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Doctorado en Oceanografía

Tesis

Distribución y transporte de larvas de crustáceos decápodos en la zona de surgencia costera de Chile Central: interacciones entre el comportamiento, tolerancias fisiológicas y períodos de liberación.

Beatriz Yannicelli

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La Tesis Doctoral en Oceanografía "Distribución y transporte de larvas de crustáceos decápodos en la zona de surgencia costera de Chile Central: interacciones entre el comportamiento, tolerancias fisiologicas y períodos de liberación" de la Srta Beatriz Yannicelli., realizada en el Departamento de Oceanografía, Facultad de Ciencias Naturales y Oceanográficas, Universidad de Concepción, ha sido aprobada por la siguiente Comisión de Evaluación:

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Curriculum Vitae

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AREAS DE INTERES

Principal : Procesos pre reclutamiento en organismos de ciclos de vida complejo.
Secundaria: Acoplamiento de procesos biológicos y físicos.
Otras: Ecología intermareal y de comunidades bentónicas.

Resumen

Distribución y transporte de larvas de crustáceos decápodos en la zona de surgencia costera de Chile Central: interacciones entre el comportamiento, tolerancias fisiológicas y períodos de liberación.

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Los procesos de transporte y producción, que resultan del acoplamiento entre ciclos físicos y biológicos durante el período larval de organismos con ciclos de vida complejo, son determinantes de la disponibilidad y condición de los propágulos al asentamiento y juegan un rol preponderante en la evolución temporal y espacial de la distribución adulta. La hipótesis general de este trabajo es que la estacionalidad en el período de predominio de surgencia costera y ascenso de aguas pobres en oxígeno sobre la plataforma de Chile Central, influyen sobre los períodos reproductivos de los organismos bentónicos con ciclos de vida complejo. El grado de influencia dependerá de la capacidad natatoria y fisiológica específica de las larvas. De acuerdo a esta idea, especies que liberan sus larvas en rangos batimétricos afectados diferencialmente por el proceso de surgencia costero, presentarán desfases conspicuos en sus períodos de liberación larval y en el comportamiento de las mismas, que favorecerían su retención sobre la plataforma continental, dependiendo de sus tolerancias fisiológicas. Utilizando datos de cruceros llevados a cabo en la plataforma de Chile Central (35-37ºS) se determinó la distribución horizontal y vertical de estadíos larvales de seis especies de crustáceos decápodos de diferentes ambientes y se estimaron los períodos de liberación larval; se determinó el efecto de los factores: temperatura, oxígeno disuelto y disponibilidad de alimento en la sobrevivencia, tiempo de desarrollo y crecimiento de las zoeas tempranas de *P. monodon* y se determinó el efecto del nivel de oxígeno sobre varias respuestas metabólicas de las mismas; finalmente, se construyó un modelo conceptual que relacionó los períodos reproductivos, la distribución vertical larval y las tolerancias fisiológicas.

En estudios de procesos a pequeña escala, se encontró que tanto la abundancia como el transporte de larvas a través de la boca chica del Golfo de Arauco (37ºS; 73ºW) dependieron de las mareas, del ciclo día/noche y la posición de las larvas en la vertical. En la entrada profunda (boca grande), hubo dos grupos de larvas que se encontraron asociadas a dos tipos de agua diferente (alto oxígeno superficial y bajo oxígeno sub-superficial). El movimiento de estas capas está determinado en forma remota por la dinámica de la surgencia y es modificada localmente por el viento (brisa marina) y las mareas. Los resultados indicaron que el transporte de organismos es afectado por procesos de diferentes escalas y por el comportamiento larval.

Se realizaron además dos cruceros para analizar la distribución en mesoescala de los estadíos larvales de las especies objetivo, durante noviembre (periodo de surgencia) y marzo (periodo de reversión) en la plataforma de Chile central entre los 35º y 37° S. Las especies liberadas fuera de fase con el periodo de máxima surgencia (*Emerita analoga*) se distribuyeron en aguas superficiales, aquellas que fueron liberadas durante períodos más intensos (*Pleuroncodes monodon* y *Libidoclaea granaria*), fueron más profundas y ampliamente distribuidas en la vertical. Aquellas especies que migraban verticalmente tuvieron un periodo de liberación más amplio (Paguridos, *Neotrypaea uncinata*). El periodo de liberación larval puede ser afectado por el ambiente advectivo al cual están sujetas las larvas, sin embargo, aquellas larvas que son capaces de regular su posición en la vertical, se pueden volver más independientes de este factor. En este caso, la tolerancia a las bajas concentraciones de oxígeno para ocupar aguas sub-superficiales se vuelve relevante.

Las zoeas I de *P. monodon* en particular, fueron más abundantes en aguas sub-superficiales, y las tardías en aguas más someras. Las megalopas y juveniles migraron hacia la superficie en la noche y, durante marzo aparecieron solamente sobre al área donde se encuentran los reclutas. De acuerdo a experimentos de simulación, el mayor potencial de retención se da a media agua, y tanto organismos originados hacia el N y S de la plataforma del Itata pueden contribuir al reclutamiento. La sobrevivencia de zoeas I que mudaron a zoea II mantenidas en bajas concentraciones de oxígeno (1 and 0.5 $ml \cdot l^{-1}$) en laboratorio, es reducida en comparación con aquellas larvas en normoxia. La exposicion inicial a bajas concentraciones de oxígeno también disminuyó la sobrevivencia aproximadamente en un 3% por día de exposición inicial. La edad a la cual mudan a zoea II también se incrementa

linealmente con los períodos de exposición inicial a bajo oxígeno y sin alimentación. Los períodos iniciales de inanición y bajo oxígeno tienen mayor efecto que solamente la inanición inicial. La tasa de crecimiento tambien se redujo en bajo oxígeno. Las bajas temperaturas (11°C) también afectaron negativamente la sobrevivencia, desarrollo y crecimiento. El consumo de oxígeno de larvas de *P. monodon* es menor en hipoxia (1.3 $mgO_2 \cdot l^{-1}$) para todas la zoeas, sin embargo el porcentaje de reducción es menor en megalopas. Mientras que las zoeas tardías oxiconformaron, las megalopas mantuvieron altas tasas respiratorias hasta muy bajas concentraciones de oxígeno. La cantidad de nitrógeno (proteínas) acumuladas en hipoxia es menor a la acumulada en normoxia. El potencial aeróbico se reduce despues de varios días de exposición a bajo oxígeno así como el potencial metabólico total. Por lo tanto, las condiciones ambientales donde son liberadas las larvas de *P. monodon* son sub-optimas para su desarrollo y su sobrevivencia puede ser potenciada al dejar las aguas Ecuatoriales Sub-Superficiales, o ser transportadas hacia la superficie y costa.

Los últimos experimentos de simulación demostraron que las condiciones de mayor retención sub-superficial solamente resultan en mayores sobrevivencias larvales cuando éstas tienen una alta tolerancia a la hipoxia.

Palabras Claves: larvas de crustáceo, zona de surgencia costera, estrategias reproductivas, metabolismo, desarrollo, zona de mínima de oxígeno, distribución de meroplancton.

Abstract

Distribution and transport of decapod crustacean larvae in the coastal upwelling area of Central Chile: interaction between behavior, physiological tolerance and larval release period.

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Transport and production processes during the larval stages of organisms with complex life cycles result from the coupling of physical and biological cycles and determine the availability and condition of the settlers. Therefore, they play an important role in the spatial and temporal evolution of adult populations. The general hypothesis of this thesis is that the seasonality of coastal upwelling dynamic together with the seasonal ascent of poor oxygen waters over the Central Chile continental shelf, influence the reproductive periods of benthic organisms with complex life cycles. The influence would depend on the swimming and physiological characteristics of the larvae. According to this idea, species that hatch larvae in bathymetric ranges affected differently by the upwelling circulation (e.g. offshore surface advection, inshore deep advection), would present hatching peaks offphase and different larval behavior that would favor retention over the shelf depending on their physiological tolerance. Using data from two cruises in the Central Chile continental shelf (35-37ºS) the horizontal and vertical distribution of six species of decapods crustacean larvae from different bathymetric ranges were analyzed, and the hatching periods were inferred. The effect of temperature, dissolved oxygen and food availability on the survival, developmental time and growth of early *Pleuroncodes monodon* larvae were measured in laboratory. The effect of dissolved oxygen on several metabolic responses of P. monodon larvae was also determined. Finally, a conceptual model relating reproductive periods, larval vertical distribution and physiological tolerances was created.

In studies of small scale processes, it was found that both the abundance and transport of larvae through the small entrance of the Gulf of Arauco (37ºS; 73ºW) depended on tides and day/night cycle. In the deep entrance of the Gulf, there were two groups of larvae associated with two different water types (high oxygen surface waters and low oxygen sub-surface ones). The motion of these two layers of water is remotely determined by the upwelling dynamic and locally modified by local wind (sea breeze) and tides. The results indicated that organisms transport is affected by different scale processes and their behaviour.

Two cruises were conducted in order to analyze the mesoscale distribution of larval stages of six target species during november (upwelling period) and march (typical period of reversal) over the continental shelf of central Chile central (between 35° and 37° S). The species liberated off-phase with the period of maximum upwelling (*Emerita analoga*) distributed in surface waters. Those that were hatched during periods of maximum upwelling intensities (*Pleuroncodes monodon* and *Libidoclaea granaria*), were deeper and widerspread vertically in the water column. Those species that migrated vertically had a widerspread hatching period (Paguridos, *Neotrypaea uncinata*). Larval hatching period can be affected by the advective environment to which larvae are subject. However, those larvae that are capable of regulating their vertical position might become more independent of this factor. In the latter case, the tolerance to low oxygen to occupy sub-surface waters becomes relevant.

The zoea I *P. monodon* in particular, they were more abundant in sub-surface waters, and the late zoea were more abundant in shallower strata. The megalopa and juveniles migrate to the surface during the night and during march, they appeared only in the area where recruits are found. The larval tracking experiments conducted showed that the higher retention potential occurs at mid-depth and that organisms originated to the north and south of the nursery area migh contribute to recruitmet. The survival of zoea I until zoea II, was reduced when they were raised in low oxygen waters (1 and 0.5 $ml \cdot l^{-1}$) in the laboratory. The initial exposure of larvae to low oxygen concentrations also diminish survival and increase the age at which zoea I moults to zoea II. The initial periods of starvations and low oxygen have larger effect that only initial starvation.The growth rate was also lower in low oxygen. Low temperatures (11°C) also decreased survival,

augmented developmental time and reduced growth rates. Oxygen consumption was lower for *P. monodon* larvae in hypoxia (1.3 $mgO_2 \cdot l^{-1}$) than in normoxia, the reduction was lower in megalopas. While late zoeas oxyconformed, the megalopas mainteined high respiration rates down to very low oxygen tensions. The quantity of nitrogen (proteins) accumulated in hypoxia is lower than the accummulated in normoxia. The aerobic potential, is reduced after several days exposed to hypoxia as well as the total metabolic potential. The environmental conditions under which *P. monodon* larvae are hatch are sub-optimal for its development and, survival can be enhanced by leaving the Equatorial Sub-Surface waters by their own swimming means and/or the coastal upwelling.

The last simulation experiments showed that the larger sub-surface retention can only result in higher larval survival when they are highly tolerant to hypoxia.

Key Words: crustacean larvae, coastal upwelling zone, reproductive strategies, metabolism, development, minimum oxygen layer, meroplankton distribution.

PARTE A

1.- INTRODUCCION

La interacción entre procesos físicos y biológicos a diferentes escalas durante el período de vida planctónico en organismos adultos bentónicos, determina la disponibilidad y condición de los propágulos al asentamiento y juega un rol preponderante en la evolución temporal y espacial de la distribución adulta. Al menos dos tipos de procesos durante la fase larval dependen de su acoplamiento con el ambiente de desarrollo: 1) el proceso de transporte, que resulta de la interacción entre las características advectivas ambientales y el comportamiento larval, y 2) el proceso productivo, en que las tasas metabólicas larvales variarán en función de la temperatura, oxígeno disuelto y disponibilidad de alimento (entre otros). El éxito individual y también poblacional estarán dados entonces por el acomplamiento de los ciclos biológicos (por ej. reproductivos, migratorios) a aquellos ciclos físico-biológicos relevantes para las etapas larvales. Este acoplamiento tiene dimensiones espacio-temporales y se da tanto en la etapa de reproducción adulta y liberación larval (aporte parental), como en el mismo periodo larval. Dentro del paradigma evolutivo, un organismo es exitoso cuando su progenie es exitosa. Por lo tanto no es extraño encontrar que los organismos adultos realicen migraciones a sitios de resguardo para liberar su progenie, o sincronicen su puesta con ciclos de producción o advección propios de su ambiente que potencien una mayor sobrevivencia larval, siendo que esta etapa es considerada la más susceptible a los cambios ambientales.

En el caso de los organismos bentónicos, su movilidad tiene en general ciertas restricciones, y por lo tanto también tiene limitaciones la habilidad parental para liberar a los propágulos en sitios más adecuados. Sin embargo, existe una gran variedad de estrategias de acoplamiento temporal de la liberación larval con ciclos mareales, día/noche, quincenales, estacionales; que permitirían aumentar las posibilidades de alimentación, evasión de predadores o aprovechar condiciones de advección favorables para las larvas. Este acoplamiento no es independiente de las capacidades natatorias y requerimientos fisiológicos y nutricionales de los propágulos. Así, larvas con altos requerimientos nutricionales pueden ser liberadas durante períodos de máxima producción estacional. En otros casos, algunos organismos sincronizan la liberación larval con períodos de mínima presencia de predadores, mientras que en el ambiente intermareal la liberación durante

períodos de marea vaciante tendería a evitar el varamiento de las larvas en la costa. Durante las primeras etapas de desarrollo, los organismos pueden tener control sobre su natación vertical, como ocurre frecuentemente en larvas de crustáceos. Dado que los mayores gradientes ambientales (alimento, temperatura, oxígeno disuelto, distribución de competidores/predadores, dirección de corrientes) se encuentran generalmente en profundidad, la capacidad natatoria vertical de las larvas les otorga una posibilidad propia de influir en su sobrevivencia y retorno al hábitat donde asentarse.

Concretamente en sistemas de borde oriental, como el de la Corriente de Humboldt, existen eventos de surgencia costera discontinuos con mayor frecuencia e intensidad estacional, forzados por el viento. El ascenso de aguas con altas concentraciones de nutrientes enriquece la capa fótica dando lugar a un aumento de la producción durante esos períodos. Sin embargo, en la capa superficial o capa Ekman, la advección costa afuera predomina durante estos períodos, pudiendo alejar a los propágulos de las zonas adecuadas para su desarrollo. Este concepto de efectos contrastantes entre producción y advección en sistemas de surgencia costeros, se ha desarrollado considerando organismos de liberación larval pelágica y costera con distribución superficial de huevos y/o larvas. En estos organismos, se observan períodos de liberación desfasados con las máximas intensidades de surgencia, o migraciones en peces pelágicos hacia zonas de resguardo. Se ha reconocido, además, que la profundidad de liberación de huevos tiene una influencia importante en su transporte. En adultos bentónicos de diferentes ambientes (intermareal, submareal, profundo), la profundidad de liberación influirá sobre las condiciones iniciales que encuentren los propágulos en el ambiente. De la misma manera, la capacidad natatoria de los organismos puede mantener/alterar ese contraste.

Al igual que en otros sistemas de borde oriental, en el sistema de la Corriente de Humboldt existe una zona mínima de oxígeno sub-superficial. Estas zonas, en general, tienen gran influencia sobre la estructuración de comunidades pelágicas y bentonicas (Madhu et al., 2003; Gallardo et al., 2004). Durante su ciclo de vida complejo, los crustáceos adultos bentónicos que habitan sedimentos bañados por las Aguas Ecuatoriales Sub-Superficiales (AESS), tales como el langostino colorado *Pleuroncodes monodon* (Gallardo et al. 2004), liberan larvas pelágicas en un ambiente hipóxico. Durante los primeros días de vida libre, las larvas planctotróficas requieren fuentes de energía externa

(alimento) y la posibilidad de metabolizarlas. Se desconocen las capacidades fisiológicas de los primeros estadíos larvales de estos grupos profundos y es posible que el hábitat parental no sea el más propicio para su desarrollo. Existen muchas hipótesis a cerca de por qué los estadíos larvales son mantenidos en organismos de ciclo de vida complejo (Pechenick, 1999; Strathmann et al., 2002), pocas de ellas tienen sólido soporte empírico y en todo caso, no se han encontrado explicaciones generales. Una de estas hipótesis es que la migración ontogenética hacia el hábitat pelágico durante el estadio larval, permite encontrar un hábitat más propicio de desarrollo. Las larvas de muchos crustáceos decápodos parecen especialmente sensibles a las bajas concentraciones de oxígeno (Miller et al., 2002 entre otros) por lo que la presencia de la zona de mínimo oxígeno puede limitar la distribución y migración vertical imponiendo restricciones al uso de esta estrategia que permite evitar advección direccional durante períodos de surgencia (Peterson et al., 1979; Wing et al., 1998).

La hipótesis general de este trabajo es que la estacionalidad en el período de predominio de surgencia costera y ascenso de aguas pobres en oxígeno sobre la plataforma de Chile Central, influyen sobre los períodos reproductivos de los organismos bentónicos con ciclos de vida complejo. El grado de influencia depende de la capacidad natatoria y fisiológica específica de las larvas. De acuerdo a esta idea, especies que liberan sus larvas en rangos batimétricos afectados diferencialmente por el proceso de surgencia costero, presentarán desfases conspicuos en sus períodos de liberación, distribución (vertical y horizontal) y características fisiológicas de las larvas.

A fin de inferir las relaciones entre las características físicas de la zona, el ciclo de vida de las especies y sus implicancias para el transporte y retención larval, se plantearon los siguientes objetivos:

1) Determinar la distribución horizontal y vertical de estadíos larvales y estimar los períodos de liberación larval de especies de crustáceos decápodos de diferentes ambientes, en la plataforma de Chile Central,

2) Determinar el efecto de los factores: temperatura, oxígeno disuelto y disponibilidad de alimento en la sobrevivencia, tiempo de desarrollo y crecimiento de las zoeas tempranas de *P. monodon*.

3) Construir un modelo conceptual que relacione los períodos reproductivos, la distribución vertical larval y sus tolerancias fisiológicas, para el sistema de la Corriente de Humboldt en Chile central.

El primer objetivo fue abordado mediante el análisis de la distribución vertical de pequeña escala en larvas de seis grupos de crustáceos decápodos en el Golfo de Arauco (Manuscrito (Ms.) 1), y el análisis de la distribución de estadíos de los mismos grupos, en el plano horizontal y vertical a escala de la plataforma de Chile Central entre los 35 y 37° S (Ms. 2). Se colocó un énfasis especial en el caso de *P. monodon* (Ms. 3).

El segundo objetivo fue abordado mediante el desarrollo de experimentos de laboratorio con larvas de *P. monodon*, en los cuales se mantuvieron cultivos bajo diferentes condiciones y se midieron tasas de desarrollo, crecimiento, respiratorias (entre otras) (Ms. 4 y 5).

El tercer objetivo se abordó con experimentos de simulación (Ms. 3 y Ms. 6). La información obtenida en estos ejercicios se utilizó para construir un modelo conceptual que se presenta en la discusión de esta tesis.

2.- MATERIALES Y METODOS

2.1.- Generalidades del área de estudio.

La zona de estudio comprende la plataforma costera de Chile centro-sur entre los 35 y 37°S y forma parte del sistema de borde oriental de la Corriente de Humboldt (SCH) (ver Strub et al., 1998 para mayor detalle acerca de la circulación general del sistema). En estas latitudes, el período de primavera-verano, es caracterizado por la presencia de surgencias forzadas por viento S-SW, con transporte superficial fuera de costa de aguas de orígen subantártico (Agua Sub Antartica, ASAA), e intrusión sub-superficial de aguas con bajo contenido de oxígeno y alta salinidad (Alarcón, 1970; Ahumada y Chuecas, 1979; Arcos y Navarro, 1986; Strub et al., 1998; Leth & Middleton, 2004). Durante el invierno, existe dominancia de viento N, con corrientes superficiales de subducción hacia la costa, y retiro de las Aguas Ecuatoriales Sub-Superficiales (AESS) de la zona costera (Alarcón 1970, Ahumada & Chuecas, 1979).

La localización específica de los centros de surgencia y alta productividad en la costa de Chile, así como los patrones de circulación y transporte a menor escala, dependen de la forma de la costa y su batimetría (Figueroa & Moffat, 2000; Sobarzo, 1999). Concretamente en el área de estudio, la orientación de la costa y la topografía de fondo cambian marcadamente en un pequeño rango latitudinal. La plataforma continental frente al Golfo de Arauco y la plataforma del Itata (figura 1), es la más ancha a lo largo de Chile. Esta se haya atravesada transversalmente a la costa por el profundo y angosto cañón del BioBio. Frente al Golfo de Arauco se presenta un importante cambio en la orientación costera (Figueroa & Moffat, 2000). Al norte de la plataforma del Itata, el cañón del Itata, más ancho y menos profundo limita la zona de amplia plataforma. Hacia el norte, esta última vuelve a tener menos de 30 kilómetros de ancho. Un importante centro de surgencia casi permanente se ubica al este (E) del Golfo de Arauco, en Punta Lavapié. Tanto dentro del Golfo de Arauco durante períodos de verano, como sobre la Plataforma del Itata se han detectado aguas AESS ricas en nutrientes (Alarcón, 1970; Djurfeldt, 1989; Sobarzo et al., 1993). La intrusión de esta agua hacia la costa y su extensión sobre la plataforma podría realizarse desde los cañones (Sobarzo & Djurfeldt, 2004) y es la responsable de la alta productividad de la zona. Las características topográficas y geográficas descritas parecen

estar relacionadas, además, a la formación de giros y filamentos recurrentes que se desarrollan en el área (Cáceres, 1992). Todo este conjunto de características tiene la potencialidad de generar intrincados patrones de transporte.

2.2.- Especies objetivo.

El comportamiento larval difiere entre especies de crustáceos e incluso entre diferentes estadíos dentro de una especie. Por lo tanto en este estudio, se eligieron seis taxa con diferentes características, tanto en los estadíos larvales (tiempos de desarrollo y número de estadíos) como en el hábitat que ocupan los adultos. Se incluyó en el análisis cinco especies y una familia. La identificación a nivel de grupo (familia) en lugar de identificación específica puede ser útil cuando los componentes comparten ciertas características como el rango de hábitat adulto (Wing et al., 1998) y no se cuenta con claves de identificación más detallada. Se estudiaron dos especies cuyos adultos provienen del intermareal arenoso (Jaramillo, 1978): *Emerita analoga* (identificadas de acuerdo a Johnson & Lewis, 1942) y *Blepharipoda spinimana* (identificada de acuerdo a Concha (1978) y la colección de ejemplares proveniente de las campañas descritas a continuación). Del sub-mareal somero se eligieron las larvas del 'nape' *Neotrypaea uncinata* (descritas por Aste, 1982). El desarrollo larval de *N. uncinata* consta de cinco estados larvales y una postlarva. Se identificó *Pagurus* spp en base a las descripciones de larvas de *Pagurus edwardsi* (Lavados, 1982); este grupo presenta cuatro zoeas y una postlarva. Los adultos de *Libidoclaea granaria* (Majidae), quinta especies estudiada, se han reportado debajo de los 50 m y hasta 400 m de profundidad (Retamal, 1981), donde son comúnmente capturados como pesca incidental en la pesquería del langostino colorado *Pleuroncodes monodon*, sexta especie analizada. *L. granaria* presenta dos estadíos larvales únicamente y una megalopa (Faguetti, 1969). Especial énfasis se hizo en larvas de *Pleuroncodes monodon* (Galatheidae), con adultos también provenientes de profundidad, cuyos estadíos larvales son numerosos (Faguetti y Campodonico, 1971), y su desarrollo en el plancton dura más de dos meses (Cañete, 1994). La fracción adulta de *P. monodon* es posiblemente el crustáceo más estudiado de la plataforma de Chile Centro Sur dada la pesquería que ha sustentado desde los años sesenta (Roa & Bahamonde, 1993), y las múltiples evaluaciones de las cuales ha sido objeto (Roa et al., 1997; Canales et al., 1997, 2002). Los estudios de

prospección de la población indican que frente a Concepción (al N del Cañon del río BioBio), se haya un área donde predominan los reclutas que ingresarían a la población bentónica principalmente en los meses de marzo y abril (Gallardo et al., 1994). Hacia el N y S se extienden dos ramas, siendo la rama N la que ha experimentado mayores variaciones en abundancia y extensión a lo largo de la historia de la pesquería. En esta rama N, los promedios de talla de los organismos (y por tanto su edad) aumenta hacia el norte, habiéndose postulado que existe una migración desde el área de reclutamiento hacia esa zona (Roa & Tapia, 2000). No se ha registrado una asociación estadística significativa del recurso con las concentraciones de oxígeno de fondo (Ernst, 1996), pero sí se ha determinado que ocurren en concentraciones bajas, conjuntamente con praderas de *Thioploca* sp (Gallardo et al., 1994). En la zona de Concepción las larvas se han encontrado en el plancton entre los meses de junio y diciembre, con abundancias máximas en noviembre (Palma, 1994; Cañete, 1994). La duración de la fase larval fue estimada por Fagetti y Campodonico (1971) quienes hicieron la primera descripción del desarrollo de *P. monodon* en laboratorio (a 15 y 20º C) y estimaron períodos cercanos a los dos meses hasta el estadío de zoea V para condiciones de alimentación *ad libitum.*

2.3.- Muestreos.

Se realizaron cruceros oceanográficos que permitieron analizar dos diferentes escalas espacio-temporales. Los muestreos en pequeña escala fueron desarrollados en el Golfo de Arauco durante Diciembre 2000 (Fig. 1 MS. 1). El fin fue identificar cómo la distribución vertical de las especies objetivo y la circulación predominante (surgencia y marea semidiurna) resultaban en transportes larvales de entrada o salida del Golfo de Arauco. La presencia de la isla Santa María divide la entrada de aguas al golfo en dos: la Boca Chica, angosta y somera al oeste, y la Boca Grande, más ancha y profunda al N por donde ocurre el mayor intercambio de agua. El primer muestreo de pequeña escala consistió en una estación fija en la Boca Chica, donde durante los días 8 y 9 de diciembre se colectaron muestras de plancton en tres profundidades (5, 10 y 15 m). Las muestras fueron colectadas desde la embarcación anclada, con una red tacker trawl (1 m^2 de apertura de boca y malla de 300 μm) a la deriva, provista con un flujómetro calibrado General Oceanics. Los lances fueron realizados en intervalos de 2 horas aproximadamente durante

22 horas. Las muestras se fijaron en formalina al 10%, y en laboratorio fueron identificadas las larvas de las especies objetivo. Además, se obtuvieron perfiles horarios de temperatura, conductividad y densidad con un perfilador SeaBird SBE19, equipado con un sensor de oxígeno. Los perfiles de corriente fueron obtenidos con un perfilador de corrientes acústico doppler (ADCP, 307 KHz workhorse RD Instruments). Se obtuvieron datos cada 1m de profundidad y se tomaron las velocidades medias cada media hora. También se obtuvieron datos de viento en Punta Lavapié a intervalos de 10 minutos (37º10'S, 73º35'W).

El segundo muestreo de pequeña escala se realizó en la Boca Grande del Golfo de Arauco, y se obtuvieron tres perfiles adaptativos de zooplancton, cuyos estratos se definieron en base a los perfiles hidrográficos obtenidos previamente. Fueron realizados principalmente durante horas del día y su objetivo fue identificar la asociación de diferentes organismos con los tipos de agua que circulan a través de esta boca del Golfo de Arauco durante un período de surgencia. Las muestras de zooplancton se procesaron como las anteriores y también se obtuvieron perfiles de corrientes conjuntamente con los perfiles hidrográficos.

A mayor escala de cobertura espacial, se realizaron dos cruceros en noviembre 2001 y marzo 2002 (Figura1-MS 2). Dichos cruceros tuvieron como fin determinar la distribución de estadíos larvales en el espacio (tridimensional), su relación a las estructuras físicas presentes e inferir los períodos de máxima liberación larval en base a la estructura de estadíos de cada especie. Los cruceros correspondieron al período de surgencia intenso (noviembre) y período de reversión (marzo). Las estaciones se distribuyeron en una grilla de 69 puntos, con 6/7 estaciones equidistantes sobre 11 transectos entre los 35 y 37ºS. Estos se extendieron entre la costa y las 35 mn. Las muestras de zooplancton estratificadas se tomaron con red tucker trawl. Se tomaron muestras en estratos 0-25; 25-50; 50-75; 50-100; 100-150; 150-250m (dependiendo de las profundidades de las estaciones) que fueron fijadas en formalina. En ambos cruceros y estaciones se midió T, S, oxígeno disuelto, pigmentos fotosintéticos (CTDO SBE19). Durante el segundo crucero se navegó con un perfilador acústico (ADCP) que permitió medir perfiles de corriente hasta los 150m de profundidad. Las larvas de las seis especies objetivo fueron separadas e identificadas a nivel de estadio.

2.4.- Análisis de datos de terreno.

Estudios de pequeña escala espacio temporal. En la Boca Chica del Golfo de Arauco, se analizó la distribución horaria de abundancia y la distribución vertical de las larvas objetivo a lo largo del día, en relación al ciclo diario y de marea, mediante el ajuste de funciones sinusoidales uni o bimodales. Se estimaron sus parámetros y se analizó la contribución de cada componente al patrón observado. Estas mismas funciones fueron aplicadas a los datos de corriente *u* a diferentes profundidades. Además, se calculó un índice de flujo larval, y de movimiento relativo de los organismos en relación a la columna de agua.

En la Boca Grande del Golfo, alternativamente, se obtuvo un índice de preferencia de los organismos por un determinado tipo de agua, definido en base a la concentración de oxígeno del estrato, mediante el análisis de cuociente (Van der Lingen et al., 1999).

Estudios de mayor escala espacio temporal. Este estudio se focalizó en el análisis de la estructura de 'edades' (estadíos larvales) de las zoeas de cada especie objetivo y la distribución espacial de las larvas y estadíos en función de las estructuras hidrográficas y potencial transporte. Para los grupos estudiados (por estadío) se estimó: 1) Número de individuos por $100m^2$ para cada estación (integrado a la profundidad máxima muestreada en cada una); 2) densidad media y varianza en el área de estudio; 3) El porcentaje de estaciones positivas del total analizado; 4) el coeficiente de variación: desviación estándar/media (agregación). 5) distancia media fuera de costa en cada transecta; 6) profundidad media en cada estación; 7) centro de masa latitudinal.

En el caso del langostino colorado, se construyeron mapas geoestadísticos de distribución en dos estratos 0-50 y 50-100 y se aplicó adicionalmente este método para estimar abundancias totales.

Se aplicaron modelos lineales (LM) para evaluar el efecto del ciclo día/noche, el área y la distancia a la costa sobre la profundidad media de distribución de los diferentes estadíos larvales considerados.

Se calcularon anomalías geopotenciales y velocidad geostrófica corregida para zonas someras en base a los datos hidrográficos de ambos cruceros.

2.5.-Simulaciones: experimentos de advección.

Para estimar si existe una alta pérdida de organismos por advección en la superficie durante períodos de surgencia, se llevaron a cabo las simulaciones utilizando la escasa información de corrientes con que se cuenta en la zona.

Se realizaron experimentos de seguimiento de larvas en campos de advección superficial en la zona de Chile Centro Sur. Estos campos de adveccion superficial fueron obtenidos por Navarro el al. (2004) y utilizados como malla para estimar el potencial dispersivo de larvas liberadas en diferentes zonas sobre la plataforma, en superficie. En el caso del langostino colorado, se evaluó el potencial éxito de reclutamiento en el área de asentamiento bentónico (detalles en manuscrito 3).

También se utilizaron datos publicados de corrientes medias y desvíos estándares para hacer el seguimiento de larvas sobre la plataforma del Itata a tres profundidades diferentes (superficie, media agua y fondo) que presentan importantes diferencias en sus componentes *u* y *v*.

2.6.- Experimentos de sobrevivencia, desarrollo y crecimiento de larvas de *P. monodon.*

Dos factores hidrográficos que tienen marcados gradientes en el ambiente ocupado por larvas de *P. monodon*, son la temperatura y el oxígeno disuelto. Su efecto, conjuntamente con el de la carencia de alimento durante los primeros días de vida fue evaluado a través de experimentos de laboratorio.

El cultivo de larvas de *Pleuroncodes monodon* se realizó a partir de hembras ovíferas capturadas en el ambiente natural por barcos pesqueros que realizaron pescas de investigación durante los años 2002 y 2003 en la zona Centro-Sur de Chile. Las hembras ovíferas se transportaron hasta la Estación de Biología Marina de Dichato, donde se mantuvieron en oscuridad, salinidad constante (34 ± 0.5) y temperatura controlada (11ºC). Un mayor detalle acerca de los métodos de cultivo y estricto diseño experimental se encuentra en el manuscrito 4.

Con las larvas recién eclosionadas, se realizaron experimentos de crecimiento, desarrollo y sobrevivencia a temperaturas de 11, 15 y 20 ºC. Existían datos publicados acerca del tiempo de desarrollo y mortalidad de larvas de langostino colorado a 15 y 20°C

(Faguetti & Campodonico, 1971), pero no datos de crecimiento. Las curvas de dependencia del tiempo de desarrollo en función de la temperatura en crustáceos suele ser exponencial (Anger, 2001). Para poder parametrizar esa dependencia en *P. monodon* fue necesario ampliar el rango de temperaturas hasta 11° C, porque es a esa temperatura en que eclosionan los organismos en las Aguas Ecuatoriales Sub-Superficiales.

También se cultivaron larvas recién eclosionadas en tres condiciones de oxígeno: normoxia, $1ml \cdot l^{-1}$ y $0.7\ ml \cdot l^{-1}$ a 11ºC, hasta el estadio de zoea II. Cada día, se revisaron los cultivos para anotar número de sobrevivientes y número de larvas que mudaron. Se calculó la mortalidad acumulada de organismos al segundo estadío, y también la edad a la cual el 50% de los individuos mudo a zoea II en 1 ml/l y en normoxia a través de modelos logísticos. Éstos fueron comparados para determinar si un único modelo podía explicar ambos comportamientos o cada uno obedecía a su propia curva. Estas comparaciones fueron realizadas a través del índice de Akaike (Akaike Information Criteria-AIC, Burnham & Anderson, 2002).

Las larvas de *P. monodon* son liberadas en bajas concentraciones de oxígeno aunque no necesariamente pasen todo su desarrollo de zoea I en esas condiciones. Se condujeron experimentos de exposición inicial a bajas concentraciones de oxígeno, para evaluar su efecto en el tiempo de desarrollo y sobrevivencia hasta el estadio II. Las larvas fueron mantenidas 1, 2, 3, 4 ó 5 días en 1 ó 0.5 ml/l y posteriormente transferidas a normoxia, donde fueron seguidas hasta su muerte o metamorfosis. Se calculó la frecuencia de muda bajo cada tratamiento, y luego se identificó si la sobrevivencia al segundo estadio dependía linealmente de la cantidad de días de exposición inicial a bajo oxígeno. También se analizó la forma y magnitud de la relación entre la edad de muda y los días de exposición inicial a bajo oxígeno.

El tiempo de desarrollo y sobrevivencia de larvas bajo inanición inicial fue estudiado mediante experimentos de puntos de no retorno. Las larvas fueron mantenidas inicialmente sin alimento y secuencialmente trasferidas a contenedores con alimento donde fueron seguidas para determinar su sobrevivencia y la edad a la cual mudaban al segundo estadío. El día al cual el 50% de las larvas no sobrevivían al segundo estadío fue determinado a través de una función logística.

Este tipo de experimento también se realizó en combinación con el factor oxígeno sometiendo grupos de organismos a inanición y bajas concentraciones de oxígeno inicial, para luego ser transferidas a normoxia y brindarles alimento. Se ajustaron modelos logísticos para determinar si el punto de no retorno dependía también de la concentración de oxígeno.

El crecimiento fue cuantificado en función del incremento en peso seco a lo largo del tiempo. Se obtuvo a partir de organismos tomados en diferentes tiempos, liofilizados y luego pesados en microbalanza. Las condiciones de cultivo fueron: 3 temperaturas (11, 15 y 20° C), y tres condiciones de oxígeno a 11°C: normoxia, 1 y 0.7 ml/l. Se parametrizaron las tasas de crecimiento y se compararon los modelos de los diferentes tratamientos a través del AIC.

2.7.- Composición elemental, respiración y actividades enzimáticas en larvas de *P. monodon.*

Los aspectos observados a un nivel de sobrevivencia y tiempos de desarrollo, tienen su base en mecanismos fisiológicos. Estos experimentos permitieron explorar algunas respuestas fisiológicas y bioquímicas de *P. monodon* a corto y largo plazo a bajas concentraciones de oxígeno.

Grupos de larvas cultivadas en normoxia y 1 ml/l fueron congeladas en nitrógeno líquido a diferentes días de su desarrollo a través del estadío de zoea I y hasta recién mudadas a zoea II. Se liofilizaron y se analizó el contenido de C y N y la razón C/N.
El efecto de la edad y el nivel de oxígeno de cultivo sobre el contenido en µg de N y C fue estudiado con un análisis de varianza ortogonal de dos vías.

Se midió la respiración de zoeas I a megalopa en normoxia y bajo oxígeno tanto en organismos sin alimentarse por 24 hrs como en organismos recién alimentados. Se estimo el consumo de oxígeno peso-específico para cada uno y, en el caso de megalopas y zoea V, el grado de oxiconformación / oxiregulación (ver procedimientos detallados en el manuscrito 5).Un análisis de varianza de dos factores: nivel de oxígeno y condiciones de alimentación fue realizado para comparar las tasas medias de respiración individual para cada estadio. Este análisis se siguió con un análisis post-hoc de mínimas diferencias cuadradas (LSD) (Sokal & Rholf, 1995). También se ajustaron curvas de tasas respiratorias

peso-específico en función del peso seco individual para organismos cultivados en normoxia y en hipoxia.

La actividad específica aparente de lactato dehidrogenasa (LDH) y malato dehidrogenasa (MDH) fue obtenida en triplicados para grupos de larvas cultivadas en alto y bajo oxígeno, y congeladas en nitrógeno líquido en diferentes días durante su desarrollo. Los procedimientos utilizados fueron detallados por González (2002) y citados en el manuscrito 5. La actividad específica aparente fue expresada en μmol min^{-1} (UI) y estandarizada por peso seco y mg de proteína. El peso seco había sido determinado en paralelo para cada muestra, ya que para cada día en que se muestrearon los cultivos se tomaron dos muestras. El contenido de proteína fue calculado a partir de los valores de N obtenidos del análisis de composición elemental y ecuaciones publicadas para esta relación en crustáceos anomuros (Anger & Harms, 1990). Estas determinaciones habían sido realizadas en las muestras pareadas de las determinaciones enzimáticas.

Un diseño de análisis de pendientes separadas se utilizó para comparar la actividad de citrato sintasa (CS), LDH y MDH en función del nivel de oxígeno (predictor categórico), y edad/peso seco individual (como predictores continuos). Se utilizaron datos transformados con un logaritmo y estandarizados por peso seco/ contenido de proteínas/ individuo dependiendo del caso. El mismo diseño se aplicó para comparar la relación entre LDH y MDH y la dependencia de la relación LDH: CS con la edad y nivel de oxígeno, y LDH:CS en función del peso individual y oxígeno.

2.8.- Simulacion energética/pérdida.

Finalmente, si dependiendo de la capacidad larval de sobrellevar las bajas concentraciones de oxígeno, el retardo en el desarrollo y el crecimiento reducido debido a menores temperaturas y menor oxígeno es balanceado por una mayor retención a nivel sub-superficial, entonces, se espera que la sobrevivencia general en cualquier escenario será mayor para organismos con menores puntos críticos. Solamente en estos organismos, el periodo de 'verano' de surgencia resultará en mayor sobrevivencia.

Para explorar esto, se generaron escenarios que consistieron en secciones hipotéticas de temperatura (T), oxígeno disuelto (OD) y K (‘difusion turbulenta’) (ver detalles en MS 6) con valores típicos de verano e invierno y se realizaron experimentos de simulación

individuo basados (IBMs), donde se liberaron larvas (400) al azar en el espacio de la sección oceanográfica, y se las dejó desarrollar en ese ambiente para evaluarse al final del desarrollo la sobrevivencia en las diferentes capas. Cada larva tenía como cualidades iniciales una determinada biomasa y su punto crítico: la concentración de oxígeno por debajo de la cual, a una determinada temperatura, su tasa respiratoria se reduce. Aquí lo utilice para definir una variable de la cual depende el crecimiento y la mortalidad.

Los procesos biológicos simulados fueron:

1) movimiento y pérdida de organismos (se utilizaron los coeficientes de difusión turbulenta para generar un 'camino aleatorio'. Los coeficientes fueron mayores en superficie).

2) crecimiento (función del peso individual, la temperatura y el oxígeno).

3) desarrollo (dependiente de la temperatura).

4) mortalidad (función exponencial negativa del peso, y dependiente del punto crítico (PC)).

En cada paso de tiempo *t*, cada larva toma las características del ambiente en el sitio donde se encuentra, se mueve, se desarrolla, crece y muere o sobrevive. En el tiempo t_{+1}, vuelve a tomar las características del nuevo sitio donde se haya y sucesivamente hasta completar el desarrollo o morir/perderse del campo del modelo.

Las variables monitoreadas son el peso de las larvas sobre o debajo de la termoclina a la metamorfosis, más la cantidad total de larvas perdidas o muertas sobre o debajo de la termoclina, y el número de larvas metamorfoseadas en un tiempo dado sobre o debajo de la termoclina.

Cada experimento consistió en liberar 400 larvas, con un punto crítico (que varió entre experimentos) en cinco escenarios oceanográficos (EO) diferentes. Se condujo un total de 55 experimentos. Los escenarios utilizados se detallan en el Manuscrito 6 (tabla 1). Los resultados se analizaron con modelos generales lineales.

3.- RESULTADOS

3.1.- Distribución vertical de larvas de decápodos en las entradas de una bahía abierta hacia el ecuador en Chile central: implicancias para el transporte. (Ms.1).

Durante el estudio conducido en el Golfo de Arauco los vientos fueron favorables a surgencia durante todo el período. En la entrada somera del Golfo de Arauco (Boca Chica), las velocidades de corrientes superficiales y a media agua mostraron una clara señal mareal semidiurna con salidas más fuertes en la superficie y entradas más fuertes en el fondo. La señal semidiurna dominó en superficie y mediagua y la diurna en profundidad. Las máximas velocidades negativas (salida del golfo) ocurrieron a medianoche y mediodia. En superficie y media agua, la temperatura y densidad mostraron variabilidad diurna con calentamiento superficial durante el día, pero gradientes débiles (Valle-Levinson et al., 2003).

La abundacia de *P. monodon* fue muy baja en Boca Chica y *L granaria* no estuvo presente. La abundancia de *B. spinimana* y pagúridos mostró un patrón bimodal a lo largo del muestreo (semidiurno con modulación diurna), los máximos ocurrieron durante la vaciante nocturna, y un segundo pequeño aumento ocurrió durante la vaciante diurna. *E. analoga* también mostró un patrón bimodal a lo largo del día con máximos en las vaciantes, pero sin modulación diurna. Para *N. uncinata*, solo la componente diurna fue significativa, con máximos en la noche. En general todos los estadíos de *N.uncinata* siguieron la misma forma (Fig. 3 Ms. 1).

En la vertical, *B. spinimana* se encontró más profunda durante las mareas vaciantes. Los pagúridos y *N. uncinata* por el contrario, se encontraron más someros en la columna de agua durante la noche. Los estadíos individuales de *N. uncinata* siguieron esa tendencia, aunque los últimos (IV y V), tendieron a ser más profundos que los tempranos. *E. analoga* no tuvo un patrón distinguible de distribución vertical a lo largo del día, pero fue la más somera de las especies consideradas en este caso.

Hubo un efecto significativo de la fase mareal en el flujo de larvas, con mayor influjo durante mareas llenantes y negativos durante las vaciantes en pagúridos y *N. uncinata*. Los estadíos I a IV individualmente fueron consistentes con ese comportamiento, mientras que el estadio V permaneció fluyendo hacia dentro durante todo el periodo. *B.*

spinimana mostró mayor influjo durante la vaciante nocturna (cuando estuvo más profunda), y *E. analoga*, mantuvo mayores velocidades negativas que la velocidad media integrada en la columna de agua durante la mayor parte del período de muestreo.

En Boca Grande (la entrada más profunda), la termoclina y haloclina estuvieron bien definidas en las tres ocasiones, los gradientes fueron más marcados que en la boca chica. La termoclina y oxiclina coincidieron entre los 10 y 20 m y la capa inferior tuvo características de Aguas Ecuatoriales Sub-Superficiales (Strub et al., 1998; Sobarzo et al., 2001). Durante los muestreos, la capa superior fluyó principalmente a favor del viento hacia el N, mientras que la subsuperficial lo hizo principalmente hacia el sur (Fig. 5 Ms. 1).

En esta boca *L. granaria* sí estuvo presente, y *P. monodon* comprendió una buena proporción de las larvas de crustáceos decápodos. Los grupos de larvas mostraron marcadas diferencias en la distribución vertical. *E. analoga* se encontró principalmente en la termoclina al igual que *B. spinimana*; *P. monodon* y *L. granaria* fueron más abundantes en aguas con bajo oxígeno y más profundas. *N. uncianta* estuvo en aguas intermedias al igual que los pagúridos. *N. uncianta* mostró diferencias entre sus estadíos: los tardíos se encontraron más profundos (Fig. 6 Ms. 1).

3.2.-Distribución horizontal a mesoescala y estructura de edades.

3.2.1 Oceanografía durante los cruceros. (Resumen físico de los Ms. 2 y 3).

El crucero de Noviembre 2001, comenzó luego de varios días de vientos favorables a surgencia. El viento predominantemente S persistió durante los primeros días de la campaña, y luego revirtió a N hasta el último día de muestreo (Fig. 2 Ms. 2). La presencia de surgencia hacia el norte de los 36°S se evidenció superficialmente en aguas más saladas y frías hacia la costa y en las secciones de densidad en la inclinación hacia la superficie de las picnoclinas en la zona costera. La velocidad geostrófica corregida para zonas someras durante noviembre, indicó flujos superficiales hacia el N en los transectos 1, 2 y 3, intensificados en el frente de quiebre de plataforma (Fig. 3 Ms. 3). Velocidades hacia el sur fueron estimadas a profundidades intermedias debajo de la picnoclina. En el cañón del BioBio se estimó un flujo hacia el N en la boca, y sur en la cabeza intensificado en la sub-superficie. Durante ambos cruceros la profundidad de la capa de mezcla fue más somera sobre la plataforma del Itata. Además, en ambos cruceros fue evidente la presencia de

Aguas Ecuatoriales Sub Superficiales. En superficie, a la altura del cañón del Itata, las aguas oceánicas siguieron el contorno de la plataforma continental. Las temperaturas medias indicaron condiciones más cálidas durante marzo 2002 (Fig. 3 Ms. 2). En este segundo crucero solamente dos días de viento norte interrumpieron los vientos dominantes del sur. Se observaron gradientes fuera de costa de temperatura superficial del mar en toda el área, haciendo evidente la surgencia costera con mayor intensidad en la terraza del Itata. La velocidad geostrófica en el área N indicó nuevamente dominancia de flujo hacia el norte en superficie, intensificado en el quiebre de plataforma (Fig. 3 Ms. 3). En la sub-superficie y fuera de costa (transecto 4) las corrientes mostraron signos negativos. El rango de velocidades v durante marzo fue mayor que en noviembre. Los rasgos generales de circulación observados con ADCP fueron congruentes con los patrones geostróficos aunque los valores absolutos difirieron y los valores no fueron tan homogeneos (Fig. 4 Ms. 3). Los valores de *v* en superficie (transecto 1) fueron positivos y en la sub-superficie negativos. Sobre el quiebre de plataforma (transecto 4) la dirección fue N, mientras que sobre la plataforma del Itata, *v* varió a lo ancho en una y otra dirección (Fig. 4 Ms. 3).

3.2.2 Distribución del meroplancton en la zona de surgencia costera en Chile Central (Ms. 2).

L. granaria fue liberada principalmente en primavera durante el período de surgencia intensificada. Durante noviembre las abundancias fueron máximas para la zoea I, y durante marzo dominó la zoea II (Tabla 1 Ms. 2). Este período de puesta coincide con el de *Pleuroncodes monodon*. Las zoeas I aparecieron ampliamente distribuidas especialmente durante la relajación y la zoea II apareció más próxima a la costa, especialmente durante vientos de surgencia (Fig. 8 Ms. 2). La aproximación hacia la costa de las zoeas II fue realizada en aguas sub-superficiales (Fig. 9 Ms. 2). No mostraron migraciones verticales y fue la especie más profunda de las estudiadas (Fig. 10 Ms. 2), conjuntamente con *P. monodon*.

E. analoga presentó mayores abundancias durante marzo, donde dominó la zoea I indicando un máximo de puesta fuera de fase con el período de surgencia (Tabla 1 Ms. 2). Las mayores concentraciones del estadio I se encontraron cercanas a la costa, los estadíos

intermedios fueron advectados fuera de costa (y desagregados) y el estadio V se acercó a la costa donde se agregó nuevamente (Tabla 1 y Fig. 9 Ms. 2). *E. analoga* fue muy somera y no presentó migraciones verticales, fue la especie más ampliamente distribuida en la horizontal (Figs. 4 y 10 Ms. 2).

B. spinimana estuvo restringida a las estaciones costeras. En noviembre se encontraron muy pocas zoeas, y durante marzo, todos los estadíos aparecieron en abundancias similares, indicando una liberación durante el verano (Tabla 1 Ms 2). Fue la especie más somera, pero solamente ocurrió en estaciones costeras (Fig. 7 y 10 Ms. 2) y la distribución en profundidad estuvo correlacionada con la profundidad de las estaciones.

Tanto los pagúridos como *N. uncinata* aumentaron su abundancia hacia marzo Tabla 1 Ms. 2). La distribución de estadíos indica que la máxima puesta ocurrió durante verano, ya que los estadíos intermedios dominaron marzo. En *N. uncinata*, la migración vertical con zoeas II a V más cercanas a la superficie durante la noche fue evidente en noviembre, así como la profundización de los estadíos de I a IV. Los pagúridos, mostraron la misma tendencia que fue significativa solamente para el estadio IV (Fig 10 Ms. 2). Los estadíos tardíos se acercaron a la costa en ambas estaciones (Fig. 9 Ms. 2) y además, en ambas especies y cruceros los estadíos tardíos fueron dominantes en la zona norte (Fig. 5 y 6 Ms. 2). *B. spinimana* durante noviembre mostró el mismo patrón latitudinal. La distribución vertical durante la ontogenia de *N. uncinata* y pagúridos, implica que estas dos especies más *L. granaria* y *P. monodon* deben ser altamente tolerantes a las bajas concentraciones de oxígeno. Más del 50% de los centros de distribución vertical de *N. uncinata*, pagúridos *L. granaria* y *P. monodon* ocurrieron en concentraciones de oxígeno y temperaturas debajo de $1ml \cdot l^{-1}$ y 11°C, respectivamente. En general, las zoeas ocurrieron entre la costa y el frente de surgencia (sobre el quiebre de plataforma) en la zona al N del cañón del Itata, excepto *E. analoga*. En resumen, las especies liberadas fuera de fase con las máximas intensidades de surgencia están distribuidas en aguas superficiales y aquellas liberadas durante la estación de surgencia más intensa se encuentran más profundas y ampliamente distribuidas en la columna de agua. Aquellas que migran verticalmente tienen un amplio periodo de liberación larval.

3.2.3.- Infiriendo el origen y destino de las larvas del langostino colorado de las distribuciones larvales observaciones y simulaciones de transporte basadas en el individuo. (Ms. 3).

En el caso de *P. monodon*, la abundancia larval encontrada en terreno fue casi dos ordenes de magnitud mayor en Noviembre que en Marzo. Durante Noviembre, hubo máximos de zoeas I y IV, se encontraron megalopas pero no juveniles mientras que en marzo, estos últimos fueron dominantes (Fig. 5 a y b Ms. 3). La liberación larval ocurrió a fines de-invierno-primavera, posiblemente con dos picos principales de liberación extendidos en esas estaciones. Ese patrón de liberación podría explicar conjuntamente con los períodos de desarrollo de las zoeas, las abundancias relativas de noviembre. En marzo, es el fin del período larval. Los máximos de zoea I en la plataforma del Itata durante noviembre, coincidieron aproximadamente con los focos de adultos descritos en la evaluación del recurso (Canales et al., 2002). Su abundancia fue mayor en capas profundas donde son liberadas. El estadio II, mostró un corrimiento hacia el sur de los parches. Hubo evidencia de concentración larval hacia el N de la plataforma del Itata, donde coincidieron máximos de todos los estadíos. Los estadíos posteriores mostraron un corrimiento hacia el norte de sus núcleos de abundancia, especialmente en superficie. En el área N, todos los estadíos se concentraron en las estaciones costeras, limitados por el frente de surgencia, mientras que al sur del cañón del Itata, las larvas se encontraron más esparcidas en la dirección costa-océano (Fig. 6a Ms. 3). En marzo, todos los núcleos importantes ocurrieron sobre la terraza del Itata. Las megalopas y juveniles mostraron migraciones verticales nocturnas, los juveniles en particular desaparecieron de la columna de agua durante el día. En el N nuevamente, los pocos estadíos tempranos encontrados ocurrieron hacia la costa del frente de quiebre de plataforma (Fig. 6b Ms. 3).

Las simulaciones espaciales, si bien fueron realizadas en el contexto de contestar preguntas acerca de la dinámica poblacional del langostino colorado, tienen implicancias para comprender la dispersión potencial de larvas en la superficie de este sistema.

Con respecto al éxito de llegada de larvas de langostino a la zona de asentamiento, tanto el sitio de liberación como el tiempo de liberación fueron importantes. Tanto el área sur como central resultaron en mayor cantidad de larvas exitosas, aunque hubo grandes variaciones en el éxito con pequeñas diferencias en la distancia a la costa a la que fueron

liberadas. Las larvas exitosas se originaron más bien en zonas cerca de la costa, y no reclutaron individuos originados hacia el N de los 35°S. Hubo también grandes diferencias dependiendo del momento de la liberación, el número de éxitos aumentó cuando la liberación se realizó más avanzado el período de verano. Sin embargo, la tendencia no fue constante en todos los sitios. Entre Achiras y cañón BioBio se produce la mayor parte de las megalopas exitosas, aunque incluso Carranza puede aportar hasta un 10% (Tabla 6. Ms. 3).

Los tiempos de desarrollo temperatura dependiente indicaron desarrollos más cortos en el N, y una reducción del peso a la metamorfosis (Fig. 10 Ms. 3). En el sur especialmente hubo mucha variabilidad tanto en los tiempos de desarrollo y el peso a la metamorfosis ya que existió una gran variabilidad posiblemente asociada a la heterogeneidad del proceso de surgencia de aguas frías en la costa y su distribución en plumas de surgencia.

El seguimiento de partículas en varias profundidades indicó que sobre la plataforma del Itata, larvas en superficie serían advectadas mayormente fuera de costa y hacia el N, mientras que en profundidad lo serían hacia el S y fuera de costa. En profundidades intermedias ocurriría la mayor retención (Fig. 7 Ms. 3).

3.3.- Contribución relativa del oxígeno, la temperatura y los esquemas de alimentación a las tasas de crecimiento, sobrevivencia y tiempo de desarrollo de larvas de *Pleuroncodes monodon* cultivadas en laboratorio. (Ms. 4)

Las bajas temperaturas (11°C) redujeron la sobrevivencia larval un 40% en relación con la sobrevivencia a 15 y 20 ºC (Fig. 1a Ms. 4). La edad media de la muda al segundo estadío, se relacionó con la temperatura de acuerdo al modelo exponencial propuesto (Fig. 1b Ms. 4). Si bien la relación presentó para cada estadío diferentes interceptas, la pendiente fue común: -1.53. Mientras que la muda a zoea II ocurrió a una edad media de 7 días a 20°C, a 11°C ocurrió a los 17 (Tabla 3 Ms. 4). Utilizando estos resultados e información publicada, se estimó que la duración del período de desarrollo del langostino colorado, duraría más de 97 días a 11°C (edad a la cual llegaría al estadío V *sensu* Faguetti & Campodonico, 1971). El peso seco individual para cada temperatura estuvo relacionado a la edad por una función exponencial (Fig. 8 Ms. 4). La tasa de crecimiento (el exponente de la

función) fue similar para individuos criados a 15 y 20ºC, y menor en aquellos cultivados a 11°C. Sin embargo, el peso seco de las zoeas recién mudadas mostró mínimas diferencias entre temperaturas, los pesos obtenidos (media y desviación estándar) fueron: 23.4(5.1), 22.8(1.7) and 22.9(0.5) µg a 20, 15 y 11°C, respectivamente .

Cuando la zoea I se mantuvo en concentraciones de 1 y 0.5 $ml \cdot l^{-1}$, la sobrevivencia fue menor que en condiciones de normoxia. Menos del 15% mudó exitosamente al segundo estadío en concentraciones de oxígeno de 1 $ml \cdot l^{-1}$ mientras que a 0.5 $ml \cdot l^{-1}$ la sobrevivencia fue menor a 1% (Fig. 3b Ms. 4). Sin embargo, a pesar de que pocas zoeas pudieron mudar efectivamente al segundo estadio en estas condiciones, la tolerancia fue alta, algunas zoea I pudieron sobrevivir hasta 23 días en 0.5 $ml \cdot l^{-1}$, y aproximadamente el 50% pudo sobrevivir más de la mitad del estadio (Fig. 3a Ms. 4). La duración del desarrollo también se prolongó bajo condiciones de hipoxia, en concentraciones de 0.5 y 0.7 $ml \cdot l^{-1}$ los organismos mudaron a zoea II después del día 20, mientras que a 1 $ml \cdot l^{-1}$ la edad de muda el 50% de los organismos sobrevivientes fue de 21.3 días.

La sobrevivencia hasta el segundo estadío, disminuyó a mayor período de exposición inicial a bajo oxígeno (Fig. 4 y Tabla 4 Ms. 4). La tasa de mortalidad aumentó linealmente con el número de días de exposición, en un 3% por día. La edad a la muda hasta el segundo estadío, aumentó positiva y linealmente con el período inicial de exposición a bajo oxígeno (Fig. 5 Ms. 4). La edad a la muda, también dependió exponencialmente del valor de oxígeno al cuál eran originalmente expuestos los organismos: menor oxígeno, mayor tiempo de desarrollo (Tabla 5 Ms. 4).

Durante el primer estadío larval, el PNR_{50} fue estimado en 3.06 días. En este caso, la dependencia de la mortalidad con respecto a los días de exposición inicial fue logística. El tiempo de desarrollo también se alargó a mayores períodos de inanición. La inanición por sí misma tuvo un mayor efecto que las bajas concentraciones de oxígeno utilizadas como estresante inicial. Sin embargo, la inanición en bajo oxígeno, fue más perjudicial que la inanición en normoxia. El PNR_{50} fue, a 1 y 0.7 $ml \cdot l^{-1}$, 0.46 y 1.82 respectivamente. La edad media a la muda al segundo estadío se incrementó linealmente con el período de inanición-hipoxia original (Tabla 6 Ms. 4). Las tasas de crecimiento fueron más bajas progresivamente a menores concentraciones de oxígeno (Fig. 9 Ms. 4). El peso de las larvas

que sobrevivieron al segundo estadío difirió en más del 50% de aquellas criadas en condiciones de normoxia.

3.4.- Respuestas del metabolismo de larvas de *Pleuroncodes monodon* a los bajos niveles de oxígeno. (Ms. 5).

Tanto las tasas respiratorias de rutina como las post-prandiales fueron menores en bajas concentraciones de oxígeno para todos los estadíos larvales. El porcentaje de diferencia fue mayor en zoeas más jóvenes (87% zoea I, 47% megalopa). La respiración individual se incrementó en sucesivos estadíos pero la tasa respiratoria peso-específica decreció (Fig. 2 a y b Ms. 5). El último estadío de zoea mostró una disminución de la tasa respiratoria relativamente continua en relación al decline de la tensión de oxígeno, sin embargo la megalopa, mostró una tasa constante de consumo hasta niveles muy bajos, por debajo de los cuales disminuyó abruptamente.

Tanto el contenido de N como de C fue mayor en organismos cultivados en normoxia. Los porcentajes de N (6-9%) y C (31-35%) estuvieron dentro de los rangos esperados en larvas de crustáceos. La razón C:N en función de la edad, también siguió la forma de campana típica de este grupo de organismos, en valores también típicos alrededor de 4. La tasa de acumulación de N (proteínas) fue más baja en condiciones hipóxicas que normoxicas. A partir de la mitad del ciclo de muda aproximadamente, la acumulación de N se detuvo. Además, la razón C:N fue mayor en organismos criados en hipoxia (Fig. 1 Ms. 5).

El potencial aeróbico de organismos criados en hipoxia (actividad de CS estandarizada ya fuera por peso seco o contenido de proteínas), decreció solamente luego de la mitad del ciclo de muda (Fig. 4 Ms. 5), de la misma manera que el potencial metabólico máximo (utilizando la actividad de MDH como índice). El potencial anaeróbico: aeróbico, sin embargo, se tornó mayor en organismos cultivados bajo hipoxia, escalando positivamente con la edad y el peso seco en este grupo. La actividad de LDH aumentó con la edad y con el peso seco individual solamente en organismos criados en normoxia. Si bien dicha relación no se observó en organismos bajo hipoxia, su actividad fue en general mayor en este grupo (Fig. 5 a-d Ms. 5). MDH fue mayor en organismos en normoxia que en hipoxia independientemente de las unidades de estandarización (Fig. 5 e-h

Ms. 5). Las actividades MDH y LDH estuvieron positivamente correlacionadas (Fig. 6 a y b Ms. 5).

3.5.- Contrastes del desarrollo larval en una zona de surgencia: una aproximación basada en el individuo utilizando diferentes escenarios oceanográficos (MS 6).

Considerando todas las larvas en conjunto, la sobrevivencia en superficie fue mayor que debajo de la termoclina para todos los escenarios, excepto el escenario con alto oxígeno sub-superficial, en que la mayor sobrevivencia fue en profundidad (Fig. 1 Ms. 6) .

Los factores que más contribuyeron fueron el 'escenario oceanográfico' (EO), el punto crítico (PC) y la interacción EO-profundidad. En superficie, siempre se perdieron más larvas de las que murieron por mortalidad natural, mientras que en sub-superficie ocurrió lo contrario (Fig. 2 Ms. 6).

Los organismos tuvieron tiempos de desarrollo mayor en profundidad y durante los períodos de surgencia (Fig. 3 Ms. 6). El peso fue afectado por el punto crítico y la profundidad (Fig. 4 Ms. 6), resultando menor en profundidad. Los mayores pesos se encontraron en el escenario de alto oxígeno sub-superficial. La sobrevivencia dependió principalmente del PC, pero también del EO (Tabla 2 y 3 Ms. 6). Organismos con muy bajos PC tuvieron mayores sobrevivencias en todos los escenarios. En niveles intermedios de PC, la sobrevivencia mostró una dicotomía, mientras que en PC altos, la mortalidad fue mayor (Fig. 5c Ms. 6).

En invierno, considerando todos los organismos, la sobrevivencia fue mayor. Sin embargo, excluyendo aquellos con alto PC, la sobrevivencia se incrementó en todos los escenarios y se hizo mayor incluso en un escenario de surgencia de verano.

4.- DISCUSION.

4.1.- Patrones de distribución espacio-temporales.

El principal resultado de esta tesis, que ha sido integrado a partir de datos de campo, laboratorio y experimentos de simulación, es que el período de liberación larval en crustáceos decápodos en la zona de surgencia de Chile Central está estrechamente relacionado con el hábitat de liberación larval y la capacidad fisiológica y natatoria de las mismas. Si bien esto se ha planteado y discutido en la literatura actual, pocas veces se encuentra una compilación de resultados multidisciplinarios que apoyen estas afirmaciones en forma unificada. Paralelamente, se aportan resultados que sustentan: a) que a pequeña escala, procesos diarios como mareales juegan roles importantes en el transporte de organismos, incluso durante época de dominio de vientos sur; y que la interacción larval con procesos intra-diarios de pequeña escala podrían influir en la generación de patrones espaciales de mesoescala; b) que incluso a nivel superficial puede generarse retención sobre la plataforma de Chile Central durante períodos de surgencia; c) que las larvas liberadas en aguas sub-superficiales pobres en oxígeno, pueden depender de su rápido alejamiento del hábitat parental para su supervivencia, esta restricción quedaría liberada en organismos con capacidades de tolerancia mayores.

Los resultados obtenidos a partir de los experimentos conducidos a pequeña escala en el Golfo de Arauco tienen implicancias tanto para la comprensión de los mecanismos que están actuando sobre el transporte hacia dentro y fuera en el golfo, así como para las especies particulares estudiadas debido a sus comportamientos específicos.

El hecho de que el flujo de larvas hacia dentro y fuera del Golfo de Arauco a través de la Boca Chica, estuviera acoplado a las mareas, revela que a pequeña escala, intra-diaria, la marea superimpuesta en un patrón de circulación de dos capas generado durante vientos sur, jugaría un rol en la dispersión larval. El resultado de la advección hacia dentro o fuera, está fuertemente asociada a la profundidad de la larva ya que una regulación fina de la profundidad resulta en exportación o retención preferencial. La regulación a pequeña escala de la profundidad ha sido ampliamente estudiada en ambientes estuarinos, dominados por mareas, y se ha visto que resulta en transportes netos de entrada o salida (ver revisión por Epifanio & Garvine, 2001). Esto, finalmente se traduce en patrones espaciales conspicuos

de los estadíos larvales (Queiroga, 1996). En ambientes de surgencia costera, este proceso ha recibido poca atención aunque Parada (1999) y Valle-Levinson et al.. (2003) ya habían propuesto que los patrones de vientos diarios y el régimen mareal, forzarían patrones de recirculación en el Golfo de Arauco, que promoverían la retención de partículas. La circulación a través de las bocas del Golfo de Arauco se da en dos capas, sobre las cuales se imponen estas fluctuaciones intra-diarias. En la Boca Grande se vio que los organismos se segregan en diferentes capas de agua, que tienen en la sub-superficie la marca de AESS. La distribución específica a meso escala, refleja los patrones encontrados a pequeña escala.

Libidoclaea granaria y *P. monodon* (Palma & Arana 1997, presente trabajo) fueron liberadas principalmente en primavera (surgencia intensificada). Existe poca información acerca de la distribución larval de crustáceos profundos. Para estas larvas, la estación de surgencia podría implicar transporte hacia la costa, y por tanto retención y mayor alimentación. Esto es apoyado por los resultados: *L. granaria* zoea II se halló más cerca de la costa que la zoea I, especialmente durante el predominio de viento S. Además, en la Boca Grande del Golfo de Arauco, *L. granaria* apareció en aguas de bajo oxígeno, entrantes al golfo, y estuvo ausente de la Boca Chica. Su profundidad en general fue similar a la de *P. monodon* y mayor a la de los otros grupos. Garland et al. (2002), mostró que larvas de adultos bentónicos profundos podrían ser transportados a la costa en aguas sub-superficiales durante condiciones de surgencia en la plataforma continental del Atlántico N, y esto mismo se ha sugerido para larvas de peces y copépodos mesopelagicos en la corriente de Humboldt (Castro et al., 1993; Vargas & Castro, 2001; Landaeta & Castro, 2002).

Del otro extremo del hábitat adulto, *E. analoga* y *B. spinimana* mostraron patrones contrastantes. *E. analoga* se distribuyó ampliamente en la horizontal, incluso la zoea I aunque tuvo máximos costeros, se esparció ampliamente en el área de estudio. Fue somera, con una amplitud vertical limitada. *B. spinimana* estuvo restringida a las estaciones costeras, el rango de profundidad para esta última fue el más somero en el muestreo de meso-escala. Sin embargo, ambas especies, mostraron contrastes en su distribución vertical a pequeña escala, lo cual podría subyacer en parte este contrastante patrón horizontal. En la boca chica del Golfo de Arauco, *E. analoga*, fue transportada principalmente costa afuera en las aguas superficiales, mientras que *B. spinimana* fue transportada principalmente hacia dentro del golfo, al profundizarse durante horas de marea vaciante. *B. spinimana* tiene

larvas grandes y podría tener mayor capacidad natatoria que las jóvenes *E. analoga*. En estratos de muestreo amplios, pequeños ajustes en la posición vertical no serían evidentes. Se ha discutido que el transporte neto debido a la marea en organismos que no sincronizan su comportamiento con ella, sería bajo o nulo. Sin embargo, se ha visto que cuando se agregan componentes de difusión a la advección media, emergen patrones complejos de distribución (Richards et al., 1995). Esto recalca la necesidad de entender procesos en pequeña escala también y la relación de éstos con los patrones que emergen a mesoescala.

Adicionalmente, mientras que en marzo *E. analoga* liberó gran cantidad de larvas, *B. spinimana* no mostró dominancia de zoeas I. No está claro cuál es el mecanismo utilizado por *E. analoga* para que sus zoeas tardías retornen a la costa, como evidentemente ocurre. Sin embargo, se sugiere que el comportamiento debe jugar un rol, ya que la agregación decrece de la zoea temprana a las intermedias para luego aumentar nuevamente como zoea V. Este patrón de agregación a través de la ontogenia de peces ha sido atribuido a comportamiento activo (Bradbury et al. 2003). Los patrones contrastantes entre estas dos especies sugieren la fuerte presión que el ambiente advectivo, dadas características intrínsecas de la larva, puede ejercer sobre los patrones reproductivos y dispersivos de las especies.

Tanto a pequeña como a mediana escala, los análisis de distribución vertical mostraron ciertas tendencias en *N. uncinata* y pagúridos: al menos una migración vertical en los estadíos mayores y una profundización de los mismos. Además, la distribución temporal de sus estadíos no mostró predominancia de zoeas I en ninguno de los dos períodos de muestreo, pero sí un aumento en el número total de noviembre a marzo: la máxima liberación podría ocurrir durante el verano. Estas especies podrían ser objeto de transporte temprano fuera de costa y luego un retorno. Esto se resalta de los datos tanto de pequeña como de meso-escala.

El período de liberación larval observado para las presentes especies, conjuntamente con su distribución vertical, concuerda con la presentada por larvas de peces en áreas de surgencia (Olivar, 1990). Aquellas liberadas fuera de fase con la máxima intensidad de surgencia se distribuyen en la superficie, y aquellas que se liberan durante el período de surgencia más intenso, son más profundas y más ampliamente distribuidas en profundidad. Este patrón, se ve repetido entonces en taxones muy diferentes y denotarían el efecto que el

ambiente advectivo, a través de su influencia sobre la historia de vida temprana, puede ejercer sobre la dinámica de poblaciones adultas bentónicas. Además, las especies que muestran ciclos aparentes en su distribución vertical, parecen tener un período de liberación menos asociado con el principio o el fin del período de surgencia, quizás, su capacidad de regular su advección horizontal, a través del comportamiento vertical, les permita ser más independiente de los procesos físicos descritos, y por lo tanto, el período de puesta pueda sufrir mayor influencia de otros factores como temperatura y productividad. Además de la capacidad de migración vertical grupo específico, una gran tolerancia a las bajas concentraciones de oxígeno y temperatura, potenciarían estas estrategias.

Al menos *L. granaria*, *P. monodon*, pagúridos y *N. uncinata* deben ser altamente tolerantes a condiciones de hipoxia. Aunque en general pocas larvas han sido reportadas en zonas de mínima de oxígeno, que en general son consideradas perjudiciales (Anger, 2001). Los talasinideos adultos (Talassinidae) habitan en general tubos y sedimentos poco oxigenados (Miller et al., 1976), mientras que *L. granaria* y *P. monodon* lo hacen en la plataforma continental bañada por AESS. Aunque los estadíos tempranos no desplieguen el mismo rango de tolerancia fisiológica que los adultos, es probable que estos grupos hayan desarrollado alta tolerancia a la hipoxia ya que son liberados en aguas hipóxicas, luego de una incubación en aguas hipóxicas también. Esta capacidad les permitiría penetrar eventualmente, ocasionalmente o regularmente en aguas sub-superficiales, donde, a mayor profundidad la retención es aparentemente mayor. Solamente siendo altamente tolerante a esas condiciones, es que la retención sub-superficial proporcionaría ventajas de supervivencia.

4.2.- *P. monodon*: ecofisiología.

A pesar de eclosionar a bajas temperaturas y bajas concentraciones de oxígeno, *P. monodon* parece no poder completar su desarrollo bajo esas condiciones. El mayor tiempo de desarrollo que se encuentra en esas condiciones pueden prolongar además el tiempo de dispersión y la exposición a predadores pelágicos.

Las larvas deben adquirir energía externa para poder completar su ciclo de muda, si la cantidad de alimento no es limitante, entonces la disponibilidad de oxígeno podrá limitar

su capacidad de metabolizarla. La capacidad de permanecer en ambientes poco oxigenados depende de la eficiencia para mantener el suplemento de oxígeno, disminuir el metabolismo o utilizar metabolismo anaeróbico. Las estructuras necesarias para mantener un alto aporte de oxígeno parecen no estar desarrolladas en las zoeas tempranas; y el metabolismo anaeróbico en crustáceos recae principalmente en las vías glicolíticas que son poco eficientes en comparación con las vías aeróbicas como para suplir toda la energia necesaria en un período de crecimiento intenso como es el primer estadío larvario. Por lo tanto, una baja metabólica debe ocurrir, como es sugerido por las reducidas tasas de crecimiento y prolongación del desarrollo. La muerte puede ocurrir por la inhabilidad de conseguir suficientes reservas para mudar hasta el próximo estadío. En Chile central, donde eclosiona *P. monodon,* el alimento no es considerado limitante, por lo tanto, las bajas temperaturas y oxígeno encontrados en la zona de liberación pueden ser los mayores impedimentos para crecer y desarrollarse. Cuando las larvas de *P. monodon* son liberadas, existe ascenso de aguas sub-superficiales a la superficie. Esto, más la capacidad larval propia de nadar verticalmente, pueden asistir en la evasión de aguas muy hipóxicas. Las zoeas I en noviembre fueron encontradas en mayores cantidades en la sub-superficie, mientras que la zoea II y estadíos más adelantados aparecían preferentemente encima de los 50m de profundidad. Sin embargo, una considerable proporción de larvas aun se encontraban en aguas hipoxicas (debajo de los 50m). Los ciclos de surgencia-relajación o zonas de convergencia podrían transportar zoeas temporariamente entre las dos capas sin mayores consecuencias negativas inmediatas, pero sí probablemente alargando el desarrollo.

El predominio de procesos de surgencia durante el periodo de liberación larval de *P. monodon* podría resultar en la potenciación del transporte hacia la costa y superficie durante el desarrollo temprano. Como las velocidades de ascenso de aguas son bajas, la capacidad de soportar bajas concentraciones de oxígeno es necesaria en las zoeas tempranas. Si bien en superficie, la advección horizontal de las zoeas mayores puede exportar a las larvas de la zona costera, la duración de estas zoeas en el plancton puede ser reducida por mayores temperaturas y disponibilidad de alimento. Además, una moderada advección fuera de costa no sería perjudicial para especies de reclutamiento en plataforma, especialmente porque ésta puede estar limitada por frentes de surgencia o de borde de plataforma, y porque esta

especie en particular, parece poder prolongar su período de vida pelágico durante los estadíos juveniles.

Las zoeas I de *P. monodon* parecen depender fundamentalmente de la producción de energía aeróbica durante hipoxia, cuando las tasas respiratorias ya sea de rutina como post-prandiales aparecen reducidas con respecto a aquellas alcanzadas en normoxia. Además, la acumulación de proteínas se ve reducida en condiciones hipóxicas, lo cual sugiere o un decaimiento de la síntesis de proteínas general, o una utilización de las mismas para suplir el metabolismo anaeróbico. Esto es consistente con el hecho de que las larvas pueden sobrevivir varios días bajo hipoxia, e incluso crecer con lentitud, pero que la muda es altamente impedida a muy bajos niveles de oxígeno, y que deben salir de la zona de mínimo oxígeno, como se discutió anteriormente. Aun más apoyo para esta conclusión aparece en los resultados enzimáticos ya que el potencial aeróbico en hipoxia (medido como CS) baja solamente pasado la mitad del periodo de intermuda, al igual que el potencial metabólico máximo (MDH). El potencial anaeróbico/aeróbico sin embargo, se volvió mayor en organismos cultivados en hipoxia. Esto puede permitirles períodos de alta actividad muscular y natatoria. Este efecto, podría ser más perjudicial en zoeas tempranas que en megalopas, cuando aparecen compensaciones respiratorias.

4.3.- *P. monodon*: perspectiva ecológica.

Una de las potenciales causas de que los ciclos de vida complejo hayan sido mantenidos a lo largo de la evolución es el potencial aumento en la sobrevivencia que brindaría el desacoplamiento entre el hábitat adulto y el larval. Para eso, el ambiente larval debería ofrecerle ventajas a los propágulos con respecto al hábitat parental. Estas ventajas no son en general óptimas sino que tienen aparejadas costos. *P. monodon* es un claro ejemplo en el cual, para las larvas, evitar el hábitat adulto le proporciona mayor probabilidad de sobrevivencia ya que las condiciones ambientales en el fondo son fisiológicamente intolerables para las jóvenes zoeas. Si bien existen factores imponderables a los cuales estarán sometidas en el ambiente pelágico (i.e. el encuentro con parches de alimento, con predadores, con eventuales filamentos que las advecten fuera de costa, etc), en el ambiente adulto las condiciones hidrográficas son claramente letales para los estadíos

tempranos si están sometidos a ellas permanentemente. Breves exposiciones a estas condiciones ambientales no tendrían mayores consecuencias de sobrevivencia inmediata.

Algunos investigadores han sostenido que la selección natural mantiene largos períodos de vida larval en las historias de vida por sus ventajas dispersivas. Inspecciones más cercanas, han evaluado a la dispersión más bien como un producto secundario de la existencia de ciclos de vida complejo, y no como el resultado de selección *per se*. En el caso de *P. monodon*, la dispersión puede remover a las larvas de las áreas bentónicas donde pueden sobrevivir como juveniles o reproducirse como adultos, pero no como larvas. La dispersión, producto secundario o no, de la migración ontogenética hacia la columna de agua en este caso, le confiere la capacidad de colonizar hábitats extremos. Los largos períodos de desarrollo con numerosos estadíos larvales, son una característica frecuente en los galateidos, familia de anomuros a la cual pertenece *P. monodon*. Los galateidos, son conspicuos componentes de zonas de surgencia de borde oriental, mares con zonas mínimas de oxígeno (mar de Arabia) y 'vents' (fuentes termales). La permanencia en estos sistemas estaría dada por las tolerancias adultas, sin embargo, el desarrollo ontogenético temprano, cuyas limitantes fisiológicas estadío específico tienen componentes estructurales y filogenéticos, debe permanecer desacoplado del ambiente adulto.

De acuerdo a la regla de Thorson (Thorson, 1950), la duración larval se acortaría en organismos más profundos donde también habría mayor incidencia de lecitotrofía. Esa tendencia respondería a la escasez de alimento en profundidad para larvas pelágicas. Vemos aquí, que otros gradientes ambientales podrían jugar un rol justamente inverso, potenciando la colonización de hábitats profundos por especies con períodos larvales prolongados.

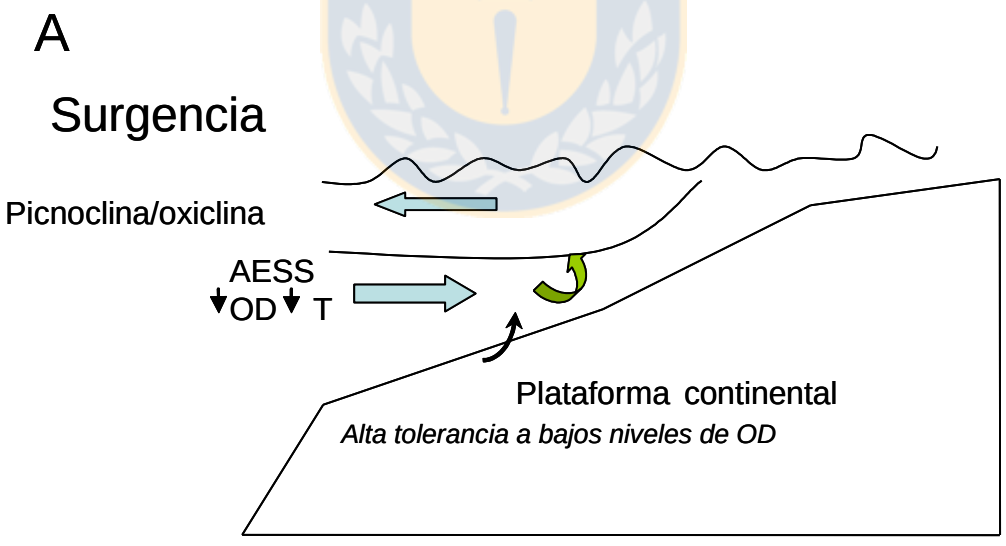
4.4- Modelo conceptual.

En la figura 1 se resumen los procesos considerados en esta tesis en el acoplamiento de los ciclos biológicos pre-reclutamiento y físicos.

Durante el periodo de surgencia, la liberación larval en profundidad se realizaría en aguas pobres en oxígeno y baja temperatura pero el transporte hacia la costa en la sub-superficie y su ascenso a la superficie durante dominancia de viento sur, potenciaría el escape de la zona de mínimo oxígeno hacia aguas también menos frías y ricas en alimento (Fig. 1 A). Estas larvas, tendrían distribución amplia costa afuera (amplia liberación en

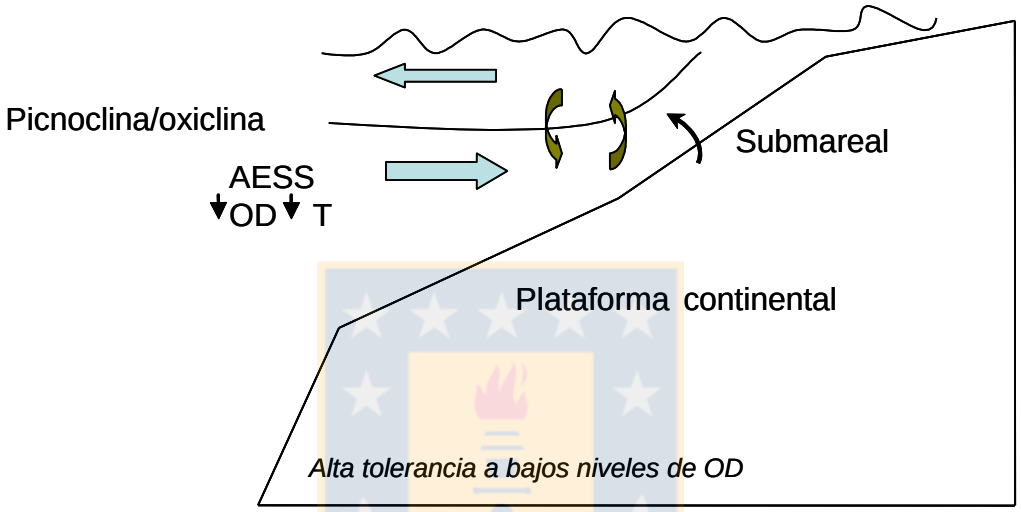
plataforma) también en la vertical, y no necesariamente deberían presentar migraciones verticales diarias durante toda su ontogenia. Las larvas liberadas en diferentes zonas del submareal, pero con migración vertical a través de la picnoclina, podrían ser retenidas sobre la plataforma. Durante los períodos de surgencia, deberían ser tolerantes a las bajas concentraciones de oxígeno, mientras que durante la relajación, al profundizarse la oxiclina, esta limitante también se relajaría (Fig. 1B).

Aquellos organismos liberados en el intermareal, pueden ser retenidos cerca de la costa durante la surgencia utilizando también migraciones verticales, mientras que las que permanecen en superficie, serían beneficiadas por los períodos de relajación que las retendrían cerca de la costa (Fig. 1C). Podrían ocurrir variaciones verticales de menor escala que retuvieran organismos cerca de la costa ya sea en fase o no con la surgencia. Estos procesos de menor escala no implicarían intrusión en aguas más frías o poco oxigenada. Poco se conoce aun acerca del acoplamiento de estas escalas.

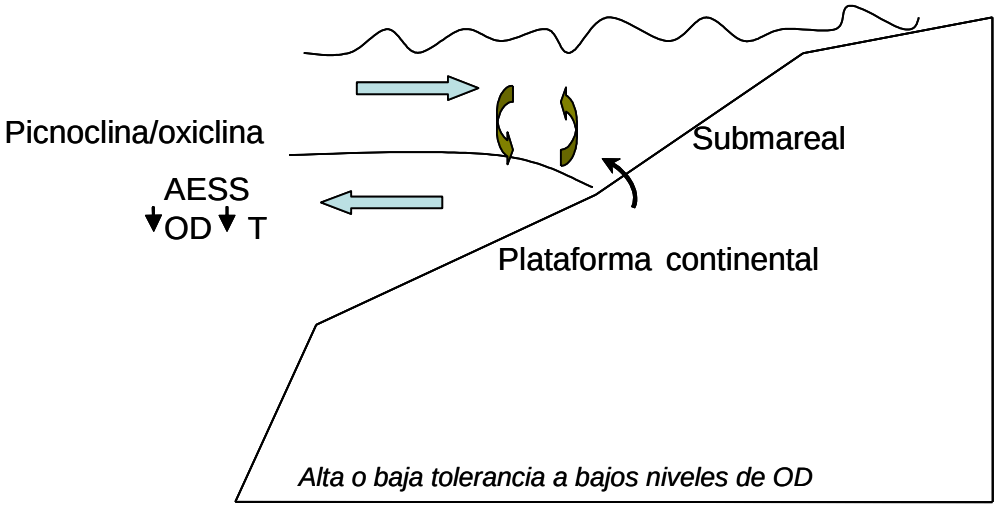


B

Surgencia



Relajacion



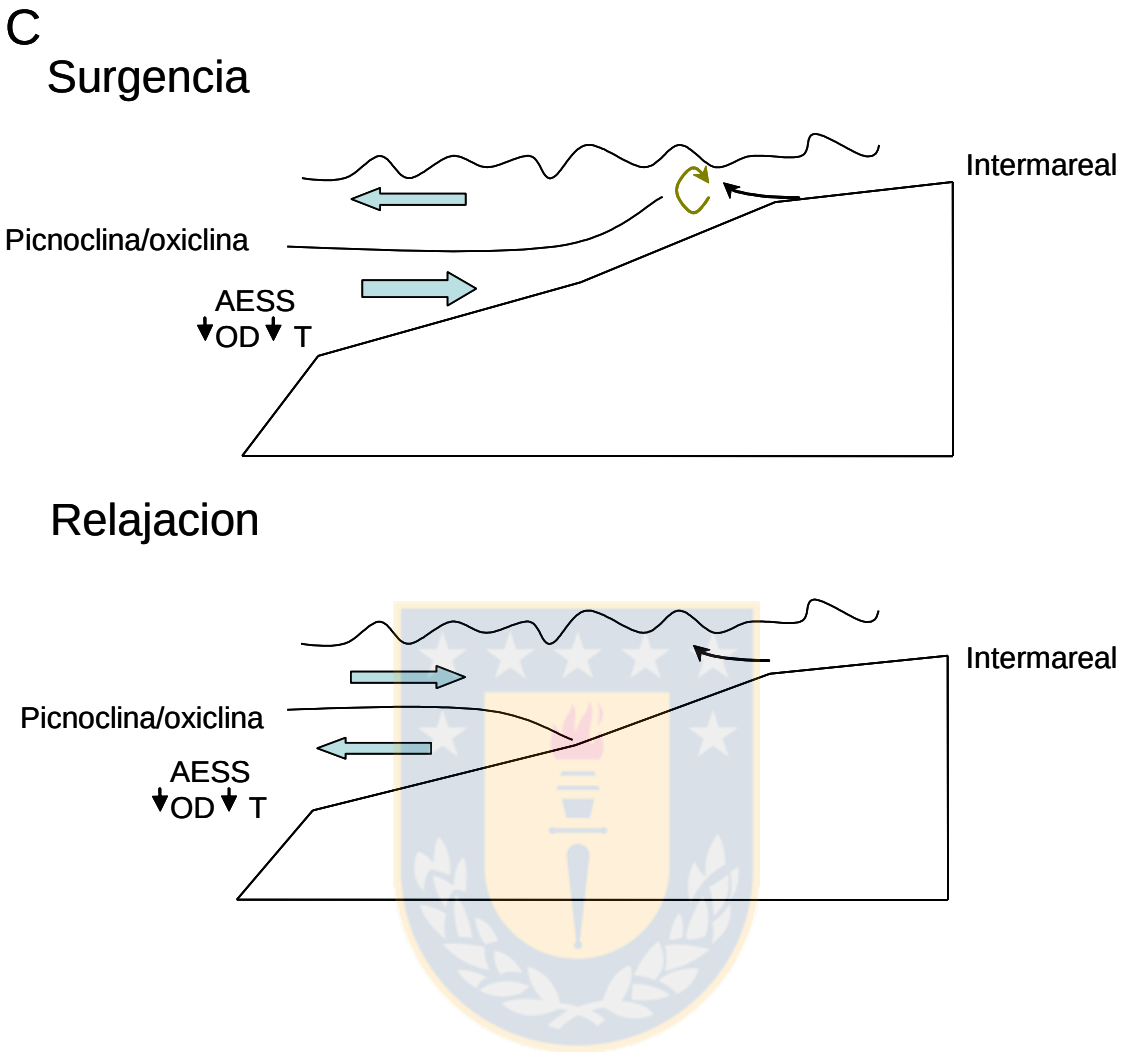


Fig. 1 Modelo conceptual del acoplamiento entre los ciclos de liberación larval y los ciclos de surgencia/relajación en relación a la zona de liberación larval, su comportamiento vertical y sus tolerancias fisiológicas. A) Liberación de organismos en profundidad, en Aguas Ecuatoriales Sub-superficiales (AESS). B) Liberación de organismos submareales, que despliegan migraciones verticales a través de la picnoclina y pueden ser retenidas cerca de la costa. C) Liberación de organismos intermareales con o sin migración vertical durante diferentes períodos. Flechas negras: zonas de liberación de larvas. Flechas verdes: movimientos verticales de las larvas. Más explicaciones en el texto.

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PARTE B

Manuscrito 1:

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Vertical distribution of decapod larvae in the entrance of an equatorward facing bay of central Chile: implications for transport

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Abstract

Two short biophysical surveys were carried out in order to assess the interaction between decapod crustacean larvae vertical distribution and circulation patterns in determining larval transport into and out of an equatorward facing embayment (Gulf of Arauco, 37ºS; 73ºW). The embayment is located at the upwelling area of South Central Chile and features a deep (~60 m) and a shallow (~25 m) pathway of communication with the adjacent coastal ocean. Profiles of zooplankton, temperature, salinity, dissolved oxygen and current velocity were measured during a 22 h period at the shallow entrance to the gulf. In addition, three zooplankton profiles were carried out at the deep entrance to the gulf on the basis of observed changes in hydrographic structure. At the shallow entrance to the gulf, current velocities showed a clear semidiurnal tidal signal, with stronger ebb than flood at the surface and stronger flood at depth. Decapod larval abundances showed a bimodal distribution through time, with a maximum during night-time ebb, and a smaller, second peak at day-time ebb. At the deep entrance, two groups of crustacean larvae were found associated with two different water types: a surface high oxygen water and a deeper suboxic equatorial sub-surface water. The motion of these layers was determined remotely by upwelling dynamics and modified by local wind (sea breeze) and tides.

Key words: larval transport, upwelling area, vertical distribution, crustacean larvae.

INTRODUCTION

It has been recognized that the heterogeneity of coastal topography creates hydrodynamic circulation features that affect the transport of inert particles and plankton (Archambault et al., 1998, Archambault and Bourget, 1999). Because gulfs and bays have higher residence times than the adjacent open coast, they have been identified as nursery areas in continental shelves under the influence of different physical forcing. This is particularly evident at embayments in upwelling regions, where high vertebrate and invertebrate larval concentrations have been reported (Pedrotti and Fenaux, 1992; Castillo et al., 1991; Graham et al., 1992; Wing et al., 1998). In these upwelling regions, an increase in plankton concentration occurs even in open gulfs, particularly downwind of headlands. Upwelled water at headlands frequently intrudes into the adjacent embayment, where cyclonic re-circulation increases the residence time and facilitates retention and concentration of organisms (Graham and Largier, 1997).

In conjunction with water circulation features, specific larval behaviour might enhance or preclude their retention. Decapod crustacean larvae can regulate their vertical position in the water column following diverse environmental cues and/or endogenous biological rhythms (Forward et al., 1997). This ability allows the use of depth-varying current fields to regulate horizontal position in dissimilar environments as has been shown in tidally dominated estuaries (Epifanio, 1988, Queiroga et al., 1997), as well as in buoyancy-driven and in wind-forced continental shelf waters (Epifanio and Garvine, 2001; Wing et al., 1998). Therefore, the combination of physical forcing and specific animal behaviour is believed to determine the fate of meroplankton in an embayment like the Gulf of Arauco.

The Gulf of Arauco (37º10'S-36º45'S) is the largest equatorward facing bay in South-Central Chile where southerly (S) to southwesterly (SW) upwelling favourable winds dominate from mid-spring through late summer. During this season, large concentrations of Chl-a and zooplankton have been recognized in the gulf in the past decades (Castillo et al. 1991; Castro et al., 1993). Djurfeldt (Djurfeldt, 1989) proposed that a wind-induced sub-surface advection of the nutrient-rich Equatorial Sub Surface Waters (ESSW) into the gulf through Boca Grande (Fig. 1) and mixing due to an internal wave breaking at the head of the gulf during upwelling reversals was responsible for the high productivity of the gulf. More recently, Parada et al. (Parada et al., 2001) and Valle-Levinson et al. (Valle-Levinson et al., 2003) have proposed that the diel wind pattern and

tidal regime would lead to recirculation processes that would also enhance chlorophyll-a and plankton concentrations.

To date, only few studies have addressed questions on meroplankton transport mechanisms in the highly productive upwelling area of South-Central Chile. A sub-surface transport of mesopelagic organisms to the shelf and gulf during the upwelling season has been suggested to result from the diel vertical migration of larvae embedded in the two layer wind-driven circulation (Castro et al., 1993; Vargas and Castro, 2001; Landaeta and Castro, 2002). In the adjacent Concepción Bay (36º40'S), an increase in crustacean larval abundance has been observed during flooding tidal currents, and a decrease during southerly (upwelling favourable) winds (Carbajal, 1997). However, coupled bio-physical studies directed to understand the interaction between crustacean larvae vertical distribution and circulation patterns in determining transport to or retention in coastal environments have not been carried out yet in this area.

During the second week of December 2000, a field study aimed to characterize the wind-induced influence in the general circulation of equatorward facing bays in eastern boundary currents was carried out in the Gulf of Arauco. The present study was conducted within that framework. We selected groups of decapod larvae in order to i) assess the relationship between the pattern of larval abundance with diurnal and semi-diurnal environmental cycles and, ii) infer how the larval vertical distribution and the prevailing circulation influenced their transport into and out of the gulf during upwelling favourable winds through the small entrance to the Gulf of Arauco. At the deeper Boca Grande we aimed to determine which groups of larvae prevailed at surface or sub-surface waters and its relationship with adult habitat depth range and life history.

METHODS

Study site

The Gulf of Arauco is located in the area of widest continental shelf in the Chilean coast. A large change in coastal orientation, and an enhancement of coastal upwelling occurs at the western cape of the gulf (Punta Lavapie, Fig. 1). The presence of Santa Maria island in the west, divides the gulf in two openings: Boca Chica (BC) a narrow and shallow opening on the west, and Boca Grande (BG) a wider and deeper one in the north where most water exchanges with the adjacent ocean. Although the bathymetry is smooth within the gulf, the narrow and deep BioBio submarine canyon cuts the shelf from east to west just a few kilometres to the north of the gulf. The BioBio river, the

largest in the region, discharges mainly during winter so no important fresh-water buoyancy forcing was expected during this study. Tides are semi-diurnal with a fortnight cycle (amplitude range from 0.5 to 1.5 m in neap and spring tides respectively).

Boca Chica Survey

An intensive 22 h hydrographic and biological sampling was conducted at Boca Chica over a fixed, 17-m deep station, on December 8th and 9th, 2000. This survey sought to assess the daily pattern of abundance of larvae and the interaction between the diel larval vertical distribution and the prevailing flow patterns during an active upwelling period. The survey was designed to collect data during two semidiurnal tidal cycles (25 h). However unfavourable weather conditions hindered completion of the full period. Sampling was carried out onboard the R/V Kay Kay of Universidad de Concepción. Currents were measured with an RD Instruments 307 kHz workhorse Acoustic Doppler Current Profiler (ADCP). The ADCP was positioned at 1 meter below the surface, pointing downward. Water velocity data were collected at 5 s intervals (pings) and averaged over 30 min at vertical resolutions (bin size) of 1 m. Water velocity was decomposed into east (u) and north (v) components. Positive signs corresponded to eastward and northward flow, respectively. Profiles of temperature, conductivity and dissolved oxygen were also obtained every 30 min with a Sea Bird SBE-19 conductivity-temperature-depth recorder with an oxygen sensor (CTDO). These data were converted to salinity and density and further processed to 1-m bins using the manufacturer's software (Seasoft). Additional field data included winds at Punta Lavapie (37º 10' S, 73º 35' W) recorded every 10 min and solar radiation, collected at Bella Vista (Fig. 1). These data were averaged to 30 min intervals. Sea surface temperature (SST) and SeaWifs images were also available. To identify diurnal and semidiurnal harmonic constituents of current velocity in u, we fitted a sinusoidal function:

$$u = A_c + A_s \cdot \sin(\frac{t}{2} + f_s) + A_d \cdot \sin(\frac{t}{4} + f_d)$$

for depth averaged u, and also for u at 5, 10 and 15 m depths, where t is local time , f_s and A_s are phase and amplitude of semidiurnal oscillation, f_d and A_d the respective parameters for diurnal and tidal constituent and A_c accounts for the mean current. We fitted functions for averaged u in order to analyse the main flow cycles explaining variability at BC and for u at every depth in order to determine whether cyclic behaviour was maintained through the water column. The semidiurnal component had a 12 h period (only longer time series would allow the separation of different semidiurnal tidal components). The diurnal component had a 24 hr period, and it would reflect diurnal fluctuation other than those due to diurnal tide, since diurnal tide was negligible in the area during the period sampled (Valle Levinson et al., 2003).

Zooplankton samples were collected at three fixed depths (z_1=5, z_2=10 and z_3=15m) with a 1m^2 mouth Tucker trawl net (300 μm mesh), equipped with a calibrated flow meter. The Tucker trawl medium net was opened and closed from the deck, so at each sampling time and depth, it was towed, opened at a desired depth and allowed to drift for approximately 10 min, then closed and retrieved. Filtered volume ranged from 50 to 100 m^3 per sample, which represented between 10 and 50 times larger volumes than those usually reported in studies with comparable objectives. Zooplankton sampling began at 17:15 h (on the 8th of December) and continued until 14:40 (December 9th). Profiles (each constituted by 5, 10 and 15m depth samples) were conducted at approximately 2 h intervals. A total of 12 zooplankton profiles were obtained (Table Ia). Once the zooplankton samples were on board, they were preserved in 5% formalin for later analyses. In the laboratory, samples were fractionated and subsamples were used for analysis under a stereo-microscope following standard procedures (Boltovskoy, 1981). Zoeas of Brachyura, Anomura and Thalassinidea (Crustacea, Decapoda) were separated.

Target species

Larval behaviour might differ among crustacean species, even among larval stages within a species. Therefore in the present study we analysed several taxa with different characteristics (both at the larval and adult stages) to compare individual patterns in the same hydrodynamic setting. We included in our study species with different life cycles (adult habitats type and depth range, and larval developmental times). In addition, abundance and frequency of occurrence should be enough to allow statistical analysis. The degree of detail in taxonomic identification was constrained by the reduced availability of larval descriptions (Wehrtmann and Báez, 1997), compared to the high diversity of decapod crustaceans in the area (Retamal, 1981). The analysis included several species and also groups of species (belonging to the same genus or family) when it was not possible to identify individual species and adults were known to present similar depth range. The use of groups of similar species is useful when the components share certain characteristics (Wing et al, 1998). The list of species and their characteristics are presented in Table II. In addition to groups and species identified (Table II), individual stages of *Neotrypaea uncinata* were recognized (Aste, 1982)

Boca Chica data analysis

At Boca Chica, larval concentration at each profile (i) and depth (z) was expressed as density D_{iz} (individuals per 100m^3). In this analysis only taxa present in over 65% of samples were included (Table II).

In order to identify whether organisms density fluctuations throughout the day followed a semidiurnal and/or diurnal pattern, we averaged density for each profile, to obtain $\overline{D_i}$, and fitted a sinusoidal function to the log ($\overline{D_i}$ +1), similar to the one used for current measurements:

$$\log(\overline{D}_i + 1) = A_c + A_s \cdot \sin(\frac{t}{2} + f_s) + A_d \cdot \sin(\frac{t}{4} + f_d)$$

where t is local time, f_s and f_d are phases of semidiurnal and diurnal oscillations, A_s and A_d are the corresponding amplitudes, and A_c is a constant that accounts for the mean larval concentration. The logarithm of mean density was taken since zooplankton samples displayed a log-normal distribution, and least square procedures used to estimate model parameters rely on normally distributed residuals. When the model as a whole did not apply to data, but just one of the terms (semi-diurnal or diurnal) we removed the non significant cyclic component, and re-calculated the percentage of variance explained by the semidiurnal or diurnal component. Both phase and amplitude parameters of the diurnal and/or semidiurnal components had to be significant in order to be included in the model and considered indicative of the presence of such a cycle. Once the parameters of the regression were estimated (a least squares procedure was followed with both Excel Solver and STATISTICA packages), the time and magnitude for expected maxima during the 24 hr cycle where calculated. The rationale behind this approach is that if organism density fluctuates conspicuously according to a natural physical cycle, we could expect a density curve to follow the cycle despite smaller scale variability. Therefore, density could be modelled as a continuous non-linear function of time. Our short Eulerian study allowed us to performe this type of analysis because of the large sampling unit (filtered volume) and a two-hour time resolution. The successful fitting of parameters allowed estimation of the expected time of largest concentration. The approach of using continuous trigonometric functions to describe cyclic biological changes, has recently been used for analysing seasonal plankton abundance patterns and tendencies, for longer time series, robust methods have been developed (Dowd et al, 2004).

In order to identify whether vertical distribution of organisms during the present study followed a semidiurnal and/or diurnal pattern we first calculated the mean weighted distribution depth (*MD*) for each profile (*i*):

$$MD_i = \frac{\sum_{z=1}^{3}(D_z \times Z)}{\sum D_z}$$

where Z stands for sampled depth, and a 2-way ANOVA was performed to test the effects of factors: light (day light, no light) and tidal phase (ebb, flood). Our design was orthogonal but unbalanced (check Table II). The timing of some profiles coincided with slack tides (high and low

waters) so the level 'ebb' in fact included ebbing plus low water, and the level 'flood' referred to flooding plus high water. An alternative would have been to leave slack tide profiles out of the analysis, which would have meant discarding one third of the observations (4 out of 12). This alternative was therefore not explored. The categorical division of a continuous phenomenon (such as mean weighted depth distribution temporal changes should be if they are in fact displayed by taxa) might be rather arbitrary, hence we also applied the previously described harmonic function to mean weighted depth distribution data. The analysis and visualization of data supplied by the trigonometric function fitting complemented that of the 2-way ANOVA.

Transport depends on the quantity of organisms present in a given water parcel and the magnitude and direction of flow they are subject to. In the present study, tidal signals on current velocity were superimposed to other signals so even ebbing tides could result in positive (inflowing) velocities at some depths. In order to understand how larvae were being transported we estimated the larval flux (*LF*) for each depth and profile as the number of larvae crossing a one m^2 section per hour:

$$LF_{iz} = \frac{D_{iz} \cdot u_{iz}}{100}$$

where u_{iz} is the east velocity component (mh^{-1}) at each profile (i) and depth (z). We analysed whether the larval flux at profile (ΣLF_z for each profile *i*) depended on light or tidal phase with a 2-way ANOVA. Factor levels were defined as in the previous analysis on mean weighted depth.

If the water velocity at any given time differs between depths, larvae might gain or lose velocity by concentrating at the bottom, middle or surface layers (Queiroga et al. 1997). The larval velocity relative to the water column (relative larval velocity) provides a quantification to understand whether larval flux results from net gain or loss of velocity. For each profile, the vertically integrated net larval velocity (LV_i) in m h^{-1} was calculated following Queiroga et al. (Queiroga et al., 1997):

$$LV_i = \frac{\sum D_{iz} \cdot u_{iz}}{\sum D_{iz}}$$

We averaged u_5, u_{10} ,u_{15} for each tow (AV_i), and we calculated the difference between LV_i and AV_i which resulted in the relative larval velocity (RLV_i). We did no performed statistical analysis in this last index, but used it as a descriptive tool.

Boca Grande Surveys

At Boca Grande (Fig. 1), an adaptive stratified sampling strategy was followed in order to identify groups of crustacean larvae that were potentially associated with the previously described

two-layer wind-driven circulation in the gulf. Current velocity and water temperature, conductivity and dissolved oxygen concentration, were measured with the same instruments as in Boca Chica, along transects from Coronel to Santa Maria Island in five different dates between December 4th and 10th. In the middle of the Boca Grande transect, zooplankton was collected by means of Tucker trawl oblique tows on three different dates (4^{th} , 6^{th} and 10^{th} of December). Organisms were collected at 3 or 4 adaptive strata (Table Ib). The depth and width of the sampled strata was determined after CTDO and ADCP data inspection at the station, which allowed the identification of thermocline, oxycline, water types and changes in flow direction. Physical data is reported only for the stations where zooplankton was collected. Zooplankton samples followed the same preservation and processing routine as those from Boca Chica. Number of individuals was first standardized to $100m^3$ and then the number of individuals per 100 cubic meter were multiplied by the depth of the corresponding stratum (and divided by 10) to obtain individuals per 10 square meter (m^2). We used these units to express our data because we were interested in the quantity of individuals that were subject to a given condition and water mass (e.g. flow direction, oxygen level).

Boca Grande data analysis

It has been reported that Equatorial Sub-surface waters characterized by low oxygen, high salinity and low temperatures are found below the surface in the Gulf of Arauco during the upwelling season (Djurfeldt, 1989; Valle-Levinson et al, 2003). Therefore we used low oxygen concentrations (<2 mL/L) as an indicator of deeper waters. In order to assess whether individual species were associated with a water type we performed a quotient analysis (q) following van der Lingen (1999). Samples from the three sampling dates were sorted into four classes of oxygen concentration. We calculated the mean oxygen concentration for each stratum and samples were sorted in classes: 0-1, 1-2, 3-4 and >4 mL/L. Class 2-3 mL/L was left aside because no sample had that average dissolved oxygen concentration. For each species, observed frequencies (f) in each class (i) were calculated by adding up the number of individuals per $10m^2$ of all samples that fell within class i. For each oxygen class, f_i was divided by the total number of individuals per $10m^2$ caught in Boca Grande, to find the percentage frequency of occurrence (pf_i) of individuals within a class. Also, the number of samples (s) within each class was determined, and the percentage frequency of samples (ps_i) within a class was calculated. Estimates of q were determined between those two quantities (pf_i / ps_i) because values of q over 1 have been used as indicative of environmental preference (van der Lingen, 1999).

We also aimed to identify groups of species that behaved similarly regarding vertical distribution, so we produced a similarity matrix for species, using oxygen classes as samples, and the quotient pf_i / ps_i as raw data. Similarity was calculated with the Bray Curtis index on the untransformed quotient, and species were clustered with a complete linkage procedure.

RESULTS

Overall, the wind regime throughout the period was dominated by northward upwelling winds with diurnal variations. Winds increased in intensity during daylight hours, being highest near local sunset (Fig. 2a). Satellite images (SST and color, not shown) indicated an active upwelling center off Punta Lavapie, and high chlorophyll a concentrations inside the gulf and along the coast of the entire region.

Boca Chica

Current velocities (in *u*) during the 22 hr sampling at Boca Chica showed a semidiurnal tidal signal of ~10 cm s^{-1}. Strongest outflow appeared at the surface (negative *Ac* Table III) and strongest inflow occurred at mid and bottom depths (positive *Ac* Table III, and Fig. 2c). Together, semidiurnal and diurnal tidal constituents explained over 90% of *u* variability throughout the day (Table III). At 5 and 10 m depth, the semi-diurnal amplitude was larger than the diurnal amplitude, while at 15 m depth, it was the amplitude of the diurnal component that dominated *u* variability. Maximum outflow (negative velocities) coincided with biological profiles 5 (night) and 11 (day) (see Table Ia), approximately midnight and noon respectively. Maximum inflow (positive velocities) appeared at profiles 2 (afternoon) and 8 (morning). Surface and mid-depth temperature and density (as sigma-t Fig. 2b) showed diurnal variability with surface heating and deepening of the pycnocline during daylight hours. At 15m, temperature and oxygen concentration increased while density decreased during the sampling period. Vertical gradients however, were relatively weak (Fig. 2b), but not correlated with wind speed (Valle-Levinson et al, 2003).

At the shallow Boca Chica, the larval density of several taxa fluctuated throughout the day following a bimodal pattern (Fig. 3 aI,cI,iI,jI,kI; Table IV), with a peak larval abundance at the night ebbing tide. *Cancer* spp. (Cancridae), *Neotrypaea uncinata* (Callianassidae), *Pagurus* spp. (Paguridae), *Pinnixa* spp. (Pinnotheridae) and Porcellanidae presented the higher densities at both (Table II). The harmonic model fitted to *Pagurus* spp., Porcelanids, *B. spinimana* and *H planatus* showed a good agreement with data (Table IV). Larger densities for these taxa, were estimated to occur past midnight, and a smaller secondary peak before midday. Both peaks were coincident with

ebbing tides: the local maximum negative velocities for *u* at 5 and 10m depth and integrated velocity (Table III). During night hours they were more abundant at late ebb, while during the day they mainly peaked at early ebb. *Emerita analoga* presented also two peaks of density at ebbing tides, without diurnal variability (Table IV, Fig. 3jI). For *N. uncinata* (Fig. 3fI) and *P. edwardsi* (3eII) only the diurnal component was significant, and both peaked at night ebb. Overall abundance of *N. uncinata* individual stages decreased with stage (Fig. 4), and they mostly followed the same pattern, except zoea IV that also showed a significant second peak at day ebb Table IV). Table IV shows that it was not possible to identify reliable cyclic components in *Cancer* spp. (Fig. 3hI) and *Pinnixa* spp. (Fig. 3bI) which showed a less clear bimodal pattern and Grapsids and *P. politus* that were spread along the day (Fig. 3dI and 3gI respectively).

The mean weighted depth at profile varied along the day for different species. *B. spinimana* was significantly deeper in the water column during ebbing tides, while *H. planatus* was significantly shallower at ebb (significant ANOVA for factor tidal phase at alpha=0.05; Fig 3iII). *Pinnixa* spp was significantly shallower during night hours (significant ANOVA for factor day/night at alpha=0.05; Fig. 3bII). Porcelanids, Pagurus spp and *N. uncinata* showed a very similar pattern: they were shallower in the water column during night hours, and at some point of day ebb although no significant differences (ANOVA) were found between night and day mean weighted depth. For the first two however, significant parameters were found for the diurnal cycle (Fig. 3 aII, cII, and fII respectively). Individual stages of *N uncianta* tended to be shallower during night (ebb) though probability was only lower than 0.1. *N uncinata* stages 4 and 5 showed an overall trend to be deeper than earlier stages (Fig.4). Although *P. politus* showed a significant interaction between tide and day/night cycle factors, the plot of MD against time shows no clear pattern through the day, except that they were shallower during night-ebb (Fig. 3gII). Grapsids showed a clear diurnal cycle though it was not in phase with the categories defined for ANOVA analysis (Fig. 3dII). They were shallower at evening high water and deeper at morning high water. *E. analoga*, *Cancer* spp and *P. edwardsi* showed no distinguishable pattern of vertical distribution though the day (Fig. 3 jII, hII and eII respectively). Taking the average mean weighted depth for each species, shows that *Pinnixa* spp. was the deepest, followed by Porcelanids, *H. planatus, P. politus* and *B. spinimana*; and *N uncinata*, *Pagurus* spp , *Cancer* spp and *E. analoga* were the shallower species (Fig. 3).

Except for *B. spinimana*, *H. planatus* and *E. analoga*, there was a significant effect of tidal phase on larval flux (Table V), inflow was larger during floods; negative fluxes appeared during day and night ebb, when the relative larval velocities were negative (Fig. 3 a through h III). Among the group of organisms that showed significant effect of tidal phase, *P. edwardsi* and Porcelanids did not show negative flux (outflowing) during night ebb (Fig 3 aIII and eIII). For Porcelanids,

Pinnixa spp. and Grapsids that showed a conspicuous deepening in the water column during day hours the relative larval velocity at day ebb was positive in spite of the absolute negative flux at this time (Fig. 3a,b and d III). *B. spinimana* larger inflow occurred during night ebb. *E. analoga* showed a negative relative velocity during most of the sampling period in spite of showing strong negative flux only during day ebb. *N. uncinata* stages from I through IV showed the same outflowing pattern at night and day ebb, on the contrary *N. uncinata* V remained inflowing (data not shown).

Boca grande

The thermocline and halocline were well defined at Boca Grande in the three sampling occasions (Fig. 5). In general, hydrographic vertical gradients were much steeper than those at Boca Chica. The thermocline and the oxycline coincided between 10 and 20 m (Fig 6 a,b and c). The upper layer temperature reached 14ºC from atmospheric heating and surface water was well oxygenated. The lower layer, in contrast, was <10ºC and <1 mL L^{-1}. These water characteristics are typical of the ESSW (Strub et al. 1998; Sobarzo et al. 2001). These hydrographic conditions were indicative of an active upwelling situation. At the time of biological sampling, surface flow was downwind (positive *v:* towards the N), while sub-surface flow was mainly upwind (negative *v:* towards the S) (Fig 5e).

At Boca Grande, larval highest concentration was not as high as in Boca Chica (Table II). However, *L granaria* was found at this entrance and not at Boca Chica, and *P. monodon* comprised a larger proportion of total organisms. Taxa showed marked differences in vertical distribution in the water column (Fig. 6 for contrasting examples). *Cancer* spp. were either more abundant at surface waters (higher temperature, higher oxygen) or at the thermocline. *Emerita analoga* peaked at the thermocline also. *Pleuroncodes monodon* was always more abundant in low oxygen, deeper waters and *Neotrypaea uncinata* showed differences for all stages (Fig.6 d and e). Late stages were found deeper than early stages. It is important to note that sampling at Boca Grande was always during daylight hours (6^{th} and 10^{th} of December) or twilight (4^{th} of December).

Cluster analysis (Fig. 7) yielded two groups. One group comprised those species/groups that mainly occupied depths above or at the pycnocline (i.e. *Cancer* spp. and *E. analoga*), of high oxygen and mainly downwind flowing waters. A second group was made of organisms that appeared at depth (eg. *Callichirus garthi*, *P. monodon*, *Taliepus* spp., *L. grenaria*) in low oxygen, upwind flowing waters. For group one (*B. spinimana*, *Cancer* spp., *P. edwardsi*, *E. analoga* and *P. politus*) quotient *q* was greater than one in well oxygenated waters. While for group 2 the quotient *q* was greater than one in less oxygenated deeper waters (Table VI).

DISCUSSION

The results obtained with this sampling snapshot, shed some insights into the role of tidal and wind-driven larval advection at the shallow and deep entrance of the equatorward facing Gulf of Arauco, and the larval flux dependence on larval vertical distribution. At the shallow entrance, peak larval densities coincided with ebbing tides (especially night ebb) and larval flux was driven by tidal oscillations superimposed on a pattern of outflowing surface waters and inflowing deep waters. Most groups were outflowing during ebb and inflowing during flood. However, larval flux was related to species vertical distribution: those that remained deeper experienced larger inflow and those that dwelled in shallower waters were influenced by larger outflow. Also for those groups that showed differences in vertical distribution between ebbing/flooding periods, average flux was independent of tidal oscillations. At the deep entrance, the regional wind-induced upwelling dynamics dominated the exchange pattern of two different water types, one above the pycnocline, outflowing from the gulf at the flanks, and another low oxygen water below the pycnocline, inflowing (Valle Levinson et al., 2003). Larvae were vertically segregated into these water types. The last finding supports the hypothesis related to a subsurface transport of organisms into the gulf during upwelling events.

Boca Chica

At Boca Chica, net flow was westward (outflow) at the surface and eastward (inflow) at the subsurface. This pattern was also found by additional current measurements taken in transects across this entrance (reported by Valle-Levinson et al., 2003) during the same week, so it seems to be a typical condition for the Boca Chica entrance during upwelling periods. In nearby Concepción Bay (36°40’S, 73°02’W), a two layer circulation also develops under upwelling winds (Sobarzo et al, 1997; Valle Levinson et al, 2004). Despite Boca Chica shallowness, intense southerly (upwelling) winds and net water inflow at Boca Grande, outflow at Boca Chica was hardly found throughout the water column at any given time. The bi-directional water exchange pattern, which might be the result of regionally forced Ekman dynamics and/or curvature effects related to the presence of a nearby headland (ValleLevinson et al, 2003), has a decisive influence on larval advection through Boca Chica. Larvae with deeper distribution were influenced mainly by positive relative velocity, and influx (e.g. Porcellanids.), and those that dwelt shallower experienced mainly negative relative velocity (e. g. *E. analoga*). Also, the semidiurnal water velocity modulation had an influence on larval advection. Many groups experienced outflux during ebb and influx during flood. However, the amplitude of the semidiurnal constituent was larger at surface (5m) and subsurface

(10m), than at 15m depth, where the amplitude of a diurnal cycle dominated the *u* variability. Organisms with vertical migrations would be subject to different amplitudes of semidiurnal fluctuations that, in this shallow entrance, could be the result of overtides (Valle-Levinson et al 2003). In nearby Concepción Bay, which has the same geographical orientation with two openings as the gulf of Arauco, semidiurnal tides also exert a larger influence on the flux through the smaller and shallower opening (Valle Levinson et al, 2004).

Several organisms showed two peaks of density and had a semidiurnal periodicity in phase with tidal currents. A coupling between meroplanktonic larval density fluctuation and tidal cycles has been reported in channels (Drake et al, 1998); estuaries (Garrison, 1999), tidal flats (Levin, 1986), and bays (Belgrano y Dewarumez, 1995; Carbajal, 1997). Such coupling has been associated with larval hatching and tidal cycle synchronization (Levin, 1986; McCounagha, 1988), larval transport, and larval active behaviour. For example some larvae are found in the water column when they leave the water-bottom interface during ebbing tide (DiBacco et al., 2001). In this case, zoeas from different stages were included in the analysis so larval hatching synchronization was excluded as a general explanation for the observed pattern. For those species with bimodal distribution over time (except *E. analoga*) densities were always higher at night. However, outflow comprised a larger part of the water column during day ebb, and not all groups peaked in surface outflowing waters. Therefore it seems that the advective effect of ebbing tides plus the availability of larvae, should be responsible for the observed density patterns.

B spinimana in particular showed semidiurnal and diurnal density peaks. They were found deeper in the water column during ebbing tides. Deepening during ebbing tides resulted in positive larval relative velocity in which case larval flux did not follow the semidiurnal tidal cycle. The vertical distribution of this group translated into influx toward the gulf. Active swimming during flooding phases has been described in megalopas of estuarine species (Forward et al., 1997), and has been related to re-invasion of coastal habitat. *B. spinimana* is an intertidal species, the larva itself is big in relation with most of the other studied taxa. The capacity to deal with currents that vary in a small depth range is consistent with the fact that offshore larval distribution is restricted to coastal waters and large concentrations have been found within the gulf of Arauco (unpublished data).

Daily fluctuations in vertical distribution, their amplitude, and the mean depth of occurrence had an influence on organisms flux through Boca Chica. For example the deepening of *Pinnixia* spp and Porcelanids during day hours resulted in a reduction of out flux from the gulf (positive relative velocity) and the opposite during night hours. *Pagurus* spp, which also was shallower during night hours, was closest to surface among the mentioned species, and experienced larger outflux than

them. Porcellanids (whose mean depth distribution was deeper) remained inflowing during night ebb. *Pinnixa* spp. was the deepest of the three, but the amplitude of diurnal cycle was larger, so outflow occurred during night ebb. In this environment, larval displacement between waters flowing in opposite directions at Boca Chica would reduce net inflow or outflow. Larvae of the genus *Pinnixa* have been found to be exported from mixed estuaries, concentrated in surface waters during night ebb, but later, daily migration would induce their retention within the inner shelf (McConaugha, 1988). In the California current, *Pinnixa* spp. are also strong vertical migrators and tend to distribute downwind with respect to non vertical migrators such as *Cancer* spp. (Wing et al., 1998). In this case, larvae were found shallower during night time (enhancing outflow) but they were found deeper during the rest of the day, resulting in inflow to the Gulf of Arauco. Since currents diurnal variability is not related to tidal influence in this area, day/night cycle and diurnal fluctuations could remain in phase, in contrast with the phase of day/night cycle and semidiurnal fluctuations that changes along time. The maintenance of phase for different cycles is necessary to enhance/reduce transport if behaviour is triggered by one factor (e.g. light) and transport by the other (e.g. daily currents fluctuations)(Criales et al., 2005).

The vertical distribution during the time of higher abundance had an important effect on larval flux. For example *P. edwardsi* and *N uncinata*, both showed one peak of abundance and neither had a significant vertical distribution pattern. However, *P. edwardsi* remained deeper during night hours (larger densities) resulting in positive fluxes, while *N . uncinata* remained above their mean distribution depth during night hours, resulting in negative relative velocities and export from the gulf during those hours. In adition, there was a tendency for late stages of *N. uncinata* to be deeper in the water column, and while stages I though III had a significant outflow during ebbing tides, stage V kept inflowing during night ebb. There is no information in the literature regarding larval vertical distribution of the species involved in this study, however it has been shown that early stages of the thalasiniid *Callianassa californiensis* occurs in surface ebbing flows in estuaries, and their megalopa in inflowing waters (Johnson and Gonor, 1982). We did not include their megalopa in this study, but even *N. uncinata* later stages gained influx by being positioned deeper in the water column. In a two layer circulation pattern characteristic of upwelling periods, ontogenetic shifts in mean distribution depth could result in early export of larvae, enhanced offshore distribution of intermediate stages, and later re-invasion of the coastal habitat where they must settle.

Also, when larval density peaks did not occur in phase with neither diurnal nor semidiurnal cycle, larval flux became independent of tidal cycle. Such effect of the density pattern throughout the day on larval flux is exemplified by *E. analoga*. It peaked during early ebbs without diurnal

modulation and such a density pattern resulted in a flux independent of tidal phase. The concentration of larvae around two semidiurnal peaks, soon after high water could be the result of continuous hatching. In adition, no pattern of vertical distribution could be distinguished for *E. analoga*, although its shallowness resulted in negative relative velocities spread throughout the day. Experiments conducted with *E. analoga* from the California Coast, have shown a behavioural response to the interactive effect of pressure and light that would position larvae in surface layers (Burton, 1979). Its surface distribution plus long larval development result in a widespread horizontal distribution (Sorte et al., 2001). Species like *E. analoga*, which show little capacity to regulate their horizontal distribution through vertical migrations, could be favoured by enhanced hatching during downwelling periods. In fact, a peak hatching period occurs during upwelling reversal in south central Chile (Contreras et al, 1999).

When organisms showed neither a clear pattern of abundance, nor a vertical distribution pattern, larval flux followed the tidal cycle, as exemplified by *Cancer* spp. and *P. politus*. *Cancer* spp. was among the shallower species in this study. Species of *Cancer* in the California current also dwell in surface waters, and tend to be more widespread in the horizontal plane than vertically migrating larvae. Estuarine pinnotherids have also been reported outflowing during ebbing tides (Garrison, 1999).

In summary, larval flux at Boca Chica followed tidal currents except for groups that showed deeper distribution during ebbing than flooding (*B. spinimana*), the deepest (stage V *N. uncinata*), and a shallow species whose peak density occurred during early ebb (*E. analoga*). In addition, shallow mean depth position at night enhanced export of larvae. The vertical patterns observed suggest at least certain control of depth by organisms, however it is not possible to speculate from our short time series, whether vertical fluctuations emerge from endogeneous behaviour, responses to external stimuli, or are influenced by other factors such as wind stress and vertical mixing. In this case, ebbing flows coincided almost entirely with night time (except at two vertical profiles), so we can not clearly separate the effects of tides and day-night cycle. In addition, wind intensity was lower during night time, and so was stratification. Nonetheless, whether vertical fluctuations were behaviourally or mechanically generated, larvae experienced bi-directional advection at Boca Chica, with a net inflow probably enhancing retention within the gulf. Again, whether the changes in current field with depth are a result of wind forcing and/or different amplitude of tidal (overtide) currents in Boca Chica, variations in vertical distribution of organisms allow group/species/stage regulation of horizontal transport.

Boca Grande

In Boca Grande, surface waters displayed maximum hydrographic and velocity amplitudes in response to daily heating and sea-breeze wind regime. Below the pycnocline, upwind flow (southward) dominated the core of the flow. The observed pattern was consistent with continental shelf Ekman dynamics plus local modifications (at higher -daily- frequencies) (Valle Levinson et al, 2003). Therefore, the larval assemblages present in each layer were subject to velocity variations of different frequencies.

During day hours at Boca Grande, two groups of larvae were differentiated, those that peaked in abundance at the pycnocline, where flow was minimal, (and high to intermediate oxygen concentration); and those in the deep layers where waters were mainly entering the gulf (and low in oxygen concentration). The accumulation of organisms in layers of reduced horizontal advection associated with pycnoclines has been documented for fish larvae and other planktonic organisms (Peterson et al. 1979). Among taxa found in sub-surface waters, *P. monodon* and *L. granaria* are especially interesting since adults have a deeper distribution than most of the other groups studied. Garland et al. (Garland et al., 2002) found a similar pattern for groups of larvae associated with cold upwelling waters (adults from the shelf) and warm downwelling waters (intertidal and shallow subtidal adults). *N. uncinata* (stages IV and V), *P. monodon* and *L. granaria* larvae should approach the coast in upwelling waters and even intrude into the gulf as reported for the larvae of some mesopelagic and demersal fishes (*Maurolicus parvipinnus* and *Merlucius gayi*) (Vargas and Castro, 2001; Landaeta and Castro, 2002). The demersal *M. gayi*, *L. granaria* and *P. monodon* overlap their depth distribution. Therefore our results support the idea that convergent strategies have evolved in benthic invertebrates and demersal fish co-occurring populations (Bradbury and Snelgrove 2001).

One of the particularities of this area, is the low oxygen concentration in shallow subsurface waters, which might impose restrictions on vertical migrations for non-tolerant species. Sampling at Boca Grande took place during day hours, when most of the described groups might be at the deepest layers they reach. However, most seemed to be excluded from the bottom layer. The deeper influence of ESSW on 10th December compared to 4th December at Boca Grande, might explain the somewhat deeper distribution of the "surface" group during the 10th. This group of organisms in the upper layers might be subject to the more variable hydrography above the pycnocline (Valle-Levinson et al, 2003). However, sampling during the 4th of December was conducted during evening hours, and a possible shift of some organisms in the surface groups towards the surface can not be discarded.

Results regarding larval vertical distribution from Boca Chica and Boca Grande were fairly consistent. Larvae that were 'surface' larvae at Boca Chica remained as surface or pycnocline

larvae at Boca Grande (*Cancer* spp., *E. analoga*, *P. politus*). Those that showed a tendency for deepening during day time at Boca Chica (although not always significantly), such as *Pagurus* spp., Porcellanids, *Pinnixa* spp. and *N. uncinata*, were found at intermediate to lower-intermediate depths at Boca Grande as could be expected for day-time. Also at Boca Grande, a completely different 'deep' group of organisms appears, which were less abundant or not present at all at Boca Chica (*P. monodon*, *L granaria*, *Taliepus* spp.). However, *B. spinima*, which tended to be deeper at Boca Chica and showed changes in vertical distribution throughout the day, appeared as surface or pycnocline organisms at Boca Grande. There were several aspects to take into account when comparing both entrances. For example, surface strata sampled at Boca Grande sometimes comprised the entire water column at Boca Chica, therefore 'shallow' has two different meanings for Boca Chica and Boca Grande, and the scale did have an effect on the definition of preferred depths. *E. analoga* for example at Boca Chica did have an overall shallow mean weighted depth distribution, however, organisms density was not significantly different between 5, 10 and 15 m. At Boca Grande it was clearly a 'surface to pycnocline' species. On the contrary, *B. spinimana* had an average mean weighted depth below 10 m deep at Boca Chica, while it was a 'shallow' species at Boca Grande. But the different scale not only has an effect on the definition of species as shallow or deep, but it reflects the scale at which the pattern of flow in opposite directions occurs at each entrance. While regulating vertical positioning in a range of 10 m at Boca Chica would expose larvae to opposite fluxes, in Boca Grande such an extent may represent unidirectional flux. In this respect, shallow parts might also allow the exploitation of the benthic-pelagic layer during certain periods of day/tidal cycle. This layer might not be available at Boca Grande because low oxygen waters in the bottom can restrict vertical distribution for certain organisms. In addition, hydrographic stratification was higher at Boca Grande, which could also contribute to the much sharper vertical contrasts found in this entrance.

The overall picture emerging from this study is that high frequency processes (semidiurnal tides, diurnal fluctuations) over imposed onto others of lower frequency (3-8 d period of wind-driven upwelling), may contribute significantly to variations in the transport of individuals from bays in upwelling areas. Larval vertical distribution in the two layer circulation at the embayment entrances can enhance or preclude retention within the bay. From the present study, we hypothesize that during upwelling periods, the group of surface dwelling larvae (eg. *Cancer* spp., *E. analoga*) would be exported offshore from the gulf. A second group (e.g. *Pagurus* spp., Porcellanids and *Pinnixa* spp.) would be retained nearshore; *N. uncinata* would be widespread during the first stages, and later stages should tend to concentrate nearshore. A third group (e.g. *P. monodon*, *L granaria*)

would possibly approach the coast in the sub-surface in successive instars from their offshore hatching areas.

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TABLE LEGENDS.

Table I. Sampling schedule followed at a) Boca Chica and b) strata sampled at Boca Grande. D: day (solar radiation>0); N: night (solar radiation=0). F: flooding tidal phase; E: ebbing tidal phase. High and low water are followed by an 'F' or 'E' to indicate their inclusion as a flooding or ebbing phase in variance analysis.

Table II. Decapod crustacean larvae identified at Boca Chica, and Boca Grande, average density (individuals/100m^3) out of non-zero samples for each site, and frequency of occurrence (number of positive samples out of the 35) at BC. The habitat type and depth range of adult species is also shown: I: intertidal, S: subtidal, D: deep, Ro: rocky bottoms, Sa: sandy bottoms, Mu: muddy bottoms, Co: commensal. Also, number of stages during larval development (when it has been described).

Table III. Parameter estimates for the sinusoidal function fitted to *u* current velocity component. Results for average velocity, 5, 10 and 15m depth and standard error (between brackets) VE: percentage of variance explained by the fitted model. H(min): real time estimates for the minima (maximum negative) velocities according to the fitted model.

Table IV. Parameter estimates of sinusoidal function fitted to mean organisms density for each tow over the 22 h sampling at BC and standard error (between brackets). H(max), estimated local time of maximum organisms abundance. VE: percentage of variance explained. Significant parameters ($p<0.05$) are indicated as bold numbers. n=12.

Table V. Summary of results for two-way ANOVA on mean organisms larval flux at profile (ind·m^{-2}·h^{-1}) for factors 1) light level (day and night) and 2) tidal phase (ebb/flood), n=10.

Table VI. Quotient (*q*) for species collected at Boca Grande in different oxygen concentration ranges.

FIGURE LEGENDS

Fig. 1. Gulf of Arauco at Central Chile and location of sampling stations Boca Chica and Boca Grande.

Fig. 2. Time and depth distribution of physical variables during the 22 h sampling at Boca Chica. a) predicted sea level (m) and wind intensity ($m \cdot s^{-1}$); b) sigma-t depth/time section ; c) E-W current component (*u*) at 5, 10 and 15m depth.

Fig. 3. For each species (indicated from a through k), the upper graph (I) refers to the average species density (individuals$\cdot 100m^{-3}$) at profile. The middle graph (II) shows the mean organism depth (m) at each profile (dots) and fitted sinusoidal model (black line) when parameters were significant. Significant models are shown. Otherwise the mean depth throughout sampling is drawn. In the bottom graph (III), the larval flux at profile (individuals $m^{-2}h^{-1}$) is plotted as a thick line refered to the left axis, and the mean current velocity is plotted with a thin line and refered to the right axis. Vertical profiles 3 through 7 corresponded to night hours. Negative signs in the bottom (III) graph of each section, indicates hours of larval negative relative velocity.

Fig. 4. a) Average *N. uncinata* stages mean weighted depth through the 22 hr sampling at Boca Chica (small squares), standard error (large squares) and standard deviation (error bars); b) Stage composition of *N. uncinata* total abundance.

Fig. 5. Hydrographic profiles at BG during the 4^{th}, 6^{th} and 10^{th} December 2000. a)salinity; b) temperature; c) oxygen concentration; d and e) N-S (*v*) and E-W (*u*) current velocity components.

Fig. 6. Vertical distribution of larvae at Boca Grande during the 4^{th}, 6^{th} and 10^{th} December 2000. Abundance at each strata (individuals$\cdot 100m^{-2}$) is shown for Cancrid, *E. analoga* and *P. monodon* larvae (first three columns) and depth percentage of *N. uncinata* stages I though III, and IV-V (columns 4 and 5 respectively). Shaded areas: thermocline at sampled date.

Fig. 7. Results of cluster analysis that grouped species from Boca Grande based on their oxygen quotient (*q*) over three sampling occasions and different strata. We used the Bray-Curtis index for constructing the distance matrix and a complete linkage procedure for clustering species.

Table I.

(a)

Tow	Local time	Light	Tidal phase
1	17:15 – 17:45	D	F
2	18:55 – 19:25	D	F
3	20:55 – 21:25	N	High water (F)
4	22:45 – 23:15	N	E
5	1:02 - 1:32	N	E
6	2:53 - 3:23	N	Low water (E)
7	4:45 - 5:15	N	F
8	6:35 - 7:35	D	F
9	8:40 - 9:10	D	High water (F)
10	10:35-11:30	D	E
11	12:50 - 13:20	D	E
12	14:40 - 15:10	D	Low water (E)

(b)

Date	Time	Strata
12/4/00	19:50	0-6
12/4/00	19:50	6-18
12/4/00	20:10	18-30
12/4/00	20:40	30-40
12/6/00	12:30	0-18
12/6/00	12:30	18-25
12/6/00	12:45	25-35
12/10/00	13:10	0-5
12/10/00	13:10	5-20
12/10/00	13:40	20-25
12/10/00	14:15	25-40

Table II.

Species/group and family	Infraorder	Mean density Ind/100m^3 Boca Chica	Frequency Boca Chica	Mean density Ind/100m^3 Boca Grande	Adult habitat type	Larval stages
Cancer spp. (Cancridae)	Brachyura	706	35	48	Ro S/ D	5 (a)
Neotrypaea uncinata (Callianassidae)	Thalassinidea	584	33	271	Mu S	5 (b)
Pagurus spp. (Paguridae)	Anomura	208	35	62	S	4 (c)
Pinnixa spp. (Pinnotheridae)	Brachyura	147	33	22	Co S	
Porcellanidae	Anomura	117	35	14	Ro I	2 (d)
Blepharipoda spinimana (Albuneidae)	Anomura	54	33	6	Sa I	
Pinnotheres politus (Pinnotheridae)	Brachyura	51	34	3	Co S	5 (e)
Halicarcinus planatus (Hymenosomatidae)	Brachyura	37	31	2	S	2 (f)
Pisoides edwardsi (Majidae)	Brachyura	14	29	3	S	2 (g)
Emerita analoga (Hippidae)	Anomura	4	29	34	Sa I	5 (h)
Grapsidae	Brachyura	6	23	3	I	5
Callichirus garthi (Callianassidae)	Thalassinidea	8	17	30	Mu S	5 (i)
Pleuroncodes monodon (Galatheidae)	Anomura	5	21	50	D	8 (j)
Taliepus spp. (Majidae)	Brachyura	0	0	2	S	2 (k)
Libidoclaea granaria (Majidae)	Brachyura	0	0	4	D	2 (l)

(a) Quintana 1981; (b) y (i) Aste 1982; (c) Lavados 1982; (d) Saelzer et al. 1986 ; (e) Saelzer and Hapette 1986; (f) Boschi et al. 1969; (g) Faguetti 1969a; (h) Johnson and Lewis 1942; (j) Faguetti and Campodonico 1971a (k) Faguetti and Campodonico 1971b; (l) Faguetti 1969b.

Table III.

Depth	F_s	F_d	A_s	A_d	A_c	H(min)	H(min)	VE
Average	**4.83(0.13)**	**2.09(0.2)**	**47.3(6.4)**	**32.7(6.18)**	**15.1(4.48)**	0.13	12.06	94%
5m	**4.71(0.13)**	**13.3(0.81)**	**60.4(7.2)**	10.9(9.1)	**-19.8(5.3)**	0.0	12.56	80%
10m	**4.75(0.07)**	**2.7(0.16)**	**81.61(6.08)**	**38.7(5.8)**	**51.7(4.3)**	0.3	12.0	93%
15m	5.4(3.13)	**1.77(0.27)**	3.77(13.4)	**48.45(11.5)**	**47.48(8.3)**		11.86	54%

Table IV.

Species	F_s	A_s	F_d	A_d	A_c	H(max)	H(max)	VE (%)
Cancer spp.	-0.64 (0.93)	-0.18 (0.17)	0.03 (0.4)	**0.4 (0.17)**	**3.77 (0.12)**			55.6
N. uncinata			**1.26 (0.22)**	**0.72 (0.15)**	**2.92 (0.11)**	1.24		73.7
Pagurus spp.	**-1.23 (0.48)**	**-0.25 (0.11)**	**0.76 (0.32)**	**0.37 (0.11)**	**2.72 (0.08)**	0.34	10.25	72.7
Pinnixa spp.	-0.7 (1.99)	-0.06 (0.12)	0.27 (0.25)	**0.52 (0.12)**	**2.53 (0.08)**			76.7
Porcelanids	**-0.92 (0.37)**	**-0.4 (0.14)**	**0.78 (0.32)**	**0.48 (0.13)**	**1.95 (0.1)**	0.78	9.9	75
B. spinimana	**1.74 (0.22)**	**0.68 (0.14)**	**0.88 (0.26)**	**0.59 (0.14)**	**1.91**	1.32	11.5	86.8
P. politus	-0.58 (0.95)	-0.11 (0.1)	0.25 (0.22)	**0.51 (0.1)**	**1.64 (0.07)**			81.4
H. planatus	**0.93 (0.38)**	**0.53 (0.2)**	**1.36 (0.4)**	**0.51 (0.2)**	**1.22 (0.14)**	2.3	13.98	67.4
P. edwardsi			**1.32 (0.2)**	**0.59 (0.12)**	**0.86 (0.08)**	2.14		75.9
E. análoga	**-0.8 (0.3)**	**-0.44 (0.13)**			**1.76 (0.09)**	23.59	11.02	57.5
Grapsids	-0.85 (0.6)	-0.14 (0.08)	0.61 (0.34)	**0.26 (0.07)**	**0.71 (0.06)**			68.6
N. uncinata Stages								
I			**1.39 (0.31)**	**0.49(0.15)**	**2.75 (0.11)**	1.85		56
II			**1.62 (0.26)**	**0.69 (0.19)**	**2.19 (0.13)**	0.93		60
III			**1.5 (0.16)**	**0.95 (0.16)**	**1.6 (0.12)**	0.41		80.6
IV	**-1.68 (0.41)**	**-0.4 (0.16)**	**1.53 (0.15)**	**1.02 (0.17)**	**1.37(0.12)**	1.33	12.88	87.2
V			**1.29 (0.11)**	**1.32 (0.15)**	**1.12 (0.11)**	2.25		90

Table V

Species/group	df	Ebb/Flood		Day/Night		Interaction	
		F	p	F	P	F	p
Cancer spp.	6	21.92	**0.0033**	3.22	0.12	0.13	0.72
N. uncinata	6	9.77	**0.020**	0.55	0.48	1.1	0.33
Pagurus spp.	6	12.24	**0.012**	0.09	0.77	1.14	0.32
Pinnixa spp.	6	18.89	**0.004**	0.40	0.54	0.06	0.8
Porcelanids	6	13.79	**0.009**	3.01	0.13	1.09	0.33
B. spinimana	6	0.77	0.41	5.56	0.056	3.7	0.10
P. politus	6	17.43	**0.005**	0.08	0.77	0.013	0.91
H. planatus	6	2.15	0.19	2.51	0.163	0.09	0.76
P. edwardsi	6	10.22	**0.018**	6.9	**0.03**	0.001	0.97
E. analoga	6	3.13	0.12	3.8	0.09	0.48	0.51
Grapsids	6	8.32	**0.027**	0.04	0.84	0.03	0.85
N. uncinata stages							
1	6	8.73	**0.025**	0.514	0.5	0.95	0.36
2	6	6.01	**0.049**	0.0009	0.97	0.61	0.46
3	6	9.3	**0.02**	1.5	0.26	0.28	0.61
4	6	5.04	0.065	0.43	0.53	1.02	0.35
5	6	0.14	0.71	8.66	**0.025**	0.033	0.86

Table VI.

	Range of oxygen concentration ($mL \cdot L^{-1}$)			
Species	0-1	1-2	3-4	>4
Cancer spp.	0.51	0.49	1.74	1.25
N. uncinata	0.77	1.66	1.76	0.46
Pagurus spp.	0.76	2.18	0.70	0.75
Pinnixa spp.	0.92	3.36	0.63	0.07
Porcelanids	0.39	1.59	1.38	0.97
B. spinimana	0.16	0.63	1.03	1.80
P. politus	0.00	1.26	1.43	1.40
H. planatus	0.38	1.21	1.98	0.87
P. edwardsi	0.99	0.48	0.79	1.38
E. analoga	0.11	0.31	3.78	0.62
Grapsids	0.99	3.12	0.64	0.13
P. monodon	2.77	0.37	0.18	0.40
Taliepus spp.	1.94	0.44	1.10	0.53
L. granaria	1.49	1.90	0.17	0.60

Fig. 1

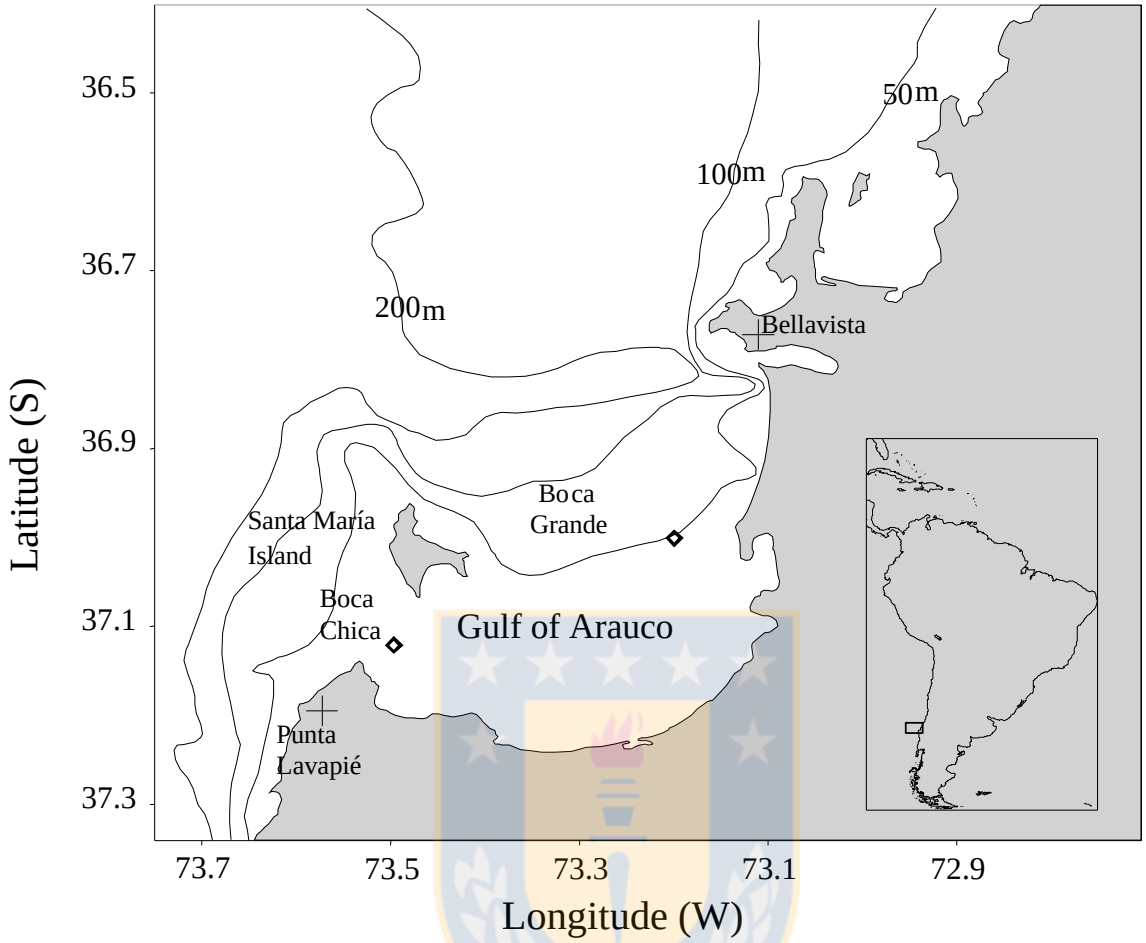

36.5
36.7
36.9
37.1
37.3
Latitude (S)
73.7
73.5
73.3
73.1
72.9
Longitude (W)
50m
100m
200m
Bellavista
Boca
Grande
Santa María
Island
Boca
Chica
Gulf of Arauco
Punta
Lavapié

Fig. 2

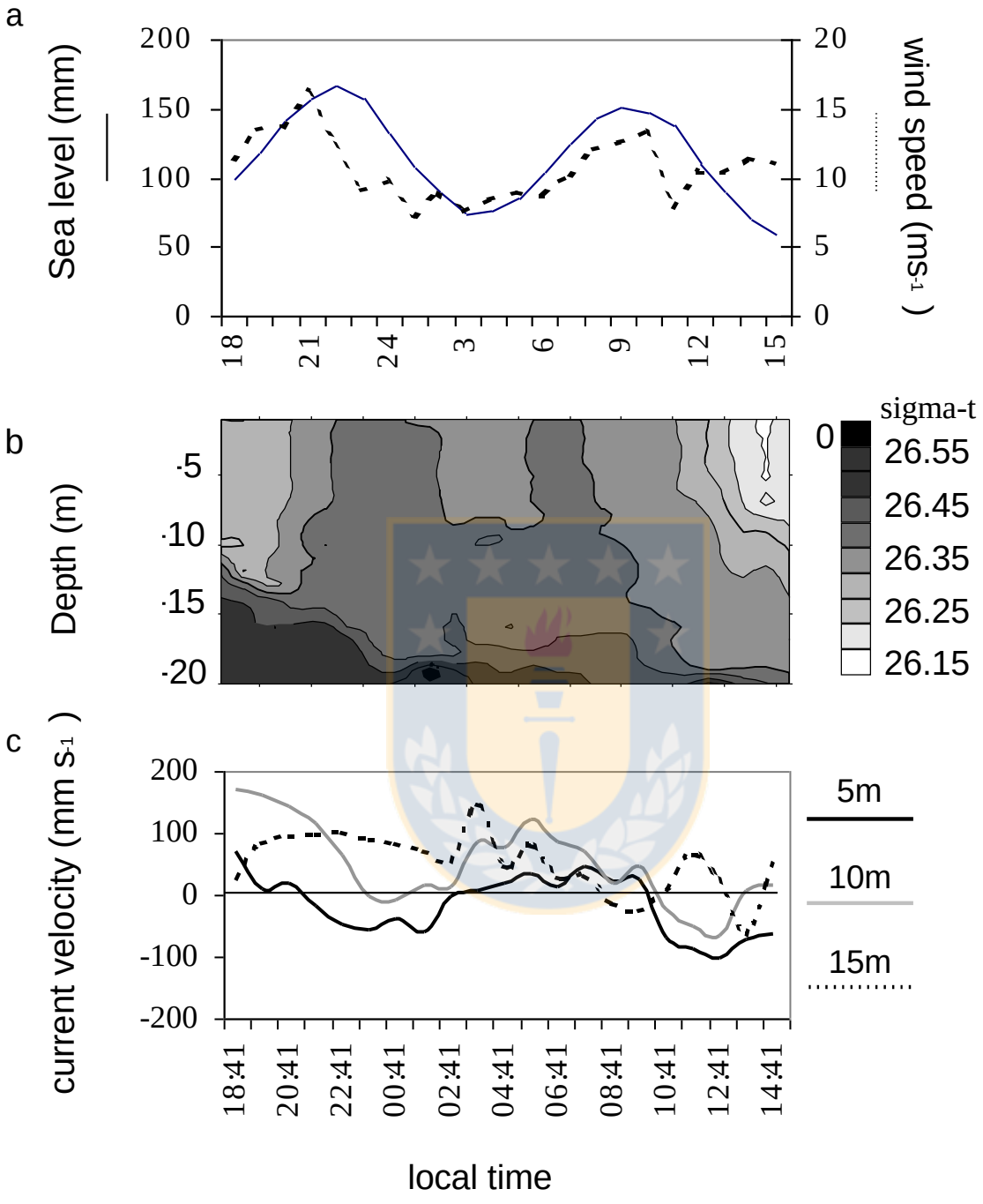

a
Sea level (mm)
200
150
100
50
0
20
15
10
5
0
wind speed (ms-1)
18
21
24
3
6
9
12
15
b
Depth (m)
-5
-10
-15
-20
sigma-t
0
26.55
26.45
26.35
26.25
26.15
c
current velocity (mm s-1)
200
100
0
-100
-200
5m
10m
15m
18:41
20:41
22:41
00:41
02:41
04:41
06:41
08:41
10:41
12:41
14:41
local time

Fig. 3

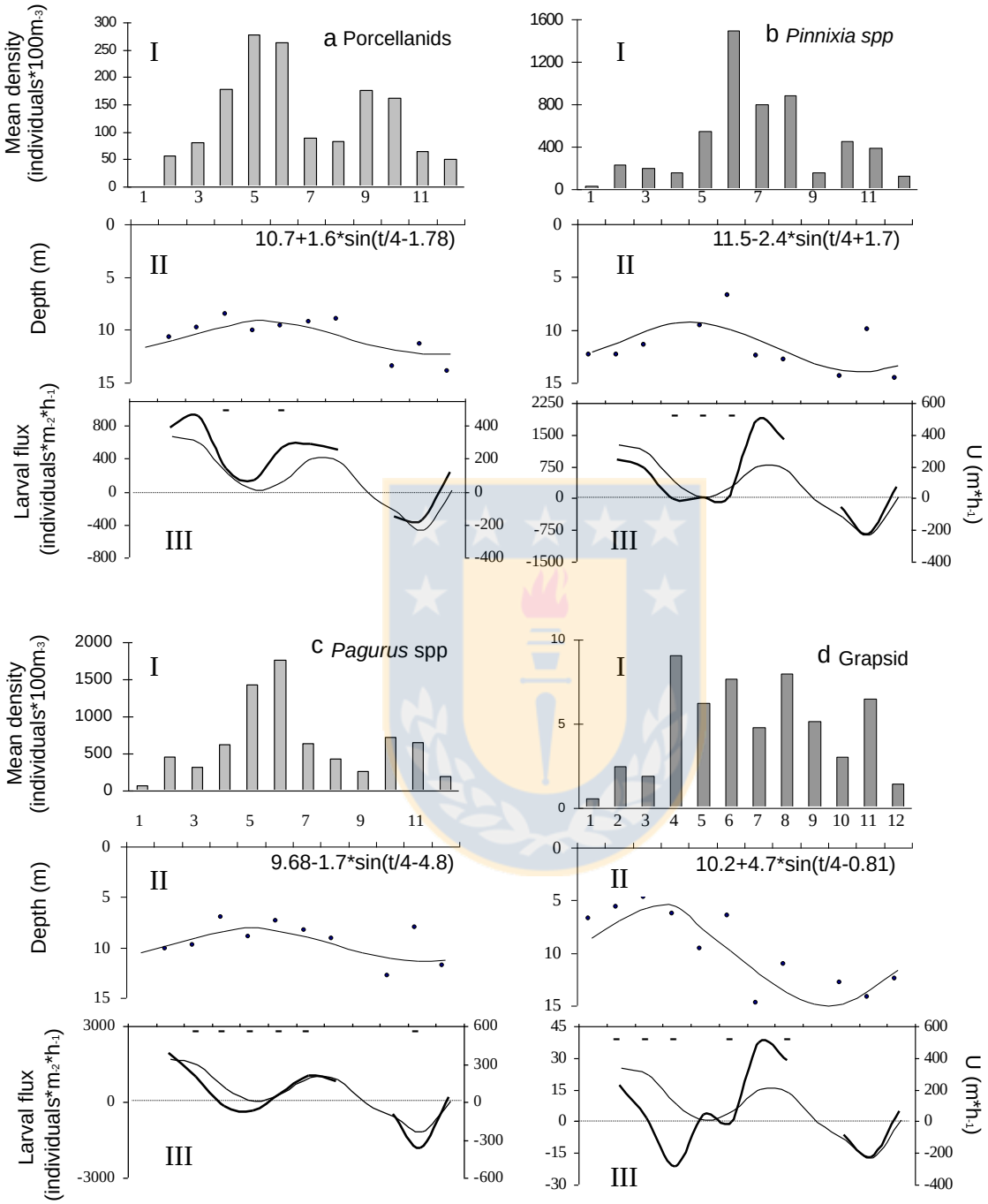

a Porcellanids
b Pinnixia spp
c Pagurus spp
d Grapsid
Mean density (individuals*100m-3)
Depth (m)
Larval flux (individuals*m-2*h-1)
U (m*h-1)
I
II
III
10.7+1.6*sin(t/4-1.78)
11.5-2.4*sin(t/4+1.7)
9.68-1.7*sin(t/4-4.8)
10.2+4.7*sin(t/4-0.81)

Fig 3 cont.

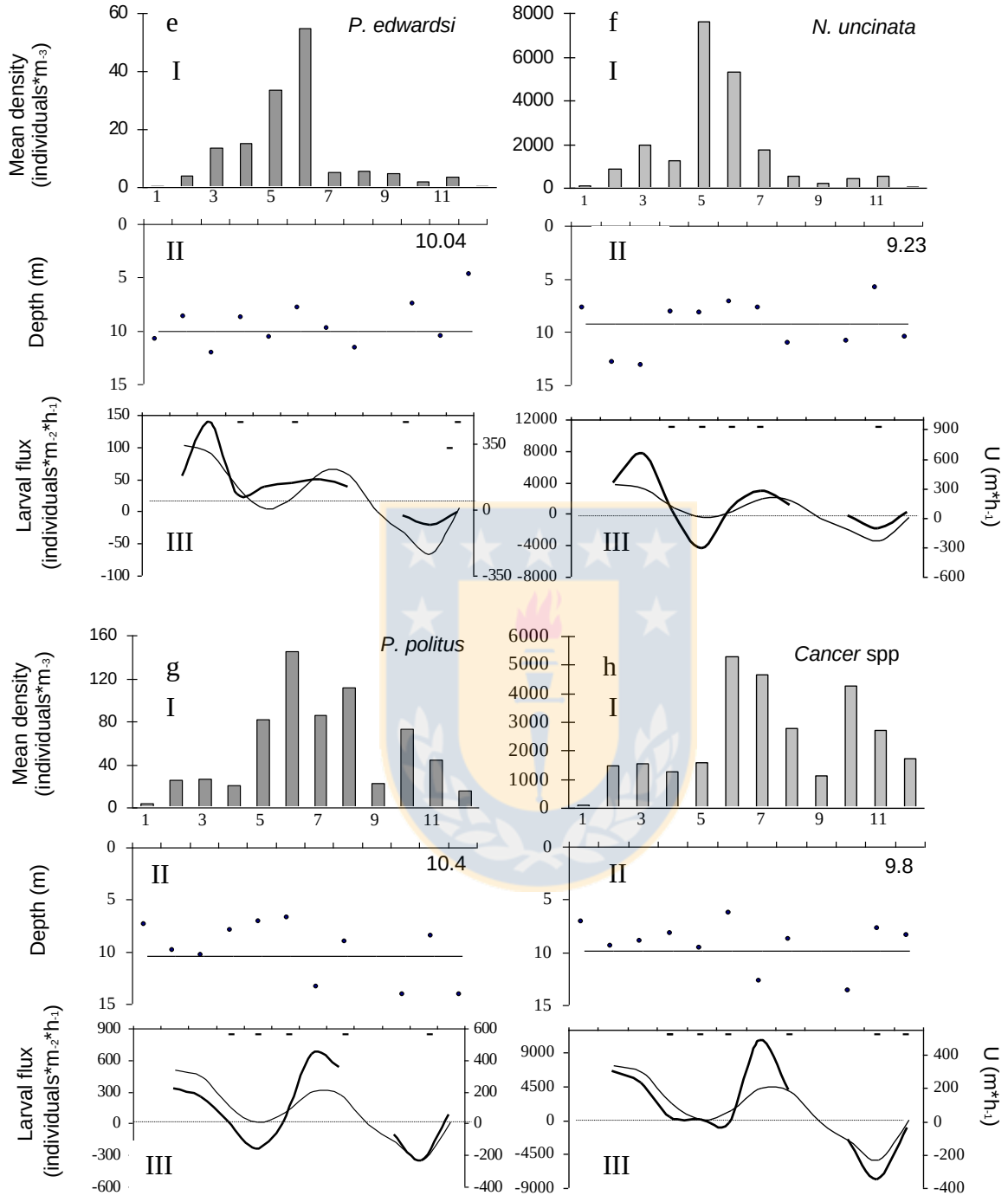

e
P. edwardsi
f
N. uncinata
g
P. politus
h
Cancer spp
I
II
III
Mean density (individuals*m-3)
Depth (m)
Larval flux (individuals*m-2*h-1)
U (m*h-1)
10.04
9.23
10.4
9.8

Fig. 3 Cont.

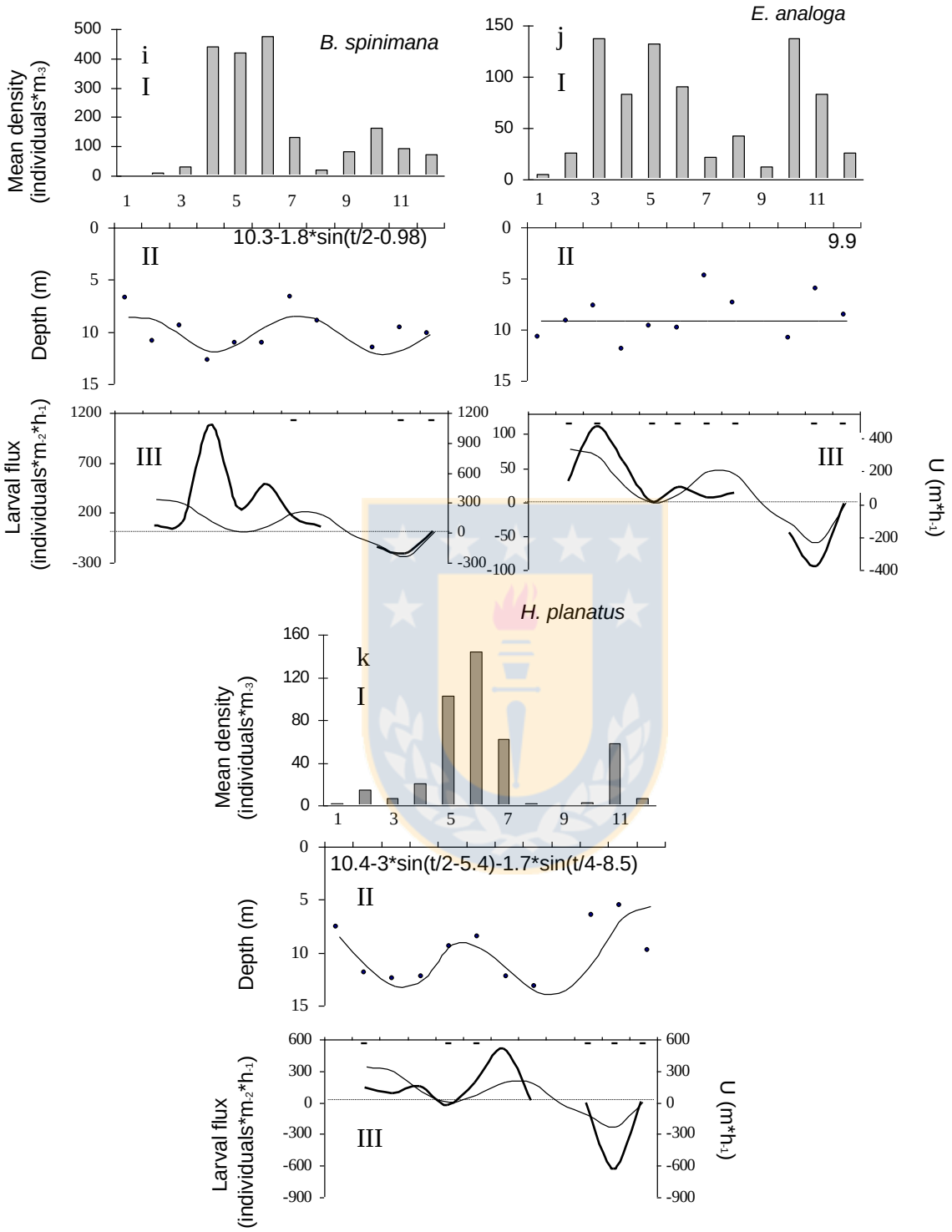

B. spinimana
E. analoga
H. planatus
i
j
k
I
II
III
Mean density (individuals*m-3)
Depth (m)
Larval flux (individuals*m-2*h-1)
U (m*h-1)
10.3-1.8*sin(t/2-0.98)
9.9
10.4-3*sin(t/2-5.4)-1.7*sin(t/4-8.5)

Fig. 4

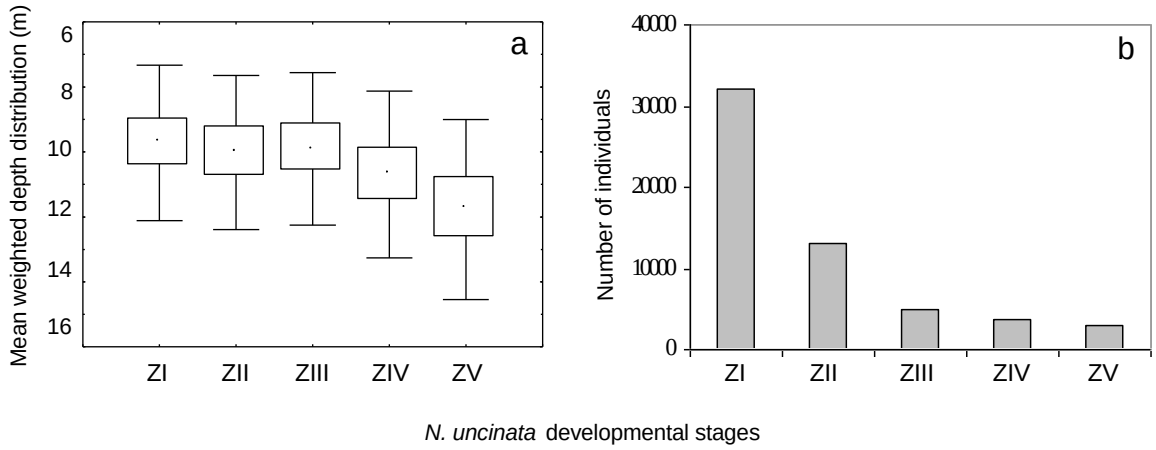

Mean weighted depth distribution (m)
6
8
10
12
14
16
a
ZI
ZII
ZIII
ZIV
ZV
Number of individuals
40000
30000
20000
10000
0
b
ZI
ZII
ZIII
ZIV
ZV
N. uncinata developmental stages

Fig. 5

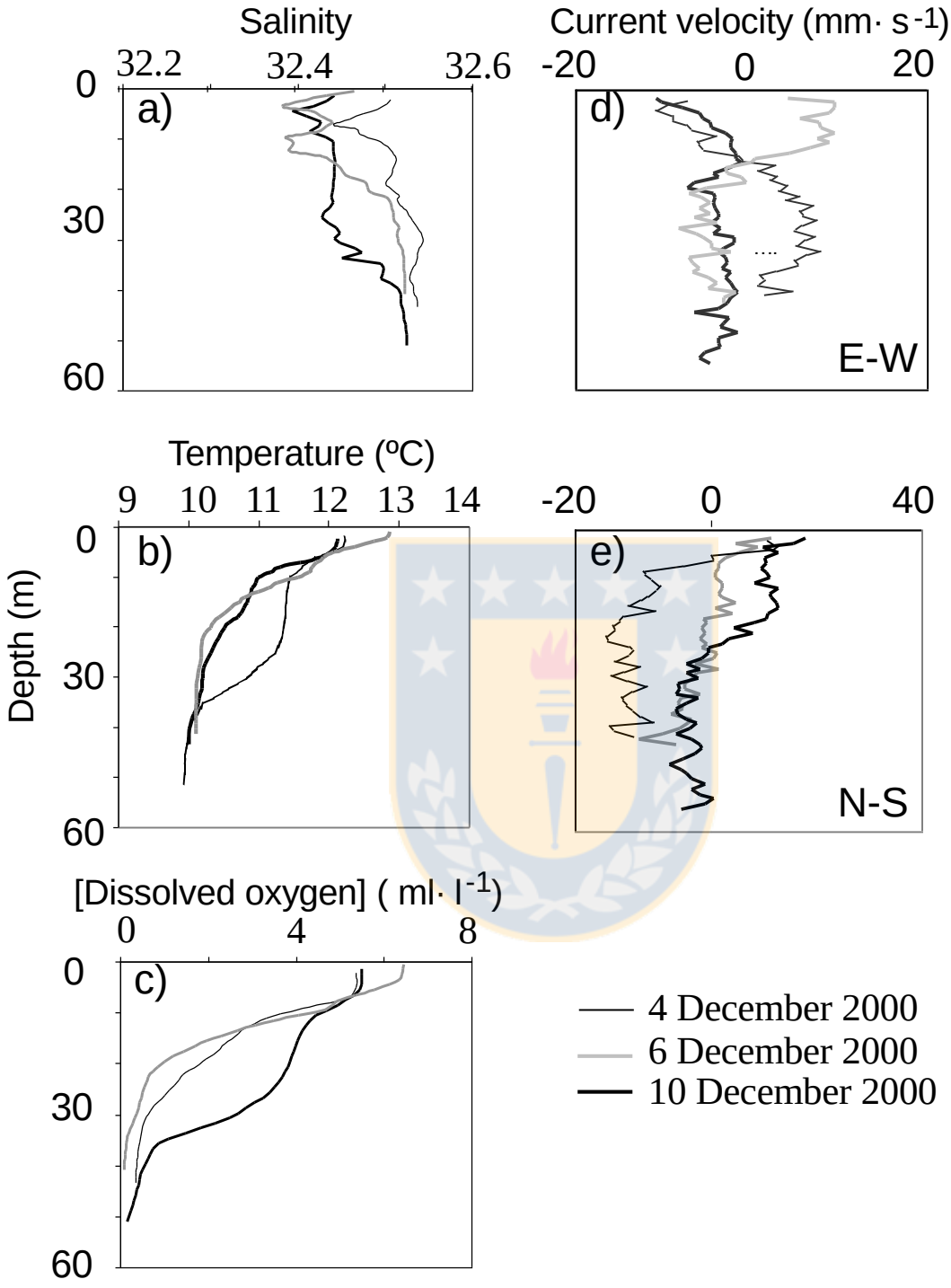

Salinity
32.2
32.4
32.6
a)
Current velocity (mm· s-1)
-20
0
20
d)
E-W
Temperature (ºC)
9
10
11
12
13
14
b)
-20
0
40
e)
N-S
Depth (m)
0
30
60
[Dissolved oxygen] (ml· l-1)
0
4
8
c)
4 December 2000
6 December 2000
10 December 2000

Fig. 6

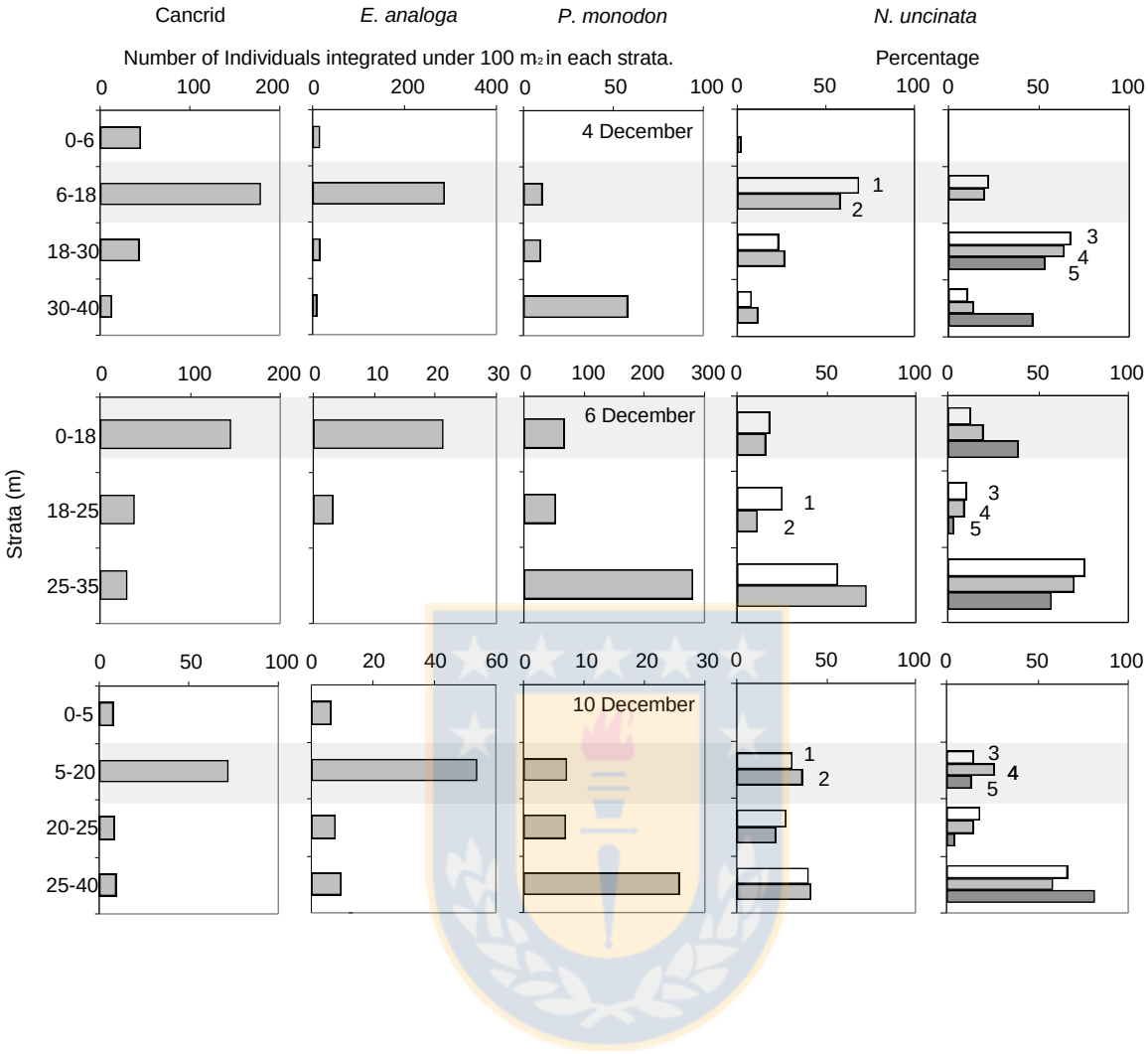
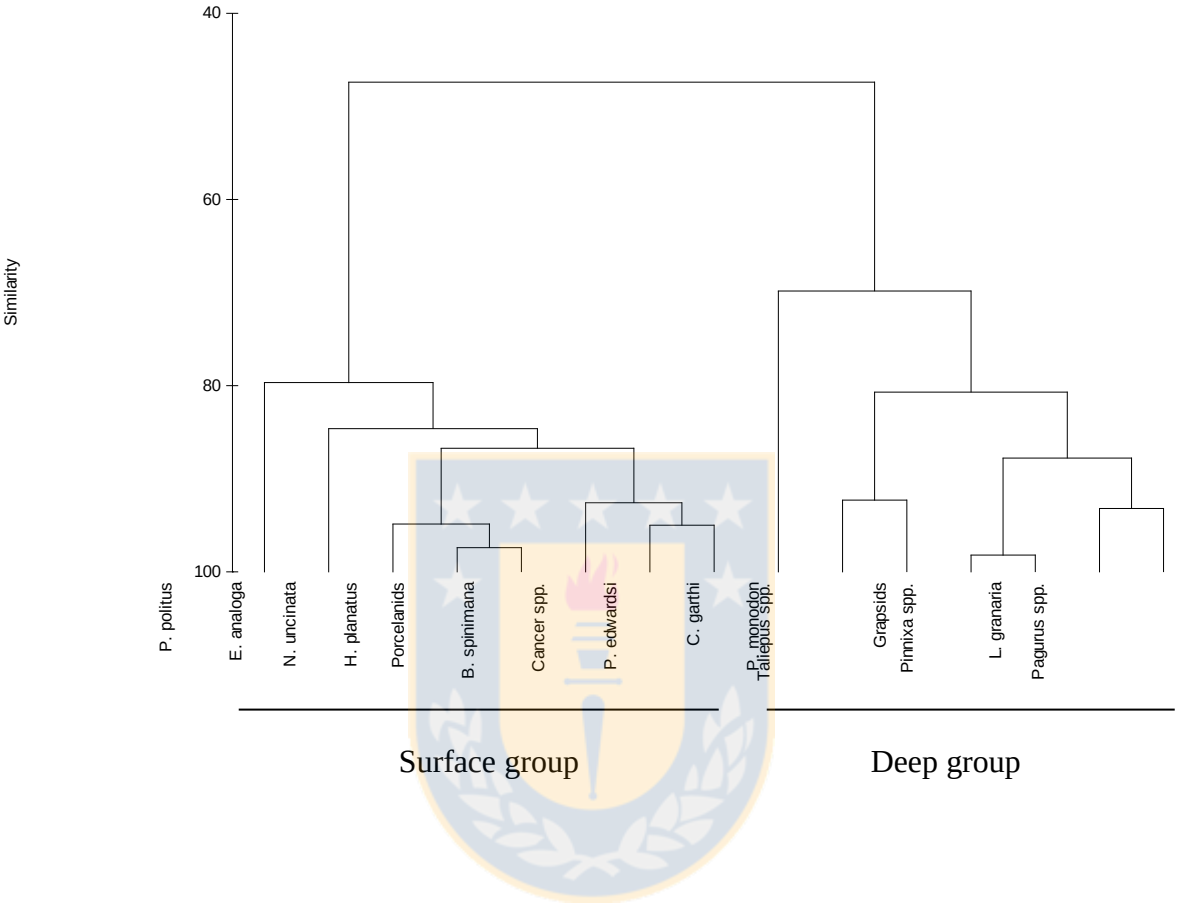

Cancrid
E. analoga
P. monodon
N. uncinata
Number of Individuals integrated under 100 m2 in each strata.
Percentage
Strata (m)
4 December
6 December
10 December
0-6
6-18
18-30
30-40
0-18
18-25
25-35
0-5
5-20
20-25
25-40
1
2
3
4
5

Fig. 7


Similarity
40
60
80
100
P. politus
E. analoga
N. uncinata
H. planatus
Porcelanids
B. spinimana
Cancer spp.
P. edwardsi
C. garthi
P. monodon
Taliepus spp.
Grapsids
Pinnixa spp.
L. granaria
Pagurus spp.
Surface group
Deep group

Manuscrito 2:

B Yannicelli, L Castro, W Schneider, M Sobarzo

Crustacean larvae distribution in the coastal upwelling zone off Central Chile

Marine Ecology Progress Series (re-enviado)

ABSTRACT

In this paper, we present data on the stages abundance and spatial distribution of five taxa of crustacean larvae whose adults inhabit different depth ranges, during late spring and late summer in the coastal upwelling area of central Chile (-35 to -37). We aimed to identify the relationship between the timing and depth range of larval release, with larval depth distribution (and behavior), and offshore transport during the main upwelling season. If advection is a relevant determinant for the success of larvae, then, species specific life history traits might be tuned with advective environmental cycles at the adult stage, or might display appropriate swimming behavior at the larval stage. In central Chile, the temporal costal upwelling dynamics, involves intra annual cycles of upwelling intensity that affect offshore/onshore seasonal advection of plankton and, nutrient surface enrichment. *Libidoclaea granaria* zoea I, whose adults occupy the continental shelf, was mainly found during late spring (November) coincident with intensified upwelling. Especially during upwelling relaxation, zoea I were widespred horizontally and zoea II appeared closed to the coast. They did not show day/night vertical migration, and they were the deeper larvae from this study. *Emerita analoga* zoea I are hatched in the intertidal, and their maximum occurred during late summer, (march: upwelling reversal season) in coastal stations, although they also spread out in the studied area. They outnumbered any other stage/species analyzed. Intermediate stages were found farther offshore, and the latest zoea stage (V) were again aggregated closer to the coast. *E. analoga* was the shallower larvae, the more widespread and no diel vertical migrations were observed. *Blepharipoda spinimana*, another intertidal species was always restricted to coastal stations, and larger numbers of all stages occurred during March. It was a shallow species and, at our sampling scale, we could not identify vertical behavior. Both paguridae and *Neotrypaea uncinata* had higher abundances of all stages in March. Maximum hatch should have occurred during summer. During November *N. uncinata* stages II to V were shallower during night time and zoeas I to IV were consecutively found deeper. Later stages were found closer to the coast. The same general pattern was found for Pagurids. Overall, the larval release timing observed for the present species together with their vertical distribution, indicate that species released off phase with maximum upwelling intensities are distributed in surface waters, and those that are

released during more intense upwelling periods are deeper and more widespread in the water column. Vertical migrating species, had a wide larval release period during upwelling seasons. In this area, besides phylogenetic development of migratory capacity, the vertical range of some taxa might be constrained by physiological tolerances to sub-surface low oxygen and temperature. The resulting spatial distribution displayed by larvae were influenced by the spatial structure of upwelling circulation, larval behaviour and physiological tolerances as well as by depth and timing of release.

KEY WORDS: crustacean larvae, upwelling area, vertical distribution, larval transport, alongshore distribution, topographically induced variability, spawning timing.

INTRODUCTION

Advection during the larval phases of benthic organisms, which results from the interaction of physical oceanographic processes and larval behaviour, has been suggested as a key factor determining site specific settlement strength therefore influencing benthic population and community dynamics (see discussions by Connolly & Roughgarden 1998, Wing et al. 1998). In eastern boundary current systems (EBC), the temporal and spatial dynamics of wind-driven coastal upwelling is thought to influence larval retention, concentration, survival, transport and hence recruitment in both vertebrates and invertebrate populations (Lundquist et al. 2000, Botsford 2001, Guisande et al. 2001, Flores et al. 2002). Temporal upwelling dynamics, involve intra annual cycles of upwelling intensity that affect offshore/onshore seasonal advection of plankton and, nutrient surface enrichment. Inter-annually, varying upwelling intensities correlate with both the expansion/contraction of the latitudinal range of species (Sorte et al. 2001) and the recruitment strength (Connolly et al. 2001). Spatially, the local topography and coastline geometry enhance the development of mesoscale circulation features such as jets, filaments, eddies, and upwelling shadows. Upwelling shadows for example, might lead to the retention of organisms in coastal sites down current of capes and points during upwelling events (Wing et al. 1998b). Upwelling fronts influence meroplankton coastal distribution (Bjorkstedt et al. 1997). Eddies can retain organisms and/or maintain production sufficiently high for larvae to feed (Kasai et al. 2002, Nishimoto & Washburn 2002) and shedding filaments might transport larvae offshore (Rodriguez et al. 2001, Hernández-León et al. 2002).

The coupling of timing and location of larval release with favourable spatio-temporal environmental conditions enhance larval survival and recruitment (Cury and Roy 1989, Hinkley et al. 2001, Stenevik et al. 2003). If advection is a relevant determinant for the success of larvae, then, species specific life history traits such as spawning timing and location can be tuned with advective environmental cycles (e.g. seasonal upwelling cycle). The release however, usually occurs within a species specific depth range and, an overall oceanographic condition (e.g. upwelling season) might affect larvae released from different depths during the same season in opposite ways.

In addition to the adult control of spawning timing and location, larval behaviour plays a role in its own advection. Behaviour regarding vertical distribution control interacts with prevailing hydrodynamics to shape distribution patterns, transport dynamics and settlement patterns (Sponaugle et al. 2002). While surface dwelling organisms might be advected offshore during upwelling events and they might later be transported shoreward during relaxation, the opposite is true for deep dwelling zooplankton (Shanks 2000, Sundby et al. 2001, Garland et al. 2002, Shanks et al. 2002, Shanks et al. 2003). Those organisms that vertically migrate between water layers flowing in opposite directions might be more concentrated nearshore (Peterson et al. 1979; Castro et al. 1993) reducing offshore loss and remaining at a food rich media. Crustacean larvae in particular might show complex vertical swimming behaviours. Semi-diurnal, diurnal or ontogenetic depth regulation has been described in a large set of crustacean species, and might arise from endogenous biological rhythms or environmentally induced behaviours (Forward and Tankersley 2001, Naylor 2005). Mechanisms favoring adequate larval advection could appear during either the adult or larval phase. A relationship between larval vertical distribution and time of spawning has been suggested for larval fish, those that are spawn during the main upwelling season are widespread in the water column (Olivar, 1990); those organisms that release eggs (larvae) in surface waters might show a peak of spawning off phase with the main upwelling season (Castro et al. , 2000).

In this paper, we analyze the spatial distribution and abundance of the stages of five species/genus of decapod crustacean larvae in the upwelling area of south central Chile (Fig. 1). Species were chosen according to contrasting depth ranges of adult habitat. We aim to identify the relationship between larval depth distribution, offshore distribution and the timing and depth range of larval release. We expect to find strategies either at the adult or larval phase that tend to minimize offshore advection, for example: i) larger numbers of early stages of non migrating surface larvae should be found offphase with main upwelling season; ii) extended spawning periods in species with migrating larvae. The ontogenetic distribution (acrosshore, spreading, clumping) as a consequence of vertical distribution and hatching period and depth, should evidence contrasting mechanisms of shelf retention (eg.: surface stages that show limited depth

control should appear more widespread, less clumpy and farther offshore during upwelling than species that do show depth control; deeper larvae should approach the coast during upwelling favourable winds).

We present data from two surveys conducted in spring and late summer along central Chile. Hydrographic data, and zooplankton stratified samples allowed the identification of major oceanographic features in the area, and crustacean larval stages vertical and horizontal distribution patterns. Our data effectively suggest a relationship between larval across-shore and vertical distribution, and adult spawning timing and depth range. However, offshore distribution is strongly influenced by coastal geography.

METHODS

Studied area:

The study area (-35 to -37°) is embedded at the coastal (shelf) edge of the Humboldt Current system, a major eastern boundary current (EBC) with an equatorward surface flow. Prevailing winds during austral spring and summer are southwesterly, and hence favor coastal upwelling conditions. Winds reverse to northerly and northwesterly directions during austral autumn and winter with some transitional periods in April and August (Arcos and Navarro, 1986). The coastline of Chile within our study area (35-37˚S) points towards 25˚ (looking from south to north). However both coastal orientation and bottom topography change markedly within a small latitudinal range (Fig. 1). The continental shelf between -35 and -35.6° is relatively narrow (15 nautical miles (nm) offshore). Around -36º the wide Itata Canyon interrupts the continental shelf. South of this Canyon the shelf widens to 50 nm (Itata terrace) and it is interrupted again by the narrow but deep Bio-bio Canyon at -36.9° S. Coastline orientation changes sharply at -37.1°, forming the equatorward facing Gulf of Arauco, and causing a 90° local divergence of the coast and isobath at the flanking eastern point (Punta Lavapie) (Fig. 1). During spring-summer period, the high salinity (34.5), low oxygen (<1 $ml \times l^{-1}$) and high nutrient Equatorial Sub-Surface Waters (ESSW), shoal over the continental shelf in this area (Djurfeldt, 1989). These waters are considered responsible for the coastal fertilization that sustains one of the most productive areas in the world's coastal ocean (Daneri et al, 2000). A strong influence of topographical and geographical features on the

upwelling process in this area has been described (Sobarzo and Djurfeldt, 2004) and modelled (Mesias et al, 2003).

Target species:

We chose for our analysis five species (groups) of crustacean larvae because of their contrasting characteristics regarding adult habitat depth range and abundance in the plankton. They differ in number of larval stages but details of their reproductive and larval ecology are not well known. Adult *Emerita analoga* and *Blepharipoda spinimana* reside in intertidal sandy bottoms. *E. analoga* has a long larval development (several months depending on water temperature) and, although ovigerous females are found year round, two main hatching periods occur during austral spring and late summer (Contreras et al, 1999). There is no information on *B. spinimana* larval developmental time and we identified four larval stages. From below 100m depth, the majid *Libidoclaea granaria*, with two zoeal stages (Faguetti, 1969) was selected , its larval development is shorter than that of *E. analoga* (about a month at 15ºC). *Neotrypaea uncinata* and Pagurids inhabit subtidal bottoms, they have five and four stages respectively, and longer larval development than *L. granaria*. At ambient temperature they developed through their last zoea stage in between 40-50 days (Aste 1982, Lavados 1982).

Sampling

During November 7th to 13th 2001 and March 7th to 12th 2002 we conducted hydrographic and zooplankton sampling over the continental shelf of Central Chile between -35 and -37º. The hydrographic survey consisted in a total of 69 profiles, in 11 equally spaced transects (from N to S transects I through XI) (fig. 1). Along each transect the stations were numbered 1 to 6 (or 7) from the coast to the ocean. Distance between stations was approximately 5 nautical miles, and began at approximately 2 to 3 nm from shore. Hydrographic casts where undertaken with a Neill Brown Mark III CTDO (November cruise) and a SeaBird SBE-25 (March cruise). Sampling was conducted throughout the 24 hours. Stratified zooplankton samples (oblique tows) were taken with a tucker trawl sampling gear. The net had a 1 m^2 frame, a 300 micrometer mesh size and it was equipped with a calibrated general oceanic flow meter. The vessel navigated at approximately 2 knots during tucker trawl deployment. Cable length was adjusted

according to the desired depth and the angle between the cable and the vertical. In the northern zone of our sampling area (from -36 to -35 ºS), strata were 0-50, 50-100, 100-150, 150-250 depending on the station depth. In the southern area, vertical resolution was higher, and we took samples every 25 m down to 100 m, and every 50 m in the deeper layer depending on station depth. The definition of strata to be sampled resulted from a compromise between cruise time and information to be gathered. Based on previous cruises in the area it was known that water properties usually show stronger vertical gradients in the southern sampled area, and therefore a more intensive sampling regarding vertical resolution should be conducted there. Samples were fixed in buffered formalin (10 %) and brought to the laboratory for identification and counting. Winds for the period were obtained from Carriel Sur Airport of Concepcion (36°46'22''S; 73°03'47''W).

The selected crustacean larvae from transects III, and V through XI were sorted and identified from November 2001 cruise, and from transects III, V, VII, X and XI from March 2002 cruise. Under stereo-microscope, *Neotrypaea uncinata* stages were identified following Aste (1982); Pagurids, following Lavados (1982), and *Libidoclaea granaria* following Faguetti (1969); the species *Blepharipoda spinimana* was identified following Concha (1978) and their stages were classified based on our own inventory; *Emerita analoga* was sorted into stages following Johnson and Lewis (1942).

Data analysis

In order to reveal oceanographic features taking place during the sampling period, we constructed horizontal maps of temperature and salinity. Wind data for the period was used to estimate the Ekman transport (U_E in m^2s^{-1}) following Gill (1982):

$$U_E = \frac{Y_s}{\rho_w f}$$

where,

Ys = along shore wind stress

ρ_w = water density (1025 kg/m^3)

f = Coriolis parameter (= - 8.69E^{-5} s^{-1}).

Larval numbers were first standardized according to filtered volume by each net, and expressed as number of individual under 100 m^2 for each strata. They were also integrated down to the maximum sampled depth at each station to construct horizontal distribution maps. Due to the patchy distribution of plankton, surveys are characterized by a large proportion of 0 counts and few high values, therefore, mean stages density, variance of the mean estimate and variance over the sampled area were calculated based on a delta distribution (Pennington, 1983). Stages abundance within each species should provide a gross estimate of main hatching period.

Three analysis of spatial distribution were performed in order to summarize and compare each stages/species spatial information: i) The spreading of larvae in the sampled area was analysed through the percentage of positive stations out of the total analysed in each cruise. ii) In spite of the availability in the literature of several patchiness indexes developed by terrestrial ecologists and/or counts data, their applicability in plankton density data is not straight forward (see Bez 2000 for a discussion). Therefore, in order to reveal the degree of clumping in the species/stages in this study and to be able to compare among stages and species, we calculated the coefficient of variation (CV: standard deviation/mean) based on the delta distribution. This index was calculated for the March cruise when most species and stages were in larger quantities as to allow comparisons. iii) Across-shore distribution in each transect was analyzed with the weighted mean offshore distance (*OD*) in each transect:

$$OD = (\sum d_i * n_{i)})/N$$

where d_i is the distance offshore of station i, n_i is the integrated number of organisms at station i, and N is the total number of organisms in the transect ($\sum n_i$).

In order to characterize the vertical distribution of larvae, we calculated the mean weighted depth (*MD*) distribution (also called 'centroid') for each group of organisms at each station as:

$$MD = \sum (p_k * z_k) / \sum p_k$$

where p_k is the number of organisms at stratum k, and z_k is mean depth of stratum k. Also across-shore modal position was registered for each group. Plots of mean weighted *MD* for each stage at day and night time were constructed, and day/night differences were

analysed in the following way. When *MD* was significantly correlated with station depth we carried out analysis of covariance on *MD* for factor day and night, with maximum station depth as a covariate (separate or homogeneous slopes depending on parallelism). When such a correlation was not found a *t* test for independent samples was applied. Whenever variance was heterogeneous (Levene test), *t* test with independent variances was applied. We also compared overall species depth distribution (*MD*) with Kruskall-Wallis analysis of variance and Mann-Whitney *U*-test to compare pair of samples. Both temperature and oxygen concentration at *MD* for each species (all stages pooled) were obtained. The frequency of centroids occurring within certain temperature and oxygen ranges was calculated and the cumulative percentage frequency as a function of temperature and oxygen was used to characterize the environment for each species.

RESULTS

Meteorological forcing and general along-shore hydrographic conditions

Just before the November cruise and during the first two days (sampling transects I through III), southerly winds were moderately favourable for upwelling. Later (sampling over the Itata Canyon: transects V, VI and VII) winds changed to northerly, (downwelling favourable) and they were only interrupted by one day of southerly winds (sampling of transects X and XI). During March 2002 wind was again mildly favourable for upwelling during the week before and during the cruise, with only two daily reversals (sampling transects IV and V, and the end of transect XI) (Fig. 2).

During November 2001, temperature at 10 m depth was colder than March 2002 with an upwelling event in the northern region (transects I, II and III) and a coastward intrusion of more warm oceanic water probably associated with the Itata canyon (transects V, VI and VII) (Fig. 3a). Equatorial Sub-Surface Waters (ESSW) with temperatures between 11.0 and 12.0 °C and salinities between 34.4 and 34.5 showing coastal upwelling at the north of the Itata Canyon. Higher surface temperatures (around 13ºC) associated with lower salinities (34), dominated the Itata terrace at the south of the Itata Canyon. On this shelf the base of the thermocline was located between 40 to 60 m depth (not shown). The base of the thermocline within of the Itata canyon (10.5ºC isotherm) was found at around 100 m depth (transect VI and also V). Major horizontal

gradients of temperatures appear at the head of the Itata canyon and northward of this canyon. The Itata terrace was more homogenous and onshore-offshore gradients almost vanished. The freshest surface water is found in the south related to fresh water runoff from the Bio-bio river. In the deeper layer the contours of salinity and oxygen closely followed the bottom topography showing the intrusion of Equatorial Sub-Surface Waters (ESSW) into the Bio-bio and the Itata Canyons (not shown).

During march 2002, all coastal region showed cold waters (11-12 °C) and salinities around 34.4 to 34.5. In the north, the strongest onshore-offshore surface temperature gradients occurred, warm waters were found offshore and surface salinity showed the influence of Rio Maule. The base of the thermocline was located at about 60m (11.5ºC isotherm) in the north, shallower over the Itata terrace at transect VII (20 to 25 m depth), and deeper in the Itata Canyon (transects VI and V, 50m). Again, a coastward intrusion of surface water more warm (15 to 16°C) and with salinities near to 34.2 appear related with the Itata canyon, resembling the importance of the bathymetry.

Abundance and stage composition

Overall species abundance was larger in March than in November, except for *Libidoclaea granaria*. *L. granaria* showed a marked peak in stage I abundance during November, while it was less than tenfold lower in March. In March, the largest proportion was comprised of stage II larvae, indicating that major spawning occurred during spring (Table 1). Overall abundance of *Emerita analoga* was a hundred fold larger in March than in November. Stages I and V were dominant during November while intermediate stages were scarce. During March, zoea I by itself outnumbered by far all other present stages/species, indicating a peak larval release during late austral summer (Table 1).

Intermediate stages of *Neotripaea uncinata* and Pagurids were more abundant than early and late stages during both seasons. Larger quantities of larvae of both species were found in March, suggesting peak larval release during summer. The same is suggested for *Blepharipoda spinimana*, it was barely present in November while in March there was an even distribution of younger and older stages (Table 1).

Neither in November nor in March the percentage of positive samples (*PS*) occupied by each stage was linearly correlated with density. Very low *PS* co-occurred with very low organism numbers, however, large *PS* occurred over a wide range of abundances both inter and intra-specifically.

In November 2001, the more widespread distribution was that of *Libidoclaea granaria* I (PS 89%), followed by *Emerita analoga* V and Pagurids III (Table 1). *Neotrypaea uncinata* showed intermedite *PS* (from 49-69%) and *Blepharipoda spinimana* was circumscribed to a few (coastal) stations (*PS* 0.2-13%). During March, all stages of *E. analoga* were more widespread than the other groups (*PS* above 85%), followed by *L. granaria*. *B. spinimana PS* ranged over 20% without changes in abundance from stage to stage, and it was again the least spread (Table 1).

During March *Libidoclaea granaria* and *Emerita analoga* showed the least heterogeneous spatial distribution (as denoted by the lower overall coefficient of variation, Table 1). However, *E. analoga* showed marked contrasts between stages, heterogeneity decreased from stage I to IV and increased again at stage V. *Blepharipoda spinimana* and *L. granaria* also spread out with increasing stages. On the contrary, Pagurids and *Neotrypaea uncinata* showed higher aggregation at intermediate stages.

Acrosshore distribution

During both cruises, most larvae were found at or coastward of station 3 in the northern area (except *Emerita analoga).* During March 2002, some stage II *Libidoclaea granaria* were spread offshore in this area. During November, in the central area, *Libidoclaea granaria* was found along the entire transect V and further offshore at trasnect VII (Fig. 9). *B. spinimana* peaked at the coastal stations (tr V, VI and VII). *E. analoga, Neotrypaea uncinata* and Pagurids also peaked at the coast at the transects over the Itata Canyon but also spread offshore in transect VII. During March 2002, in contrast, all groups OD over transect VII were shifted coastward compared to November. In the south, transects at the northern and southern flanks of the Bio-bio Canyon (X and XI) were sampled under upwelling favourable winds and the beginning of a reversal during both cruises. During November, *Emerita analoga*, Pagurids, *Blepharipoda spinimana* and *Neotrypaea uncinata OD* ranged from 3 to 4.5 (offshore, compared to the northern region). *Libidoclaea granaria* in contrast, was more concentrated towards the coast. During

March, *OD* at transects X and XI were offshore with respect of those in other transects (Fig 4-8).

Overall, *Emerita analoga*, Pagurids, *Blepharipoda spinimana* and *Neotrypea uncinata* (except stag II) were distributed further offshore in the southern area compared to the north and Itata Canyon areas. *Libidoclaea granaria*, instead, was farther offshore at the Itata Canyon (sampled during donwelling winds), and approached the coast in the north and south (sampled during upwelling winds in November). *E. analoga* stage I and V concentrated closer to the shore than intermediate stages during both cruises. Late stages *N. uncinata*, *L. granaria* and Pagurids approached the coast with increasing stages, however across-shore range was more restricted during March. *B. spinimana* was during both cruises the species closest to shore, while *L. granaria* and *E. analoga* the furthest offshore.

Depth range distribution

During November 2001, *Neotrypaea uncinata* stages II, III and V were significantly deeper during day time than during night time (Fig 10). Stages I and IV showed no significant differences in depth distribution (Table 2). Only *Emerita analoga* stages I, IV and V were analyzed during November 2001 because of the low number of positive stations of the other stages, and there were no significant differences for day/night mean depth distribution in any of these 3 stages. The same was true for *Libidoclaea granaria* stages I and II. Pagurids were deeper in the water column during daytime, but differences were significant only for stage IV (ANCOVA, Table 2). *E. analoga* distributed significantly shallower than *L. granaria*, Pagurids and *N. uncianta* (Kruskall-Wallis for factor species, and Mann-Whitney *U*-test for pair comparisons, $p<0.01$). *Blepharipoda spinimana* was not considered since they occurred in very few stations only.

In November, as shown by the cumulative percent frequency of *MD* occurrence at temperature/oxygen concentration (Fig. 11), more than 50% of *Neotrypaea uncinata*, Pagurids and *Libidoclaea granaria* centroids occurred at temperatures below 11ºC and oxygen levels lower than 1 ml/l which characterize ESSW. On the contrary *Emerita analoga*, the shallowest species, occurred mainly above 3 ml/l and 11ºC.

During March 2002, *Neotrypaea uncinata* and Pagurids showed the same trend as in the November cruise (shallower position during nighttime except *N. uncinata* stage I) although differences were not significant for the March cruise (Table 2). During March, not only less stations were considered in the analysis but it coincided that shallower stations were sampled during day time. The mean weighted species depth showed that *B. spinimana* was the shallowest followed by *E. analoga* and that *L. granaria* was the deepest.

DISCUSSION

Larval distribution: topography and upwelling phase

Different patterns of across-shore larval distribution were observed among areas and species. In the northern area, where upwelling was circumscribed over the narrow shelf most species of crustacean larvae were concentrated between the coast and the shelf break, offshore the marked temperature gradient that coincided with the shelf break few larvae where found. The species located nearshore were those that were mainly released during summer, and/or displayed vertical migrations. Surface dwelling larvae (*Emerita analoga*) and early stages of deeper released larvae (*Libidoclaea granaria*), were not circumscribed to coastal stations in this oceanographic setting. These are expected patterns of across-shore distribution in a coastal upwelling area (Peterson et al. 1979).

In the southern area, in spite of prevailing upwelling winds as in the north, and during both cruises, larvae were widespread between the coast and the offshore stations, with maximums at intermediate distance from shore. Evidence from direct current measurements (Atkinson et al. 2002, Valle Levinson et al. 2003), modelling simulations (Mesias et al. 2001) and surface advection (Navarro et al. 2004) indicate that recirculation features develop at the Gulf of Arauco area as a consequence of coastal geometry. These recirculation features could have the potential for retaining organisms over the shelf, even during upwelling prevailing winds although not necessarily at the coast. Large concentrations of plankton have been reported repeatedly in the in the gulf of Arauco-Bio-bio area (Valle-Levinson et al. 2003) as well as high levels of production (Daneri et al. 2000), diminishing mainly offshore the shelf break. In this area, there were not conspicuous differences between the horizontal larval distribution of the different

species, probably indicating that local circulation might have disrupted any potential inshore-offshore gradient over this part of the shelf.

In the central area (Itata Canyon), when surface warmer waters were closer to the shore during downwelling, larval peaks occurred in the coastal station, except *Libidoclaea granaria*, the deeper larvae that were spread offshore.. However, to the south of the Canyon (transect VII), while most groups spread offshore during downwelling (November) they remained closer to the coast during upwelling (March). These alternating situations, were evident for all taxa, and differ from our expectations (specially the downwelling situation for vertically migrating and surface larvae that should have been concentrated at the coast). However, the modelling of coastal upwelling process between the -38 and -35º indicates that a strong equatorward jet develops and meanders along the shelf break north of Punta Lavapie, shedding cyclonic eddies at about -36 (Mesias et al., 2003). In our study, a northward flow along the shelf break would bend coastward at the Itata Canyon during November (as inferred from the geopotential anomalies: Sobarzo et al. 2004, Yannicelli et al. in prep.), thus, if organisms are entrained into this type of features, their across-shore distribution would respond oddly to upwelling/downwelling dynamics.

Larval distribution and species ecology

Libidoclaea granaria larvae were mainly released during spring, the period of intensified upwelling. *L. granaria* occupies the continental shelf in south-central Chile, and usually turns up as bycatch of the squat lobster *Pleuroncodes monodon* fishery trawling at depths around 100 m. This seasonal timing of larval release also appears to coincide with that of *Pleuroncodes monodon* (Palma & Arana 1997). There is very little information regarding larval distribution of crustaceans whose adults inhabit depths around 100m, and their association with upwelling cycles. For larvae released over the shelf area, the upwelling season might imply onshore transport, and enhanced feeding conditions. The facts that zoea I appeared widely distributed (especially during downwelling winds) and zoea II approached the coast (especially during upwelling) support that idea. Across-shore distribution was apparently accomplished by occupying deeper waters than the other species and without displaying diel changes in their vertical

position. In fact, mean depth was strongly associated with station depth, and a large proportion of larvae occurred in low oxygen ESSW. In a spatially limited study, *L. granaria* larvae had been reported in deep intruding waters in the gulf of Arauco during upwelling conditions (Yannicelli et al. submitted). These results are in agreement with Garland et al (2002), who showed that larvae of deep dwelling benthic adults could be transported shoreward in sub-surface waters during upwelling conditions over the continental shelf. Similar patterns have also been observed for mesopelagic fishes spawning at the subsurface in the shelf break of this upwelling area (Vargas & Castro 2001, Landaeta & Castro, 2002) and for mesopelagic copepods that approach the coast to reproduce during the upwelling season (Castro et al. 1993).

From the other extreme of adult habitat depth range, *Emerita analoga* presented a contrasting pattern. *E. analoga* was also widespread horizontally, but it was very shallow, and its depth range was more restricted than that of *L. granaria*. Larvae are released in the intertidal and although highest concentrations of stage I were found close to shore, high offshore advection was evident even for this first stage. *E. analoga* spawning in late summer is strongly opposed to the pattern of larval release of *Emerita brasiliensis* from the Atlantic coast at approximately the same latitude (Gimenez and Yannicelli unpublished data). Since closely related species from the same habitat tend to develop convergent strategies when faced to similar environmental pressures, we proposed that the oceanographic setting (seasonal upwelling dynamics) in south central Chile is shaping *E. analoga* timing of larval release. *E. analoga* increases its offshore distance at intermediate stages and approaches the coast again during the last stage V during both cruises. The mechanism by which this transition is accomplish is not clear from our study, behaviour is strongly suggested by the fact that clumping decreases from early zoea to intermediate ones, and then it increases again, a pattern that has been discussed in larval fish and attributed to behavioural concentration as larvae progress through ontogeny (Bradbury et al. 2003). Usually the behavioural mechanism that accomplishes return to coastal habitat, is a deepening of late zoeas. However that is not evident from the November cruise and slight evidence appeared during March.

Blepharipoda spinimana, the second intertidal species, was always restricted to very coastal stations and it spawned mainly during summer. Although development should be

shorter than *Emerita analoga*, this fact alone can not account for the differences in spatial distribution observed, since even stage I *E. analoga* was distributed further offshore than *B. spinimana*. The depth range and absolute depth were lowest for *B. spinimana*, however, since they were sampled only over shallow strata, and depth distribution is correlated with station depth, this comparisons might not be informative. Yannicelli et al. (submitted) showed that *B. spinimana* could be retained within the gulf of Arauco during the upwelling season by finely regulating their depth distribution. Such a regulation can not be assessed by the present sampling scheme; however, its restricted coastal distribution is in agreement with the prediction from previous results. Since the vertical structure of flow changes within a few meters in the coast, it should not be necessary for larvae to perform large vertical excursions in order to remain nearshore.

Larvae of Pagurids and *Neotrypaea uncinata*, the two subtidal taxa, showed quite remarkable similarities regarding spatial and temporal distribution patterns. Their larval abundance was higher in March and their stage temporal distribution indicate that peak spawning occurred during summer. For *N. uncinata*, diel vertical migration was evident as well as a deepening from stage I to III and IV during day time. Yannicelli et al (submitted) reported ontogenetic deepening of zoea IV and V *N. uncinata*, which can not be fully addressed in the present study because their late stages are concentrated in coastal shallow stations. They also reported a tendency of Pagurids to present diel vertical movements and the present study confirms a vertical migration for zoea IV. The ontogenetic pattern of vertical distribution caused offshore early larval transport and late stages inflow to the gulf of Arauco. Accordingly, during the present study, older stages of both groups were closer to the shore.

In order to utilize the ontogenetic and dial vertical migration strategy for transport as well as that of *Libidiclaea granaria*, species should be highly tolerant to hypoxic conditions prevailing during the upwelling season even at the nearshore stations. Crustacean larvae have hardly been reported in very low oxygen concentrations, which is usually considered detrimental (Anger, 2001). Adults Thallasinids might inhabit poorly oxygenated tubes and sediments, and are known to be highly tolerant to hypoxic conditions (Miller et al. 1976). Adult *L. granaria* can also inhabit sediments bathed in hypoxic waters. In spite of the fact that larvae not necessarily display the same

physiological capabilities as adults, it is probable that these groups have developed high tolerance to hypoxia since they are released in hypoxic waters overlaying adult habitat.

It has been suggested that species that spawn before the main upwelling season would spread farther because they would be displaced offshore (Wing et al. 1998). In this case, *Libidoclaea granaria*, spawns earlier during the upwelling season and stage I was in fact distributed further offshore. However, *Emerita analoga* whose main peak occurred during march, at the transition period, also distributed offshore in spite of its coastal release site. Therefore other factors should be recognized. For *L. granaria* wide distribution should be due to its wide release site over the shelf, and for *E. analoga*, to its surface preference and long larval development.

Overall, the larval release timing observed for the present species together with their vertical distribution, agrees with that presented for larval fish in upwelling areas (Olivar 1990). Species released off phase with maximum upwelling intensities are distributed in surface waters, and those that are released during more intense upwelling periods are deeper and more widespread in the water column. Such a pattern repeated in so different taxa, denotes the strong effect that the advective environment through its influence on early life history, might exert on adult benthic populations dynamics. Also, vertical migrating species, seem to have a wide larval release period during upwelling seasons, enhancing onshore retention. Organisms that have the capability of regulating their positioning in the water column could become more independent of physical oceanographic processes and therefore spawning timing could be more influenced by other factors such as temperature or productivity. In this area, besides phylogenetic development of migratory capacity, the vertical migratory capability of some taxa might be constrained by physiological tolerances to low oxygen and temperature. Therefore, the resulting spatial distribution displayed by the larvae are influenced by the spatial structure of upwelling circulation, the larval tolerances to the chemical environment and the larval own behavioural responses as development progresses.

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Table 1. Abundance and distribution of *Neotrypaea uncinata*, *Libidoclaea granaria*, *Emerita analoga*, Pagurids and *Blepharipoda spinimana* larval stages in south central Chile continental shelf during November 2001 and March 2002. PS: positive stations; Var mean: variance of the mean estimate; Var: variance; CV: coefficient of variation. Mean number of individuals integrated per 100 m^2.

		NOVEMBER 2001				MARCH 2002				
Species	**Stage**	**PS**	**Mean**	**Var mean**	**Var**	**PS**	**Mean**	**Var mean**	**Var**	**CV**
N uncinata	I	0.69	556	41600	3837988	0.53	236	10841	393270.2	2.66
	II	0.53	922	150300	13665233	0.60	4620	9219800	6.36E+08	5.46
	III	0.49	450	38000	3382600	0.60	2156	1964635	1.32E+08	5.33
	IV	0.58	385	32300	2714652	0.47	549	54591	1840836	2.47
	V	0.53	606	89000	10083285	0.43	134	2948	95289.12	2.30
L. granaria	I	0.89	1580	318000	31294697	0.60	93	733	23063.31	1.63
	II	0.33	50	500	44363.96	0.67	219	2983	92686.18	1.39
E. analoga	I	0.56	212	5100	437204.5	0.93	12164	31825190	1.8E+09	3.49
	II	0.09	23	280	35513.76	0.87	2418	1025250	47546913	2.85
	III	0.07	14	130	18473.15	0.87	804	47471	1616605	1.58
	IV	0.31	19	300	1346.1		1379	49921.52	1551264	0.90
	V	0.80	173	1400	87053.06	0.87	330	9415	332048.6	1.75
Pagurids	I	0.16	26	200	14490.84	0.30	87	1825	57944.91	2.77
	II	0.24	46	400	33168.01	0.60	1218	296633	11546060	2.79
	III	0.76	262	3100	180570.7	0.60	1847	752135	30605569	3.00
	IV	0.22	78	1600	120668.7	0.57	1196	304063	11768648	2.87
	M	0.13	8	10	1086.838	0.53	269	11645	399152.2	2.35
B. spinimana	I	0.13	20.3	141.8	9678.26	0.37	322	32145	1166296	3.35
	II	0.04				0.50	313	19331	685053.3	2.64
	III	0.07	5.6	18	1234.986	0.53	324	20177	729336.8	2.64
	IV	0.02				0.57	329	17415	610974.2	2.38

Table 2. Summary of analysis comparing day and night mean depth of each species stage. Dependence: mean depth dependence on station depth (linear regression). Paralelism: when the day and night mean depth depended on station depth, the parallelism for regression lines for day and night samples. CoVar: analysis of covariance, performed if dependece and parallelism were significant. t-test: between day and night samples when covariance analysis was not proper or could not be performed.

		NOVEMBER 2001				March 2001			
Species	**Stage**	**Dependence**	**Paralelism**	**CoVar**	**t-test**	**Dependence**	**Paralelism**	**CoVar**	**t-test**
N uncinata	I	n.s.			n.s.	n.s.			n.s.
	II	*	n.s.		**	n.s.			n.s.
	III	*	n.s.		**	n.s.			n.s.
	IV	n.s.			**	n.s.			n.s.
	V	*	n.s.		**	n.s.			n.s.
L. granaria	I	n.s.			n.s.	*	*	n.s.	
	II	n.s.			n.s.	*	*	n.s.	
E. analoga	I	n.s.			n.s.	n.s.			n.s.
	II					n.s.			n.s.
	III					n.s.			n.s.
	IV	n.s.			n.s.	n.s.			n.s.
	V	n.s.			n.s.	n.s.			n.s.
Pagurids	I					*	*	n.s.	
	II	n.s.			n.s.	*	*	n.s.	
	III	n.s.			n.s.	*	*	n.s.	
	IV	*	*	*		*	*	n.s.	
B. spinimana	I					*	n.s.		n.s.
	II					*	n.s.		n.s.
	III					n.s.			n.s.
	IV					n.s.			n.s.

n.s. non significant differences
* significant at $\alpha = 0.05$
** significant at $\alpha = 0.01$

FIGURE CAPTIONS

Fig. 1. South Central Chile coastal area and position of CTD stations during November 2001 cruise. Transects are indicated in roman numbers.

Fig. 2. Ekman transport (in m^2s^{-1}). a) November 2001 and, b) March 2002. Box shows the date of the hydrographic cruises. Negative values indicate offshore transport resulting from upwelling favorable winds (southwesterly).

Fig. 3. Horizontal distribution of temperature (a and b) and salinity (c and d) during the November 2001 (left column) and March 2002 (right column) surveys. Dotted lines indicate the isobaths of 200m (approximately the shelf break zone).

Fig. 4. Spatial distribution of larval *Emerita analoga* stages I to V during November 2001 and March 2002 at south central Chile continental shelf. Numbers are in individuals integrated per m^2.

Fig. 5. Spatial distribution of larval Pagurids stages I to IV and megalopas, during November 2001 and March 2002 at south central Chile continental shelf. Units as in Fig. 4.

Fig. 6. Spatial distribution of larval *Neotrypaea uncinata* stages I to V during November 2001 and March 2002 at south central Chile continental shelf. Units as in Fig. 4.

Fig. 7. Spatial distribution of larval *Blepharipoda spinimana* stages I to IV during November 2001 and March 2002 at south central Chile continental shelf. Units as in Fig. 4.

Fig. 8. Spatial distribution of larval *Libidoclaea granaria* stages I and II during November 2001 and March 2002 at south central Chile continental shelf. Units as in Fig. 4.

Fig. 9. Mean distance offshore (km) and standard deviation during November 2001 and March 2002 cruises for each stage of the five studied species.

Fig. 10. Mean day and night depth distribution of larval stages of the five studied species during November 2001 cruise (first column) and March 2002 cruise (second column). Vertical lines indicate one half standard deviation. Numbers highlighted in each graph correspond to the overall species mean depth during each cruise.

Fig. 11. Cumulative frequency of centroids at temperature and oxygen concentration.

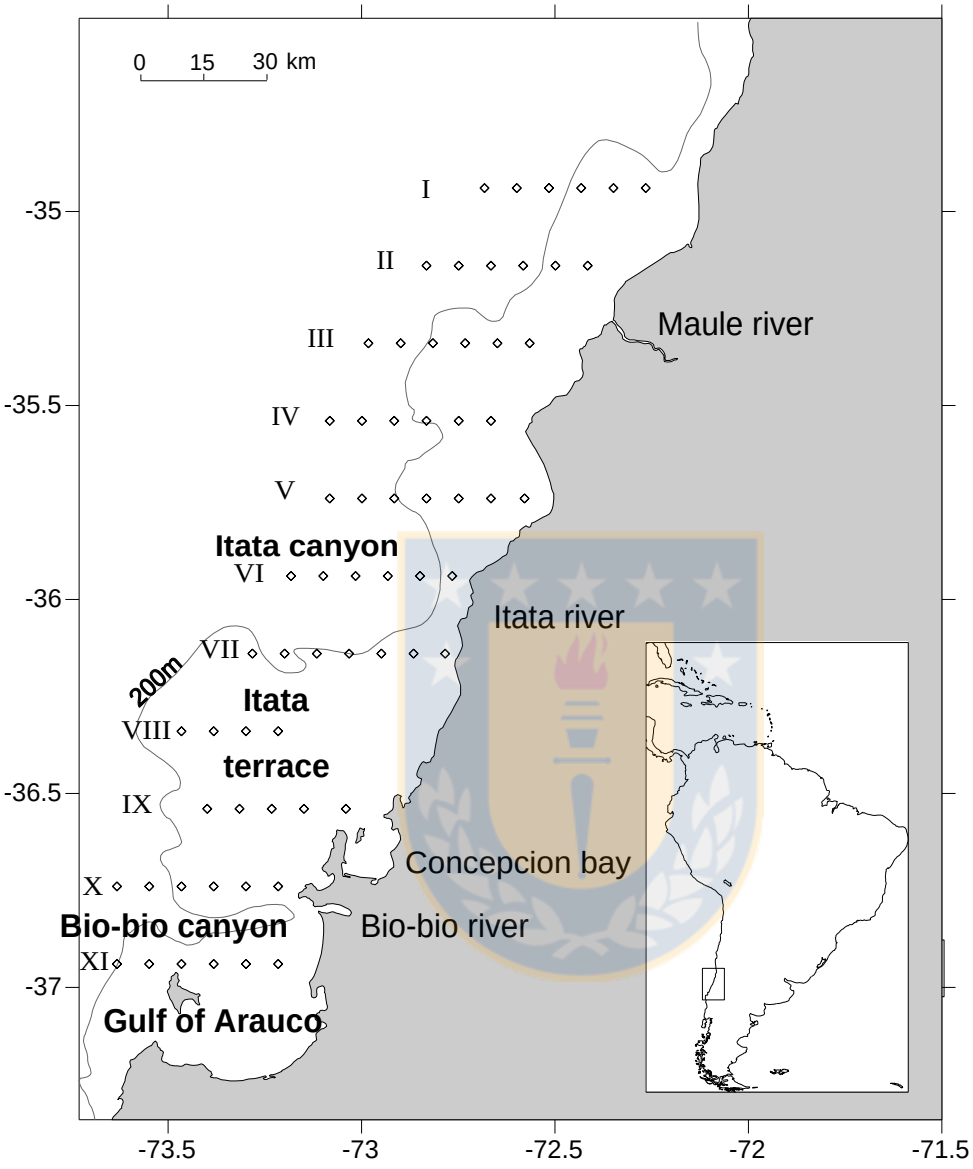
0 15 30 km
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
Maule river
Itata canyon
Itata river
200m
Itata
terrace
Concepcion bay
Bio-bio canyon
Bio-bio river
Gulf of Arauco
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5

Fig. 1

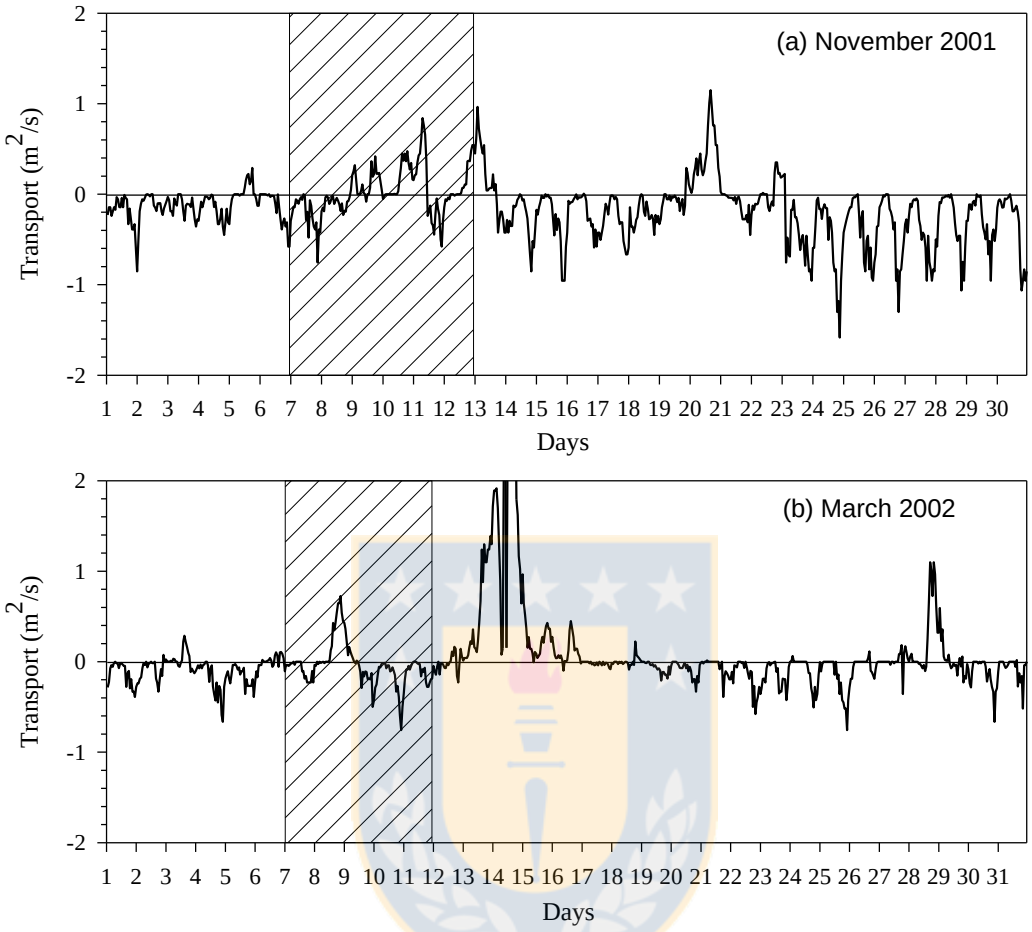
(a) November 2001
Transport (m^2/s)
2
1
0
-1
-2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Days
(b) March 2002
Transport (m^2/s)
2
1
0
-1
-2
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31
Days

Fig. 2

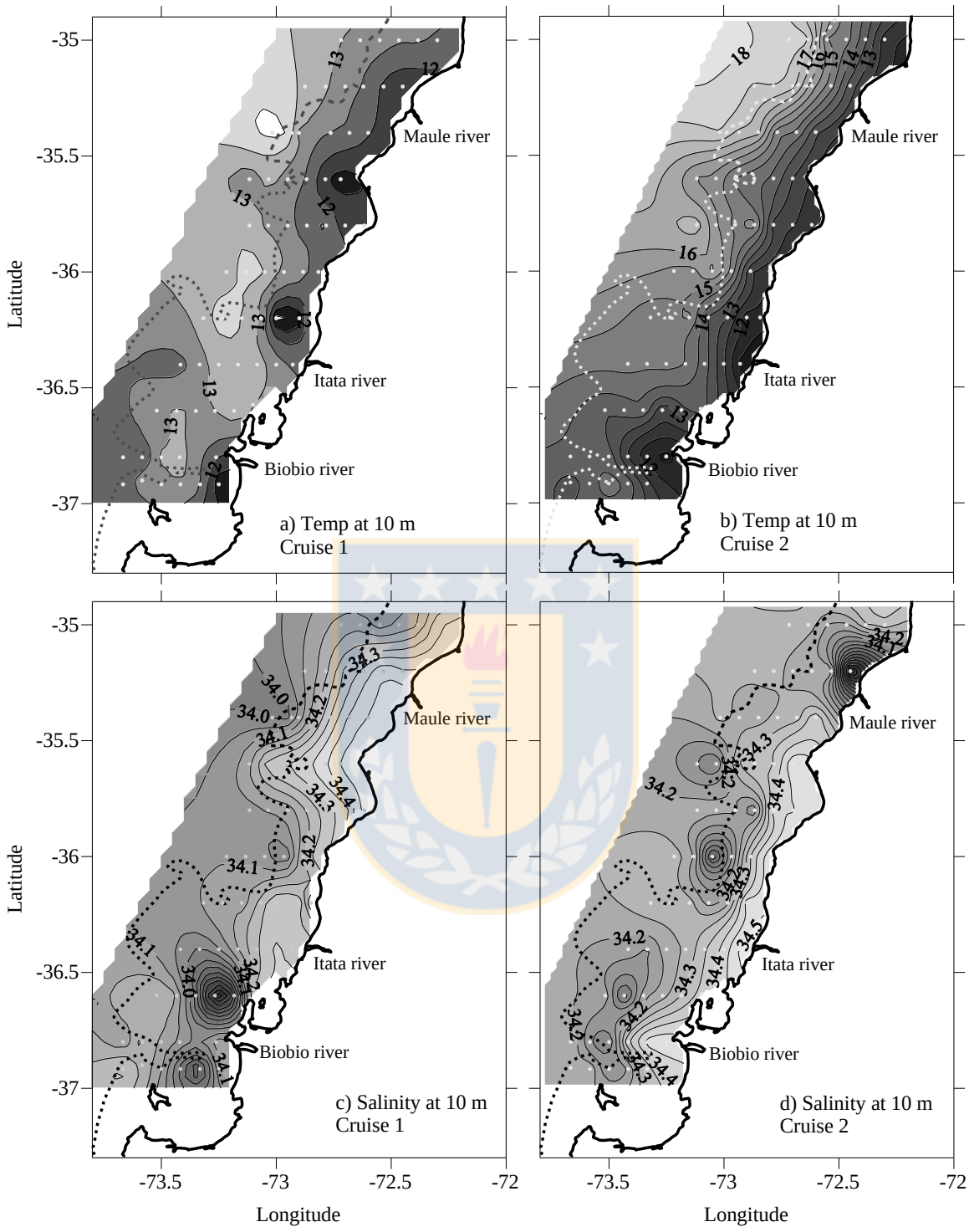
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
Maule river
Itata river
Biobio river
a) Temp at 10 m
Cruise 1
b) Temp at 10 m
Cruise 2
c) Salinity at 10 m
Cruise 1
d) Salinity at 10 m
Cruise 2

Fig. 3

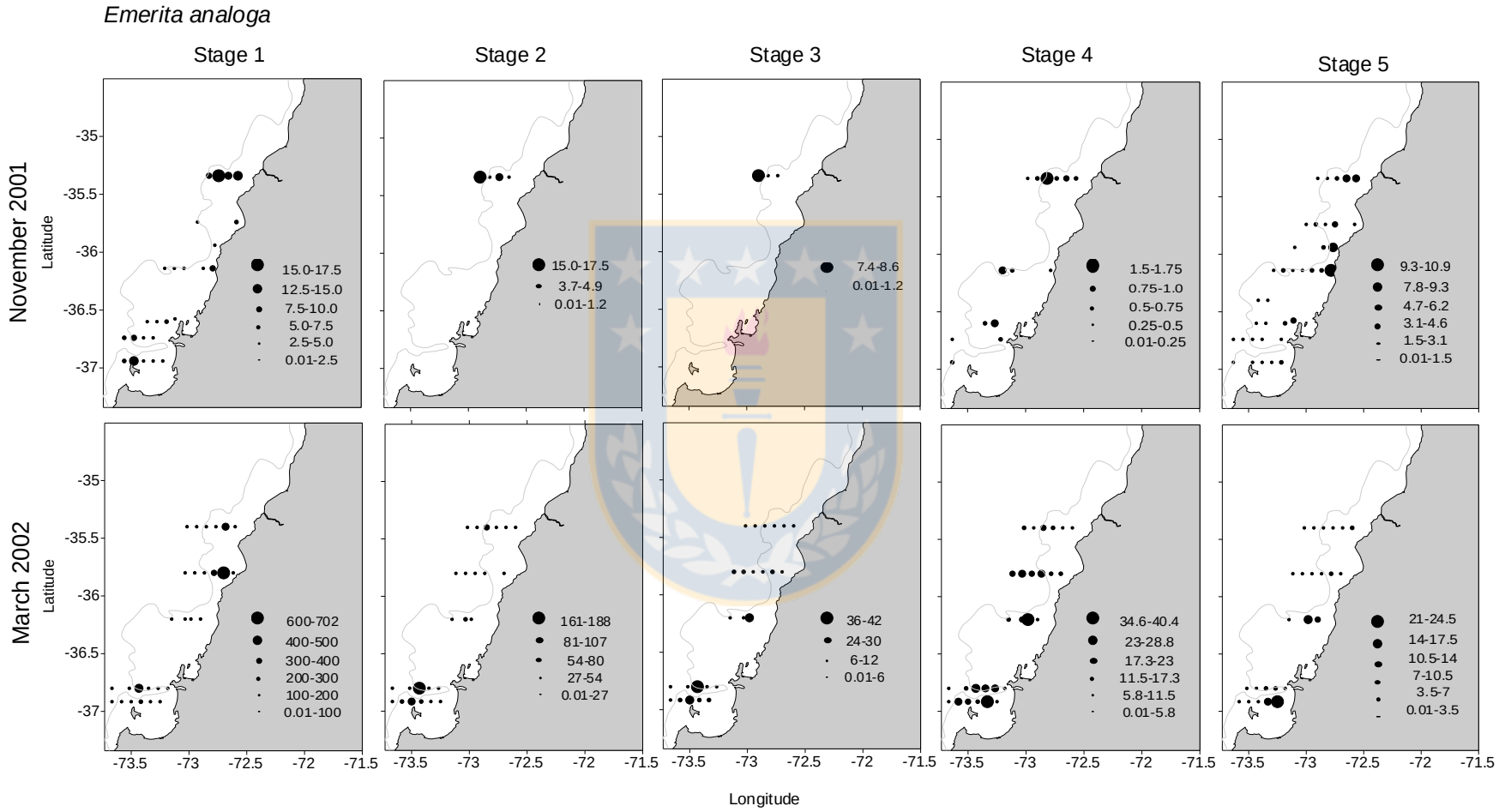
Emerita analoga
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
November 2001
March 2002
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
15.0-17.5
12.5-15.0
7.5-10.0
5.0-7.5
2.5-5.0
0.01-2.5
15.0-17.5
3.7-4.9
0.01-1.2
7.4-8.6
0.01-1.2
1.5-1.75
0.75-1.0
0.5-0.75
0.25-0.5
0.01-0.25
9.3-10.9
7.8-9.3
4.7-6.2
3.1-4.6
1.5-3.1
0.01-1.5
600-702
400-500
300-400
200-300
100-200
0.01-100
161-188
81-107
54-80
27-54
0.01-27
36-42
24-30
6-12
0.01-6
34.6-40.4
23-28.8
17.3-23
11.5-17.3
5.8-11.5
0.01-5.8
21-24.5
14-17.5
10.5-14
7-10.5
3.5-7
0.01-3.5

Fig. 4

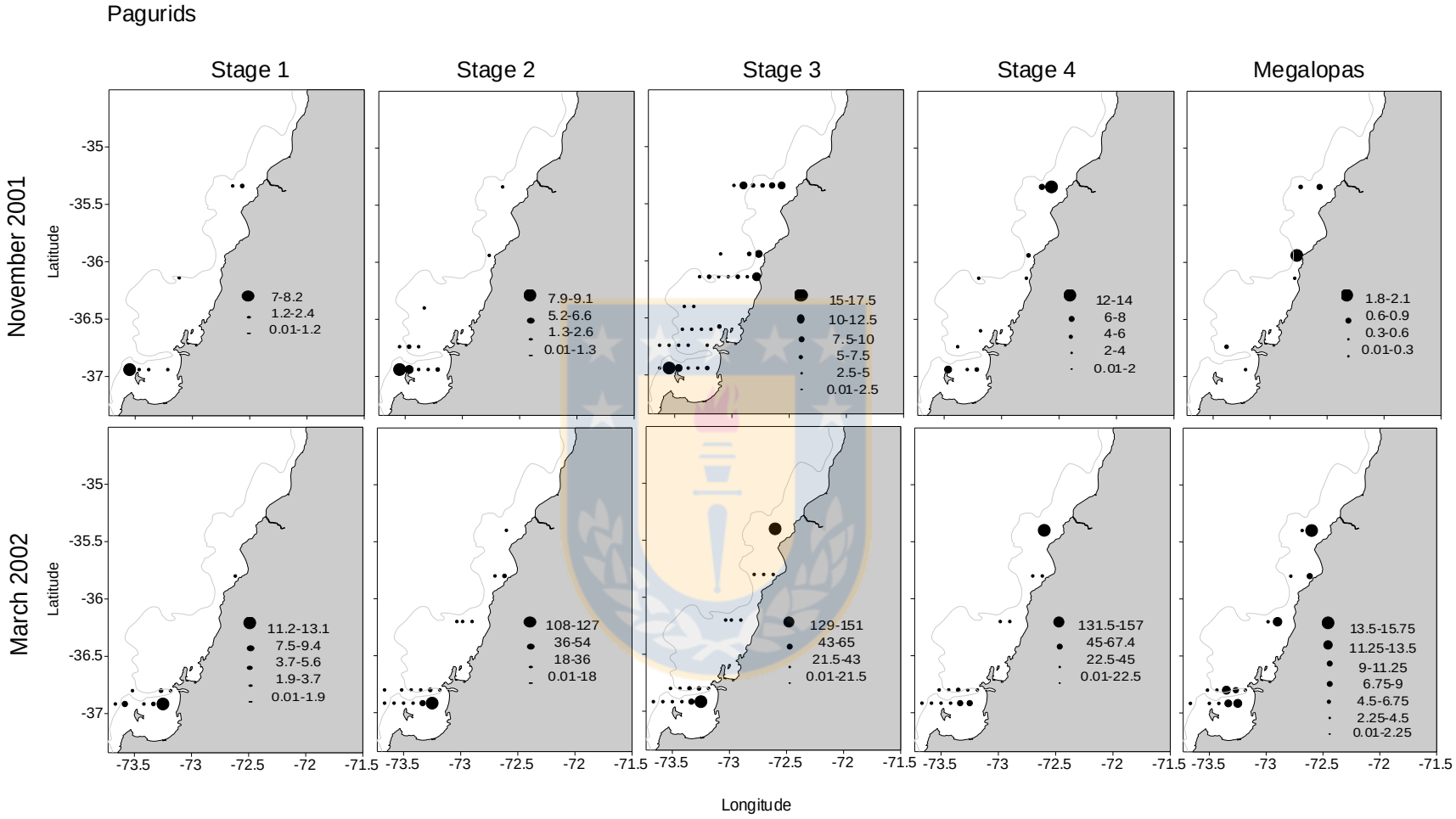
Pagurids
Stage 1
Stage 2
Stage 3
Stage 4
Megalopas
November 2001
March 2002
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
7-8.2
1.2-2.4
0.01-1.2
7.9-9.1
5.2-6.6
1.3-2.6
0.01-1.3
15-17.5
10-12.5
7.5-10
5-7.5
2.5-5
0.01-2.5
12-14
6-8
4-6
2-4
0.01-2
1.8-2.1
0.6-0.9
0.3-0.6
0.01-0.3
11.2-13.1
7.5-9.4
3.7-5.6
1.9-3.7
0.01-1.9
108-127
36-54
18-36
0.01-18
129-151
43-65
21.5-43
0.01-21.5
131.5-157
45-67.4
22.5-45
0.01-22.5
13.5-15.75
11.25-13.5
9-11.25
6.75-9
4.5-6.75
2.25-4.5
0.01-2.25

Fig. 5

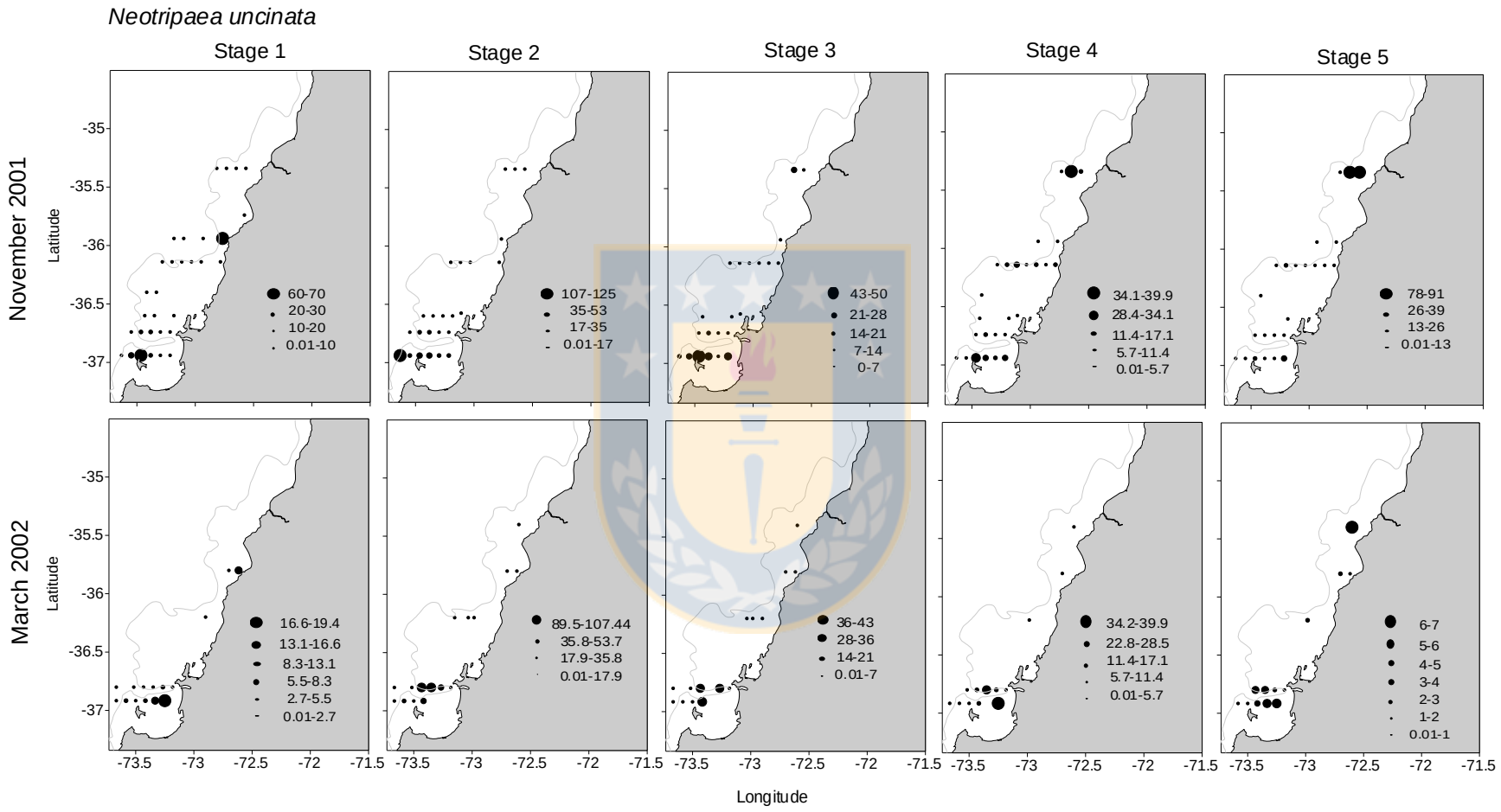
Neotripaea uncinata
Stage 1
Stage 2
Stage 3
Stage 4
Stage 5
November 2001
March 2002
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
60-70
20-30
10-20
0.01-10
107-125
35-53
17-35
0.01-17
43-50
21-28
14-21
7-14
0-7
34.1-39.9
28.4-34.1
11.4-17.1
5.7-11.4
0.01-5.7
78-91
26-39
13-26
0.01-13
16.6-19.4
13.1-16.6
8.3-13.1
5.5-8.3
2.7-5.5
0.01-2.7
89.5-107.44
35.8-53.7
17.9-35.8
0.01-17.9
36-43
28-36
14-21
0.01-7
34.2-39.9
22.8-28.5
11.4-17.1
5.7-11.4
0.01-5.7
6-7
5-6
4-5
3-4
2-3
1-2
0.01-1

Fig. 6

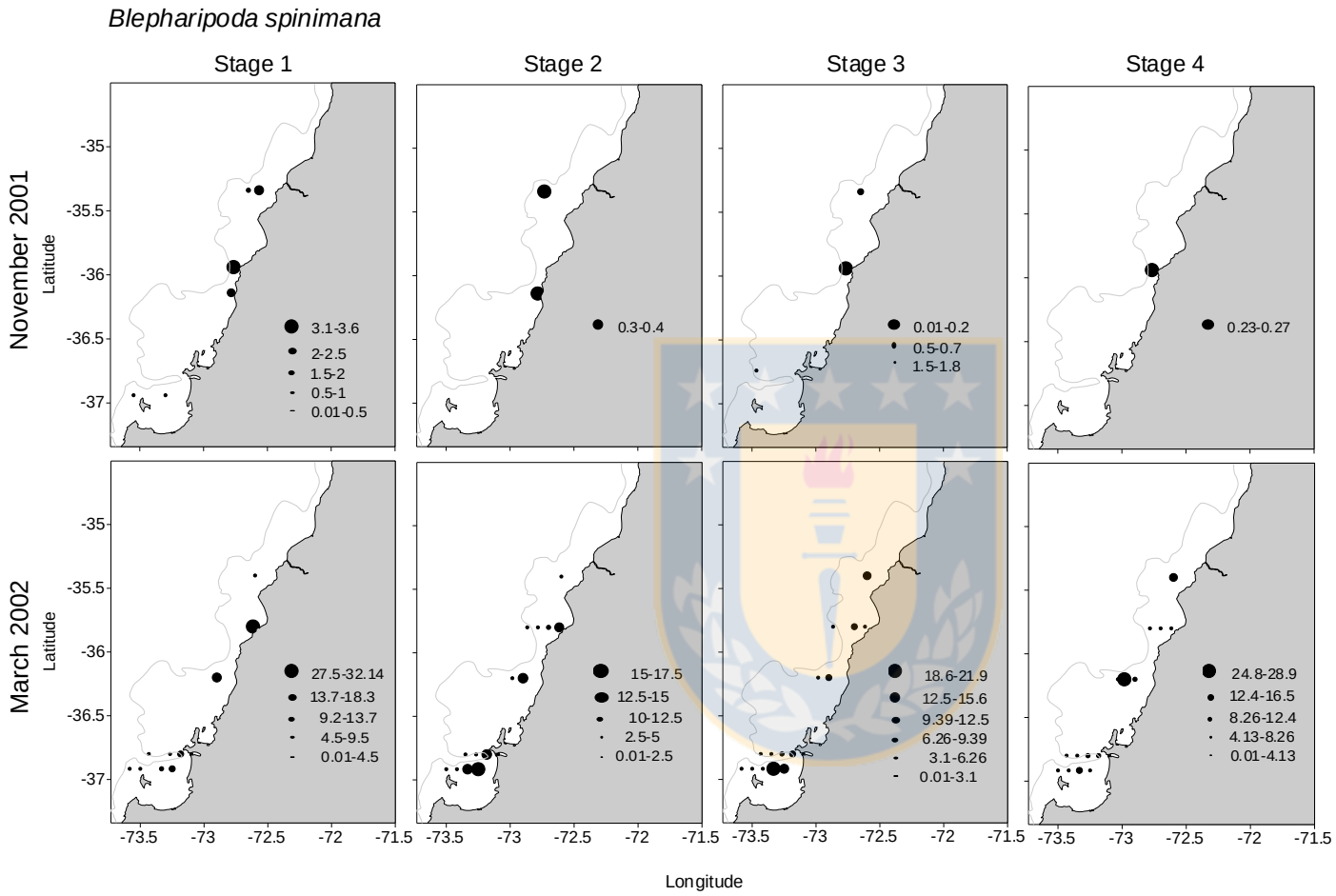
Blepharipoda spinimana
Stage 1
Stage 2
Stage 3
Stage 4
November 2001
March 2002
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
3.1-3.6
2-2.5
1.5-2
0.5-1
0.01-0.5
0.3-0.4
0.01-0.2
0.5-0.7
1.5-1.8
0.23-0.27
27.5-32.14
13.7-18.3
9.2-13.7
4.5-9.5
0.01-4.5
15-17.5
12.5-15
10-12.5
2.5-5
0.01-2.5
18.6-21.9
12.5-15.6
9.39-12.5
6.26-9.39
3.1-6.26
0.01-3.1
24.8-28.9
12.4-16.5
8.26-12.4
4.13-8.26
0.01-4.13

Fig. 7

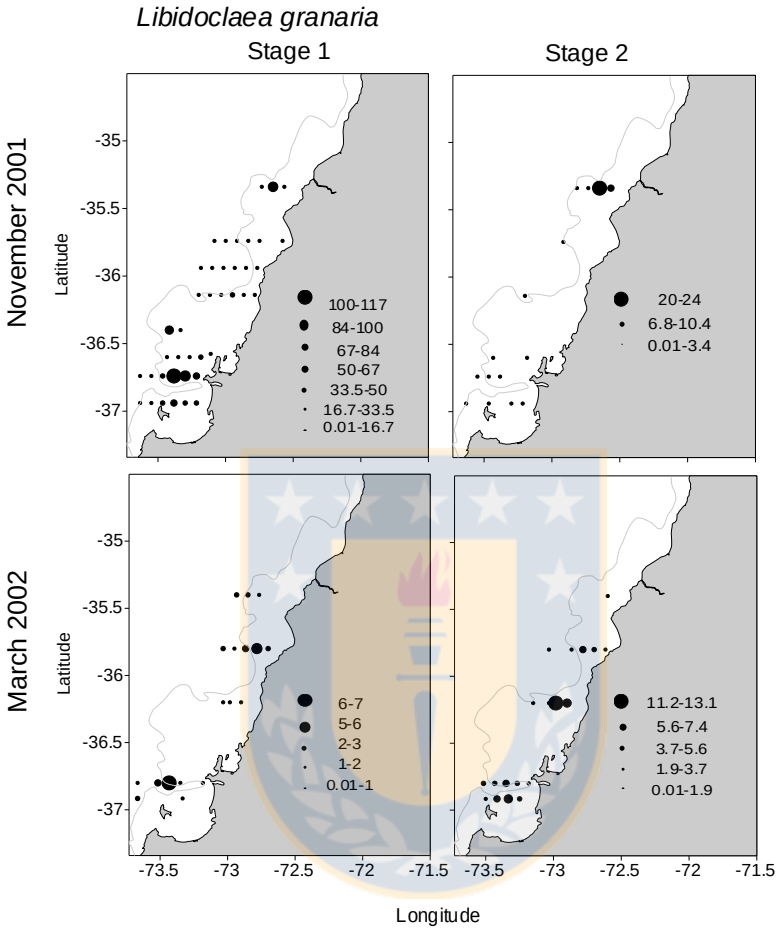
Libidoclaea granaria
Stage 1
Stage 2
November 2001
March 2002
Latitude
Longitude
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
100-117
84-100
67-84
50-67
33.5-50
16.7-33.5
0.01-16.7
20-24
6.8-10.4
0.01-3.4
6-7
5-6
2-3
1-2
0.01-1
11.2-13.1
5.6-7.4
3.7-5.6
1.9-3.7
0.01-1.9

Fig. 8

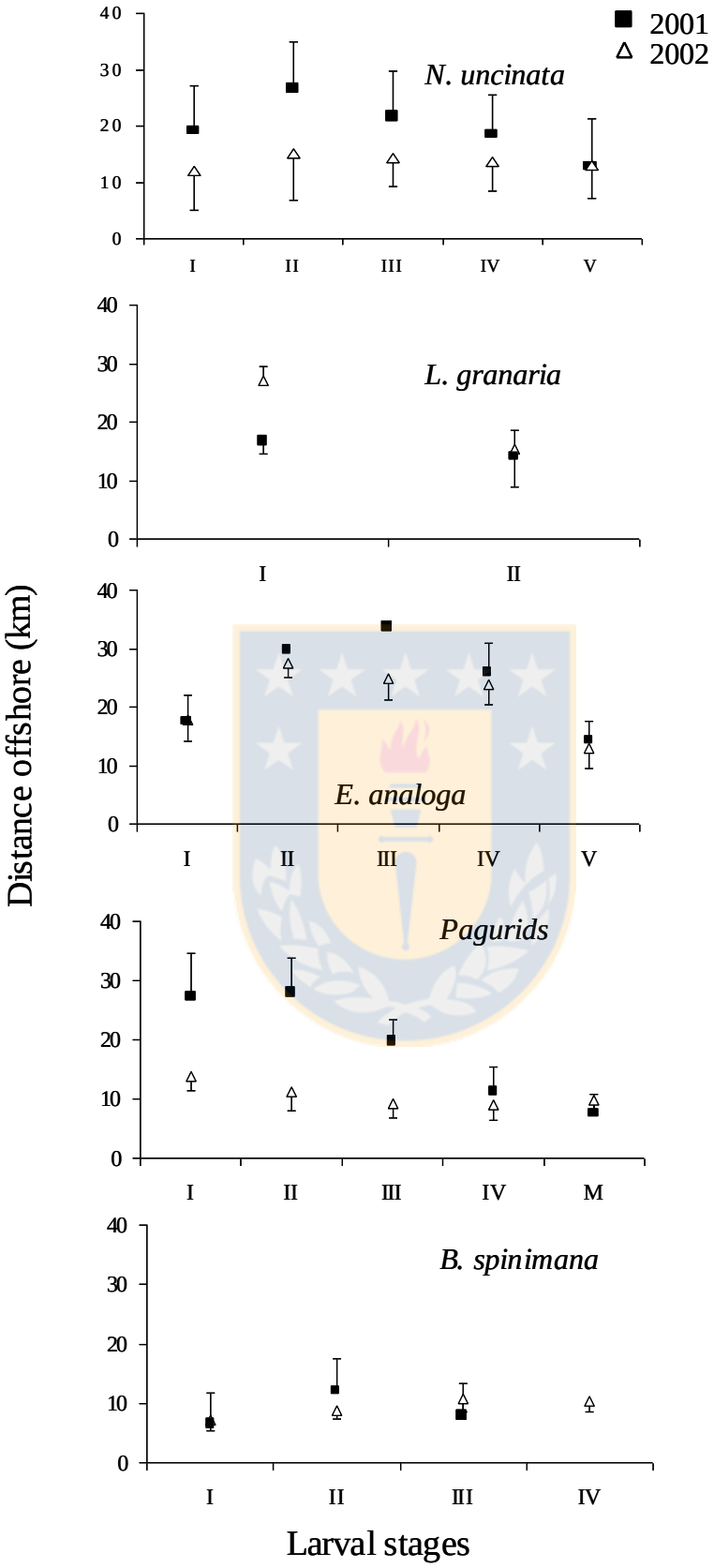
2001
2002
N. uncinata
L. granaria
E. analoga
Pagurids
B. spinimana
Distance offshore (km)
Larval stages
I
II
III
IV
V
M

Fig. 9

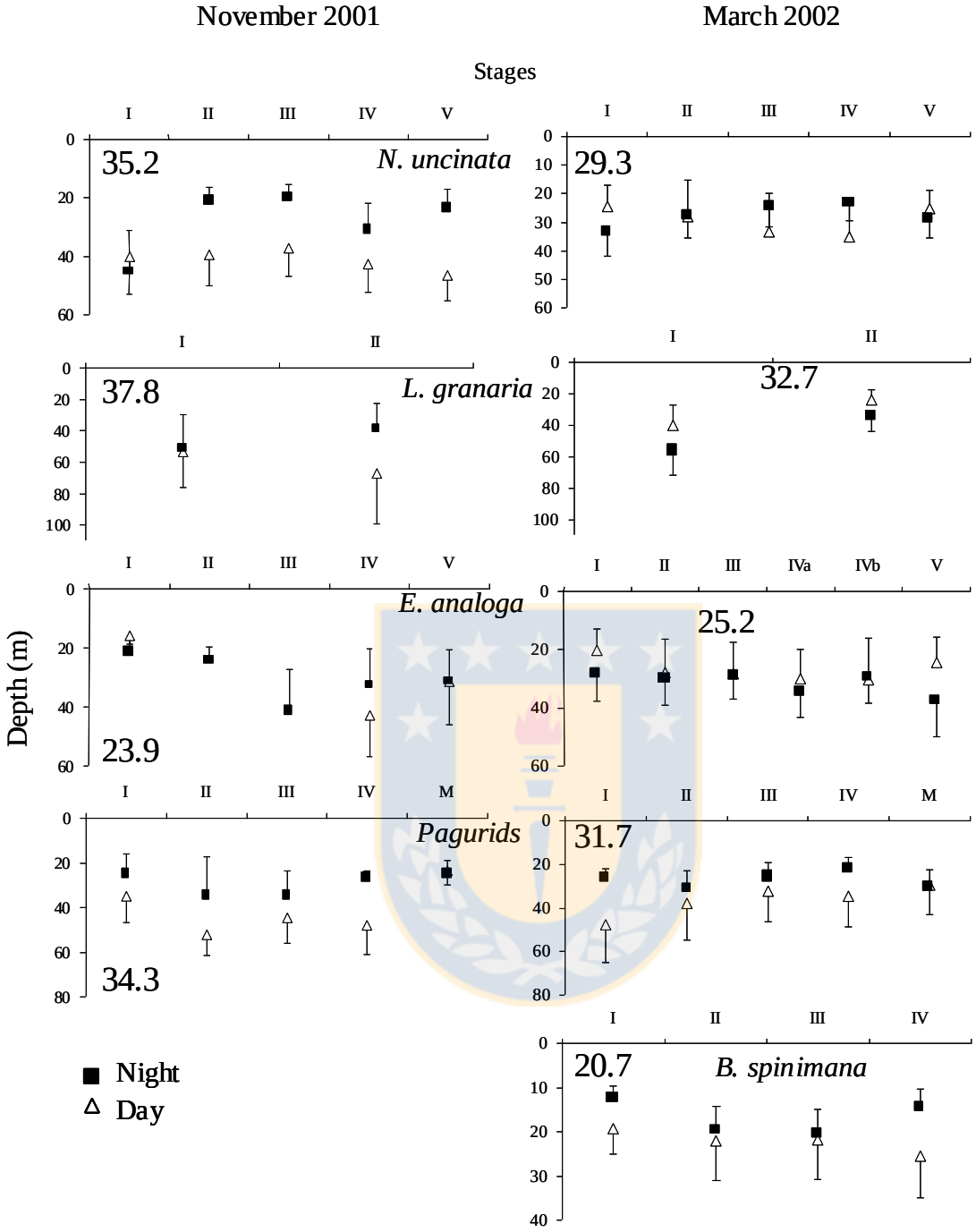
November 2001
March 2002
Stages
Depth (m)
N. uncinata
35.2
29.3
L. granaria
37.8
32.7
E. analoga
23.9
25.2
Pagurids
34.3
31.7
B. spinimana
20.7
Night
Day

Fig. 10

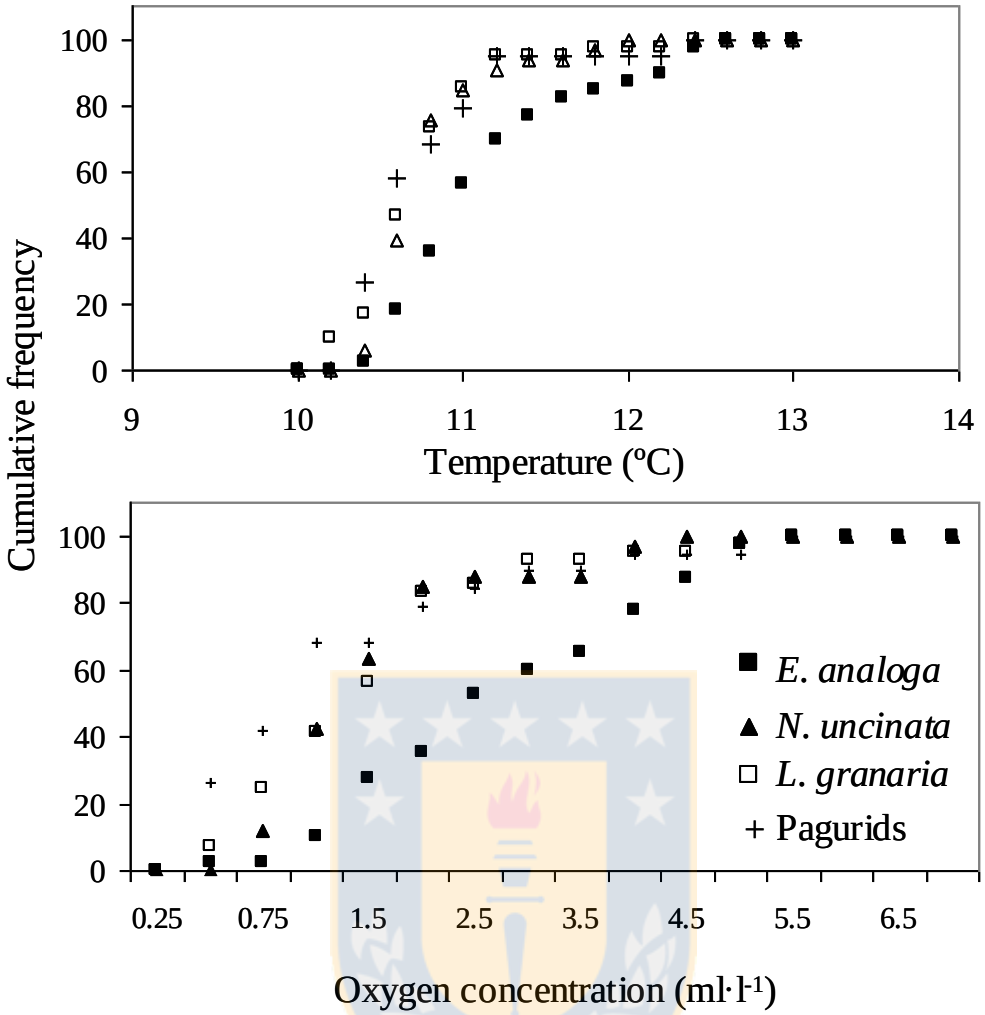
Cumulative frequency
100
80
60
40
20
0
9
10
11
12
13
14
Temperature (ºC)
0.25
0.75
1.5
2.5
3.5
4.5
5.5
6.5
Oxygen concentration (ml·l-1)
E. analoga
N. uncinata
L. granaria
Pagurids

Fig. 11

Manuscrito 3:

B Yannicelli, L Castro, W Schneider, E Navarro, J Letelier, R Roa-Ureta

Inferring the origin and fate of squat lobster larvae from observed larval distribution and individually-based transport simulations

Fisheries Oceanography (terminado)

ABSTRACT

The spatial distribution of the squat lobster larvae in the coastal upwelling area of south Central Chile (35-37°S), where the adult population has a clear spatial structure with known focus of larval release and a central conspicuous nursery area was analyzed. Larvae were released during late winter-spring from two spots to the north and south of the nursery, zoea I appeared in larger quantities in sub-surface (southward flowing) waters, while later stages dominated surface (northward flowing) ones. While zoea II appeared to be transported south in sub-surface waters, later stages, showed shallower maximums in the northern area. Megalopas and juveniles migrated daily, ascending during night hours. In March, juveniles and megalopas were only found over the wide shelf where the nursery occurs (Itata terrace: IT). Pelagic areas of retention over the IT were recognized, where all larval stages aggregated. In the north, larvae were always circumscribed between the coast and the shelf break front. Simulation experiments that tracked larvae in the studied area, forced with mean measured currents, indicated that the mid depth had the highest potential for retention, and that maximum exportation should be expected in surface waters. Larval tracking experiments forced with surface advective fields showed that both northern and southern branches of the population were conribuiting to next year recruits, but the three historial spots furthest north, did not. Sucessful recruitment was affected by the period of larval release and the place of larval release. Also, developmental time and weight at moulting to megalopa were dependent on release site. The implementation of novell management for spatially structured populations might be strongly dependent on this type of combined field and simulation results.

KEY WORDS: Coastal upwelling, crustacean larvae, vertical migration, bio-physical coupling, sea surface advection, larval tracking.

INTRODUCTION

The squat lobster *Pleuroncodes monodon* (Crustacea: Galatheidae) is a conspicuous component of the Humbolt Current upwelling ecosystem. While in Peruvian waters it comprises one of the main micronektonic species (Ayon, com pers), in South-Central Chile, it is a dominant benthic species (Gallardo et al. 2004). The southern population, that actually extends on the Chilean continental shelf between the 36 and 37° S, has been subject to strong fishing pressure since mid 60s, and sustained during the mid 70s and mid 90s one the largest invertebrate fisheries in the area. Catches, biomass, mean adult carapace length and, in particular the latitudinal range of the population have undergone contraction/expansion cycles in the last decades, probably responding to cycles of fishing pressures as well as natural fluctuations (Roa and Bahamonde, 1993, Canales et al, 1997, Roa and Tapia, 2000).

P. monodon in its persistent southern area aggregates in two main branches: around the the Bio Bio canyon (branch 1) and the Itata canyon (branch 2) (Fig. 1) The population has a clear age spatial structure. Only one nursery area has been described between the mentioned branches, over the Itata terrace where the shelf is widest (Roa et al. 1995). From there, the two adult population branches extend to the south and north respectively (Roa and Tapia 2000). The population consists of annual cohorts (Gallardo et al. 1994) that recruit from the pelagic environment to the benthos and then perform a lifelong latitudinal migration, radiating away from the nursery ground (Roa and Tapia 2000). The southern branch is smaller in biomass and does not radiate further south than the gulf of Arauco (Fig. 1). However, the northern larger branch might radiate as far as 34° S where mainly older (~ age 6) individuals had been found. It is this expantions futher north of 36°S that had been cyclically depleted and reoccupied. Although the southern and northern adult branches originate in the common nursery ground, they differ in life history parameters: southern squat lobsters mature earlier and die younger than their northern counterparts (Roa and Tapia 1998).

The dynamics of marine populations with complex life cycles might be strongly dependent on pre-recruitment processes. The larval dispersal phase and the probability of succesfully arriving to the nursery area will determine the relative contribution of each population branch to the following year recruitment, and therefore it should have important

management implications. *P. monodon* clear age-spatial structure raises several questions. Which branch contributes more to population renewal? Does one branch subsidize the other? Is a large fraction of the larval population lost to the open ocean, or to the north from any specific branch? It would not be surprising that recruitment variability responded not only to total adult stock biomass and oceanographic variability, but also to the spatial distribution of age groups. The southern branch is smaller and could be subsidized by the northern branch. However, southern squat lobsters remain closer to the nursery grounds and mature earlier than northern ones. Thus, it is not clear how this spatially structured population sustains itself. The interdependence of the two branches could be affected by the spatial distribution of the reproductive potential, as well as by the circulation characteristics and the larval behaviour and duration in the plankton.

The role of propagules dispersal in the functioning of spatially structured populations is one of the central problems in marine ecology, and largely unanswered so far (Largier 2003). In general, the spatial origin of the larvae and its final destination are unknown. In the case of squat lobster, larvae must reach a specific well-defined area for successful recruitment, so the question is somehow reduced to the potential origin of larvae. Here, we approach the questions posed above by studying the larval distribution of the squat lobster in two extense across-shelf plankton research surveys, one at the start (November 2001) and the other at the end (March 2002) of the period of pelagic larval life, together with hydrographic features and main circulation characteristics. We also studied potential larval dispersal by inferring surface advection (during the reproductive period) from satellite image data, and sub-surface transport by using published mean current measurements. An individual-based model of temperature-dependent larval transport, development, and growth was coupled to current information, in order to estimate the relative contribution of each branch to the squat lobster nursery ground.

MATERIALS AND METHOD

Study site

The study area (Fig. 1) is part of the Humboldt Current system, a major eastern boundary current system (EBC) with an equatorward surface flow, and a subsurface poleward undercurrent characterized by Equatorial Sub-Surface Waters (ESSW) (Leth and Shaffer, 2001). The circulation over the continental shelf off central Chile presents a seasonal coastal upwelling forced by the prevalence of southerly wind during late spring-summer months (Arcos and Navarro, 1986). During spring-summer upwelling periods, cold, low oxygen and high nutrient ESSW ascent over the continental shelf fertilizing shallower strata. The continental shelf is relatively narrow except in the Itata Terrace, where the squat lobster nursery ground is found, and the Gulf of Arauco (Fig. 1). The complex local topography and shoreline configuration influence the formation of northward flowing shelf break meanders, offshore spreading filaments and cyclonic eddies (Hormazabal et al. 2004, Mesias et al. 2001, 2003, Sobarzo and Djurfeldt 2004, Strub et al. 1998), and generate dynamic exporting/retaining features within a small geographical range.

Data collection and analysis

Hydrographic and plankton Surveys. During November 7th to 12th 2001 and March 7th to 12th 2002 we conducted hydrographic and zooplankton sampling over the continental shelf of Central Chile between 35 and 37ºS. The hydrographic survey was planned as a total of 69 profiles, in 11 equally spaced transects (from N to S transects I through XI) (Fig. 1). Ocationally weather hindered sampling some stations. Along each transect, casts were undertaken with a Neill Brown Mark III CTDO (November cruise) and a SeaBird SBE-25 (March cruise) in stations 1 to 6 (or 7) from the coast to the ocean. Distance between stations was approximately 5 nautical miles. Stratified zooplankton samples were taken with a tucker trawl sampling gear. The net had a 1 m^2 frame, a 300 micrometer mesh size and it was towed with a calibrated General Oceanic flow meter. In the northern zone of our sampling area (35-36°S), sampled strata were 0-50, 50-100, 100-150, 150-250 depending on the station depth. In the southern area (36-37), vertical resolution was higher, and we took samples every 25 m down to 100 m, and every 50 m in the deeper layer depending on

station depth. Samples were fixed in buffered formalin (10 %) and brought to the laboratory for identification and counting. In each sample of the November-2001 survey, squat lobster early stages were identified as belonging to any of six developmental stages: zoea I to V plus megalopae, while in the March-2002 survey squat lobster was classified into 8 stages: zoea I to V with the IV stage splittted into early IV and late IV, plus megalopae and juveniles. Stage identification under stero-microscope followed Fagetti and Capodonico (1971) and Palma (1994). Larval density at station and strata was estimated as number of individuals per 100 cubic meter.

Currents measurements and estimations. Two sections of water velocity were obtained with an Acoutic Dopler Current Profiler (ADCP) carried during the March cruise at the side of the vessel. In addition, we used CTD data to estimated geostrophic velocity (South-North, *v* component) through the dynamic height methodology adjusted for shelf areas (Reid and Mantyla, 1978). Only data for transects in which offshore stations were deeper than 450m were used. Geopotential anomaly was corrected over the shelf relative to the 450m offshore station, and therefore *v* was estimated relative to 450m.

Surface Currents Analysis. Between November 2001 and March 2002, NOAA-AVHRR sequential thermal satellite images were obtained twice a day for the region between 30-40ºS and 70-80ºW. The geo-referenced horizontal resolution for each SST-pixel resulted in 2 km, in east-west and north-south direction. To derive the surface current field from the sequential images from NOAA-AVHRR the maximum cross-correlation method of Emery et al. (1986) was used. This method produces both velocity and direction of the current in pixels representing square blocks of surface water. The time separation of sequential images (12 h) was just within the range in which rotational motion could be seen as translational motion. The search window (second image) was 20 pixels (km) and the template window (first image) was 10 pixels (km), further details of the exact procedure applied are found in Navarro et al, 2004. Not all the images for the entire period could be used due to cloud cover. Only those periods when the cloud cover was less than 50% after 3D krigging were used.

Horizontal distribution of larvae. In order to infer spatial distribution of larvae, location and larval density data were analyzed using goestatistical techniques (Isaaks and Srivastava 1999). The number of larvae belonging to each stage, at each station, was

integrated into two strata: a surface stratum of 0-50 m, and a deep stratum of 50-100 m. This procedure resulted in 64 and 49 samples in the surface and deep strata respectively in November 2001, and 60 and 46 samples in March 2002. The analyses were based on the intrinsic second-order stationary model (Isaaks and Srivastava 1999). The unitary database for analysis was the combination of larval stage, depth stratum, and survey. Thus, we performed 12 analyses for the November-2001 survey and 16 analyses for the March-2002 survey. For each analysis, the experimental variogram was either of the classical Matheron form or the standardized form (Table 1). The model variogram was fitted by weighted least squares and it was mostly of the exponential form though for some cases the Gaussian or spherical models gave better fits (Table 1). Spatial interpolation was completed using point ordinary kriging. Cross validation was employed to fix the search radius and the number of points for kriging. Computation of experimental variograms, cross-validation, and kriging were done in Surfer 8.0, while the fit of model variograms was performed using Solver in Excel spreadsheets. Stations location in latitude and longitude were converted into UTM units in order to estimate areas and abundances. UTM conversion was conductred with GeoConv (Uikkanen, 2004). Two methods were used to estimate larval stage abundance in the sampled area: 1) integration of predicted (by kriging) organism numbers based on the geostatistical analysis (Surfer 8) and, 2) estimation of mean density obtained assuming a delta distribution as a model for the frequency distribution of larval density (Pennington, 1983).

Vertical distribution of larvae. Weighted mean depth was calculated for each stage, at each non 0 station and date from density values (individuals$\times 100 m^{-3}$). A Covariance analysis (separate or homogeneous slope models when appropriate), was conducted on mean organisms depth, to analyze day/night mean depth differences, controlling for the co-variable station depth.

Individual-based simulations

Larval tracking experiments (Simulation 1). This simulation aimed, with very basic oceanographic and biological information, to analyze how the potential for dispersion/retention varies with depth over the Itata terrace. This was in the spirit of Largier (2003) and Siegel et al (2003) whom argued that mean currents (advection term) plus

currents standard deviation (diffusion term) in addition to larval period in the plankton would provide the basic information needed as a first approach to understand larval dispersal potential. The alongshore and acrosshore flux over the Itata terrace have a strong vertical shear. Sobarzo and Durjfeldt (2004) published current data obtained from six moorings (3 at the coast and 3 at the shelf edge of the Itata terrace) at three depths. The published currents were averages (and standard deviations) calculated over a period of a month when both upwelling and downwelling events were identified. We defined a grid based on the approximate Itata terrace area, where mean current and current standard deviation (as measured by Sobarzo and Durjfeldt, 2004) took locations corresponding to the real mooring setting. We liberated 10000 larvae randomly in the Itata terrace, at each depth, and their fate was tracked according to mean surface, mid-water and bottom velocities respectively (three two dimensional simulations were conducted). Velocities at each point (larval position) and each time step, were interpolated linearly from the closest four current-meters, as well as the standard deviations. Cross-shore and alonshore diffusivity may increase exponentially with distance offshore (Largier, 2003). However, there was no clear pattern of increase or decrease of velocities standard deviations from coastal to oceanic moorings in Sobarzo and Durjfeldt (2004) data. Therefore, we chose to interpolate the values of standard deviation linearly. Larval velocities were given by a mean current (U,V) (interpolated value) plus a fluctuating term at each time step t (u_i^t , v_i^t):

$$u_i^{t+1} = u_i^t \times \left(1 - \frac{\Delta t}{\tau}\right) + \sigma_u \sqrt{\frac{2 \times \Delta t}{\tau}} RN$$

where RN is a normal random number with 0 mean and variance=1. τ is the decorrelation time, chosen to be 1.5 days for the area considered. σ_u is the standard deviation of velocity component *u*, at each site. Δt was 0.1 days. The simulation lasted 85 days based on the expected time of larval development until megalopa at 12°C (Yannicelli and Castro, in review). The same formula was applied to velocity component *v*. The *u* and *v* obtained by these methods were used to relocate organisms at each time step following the first order Euler method.

We quantified and compared the number of larvae that remained in the recruitment area at different time intervals, and the mean direction of loss (N; S; W;E) between each of the experimental depths.

Larval tracking experiments (Simulation 2). The basic template for the individual-based simulations of spatio-temporal movement and development were the surface current fields and SST images. Since there were two images/fields a day, we used a time step of half a day for the update of larval location and temperature for growth and development. The individual langragian larval drift was computed with a first order Euler method. We performed several experiments to identify the effect of hatching area and period on larval success. We defined 12 areas (Table 2) based on historical patches of adult *P. monodon* distribution. From each area 10000 larvae were liberated. We defined three time periods for the beginning of the simulations: Days 3 through 8, 15-20 and 30-35. The 10000 larvae were placed randomly within each of the defined areas, and assigned randomly to a hatching day within the defined time period for each simulation. Therefore 36 experiments were conducted (12 areas × 3 periods of time). We performed each of those experiments over the sequential velocity fields. The actual velocity and temperature of a particle *i* at time *t* were obtained by linear interpolation from the four closest nodes in the velocity fields, and temperature images respectively. Each node was 6 km appart. Temperature was used to drive larval temperature dependent development and growth following the equations parameterized by Yannicelli and Castro (in review). Developmental time (*DT*) until megalopa is related to temperature (*T*) following an exponential model:

$$DT = a + T^b \quad \text{where } a = 4534 \text{ and } b = -1.53.$$

Weight increment (*WI*) at each time step was calculated as

$$WI = gr \times ts^{gr-1}$$

Where *ts* is time step and *gr* is the temperature dependent growth rate:

$$gr = 0.31 + 0.046 \times T;$$

The percentage of larvae moulting into megalopa at the nursery area over the Itata terrace was recorded as main outcome of the individually-based simulation. The total time requiered to complete development, and the average final weight at metamorphosis were compared for releases from different periods and areas using linear models. The programs for the larval tracking simulations were written and run in Java (J Builder 8), making use of the library 'Matrix' and building over the codes originally developed by C Mullon (Mullon et al, 2003).

RESULTS

Surveys

Oceanographic setting. The oceanographic conditions during the cruises were described in detail in Yannicelli et al (in review). In the present paper, we will briefly describe general conditions to concentrate on current estimates. The november cruise began after several days of upwelling favourable winds that persisted during the first couple of days, then wind reversed to northerly until the last sampling day (data not shown). North of 36°S, colder (Fig. 2) and saltier coastal waters towards the coast evidenced upwelling, and a shelf break front was noticed during both cruises. Geopotential velocity corrected for shallow areas during November indicated a surface northward flow in the northern transects, intensified at the shelf break front, and evident in the surface and subsurface (Fig. 3). Negative (southward) velocities were estimated at intermediate depths bellow the pycnocline. In the BioBio canyon northward flow was found in the mouth, and a southward flow in the head, the later was intensified in the sub-surface. During November, estimated v ranged from 40 to -5 cm×s-1 (Fig. 2).

Average temperature, as estimated from SST, indicated warmer conditions during March than during November (Fig. 2). During March 2002 only two days of northerly winds interrupted the prevailing southerly, upwelling favourable winds. During March, offshore gradients of SST developed throughout the area evidencing coastal upwelling with more intensity over the inner shelf of the Itata terrace (Fig. 2). Stronger gradients though, occurred just north of the Itata canyon (36ºS, Fig. 2). Geostrophic velocity in the northern area again indicated a dominance of northward flow in the surface, intensified at the shelf break (Fig. 3). In the BioBio canyon, as in November, northward flow was circunscribed to the mouth of the canyon. The range of v during march was larger than during November, positive magnitudes were similar, but negative velocities reached -20 cm×s-1. ADCP data was congruent with geostrophic velocity patterns, although absolute values differed as expected, and velocity contours were not so smooth (Fig. 4). Transect I showed positive northward velocities in the surface, reaching 40 cm×s-1, and negative ones in the sub-surface (Fig. 4, Table 3). In the north, the S-N component showed larger magnitudes than E-W. Mean E-W flow was offshore except below 75m depth (Table 3). In transect IX, current direction alternated several times within the same depth, southward velocities were

stronger reaching -45 $cm \times s^{-1}$, and mean flow was southward (Table 3). In the surface, coincidently with geostrophic estimations, southward flow dominated near the coast, and northward flow towards the ocean (Fig. 4), becoming more intense with depth. Onshore-offshore velocities reached larger magnitudes than the S-N component, and it was mainly inflowing at 10m depth and outflowing below (Fig. 4, Table 3).

Larval Distribution. Larval abundance was from one to two orders of magnitude larger in November 2001 than in March 2002. Two peaks of larvae were conspicuously distinguished during November (Fig 5 and Table 4): zoea I and zoea IV. During November, no juveniles were found, and megalopa was the latest stage found in the water column whereas in March, juveniles and megalopae dominated the pool of *P. monodon* pelagic stages, indicating the end of the seasonal cycle of pelagic larval development. The carapace length of the planktonic juveniles coincided with that measured in recruited juveniles from benthos trawling.

During November, large aggregations of zoea I and II both in the surface and sub-surface were found over the Itata Terrace. Zoea I distribution closely resembled that of reported adult population (Fig 6a). In addition, larger numbers were found in deeper waters, where they were being hatched. Zoea II showed a shift southward especially at the sub-surface (Fig. 6a), consistent with expected under current (see later). There was also evidence of a higher larval concentration at the northern area of Itata terrace (transect VII), where aggregations of several stages were found both in the subsurface and surface. Later stages showed a shift towards the north, specially in the surface. From stage III to V, aggregations were found progressing to the north, and larger concentrations were found in the northern sampled area (Fig. 6a). Surface peaks were northward than sub-surface ones (Fig. 6a). In the northern area (between 35 and 35.8ºS) all stages were concentrated in coastal stations, coastwards of the upwelling front. From 36º to the south (transects VII to XI), larvae where more spread in the offshore direction (Fig. 5a).

During March all important larval aggregations occurred over the Itata terrace both at the surface and sub-surface, especially megalopas and juveniles (Fig. 6b). The later, were found in the water column mainly during night time. The aggregations found over the Itata terrace in transects eight and ten corresponded to night sampling, while transect 7 and 9

were sampled maily during day time and no juveniles were found. Again, the few larvae found in the northern area occurred coastward of the shelf break, and mainly at the surface.

Larval vertical distribution. During November, mean depth distribution did not vary from day to night hours for any stage. Mean organisms depth distribution was mainly influenced by the depth of the sampled station. During March 2002, only megalopas and juveniles differed in depth distribution from day to night hours. They were shallower during the day and, in the case of megalopas, the mean organisms depth was also dependent on station depth. Depth distribution of earlier stages only depended on station depth (Table 5).

Simulations

Simulation 1 Larval tracking over the Itata Terrace showed that they were lost faster and to a larger extent in surface waters (Fig 7a and d). The majority of larvae were advected northward, and offshore and Few larvae were lost to the south in surface waters (Fig. 7a). The larger retention was accomplished in mid-water, where about 10% of released larvae remained in the target area after 85 days of simulation. In this case, over 50% of larvae were lost offshore, and to the south (Fig. 5b and d) . Few larvae were retained in bottom waters (approximately 2%), and over 90% of larvae were lost through the southern border (Fig 7 c, d).

Simulation 2 Overall picture for the surface circulation on the studied period (from November 2001 to March 2002) In spite that the coastline does not point northward exactly by leans to the east about 20 degrees, we will refer to *u* as acrosshore current component, and *v* as alonshore current component. The mean acrosshore velocity was negative (offshore direction) over the shelf and it intensified towards the inner shelf (Fig 8a). From the ocean towards the coast, tongues of low positive (shoreward) velocities are found, and coicide (from north to south) with the Itata canyon (36°S; where warmer waters also form an intrussion towards the coast Fig 8 c), the northern Itata terrace and the BioBio canyon mouth (37.8° S). The mean alongshore current flowed northward, coinciding almost entirely with the shelf break; its intensity increased toward the inner shelf, and became negative (southward flux) beyond the shelf (fig. 8b). Over the Itata terrace and Arauco Gulf, a wide area with very low mean northward velocity component was observed

(0-2 cm s^{-1}). However, a spot of southward advection appeared at the entrance of the Gulf of Arauco (coinciding with Valle Levinson et al, 2003 observations). Absolute mean magnitudes were larger at the coast, and they were also more consistent through time, which is reflected in the small standard deviations (Fig. 8d). The alongshore velocity had a larger overall standard deviation (6.5) than the acrosshore velocity component (5.5).

Both the site of release and the time of release were important determinants of the quantity of succesfully moulting megalopas in the recrutiment area, according to simulations conducted on surface advection fields. Overall, the southern and central sites of release rendered more succesfull megalopa than the northern ones (Fig. 9, Table 6) although there were large variabilities within areas few decimals of degree apart. Specially notorious is the difference between inshore and offshore sites at almost the same latitude (eg. Achiras 'coast' and 'offshore'; shelf 'coast' and 'break', Table 2 and 6). Succesfull larvae originated mainly from inshore sites. From Constitucion to the North there were no individuals recruiting (Table 2 and 6).

The overall number of succesfull megalopas resulting from the release between days 30-35 was three times larger than the success of larvae being released earlier (eg. between days 3-8) (Table 6). Although the trend of increasing success towards the later releases is common and observable in more sites, there is some variability, and some times of release within a site are notoriously poorer (eg. release time 2 within BioBio N), when most larvae are lost offshore. Achiras coast and BioBioS are the most relevant southern spots of release according to literature (persistent over time), and when we exclude other southern areas which are not historically important release sites (where adults have just occationally being found; i.e. BBNorth), both account for over 50% of larvae reaching the nursery area as megalopas. Nevertheless, depending on release timing, even Carranza might account for over 10% of succesfully mouting megalopas (Table 6).

Both the time larvae took to molt until megalopa, and the weight at moulting varied with the sites and dates of larval release. Shorter developmental time and smaller megalopae resulted from zoeas originated in the northern sites (Fig 10). There was no overall trend regarding time of release when all release spots were considered together.

DISCUSSION

According to our observational and simulation results, both population branches contribute to population renewal but most of the larvae from the northern branch would be lost to areas outside the nursery ground in the Itata terrace.

Inferences from field observations.

Field observations indicate that larvae were released south of the Itata canyon, and as later stages became shallower, they could be either retained locally or transported north. The spatial distribution of zoea I resembled that of the adult reproductive population during the larval release season 2001 as described by Canales et al. (2002). They were hatched over the Itata terrace in the north and south. The aggregations of zoea II were found in the south, indicating either local retention and/or southern concentration due to the advection of zoeas from the northern terrace in the subsurface waters where both zoea I and II were more abundant. Stages III, IV and V were found shallower than earlier ones due to either active swimming, buoyancy or the effect of active upwelling raising larvae at the coast. As larvae occupy shallower strata (where higher temperatures and better feeding conditions are encountered) transport north could be enhanced. Current estimations and measurements indicated overall surface northward advection and an intensification of such a flow in the shelf break at least from the Itata terrace to the north where this pattern is observed. The fact that zoeas III, IV and V northern sub-surface aggregations were always southwards than the surface ones, indicate that northward transport might have occurred mainly in the surface but that it can be reduced by occupying, at least partially, sub-surface layers. The idea of northern transport, is also supported by the coinsidence with other crustaceans, whose later stages were also found in our northern sampling area during the november cruise (Yannicelli et al, in review). Although the conclusions that can be drawn from the simulations conducted with current measurements at different depth are limited due to their nature (Simulation 1), sub-surface retention and even southward transport are likely, and coincident to larval distribution patterns.

The spatial coincidence of different stages in the same spot should respond to the formation of mesoscale features that retain or (more likely) concentrate larvae. Once organisms are released in the water column, advection and diffusion tends to spread them

out. When larvae develop over months in the pelagos, like the squat lobster does, no cohesion of a larval patch should be expected throughout development (see Natunewits and Epifanio 2001). Therefore, the high spatial coinsidence of nucleous of high density of different stages can be only explained by mesoscale features that concentrate organisms. Such a case occurs for example in the northern Itata terrace. The simple simulations conducted with current measurements also tended to concentrate larvae at that site. If the meandering flow along the shelf break bends coastward following the bathimetry, it could result in an area of high retention at least when this feature dominates. The high concentration is found both in surface and sub-surface waters, so: i) the structure occupies more than a single strata in the water column and ii) even in surface waters, retentive structures can develop over the shelf due to complex topography (Sobarzo and Djurfeldt, 2004).

Megalopae were found in November and March migrating vertically within the water column (in agreement with results from Cañete et al. 1996). The juveniles were found in the water column only during March over the Itata terrace. They had the same mean carapace length as juveniles recruited to the benthos, and they disappeared from the water column during day time. Therefore juveniles seem to migrate from the benthic nursery ground into the water column daily during the recruitment period. The appearance of megalopas in the water column as early as November and the long larval release period, contrasts with the known short period of recruitment of squat lobster (Gallardo et al, 1994). Thus, two processes might account for the short length of settling period previously reported. First, during the begginning of the larval release period, water temperatures are colder than in summer, and the development of those organisms released earlier should be longer. Second, crustaceans do have the capacity to retard settlement until favourable conditions arise. Some crustaceans can retard metamorphosis until they are about to settle but *P. monodon*, seems capable of maintaining a semi-pelagic juvenile period until settling definitely in South Central Chile when, according to Gallardo et al. (1994) bottom conditions become favorable for settlement. Since these late stages are strong swimmers, they might no longer be prone to horizontal passive advection, and the successful retention until zoea V within the recruitment area would be as far in development as currents might influence early stages loss.

Another question regarding late stages found during November is whether late stages found in November can return to the southern nursery area. Larval tracking in surface advective fields suggest that larvae can be transported in surface waters at least one and a half degrees of latitude, even to the south. There is truly a high variability and possibly a strong sensitivity to spatial and temporal 'events' that might cause loss and return. There are examples in the literature where crustacean larvae have been shown to mix along several degrees of latitude (Caputi et al. 2003), so far, we can draw no conclusion in this respect.

Insights from simulation results

Random release over the Itata terrace is not realistic, however, simulation 1 allowed to gain a first picture on the spectrum of larval fates that would result from considering larval depth distribution and depth specific advection over the Itata terrace, based on real, measured velocity ranges. Consider that 2/3 of the zoea I pool occured below 50m (at mid-depth) and 1/3 above. We can grossly estimate that the surface third would mainly be advected north and offshore, while a 20-30 per-cent of the total larval pool would be retained within the shelf (due to the 2/3 at mid-depth).

The reliability of a larval tracking experiments, depends on the quality of the advective template used. The surface advective fiels used for simulation 2 describe the displacement of thermal structures, so, if advection occurs within a water parcel that does not imply changes in the shape of a thermal structure (eg. along a frontal structure), the method would not solve it. For example, the surface advective fields used, showed an overall similarity with geostrophic calculations: northward surface advection over the shelf and the decrease of northward advection offshore. However, it did not show the intensification over the shelf break front. If water flows along a front and if there is no transport of thermal structures, this component of advection may not be 'seen' by the mcc method yieling a discrepandy. The high offshore overall mean costal advection can result also because at the very cost, there is no possible landward movement, therefore, means are always positive and standard deviation low. So, the overall picture presented by mcc advective fields do have an agreement with expected circulation, but we do know that

smaller features are lost. For larval tracking, a general idea of larval dispersal potential and mean direction can be gained from these simulations (Kim and Sugimoto, 2002).

In spite of the prevailing northward transport of surface waters, the high temporal variability that characterizes the area of squat lobster release sites, precluded a constant northward transport of larvae. Release sites to the south and north of the nursery area (BioBio and Achira respectively), could be the main contributors to the nursery area replenishment. However, the relative importance is highly dependent on time of release, which indicates that short time variability (scale of few days) plus, trends of coastal upwelling within the season, also have an influence. Sites north of Constitucion would not contribute larvae (at least in surface waters) to the nursery, in any time of release. However, the contribution of Carranza, north of Achira, would depend on intraseasonal variability. Its relative importance would also depend on the spawning potential found in the spot. A more detailed study of the coastal upwelling process intraseasonal variability, based on longer time series, would enlight the comprehension of the physical environment that underlay these results.

Another relevant aspect outlined by simulations is that small differences in spatial distribution of releases, from the coast to offshore, might have very important consequences for recruitment. The higher retention was accomplished in larvae that were released in the coast. Particles in our simulations were released in the surface, and there are two non exclusive ways for *P. monodon* larvae to reach the surface. One is upward swimming, a behaviour that has been observed in laboratory in recently hatched larvae (per. obs.). The other, is larval onshore and upward transport with upwelling waters. Both a processes would be highly advantageous for *P. monodon* early zoeas, since they would also be placed in higher oxygen, warmer and food richer waters without too much energy expenditure on swimming.

Finally, the simulations over surface advective fields showed a shortening of development in the zoeas originated from the northern spots. That is because warmer waters usually reach closer to the shore, and also stronger offshore advection placed larvae in those warmer waters. The spatial heterogeneity of the coastal upwelling process reflected on temperature fields, caused marked spatial heterogeneity in the larval duration and growth. Such spatial heterogeneity arising from thermal fields is likely to reflect

reality. Giraldo et al. (2002) have shown with field sampling that spatial differences in copepod growth rates at a given time, arise from the heterogeneity of coastal upwelling process.

Management implications and future research

The historical northernmost patches of adult *P. monodon*, do not appear to contribute to the renewal of the population, however, other historical fishing grounds such as Achira appear to have a strong role in replenishing the nursery area. This simulation result emerged in spite that Achira is located to the north of the nursery, and that we were only considering surface advection. Setting up an spatially explicit fishing regulation, where efforts are spatially allocated to spots such as Pichilemu, can be based in this type of study. Also, fishing periods in each site could be managed after we understand the spatially explicit intra-seasonal variability of upwelling events. Future studies will make use of hydrodynamic models to further explore and understand how the spatial structure of this population influences its maintenance.

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FIGURE CAPTIONS

Fig. 1 Map of the studied area highlighting the main local topographic features, the sampling design and the gross distribution of *P. monodon* benthic population. Gray areas 1 and 2 correspond to locations where squat lobsters have persisted through time. Gray areas 3 and 4 correspond to northern expansions of larger squat lobsters, where they are no longer found according to latest stock assessments. Circle marked NA: nursery area. Smaller dots indicate positions of zooplankton and CTD stations.

Fig. 2. Temperature at 10m depth for November 2001 and March 2002 cruise.

Fig 3 Sections of geostrophic velocity for both November 2001 and March 2002 cruises. Geostrophic velocity estimations were based on the dynamic height correction for shallow areas. All sections are drawn from 0 to 150 m depth. Arrows indicate shelf break position.

Fig. 4. Current velocities measured with ADCP during March 2002 cruise, in transects I and IX. Data represent values for the given depth.

Fig 5. a) and b) Larval stages abundance for November 2001 and March 2002 cruises, respectively.

Fig. 6. Spatial distribution of larval stages of the squat lobster *Pleuroncodes monodon* off central Chile in two depth strata, as inferred from geostatical models. In panels with circles it was not possible to fit a model. Variogram models are shown as inserts. The plankton stations surveyed are shown as dots. a) November 2001 and b) March 2002.

Fig. 7. Results from larval tracking simulation experiments over the Itata terrace (simulation 1). Temporal evolution of the number of larvae retained and the direction of loss from the Itata Terrace, in the surface (a), midwater (b) and bottom waters (c). LA: larvae at the area (retained); LO: larvae lost to the ocean; LC: larvae lost to the coast; LS: larvae lost to the south; LN: larvae lost to the north. d) mean and standard deviation of the

quantity of larvae retained at day 85 of the simulations in each of the considered depths. 10000 particles were released for each simulation.

Fig. 8 Mean surface properties derived from SST images between Novembre 2001 and March 2002 at South Central Chile. a and b: acrosshore (*u*) alongshore (*v*) surface velocity components respectively, inferred from maximum cross correlation of pixels of two sequential thermal satellite images; c) SST; and d) spatial distribution of currents standard deviation (absolute magnitude).

Fig. 9 Percentage of succesful megalopa in relation to pooled focus of larval release into areas S:south, C: center, N:north (see table 2), and release timing: 1: days 3-8; 2: days 15-20; and 3: days 30-35.

Fig. 10 Mean weight at metamorphosis (a) and mean time taken until metamorphosis (b) by larvae mouting to megalopa, as a function of area of release. Areas are numbered 1 through 12 in the same order as indicated in table 2 . t1, t2 and t3 refer to release timing (3-8; 15-20; 30-35).

Table 1. Best model fitted to empirical semi-variograms (SV) for each set of data, and parameters estimated. Range is expressed in m.
Shape: O omnidirectional; A: anisotropic
Model: E exponential; G: gaussian; S: spherical

Noviembre 2001

	Type	Shape	Model	nugget	sill	Range	R^2
0-50m							
Zoea I	Standardized SV	O	E	0.16	0.86	20120	0.89
Zoea II	Standardized SV	O	E	0.31	0.70	19750	0.80
Zoea III	Standardized SV	O	E	0.079	1.04	20200	0.91
Zoea IV	Standardized SV	O	E	0.18	0.83	20000	0.91
Zoea V	Semivariogram	O	E	2700	14300	29600	0.99
Megalopas	Semivariogram	O	E	0	4070	17300	0.98
50-100m							
Zoea I	Standardized SV	O	E	0	0.96	15630	0.98
Zoea II	Standardized SV	O	E	0	0.84	31370	0.99
Zoea III	not valid						
Zoea IV	Standardized SV	O	E	0	0.96	20500	0.99
Zoea V	Standardized SV	O	E	0.01	1.35	31606	0.99
Megalopas	Standardized SV	O	E	0	1.08	17800	0.00

March 2002

	Type	Shape	Model	nugget	sill	Range	R^2
0-50m							
Zoea I	Semivariogram	O	E	0	2.94	25000	0.98
Zoea II	Semivariogram	O	E	0.14	6.72	25000	0.98
Zoea III	Standardized SV	O	E	0	0.99	25000	0.99
Zoea Iva	Standardized SV	O	G	0	0.96	10500	0.99
Zoea IVc	Standardized SV	O	G	0	1.4	13500	0.99
Zoea V	Standardized SV	O	E	0	0.93	13148	0.98
Megalopas	Standardized SV	O	E	0	1.26	34000	0.99
Juveniles	Standardized SV	O	E	0	1.4	49900	0.99
50-100m							
Zoea I	Standardized SV	A 70/ 160	E	0.1	1.37	64500/ 35065	0.92
Zoea II	Standardized SV	O	E	0	1.06	26520	0.99
Zoea III	Semivariogram	O	E	1.44	2.21	31110	0.7
Zoea IVa	Standardized SV	O	E	0	1.18	26033	0.99
Zoea IVc	not valid						
Zoea V	Semivariogram	A 0/ 90	G	0.05	0.82	37900/ 27350	0.95
Megalopas	Standardized SV	O	S	0	1.11	24450	0.99
Juveniles	not valid						

Table 2. Areas of larval release for simulations using surface velocity fields. Mean latitude and mean longitude indicate the coordinates in degrees, where the area is centered. The amplitudes (lat and lon respectively for the y and x axis) indicate haf the latitudinal and longitudinal extension of the area. Zones are defined according to the latitudinal position relation to the nursery area S: south; C: center; N:north.

Hatching areas	Mean latitude	Amplitude lat	Mean longitude	Amplitude lon	Zone
1) South BioBio	-36.87	0.07	-73.4	0.1	S
2) North BioBio	-36.675	0.12	-73.4	0.1	S
3) Shelf coast	-36.5	0.1	-73.2	0.07	C
4) Shelf break	-36.6	0.1	-73.55	0.05	C
5) Achira offshore	-36.3	0.1	-72.875	0.15	C
6) Achira coast	-36.3	0.1	-73	0.15	C
7) Nugurme	-36	0.07	-72.875	0.075	N
8) Nugurme N	-35.875	0.075	-72.9	0.07	N
9) Carranza	-35.6	0.1	-73.4	0.03	N
10) Constitución N	-35	0.2	-72.35	0.15	N
11) Serena	-34.7	0.1	-72.15	0.07	N
12) Pichilemu	-34.4	0.2	-72.1	0.02	N

Table 3. Maximum, minimum and mean current velocity components (u and v) at specific depths, as measured by ADCP in transects 1 and 9 during March 2002.

Transect	Depth (m)	u (cm s^{-1})			v (cm s^{-1})		
		max	min	mean	max	min	mean
I	10	26	-24	-2.4	55.2	0.43	27.2
	25	17.86	-16.7	-2.9	27.4	-12.6	11.5
	50	15.25	-16.1	-2.54	19	-38	-3.3
	75	35.56	-19.19	3.58	26.7	-55.2	-4.3
	100	28.39	-37.9	0.45	31.14	-30.57	2.9
IX	10	46.1	-15.9	6.01	40.04	-29.04	-0.3
	25	20.68	-18.37	-3.89	18.81	-16.12	-2.3
	50	5.8	-33.65	-7.71	12.23	-24.65	-3.86
	75	7.4	-24.9	-8.5	6.32	-20.7	-6.2
	100	27.4	-48	-3.37	15.0	27.8	-6.3

Table 4. Total number of individuals estimated in the studied area during November 2001 and March 2002. AM: arithmetic mean, DM: delta mean; GS: geostatistical total estimate, also estimates based on the arithmetic mean and the delta mean are shown. Total area was estimated after krigging.

Noviembre 2001

Zoea	**Area (GS) ($m^2 \times 10^9$)**	**Ind×10^9 (GS)**	**AM ind×m^{-2}**	**Ind ×10^9 (AM)**	**DM Ind×m^{-2}**	**Ind×10^9 (DM)**
I (0-50)	10.37	200	20	206	21	221
I (50-100)	10.13	367	30	309	40	408
II (0-50)	10.44	126	15	156	13	138
II (50-100)	10.25	200	14	147	13	141
III (0-50)	11.01	84	7	74	7,6	84
III (50-100)	11.01	-	5	55	4	46
IV (0-50)	11.76	299	32	379	42	490
IV (50-100)	10.64	319	23	242	27	291
V (0-50)	11.96	218	27	317	25	303
V (50-100)	10.32	168	11	114	10	99
M (0-50)	11.35	110	11	127	12	132
M (50-100)	11.98	71	7	86	8	95
March 2002						
I (0-50)	10.50	3	0.3	3,28	0,3	3,1
I (50-100)	10.51	2	0.2	1,96	0,2	1,9
II (0-50)	11.94	8	0.6	7,63	0,7	7,9
II (50-100)	8.86	1	0.1	1,22	0,1	1,2
III (0-50)	10.41	9	0.8	8,87	1,0	10
III (50-100)	11.60	4	0.2	2,8	0,2	2,4
IV (0-50)	9.64	4	0.5	4,69	0,5	4,4
IV (50-100)	9.85	1	0.1	1	0,1	1,1
IV c (0-50)	9.34	2.9	0.3	2,65	0,3	2,5
IV c (50-100)	9.34	-	0.1	1	0,1	1,0
V (0-50)	11.04	6	0.6	6,48	0,6	6,7
V (50-100)	10.03	1.8	0.1	1,4	0,1	1,3
M (0-50)	10.78	13	1.2	13,5	1,4	15,6
M (50-100)	10.73	10	0.9	10,1	1,0	10,4
J (0-50)	9.69	13	1	9,88	0,9	8,7
J (50-100)	9.69	-	2	20	2,6	24,8

Table 5. ANCOVA results for organisms mean depth distribution. Only significant factors at the 0.05 level are included in the table.
SD: station depth (maximum sampled depth at the station), continuous predictor; H: hour (day or night), categorical factor; N: night hours.

2001

Zoea	**November 2001**	**March 2002**
I	N×SD	SD
II	SD	SD
III	SD	---
IV	---	---
V	---	---
Megalopas	SD	SD+H
Juveniles		H

Table 6 Results from larval tracking and development on surface advection fields. % Successful megalopas: the percentage of megalopa moulting at the nursery area out of the 10000 initial larval released in each area and date. Total: total number of larvae from each area that reached the nursery (3 releases included). Average: percentage of successful megalopa in four areas after tracking on average current fields. Percentage of succesfull megalopa incomming from each area: out of the total number of succesful megalopa from a given time of larval release, the percentage that proceeded from each area. The percentage of succesfull megalopas incoming from selected areas, the same but only for selected areas. Below('Total number'), the total number of succesful megalopa from the selected areas as a function of release date.

	% Successful megalopas			% of succesfull megalopas incoming from each area			% of succesfull megalopas incoming from selected areas				
Zones of origin	**3-8**	**15-20**	**30-35**	**3-8**	**15-20**	**30-35**	**3-8**	**15-20**	**30-35**	**Total**	**Average**
BioBio South:	5.8	16.4	27.62	7.19	15.88	18.37	19.99	24.58	25.40	4982	0
BioBio North:	18.55	5.38	20.02	23.01	5.21	13.32				4395	
Shelf coast:	28.77	30.23	17.89	35.69	29.26	11.90				7689	
Shelf break:	4.29	0.98	3.68	5.32	0.95	2.45				895	
Achira coast:	11.69	14.62	19.35	14.50	14.15	12.87	40.30	21.92	17.79	4566	21.88
Achira break:	3.32	12.43	19.52	4.12	12.03	12.98	11.44	18.63	17.95	3527	
Nugurme	3.15	2.98	7.6	3.91	2.88	5.06	10.86	4.47	6.99	1373	
Nugurme N	2.36	8.39	23.96	2.93	8.12	15.94	8.14	12.58	22.03	3471	50.52
Carranza	2.69	11.89	10.69	3.34	11.51	7.11	9.27	17.82	9.83	2527	
Constitucion N	0	0	<0.01	0	0	0	0	0	0	0	0
Pta Serena	0	0	0	0	0	0	0	0	0	0	
Pichilemu	0	0	0	0	0	0	0	0	0	0	
Total Number:							2901	6671	10874		

Fig. 1

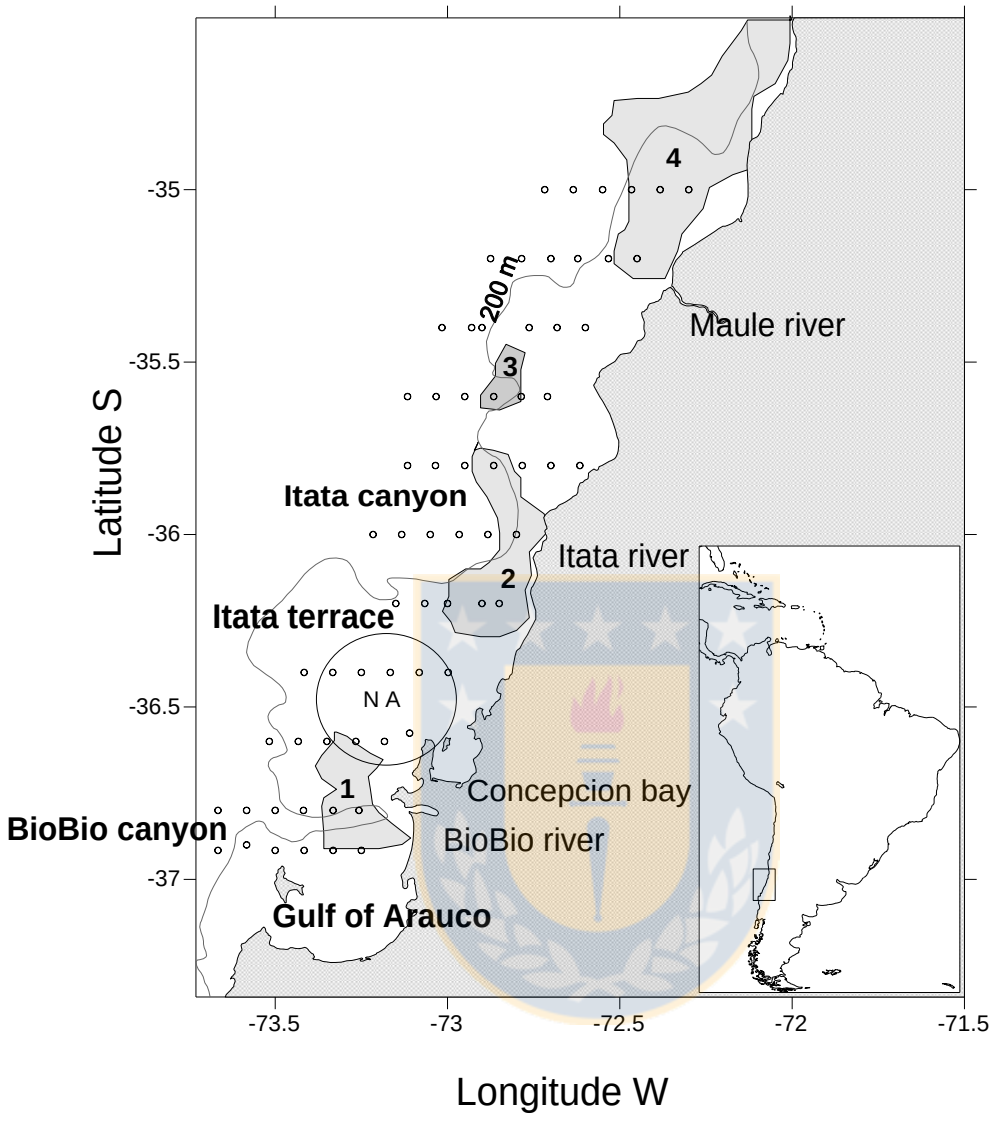

4
200 m
Maule river
3
Itata canyon
Itata river
2
Itata terrace
N A
1
Concepcion bay
BioBio river
BioBio canyon
Gulf of Arauco
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5
Latitude S
Longitude W

Fig. 2

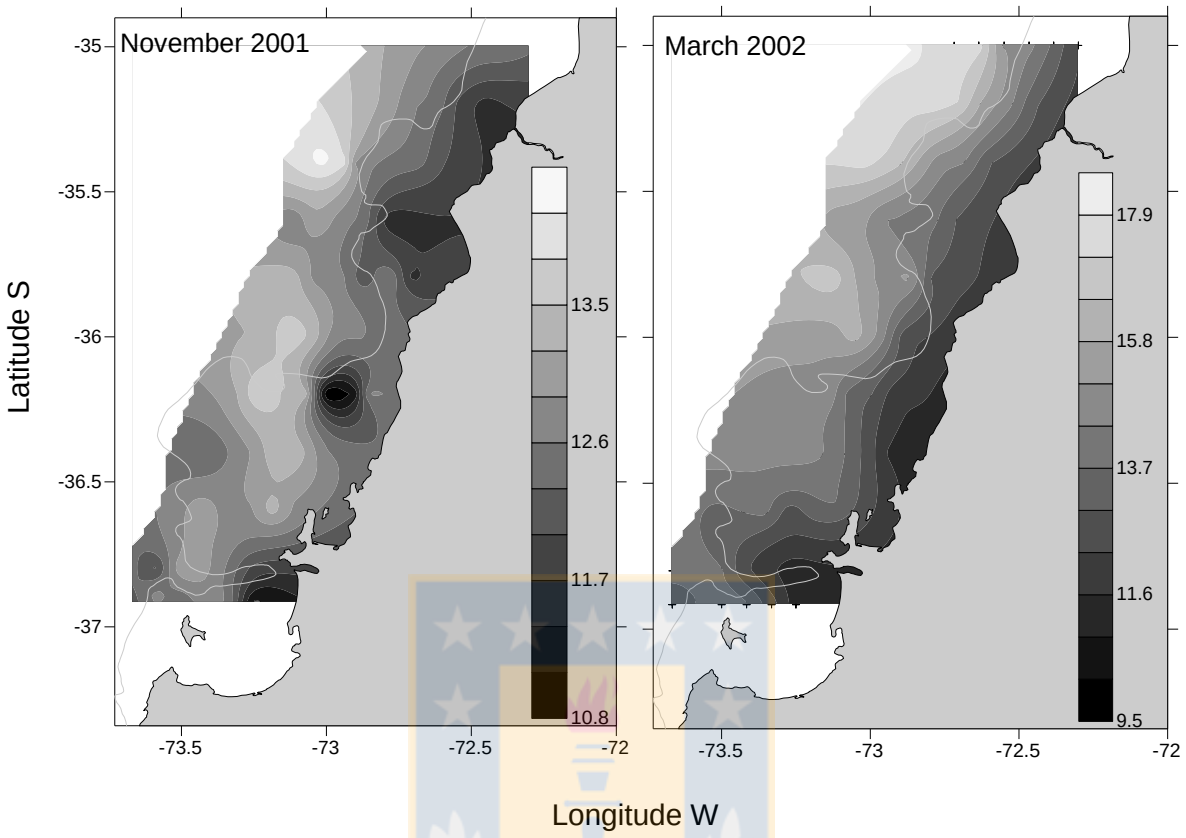

November 2001
March 2002
Latitude S
Longitude W
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
13.5
12.6
11.7
10.8
17.9
15.8
13.7
11.6
9.5

Fig. 3

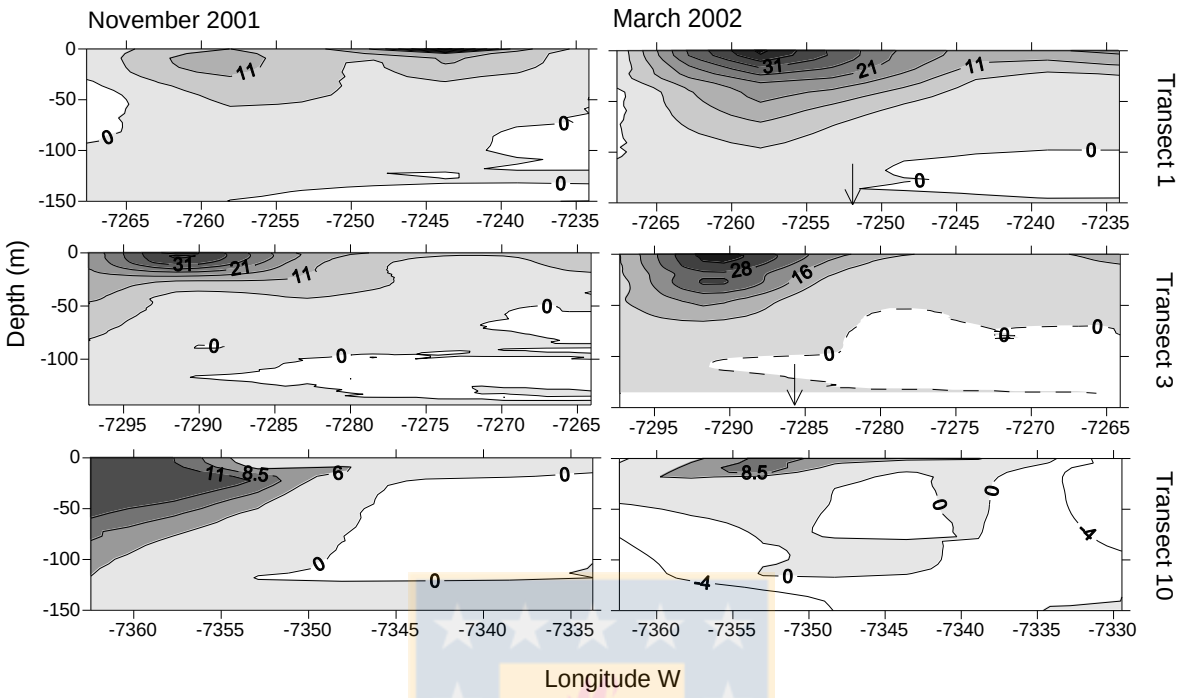

November 2001
March 2002
Transect 1
Transect 3
Transect 10
Depth (m)
Longitude W

Fig 4

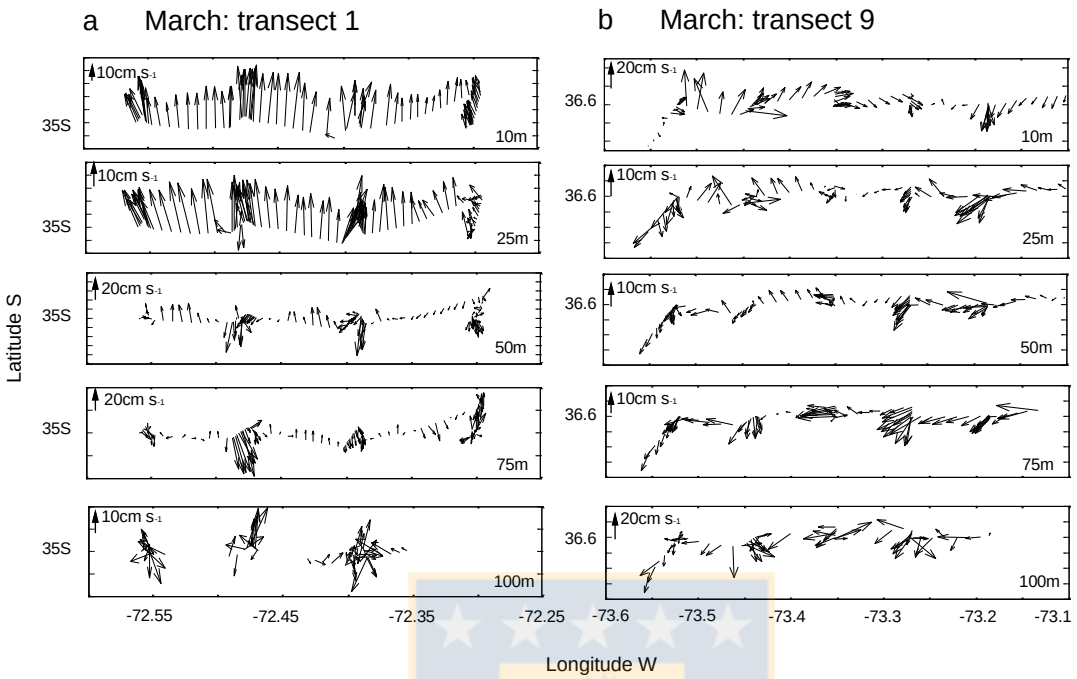
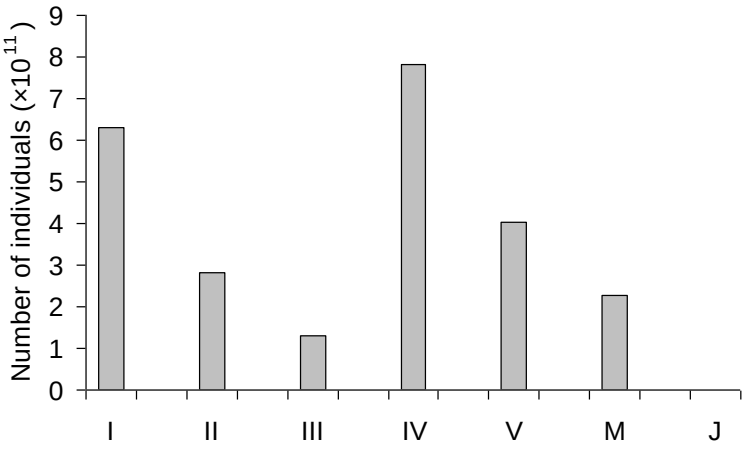
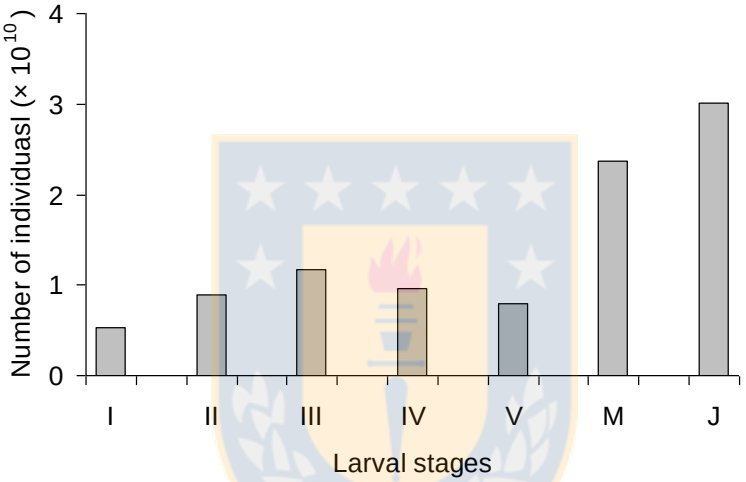

a March: transect 1
b March: transect 9
Latitude S
35S
36.6
10cm s-1
20cm s-1
10m
25m
50m
75m
100m
-72.55
-72.45
-72.35
-72.25
-73.6
-73.5
-73.4
-73.3
-73.2
-73.1
Longitude W

Fig. 5

a)

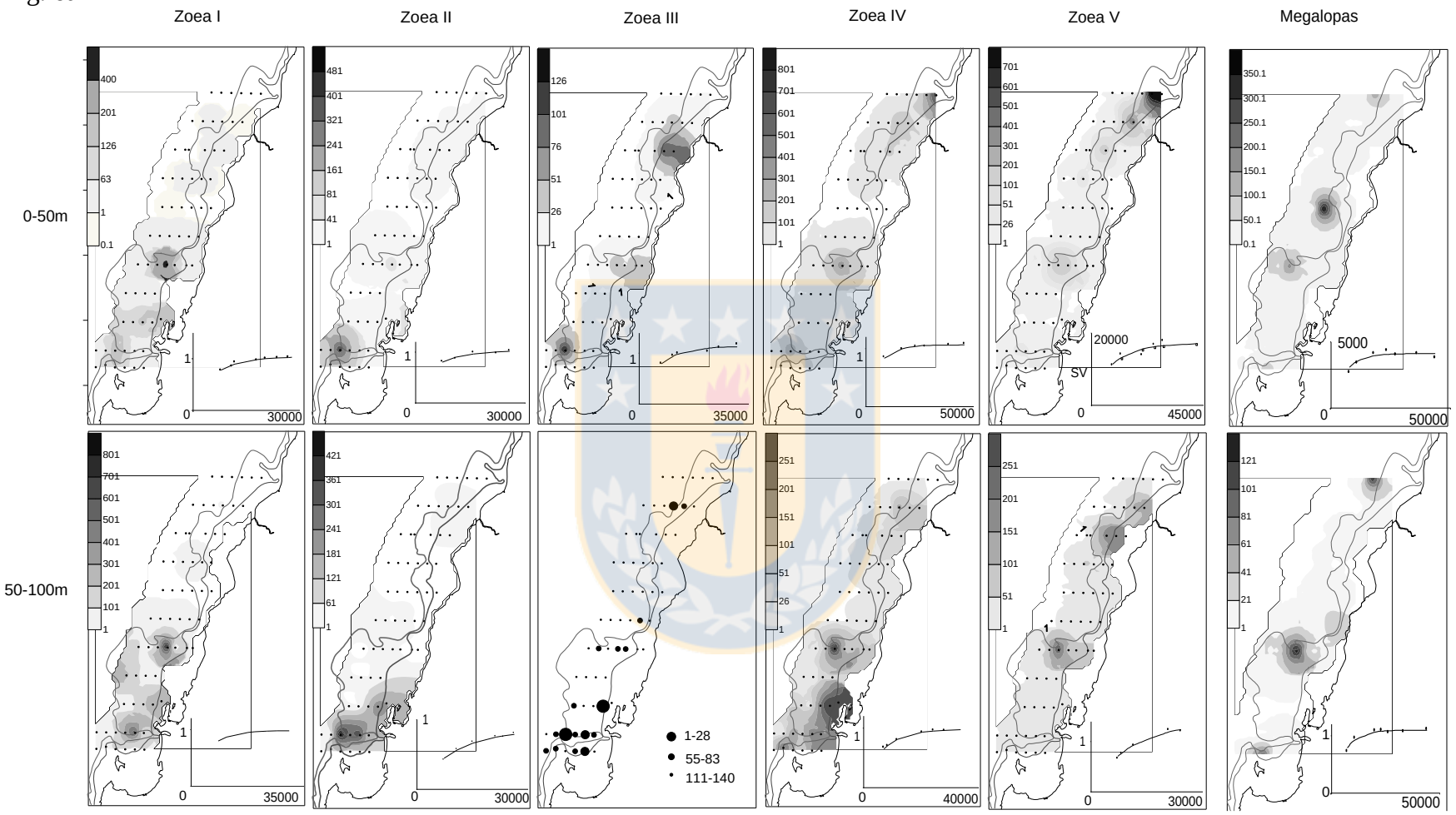

Number of individuals (×10^11)
0
1
2
3
4
5
6
7
8
9
I
II
III
IV
V
M
J

b)


Number of individuasl (× 10^10)
0
1
2
3
4
I
II
III
IV
V
M
J
Larval stages

A

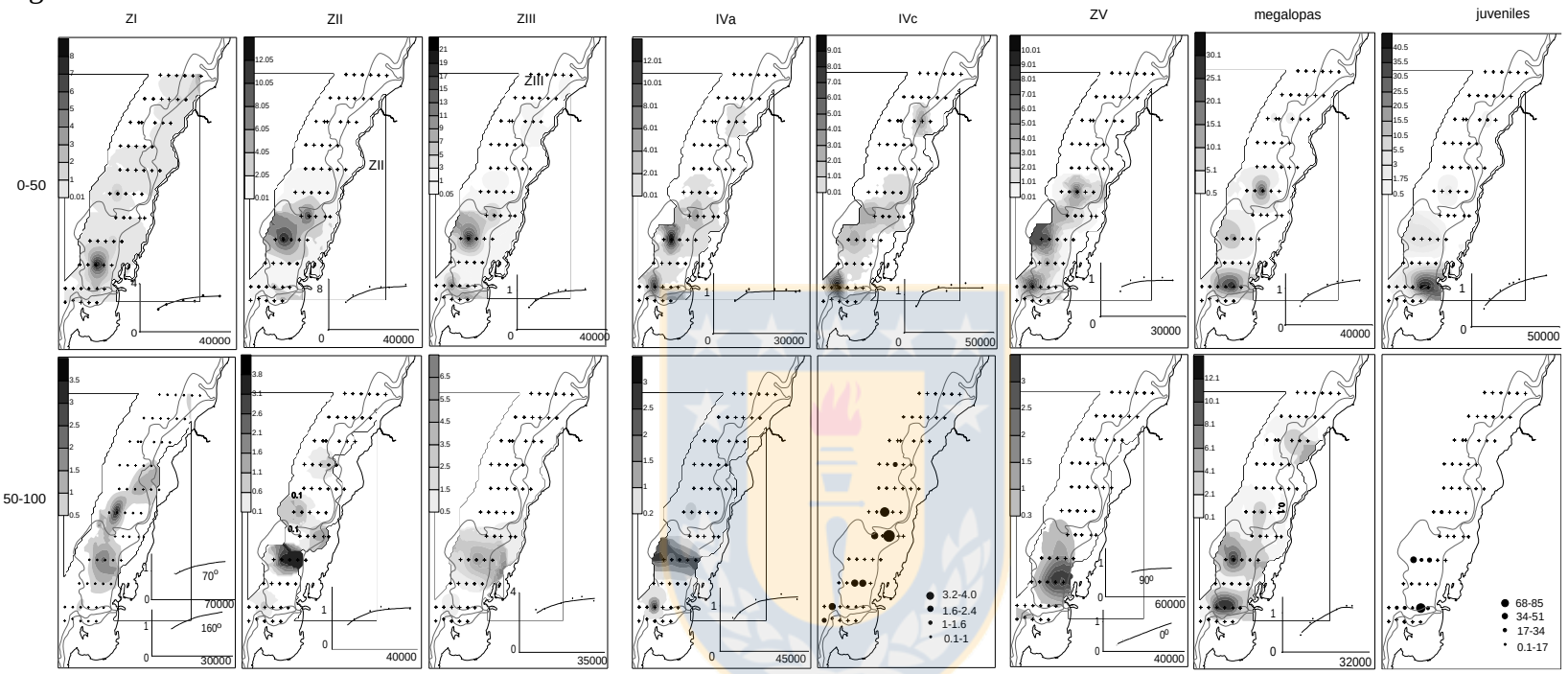
Fig. 6a


Zoea I
Zoea II
Zoea III
Zoea IV
Zoea V
Megalopas
0-50m
50-100m
SV
1-28
55-83
111-140

E

A

Fig. 6 b


ZI
ZII
ZIII
IVa
IVc
ZV
megalopas
juveniles
0-50
50-100
3.2-4.0
1.6-2.4
1-1.6
0.1-1
68-85
34-51
17-34
0.1-17

E

Fig. 7

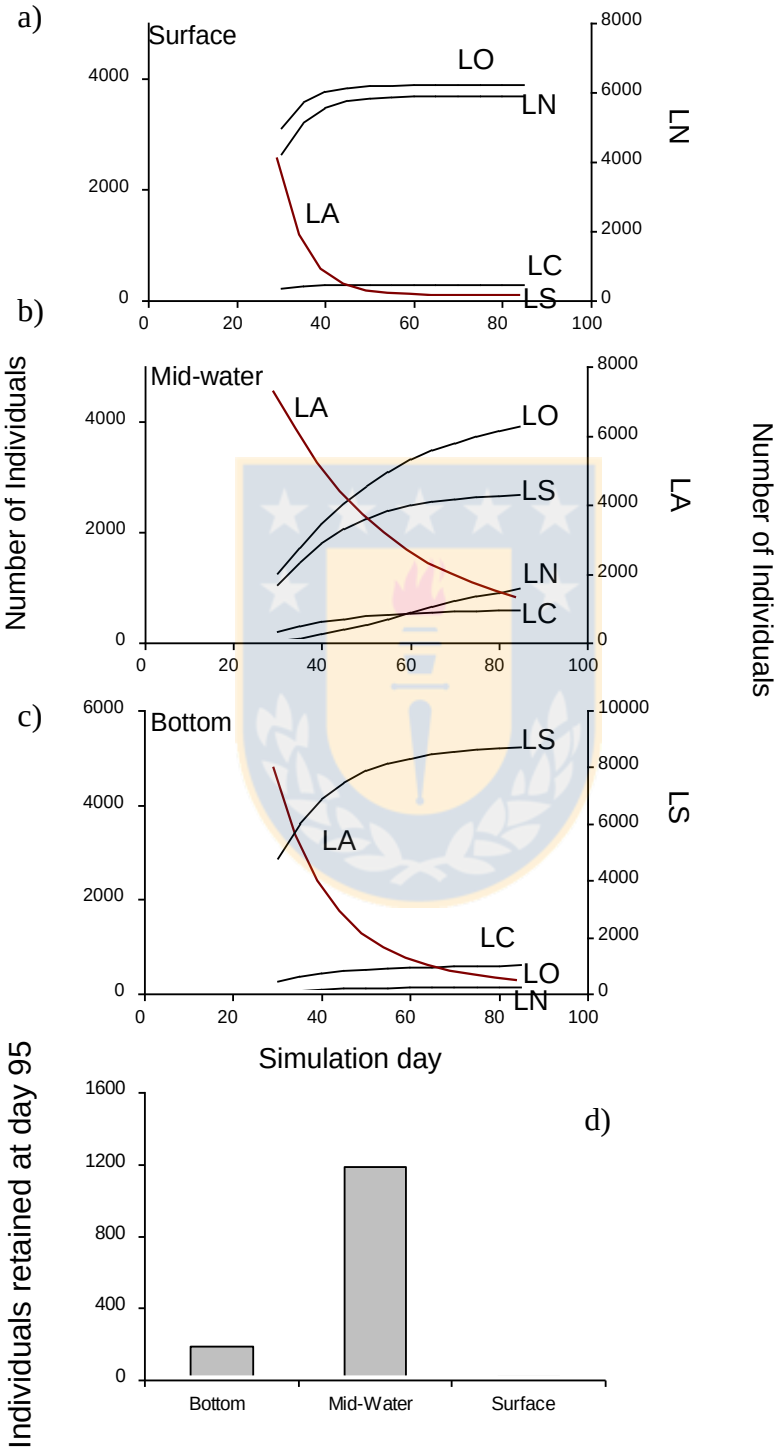

a)
Surface
LO
LN
LA
LC
LS
b)
Mid-water
LA
LO
LS
LN
LC
c)
Bottom
LS
LA
LC
LO
LN
Number of Individuals
LN
LA
LS
Number of Individuals
Simulation day
d)
Individuals retained at day 95
Bottom
Mid-Water
Surface

Fig. 8

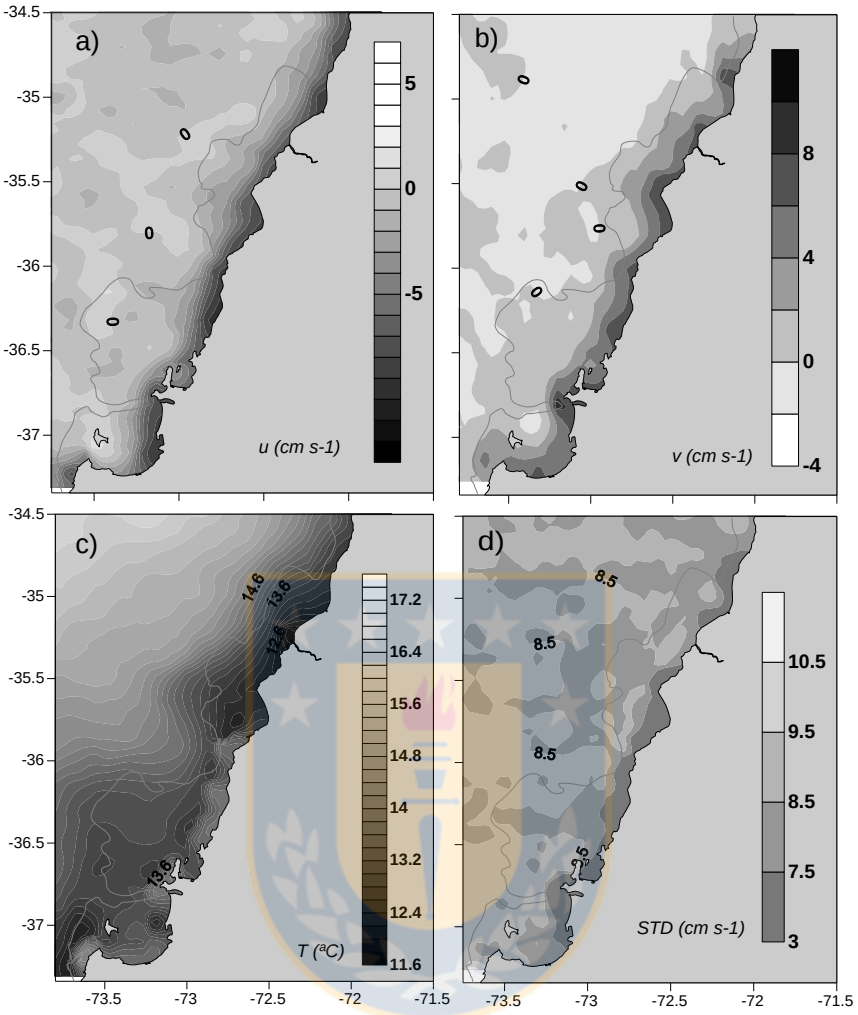

a)
u (cm s-1)
5
0
-5
b)
v (cm s-1)
8
4
0
-4
c)
T (ºC)
17.2
16.4
15.6
14.8
14
13.2
12.4
11.6
d)
STD (cm s-1)
10.5
9.5
8.5
7.5
3
-34.5
-35
-35.5
-36
-36.5
-37
-73.5
-73
-72.5
-72
-71.5

Fig. 10

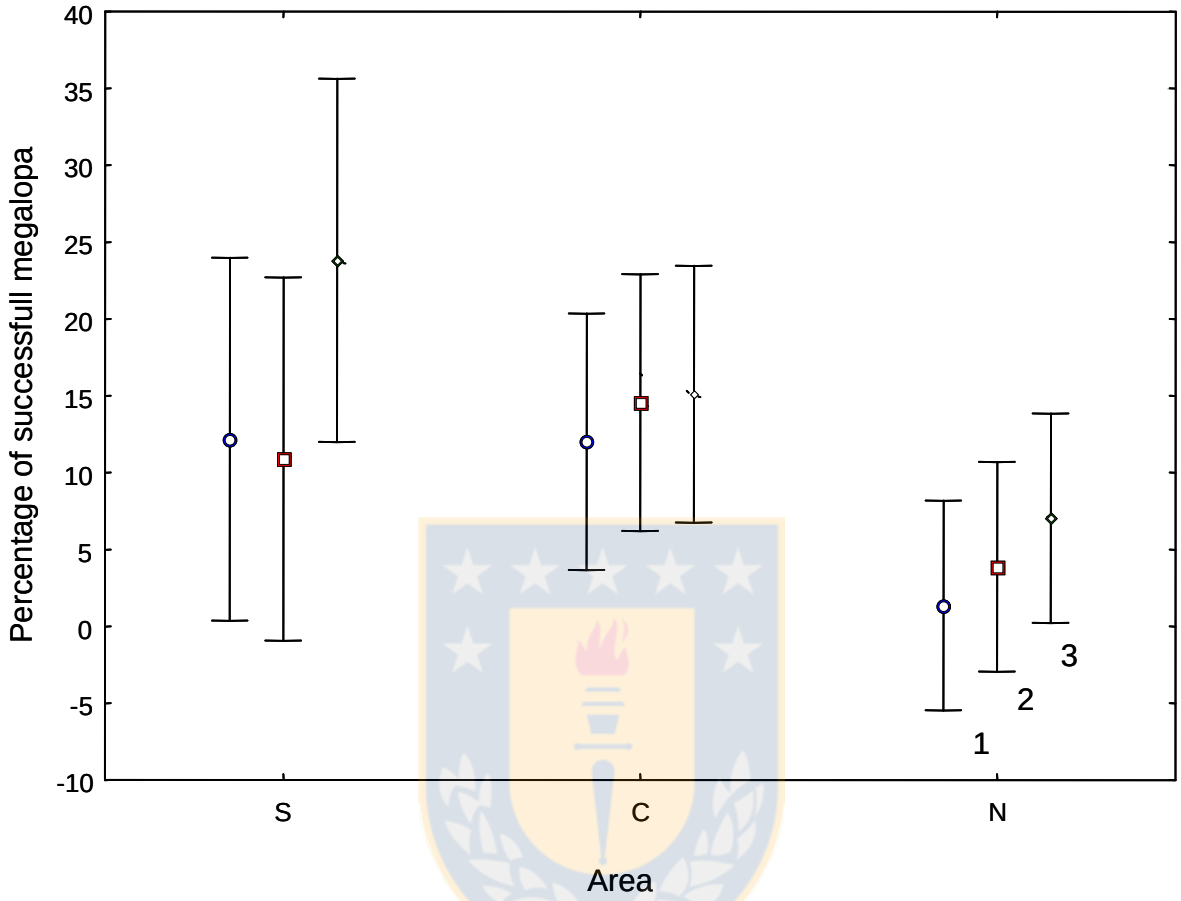
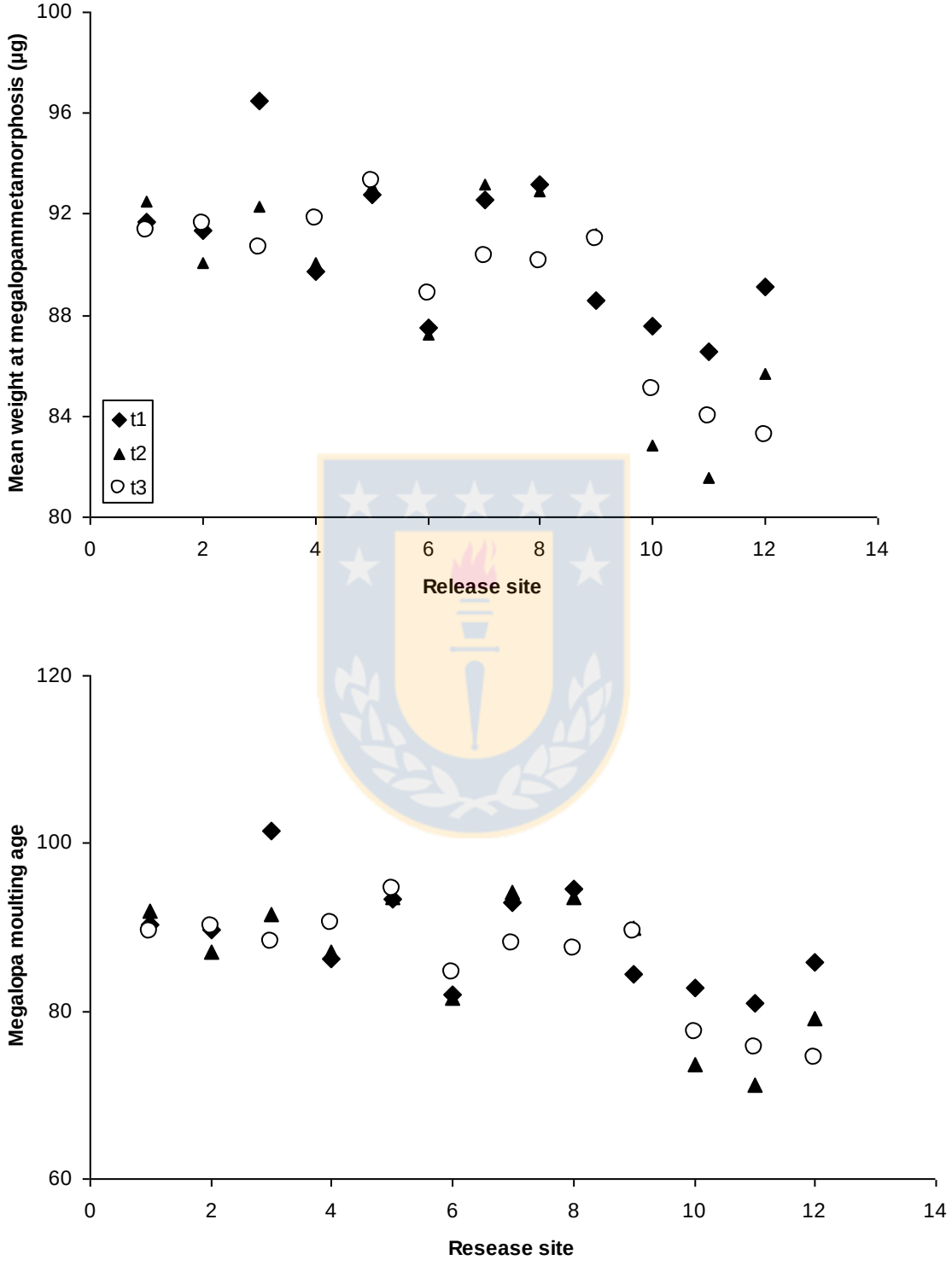

Percentage of successfull megalopa
40
35
30
25
20
15
10
5
0
-5
-10
S
C
N
Area
1
2
3

Fig. 9


Mean weight at megalopammetamorphosis (µg)
100
96
92
88
84
80
0
2
4
6
8
10
12
14
Release site
t1
t2
t3
Megalopa moulting age
120
100
80
60
0
2
4
6
8
10
12
14
Resease site

Manuscrito 4:

B Yannicelli & L Castro.

Relative contribution of oxygen, temperature and feeding schemes on growth rate, survival and developmental times of *Pleuroncodes monodon* larvae reared in laboratory.

Journal of Experimental Marine Biology and Ecology (re-enviado)

Abstract

The galatheid Pleuroncodes monodon inhabits the soft sediments of South Central Chile at depths between 50 and 350m where the bottoms are bathed by the low oxygen Equatorial Sub-Surface Waters. Thus, larvae of this species hatch in water characterized by low temperature (11°C) and low oxygen concentration (<0. 5 $ml \cdot l^{-1}$). We studied the effect of low oxygen levels, temperature, and initial deprivation of food on survival, developmental time and growth of larvae of P. monodon.

When zoea I was maintained at oxygen concentrations of 1 and 0.5 $ml \cdot l^{-1}$, survival was reduced as compared to normoxic conditions. Less than ten percent moulted successfully to the second stage at oxygen a concentration of 1 $ml \cdot l^{-1}$. However, tolerance to low oxygen conditions was high since zoea I raised in 0.5 $ml \cdot l^{-1}$ survived up to 23 days. Survival to the second stage, decreased with increasing periods of initial exposure to low oxygen (0.7, 1 and 2 $ml \cdot l^{-1}$): the mortality rate of larvae exposed to initial periods of low oxygen from 1 to five days increased 3% per day in the three cases. The age at moulting to the second stage increased linearly with increasing periods of initial exposure to low oxygen. Age at moulting also depended exponentially on oxygen concentration: lower oxygen concentrations, larger development. During the first larval stage, an initial starvation period of 3.5 days caused 50% mortality. Initial starvation alone had a more pronounced effect than initial low oxygen. Initial starvation in low oxygen conditions however, was even more detrimental to survival than only starvation. Growth was reduced at lower oxygen concentrations; body weight of larvae surviving to the second stage differed in more than 50% from those reared under normoxic conditions.

Low temperature (11°C) reduced survival about 40% as compared with higher temperatures (15 and 20ºC). The mean age at moulting to the following stage was also related to temperature following an exponential model. Individual dry weight for each temperature was related to age by an exponential function. The growth rate (constant exponent of the growth function) was lower at 11°C as compared with 15 and 20ºC. However, dry weight of recently moulted zoea II showed minimal differences between temperatures. The natural hypoxic and cold water conditions where the larvae of Pleuroncodes monodon are hatched are sub-optimal for development. Larval survival may

be enhanced if upwelling circulation in the hatching area transport larvae to normoxic waters also characterized by higher temperatures.

Key words: crustacean larvae, developmental time, growth, hypoxia, Pleuroncodes monodon, point of no return, survival, temperature

1. Introduction

The larval planktonic phase of marine invertebrates is considered the most vulnerable part in their complex life cycle. In fact, it is believed that pre-recruitment processes are one of the main responsible for regulating population and community dynamics (Connoly and Roughgarden, 1998). Developmental times, growth rates, and physiological conditions affect the expected larval survival while they are influenced by the bio-physical environment where larvae developmet takes place (Hinckley et al., 1996). Longer developmental times, reduced growth rates, or poorer physiological conditions render larger vulnerability to predation (Frank and Legget, 1982), advective loss, and lower post-settlement survival and growth (Pechenik et al., 2002; Shima and Findley, 2002; Giménez, 2004).

The pelagic larval development of decapod larvae involves successive moulting cycles, affected by a conmbination of intrinsic (e.g. endocrine) and extrinsic factors (e.g. temperature, nutrition). The effect of an extrinsic factor would depend not only on the type and strength of environmental influence but also on the timing of occurrence during the moult cycle (Anger, 2001). Temperature influences the duration of larval stages, growth rate and the size at the end of larval development; the degree of dependence varies among species (Anger, 1983; Lindley, 1998; Anger et al., 2003). Diet and feeding schedule also affect growth and development (Wehrtmann, 1991; Sulkin et al., 1998; Anger, 2001). Initial periods of starvation (in several planktotrophic crustacean larvae) delay or might preclude moulting to successive instars after a point of no return (PNR: Anger and Dawirs, 1981; Anger, 2001). This fact, indicates the importance of replenishing internal reserves soon after hatching. Given the necessity to build up reserves in a short period of time, the availability of environmental dissolved oxygen, and the metabolic changes that are caused by its reduction, might be of special significance during early development.

So far, it is known that low oxygen concentrations result in reduced growth and developmental lengthening in juveniles of decapod crustaceans (Seidman and Lawrence, 1986; Rosas et al., 1998; Coiro et al., 2000). Low oxygen conditions may cause high mortalities during the larval life (Miller et al., 2002). Low rate of development and growth under hypoxia have been related to reduced oxygen uptake rates (Tankersley and Wieber,

2000), substrates used as energy supply (Rosas et al., 1999), and low assimilation efficiencies (Rosas et al., 1998).

Several species of decapod crustaceans inhabit and release their larvae in the oxygen minimum layers in the world ocean. Species that are common inhabitants of naturally occurring hypoxic waters, display well-known mechanisms to withstand environmental oxygen deficiency (Childress and Seibel, 1998). For example, adults of the squat lobster Pleuroncodes planipes oxyregulates respiration rates down to a very low critical point (Quentin and Childress, 1976). However, there is till limited information about the capabilities of centhic crustaceans during the early life stages. Physiological (and morphological) capacities evolve during ontogeny in species with complex life cycles, as has been shown in crustacean larval osmoregulatory capacity (Charmantier et al., 2002) and oxyregulation in artemia nauplii (Spicer and El-Gammal, 1999) and Cancer magister (Terwilliger and Dumler, 2001) among others.

The squat lobster Pleuroncodes monodon (Crustacea: Galatheidae) distributes from Perú to South Central Chile (38º S). In a southern population (34-37S: Roa et al., 1995; Roa and Tapia, 1998) adults are found in the continental shelf and slope between 50 and 350 m depth (Retamal, 1981). They are usually associated with Equatorial Sub-Surface Waters (ESSW), characterized by poor oxygen content (<0.05 $ml \cdot l^{-1}$), low temperatures (10-11ºC) (Roa et al., 1997). For this population, the reproduction has a seasonal pattern: the larvae are released mainly during spring; they develop in the water column during the period of most intense upwelling, and then recruit in a well-established nursery area over the continental shelf, located between 36.2 and 36.6S (Gallardo et al., 1994; Roa et al., 1995). Recruitment occurs mainly during early austral autumn, when bottom water characteristics are those of ESSW (Gallardo et al., 1994). Juveniles exhibit daily vertival migrations before settling definitely (Yannicelli et al., in prep.). Egg-carrying females of P. monodon are found within ESSW; larvae distribute in a very wide depth range (Yannicelli et al., in prep.). Large concentrations of larvae are found both above and below the marked oxycline that develops seasonally during summer months in central Chile as ESSW shoals over the continental shelf.

The larval development of P. monodon has been studied in the laboratory under ad libitum food conditions, normoxia and at a range of temperatures 15 and 20°C (Faguetti

and Campodonico, 1971). However, they develop under wider rages of temperature (10 and 13.5 degrees C) and oxygen concentrations (0.5 ml l^{-1} to normoxia: Yannicelli et al., in prep). In the present paper, we quantified the influence of oxygen concentration, temperature and food availability on survival, developmental times, and growth of P. monodon larvae in laboratory cultures.

2. Materials and Method

Ovigerous females where collected during direct stock-assessment campaigns by trawling between depths of 100 to 150m, during austral spring in the South-Central Chilean continental shelf. Ovigerous females were brought alive to the Marine Biological Station of the University of Concepción in Dichato where experiments were carried out. Since spawning season and also stock assessment occur during a short period within the year, the experiments where carried out in two consecutive years (yr 1 and 2). In each year the females were placed in large containers at constant salinity (33±1) and temperature (11ºC), and complete darkness. Water was permanently aireated; water and food were changed every two days. Females were fed with commercial pellets for salmon alevins. When the eggs where close to hatch, females were placed in individual containers without food; swimming larvae were picked with pipettes and used for experiments. Unless specifically stated, all experiments were conducted in a controlled temperature room (11ºC ± 1ºC), salinity (33±1), with filtered seawater (1 / 0.5 µm +UV filters) under a photoperiod 12:12. The temperature and salinity were chosen based on field data that indicated that about 50% of zoea I larvae from the south-central Chile population were found in those conditions (Yannicelli et al., in prep).

2.1 Developmental time and survival in different temperatures: 11, 15 and 20

Freshly hatched larvae from two females, were kept under three different temperatures: 11, 15 and 20ºC. For 15 and 20ºC, larval containers were placed in thermo-regulated baths in the controlled temperature room. A total of eight groups per treatment were followed. Four groups from each of the two females. Since it has been demonstrated that larval initial conditions might influence post-hatching performance (Giménez, 2002), througout this work we split groups of larvae from each female into the different

treatments. The total number of individuals in each container ranged from 10 to 21. Density was not biased to any treatment. The homogeneity of survivors per replicate (G test, Sokal and Rohlf, 1995) was checked in order to discard density-dependent effects on survival. In addition, density effects were discarded by comparing the percentage survival (and standard deviation) from these experiments with those of preliminary trials, in which groups of larvae from different females were raised in homogeneous densities at 11ºC (see results). We used 250 ml containers for 11ºC, and 125 ml containers (not sealed winkler bottles) for higher temperatures. Preliminary trials showed that survival did not depend on the types of containers (see results). Water was changed every two days, because preliminary trials had also shown that survival was not enhanced by daily water changes. Larvae were fed ad libitum with freshly hatched Artemia sp. nauplii. The containers were inspected every day to check for dead larvae or moultings.

We modelled larval survival and growth in response to oxygen, tenperature and food levels. Data analysis in this paper was orientated towards a multi-model comparison and parameter estimation; we were concerned about quantifying magnitudes and shapes of responses rather than determining whether response variables differ significantly among treatments. Thus, we followed the ideas of Hilborne and Mangel (1997) and Burnham and Anderson (2002). Therefore, the hypothesis testing approach was avoided in favor of finding which of the proposed models (chosen a priori) had the greatest support from data. We used Akaike Information Criteria (AIC), Akaike differences and Akaike weights (Burnham and Anderson, 2002) to select the best-fitting model. Sakamoto et al. (1986) suggest that an AIC larger than 2 provides good evidence in favor of a particular one. Agresti (2002), McCullagh and Nelder (1999) provided basis for model construction and (maximum likelihood) estimation procedures. Model fitting was conducted in Microsoft Excel Solver and Statistica 6.1 non linear estimation module (user-specified regression).

In order to analyze temperature effects on developmental time, we considered that the proportion of individuals, “y” at age “d”, stage “j”, and temperature “i” followed a logistic model with binomial distribution. We found the age in which 50% of the individuals moulted (d50ij), and its standard deviation:

$$y_{ij}(d_{ij}) = \frac{1}{1 + e^{(\log(1/19) * \frac{(d_{ij} - d_{50_{ij}})}{(d_{95_{ij}} - d_{50_{ij}})})}}$$

In crustacean larvae, stage duration (D) relates to temperature as: D=a·Tb (Anger, 1983; Ismael et al., 1997; Anger et al., 2003). We were interested on finding the slope 'b' of this relationship and so published information (Faguetti and Campodonico, 1971) could be used to determine developmental times at 11º C. We compared two models including data from stages (j) I to III:

$$(1) \qquad D = a_j \cdot T^{b_j} \qquad (2) \quad D = a_j \cdot T^{b}$$

the values of D used in these models were the estimated d50ij from the previous equation; the models were adjusted by minimizing the negative log-likelihood of a normal distribution at each temperature, using the variance associated with d50ij, estimated from the logistic model. Afterwards, Akaike Information Criteria (AIC) was used to choose between the two models (see Burnham and Anderson, 2002).

2.2 Developmental time and survival in different oxygen concentrations. Ambient, 1.0 and 0.5 ml/l

Freshly hatched larvae were placed under three different oxygen regimes: normoxia, 1ml·l^{-1} and 0.5 ml·l^{-1} at 11ºC. During year 1, the experiment was done with larvae from three females. From each female, two groups of larvae were kept in normoxia (control) and four groups of larvae were kept in 1 ml·l^{-1} [OD]; 9 to 15 individuals were placed in each group. Density differences did not introduce an additional bias. During the second year, experiments where carried out with larvae from four females. We kept four groups from each female in normoxia, four groups in 1 ml/l oxygen water and four groups in 0.5 ml/l oxygen water. In all cases, they were fed daily with freshly hatched Artemia sp. nauplii. We changed water every two days under normoxia, and daily under hypoxia. Water exchange under normoxia every two days rendered no differences compared with daily water exchange. However, water in hypoxic treatments had to be changed daily in order to avoid oxygen reduction below the desired levels. To accomplish the low oxygen condition, we bubbled N_2 into the sea water until the desired concentration was reached. We used an oxymeter (Schott Handylab OX1) to measure experimental oxygen concentrations. For the low oxygen conditions, larvae were reared in 125 ml Winkler bottles, which were carefully filled with low oxygen water. Potential effect of the type of container was previously tested

but it was insignificant (see results: section 1). The pH was checked at the start and the following day before water exchange: no decrease was observed. Every day, we checked larvae to record the number of survivors and the number of larvae that moulted to the next stage.

Binomial mean and standard deviations were obtained for the cumulative mortality and the number of survivors to the second zoeal stage. Few individuals moulted to the second stage under low oxygen conditions. However, we compared the age (d_{50}) at which 50% of individuals moulted for 1 ml/l and the normoxic condition: a single logistic equation was fitted for both conditions and compared with an alternative model where that equation was fitted separately for each treatment. The comparison was made with AIC. Since the groups of larvae took several days to moult each curve was supported by at least 4-5 points in time.

2.3 Initial exposure to low oxygen

Although larvae of P. monodon hatch in low-oxygen waters, they might not experience hypoxic conditions more than a few days during the first zoeal stage. We conducted experiments to evaluate the effect of the initial exposure to low oxygen concentration on developmental time and survival of zoea I to zoea II. Two different experimental settings were used. In the first year freshly hatched larvae were sorted in groups of 20 ind. in 10 containers with sea water at 2 $ml \cdot l^{-1}$[OD] and in ten other containers with 1 $ml \cdot l^{-1}$ [OD]. Daily, for up to five days, two groups were transferred and maintained thereafter in normoxic water. In the second year, freshly hatched larvae were kept in containers of 1 l volume at 1 $ml \cdot l^{-1}$ (1 hatch) and 0.7 $ml \cdot l^{-1}$ (3 hatches), then groups of ten organisms were transferred to normoxic conditions as for the first year. The frequency and day of moulting was registered. Water and food were changed daily at low oxygen conditions, and every two days afterwards. The rationale for a different experimental setting during the second year was to homogenize the initial conditions. In the first approach, groups of individuals had different histories: the initial containers were individually filled with low oxygen waters. In the second approach all organisms were subject to exactly the same oxygen levels in the same container. We evaluated if results from year 1 and 2 were producing consistent results: we identifiyed, through AIC, if the

survival was better explained by a single lineal model, or by a two models, one for each year.

The frequency of moulting was calculated based on the quantity of larvae that were alive at the sixth day of experimental run, i.e. after the larvae from all treatments were at least at nomroxic conditions for at least one day. Several models were contrasted in order to identify if survival to the second stage depended linearly on the days of initial exposure and oxygen level. AIC was always used to contrast models. For each treatment (oxygen level and number of days of initial exposure), the percentage of moulted individuals at an age were compared. We first fitted a logistic models to the data of each treatment to estimate the d_{50} and its standard deviation (variance). Based on those estimates, a series of models were fitted to analyze the shape and magnitude of age at moulting as a function of days of initial exposure and oxygen level (see results).

2.4 Developmental time and survival under initial starvation

Freshly hatched larvae were used in experiments of "point of no return"; they were incubated in groups of 10 individual, in 20 containers without food. Each day, we added Artemia to two containers with previously starved larvae; in the 10^{th} day, larvae form all containers had been fed at least once. Water was changed every two days in all containers; in those with Artemia it was exchanged daily. The procedure was followed with larvae from three females.

We calculated the point of no return (PNR_{50}) by fitting a logistic function to the survival as a response to days of initial starvation. In this case, the numerator was chosen to be a parameter, since maximum survival at 11ºC was not 100%.

2.5 Initial starvation in low oxygen

This experiment was performed with larvae from two consecutive year. For the first year, freshly hatched larvae were collected and placed in groups of ten (twenty) larvae in 20 winkler bottles without food. Winkler bottles were filled with 1.0 $ml \cdot l^{-1}$ (exp 1) and 0.7 $ml \cdot l^{-1}$ (exp 2) oxygen water. Each day we removed larvae from two containers with previously starved larvae, and placed them in normoxic water with Artemia sp nauplii. Water and food was changed daily in all containers. We recorded mortality and

developmental time. The procedure was followed separately with larvae from two different females.

For the second year the initial exposure to low oxygen and starvation conditions was conducted in a common container. Groups of larvae were daily taken from this container and incubated under normoxic conditions and ad libitum food conditions as explained for the first year.

We fitted a logistic model for survival data as a function of days of initial exposure to low oxygen and starvation and determined whether the PNR_{50} was dependent on oxygen level. The analysis of the effect of decreasing food and oxyc stress on the duration of development followed the procedure described in the Section 'Initial exposure to low oxygen'.

2.6 Growth:

2.6.1 Temperature

Larvae from three different females were mass-reared in 2 l containers, under three different temperatures: 11, 15 and 20°C. Water was exchanged every two days, and freshly hatched Artemia nauplii were added after water change. Groups of 15 zoea I were removed at different time intervals; we also took samples of freshly moulted zoea II. Samples from each brood were taken from either of the two containers maintained for each brood. Larvae were rinsed in distilled water in a sieve, dried in 'klinex' and frozen in liquid nitrogen. Later, each sample was freeze dried, and weighted to the nearest 0.001 mg in a high precision balance. Individual dry weight was used to construct power growth curves: $a \cdot t^b$ where t is time (age in days) and a and b are parameters. We compared models with single a and b parameters for all temperatures, models with independent a and b parameters for each treatment, and combinations of both. Models were compared with AIC; the log-likelihood, based on the normal distribution, was used to estimate the different combinations of parameters a and b.

2.6.2 Oxygen concentration

We also analyzed growth for larvae maintained under different oxygen concentrations at 11ºC. Individuals in normoxia were reared as in 6. Those in hypoxic

conditions, were raised in 1 l Schott bottles. Water was bubbled with N_2 in large containers. When the desired oxygen concentration (around 1 or 0.7 ml/l) was reached, the bottles were filled with a silicone hose without bubbling; oxygen concentration was then checked for each bottle, the larvae were introduced with pipette under the water surface, and the bottles were sealed with parafilm leaving no air bubbles. The oxygen concentration was also checked in each bottle at the daily time of water exchange, so we recorded daily initial and final oxygen concentration. The fact that parafilmn is not completely hermetic to gas exchange compensated for respiration inside the bottles. Table 1 shows the mean, maximum and lowest oxygen level reached in each of the containers. Each bottle would have therefore a unique history of oxygen concentration, but since groups were similar in a reasonable range they were treated as a category for model construction.

To find the dependence of growth rate on oxygen level we followed the same procedure as for weight data obtained under different temperatures (see Section 3.5.1: Growth: temperature).

3. Results

3.1 Developmental time and survival in different temperatures.

Survival increased with temperature (Fig. 1a), with a survival of 56% at 11°C (95% CI 0.66-0.50); 86% at 15°C and 98% at 20°C. Under each treatment, and for each female, the proportion of survivors was homogeneous between replicates and therefore independent from the density of organisms used (Table 2a). In fact, the only group of replicates that were not homogeneous was one in which the differences in densities were low (female 2, 15°C). The mean number of survivors from two broods raised at 20 and 10 individuals per container (Table 2b), were similar. There was not any relationship between the survival of organisms and the density (Fig. 2). Another preliminary trial was conducted at 15°C to check if the type of containers affected larval survival. The proportion of surviving zoea II from zoea I reared in 3 plastic containers of 250 ml was 0.83 (Std=0.06); it did not differ from the one obtained for larvae reared in winkler bottles.

We did not attempt to fit a model describing survival as a function of temperature. Our experiments did not include high temperatures, where survival most likely decreases as a consequence of thermal stress.

The average moulting age depended on temperature (Fig. 1b). Model (2), i.e. with intercepts varying between stages but with common slope (b= -1.53), was the most parsimonious (AIC difference = 3.7). The mean moulting age ($\underline{d}_{50ij}$) and standard deviations are presented in Table 3a. Moulting to zoea II took between 7 days (20°C), and 17 days (11°C), and stage durantion tended to decrease in succesive stages from I to III. The age at moulting from zoea I to III at 15 and 20°C was in agreement with published results, so we used published data and *b*=-1.53 to estimate later stages duration at 11º C (Table 3b): the average time to moult to the eighth instar was estimated in 97 days (± 12)

3.2. Developmental time and survival in different oxygen concentrations

The survival to the second stage decreased at lower oxygen concentrations (Fig. 3a and b). While over 40% of larvae survived to the second stage under normoxic conditions, 13% survived in 1 $ml \cdot l^{-1}$ and less than 1% in 0.5 $ml \cdot l^{-1}$ (all experiments from period 1 and 2 combined). In spite that under very low oxygen conditions only a few zoeae I moulted to the second stage, approximately 50% were capable of surviving half the stage duration (Fig 3a).

The duration of development was lengthened by hypoxic conditions. At 0.5 $ml \cdot l^{-1}$ the surviving individuals moulted after 20 days. At 1 $ml \cdot l^{-1}$ the age at 50% moulting to the second stage was 21.3 days, while at normoxic conditions it was 17.6 days. The AIC favored one model for each condition (AIC= 47.2).

3.3. Initial exposure to low oxygen.

The lineal decrease of survival with increasing initial period of exposure to 1 ml/l for the experiments conducted in the different initial settings (and years), was better explained by a single equation (AIC=88), while the worst explanation was that given by a single model adjusted to each treatment (AIC=93). Therefore, we used the model with a single equation for the treatment.

The experiments conducted under the different initial oxygen concentrations, showed that survival to the second stage decreased with increasing days of initial exposure (Table 4). Survival, decreased linearly as the initial period of oxygen increased; the intercepts of the linear functions depended on the oxygen level. The common slope indicated that each

day of exposure reduced survival approximately 3% (Fig. 4). The duration of development was lengthened as the initial period of stress increased. A model using the pooled data (common intercept and slope) suggested that the age at 50% moulting to the second stage depended linearly on the initial period of stress, and exponentially on oxygen concentration (Table 5 and Fig. 5a). However, another model suggested that the age at 50% moulting to the second stage depended exponentially on the initial period of stress, it was not possible to select a particular model since both of them satisfactorily represented the shape of the relationship and the number of the estimated parameters was the same (Table 5, Fig. 5b).

3.4. Developmental time and survival under initial starvation and initial starvation in low oxygen

Survival decreased with increasing initial starvation period. The estimated PNR_{50} was 3.06 days (Fig. 6). The duration of development was lengthened with increasing initial starvation period (Fig. 7).

The effect of starvation under low oxygen had a stronger detrimental effect on survival that initial starvation under normoxia (Fig. 6): the PNR_{50} were, at 1 and 0.7 $ml \cdot l^{-1}$, 0.46 and 1.82 respectively. We chose to adjust one logistic model to each experimental oxygen level (AIC=1110.4), since adding oxygen in the equation, or considering a single model for all data had substantially higher AIC (1121.74 and 1156.42 respectively). Our estimates did not decreased monotonically with decreasing oxygen levels. One experiment at oxygen concentrations of 0.7 ml/l was conducted during year 1 and two were run during year 2. However, one experiment from year 2 was more similar to the experiment during year 1, than the two experiments conducted during year 2 among them (AIC=69.1 and AIC=80.1 respectively).

Mean age at moulting to the second stage, increased linearly with increasing initial starvation-hypoxic period (Fig. 7). Some of the compared models could not be distinguished from each other. A model with individual parameters for each condition (three independent curves) showed the lowest AIC. So far, it is clear that the number of days of exposure had a linear influence on moulting date, but we could not identify the shape of response to oxygen concentration from our data since Akaike differences were not large enough. No effect (a single equation for each treatment) and linear effect were the

best models, and had almost the same Akaike weight. (Table 6a). The effect of initial starvation in low oxygen on survival or age at moulting was larger than that of initial exposure to low oxygen alone as shown by the higher slope of linear relationship (Fig. 7 Table 6b).

3.5 Growth

3.5.1 Temperature

Body weigth of zoea I increased exponentially with age. For growth under 15 and 20 °C a single equation was used to describe dry weight as a function of age: the AIC difference between a single model and a model for each temperature was 2.6. The growth rate parameter at 15 and 20°C (b=1.19) was larger than the growth rate parameter at 11°C (b=0.85) (Fig. 8). A single model could not explain all three curves: the AIC difference between one model for 15 and 20°C and one for 11°C, vs a single model for all temperatures pooled was 34. For 15 and 20°C a lineal model could also be adjusted to growth data, however at 11°C the exponential model was better (AIC difference > 2). The effect of temperature on the dry weight reached after moulting to zoea II was not as large as for the growth rate. Mean weight (and standard deviation) of zoea II recently moulted were: 23.4(5.1), 22.8(1.7) and 22.9(0.5) µg at 20, 15 and 11°C respectively.

3.5.2 Oxygen concentration

Growth under hypoxia was lower than under normoxia (Fig. 9). A different model was fitted for each oxygen level (Table 7), and growth rates were progressively lower at lower oxygen concentration. At 11°C, the difference in the growth rates between larvae developing in 0.7 ml l^{-1} of oxygen and normoxic conditions was about the same as differences in slopes between those at 11 and 20° C. In addition, the mean dry weight of freshly moulted zoeae II in normoxic water was 23 µg, while in 0.7 ml l^{-1} oxygen water it was 14 µg. By the end of the experiment, the larvae under 0.7 ml l^{-1} showed a decline in dry mass.

4. Discussion

Pleuroncodes monodon larvae hatch at very low oxygen concentrations and low temperature. Although they tolerate low oxygen concentrations, our laboratory experiments showed that the zoea I moulting is precluded or delayed at the constant oxygen and temperature levels that characterize adult habitat.

4.1 Evaluation of experimental setting

Our experimental setting did not allow the maintenance of organisms under low oxygen in exactly constant conditions. Certain reduction of oxygen concentration during each day was monitored and recorded in common containers. However, we were able to maintain different mean oxygent concentrations throughout the experiments for each of the treatments: in the common containers, the standard deviations were very low (< 0.3 for both hypoxic conditions. This values are not far from those reported from more sophisticated flow through systems, in which standard deviations might vary between 0.27 and 0.14 depending on the oxygen level and treatment (Grecay and Stiefhoff, 2002). In the case of the small containers used for rearing larvae at 0.5 ml/l, we calculated based on respiration rates for feeding larvae under hypoxic conditions (Yannicelli et al., in prep.) that it would not be reduced below 0.43.

Neither differences in larval rearing densities nor different types of containers among treatments affected our results. Previous experiments showed that a density of 40 larvae per 250 ml container was detrimental for survival, but we maintened rearing densities at much lower levels. Several preliminary trials were conducted before choosing versatile containers needed for the different experiments. Previously to choose winkler bottles and 250 ml plastic containers, we had discarded two other types of glass containers that led to high mortality. After the chosen containers were proven to be equally effective for rearing, we used them indistinctly depending on the availiabity of each container.

4.2 Effects of oxygen, food and temperature on development

Higher larval mortality registered at low water temperature is consistent with the fact that Pleuroncodes monodon is at its southern distribution limit at central Chile. Northern populations which develop at whigher temperatures, tend to be pelagic (Ayon

pers. com.). Reduced swimming activity was observed at 11°C as compared to 15 and 20 C (pers. obs.). Although food was added ad libitum, reduced swimming could potentially reduce prey encounter rates and contribute to larval mortality. This type of considerations should be taken into account when extrapolating laboratory results to field estimates, since natural levels of turbulence and upwelling currents may enhance encounter rates. The fact that the duration of development was lengthened at 11°C, may account for a large part of decoupling between the expected duration of development inferred from previous laboratory studies using higher temperatures (Faguetti and Campodonico, 1971) and those estimated from field sampling (Cañete, 1994). Still there is not any information on the stage duration of the megalopae.

The lenghteninig of the larval development at early stages might increase the time of larval advection as well as the exposure to pelagic predators. While advection during upwelling events might render greater loss of larvae from the adult population (Castro, umpublish info), it could also increase the potential for alongshore transport (Siegel et al., 2003).

Planktotrophic decapod larvae need to rapidly acquire external energy after hatching in order not to deprive their own energy reserves and build up enough new reserves to moult successfully (Anger, 2001; Gimenez, 2002; Paschke et al., 2004). If food is not limiting, the capacity to metabolize depends on oxygen availability among other factors. The capacity of withstand reduced environmental oxygen concentration lies on the efficiency of maintaining oxygen supply, diminish metabolism and/or utilize anaerobic capacity. Maintaining high oxygen supply is based on body structures which are not ussualy present during early ontogeny, or on high hemocyanin concentration (Spicer and El Gammal, 1999). Anaerobic metabolism in crustaceans mainly relies on glycolitic pathways (Griesharber et al., 1994) but the glycogen reserves of zooplankton are not usually enough to maintain normal metabolism for several days. Therefore, when organisms spend prolonged periods of time under low oxygen concentrations, down regulation of metabolism is likely to occur.

Hypoxic waters do not cause immediate death in zoea I of Pleuroncodes monodon, but growth is reduced. Thus, mortality may result from the inability to obtain enough energy reserves, but not from asphyxia. Further support comes from the fact that few larvae

could even moult under low oxygen, although they took much longer time to do so than organisms under normoxia. In addition, reduced swimming shoud reduce the capture and handling of prey (Breitburg et al., 1994). If crustacean larvae are encounter-feeders such changes could also influence the present results in spite of high food availability in culture containers.

When low oxygen does not reach lethal levels, short periods of exposure to hypoxia do not lead either to higher mortality or to growth reduction. Low oxygen by itself is not a cause of damage, except that exposure lead to a deficit in reserve gain during critical periods of the larval moulting cycle. In this case an extra period of time should be needed to accumulate enough reserves to moult successfully to the following stage. This might be the explanation for the longer duration of development of zoea I under initial conditions of low oxygen. Besides, an increase in mortality was observed. The initial reserves carried by freshly hatched larvae vary among broods (Gimenez and Anger, 2001; Anger et al., 2003), and season (Paschke et al., 2004). Within a brood, developmental conditions vary as a function of the oxygen availability (Fernandez et al., 2003), or genetic individual variability (discussed by Moran and Manahan, 2004). Mild stress during the beginning of development could enhance those individual differences rendering mortality in weaker individuals, (e.g. Gimenez and Anger, 2003). The adult females used in our experiments spent most of their breeding time in hypoxic waters of the continental shelf of south central Chile; in the laboratory they continuously shaked the abdomen and egg mass (pers. obs.). Low oxygenation during embryogenesis could also reduce the capability to deal with energetic constraints.

Lipids are consumed from the hepatopancras after several days of starvation in normoxia. Further starvation leads to irreversible cytological damage even in presence and ingestion of food (Anger, 2001). Such a point in larval development has been described in several crustacean and fish larvae (Blaxter and Hembel, 1963; Gisbert et al., 2004) and it is known as the point of no return (PNR). Pleuroncodes monodon is not an exception showing a clear point of no return. The PNR occurs sooner at high temperatures (Bisbal and Bengstson, 1995). Since energy reserves are used up faster at higher temperatures, lower metabolic rates under hypoxia would perhaps delay de PNR. Lower respiration rates have been effectively measured in P. monodon zoeae under hypoxia (Yannicelli et al., in prep).

However, the contrary was observed in our experiments, and lack of food plus hypoxia led to larvae reaching the PNR sooner. Under hypoxic conditions proteins instead of lipids may be used to fuel bioquemical reactions (e.g. Penaeus setiferus juveniles: Rosas et al., 1999). In this case, the depletion of lipid reserves should not account for increased deterioration. Larval death did not occur necessarily during the period of initial exposure to stress conditions, indicating that larval death was not a consequence of immediate depletion of reserves. Another type of damage may have occurred. In our experiments, the PNR not did not vary monotonically with oxygen availability. We did not study the oxygen level at which the onset of anaerobic metabolism occurs, so we can only speculate that maybe the usage of alternative metabolic pathways at very low oxygen levels can also alter the PNR. In addition, we found that different broods rendered large response differences. An evaluation of the maternal effects was not among our objectives, but undoubtedly, from our results and compiling literature evidence, it should be specifically addressed in future studies.

The shape of dry mass/age curve for larvae raised at 11°C followed the same curvature that has been previously described for brachyuran crustaceans with a slight decrease of dry weight close to moulting compared to that at intermoult (see Anger, 2001 for review). Freshly hatched zoea II raised under hypoxic conditions had a lower dry mass than those raised in normoxia. Further survival or weight increase were not monitored in those larvae and their viability can not be assessed from our results. However, even if they are able to develop further, they are in evident disadvantage since their food and predator size spectrum would remain almost the same as when they hatched. Temperature had a strong effect on instantaneous growth rate, but the dry mass of freshly moulted zoea II did not vary among treamtments. Thus, accelerated development within the studied range, affects size at age, but not size at stage.

4.3 Ecological implications

In the continental shelf of South Central Chile, where larval Pleuroncodes monodon hatch, food for the maintenance of pelagic planktotrophic fish larvae is considered to be enough year-around (Castro et al., 2000) and peaks of primary production coincide with the months of larval production (September-October, Daneri et al., 2000). Therefore, in the

subsurface waters where larvae are released, the lower availability of oxygen as well as the low temperature may be the major impediment for growth and development. When larvae of *P. monodon* are released, during upwelling periods, sub-surface waters may ascend from 80 m to the surface in 4 days (calculated from vertical velocities taken from Sobarzo et al., 2001). This and the larval own swimming capacity may lead to avoidance of highly hypoxic cold waters. Zoea I is mainly found in the sub-surface, while higher abundances of zoea II and advanced stages appear in surface waters (Yannicelli et al., in prep). However, a considerable proportion of larvae are found below 50m depth suggesting that, natural upwelling-downwelling cycles or convergence zones temporarilly transport larvae towards sub-surface waters, without major negative consequences. It is known that alongshore and acrosshore current velocities can be reduced at the thermocline. In P. monodon distribution area the thermocline and oxycline are usually coincident during upwelling periods. Therefore larvae could even take advantage of the potential (latitudinal, cross-shore) retention at thermocline depths. However, deeper waters should be avoided.

Upwelling prevalence during Pleuroncodes monodon larval hatching period, may provide the mean of reaching more coastal and surface waters during early development. Since upwelling velocities are slow, the capability to withstand low oxygen levels for a longer period is necessary for the first zoea stages. Once the larvae have reached the surface layer, horizontal advection in the surface Ekman layer might export larvae from the coastal zone. However, the duration of the later zoea stages might be shortened since the overall environmental conditions become more benign in the surface (higher oxygen concentrations, higher temperatures and increased food availability). Since recruitment occurs on the shelf, the later offshore surface advection should not cause great larval loss if, additionally, it is limited by upwelling fronts or shelf break fronts that normally occur in most coastal upwelling areas. The interplay of specific ecophysiology, hydrography and circulation, can only be further analyzed by coupled biophysical models. The southern portion of the population has suffered large biomass shifts during the last two decades (Roa et al., 1997) due to either an environmental or a fishery effect. Detailed larval ecophysiological studies should provide new information to evaluate species-specific responses to environmental fluctuations, and re-evaluate the role of upwelling circulation for particular species.

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Tables

Table 1

Oxygen rearing conditions for experiments in common containers. Mean concentration during the experiment, DMV: daily mean variation, maximum and minimum values measured. Treatment 1: moderately low oxygen concentration (at or above 1 $ml \cdot l^{-1}$), treatment 2: low oxygen concentration (between 1 and 0.5). treatment EI: initial exposure to low oxygen (moderate: 1EI, low: 2EI).

Female	treatment	replicate	Mean concentration ($ml \cdot l^{-1}$)	DMV	Max	Min
16	2	1	0.75	0.34	1.00	0.43
17	2	1	0.85	0.17	1.21	0.39
17	2	2	0.68	0.21	1.07	0.49
17	1	3	1.06	0.20	1.25	0.71
18	2	1	0.79	0.16	1.63	0.53
18	2	2	0.66	0.14	1.07	0.49
18	1	1	1.01	0.16	1.36	0.79
20	2	2	0.77	0.12	0.94	0.57
20	2	1	0.69	0.12	1.07	0.50
20	1	1	0.99	0.22	1.29	0.71
20	2EI	1	0.75	0.06	1.07	0.62
21	2	1	0.66	0.07	0.86	0.50
21	2	2	0.70	0.15	1.00	0.50
21	2	3	0.64	0.14	0.86	0.43
21	1	1	0.97	0.22	1.50	0.57
21	1	2	0.99	0.18	1.29	0.71
23	2	1	0.62	0.17	1.36	0.29
23	2	2	0.58	0.14	0.86	0.31
23	1	1	0.93	0.27	1.64	0.47
23	1	2	0.95	0.23	1.21	0.64
24	2	1	0.64	0.20	0.97	0.48
24	1	1	1.06	0.15	1.43	0.86
24	2EI	1	0.73	0.15	0.93	0.57
24	2EI	1	0.76	0.18	1.00	0.64
24	1EI	1	1.04	0.23	1.36	0.79
24	1EI	1	1.14	0.36	0.79	1.57

Table 2

A) Maximum and minimum number of individuals used in replicates from females 1 and 2 cultivated at three temperatures. Also the heterogeneity statistic G is shown for each female and temperature treatment. (*) indicates that replicates were not homogeneous (0.05>p>0.01). B) Mean proportion of survivors (MPS) from replicated trial experiments with densities of 20 (4 replicates) and 10 (3 replicates) individuals per container. Also standard deviation (std) is shown.

A)

		Temperatures		
		11	15	20
Female 1	Max.	23	19	21
	Min.	10	15	12
	Heterogeneity G	7.53	7.5	0
Female 2	Max	15	21	15
	Min	9	20	21
	Heterogeneity G	2.08	9.76*	1.78

B)

Initial number	Number of Replicates	MPS	std
20	4	0.525	0.12
10	3	0.533	0.21

Table 3

A) Age at molting (days) estimated as d_{50} and standard deviations (between brackets) for *Pleuroncodes monodon* larvae at different stages and temperatures. Stage duration (in days) according to present experiments (+) and Faguetti and Campodonico (1971) (FC). *a* and *b* are the parameters of the power function that relates mean stage duration and temperature. B) Mean stage duration for later stages according to Faguetti and Campodonico, 1970 (FC) at 15 and 20 °C, and estimated duration at 11°C according to our model.

A)

	Age at molting (+)			Stage duration (+)			Mean age at molting (FC)		Fitted model parameters		
Stage	11	15	20	11	15	20	15	20	a_j	b	AIC
I	17.41 (0.65)	9.55 (0.74)	7.36 (0.41)	17.41	9.55	7.36	10.3	8.3	679		
II	29.43 (0.82)	17.17 (0.87)	12.51 (1.18)	12.02	7.62	5.15	17.3	13.6	1143	-1.53	20.94
III	40.9 ----	23.19 (1.31)	16.5 (0.53)	11.47	6.02	3.99	24.8	19.1	1595		

B)

	Mean age at molting (FC)		Estimated age at molting
Stage	15	20	11
IVa	31.6	24.9	48 / 56 (54.14)
IVb	39.3	31.2	60 / 70 (67.5)
IVc	47.2	38.6	72 / 87 (81.9)
IVd	54.7	48.3	83 / 109 (97.5)

Table 4

Models analyzed to explain proportion of survival (S) to the second stage after initial exposure to low oxygen concentrations. x = days under low oxygen exposure; OD, oxygen concentration, *a*, *b* and *c* are parameters, and sub fix *i* denotes a different parameter was estimated for each oxygen level. Below, parameter estimates for the best two models.

MODEL S=	L	AIC	K	AICi	AICw
a	-838.373	1678.74	1	23.51697	0.000
a_i	-833.84	1673.68	3	18.45712	0.000
a+bx	-827.561	1659.12	2	3.898108	0.078
a_i+bx	-823.612	1655.22	4	0	0.549
a+b_ix	-826.049	1660.1	4	4.874926	0.048
a_i+bx+cOD	-823.612	1657.22	5	2	0.202
a_i+cOD	-833.84	1675.68	4	20.45712	0.000
A+bx+cOD	-827.55	1661.1	3	5.876099	0.029
a_i+b_ix+cOD	-822.376	1658.75	7	3.529368	0.094

	a_i+bx		a_i+bx+cOD
$a_{2.0}$	0. 5	()	0.39
$a_{1.0}$	0.56	()	0.51
$a_{0.7}$	0.47	()	0.45
b	-0.03	()	-0.03
c			0.039

Table 5

A) Models fitted for developmental time dependence on days of exposure to low oxygen and oxygen concentration. B) Parameter estimates for best models.

A)

MODEL: d_{50} =	K	AIC	AIC dif	AICw
1) h_i	3	55.38	31.19	0.00
2) $h_i + f_i \cdot DE$	6	31.02	6.83	0.02
3) $h_i + f_i \cdot e^{(1-DE)}$	6	32.73	8.55	0.01
4) $H + f \cdot DE + k \cdot e^{(-lo)}$	3	24.19	0.00	0.57
5) $H + f \cdot DE$	2	34.04	9.85	0.00
6) $H + f \cdot e^{(1-DE)} + k \cdot e^{(-lo)}$	3	25.00	0.81	0.38
7) $H + f \cdot DE + k \cdot lo$	3	30.43	6.24	0.02

h, *f* and *k* are parameters
DE: days of initial exposure
lo: oxygen concentration

B)

Model 4		Model 6	
h	18.24	h	19.41
f	0.25	f	-0.73
k	0.85	k	0.93

Table 6

A) Model fitted for developmental time dependence on days of exposure to starvation and low oxygen concentration. B) Parameter estimates for the best models.

A)

MODEL: d_{50} =	K	AIC	AIC dif	AICw
1) h	1	123.94	96.94	0.00
2) h_i	3	124.31	97.32	0.00
3) $h_i + f_i \cdot DS$	6	27.00	0.00	0.29
4) $h + f \cdot DS$	2	28.49	1.49	0.14
5) $h_i + f_i \cdot DS + k \cdot oc$	7	29.00	2.00	0.11
6) $h_i + f_i \cdot DS + k \cdot \exp(-oc)$	7	29.00	2.00	0.11
7) $h + f \cdot DS + k \cdot e^{(-oc)}$	3	28.45	1.45	0.14
8) $h + f \cdot DS + k \cdot oc$	3	27.47	0.47	0.23

h, *f* and *k* are parameters
DS: days of initial starvation
oc: oxygen concentration

B)

Model 3			Model 8		Model 4	
7.5	h	19.2	h	18.41	h	18.77
7.5	f	0.71	f	0.76	f	0.77
1	h	18.3	k	0.07		
1	f	0.76				
0.7	h	16.89				
0.7	f	1.39				

Table 7

Results of model selection relating dry weight at age, for organisms grown under normoxia, 1 and 0.7 ml l^{-1} oxygen waters. Below, parameter estimates under the 'best' model (2) and the following one.

	Model	-ln(L)	k	AIC	Dif AIC	AICw
1)	$a+t^{bi}$	-93.95	4	195.90	3.42	0.181105
2)	a_i+t^{bi}	-90.24	6	192.48	0	1
3)	$a+t^{b}$	-134.82	2	273.64	81.16	2.38E-18

2) $a_{norm}=13.4$ $b_{norm}=0.78$
$a_1=12.2$ $b_1=0.63$
$a_{0.5}=11.4$ $b_{0.5}=0.41$

1) $a=13.4$
$b_{norm}=0.82$
$b_1=0.62$
$b_{0.5}=0.25$

Figure captions

Fig. 1. Temperature effect on Pleuroncodes monodon zoea I survival to zoea II (A) and, (B) duration of stages I, II and III at temperature. Mean values (dots), standard deviation (vertical lines) and the adjusted logarithmic model.

Fig. 2. Proportion of Pleuroncodes monodon zoea I surviving to zoea II as a function of rearing density (individuals per container).

Fig. 3. Survival to the II stage of Pleuroncodes monodon larvae reared under different oxygen concentrations. A) mean percentage of survivors at age, and standard deviation between different females ____ (normoxia); …… (1 $ml \cdot l^{-1}$); ````````` (0.5 $ml \cdot l^{-1}$). Circles indicate molting dates. Data from the second experimental period was included. B) Mean proportion of P. monodon survivors to the second stage at different oxygen concentrations and standard deviation. Data from first and second experimental periods are included.

Fig. 4. Proportion or Pleuroncodes monodon larvae surviving to the second stage (zoea II) as a function of days of initial exposure to low oxygen concentrations (1 to 5 days, and three oxygen levels).

Fig. 5. Mean age at molting from stage I to stage II for Pleuroncodes monodon larvae subject to initial periods (1 to 5 days) of low oxygen concentrations. In *a* and *b* the markers indicate mean values, the vertical lines the standard deviation and continuous lines indicate the mean molting date as a function of time of initial exposure, estimated from the two best models analyzed (a) (b).

Fig. 6. Proportion of surviving larvae to the second stage after initial periods of starvation in normoxia, 1 and 0.7 $ml \cdot l^{-1}$ (markers), and the fitted logistic models for each treatment.

Fig. 7. Day at which 50% larvae molted to the second stage after being subject to initial starvation in normoxic, 1 and 0.7 ml l^{-1} oxygen waters, calculated with individual re-

parameterized logistic models and its standard deviation. Also, the most likely model to explain dependence on initial exposure for each oxygen level is presented as continuous line.

Fig. 8. Dry weight at age of Pleuroncodes monodon larvae raised under 20, 15 and 11°C (markers). Also, adjusted power models are shown (lines).

Fig. 9. Dry weight at age of Pleuroncodes monodon zoea I in normoxic 1 and 0.7 ml l^{-1} oxygen waters (markers). Also, adjusted power models are shown (lines).

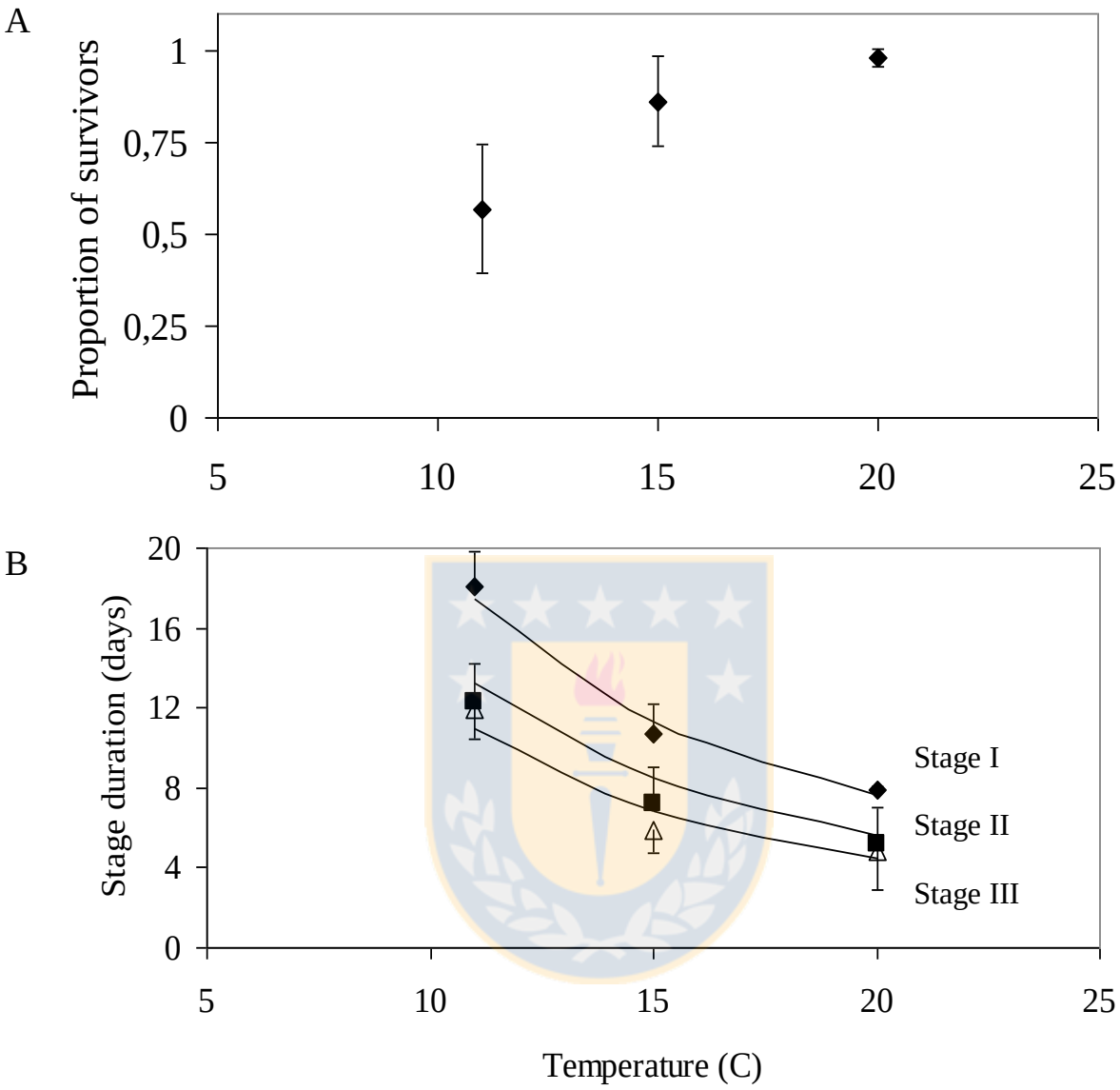

A
Proportion of survivors
1
0,75
0,5
0,25
0
5
10
15
20
25
B
Stage duration (days)
20
16
12
8
4
0
Stage I
Stage II
Stage III
5
10
15
20
25
Temperature (C)

Fig. 1.

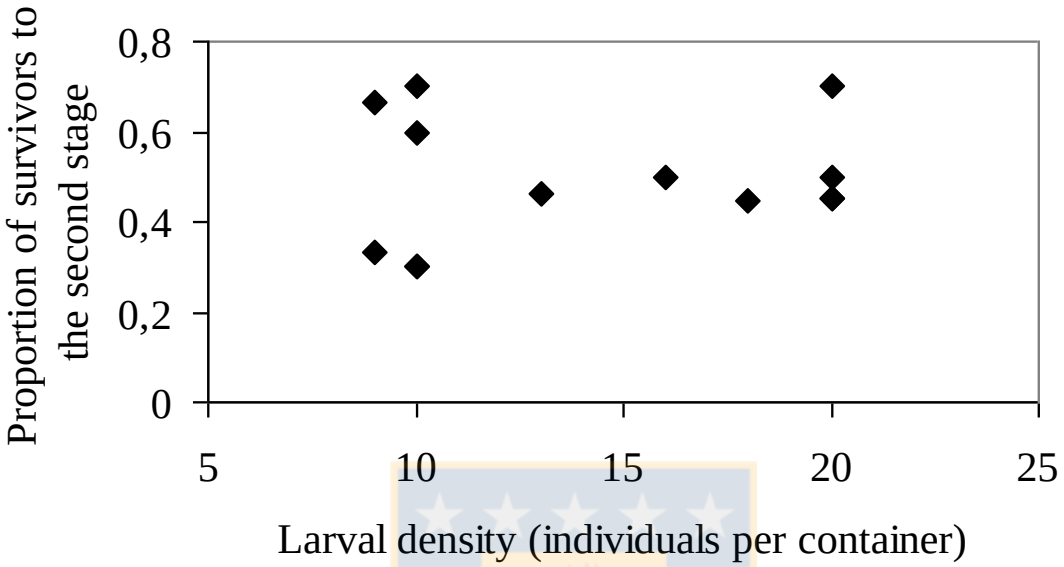
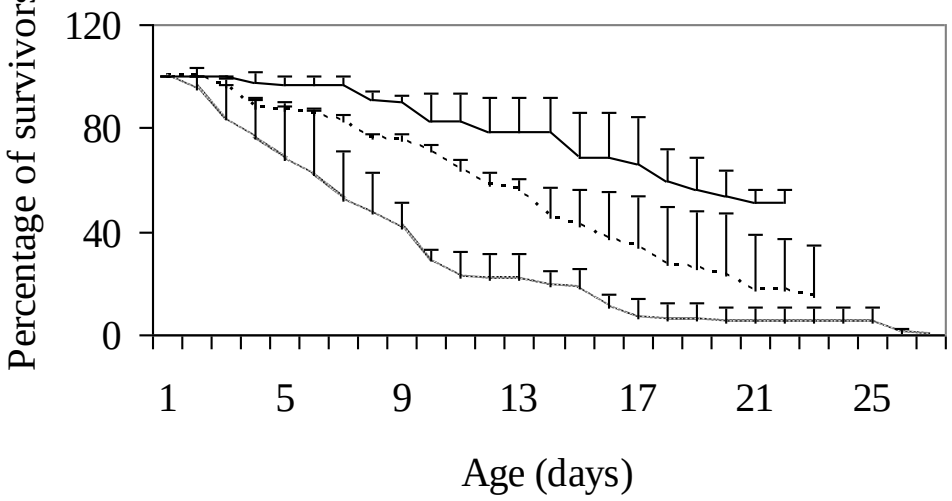

Proportion of survivors to the second stage
0,8
0,6
0,4
0,2
0
5
10
15
20
25
Larval density (individuals per container)

Fig. 2.

A)


Percentage of survivors
120
80
40
0
1
5
9
13
17
21
25
Age (days)

B)

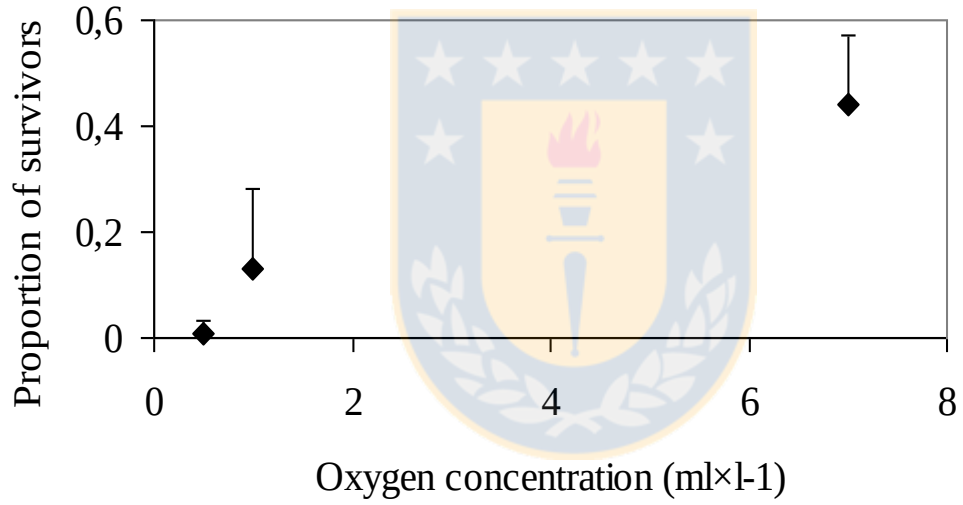

Proportion of survivors
0,6
0,4
0,2
0
0
2
4
6
8
Oxygen concentration (ml×l-1)

Fig. 3.

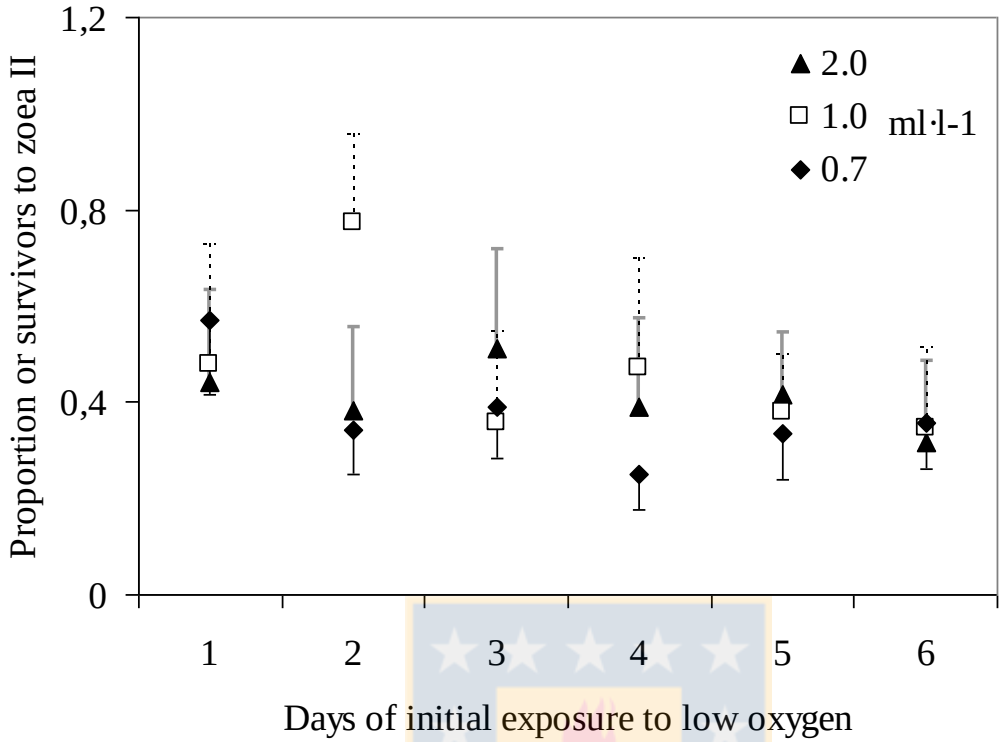

1,2
0,8
0,4
0
Proportion or survivors to zoea II
2.0
1.0
0.7
ml·l-1
1
2
3
4
5
6
Days of initial exposure to low oxygen

Fig. 4.

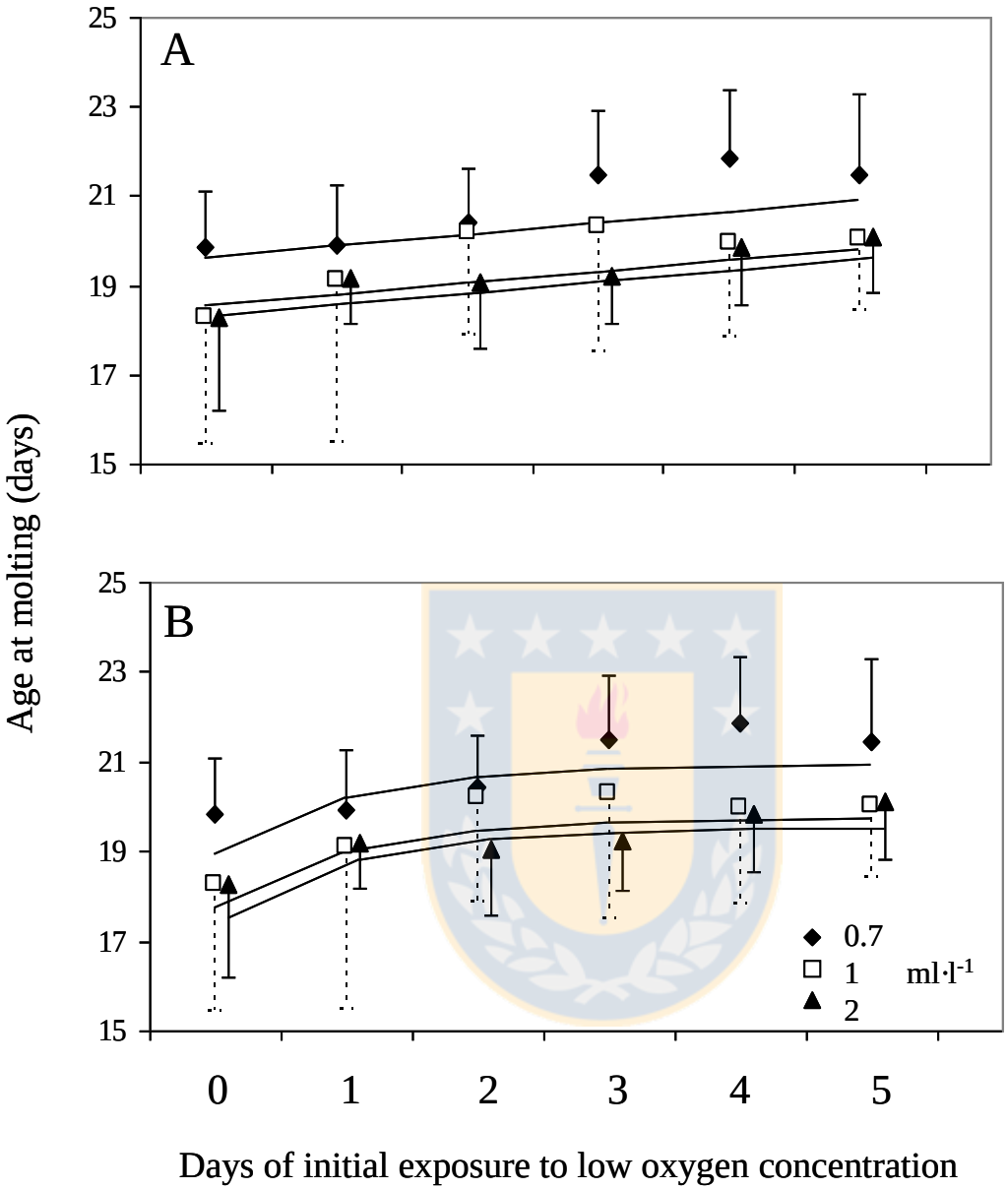

A
B
25
23
21
19
17
15
Age at molting (days)
0.7
1
2
ml·l-1
0
1
2
3
4
5
Days of initial exposure to low oxygen concentration

Fig. 5.

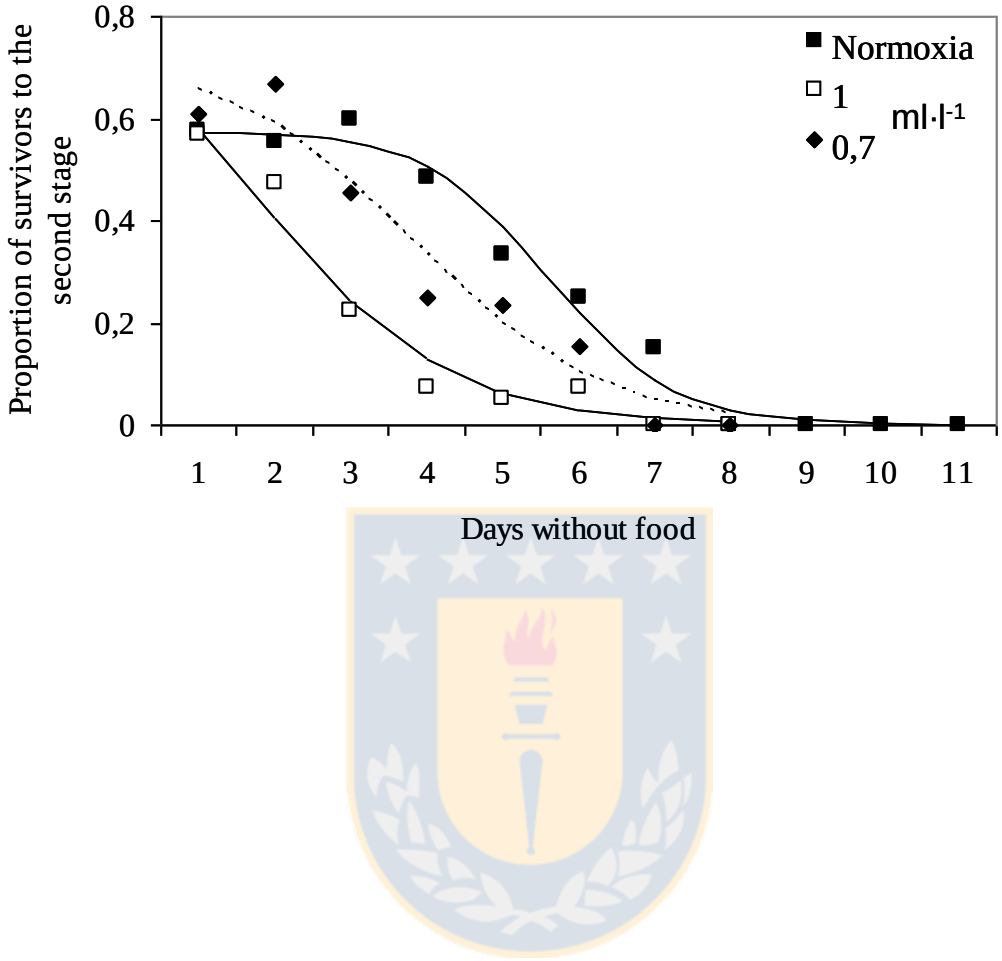

Proportion of survivors to the second stage
0,8
0,6
0,4
0,2
0
1
2
3
4
5
6
7
8
9
10
11
Days without food
Normoxia
1
0,7
ml·l-1

Fig. 6.

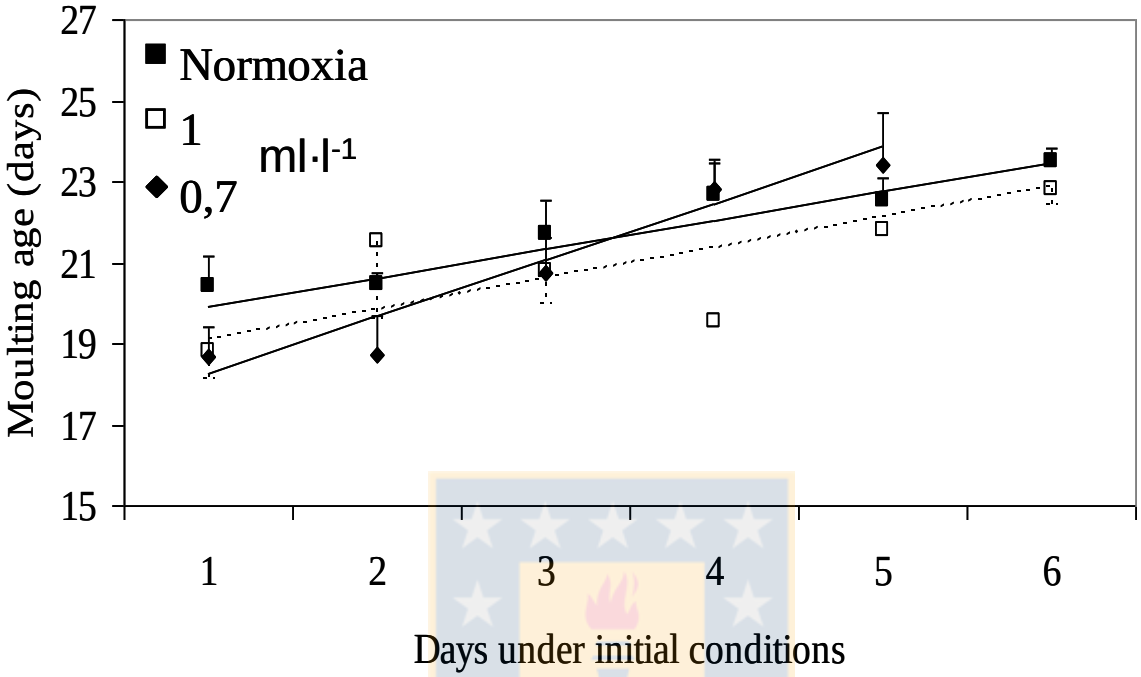

Normoxia
1
0,7
ml·l-1
Moulting age (days)
27
25
23
21
19
17
15
1
2
3
4
5
6
Days under initial conditions

Fig. 7.

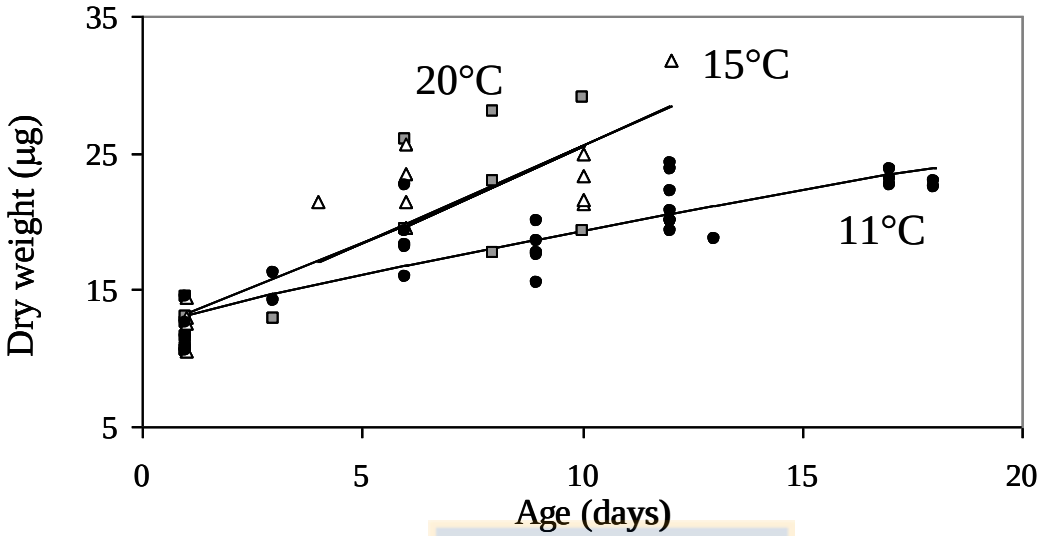


Fig. 8

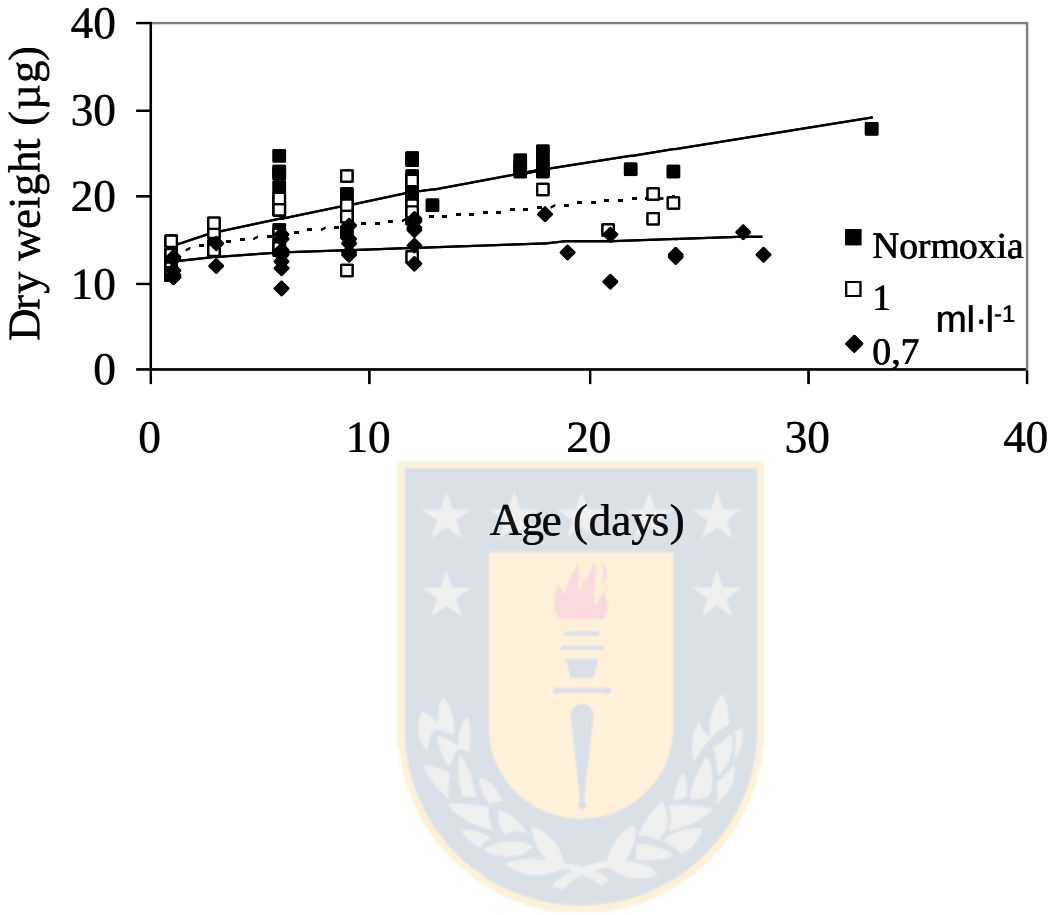

Dry weight (µg)
40
30
20
10
0
0
10
20
30
40
Age (days)
Normoxia
1
0,7
ml·l-1

Fig. 9

Manuscrito 5:

B Yannicelli, K Paschke, R González, L Castro, C Rosas.

Metabolic responses of the squat lobster (*Pleuroncodes monodon*) larvae to oxygen concentration.

Marine Biology (terminado)

ABSTRACT

The squat lobster *Pleuroncodes monodon*, a galatheid that inhabits sediments of the Chilean continental shelf, releases pelagic larvae in a hypoxic environment. Larvae tolerate relatively long periods of exposure but for the most part fail to molt to subsequent stages at oxygen concentrations typical of the adult habitat. In the present paper we analyzed some short term and long term responses of *P. monodon* larvae to hypoxia. In the short term, respiration in low oxygen concentrations is compared with respiration under normoxic conditions both in fed and unfed organisms. We also quantify the progress of apparent specific activity of enzymes of intermediate metabolism (lactate dehydrogenase, LDH, citrate synthase CS and malate dehydrogenase MDH) and the body composition (carbon and nitrogen: CN) through the development of zoea I raised in normoxia and hypoxia (between 1 and 0.7 $mlO_2 \cdot l^{-1}$).

Routine and post-prandial aerobic respiration rate (RRR, and PRR respectively) were lower in hypoxia (1.3 $mgO_2 \cdot l^{-1}$) in all zoeal stages. The percentage reduction in PRRwas larger in younger zoea (87% zoea I, 47% megalopa). Also, zoea VIII oxyconformed with oxygen reduction, while megalopa showed a tendency to oxyregulate down to very low oxygen concentrations. The rate of nitrogen (protein) accumulation was first reduced in hypoxic conditions (compared to normoxic ones), and later arrested, suggesting a decrease in overall protein synthesis. In addition C:N ratio was higher in organisms sampled from hypoxia. This is consistent with larvae being able to survive for several days under hypoxia, but that moulting is largely precluded at this level of hypoxia. In addition, the aerobic potential in hypoxia (CS), decreased only after the half moult cycle, as did the maximum metabolic potential (MDH). Anaerobic/aerobic potential (LDH:CS) however was higher in organisms reared under hypoxia, scaling positively with age and dry weight. Overall, our obsevations indicate that the moderate capacity of zoea I to tolerate the low oxygen levels that characterize adult habitat, allow the species to colonize this habitats types, but they can not inhabit within the adult habitat environmental throughout development.

INTRODUCTION

Several areas at intermediate depths in the world's oceans are known as 'oxygen minimum layers', where the naturally occurring low oxygen level shapes the pelagic and benthic communities (Madhu et al. 2003; Gallardo et al. 2004; Levin and Atkinson 2004), restricting (above or below) the distribution of aerobic organisms incapable of coping with low oxygen tensions. These areas present stable low oxygen levels, temperature and salinity, and low diversity (Gallardo et al. 2004). The key to inhabit an OMZ permanently is the organisms' capacity to keep a constant level of oxygen consumption, whatever the mechanism (Childress and Seibel 1998). Nevertheless, anaerobic pathways might also compensate for oxygen deficiency to sustain energy supply when environmental oxygen concentration is lower than the critical tension at which organisms are capable of maintaining aerobic metabolism (Griesharber et al. 1994; González and Quiñones 2000; Levin and Atkinson 2004).

During their complex life cycle, adult benthic crustaceans inhabiting sediments bathed by the Equatorial Sub-Surface Waters (Central-South Chile, fig. 1), such as the squat lobster (Pleuroncodes monodon Milne Edwards, 1837) (Gallardo et al. 2004), liberate pelagic larvae in a hypoxic environment. Within the first days from hatching, the requirement for external source of energy (food) is paramount and the acquisition of a minimum energy reserve to survive to the following developmental stage must be achieved a sufficient time before moulting (Anger and Dawirs 1981). Weight specific respiration rates diminish with increasing size. In the few crustaceans studied, both respiratory regulation (Spicer and El-Gamal 1999) and anaerobic capacity develop through ontogeny. This suggests that low oxygen concentration could have very different consequences for the individual larval of a hatching than it does for the benthic adults that spawned them Yannicelli and Castro, (submitted) indicate that zoea I P. monodon are able to survive for several days under hypoxia but that moulting to the following stage is largely precluded. The aim of this work is to understand some metabolic features underlying such an observation.

So far, it has been shown in crustacean larvae, that oxygen deficiency limits biomass production (Coiro et al. 2000). However, some compensatory mechanisms, such as higher allocation to production per unit of assimilated food, have been found in juvenile crustaceans raised under hypoxia compared to those raised in normoxia (Rosas et al. 1998). Also, it has been

observed that juvenile crustaceans change their metabolic substrates when they are raised under hypoxia (Rosas et al. 1999). Respiration responses to decreasing oxygen tension have been widely analyzed and reviewed (Pörtner and Grieshaber 1993). Recent advances have shown that for some invertebrates oxyconformity and oxyregulation at the organism level, is accompanied by tissue, cell and mitochondria oxyconformity or oxyregulation respectively (Chandel et al. 1996; Tschischka et al. 2000; Buchner et al. 2001). Hochachka (1997) indicates that, besides immediate responses to hypoxia, cells can activate or silence batteries of genes to decrease the effects of long term periods of hypoxia. Organisms are not only capable of adjusting rates of ATP turnover, but also to recognize pathways of ATP demand and supply. For example, lactate dehydrogenase (LDH) is the most widespread enzyme involved in anaerobic pyruvate reduction to lactate across taxa. The synthesis of this enzyme in mammals, is activated during hypoxia by a mechanism that senses low oxygen levels. Suppressed loci can include mitochondria enzymes involved in oxygen dependent metabolism (e.g. citrato sintase, CS) (Hochachka 1997).

In the present paper we analyzed some short term and long-term responses of *P. monodon* larvae to hypoxia. In the short term, the respiration in low oxygen concentrations is compared with respiration under normoxic conditions both in fed and unfed organisms through several zoeal stages until megalopa. We also quantify the apparent specific activity of enzymes of intermediate metabolism (lactate dehydrogenase, LDH, citrate synthase CS and malate dehydrogenase MDH) and the body composition (carbon and nitrogen: CN) through the development of zoea I raised in normoxia (treatment I) and hypoxia (treatment II). Our purpose was to determine whether zoea I exposed to hypoxia during all its development is able to induce/suppress the maximum activity of the measured metabolic enzymes, which could indicate enhancement/suppression of metabolic pathways under the effect of oxygen concentration. In addition we evaluated if the potential changes is substrates metabolized could be detected in organisms' body composition.

METHODS

Female collection and maintenance

Ovigerous females where collected by trawling at depths of 100 to 150m during austral spring in the South Central Chile continental shelf. Ovigerous females were brought alive to the Marine

Biological Station of the University of Concepción in Dichato where experiments were carried out. Females were placed in large containers in constant salinity sea water (33+/-1) and temperature (11ºC), and complete darkness. Water was permanently aerated and exchanged every two days. When the eggs where in advanced developmental stage, females where placed in individual containers, without food, from where larvae were picked with pipettes to be distributed between different experimental conditions.

Larval rearing

Larval rearing was conducted in a controlled temperature room at 11ºC (+/- 1ºC), with filtered (1 µm +UV filters) constant salinity sea water (33+/-1), and photoperiod 12:12. The chosen temperature and salinity are similar to the ambient where ovigerous females liberate larvae.

Larvae from three different females were reared in two different oxygen levels. Under normoxia, they were massed reared in 2 l containers. Water was exchanged every two days, and freshly hatch artemia nauplii were added daily. Those in hypoxic conditions were raised in 1 l Schott bottles. Water was bubbled with N_2 in large containers, when the desired oxygen concentration (between 1 and 0.7 $ml \cdot l^{-1}$) was reached, bottles were filled with a silicone hose without bubbling, oxygen concentration was checked for each bottle, 300 larvae were introduced with pipette under the water surface, and bottles were sealed with Parafilm leaving no air bubbles between the water and the Parafilm. Oxygen concentration was also checked daily in each bottle at the time of water exchange, so we recorded daily initial and final oxygen concentration. In case the Parafilm might allow a certain degree of gas exchange, respiration compensated possible oxygen enrichment of the low oxygen bottles. Table 1 shows the mean, maximum and lowest oxygen level reached in each of the containers for the hypoxic bottles.

From the two different rearing conditions, two groups of 15 larvae were removed at different time intervals within zoea 1 stage (and under normoxia in later stages also). Two samples from each brood were taken from either of the two containers maintained for each brood. Larvae were rinsed in distilled water on a sieve, dried in tissue paper and frozen in liquid nitrogen. One of the samples was assigned to carbon (C) and nitrogen (N) content determination; the other was destined to enzymatic determinations. Dry weight (dw) was determined for all organisms analyzed for enzymes, but it was not possible to analyze all samples for carbon and nitrogen (CN). Samples used for CN determinations were freeze dried prior to analysis, and the

determinations were conducted in a Thermo Finnigan Flash EA 1233, and weights determined with a Thermo Cahn balance with a precision of 0.1µg. The standard used for elemental composition determination was acetanilida (71.09 %C y 10.36 %N).

Respiration experiments

All respiration measurements were conducted at 11ºC. Measurements were conducted with a TH connected to a mixrox TX3 oximeter introduced through a needle and without agitation into plastic syringe (closed system) where larvae were kept. For zoeas I to V determinations were conducted in a volume of 3.3 ml. For megalopas the volume used was 5.7 ml. Stage I, II, III and IV were measured either individually or in couples, while later stages were measured individually. Water for both incubation and acclimation was filtered through 0.5 µm mesh plus UV irradiation. Immediately prior to measurement, water was re-filtered through Whatmann 0.22 µm filters. No background respiration was detected.

We measured respiration under four different conditions: a) normoxia and starved for 24 h, b) normoxia and fed for 12 h, c) hypoxia and starved for 24 h, d) hypoxia and fed for 12 h. Four replicate measurements were conducted in each condition, and stages I to megalopa (a total of 96 measurements). The same group of individuals within a stage was used for the four treatments.

Organisms were placed in acclimation containers in a controlled temperature bath (11°C) for 24 h without food. Organisms were transferred to the experimental syringe filled with high oxygen for an hour before recording oxygen consumption. After measurement, they were placed in a second syringe filled with low oxygen water and were acclimatized for one hour before measurements were conducted. Low oxygen condition was 1.3 ± 0.3 mg·l^{-1}. In order to check if there was an aerobic scope due to dynamic action at low oxygen levels for larval *Pleuroncodes monodon,* organisms were transferred back to the acclimation container, and they were fed with artemia nauplii for 12 h. After this period of time, the recording of oxygen consumption proceeded as described above.

In addition, three long experiments were conducted with non fed organisms, two for zoea VIII and one for megalopa. These experiments started from normoxic condition and the organisms were allowed to consume all available oxygen. Data from zoea VIII and megalopa were used to construct oxygen consumption vs oxygen concentration curves and determine the

degree of oxyconformity in each stage. Following the procedure used by Tankerley and Weiver (2000) and Van Winkle and Mangum (1975) we adjusted a typical quadratic polynomial model to the data $y = a + bx + cx^2$, where *y* is the respiration rate (in mg $O_2 \times h^{-1} \times individual^{-1}$). The coefficient of the quadratic term (c) is an index of the shape of the response curve: coefficients equal to 0 indicate oxyconformity; coefficients larger than 0 indicate oxyregulation, although clearly respiration does not decrease at higher oxygen levels.

Respiration rates were expressed as mg $O_2 \times h^{-1} \times individual^{-1}$, and also as mg $O_2 \times h^{-1} \times$ mg dw^{-1}. First stages could not be weighted individually after dry freezing, therefore we used the mean weight at stage: for zoeas I and II we used groups of 15 individuals; for later stages we pooled the organisms used in the experiment to weight them. In the case of megalopas, individual dry weight was possible. Therefore, since we were mostly working with mean weight at stage, we calculated a mean weight specific respiration rate for each stage.

Enzymatic assays

Each enzyme apparent specific activity was determined in triplicates (LDH, MDH) and duplicates (CS) for each group of 15 larvae. Larvae were weighted (wet weight) and homogenized with an Ultra-Turrax in a homogenate buffer at pH 7.9. The homogenate was centrifuged at 3000 rpm for 10 minutes at 4ºC, and the supernatant was added to the reaction mixture to measure kinetically the enzymatic activity. The NADH decay was measured at 340 nm to calculate LDH and MDH activity (González, 2002). For CS activity, the increase in absorbance was followed at 412 nm. All measurements were conducted at an ambient temperature of 20ºC. Previous trials indicate that both LDH and MDH activity in *Pleuroncodes monodon* do not vary significantly from 11 to 20ºC (González, 2002). All determinations were conducted based on methods modified and described in detail by González (2002).

LDH assay was conducted on a mixture of K_2HPO_4 buffer (80mM), NADH (0.2 mM) and pyruvate (3.2 mM), pH 7.9. MDH determinations used a mixture of K_2HPO_4 buffer (80mM), NADH (0.2 mM), $MgCl_2$ 1.5 mM, and oxalacetate 0.2 mM, pH 7.9. Also, the inespecific NADH oxidation was determined as a blank.

For CS, we used Imidazol (50mM), $MgSO_4$ (1.5 mM), 5.5 dithiobis (2-nitrobenzoic) acid DTNB (0.1mM), oxalacetate (0.2mM) and Acetyl CoA (0.06mM). All measurements were conducted in parallel with a blank containing supernatant but without oxalacetate.

Potential enzymatic activities expressed as apparent specific activity, have units of μmol min^{-1} (UI), standardized per g of dry weight or mg of protein. Dry weight was determined in parallel for each sample. Protein was calculated using N values obtained from elemental composition analysis and the equation for anomuran larvae:

$$Log(P) = 0.48 + 1.076 \cdot log(N)$$

from Anger and Harms (1990), in which P are proteins in mg and N is nitrogen in mg. Standardizations with protein content were conducted only when CHN had been determined for the paired sample.

Data analysis

The effect of age (factor 1) and oxygen rearing level (factor 2) on mean carbon and nitrogen content (μg) and C:N ratio were analyzed with a two way ANOVA orthogonal design.

We also analyzed the mean individual respiration rates for each zoea stage with a two way ANOVA. Factors were: i) oxygen level (high or low) and ii) feeding conditions (fed or unfed). A post hoc LSD test was also conducted afterward to check for specific effects (Sokal and Rholf 1995). Also, two curves of weight specific respiration rate (*wsRR*) of unfed organisms and individual dry weight (*idw*) were obtained according to the equation:

$$wsRR = a \times idw^{b}$$

for measurements conducted in i) normoxia, and ii) hypoxia.

A lineal model with separate slope design (Statistica 6.1), was used to statistically analyze the maximum apparent specific activity of the three measured enzymes (CS, LDH, and MDH), as a function of oxygen level (OL, categorical predictor) and age/individual dry weight (continuous predictor). Oxygen had two levels: high and low. Since a log/log relationship has been described in the literature for enzymatic activities standardized by individual dry weight as a function of individual dry weight, logarithmically transformed data was used in the relationships activity/weight. Also, the logarithmically transformed LDH and MDH activity was used against age to homogenize the variance. Analysis were performed for enzymatic activities

(UI) standardized by individual dry weight (g) and also UI per individual protein content (mg). The same design was used to analyze the relationship between LDH and MDH activity (as dependent and independent continuous variables respectively) and OL. Also, the ratio LDH:CS activity was calculated (using measurements standardized by dry weight). The separate slope design was used again to analyze the dependence of log (LDH:CS ratio) on age and OL, and LDH:CS ratio dependence on individual dry weight and OL.

RESULTS

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As expected, carbon and nitrogen content increased with age in both high and low oxygen reared larvae. However, a small decrease in both elements was detected in pre-molt individuals (~ 17 days old) under the two oxygen experimental conditions. Both carbon and nitrogen contents were higher in individuals reared in normoxia (Fig. 1 a and b) (Table 2). Carbon conformed a 31 to 35% of organisms dry weight, and nitrogen between 6 and 9 %. C:N ratio during zoea I development under normoxia followed a bell shape curve (Fig 1 c), with maximum between 6 and 9 days old larvae and later decrease until molting. The ratio augemented again in recently molted zoea II (the 23 days old larvae have molted to zoea II in normaxia but not in hypoxia). C:N ratio in organisms reared under hypoxia also showed a bell shape curve and in pre-molt individuals a sharp C:N drop was observed (Fig 1 c). The ratio at age, was significantly higher in normoxia reared organisms than in the ones reared in hypoxia (Fig 1c and Table 2). An exception occurred when pre-molt was reached by organisms in normoxia but not by organisms under hypoxia. The C:N ratio fluctuated above 4 (Fig. 1c).

Respiration

Individual respiration rates (IRR) were significantly ($p < 0.05$) lower in low oxygen levels (Fig. 2a, table 3). Particularlly, , both fed or unfed zoeas II, III, VIII and megalopas under hypoxia had a lower mean respiration rate than organisms in normoxia. Fed zoea I (hypoxia), IV (normoxia and hypoxia) and megalopa (hypoxia), showed significantly higher IRR than the unfed organism in the same oxygen level (Table 3: LSD). That tendency was repeated in almost

all cases, although differences were not significant. There were no significant interaction between the factors oxygen level and feeding condition. The IRR reduction detected from normoxia to hypoxia had a maximum in zoea I (87%) and a minimum in megalopas (47%).

IRR increased in succesive larval stages but, as expected, weight specific respiration rate (wsRR), decreased with increasing dry weight (Fig. 2b). An exception occurs between zoea I and II, because zoea I had a lower wsRR than zoea II. A higher significant slope for the relationship between wsRR and individual dry weight was found for organisms measured in normoxic waters (b_{norm}= -0.66; b_{hyp}= -0.52), excluding zoea I.

Pooled data for Zoea VIII indicated that at this stage organisms tend to oxyconform (Fig. 3 a) since oxygen consumption decreases with decreasing oxygen tension without showing a plateau at higher oxygen levels. The coeficient of the quadratic term in the polynomial equation that related oxygen consumption and oxygen concentration (c) was not significantly different from 0. In megalopas, c was significantly higher than 0. There was a tendency to regulate oxygen consumption to lower oxygen concentrations although we did not attempted to calculate a critical point.

Enzyme activity

CS aparent specific activity ($UI \cdot gdw^{-1}$) decreased with age only for organisms reared in low oxygen (Fig. 4a, Table 4). When the log(CS) was analyzed as a function of dry weight, only organisms reared in hypoxia showed decreasing activities with increasing body weight (Fig 4b). The same pattern was observed when CS activity was standardized per mg of protein (Fig 4 c and d). No relationship of CS activity with age or body weight was found for organisms raised in normoxia (Fig. 4, table 4).

Only in organisms reared in normoxia, the LDH aparent specific activity (standardized by g of dw or mg of protein) augmented with age and individual dry weight (Fig. 5a to d). In addition, the overall LDH activity per mg of protein was significantly higher in organisms raised in hypoxia than in those raised in normoxia (Fig. 5c, Table 4).

MDH apparent specific activity had a very similar pattern to LDH. MDH activities standardized by g of dry weight or mg of protein, increased with age and individual dry weight only in high oxygen reared organisms (Fig. 5e to h, Table 4) . No significant trend was found for

individuals raised in low oxygen. In addition, significant differences between overall activity in the two groups of individuals were found: MDH activity was higher in normoxia reared organisms regardless of standarization units (Table 4).

LDH and MDH aparent specific activities showed a significant positive correlation for organisms raised in either oxygen level but the differences between groups were not significant (Fig. 6a and b, table 4). MDH:LDH ratio in hypoxic conditions was independent from age and individual dry weight (Fig. 7a and b, table 4). MDH/LDH ratio in normoxic conditions was positively correlated with age (Fig. 7a, table 4). LDH:CS ratio increased significantly with age (logarythmically) and individual dry weight (linearly) for organisms reared in hypoxia, but not for those raised in normoxia (Fig. 8a and b, table 4). During the first half of the molt cycle the ratio was indistinguishable from hypoxia and normoxia reared organisms, in older larvae it became higher in those coming from hypoxic conditions.

DISCUSION

In this study we showed that both the amount and rate of accumulation of carbon and nitrogen were lower in organisms raised under hypoxia than in organisms raised in normoxia. This result is consistent with previous findings, since the growth rate of zoea I *P.monodon* measured as individual dry weight was lower in hypoxia (Yannicelli and Castro submitted), and the further viability of larvae was also greatly reduced in this condition.

Mean percentages of C and N content as well as mean C:N values reported in this study are very similar to those reported in the literature for crustacean larvae (Anger 1988; 1989; 1998; among others). The increase of C:N ratio during early development of zoea I, and its later decrease until molting in normoxia is also consistent with patterns found in brachyuran larvae (Anger 1998). Such a pattern has been interpreted as a higher accumulation rate of lipids at the begginning of the moult cycle and a later shift to protein accumulation (Anger 1988). However, among the organisms raised in hypoxia, only those recently molted to zoea II showed a decrease in C:N ratio. The build up of tissue with high proportion of protein during the second half of the molt cycle in hypoxia is therefore unlikely. In fact, no further accumulation of either nitrogen or carbon were found beyond larvae aged 12 days in hypoxia. Later fluctuations in C:N ratio (12-16 d) could be due to differential loss rather than gain of tissue proteins. Under hypoxic conditions

organisms might rely on protein catabolism for energy production, reducing the accumulated proportion of nitrogen (Fig. 1). In *Crassostrea gigas* larvae, molting sucess was not related to the pre-molt accumulation of lipids, but a high catabolism of proteins was infered from O/N ratios during metamorphosis (García-Esquivel et al. 2001). A closer look to the protein requirements prior to molting in crustacean larvae should be undertaken in order to elucidate the mechanisms behind moulting failure in hypoxia reared organisms such as *P. monodon* which shows certain capacity to cope with low oxygen conditions.

Respiration

The respiration experiments determined the routine metabolic rate (unfed organisms) at high and low oxygen levels. Those experiments conducted with fed organisms assessed whether larvae conserved an aerobic scope at the low oxygen levels utilized, although we were not able to quantify it precisely. With this analysis we aimed to determined whether *P. monodon* larvae at oxygen levels that characterize adult habitat would still have the capacity to increase their oxygen consumption over routine levels as a response to food availability (post-prandial respiration), identifying that they were subject to oxygen tensions above the critical point. The concept of a critical point in organisms respiration as a function of ambient oxygent was introduced to characterize oxygen tensions below which an oxyregulating animal was no longer able to maintain its rate of oxygen consumtion independent of ambient oxygen tension, but showed a decreasing oxygen uptake (Pörtner and Griesharber 1993). But this critical point, also characterizes the onset of an anaerobic energy metabolism and results from a failure of oxygen provision to mitochondria. In that sense a critical point can also be found in both oxyregulating and oxyconforming organisms (Pörtner and Griesharber 1993). The metabolism of oxyregulating animals show an obligatory high rate of energy consumption even in hypoxia (e.g. adult crustaceans like *Pleuroncodes planipes,* Quentin and Childress 1976), while in oxyconformers, the energy demands decrease with decreasing oxygen availability (Boutilier 1001). The reduction of oxygen consumption as a function of oxygen level (below the critical point) in oxyconformers does not necesarily reflect a systemic failure to provide enough oxygen to the cells, since oxygen consumption can be increased during feeding and activity (Rosas et al, 1998; Leferancois and Claireaux 2003). Not only routine respiration rates are oxygen dependent but also the maximum

oxygen consumption, and therefore the aerobic metabolic scope (Leferancois and Claireaux 2003).

Our results showed that larval *Pleuroncodes monodon* through its different stages until megalopa respire less at oxygen levels of about 1.3 $mg \cdot l^{-1}$ than they do in normoxia, although oxygen supply was not limiting since they have a positive aerobic scope, as shown by the higher respiration after feeding. In all cases, the respiration rate in low oxygen (fed and unfed) was lower than in normoxia. This is consistent with the fact that production (carbon and nitrogen acumulation in zoea I) is lower in hypoxia reared organisms. Previous studies have indicated that *P. monodon* zoea I is able to survive prolongued periods in hypoxic waters, but that survival to the following stage is greatly reduced (Yannicelli and Castro submitted). Such a pattern might be a consequence of low capacity to build up reserved at this level of hypoxia. These two results point out that oxygen levels found in the natural environment where larvae are hatched, allow the maintenance of aerobic metabolism, and new tissue production, although at a lower rate than normoxic conditions. At a cellular level two major processess are responsible for the standard energy consumption: protein synthesis and ion-motive ATPases. The decrease in the demand of this two processess are thought to be responsible for downregulating the energy turnover that should be brought about by oxygen reduction (Boutilier 2001; Wu 2002). Complex cellular signaling translate low oxygen concentrations into biochemical responses (Chandel and Schumacker 2000). The short term response of *P. monodon* larval respiration to surounding oxygen tension, plus growth data (dry weight, carbon, nitrogen and C:N evolution during zoea I development), indicate that cellular metabolism is reduced to withstand adult habitat oxygen tensions. It seems that larvae are not able to build up enough reserves to moult succesfully under the tested oxygen levels, although they can withstand them.

The slope of the relationship between the weight specific routine respiration rate and individual dry weight had a higher absolute value for oxygen consumption measurements in normoxia than in hypoxia. Therefore, the decrease in respiration rate from hypoxia to the level of hypoxia analyzed was proportionally larger in young zoea. Consistently, it has been shown in other crustaceans with indirect development, that oxygen uptake and regulatory capacity develops during ontogeny (e.g. Spicer 1995; Spicer and Stroemberg 2003, Terwillinger and Ryan 2001). Towards the megalopa stage *P. monodon* start migrating from the surface to the bottom low oxygen waters (Cañete 1994; Yannicelli et al. in prep), coinsidently with their increase in

oxyregulatory capacity. A shift in oxygen regulatory capacity might be expected during the transition from pelagic to benthic habitat although prolongued environmental low oxygen levels might bring regualtion forward (Spicer and Eriksson 2003) or enhance oxygen delivery through changes in hemocyanine oxygen afinity (Mangum 1997). While the maximum metabolic scope in adult individuals might be related to maximum active metabolism, in young zoea it can be related to post-prandial metabolism (Goolish 1991), consequently the effects of reduced oxygen tensions on young zoea energy allocation to growth should be more severe than in megalopas.

Enzymatic activity

The present study shows that the relationship between enzymatic activities (expressed as aparent specific activities) and age/dry weight, differ between individuals reared in normoxia or hypoxia.

During the development of *P. monodon* zoea I reared in normoxia, CS activity remained constant: neither age, nor dry weight correlated significantly with CS activity. However, a negative trend in CS activity with incrasing age and dry weight was found in organisms raised under hypoxia. In this case, there are at least two possible interpretation of our results. First, the decrease of CS activity is as a downregulation response to ambient oxygen level as the period of hypoxia exposure augments. Second, CS activity decrease with age indicates a poor physiological condition. Both interpretations are based on CS metabolic role. It is a mitochondrial enzyme that plays a rate limiting role in the cytric acid cycle (Lehninger 1875) it has been used as indicative of aerobic potential (Somero and Childress 1980). Some studies have shown that CS activity follow the same trend that oxygen consumption in normoxic conditions (e.g. Sukhotin and Pörtner 2001), and that the correlation holds through ontogeny of crustacean larvae, decreasing with age (Lemos et al. 2003). It is therefore among the enzymes prone to be downregulated during periods of prolonged hypoxia. The response of enzyme activities to ambient stressors vary from hours to days (Geiger 1999). So In this case, over 9 days of exposure should be needed to measure effects on maximum aerobic capacity. Cooper et al. (2002) found no changes on CS activity for estuarine fish exposed during 12 hr to hypoxia. CS has also been used as a condition index since it has been positively correlated with growth rates (Clarke et al. 1992). Concordantly, carbon and nitrogen accumulation during hypoxia were lower than under normoxia in *P. monodon* zoea I. A second explanation, is that CS is not being downregulated by

an oxygen dependent mechanism, but that its activity decreased with age indicates a poor physiological condition. The maintenance of a high aerobic potential in spite of low environmental oxygen concentrations should be specially relevant in *P. monodon* zoea I, since they are hatched in a low oxygen environment, but later zoea are found in more oxygenated waters. Both active behavior plus the upwelling circulation at their hatching areas should bring zoea I towards more surface and oxygenated waters, where maintaining a high aerobic potential for growth and development should be advantageous. Yannicelli and Castro (submitted) showed that mortality of *P. monodon* zoea I was largely increased in hypoxic rearing conditions, and that weight increment became neglegible in surviving larvae after mid-zoea I development. Therefore it is likely that an overall inhability to store carbon and nitrogen under hypoxia undeerlies growth and CS activity diminishment in older zoea I rather than metabolic downregulation of this metabolic pathway.

LDH is by far the most studied enzyme from the anaerobic glycolitic metabolic pathway. It has been repeatedly used as indicator of anaerobic potential, and it should be specially so in crustaceans, where this is the main (or only) enzyme of the glycolitic pathway (Griesharber et al. 1994). LDH activity was higher in *P. monodon* larvae compred to other crustacean larvae: for zoea I, Sastry and Ellington (1978) reported a mean of 0.025UmgP in *Cancer irroratus*, while Marsh et al. (2001) reported 0.05-0.1 UmgP in *Hemigrapsus sanguineus*. Our values ranged from 0.1UmgP to 0.35. High LDH activities have been reported in organisms that inhabit or intrude into the oxygen minimum layer (Yang et al. 1992; González and Quiñones 2002). Flint et al. (1991) showed that LDH activity was undetectable in surface copepods but was rather high in copepods that agregated in low oxygen oceanic layers.

In crustaceans not only is the ontogeny of enzyme expresion scarcely studied but few studies have addressed the specific effect of oxygen rearing conditions on metabolic enzyme activities within this group. A positive scaling in anaerobic potential has been analyzed in several vertebrates and invertebrates (Somero and Childress 1980; Childress and Somero 1990; Overnell and Batty 2000; González 2002). Our results show that within zoea I, LDH (as well as MDH) weight specific and protein specific activities increased with age and dry weight as expected when larvae were reared in normoxia, but in hypoxia the relationship is lost: we found a high variability and higher activities at lower dry weights. When LDH activity was standardized per mg of protein, hypoxia reared organisms showed a higher activity than normoxia reared ones as a

function of age. In this case, LDH activity was estimulated at low oxygen levels, and it became evident during the first days of exposure. The glycolitic enzyme genes are known to be regulated by hypoxia from bacteria to higher metazoan, in the later, regulation is mediated by an hypoxia inducible factor (Webster 2003). The LDH activity curve as a function of age, showed a bell shape, decreasing towards the last days of zoea I. As previously explained for CS, LDH has also being correlated with physiological condition indices, so a two step process could be taking place. Initially, during the first days of hypoxia an enhancement of LDH maximum activity during hypoxia could increase the ability of larvae to undertake swimming excursions out of the oxygen minimum layer, however, passed a thereshole time limit, after which most larvae are mainly bound to die, LDH activity, as CS activity, weight and overall condition decrease. It is relevant to point out that during the first stages of planktotrophic larvae, maintenance is not enough for survival, but the adquisition of reserves to undergo moulting is required (Anger 2001). In crustaceans (Mugnier and Soyez 2005) as well as in fish larvae (Ishibashi et al. 2005), respiration rates increase prior to moulting and tolerance to hypoxia diminishes. Higher metabolic demands acompany this period, which organisms might not be able to met at this levels of hypoxia.

In spite that LDH has also being related to growth rates (e.g. Geiger 1999 among others), when oxygen concentrations is playing a role in its activity modulation, such tendencies might not apply. The large variability of LDH activity as a function of dry weight might be related to the fact that in hypoxia, weight did not increase monotonically with age. In addition individual metabolic enzyme activities have been shown to be highly variable (Marsh et al. 2001). In addition to provide energy anaerobically for swimming excursions, LDH end product, lactate, also enhances O_2 blood affinity in crustaceans during severe hypoxia (Mangum 1997), and might signal behavioral search for colder waters (De Wachter et al. 1997).

MDH has been used as an overall indicator of metabolic potential (e.g. Geiger 1999; González 2002) since it participates in both mitochondrial and cytosolic cycles, and the transport of NAD^+ and oxaloacetato through the mitochondrial membrane (Lehninger 1975). MDH activity clearly support the previous conclusions. There was a positive correlation of weight specific and protein specific MDH activity with age and dry weight for organisms raised in normoxia, but such a relationship was not found in organisms raised under hypoxia. In addition, mean values were higher in normoxia, indicating a higher metabolic potential in this condition

The younger larvae, apparently had a higher MDH activity under hypoxia, but the situation is reversed towards the second half of the moulting cycle, consistently with the idea of an empoverished condition towards the end of zoea I. MDH showed a positive correlation with LDH activity for both oxygen levels. Although we could not differentiate both equations, the slope is higher for low oxygen reared organisms, which means larger increases in LDH for the same amount of MDH increase.

A notable effect of hypoxia on enzyme activities is depicited by the ratio of LDH/CS activity, which was positively correlated with dry weight and age in organisms raised in hypoxia. The anaerobic to aerobic potential was enhanced through zoea I development as a consequence of low oxygen exposure.

In summary, *P. monodon* zoea I seems to rely mainly in aerobic energy production even during hypoxia, when routine and post-prandial aerobic respiration are reduced below levels attained in normoxia. The rate of protein accumulation is first reduced in hypoxic conditions, and later arrested, suggesting a decrease in overall protein synthesis. This is consistent with the fact that larvae are able to survive for several days under hypoxia, but that moulting is largely precluded at this level of hypoxia. In addition, the aerobic potential in hypoxia (CS), decreased only after the half moult cycle, as did the maximum metabolic potential (MDH). Anaerobic/aerobic potential however became higher in organisms reared under hypoxia. This effects should affect young zoea more than megalopa when oxygen compensation appears. Overall, our obsevations agree with the idea that releasing larvae during upwelling periods in the Central Chile continental shelf, can increase the survival of early zoea by enhancing their coastal and upward transport, to more oxygenated waters. The moderate capacity of zoea I to tolerate the low oxygen levels that characterize adult habitat, should be enough as to ensure the colonization of this habitats types, but not to live in them throughout larval development.

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Table 1. Oxygen rearing conditions for larvae in low oxygen water. Mean concentration during the experiment, DMV: daily mean variation, maximum and minimum values measured.

Female	Mean concentration ($ml \cdot L^{-1}$)	DMV	Max	Min
18	0.79	0.16	1.63	0.53
18	1.01	0.16	1.36	0.79
20	0.77	0.12	0.94	0.57
20	0.99	0.22	1.29	0.71
21	0.70	0.15	1.00	0.50
21	0.97	0.22	1.50	0.57
23	0.62	0.17	1.36	0.29
23	0.95	0.23	1.21	0.64
24	1.06	0.15	1.43	0.86
24	0.76	0.18	1.00	0.64

Table 2 Results of two way ANOVA on carbon and nitrogen content and C:N ratio for factors: Age (6 levels) and rearing oxygen concentration (OL): normoxia and hypoxia.

	C:N		**Carbon**		**Nitrogen**	
	MS	F	MS	F	MS	F
Age	0.02	0.83	2×10^{-6}	6.04**	$<1\times10^{-6}$	4.85*
OL	0.27	10.7**	1.8×10^{-5}	55.2**	1×10^{-6}	63.33**
Age×OL	0.03	1.44	$<1\times10^{-6}$	1.28	$<1\times10^{-6}$	1.03

* $0.01<p<0.05$
** $p<0.01$

Table 3 Two-way anova on individual respiration rate. Factors were oxygen (OL) with levels high and low; and feeding (FL) with levels fed or unfed. Post-hoc test result: LSD.
lu: low oxygen unfed larvae
lf: low oxygen fed larvae
hu: high oxygen unfed larvae
hf:high oxygen fed larvae

Zoea	OL		FL		OL×FL		LSD
	MS	F	MS	F	MS	F	
I	2.97	20.87**	1.32	9.26**	0.36	2.56	lu<lf=hf=hu
II	0.46	8.09*	0.08	1.46	0.006	0.12	lu<hf
III	40.7	45.1**	0.22	2.41	0.008	0.088	lu=lf<hu=hf
IV	0.74	53.24**	0.01	1.02	0.06	4.7	lu=lf<hu=hf
VI	0.2	12.1**	0.71	42.4**	0.0001	0.007	lu<lf=hu<hf
VIII	0.79	38.1**	0.015	0.76	0.003	0.013	lu=lf<hu=hf
Megalopa	0.62	22.19**	0.034	1.36	0.095	3.4	lf<hu<hf lf=lu; lu=hu

* 0.01<p<0.05
** p<0.01

Table 4 Results of covariance analysis (single or separate slope design) of enzymatic activity dependency on rearing oxygen level (categorical predictor) and organisms age/dryweight/protein content (continuous predictors). OL: oxygen level; HO: high oxygen; LO: low oxygen; Idw: individual dry weight (g) HM: mean for organisms raised in high oxygen. LM: mean for organisms raised in low oxygen. Significant effects and significant parameters were determined at the 0.05 level.

	Dependent variable	Categorical predictor	Continuous predictor	Significant effects	Significant parameters		Means
					Factor	Estimate (β)	
CS	UI·gdw^{-1}	OL	Age	OL×Age	LO×age	-0.67	
	UI·ind^{-1}	OL	log (idw)	OL×log(idw)	HO×log(idw)	24	
	UI·mgP^{-1}	OL	Age	OL×Age	LO×age	-0.9	HM: -0.1
				OL	OL	0.9	
	log(UI·mgP^{-1})	OL	log (idw)	OL×log(idw)	LO×log(idw)	-35	LM: -0.12
				OL	OL	-39	
LDH	log(UI·gdw^{-1})	OL	Age	OL×Age	HO×Age	0.95	
	UI·ind^{-1})	OL	log (idw)	log(idw)	log(idw)	0.004	
	log(UI·mgP^{-1})	OL	Age	OL×Age	HO×Age	0.34	HM: -0.75
				OL	OL	0.4	
	log(UI·mgP^{-1})	OL	log (idw)	OL×log(idw)	HO×log(idw)	10.24	LM: -0.67
MDH	log(UI·gdw^{-1})	OL	Age	OL×Age	HO×Age	1.29	HM: 1.94
				OL	OL	-0.86	
	UI·ind^{-1})	OL	log (idw)	OL×log(idw)	HO×log(idw)	0.02	LM: 1.87
				OL	OL	0.07	
	log(UI·mgP^{-1})	OL	Age	OL×Age	HO×Age	1.24	HM: 0.46
				OL	OL	-1.11	
	log(UI·mgP^{-1})	OL	log (idw)	OL×log(idw)	HO×log(idw)	9.3	LM: 0.44
				OL	OL	12.07	
MDH	log(LDH) (UI·gdw^{-1})	OL	log(MDH) (UI·gdw^{-1})	OL×log(MDH)	HO×log(MDH)	1.02	
					LO×log(MDH)	1.05	
	log(LDH) (UI·mgP^{-1})	OL	log(MDH) (UI·mgP^{-1})	OL×log(MDH)	HO×log(MDH)	3.4	
					LO×log(MDH)	4.85	
MDH:LDH	MDH:LDH	OL	Age	OL×Age	HO×Age	0.99	
	MDH:LDH	OL	idw	n.s.			
LDH:CS	log(LDH:CS)	OL	Age	OL×Age	LO×Age	0.02	
	LDH:CS	OL	idw	OL× idw	LO×idw	0.2	

FIGURE LEGENDS

Fig. 1 Individual Nitrogen and Carbon content in *Pleuroncodes monodon* Zoea I (a,b), and C:N ratio (c) as a function of age. For carbon and nitrogen actual values are shown. For C:N ratio, mean and standard deviations were ploted. Dark squares: organisms raised under normoxia. Open symbols: organisms reared in hypoxia. Arrows in the lower pannel indicate pre-molt individuals from each treatment.

Fig. 2 Individual respiration rate for larval stages of *P. monodon*, measured in high and low oxygen, with individuals fed and unfed (a). (b) Weight specific respiration rate for each zoea stage as a function of individual dry weight. SMR: Standard metabolic rate; MR: metabolic rate.

Fig. 3 Individual respiration rate for *P. monodon* stage VIII (A) and Megalopa (B) as a function of oxygen concentration ($mg \cdot l^{-1}$) in unfed organisms.

Fig. 4 CS aparent specific activity as function of age and dry weight In organisms reared in normoxia (filled symbols) and in organisms reared in hypoxia (open symbols).

Fig. 5 LDH and MDH aparent specific activity as function of age and dry weight from organisms reared in normoxia (open symbols) and hypoxia (filled symbols).

Fig. 6 LDH aparent specific activity as a function of MDH aparent specific activity for UI values standardized per g of dry weight (a) and mg of protein (b) Symbols as in Fig. 5.

Fig. 7 MDH:LDH ratio as a function of age (a) and individual dry weight (b). Symbols as in Fig. 5.

Fig. 8 LDH:CS ratio as a function of age (a) and individual dry weight (b). Symbols as in Fig. 5.

Fig. 1

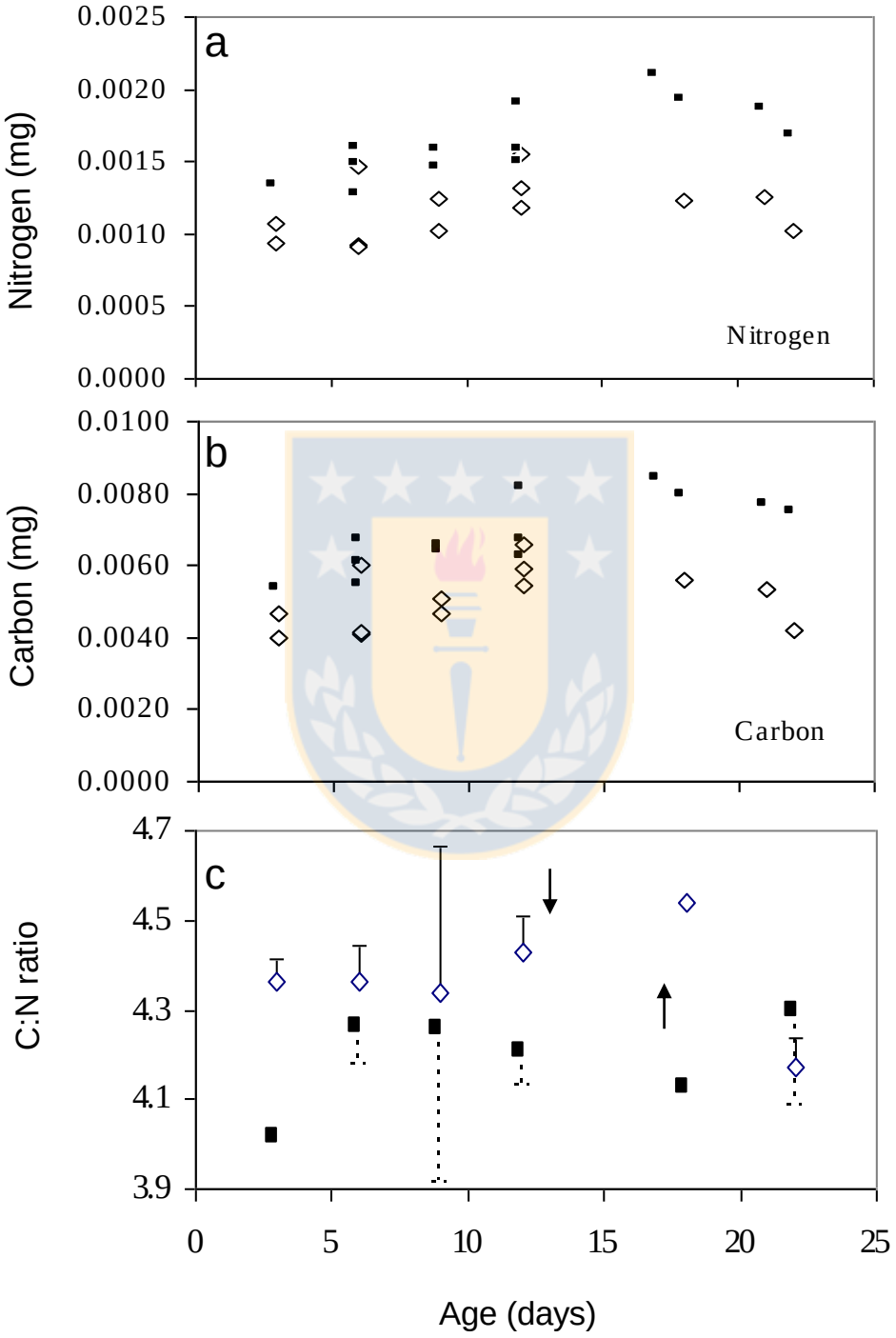

a
Nitrogen (mg)
0.0025
0.0020
0.0015
0.0010
0.0005
0.0000
Nitrogen
b
Carbon (mg)
0.0100
0.0080
0.0060
0.0040
0.0020
0.0000
Carbon
c
C:N ratio
4.7
4.5
4.3
4.1
3.9
0
5
10
15
20
25
Age (days)

Fig. 2

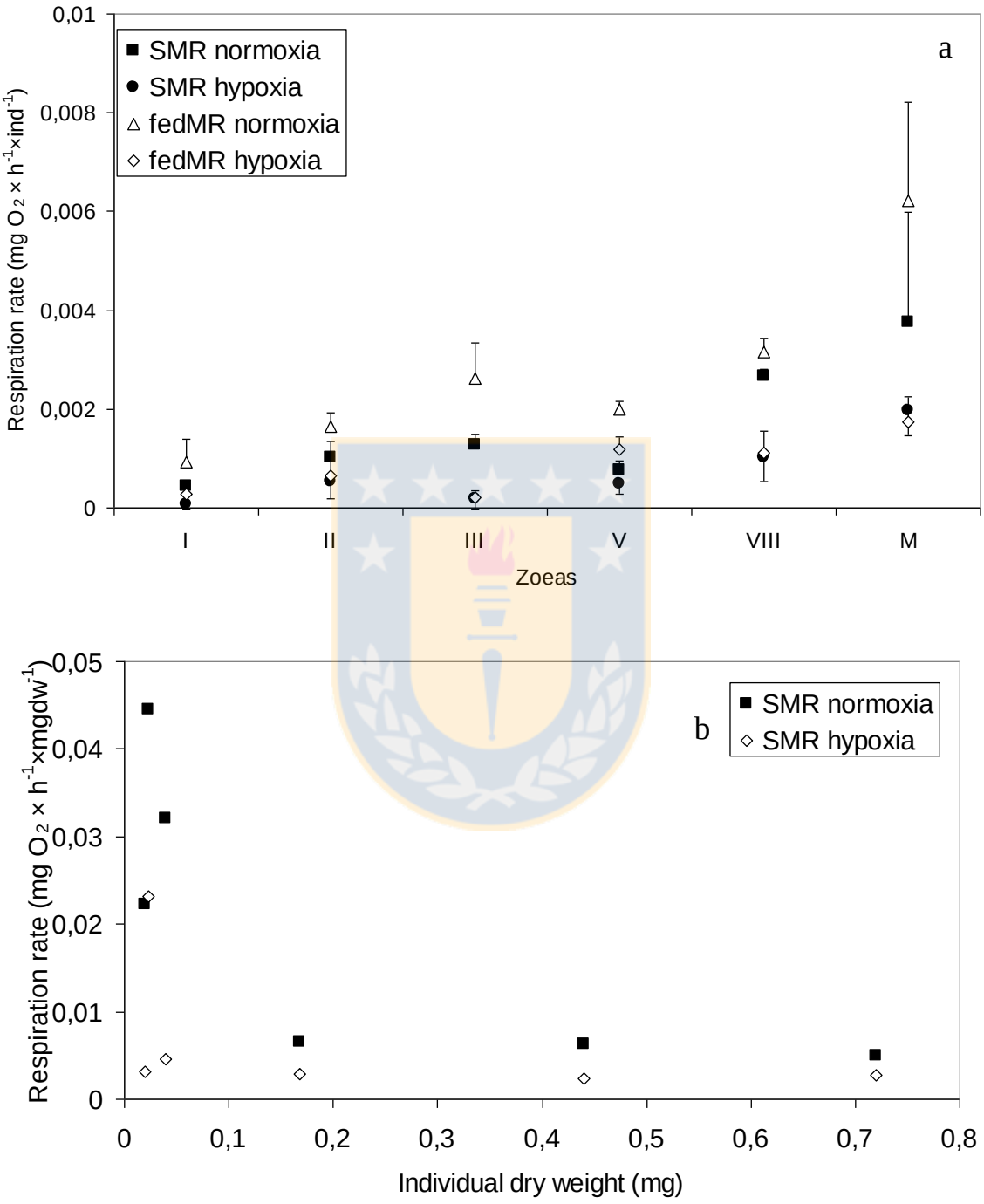

a
SMR normoxia
SMR hypoxia
fedMR normoxia
fedMR hypoxia
Respiration rate (mg O2 × h-1×ind-1)
0,01
0,008
0,006
0,004
0,002
0
I
II
III
V
VIII
M
Zoeas
b
SMR normoxia
SMR hypoxia
Respiration rate (mg O2 × h-1×mgdw-1)
0,05
0,04
0,03
0,02
0,01
0
0
0,1
0,2
0,3
0,4
0,5
0,6
0,7
0,8
Individual dry weight (mg)

Fig. 3.

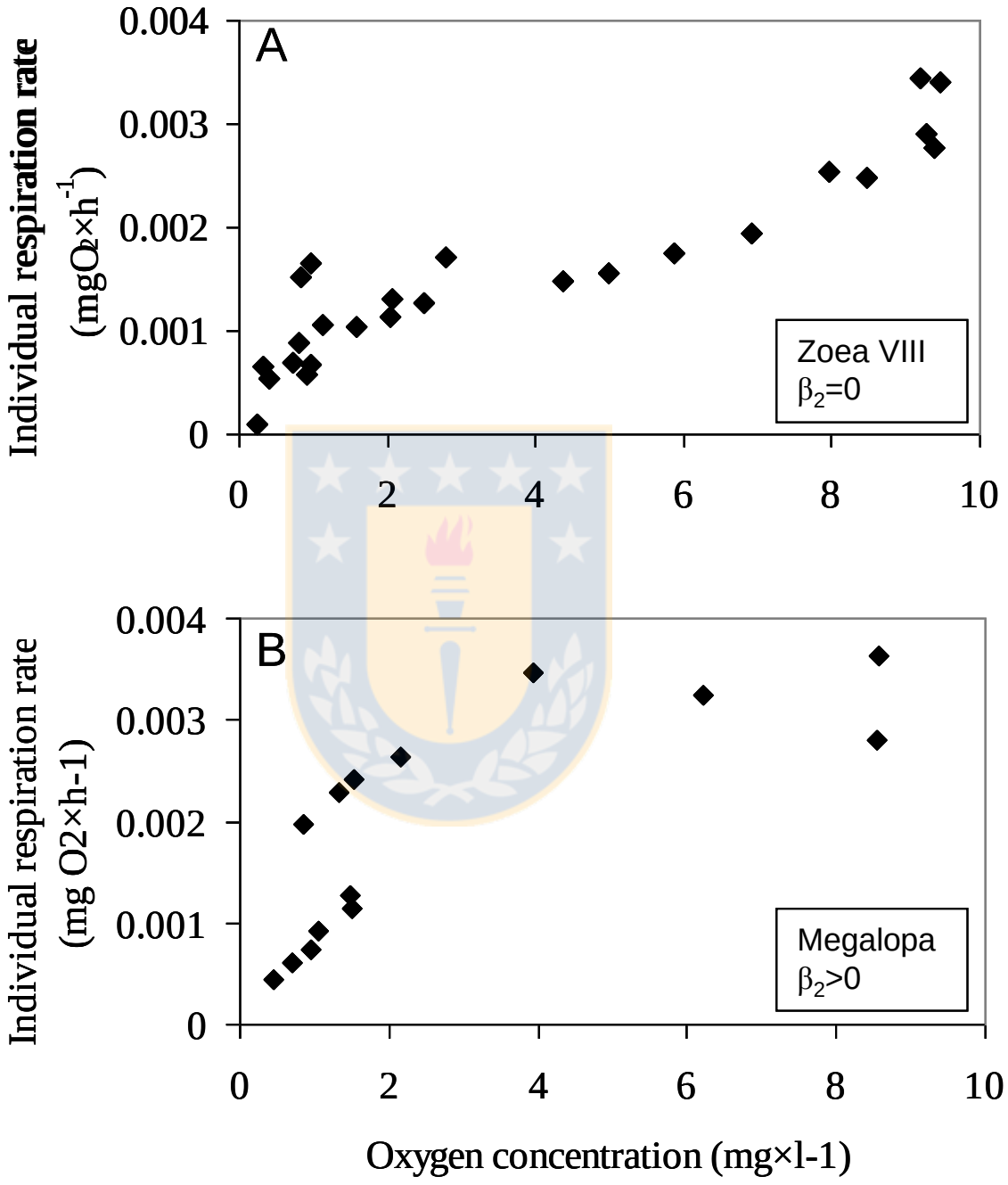

A
Individual respiration rate
(mgO2×h-1)
0.004
0.003
0.002
0.001
0
0
2
4
6
8
10
Zoea VIII
β2=0
B
Individual respiration rate
(mg O2×h-1)
0.004
0.003
0.002
0.001
0
0
2
4
6
8
10
Megalopa
β2>0
Oxygen concentration (mg×l-1)

Fig. 4

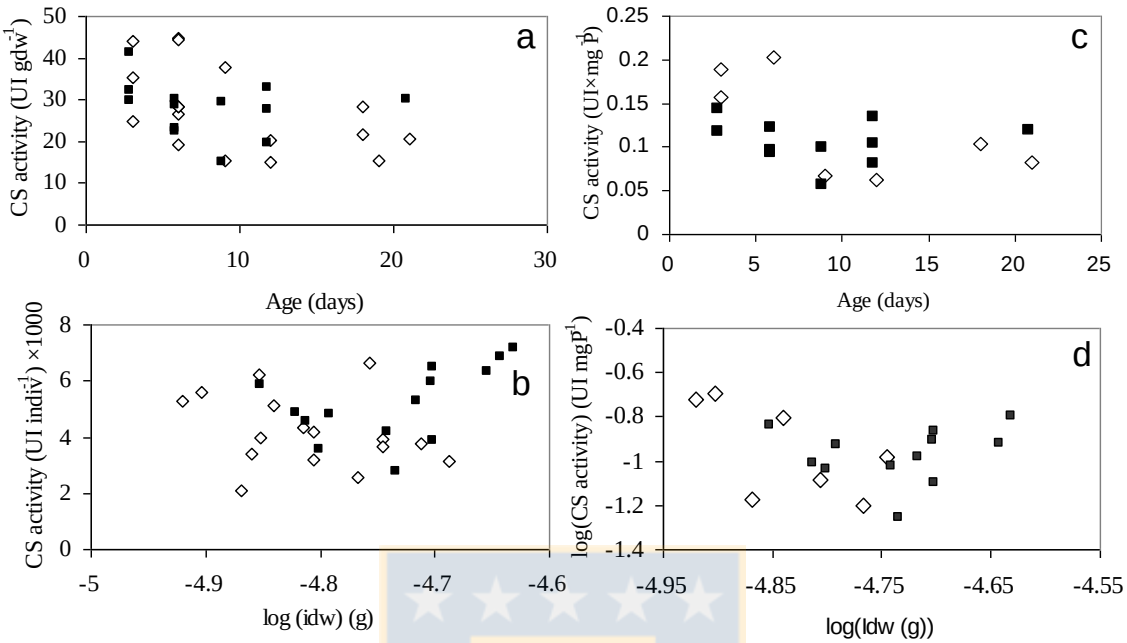

a
CS activity (UI gdw-1)
Age (days)
c
CS activity (UI×mg P-1)
Age (days)
b
CS activity (UI indiv-1) ×1000
log (idw) (g)
d
log(CS activity) (UI mgP-1)
log(ldw (g))

Fig. 5

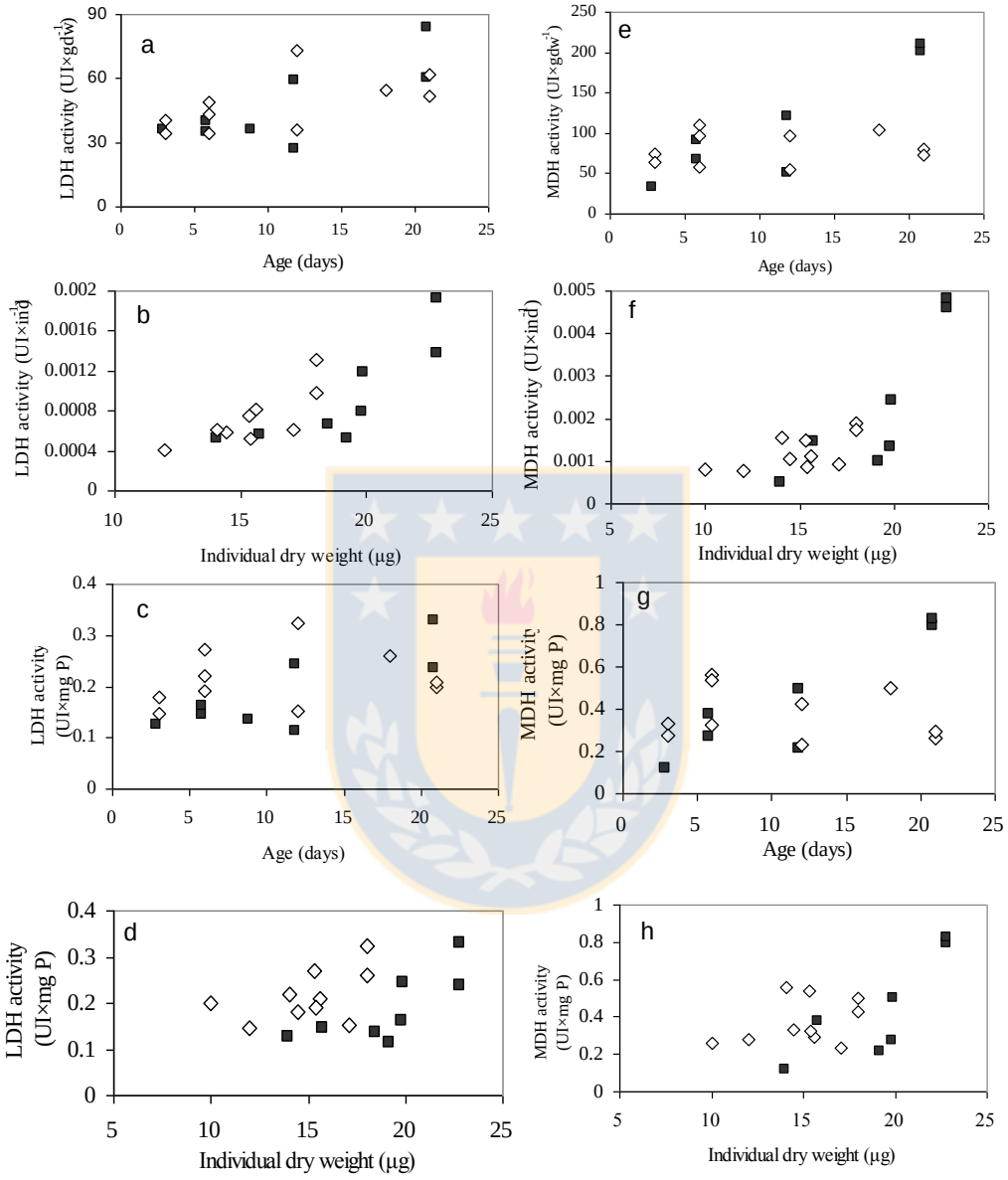
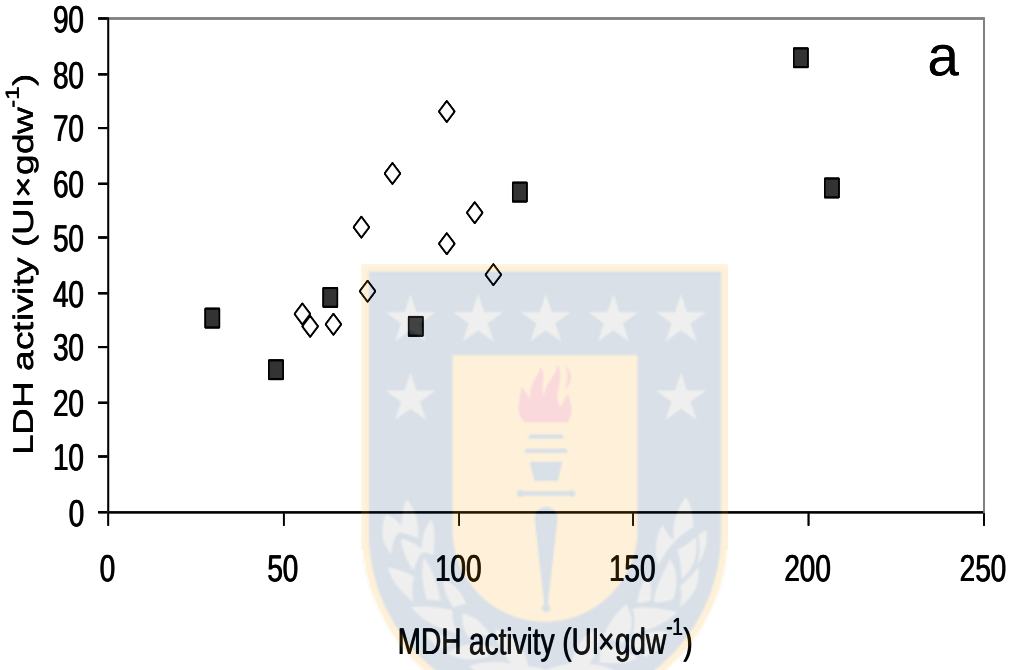

a
LDH activity (UI×gdw-1)
Age (days)
e
MDH activity (UI×gdw-1)
Age (days)
b
LDH activity (UI×ind-1)
Individual dry weight (µg)
f
MDH activity (UI×ind-1)
Individual dry weight (µg)
c
LDH activity (UI×mg P)
Age (days)
g
MDH activity (UI×mg P)
Age (days)
d
LDH activity (UI×mg P)
Individual dry weight (µg)
h
MDH activity (UI×mg P)
Individual dry weight (µg)

Fig. 6


a
LDH activity (UI×gdw-1)
MDH activity (UI×gdw-1)
0
10
20
30
40
50
60
70
80
90
0
50
100
150
200
250

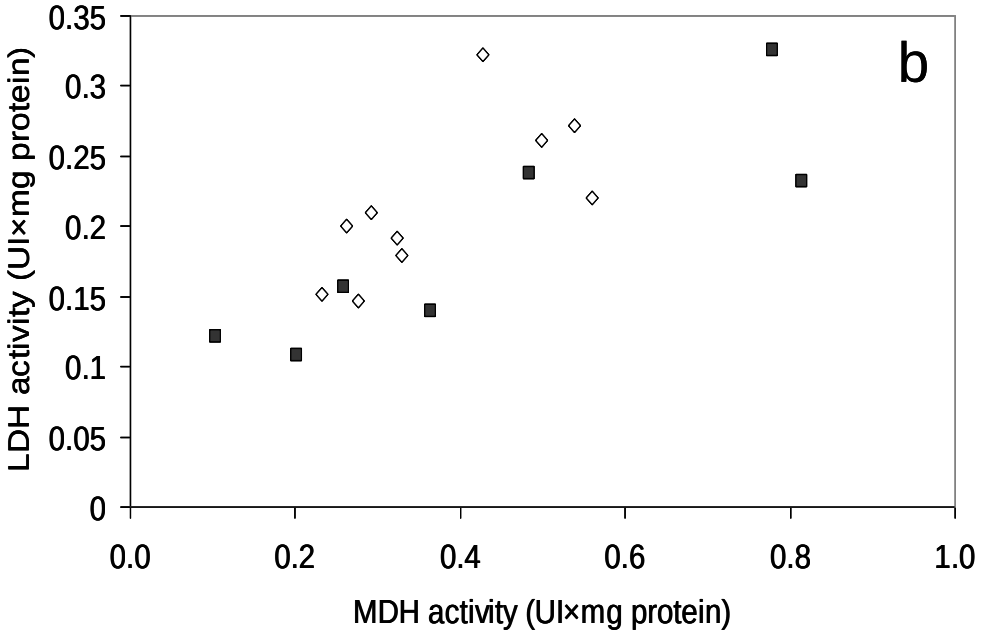

b
LDH activity (UI×mg protein)
MDH activity (UI×mg protein)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.0
0.2
0.4
0.6
0.8
1.0

Fig. 7

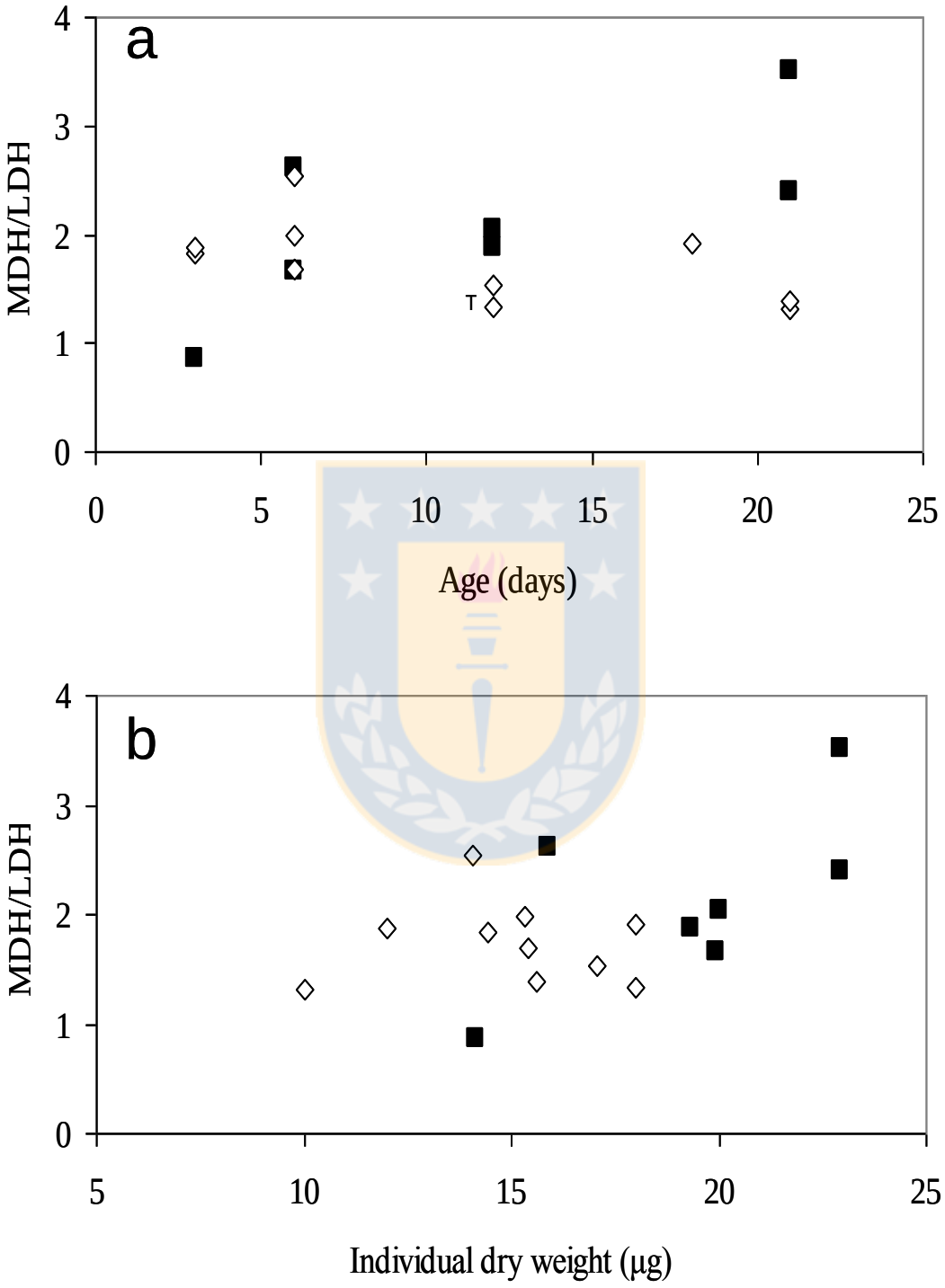
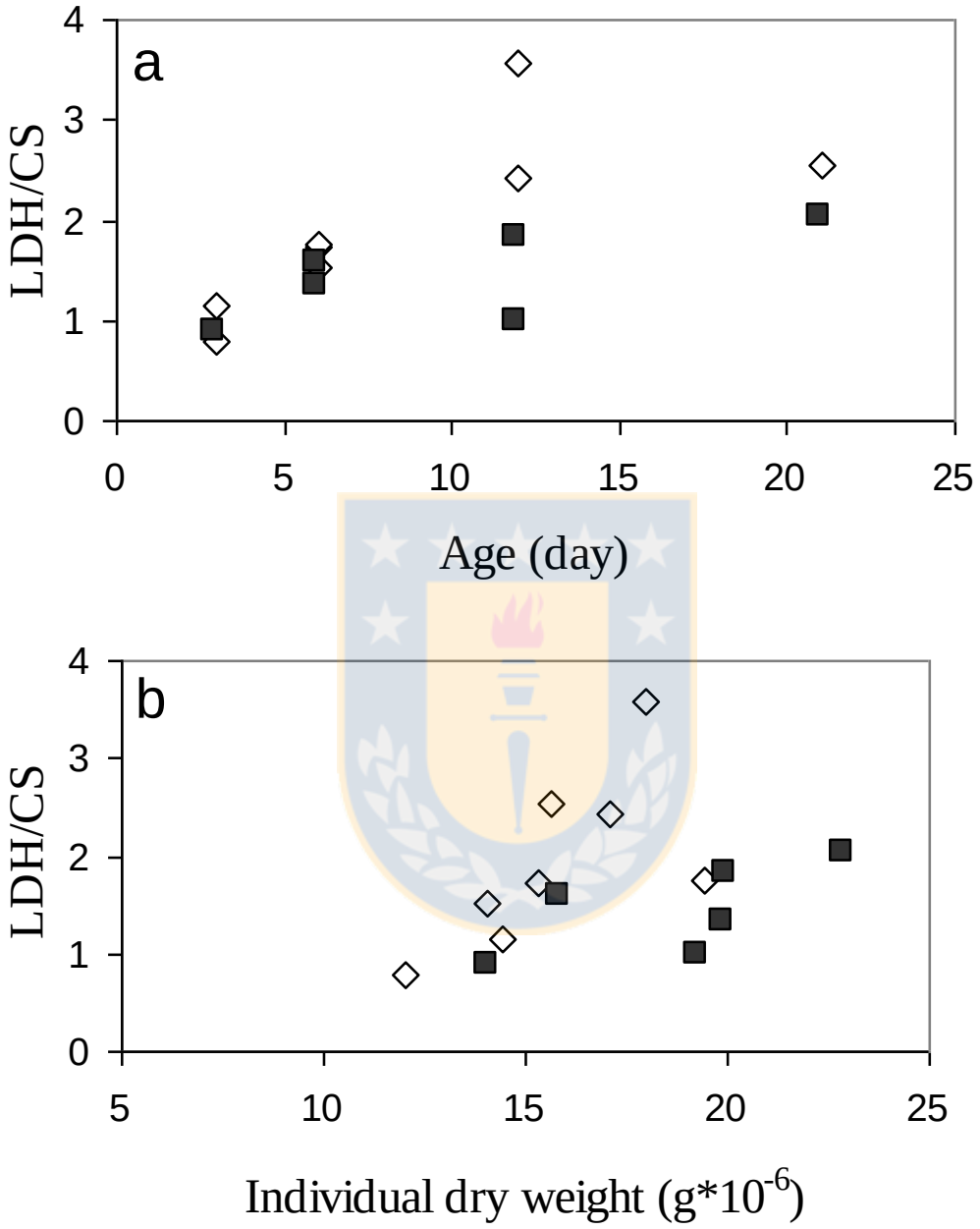


Fig. 8


a
LDH/CS
0
1
2
3
4
0
5
10
15
20
25
Age (day)
b
LDH/CS
0
1
2
3
4
5
10
15
20
25
Individual dry weight (g*10-6)

Manuscrito 6:

Beatriz Yannicelli

Larval developmental trade offs in an upwelling area: an IBM approach considering simulated hydrographic conditions

(Nota en preparación)

INTRODUCTION

It has been recognized that the dynamics of marine populations with complex life cycles largely depend on recruitment intensity, which has driven enormous interest on larval survival and transport processes over many decades. Since the early nineteen hundreds, starvation, advection and predation were proposed as the main causes of larval loss from a population. Different hypotheses have risen since; they support either one or a combination of those processes as main driving agents in the evolution of spawning strategies in varying oceanographic contexts (Sinclair, 1988; Parrish et al, 1981). In Eastern Boundary Currents, larvae can benefit from the enhanced production of coastal upwelling during upwelling seasons, however, they risk wind forced offshore advection in surface waters. Therefore, different strategies seem to have evolved to cope with that trade-off in species with planktotrophic development, eg.:

i) Spawning at specific locations where they can benefit from the tri-dimensionality of costal upwelling (e.g. enhanced larval retention at upwelling shadows).

ii) Extending spawning season, or spawning out of phase with upwelling season.

iii) Spawning in phase with upwelling season, but displaying vertical migrations at the larval stage (allowing for drifting on depth varying current fields).

Along South Central Chile, S-SW upwelling favorable winds dominate cyclically from mid-spring and throughout summer. In a simplified picture, the nutrient rich and oxygen poor Equatorial Subsurface Waters (ESSW) shoal over the continental shelf, and the Sub Antarctic Surface Waters (SASW) are transported seaward in the surface Ekman layer during the upwelling season (Strub et al. 1998). A small scale study carried out at the Gulf of Arauco (37S) showed that crustacean larvae segregation into different water types might dominate exchange pattern in this coastal embayment, enhancing the coastal retention and intrusion of deep larvae (Yannicelli et al. accepted). However, larval interaction with water mass characteristics (temperature and salinity) imply variations in developmental and growth rates, and therefore on expected survival (Hinckley *et al.*, 1996). It is believed that reduced growth rates and longer developmental times increase larval mortality due to predation, and that juveniles survival at settlement also depend on

the previous pelagos experience (Ouellet & Allard, 2002; Shima & Findley, 2002). Dissolved oxygen, which in not considered a limiting factor except in highly polluted semi-closed areas, can reach levels of 0.5 ml/l at 50-60m depth in the study area. According to laboratory experiments, these levels are likely to produce important metabolic changes and growth reduction in most crustacean larvae and juveniles (Coiro *et al.*, 2000; Rosas *et al.*, 1998; Seidman & Lawrence, 1985; Yannicelli and Castro, in review). Therefore, it is likely that the low dissolved oxygen found in shallow ESSW, play an important role in shaping reproductive and larval strategies in South Central Chile, by limiting the larval vertical migration strategy only to tolerant species. In this note, I followed an individually based modeling approach, to determine the combined effect of spatially explicit temperature, oxygen concentration and loss potential in the developmental times, survival, mean weight at metamorphosis and mortality cause, of hypothetical crustacean larvae in simulated environmental scenarios. Those idealized scenarios, mimicked hydrographic sections in the Chilean coast within expected temperature and oxygen values for particular situations. The main hypothesis is that depending on the larval capacity to cope with oxygen deficient waters, the delayed sub-surface development and reduced growth due to lower temperatures and low dissolved oxygen is balanced by increased subsurface retention. For this hypothesis to be true, we expect that the differences in survival to the megalopa stage observed between scenarios, will decrease for organisms with high tolerance to low oxygen concentration, and that overall survival in a given scenario will be larger for low critical point individuals.

THE MODEL

Simulated environment:

The simulated space was a vertical section (rectangular in shape) across the continental shelf of "Central Chile", 150m depth (z), and an arbitrary inshore to offshore x axis set to 1.

Each section was characterized by four environmental variables: temperature (C), dissolved oxygen concentration (ml/l), and two dimensionless parameters standing for horizontal diffusion (*Kx*), and vertical diffusion (*Kz*). The depth of the thermocline for each scenario was also given.

Temperature field. We generated a continuous field of temperature from initial values of surface inshore temperature (*Tsi*), surface offshore temperature (*Tso*), and fixed values of bottom temperature (9°C). At the surface, the across-shore gradient of temperature was generated linearly as a function of x from the inshore values of *Tsi* to the offshore values of *Tso*. For each larva at location x, z the surface temperature at x (*Tsx*) was calculated as:

$$Tsx = (1 - di) \times Tso + di \times Tsi$$

where *di* is the distance to the inshore border of the simulated space. After this calculus, surface and bottom temperatures corresponding to x are known and the temperature at the thermocline is set to the arithmetic mean of those two values. Temperature (*T*) at each point (x,z) can then be calculated as an arctan function of depth, and the temperature at the thermocline.

Dissolved oxygen. The generation of the oxygen field followed the same procedure of temperature, except that the initial parameters were deep oxygen inshore (*Odi*), and deep oxygen offshore (*Odo*), and the fixed level was surface oxygen (6 ml/l).

Diffusion. Surface (0m) and bottom values (150m) of the two variables *Kx* and *Kz* were constant across-shore, and given as a characteristic of each simulated scenario. In depth, Kx and Kz at z were calculated as an arctan function of depth, and *Kx* and *Kz* at the thermocline.

Thermocline depth (thd), was constant across-shore, and was fixed for each experiment.

Larvae:

The initial number of larvae to run the experiments was chosen to be 400. Larvae were placed randomly in space with an initial biomass (*ib*) that was set at the beginning of the experiment. *ib* was equal for all of the larvae in a given experiment, but could vary in between experiments (ranging from 5 to 8 μg/individual). Another attribute of larvae was the 'critical point' (*cp*). The critical point in respiration experiments refers to that oxygen concentration below which, at a given temperature, organism oxygen consumption becomes dependent on environmental oxygen concentration. Here, we used the critical point, as an oxygen level that determines the point at which growth and death become oxygen dependant.

Biological processes:

Movement and loss: equations and border conditions

The distance and direction an individual larval would move at each time step were determined as a modification of Batchelder et al (2002) random walk equation for diffusion:

$$ux = (0.5 - RN) \times \sqrt{2 \times Kx}$$

$$wz = (0.5 - RN) \times \sqrt{2 \times Kz}$$

where *ux* and *wz* are the horizontal and vertical velocity components respectively, *RN* is a random number between 0 and 1, and *Kx* and *Kz* are the turbulent coefficients defined above.

After the new positions in the section were obtained (actual *x* and *z* plus *ux* and *wz* respectively), they were evaluated. If the "*x*" exceeded the section dimensions, larvae were considered "lost" from the system. If the "*z*" exceeded the dimensions of the section (above surface, or bellow bottom, *wz* reverted its sign in order to maintain larvae within the section.

The surface and bottom values for *Kx* and *Kz* were set in advance, and are not realistic. They were set in order to generate random movement of larvae in the system following certain criteria: i) *Kx* was always an order of magnitude lower than *Kz*, since

horizontal turbulent diffusion coefficients in the sea are always larger in the horizontal plane; ii) surface values were an order of magnitud larger than the bottom ones for both parameters, since surface turbulence is enhanced due to wind forcing.

Growth: equations

Growth rate was a function of individual dry weight (µg) and temperature. The parameters were adjusted to pooled weight increment data from different crustaceans, obtained from Anger 2001 and references therein:

$$GR = 4.495 \times W^{0.096 \times Tf}$$

where *GR* is growth rate in µg/dia, *W* is individual weight (µg) and *Tf* is the temperature factor. The temperature factor is given by the parabolic equation:

$$Tf = -3.8 + 40.64 \times T - 0.02 \times T^2$$

where T is temperature (°C). Since the time step in the simulation was 1 day, the daily weight increment (*Wi*) is equal to GR/1.

Very little work has been carried out on sub-lethal effects of environmental low oxygen concentration on larval growth and/or development, so there is to my knowledge no general function in the literature to relate growth rate and oxygen concentration in crustacean larvae (but see Coiro et al, 2000). Based mainly in the results of Coiro et al. (2000) I calculated a proportion of growth that would be reduced as a consequence of oxygen concentration. This proportion, allowed the inclusion of environmental oxygen concentration effect on growth, when the critical point (*cp*) was above environmental level.

$$G\,\mathrm{Re} = \frac{100 - \frac{100 \times O}{Ks + O}}{100}$$

where *GRe* is growth reduction, O stands for Oxygen concentration (ml/l) and Ks is a parameter given by:

$$Ks = \frac{cp}{4} \times \frac{T}{15}$$

Therefore, for every time step when cp>O:

$$W(t+1) = W(t) + Wi - Wi \times G\,\mathrm{Re}$$

Wi is weight increment (defined above).

Development: equation

Development and growth are, to a certain extent, rather independent processes in crustacean larvae, and the effect of oxygen on development is even less studied than that on growth. So we set development dependent only on temperature. We considered the total time of development from hatching until metamorphosis to postlarvae (when they become powerful swimmers), ignoring zoeal stages. We considered that developmental time dependence on temperature was lineal for the range of temperatures used in the simulations according to the following equation:

$$TDt = 106 - 2.7 \times T$$

TDt in days, is the total developmental time at a temperature T.

So:

$$S(t+1) = S(t) + \frac{1}{TDt}$$

S is stage, and it stands for the fraction of the complete development that has been reached by the individual at time. An *S*=1 indicates the larvae has become a megalopa. At every time step (1 day), the larva advances its development according to a fraction of the total developmental time at the *in situ* temperature. The approach followed Anger, 2001; and the function parameters were adjusted as a simplification of Yannicelli and Castro (in review) results.

Death: equations

Earlier larval stages are more susceptible to die, so 'natural death rate' was set as a negative power function of weight:

$$Pd = 0.03 \times W^{-0.358}$$

where *Pd* is the probability of dying in each time interval.

Besides, depending on an organism critical point, death rate might increase at low environmental oxygen concentrations (O). When *cp* was larger than O, the probability of dying depending on the organism tolerance to hypoxia (*PdO*) was set equal to:

$$PdO = 1 - \frac{O}{\frac{Ks}{10} + O}$$

Both probabilities of dying were added up. A random number was generated between 0 and 1, and if it was lower than the added probability of dying, then the organism was removed from the population.

Bio-physical coupling

For every time step, each larvae gained a new position in depth and distance inshore, for which temperature, oxygen, *Kx* and *Kz* value were calculated. Each value was used during the corresponding time step to grow, develop, die, and move to the following time step.

Assumptions and limitations:

The model was built assuming:

a) there is no food limitation of growth, or development.
b) all individuals within an experiment were born with the same conditions (no genetic, physiological or prehatching experience differences).
c) There are no differences in what has been defined as "natural mortality" in this model above or below the thermocline.
d) We assumed a continuous growth function for a 'crustacean', which is obviously an oversimplification of their molting life cycle.

Monitored variables

The mean weight of larvae above and below the thermocline until metamorphosis (μg/ind), plus the lost and dead larvae above and below the thermocline, and the number of larvae metamorphosed at a given time above and below the thermocline were monitored during the simulations.

EXPERIMENTS AND STATISTICAL ANALYSIS

The described model was coded in JAVA programming language, and it was debugged and run from JBuilder 7 platform. The original codes that were modified to implement the present simulation were developed by C Mullon.

Five scenarios corresponding to five different oceanographic conditions were defined to conduct experiments. The parameters used to define the fields of temperature, oxygen, diffusion and thermocline depth for each scenario (oceanographic condition) are listed in Table 1. For each of these scenarios, 11 experiments were conducted. In each experiment, 400 larvae were allocated randomly in the across-shore section, with an initial larval biomass of 6 μg. All larvae within an experiment were initialized with a common critical point, but between experiments, the critical point took values from 0.7 to 6 in 0.5 steps (11 values in total). In summary, 55 experiments were carried out (5 scenarios × 11 critical points). The 400 larvae were allowed to move, grow, and develop until they molted to megalopa or disappeared (due to loss or death).

The monitored variables during the simulation were used to further calculate these response variables for each scenario:

i) Overall mean weight at metamorphosis

ii) Proportion of overall lost to dead larvae

iii) Overall surviving larvae

In addition: i) the mean weight at metamorphosis (surface organisms vs. deep organisms), ii) the proportion of lost to dead larvae in the surface and depth, iii) the proportion of surviving larvae at surface and depth and, iv) the total time to metamorphosis of all larvae at surface and depth, were also calculated in order to identify

different processes acting above and below the thermocline in the simulated environments.

The results were analyzed with Linear Models (Statistica 6.0). The variables lost:death ratio, mean weight at metamorphosis, the proportion of survival and time to metamorphosis, were analyzed as functions of two categorical independent variables: Oceanographic conditions (scenario) and depth (release above/below the thermocline), one continuous variable (critical point) and the interaction term Oceanographic condition×depth. The analysis aimed to determine the percentage of variance of each variable explained by the factors considered. A second analysis, included only Oceanographic condition and critical point as predictor variables for lost: dead ratio, mean weight at metamorphosis and proportion of larvae that survived to metamorphosis.

RESULTS

Considering larvae with different critical points together, the proportion of larvae that survived in the surface was higher than that of larvae at sub-thermocline waters for all oceanographic scenarios, except, OC 4 (fig. 1), where sub-surface survival was the largest. OC4 corresponds to the only scenario with high oxygen concentration below the thermocline (winter conditions). The factors that contributed the most to survival proportion were oceanographic scenario (OC), critical point, and the interaction OC-depth (Table 2). The lost to dead ratio above the thermocline was always higher than 1 (fig 2), and augmented in OC4. In sub-thermocline waters, lost larvae were usually less than a fifth of dead larvae, except in deep OC4 waters where, it was almost half. Since the time to metamorphosis in this waters was not significantly larger than that of other scenarios sub-thermocline waters (fig. 3), it indicates reduced death. Depth and critical point were the most important factors explaining lost to death ratio variance.

In general, metamorphosis always took longer at depth where waters were colder, and during upwelling seasons (1 and 2), development was probably retarded in the surface due to upwelling cold waters. Mean weight at metamorphosis was affected by

critical point and depth maily (Table 2). Overall, weight was lower at depth, but variance was reduced when organisms with very high (unrealistic) critical points are excluded (fig. 4). The higher weights were achieved under winter conditions (OC4).

The overall number of dead to lost individuals was inversely related to critical point (fig. 5a), except for the oceanographic scenario where oxygen was never limiting (OC 4). The percentage of variance in lost/death ratio was mainly explained by critical point (more than 50 %), and also by oceanographic scenario (28%)(Table 3). Mean weight at metamorphosis does not show a clear relationship with critical point (Figure 5b), except at very high critical points. Oceanographic scenario and critical point explained together less variance than the error term (Table 3). Overall survival depended mainly on critical point, but also on oceanographic scenario. At very low critical points survival was high in all oceranographic scenarios. At intermediate critical points there was a dichotomy in survival probably due to different oxygen conditions at depth. At very high critical points survival was always reduced (fig. 5c).

Overall survival was higher at OC4 (fig. 6a), however, when we exclude organisms with high critical points, and only the three lowest ones are kept, survival increases at all other scenarios, and becomes greatest at OC2 (fig. 6b). The variance of survival values are expected to be further reduced in each treatment if even fewer critical points are considered.

CONCLUSIONS

The results from this simulations show effectively, that the survival of larvae in the oceanographic scenarios considered is dependent on critical point (which indicates dependence on environmental oxygen concentration). In the range of environmental conditions considered, the fact that critical point and depth significantly affected the variance of response variables, and that they interacted significantly, indicate that dissolved oxygen is an important characteristic in the upwelling area of Central Chile, which could have the potential to influence spawning patterns, even more than latitudinal differences in temperature. While winter conditions seem the best when considering all

larvae, removing high critical point larvae reduced such difference, and including only the lowest critical point larvae (data not shown), indicates that summer conditions result in higher survival. The possibility of entering low oxygen waters without death increment and a weight at metamorphosis approximately 15% lower than that achieved at surface waters, results in increase retention in spite of a slightly longer developmental times.

This is the first and most simple individual based model that could be designed to approximate the hypothesis posed, however, these first results highlight the relevance of the topic and the potential of the tool. Therefore, in order to improve the approach, the model and gain further insight into the effect of the low oxygen layer on early life stages of organisms in this upwelling area, future simulations should proceed according to the following steps:

i) Couple the biological model with environmental variables gathered from 2-D or 3-D hydrodynamic model outputs with velocity fields, temperature and salinity (we could estimate oxygen concentration from specific T-S values, since we know low concentrations are characteristic of certain water mass).

ii) Construct more accurate biological functions, based on larger literature set.

iii) Test the sensitivity of the model to the biological parameters that were taken as 'facts' (growth rates, mortality rates, developmental rates and their dependance on T and O).

iv) Include organisms behavior and more realistic depth distributions gathered from field data.

v) Include predator-mediated mortality rates above and below the oxycline.

vi) Include food fields.

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Table 1. Initial parameters used to simulate 5 experimental environments. Tsi (Tso): surface inshore (offshore) temperature (°C); Odi (Odo): deep inshore (offshore) oxygen concentration ($ml \times l^{-1}$); Thd: thermocline depth (m)

	SSU	SCU	SNU	WC&SD	WN
Tso	15	15	19	14	17
Tsi	11	11	13	14	15
Odo	1	2	1	5	2
Odi	0.5	1	0.5	4	1
Thd	50	60	40	50	50

SSU: Summer, southern upwelling (Oceanographic Condition 1)

SCU: Summer, central upwelling (OC 2)

SNU: Summer, northern upwelling (OC 3)

WC&SD: Winter, central and southern downwelling (OC 4)

WN: Winter, northern condition (OC 5)

Table 2. Percentage of variance explained by each factor, for the four response variables considered. All the results shown are significant at the 0.05 level. *OC*: oceanographic condition (scenario), depth (above/below thermocline), *cp*: critical point.

	Lost/death ratio	**Mean weight**	**Survival proportion**	**Time to metamorphosis**
OC	2.41	2.39	20.68	13.99
depth	63.93	47.30	1.82	54.30
cp	11.56	25.21	32.40	5.11
OC*depth			14.96	9.37
model r2	**0.78**	**0.74**	**0.70**	**0.83**
error	22.10	25.10	30.14	17.22

Table 3. Percentage of variance explained by each factor, for the three response variables considered. All the results shown are significant at the 0.05 level.

	lost/death ratio	mean weight	Surviving larvae
OC	28.58	3.93	22.80
cp	52.54	19.56	63.78
model r2	**0.81**	**0.23**	**0.87**
error	18.88	76.51	13.42

FIGURE CAPTIONS

Fig. 1. Proportion of surviving larvae at surface (continuous line) and deep (dashed line) waters in the 5 different simulated scenarios.

Fig. 2. Ratio of lost to death larvae in different oceanographic scenarios, at surface (continuous line) and depth (dashed line).

Fig. 3. Time to metamorphosis (days) for the different oceanographic scenarios at surface waters (red) and sub-thermocline waters (red).

Fig. 4. Mean weight of larvae above or below the thermocline. a) mean weight of all surviving larvae. b) larvae with critical points below 5.

Fig. 5. Overall response variables as a function of critical point (x axis). A) Proportion of lost to death larvae. B) Mean weight of surviving larvae at metamorphosis (micrograms/individual). C) Surviving larvae (number of individuals).

Fig. 6. Surviving larvae as a function of Oceanographic scenario. A) All larvae included. B) Values for larvae with the lowest three critical points.

Fig. 1

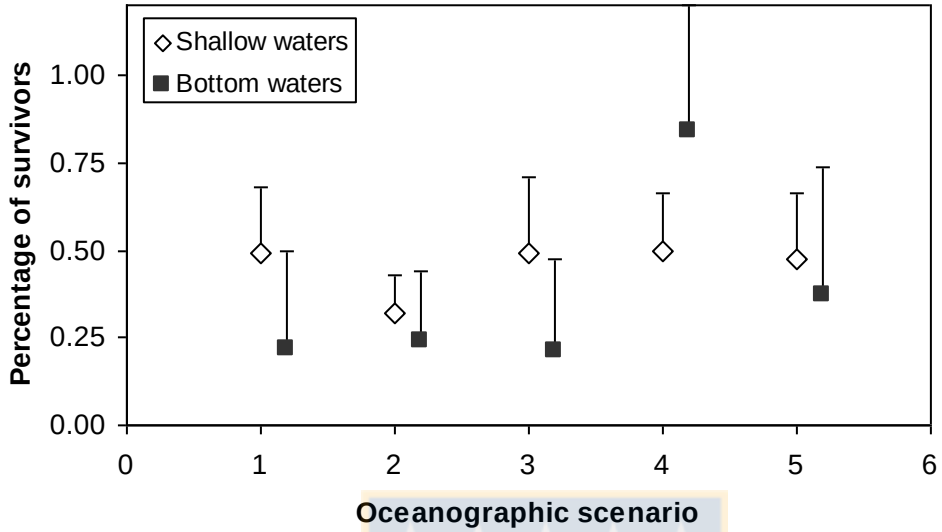

Shallow waters
Bottom waters
Percentage of survivors
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
6
Oceanographic scenario

Fig. 2

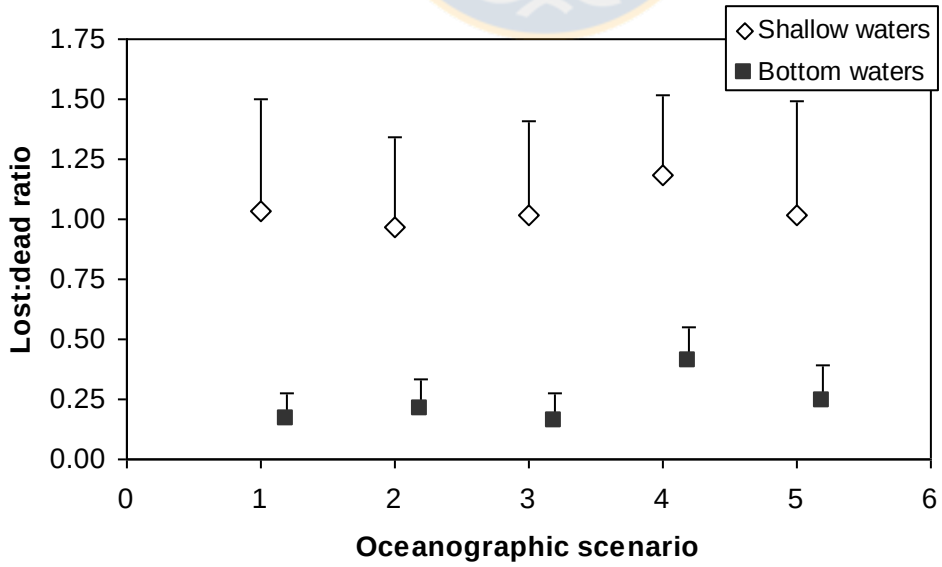

Shallow waters
Bottom waters
Lost:dead ratio
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
1
2
3
4
5
6
Oceanographic scenario

Fig. 3

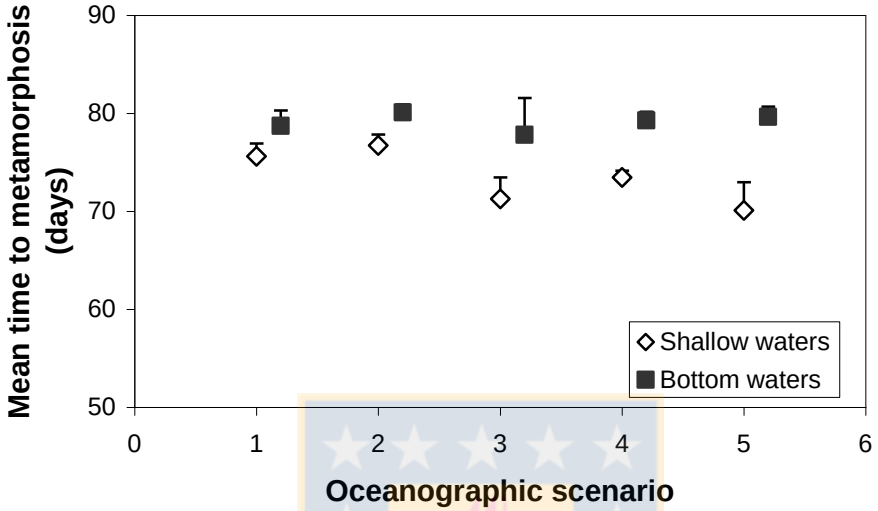

Mean time to metamorphosis (days)
90
80
70
60
50
0
1
2
3
4
5
6
Oceanographic scenario
Shallow waters
Bottom waters

Fig. 4

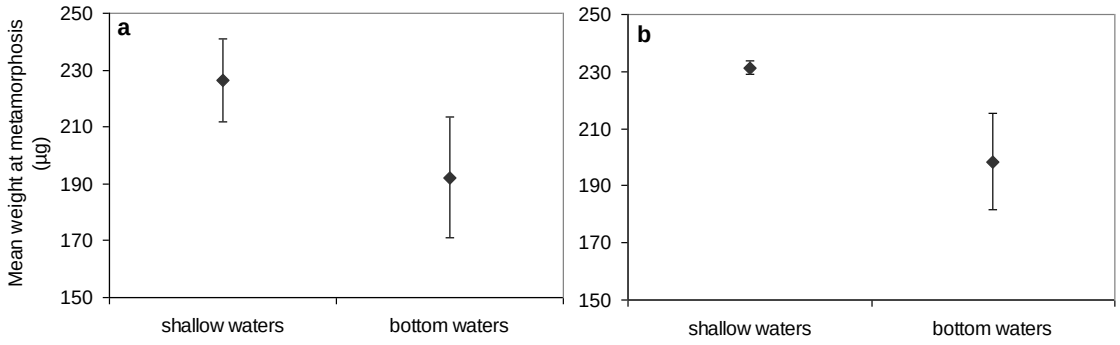

Mean weight at metamorphosis (µg)
250
230
210
190
170
150
a
shallow waters
bottom waters
b
shallow waters
bottom waters

Fig. 5

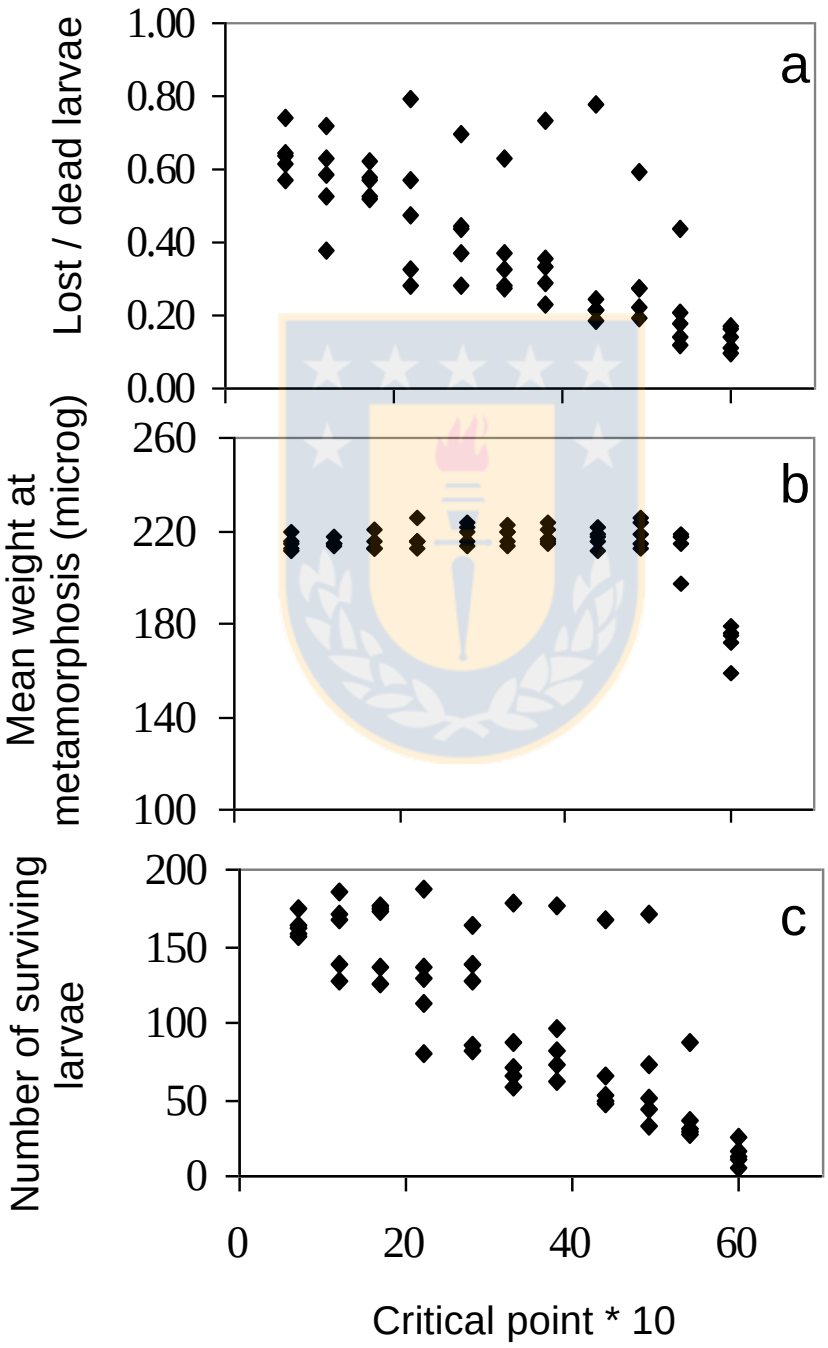
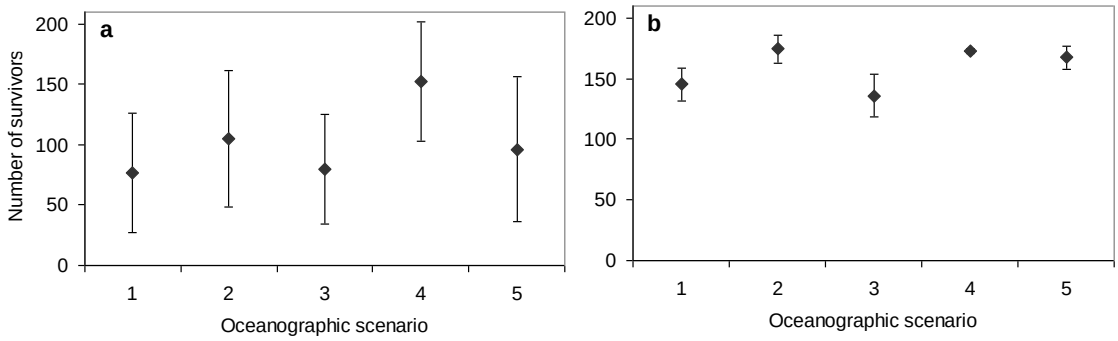

Lost / dead larvae
1.00
0.80
0.60
0.40
0.20
0.00
a
Mean weight at metamorphosis (microg)
260
220
180
140
100
b
Number of surviving larvae
200
150
100
50
0
c
0
20
40
60
Critical point * 10

Fig. 6


a
b
Number of survivors
200
150
100
50
0
1
2
3
4
5
Oceanographic scenario