variation as well. This partially agrees with the variation in elemental chemistry in *Acanthochromis polyacanthus,* in the Great Barrier Reef, that was explained, to a large degree at the site level, however Ba/Ca ratios were higher where upwelling occurred (Kingsford et al. 2009). Contrastingly in the GMR it is the opposite, Low upwelling sites had greater Ba/Ca concentrations, which agrees with the experiment conducted by Walther et al. (2010) who reported significant interactive effects between life history stage, temperature and food quantity were observed for otolith Ba/Ca ratios for *A. polyacanthus* ; sub-adults had higher ratios than juveniles or adults, and juveniles reared with low quality food had higher Ba/Ca concentrations than those with high quality diet, which corresponds with my findings in wild fish.

Significant differences were observed for Ba/Ca ratios related to temperature, for juvenile black bream, *Acanthopagrus butcheri* in a manipulative experiment, where Ba/Ca concentration ratio increased with temperature but the upper and lower temperature treatments showed no significant differences (Elsdon & Gillanders, 2002).

The relation of Sr/Ca and temperature was evident in this study when compared to SST and supported by the findings in the manipulative experiment, *S. arcifrons* Sr incorporation into its otolith increases with temperature, and apparently food consumption is not an important mechanism for Sr incorporation. However, due to a low resolution in the satellite imagery data, which failed to discriminate the patchiness of upwelling events in the archipelago, sites that are close but are known to have contrasting upwelling regimes (e.g. La Botella and Champion, in Floreana) were considered to have the same SST average, this would have added unknown residual to the analysis.

In the experiment mentioned above with juvenile black bream, *A.s butcheri* Sr/Ca ratios was significantly greater at low and high temperatures (12 and 16°C and 28°C, respectively) (Elsdon & Gillanders, 2002); while significant inter- actions between stage and food were observed for Sr/ Ca in *A. polyacanthus* (Walther et al., 2010). In another experiment it was demonstrated that genetic component (stock) has a strong interactive effect with salinity and temperature for Sr incorporation, but salinity interacted just with the genetic factor for Ba incorporation for the mulloway (*Argyrosomus japonicus*) reared in captivity (Barnes et al., 2013).

When ploted, Sr/Ca LA-ICP-MS transects, two peaks are easily recognizable, these peaks may correspond to 2010 and 2015 when El Niño events were reported, however further data analysis is needed to confirm this, despite that high correlation between the Sr/Ca ratio and SST, with Sr load increasing with higher temperature, suggests this may be the case. A possibility that can be supported by the fact that seasonal variations in otolith elemental composition is thought to reflected physiological controls, which are likely moderated by ambient temperature. This was particularly evident in Sr/Ca ratios in the European plaice *Pleuronectes platessa* (Sturrock et al. 2015).

Liu et al. (2013) findings demonstrate that throughout the Archipelago different current systems affect some areas in a different fashion, in some areas are affected by only one current, and in other regions the influence of several currents occur, seasonality plays an important role in the interactions of environmental conditions. In this sense the use of multivariate analysis for the elemental fingerprints in the otoliths of territorial fish can be a very useful tool helping to discriminate/identify areas with contrasting environmental conditions (upwelling regime that affects nutrient input); the great variation at the site level from our results supports the idea that seasonality as well as upwelling regime play and important role in damselfish ecology.

In structurally complex areas, where fish are more abundant and resources are often scarce, herbivorous fish may be forced to consume less-optimal resources relative to those in low-complexity areas, which are less-preferred habitats and may have better quality food sources.(Catano, Shantz, & Burkepile, 2014). In the Galapagos rocky reefs a territorial fish has to consume whatever is available, and at the same time there is the factor of inter specific competition since yelow-tail damselfish are quite abundant where it occurs. Vinueza et al. (2014) have already point out that at low productivity areas, there is a strong top down effect on algal cover in the intertidal zone, where high quality algae are scarce, and such mechanism is probable to happen in the shallow sub tidal zone as well. Edgar et al. (2010) noted that sea urchin numbers are increasing in numbers probable because top predators that feed on urchin’s predators have been removed from the system, therefore increasing competition in Low upwelling areas, where resources are already scarce, which implies that territorial fish may experience starvation occasionally. The potential impact of El Niño events is also a source of disruption in the system, there are well documented evidence of areas that use to harbor abundant microalgae populations that no longer exist (Tompkins & Wolff, 2017), and otolith elemental analysis can be a reliable tool to reconstruct the life history of reef fish.

Biological factors, such as metabolism, kinetics, and ontogeny, are thought to influence otolith microchemistry. Sadovy & Severin (1994) reported that the concentration ratio of Sr/Ca in fish otoliths was inversely related to the growth rate of the fish, which can be an explanation of my findings, since Sr/Ca ratios were higher where growth rate was slower in *S. arcifrons.* Physiological processes may be contributing to an important extent in the recording of different elements and therefore establishing unique fingerprints for different sites. Recent studies on the European plaice *Pleuronectes platessa* strongly suggest that growth and reproduction have a strong effect in element incorporation into the otolith, and the seasonal variation in environmental conditions trigger those physiological responses (A. M. Sturrock et al., 2014); (A. M. Sturrock et al., 2015). There is the need for more exploration on this trends in tropical species, since elemental incorporation has being assumed to have primarily an environmental drive (Campana 1999; Doubleday et al. 2013). A recent study (Grammer et al., n.d.) on the reef ocean perch (*Helicolenus percoides*), confirmed that Sr/Ca is mainly controlled by physiological processes, while Ba/Ca is more environmentally influenced. This supports our findings regarding Sr/Ca and its relation with temperature, both in the wild and in the experimental phase.

*Stegastes arcifrons* is a reef fish species that live in fairly shallow waters, during our study we were no able to find this species deeper than 7 m, and therefore similarities to intertidal habitats can be drawn to some extent, however further studies are needed to corroborate this idea. However in periods of low food or even starvation any organism will have a somatic response, and growth will have a slower rate than in conditions of food surplus. As Sturrock et al. (A. Sturrock et al., 2012) have stated physiological hypothesis is based on the coupling of somatic growth rates with protein synthesis, with significant changes in the protein composition of biological fluids affecting the availability of ions for uptake into the otolith, which can be reflected both in element incorporation and therefore annual increments. The abundance and wide distribution of *S. arcifrons* in the GMR provide the opportunity to continue to study this species, the analysis of otoliths for aging and microelement incorporation has been useful to understand the ecological differences between High and Low upwelling sites. A manipulative experiment for a longer period would be adequate to deepen in the understanding of the relation of environmental factors in fish growth and element incorporation into the otolith.

# 5. CONCLUSION

It can be concluded that upwelling regime strongly affects patterns of growth and elemental concentrations in otoliths.  In this sense it was demonstrated that otoliths are excellent environmental loggers. Even though there was significant variation in elemental load between upwelling treatments, most of the variation occurred at the site level. In an environmental patchy archipelago such as the Galapagos I advise against pooling age length data; a greater sample size would be needed to explore the possibility of age/length relation occurring at site level.

Ba and Sr proved to be the more reliable elements to discriminate upwelling strength, Mn also seem to be useful when it comes to site level identification, and not so much at upwelling regime discrimination, however the three elements combine as elemental fingerprints seem to explain most of the variance, and may be useful to discriminate between sites in further otolith elemental chemistry studies.

I also found that temperature plays a strong role in determining elemental patterns, especially for Sr.  There is considerable evidence that Ba signatures are heavily influenced by how well fish feed.  Where variance is high among individuals could be an index of competition (e.g. Cabo Ibbetson, Pinta).  It is likely that territorial herbivores may be competing aggressively for food – resulting in winner and loser, having data of fish densities would help to test this assumption.  Patterns of growth and elemental chemistry provide a tool for diagnosing upwelling regimes and provide a hypothesis generating platform for ecological processes that could be occurring by site.

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# 7. TABLES AND FIGURES

**Table. 1** Sites where adult *Stegastes arcifrons* were collected in the GMR

| **Site** | **Island** | **Upwelling Regime** | **Reference** |
|---|---|---|---|
| La Botella | Floreana | High | Witman et al. 2010 |
| Punta Mangle | Fernandina | | Edgar et al. 2004 |
| Cerro Brujo | San Cristóbal | | Schaeffer et al. 2008 |
| North Baltra | Baltra | | Witman et al. 2010 |
| Champion | Floreana | Low | Witman et al. 2010 |
| Cabo Ibettson | Pinta | | Edgar et al. 2004 |
| Tijeretas | San Cristóbal | | This study |
| Beagle Rocks | Santiago | | Witman et al. 2010 |

**Table 2.** Summary of von Bertalanffy growth parameters for *S.arcifrons* from High and Low upwelling sites.

| Upwelling regime | *n* | $L_\infty$ | *K* |
|---|---|---|---|
| High | 36 | 120.63 | 0.807 |
| Low | 40 | 118.24 | 0.546 |
| All fish | 76 | 119.06 | 0.654 |

**Table 3.** RM-ANOVA for annual band increments

a) Upwelling

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Upwelling | 1 | 2548.9 | 5.05 | 0.066 |
| Year | 6 | 8128.2 | 16.11 | 0.002 |
| Interaction | 6 | 504.6 | 1.33 | 0.244 |
| Residual | 210 | 378.7 | | |

b) Site

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Site | 7 | 3355.8 | 5.97 | 0.000 |
| Year | 6 | 8128.2 | 14.47 | 0.000 |
| Interaction | 42 | 561.8 | 2.48 | 0.000 |
| Residual | 168 | 226.3 | | |

Data correspond to the four oldest fish from all sites. n=16 fish per upwelling regime.

**Table 4**. Nested ANOVA for elemental ratios of otoliths of adult fish with variance components for each factor expressed as the percentage (%) of the total variation

| | Ba137/Ca43 | | | | |
|---|---|---|---|---|---|
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 9351.63 | 7.12 | 0.009 | 42.6 |
| Site (Upwelling Regime) | 6 | 1314.25 | 8.51 | 0.000 | 24.6 |
| Residual | 70 | 174.28 | | | 32.8 |
| | Mn55/Ca43 | | | | |
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 16.02 | 6 | 0.017 | 25.7 |
| Site (Upwelling Regime) | 6 | 5.32 | 19.83 | 0.000 | 48.5 |
| Residual | 60 | 0.27 | | | 25.8 |
| | Mg25/Ca43 | | | | |
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 1298.32 | 0.39 | 0.532 | 0 |
| Site (Upwelling Regime) | 6 | 3302.93 | 3.34 | 0.006 | 19.1 |
| Residual | 66 | 986.73 | | | 80.9 |
| | Sr88/Ca43 | | | | |
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 44060800 | 1.32 | 0.254 | 5.3 |
| Site (Upwelling Regime) | 6 | 33331333.33 | 20.74 | 0.000 | 62.9 |
| Residual | 69 | 1606492.75 | | | 31.8 |

**Table 5.** Nested ANOVA for values for the Principal Components PC1 and PC2 of otoliths of adult fish with variance components for each factor expressed as the percentage (%) of the total variation

| | Values for PC1 | | | | |
|---|---|---|---|---|---|
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 5080.63 | 3.251 | 0.121 | 23.79 |
| Site (Upwelling Regime) | 6 | 1562.87 | 11.211 | 0.000 | 38.50 |
| Residual | 72 | 10037.19 | | | 37.71 |
| | Values for PC2 | | | | |
| | df | MS | *F* | *P* | % |
| Upwelling Regime | 1 | 11.58 | 0.006 | 0.942 | 0.0* |
| Site (Upwelling Regime) | 6 | 1997.81 | 2.542 | 0.027 | 13.36 |
| Residual | 72 | 785.99 | | | 86.64 |

*The variance component value was negative, and was estimated by zero.

**Table 6**. Two-Way ANOVA for elemental ratios in the marginal edge of the otolith of juvenile fish

Ba137/Ca43

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 0.000034 | 0.32 | 0.728 |
| Temperature | 2 | 0.000192 | 1.85 | 0.173 |
| Interaction | 4 | 0.000181 | 1.75 | 0.161 |
| Residual | 26 | 0.000103 | | |

Mg25/Ca43

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 0.3574 | 0.41 | 0.666 |
| Temperature | 2 | 1.8040 | 2.07 | 0.141 |
| Interaction | 4 | 2.1347 | 2.45 | 0.064 |
| Residual | 26 | 0.8699 | | |

Mn55/Ca43

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 0.00193 | 0.74 | 0.484 |
| Temperature | 2 | 0.00207 | 0.79 | 0.461 |
| Interaction | 4 | 0.00231 | 0.88 | 0.486 |
| Residual | 26 | 0.00261 | | |

Sr87/Ca43

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 0.00938 | 0.12 | 0.887 |
| Temperature | 2 | 0.32233 | 4.06 | 0.026 |
| Interaction | 4 | 0.02810 | 0.35 | 0.842 |
| Residual | 26 | 0.73425 | | |

Cu65/Ca43

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 7.1715 | 3.19 | 0.053 |
| Temperature | 2 | 3.5735 | 1.59 | 0.218 |
| Interaction | 4 | 3.1885 | 1.42 | 0.248 |
| Residual | 26 | 2.2466 | | |

Zn66/Ca43

| Source | df | MS | F | P |
|---|---|---|---|---|
| Food | 2 | 1.103 | 2.89 | 0.069 |
| Temperature | 2 | 0.606 | 1.58 | 0.221 |
| Interaction | 4 | 0.599 | 1.57 | 0.204 |
| Residual | 26 | 0.381 | | |

**Table** 7. Two-way ANOVA for elemental ratios for the Principal Components PC1 and PC2 of otoliths of juvenile fish

Values for PC1

| Source | df | MS | *F* | *P* |
|---|---|---|---|---|
| Food | 2 | 4.953 | 2.30 | 0.119 |
| Temperature | 2 | 4.139 | 1.92 | 0.165 |
| Interaction | 4 | 4.053 | 1.88 | 0.142 |
| Residual | 27 | 2.150 | | |

Values for PC2

| Source | df | MS | F | P |
|---|---|---|---|---|
| Food | 2 | 0.2291 | 2.09 | 0.143 |
| Temperature | 2 | 0.0569 | 0.52 | 0.601 |
| Interaction | 4 | 0.0876 | 0.80 | 0.536 |
| Residual | 27 | 0.1095 | | |

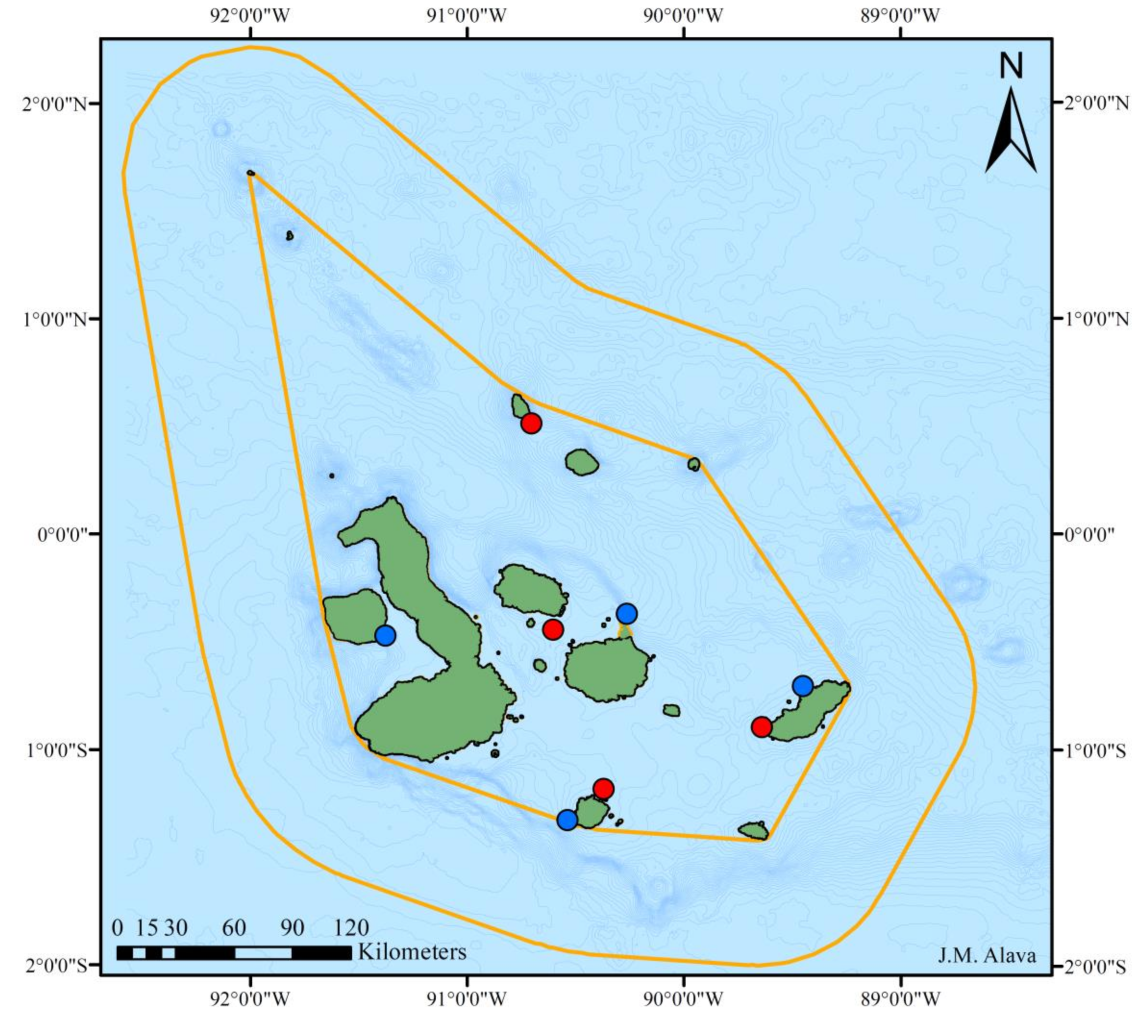

**Figure 1**. Study sites with High upwelling (blue dots) and Low upwelling (red dots) in the GMR. Orange lines show the limits of the reserve; the inner line is a baseline used to project the 40 nm boundary of the protected area.

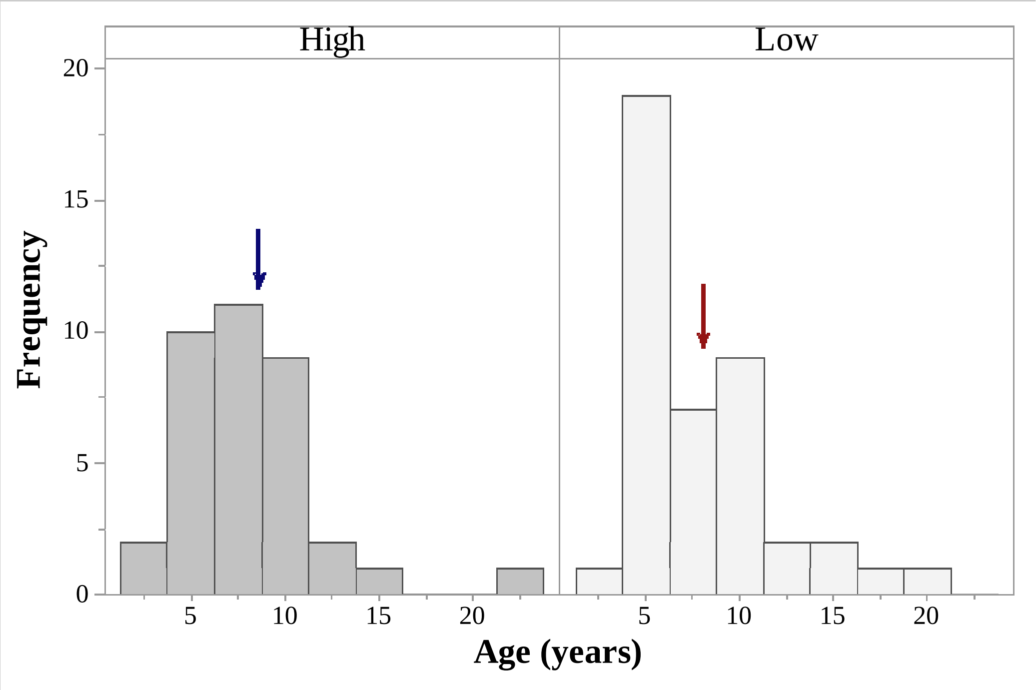

**Figure 2**. Age-frequency distributions for *S. arcifrons*. Data pooled by Upwelling regime. High Upwelling n=36; Low Upwelling n=40. All age estimates were derived from counts of otolith annuli. Arrows indicate means.

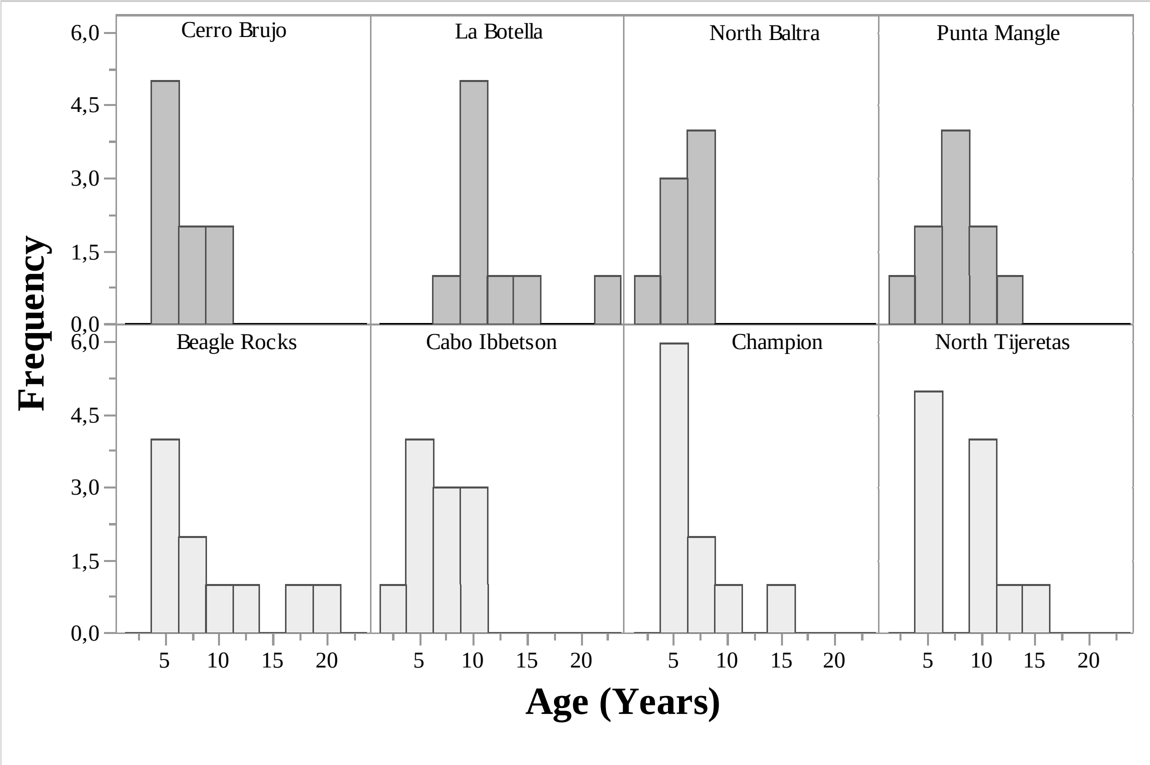

**Figure 3**. Age-frequency distributions for *S. arcifrons* across four High Upwelling Sites (Dark grey) and four Low Upwelling Sites (light grey). For all sites n=10, except for Cerro Brujo (n=9), La Botella (n=9) and North Baltra (n=8). All age estimates were derived from counts of otolith annuli.

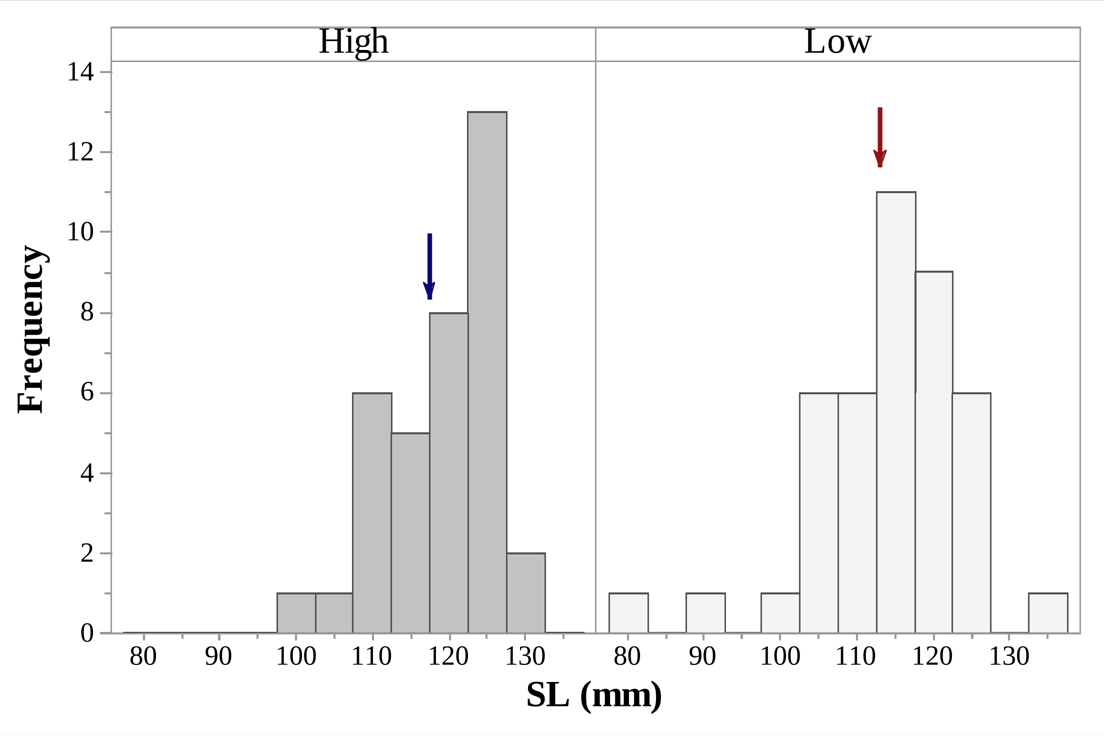

**Figure 4**. Size-frequency distributions for *S. arcifrons*. Data pooled by Upwelling regime. High Upwelling n=36; Low Upwelling n=40. Arrows indicate means.

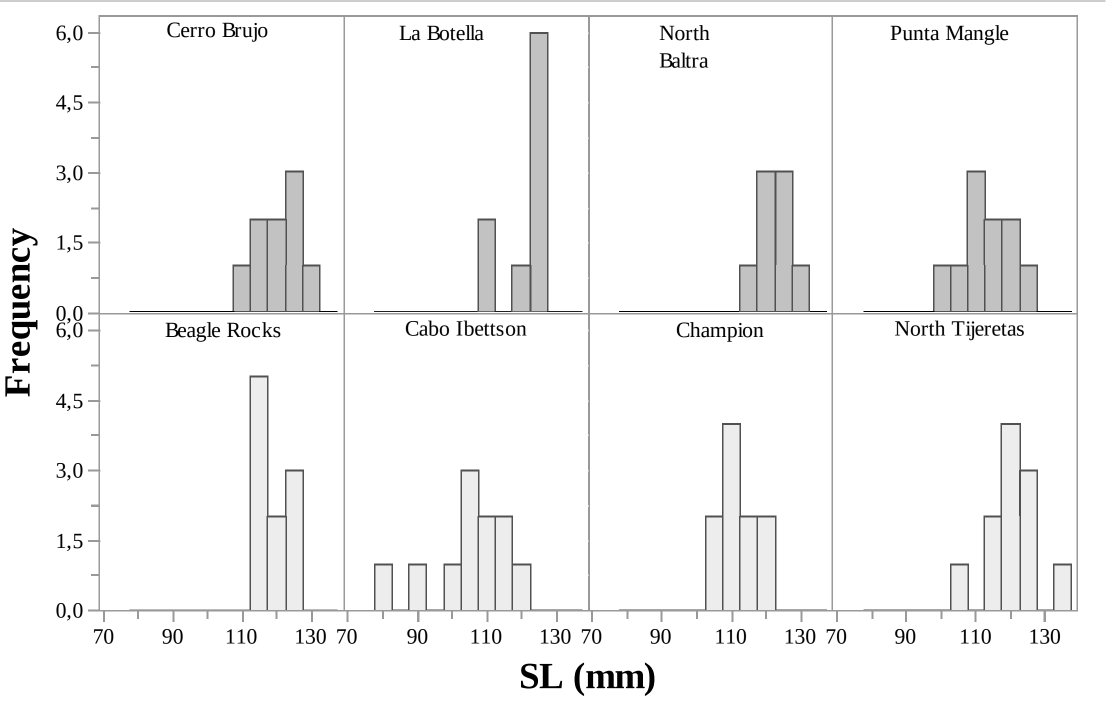

**Figure 5.** Size-frequency distributions for *S. arcifrons* collected across four High Upwelling Sites (Dark grey) and four Low Upwelling Sites (light grey). For all sites n=10, except Cerro Brujo (n=9), La Botella (n=9) and North Baltra (n=8).

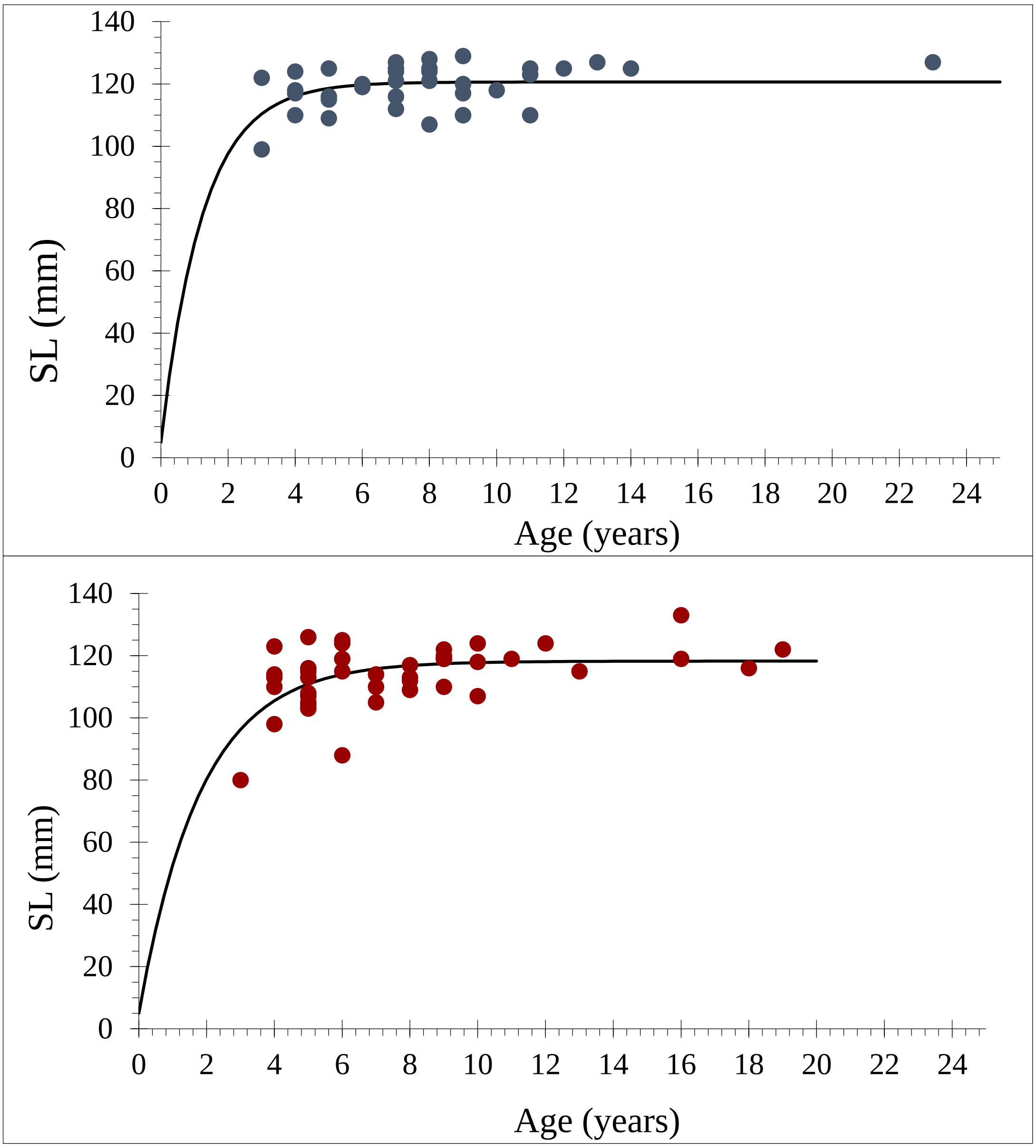

**Figure 6.** Von Bertalanffy growth curves fitted to length-at-age data of all age classes of *S. arcifrons* at High upwelling (blue dots) and Low upwelling (red dots) sites. SL = Standard length. High Upwelling n=36; Low Upwelling n=40.

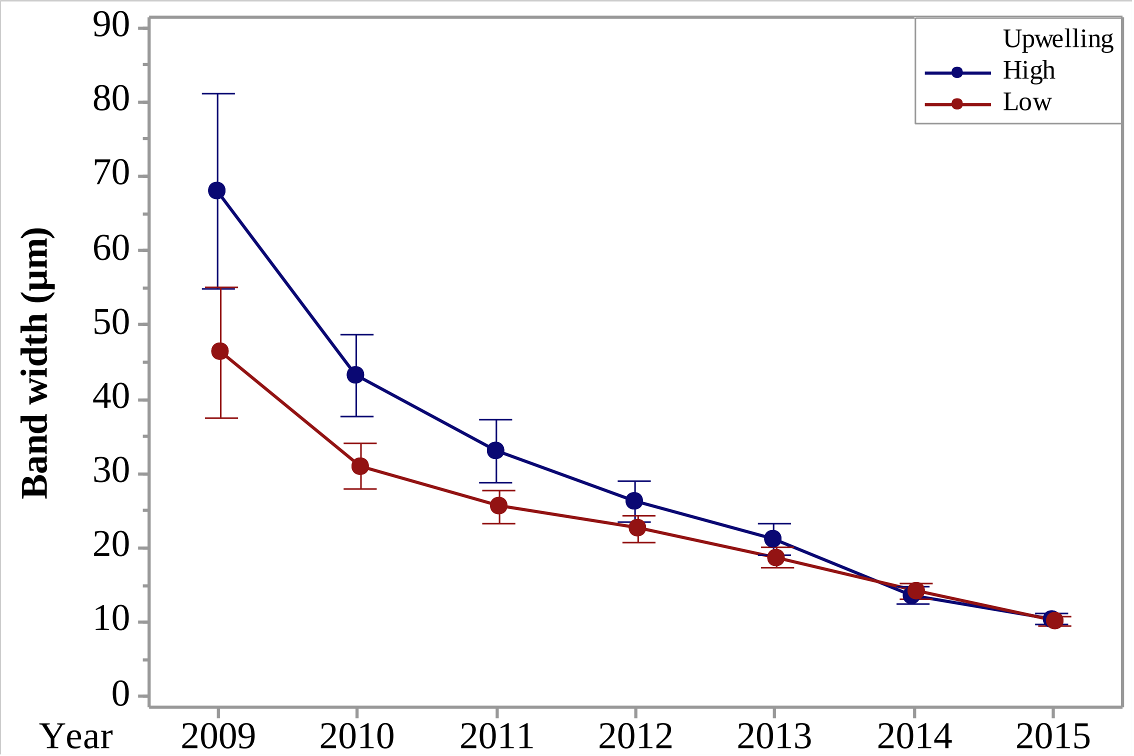

**Figure 7**. Comparison of mean ± SE band widths between annuli of *S. arcifrons*, from High and Low upwelling regimes. Data correspond to the four oldest fish from all sites. n=16 fish per upwelling regime. For High upwelling sites minimum age was 7 yrs. and maximum age was 23 yrs., for Low upwelling sites it was 7 and 19 yrs., respectively.

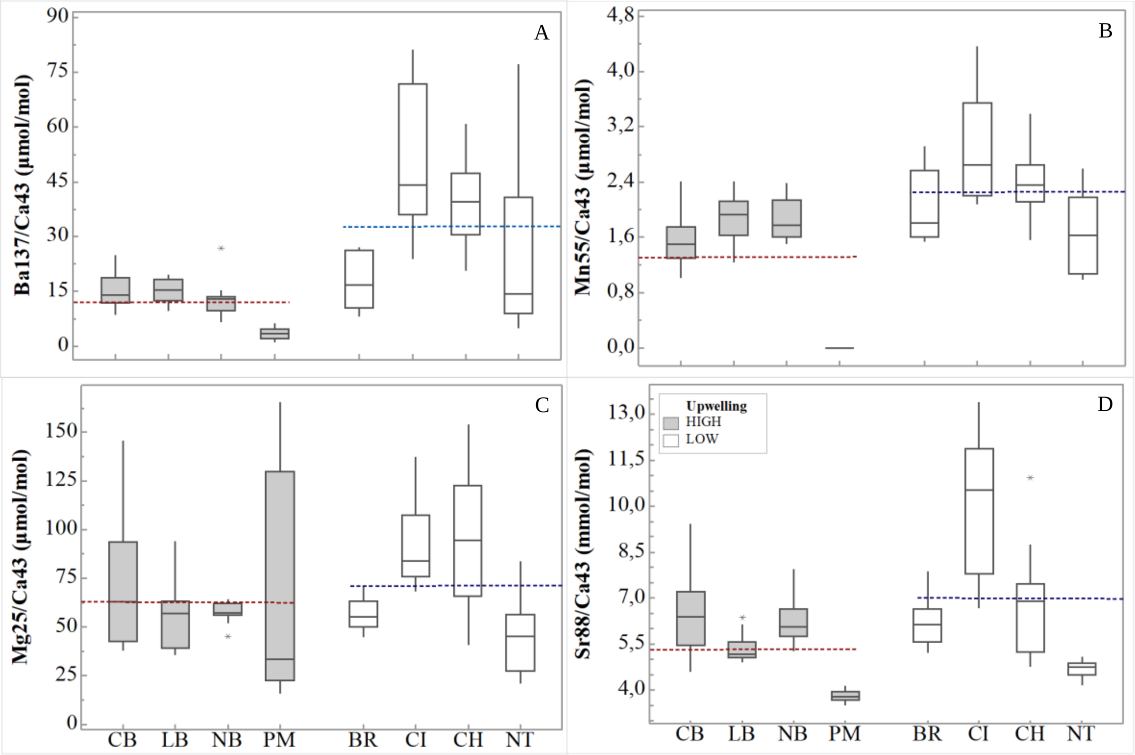

**Figure 8.** Variation in metal-to-calcium ratios in otolith of adult fish among High Upwelling Sites (Cerro Brujo [CB], La Botella [LB], North Baltra [NB] and Punta Mangle [PM]) and Low Upwelling Sites (Beagle Rocks [BR], Cabo Ibettson [CI], Champion [CH] and North Tijeretas [NT]). Standard error bars, n = 10 fish. Dotted lines represent the mean. For Mn55/Ca 43 there was not a detectable amount by the ICP-MS in PM. Asterisks represent outliers within acceptable levels.

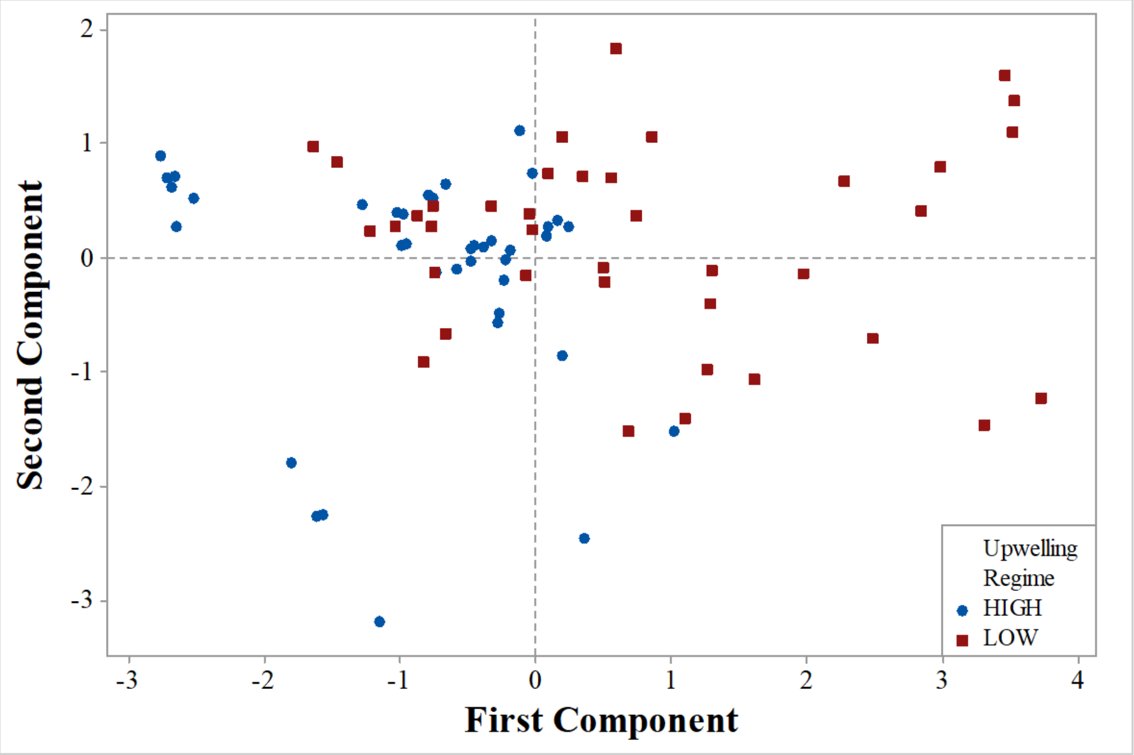

**Figure 9**. Principal Components Analysis Score plot of elemental loads in otoliths of adult fish by upwelling regime. Each point represents a single otolith. PC1 loads are 0.562 Sr88/Ca43, 0.556 Ba137/Ca43, 0.519 Mn55/Ca43 and 0.325 Mg25/Ca43, while PC2 ratios are -0.917 Mg25/Ca43, 0.289 Ba137/Ca43, 0.274 Sr88/Ca43 and -0.032 Mn55/Ca43.

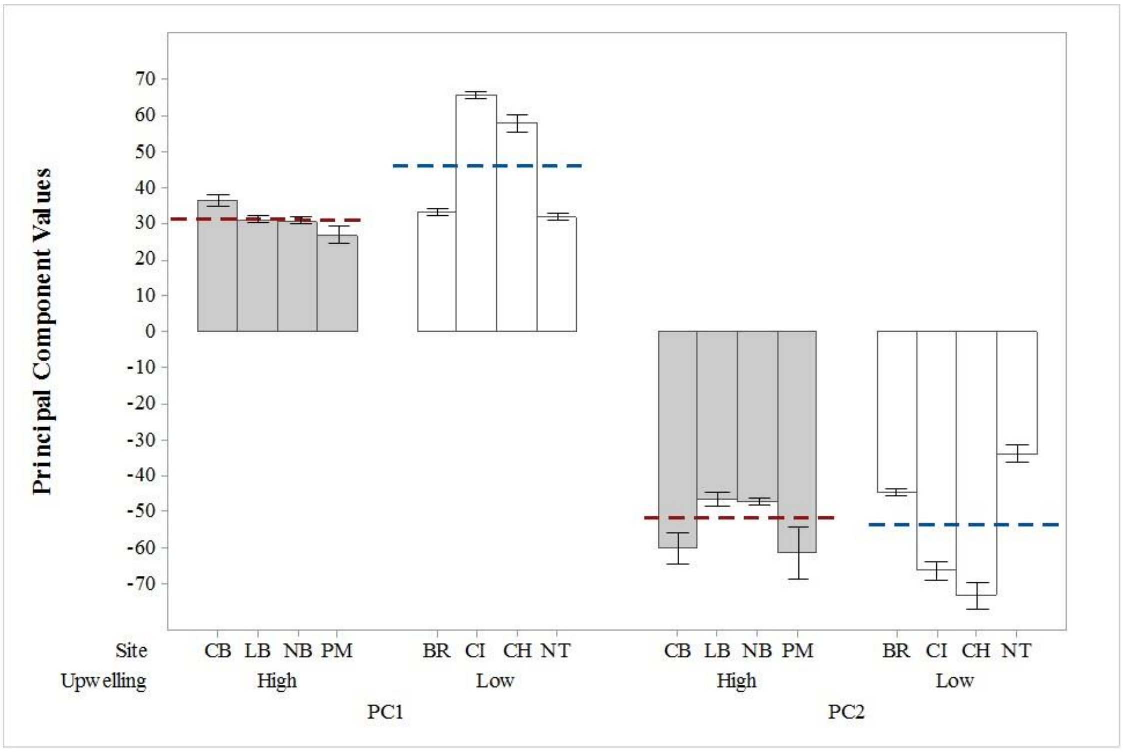

**Figure 10**. Variation in Principal Component Values in metal-to-calcium ratios in otolith of adult fish among High Upwelling Sites (Cerro Brujo [CB], La Botella [LB], North Baltra [NB] and Punta Mangle [PM]) and Low Upwelling Sites (Beagle Rocks [BR], Cabo Ibettson [CI], Champion[CH] and North Tijeretas [NT]). Standard error bars, n = 10 fish. Dotted lines represent the mean.

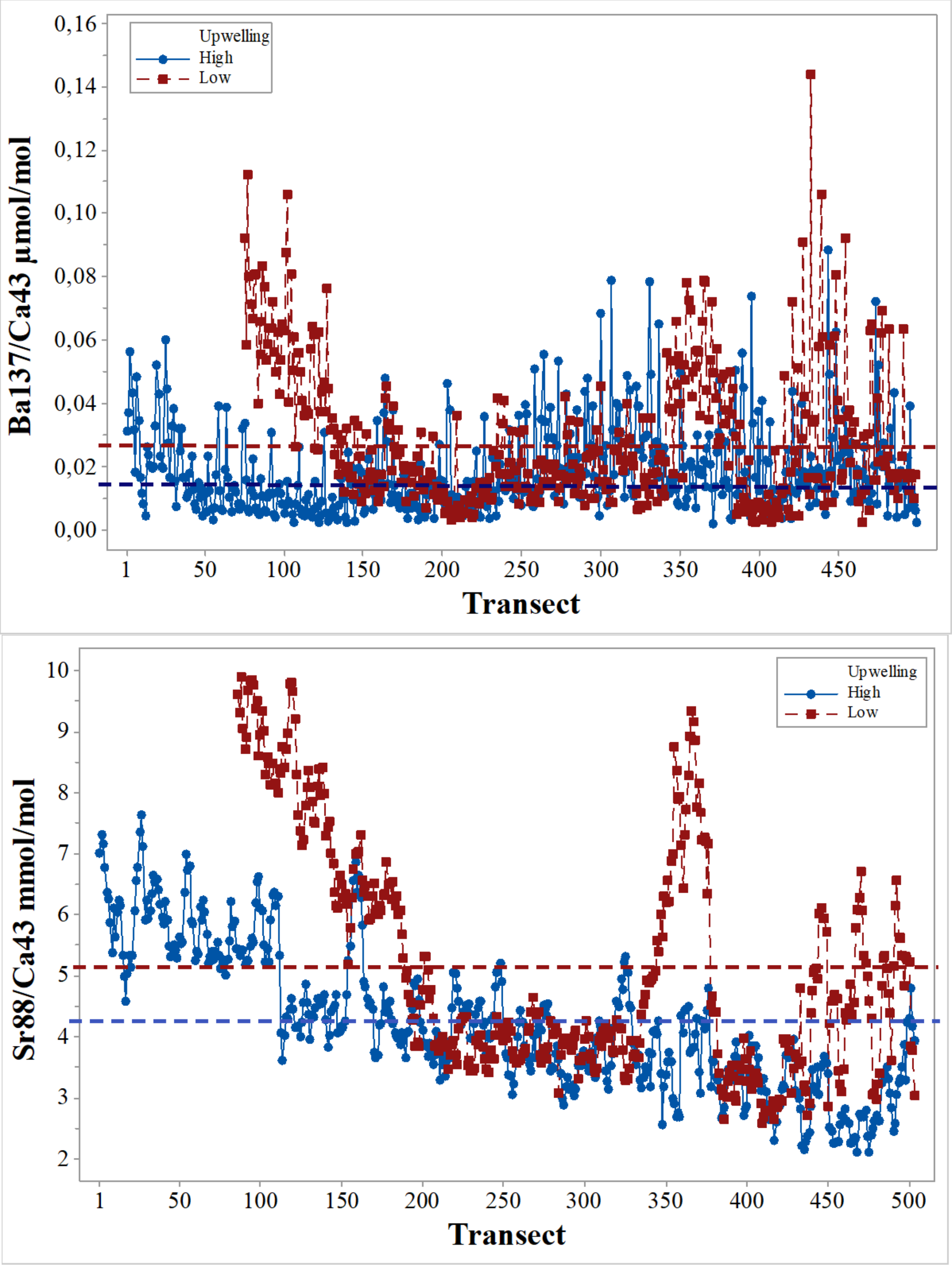

**Figure 11**. Mean Ba/Ca and Sr/Ca profiles for LA-ICP-MS data of otoliths of adult fish. The values in the *x*-axis represent the number of Laser Ablation spot; the higher values coincide with the marginal edge of the otoliths. Dotted lines represent the mean.

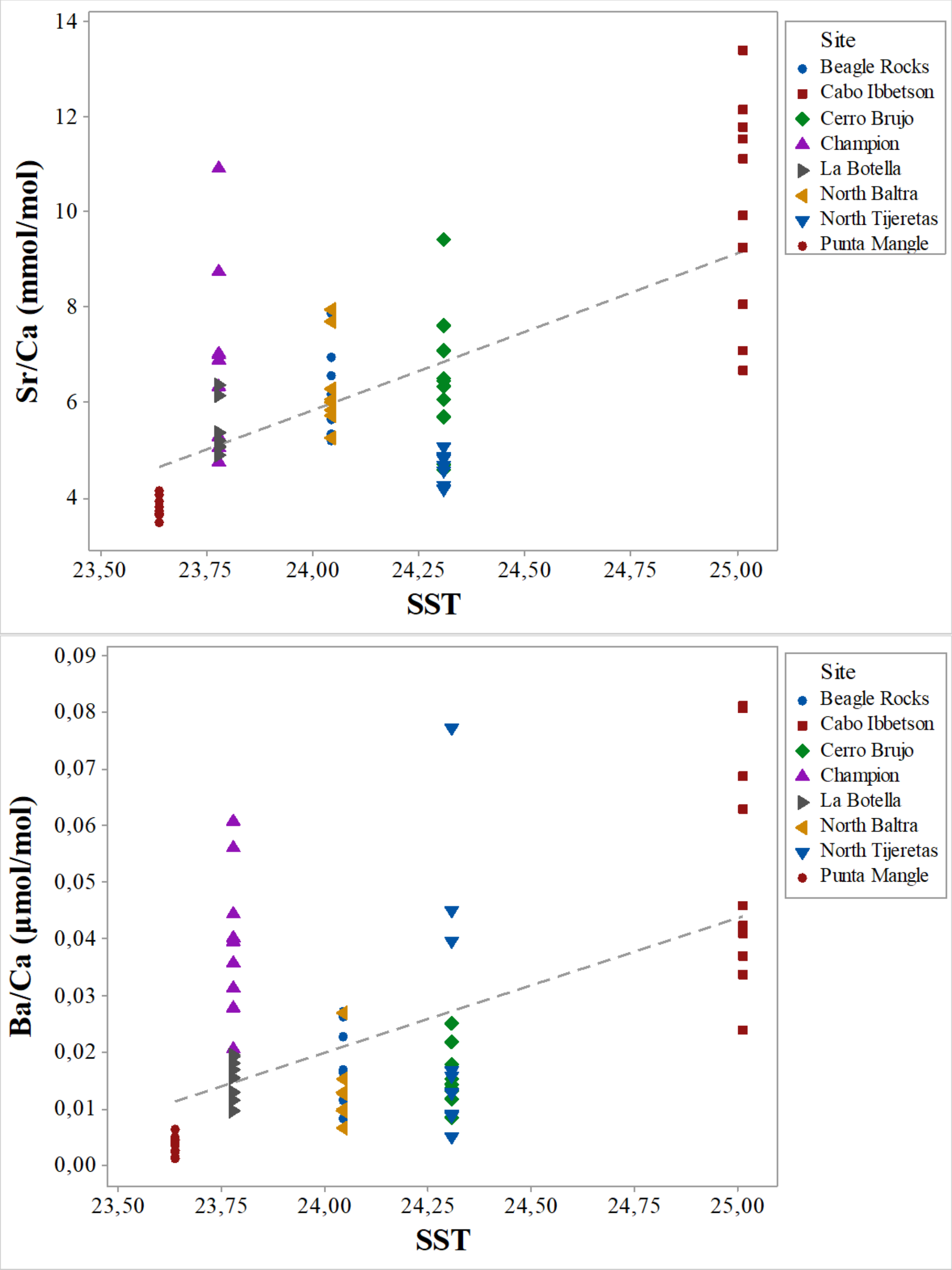

**Figure 12.** Correlation between Sea Surface Temperature (SST) and metal-to-calcium ratios of otoliths of adult fish (Sr/Ca $r^2$=0.411, Ba/Ca $r^2$=0.269).

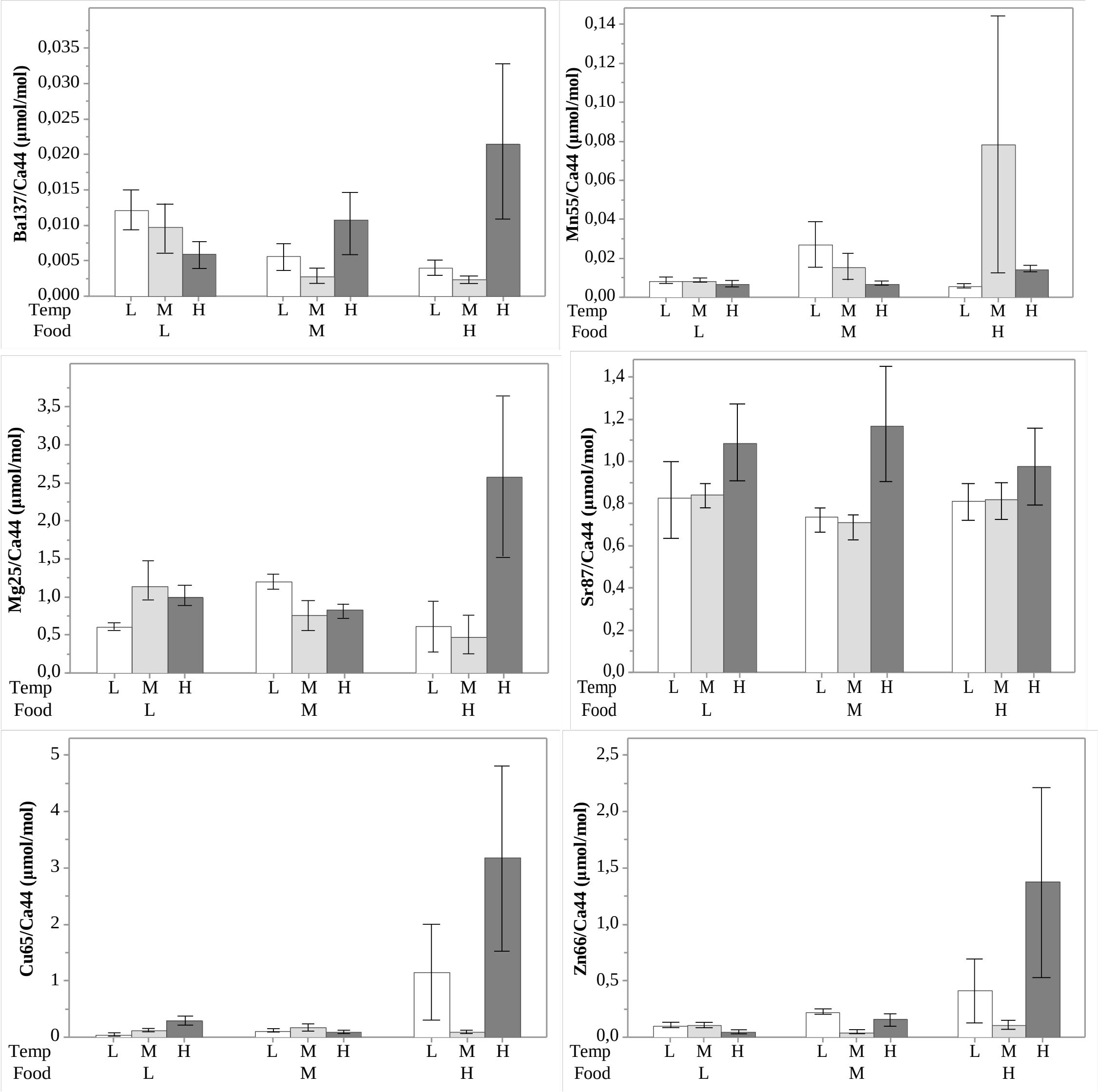

**Figure 13**. Variation in metal-to-calcium ratios from the edge of the otolith of juvenile fish among experimental treatments: Temperature (Low [L], Medium [M] and High [H]) and Food (Low [L], Medium [M] and High [H]). Standard error bars.

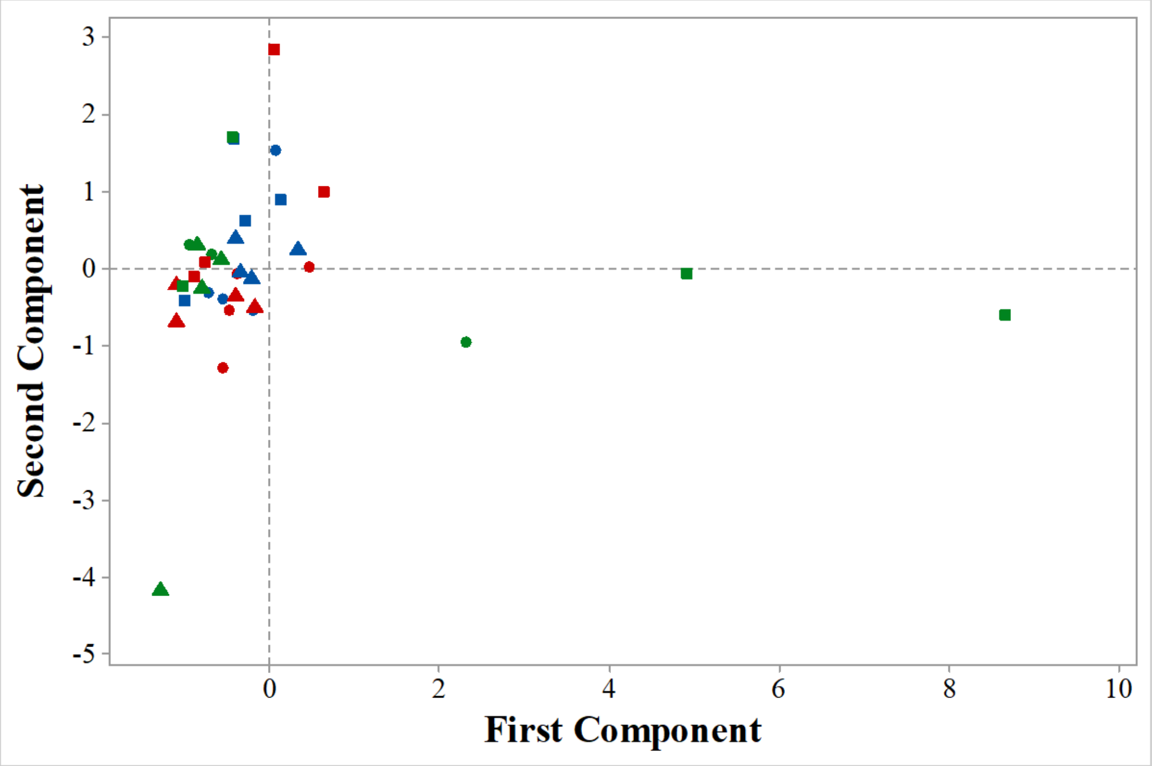

**Figure 14**. Principal Components Analysis Score plot of elemental loads in the margin of otoliths of juvenile fish by treatment (Temperature or Food). Each point represents a single otolith. Circles=Low Temp., triangles=Mid Temp., squares=High Temp.; blue=Low food, red=Mid Food, green=High Food. PC1 ratios are 0.524 Zn66/Ca43. 0.516 Cu65/Ca43, 0.496 Mg25/Ca43, 0.460 Ba137/Ca43, -0.038 Mn55/Ca43 and -0.007 Sr87/Ca43, while PC2 ratios are 0.737 Sr87/Ca43, -0.638 Mn55/Ca43, 0.135 Ba137/Ca43, -0.132 Cu65/Ca43, -0.096 Zn66/Ca43 and 0.074 Mg25/Ca43.

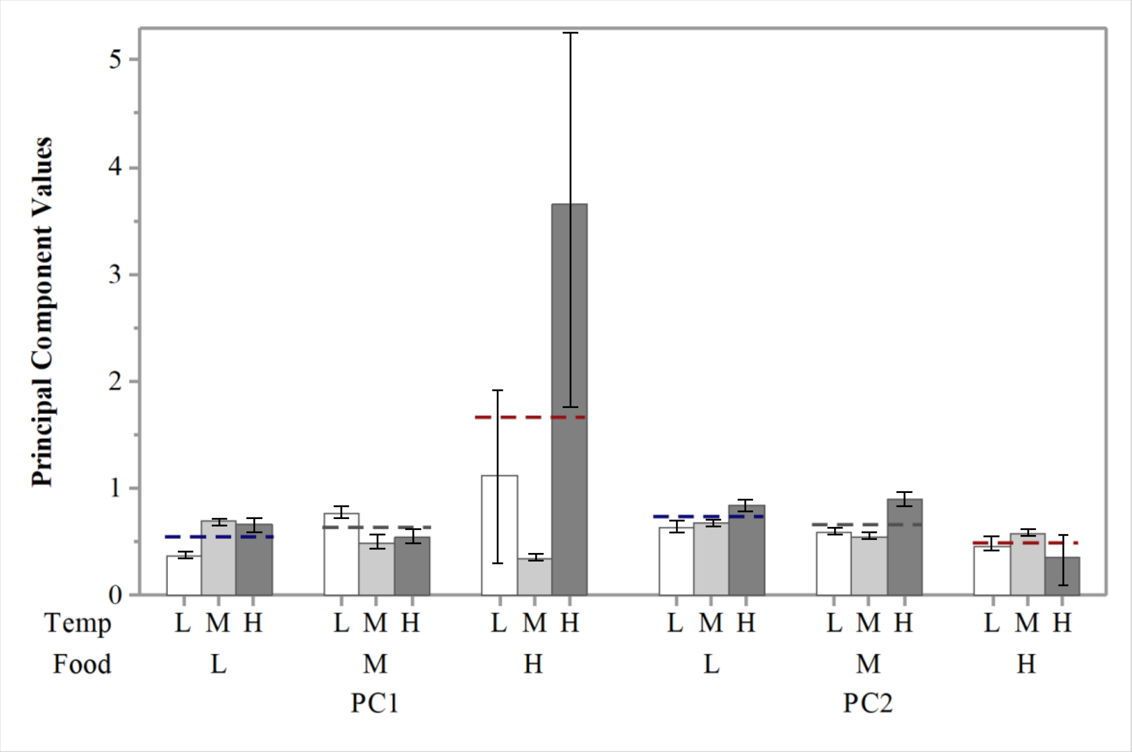

**Figure 15**. Variation in Principal Component Values for metal-to-calcium ratios in the margin of otoliths of juvenile fish among experimental treatments: Temperature (Low [L], Medium [M] and High [H]) and Food (Low [L], Medium [M] and High [H]). Standard error bars, dotted lines represent the mean.