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# The use of otoliths and larval abundance for studying the spatial ecology of the blenny *Scartichthys viridis* (Valenciennes, 1836) in coastal central Chile

El uso de otolitos y abundancia larval para el estudio de la ecología espacial de *Scartichthys viridis* (Valenciennes, 1836) en la zona costera de Chile central

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**Resumen**.- Numerosos estudios en ecología marina se han enfocado en evaluar qué determina la estructura espacial y variabilidad temporal de los organismos que habitan la zona costera e intermareal. Nuestros resultados sugieren que individuos de *Scartichthys viridis*, separados espacialmente por una escala mayor a los 200 km, deberían ser demográficamente independientes, manteniendo actividades reproductivas y dinámicas poblacionales propias. Esta segregación espacial es sugerida a partir del análisis de elementos traza en los otolitos de reclutas, obtenidos simultáneamente de las localidades de Los Molles e Isla Negra, Chile central. Considerando el potencial de dispersión (92-106 días), basado en el conteo de micro incrementos diarios en otolitos, ambas poblaciones podrían estar conectadas. Sin embargo, la distribución vertical selectiva de sus estadios larvales favorecería su retención cerca de las áreas donde eclosionaron. Aunque el presente estudio no determina en particular los mecanismos biofísicos involucrados en la distribución larval, propone alternativas que pueden responder a tal interrogante. Estos resultados sientan un precedente para futuros estudios que permitan determinar explícitamente segregación poblacional a escalas espaciales menores, así como él o los mecanismos biofísicos que determinan el transporte, dispersión larval y conectividad poblacional de peces costeros intermareales.

Palabras clave: Microquímica, larva de pez, intermareal, dispersión

**Abstract**.- Several studies in marine ecology have focused on evaluating what determines the spatial and temporal structure of organisms within the intertidal and coastal zone. Our results suggest that individuals of *Scartichthys viridis*, separated by spatial scales greater that 200 km, would also be demographically separated, having independent reproductive activity and population dynamics. Such spatial difference is suggested by analyzing trace elements found in the otoliths of recruited specimens, which were obtained simultaneously from Los Molles and Isla Negra, central Chile. Considering larval dispersion potential (92-106 days), based on daily micro increments of otoliths, both populations could be connected. However, this may not be the case, since the behavioral trend of these fish during larval stages seems to be near the areas where they hatched. Although this study does not fully reveal the specific biophysical mechanisms involved, it proposes some alternatives that may address such questions. These results may assist in guiding further studies towards explicitly determining population segregation at minor spatial scales, as well as the specific biophysical mechanisms that determine transport, larval dispersion and population connectivity of fishes in intertidal environments.

Key words: Microchemistry, fish larvae, intertidal zone, dispersal

## Introduction

Since the 1970's, numerous studies in marine ecology have focused on evaluating what determines the spatial and temporal structure of organisms in the intertidal and coastal zone (*e.g.* Gaines & Roughgarden 1985, Roughgarden *et al.* 1987, Blanchette *et al.* 2006). Consequently, two types of processes have been described: those that directly affect juvenile and adult stages in the intertidal zone (post-recruitment processes), and those that affect larval stages in the water column (pre-recruitment processes) (Caley *et al.* 1996). The structure of the marine populations results from a combined effect of these processes (*e.g.* Underwood & Fairweather 1989). Moreover, pre-recruitment processes have become a target of considerable research efforts,

being larval supply defined as the arrival of new specimens or recruits to environments where adults reside (Lewin 1986, Sale 1990). Larval supply and the arrival of new individuals to the settlement ground are mainly determined by the availability of food, predation and local advective and/or mesoscale transport (Roughgarden *et al*. 1988, Sponaugle *et al.* 2002, Pineda *et al*. 2007). Particularly, the loss of individual specimens from the population due to advective processes would be offset by the biological mechanisms of the larval stages, such as vertical migration and the use of discrete portions in the water column, which allow them to avoid offshore currents and/or select currents towards the natal origin of the larvae (Neilson & Perry 1990, Kingsford *et al.* 2002, Poulin *et al.* 2002).

Accordingly, prior to evaluating the effect of post-recruitment factors on population dynamics in marine organisms, it is necessary to quantify the degree of spatial independence existing among the different populations. This can be done, for instance, by evaluating dispersal processes and their consequences on genetic structure and spatial demography (*e.g*. Jones *et al.* 2005, Cowen *et al.* 2006). Basically, the idea is to determine whether adult individuals of a population come from the same area where they currently reside (self-recruitment, Sponaugle *et al.* 2002), or if they have arrived from other populations due to dispersal processes. Ultimately, this information will allow us to predict whether the presence of a population in a determined area is self-sustainable, or rather depends on another spatially segregated population. Despite the importance of this point, there are only a few empirical estimates of larval dispersal or population connectivity in ocean environments (*e.g*. Swearer *et al.* 1999, Thorrold *et al.* 2002, Gillanders 2005, Thorrold *et al.* 2007).

The majority of marine fish species have planktonic larvae, which represents a dispersive stage. This life history stage, following a variable amount of time in the water column (Doherty *et al*. 1995, Raventos & Macpherson 2001), will lead fish to recruit in the same areas as their parents, located at a few meters or up to hundreds of kilometers from their birthplace (Cowen *et al*. 2006). The degree of dispersal prior to recruitment will, therefore, determine the degree of independence between different populations and the spatial scale on which they operate (Sponaugle *et al.* 2002). In this sense, it would be crucial to understand the how far the specimens could be dispersed in a determined area, the biological mechanisms, as well as the associated advective processes undergone (Thorrold *et al.* 2002). Resolving the latter would allow us to evaluate the demographic processes, considering a restricted spatial scale in which the persistence of a population in a determined area would mainly result from birth and mortality processes.

Some studies have recently been developed to quantify the degree of spatial connectivity between marine fish populations. These studies suggest that self-recruitment is a more important process than it originally had been thought to be (Jones *et al*. 1999, Cowen *et al.* 2000, Sponaugle *et al.* 2002, Swearer *et al.* 2002, Jones *et al.* 2005, Cowen *et al.* 2007). Moreover, low levels of larval transport and dispersal have been found in field studies (Marliave 1986, Swearer *et al.* 2002, Hernández-Miranda *et al.* 2003; Miller & Shanks 2004, Gillanders 2005, Jones *et al.* 2005, Miller *et al.* 2005, Shanks & Eckert 2005, Almany *et al.* 2007) and through modeling (Cowen *et al.* 2000, Irisson *et al.* 2004, Gerlach *et al.* 2007). In other words, the empirical evidence indicates that coastal and littoral marine fish populations would be demographically more closed than what has been theoretically hypothesized. It has also been recognized that diverse biophysical coupling mechanisms that develop during larval stages (*e.g.* vertical migration) underlie these low patterns of dispersal (Kingsford *et al.* 2002, Sponaugle *et al*. 2002), offsetting the inherent transport effect which is generated through physical and advective processes. The most common methodologies used for inferring larval fish transport and/or dispersal include estimates of pelagic durations of larval dispersive stages (Pelagic Larval Duration, PLD), based on modeled movements of passive particles by ocean currents, analyses of variation in allele frequencies of mitochondrial or nuclear genes, or natural and artificial tags (Scheltema 1986, Planes 2002, Macpherson & Raventos 2005, Thorrold *et al.* 2007).

Littoral fishes are defined as the species that occupy the narrow band of habitat between the tidemarks on marine shores, at least during part of their life history (Horn *et al.* 1999). The rocky shore along the coast of central Chile gives rise to tidal pools which are inhabited by littoral fish, at least during the juvenile stage (Varas & Ojeda 1990). Due to their low mobility and lack of planktonic eggs, most littoral fish are good candidates as models for testing hypotheses regarding marine population connectivity and self-recruitment. In this study, we hypothesized that the use of discrete portions of the water column by littoral fish during larval stages may offset the effect of advective oceanographic processes (Hill 1991). These mechanisms could explain the high abundance of littoral fish larvae at a very close distance to the spawning area (Hernández-Miranda *et al.* 2003), which in the end could greatly contribute to recruitment in areas adjacent to those occupied by their

parents. We used a combination of field and laboratory methods, including ichthyoplankton sampling, estimation of the time before recruitment by counting otolith microincrements, and natal origin discrimination from otolith microchemistry. The blenny *Scartichthys viridis* (Valenciennes, 1836) was taken as a model, due to the high abundance of juveniles belonging to this species in tidal pools, their non-pelagic spawning behavior, and the higher amount of information known about their biology and demography processes (Varas & Ojeda 1990, Muñoz & Ojeda 1997, Horn & Ojeda 1999, Ojeda & Muñoz 1999, Muñoz & Ojeda 2000, Hernández-Miranda & Ojeda 2006, Hernández-Miranda 2007).

The general aims of this study were to evaluate for *S. viridis*: i) time from hatching to recruitment to tidal pools, ii) if individuals of two localities occurring 200 km apart show differences in natal and recruitment otolith microchemistry signals, and iii) if larval distribution in the water column is homogeneous in different day-light and tidal-cycle scenarios.

## Material and methods

### Study area

This study was conducted along the coastal zone of central Chile, where two areas separated by approximately 200 km were considered. Towards the north, we selected Los Molles (LM), 32º13'S, 71º30'W. Towards the south, different experiments were conducted in El Quisco (EQ), 33º24'S, 71º43'W and Las Cruces (LC), 33º30'S, 71º37'W, (Fig. 1). The area between EQ and LC also includes Isla Negra (IN) and El Tabo (ET), which are both rocky zones separated by about 10 km of sandy beaches. EQ is considered to be within a Management and Exploitation Area for Benthic Resources (MEARB); LC is besides the Estación Costera de Investigaciones Marinas (ECIM), which is a Marine Protected Area (MPA); and finally IN and ET are free access areas. On the other hand, LM is located at the northern end of the Los Molles Bay. The intertidal and subtidal zones are formed by continuous rocky areas, and currently lie within the MEARB.

The 200 km separating the two major study areas share similar meteorological and oceanographic conditions (Strub *et al*. 1998). The oceanography and coastal hydrography have mainly been described for the area between EQ and LC (Poulin *et al.* 2002, Hernández-Miranda *et al.* 2003, Kaplan *et al*. 2003, Wieters *et al*. 2003, Narváez *et al*. 2004, Vargas *et al.* 2004, Piñones *et al*. 2005, Aiken *et al*. 2007, 2008).

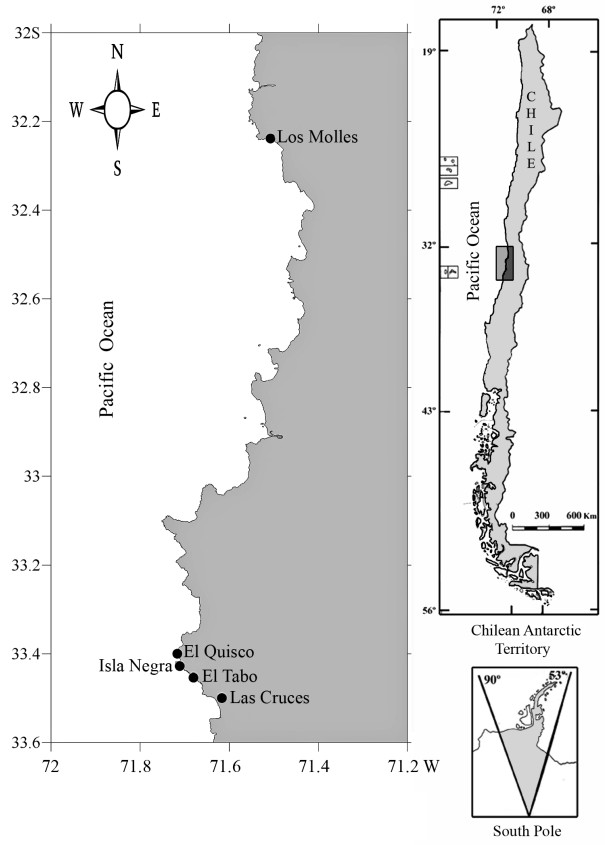


**Figure 1**

**Map of the study zone, showing the sampled local areas. Los Molles is approximately 200 km away from Isla Negra and El Quisco**

Mapa del área de estudio, señalando las localidades muestreadas. Los Molles se encuentra aproximadamente a 200 km de Isla Negra y el Quisco

### Estimation of time before recruitment

In order to estimate the dispersion potential of *S. viridis* (time spent in the water column before recruitment), the time that elapsed between hatching and the arrival of the fish to the intertidal zone was estimated by counting the rings on sagitta otoliths of young specimens that had just arrived to the intertidal pools, as well as by experimentally validating the frequency of microincrement deposition (Panella 1971). At LM and IN, samples of *S. viridis* recruits were collected during the recruitment window in 2004, 2005 and 2006. The recruitment signal of *S. viridis* in the intertidal pools occurs in a defined window of time between January and February every year. Once in the pools the recruits are highly territorial and with a high fidelity to the pool in which they have arrived (Hernández-Miranda 2007). Each sample consisted of

specimens that had recently arrived to different intertidal pools, which were disconnected during low tide periods. The collected specimens were fixed and kept in 95% ethanol, until they were manipulated in the laboratory. Each specimen was weighed (wet weight in g) and measured (total length in cm) before preservation.

In the laboratory, each specimen was dissected under the stereomicroscope, and sagitta, asteriscus and lapillus otoliths were extracted. The latter were kept in Eppendorf tubes with distilled water until they were analyzed. *Sagitta* otoliths were used for microincrement analysis, and were individually mounted on slides with adhesive (Eukitt®). Otoliths were polished with 30 μm and 1 μm grit paper, so as to count the number of rings on each of them. Sequential images were obtained by means of an Olympus® CX31 optic microscope with an Olympus® Camedia C-5050 digital camera. Finally, based on the photograph sequences, the numbers of rings were quantified from the nucleus to the edge with the aid of the Image Pro-Express Software, version 4.5, 2002, by Media Cybernetics. Differences in the number of rings among years and between localities were tested by two way factorial ANOVA and HDS Tukey post hoc test. Somatic growth rate differences among years in each locality were tested by slope comparison and Tukey post hoc test. The assumptions of normality and variance homogeneity were tested by graphical analysis of the residuals (Zar 1999).

To validate the number of rings deposited daily on the otoliths, a laboratory experiment was carried out, which involved capturing 40 *S. viridis* specimens that had just arrived to the intertidal pools at ET in February 2006. These individuals had an average size of 5.64 ± 0.36 cm. To acclimate the fishes, they were transferred to the lab in ECIM, where they were kept in several 0.25 $m^3$ aquariums for seven days at natural light photoperiod, with circulating water, constantly aerated, and fed with seaweed (*Ulva* sp.) and Tetra Pond® Power food nutritional supplement on a daily basis. After the seven days of acclimation were over, the specimens were subject to three treatments, in which Oxytetracycline Hydrochloride (OTC) was incorporated. This compound was assimilated and incorporated into the calcareous structure of the otoliths, which, by means of epifluorescence microscopy techniques, reveals the number of rings deposited after they have been marked (Campana 1999, 2001). The first treatment consisted of submerging 5 individuals into an aquarium with OTC, at a concentration of 500 mg $L^{-1}$, for 5 hours. Then, they were put in an aquarium with circulating water. The second treatment involved injecting OTC at a concentration of 20g $kg^{-1}$ into 30 individuals, which were subsequently sorted at random into five aquariums. The third treatment consisted of injecting 5 individuals with OTC at a concentration of 20g $kg^{-1}$ and then putting them into an aquarium with Power food® nutritional supplement, previously immersed in OTC. The three treatments followed techniques that have been described by Vigliola (1997) and Campana (2001). The individuals (average total length = 5.64 ± 0.36 cm, average total weight = 1.75 ± 0.38 g) were kept in aquariums that were constantly aerated and had a constant water flow. Temperature and oxygen were recorded daily and the fishes were given food made of seaweed and nutritional supplement *ad libitum*. The individuals that died during the experiment were weighed and measured before being preserved with 95% ethanol. Death dates were labeled. A total of 18 individuals survived throughout the experiment. These surviving fishes were weighed, measured and subsequently fixed in ethanol. They were dissected in the laboratory and their sagitta, asteriscus and lapillus otoliths were kept in an aqueous solution. The lapillus otoliths of the individuals that survived the 14 days were mounted on slides with Eukitt® and observed under an epifluorescence microscope (Zeiss®, Axioskop 2 plus with a magnification of 40x15). The otoliths were exposed to UV light excitation and the further light emission, under specific filter, made it possible to quantify the number of rings between the OTC mark and the otolith edge.

**Otolith microchemistry**

Differences between larval origin and the arrival of recruits were explored between LM and IN by using otolith chemistry (Thorrold *et al*. 2001, Campana & Thorrold 2001). Elemental composition analysis was carried out for a total of 21 *S. viridis* recruits (10 from LM and 11 from IN), which were captured in February, 2005. In the laboratory, a sagitta otolith was removed from each individual, cleaned with ultrapure water (milli-Q Water) and subsequently mounted on petrographic slides with Krazy Glue®. Each otolith was dried and polished, alternatively with 30μm and 3μm grit paper, until the nucleus was reached. After being polished, the otoliths were decontaminated in a Clean Room prior to analysis in LAICP-MS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry). The thin sections were washed with ultra pure water (milli-Q Water), using a nylon brush, then ultrasound was applied for 2 min and finally they were washed again with ultra pure water. Subsequently, the sections were mounted on new slides and were left to dry under a positive flow hood for 24 h, so they could then be stored on petri dishes in Ziploc® bags.

Element analysis was carried out with an ICP-MS Finnigan MAT Element 2 and a Merchantek EO LUV 266X laser ablation system (Thorrold & Shuttleworth 2000). Nuclei sampling was carried out by drawing a 70 µm x 70 µm raster. $^{48}$Ca, $^{86}$Sr, $^{138}$Ba, $^{25}$Mg, $^{55}$Mn and $^{208}$Pb were quantified for each otolith. Element comparison between LM and IN was determined, based on the ratio between each of the rest of the elements (Me) and $^{48}$Ca. Differences between the ratios of elements from both local areas were identified by analyzing the elemental composition of the nuclei and edges of the *S. viridis* otoliths separately, by means of two approximations. The first was done by performing a non-metric multidimensional scaling analysis (MDS), based on a Bray-Curtis similarity matrix from log-transformed data (Ashford *et al*. 2005, 2006). Through this method, similarities between each sample were represented in a two-dimensional graph. The distance between each of them on the graph is inversely proportional to their similarity, that is, samples that are closer to each other on the graph display a higher degree of similarity. The values of Stress less than 0.2 indicated a good representation in two dimensions (Clarke 1993). Another method used to compare the areas was that of a non-parametric multivariate analysis of variance (NP-MANOVA), using such local area as a fixed factor. This method was based on a distance measure, which in this case was the Bray-Curtis distance measure (Anderson 2001).

### Ichthyoplankton sampling design

In order to describe the vertical distribution of *S. viridis* larval stages, plankton samples were collected on an intra-daily basis within the coastal zone off EQ (Fig. 1). Sampling area was selected, based on Hernández-Miranda *et al*. (2003), who found that the greatest abundance of ichthyoplankton of littoral species, including *S. viridis*, occurs near the coast during the southern Spring-Summer months (< 1 nautical mile, nm, from the coast). Spatial and temporal sampling strategies were used in this study.

Spatial sampling. Transects were parallel to the coast, with a length of 2.5 km located at 0.1, 0.5, 1 and 2 nm from the coast, with each transect being composed of two ichthyoplankton tows, one at each extreme of the grid. Plankton samples were collected by horizontal towing at two depths (surface and 5-10 m strata) using a floating neustonic net (700 µm mesh size) and Bongo net (60 cm mouth diameter, 250 and 500 µm mesh size) respectively, both equipped with a flow meter to quantify the volume of water filtered. This was carried out in 1999 on two respective dates, from September 23$^{rd}$ at 23:00 PM to September 24$^{th}$ at 15:30 PM, (Night: 23:00 PM to 03:00 AM and Day: 09:00 AM to 15:30 PM) and from December 9$^{th}$ at 23:00 PM to December 10$^{th}$ at 16:30 PM (Night: 23:00 PM to 04:00 AM and Day: 11:00 AM to 16:30 PM).

Temporal sampling. A continuous sampling following transects at 0.1 and 0.5 nm from the coast were carried out in October 23$^{rd}$ (From 13:00 PM to 22:00 PM) and November 11$^{th}$ (from 02:00 AM to 09:30 AM) 2000 and, June 30$^{th}$ 2001 (From 02:00 AM to 10:00 AM). Samples were collected simultaneously with the use of a floating neustonic net (700 µm mesh size) at surface and two non-closing conical nets (0.7 m diameter and 350 µm mesh size) were towed at 5 and 15 m depth. Both kinds of net were equipped with a flowmeter for estimating water volume filtered. In order to minimize the probability of capturing organisms from other stratums, net tows did not begin until the nets reached the required depth, and when tow finished the nets were raised only after the boat having been stopped completely.

Both spatial and temporal collected samples were fixed in 10% formalin and the fish larvae were separated and identified under a stereomicroscope in the laboratory. Based on what was obtained, the vertical distribution of *S. viridis* larvae was explored by graphical analysis with respect to the day-light time and daily tidal cycle.

## Results

### Time before recruitment

The experiment used to validate daily increments gave a total of 14 fish with clear OTC marks and an average of 13.57 ± 0.51 rings, between the day on which they were marked and the last day of the experiment. The *t* test that was used to contrast the number of rings that appeared after marking and the days that elapsed didn't show significant differences ($t_{calc}$ = -1.8829, g.l. 13, $P$ = 0.083). This validated the daily frequency of microincrement deposition in *S. viridis* specimens.

Given that the exact timing of increment deposition initiation in this species is unknown, quantification may over- or underestimates the time before recruitment by a few days (Raventos & Macpherson 2001, Beldade *et al.* 2007). However, if we assume that first ring deposition occurs at hatching, then the pooled average time (± standard error) that the *S. viridis* individuals took to get to the intertidal zone after hatching were 96.03 ± 1.36 days in 2004, 107.12 ± 2.10 days in 2005 and 113.81 ± 2.63 days in 2006. The period of time tends to increase each year (Fig. 2a), showing significant differences among 2004 and the other two years (Factorial ANOVA,

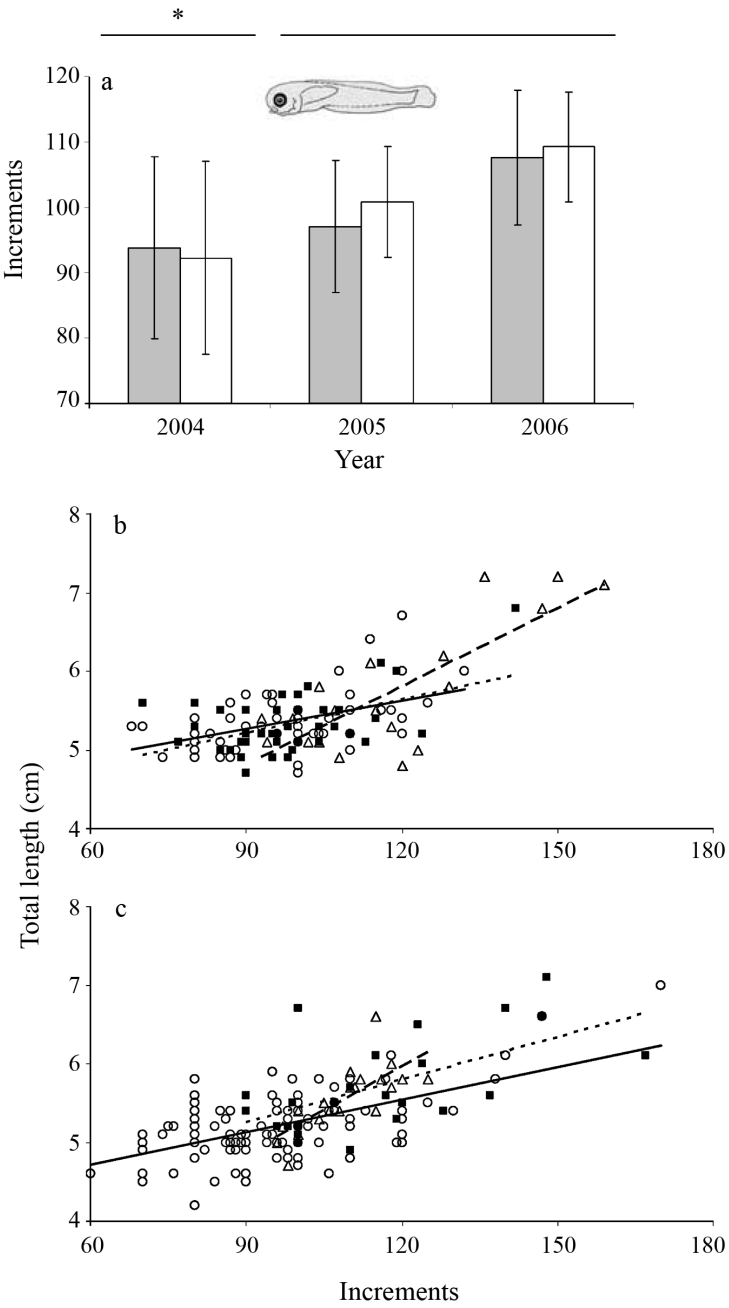


**Figure 2**

**a) Number of otolith daily increments at Los Molles (grey) and Isla Negra (white); b) somatic growth rate at Los Molles; c) somatic growth rate at Isla Negra for *S. viridis*. The continuous line (circles) represents the year 2004 in each case, the dotted line (black squares) represents the year 2005 and the segmented line (triangles) stands for the year 2006. The continuous line at a) indicated that there are significative differences among 2004 and the other two years (Tukey HDS $P < 0.05$)**

a) Número de incrementos diarios en otolitos de Los Molles (gris) e Isla Negra (blanco), b) curvas de crecimiento somático en Los Molles, c) curvas de crecimiento somático en Isla Negra, para *S. viridis*. La línea continua (círculos) representa el año 2004, la línea punteada (cuadrados negros) el año 2005 y la línea segmentada (triángulos) el año 2006. La línea continua en a) indica que el año 2004 presenta diferencias significativas con respecto de los otros dos años (Tukey HDS $P < 0,05$)

**Table 1**

**Simple linear regression analysis between the number of rings (relative age) and the body size (total length) of recruited *S. viridis*. The parameters are indicated, as well as the sample size and estimated statistics. LM: Los Molles, IN: Isla Negra and El Tabo**

Análisis de regresión lineal simple entre el número de anillos (edad relativa) y el tamaño corporal (longitud total) de reclutas de *S. viridis*. Se indica el valor de los parámetros, así como el tamaño de muestra y los estadísticos estimados. LM: Los Molles, IN: Isla Negra y El Tabo

| Year | Locality | Intercept | Slope | $r^2$ | N | *P* |
|---|---|---|---|---|---|---|
| 2004 | LM | 4.21 | 0.0118 | 0.23 | 55 | 0.0002 |
| | IN | 3.89 | 0.0138 | 0.34 | 108 | <0.0001 |
| 2005 | LM | 3.94 | 0.0142 | 0.25 | 42 | 0.0007 |
| | IN | 3.65 | 0.0179 | 0.36 | 24 | 0.0021 |
| 2006 | LM | 1.82 | 0.0332 | 0.63 | 19 | <0.0001 |
| | IN | 1.37 | 0.0384 | 0.57 | 20 | 0.0002 |

$F$ = 22.61, d.f. = 2, 267, $P$ < 0.0001), 2004 being significantly lower (Tukey HDS $P$ < 0.01), however there are non significant differences between the two localities (Factorial ANOVA, $F$ = 0.97, d.f. = 1, 267, $P$ = 0.325). By estimating the somatic growth rates of the pre-recruited fish, an increase of two-fold in the year 2006 was found for both areas compared to the previous years (Table 1, Fig. 2b and c; LM slope test, $F$ = 7.93, d.f. = 2, 110, $P$ = 0.0006; EQ slope test, $F$ = 3.85, d.f. = 2, 146, $P$ = 0.023), being 2006 significative higher in both localities (Tukey HDS $P$ < 0.01).

### Otolith microchemistry

MDS analysis between the LM and IN local areas was based on the following ratios for *S. viridis* otolith nuclei: $^{86}Sr/^{48}Ca$, $^{135}Ba/^{48}Ca$, $^{25}Mg/^{48}Ca$, $^{55}Mn/^{48}Ca$ y $^{208}Pb/^{48}Ca$. This analysis suggests that there are differences between the specimens obtained from both areas (Fig. 3a). This was confirmed by non-parametric MANOVA, which indicated significant differences between these local areas ($F$ = 3.337, $P$ = 0.049). Complementary to this, when conducting the same analysis using otolith edge measurements, differences between specimens obtained from both local areas were also evident, but with some mixing (Fig. 3b), which was also significant by non-parametric MANOVA ($F$ = 11.36, $P$ = 0.002). These results suggest at least three aspects of the recently recruited fish in the intertidal pools at any determined site (*e.g.* LM), i) that they originated in the same location,

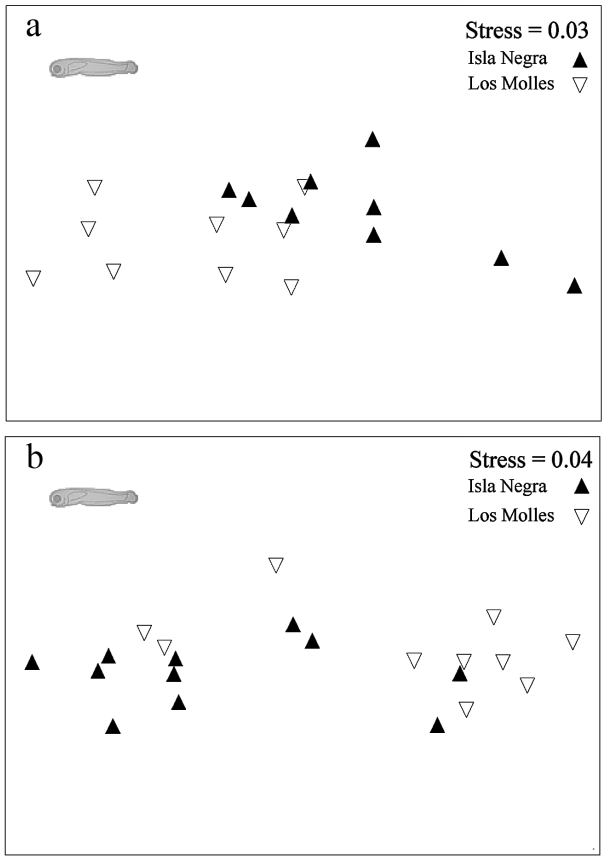


**Figure 3**

**Non-metric multidimensional analysis (nMDS) using micro-chemical measurements of otoliths from *S. viridis* collected at Isla Negra and Los Molles. a) otolith nuclei, and b) otolith edges**

Análisis no métrico multidimensional (nMDS), utilizando mediciones microquímicas en otolitos de *S. viridis* recolectados en Isla Negra y Los Molles. a) núcleo, y b) bordes

ii) that this location is different from the location where the recruited fish from IN originated and iii) that in their recent history the recruited fish at LM were at a different location than the recruited fish from IN.

On the other hand, the temporal profile of the different Me/Ca ratios obtained from the nuclei to the edges of each otolith shows a general pattern for the specimens that come from the IN local area. The maximum Me/Ca ratio (except for Mn and Pb) was found at the nucleus, gradually decreasing away from the nucleus, until the ratio began to increase again from the mid point of the otolith towards the edge, displaying similar values as those obtained at the nucleus (Fig. 4). In the case of the specimens that came from LM, the U-like pattern was

relatively maintained. However, the difference between the ratios found at the edge and nucleus with respect to the midpoint is less than that found in specimens coming from IN (Fig. 4).

### Larval vertical distribution

During the day-night spatial sampling (September and December) it was found that the greatest density of larvae was found nocturnally and during ebb tide (Fig. 5). In none of these samples were larvae found in the superficial stratum. With respect to the night-day temporal samples (November and June) and day-night samples (October), the results did not present a common pattern. In October the increase in larval density presented itself during the nocturnal period. In November, however, it was associated with the daytime period, while in June no changes were observed, which is principally explained by the low larval densities (Fig. 6 a, b, c). On the other hand, during October and November, the greatest densities were associated with ebb tide (Fig. 6 d, e, f). Regarding the greater densities, these were given in the 5 m stratum, followed by the 15 m stratum. In the

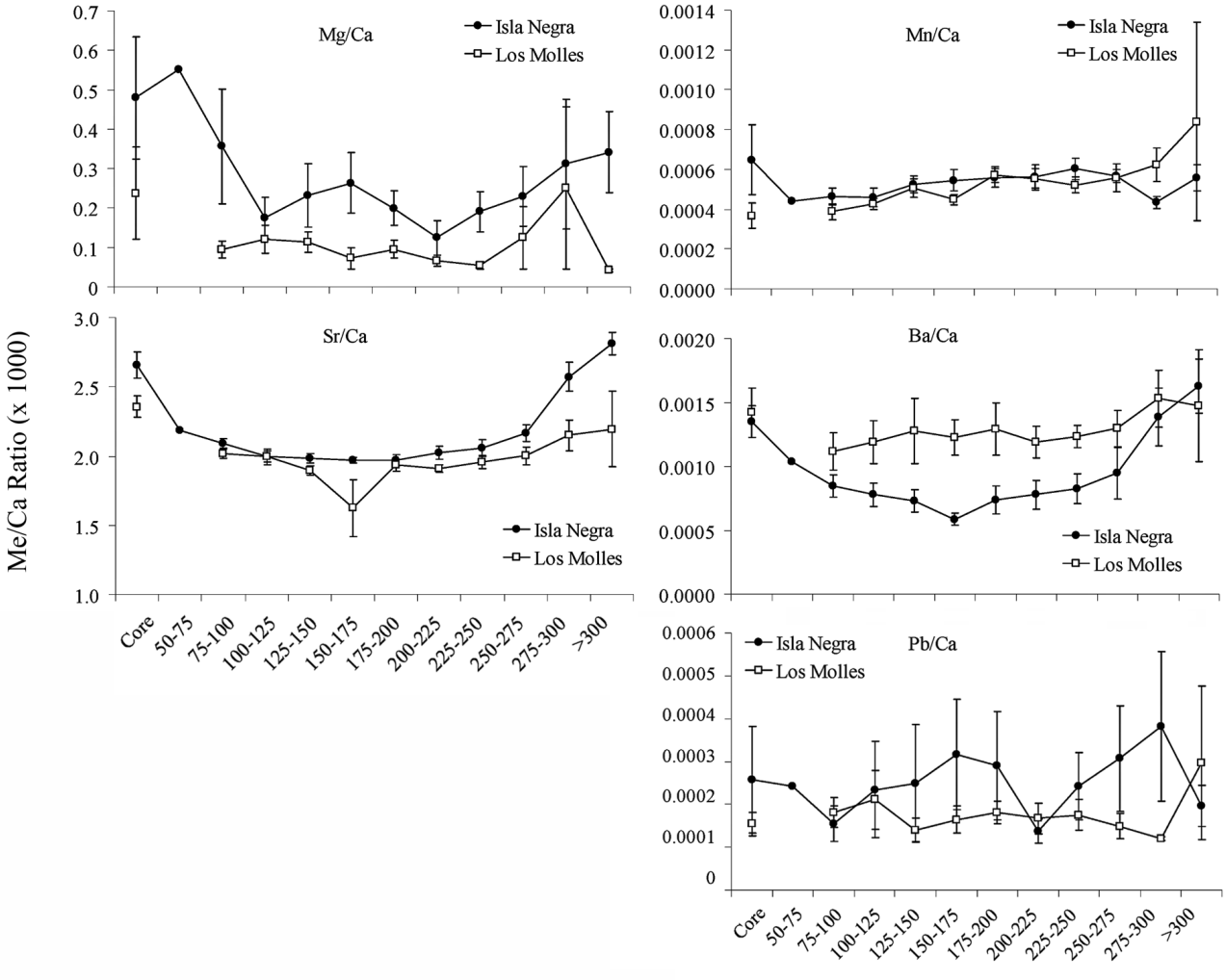


**Figure 4**

**Profiles of element/calcium ratios determined by LAICP-MS, from the nucleus to the edge of each *S. viridis* otolith. Individual points are mean (± SE) values grouped at 25-µm intervals**

Perfiles de razones elemento/calcio determinadas por LAICP-MS, desde el núcleo hasta el borde de cada otolito de *S. viridis*. Los puntos corresponden a los valores medios (± EE) agrupados en intervalos de 25 µm

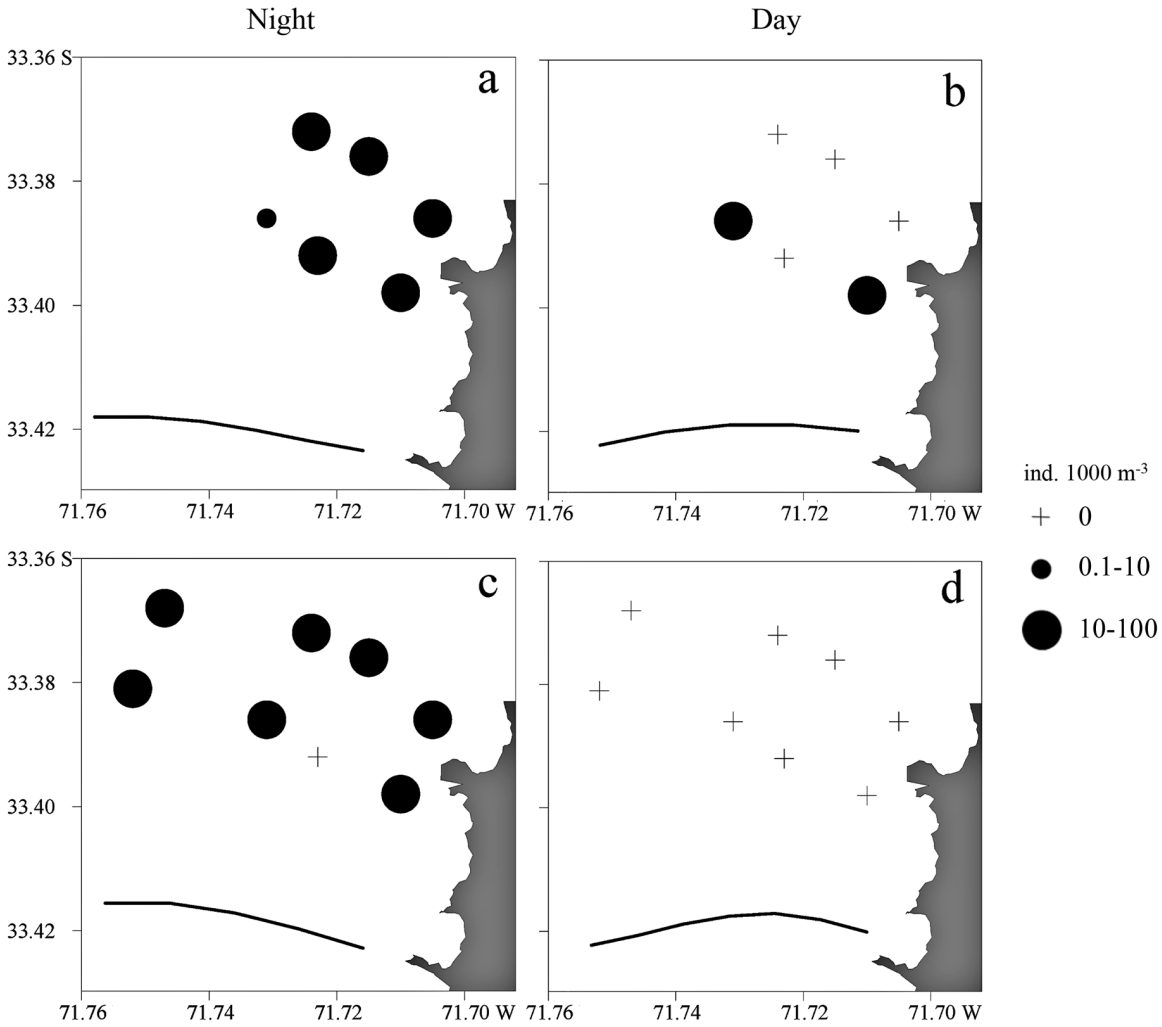


**Figure 5**

**Larval density of *S. viridis* during night-day continuous sampling in September (a, b) and December (c, d) at 1999 off El Quisco. Continuous line inside of each panel show the signal of tidal cycle during the development of each cruise sampling**

Densidad larval de *S. viridis* durante muestreos continuos noche y día realizados en septiembre (a, b) y diciembre (c, d) de 1999, frente a El Quisco. La línea continua dentro de cada panel indica la tendencia de la altura de mareas durante el desarrollo de cada muestreo

superficial stratum, there were only *S. viridis* larvae present in 2 out of 29 catches carried out during the three samplings.

## Discussion

The average time in which *S. viridis* pre-recruited fish stay in the pelagic and/or subtidal zone before arriving to the intertidal pools is between 90 and 100 days, depending principally on the year. The inter-year differences could be associated with the estimates of somatic growth, and thus, years with large growth rates could determine a smaller pre-recruitment period. In this context, the year 2006 presented greater rates at both locations. These values are consistent with those recorded through capture-mark–recapture tools and others, with the variability proposed by Hernández-Miranda & Ojeda (2006), in relation to the Southern Oscillation Index (Spearman correlation; LM = 0.93, $P < 0.01$; IN = 0.91, $P < 0.01$), however they would not explain the smaller pre-recruitment period in the years 2004 and 2005.

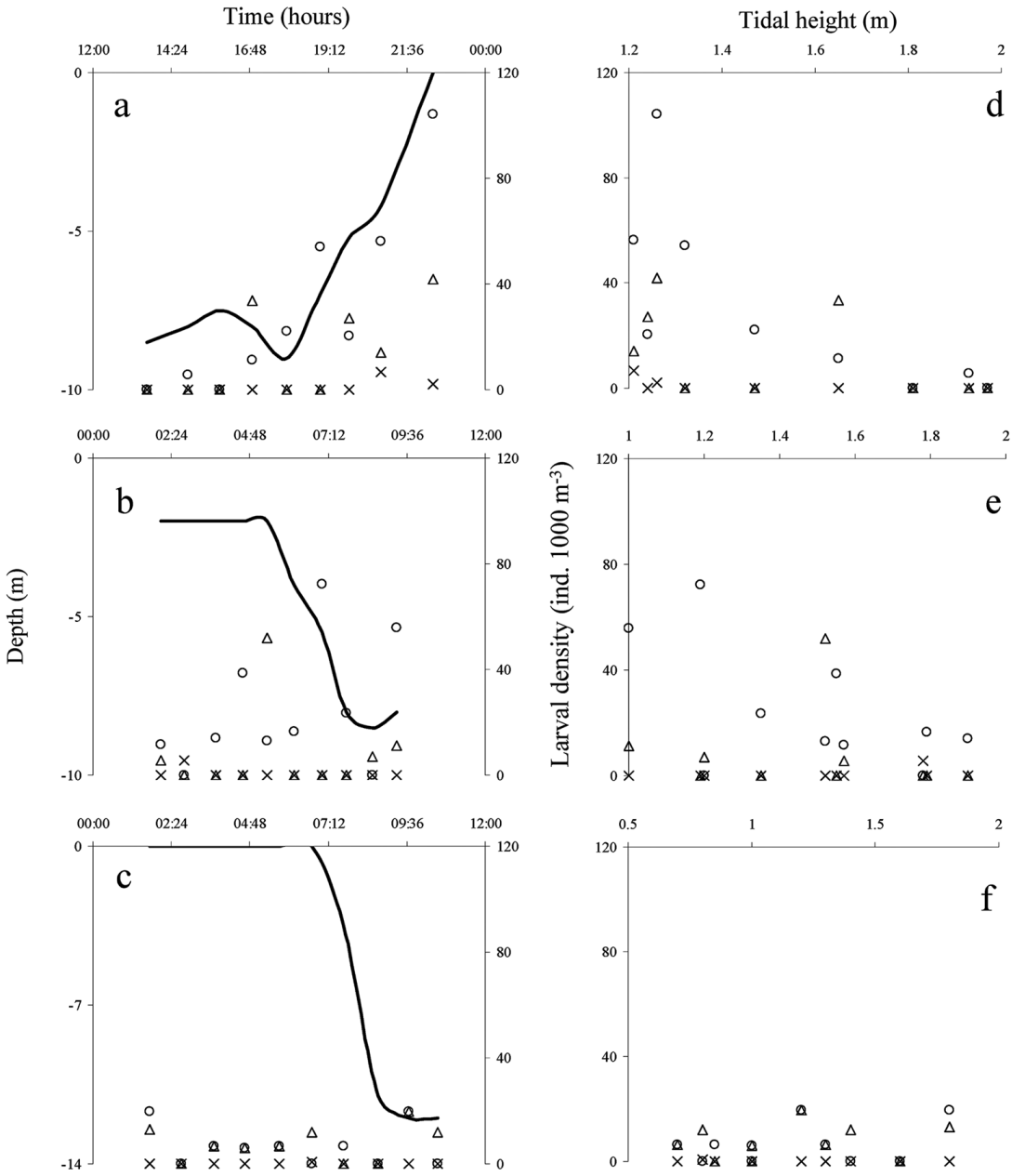


**Figure 6**

**Larval density of *S. viridis* during continuous sampling for three depths in the day-night transition (a) and night-day (b, c) off El Quisco. The continuous line from each panel indicates the tendency for light penetration (measured with a Secchi disc) during the development of each sample. It signals for every sample the relation between the larval density and tidal depth at the moment of sampling (d, e, f). Crosses (superficial), circles (5 m) and triangles (15 m)**

Densidad larval de *S. viridis* durante muestreos continuos para tres profundidades en la transición día-noche (a) y noche-día (b, c) frente a El Quisco. La línea continua de cada panel indica la tendencia de la penetración de la luz (medida con disco Secchi) durante el desarrollo de cada uno de los muestreos. Se señala para cada muestreo la relación entre la densidad larval y la altura de marea al momento del muestreo (d, e, f). Cruces (superficial), círculos (5 m) y triángulos (15 m)

Although the exact period in which *S. viridis* larvae stay in the water column (PLD) is unknown, since it was not possible to detect the settlement marks on the otoliths, the total time of arrival (time from hatching to recruitment in the tidal pools) would be between two or three times longer than what has been recorded for other blennid species in the Mediterranean Sea (26-43 days, Raventos & Macpherson 2001, Beldade *et al.* 2007).

From the point of view of population connectivity, Pineda *et al.* (2007) indicated that this occurs when three components are fulfilled: i) transport ii) dispersion and iii) reproduction. Larval transport is understood only as the displacement between one site and another, the dispersion is defined as the settlement and/or recruitment of one larva produced in one place to another, while the reproduction of this new recruit in its new place would permit the connection. In this context, if it is assumed that the coastal waters of the zone under analysis have a net flow to the north (which is a typical condition of the coastal zone along the central region of Chile), then hatched larvae in Isla Negra, Las Cruces or El Quisco transported at a spring-summer typical speed of 0.2 m/s (Strub *et al.* 1998, Cowen *et al.* 2000), may be transported 17.3 km/day, allowing them to reach Los Molles in a period of approximately 12 days. This suggests that *S. viridis* may have a greater transport-dispersion potential compared to other species of this family (*e.g.* Raventos & Macpherson 2001).

Given the above consideration, pre-recruitment time and potential transport (which is a tenth of the 90-100 days estimated as their real transport potential), the two localities in our study should be connected. However, by using the ratio between trace elements and the calcium present in the nuclei and edges of *S. viridis* otoliths (Gillanders 2002, Thorrold *et al.* 2002, Gillanders & Kingsford 2003, Gillanders 2005), evidence was found supporting spatial segregation between the specimens collected in the intertidal zone off Los Molles and Isla Negra (at a distance of 200 km). These results suggest that both groups of recruited fish originated from different areas at this spatial scale could act as demographically independent entities with distinct dynamics (*i.e.* different populations). Nevertheless, the occasional arrival of specimens from separate sources within these distances would allow both populations to maintain the characteristic features of the species.

The apparent spatial segregation of *S. viridis* could result mainly from hydrodynamic and/or behavioral causes. The former refers to the fact that the coast is not linear and, therefore, the net transport of the current is not necessarily unidirectional towards the north, since there is a large number of bays and headlands within the area under analysis that could modify the flows due to the presence of Coriolis effect and inertial motion (Sobarzo *et al.* 2007), eddies or other oceanic structures (Strub *et al.* 1998). Thus, transport is limited and, therefore, restricts dispersion potential.

On the other hand, certain behavioral mechanisms in early ontogenetic stages of *S. viridis* could determine their location in the water column, offsetting advective transport through biophysical coupling (Neilson & Perry 1990, Sponaugle *et al.* 2002, Pineda *et al.* 2007, Gawarkiewicz *et al.* 2007). Our results cannot explicitly define the biophysical mechanisms associated with the vertical displacements during the day-night or tidal cycles studied. However, the acknowledgement that the greatest densities (> 99%) were consistently found in the subsurface (5 to 15 m) would suggest that *S. viridis* larvae may be able to select their location in the water column, minimizing the probability of being transported away from the coast in the Ekman layer, for example, during upwelling periods that are very common during the period of greatest larval abundance (Hernández-Miranda *et al.* 2003). Complementary to this, our observations indicate that density and vertical distribution seem to be modified during the tidal cycle, also suggesting that *S. viridis* larvae should have a mechanism (*e.g.* high swimming capacity, Kingsford & Suthers 1996, Kingsford *et al.* 2002) capable of compensating advective transport, which in this case is related to the tides.

Population connectivity of marine species with complex life cycles has been one of the most discussed and analyzed research issues during the last decades (Roughgarden *et al.* 1988, Cowen *et al.* 2007). Considering coastal fish, there have been important advances in empirical and theoretical studies (Irisson *et al.* 2004, Jones *et al.* 2005, Cowen *et al.* 2006). In this context, identifying the spatial scale in which populations are demographically disconnected is crucial for determining, for example, protected areas or management strategies (Botsford *et al.* 1997, Fogarty & Botsford 2007). Camus & Lima (2002) indicate that such separation should mostly be defined as from where birth and mortality processes occur rather than from where migration originates. In other words, the question comes down to what spatial scale demographic discontinuities can be identified, allowing each population to function according to the factors that determine their own dynamics, that is, considering feedback mechanisms or environmental factor control (Berryman 1999, Royama 1992). Considering *S. viridis* fish, the results obtained from the micro-increment analysis suggest a demographic spatial segregation of at least 200 km. However, there

could be a small link within this spatial scale if the fact that a few migrants could arrive to one zone from another is considered, thus maintaining the attributes relevant to the species.

Our results suggest that although simultaneous reproductive events could occur at the LM and IN local areas, recruitment would be separated at this spatial scale, because larval behavior and coastal retentive hydrodynamics structures would play a fundamental role in maintaining spatial segregation.

Although this study does not expose the specific biophysical coupling mechanisms that may determine *S. viridis* larval stage distribution, the results suggest the hypothesis that larval behavior plays a crucial role in population segregation at spatial scales up to hundreds of kilometers. The only published study on intertidal fish larval stages, including *S. viridis*, within this area indicates that larvae of this species are preferably located at least 1 nm away from the coast (Hernández-Miranda *et al.* 2003). This agrees with the above-mentioned hypothesis and consequently implies that larvae remain in areas near adult habitats during the pre-recruitment stages, favoring self-recruitment (Sponaugle *et al.* 2002). Moreover, the analysis of trace elements deposited on the otoliths suggests a similar pattern, *i.e.* the Me/Ca ratios show similar values at the otolith nuclei and edges, indicating the settlement or arrival of the specimens in areas which are similar to where they hatched. Finally, according to the somatic growth rates and microelement ratios, larvae seem to be transported away from the adult habitat until they reach a size of a 2.5 cm (about 30-45 days). After this and as the fish increase their swimming capacity, they begin to go back to their place of origin until they finally reach the hatching sites (Gerlach *et al.* 2007).

In brief, based on what was found in terms of larval stage distribution and abundance, as well as *S. viridis* demographic spatial segregation according to natural tags, this study reveals elementary information that gives rise to a better understanding of the ecology of coastal fish in central Chile. Based on this study, it is possible to formulate the general hypothesis claiming that coastal fish recruitment in central Chile would depend on larval behavior and local hydrodynamics, at scales shorter than hundreds of kilometers. This could help to guide future studies toward explicitly determining population segregation at minor spatial scales, as well as the specific biophysical mechanisms that determine transport, larval dispersion and connectivity of resident fish in intertidal environments.

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