

DISPERSIÓN LARVAL Y PATRONES DE
CONECTIVIDAD EN PECES LITORALES DE CHILE
CENTRAL



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DISPERSIÓN LARVAL Y PATRONES DE CONECTIVIDAD EN PECES LITORALES DE CHILE CENTRAL

Por

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Las palabras, ya ves, jamás alcanzan si lo que hay que decir desborda el alma

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Resumen

En estudios de ecología marina, uno de los temas centrales es la conectividad o intercambio de individuos entre poblaciones marinas. Para muchas especies bentónicas con ciclos de vida complejo, este intercambio ocurre en los estadios larvales pelágicos. El pequeño tamaño de las larvas y su comportamiento, la dificultad en realizar seguimientos desde la eclosión de huevos hasta el asentamiento y las condiciones oceanográficas en las cuales se desarrollan estas especies, dificultan el entendimiento del fenómeno. No obstante esto, en las últimas décadas, a partir de aproximaciones directas e indirectas utilizando técnicas geoquímicas y genéticas, es posible determinar el rango geográfico de las poblaciones de especies bentónicas.

Considerando los peces costeros, numerosos estudios sugieren que el rango de las poblaciones varía desde pocos a cientos e incluso miles de kilómetros. En este contexto, identificar la escala espacial en la que las poblaciones están demográficamente desconectadas es crucial para determinar, por ejemplo, áreas protegidas o estrategias de manejo. En el ambiente intermareal rocoso de la costa central de Chile, el ensamble de peces está caracterizado por dos componentes, especies “residentes” (luego de reclutar permanecen en el intermareal) y “temporales” (luego de alcanzar cierto tamaño migran al ambiente submareal). Considerando que representa un ambiente perturbado y que se encuentran especies de interés para la pesca artesanal, se plantea la necesidad de abordar estudios de conectividad. Para lo cual, se hace necesario conocer previamente la biología y ecología en la fase larval y recluta; como así también, realizar estudios que involucren una larga extensión espacial.

El objetivo central de la tesis es analizar la capacidad de dispersión de peces litorales con diferentes estrategias de vida (residentes y temporales), de Chile central y determinar cuáles son los mecanismos biofísicos que subyacen al fenómeno. Se seleccionaron las especies más representativas de ambas estrategias de vida, *Helcogrammoides chilensis* (especie residente) y *Girella laevis* (especie temporal).

En primer lugar se estimó la dispersión larval a partir de la determinación del período de permanencia larval en el plancton (PLD), la que permitió establecer como distancia máxima a la que podrían dispersar en 200 km. A su vez se determinó la sincronía de los eventos de reclutamiento con las fases lunares. Considerando la distancia de dispersión, se realizó el análisis de la estructura genética en seis localidades ubicadas en el rango de dispersión a partir del análisis de ADN nuclear (AFLP). Se pudo determinar que los peces residentes presentan estructuración a distancias de hasta 10 km y que la dispersión está limitada por barreras oceanográficas principalmente la presencia de cuerpos de agua dulce. Por el contrario, las especies temporales con huevos y larvas pelágicos no presentan estructuración en el rango geográfico del estudio. Dicho fenómeno se explica en que las larvas al ser liberadas al plancton lejos de la costa, no se ven afectadas por eventos o barreras oceanográficos costeros que producen retención larval. Por último se analizó una serie temporal de 15 años en 3 localidades para determinar cuáles son los factores (exógenos o endógenos) que explican la dinámica de las poblaciones. Como resultado fue posible generar modelos simples para ambas especies y corroborar lo encontrado en el análisis genético en cuanto a la escala en que se pueden individualizar poblaciones.

I. Introducción General

Marco teórico

En ecología, desde la década del 50 trabajos como el de Thorson (1950) y Mileikovski (1971) dan cuenta de como el modo de reproducción determina los patrones de diversidad y capacidad de dispersión de las especies. A partir de los ochentas se comienza a considerar al reclutamiento y dispersión procesos estructuradores de comunidades (Roughgarden *et al.*, 1985; Leis, 1986; Marliave, 1986; Greene & Johnson, 1989). Actualmente se considera que la escala a la cual las especies interactúan con el ambiente determina la forma en que responden a perturbaciones y evolucionan. A su vez, conocer los patrones de conectividad y la escala a la cual ocurre, es determinante para abordar estudios desde dinámica de poblaciones (Roughgarden *et al.*, 1988; Hanski, 1998), interacciones entre especie (McCauley *et al.*, 1996; Gaines *et al.*, 2007), patrones espaciales y temporales de distribución (Sousa, 1984), estructura genética de poblaciones (Loveless & Hamrick, 1984; Grosberg & Cunningham, 2001), macroevolución (Valentine, 1986; Palumbi, 1994), e incluso patrones biogeográficos (Brown and Lomolino 1998, Gaylord & Gaines 2000, Navarrete *et al.*, 2005).

En este contexto, el estudio de las poblaciones naturales ha presentado a lo largo de la historia la problemática de cómo y cuando considerar a un grupo o conjunto de individuos pertenecientes a una misma población (Roughgarden *et al.*, 1988). Una población es definida como el grupo de individuos de la misma especie que viven en un área lo suficientemente grande que permite la dispersión o migración normal y en donde la dinámica poblacional resulta de los procesos de natalidad y mortalidad (Berryman, 2002). Por lo tanto, determinar adecuadamente y sin ambigüedad la escala espacial en donde los procesos de natalidad ocurren resulta de suma importancia para entender la dinámica temporal de las poblaciones.

Conceptos como meta-población (Hanski, 1998; Thorrold *et al.*, 2001), población local, sub-poblaciones, poblaciones abiertas o cerradas (Roughgarden, *et al.*, 1985; Caley *et al.*, 1996) han sido propuestos y utilizados para dar cuenta de la conectividad entre grupos de individuos de una misma especie. De todas maneras, la falta de definición explícita de la escala espacial de la población y los criterios biológicos subyacentes, ha limitado un

entendimiento más acabado de poblaciones en ecosistemas marinos. Por otra parte, el concepto de auto-reclutamiento ha surgido para explicar casos en donde los individuos adultos de los ambientes intermareales provienen de larvas producidas en el mismo ambiente y no de zonas lejanas arribadas debido a procesos dispersivos (Kingsford *et al.*, 2002). Si bien es un concepto operativo que permite identificar relaciones de dependencia entre áreas de desove y posterior reclutamiento, éste no permite determinar la escala espacial del funcionamiento demográfico poblacional. Considerar la información completa permitirá entonces, reconocer los factores que determinan los patrones de distribución y abundancia de las poblaciones, así como también la estructura genética y la demográfica espacial (Cowen *et al.*, 2006), información clave a la hora de tomar decisiones de manejo o conservación, como por ejemplo diseñar áreas marinas protegidas (Agardy, 2000; Camus & Lima, 2002; Palumbi, 2003; Levin, 2006).

A partir de la década del noventa, numerosos estudios han demostrado que un porcentaje significativo de las larvas que llegan a una determinada localidad proceden de progenitores que viven en dicha zona (Jones *et al.*, 1999; Gillander *et al.*, 2003; 2005; Thorrold, 2001, Gerlach *et al.*, 2007). La creciente evidencia, sugiere que las poblaciones de peces no son necesariamente “abiertas” y que la inter-conectividad entre poblaciones es inferior a lo que sugerían estudios anteriores (Cushing, 1996; Swearer *et al.*, 2002). Es decir, la posibilidad de que una población deteriorada pueda recuperarse a partir de las larvas de otras poblaciones próximas, debe analizarse con cautela. Diversos autores sugieren que las reservas de cualquier red de conservación deben situarse a cortas distancias entre las mismas (máximo 50-100 km) y conteniendo tanto áreas de reproducción como de asentamiento (Lubchenco *et al.*, 2003). Sin embargo, dado la escasez de datos sobre estos aspectos es que se hace necesario recoger información de distintas especies, y con diferentes capacidades de dispersión, para conocer el grado de generalización de este interesante problema. A su vez, es importante analizar desde el punto de vista evolutivo si las especies con largos períodos larvales tienen amplios rangos geográficos y estratigráficos y son menos propensas a la extinción y especiación que aquellas que tienen larvas “non-feeding” o que requieren incubación (Strathmann *et al.*, 2002).

Durante las últimas décadas, gran cantidad de estudios en ecología marina se han centrado en evaluar qué determina la configuración espacial y temporal de las poblaciones en las zonas intermareales rocosas (Gaines *et al.*, 2007). En este tipo de estudios se reconocen dos tipos de procesos: post reclutamiento (estadio juveniles y adultos en la zona intermareal propiamente dicha) y pre-reclutamiento (estadios larvales en la columna de agua). Respecto a los primeros, se ha documentado que los mecanismos más importantes están asociados a competencia por espacio, depredación, perturbaciones físicas y el grado de tolerancia fisiológica (Connell, 1985; Menge & Sutherland, 1987). En cuanto a los mecanismos relacionados a los procesos pre-reclutamiento, se ha descrito que el suplemento larval (Lewin, 1986; Sale, 1990), esto es, la llegada de reclutas, está determinado por la disponibilidad de alimento, la depredación y el transporte advectivo-difusivo local y/o de meso-escala impuesto por procesos oceanográficos (e.g Roughgarden *et al.*, 1988; Poulin *et al.*, 2002; Shanks *et al.*, 2003; Siegel *et al.*, 2003; Menge *et al.*, 2004). A su vez se conoce que, para especies bentónicas (demersales o sésiles), la fase de dispersión primaria y dominante, se asocia a estadios tempranos de la historia de vida (esporas, huevos o larvas), en tanto que para especies móviles, como peces, los estadios juveniles y adultos son los que se asocian a la dispersión (Cowen & Sponaugle, 2009).

Las principales variables biofísicas que influyen la fase larval y el reclutamiento son: fecundidad de adultos, patrones espaciales de desove, patrones temporales de desove, inversión en cuidado parental, duración del periodo larval pelágico (Pelagic Larval Duration: PLD), migración vertical, capacidad natatoria, aislamiento geográfico, complejidad costera, variabilidad en flujo y estratificación de la columna de agua (Werner *et al.*, 2007; Palma *et al.*, 2009; Cowen & Sponaugle 2009).

La mayoría de las especies de peces costeros tienen ciclos de vida complejos, con diversos estadios de desarrollo. La fase larval planctónica, es el estadio principalmente dispersivo, y el tiempo de duración es variable. Las larvas, luego de pasar un tiempo en la columna de agua pueden reclutar en la misma área de sus progenitores, distante algunos metros o bien cientos de kilómetros lejos de su lugar de nacimiento (Cowen *et al.*, 2002). Diversos estudios han tratado de cuantificar el grado de conectividad espacial de poblaciones marinas de peces a partir de la estimación del PLD (Jones *et al.*, 1999;

Thorrold *et al.*, 2001; Hamer *et al.*, 2005; Fogarty & Botsford, 2007). Recientes meta-análisis han mostrado correlación positiva y fuerte entre el potencial de dispersión, estimado a partir del PLD y la dispersión realizada en especies de invertebrados. Para peces, donde las larvas tienen gran capacidad natatoria y donde la distancia de dispersión es influenciada por una gran variedad de variables biofísicas, utilizar la dispersión como función directa del PLD es insuficiente (Sponaugle *et al.*, 2002; Weersing & Toonen, 2009). Respecto a la intensidad de reclutamiento de juveniles, típicamente fluctúa en función del ciclo de mareas y del régimen de vientos. Las variaciones pueden ser detectadas a diferentes escalas: diaria, fase lunar, mensual, estacional e incluso anual (Robertson *et al.*, 1999). En peces de arrecifes, se ha encontrado que tanto el viento como los ciclos de mareas tienen efecto de intensidad variable respecto a la dinámica de asentamiento (MacKenzie & Leggett, 1991).

En la actualidad, se utilizan métodos directos e indirectos y modelos biofísicos capaces de determinar la escala espacial a la cual las poblaciones tienen conectividad. Dependiendo del método utilizado, eventos individuales o patrones a largo plazo pueden ser explicados, pero generalmente con poca resolución espacial debido a limitaciones logísticas en la toma de datos. Se reconoce que la utilización de modelos biofísicos de alta resolución para calcular las funciones de distribución de dispersión larval (kernels) es muy ventajoso respecto a métodos directos e indirectos (James *et al.*, 2002; Cowen *et al.*, 2006; Aiken *et al.*, 2007). De todas maneras los métodos indirectos son ampliamente utilizados, ya que consideran marcas naturales, descartando la necesidad de aplicar marcas individuales artificiales. Las marcas pueden ser de dos clases: marcas microquímicas en partes duras (e.g., otolitos y valvas) ó marcas genéticas (e.g., ADN mitocondrial, microsatélites). Marcas geoquímicas (basadas en concentración de isótopos estables, razón de concentración de elementos ó ambas) en las partes duras de ciertos taxa (otolitos en peces, valvas y estatolitos en moluscos) han sido utilizadas para identificar potenciales sitios fuentes de aporte larval, particularmente en ambientes de estuario (Thorrold *et al.*, 2000, 2001, 2002, 2007) e incluso sitios de guardería a escalas espaciales relativamente pequeñas (Gillanders & Kingsford, 2000).

Los otolitos, son tres estructuras pares (*sagitta*, *asteriscus* y *lapillus*) compuestas por carbonato de calcio biogénico, en forma de aragonita inmersas en una matriz proteica. Forman parte de la estructura del oído interno de los peces teleósteos y están incluidos en la endolinfa (Campana, 1999; Campana & Thorrold, 2001; Jolivet *et al.*, 2008). Los otolitos adicionan diariamente nuevas capas de material cristalino y proteico, principalmente compuesto por C, O y Ca pero también adiciona elementos trazas (e.g.: ^{48}Ca , ^{86}Sr , ^{138}Ba , ^{25}Mg , ^{55}Mn y ^{208}Pb). Debido a la dinámica de depositación diaria de elementos en el otolito, es posible reconstruir la historia de vida de peces teleósteos (Campana, 1997; Elsdon & Gillander, 2004; Elsdon & Gillander, 2006).

A partir de la década del setenta, el estudio de otolitos (otolimetría) ha tenido un gran desarrollo, aunque el estudio de la micro-estructura y de incrementos periódicos ha tenido su gran desarrollo a partir de la década del noventa (Panella, 1971; Fossum *et al.*, 2000; Campana *et al.*, 2001; 206 trabajos, 1996 – 2005 en Campana, 2005; Begg *et al.*, 2005; Morales-Nin & Panfili 2005, Elsdon *et al.*, 2008). Para realizar la reconstrucción de historia de vida, en primer lugar se estima el tiempo de permanencia en la columna de agua (PLD). La estimación se realiza a partir del conteo de anillos de crecimiento diario. Debido a que las marcas diarias varían con las condiciones ambientales, temperatura y salinidad asociadas a ciclos de marea, en otolitos de especies con ciclo de vida que incluye, fase larval pelágica y fase adulta bentónica, se encuentran marcas distinguibles asociadas al asentamiento y es posible cuantificarlas. En segundo lugar, se determina la vía migratoria de un individuo pre-recluta, a partir de la presencia de macro elementos y elementos traza asimilados e incorporados diariamente, desde la columna de agua en la estructura calcárea de los otolitos (Campana, 1999; Thorrold *et al.*, 2001; Thorrold *et al.*, 2002). El principio que subyace en este análisis es considerar que la disponibilidad de elementos y la concentración de isótopos estables son variables en el ambiente marino, tanto en escala temporal como espacial. Variación en las marcas geoquímicas a través del tiempo podría limitar el éxito en predecir localidades fuente en años sucesivos (Popper *et al.*, 2005; Cowen & Sponaugle, 2009). Por tanto, considerar la información en conjunto, permite definir el grado de segregación espacial de poblaciones de peces de la misma especie (Gillanders, 2002; Hamer *et al.*, 2005).

Otra aproximación para medir conectividad entre poblaciones es la integración de información genética y geográfica. Esta aproximación ha tenido una gran proliferación en las últimas décadas debido al incremento en herramientas genéticas y estadísticas. (Palumbi, 2003; Hedgecock *et al.*, 2007; González-Wangüemert *et al.*, 2010). El modelo más utilizado en estos estudios es de aislamiento por distancia y permite definir flujo génico entre poblaciones distantes desde 10 km. Para peces Kinlan & Gaines (2003) documentan que la dispersión genética de peces en general puede ser encontrada entre unos metros a centenas de kilómetros, sin embargo, el promedio sería cercano a los 100 kilómetros, encontrándose en estrecha relación con su PLD. En el caso de peces costeros el potencial de dispersión espacial ha sido estimado, por ejemplo, en 200 km en peces tropicales en Barbados (Cowen *et al.*, 2000), entre 10 y 100 km en *Thalassoma bifasciatum* en Islas Virginia (Swearer *et al.*, 1999), menor a los 120 km en *Sebastes melanops* en la costa de Oregon (Miller & Shanks, 2004), cercana a 200 km en *Pagurus auratus* en el sureste de Australia (Hamer *et al.*, 2005), pudiendo incluso alcanzar entre 200 y 500 km en peces que poseen un período pelágico larval largo (Siegel *et al.*, 2003).

En la costa central de Chile, la conectividad entre poblaciones marinas, ha sido abordada a partir de estudios de reclutamiento y de los factores que lo influyen a escala local y meso-escala, utilizando como modelos de estudio invertebrados (Lagos *et al.*, 2005; Narváez *et al.*, 2006; Aiken *et al.*, 2007). Si bien en la costa central de Chile, se reconoce una gran diversidad íctica, 44% de endemismo del pacífico sudoriental (Ojeda *et al.*, 2000), estudios que aborden la conectividad o dispersión son escasos, pudiendo citar como trabajo pionero a Hernández-Miranda *et al.*, 2009. En el ambiente intermareal rocoso de la costa central de Chile, el ensamble de peces está caracterizado por dos componentes, especies “residentes” (luego de reclutar permanecen en el intermareal) y “temporales” (luego de alcanzar cierto tamaño migran al ambiente submareal). Las especies más abundantes de cada componente son, residentes: *Helcogrammoides chilensis* y *Helcogrammoides cunninghami* y temporales: *Scartichthys viridis* y *Girella laevis* (Varas & Ojeda, 1990; Muñoz & Ojeda, 1997).

Hoy en día se ha planteado que para el diseño de una red de reservas y/o parques marinos es crucial el conocimiento de los procesos oceanográficos costeros, de las áreas de

“nursery” y de la capacidad de dispersión de las especies que permitirían construir un sistema conectado entre cada una de las poblaciones locales adultas (Palumbi, 2003; Fernández & Castilla, 2005; Aiken *et al.*, 2007; Jones *et al.*, 2007; Cowen & Sponaugle, 2009; White *et al.*, 2010).

La conectividad de poblaciones de especies marinas con ciclo de vida complejo ha sido en las últimas décadas muy discutida y analizada (Roughgarden *et al.*, 1988; Cowen *et al.*, 2007). Considerando a los peces costeros, ha habido un gran avance en estudios teóricos y prácticos. En este contexto, identificar la escala espacial en la que las poblaciones están demográficamente desconectadas es crucial para determinar, por ejemplo, áreas protegidas o estrategias de manejo

Respecto a peces residentes en las costas de Chile, si bien se cuenta con antecedentes de la biología y ecología, aún son escasos estudios de la biología pre-reclutamiento. Para estudios de conectividad de peces litorales, se hace necesario entonces en primer lugar conocer la biología y ecología en la fase larval y recluta. En segundo lugar, realizar estudios que involucren una larga extensión espacial, esto debido a la alta heterogeneidad espacial del litoral, la disminución de la riqueza de especies en función de la latitud (Ojeda *et al.*, 2000) y la heterogeneidad oceanográfica latitudinal de la zona costera, con áreas definidas de afloramiento/surgencia, interrumpidas por áreas poco productivas (Wieters *et al.*, 2003; Narváez *et al.*, 2004; Aiken *et al.*, 2007).

Estructura de la tesis

En consideración a lo expuesto, en este proyecto de tesis se plantea como objetivo central analizar la capacidad de dispersión de peces litorales con diferentes estrategias de vida de Chile central y determinar cuales son los mecanismos biofísicos que subyacen al fenómeno.

Se eligieron como modelo de estudio las especies representativas de cada estrategia de vida, siendo *Helcogrammoides chilensis* como especie residente y *Girella laevis* como especie temporal. En primer lugar, previo al análisis de otolimetría se realizó la validación de la periodicidad de los incrementos de los otolitos de ambas especies (véase capítulo 1,

sometido a la Revista de Biología Marina y Oceanografía). El estudio de la microestructura de los otolitos, permitió establecer el período planctónico larval (PLD), momento de eclosión, momento de reclutamiento y sincronía de dichos eventos con el ciclo lunar.(véase capítulo 2, sometido a Marine and Freshwater Research; capítulo 3, en preparación). A partir del PLD se estimó la distancia máxima a la que las larvas podrían dispersar al considerarlas como partículas inertes inmersas en la corriente. A partir de lo cual se analizó la diversidad genética en 6 localidades de Chile central, distantes entre sí entre 10 y 50 km. El análisis se realizó con la técnica de AFLP y se pudo establecer que *H. chilensis* (especie residente) presenta estructuración a escala de 10 km. en tanto que *G. laevifrons* (especie temporal) no presenta estructuración en el rango geográfico estudiado (véase capítulo 4, artículo a ser sometido a la Revista Chilena de Historia Natural). Por último se analizó la dinámica de las poblaciones de ambas especies en tres localidades, utilizando una serie temporal de 15 años. Se pudo determinar que sólo *H. chilensis* presenta dinámicas independientes en escala de poca distancia. A su vez fue posible establecer las interacciones que determinan las dinámicas para ambas especies (véase capítulo 5, artículo sometido a Oecologia).

Área de estudio

El estudio se realizó en siete intermareales rocosos de localidades costeras de Chile central entre 33,5° y 32,2° de latitud Sur. De sur a norte las localidades seleccionadas fueron: Las Cruces (**LC** 33° 30' S; 71° 37' O), El Tabo (**ET** 33° 31' S, 71° 40' O), El Quisco (**EQ** 33° 24' S, 71° 43' O), Valparaíso (**V** 32° 57' S; 71°34' O), Maitencillo (**MA** 32° 30' S; 71° 27' O) y Los Molles (**LM** 32° 13' S, 71° 30' O).



La selección de los sitios de estudios se fundamenta en los siguientes aspectos:

1.- Considerando las dimensiones de las larvas, estimando el período pelágico larval y considerando el modelo de dispersión advectivo-difusivo descrito en Hernández-Miranda 2009 las larvas podrían recorrer hasta 200 Km.

2.- De acuerdo a Swearer *et al.*, 1999; Cowen *et al.*, 2000; Siegel *et al.*, 2003; Miller & Shanks, 2004, Hamer *et al.*, 2005 y Gaines *et al.*, 2007, especies de peces marinos muestran estructuración genética de sus poblaciones en una escala que va desde los 10 km a más de 200 km de distancia.

3. Las Cruces, el Tabo e Isla Negra, corresponden a zonas rocosas separadas por 10 km de playas de arena. Las localidades al encontrarse entre 20 y 50 km al norte de la desembocadura del río Maipo, presentan un enriquecimiento de microelementos en el agua que decrece conforme aumenta distancia al río.

4-. De la región de Valparaíso, se considerará la localidad Montemar, que corresponde a una zona de rocas, rodeada de playas de arena en el centro de la bahía. Se encuentra a menos de 10 km al sur de la desembocadura del río Aconcagua.

5-. Maitencillo se corresponde con una zona rocosa, rodeada de playas de arena y acantilado, sin influencia de desembocadura de ríos a menos de 50 km.

6-. Los Molles está emplazado en la cabeza norte de la bahía del mismo nombre. La zona intermareal está compuesta por continuas áreas rocosas, sin influencia de desembocadura de ríos a menos de 50 km.

Finalmente, considerando que por las características de las larvas de las especies en estudio podrían dispersar como máximo 200 km, se eligió dicha distancia como máxima limitando el estudio entre las localidades de Las Cruces y Los Molles. A su vez, considerando los antecedentes bibliográficos que dan cuenta de estructuración genética en poblaciones de peces intermareales en distancias de 10 km, se seleccionaron dos escalas de estudio. La primera escala considera localidades distantes entre si aproximadamente de 10 km (Las Cruces - El Tabo - Isla Negra) y una segunda escala que considera separaciones entre 30 y 50 km (Valparaíso – Maitencillo – Los Molles).

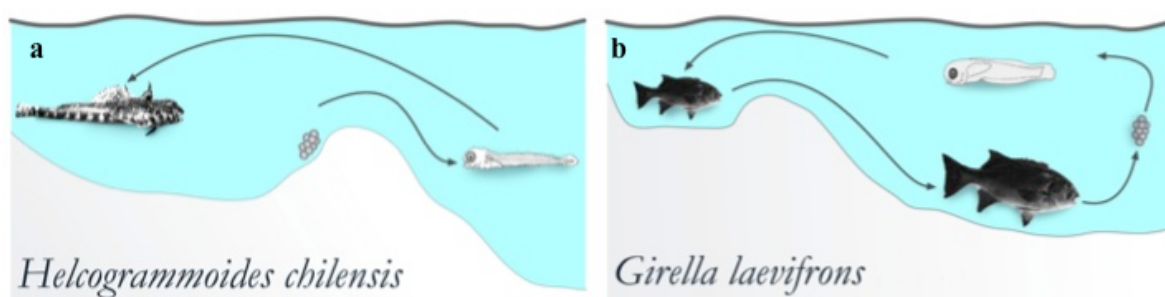
La oceanografía costera para las localidades a muestrear ha sido descripta por Montecinos & Balbontín, 1993; Sievers & Vega, 2000; Poulin *et al.*, 2002; Shanks, 2002; Sobarzo, 2002; Hernández-Miranda *et al.*, 2003, 2009.

Modelo de estudio

Se consideran *especies residentes* aquellas que su ciclo de vida consta de las siguientes etapas: larva pelágica, juveniles y adultos intermareales, puesta de huevos en forma de racimo (Figura a). Los desoves son pequeños y las puestas generalmente están ubicadas en grietas u oquedades de las pozas de marea. Para las especies del litoral de Chile no se tiene información respecto a cuidados parentales. La duración de la fase larval pelágica generalmente es corta y reducida respecto a especies submareales (Horn *et al.*, 1999). La especie seleccionada para el presente trabajo es la dominante del ensamble, *Helcogrammoides chilensis*. Antecedentes de la biología y ecología se encuentran en Pérez-Mongard (1979), Castillo & Pequeño (1998), Muñoz & Ojeda (1997), Muñoz & Ojeda (1998), Williams & Springer (2001); Hernández-Miranda *et al.* (2003).

Se consideran *especies temporales* aquellas que su ciclo de vida consta de las siguientes etapas: Juveniles intermareales, adultos submareales, huevos y larvas pelágicas. Tienen grandes desoves y los huevos son liberados a la columna de agua. Generalmente no presentan claro comportamiento de homing (Varas & Ojeda, 1990), ni de inversión en

cuidado parental (Horn *et al.*, 1999). La especie seleccionada es la mas abundante en el ensambles de peces de Chile central. Antecedentes de la biología y ecología de *Girella laevisfrons* se encuentran en Varas & Ojeda (1990), Muñoz & Ojeda (1997), Ojeda & Muñoz (1999), Cáceres & Ojeda (2000), Muñoz & Ojeda (2000), Fariña *et al.* (2000); Hernández-Miranda *et al.* (2003), Pulgar *et al.* (2003), Hernández-Miranda *et al.* (2006), Pulgar *et al.* (2006) y Hernández-Miranda *et al.* (2009).



Visión general

En la costa central de Chile, la conectividad entre poblaciones marinas, ha sido abordada a partir de estudios de reclutamiento y de los factores que lo influyen a escala local y meso-escala, utilizando como modelos de estudio invertebrados. Si bien en la costa central de Chile se reconoce una gran diversidad íctica, 44% de endemismo del pacífico sudoriental, estudios que aborden la conectividad o dispersión son escasos. En el ambiente intermareal rocoso de la costa central de Chile, el ensamble de peces está caracterizado por dos componentes, especies “residentes” (luego de reclutar permanecen en el intermareal) y “temporales” (luego de alcanzar cierto tamaño migran al ambiente submareal).

En esta tesis se analizó la dinámica de las poblaciones de *Helcogrammoides chilensis* (especie residente) y *Girella laevisfrons* (especie temporal), para determinar cuáles son los factores (exógenos o endógenos) que las explican. Mediante el estudio de los anillos de crecimiento periódico de los otolitos para la estimación del período de permanencia larva en el plancton (PLD) y el estudio del ADN nuclear (AFLP) para un análisis de la

estructura genética de las seis localidades, se pudo determinar que los peces residentes presentan estructuración genética y dinámicas independientes a distancias de hasta 10 km. y que la dispersión está limitada por barreras oceanográficas principalmente la presencia de cuerpos de agua dulce. Por el contrario, las especies temporales con huevos y larvas pelágicos no presentan estructuración en el rango geográfico del estudio y presentan una dinámica común, fenómeno que se explicaría en que las larvas al ser liberadas al plancton no se verían afectadas por eventos o barreras oceanográficos costeros que puedan producir retención larval.

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CAPÍTULO I

II. Validations of the daily periodicity of increment deposition of eight species of intertidal rocky fishes in the South-eastern Pacific Ocean

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**Validations of the daily periodicity of increment deposition of
eight species of intertidal rocky fishes in the South-eastern Pacific
Ocean**

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Abstract

Daily deposition of otolith increments was validated for juvenile of eight intertidal fish species in central Chile, *Helcogrammoides chilensis*, *Helcogrammoides cunninghami* (Tripterygiidae), *Auchenionchus microcirrhys*, *Auchenionchus crinitus* (Labrisomidae), *Hypsoblennius sordidus* (Blenniidae), *Gobiesox marmoratus*, *Sygiacis sanguineus* (Gobiesocidae) and *Myxodes viridis* (Clinidae). Validation was performed by 2 successive Alizarin red-S immersion.

Key words: otolith daily increments, alizarin red S, intertidal fishes

Introduction

The increment patterns of otoliths have permitted great progress in studies on the growth dynamics of fishes. In addition to age and growth, fish otoliths may also record life history events such as metamorphosis, settlement and migration (Victor 1982, 1986, Kingsford & Milicich 1987, Sponaugle & Cowen 1994, Hamer & Jenkins 1996, Jenkins *et al.* 1996, Linkowski 1996, Wilson & McCormick 1997, Thorrold *et al.* 2001). Age estimates can be obtained by the enumeration of growth increments observed in the otoliths. Growth increments are formed during otolith deposition, and these increments can be deposited annually (e.g. Fowler 1990), daily (e.g. Campana & Neilson 1982, Schmitt 1984, Pitcher 1988, Radtke *et al.* 1988, Iglesias *et al.* 1997), or even sub- daily (e.g. Pannella 1980, Campana & Neilson 1982).

Because of the potential variation in the temporal pattern of increment formation, it is important to validate otolith increment periodicity when using otoliths to age fishes. The manipulation of temperature, light or food ration can produce a natural structural mark in the

otolith which may be used in age validation (e.g. Victor 1982), however, a chemically induced mark is preferred because it is more easily distinguished from naturally occurring variation in increment structure. Therefore, validation of increment periodicity is commonly achieved by inducing a mark in the otolith, then sacrificing the fish after a number of days, months or years. The number of growth increments deposited between the induced artificial mark and the otolith edge is then compared to the known time period the fish was alive since mark induction (Hernaman *et al.* 2000).

Fluorescent chemicals such as tetracycline and calcein have been widely used to induce a mark in the otoliths of species from many families of tropical and temperate fishes (e.g. Hettler 1984, Schmitt 1984, Fowler 1990, Secor *et al.* 1992, Lou 1992, Monaghan 1993, Newman *et al.* 1996, Wilson & McCormik 1997). Recently Alizarin Red-S have been used as an effective chemical marker in a number of studies (Geffen 1998, Campana 1999, Skov 2001; Fitzpatrick *et al.* 2010). (Geffen 1998, Campana 1999, Skov 2001). This compound is 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 central coast of Chile comprises large fish diversity, including the 44% of the all endemic recorded in southeast Pacific (Ojeda *et al.* 2000). Typically, fish assemblages observed in intertidal rocky shores are characterized by 2 components, resident and transient species. While residents remain in the intertidal area after recruitment, transient species migrate to the subtidal once they reach a certain body size (Varas & Ojeda 1990, Muñoz & Ojeda 1997). While numerous studies focused on the biology and ecology of the most conspicuous species found in the rocky intertidal shores (Pérez 1979, Varas & Ojeda 1990, Muñoz & Ojeda 1997, 1998, Castillo & Pequeño 1998, Ojeda & Muñoz 1999, Cáceres & Ojeda 2000, Fariña *et al.*

2000, Muñoz & Ojeda 2000, Williams & Springer 2001, Hernández-Miranda *et al.* 2003, 2009, Hernández-Miranda & Ojeda 2006, Pulgar *et al.* 2003, 2006), most of this research was concentrated in the juvenile and adult phase. However, in recent years more attention has been paid to the pre-recruitment biology and dynamics (Plaza-Pasten *et al.* 2003, Hernández-Miranda *et al.* 2009, Palacios-Fuentes *et al.* 2012, Contreras *et al.* 2013). Most of the research conducted on pre-recruits heavily relied on the information obtained from otolith's reading, usually assuming the number of observed rings correspond with a daily frequency of deposition. Hence, to contribute to fill this gap the current study aimed to validate the periodicity of increment deposition in post-settlement juveniles of eight intertidal fish of central Chile using Alizarin Red-S.

Materials and Methods

Young-of-the-year (YOY) of triplefins *Helcogrammoides chilensis*, *Helcogrammoides cunninghami* (Tripterygiidae), labrisomids *Auchenionchus microcirrhis*, *Auchenionchus crinitus* (Labrisomidae), blenny *Hypsoblennius sordidus* (Blenniidae), clingfishes *Gobiesox marmoratus*, *Syngnathus sanguineus* (Gobiesocidae) and clinid *Myxodes viridis* (Clinidae) were collected from rocky intertidal pools at 3 localities separated by approximately 10 km of sandy beaches along the central Chilean coast: Las Cruces (LC: 33° 30' S; 71° 37' W), El Tabo (ET: 33° 31' S, 71° 40' W), and Isla Negra (IN: 33° 24' S, 71° 43' W). YOY were captured with hand nets aided by the use of 20% benzocaine (BZ-20) from April 2010 to December 2011. Captured YOY were placed in labelled plastic bags and transported to the laboratory, where they were measured to the nearest mm (total length=TL). The collected specimens (Table 1) were transferred to the lab in Estación Costera de Investigaciones Marinas (ECIM). To acclimatize the fishes, they were kept in several 0.015 m³ aquaria for 7 days under natural

light photoperiod, with circulating water, constantly aerated, and fed *ad libitum* with TetraMarine Saltwater Flakes® food nutritional supplement on a daily basis.

The Alizarin red S, was dissolved in 2 tanks (0.015 m³) with seawater at concentrations of 150 mg l⁻¹. The tanks remained heavily aerated to maintain the pH near 7. Individuals were kept in these conditions for 24 h. Then, individuals were placed in small aquaria (5 ind. aquarium⁻¹), with circulating water, constantly aerated, and fed *ad libitum* for 6 days. After the day 6 of acclimation, the specimens were subject to fasting one day and the second staining with Alizarin Red-S was then carried out.

The surviving fishes were sacrificed with benzocain BZ-20 and were measured and subsequently fixed in ethanol 96%. They were dissected in the laboratory and their sagitta, asteriscus and lapillus otoliths were kept in an aqueous solution. The sagitta otoliths were mounted on slides with epoxy resin, polished with 30 µm and 1 µm grid paper and observed under an epifluorescence microscope (Nikon Eclipse E4000, blue filter Nikon B-2A). Images were obtained with an Olympus® Camedia C-5050 digital camera. Finally, based on the photograph, the numbers of rings were counted between the Alizarine red-S marks with the aid of the Image Pro-Express Software, version 4.5, 2002 (Media Cybernetics)(Figure 1). The number of rings observed was compared with the expected number of rings (6) and analyzed with the chi square test (Zar 1999).

Results and Discussion

Survival rates to the Alizarin red-S treatment was high, being at least 85% for 6 of the 8 species under study. The lowest survival rates were recorded for *M. viridis* (45%) and *S. sanguineus* (15%; Table 1). A fluorescent mark was clearly visible under UV light in the sagittae of all fishes. Daily increments following the fluorescent mark (VZ) were clearly

discernible in all of the sagittae examined (Figure 2). There was agreement between the expected and observed numbers of daily increments between the alizarin red-S marks for all species (Table 1).

The present research confirmed that primary increments in young-of the year of 8 intertidal fishes studied were deposited on a daily basis. The results are consistent with what is known to date. The daily periodicity of otolith increments has been confirmed in the larvae and juveniles of many teleost fishes (Oxenford *et al.* 1994, Kokita & Omori 1998, Hernaman *et al.* 2000, Joh *et al.* 2005, Parkinson *et al.* 2012) including some intertidal species (Hernaman *et al.* 2000). Besides, results obtained in this paper also agrees with recent work in which daily increments were validated in 4 species of New Zealand intertidal triplefins using injected tetracycline (Kohn & Clements 2011). In the current research, Alizarin red-S appeared as clear fluorescent marks in almost all of the YOY analyzed, which agrees with recent studies demonstrating Alizarin red-S to be a very effective chemical marker for validating growth bands in larval, juvenile and adult teleost fishes, with a similar performance to alizarin complexone, calcine and other tetracycline-derived markers (Bashey 2004, Meisfjord *et al.* 2006, Crook *et al.* 2007, Liu *et al.* 2009, Fitzpatrick *et al.* 2010).

Finally, this work provides a simple technique to validate the increment patterns of otoliths in pre-recruits individuals of the most common species inhabiting the rocky intertidal cost of central Chile. Moreover, our research provides information necessary to validate results founded in previous research and for future studies of pre-recruitment biology of the main components of intertidal fish assemblage in central Chile.

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Table 1. Survival resulting from the experiment (% sur.), number of analyzed sagittae (n), total length average in millimetres (TL), number of rings/sagitta (r) and chi-square statistical results (χ^2), degrees of freedom (df), probability (p) of the 8 species evaluated.

Tabla 1. Porcentaje de sobrevivencia resultante del experimento (% sur.), número de sagitta analizados (n), longitud total promedio de los individuos en milímetros (TL), número de anillos/sagitta (r) y resultados estadísticos chi-cuadrado (χ^2), grados de libertad (df), probabilidad (p), de las ocho especies evaluadas.

Family/ Species	% sob.	n	LT (mm.)	r	χ^2	g.l.	p
Labrisomidae							
<i>A. crinitus</i>	81.81	9	75.11 ± 17.17	5.22 ± 0.44	1.17	8	0.997
<i>A. microcirrhis</i>	81.81	9	135.39 ± 36.33	5.22 ± 0.44	1.17	8	0.997
Tripterygiidae							
<i>H. chilensis</i>	88.63	15	37.60 ± 12.40	5.20 ± 0.68	2.67	14	0.999
<i>H. cunninghami</i>	83.33	5	32.17 ± 2.96	6.00 ± 0.0	0.21	4	0.977
Blenniidae							
<i>H. sordidus</i>	87.18	15	38.50 ± 5.95	3.87 ± 0.83	13.00	18	0.527
Clinidae							
<i>M. viridis</i>	45.45	4	91.75 ± 2.30	4.75 ± 0.50	1.17	3	0.761
Gobiesocidae							
<i>G. marmoratus</i>	87.50	7	40.91 ± 9.47	4.71 ± 0.49	2.17	6	0.904
<i>S. sanguineus</i>	14.29	5	24 ± 5.21	4.60 ± 0.89	2.17	4	0.705

Figure 1: Photos of sagittae used to validate the daily periodicity of otolith increments in *H. chilensis*. A, B fluorescence microscope, C light microscope, D Scheme used for counting rings.

Figura 1: Secuencia de lectura de un otolito sagitta de *H. chilensis*. A y B Imágenes en microscopio de fluorescencia, C Imagen en microscopio óptico D Esquema utilizado para el recuento de anillos.

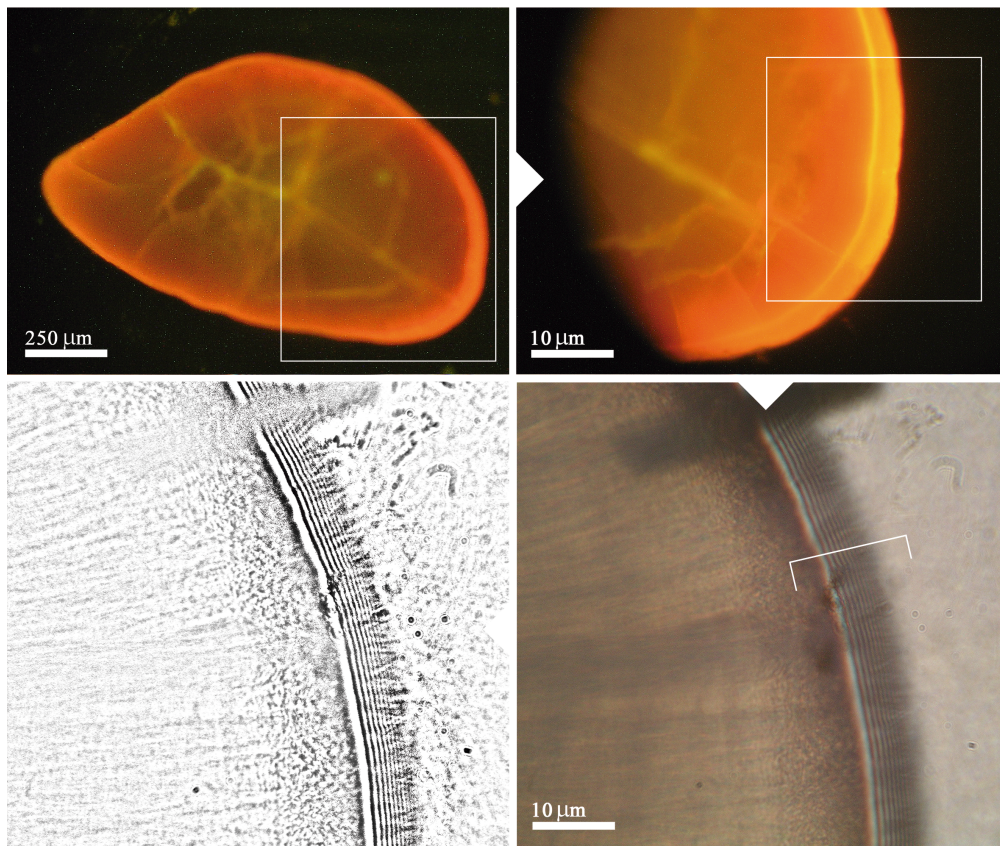
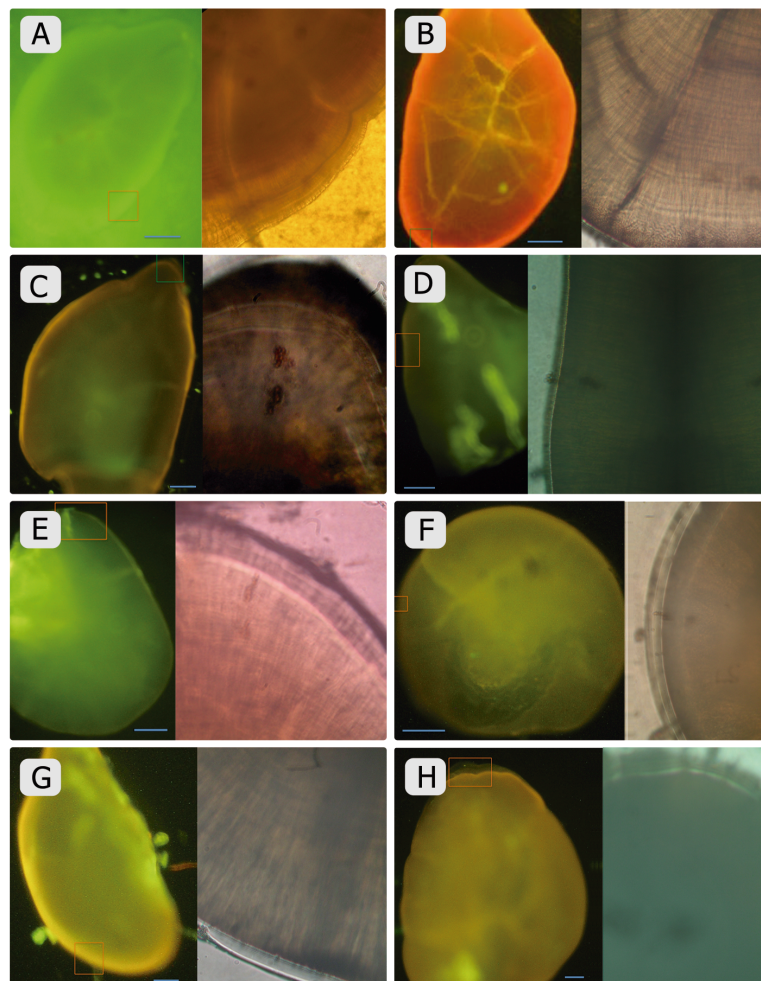


Figure 2: Photographies of sagittae used to validate the daily periodicity of otolith increments, fluorescence microscope (left) and light microscope (right). A: *Helcogrammoides cunninghami*, B: *H. chilensis*, C: *Auchenionchus crinitus*, D: *A. microcirrhis*, E: *Gobiesox marmoratus*, F: *Sicyases sanguineus*, G: *Myxodes viridis*, H: *Hypsoblennius sordidus*. Scale bar: 250 μm .

Figura 2: Fotografías de los otolitos sagitta utilizado para la validación de la periodicidad de los incrementos, utilizando microscopio de fluorescencia (izquierda) y microscopio óptico (derecha). A: *Helcogrammoides cunninghami*, B: *H. chilensis*, C: *Auchenionchus crinitus*, D: *A. microcirrhis*, E: *Gobiesox marmoratus*, F: *Sicyases sanguineus*, G: *Myxodes viridis*, H: *Hypsoblennius sordidus*. Escala 250 μm .



Capítulo II

III. Planktonic duration in fourteen species of intertidal rocky fishes in the south-eastern Pacific Ocean

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Marine and Freshwater Research (submitted)

**Planktonic duration in fourteen species of intertidal rocky fishes from
the south-eastern Pacific Ocean**

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Abstract

The planktonic duration (PD) was determined in fourteen intertidal rocky fishes from the south-eastern Pacific Ocean by counting the otolith micro-increments from hatch check to a transition mark (TM). TMs were validated by comparing the PDs estimated from post-settlers with the total increment counts from otoliths of more recent (new) settlers. In 11 species (79%), the most common TM corresponded to a notable decrease in increment width after settlement. The PDs ranged from 45 days in the kyphosid *Girella laevis* to 135 days in the labrisomid *Auchenionchus variolosus*, and 8 species (57%) had mean PDs of longer than 3 months. The mean observed size-at-settlement (SAS_M) values ranged from 17.5 ± 7.2 mm TL in *Gobiesox marmoratus* (Gobiesocidae) to 40 ± 5.5 mm TL in *Calliclinus geniguttatus* (Labrisomidae) and showed low variability within species of the same family. PDs were markedly longer in intertidal rocky fishes than in other littoral and reef fishes in tropical and temperate waters. A lengthy and variable duration of the pre-settlement phase, along with a relatively consistent size at competence within closely related species, are suggested as mechanisms through which these fish may increase their probability of survival in the spatially and temporally restricted habitat of exposed rocky pools.

Additional keywords: otolith increment, planktonic duration, settlement, intertidal fishes

Introduction

Most demersal fishes have a pelagic larval phase that, in combination with dispersal and settlement processes, can influence their biogeographic ranges and recruitment patterns as well as the connectivity among populations (Victor 1986; Cowen and Sponaugle 1997; Hickford and Schiel 2003; Geffen *et al.* 2011; Kingsford *et al.* 2011; Kohn and Clements 2011). The information gathered to date has demonstrated a significant variability in larval duration in these fishes, ranging from a few days to several months within and among species (Bay *et al.* 2006; Kingsford *et al.* 2011; Macpherson and Raventós 2006; Kohn and Clements 2011). Most studies have suggested that the distribution ranges of fishes are likely controlled by factors other than the duration of the planktonic phase (Victor and Wellington 2000; Macpherson and Raventós 2006; Kingsford *et al.* 2011). In other studies, a fixed larval duration has been associated with synchrony between the timing of spawning and subsequent settlement (Robertson *et al.* 1990), whereas a variable larval duration appears to be associated with specific environmental conditions (e.g., lunar or tidal periods) to maximise settlement at a competent size (Cowen and Sponaugle 1997). On the other hand, a positive correlation between larval duration and growth rate has been reported in a number of studies (Shima and Findlay 2002). Thus, the extent to which PD is either ecologically based or mediated by the pivotal influence of growth rate, which is itself associated with the concomitant effects of variations in temperature and productivity, is unclear (Victor 1986a).

Studies of the pelagic larval duration of demersal fishes have been carried out mainly in tropical areas and temperate waters in the Northern Hemisphere using settlement marks recorded in their otoliths, which are associated with the change from pelagic to benthic life (Victor 1982; Victor 1986a; Victor 1986b; Kingsford and Milicich 1987; Wellington and Victor 1989; Sponaugle and Cowen 1994; Wilson and McCormick 1997; Jenkins *et al.* 1996;

Raventós and Macpherson 2001; Soeparno *et al.* 2012). This method, developed by Victor (1982), allows PD to be determined by counting the number of primary increments from hatching to the settlement mark. PD data collected using this methodology in the Southern Hemisphere are more scarce (but see Kingsford and Milicich 1987; Kingsford 1990; Jenkins and Black 1994; Hamer and Jenkins 1996; Buratti and Santos 2010; Kohn and Clements 2011). The lack of studies is particularly evident in intertidal fishes that after a pelagic life recruit to intertidal areas to use rocky pools either as a transitory habitat during their juvenile phase or as a permanent residence over their entire life. New recruits must cope with many biotic and abiotic factors due to the temporal and spatial limitations of rocky pools (Gibson 1982; Metaxas and Scheibling 1993; Pulgar *et al.* 2006). Hence, it is reasonable to hypothesise that such a dramatic change in environment would be recorded as transition marks in their otoliths, similar to those reported to characterise the settlement process from pelagic to subtidal benthic life in other reef fishes; consequently, it should be possible to determine the duration of the pre-settlement phase before intertidal fishes are recruited into rocky pools. These groups of fishes are good models with which to provide a wider framework of PD for comparative purposes because they encompass a variety of phylogenetically related families of fishes that are distributed worldwide. Therefore, this study aimed to determine the durations of the pre-settlement phases of fourteen species of intertidal rocky fishes in the south-eastern Pacific Ocean using the transition marks recorded in their otoliths.

Materials and methods

Study area and sampling methods

The juvenile fish used in this study were collected from rocky intertidal pools at 3 localities separated by approximately 10 km of sandy beaches along the central Chilean coast: Las Cruces (LC: 33° 30' S; 71° 37' W), El Tabo (ET: 33° 31' S, 71° 40' W), and Isla Negra (IN: 33° 24' S, 71° 43' W). The oceanography and coastal hydrography of the study areas have primarily been described based on the area between IN and LC (Hernández-Miranda *et al.* 2003; Aiken *et al.* 2007). Juveniles were captured with hand nets, aided by the use of 20% benzocaine (BZ-20), on a monthly basis from April 2010 to December 2011. The captured fish were placed in labelled plastic bags and transported to the laboratory, where they were measured to the nearest 0.1 mm in total length (TL). The collected specimens were fixed and stored in 95% ethanol until they were manipulated in the laboratory. Overall, 1250 juveniles ranging from 13 to 175 mm TL and belonging to fourteen species were collected during the study period.

Otolith extraction and preparation procedures

The left and right sagittae and lapilli from all species were removed from preserved juveniles under a dissecting microscope. Otoliths were stored in polyethylene microvials, mounted in epoxy resin on slide glasses and then polished with 800- to 2000-grit lapping films and 4000-grit grinding paper until the nucleus became clearly visible. Once extracted, the left and right lapilli were immediately fixed on a small drop of transparent fingernail varnish and then smoothly polished on only one side for approximately 30 s with a fine lapping film (400 grit; 3 µm). Counts of the daily increments were made across an area of distinctive primary increments from the first check surrounding the primordium along the otolith edge. All

counting and measurements of primary micro-increments were carried out using an image analysis system composed of a light microscope, a CCD camera and measurement software (Leica Application Suite; LAS EZ ver 1.8.0) at magnifications of 400-1000 $\times$. The primary increments in both the sagittae and lapilli were distinctive in most species, but in clinids and gobids, the increments in the lapilli become very narrow. Hence, measurements from the sagittae were used throughout this study to ensure consistency among species. The daily periodicity of primary increments has been only validated for *Scartichthys viridis* (Hernández-Miranda *et al.* 2009). For the remaining species, the periodicity of the formation of primary increments was assumed to be daily based on previous studies of other intertidal fishes (Soeparno *et al.* 2012; Kohn and Clements 2011). No significant differences in increment number between the left and right sagittae were found in six species for which more data were available (*Auchenionchus microcirrhys*, *Girella laevisfrons*, *Helcogrammoides chilensis*, *S. viridis*, *Gobiesox marmoratus* and *Sicyases sanguineus*; Table 1), and this hypothesis was tested. Thus, this condition was assumed to apply to all of the species analysed. The mean counts from three replicates, taken from high-resolution otolith sections made by the same observer, were used for age determination; when counts differed by more than 5%, the sample was rejected. A total of 908 otolith samples satisfied our criteria and were used for further otolith analyses (Table 1).

Planktonic duration (PD) was defined as the number of days between the first well-defined increment near the primordium to a transition mark recorded in the sagittal otoliths of each species, which is linked to the recruitment of juveniles into intertidal rocky pools. The term "planktonic duration" was used instead of "pelagic larval duration PLD" because the juveniles of these species settle into intertidal pools as already transformed juveniles. In

addition, it is unclear whether these fishes are exclusively pelagic during their entire pre-settlement phase or whether they have a temporal subtidal permanence before settling the intertidal pools. To determine whether the transition zone was linked to the settlement of juveniles into intertidal pools, the otolith microstructure features of recently settled fish were characterised to identify the presence and/or absence of settlement marks. Because fish that have recently settled into intertidal pools are expected to be already transformed juveniles, they were identified using a quantitative approach rather than the description of their morphological characteristics. The juveniles collected were considered recently settled fish if they were shorter than the observed overall mean size-at-settlement (SAS_M). The SAS_M for each species was determined using a historical database of monthly fish size surveys taken from 1997 to 2012 using catch-and-release procedures described by Hernández-Miranda and Ojeda (2006) as part of a protocol to monitor growth in the intertidal pools in the study area. These data were used to calculate SAS_M as follows:

$$SAS_M = \frac{1}{n} \sum_{i=1}^n a_i$$

where "a" corresponds to the size of the shortest fish collected in each year and "n" is the number of years from which data are available. To validate the transition mark, the increment number (*i.e.*, total age) of recently settled fish was related to the duration of the pre-settlement phase of older fish, calculated based on the transition marks in the otoliths. A significant linear regression of the form $Y = a + bX$ with slope ($b=1$) was used as proof that otolith-based determinations of the pre-settlement phase using transition marks are reliable. The PDs of the fourteen species were compared using a one-way ANOVA along with a post-hoc Tukey's test after confirming the assumptions of normality and homogeneity of variance.

Results and discussion

Transition mark types in otoliths

One characteristic feature of the sagittal otoliths of fishes collected in intertidal pools, irrespective of species and families, is the existence of a marked shift from opaque to hyaline deposition when otoliths are viewed under induced light using a stereomicroscope. This macro-structural feature was either absent or present very close to the edges of the otoliths in recently settled fish of the 9 species for which recently settled juveniles were available (Table 1). At the micro-structural level, the shift from opaque to hyaline deposition coincided with the presence of transition marks (TM). The most common TM was either a prominent concentric check or a very wide perturbation followed by a marked decrease in increment width. This transition mark type occurred in 11 (79%) of the 14 species, irrespective of fish size. Type III transition marks were restricted to bleniids and tripterygids (27%) and were characterised by a gradual decrease in increment width. For the kyphosids *G. laevisfrons* and *G. nigra*, the shift from opaque to hyaline deposition was more irregular due to the occurrence of new growth centres (accessory primordia; AP) from which wider primary increments were initiated. Because no recently settled fish of this species were available for analysis, the position of the innermost AP was used to determine the duration of the pre-settlement phase.

Planktonic duration

The relationship between the mean total age of recently settled fish (independent variable) and the mean duration of the pre-settlement phase (dependent variable) across species was significantly linear ($Y = a + bX$; $a = 1.03 \pm 0.059$; $b = -7.26 \pm 5.84$; $r^2 = 0.97$; $n = 9$ species; $F_{(1,7)} < 0.001$), showing an equivalence between estimations of the pre-settlement phase made by ageing recently settled fish and using the otolith transition marks (t-test; $t_{\text{cal}} = 0.40 < t_{\alpha} = 0.05$; d.f. = 7 = 3.59). The length of the pre-settlement phase calculated based on otolith transition marks was highly variable across species, ranging from 55 days in *G. laevisfrons* to 135 days in *Auchenionchus variolosus* (VC 19-31%; Table 1). The highest and lowest mean values were restricted to labrisomids (102.6 ± 14.2 days) and kyphosids (67.0 ± 2.3 days), respectively. A one-way ANOVA carried out in 10 species (with sufficiently large sampling sizes) showed significant differences in the mean duration of the pre-settlement phase, although members of the same family tended to exhibit similar mean values (Tukey t-test, Table 1). SAS_M ranged from 17.5 mm TL in *Gobiesox marmoratus* to 40 mm TL in *Calliclinus geniguttatus*. There was no clear pattern in SAS_M across families, although species with larger mean SAS_M values (independent variable) tended to have longer mean pre-settlement phases (dependent variable) ($Y = a + bX$; $a = 32.21 \pm 12.49$; $b = 1.89 \pm 0.43$; $r^2 = 0.61$; $n = 14$ species; $F_{(1,12)} < 0.001$).

Settlement mark types

One distinctive finding that emerged from the current study is that the presence of a very distinctive transition mark in both the macro and micro-structure of otoliths is linked to fish settlement in intertidal pools, irrespective of otolith type or species. A sharp decrease in

increment width after a transition mark in otoliths was the most common TM pattern found across species. This pattern was similar to the Type I (a & b) settlement marks described by Wilson and McCormick (1999) in tropical reef fishes and also similar to findings from other studies that aimed to determine planktonic larval duration in intertidal and tidal habitats in temperate waters (Raventós and Macpherson 2001; Wilson and McCormick 1999; Ahrenholz *et al.* 2010; Beldade *et al.* 2007; Kohn and Clements 2011). The reduction in increment width after fish arrival in intertidal rocky pools is expected because these ontogenetic movements involve a drastic change in lifestyle, during which fish must cope with changing environmental conditions and the limited, enclosed nature of intertidal pools (Gibson 1982; Jordaan *et al.* 2011). Moreover, the transition mark coincided with a change from opaque to hyaline deposition irrespective of otolith type and species. Because this feature can be easily measured using a stereomicroscope, size-at-settlement can be back-calculated without polishing the otoliths. This finding could be very useful for further studies related to the characterisation of size-at-settlement in these species on larger spatial and temporal scales. For the kyphosids *G. laevis* and *G. nigra*, the opaque-hyaline transition was somewhat more variable due to the occurrence of accessory primordia, and this feature was used to determine the duration of the PD. Similar phenomena have been reported in other demersal fishes, in which similar structures were observed (Toole *et al.* 1993; Modin *et al.* 1996; Gunnarsson *et al.* 2010).

Planktonic duration size and size-at-settlement

In the present study, the PD was highly variable across species, ranging from 50 to 135 days, with overall mean values close to 3 months. The observed high variability in PD is

consistent with previous works on coastal fishes from tropical and temperate waters, which have also reported high variability in the duration of the pelagic phase using similar methodologies. However, the PDs obtained in the present study sometimes extended far beyond the upper limit of the pelagic larval duration (PLD), which were previously reported as between 5-70 days for reef fishes in tropical waters (Victor 1986a; Wellington and Victor 1989). In a later study, Raventós and Macpherson (1991) reported PLDs for 42 species of Mediterranean littoral fishes, showing a high variability within species and families and ranges from 9 to 77 days, while blenniids and tripterygids had mean PLDs < 20 days. Similarly short PLDs (< 30 days) have also been reported in other triplefin fishes (Longenecker and Langston 2005; Riginos and Victor 2001).

Some recent studies have also reported relatively short PLDs for species of tropical gobiids (15.25 ± 2.5 d; Bay *et al.* 2006) and a temperate pomacentrid (15-17 days, Kingsford *et al.* 2011). Additionally, Beldade *et al.* (2006) reported PLDs ranging from 11 to 39 days for 10 species of cryptobenthic fishes (Gobiidae, Gobiesocidae and Blenniidae). A more recent study of 35 species of tropical fishes in Japanese waters also reported relatively short PLDs, ranging from 13 to 36 days (Soeparno *et al.* 2012). Although the PD *per se* can vary within individual and species, there is sufficient evidence that the PDs of the intertidal rocky fishes in the current study are longer than those of their counterparts in tropical and warmer temperate waters. A comparison of our results with data from the Southern Hemisphere demonstrates that our recorded PDs are similar to those from a previous study on triplefin fishes from cold temperate waters in New Zealand, where PDs of more than 2 months were reported for the juveniles of some species collected in intertidal pools (Kohn and Clements 2011). Further evidence supporting the long planktonic durations found in the present study may be found in

two recent studies on early life history traits determined using otolith microstructure analysis for the planktonic larvae of two clingfishes (*G. marmoratus*, *Sicyases sanguineus*; Contreras *et al.* 2013) and the triplefin *Helcogrammoides chilensis* (Palacios-Fuentes *et al.* 2012) in the same geographic location off the coast of central Chile. The oldest and largest larvae aged for both clingfish and the triplefin fish were ~1 month at ~7 mm TL and ~57 days at ~16 mm TL, respectively. These size values are markedly shorter than the mean sizes-at-settlement observed in the current study (18-20 mm TL), suggesting that these species can indeed remain in pelagic or subtidal waters for longer periods before settling into intertidal rocky pools as early juveniles.

The long PDs of intertidal fishes reported here could be linked to the oceanographic conditions associated to the Humboldt Current System (HCS). In the HCS, Ekman transport is an intrinsic driving force for the dispersal of eggs and larval fishes at speeds of approximately 0.2 m s^{-1} in central Chile (Strub *et al.* 1998; Cowen *et al.* 2000). Under the influence of such a mechanism, it is reasonable to expect that larvae and/or early juveniles require a long time to reach intertidal areas, even with active swimming. Furthermore, the planktonic duration of any larva will be a product of the conditions encountered by the larvae in the plankton, such as food and temperature (McCormick and Molony 1992; Meekan *et al.* 2003; Bay *et al.* 2006), and temperature alone is considered a good predictor of PD (Sponaugle 2010). In this scenario, the early life stages of these species are subjected to the effects of both dispersal and low temperatures associated with the cold-water HCS, which would be consistent with the long PDs observed in the present study. However, most of the intertidal fishes analysed are benthic or demersal spawners (Herrera 1984; Table 1) with a marked inshore distribution of

their larval stages < 500 m from shore (Hernández-Miranda *et al.* 2003), which would favour settlement at relatively short PDs and small size-at-settlement. Given these factors, the mechanisms underlying the long PD observed in this study remain unclear.

An extended planktonic period would also suggest that the YOYs of these species delayed settlement, perhaps to increase the chance of finding suitable conditions (e.g., onshore tidal flow) within rocky pools, as has been suggested for other reef fishes (Victor 1986b, Victor 1986c, Sponaugle and Cowen 1994, Jenkins and May 1994; McCormick 1999). For example, Cowen (1991) proposed that a long competence period would occur when settlement sites are very restricted, as is the case of rocky pools in the exposed Chilean coast. A similar scenario was proposed by Jenkins *et al.* (1994) to explain the PDs between 100 and 150 days for the King George whiting *Sillaginoides punctata*, which settles very restricted seagrass sites in a coastal area in southern Australia. A restricted settlement site would not only impose limitations on finding a suitable onshore circulation event but also trigger intra and site-specific competence after settlement in species that must share a temporally and spatially limited habitat. In addition, after settlement, the YOY of these species must cope with drastic changes in salinity and temperature on a daily basis due to the tidal cycle. Hence, it is reasonable to hypothesise that long and flexible PDs, which may be a result of the pivotal influence of hydrographical conditions associated with the HCS, combined with a strategy of reaching a specific size at competency, would enhance the probabilities of successfully arriving and adapting to the conditions of rocky pools. In the current study, the mean sizes of recently settled fish showed little variation within species of the same family, arguing in favour of a specific size for competence to settle in closely related species. This finding is

consistent with previous studies in other demersal fishes, where size at competency was reported when long PDs were observed (Jenkins and May 1994; Plaza-Pastén *et al.* 2003).

As a corollary, this study showed evidence of long but variable PDs along with relatively low variation in size-at-settlement in fourteen species of intertidal fishes in the south-eastern Pacific Ocean. It is unclear whether the existence of a lengthy PD is a generalised process across intertidal fishes associated with settlement in rocky pools and/or similar restricted habitats. This question deserves further research, which will ultimately reveal the ecological mechanisms that intertidal fishes have developed to successfully colonise new habitats and extend their geographic distribution ranges.

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Figure captions

Fig. 1. Transition zones (TM: filled arrows) in the otoliths of intertidal fishes collected in rocky pools in Central Chile. Illustration of the presence (**a**) and absence (**a₁**) of TMs (Type) in sagittae from a 95.8 and 22.3 cm TL *Auchenionchus microcirrhys*, respectively, viewed under a stereomicroscope. A magnification of micro structural feature of TM (**b**) Microstructure view (**c & c1**) and increment width profile (**d**) of TMs in a lapillus from a 39.5 cm TL *Helcogrammoides cunninghami*. Accessory primordia (**e**: enclosed circles) in a juvenile *Graus nigra*. Absence of TM in a recently settled juvenile *Sicyases sanguineus* (**f**: 18.5 cm TL). **Pr**: primordium; **Pd**: planktonic duration; **Ds**: discontinuities.

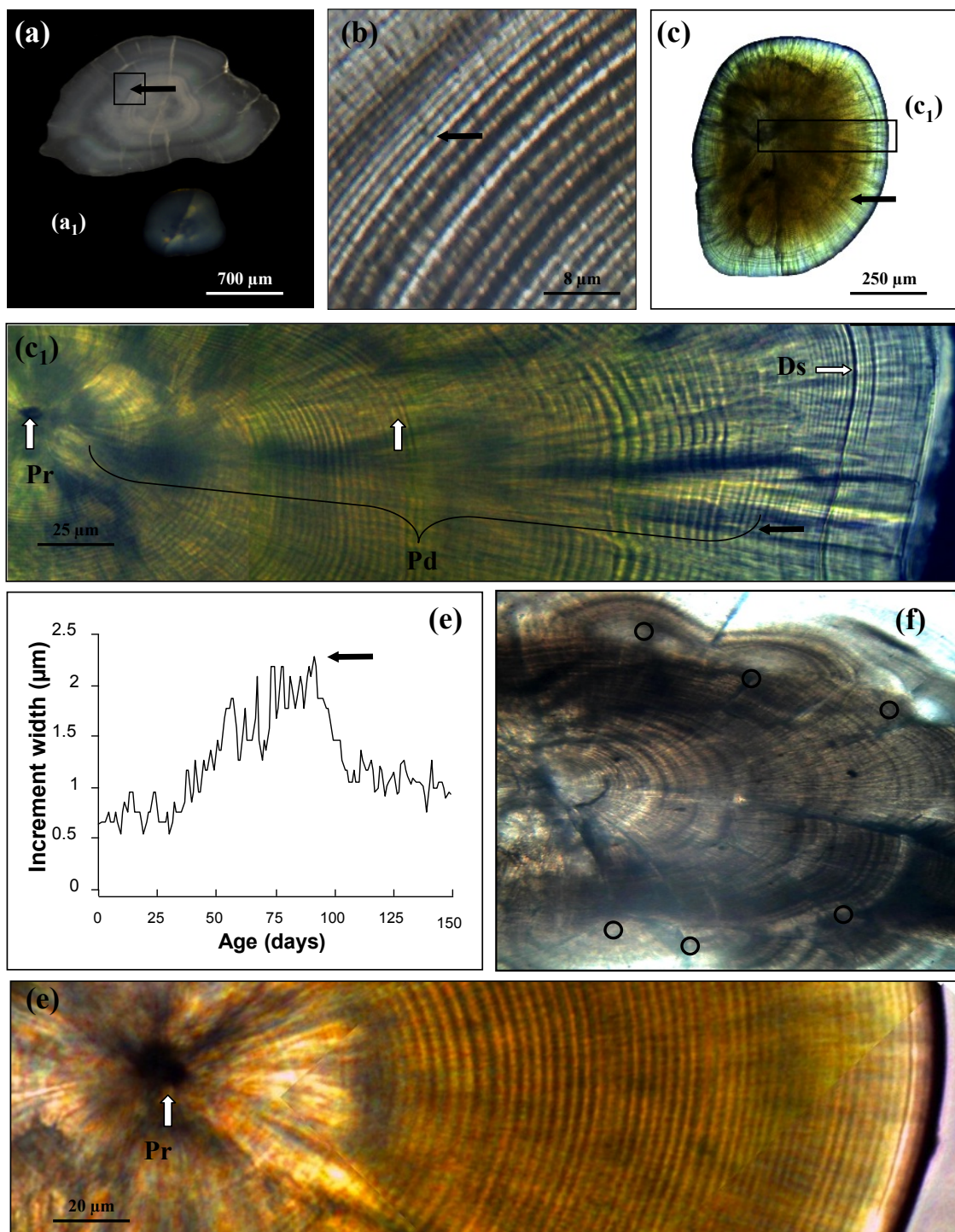


Table 1. Overall statistics (mean, range and variation coefficient, **VC**) of total length, planktonic duration (**PD**) and observed size-at-settlement (**mSAS**) of fourteen species of intertidal fishes collected in Central Chile from April 2010 to December 2011. **n**: number of otoliths analysed; **Years**: the number of monitoring years from which mSAS was obtained; **Tn**: total number of fish collected and released across years as part of the monthly monitoring procedures. **Tk**: denotes Tukey's post-hoc tests after rejecting the null hypothesis of the absence of significant differences in mean PD among species. Different letters denote significant differences at $\alpha=0.05$; **SpS**. Spawning strategy.

	Total length (mm) of all fish collected in rock pools			Planktonic duration (days) based on transition marks					Planktonic duration (days) based on total age of new settlers				Transition marks in otoliths		Observed size-at-settlement (mm) based on an historical database					
Family/ Species	Mean	Range	Vc	Mean	Range	VC	n	Tk	Mean	Range	VC	n	Tm	Ot	Mean	Range	Vc	Years	Tn	SpS
Labrisomidae																				
<i>Auchenionchus crinitus</i>	50.5	25 - 117	52	73.0	56 - 92	19	13		75.3	65 - 83	12	3	I	S	26.5	26 - 28	5.3	2	31	Benthic
<i>Auchenionchus microcirrh</i>	46.4	22 - 165	73	71.5	57 - 89	21	80	a	73.5	52 - 89	20	7	I	S	28.2	21 - 45	19	16	4,311	Benthic
<i>Auchenionchus variolosus</i>	86.1	40 - 120	34	110.6	82 - 138	26	6		112.2			1	I	S	39.5	30 - 49	21	4	229	Benthic
<i>Calliclinus geniguttatus</i>	48.7	34 - 87	41	107.5	83 - 135	21	7		110.6	92 - 120	10	4	II	S	40.0	30 - 49	14	3	222	Benthic
Bovichtidae																				
<i>Bovichtichys chilensis</i>	61.8	49 - 121	20	88.0	60 - 95	13	56		-				I	S	34.5	20 - 48	26	15	3,199	Pelagic
Kyphosidae																				
<i>Girella laevisfrons</i>	82.3	27 - 141	40	68.6	55 - 77	15	70	b	-				AP	S	24.2	15 - 32	26	13	8,095	Pelagic
<i>Graus nigra</i>	98.9	60 - 151	26	65.4	58 - 76	13	26	b	-				AP	S	25.8	15 - 39	28	13	5,257	Pelagic
Tripterygiidae																				
<i>Helcogrammoides chilensis</i>	35.0	25 - 73	25	95.2	78 - 115	25	190	a	96.3	80 - 113	11	10	I	S	25.5	25 - 3	13	16	15,858	Benthic
<i>Helcogrammoides cunninghami</i>	35.0	29 - 50	17	75.3	59 - 95	15	27	c	-				I	L	25.4	2 - 3	12	16	4,194	Benthic
Blenniidae																				
<i>Hypsoblennius sordidus</i>	45.3	28 - 86	31	88.0	76 - 100	19	70	e	92.5	83 - 108	10	6	I	S	28.4	22 - 35	15	16	3,346	Benthic
<i>Scartichthys viridis</i>	83.5	42 - 175	33	97.8	72 - 124	22	222	a	-				I	S	30.2	12 - 45	28	16	39,456	Benthic
Clinidae																				
<i>Myxodes viridis</i>	69.3	29 - 112	43	95.0	69 - 118	24	13		98.2	92 - 105	6	4	I	S	29.5	27 - 32	7.8	4	28	Benthic
Gobiesocidae																				
<i>Gobiesox marmoratus</i>	43.7	13 - 102	44	72.5	61 - 98	29	105	c	74.5	65 - 88	10	8	I	S	17.5	12 - 40	41	16	2,728	Benthic
<i>Sicyases sanguineus</i>	43.7	13 - 102	44	76.6	59 - 100	31	115	c	78.2	66 - 99	12	12	I	S	22.0	11 - 35	32	11	743	Benthic

Capítulo III

IV. Patterns of lunar Recruitment in Intertidal Fish Species on the Temperate Coast of Central Chile.

Lidia Mansur, Daniela Catalán S. & F. Patricio Ojeda

(En preparación)

Patterns of lunar Recruitment in Intertidal Fish Species on the Temperate Coast of Central Chile

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Introduction

For marine organisms with complex life cycles, identifying the patterns and processes associated with the supply (or delivery) of settlement stage larvae to juvenile habitats is critical to our understanding of how populations are replenished over time. (D'alessandro et al. 2007).

Fish living in coastal areas often spawning and settlement in synchrony with the lunar cycle, either at a new or full moon (Johannes 1978; Robertson et al. 1990, 1999; Courtney et al. 1996; Yamahira 1997; Takemura et al. 2004; Contreras et al 2013). The fact that different marine species present reproductive activities associated with lunar cycles can be explained by various hypothesis, which focus primarily on offspring survival. These studies suggested that the new moon provides suitable conditions for survival because darkness would reduce the predation pressures on spawned eggs, whereas moonlight can be advantageous when adults migrate to spawning sites (Robertson et al. 1990, 1999; Takemura et al. 2004). However, spawning that occurs on a semi-lunar cycle, either in synchrony with a new full-moon period or associated with the quarter-moon phases reported in several studies, is more controversial (Noichi et al. 1994; McIlwain 2002; Sponaugle and Pinkard 2004; Gladstone 2007). Some authors have suggested that spawning can be continuous over the lunar cycle and larvae derived from eggs spawned during either the spring or neap tides would have a better chance of survival (Noichi et al. 1994; Sponaugle and Pinkard 2004), which is presumably linked to an adaptive strategy to enhance offshore dispersal or larval retention, respectively. Furthermore, the semi-lunar spawning patterns could be a result of a strategy to spawn in synchrony with the tidal cycle because neap tides are coupled with quarter moons and spring tides are coupled with full

moons, which has been reported in the settlement patterns of several demersal fishes (Sponaugle and Cowen 1994; Robertson et al. 1999; Plaza-Pasten et al. 2003).

In the past two decades, several studies have addressed the link between hatching patterns and the lunar cycle, by using otolith microstructure analysis (Sponaugle and Cowen 1994; Noichi et al. 1994; Plaza-Pasten et al. 2003; Sponaugle and Pinkard 2004). This technique obtains precise estimations of the hatch date by subtracting the number of daily rings from the capture date. However, most otolith-based studies of hatch patterns have remained restricted to either tropical areas or temperate zones in the northern hemisphere. Conversely, studies on lunar spawning synchrony in the southern hemisphere are scarce and remain non-existent in some areas (Contreras et al. 2013). This is true for the south-eastern Pacific Ocean where several families of intertidal fishes occur (e.g. Blennidae, Labrisomidae, Clinidae, Gobiidae, Gobiesocidae). Members of these families are demersal spawners, a reproductive mode that has been traditionally viewed as a distinct, alternative strategy to maximise larval survival and return to the adult population (Sponaugle and Cowen 1994).

In the rocky intertidal environment of the central coast of Chile, the fish assemblage has two components, "resident" and "temporary" species (Fig. 1). Resident species are those whose life cycle consists of the following stages: pelagic larvae, intertidal juveniles and adults, and egg laying in clusters. The spawns are small and the egg laying sites are generally located in cracks or crevices of the tide pools. There is no information about parental care for Chilean coastal species. The duration of the pelagic larval phase is generally brief and shorter than that of subtidal species (Horn *et al.* 1999). The species selected for this work are the dominant ones of the *Helcogrammoides chilensis* and *Helcogrammoides cunninghami* assemblage. Biology and ecology background information

is found in Pérez-Mongard (1979), Muñoz and Ojeda (1997, 1998), Williams and Springer (2001) and Hernández-Miranda *et al.* (2003). Temporary species are those whose life cycle consists of the following stages: intertidal juveniles, subtidal adults, eggs and pelagic larvae. They have large spawns and eggs are released into the water column. They do not usually show clear homing behavior (Varas and Ojeda, 1990) or investment in parental care (Horn *et al.* 1999). The species selected are abundant in the fish assemblages in central Chile. Background information on the biology and ecology of *Scartichthys viridis* and *Girella laevisfrons* is found in Varas and Ojeda (1990), Muñoz and Ojeda (1997, 2000), Ojeda and Muñoz (1999), Cáceres and Ojeda (2000), Fariña *et al.* (2000), Hernández-Miranda *et al.* (2003, 2006, 2009), Pulgar *et al.* (2003, 2006).

For the reasons the main goal of this research was to establish the recruitment patterns of two intertidal species, *Helcogrammoides chilensis* (resident species) and *Girella laevisfrons* (temporary species) in central Chile, on the basis of the analysis of otolith microstructure.

Study area and sampling methods

Young-of-the-year (YOY) of *H. chilensis* and *G. laevisfrons* were collected from rocky intertidal pools at 6 localities along the central Chilean coast: Las Cruces (LC: 33° 30' S; 71° 37' W), El Tabo (ET: 33° 31' S, 71° 40' W), and Isla Negra (IN: 33° 24' S, 71° 43' W), Valparaíso (VA: 33° 05' S, 71° 61' W), Maitencillo (MA: 32° 38' S, 71° 26' W) and Los Molles (LM: 30° 44' S, 70° 35' W) (Figure 1). The oceanography and coastal hydrography have primarily been described for the area between IN and LC (Poulin *et al.* 2002; Hernández- Miranda *et al.* 2003; Aiken *et al.* 2007). YOY were captured with hand nets aided by the use of 20% benzocaine (BZ-20) on a monthly basis from April 2010 to

December 2011. Captured YOY were placed in labelled plastic bags and transported to the laboratory, where they were measured and weighed to the nearest mm (total length=TL) and 0.01 g., respectively. The collected specimens were fixed and stored in 95% ethanol until they were manipulated in the laboratory.

Otolith extraction and preparation procedures

The left and right *sagittae*, *lapilli* and *asterisci* were removed from preserved YOY under a dissecting microscope. *Sagittae* were stored in polyethylene microvials, mounted in epoxy resin on slide glasses and then polished with 800-2000 grit lapping films and 4000-grit grinding paper until the nucleus became clearly visible using the slide-glass embed method (Plaza *et al.* 2005). The otoliths were observed using a compound microscope under transmitted light at 1000X magnification (oil-immersed lens) due to their smaller size, whereas for *sagittae*, a magnification between 200 and 1000X was used depending on fish size. Counts of the daily increments were made across an area of distinctive primary increments from the first check surrounding the primordium along the otolith edge. All measurements of increment width were made through a straight line from the primordium to the posterior edge (postrostrum) of *sagittae* otoliths. All counting and measurements were carried out using an image analysis system composed of a light microscope, a CCD camera and measurement software (Leica Application Suite; LAS EZ ver 1.8.0) at magnifications of 400-1000X. Otolith Morphometry

Validation of transition mark and planktonic duration

A transition mark characterized by a shift from wider to narrower increments was observed in *sagittae* otoliths. This mark was linked to settlement from pelagic life to the

intertidal pools in rock shores. This approach, firstly developed by Victor (1982), has been used in a number of studies to characterize settlement patterns (e.g., Kingsford and Milicich 1987; Wellington and Victor 1989; Wilson and McCormick 1997; Raventós and Macpherson 2001; Ahrenholz *et al.* 2010; Kohn and Clements 2011). To determine if the transition mark was linked to settlement, the transition-centered method described by Wilson and McCormick (1997) was used. Validation of the daily periodicity of increment deposition was performed by *G. laevisfrons*: Landaeta (Pers. comm.) and *H. chilensis*: Mansur *et al.* 2013.

Data analysis

The back-calculated hatching dates were related to the lunar cycle. For each sampling date, the days since the new moon were counted (DNM), and thereby assigned DNM values from 0 to 29 for each date, in which 0 represented the new moon. The DNM values were converted to angles ($^{\circ}$) by dividing by 29 (the length, in days, of the lunar cycle) and then multiplying by 360° , so that the data could be analysed using circular statistics. To assess whether the hatching events showed lunar periodicity, the data were analysed using the Rao's spacing test (Batschelet 1981). The Rao's spacing test is more powerful and robust than many other circular goodness-of-fit-tests and is able to analyse bi- and multimodal distributions, whereas other tests, such as the Rayleigh test and Watson's U_2 , cannot (Bergin 1991). The Rao's spacing test is robust even for small sample sizes but also shows a low frequency of Type I errors when analysing data that display no pattern. We also used the Rayleigh test for a departure from randomness. The null hypothesis that the recruitment events would be equally or randomly spaced throughout the lunar cycle was tested for each dataset. The angular means and 95% confidence intervals were also calculated. Finally, comparisons between the lunar hatching distributions of both species

were conducted using the non-parametric Mardia–Watson–Wheeler test (W) for equal distributions (Mardia 1972).

Results

A total of 43 pairs of *sagitta* of *H. chilensis* and 24 of *G. laevisfrons* were read and measured under the microscope. *H. chilensis* otoliths showed a slightly oval shape, symmetric and laterally compressed. This shape facilitated the preparation in sagittal position, where the micro-increments were distinguishable and transition mark is clearly. Instead *G. laevisfrons* has concave otoliths and due to the poor readability of increments in most specimens.

Two important recruitment pulses were detected by the back-calculated dates of *H. chilensis*, and both occurred near the full moon (Fig. 2) (i.e. semi-lunar periodicity), for *G. laevisfrons*, only one pulse was detected near new moon (Fig. 3) (i.e. lunar periodicity).

The angular mean (95% confidence interval) corresponded to Day 24 (18-27) of the lunar cycle for *H. chilensis*; for *G. laevisfrons*, the angular mean was approximately Day 17 (14–21) of the lunar cycle (Fig. 4). Rayleigh and Rao's spacing tests indicated that the hatching patterns of young-of-the-year of *H. chilensis* and *G. laevisfrons* were not uniform over the lunar cycle (*H. chilensis*: $r = 0.194$, $p = 0.204$, Rao's $U = 171.4$, $p = 0.002$; *G. laevisfrons*: $r = 0.123$, $p = 0.625$, Rao's $U = 127.7$, $p = 0.554$).

Discussion

The lunar cycle provides a strong, predictable set of environmental cues for marine species. Environmental cycles (e.g., tidal water movement, moonlight) entrain endogenous reproductive cycles, synchronizing gamete release within a population and ensuring that movement, feeding and reproduction occur under favourable conditions (Taylor 1984, Omori 1995). Lunar-synchronized spawning, for example, is commonly documented for

species of shallow waters with large tidal fluctuations (Korringa 1947, Taylor 1984). Reef fishes often mass in spawning aggregations on a specific lunar and seasonal cycle (Johannes 1981, Robertson et al. 1990). In addition, lunar cycles have been detected in spawning and settlement of intertidal (Taylor 1984) and pelagic-spawning fish and invertebrates (Crabtree 1995, Robertson et al. 1999).

While the results are preliminary they agree with Plaza-Pasten et al. 2003, lunar and semi-lunar hatching patterns are frequent in fishes with benthic eggs. A recruitment pattern related to the full moon as was detected for *H. chilensis* is associated to spring tides (Robertson et al 1990) and these results agree with those found in most of the Caribbean reef fish (Robertson 1992) and Chilean intertidal fish (Contreras et al. 2012, Palacios et al. 2013). This phenomenon is related to the use of more extensive tidal to recruit higher intertidal pools.

In the case of *G. laevisfrons* having a lunar pattern between the third-quarter and new moon. Synchronization of settlement with the third-quarter moon is a widespread phenomenon among fishes and crustaceans at Barbados (Sponaugle & Cowen 1996). Minimum amplitude tides may be easier to swim against and nights become progressively darker between the third-quarter and new moon, potentially reducing losses to predation (Sponaugle & Cowen 1997, Sponaugle & Pinkard 2004). We believe that because *G. laevisfrons* recruit in schools, darkness is the most important factor as reducing the risk of predation.

Finally, it needs to be increased the number of individuals analysed in order to have strong conclusions and to analyse recruitment patterns in different locations that will identify synchrony of such events.

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Figures

Figure 1: Lifecycle of *Helcogrammoides chilensis* (resident species) b: lifecycle of *Girella laevis* (temporal species)

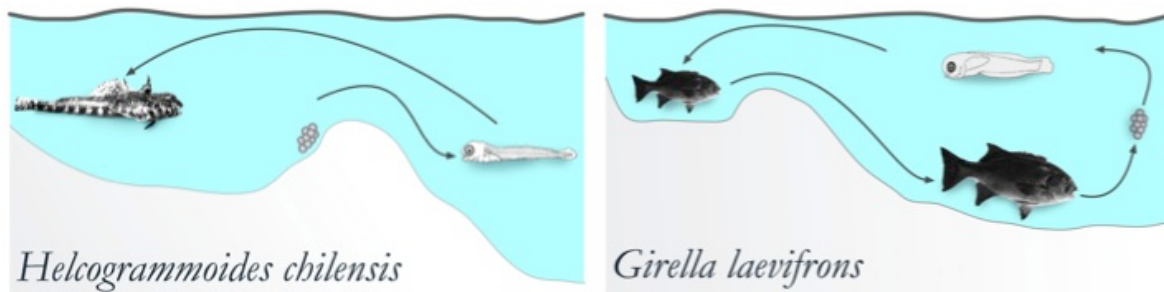


Figure 2: Distribution of recruitment abundances of *H. chilensis* throughout the lunar calendar. Filled circles represent new moon and open circles, full moon.

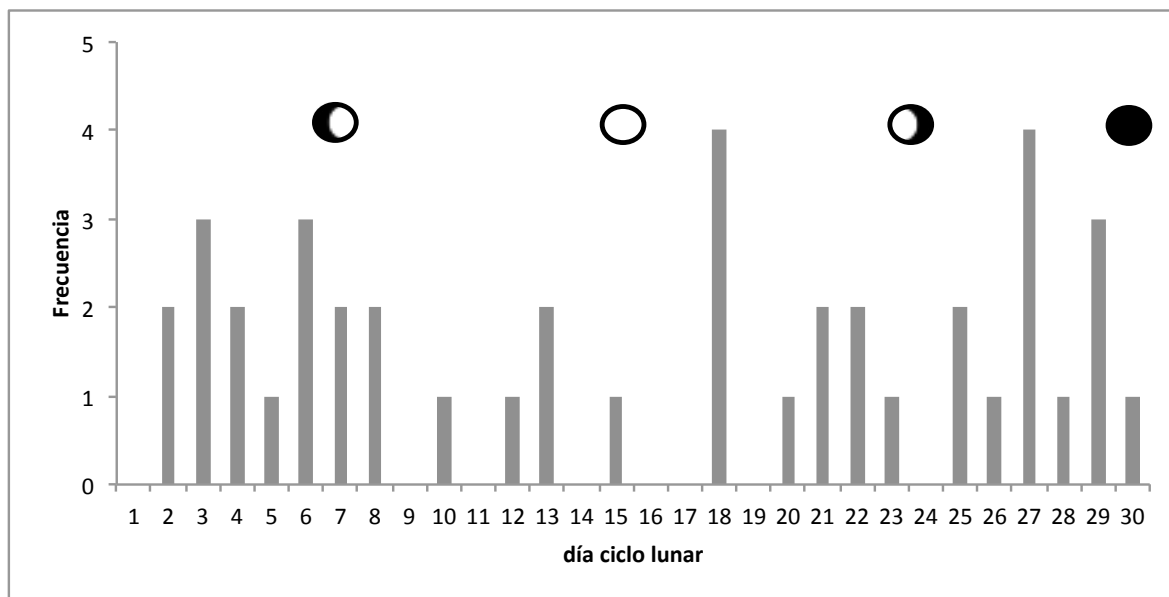


Figure 3: Distribution of recruitment abundances of *G. laevifrons* throughout the lunar calendar. Filled circles represent new moon and open circles, full moon.

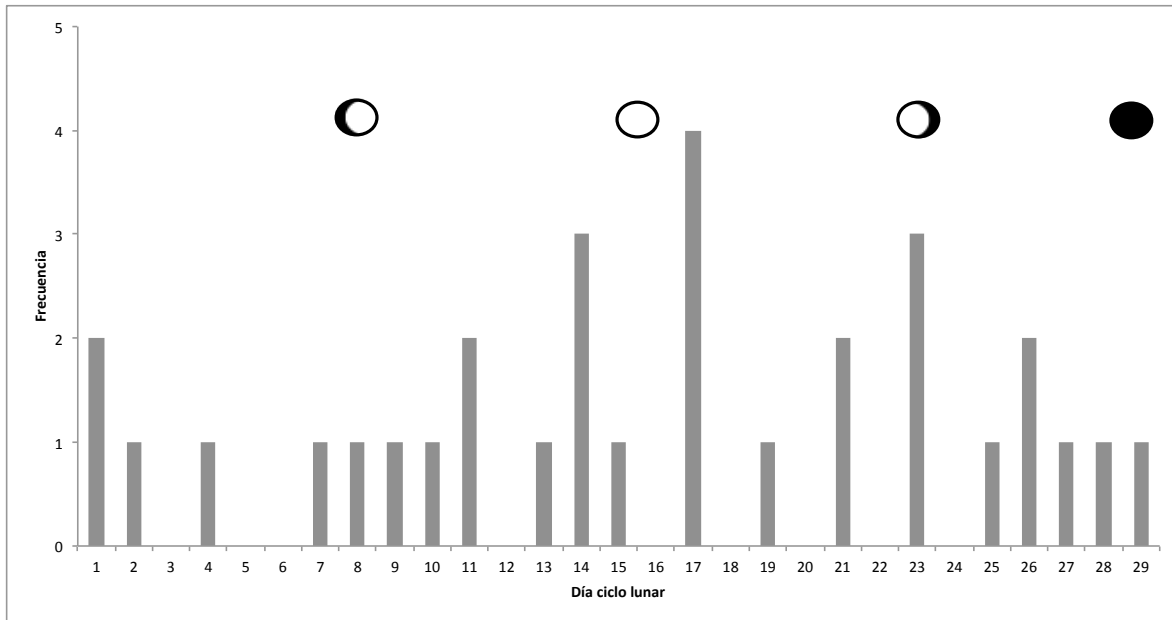
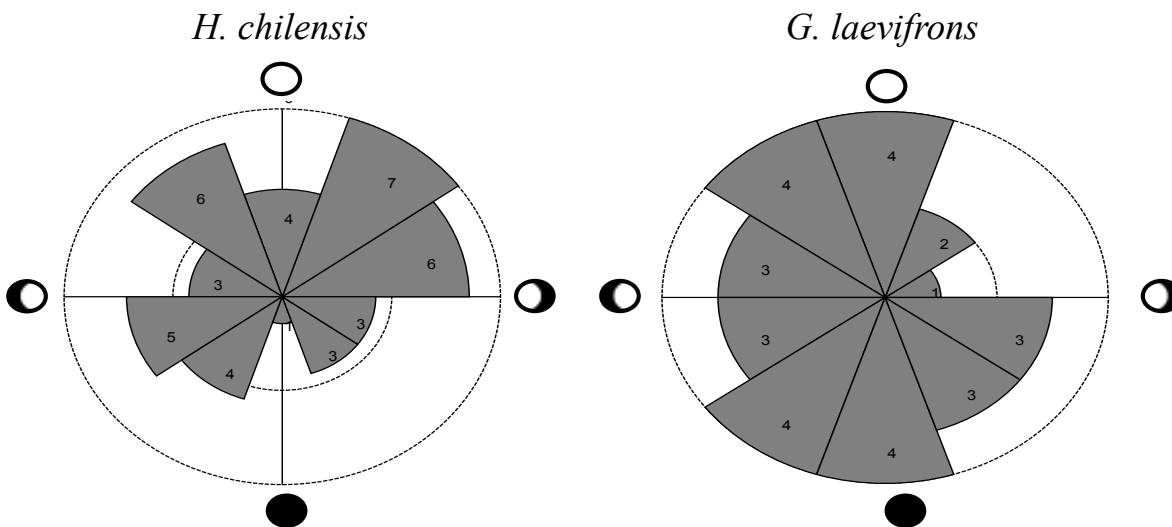


Figure 4: Recruitment patterns of intertidal fishes from central Chile, based on the lunar cycle. Note that *Helcogrammoides chilensis* shows a lunar periodicity and *Girella laevifrons* shows a semi-lunar periodicity.



CAPÍTULO IV

V. Genetic approach to measure connectivity in intertidal fishes with different life cycle in central Chile

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Genetic approach to measure connectivity in intertidal fishes with different life cycle in central Chile

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Abstract

In studies of marine ecology, one of the central themes is connectivity. In fish with complex life cycles, dispersal occurs in pelagic larval stages. The small size of the larvae, their behavior and oceanographic conditions in which they develop, hinder the understanding of the phenomenon. Currently, it is possible to determine the geographic range of coastal fish using genetic techniques. In Chile, studies addressing connectivity or dispersion in fish are scarce. In this paper we analyzed the genetic structure of *Helcogrammoides chilensis* (resident species) and *Girella levifrons* (temporary) in six rocky intertidal of central Chile. We used the technique "Amplified fragment lenght polymorphis" (AFLP), with eight combinations of primers. Polymorphic DNA fragments obtained are compiled in a matrix of binary data and calculated rates of genetic similarity and global FST differentiation between pairs of locations. We assessed whether the overall FST is significantly different from zero and the correlation between population genetic differentiation [$FST / (1-FST)$] and geographic distance using the Mantel test. From the results, we discuss the relevance of the strategy of life in the distance as a determinant of genetic structure in intertidal fish, and the need for these analyses for making management decisions or in marine conservation.

Introduction

Throughout the history of ecology, the study of natural populations has presented the question of how and when a group or set of individuals is to be considered part of the same population (Roughgarden *et al.* 1988). To date, a population has been defined as a group of individuals of the same species living in an area large enough to allow normal dispersal or migration, where population dynamics results from birth and death processes (Berryman 2002). Therefore, properly and unambiguously determining the spatial scale at which processes occur, birth is extremely important in understanding the temporal dynamics of populations. Concepts such as meta-population (Hanski 1998, Thorrold *et al.* 2001), local population, sub-populations, and open or closed populations (Roughgarden *et al.* 1985, Caley *et al.* 1996) have been proposed and used to account for connectivity between groups of individuals within a species. However, the lack of an explicit definition of the spatial scale of the population and the underlying biological criteria has limited a more thorough understanding of populations in marine ecosystems. Moreover, the concept of *self-recruitment* has emerged to explain cases where adults of intertidal environments form larvae produced in the same environment and not in remote areas due to dispersive arrival processes (Kingsford *et al.* 2002). While it is an operational concept that identifies the dependency of relationships between spawning and subsequent recruitment, it is not possible to determine the spatial scale of population demographic performance. Considering the complete information will then reveal the factors that determine the distribution patterns and abundance of populations, as well as population genetic and spatial structure (Cowen *et al.* 2006), which constitute key information when making decisions about management or conservation, such as designing marine protected areas (Agardy 2000, Camus and Lima 2002, Palumbi 2003, Levin 2006).

The application of genetic markers has escalated thanks to improved laboratory protocols for analyzing genetic data. Hedgecock *et al.* (2007) and Hellberg (2007) have provided recent reviews of these techniques, aiming at promising new approaches (e.g., Monte Carlo, maximum likelihood, and Bayesian analyses; Nielsen & Wakeley 2001, Manel *et al.* 2005, Beerli 2006). These researchers also note important limitations of using genetic markers to differentiate population structure at various spatial scales. Two primary analytical approaches that use demographic rates of exchange in marine organisms have

dominated the recent literature: the utilization of the stepping-stone model (Wright 1943, Slatkin 1993) via estimation of isolation-by-distance, and more recently, the use of assignment tests (Manel *et al.* 2005) to more directly link dispersal of youth to known adult populations (Cowen and Spounagle 2009). In studies of connectivity between marine populations, the most frequently used model is isolation by distance (stepping stone), which integrates genetic and geographic information (Kinlan and Gaines 2003, Gaines *et al.* 2007, Hellberg 2007, Lin *et al.* 2009). This approach makes it possible to define gene flow between distant marine populations, from 10 km (quote). For fish, it is documented that genetic dispersion in general can be found anywhere from a few meters to hundreds of kilometers; however, the average would be around 100 kilometers, being closely related to the PLD (planktonic larval duration).

Many molecular genetic techniques have been successfully used to understand the structure of marine fish species (Viñas *et al.* 2004; Kochzius and Blohm 2005; Durand *et al.* 2005; Han *et al.* 2008). One of the most used is Amplified fragment length polymorphism (AFLP) analysis (Vos *et al.* 1995), a multilocus marker technique, is a PCR-based multilocus fingerprinting technique that combines the strengths and overcomes the weaknesses of PCR-RFLP and RAPD (Bensch and Akesson 2005). The major strengths of the AFLP method include simultaneous screening of a large number of polymorphic loci, high reproducibility due to high stringency of PCR, and relative cost effectiveness (Liu and Cordes, 2004). Because of such advantages, the use of AFLP markers has been advocated as a powerful genetic system to assess population structure and individual identity (Gerber *et al.* 2000; Bensch and Akesson 2005) for a wide variety of organisms, including fishes and shrimp (Liu and Cordes 2004; Wang *et al.* 2004; Kakehi *et al.* 2005; Kassam *et al.* 2005; Gwo *et al.* 2008, Lin *et al.* 2009).

In the rocky intertidal environment of the central coast of Chile, the fish assemblage has two components, "resident" and "temporary" species (Fig. 1). Resident species are those whose life cycle consists of the following stages: pelagic larvae, intertidal juveniles and adults, and egg laying in clusters. The spawns are small and the egg laying sites are generally located in cracks or crevices of the tide pools. There is no information about parental care for Chilean coastal species. The duration of the pelagic larval phase is generally brief and shorter than that of subtidal species (Horn *et al.* 1999). The species

selected for this work are the dominant ones of the *Helcogrammoides chilensis* and *Helcogrammoides cunninghami* assemblage. Biology and ecology background information is found in Pérez-Mongard (1979), Muñoz and Ojeda (1997), Muñoz and Ojeda (1998), Williams and Springer (2001) and Hernández-Miranda *et al.* (2003) (Figure 1). Temporary species are those whose life cycle consists of the following stages: intertidal juveniles, subtidal adults, eggs and pelagic larvae. They have large spawns and eggs are released into the water column. They do not usually show clear homing behavior (Varas and Ojeda, 1990) or investment in parental care (Horn *et al.* 1999). The species selected are abundant in the fish assemblages in central Chile. Background information on the biology and ecology of *Scartichthys viridis* and *Girella laevisfrons* is found in Varas and Ojeda (1990), Muñoz and Ojeda (1997, 2000), Ojeda and Muñoz (1999), Cáceres and Ojeda (2000), Fariña *et al.* (2000), Hernández-Miranda *et al.* (2003, 2006, 2009), Pulgar *et al.* (2003, 2006).

For the reasons stated above and under the hypothesis that there is population structure in accordance with the stepping stone model, the objective of this project is to evaluate the genetic structure of *Helcogrammoides chilensis* (resident species) and *Girella laevisfrons* (temporary species) in six locations in central Chile, between 33.5°S – 32.2°S.

Materials and methods

Area of study

Since characteristics of the larvae of the species under study limit the larvae's dispersion to a maximum of 200 km. (Mansur *et al.* 2012), this distance was chosen as the maximum distance, confining the study between Las Cruces (LC), 33°30'S, 71°37'W and Los Molles (LM), 32°13'S, 71°30'W of central Chile. Based on the background literature that shows genetic structure in populations of intertidal fish at distances of 10 km, two scales were selected for study. The first scale includes three locations, Las Cruces (LC), El Tabo (ET) and Isla Negra (IN), which are rocky zones separated by about 10 km of sandy beaches, and a second scale includes three other locations with separations between 30 and 50 km (Valparaiso [VA]- Maitencillo [MA] - Los Molles [LM]) (Fig. 2).

All along this 200 km stretch there are 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, Hernández-Miranda *et al.*, 2009).

Sampling

Ten specimens of each *Helcogrammoides chilensis* species (resident species) and *Girella laevis* species (temporary species) were extracted from tidepools in each location. The specimens were captured with hand nets aided by the use of 20% benzocaine (BZ-20). All specimens were preserved in ice and brought back to a laboratory for meristic measurement and molecular study.

Nucleic acid extraction

H. chilensis and *G. laevis* DNAs were extracted from 10 to 25 mg of fish tissue with DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany), according to the instructions of the manufacturer. Elution was done in 100 µl of elution buffer.

Procedures of AFLP analysis were based on Vos *et al.* (1995) with slight modifications.

DNA digestion: 250 ng DNA (10 µL), 2.5 µL Buffer 4 10X (), 0.25 µL BSA 100X, 12 µL distilled water, 2.5 U MseI (0.25 µL) and 20 U EcoRI (1 µL). This was incubated at 37°C overnight, and then the enzymes were rendered inactive at 65°C for 20 minutes.

Ligation of adapters: After the digestion, the fragments obtained were ligated to a mixture of specific adapters for each restriction site. These adapters contain a region complementary to the restriction sequence and another that anchors the AFLP primers (Table 1). Prior to ligating, the digested DNA adapters were incubated for 15 min. at 65°C to facilitate annealing. The ligation mixture requires 20 µL of digested DNA, 0.5 µL EcoRI adapter 50 pmoles/µL, 0.5 µL MseI adapter 50 pmoles/µL, 2.5 µL 10X T4 DNA ligase buffer (Tris-HCl 500 mM pH 7.5, MgCl₂ 100 mM, DTT 100 mM, ATP 1 mM, 250 µg/ml BSA), 0.3 µL T4 DNA ligase (100 U enzyme) and 1.2 µL distilled water. This was incubated overnight at 16°C. Subsequently it was diluted 10 times and the stock was stored at -20 for later use.

Preamplification reaction (AFLP +1): Pre-amplification, which reduces the "dirt" by amplifying only those fragments that were properly digested and ligated, used primers with a sequence complementary to that of the EcoRI and MseI adapters with an extra or

selective nucleotide in position 3' (EcoRI+1 and MseI+1). (Table 1) Each reaction should contain 5 µL of diluted product 1/10, 13.2 µL distilled water, 2.5 µL 10X PCR buffer, 2 µL MgCl₂ 25mM, 1 µL dNTPs 10mM, 0.6 µL EcoRI+1 primer 10 pmoles/µL, 0.6 µL MseI+1 primer 10 pmoles/µL, 0.1 µL Taq Polymerase 5U/µL. The following amplification program was used: initial denaturation at 94°C for 2 min., followed by 20 cycles of 30 seconds at 94°C, of 1 min. at 55°C and of 1 min. at 72°C, with a final extension for 5 min at 72°C. The mixture is kept at 4°C. Then the PCR reactions were diluted to the ratio 1:50.

Selective amplification reaction (AFLP +3): Each tube should contain 5 µL of diluted preamplified DNA, 7.6 µL distilled water, 2 µL 10X PCR buffer, 1.6 µL MgCl₂ 25 mM, 1.6 µL dNTPs 10mM, 1 µL EcoRI+3 primer 1 pmol/µL, 1 µL MseI+3 primer 5 pmol/µL and 0.2 µL Taq Polymerase 5U/µL. The PCR program for selective AFLP amplification consisted of an initial denaturation at 94°C for 4 minutes, followed by 30 seconds at 94°C, 65°C for 30 seconds and 72°C for one minute, in the first cycle. In subsequent cycles, the annealing temperature decreased by 0.7°C per cycle, until reaching 56°C. Then 25 cycles were repeated with the same conditions (annealing at 56°C), ending with an extension at 72°C for 5 minutes. At the end it was kept at 4°C. The primer sequence can be seen in Table 2. 10 µL of formamline solution (Formaline 95% v/v, 20mM EDTA, 0.05% bromophenol blue w/v, 0.05% Xylene cyanol w/v) were added to the PCR products. The products were separated by electrophoresis in polyacrylamide gel at 6% as described below. 8 µL of product were loaded in the sequencing chamber, which was run for approximately 90 min.

Polyacrylamide gel electrophoresis: polyacrylamide gels contained 6% acrylamide/bisacrylamide 19:1, 0.5X TBE buffer and 7.5 M urea in 35x45 cm pieces of glass. These gels were prepared between two tempered pieces of glass, one of which retained the adhered gel. To achieve this, the pieces of glass are washed with water and ethanol and then subjected to differential treatment. While one glass was impregnated with 600 µl of Repel-Silane ES, the other, where the gel was adhered, received 1ml of absolute ethanol plus 5 µl of Silane A-174 and 5 µl of 10% acetic acid. Once the glass was dry, the treated surfaces were placed facing each other with a system of separators and clamps, plus an inverted shark tooth comb. The mixture to be polymerized had 70 ml of 6% acrylamide-urea solution, 190 µl of 10% APS and 95 µl of TEMED. The solution was injected with a

syringe, and then advances through capillary action. It is allowed to polymerize for one hour.

After the polymerization time, the PCR product was denatured by adding formamide loading buffer (Formamide 95% v/v, EDTA 20mM, 0.05% bromophenol blue w/v, 0.05% Xylene cyanol w/v) in a 1:1 ratio and heating it for 5 min at 95°C. After this process, the samples were immediately placed in cooling before loading. The load volume depends on each PCR; generally, between three and five microliters is sufficient.

To save the amplification patterns, the gels were scanned in Epsilon Perfection Photo 3490 equipment. Polymorphic DNA fragments obtained are compiled in a matrix of binary data. To analyze the location grouping, dendrograms were created with the neighbor-joining algorithm (PAUP 4.0) and indices of genetic similarity and global F_{ST} differentiation were calculated between pairs of locations. We assessed whether the overall F_{ST} is significantly different from zero and the correlation between population genetic differentiation [$F_{ST} / (1-F_{ST})$] and geographic distance using the Mantel test (Genetix v 4.05).

Results

Twelve primer combinations were tested and only eight were informative and show polymorphisms (Table 3). The dendrogram using parsimony algorithm (bs: 1000, PAUP 4.0), created based on the polymorphic fragments, suggested that for *H. chilensis* there are three groups: Las Cruces, El Tabo - Isla Negra and Valparaiso - Maitencillo - Los Molles (Figure 3a), whereas for *G. laevifrons*, it was not possible to determine groupings of locations (Figure 3b).

Regarding the genetic diversity analysis, it could be determined that:

H. chilensis: a total of 47 putative loci were detected by the eighth primer combinations. On average 12 of 47 of which were polymorphic (27.8%, Table 3). The population with the highest proportion of polymorphic loci (32,5%) was population ET, whereas that with the lowest value was population LM, in which the proportion of polymorphic loci and number of polymorphic loci was 26% (Table 3). Anyway, all populations showed the similar and low proportion of polymorphic loci. Results of the analysis of population pairwise F_{ST} s are shown in Table 5. It can be noted that in all cases the values were higher than 0.6 the only exception is between MA and LM where F_{ST} is 0.

G. laevisfrons: a total of 38 putative loci were detected by the four primer combinations. . On average 16 of the 38 were polymorphic (42.1%, Table 4). The population with the highest proportion of polymorphic loci (78,7%) was population LM, whereas that with the lowest value was population ET, in which the proportion of polymorphic loci and number of polymorphic loci was 24.1% (Table 4).

Among sample sites for *H. chilensis*, a Mantel test indicated a no significant relationship ($Z=96.11$, $p= 0.48$) between $F_{ST}/(1-F_{ST})$ and geographic distance, indicating no isolation by distance. Finding no significant differences in the diversity index and seeing as how F_{ST} values tend to 0 in all cases, the Mantel test was not performed for *G. laevisfrons*.

Discussion

One of the benefits of AFLPs over other molecular techniques is their sensitivity for stock and population identification without any previous knowledge of the species' genome, provided a large number of independent markers can be rapidly surveyed (Bensch and Akesson 2005; Bonin *et al.* 2007; Gwo *et al.* 2008; Dasmahapatra *et al.*, 2009). Although, the primary reason for rapid acceptance of AFLP technology is its ability to detect a large number of polymorphic DNA markers rapidly and in a reproducible manner (Féral 2002; Bensch and Akesson 2005), species analyzed had low percentages of polymorphic loci (*H. chilensis*: 27.8 %, *G. laevisfrons*: 42.1 %). Anyway, the current study offers the first evidence of structuring in intertidal fish populations in central Chile. Contrary to expectations, the hypothesis of isolation by distance was rejected for both species, but for different reasons.

First, the failure to obtain real groups in *G. laevisfrons* and lack of genetic differentiation observed in the six locations reflects the dispersion capacity of the eggs and pelagic larvae that this species produces. As a species that inhabits the sub tidal when adult, the release of eggs and larvae does not occur on the coast (Hernandez Miranda *et al.* 2003). In this way the barriers or coastal oceanographic events do not prevent the free dispersion through mainstream Humbolt. Hence, it is possible that for this species the dispersion distance is much greater than expected according to their PLD (110 days, Mansur *et al.* 2012), and to analyze the structure, a larger geographic range has to be covered. Note that

the results do not reflect an artifact of the analysis and that adding more markers to the analysis would not reverse the trend.

In the case of *H. chilensis*, coastal geomorphology and oceanographic events represent barriers that limit the dispersion of larvae from the site of origin, consistent with what is already described for the area (Hernández-Miranda *et al.* 2003, Aiken *et al.* 2007, Zakas *et al.* 2009, Gómez-García 2012). These results are consistent with the importance of self-recruitment for intertidal fish species (Cowen *et al.* 2000, 2007, Sponaugle *et al.* 2002, Swearer *et al.* 2002, Jones *et al.* 2005, Hernández Miranda *et al.* 2009, Lin *et al.* 2009). 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 and Shanks 2004, Gillanders 2005, Jones *et al.* 2005, Miller *et al.* 2005, Shanks and Eckert 2005, Almany *et al.* 2007) and through modeling (Cowen *et al.* 2000, Irisson *et al.* 2004, Gerlach *et al.* 2007).

It is important to note that *H. chilensis* spawn in the pools, eggs sticking to the intertidal rocky pools walls. Therefore, the larvae must cross the coastal oceanographic barriers to reach the main circulation and disperse to distant locations. In turn, *H. chilensis* spawns and recruits in the intertidal (Mansur *et al.* 2012) with peaks in the spring and summer months, coinciding with the period where the water column has the greatest stratification (Hernández-Miranda *et al.* 2003). Furthermore, these localities have geographical particularities are reflected in the genetic differentiation. Las Cruces shows genetic differentiation with other five localities. This is attributed to larvae dispersion being impeded by the proximity of the mouth of the Maipo River. The plume of the river intensifies in the summer months and represents the physical barrier that favors self-recruiting (Gómez G 2012). The "Quebrada de Córdoba" is found between the El Tabo and Isla Negra locations. This body of fresh water intensifies its flow in the winter months as a result of seasonal rains, diluting its effect in the summer. This phenomenon represents a weak seasonal oceanographic barrier allowing the flow of larvae between locations, which is reflected in intermediate F_{ST} values. The significant difference found between the three locations north to south of Valparaíso is due to Punta Curaumilla upwelling event (Silva and Valdenegro 2003, Navarrete *et al.* 2005, 2008), which represents an oceanographic barrier to marine species with pelagic larvae (Aiken *et al.* 2007). Considering that the event

is intensified in the summer represents a strong barrier to the flow of oceanographic larvae. Regarding the differentiation between localities LM and MA with VA, is attributable to the presence of the Aconcagua River plume. Since it is also seasonal and increases flow in the summer months, it appears that has the same effect described for the Feather River Maipo. Finally it should be noted that between the towns of Maitencillo and Los Molles are not freshwater bodies and this is reflected in the lack of distinction between them (FST = 0).

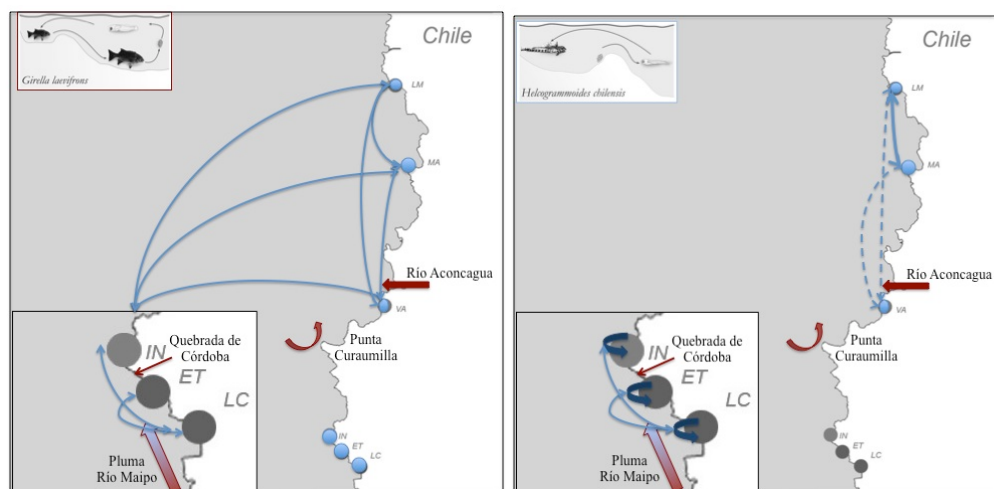
In other words, this work represents a new empirical evidence that coastal and littoral marine fish populations would be demographically more closed than what has been theoretically hypothesized. It has also been recognized that diverse biophysical coupling mechanisms that develop during larval stages underlie these low patterns of dispersal (Kingsford *et al.* 2002, Sponaugle *et al.* 2002), offsetting the inherent transport effect which is generated through physical processes.

Conclusions

The AFLP technique turned out to be suitable for analyzing the genetic structure of populations of *H. chilensis* and *G. laevisfrons*, although analysis of microsatellites is recommended to corroborate these results.

The life cycle is a crucial feature in the dispersion distance for intertidal fish species in central Chile, as it is a determining factor in the ability of larvae to overcome oceanographic events and barriers. Temporary species disperse farther than expected from their PLD and show no structuring in the geographic range studied (200 Km), while resident species disperse at a closer distance than expected from their PLD and show structuring at a short distance (less than 10 Km).

Finally, a graphical model is given that explains connectivity in both lifecycles:



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Table 1: Nucleotide sequence of the primers used in the AFLP protocol

Adapters		
EcoR1	Oligo 1	5' - CTC GTA GAC TGC GTA CC - 3'
	Oligo 2	5' - AAT TGG TAC GCA GTC TAC - 3'
Mse1	Oligo 1	5' - GAC GAT GAG TCC TGA G - 3'
	Oligo 2	5' - TAC TCA GGA CTC AT - 3'
Preamplification		
EcoR1	(+1)	5' - GAC TGC GTA CCA ATT CA - 3'
Mse1	(+1)	5' - GAT GAG TCC TGA GTA AG - 3'

Table 2: Nucleotide sequence of the primers used in the selective amplification and combinations were show polymorphisms.

Selective Amplification		
EcoR	PE1B (+3)	5' - GAC TGC GTA CCA ATT C ACG - 3'
	PE1C (+3)	5' - GAC TGC GTA CCA ATT C AGT - 3'
	PE1E (+3)	5' - GAC TGC GTA CCA ATT C AAT - 3'
	PE1F (+3)	5' - GAC TGC GTA CCA ATT C ATG - 3'
	PE1G (+3)	5' - GAC TGC GTA CCA ATT C ATC - 3'
	PE1H (+3)	5' - GAC TGC GTA CCA ATT C AGA - 3'
Mse1	PM1A (+3)	5' - GAT GAG TCC TGA GTA A GTA - 3'
	PM1B (+3)	5' - GAT GAG TCC TGA GTA A GCG - 3'
	PM1C (+3)	5' - GAT GAG TCC TGA GTA A GAC - 3'
	PM1D (+3)	5' - GAT GAG TCC TGA GTA A GGT - 3'
	PM1F (+3)	5' - GAT GAG TCC TGA GTA A GTC - 3'
	PM1H (+3)	5' - GAT GAG TCC TGA GTA A GAG - 3'
Mse1-EcoR	GTA-AAT; GCG-AGA; GTC-AGT; GGT-AAT; GAC-ATC; GAG-ACG; GCG-AAT; GAG-AGA	

Table 3. Parameters of genetic diversity for populations of *Helcogrammoides chilensis*.

Population	N	Date of collection	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci
LC	10	Feb 11	32	9	28 %
ET	11	Sep 10	43	14	32.5 %
IN	10	Apr 10	44	12	27.2 %
VA	10	Apr 11	44	12	27.2 %
MA	10	Apr 11	47	13	27.6 %
LM	10	Apr 11	46	12	26 %

Table 4. Parameters of genetic diversity for populations of *Girella laevis*.

Population	N	Date of collection	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci
LC	10	Feb 11	30	17	56.6
ET	10	Oct 10	29	7	24.1
IN	10	Nov 10	31	12	38.7
VA	10	Oct 10	37	11	29.7
MA	11	Apr 11	33	26	78.7
LM	10	Apr 11	34	24	70.5

Table 5. Pairwise FST (below) between *H. chilensis* populations.

	LC	ET	IN	VA	MA	LM
LC	0					
ET	0.78584	0				
IN	0.68423	0.70531	0			
VA	0.92429	0.72543	0.92363	0		
MA	0.92379	0.70311	0.92542	0.53583	0	
LM	0.91972	0.69040	0.92048	0.52431	0	0

Figure 1: Lifecycle of *Helcogrammoides chilensis* (resident species) b: lifecycle of *Girella laevisfrons* (temporal species)

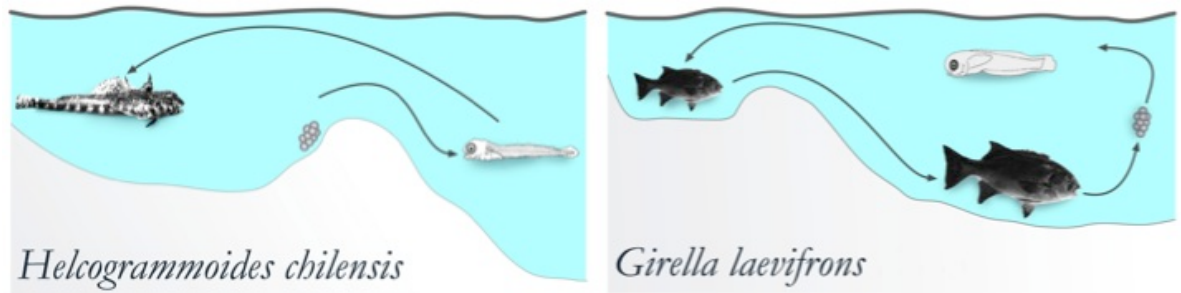
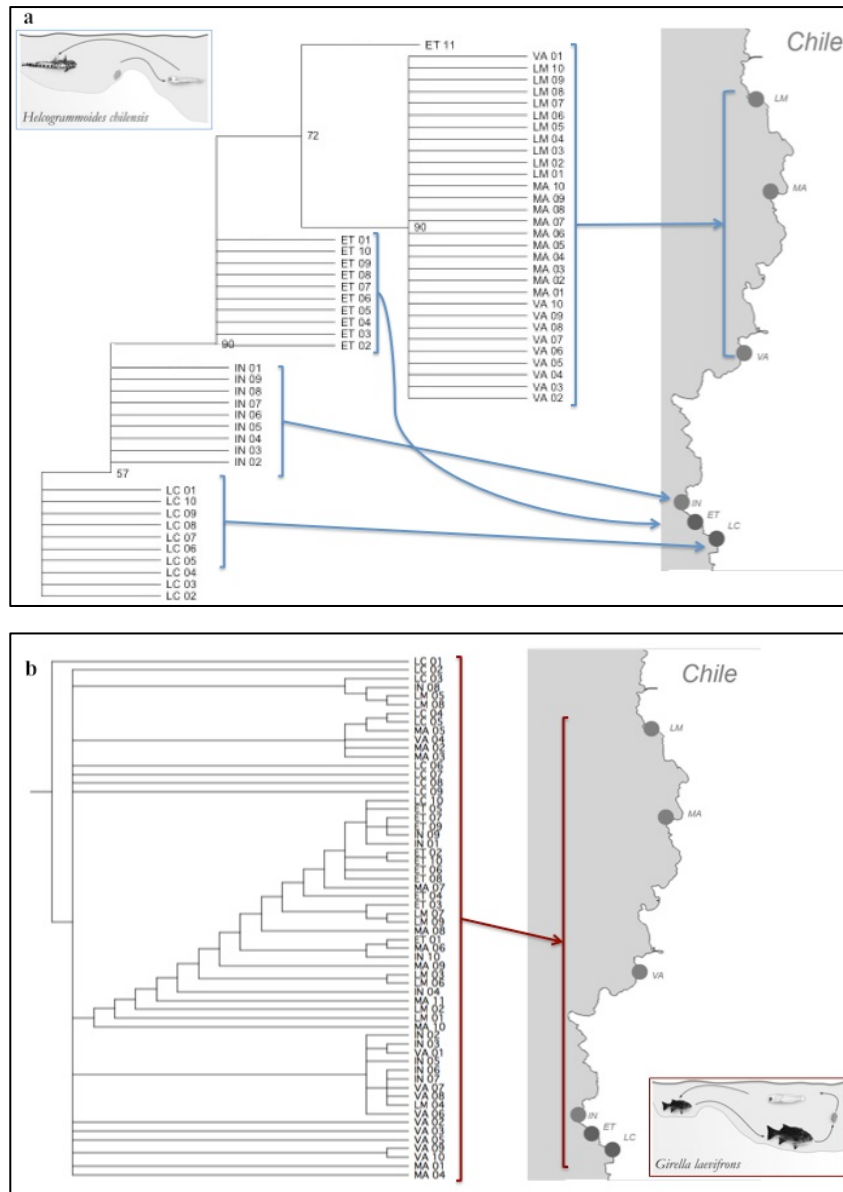


Figure 2: Area of study. Las Cruces (LC), El Tabo (ET), Isla Negra (IN), Valparaíso (VA), Maitencillo (MA) and Los Molles (LM)



Figure 3: a. *Helcogrammoides chilensis* b. *Girella laevisfrons*



CAPÍTULO V

VI. Population dynamics of intertidal fish species on the temperate coast of central Chile

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Oecologia (Submitted)

**Population dynamics of intertidal fish species on the temperate coast of central
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Abstract

Population connectivity of marine species with complex life cycles has been one of the most discussed and analyzed research issues in ecology. Today, most ecologists recognize that endogenous and exogenous factors interact in every process affecting natural populations. However, an understanding of how each factor acts directly and indirectly on any given ecological process is a critical goal to be determined.

Although there are many studies that address the dynamics of intertidal fish functions from a community approach, those that explain the population dynamics of its components are still scarce. Here, we propose a simple but realistic model for the exploration of the combined effects of the different factors that determine fish population dynamics for the rocky, intertidal fish fauna of central Chile. This is a well-studied assemblage, and monitoring of the ichthyofauna has taken place in ten intertidal pools for fifteen years. This monitoring and analysis included the most representative species of central Chile, *Helcogrammoides chilensis* (resident) and *Girella laevisfrons* (transient). We found that the transient or temporal condition is determinant for establishing the scale at which analysis of the population dynamics takes place. We also found that dynamics of both species are explained mainly by intraspecific competition. ENSO does not represent a significant disturbance, while the presence of fresh water and mud affect the availability of shelter in the intertidal pools. These approaches allowed us to incorporate the population dynamics theory in our models in order to better understand the underlying causes of intertidal fish population dynamics. The new approaches we are postulating deduce the ecological interactions and exogenous factors very well. Thus, this long-term study provides a theoretical, yet simple, way for deciphering intertidal system dynamics, acting as a new framework for evaluating intertidal fish populations in a temperate or tropical coastal ecosystem.

Keywords: intertidal fish, resident and transient fish, dynamics theory, ENSO

Introduction

Population connectivity of marine species with complex life cycles has been one of the most discussed and analyzed research issues of ecology, with several lines of research aiming to identify factors underlying the regulation of its structure and organization (Gibson 1982; Horn and Gibson 1988; Roughgarden et al. 1988; Varas and Ojeda 1990; Muñoz and Ojeda 1997; 1998; Angel and Ojeda 2001; Wennhage and Pihl 2001; Boyle and Horn 2006; Cowen et al. 2007).

For 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 and Botsford 2007). Camus and Lima (2002) indicate that such a separation should be mostly 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 forcing (Berryman 1999; Royama 1992).

Today, most ecologists recognize that the endogenous and exogenous factors that interact in every process determine natural population dynamics. However, an understanding of how each factor directly and indirectly acts upon any given ecological process is a critical goal to be determined (Lima et al. 2001, 2002; Stenseth et al. 2002, 2003). The natural populations inhabiting marine ecosystems, particularly intertidal assemblages, are strongly affected by exogenous factors which operate at all levels of ecological organization (Hayward 1997; Attrill and Power 2002; Walther et al. 2002). For example, the El Niño Southern Oscillation (ENSO) is a coupled ocean-atmosphere event, and it is one of the most exogenous factors studied in South America. It is a strong, natural inter-annual climate forcing event that affects natural populations on the coastal areas of the eastern Pacific Ocean (Aceituno 1992; McPhaden 1999; Davis 2000; Kayano and Andreoli 2007; Barros et al. 2008; Garreaud et al. 2009; Estay and Lima 2010). The effects of ENSO on marine ecosystems have been widely documented, principally of which are changes in

population sizes and community structure (Guzmán and Cortés 2001; Jaksic 2001). On rocky shores, the most significant impact of El Niño is in reducing the frequency and extent of upwelling in cold, nutrient-rich water along the western coast of the Americas. Relatively warm, nutrient-poor surface waters replace these usually productive coastal waters. ENSO events also change currents and storm regimes, and have been associated with changes in species distributions, prevalence of marine diseases, and the primary production of marine systems (Fields et al. 1993; Harvell et al. 1999; Thompson et al. 2002).

Most studies show that intertidal communities may be sensitive to ENSO climate forcing, especially sessile invertebrate communities. The intertidal habitat marks the intersection of terrestrial and marine environments, and therefore it is exposed to the environmental changes of both realms. Although intertidal organisms are adapted to fluctuating environments, some may exist close to their physiological tolerances, bringing consequences on their geographical distribution (Tomanek Somero 1999; Davis 2000). In the case of intertidal fish, there is little information on ENSO effects for fish population dynamics. This is due in part to the difficulty of monitoring mobile organisms and for the lack of long-term intertidal fish assemblage studies.

In general, intertidal fish population dynamics have been evaluated from a community approach (Gibson and Yoshiyama 1999). However, these fail to provide explanations based on general and basic population dynamics' principles (Turchin 2003; Ginzburg and Colyvan 2004). There are few analytical models that incorporate the population dynamics theory in order to better understand the underlying causes of fluctuations in natural populations (Hernández and Ojeda 2006). The use of the population dynamics theory provides a simple way for deciphering system dynamics as described not only through the most important ecological interactions but also through exogenous forces, such as climate (Berryman 1999; Stenseth et al. 2002). This approach demonstrates that simple models can offer reasonable explanations and accurate predictions of marine fish populations provided that they are based on a sound theoretical framework (Lima and Naya 2011).

In this paper, we had the unique opportunity of analyzing fish information monthly for fifteen years in ten intertidal pools of central Chile. This long term data allowed us to enunciate a theoretical approach for evaluating intertidal fish population dynamics.

The intertidal fish fauna of central Chile (30 – 33° S) present a useful model for study given a fair diversity of 18 to 20 species (Varas and Ojeda 1990; Stepien 1990; Muñoz and Ojeda 1997). This assemblage is composed of both resident species (Blenniidae, Tripterygiidae, and Clinidae) and transient or temporary species that inhabit the intertidal pools as juveniles (e.g., Kyphosidae; Varas and Ojeda 1990). Resident species are those whose life cycle consists of the following stages: pelagic larvae, intertidal juveniles and adults, and egg-laying in clusters. The spawns are small, and the egg-laying sites are generally located in cracks or crevices of the tide pools. There is no information about parental care for Chilean coastal species. The duration of the pelagic larval phase is generally brief and shorter than that of subtidal species (Horn et al. 1999). The most representative resident species of intertidal pools is *Helcogrammoides chilensis* (Tripterygiidae). On the other hand, transient species are those whose life cycle consists of the following stages: intertidal juveniles, subtidal adults, egg-laying, and pelagic larvae. They have large spawns, and eggs are released into the water column. They do not usually show clear homing behavior (Varas and Ojeda 1990) or investment in parental care (Horn et al. 1999). The transient species with the highest abundance in intertidal pools is *Girella laevisfrons* (Kyphosidae). Background information on the biology and ecology of *G. laevisfrons* is found in Varas and Ojeda (1990), Muñoz and Ojeda (1997, 2000), Ojeda and Muñoz (1999), Cáceres and Ojeda (2000), Fariña et al. (2000), Hernández-Miranda et al. (2003, 2006, 2009), and Pulgar et al. (2003, 2006).

Our analysis of *Helcogrammoides chilensis* and *Girella laevisfrons* population dynamics was based on the following three sequential steps: a diagnostic approach to determine what kind of population dynamic model was most appropriate (Berryman 1999); the modeling of the observed dynamic through a simple theoretically-based model; and the use of a theoretical framework for analyzing exogenous perturbations (Royama 1992), such as the El Niño Southern Oscillation (ENSO).

Materials and Methods

Fish Sampling and Density of *H. chilensis* and *G. laevisfrons*

Ten intertidal pools were selected in three localities along the central Chilean coast between the 33 and 34° Southern latitude, with four pools at Isla Negra (33.4°S), three at El Tabo (33.45°S), and three at Las Cruces (33.5°S) (see Fig. 1). The pools were sampled every month from 1997 to 2012, so as to estimate abundance and size distribution of the existing intertidal fish assemblages. Sampling consisted of suctioning out the water contained in the pools during the tidal ebb by means of a portable water pump (Subaru Robin 3.5 Hp). The nozzle of the hose was placed perpendicular to and at the bottom of the pool in order to prevent fish from being sucked into the pump. Once the water was extracted, BZ 20 (benzocaine) anesthetic was applied under boulders and inside crevices, thereby facilitating the capture of fish specimens. Fish were collected with hand nets and placed in cooler tanks with fresh seawater and constant air supply. Individuals were identified and measured to the nearest mm. After the pools were replenished with seawater using the water pump, the fish were returned to their original pool. Finally, because of the high heterogeneity that intertidal pools have, estimates of species' abundances were corrected for the volume (m³) of each pool.

Climate Data

As a proxy for climatic conditions, we used the Southern Oscillation Index (SOI). SOI is one of the indexes used to represent the occurrence and strength of the El Niño Southern Oscillation (ENSO), and it compares meteorological conditions in two tropical areas of the Pacific Ocean, Tahiti (French Territory) and Darwin (Australia) (Allen et al. 1996). A standardized, annual SOI was obtained from <http://www.bom.gov.au/climate/current/soihtml1.shtml>.

Diagnosis and Modelling

To determine the endogenous structure of *H. chilensis* and *G. laevisfrons* populations, and to define the order of the feedback structure of these time series, we carried out an autoregressive analysis through the partial rate correlation function (PRCF) (Berryman and Turchin 2001). Therefore, as a starting point for modelling the reproductive

function, or R-function (Berryman 1999), we used a nonlinear version of the simple Ricker's (1954) equation and logistic based models (Lima et al. 2002) to demonstrate the basic influence of endogenous and exogenous forces on both intertidal fish population dynamics.

The nonlinear Ricker's (1954) equation is:

$$R = Rm \left(1 - \left(\frac{Nt - 1}{K} \right) \right)$$

Where N_{t-1} is the standardized density at time t-1; R is the realized per-capita growth rate $R_t = \ln(N_t/N_{t-1})$; R_m is the maximum per-capita growth rate estimated for the species; and K is the carrying capacity or equilibrium density.

We incorporated climatic perturbation (ENSO) using Royama's framework (1992). According to Royama (1992), exogenous perturbations may be vertical when the R-function is completely displaced through the y-axis, which means that the maximum reproductive capacity and the equilibrium density are modified by the action of the exogenous factor, or it may be lateral when the R-function is displaced through the x-axis, changing the equilibrium density but not the maximum reproductive capacity. Using this framework, we can build mechanistic hypotheses about the effects of ENSO on *H. chilensis* and *G. laevisfrons* populations. To assess the real impact of ENSO, we used two approaches. The first was evaluating the effect of the month in which the larvae are in the plankton (SOI1). The second was evaluating the effect of the period in which females prepare to spawn (SOI2). The months were chosen based on the results of otolith analysis obtained in Mansur *et al.* (2013) for both species.

Models were fitted by using the nls (stats) library in the R program (Bates and Watts 1998; R development Core team, 2004, available at <http://www.r-project.org>) and ranked according to the Bayesian Information Criterion (BIC or Schwarz Criterion; Schwarz 1978). The minimum BIC values were selected to determine the best model.

Results

The results from the statistical models suggest that both feedback structure and ENSO are important determinants of intertidal fish population dynamics in central Chile. However, the relative influence of these factors differs between and within species, particularly in feedback processes of population regulation.

For *H. chilensis*, we evaluated the effect on R of population density for five microcarnivore guild species (*Bovichthys chilensis*, *Auchenionchus microcirrhys*, *Graus nigra*, *Gobiesox marmoratus*, *Ophiogobius jenynsi*, and *Helcogrammoides cunninghami*) (Muñoz and Ojeda 1998). Only *B. chilensis* and *A. microcirrhys* had a significant effect on R. Therefore, models were proposed considering the interactions with these species. In turn, analyzing *H. chilensis* data from the three localities together did not yield significant results, and the models only explain 35% of the variation in population growth rates. When analyzing the sites separately we obtained that *H. chilensis* in Isla Negra shows clear first-order dynamics (Fig. 3c). First-order dynamics are characterized by saw-toothed oscillation (Fig. 2c), which is caused by positive feedback of density at time t-1 (Berryman 1999). PRCF showed that lag-1 is the main feedback process in the time series. Moreover, *H. chilensis* in El Tabo and Las Cruces show second-order dynamics (Fig. 3a, b). Second-order dynamics are characterized by positive feedback of density at time t-2 (Berryman 1999). PRCF showed that lag-2 is the main feedback process in both time series.

When analysing *G. laevisfrons* localities together or separately, the same results were obtained, with central Chile showing clear first-order dynamics (Fig. 2d). PRCF showed that lag-1 is the main feedback process in the time series. In this case, we evaluated the effect of two assembly herbivorous species, *S. viridis* and *H. sordidus*.

For both species, intraspecific competition is the main interaction that explains population dynamics. ENSO explains no more than 5% of the variance regardless of whether the assessment was in the larval period or when female adults prepared to spawn. Given that the basic structure for the analysis was a first-order Ricker model, ENSO was additively incorporated (lateral effect *sensu*, Royama 1992). The Ricker/Logistic model, plus the influence of ENSO (Table 1), had the best BIC.

A Ricker formulation described an even better model structure for *H. chilensis* populations in Las Cruces, El Tabo, and Isla Negra (Tables 1, 2, 3). In Las Cruces, the self-regulation term explains 37% of the variation in population growth rates, while the trophic

term explains 20% and SOI explains 12%. In El Tabo, the self-regulation term explains 34% of the variation in population growth rates, while the trophic term explains 2% and SOI explains 5%. In Isla Negra, the self-regulation term explains 42% of the variation in population growth rates, while the trophic term does not explain more than 1% (Table 3).

For *G. laevisfrons*, considering the three localities together presents the best model, and is one that considers variation in population growth rates as being explained by self-regulation term at 29%, trophic terms at 2%, and SOI at only 4%.

Discussion

We are aware that the time series analyzed here are not long enough, and that they are at the lower endpoint of adequate length. However, these data still present unusual results that offer a preliminary description of population dynamic patterns in intertidal fish, representing an important step towards understanding these dynamics. Our results represent further evidence that fish populations are not necessarily "open," and that the interconnectivity between populations is lower than previous studies suggest (Gillander et al. 2003, 2005; Thorrold 2001; Swearer et al. 2002; Gerlach et al. 2007). In this study we have determined the life history traits that determine the scale in which intertidal fish populations can be identified.

For *G. laevisfrons*, a species that inhabits the subtidal zone as an adult, the release of eggs and larvae does not occur on the coast (Hernandez-Miranda et al. 2003). Thus, barriers or coastal oceanographic events do not prevent free dispersion through Humboldt mainstream. Hence, it is possible that for *G. laevisfrons*, dispersion distance is much greater than expected according to its PLD (110 days) and presents synchrony in recruitment (Mansur et al. 2013). This synchrony is reflected in the dynamics of *G. laevisfrons* when the three locations are analyzed together and understood as metapopulations (Wiens 1989, Hanski 1994, Levins 2006). In turn, the little variance explained by the model would be masking determinant processes in the dynamics that occur in the subtidal zone. In other words, we consider that for both *G. laevisfrons* and temporary intertidal species, the spatial scale in which demographic discontinuities can be identified is more than 50 km. To the contrary, the scale at which we can differentiate *H. chilensis* populations is small, and is only a few kilometers. It is important to note that *H. chilensis* is a territorial fish, spawning

in the pools with its eggs sticking to the intertidal, rocky pool walls. Therefore, the larvae must cross the coastal oceanographic barriers to reach the main circulation and disperse to distant locations. *H. chilensis* spawns and recruits in the intertidal zone (Mansur et al. 2013), with peaks in the spring and summer months to coincide with the period where the water column has the greatest stratification (Hernández-Miranda et al. 2003). The features of the life cycle of *H. chilensis* together with the geographical and oceanographic particularities of the three localities studied have been reflected in three different population dynamics.

The population dynamics of *H. chilensis* in Las Cruces is mainly explained by intraspecific competition and predation by *A. microcirris*, the principal predator of the assemblage (Muñoz and Ojeda 1998; Rojas and Ojeda 2010). Since Las Cruces is a protected area and has no influence of freshwater bodies, it is considered a locality with very little disturbance. Besides this, it is in proximity to the mouth of the Maipo River, with the plume of the river intensifying in the summer months and representing the physical barrier that favors self-recruiting (Gómez 2012). However, this does not change the conditions of intertidal pools.

While the dynamics of *H. chilensis* in Isla Negra and El Tabo is explained by intraspecific competition, both intertidal zones are disturbed by the presence of the “Quebrada de Córdoba” in differing intensities. Isla Negra’s intertidal, rocky shore can be regarded as very disturbed given the input of great masses of fresh water and mud. This body of fresh water intensifies its flow in the winter months as a result of seasonal rains, and its effect diminishes in the summer. This phenomenon represents a weak seasonal oceanographic barrier allowing the flow of larvae between locations, but it modifies the structure of the intertidal pools and the availability of refuge for *H. chilensis*. In El Tabo, intraspecific competition explains the largest percentage of variance. These results are contrary to those predicted by PRCF analysis, which indicated that the dynamic order should be second. We understand that this is due to a significant and regular supply of fresh water in the pools, thus modifying the availability of shelter or food. The disturbance, however, is not as great as in Isla Negra.

The differences in the relative importance of the interactions found in the three localities corroborate the model proposed by Menge and Sutherland (1987), in which harsh

environments present competition as the main biotic interaction regulating community structure since predation is low or nonexistent. In more moderate environments, consumers persist, and it is hypothesized that they have a greater effect and that competition is low.

Finally, although this study did not evaluate recruitment in the dynamics of the species, we believe that part of the variance explained by the proposed model is because of these, as suggested by Hernández-Miranda (2006) for *S. viridis*. Therefore, further studies are necessary to consider this factor. Such works will deliver a valuable foundation for the background of this important component of coastal, central Chile and the South Pacific.

ENSO

Coastal habitats, such as rocky intertidal zones, are characterized by dynamic environmental conditions. Properties such as wave action, temperature, and water chemistry have large ranges which cycle on tidal, daily, seasonal, and interannual time scales (Metaxis and Schiebling 1993; Barry et al. 1995). Environmental variation for each scale has the potential to affect intertidal populations and assemblage structure. Intertidal communities may be sensitive to ENSO-induced environmental changes in part because the intertidal habitat marks the intersection of terrestrial and marine environments and is therefore exposed to environmental changes of both realms (Davis 2000). Intertidal fish occupy a wide range of habitats throughout their lifetimes, ranging from tens or hundreds of offshore kilometers during the planktonic larval phase and to the near terrestrial splash zone. In turn, transient fish are found in the subtidal zone reaching 50 m deep. A change in environmental conditions in any of those areas could, by influencing individual populations, affect entire intertidal systems (Sanford 1999). Although intertidal organisms are adapted to fluctuating environments, some may exist close to their upper or lower tolerances of environmental factors (Tomanek and Somero 1999; Davis 2000; Pulgar et al. 2003; Hernández-Miranda et al. 2006). The results show that SOI explains less than 10% of the variance for the population dynamics of *H. chilensis* and *G. laevisfrons*. In turn, significant results were obtained only when the SOI was evaluated in the months of the planktonic stage for both species. Therefore, we believe that the greatest influence of ENSO events on intertidal fish may not take place in the intertidal zone at all, but may be concentrated in the relatively short offshore, planktonic larval stage. Such offshore

disturbances, whether affecting larval survivorship or larval transport, result in differences in recruitment (Davis 2000).

Finally, this work represents the first description of population dynamics for the most abundant and representative species of the intertidal fish assemblage of central Chile. We propose models for explaining the dynamics of both *H. chilensis* and *G. laevisfrons* populations, thus giving considering to transient or resident status, the relative importance and strength of intraspecific and interspecific interactions, and SOI (Figure 4). We also raise the need for such analysis in all species of the assembly in order to explain and predict the response of the system to the global change scenario.

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Tables

Table 1: *Helcogrammoides chilensis* in Las Cruces, results of modeling. The best model is shown in bold face. Estimated parameters for each model are shown in the model (see Eq. 1 for reference).

Models	BIC	R ²
<i>Helcogrammoides chilensis</i> - Las Cruces		
$R = 1 * (1 - (\text{LnHch}/2.39))$	33.3948	0.3698
$R = 1 - (\text{LnHch}/2.49 + 0.02 * \text{SOI})$	34.3406	0.3854
$R = 1 - (\text{Ln } Hch / 1.80 - 0.22 * \text{Ln } Am)$	35.2148	0.3428
$R = 1 - (\text{Ln } Hch / 1.84 - 0.23 * \text{Ln } Am + 0.02 * \text{SOI})$	35.9903	0.4165
$R = 1 - (\text{Ln } Hch / 1.85 - 0.49 * \text{Ln } Am / \text{Ln } Hch)$	33.4019	0.4148
$R = 1 - (\text{Ln } Hch / 1.95 - 0.43 * \text{Ln } Am / \text{Ln } Hch + 0.01 * \text{SOI})$	34.8142	0.4655
$R = 1 - (\text{Ln } Hch / 3.90 + 0.52 * \text{Ln } Bc)$	32.4985	0.5044
$R = 1 - (\text{Ln } Hch / 5.37 + 0.67 * \text{Ln } Bc + 0.03 * \text{SOI})$	30.2061	0.6213
$R = 1 - (\text{LnHch}/2.47 - 0.25 * \text{Ln } Am + 0.54 * \text{Ln } Bc)$	33.8249	0.5098
$R = 1 - (\text{Ln } Hch / 2.92 - 0.27 * \text{Ln } Am + 0.69 * \text{Ln } Bc + 0.03 * \text{SOI})$	30.6443	0.6701
$R = 1 - (\text{Ln } Hch / 2.64 - 0.51 * \text{Ln } Am / \text{Ln } Hch + 0.53 * \text{Ln } Bc)$	31.4022	0.5795
$R = 1 - (\text{Ln } Hch / 3.39 - 0.41 * \text{Ln } Am / \text{Ln } Hch + 0.66 * \text{Ln } Bc + 0.02 * \text{SOI})$	29.4818	0.6975

Table 2: *Helcogrammoides chilensis* in El Tabo, results of modeling. The best model is shown in bold face. Estimated parameters for each model are shown in the model (see Eq. 1 for reference).

Models	BIC	R2
Helcogrammoides chilensis - El Tabo		
$R = 1.5 * (1 - (\text{LnHch}/2.69))$	35.9309	0.3409
$R = 1.5 * (1 - (\text{LnHch}/2.67 - 0.004 * \text{SOI}))$	38.4563	0.3513
$R = 1.5 * (1 - (\text{Ln Hch} / 2.91 + 0.05 * \text{Ln Am}))$	38.3636	0.3569
$R = 1.5 * (1 - (\text{Ln Hch}/2.92 + 0.06 * \text{Ln Am} - 0.005 * \text{SOI}))$	40.8006	0.3835
$R = 1.5 * (1 - (\text{Ln Hch} / 2.77 + 0.04 * \text{Ln Am}/\text{Ln Hch}))$	38.5051	0.3509
$R = 1.5 * (1 - (\text{Ln Hch} / 2.73 + 0.04 * \text{Ln Am}/\text{Ln Hch} - 0.003 * \text{SOI}))$	41.0480	0.3622
$R = 1.5 * (1 - (\text{Ln Hch} / 2.69 + 0.03 * \text{Ln Bc}))$	38.5001	0.3474
$R = 1.5 * (1 - (\text{Ln Hch}/2.67 + 0.02 * \text{Ln Bc} - 0.003 * \text{SOI}))$	41.0470	0.3573
$R = 1.5 * (1 - (\text{Ln Hch} / 3.04 + 0.07 * \text{Ln Am} - 0.03 * \text{Ln Bc}))$	40.9747	0.3571
$R = 1.5 * (1 - (\text{LnHch}/3.42 + 0.15 * \text{Ln Am} - 0.11 * \text{Ln BC} - 0.009 * \text{SOI}))$	43.2007	0.4034
$R = 1.5 * (1 - (\text{Ln Hch}/2.73 + 0.02 * \text{Ln Am}/\text{Ln Hch} + 0.02 * \text{Ln Bc}))$	41.1338	0.3498
$R = 1 - (\text{Ln Hch}/2.70 + 0.02 * \text{Ln Am}/\text{Ln Hch} + 0.01 * \text{Ln Bc} - 0.003 * \text{SOI})$	43.6810	0.3606

Table 3: *Helcogrammoides chilensis* in Isla Negra, results of modeling. The best model is shown in bold face. Estimated parameters for each model are shown in the model (see Eq. 1 for reference).

Models	BIC	R2
Helcogrammoides chilensis - Isla Negra		
R = 1.8 * (1 - (LnHch/2.26))	47.5971	0.4210
R = 1.8 * (1 - (LnHch/2.28 + 0.003 * SOI))	50.1811	0.4220
R = 1.8 *(1 - (Ln Hch / 2.32 + 0.02 * Ln Am))	50.2157	0.4227
R = 1.8 * (1 - (Ln Hch/2.31 + 0.01 * Ln Am + 0.003 * SOI))	52.8154	0.4226
R = 1.8 *(1 - (Ln Hch / 2.08 - 0.04 * Ln Bc))	50.1449	0.4222
R = 1.8 *(1 - (Ln Hch / 2.12 - 0.03 * Ln Bc + 0.002 * SOI))	52.7623	0.4231
R = 1.8 * (1 - (Ln Hch/2.1 + 0.04 * Ln Am - 0.05 * Ln Bc))	52.7406	0.4240
R = 1.8 * (1 - (Ln Hch/2.14 + 0.03 * Ln Am - 0.04 * Ln Bc + 0.001 * SOI))	55.3755	0.4242

Table 4: *Girella laevis* in Las Cruces, results of modeling. The best model is shown in bold face. Estimated parameters for each model are shown in the model (see Eq. 1 for reference). BIC and R2 are shown

Models	BIC	R2
Girella laevis - Las Cruces, El Tabo e Isla Negra		
$R = 1.2 * (1 - (\ln GI / 1.95(0.40)))$	45.3814	0.2913
$R = 1.2 * (1 - (\ln GI/1.93(0.40) + (-0.01 * SOI)))$	47.7520	0.3142
$R = 1.2 * (1 - (\ln GI / 1.62(0.80) + (-0.10 * \ln Sv)))$	47.8657	0.2758
$R = 1.2 * (1 - (\ln GI/1.47(0.72) + (-0.16*\ln Sv) + (-0.01*SOI)))$	50.0587	0.2968
$R = 1.2 * (1 - (\ln GI / 2.10(0.77) + 0.12 * \ln Hs))$	47.9417	0.3199
$R = 1.2 * (1 - (\ln GI/2.07(0.77) + 0.12 * \ln Hs + (-0.01 *SOI)))$	50.3042	0.3492
$R = 1.2 * (1 - (\ln GI/1.5(0.7) + 0.41*\ln Hs + (-0.2*\ln Sv)))$	49.9778	0.3044
$R = 1.2 * (1 - (\ln GI/1.29(0.59) + 0.57*\ln Hs + (-0.42*\ln Sv) + (-0.02 * SOI)))$	51.7142	0.3561

Figures

Figure 1: Annual time series data for intertidal fish density (individual . m^{-3}). (a) *Helcogrammoides chilensis* in Las Cruces; (b) *Helcogrammoides chilensis* in El Tabo; (c) *Helcogrammoides chilensis* in Isla Negra; (d) *Girella laevis* in Las Cruces, El Tabo and Isla Negra, for the period 1997–2012.

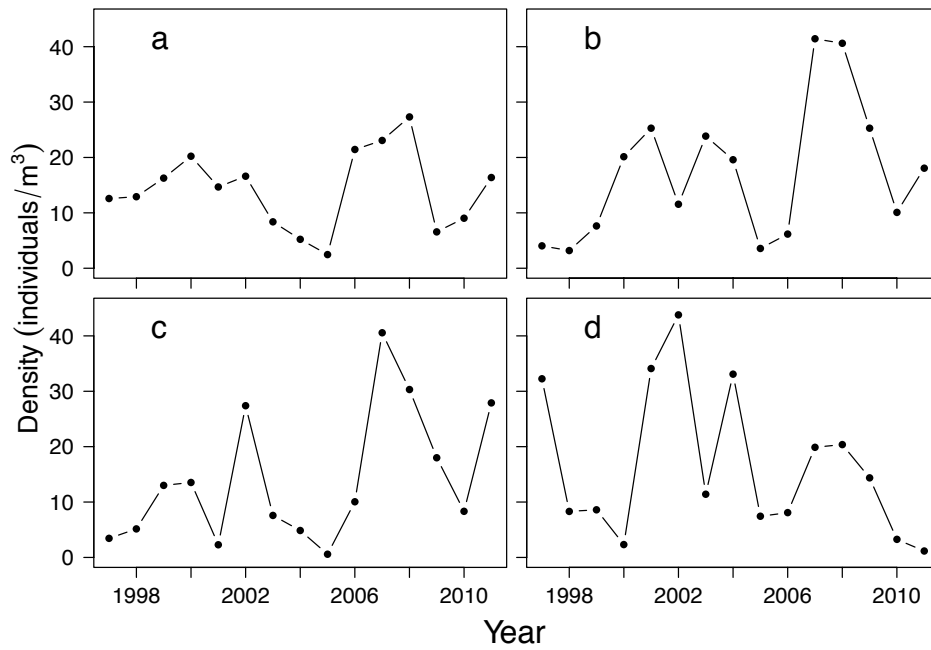


Figure 2: Partial correlation rate function (PRCF) plots for the two intertidal fishes species time series (a) *Helcogrammoides chilensis* in Las Cruces; (b) *Helcogrammoides chilensis* in El Tabo; (c) *Helcogrammoides chilensis* in Isla Negra; (d) *Girella laevisfrons* in Las Cruces, El Tabo and Isla Negra, for the period 1997–2012.

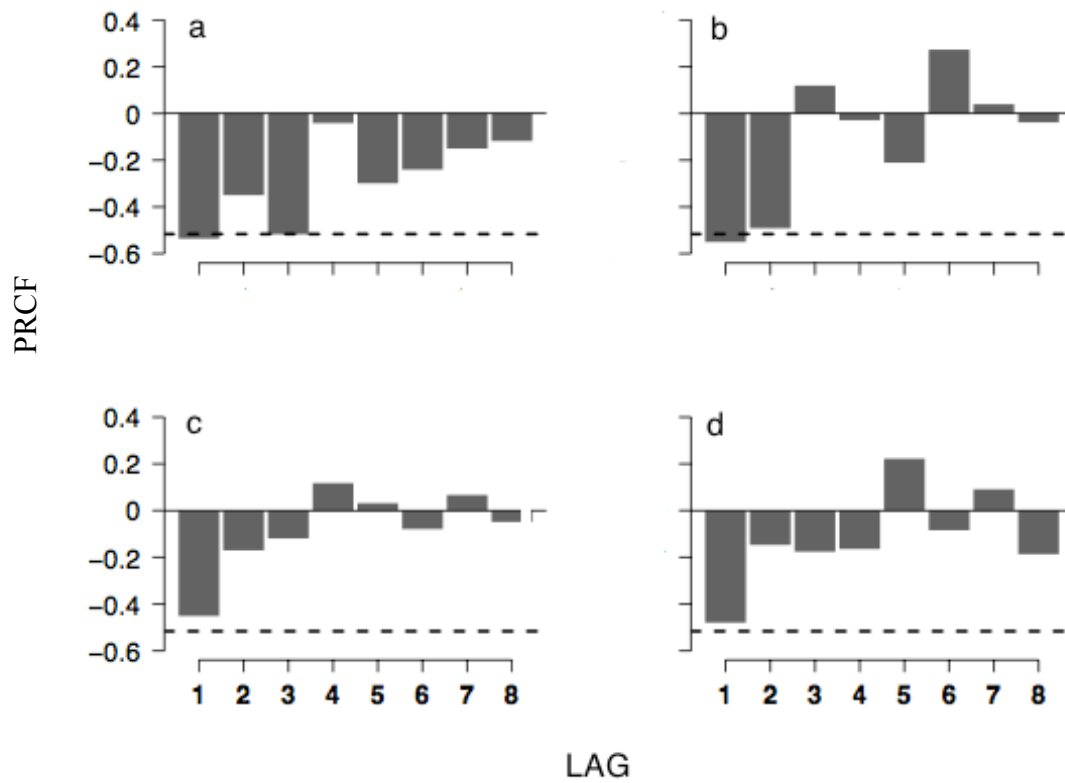


Figure 3: Hypothesized structure of the dynamics of the *Helcogrammoides chilensis* in (a) Las Cruces; (b) El Tabo; (c) Isla Negra. The relative importance and strength of the intraspecific ($\Rightarrow$), interspecific interactions: competition ($\bullet\text{---}\bullet\text{--}\Rightarrow$), depredation ($\text{--}\Rightarrow$) and ENSO ($\bullet\bullet\bullet\Rightarrow$) are represented by the thickness of the arrows.

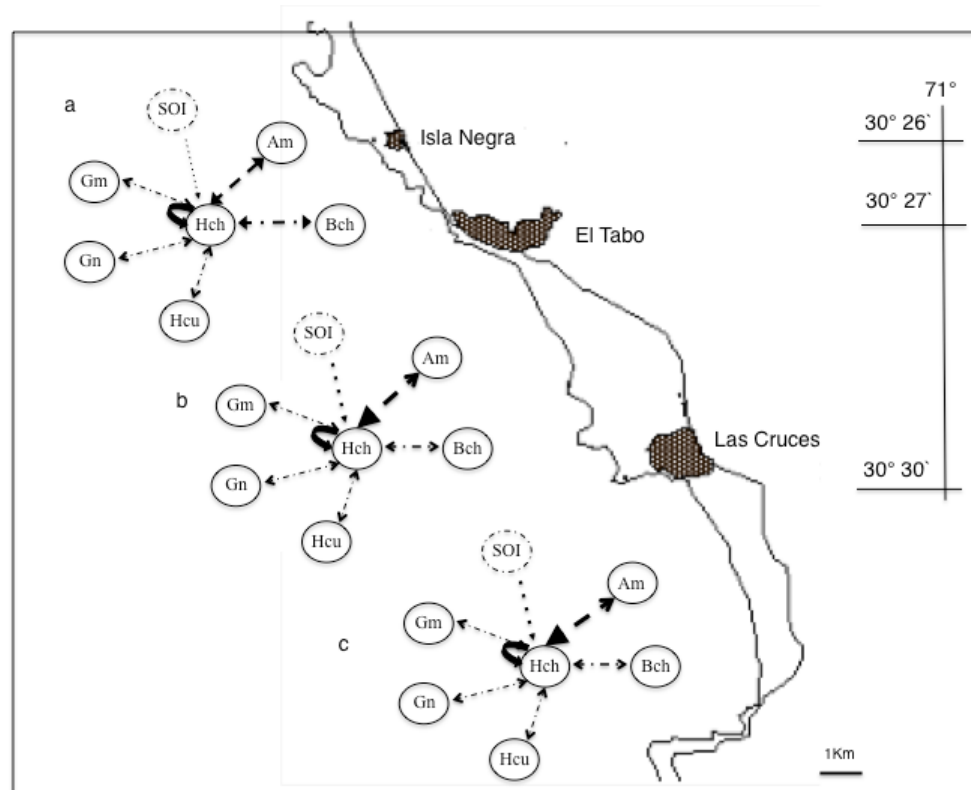
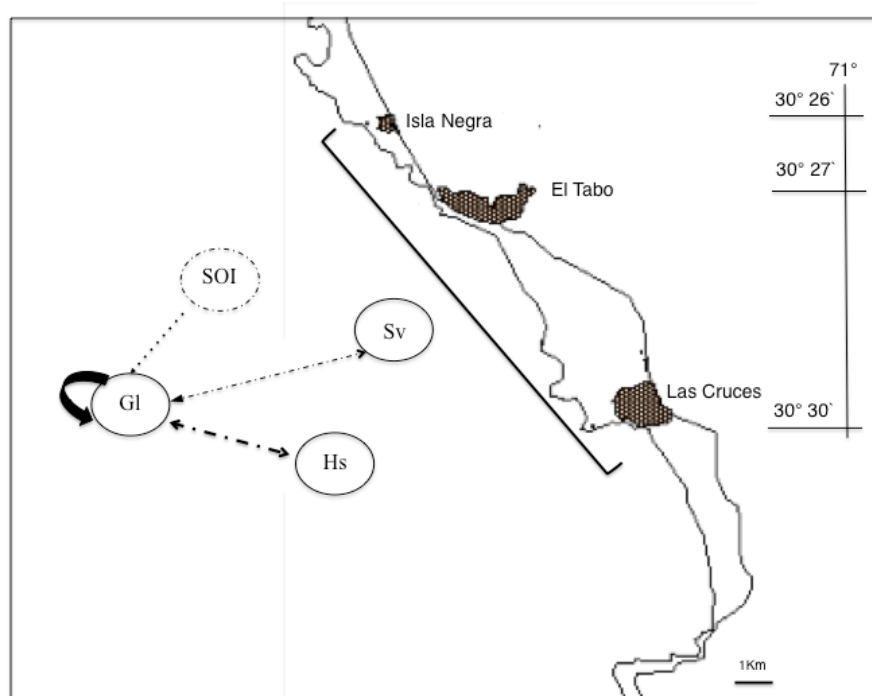


Figure 4: Hypothesized structure of the dynamics of the *Girella laevis* in Las Cruces, El Tabo and Isla Negra. The relative importance and strength of the intraspecific ($\Rightarrow$), interspecific interactions ($\bullet\text{---}\bullet\text{---}\Rightarrow$) and ENSO ($\bullet\text{---}\bullet\text{---}\bullet\text{---}\Rightarrow$) are represented by the thickness of the arrows.



VI. Conclusiones Generales

Conclusiones

La presente tesis representa el primer trabajo que aborda el estudio de la dispersión en peces litorales de Chile central de forma integral, utilizando otolimetría, análisis genético y de la dinámica de las poblaciones. Se consideraron las especies más representativas del ensamble, *H. chilensis* (residente) y *G. laevisfrons* (temporal).

En la primera parte de la tesis se realizó la descripción de la biología pre reclutamiento de las especies más representativas de la ictiofauna del intermareal rocoso de Chile central. A partir de dicho análisis fue posible determinar el momento de eclosión, asentamiento y el período planctónico larval (PLD). Para *H. chilensis* y *G. laevisfrons* el momento de reclutamiento fue correlacionado con el ciclo lunar y fue posible establecer que *H. chilensis* presenta un régimen semi-lunar en tanto que *G. laevisfrons* lunar. Estos resultados son preliminares ya que se encuentra en desarrollo el análisis de correlación de un mayor número de individuos.

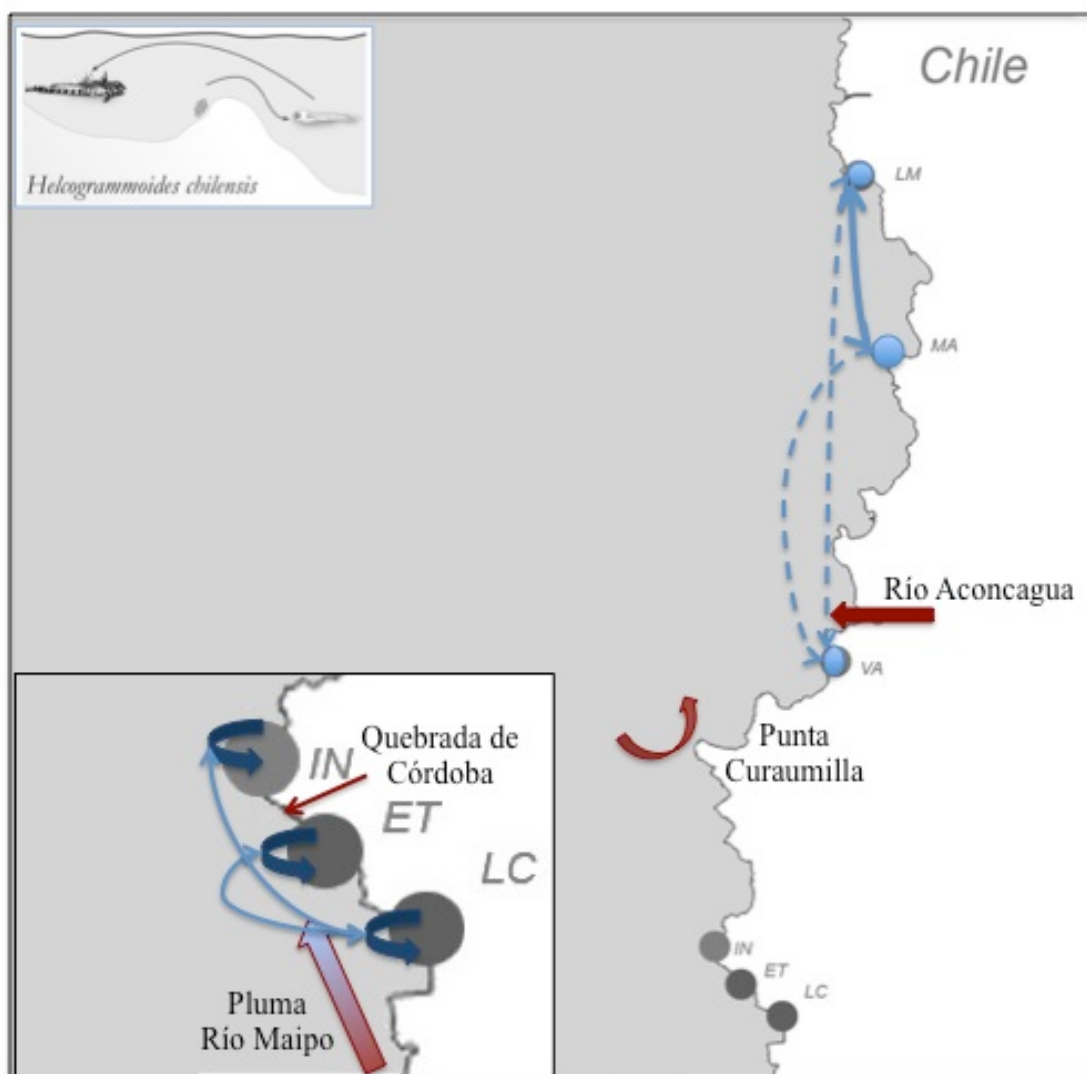
En una segunda etapa se determinó la estructura genética de *Helcogrammoides chilensis* y *Girella laevisfrons* en seis localidades de Chile central ubicadas entre 33,5°S – 32,2°S. El análisis se realizó con la técnica basada en enzimas de restricción enzimática, AFLP (amplified fragment length polymorphism). Se calculó el índice de diferenciación F_{ST} global y entre pares de localidades y se evaluó si el F_{ST} global era significativamente diferente de cero. La correlación entre diferenciación genética poblacional [$F_{ST}/(1-F_{ST})$] y distancia geográfica, se examinó utilizando el test de Mantel. Dicho análisis permitió establecer que *H. chilensis* presenta estructuración parcial a corta distancia (10 - 30 km) en tanto que *G. laevisfrons* no presenta estructuración en la escala geográfica analizada.

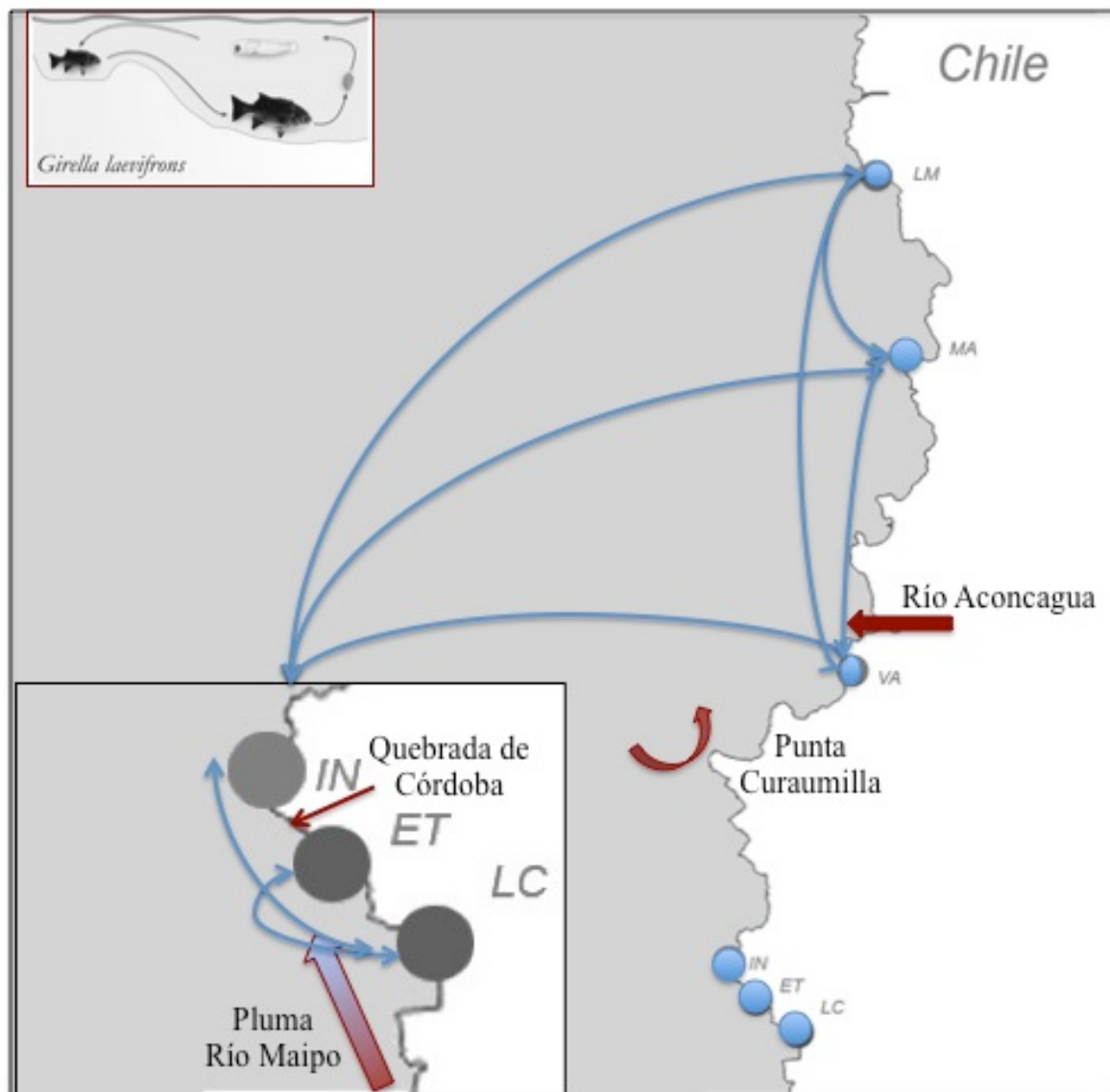
De manera general se puede concluir que el PLD no es un buen indicador de dispersión para peces intermareales. En el caso de los peces residentes que desovan en las pozas, los eventos y barreras oceanográficas costeras estarían determinando la capacidad de las larvas pelágicas para dispersarse. En este caso el PLD estaría sobre-estimando la verdadera capacidad de dispersión. En tanto que para peces temporales que desovan en el submareal y tanto los huevos como las larvas son pelágicos, el PLD estaría subestimando la capacidad de dispersión.

Finalmente al contar con una serie de tiempo de mas de 15 años, fue posible analizar la dinámica de las poblaciones de ambas especies en tres localidades contiguas de

Chile central. Se pudo generar un modelo simple para ambas especies, considerando a las tres localidades por separado en el caso de *H. chilensis* y juntas en el caso de *G. laevisfrons*. Los resultados encontrados son consistentes a los encontrados en el análisis genético ya que para la especie residente la escala a la cual se pueden individualizar poblaciones es de menos de 10 km, en tanto que para la especie temporal no es posible individualizar poblaciones en el rango geográfico estudiado.

Por lo expuesto, se presenta un modelo de conectividad para peces intermareales de Chile central fundamentado en los resultados del análisis de AFLP y las barreras oceanográficas costeras (cuerpos de agua dulce y eventos de surgencia) presentes en el área de estudio.

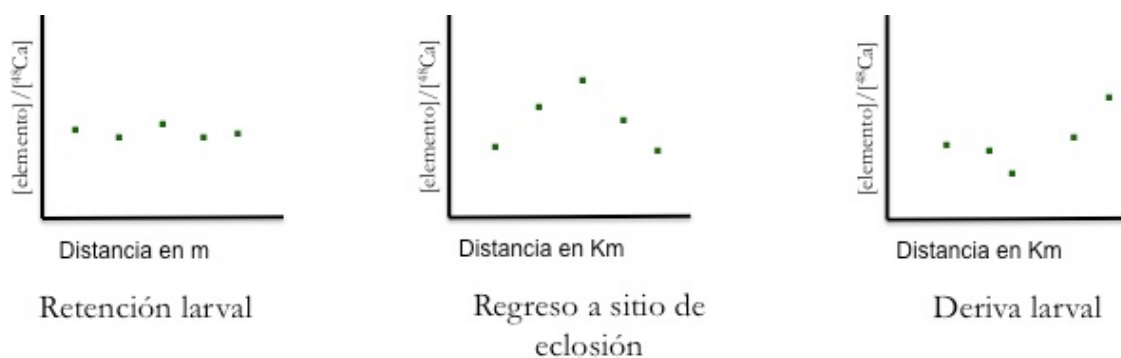




Vislumbro que nuevas direcciones de investigación en el área de esta tesis debiesen orientarse a:

- Realizar el análisis de ADN nuclear con microsatélites para corroborar los resultados obtenidos con AFLP. A su vez, en el caso de *G. laevisfrons* se debería extender el rango geográfico de 200 Km.
- Realizar análisis de microquímica de los otolitos para cuantificar el grado de diferenciación espacial de los marcadores naturales ^{48}Ca , ^{86}Sr , ^{138}Ba , ^{25}Mg , ^{55}Mn y ^{208}Pb , presentes en los otolitos de reclutas de *Helcogrammoides chilensis* y *Girella laevisfrons*.

Dicho análisis permitirá evaluar cuál de los escenarios (ver Figura) es el que explicaría el comportamiento de las larvas y si existe comportamiento de homing en *H. chilensis*.



Por último es necesario complementar con estudios oceanográficos costeros que permitan explicar acabadamente cómo afectan la conectividad de las poblaciones de peces litorales de Chile central.