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Research Article

Reproductive output of two benthic resources (*Fissurella latimarginata* and *Loxechinus albus*) under different management regimes along the coast of central Chile

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ABSTRACT. The biological and ecological benefits of fully and partially marine protected areas are well documented. However, the benefits reaching areas beyond the limits of the reserves are still emerging in spite the fact that they are essential for fishing grounds recovery and to gain support for the protection of the ocean among stakeholders. We analyzed the influence of protection on gonadosomatic index, and also body dry weight, of two economically and ecologically important species: the keyhole limpet, *Fissurella latimarginata* and the red sea urchin, *Loxechinus albus*, in order to determine the value of protected areas in directly enhancing reproduction, and therefore potential seeding on exploited areas. We compared two levels of protection in central Chile, determined by fisheries management regimes (in turn associated to fishing pressure): a) areas with fishing restrictions (low or none fishing effort; territorial use rights for fisheries and no-take areas) and b) open access areas (high exploitation rates). We also evaluated the independent influence of upwelling on both variables. Our results show for both species that a) body dry weight is not affected by management regime, b) management regime did not show a consistent impact on gonadosomatic index and c) upwelling did not affect the response variables. Our findings help disentangling the main factors determining reproductive patterns under contrasting human impact scenarios, suggesting that the selection of sites for establishing marine protected areas seems to be less relevant than efficient control of fishing effort and minimum legal size to assure natural seeding.

Keywords: *Fissurella latimarginata*, *Loxechinus albus***,** seeding, reproduction, marine protected areas, TURF, conservation, management, upwelling.

INTRODUCTION

Marine protected areas (MPA) have increased worldwide in response to increasing human impacts on the ocean (Halpern *et al*., 2008). The benefits of MPA, particularly for fished areas, have been widely reported (Roberts *et al*., 2001; Halpern, 2003; Lester *et al*., 2009). Most of the evidence focused on the benefits of protection inside the boundaries of protected areas, measured on a set of biological variables such as species richness as well as abundance, biomass, and/or adult size of exploited species (Jennings *et al*., 1996; Roberts *et al*., 2001; Halpern, 2003; Shears *et al*., 2006; Lester *et al*., 2009). It is clear that fully protected areas confer more benefits than partially protected areas on most of these biological variables (Lester & Halpern, 2008; Gelcich *et al*., 2012). However, it is also evident that establishing fully protected areas generates social resistance (West *et al*., 2006). Thus, there is a compromise between reaching higher benefits fully protecting smaller fractions of the ocean and achieving lower enhancement but partially protecting larger proportions of the world ocean. Territorial Use Rights for Fisheries (TURF), implemented for management purposes, can also provide ancillary benefits for marine conservation as partially protected areas, over large fractions of the ocean (Gelcich *et al*., 2012).

The performance of fully and partially MPAs beyond the limits of the reserve is essential for fishing grounds recovery and to gain support among stakeholders (Roberts *et al*., 2001; Gells & Roberts, 2003a; Halpern & Warner, 2003; Russ *et al*., 2004; Sale *et al*., 2005). Spillover of exploitable adult biomass from no-take reserves clearly benefits local fisheries outside the boundaries of protected areas (*e.g.*, Roberts, 2001; Goñi *et al*., 2006). The benefits may reach even

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larger distances through the export of dispersive propagules, which seem to be enhanced inside protected areas. The enhancement in egg and larval production has been associated to higher reproductive potential inside both fully protected areas due to the combined effect of increased adult size and higher density of exploited species (Roger-Bennett *et al*., 2002; Gells & Roberts, 2003a; Willis *et al*., 2003; Pelc *et al*., 2009). Empirical evidence shows increases in reproductive potential (egg or gonad production) ranging between 2 and 18 fold in protected areas with respect to fished areas (Roger-Bennett *et al*., 2002; Willis *et al*., 2003; Pelc *et al*., 2009). The benefits of partially protected areas could be lower than fully protected areas, as the increase in size and density, two critical variables affecting egg production, is higher in no-take areas than in partially protected areas (Lester *et al*., 2009; Gelcich *et al*., 2012). All the analyses, however, have focused on the indirect consequences of enhanced size and density, while the direct influence of protection on reproductive investment has rarely been assessed (Kaiser *et al.*, 2009).

Direct influence of protection on seeding is expected to be positive if sites selected for conservation or TURFs are particularly productive (an attribute often dominant in the selection of areas for TURFs). In fact, the 20 to 25% increases in gonad weight observed in the scallop *Pecten maximus* between protected and open access areas suggest that protection can directly affect reproductive investment (Kaiser *et al*., 2009). However, negative effects can also be expected under density-dependent feeding, or parasitism scenarios (Loot *et al*., 2005; Wood *et al*., 2013). For instance, infection rates of several invertebrate species, including the keyhole limpet *Fissurella crassa*, are significantly higher in marine protected areas (Loot *et al*., 2005; Wood *et al*., 2013). However, the higher abundance of parasites infecting the gonads of *Fissurella* spp. in partially protected areas (TURFs) seem to enhance the gonadosomatic index in TURFs, suggesting a potential positive direct effect of fishing restriction on the reproductive success of keyhole limpets (Aldana *et al*., 2014). Further evidence on the direct influence of protection on reproductive output are needed to better understand the role of fully and partially protected areas on seeding beyond the boundaries of protection, the influence of site selection for conservation and management, and the relevance of individual reproductive potential for conservation planning and management.

The coast of central Chile provides a good model to analyze the direct effect of protection on reproduction (*e.g.*, gonad investment). First, this section of the coast is heavily impacted by artisanal fisheries (Fernández & Castilla, 2005). Second, there is a mosaic of human impact that includes fully and partially (TURFs; Gelcich *et al.*, 2012) protected areas interspaced with open access fishing zones (Fernández & Castilla, 2005). Thus, levels of protection of the coastal ecosystem can be associated to fisheries management regimes. Third, the spatial variation in upwelling influence on coastal areas also allows assessing the effect of this environmental driver on reproduction. Finally, there is a need to advance in management and conservation plans of coastal areas, identifying the most relevant zones for propagules production (Tognelli *et al*., 2009). We compared two levels of protection, determined by fisheries management regimes (in turn associated to fishing pressure) on reproductive investment (gonadosomatic index) and body dry weight of two economically and ecologically important rocky reef species in central Chile: the keyhole limpet, *Fissurella latimarginata* and the red sea-urchin, *Loxechinus albus*. Both variables, body dry weight and gonadosomatic index, provide indication of the general physiological condition (*i.e.*, energy assigned to reserves) of the organisms. Additionally, we also evaluated the independent influence of upwelling on body dry weight and gonadosomatic index, in order to help interpreting the potential influence of environmental conditions on reproductive investment. Thus, our results have local and global relevance, as they allow defining specific management and conservation strategies for the study area but also they can help informing on the direct influence of protection on reproduction (and therefore on seeding) besides the indirect effect expected through enhanced size and density of reproductive individuals.

MATERIALS AND METHODS

The study system

Both model species are targeted by the artisanal fishery that operates along the coast of central Chile. This geographic area (30º-36ºS) is characterized by high human impact (particularly fishing), low number of fully marine protected areas (<0.001%), and large fractions of the coast under a co-management system based Territorial Use Rights for Fisheries, covering 30% of the coastal area (Fernández & Castilla, 2005). Therefore, the TURF system confers most of the protection to coastal marine ecosystems, contributing to sustainable exploitation and conservation goals (Gelcich *et al*., 2012). Since levels of protection are associated to fisheries management regimes (no-take, TURF and open access), the effect we actually evaluated, we used both terms indistinctly throughout the manuscript. The coastal landscape of the study area is also characterized by strong heterogeneity driven by spatially persistent differences in oceanographic conditions that affect temperature and determine the local supply of nutrients and hence, primary production (upwelling, Wieters *et al*., 2003; Navarrete *et al*., 2005; Wieters, 2005; Tapia *et al*., 2009, 2014). Temperature and food supply can affect energy budget of organisms, determining the energy assigned to body mass and gonads (Clarke, 1987; Leslie *et al*., 2005; Monaco *et al*., 2014). For this reason, the role of upwelling intensity in modifying reproductive investment needs to be considered in our assessment, despite the fact that we cannot simultaneously evaluate both factors (management regime and upwelling) at a given site (a site corresponds to one or the other: low or high upwelling influence).

Specifically, our study area was located on a small fraction of the coast of central Chile, between 32.6ºS and 33.5ºS. Within this ecoregion (Camus, 2001), we selected five sites (Maitencillo, Laguna Verde, Quintay, El Quisco and Las Cruces; Fig. 1). Laguna Verde and Quintay were classified as sites more strongly influenced by upwelling (see Wieters, 2005; Tapia *et al*., 2009, 2014) than the remaining three sites (low upwelling). The influence of protection was associated to the management regimes operating in this region, we sampled (a) areas with restricted access for fishing (RAA), and (b) open access areas (OAAs). In each site we sampled an OAA adjacent to a RAA (named here area or sampling area). Among the latter, we sampled one no-take area (Las Cruces) and four TURFs. Thus, altogether we sampled ten areas (Fig. 1).

In the analysis, we pooled no-take and TURFs, as in the sampling region there is only one no-take area. Otherwise we would have not been able to determine if potential differences were exclusively due to human impact or environmental variability. Previous evidence suggests that the no-take area of Las Cruces exhibits a similar response of traditional biological variables (size, density) than some TURFs for benthic resources (Gelcich *et al*., 2012).

Sampling and data analysis

At each sampling area between 13 and 46 reproductive individuals of the keyhole limpet *Fissurella latimarginata* and the red sea-urchin *Loxechinus albus* were collected during the reproductive peak reported for both species (Table 1; Guisado & Castilla, 1987; Brown *et al*., 1997). Individuals, larger than minimum size of reproduction (6 cm for limpets and 7 cm for urchins) were collected by professional divers in each site.

Sampling was repeated in 2012 and 2014. Since the same patterns in the response variables were observed between years, we pooled all the samples in order to increase sample size. In total, we sampled 295 individuals of *F. latimarginata* and 273 of *L. albus* following all bioethics protocols approved for this study (CBB-233/2012).

At the laboratory, body size of all specimens was measured using a caliper (±0.1 mm), considering peristomial length for limpets and diameter without spines for sea-urchins. After obtaining the wet weight of each individual (mg), animals were dissected and the gonads were extracted. The soft and hard (calcareous) body parts, in addition to the gonads, were placed first on towel paper for 10 min to eliminate the excess of water, and then separated on labeled containers. Subsequently, all parts were dried in a standard oven at 60ºC for 48 h. Finally, dry weights were obtained with a Sartorius BP211D digital balance to the nearest 0.01 mg. Body dry weight was considered as a proxy of body condition.

In order to compare reproductive output between fisheries management regimes, the proportion between gonad dry weight and soft body dry weight was obtained (Gonadosomatic Index; GSI). We also compared individual body dry weights between fisheries management regimes to further evaluate effect of protection levels (associated here to management regimes) on energy reserves that can be assigned either to body mass or gonads. Since body mass and gonadosomatic index are influenced by size, and size is affected by levels of protection, the first step was to assess the effect of fisheries management regime on mean individual size. A two-way analysis of variance (ANOVA) with a complete non-balanced block design without replication was used to assess the effect of fisheries management regime (two levels, fixed factor) blocked by site (five levels, random factor) on mean individual size. We used a complete block design in order to account for the spatial auto-correlation of the observations. Spatial autocorrelation might occur because observations from neighbor areas with different management regimes (within site observations) might be more similar to each other than to observations from other sites since environmental conditions might differ among sites not related to management regimes. Data are slightly unbalanced due to the slightly different number of individuals measured in each area (Table 1). Fligner-Killeen test was used to assess the homogeneity of variances and Shapiro-Wilk test was used to assess the normality of the error distributions. The ANOVA assumptions were met for *F. latimarginata* but not for *L. albus*. Thus, in this case a Generalized Linear Model (GLM) using "quasi-poisson" family error distribution was performed. The model fit was checked with chi-square test for deviance *vs* the degrees of freedom.

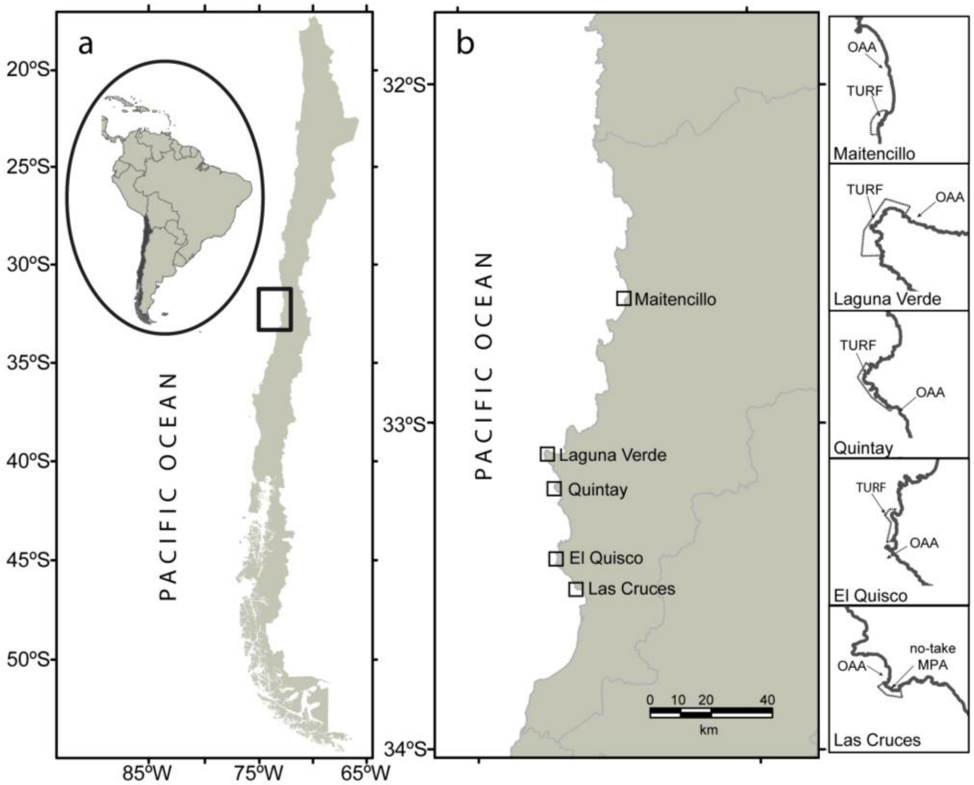

Figure 1. a) Maps of the general location of the study region showing sites, and b) specific areas where samples were collected in Fishing Restricted Access Areas (either no-take area or TURF) and adjacent Open Access Areas (OAAs).

Table 1. Sample size and body size ranges of reproductive individuals collected to estimate dry weight and gonadosomatic index for each species and fisheries management regime.

Species	Site	Fishing condition	Sample size	Body size range (cm)
F. latimarginata	Maitencillo	Restricted access	38	(6.42-10.77)
		Open access	46	(6.05-10.20)
	Laguna Verde	Restricted access	20	(8.03-9.48)
		Open access	37	(6.39-8.11)
	Quintay	Restricted access	37	(7.59-9.87)
		Open access	34	(7.17-9.86)
	El Quisco	Restricted access	15	(7.24-9.79)
		Open access	20	(7.07-9.61)
	Las Cruces	Restricted access	32	(6.60-11.41)
		Open access	16	(7.20-10.26)
L. albus	Maitencillo	Restricted access	38	(7.31-11.47)
		Open access	35	(7.03-10.90)
	Laguna Verde	Restricted access	40	(7.94-10.50)
		Open access	17	(7.02-9.34)
	Quintay	Restricted access	39	(8.60-10.80)
		Open access	38	(8.57-12.83)
	El Quisco	Restricted access	20	(7.65-11.32)
		Open access	10	(8.23-11.58)
	Las Cruces	Restricted access	23	(8.83-11.42)
		Open access	13	(7.21-9.84)

Since individual body dry weight (IDW) and the gonadosomatic index (GSI) were highly correlated with body size ($r > 0.45$ in all cases), we used body size as a covariate in our analyses to assess the effects of fisheries management regime and upwelling. Following the method of residuals analysis proposed by Ouréns *et al.* (2012), we first computed the individual linear regression model between each log-transformed response variable (IDW and GSI) and mean individual body size for each combination of levels of both factors. Then, for each significant linear model we removed the effect of the covariable (body size) on the response variable (IDW and GSI) extracting the residuals for each linear regression, and used the residuals as the new response variable in subsequent analyses. When no significant relationship was found between the log-transformed response variables and the log-transformed body size (only three models, all for *L. albus*), the new response variable were obtained by subtracting each observation from the mean. We assessed the effect of fisheries management regime on each new variable by using a 2-way ANOVA with the same non-balanced complete block design (by site) described above. Contrastingly, in order to test for the effect of upwelling (fixed factor) we used a 2-way ANOVA with a nested design (site nested within upwelling) due to environmentally driven site differences within levels of factor upwelling. Whenever ANOVA assumptions were not met, GLMs (family structure "quasi-Poisson") were used instead; again, the GLM model fit was checked with a chi-square test for deviance *vs* the degrees of freedom. Laguna Verde was excluded from the analysis of *F. latimarginata* because there was no overlap in size between the two management regimes of this site. Similarly, Laguna Verde and Las Cruces were also excluded for *L. albus*. All the statistical analyses were carried out using the free software R version 3.1.3 (R Development Core Team 2013).

RESULTS

Body size

We found a significant effect of fisheries management regime ($F_{1,289} = 56.22$, $P < 0.0001$) and site ($F_{4,289} = 12.32$, $P < 0.0001$) on body size of keyhole limpets. However, management regime did not have the same effect in all sites (Fig. 2a) in spite of a general trend towards larger body sizes in RAA. Laguna Verde and El Quisco showed the greater differences between RAA and OAA (largest individuals in RAA). Differences in body size between pairs of sites were not related to the distance (km) between them (Fig. 2a). Thus, differences in mean size of keyhole limpets were observed between El Quisco and Quintay (which are close together), but also between El Quisco and Maintencillo, or between Quintay and Maintencillo, which are further away (Fig. 1).

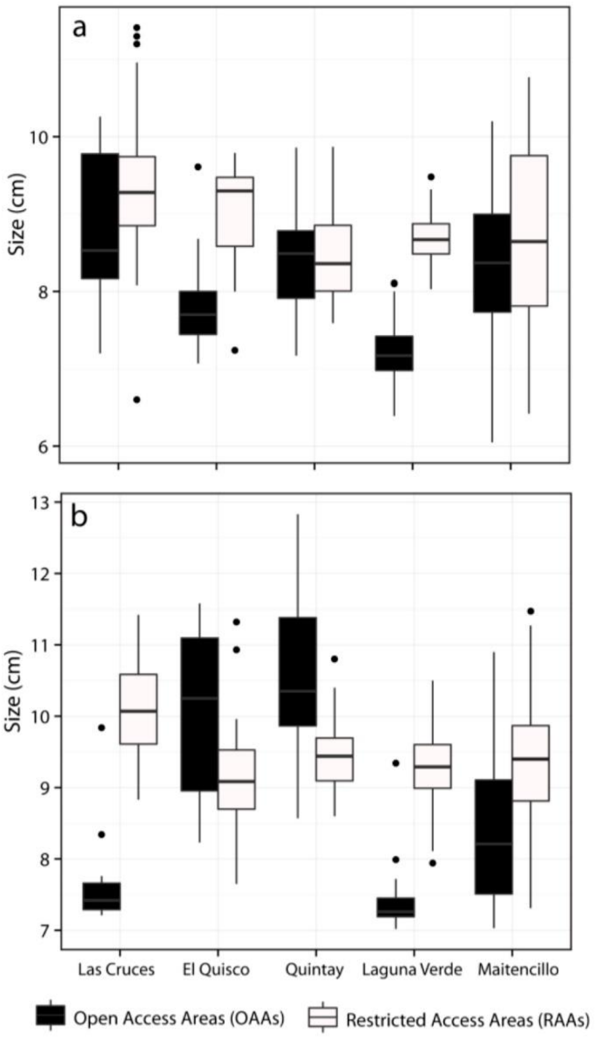

Figure 2. Boxplots showing individual body size for each sampling site in areas under different fishing management regimes. White bars represent areas with entry restriction to fishing named here Restricted Access Areas (RAAs, include one no-take area and four Territorial Use Rights for Fisheries; TURF). Black bars indicate areas with no access restrictions, called here Open Access Areas (OAAs). a) *Fissurella latimarginata* and b) *Loxechinus albus*.

We also found a significant effect of fisheries management regime ($F_{1,271} = 41.87$, $P < 0.001$) and site ($F_{4,267} = 33.99$, $P < 0.001$) on the body size of sea-urchins (Fig. 2b). Larger sea-urchins in RAA were observed in Las Cruces, Laguna Verde and Maitencillo, and the opposite trend in El Quisco and Quintay (Fig. 2b). As observed for keyhole limpets, mean differences

Table 2. Results of the relationship between body dry weight (log transformation) and size (log transformation) of the keyhole limpet *Fissurella latimarginata* and the red sea-urchin *Loxechinus albus* for the different sites and fisheries management regimes. RAA: Restricted Access Areas for fishing (which can be either no-take areas or Territorial use Rights for Fisheries; TURF), OAA: Open Access Areas.

Site	Fishing regime	*Fissurella latimarginata*			*Loxechinus albus*		
		Intercept	Slope	*P*-value	Intercept	Slope	*P*-value
Las Cruces	OAA	-5.68	3.71	<0.001	-1.63	2.73	<0.001
	RAA (no take)	-3.00	2.55	<0.001	-1.09	2.55	<0.001
Quisco	OAA	-5.02	3.29	<0.001	-1.58	2.74	<0.001
	RAA (TURF)	-5.99	3.97	<0.001	-2.29	3.05	<0.001
Quintay	OAA	-6.86	4.19	<0.001	0.15	1.99	<0.001
	RAA (TURF)	-3.28	2.49	<0.001	-1.76	2.79	<0.001
Laguna Verde	OAA	-1.03	1.44	0.02	-0.26	2.04	<0.001
	RAA (TURF)	-4.16	3.06	0.009	-1.95	2.88	<0.001
Maintencillo	OAA	-2.85	2.45	<0.001	-2.73	3.24	<0.001
	RAA (TURF)	-5.03	3.41	<0.001	-1.41	2.63	<0.001

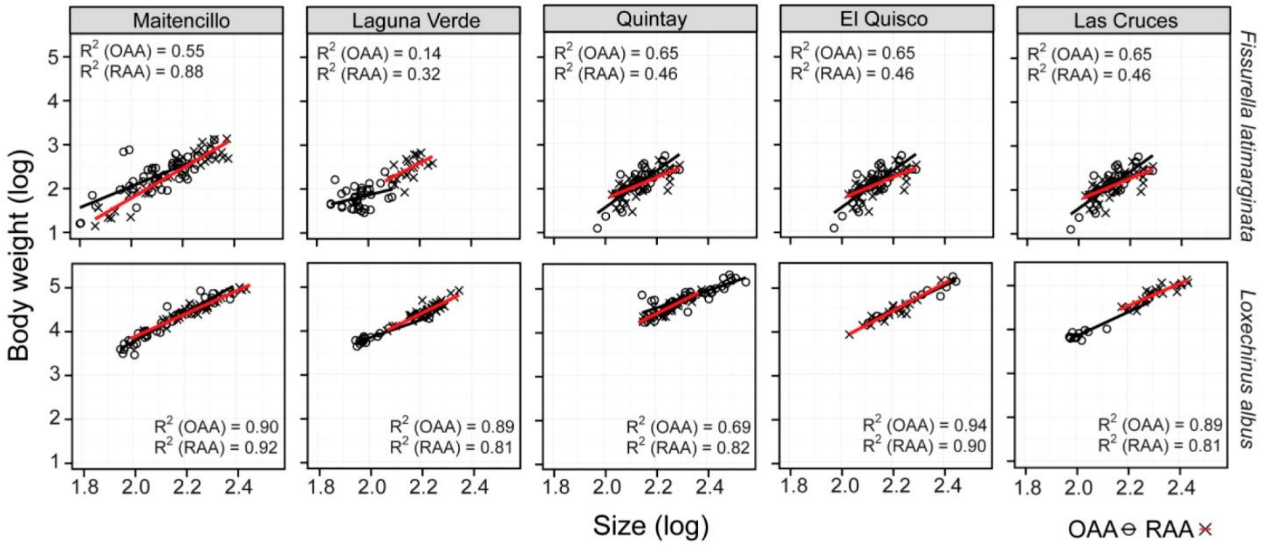

Figure 3. Relationship between body dry weight (log) and size (log; length [mm] in the keyhole limpet *F. latimarginata* and diameter [mm] in the sea-urchin *Loxechinus albus*) in the five study sites, considering one Open Access Area (OAA; circles and black line) and one Restricted Access Area (RAA: crosses and red line) per site. The regression coefficients (R^2) for each fishing regime and site are also shown. Table 2 includes the parameters of the regressions.

in body size between pairs of sites was not related to the distance between sites: differences were observed between both the closest sites (Laguna Verde and El Quisco) and the two farthest ones (Las Cruces and Maitencillo).

Since body size showed significant differences between fisheries management regimes and sites, it was treated as a covariable in the remaining analysis conducted to test for the influence of fisheries management regime and upwelling.

Body weight

Body dry weight was significantly correlated with body size in both species, regardless of the fisheries management regime or site (Table 2, Fig. 3).

After removing the influence of size on body weight (residual analysis), no influence of fisheries management regime on body dry weight of keyhole limpets ($F_{1,233}$ = 0.99, *P* = 0.32) and red sea-urchin ($F_{1,176}$ = 1.25, *P* = 0.26) were detected. Body dry weight also increased significantly with body size when sites were

Table 3. Results of the relationship between body dry weight (log transformation) and size (log transformation) for the keyhole limpet *Fissurella latimarginata* and the red sea-urchin *Loxechinus albus* for the different sites and upwelling conditions.

Site	Environmental condition	*Fissurella latimarginata*			*Loxechinus albus*		
		Intercept	Slope	*P*-value	Intercept	Slope	*P*-value
Las Cruces	Low upwelling	-4.31	3.12	<0.001	-2.19	3.02	<0.001
Quisco	Low upwelling	-8.58	5.08	<0.001	-2.04	2.90	<0.001
Quintay	High upwelling	-5.00	3.31	<0.001	-0.87	2.41	<0.001
Maintencillo	Low upwelling	-3.82	2.88	<0.001	-2.14	2.96	<0.001
Laguna Verde	High upwelling	-3.99	2.96	<0.001	-1.61	2.72	<0.001

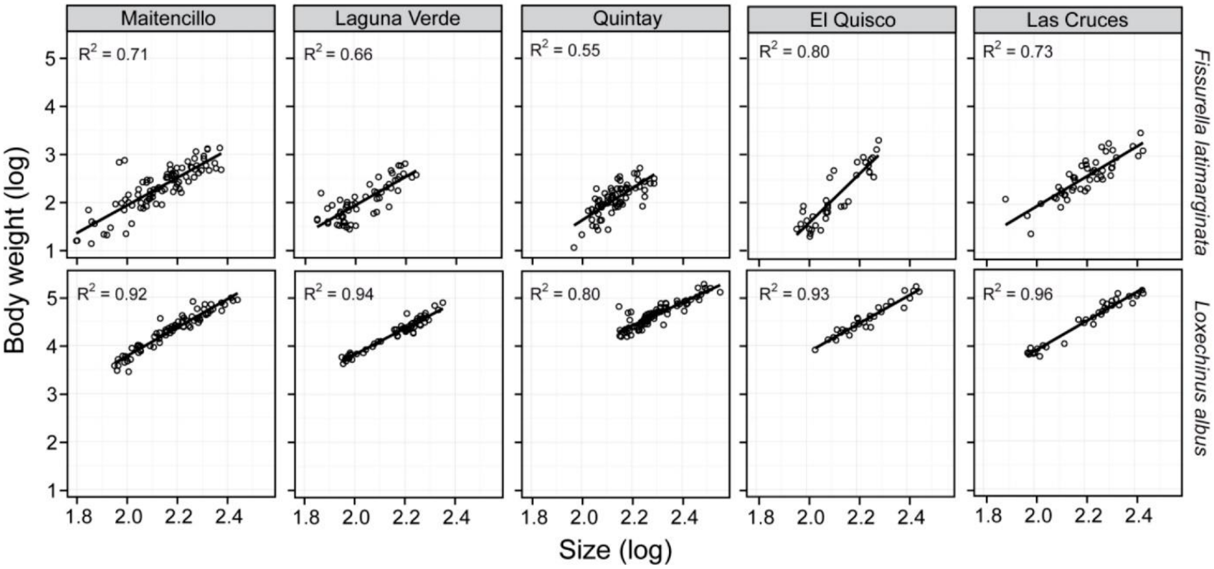

Figure 4. Relationship between body dry weight (log) and size (log: length in the keyhole limpet *F. latimarginata* [mm] and diameter [mm] in the red sea-urchin *Loxechinus albus*) in the five study sites. Two sites were labeled as high upwelling (Quintay and Laguna Verde) and the remaining as low upwelling. The regression coefficients (R^2) for each site are shown. Table 3 includes the parameters of the regressions.

analyzed considering the influence of upwelling, by pooling data from both management regimes for each site (Table 3, Fig. 4). The nested ANOVA performed on the residuals of the relationship between body dry weight and body size did not detect any influence of upwelling condition. The results were consistent between species (keyhole limpet: $F_{4,290}$ = 0.11, *P* = 0.97; red sea-urchin: $F_{4,268}$ = 0.13, *P* = 0.97).

Reproductive output

Gonadosomatic index was also significantly correlated with body size in both species for most sites and both fisheries management regimes, with some exceptions (see Table 4, Fig. 5). The residual analyses showed no effect of fisheries management regime ($F_{1,233}$ = 1.39, *P* = 0.24) or site ($F_{3,233}$ = 0.13, *P* = 0.94) on gonadosomatic index of the keyhole limpet after removing the effect of size (residuals). Although data from the site that was excluded from the analysis (Laguna Verde) cannot be statistically compared because of lack of overlap in size, the slopes between both fishing regimes showed similar trends (Fig. 5). In the case of the red sea-urchin, no differences in gonadosomatic index after removing the effect of size were found among sites (*P* > 0.9 in all cases). Although fisheries management regime showed significant effects on the residuals of the regressions between gonadosomatic index and size, no consistent pattern was detected across sites.

The gonadosomatic index significantly increased with size in both species only at the sites characterized by low upwelling influence (Table 5, Fig. 6). Nevertheless, the residual analysis of the relationships between gonadosomatic index and size did not show significant differences between upwelling conditions neither for the keyhole limpet ($F_{4, 290}$ = 0.03, *P* = 0.99) nor for the red sea-urchin ($F_{4,268}$ = 0.4, *P* = 0.81).

Table 4. Results of the relationship between gonadosomatic index (log transformation) and size (log transformation) of the keyhole limpet *Fissurella latimarginata* and the red sea-urchin *Loxechinus albus* for the different sites and fisheries management regimes. RAA: Restricted Access Areas for fishing (which can be either no-take areas or territorial use rights for fisheries; TURF), OAA: Open Access Areas.

Site	Fishing regime	*Fissurella latimarginata*			*Loxechinus albus*		
		Intercept	Slope	*P*-value	Intercept	Slope	*P*-value
Las Cruces	OAA	-12.81	5.17	0.011	-2.33	-0.10	0.922
	RAA (no take)	-4.34	1.31	0.11	-5.01	1.25	0.021
Quisco	OAA	-11.54	4.50	0.018	-2.69	0.21	0.766
	RAA (TURF)	-7.79	2.95	0.012	-9.88	3.21	0.003
Quintay	OAA	-7.72	2.71	0.034	-3.94	0.61	0.309
	RAA (TURF)	2.97	-2.37	0.11	-1.28	-0.61	0.495
Laguna Verde	OAA	-7.23	2.65	<0.001	-4.92	1.11	0.045
	RAA (TURF)	-8.51	3.08	<0.001	-5.99	1.52	0.009
Maintencillo	OAA	0.18	-0.99	0.60	-1.96	-0.34	0.773
	RAA (TURF)	-2.85	0.65	0.58	-11.39	3.91	0.001

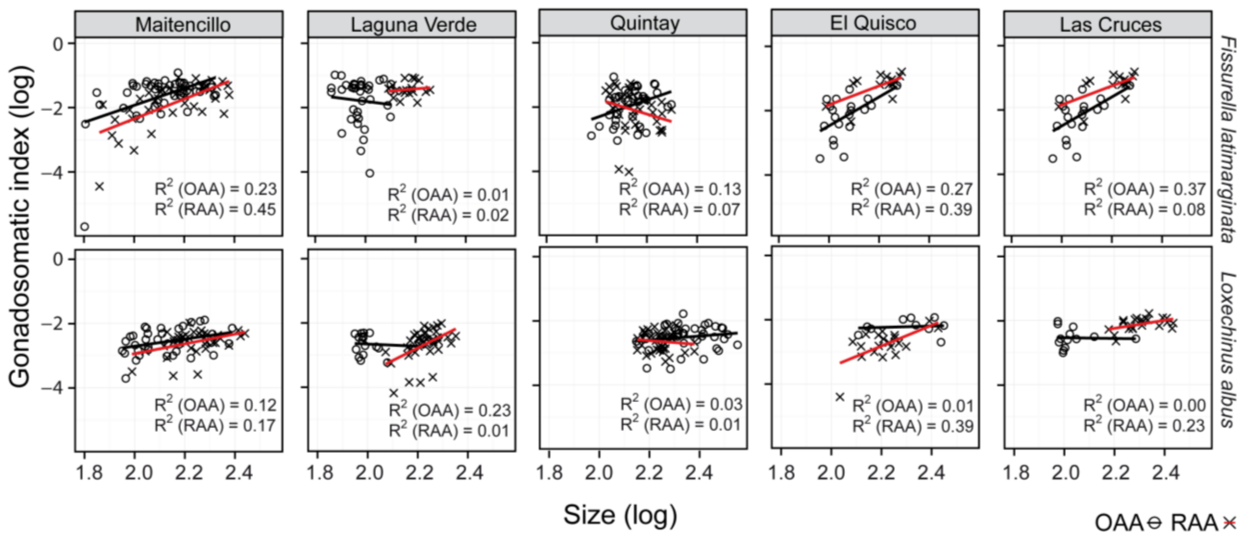

Figure 5. Relationship between gonadosomatic index (log) and size (log; length [mm] in the keyhole limpet *F. latimarginata* and diameter [mm] in the case of the red sea-urchin *Loxechinus albus*) in the five study sites, considering one Open Access Area (OAA: identified by circles and black line) and one Restricted Access Area (RAA: identified by crosses and red line) per site. The regression coefficients (R^2) for each fishing regime and site are shown. Table 4 includes the parameters of the regressions.

DISCUSSION

The main conclusions of our study are the consistent patterns evidenced by a) the lack of effect of fisheries management regime (related to protection level) on the individual body dry weight in both species, b) the poor influence of fisheries management regime on reproductive investment (*i.e.*, gonadosomatic index), suggesting that the selection of sites for sitting of marine protected areas or TURFs seems to be less relevant for seeding than efficient control of fishing effort and minimum legal size, as these two variables are major indirect determinants of egg production (Roger-Bennett *et al*., 2002; Willis *et al*., 2003; Pelc *et al*., 2009), and (c) the lack of effect of upwelling on body condition (body dry weight) and reproductive investment (gonadosomatic index). Although in general we found no effects of the study variables, the results are of local and global interest. First, because we clearly show that at the spatial scale of our analysis, the main driver of coastal dynamics in the study area (upwelling; see Wieters *et al*., 2003; Navarrete *et al*., 2005; Wieters, 2005; Tapia *et al*., 2009, 2014) does not influence critical indicators of general physiological conditions and reproductive investment in two herbivore species. And second, because our results help di-

Table 5. Results of the relationship between gonadosomatic index (log transformation) and size (log transformation) of the keyhole limpet *Fissurella latimarginata* and the red sea-urchin *Loxechinus albus* for the different sites and upwelling conditions.

Site	Enviromental condition	*Fissurella latimarginata*			*Loxechinus albus*		
		Intercept	Slope	*P*-value	Intercept	Slope	*P*-value
Las Cruces	Low upwelling	-7.43	2.69	0.001	-5.24	1.35	<0.001
Quisco	Low upwelling	-12.68	5.11	<0.001	-8.11	2.46	0.001
Quintay	High upwelling	-2.18	0.07	0.941	-4.02	0.63	0.113
Maintencillo	Low upwelling	-7.47	2.69	<0.001	-4.72	0.99	0.004
Laguna Verde	High upwelling	-4.01	1.15	0.130	-3.97	0.60	0.224

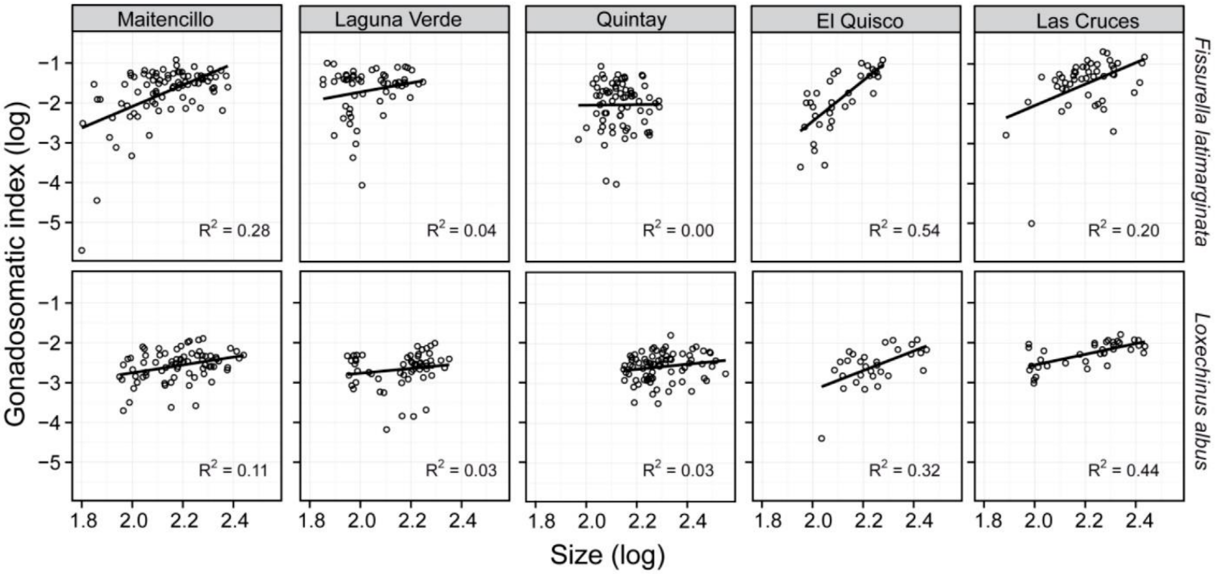

Figure 6. Relationship between gonadosomatic index (log) and size (log; length [mm] in the keyhole limpet *F. latimarginata* and diameter [mm] in the case of the red sea-urchin *Loxechinus albus*) in the five study sites. Two sites are classified as high upwelling (Quintay and Laguna Verde) and the remaining as low upwelling intensity. The regression coefficients (R^2) per site are shown. Table 5 includes the parameters of the regressions.

sentangling the main determinants of reproductive patterns observed in protected areas, which is critical for decision making (*e.g.*, site selection for conservation and management, spatial planning).

In line with existing evidence, our results show the benefits of protection for enhancing size of exploited species inside the boundaries of protected areas (Lester *et al*., 2009; Gelcich *et al*., 2012). However, a) in some sites significant larger sizes in the RAA with respect to OAA were not found, in spite the suggestive general trend towards size enhancement and b) in El Quisco and Quintay mean size of the red sea-urchin exhibited the opposite pattern (larger sizes in open access areas). A plausible explanation is that most of our RAA are only partially protected from fishing. Across all sites we observed, on average, 17 and 28% increase in body size with protection in the keyhole limpet and in the red sea-urchin, respectively. A larger average increase in size (30%) has been reported in global analyses comparing individual size between no-take and open access areas (Lester *et al*., 2009). However, smaller changes, comparable with our results (~17%), have been observed in global comparisons between partially protected and open access areas, including an broad range of species (Lester & Halpern, 2008), suggesting the higher benefits of full protection within the limits of the reserves. Nevertheless, our findings reinforce and highlight the benefits of partially protected areas, particularly in regions where excluding human activities does not seem a viable option (Tognelli *et al*., 2009). Our results suggest the need of further exploration of the influence of fishing effort on mean size of exploited species (*e.g.*, comparing TURFs across a gradient of fishing level) since exploitation seems to be the major determinant of differences in body size. Another suggestion that fishing, rather than environmental factors, determines local mean body size, is that variability in mean body size across sites was not consistent between the two grazer species, and did not show a regional effect, as differences occurred among the closest or the farthest sites. Therefore, it seems relevant to determine the level of human impact that affects body size, and as a consequence influences the variables related to reproductive investment, critical for management and conservation, such as egg production.

Enhanced size in protected areas can amplify the benefits of protection outside the boundaries of protected zones by increasing individual production of dispersive propagules (Gell & Roberts, 2003b). Empirical evidence predicts between 2 to 5 times increase in abalone egg production in protected areas with respect to fished areas, associated to 30% increase of size of adults (Rogers-Bennett *et al*., 2002). The contribution of partially protected areas to potential egg production may be less significant than no-take areas since smaller changes in size, as those observed in this study, can have tremendous impact on potential fecundity (*e.g.*, Espinosa *et al.*, 2006) However, not only size, but also density is enhanced in partially and fully protected areas (Lester & Halpern, 2008). The relative importance of increased size and abundance in protected areas on reproductive potential is yet poorly understood. Most studies report increases in egg production within protected areas due to the combined influence of size and density (Roger-Bennett *et al*., 2002; Willis *et al*., 2003; Pelc *et al*., 2009). Another element contributing to local reproductive potential is the direct influence of fishing regime on reproductive investment; however this is even less understood (Kaiser *et al*., 2009) and our results help filling this important gap. Density-dependent reproduction or parasite load can be potential mechanisms behind direct effect of protection on reproductive investment.

Our evaluation on the direct influence of protection on both reproductive investment and general condition of keyhole limpets and red sea-urchins showed a consistent lack of effect of protection on body dry weight and gonadosomatic index. This finding contrast with the results of similar comparison in the scallops *Pecten maximum*, showing that gonad weight per unit of body size increased between 19 and 24% in the areas protected from fishing, associated to a 8-fold increase in density (Kaiser *et al*., 2009). Our results showing persistent patterns in the comparison of fishing management regimes are solid as they are based on comparisons among five sites and two species. Moreover, fishing management regime consistently showed no effect regardless of upwelling condition. Contrasting results on the direct influence of protection on reproductive investment (*e.g.*, Kaiser *et al*. 2009, this study) suggest the need to advance our understanding of the mechanisms behind. Clearly, density-dependent effects (*e.g.*, feeding, parasitism, behavior such as territorialism) might influence individual energy budgets and determine differential investment in gonads in protected areas (Kaiser *et al*., 2009; Aldana *et al.*, 2014). However, the potential factors appear to be complex. For instance, parasitic biomass in gonads seems to generate contrasting patterns on gonad investment (Loot *et al*., 2005; Aldana *et al.*, 2014). External factors may also play a role. In our particular case, we expected that partially protected areas (TURFs) would exhibit larger effects on both response variables than no-take or open access areas, as fishers might select the most productive areas for TURFs. However, we cannot conclude that fishers' selection for productive sites, or density dependent factors determine the patterns found in this study. The lack of direct effect of protection on reproductive investment drives our main conclusion that the selection of particular areas for sitting marine protected areas seems to be less relevant than effective enforcement, which promotes enhancement of size and density (Halpern, 2003; Lester & Halpern, 2008; Lester *et al*., 2009).

It is also remarkable that the upwelling gradient analyzed here, including the influence of a major upwelling center (Curaumilla), did not influence reproductive output or body size condition. A five-fold increase in planktonic chlorophyll-*a* produces a 3-fold increase in larval production of the filter feeding barnacle *Balanus glandula* (Leslie *et al*., 2005). Despite macroalgal growth seems to be higher in areas under high upwelling influence (Wieters *et al*., 2003), this effect does not seem to be transmitted to herbivores in the form of body dry weight or gonadosomatic index (but see Pulgar *et al*., 2013). Our results again highlight that this environmental factor, at least at the scale of variability of our study sites, does not seem to be a major determinant for body condition of the main herbivores exploited in central Chile.

Our results strongly suggest that at the local level (Chile), major fishing regulations such as minimum legal size and quotas, need to be enforced, in order to control size and density of reproductive individuals. Recent studies have shown that illegal fishing of benthic resources can generate catch levels similar to the legally reported landing (González *et al.*, 2006), clearly suggest poor enforcement. Our results show that maintaining larger individuals in protected areas is critical for seeding. Therefore, special incentives should be created to maintain the Chilean TURF system, because of its contribution to egg production by enhancing size and density of exploited benthic species. Our analysis also allowed to extrapolate the value of fully and partially protected areas (including TURFs) on less studied variables, such as reproductive output and egg production.

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