

Impact of oceanic upwelling on morphometric and molecular indices of an intertidal fish *Scartichthys viridis* (Blenniidae)

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Upwelling is a determinant factor for intertidal community structure on all coasts where it occurs. Most of the evidence has been obtained from sessile species or those with limited mobility and it is still unknown whether nutrients are transferred from primary producers to upper trophic levels in upwelling systems. We studied a fish species from two localities in Central Chile, Quintay, and Las Cruces, the former affected by upwelling and the latter not as evidenced by sea surface temperatures. Specimens of the herbivorous fish *Scartichthys viridis*, from the two sites were compared for weight–length relationships and RNA:DNA ratio in muscle tissue. The results showed that in the upwelling zone, fish increase their weight faster and have greater RNA:DNA ratios than those from the non-upwelling zone. This suggests that nutrient subsidies can alter the performance of key intertidal vertebrates such as *S. viridis*. The consequences of this effect on community structure and dynamics are not known.

Keywords: upwelling; fish; intertidal; RNA:DNA; body size; SST; *Scartichthys viridis*

Introduction

Upwelling is an oceanographic condition that can be intermittent or persistent, upwelling waters are colder and richer in nutrients than the surface waters they replace (Strub et al. 1998; Poulin et al. 2002a, 2002b; Palumbi 2003; Narváez et al. 2004); this coastal phenomenon causes dramatic effects on nutrient availability as well as herbivore and predator activity (Menge et al. 2004; Nielsen and Navarrete 2004; Wieters 2005; Thiel et al. 2007). Upwelling can regulate rocky shore communities by modifying the strength of positive interactions between algae and mussels (Menge 2000; Menge et al. 2003; Wieters 2005). In New Zealand, intermittent upwelling on the west coast increases ecologically significant processes such as prey growth, abundance of consumers and rates of predation. Compared to the downwelling observed in the east coast, the entire intertidal ecosystem moves at a

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faster pace on the New Zealand west coast (Palumbi 2003). Upwelling on the Chilean coast has been associated with increased abundance and faster growth rates of algae (Broitman et al. 2001; Nielsen and Navarrete 2004), with no effects on the diversity of intertidal and subtidal organisms (Vasquez et al. 1998).

Dahlhoff (2004) describes the effects of environmental variability on community dynamics in terms of two complementary models; the environmental stress model and the nutrient/productivity model. Environmental stress models assume that community structure results from species interactions and their modulation by the underlying gradients of environmental stress, whereas nutrient/productivity models emphasize the role of bottom-up factors (nutrients, productivity) as the determinants of species interactions. Under both models, environments with the harshest physical conditions or the lowest productivity will have simple communities with structure determined directly by severe stress or nutrient shortage. The moderation of environmental conditions leads to increased abundance, more complex trophic structure and increased influence of species interactions on community structure (Menge 2000; Menge and Branch 2001; Menge et al. 2002). Consistent with these predictions, invertebrates grow faster and cover a wider intertidal rock surface in sectors with nutrients subsidies (e.g. upwelling) than in similar habitats on a non-upwelling coast (Menge et al. 2003, Palumbi 2003).

Among the biochemical indicators for determining nutritional condition and metabolic activity *in situ*, RNA to DNA ratio (RNA : DNA) is the most widely used index to determine field organism condition (Chícharo and Chícharo 2008). This index measures the synthetic capacity and is usually correlated with nutritional status under a given environmental condition (Buckley and Caldarone 1999). Organisms in good condition, such as species inhabiting upwelling zones, therefore tend to have higher RNA : DNA ratios (Bulow 1987; Robinson and Ware 1998). This index has been used on a wide range of marine organisms such as plankton, phytoplankton (Dortch et al. 1983), zooplankton (Sutcliffe 1965; Ikeda et al. 2007), larval fish (Bulow 1987; Caldarone et al. 2003), juvenile and adult fish (Bulow 1970; Thorpe et al. 1982), bivalves (Grémare and Vétion 1994; Chícharo and Chícharo 1995; Chícharo et al. 2001), cephalopods (Clarke et al. 1989; Sykes et al. 2004), and crustaceans (Lemos et al. 2002).

Most of the evidence showing upwelling as a determinant factor of intertidal community structure has been obtained from sessile organisms or those with limited mobility (e.g. limpets, mussels, littorines, barnacles, whelks) (Dahlhoff and Menge 1996; Menge 2000; Menge et al. 2004; Wieters 2005). Fishes have not been studied as determinants of community structure and dynamics despite the strong effects they exert on intertidal algae and invertebrates (Horn et al. 1999; Ojeda and Muñoz 1999), thus the effect of upwelling on their biology remains unknown. Evidence on the growth and mortality of intertidal fishes associated with changes in nutrient availability during the climatic variation as a result of El Niño and La Niña events indicates that irrespective of the trophic level, fish growth rates decrease with El Niño and increase during La Niña events, suggesting that bottom-up nutrients drives fish growth at multiple levels (Hernández and Ojeda 2006; Carstensen et al. 2010). Whether upwelling determines the conditional status of intertidal fishes, and hence affects the ecological performance of algae predators, is not known. To address this, we studied the physiological status of the highly mobile and abundant herbivorous intertidal fish *Scartichthys viridis* (Muñoz and Ojeda 1997, 1998; Ojeda

and Muñoz 1999; Pulgar et al. 2005; 2007) in the upwelling (Quintay) and the non-upwelling (Las Cruces) localities of the central Chile coast (Wieters 2005).

Materials and methods

Specimen collection and temperature recording

Two zones on the Central Chile coast were studied. One with reported upwelling (U = upwelling); Quintay (33°11'S, 71°43'O), and the other not affected by upwelling (NU = non-upwelling); Las Cruces (32°00'S, 71°00'W). These localities were selected because they represent extremes in nutrient availability, as indicated by Thiel et al. (1997), Wieters et al. (2003), and Wieters (2005). The selected low intertidal rock pools in both localities were located no more than 1–2 m above the low-tide mark. The seawater of these pools was consequently renewed during every tidal cycle. The temperature of this environment was recorded to allow assessment of its relationship to potential effects on the morphology and molecular response of *S. viridis*. Data on onshore sea surface temperature (SST) were obtained by recording surface water temperature at 20 min intervals with Optic Stowaway (Onset Computer ± 0.1°C precision) submersible temperature loggers placed at approximately 1 m depth below the lowest low tide at U and NU zones (Data provided by Dr S. Navarrete, ECIM). SST is often used as a proxy for nutrient concentration (Wieters 2005) and to characterize upwelling effects on coastal marine ecosystems (Barth et al. 2007). Data from recordings made in 2008 and 2009 during the same study season (winter) were pooled for analysis.

Processing of specimens

Specimens were captured at the same tide cycle in winter 2009, using BZ20 anesthetic. We used two approaches to determine fish condition in both zones; a morphometric approach based on the weight–body size relation comparisons, and a molecular approach based on RNA:DNA ratio determinations. For the morphometric analysis we used 51 fish from the upwelling zone and 143 fish from the non-upwelling zone. Total fish length (L) and body mass (W) were recorded using a caliper (0.1 cm) and an electronic balance (0.1 g). For the molecular approach we used 18 individuals per site. Individuals were captured and immediately deposited in liquid nitrogen, transported to the laboratory, and kept frozen until analysis. The extraction of RNA and DNA was performed using TRIZOL[®] Reagent, which is a ready to use reagent for the isolation of total RNA from cells and tissues (Chomczynski and Sacchia 1987). We extracted 200 mg of muscle from the back of the pectoral fin and between the high dorsal and belly of each individual. During the homogenization of the sample previously extracted, TRIZOL[®] Reagent maintains the integrity of the RNA, while disrupting cells and dissolving cell components. The addition of chloroform followed by centrifugation separates the solution into an aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After the transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol. After the removal of the aqueous phase, the DNA in the interface can be recovered by sequential precipitation (Chomczynski 1993). After extraction the RNA and DNA were reconstituted in 50 and 900 µL of nuclease-free water, respectively. Both RNA and DNA were quantified spectrophotometrically to

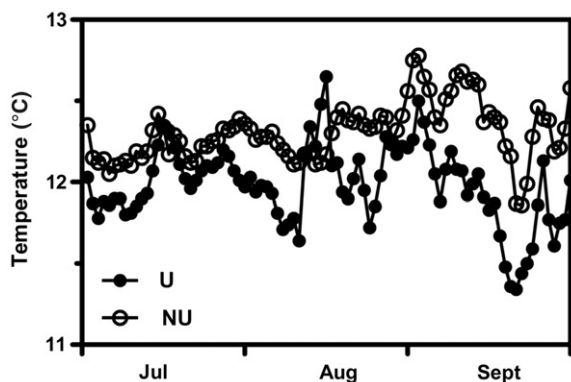


Figure 1. Average of daily SST during 2008 and 2009 in study zones from Central Chile. U, upwelling zone; NU, non-upwelling zone.

260/280 nm (Perkin Elmer Lambda Bio L7110184) and expressed as microgram per microliter, corrected for body and sample size.

Statistical analysis

A Kolmogorov–Smirnov test was used to compare body size frequency distribution of fish in the total sample and in those used for RNA : DNA ratio analysis. We used linear regression and ANCOVA to compare weight (g) : length (cm) relationships and RNA : DNA ratios between U and NU fish, in relation to fish length (Zar 1996). An ANOVAS (GLM) was used to compare the weight : length ratio between U and NU fish. A significant level of $p < 0.05$ was chosen.

Results

Temperature

Recording of SST indicated that the temperature of the upwelling site was consistently colder than that of the non-upwelling site during the study period. Figure 1 shows data from 2 years combined (2008 and 2009).

Morphometric analysis

Frequency of body size distribution for total individuals sampled, indicated no differences between upwelling and non-upwelling fish (Kolmogorov–Smirnov: $p > 0.1$, max diff. -4 , Figure 2). However, the comparisons of weight–length relationships among upwelling and non-upwelling *S. viridis*, indicated a greater weight gain rate in relation to body size in upwelling fish ($F_{1,191} = 6.66$, $p = 0.010$, Figure 3A, upwelling slope = 6.07, non-upwelling slope = 2.06). Moreover, the comparison of weight : length ratios between fish from each site revealed that upwelling fish exhibited greater weight per body size unit (W : L ratio) than those from the non-upwelling site ($F_{1,192} = 30.4$, $p = 0.001$, Figure 3B).

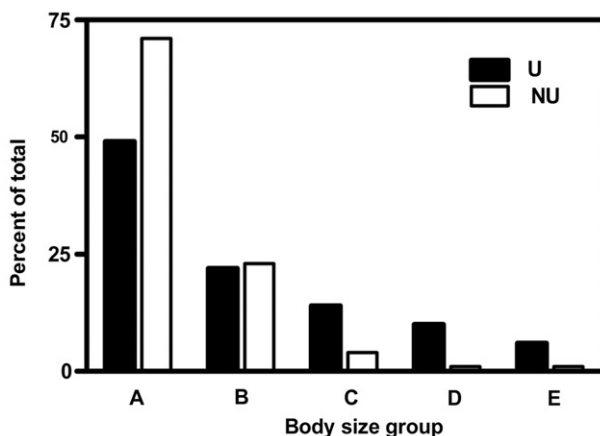


Figure 2. Body size distribution of *S. viridis* in upwelling and non-upwelling zones in Central Chile. Body lengths were distributed in size groups (A, 4.9–7.4 cm; B, 7.5–10 cm; C, 10.1–12.6 cm; D, 12.7–15.2 cm; E, 15.3–17.8 cm) for upwelling (U, ■, $n = 51$) and non-upwelling (NU, □, $n = 145$) fish and compared as percent of total animals analyzed per site.

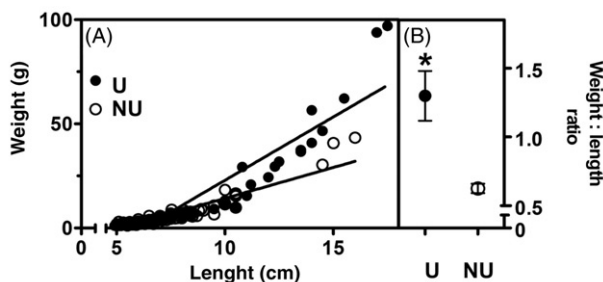


Figure 3. Weight to body size relationships in upwelling and non-upwelling *S. viridis* from Central Chile. (A) Upwelling fish (U, ●): $\text{Weight} = 6.07 \cdot \text{length} - 37.85$, $R^2 = 0.84$, $F_{(1,46)} = 244.81$; $p = 0.001$. Non-upwelling fish (NU, ○): $\text{Weight} = 2.96 \cdot \text{length} - 15.34$, $R^2 = 0.87$, $F_{(1,141)} = 913.5$; $p = 0.002$. (B) Weight to length ratio between upwelling (U, ●, $n = 51$) and non-upwelling (NU, ○, $n = 143$) fish. Bar indicate \pm SEM, * $p < 0.05$.

Molecular analysis

Frequency of body size distribution for the individuals used in the RNA : DNA ratio analysis indicated no differences between upwelling and non-upwelling fish (Kolmogorov–Smirnov: $p > 0.1$, max diff. -2). The RNA : DNA ratio in relation to body size was significantly higher in upwelling fish than non-upwelling fish ($F_{1,33} = 4.21$, $p = 0.04$, Figure 4A), with greater values for overall RNA : DNA ratio/length in upwelling compared to non-upwelling fish (2.3 ± 0.5 vs. 1.2 ± 0.2 , $p < 0.05$, Figure 4B)

Discussion

Our results support the hypothesis that the higher food availability in upwelling zones is associated with the transfer of nutrients to higher trophic level predators

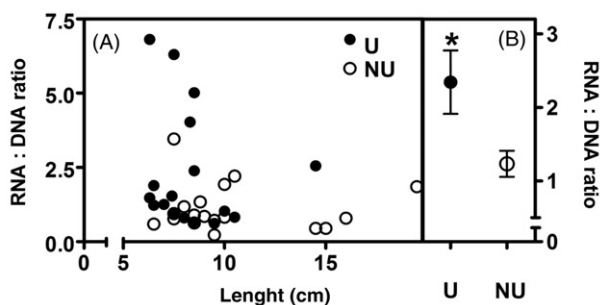


Figure 4. RNA to DNA ratio and its relationship to body size in upwelling and non-upwelling *S. viridis* from Central Chile. (A) RNA:DNA ratio and body length in upwelling (U, ●) and non-upwelling (NU, ○) fish. (B) Summary of RNA:DNA ratio values in upwelling (U, ●, $n = 18$) and non-upwelling (NU, ○, $n = 18$) fish, $*p < 0.05$.

such as fishes. At the morphological level, *S. viridis* from upwelling zones display greater weight gain, whereas at the molecular level, a greater RNA:DNA ratio was observed in fish from upwelling zones.

Upwelling intensity along the central Chile coast has been reported and a direct relationship between water temperature and nutrient availability was observed (Wieters 2005). Our temperature data showed persistent differences between both study zones: Las Cruces displays higher temperature than Quintay during the study period. This local oceanographic scenario allows the classification of Quintay and Las Cruces as upwelling and non-upwelling zones, respectively.

The morphological evaluations of fish, using frequency distribution of body size and weight–body size relationships, indicate that the increase in weight in the upwelling fish was higher compared with non-upwelling fish (Figure 3A and B). This result suggests that there was more food available in upwelling zones, for example, Chlorophytes and Rhodophytes (Caceres et al. 1994; Vasquez et al. 1998; Ojeda and Muñoz 1999), to be transferred to herbivorous fishes such as *S. viridis*. Previous studies performed in equivalent upwelling and non-upwelling zones in Central Chile reported transfer of nutrients to primary producers (Broitman et al. 2001; Nielsen and Navarrete 2004; Wieters 2005), these studies did not considered higher trophic level predators such as herbivorous fishes.

At the molecular level, upwelling *S. viridis* displays higher RNA:DNA ratio related to body size (Figure 4A). The RNA:DNA ratio is considered as an *in situ* indicator of the physiological status, because of its association to nutritional condition and growth in several marine organisms (Buckley and Caldarone 1999; Chicharro and Chicharro 2008). A higher RNA:DNA ratio in the upwelling fish suggests greater protein synthetic activity producing higher weight gain. This indicates that at the same body size the upwelling fish are in better condition (e.g. weight gain, growth rate, reproduction, and survival) compared to the non-upwelling fish. To our knowledge this represents the first evidence of the effect of an upwelling nutrient subsidy on the nutritional status of an herbivorous fish. This is particularly significant because *S. viridis* is an important ecological component of its intertidal communities (Muñoz and Ojeda 1998).

Understanding the mechanisms by which environmental variability modifies physiological performance of organisms in nature is of great interest when

considering the foundations of community dynamics (Hofmann and Somero 1995; Parmesan and Yohe 2003; Dahlhoff 2004). Given the important role that predators and herbivores play in the structure of intertidal communities (Paine 1966; Dayton 1971; Menge 1976; Lubchenco 1978), the assessment of the physiological condition of ecologically significant consumers is essential for an understanding of how the environmental variation (e.g. nutrients subsidy) affects the performance or fitness of these consumers (Menge et al. 2002). Due to higher weight gain (Figure 3B) and RNA:DNA ratio (Figure 4B) in the upwelling condition, it is expected that upwelling *S. viridis* would exert a more significant influence on intertidal community dynamics than non-upwelling fish. This is in agreement with nutrient/productivity model's predictions, where with increased productivity, both prey and their consumers will be increasingly well-off nutritionally (Palumbi 2003).

Ecological measurements such as growth and feeding rates, as well as reproductive output should therefore grow with increased productivity (Menge et al. 2003; Wieters 2005). For example, growth rates of sessile invertebrates influence community structure and prey availability by altering the outcome of competition (Menge et al. 2003). Our results indicate that upwelling fish had double the weight gain compared with non-upwelling fish (Figure 3A,B). Because individual performance (strength, activity levels, growth, feeding, survival, and reproduction) ultimately depends on physiological status, the links between ecological individual performance and physiological status need to be uncovered for a full understanding of the effect of a predator on community structure and dynamics.

Upwelling sites represent physically complex habitats for the energy balance of individuals. The higher nutrient availability increases metabolic processes (Palumbi 2003; Wieters 2005), and at the same time lower temperature has direct consequences on physiology, behavior, foraging, and general ecology, through its effects on vital rates (Sanford 2002, Pulgar et al. 2005). Non-upwelling sites may impose energetic restrictions, associated with higher temperature and lower nutrient availability. *Scartichthys viridis* in upwelling and non-upwelling zones may have different optima for maximizing metabolic scope, the amount of energy available for activity and food processing (Kelsh and Neill 1990). Such optimum temperature is often, but not always, near to that providing optimal growth (Casselman 1996). Greater growth rates in upwelling sites suggest that nutrient availability is more important than thermal variation in setting up the synthetic capacity and growth in *S. viridis*. Finally, our morphological and molecular evidence suggests that nutrient subsidy could alter the predatory performance of an herbivorous fish, which is important in intertidal communities along the Central Coast of Chile. The consequence for the structure of those communities remains unknown. Integrative approaches dealing with the effects that environment may have on individual biological responses are necessary and urgent in countries located in extreme geographic zones that are considered sentinels of the effects of global change on biodiversity.

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