

The impact of a strong natural hypoxic event on the toadfish *Aphos porosus* in Coliumo Bay, south-central Chile

Impacto de un fuerte evento de hipoxia natural sobre el bagre de mar *Aphos porosus* en Bahía Coliumo, centro-sur de Chile

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Resumen. La ocurrencia de hipoxias naturales y las áreas marinas costeras afectadas por hipoxias antropogénicas se han incrementando en las últimas décadas. En Coliumo, una pequeña bahía somera del Pacífico sur-este, se registró un intenso evento de hipoxia natural en enero del 2008, provocando una mortalidad masiva de peces y otros componentes de la comunidad bentónica y pelágica. En el corto plazo (3 meses) se observó una recuperación en la riqueza y densidad total de peces, sin embargo, en el mediano plazo (2 años) este evento tuvo efectos negativos en sus tamaños poblacionales. En particular *Aphos porosus*, especie dominante del ensamble de peces en los fondos blandos de Bahía Coliumo, presentó una disminución progresiva en su tamaño poblacional luego del evento, asociada a una disminución en el reclutamiento. Nuestra hipótesis es que la reducción del tamaño poblacional, habría disminuido la probabilidad de encuentro de adultos reproductivos, disminuyendo huevos, larvas y finalmente los reclutas, llevando a *A. porosus* hacia un umbral crítico de baja densidad, favoreciendo una condición poblacional de efecto Allee. Estos resultados entregan evidencia sobre la relación de causalidad entre eventos de hipoxia natural, mortalidad masiva, historia de vida y dinámica poblacional. La lenta recuperación numérica de *A. porosus* sugiere un proceso en el cual la población presenta una alta vulnerabilidad a nuevas perturbaciones. Esta respuesta poblacional es un ejemplo de la trayectoria que ciertas especies pudieran seguir ante el incremento de eventos de hipoxia natural, el cual se estima podría ocurrir en un escenario de calentamiento global.

Palabras clave: Peces, surgencia, hipoxia, efecto Allee, Sistema de Corriente Humboldt

Abstract. The occurrence of natural hypoxic events and coastal marine areas affected by anthropogenic hypoxia has increased over the past decades. In Coliumo Bay, a small shallow bay of the eastern South Pacific, an intense event of natural hypoxia took place in January 2008, causing the mass mortality of fish and other components of the benthic and pelagic communities. In the short term (3 months) recovery was observed in species richness as well as in total fish density. Nevertheless, in the medium term (2 years), this event produced negative effects on population size. In particular, the toadfish *Aphos porosus*, a dominant fish species in Coliumo Bay, showed a progressive decrease in population size since the occurrence of the hypoxic event, associated with a decrease in recruitment. We hypothesized that this reduction in population size lowered the probability of encounter of reproductive adults, decreasing the number of eggs, larvae and ultimately recruits, thus driving the population towards a low density threshold, and eventually, favoring an Allee effect condition. These results provide evidence on the causal relationship between coastal natural hypoxic events, mass mortality, life history and population dynamics. The slow recovery of the *A. porosus* population size suggests a process in which the population remains vulnerable to new perturbations. The observed population response is an example of the trajectory that some species may follow when faced with increasing occurrences of natural hypoxic events as predicted, for instance, by global warming scenarios.

Key words: Fish, upwelling, hypoxia, Allee effect, Humboldt Current System

INTRODUCTION

The frequency of environmental hypoxic events and the total coastal area affected by hypoxia worldwide have increased over the past decades (Díaz & Rosenberg 2008, Breitbart *et al.* 2009). Rabalais *et al.* (2010) described two origins for the generation of hypoxic water masses: (1) Human-caused and (2) naturally-caused. The former is associated with processes of coastal eutrophication, mainly due to the discharge into the coastal ocean of continental waters with high nutrient and organic matter loads. These human-caused hypoxias, in which dissolved oxygen is consumed mostly by prokaryote metabolism, are fairly common and widely studied, including their effects on populations and communities (Rabalais *et al.* 2010 and references therein, Fleddum *et al.* 2011, Kodama & Horiguchi 2011). The naturally-caused hypoxias, which are due principally to the photosynthetic generation of carbon and the associated microbial respiration, are related to permanently hypoxic water masses in the open ocean, referred to as Oxygen Minimum Zones (OMZ) (Helly & Levin 2004). The entrance of these oxygen-deficient waters into coastal areas due to upwelling processes (Giesecke & González 2004, Pavez *et al.* 2006, Sobarzo *et al.* 2007) may ultimately generate hypoxic conditions (Rabalais *et al.* 2010 and references therein). These upwelling-driven hypoxic events have been poorly documented and their chronic effects on population and community levels are unknown (but see Grantham *et al.* 2004, Hernández-Miranda *et al.* 2010, 2012).

In Coliumo Bay, a small shallow bay off central-south Chile, an intense hypoxic event was recorded in austral summer 2008, causing the mass mortality of fish (Hernández-Miranda *et al.* 2010) and other epibenthic and demersal taxa (Hernández-Miranda *et al.* 2012). The instantaneous effects on the fish assemblage were dramatic, with an abrupt drop in total density, total biomass and species richness, which was consistent with the conclusion of Vaquer-Sunyer & Duarte (2008) who, based on an extensive literature review suggested that fish are the group of organisms most affected by environmental hypoxia conditions. Nevertheless, in the short term (almost 3 months), partial recovery was observed in richness and total density (Hernández-Miranda *et al.* 2010). Despite this, questions emerge regarding the recovery of fish assemblages in the long term (Hernández-Miranda *et al.* 2012). In addition, how might have this hypoxia affected each particular species?

In the epibenthic macrofaunal community inhabiting the soft-bottom benthos of Coliumo Bay, the toadfish

Aphos porosus (Valenciennes, 1837) (Batrachoididae) is the dominant fish species (Hernández-Miranda *et al.* 2010) and is the fourth dominant species in relation to the total density and total biomass of the whole community (Hernández-Miranda *et al.* 2012). The family Batrachoididae has a wide distribution in the Atlantic, Indian and Pacific Oceans (Nelson 1994), mainly inhabiting hard and soft bottoms in shallow marine coastal environments (Varas & Ojeda 1990, Merlo *et al.* 2005, Canto-Maza & Vega-Cendejas 2007). These species are characterized by solitary and cryptic habits, parental care and larvae without a pelagic life stage (Nelson 1994). Although the members of this family are made up of species with no commercial value (Palazón-Fernández *et al.* 2010), they are recognized as important components of ichthyofauna communities in the coastal ecosystems they inhabit (Canto-Maza & Vega-Cendejas 2007, Cortés & Muñoz 2008). Historically, toadfish species have been used in laboratory studies in the field of endocrinology (Hoffman 1963), physiology (Fänge & Wittenberg 1958, Haschemeyer 1969) and animal toxicology (Alquezar *et al.* 2006), among others, and there are increasing efforts to improve knowledge of their basic biology and ecology (Price & Mensinger 1999, Palazón-Fernández *et al.* 2001, 2010; Palazón *et al.* 2003, Malca *et al.* 2009).

Of the 69 species that make up the Batrachoididae family (Nelson 1994), only *A. porosus* is found off the coast of Chile (Mann 1954, Pequeño 1989), with a distribution that extends from Puerto Pizarro (Perú) to the Strait of Magellan (Chile) (Chirichigno 1974). The ecological knowledge of *A. porosus* is scarce and mostly consists of reports on spatial and bathymetric distribution (Sielfeld & Vargas 1999, Ojeda *et al.* 2000), studies of coastal ichthyofauna (Varas & Ojeda 1990, Sielfeld & Vargas 1996, Medina *et al.* 2004), parasitological studies (Cortés & Muñoz 2008), and as part of the by-catch of industrial (Lillo *et al.* 2002) and artisanal common hake (*Merluccius gayi gayi*) fisheries (Acuña *et al.* 2007). Up to now, in central-south Chile there is no information about its population dynamics, spatial distribution, habitat selectivity or age at sexual maturity. However, Cortés & Muñoz (2008) based on the study of the infra-community of parasites of *A. porosus*, suggested that the habitat of the species was demersal and that this fish could be a prey of other demersal fishes, cetaceans, elasmobranchs and sea lions. *A. porosus* is a predator of small gastropods, fish, amphipods and gamariids (Varas & Ojeda 1990, Cortés & Muñoz 2008), and therefore a consumer of intermediate trophic level or secondary consumer.

In relation to the effects of the natural hypoxic event on *A. porosus* in Coliumo Bay, we hypothesized that the intense hypoxic event triggered negative lagged effects over time in *A. porosus*, leading to a small and critical population size. Given the characteristics of the life history of this species, the recovery could be slow and probably set the population near an Allee threshold (Stephens *et al.* 1999, Berec *et al.* 2006). The main objective of this study was to assess the inter-annual effects of hypoxia on the population of *A. porosus* inhabiting the soft-bottom benthos in Coliumo Bay. For this, we (i) evaluate the inter-annual dynamics of density and biomass; (ii) identify the inter-annual recruitment dynamics; (iii) discuss the results in the context of an Allee effect and its ecological implications.

MATERIALS AND METHODS

STUDY AREA AND DATA COLLECTION

Coliumo Bay (36°30'S, 72°56'W, Fig. 1) is a small, shallow, equator-facing bay with depths under 25 m, characterized mainly by sandy bottoms. *A. porosus* captures were carried out at 3 sampling stations within Coliumo Bay approximately every 3 months. The surveys were conducted in January, May, August and November 2007; January, April, July and October 2008 and 2009 and January 2010. Sampling was carried out using a modified Agassiz trawl (1 m wide × 1 m long × 30 cm high, lined with 5 mm “knot to knot” netting); on the average the survey distance for the whole sampling period was 378 m ± 145 m trawling for 5 min to 1.5-2 knots. Fish were collected directly from the net onboard and put in plastic bins with seawater. In the laboratory all individuals were measured (LT: 0.1 cm precision) and weighed using an analytic precision scale (MT: 0.01 g precision). All surviving fish were then returned to their environment. *A. porosus* density (ind. 500 m⁻²) and total biomass (g 500 m⁻²) was calculated by standardizing the quantity and biomass of organisms captured in each tow in relation to the swept area (*e.g.*, N° ind. × 1 m wide trawl × 500 m / Survey distance m). Vertical hydrographic profiles for each sampling period (temperature, salinity and dissolved oxygen) were sampled using a CTD Sensor Data Model SD204. Data were collected as part of the periodic monitoring program set up by the Programa de Investigación Marina de Excelencia (PIMEX) at Universidad de Concepción (UDECE), Chile. The sampling was carried out using UDECE's research vessels R.V. Kay-Kay I or R.V. Kay-Kay II.

SPECIAL *A. POROSUS* SAMPLING

On January 3, 2008, dead and dying fish were collected from Coliumo Bay (Fig. 1). 396 individuals of *A. porosus* were sampled from a sandy, rocky intertidal zone in front of Villarrica Cove, Dichato in Coliumo Bay (see Hernández-Miranda *et al.* 2010). In the laboratory, for each individual the following measurements were recorded: total length (LT: 0.1 cm precision), total weight (MT: 0.01 g precision), sex, gonad weight (MG: 0.01 g precision), and state of sexual maturity (MS). For MS, a 5-stage visual scale was used following Holden & Raitt (1975): MS I (immature), MS II (in maturation), MS III (mature), MS IV (hydrated) and MS V (spawned).

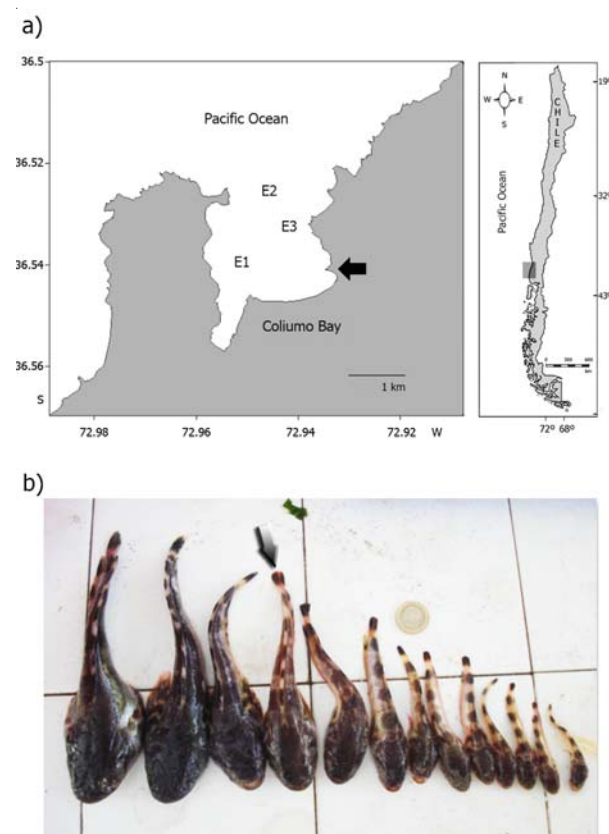


Figure 1. (a) Sampling stations within Coliumo Bay. E1, E2 and E3 correspond to the periodical sampling stations and the black arrow marks the area where the mass stranding of fish was recorded in January 2008. (b) Photographs of the stranded toadfish *A. porosus* in January 2008. The arrow shows the juvenile-adult threshold / (a) Estaciones de muestreo dentro de Bahía Coliumo. E1, E2 y E3 corresponden a las estaciones periódicas de muestreo y la flecha negra señala el lugar de muestreo durante la varazón de enero del 2008. (b) Fotografías de *A. porosus* varados en enero de 2008. La flecha señala el umbral entre individuos juveniles y adultos

DATA ANALYSIS

From the 396 individuals of *A. porosus* collected during the stranding event that took place on January 3, 2008, the following information was obtained: (i) size structure of juveniles, males and females at intervals of 1 cm length, expressed as percentage frequency; (ii) the fit for the length-mass relationship: $MT = a * LT^b$, where a and b are fitted parameters describing the allometric relationship between these variables. The fit was determined via a least squares non-linear regression, using the NONLIN module in the statistical software package SYSTAT (Wilkinson 1988); (iii) the frequency of occurrence of maturity stage (MS) for both sexes expressed as a percentage. For the analysis, juvenile individuals and MS I and II were considered immature, and MS III and IV were considered mature; (iv) the gonadosomatic index (IG), for reproductive tissue growth. For this the following expression was used: $IG = (MG/(MT-MG)) * 100$; (v) the average size at sexual maturity ($L_{50\%}$). For this parameter a logistic model was adjusted according to: $P_{(l)} = 1 / (1 + e^{\beta_1 + \beta_2 * l})$, where: $P_{(l)}$ is the mature proportion at size l , and β_1 and β_2 are the position and slope parameters, respectively; the average size at sexual maturity is defined as $L_{50\%} = -(\beta_1 / \beta_2)$. For the fitting of the logistic model, maximum likelihood was used according to Roa *et al.* (1999).

The segregation of juvenile from adult stages for the entire sampling period (January 2007- January 2010) was performed according to the average size at sexual maturity obtained during the stranding event (see Hernández-Miranda *et al.* 2010). In addition to the time series of densities, the temporal dynamics of the numerical frequency of body sizes was obtained using the ELEFAN analysis (Gayaniolo & Pauly 1997) of the FiSAT II FAO-ICLARM Fish Stock Assessment Tools Software. This allowed us to obtain graphically periods of arrival of smaller-sized individuals (*i.e.*, recruitment), an analysis that has already been used for intertidal fish in central Chile (Hernández-Miranda & Ojeda 2006).

For the entire time series of *A. porosus* catches, the length-mass relationship between calendar years, between cohorts and for the periods prior and after the mortality event were estimated using an algorithm similar to the one utilized for fish collected in the mortality event of January 2008. For this, individuals were pooled as follows: (i) January to November 2007, January 2008 (mortality event), and April 2008 to January 2010, (ii) for the calendar years 2007, 2008 and 2009 and (iii) for each cohort identified according to its recruitment period based on

the ELEFAN analysis. The differences between these relationships were analyzed with the slope test (Zar 1999). Based on these relationships, the relative condition factor (Kr) was estimated according to Le Cren (1951) and applied to toadfish following Palazón-Fernández *et al.* (2001). Kr was calculated as the percentage ratio of the observed weight of a fish to the weight expected from the calculated length-weight relationship. This factor was obtained for 3 calendar years, for each one of the cohorts identified and for the pre- and post-mortality periods. After testing for the assumptions of normality and homogeneity of variances, Kr values were compared with a one-way ANOVA.

RESULTS

HYDROGRAPHIC DYNAMICS

The hypoxic event at Coliumo Bay that took place in January 2008 was characterized by low temperature ($<11^\circ\text{C}$), increment of salinity in the surface stratum (>34) and very low dissolved oxygen concentration in the whole water column including the surface stratum (0-1 ml l^{-1} close to 2 m deep) (Fig. 2, see Hernández-Miranda *et al.* 2010, 2012). Coliumo Bay during the sampling dates in January 2009 and January 2010 also showed the presence of Equatorial Subsurface Waters (ESSW) (*i.e.*, temperatures near to $11-12^\circ\text{C}$ and salinities higher than 34 at the bottom of the water column), however, dissolved oxygen concentrations were greater than 2 ml l^{-1} in the surface stratum. This indicated that hypoxic conditions were not observed in both periods.

REPRODUCTIVE AND BIOLOGICAL PARAMETERS

The size structure of *A. porosus* individuals collected from Coliumo Bay during the massive stranding was unimodal and was composed of 376 juveniles (95.0%), 14 males (3.5%) and 6 females (1.5%). The size range was 5.2-15.2 cm LT, 8.1-15.3 cm LT, and 13.1-19.5 in juveniles, males and females, respectively (Fig. 3a). For individuals with development of reproductive structures ($n = 20$), the male:female ratio was 2:1. Ninety three percent ($n = 13$) of males were immature (MS I) and 7% ($n = 1$) in maturation (MS II); while 50% of females were mature (MS III, $n = 3$) and 50% hydrated (MS IV, $n = 3$). In immature and maturing males and immature females the average IG was less than 2%, while in hydrated females ovaries represented between 32% and 40% of body weight, with an average IG of 37%. In general terms, the IG and MS increased in larger individuals (Fig. 3b). For the construction of the

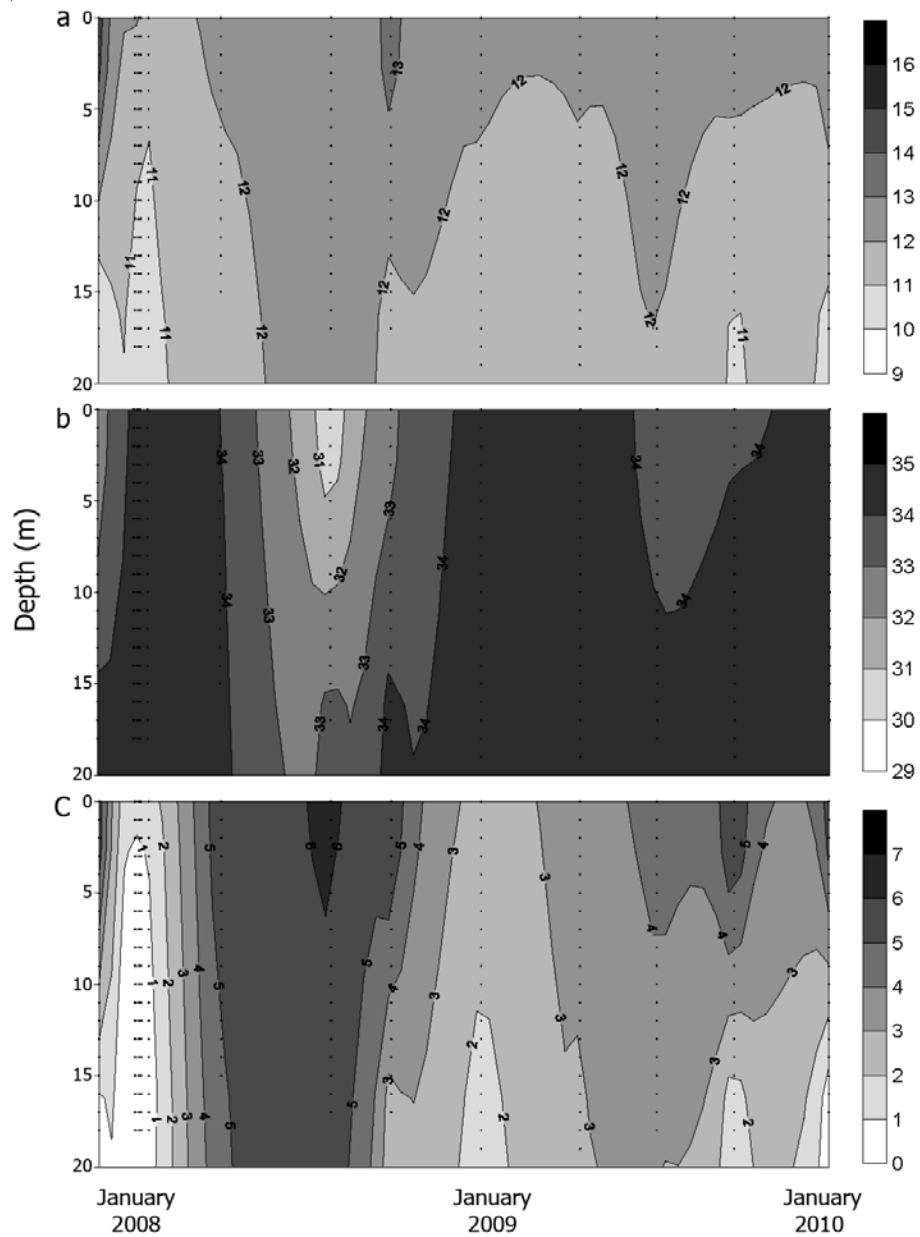


Figure 2. Temporal series of (a) temperature (°C), (b) salinity, and (c) dissolved oxygen (ml l⁻¹) for the entire water column in the period November 2007-January 2010 / Series temporales de (a) temperatura (°C), (b) salinidad, y (c) oxígeno disuelto (ml l⁻¹) en la columna de agua para el período noviembre 2007- enero 2010

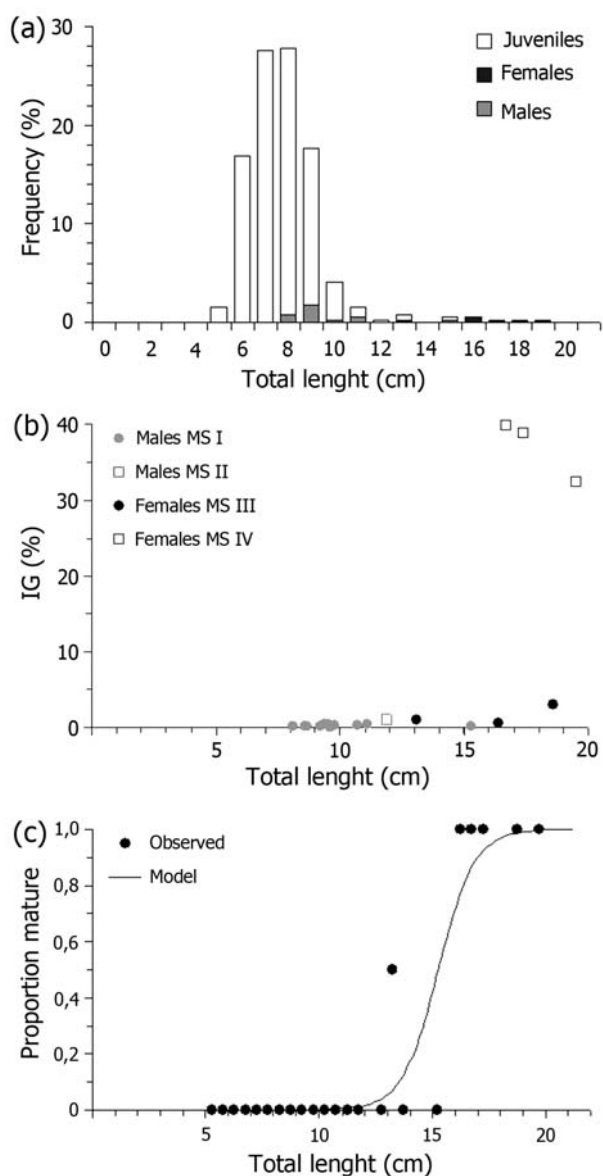


Figure 3. (a) The proportion of juveniles and adults of *A. porosus* in Coliumo Bay during the stranding event of January 2008. (b) Average gonadosomatic index (IG) per maturity stage for males ($n = 14$) and females ($n = 6$) of *A. porosus* during the stranding in January 2008. (c) Sexual maturity ogive for both sexes combined, observed (*) and estimated (-) / (a) Proporción de individuos juveniles y adultos de *A. porosus* en Bahía Coliumo durante la varazón de enero del 2008. (b) Índice Gonadosomático (IG) promedio por estado de madurez para machos ($n = 14$) y hembras ($n = 6$) de *A. porosus* durante la varazón de enero de 2008. (c) Ojiva de madurez sexual combinado para ambos sexos, observado (*) y estimado (-)

maturity ogive, information from both sexes and juveniles was used. The parameters of the logistic model adjusted to the mature proportion data were $\beta_1 = 19.46 (\pm 2.34 \text{ SE})$ and $\beta_2 = -1.27 (\pm 0.16 \text{ SE})$, with the average size at sexual maturity ($l_{50\%}$) calculated at 15.3 cm LT (Fig. 3c).

SPATIAL AND TEMPORAL DYNAMICS

The spatial density of *A. porosus* during the whole sampling period was highly heterogeneous, but it was lowest immediately after the hypoxic event (Fig. 4). In relation to body sizes, between January 2007 and January 2010 *A. porosus* presented 3 recruitment events (May 2007, April 2008, July 2009); (Fig. 4a), all of individuals close to 4 cm. Three cohorts were identified using the FiSAT II software (Fig. 4a). Temporally, one year after the hypoxic event a recovery in average density and biomass was observed (relative to the hypoxic event), but without reaching the high values observed during the pre-hypoxia period (Fig. 4b, c). The average density in 2007 was 96.98 ($\pm 93.92 \text{ SD}$) individuals 500 m^{-2} , in 2008 25.82 ($\pm 33.17 \text{ SD}$) individuals 500 m^{-2} and 2009 13.6 ($\pm 13.74 \text{ SD}$) individuals 500 m^{-2} . The ANOVA showed significant differences between the 3 periods ($F_{(2, 11)} = 4.256, P = 0.025$). The *a posteriori* tests indicated that the differences were between the densities from 2007 and 2009, and 2007 and 2008 (Tukey HDS, $P < 0.05$). The average biomass in 2007 was 195.94 ($\pm 215.03 \text{ SD}$) g 500 m^{-2} , in 2008 64.48 ($\pm 63.08 \text{ SD}$) g 500 m^{-2} and 2009 47.62 ($\pm 47.46 \text{ SD}$) g 500 m^{-2} . The ANOVA showed significant differences between the 3 periods ($F_{(2, 11)} = 4.256, P = 0.072$). The *a posteriori* tests indicated that the differences were between the biomass from 2007 and 2009, and 2007 and 2008 (Tukey HDS, $P < 0.1$).

Based on the average size at sexual maturity calculated in this study (15.3 cm, see Fig. 3c), we observed a majority of juveniles during all study periods. Adults were captured during only 3 sampling events (Nov. 2007: 0.32%; Jul. 2008: 15.78%; Jan. 2010: 25.00%). For the length-mass relationship, significant differences were encountered between slopes before and after the mortality event relative to that estimated for stranded fish in January 2008 (Fig. 5a) (ANOVA $F_{(2, 1392)} = 11.89, P < 0.001$). These differences are due to a steeper slope during the mortality event ($b = 3.22$) relative to estimates for the before ($b = 2.98$) and after ($b = 3.03$) periods (Tukey HDS, $P < 0.05$). Differences were significant between the slopes for the 3 calendar years (Fig. 5b) (ANOVA $F_{(2, 1000)} = 11.44, P < 0.001$). These differences are due to a steeper slope during 2009 ($b = 3.17$) relative to 2007 ($b = 2.98$) and 2008 ($b = 2.95$) (Tukey HDS, $P < 0.05$). For the length-mass

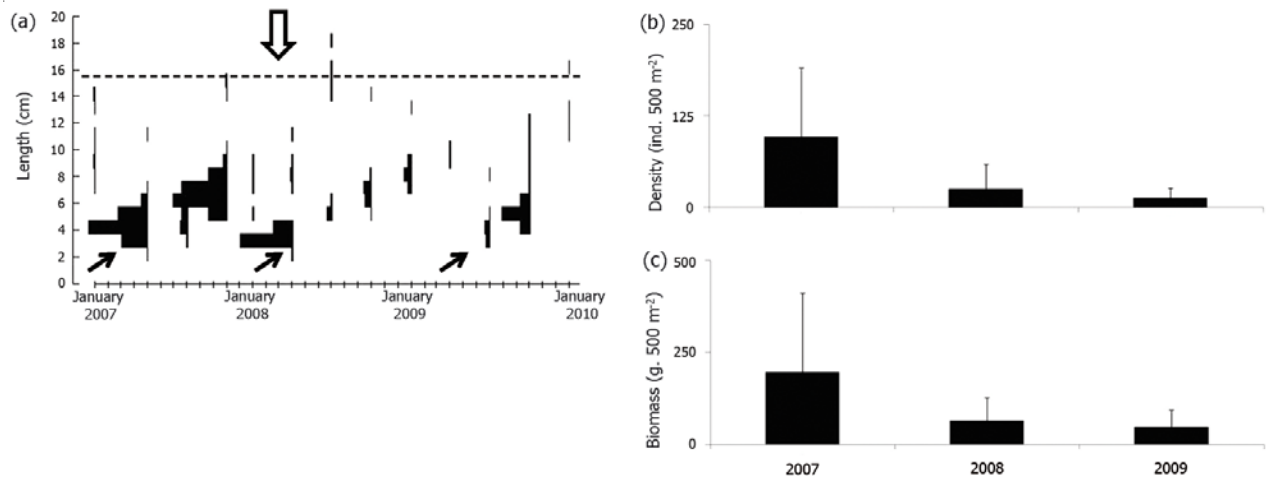


Figure 4. (a) Time series of size frequencies (LT). Black arrows indicate 3 recruitment events and the start of each new cohort. The dashed horizontal lines mark size at sexual maturity estimated for *A. porosus*. The white arrow indicates sampling immediately after the hypoxia event in January 2008. (b) Time series of the mean density and (c) biomass of *A. porosus* in Coliumo Bay, from January 2007 to January 2010. Vertical bars indicate standard deviation (SD) / (a) Serie temporal de frecuencias de tamaño (LT). Las flechas negras señalan tres eventos de reclutamiento y el inicio de cada nueva cohorte. La línea horizontal segmentada señala el tamaño de madurez sexual estimado para *A. porosus*. La flecha blanca señala el muestreo inmediatamente después de la hipoxia natural de enero del 2008. (b) Serie temporal de la densidad y biomasa promedio (c) de *A. porosus* en Bahía Coliumo desde enero 2007 hasta enero 2010. Las barras verticales señalan la desviación estándar (DS)

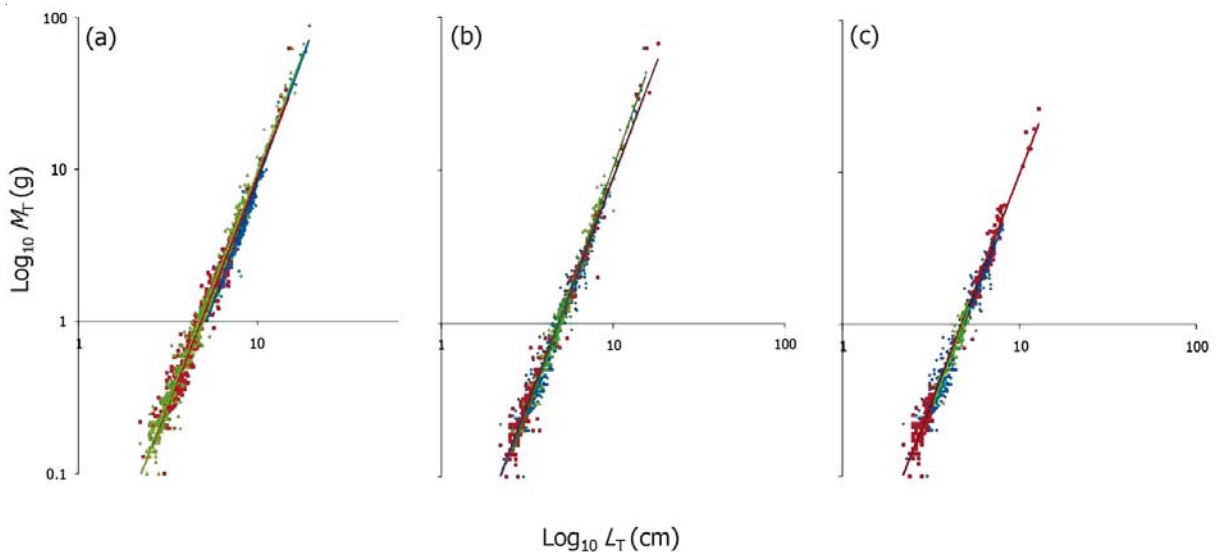


Figure 5. Length (LT) and total mass (MT) relationship (\log_{10} - \log_{10} scale) for *A. porosus*. (a) Blue marks: stranded fish; red marks: fish captured before natural hypoxia; green marks: fish captured after natural hypoxic event. (b) Blue marks: fish captured during 2007; red marks: fish captured during 2008; green marks: fish captured during 2009. (c) Blue marks: 1^o cohort; red marks: 2^o cohort; green marks: 3^o cohort (see Fig. 5a) / Relación entre el tamaño corporal (LT) y biomasa (MT) (escala \log_{10} - \log_{10}) para *A. porosus*. (a) Marcas azules: peces varados; Marcas rojas: peces capturados antes de la hipoxia natural; Marcas verdes: peces capturados después de la hipoxia natural. (b) Marcas azules: peces capturados durante 2007; Marcas rojas: peces capturados durante 2008; Marcas verdes: peces capturados durante 2009. (c) Marcas azules: 1^a cohorte; Marcas rojas: 2^a cohorte; marcas verdes: 3^a cohorte (Ver Fig. 5a)

relationships per cohort, no significant differences were found between slopes (ANOVA $F_{(2, 737)} = 2.20, P > 0.1$), with values of $b = 2.92$ (cohort 1), $b = 2.99$ (cohort 2) and $b = 3.16$ (cohort 3) (Fig. 5c).

With regard to the factors of relative condition Kr, no significant differences were found between estimated Kr values before ($Kr = 1.014 \pm 0.178$ SD) and after the mortality ($Kr = 1.013 \pm 0.197$ SD), relative to the stranded fish in January 2008 ($Kr = 1.001 \pm 0.110$ SD) (ANOVA $F_{(2, 1392)} = 0.94, P = 0.39$). Nor were there significant differences among Kr estimates for calendar years ($Kr 2007 = 1.014 \pm 0.178$ SD; $Kr 2008 = 1.025 \pm 0.213$ SD; $Kr 2009 = 1.019 \pm 0.168$ SD) (ANOVA $F_{(2, 1000)} = 0.28, P = 0.75$), or for Kr estimates per cohort (Kr cohort 1 = 1.012 ± 0.179 SD; Kr cohort 2 = 1.022 ± 0.191 SD; Kr cohort 3 = 1.010 ± 0.144 SD) (ANOVA $F_{(2, 737)} = 0.21, P = 0.81$).

DISCUSSION

REPRODUCTIVE AND BIOLOGICAL PARAMETERS

Based on the stranded fish during the natural hypoxia event of January 2008, the average size at sexual maturity, estimated for males and females (pooled), was 15.3 cm. Palazón-Fernández *et al.* (2001), reported for *Halobatrachus didactylus* (Batrachoididae; Schneider, 1801) in Cádiz Bay (Spain), that 50% sexual maturity was reached at 26.2 cm LT in females and 30.2 cm LT in males, associated with sexual dimorphism during growth, a phenomenon also reported for other species of the family Batrachoididae (Malca *et al.* 2009). More recently, Palazón-Fernández *et al.* (2010), in another study in Cádiz Bay, reported that this species was able to reach sexual maturity at 19.1 cm LT (3 years) in females and 16.0 cm LT (2 years) in males. Palazón-Fernández *et al.* (2001) suggested that the segregation between juveniles and adults may be above the minimum size at sexual maturity or at least in MS II. Thus, our first approximation for *A. porosus* established the minimum size at sexual maturity at 15.3 cm LT, within the lower range for toadfish. However, this does not account for possible differences associated with sex.

The soft bottom of Coliumo Bay may be characterized as areas mainly used by recruits and juveniles of *A. porosus* (see Fig. 4a). The absence of adult individuals may be due to their solitary behavior during this ontogenetic stage, in which individuals are territorial and move towards rocky and protected areas to carry out reproduction, where females lay eggs on the rocky substrates of intertidal and subtidal areas, in pools and

cracks (Mann 1954, Varas & Ojeda 1990), thereby facilitating the parental care of fry (Ruiz 1993) characteristic of toadfishes (Amorim *et al.* 2006).

The occurrence of mature or hydrated females in January 2008 suggests that reproduction and spawning of *A. porosus* occurs in Coliumo Bay during the austral summer period. Varas & Ojeda (1990) also reported the presence of egg masses in intertidal pools off central Chile between the period of January to February. The extension of the reproductive period possibly spans spring and summer months, as seen in coastal fish that couple their biological cycles to months of higher primary production (Castro *et al.* 2000, Hernández-Miranda *et al.* 2003, 2009). A similar reproductive strategy has been reported for the toadfish *H. didactylus* (Palazón-Fernández *et al.* 2001). The marked increase in IG from mature females to hydrated females suggests that once this stage is reached, spawning happens shortly after. This reproductive period is consistent with the presence of smaller-sized individuals in April-May in Coliumo Bay during the 3 autumn seasons sampled (2007, 2008 and 2009). Thus, the reproductive events of *A. porosus* occur mainly in the period January-February, with recruitment following in the period April-May (see Fig. 4a).

NATURAL HYPOXIC EVENT AND ITS IMPLICATIONS

The occurrence of natural hypoxic events and/or areas affected by hypoxic conditions of anthropogenic origin has increased in recent decades (Díaz & Rosenberg 2008, Breitburg *et al.* 2009, Rabalais *et al.* 2010). Vaquer-Sunyer & Duarte (2008) concluded that fish and crustaceans are the most sensitive taxa in terms of: (i) the average lethal concentration of oxygen they are able to withstand, (ii) the sublethal threshold of dissolved oxygen, and (iii) the time necessary to generate a negative response (death). Nevertheless, the response of fish populations exposed to natural hypoxic events over longer time scales is still poorly-known (Grantham *et al.* 2004, Breitburg *et al.* 2009). The intense event of natural hypoxia in January 2008 affected the entire resident community of Coliumo Bay, causing widespread mortality of organisms and a mass stranding event, with fish being one of the most affected groups (Hernández-Miranda *et al.* 2010, 2012). These authors reported a recovery in richness of the fish assemblage in a time scale of only 3 months; nevertheless, densities reached only about half their previous level. At a longer time scale negative effects on the total density of the fish assemblage, including *A. porosus*, were also detected. A similar response pattern was detected in a

reef fish assemblage exposed to hypoxia due to coral spawn 'slicks' off Coral Bay in Western Australia (Halford & Perret 2009).

From an ontogenetic point of view, the greatest percentage of dead individuals of *A. porosus* during the hypoxic event were juveniles with a mode around 8 cm (approximately 1 year old; see Fig. 1b and 3a), similar to what occurred in the stranding event occurred in Coliumo Bay in 1989 (Jara 1992). This is consistent with the reported high sensitivity of juvenile fish when exposed to hypoxia (Nilsson & Östlund-Nilsson 2008). From a physiological point of view, the length-mass relationships allowed us to determine the condition of this segment of the population of *A. porosus*. Estimations of allometric slopes from 3 analyses (pre and post mortality, inter-annual and cohorts) demonstrated some significant differences in *A. porosus* in Coliumo Bay during the massive mortality event in relation to the before and after periods, and in 2009 relative to 2007 and 2008, but not between cohorts. These results, in terms of the cube law for the length-mass relationship (Verdiell-Cubedo *et al.* 2006), suggest temporal changes in the condition of *A. porosus* within Coliumo Bay. However, in all cases this parameter was within the expected range of variability about a theoretical value of 3. Le Cren (1951) suggested that the relative condition factor K_r is of greater use in the study of fish condition. This factor corrects possible errors associated with a non-perfect fit of 3 for all fish, which could be caused by, for example, differences in sex, ontogenetic stages and differential fishing gear. The K_r is not affected by these factors, and results in a better comparative tool (Palazón-Fernández *et al.* 2001). In the case of *A. porosus*, for the 3 analyses carried out there were no significant differences in K_r . This result suggests that, independent of the density decrease and biomass that took place in Coliumo Bay, the physiological condition of *A. porosus* (mainly juveniles) was normal.

It is important to note that hypoxia may affect differentially some species with particular ecological characteristics, such as low dispersion of early life stages and low mobility in adults. *A. porosus* displays (i) a low level of dispersion due to larvae that do not possess a planktonic phase, and once hatched tend to remain close to the rocky substrate where eggs were deposited (*e.g.*, Varas & Ojeda 1990), and (ii) highly territorial adults which carry out parental care during the reproductive period (*e.g.*, Ruiz 1993, Amorim *et al.* 2006), which leads to a restricted home range. These characteristics suggest that *A. porosus* has a limited dispersal potential especially

during the juvenile life stage, given that this age group inhabits soft bottom environments. Because of this, *A. porosus* requires a longer period of time (probably inter-annual scale) for juveniles to segregate and colonize new habitats, in contrast to organisms that undergo dispersion via pelagic larvae, which can establish connection between areas on an intra-annual scale or even within days. Thus, it is likely that juveniles outside Coliumo Bay, not affected by the natural hypoxic event, colonized the bay slowly, with a stepping-stone dynamics (*e.g.*, Neal 2004). Individuals that initially survived the natural hypoxia event, or moved to areas not affected by it, were in very low densities, thus decreasing the encounter probability between reproductive males and females, which ultimately would explain the sustained decrease in population size within Coliumo Bay following the January 2008 mortality event. This is further reflected by the cohorts identified from 2007 onwards (see Fig. 4). Decrease in population size in the juvenile fraction of *A. porosus* may cause a time lag of at least 3 years until the recovery of previous population size. This inference is based on the curves of somatic growth (see Fig. 4a), where following a recruitment event the size at sexual maturity was reached approximately 3 years after the recruitment event.

APHOS POROSUS IN A POPULATION CONTEXT

In the population context, it is probable that following the natural hypoxic event the abundance of *A. porosus* within the bay shifted towards a low or unstable condition (Berryman 1999), further increasing the spatial segregation of individuals, including spawning adults. This underpopulation condition, also known as the Allee effect (Allee 1949, Berryman 2003), may currently dominate *A. porosus* population dynamics in Coliumo Bay. An Allee effect occurs when the fitness of individuals in a small or dispersed population declines when the size or density of the population decreases (Courchamp *et al.* 1999, Stephens *et al.* 1999, Berec *et al.* 2006). The causal factors are diverse, but according to Courchamp *et al.* (1999) they may be classified in 3 categories: (1) inbreeding and loss of heterozygosity which cause a reduction in fitness; (2) demographic stochasticity, including fluctuations in the proportions of sexes; and (3) reduction in cooperative interaction (obligatory or facultative) when there are few individuals, including reproduction and anti-predator strategies. Operationally, a low reproductive rate will generate low recruitment and successive low rates of reproduction will lead the population to an extinction

vortex and finally to collapse. The categories mentioned above may act individually or simultaneously as demonstrated by Berec *et al.* (2006), for instance: (i) difficulty in finding mates when population size or density is low, (ii) when population size decreases, vulnerability to predators increases, and (iii) increase in the selfing rate and/or a decrease in number of matings among small and inbred populations. In the case of *A. porosus*, given its lack of gregariousness, especially in the adult state, the proposed Allee effect may be associated with proto-cooperation mechanisms (Courchamp *et al.* 1999). Here, the effect is evident at the moment of reproduction, in which low population size decreases the rate of encounter among reproductive individuals, reducing the final success of the total reproduction of the population. Below this critical threshold of stability the population trajectory may lead to local extinction as a result of a decline in reproduction, survival or both. In marine environments this has even been documented in benthic invertebrates with pelagic larval stages (Knowlton 1992). However, it is

difficult to demonstrate an Allee effect quantitatively (Courchamp *et al.* 1999). A first step is to identify the mechanisms and factors which cause a population to cross its extinction threshold, for example, due to natural catastrophes (Lande 1993, 1998) in which population sizes are reduced drastically. Courchamp *et al.* (1999) and Stephens *et al.* (1999) developed a theoretical mathematical model of population dynamics of the necessary conditions to produce an Allee threshold in which a population is driven to local extinction. We hypothesize that after the hypoxic event the population of Coliumo bay *A. porosus* appears to have crossed this threshold. The incorporation of the estimated values of population growth for *A. porosus* for the years 2007-2010 in Courchamp *et al.* (1999) and Stephens *et al.* (1999)'s model (see Fig. 6) shows that the growth rate of the population after the hypoxia is in the unstable zone and has probably fallen under the extinction threshold.

In the absence of new severe environmental

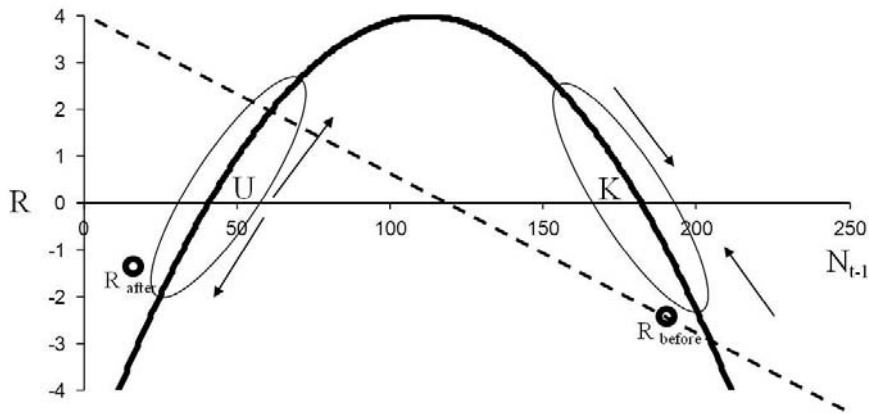


Figure 6. Conceptual model of population dynamics, modified from Courchamp *et al.* (1999) and Stephens *et al.* (1999). R is the realized per capita rate of change of the population. $R = \ln N_t - \ln N_{t-1}$ (see Berryman 1999). N_t is the density of recruits for the April-May periods of the years 2007, 2008 and 2009. U is the zone of unstable equilibrium or extinction threshold. Below this zone ($N_t < U$) the population may go extinct. K is the zone of stable equilibrium or carrying capacity, in which the population size oscillates around the value $N_t = K$. The ellipses indicate the areas of influence of each of the equilibrium points; arrows indicate the most probable direction of R when population size is in these areas. The dashed line corresponds to a theoretical negative linear relationship between R and population size (classical density-dependent model). The humped curve is a theoretical non-linear relationship between R and population size (inverse density-dependent model with Allee effect). Circles indicate the calculated R values for the periods 2007-2008 (before hypoxia: $N_{t-1}=184$, $R=-2.73$) and 2008-2009 (After hypoxia: $N_{t-1}=12$, $R=-1.39$). Modelo conceptual de dinámica poblacional, modificado a partir de Courchamp *et al.* (1999) y Stephens *et al.* (1999). R, corresponde a la tasa per cápita de cambio poblacional realizada, donde $R = \ln N_t - \ln N_{t-1}$ (Ver Berryman 1999). N_t , corresponde a la densidad de reclutas para cada periodo abril-mayo de los años 2007, 2008 y 2009. U, corresponde a la zona de equilibrio inestable o umbral de extinción. Bajo esta zona ($N_t < U$), la población puede llegar a una extinción. K, corresponde a la zona de equilibrio estable o capacidad de carga en donde la población oscila con un tamaño poblacional en torno al valor de $N_t = K$. Las elipses señalan las zonas de influencia de cada uno de los puntos de equilibrio y las flechas señalan la dirección más probable que tomará R luego de que el tamaño poblacional se ubique en dichas zonas. La línea segmentada corresponde a una relación negativa teórica lineal entre R y el tamaño poblacional (modelo clásico de denso-dependencia). La curva jorobada corresponde a una relación teórica no-lineal entre R y el tamaño poblacional (modelo de denso-dependencia inversa con un efecto Allee). Los círculos señalan los valores de R calculados para los periodos 2007-2008 (Antes de la hipoxia: $N_{t-1}=184$, $R=-2,73$) y 2008-2009 (Después de la hipoxia: $N_{t-1}=12$, $R=-1,39$)

perturbations the abundance of *A. porosus* population in Coliumo Bay should increase slowly over time. In this recovery, the connectivity of Coliumo Bay and nearby non-affected areas should play a fundamental role.

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