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# Upwelling affects food availability, impacting the morphological and molecular conditions of the herviborous limpet *Fissurella crassa* (Mollusca: Archeogastropoda)

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*Oceanographical processes, such as upwelling, induce variations in nutrient availability in marine ecosystems, and evidence indicates that nutrient input can strongly influence the physiological activities, structure, and dynamics of marine communities. Intertidal organisms have long been considered ideal study units in which to quantify the relationship of physical variations and differential energy allocations in specimens that undergo environmental variations, such as observed with nutrient availability. In habitats with differential nutrient input (upwelling versus non-upwelling), both food availability (algae abundance) and seasonal gonadal and foot weight variations were determined in the keyhole limpet* Fissurella crassa. *Gonadal weight is used as a measure of reproduction allocation whereas foot weight is an indirect indicator of energy allocation towards survival. RNA:DNA ratio in limpets was used as an indicator of biosynthetic capability. Our results indicate that, in general, algae abundance, muscular foot weight, and gonadal weight were higher in upwelling sites during all seasons studied. The same result was found for RNA:DNA ratios. Energetic allocation in animals that inhabit intertidal upwelling habitats supported a constant allocation towards reproduction and soft tissues. In contrast, animals that inhabit non-upwelling habitats showed important energetic restrictions associated with higher water temperature and lower food availability. Our results clearly show that in the keyhole limpet* F. crassa *food availability is a more important determinant of an individual's condition than a physical variation such as environment temperature.*

**Keywords:** RNA:DNA ratio, life histories, food availability, keyhole limpet, upwelling

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## INTRODUCTION

Oceanographical processes such as upwelling induce variability in nutrient availability in marine ecosystems, and evidence indicates that nutrient input can strongly influence the structure and dynamics of marine communities (Menge *et al.*, 2003).

At a local scale, biological interactions, such as competition and predation, are determinants of the intertidal communities' functioning (Paine, 1966; Dayton, 1971; Menge, 1976; Lubchenco, 1978). However, scarce evidence exists about how the physiological condition and performance of consumers is affected by environmental variations (e.g. upwelling versus non-upwelling) and the assemblage of prey. Understanding the variability in the physiological performance of consumers in nature is of great interest, given the important role that consumers play in the structure of intertidal communities (Paine, 1966; Dayton, 1971; Menge, 1976; Lubchenco, 1978; Menge *et al.*, 2002).

The upwelling phenomenon affects many physiological activities at different trophic levels (Menge *et al.*, 2004; Nielsen & Navarrete, 2004; Wieters, 2005; Thiel *et al.*, 2007). Evidence of the ecological impact of upwelling indicates that invertebrates and algae grow faster and cover far more of the rocky intertidal surface in upwelling than in non-upwelling habitats (Menge *et al.*, 2003; Palumbi, 2003). Moreover, biological interactions are modulated by upwelling, and these responses are associated with modifications on all biological levels, from molecular (i.e. biosynthetic capability) to population and community processes (i.e. growth rate and reproductive aggregations) (Menge *et al.*, 2003; Palumbi, 2003; Wieters, 2005; Pulgar *et al.*, 2011). All

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evidences are in agreement with the predictions of nutrient/productivity models stating that with increased productivity (bottom-up control), both prey (invertebrates and algae) and consumers will become increasingly well off nutritionally (Broitman *et al.,* 2001; Palumbi, 2003; Nielsen & Navarrete, 2004; Wieters, 2005).

The impact of bottom-up control represents an interesting situation as animals continuously cope with environmental fluctuations through behavioural, physiological, and structural adjustments to ensure appropriate function (Wiener, 1992; Bellard *et al.,* 2012). These adjustments imply that natural selection acts to maximize individual fitness and that trait combinations are constrained by trade-offs, as explained by life history theory (Fisher, 1930; Roff, 2002). Classic reported trade-offs indicate that an increased reproductive effort can enhance reproductive success through improved growth and survival, but at the same time, it may compromise adult survival (Dijkstra *et al.,* 1990; Roff, 2002; Hanssen *et al.,* 2005).

In the intertidal system, food supply and seawater temperature have been described as determinant factors for the physiological conditions of heterotrophic organisms (Palumbi, 2003; Wieters, 2005; Lesser *et al.,* 2010; Pulgar *et al.,* 2011). On the Chilean coast, molecular and morphological evidence has only recently been obtained concerning the impact of upwelling on the physiological responses of animals, showing that in upwelling locations, the intertidal herbivorous fish *Scarthychthys viridis* displays a higher biosynthetic capability (e.g. RNA:DNA ratio and body size) compared to conspecifics from a non-upwelling site (Pulgar *et al.,* 2011). To address the effects of upwelling on the consumer–resource interaction, we measured the temporal variation of algal cover as well as the morphological and molecular responses in the herbivorous limpet *Fissurella crassa* (Lamarck, 1882) at both upwelling and non-upwelling sites in central Chile. *Fissurella crassa* is one of the most abundant intertidal (Pulgar *et al.,* 2012), herbivorous species, and it represents the greatest catch per unit effort among keyhole limpets caught by shell fishermen (Oliva & Castilla, 1986).

## MATERIALS AND METHODS

### Localities

Two zones on the Central Chilean coast were studied. Quintay (33°11′0S 71°43′W) has reported upwelling (U, upwelling), whereas Las Salinas (32°00′S 71°00′W) is not affected by upwelling (NU, non-upwelling). These localities were selected because they represent extremes in nutrient availability (Wieters, 2005; Thiel *et al.,* 2007). In both studied sites, we had previously reported lower seawater surface temperature (SST) in U with respect to NU during all temporal evaluations (Pulgar *et al.*, 2011), and lower temperature is associated with high nutrient availability in sampled sities (Wieters, 2005). All sampling was performed in the low intertidal zone during a similar low tide.

### Food availability

Seasonal food availability and variations for the predator *Fissurella crassa* in the low intertidal zone of U and NU study sectors were estimated along 100 m long transects parallel to the coast. In these transects, the abundances of *Mazzaella* spp. and *Ulva* spp. were estimated using randomly selected 50 × 50 cm quadrats (winter U = 27, NU = 10; spring U = 6, NU = 3; summer U = 13, NU = 10). In each quadrant, the macroalgae cover of *Mazzaella* spp. and *Ulva* spp. were determined as these algal items are those most frequently consumed by *F. crassa* (Aguilera 2011; Aguilera & Navarrete, 2011).

### Limpet morphology

Individuals of *F. crassa* were sampled during the winter, spring and summer seasons. Abundances reflect availability of individuals in the field (winter U = 109, NU = 26; spring U = 119, NU = 20; summer U = 39, NU = 119), with a total of 267 U and 165 NU limpets sampled. Limpets were sampled from the low intertidal zone during the winter, spring and summer from each sector, and deposited in labelled plastic bags for transport to the laboratory. Soft tissue and gonadal biomass (g) of limpets were estimated using an analytic balance (+/− 0.01 g precision). We considered soft tissue biomass as a direct estimate of growth capacity, and gonadal biomass as an indirect estimator of the reproductive tissue investment of *F. crassa*. The maximum shell length (cm) of limpets was measured using a digital caliper (Mitutoyo) (+/− 0.01 mm).

### Molecular analysis

A total of 20 individuals of similar body size (mean= 5.03 cm (0.34 standard error of the mean) were sampled from the low intertidal zone during the spring from U and NU sites (U = 10, NU = 10) for molecular analysis. We used spring limpets because in this season upwelling is more intense on the Pacific South American coast (Hernández-Miranda *et al.*, 2003). Limpets were captured, immediately deposited in liquid nitrogen, transported to the laboratory, and kept frozen until analysis. The extraction of RNA and DNA was performed using the TRIZOL® Reagent, which is a ready-to-use reagent for the isolation of total RNA from cells and tissues (Chomczynski & Sacchi, 1987). We extracted 200 mg of muscle from the foot tissue of each limpet. During the homogenization of the previously extracted sample the 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. Exclusively RNA remains 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). Following extraction, the RNA and DNA were reconstituted in 50 and 900 ml of nuclease-free water, respectively. Both RNA and DNA were quantified spectrophotometrically at 260/280 nm (Perkin Elmer Lambda Bio L7110184) and expressed as microgrammes per microlitre, correcting for body and sample size.

### Statistical analysis

A two-way analysis of variance (ANOVA) (general linear models) test was used to compare algal abundance from study sites and seasons. As the biomass of soft and gonadal tissue increases significantly with the limpet's maximum shell length, we compared the residuals of these relationships between study sites and seasons through two-way ANOVA (general linear models). Finally, one-way ANOVA (general linear models) was used to compare the RNA:DNA ratio in similar sized limpets from the spring season. Prior to analysis, data normality was evaluated. The data expressing proportions (i.e. RNA:DNA ratio), were transformed to arcsine. A Tukey *a-posteriori* test was used to assess specific differences between factor levels. A significant level of $P < 0.05$ was selected for rejection as a null hypothesis of no significant difference (Zar, 1996).

# RESULTS

## Food availability

Food availability (% algae cover) for *Fissurella crassa* was higher at the U site during the studied seasons (Figure 1). However, a significant increase in food availability to *F. crassa* was detected in U as compared to NU areas during the spring and summer (Figure 1; Table 1).

## Limpet morphology

Throughout all seasons studied a greater gonadal weight was observed at the U site compared to the NU site (Table 2). In general, soft tissues weight and limpet's size were also higher at the U site (Table 2).

Analyses of tissues of *F. crassa* indicate that limpets from the U site showed a greater soft and gonadal tissue biomass than the NU animals (Figure 2; Table 3). The residual soft tissue biomass was higher in the U than in the NU limpets during all seasons (Figure 2A: Tukey *a-posteriori* test). Similar results were found in gonadal tissue biomass (Figure 2B: Tukey *a-posteriori* test), revealing a greater energetic investment in soft and reproductive tissue production in U than NU animals (Figure 2 A, B).

## Molecular analysis

A significantly higher RNA:DNA ratio was observed in tissues obtained from U than in NU limpets. This evidence indicates a higher biosynthetic capability of U limpets as compared to NU limpets (Figure 3; Table 4).

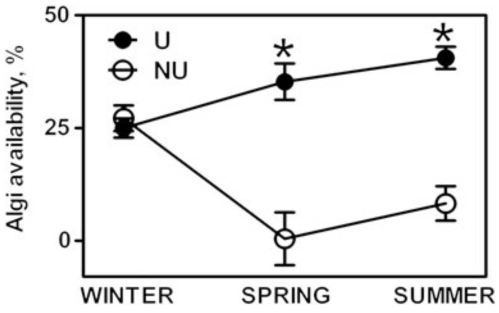

**Fig. 1.** Algal cover in the different seasons and sites. Bars indicate ± 1 standard error of the mean. *, significant differences; U, upwelling; NU, no-upwelling.

**Table 1.** General linear model (two-way analysis of variance), results comparing food availability (*Ulva* spp. and *Mazzaella* spp. coverage) between locations (upwelling and non-upwelling) and seasons (winter, spring, and summer). df, degree of freedom; MS, mean square.

| Effect | df | MS | F | *P* |
|---|---|---|---|---|
| Locality (L) | 1 | 123.3 | 0.15 | 0.6962 |
| Season (S) | 2 | 6324.6 | 7.83 | 0.0004 |
| L * S | 2 | 7250.4 | 8.98 | 0.0001 |
| Error | 269 | 806.9 | | |

**Table 2.** Basic morphological description of the keyhole limpet in sampled zones (U, upwelling; NU, non-upwelling), during the three seasons studied. Results are expressed as mean ± standard error of the mean (SEM).

| | Sampled zone/season | Mean (SEM) |
|---|---|---|
| Gonadal weight (g) | U/summer | 10.43 (0.17) |
| | U/winter | 7.18 (0.06) |
| | U/spring | 7.30 (0.05) |
| | NU/summer | 1.22 (0.03) |
| | NU/winter | 2.71 (0.25) |
| | NU/spring | 1.06 (0.33) |
| Soft tissue (g) | U/summer | 45.45 (0.36) |
| | U/winter | 27.34 (0.13) |
| | U/spring | 26.93 (0.11) |
| | NU/summer | 12.37 (0.07) |
| | NU/winter | 19.30 (0.547) |
| | NU/spring | 11.32 (0.71) |
| Limpet size (mm) | U/summer | 67.69 (0.33) |
| | U/winter | 56.67 (0.11) |
| | U/spring | 55.34 (0.10) |
| | NU/summer | 41.35 (0.06) |
| | NU/winter | 51.36 (0.50) |
| | NU/spring | 47.43 (0.33) |

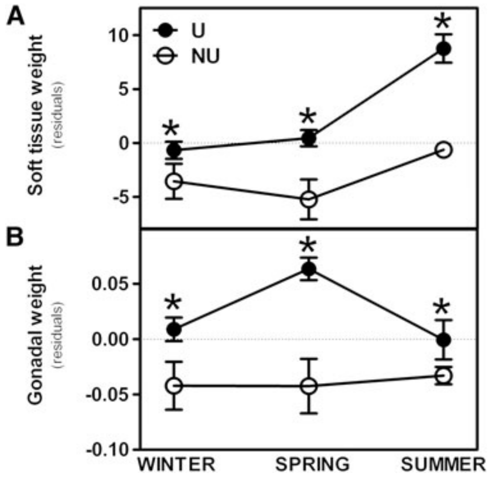

**Fig. 2.** (A) Soft tissue biomass and (B) gonadal tissue biomass of *Fissurella crassa* between locations and seasons. Bars indicate ± 1 standard error of the mean. *, significant differences; U, upwelling; NU, no-upwelling.

# DISCUSSION

Our results on the impact of upwelling on organisms showed greater algal abundance in U sectors, suggesting that upwelling supports greater food availability for the herbivorous

**Table 3.** General linear model (two-way analysis of variance), results comparing soft tissue and gonadal tissue biomass of *Fissurella crassa* between locations (upwelling and non-upwelling) and seasons (winter, spring, and summer). df, degree of freedom, MS, mean square.

| Effects | df | MS | F | *P* |
|---|---|---|---|---|
| **Sof tissue biomass** | | | | |
| Locality (L) | 1 | 2161.54 | 31.69 | 0.001 |
| Season (S) | 2 | 1316.34 | 19.29 | 0.001 |
| L * S | 2 | 185 | 2.71 | 0.067 |
| Error | 506 | | | |
| **Gonadal tissue** | **df** | **MS** | **F** | *P* |
| Locality (L) | 1 | 0.193 | 15.86 | 0.001 |
| Season (S) | 2 | 0.017 | 1.45 | 0.234 |
| L * S | 3 | 0.131 | 2.52 | 0.077 |
| Error | 505 | 0.012 | | |

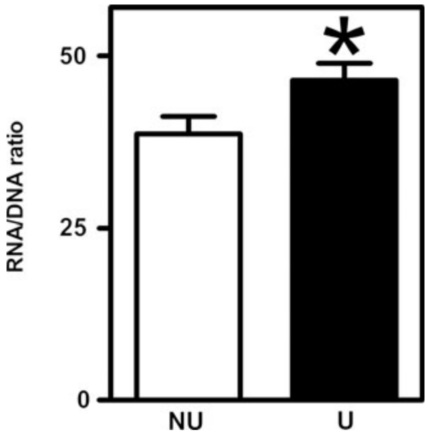

**Fig. 3.** Arcsine RNA:DNA ratio between locations. Bars indicate ± 1 standard error of the mean. U, upwelling; NU, no-upwelling.

limpet *Fissurella crassa*. In addition, the higher soft tissue and gonadal biomass shown by *F. crassa* in these U sectors suggests an increased energetic investment in growth and reproduction during the seasons evaluated. These results are strengthened through the molecular data, where a higher RNA:DNA ratio was registered in U than in NU limpets.

To characterize U zones, a direct relationship has been detected between seawater temperature and nutrient availability, with lower seawater temperature being associated with higher nutrient availability (Wieters, 2005). Our current data regarding the annual seawater temperature for the localities observed is in agreement with previous results of our group which indicate that Quintay, our U zone, displays a lower temperature than Las Salinas (Pulgar *et al.,* 2011). Upwelling is directly related to nutrient availability and evidence indicates that both prey and their consumers will be increasingly well off nutritionally in these conditions (Broitman *et al.,* 2001; Palumbi, 2003; Nielsen & Navarrete, 2004; Wieters, 2005; Pulgar *et al.,* 2011). For primary producers, it has been suggested that corticated algae are most abundant at sites of high upwelling intensity, whereas ephemeral algae predominate sites of low upwelling intensity (Nielsen & Navarrete, 2004). Our study sectors represent extremes in food availability for intertidal herbivorous invertebrates, such as the limpet *F. crassa*.

**Table 4.** General linear model (one-way analysis of variance), results comparing RNA:DNA ratio of *Fissurella crassa* between sampled locations (upwelling and non-upwelling). df, degree of freedom; MS, mean square.

| Effect | Df | MS | F | *P* |
|---|---|---|---|---|
| Locality | 1 | 302.51 | 4.80 | 0.041 |
| Error | 18 | 62.90 | | |

Animals continuously cope with environmental fluctuations through behavioural, physiological and structural adjustments to ensure appropriate function (Green, 1989; Wiener, 1992; Urrejola *et al.,* 2011; Bellard *et al.,* 2012) including variations in gene expression induced by changes in the intertidal environment (Hoffmann & Somero, 1995; Halpin *et al.,* 2002; Menge *et al.,* 2002; Lesser *et al.,* 2010). Our morphological evaluations of *F. crassa* indicate a greater body size and reproductive tissue in U than in NU animals (Figure 2; Table 3), which is in agreement with the increased food availability at the U sector. These results suggest that in U conditions, *F. crassa* displays a higher capability to acquire and allocate energy towards processes associated with survival, growth and reproduction. Moreover, estimates of the physiological condition, such as RNA:DNA ratios, reveal that in the spring season, when invertebrate reproduction and productivity is higher in South America, U animals show an increased biosynthetic capability. This result indicates that upwelling represents a key oceanographical condition that determines the physiological rate of all biological processes in intertidal, herbivorous invertebrates, such as *F. crassa*. This in agreement with evidence previously reported by us in intertidal fish species (Pulgar *et al.,* 2011).

Evidence shows that patterns of temporal variability in oceanographical conditions (e.g. upwelling) act as a determinant of major changes in the structure of biological communities, and must be incorporated into models and predictions of climate change, as well as into policies for conservation and sustainable management of marine resources (Wieters *et al.,* 2009). Consequently, the evaluation of the physiological responses of important marine predators (e.g. *Concholepas concholepas*, *Loxechinus albus* and *Fissurella* spp.) in variable oceanographical conditions is an urgency as these consumers have a strong ecological impact on marine community dynamics (Navarrete & Castilla, 2003; Castilla & Gelcich, 2008), they are of commercial interest with specific management programmes (e.g. Management and Exploitation Area for Benthic Resources (MEABRs): Gelcich *et al.,* 2008) and finally, because of their natural variability of biological interactions across environmental gradients (Menge & Sutherland, 1987; Menge *et al.,* 1999).

Fundamental to our understanding of the physiological responses to different environmental conditions is the analysis of the mechanisms that cause variation in physiological traits and the ecological consequences of these variations at different hierarchical levels (Spicer & Gaston, 1999). In this sense, the implications of different nutrient availability for marine animal's life histories are unknown; however, in habitats with low nutrient availability, life history theory predicts the presence of a trade-off between life history traits (Riklefs & Wikelski, 2002; Stearns, 2002; Monaco & Helmuth, 2011). In this context, and considering 'the barrel' model for organisms (Wiener, 1992), a higher maintenance cost and lower reproduction and growth survival investment (e.g. soft or reproductive tissue) in *F. crassa* inhabiting NU sectors would be predicted. Our results are in agreement with this prediction, revealing that NU *F. crassa* suffer energetic restrictions as compared with U animals. In NU sectors, animals may face energetic restrictions due to lower nutrient availability and a higher water temperature than for U animals. Conversely, in U sectors, *F. crassa* are exposed to higher food availability and potentially decreased thermal stress due to a lower water temperature, conditions that characterize U zones. This physical variability of habitats (e.g. SST and nutrient availability), would promote differential energetic investments of the same genotype to life history traits associated with growth and reproduction.

It is known that population density and temperature affect the body size of many organisms (Spicer & Gaston, 1999). Our U zone corresponds to MEABRs where the abundance of *F. crassa* is higher than in the NU zone, and consequently an impact of population density on organismal responses in this area would be expected. However, in addition to having the lowest temperature of all sampled zones, this U zone displays greater food availability.

In the present study only one site per treatment (U versus NU) was analysed which represents one limitation of our study; nonetheless our results on the impact of upwelling are in agreement with what has been observed at other latitudes (Palumbi, 2003). Moreover, our two study zones, Quintay and Las Salinas, have been independently characterized by other research groups (Wieters, 2005) using the same classification criteria (U versus NU), also validating our classification. Our results are also consistent with what we have previously reported for other taxonomic groups in the same sites (see Pulgar *et al.*, 2011) and thus we conclude that our observations would be not site-specific but are the expression of specific upwelling-dependent effects on the conditions of the respective organisms.

Taking into account that individual performance (activity levels, growth, feeding, survival and reproduction) ultimately depends on the physiological status, the links between ecological individual performance and physiological status need to be uncovered in order to fully understand the effects any consumer has on community structure and dynamics.

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