

Reproductive investment in rocky intertidal mussels: spatiotemporal variability and environmental determinants

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ABSTRACT: Spatial and temporal variability in the reproductive output of marine invertebrates affects individual energy balance and physiology, reproductive evolution, and population connectivity and recruitment. Multiple factors modulate reproductive investment and generate temporally varying or persistent spatial patterns across different scales. We used a hierarchical sampling design at 4 spatial scales over 2 consecutive years to characterize spatial patterns in the intertidal mussel *Perumytilus purpuratus* along 600 km of central Chile. The gonadosomatic index (GSI) and the condition index were assessed in monthly samples collected during peak reproduction. No latitudinal trends or regional breaks were observed across the region. The largest fraction of variability was explained by differences among localities, separated by 10s of kilometers, and among individuals, spaced a few centimeters to meters apart. Intermediate spatial scales, i.e. few kilometers or 100s of meters, explained only small fractions of the total variance. Persistent variability at the scale of locality is understood as resulting from topographic modulation of upwelling intensity over mesoscales. Variability among individuals is interpreted as variation in both micro-environmental and individual hormonal conditions. Sea surface temperature, chlorophyll *a*, and upwelling intensity were not correlated with GSI across space. Our results are consistent with the idea that a suite of variables, rather than a single dominant factor, modulate reproductive output and generate environmental mosaics over 10s of kilometers. Observed spatio-temporal patterns also suggest that spatial variability in larval output may have important consequences on metapopulation dynamics and that territory-based conservation of reproductive stock can be an effective management strategy.

KEY WORDS: Reproductive output · Mussels · *Perumytilus purpuratus* · Gonadal index · Condition index · Rocky intertidal · Central Chile

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INTRODUCTION

Knowledge about the scales of spatial and temporal variability in reproductive output and reproductive investment of marine invertebrates is essential to gain a better understanding of the ecological and evolutionary constraints that shape the reproductive physiology and individual performance of species

within both natural communities and human-maintained aquaculture systems (Bertness et al. 1991, Dugan et al. 1991, Hughes et al. 2000, Lester et al. 2007, Macala & McQuaid 2017). Information on spatiotemporal variability in reproductive output is also important for an understanding of the factors that determine variation in the replenishment of coastal populations and to propose strategies for con-

ervation, management, and restoration (Pulliam 1988, Cabral et al. 2016, Macala & McQuaid 2017). Many studies have shown that, in the case of marine invertebrates with obligatory pelagic larval development, reproductive investment is a main determinant of larval output and fitness (Hughes et al. 2000, Leslie et al. 2005, McQuaid & Phillips 2006), although future settlement and recruitment may be decoupled from local larval output at most spatial scales (Hughes et al. 1999, Navarrete et al. 2010). Marine bivalves of the family Mytilidae (i.e. mussels), common to most rocky shores of the world (Suchanek 1985), are a group of species whose fecundity has been shown to vary over time and space; they have also served as model species to examine the effects of multiple stressors on individual performance (Page & Hubbard 1987, van Erkom Schurink & Griffiths 1991, Archambault et al. 1999, McQuaid & Lindsay 2005, Blanchette et al. 2007, Kroeker et al. 2016). Patterns of reproductive investment and timing in marine mussels depend on multiple environmental variables (e.g. sea surface temperature [SST], variability in SST, salinity, nutrients, pH), which modulate stress and individual physiological performance, and influence food availability (i.e. phytoplankton, particulate organic matter), which is considered a bottom-up influence in benthic communities when such an effect propagates to higher trophic levels (Bayne 1976, Menge 1992). However, the relationship between a single variable and reproductive output can be blurred by conflicting individual responses to co-varying factors (e.g. lower temperatures, low pH, increased food), which form spatial mosaics of interactive variables that can alter the energetic requirements of individuals (Kroeker et al. 2016). Such costly expenditures like growth, managing competitors, or displaying defense responses may take precedence over or even impede reproduction (Zardi et al. 2007, Petes et al. 2008, Fearman & Moltschaniwskyj 2010).

In the coastal ocean, many processes produce environmental variability over a wide range of spatiotemporal scales due to the interaction of hydrodynamics and shore geomorphology (Mann & Lazier 1996, Nickols et al. 2015). For instance, persistent variation over a few meters can be produced by local topography and rock surface heterogeneity interacting with wave splash (Denny 2014, Flores et al. 2016), while variation over 10s to 100s of meters can be introduced by variability in surfzone hydrodynamics, waves, and bathymetry (Shanks et al. 2010, Lentz & Fewings 2012, Morgan et al. 2016). The variation over 10s of kilometers can be due to coastal morphology and intensification of upwelling (Wolanski &

Hamner 1988, Narváez et al. 2004, 2006, Lagos et al. 2005, Wieters 2005), and changes over 100s of kilometers can be attributed to variation in upwelling regimes, latitudinal gradients in solar radiation, or coastally trapped waves (Navarrete et al. 2005, Connolly et al. 2014, Tapia et al. 2014).

The biological response of many marine invertebrates to these dynamic oceanographic and topographic variables is to vary the allocation of energy and resources to different physiological and reproductive requirements. For example, reproductive studies of the intertidal mussel *Mytilus californianus* along the northeastern Pacific have demonstrated large variability among sites separated by 20–30 km along the coast of California (USA), with increased reproductive investment around the upwelling regime shift of Point Conception (Phillips 2007b). On the southeast coast of South Africa, gamete output of intertidal mussels was consistently higher at sites within a bay than those separated by approximately 100 km on the open coast (McQuaid & Phillips 2006), which could be attributed to embayment or other environmental/oceanographic conditions that vary over these distances. Other studies have identified large variation in reproductive output over scales of 100–500 km (Thorarinsdóttir & Gunnarsson 2003, Oyarzún et al. 2018).

In this study along the coast of central Chile, we examined a range of spatial scales accounting for variability in reproductive investment and individual condition of intertidal mussels, whether the general pattern remains similar between years, and whether environmental variation in mean SST, food availability, and upwelling intensity can, in a simple manner, explain observed spatial patterns.

Extensive literature on bivalves indicates a seasonal cycle in their biochemical composition, with accumulation of lipids, proteins, and glycogen in different tissues—including the gonads—during periods of excess nutrient/food availability and the subsequent use of these reserves during food scarcity (Bayne 1976, Bayne et al. 1983, Hawkins et al. 1985). The utilization of such reserves can vary substantially within and among mussel species (Bayne 1976, Gosling 2003, Orban et al. 2002) due to variation in the acquisition and allocation of energy (Bayne 2004). Most or all energy reserved in the gonads is channeled into gametogenesis, a process typically related to SST and food availability (Seed 1976, Kennedy 1977, Jaramillo & Navarro 1995, Gosling 2003). Temporal patterns of gametogenesis can generate annual or semiannual spawning cycles (Thorarinsdóttir & Gunnarsson 2003, Oyarzún et al. 2010,

2018). However, continuous spawning throughout the year can also be observed, and large mesoscale variability in the reproductive cycle of a single species is common among intertidal mussels (King et al. 1989, McQuaid & Phillips 2006, Phillips 2007b, Oyarzún et al. 2018).

Here, we characterized spatial scales of variation in individual reproductive investment, as measured by the gonadosomatic index (GSI), and somatic investment (body condition index, CI) of the intertidal mussel *Perumytilus purpuratus*, a common and widely distributed species that can form dense beds along large sections of the central coast of Chile (Castilla & Durán 1985, Guíñez & Castilla 1999, Fernández et al. 2000, Navarrete et al. 2005, 2010). Studies on the reproduction of *P. purpuratus* have shown that GSI is an accurate indicator of gametogenic stages and development (Oyarzún et al. 2010), and together with individual body mass, provides an estimate of per capita reproductive output. The species exhibits annual or semiannual reproductive cycles at different sites along the Chilean coast, without a clear latitudinal trend in the type of cycle (Lozada & Reyes 1981, Oyarzún et al. 2010, 2018), but probably associated with large mesoscale variability in temperature and upwelling intensity (Oyarzún et al. 2018). The number of spawns per year decreases towards increasing latitudes (lower mean temperatures), and the timing of spawns is generally associated with increases in mean SST (Oyarzún et al. 2018). Thus, while significant variation in reproductive output of *P. purpuratus*, as inferred from the GSI, has been documented among sites separated by >100 and up to 500 km (Oyarzún et al. 2018), the relative importance of this variability in comparison to other spatial scales has yet to be examined. In fact, the great majority of studies on invertebrate reproduction—mussel reproduction in particular—have fixed a single spatial scale of analysis and then correlated reproductive patterns with environmental variables. Here, we aimed to determine the spatial and temporal variability of reproductive investment and conditioning of *P. purpuratus* at 4 different spatial scales, ranging from 100s of centimeters to 10s of kilometers, across an extensive biogeographical region of central-northern Chile. Our goals were: (1) to identify the spatial scales that account for the greatest variation in GSI and CI of this intertidal mussel, (2) to assess whether spatial scales of variation were persistent over the examined temporal scales (months within reproductive season over 2 consecutive years), and (3) to examine whether simple explanatory variables such as SST and chl *a* concentration can

account for some of the spatial and interannual variability in individual performance.

MATERIALS AND METHODS

Study system

The mussel *Perumytilus purpuratus* is a dominant competitor that can monopolize space from the mid- to the low zone of rocky intertidal habitat. It is distributed from the coast of Ecuador (3° S) to the Strait of Magellan (56° S), continuing along the Atlantic coast of Argentina (Paine et al. 1985, Alvarado & Castilla 1996, Fernández et al. 2000). *P. purpuratus* is the main prey of a variety of top intertidal predators, including the sunstar *Heliaster helianthus*, the carnivorous gastropod *Concholepas concholepas*, and intertidal crabs (*Acanthocyclus* spp.) on the central coast of Chile (Castilla 1981, Castilla & Paine 1987). This mussel forms dense matrices that provide substratum and refuge for more than 90 invertebrate taxa (Prado & Castilla 2006). Thus, this filter-feeder acts not only as a biogenic habitat, but seems critical to the transfer of energy and nutrients through the intertidal ecological network (Kéfi et al. 2015, Reddin et al. 2015).

Previous studies have reported a typically bimodal size distribution and a maximum valve length of approximately 4 cm (Alvarado & Castilla 1996). Histological analyses have indicated that sexual maturity occurs from 8 to 10 mm during the first year of life and have revealed simultaneous gonadic development in both sexes, with rare cases of hermaphroditism (Lozada & Reyes 1981, Montenegro et al. 2010). Seasonal reproductive patterns vary along the Chilean coastline but show a semiannual spawning season in early austral spring and summer for the central region of Chile (Lozada & Reyes 1981, Oyarzún et al. 2018). Following spawning, larvae spend approximately 14 d in the water column with recruitment occurring throughout the year and peaking in summer along the central coast of Chile (Ramorino & Campos 1979, Navarrete et al. 2008). The Humboldt Current, which flows north along Chile to Peru, produces strong seasonal coastal upwelling which supports the highly productive open and coastal marine ecosystems of Chile (Strub et al. 1998, Hormazabal et al. 2001, Thiel et al. 2007). Spatiotemporal variability in upwelling modulates onshore nutrient input, primary production, both phytoplankton and zooplankton abundance, benthic community structure, and recruitment to rocky intertidal shores (Daneri et al. 2000, Broitman et al. 2001, Narváez et al. 2006).

Hierarchical design of field collections

To determine which are the most important spatial scales accounting for variation in reproductive variability of *P. purpuratus*, among the range of scales selected, and whether such spatial patterns were persistent between years, a hierarchical sampling design including 4 different spatial scales was implemented along the central coast of Chile over a 5° latitudinal span (600 km) during 3–4 mo of the peak austral winter reproductive season in 2012 and repeated again in 2013 (Fig. 1a). Six localities separated by a mean of 110 km (range 80–140 km) were haphazardly selected at wave-exposed rocky shores so as to capture variation along this stretch of the coast. Each locality

was considered to encompass between 3 and 6 km along the coastline, within which 3 sites separated by a mean of 2 km (range 1–5 km) were selected (Fig. 1b). Thus, sites within each locality can be assumed to be similarly exposed to upwelling intensity and regimes, yet these conditions varied widely across localities (Narváez et al. 2004, Tapia et al. 2009, 2014). Furthermore, localities encompassed 2 regions, north and south of 32°S, with distinct oceanographic regimes (Navarrete et al. 2005, Tapia et al. 2014) that are evidently associated with lower recruitment and overall abundance of *P. purpuratus* at the most northern sites (Broitman et al. 2001, Navarrete et al. 2005, 2008). Sites within locality were sufficiently far apart to exhibit differences in local circulation, water column stratification, and other hydrographic conditions (Palma et al. 2006, Bonicelli et al. 2014), which may affect overall mussel performance, including growth and reproduction. Within each site, we selected 3 wave-exposed rocky platforms separated by 100–200 m (n = 54 total platforms). While oceanographic conditions associated with upwelling and mesoscale circulation were probably similar among the 3 platforms within sites, hydrodynamic characteristics of the surf zone (e.g. modulation of waves, splash, and rip currents due to local topography) likely varied among platforms (Flores et al. 2016).

Finally, within each platform, we randomly selected 20 individuals along 10–20 m transects. This among-individual variation spaced on average 100 cm apart represents the smallest spatial scale in our design. Individuals of *P. purpuratus* were collected monthly from the mid-intertidal zone, as defined by Castilla (1981) and Broitman et al. (2001), between May and July in 2012 and again in 2013. This sampling period was selected to examine gonadic investment because it coincides with peak GSI for central Chile and precedes the typical early spring spawning (Lozada & Reyes 1981, Oyarzún et al. 2018). Sampling was extended to April for the southern sites of Los Molles, Estación Costera de Investigaciones Marinas (ECIM), and Pichilemu in 2013.

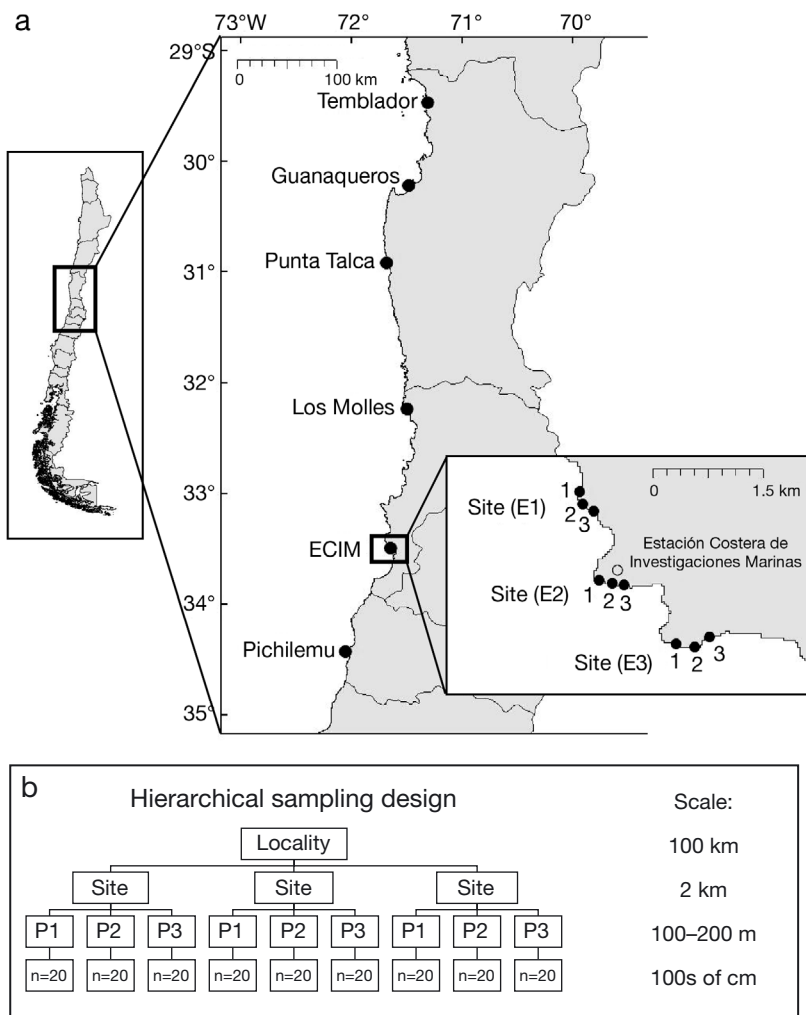


Fig. 1. (a) Central coast of Chile, showing the position of each study locality where fecundity of *Perumytilus purpuratus* was measured. Inset shows an example of the sites nested within each locality and platforms nested within sites. Platforms E2.2 and E2.3 reside within the only no-take marine reserve in the region. ECIM: Estación Costera de Investigaciones Marinas. (b) Hierarchical sampling design implemented within each locality

Laboratory processing of individuals for GSI and CI

Mussels were placed in 70% ethanol, labeled, brought to the lab, and kept for a minimum of 6 mo to facilitate dissection (see Kim et al. 2006 for methods). After this time, individuals were measured with calipers and the mantle and body tissues were dissected, dried in an oven to constant weight, and weighed in a precision balance to 0.00001 g. Shells were weighed separately. Gonadic maturation occurs inside the mantle tissue as the follicles develop, and it is therefore considered a reliable indicator of reproductive tissue in most mussels (Mathieu & Lubet 1993), and especially in *P. purpuratus* (Oyarzún et al. 2010). The body tissue is comprised of the mollusk's foot, adductor muscle, and visceral mass, thus representing the somatic energy concentration and development of a mussel (Mathieu & Lubet 1993). Variation of reproductive allocation over somatic allocation by *P. purpuratus* should then be reflected in differences of mass (i.e. GSI) at varying spatial scales.

Environmental data

To identify potential environmental factors driving spatial differences among localities and between the 2 reproductive seasons, we obtained data on SST and chl *a* as a proxy of food availability (Peterson et al. 1988, Menge et al. 1997), and calculated an index of upwelling intensity. *In situ* temperature loggers (Tidbit, StowAway®) were deployed 1–2 m deep at 1 site in each locality and are used to represent mean SST for that locality (see Tapia et al. 2014 for details). Previous studies have shown that these *in situ* temperature loggers correlate well with satellite-derived SST but show finer resolution (Lagos et al. 2005, Tapia et al. 2014). Average monthly chl *a* concentrations were obtained for each locality from AquaMODIS chl *a* 8 d composite data (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMH1chla8day.html>) and are interpreted here as a broad indicator of 'food availability' for this filter-feeder. A total of 15 pixels of 4 km each (240 km²) were defined in front of each locality and averaged to represent the monthly chl *a* values. Monthly chl *a* averages were calculated for each locality and used to examine relationships with reproduction and condition, for the same month of mussel collection, and with the change in mean chl *a* observed 1 mo before mussel collection.

A locality-specific thermal upwelling index (UI) was calculated for this same time period following Pfaff et

al. (2011), and defined as $UI = (T_{\text{offshore}} - T_{\text{onshore}}) / (T_{\text{offshore}} - T_{\text{bottom}})$. SSTs offshore (T_{offshore}) were obtained from AquaMODIS SST 8 d composite data (<https://coastwatch.pfeg.noaa.gov/erddap/griddap/erdMH1sstd8day.html>) at 350 km westward from each study locality using the average of 64 pixels (1024 km²). Onshore temperatures (T_{onshore}) were obtained from Tidbit temperature loggers deployed at 1 m depth. Bottom sea temperature around 200 m deep (T_{bottom}) was set at 10°C after data presented by Blanco et al. (2001), whose work also showed little variation around this temperature across our study region (Blanco et al. 2001).

Data analysis

Before analyses, we examined whether a linear model was appropriate to characterize the relationship between body mass and mantle mass (Fig. 2). To this end, we fitted linear and quadratic polynomial regressions using ordinary least squares (OLS) and determined that, within the mussel size range studied, the linear relationship provided a better fit to the data (see Table S1 in the Supplement at www.int-res.com/articles/suppl/m599p107_supp.pdf), with an exception at the locality of Guanaqueros. Deviation from linearity at this locality had only a small effect on the results presented here, so we used the linear approximation to calculate GSIs (see below). An analysis of covariance showed the existence of significant interactions between locality and body mass, thus rejecting the null hypothesis of homogeneous slopes among localities and suggesting the existence of locality-specific relationships between reproductive investment and size in *P. purpuratus* (Table S2). A GSI was calculated for each individual to examine the proportion of soft tissue allocated to reproduction:

$$GSI = \frac{\text{Mantle mass}}{\text{Mantle and body mass}} \times 100 \quad (1)$$

The appropriateness of gonadal indices as indicators of reproductive investment has been frequently discussed, and limitations of these methods have been noted for marine invertebrates (Ebert et al. 2011). However, previous work has found very good agreement between the GSI, the gametogenic stages, and the mature oocyte percentages for *P. purpuratus* (Lozada & Reyes 1981, Oyarzún et al. 2010); we therefore used GSI in this study.

Following the hierarchical experimental design (Fig. 1), a nested analysis of variance was used to analyze GSI data and estimate variance components

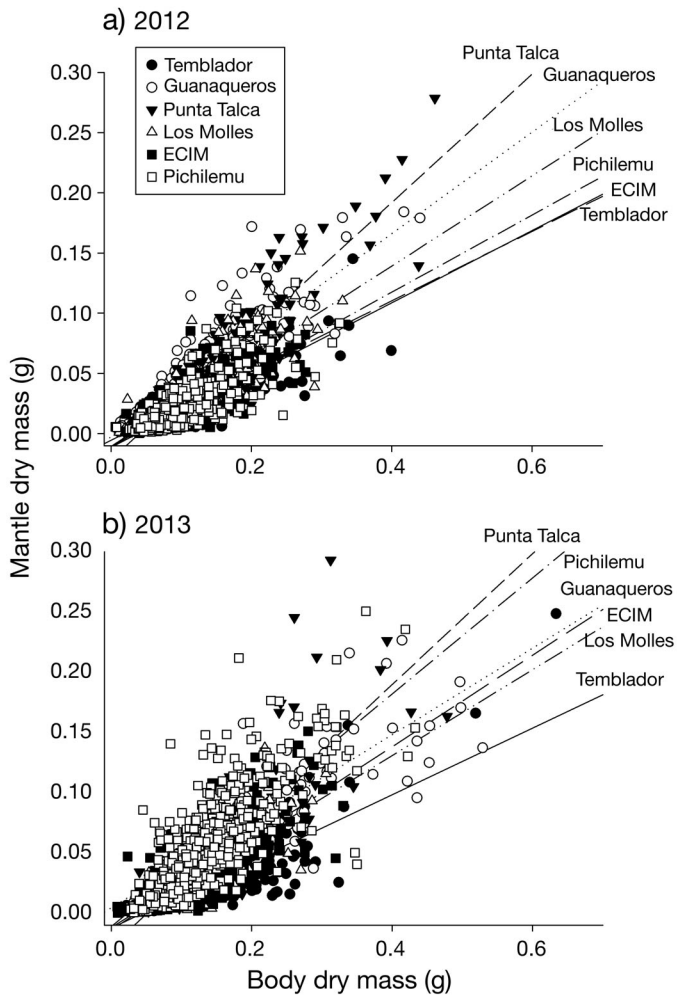


Fig. 2. Relationship between body mass and mantle mass for *Perumytilus purpuratus* at each locality for (a) 2012 and (b) 2013. Data from each year were pooled over the sampling period

for each month and reproductive season, considering individual mussels as replicates, platforms nested within sites, and sites nested within localities. Maximum likelihood was used to provide more stable estimates of variance components than those provided by the Type I method in unbalanced designs (Milliken & Johnson 2009), although both methods were highly coincident. Although significance testing was not the focus of these analyses, they are presented in Table S3, after Satterthwaite's approximation for degrees of freedom (Kuehl 1994) to correct for loss of replicates under certain combinations of factor levels. Visual inspection of variance components across sampling months was used as an indication of consistency within the selected spatial scales of variability. To determine whether the spatial pattern of GSI was

persistent, or 'geographically fixed,' between the 2 consecutive years of the study, we simply examined whether the variance contribution of a given locality, site, or platform was the same from one year to the next. Note that this approach provides weaker tests of 'pattern persistence' than the test of time invariance presented by Benedetti-Cecchi (2001) in a study of spatial variability in Mediterranean intertidal assemblages. There, the author tested the necessary assumption that assemblages did not vary between 2 sampling dates, so that spatial comparisons could be made with data collected over different dates. In our case, overall variance due to 'year,' or even variance due to a 'space × year' interaction, may be significantly different from 0 (which is the most likely scenario in natural systems), but the pattern may still be 'persistent' if such variability does not significantly alter structure across the region, breaking down the correlations.

The CI of individuals was also calculated in order to examine the spatiotemporal patterns of overall individual condition and to compare with GSI patterns. The CI is calculated as tissue content of the mussel against the total mass, including shell:

$$CI = \frac{\text{Mantle and body mass}}{\text{Total mass of the mussel}} \times 100 \quad (2)$$

This CI is commonly used in bivalve aquaculture studies for environmental, management, and monetary purposes (Orban et al. 2002, Irisarri et al. 2015). Variability of the CI and relation with environmental variables were examined in the same manner as described for GSI. Although these biological indices (GSI, CI) measured different aspects of mussel biology, they were highly correlated over space and time (see 'Results') and therefore we concentrate here on reproductive investment and then show main CI patterns.

We examined the relationship between GSI and CI with single environmental variables using OLS regressions to explore linear and non-linear (polynomial) relationships. Since SST and chl *a* show large variability at scales from 10s to 100s of km (Narváez et al. 2004, Lagos et al. 2005, Tapia et al. 2009, 2014), and we do not have reliable information at lower spatial scales, we averaged GSI values at the scale of locality before conducting these analyses. Interannual changes in mussel indices were contrasted with interannual changes in mean SST, chl *a*, and UI at each locality.

Lastly, to further characterize spatial patterns of mussel reproduction and condition (GSI, CI) and environmental variables (SST, chl *a*), we examined

their spatial autocorrelation structure through correlograms (Legendre & Legendre 1998, Rangel et al. 2010). Spatial correlograms of the yearly average of GSI and CI (Moran's I autocorrelation index) were calculated as a function of the shortest Euclidian distance separating platforms. Similar statistical analyses were performed using satellite-derived SST and chl a for the study period of each year. In this case, we obtained evenly spaced data across the study region by selecting contiguous 4 km pixels adjacent to the shore, from the northernmost to the southernmost site. To remove the effect of latitudinal variation in SST, prior to the correlogram analyses we regressed SST over latitude and constructed correlograms based on the residual of this relationship. UIs could not be calculated in a similar manner because we had only 1 *in situ* record per locality.

RESULTS

Spatial and temporal patterns

Collected individuals of *Perumytilus purpuratus* ranged in size between 10 and 50 mm shell length, and only 16 individuals were hermaphroditic (<0.01%). All individuals had clearly defined gonad tissue. Males and females did not differ significantly in GSI, which has also been shown in earlier studies of this species (Lozada & Reyes 1981, Oyarzún et al. 2018). Therefore, both sexes were pooled in the analyses.

There were no clear latitudinal trends in GSI across the region, either when averaging at the scale of locality (Fig. 3a) or when examining all sites (Fig. 3b). Note that the geographic pattern of variability in GSI

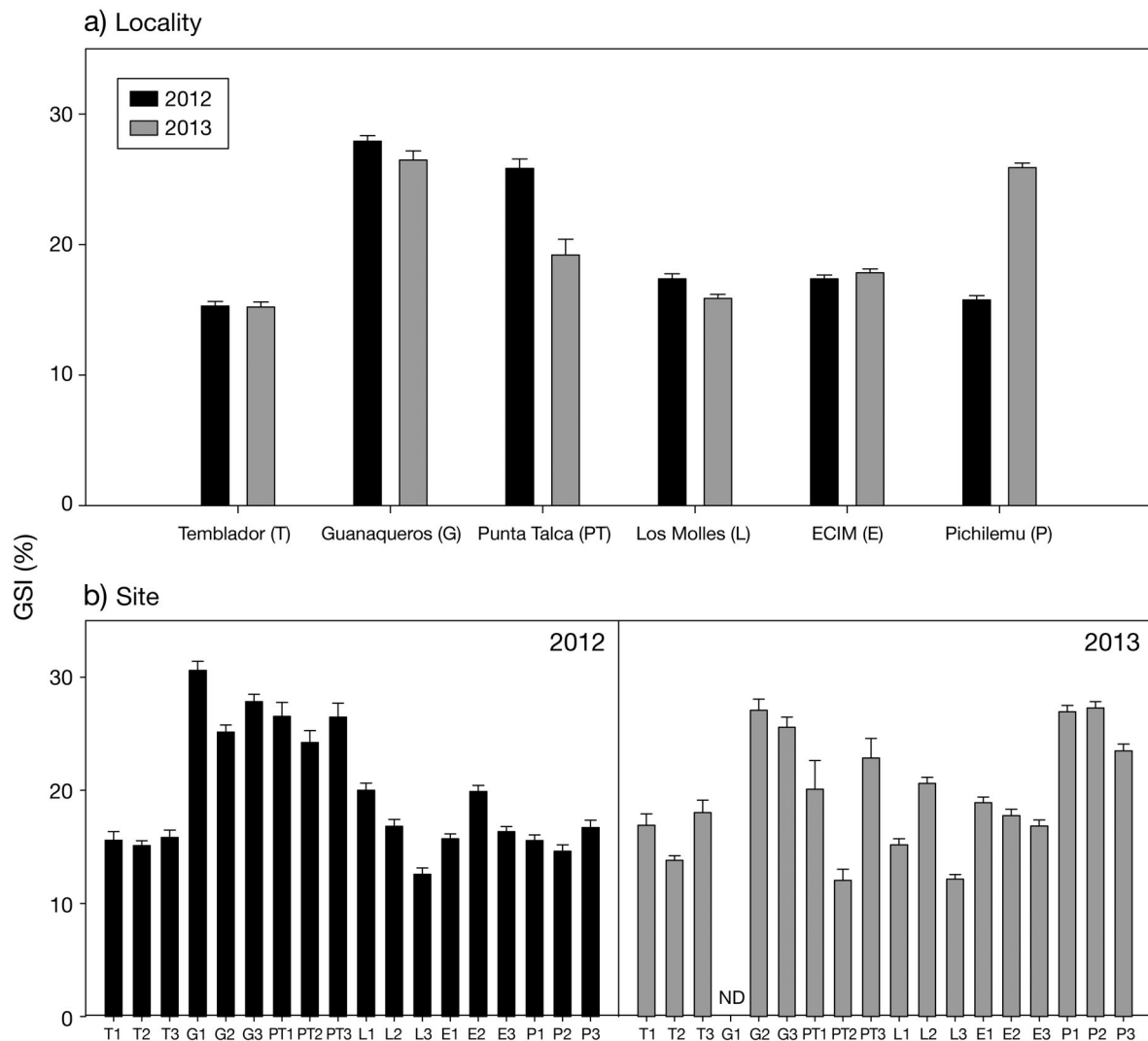


Fig. 3. Spatial pattern of the gonadosomatic index (GSI) of *Perumytilus purpuratus* averaged for the sampling period at the level of (a) locality and (b) site (see Fig. 1). Error bars are SE; ND: no data available

Table 1. Variance components of nested ANOVA for each month, indicating the proportion of the variance in the gonadosomatic index of *Perumytilus purpuratus* attributable to different spatial scales

Scale	May 2012	June 2012	July 2012	May 2013	June 2013	July 2013
Locality	15.4455	34.7076	30.1407	10.1917	5.0461	62.6741
Site	0	6.5531	2.8657	5.1930	35.8914	11.3895
Platform	10.5687	7.0967	8.8330	11.0018	7.4480	9.2813
Individual	34.6730	33.7108	45.4014	46.4585	51.5660	40.0422

observed within localities is generally well preserved and there is little variation among sites in the same locality. (Fig. 3). Within the chosen window of time for the surveys, there was no clear trend across months (Fig. S1 in the Supplement); while at most localities GSI fluctuated slightly from month to month, at 1 locality (Guanaqueros), GSI increased consistently from May through July in both years. At some localities (Temblador, ECIM, and especially Punta Talca), there was a sharp decrease in GSI between June and July of 2013, indicating that early spawning occurred between sampling dates (see Oyarzún et al. 2010). Thus, we performed analyses with and without the individuals collected in July and indicate when changes occur with respect to using all data.

Variance component analyses for each month showed a generally persistent structure of spatial variability in GSI throughout the reproductive season and between years, with locality separated by 10s of km and individual separated by centimeters to meters capturing the greatest proportion of the variance in GSI of this species (Table 1, Fig. 4). The scales of

site (2 km) and platform (100s m) together accounted for less than 22 % of the variability in GSI throughout the study period (Fig. 4). The notable exception to this pattern occurred in June 2013, when variance due to site accounted for more than 36 % of the total variance and surpassed the variance attributed by locality (Fig. 4). In part, the result for this month of the year is due to missing observations at Guanaqueros and Punta Talca. Removing these localities from the variance component analyses generates a spatial structure of variation more similar to the ones observed during other sampling times (Fig. S2).

With the exception of Pichilemu, the southernmost locality in the region, there were small interannual changes in GSI (<10 % on average), across all spatial scales (Fig. 5). Once Pichilemu was removed, high

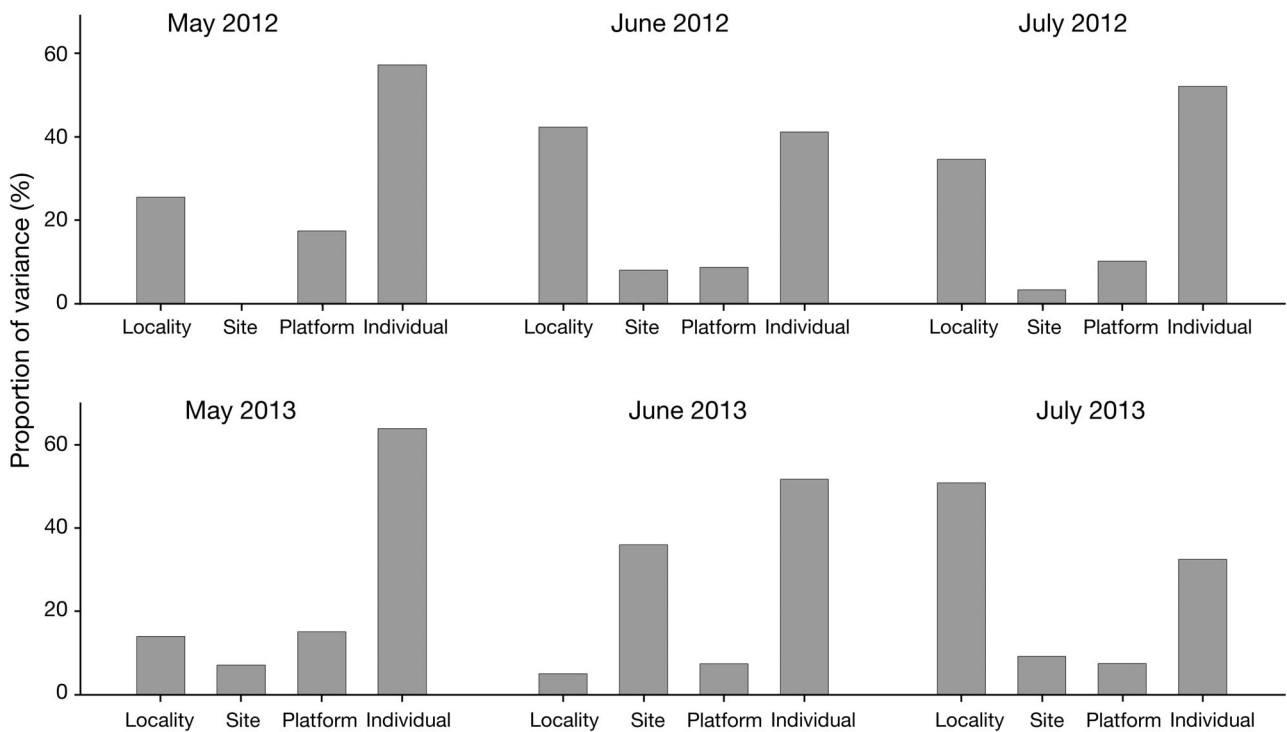


Fig. 4. Variance components of a nested ANOVA of the gonadosomatic index (GSI) of *Perumytilus purpuratus* at each level of the hierarchical sampling design (see Fig. 1)

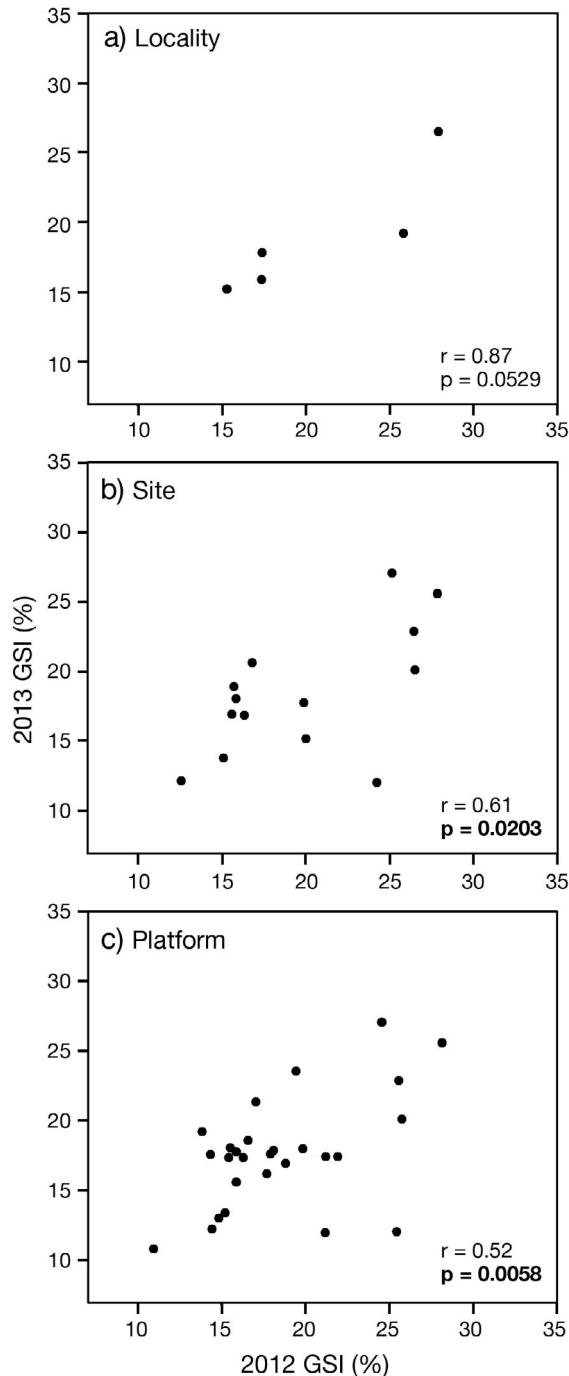


Fig. 5. Pearson correlations (r) examining the persistence of the spatial patterns in the gonadosomatic index (GSI) of *Perumytilus purpuratus* between years at the scale of (a) locality, (b) site, and (c) platform. **Bold** p-values represent a significant regression at $\alpha = 0.05$. The site Pichilemu was removed

interannual persistence of the spatial pattern (order of sites) was observed at the scale of locality (Pearson $r = 0.87$), although marginally non-significant ($p = 0.0529$) probably due to low replication ($n = 5$,

Fig. 5a), and significant at all other scales (Fig. 5b,c). At Pichilemu, we observed a marked increase in GSI in 2013, which was evident at all spatial scales within that locality (Fig. S3).

In accordance with results from variance components, spatial autocorrelation analyses showed significant positive correlations of GSI at scales up to about 100 km, switching to mostly negative and non-significant correlations at larger spatial scales in both years (Fig. 6a). Roughly coincident decorrelation scales of ca. 100 km were observed in SST after removing the latitudinal trend and in chl *a* (Fig. 6b,d), but in the case of SST another significant spatial structure was observed at about 300 km (Fig. 6b) in 2012.

Relationships with environmental variables

No simple linear, unimodal, or saturation-type relationship was observed between GSI and temperature or chl *a* concentration either in 2012 or 2013, or pooling both years (Fig. 7a,b). Changes in temperature or in the mean chl *a* concentration observed the month prior to collecting individuals were also not associated with GSI (Fig. 7c,d). However, while interannual differences in GSI were small at most sites (<10%), these changes, at each locality and month, were significantly and negatively correlated to the interannual change in mean SST observed at that locality and month (Fig. 7e); a positive interannual increase in GSI was significantly associated with a decrease in SST. These results include the stark increase in GSI observed at Pichilemu between 2012 and 2013 (Fig. S3), where the largest interannual changes in SST were also observed. In contrast, interannual variability in chl *a* concentration did not explain interannual variation in GSI. The UI, calculated for each locality and time period, showed no association with GSI within years (Fig. 8a,b), nor did the change in UI observed between years at each locality and time period coincide with GSI (Fig. 8c). Note that 2012 was on average a year of more intense upwelling than 2013, which corresponds well with the lower temperatures observed in corresponding months during that year.

Monthly values of CI remained slightly more stable than GSI among months and followed the same overall pattern across all spatial scales (Figs. S4 & S5). Slight declines in CI, at months suspected of spawning events (between June and July), were observed only at Temblador, Punta Talca, and ECIM, and only in 2013. The spatial and temporal structure of CI

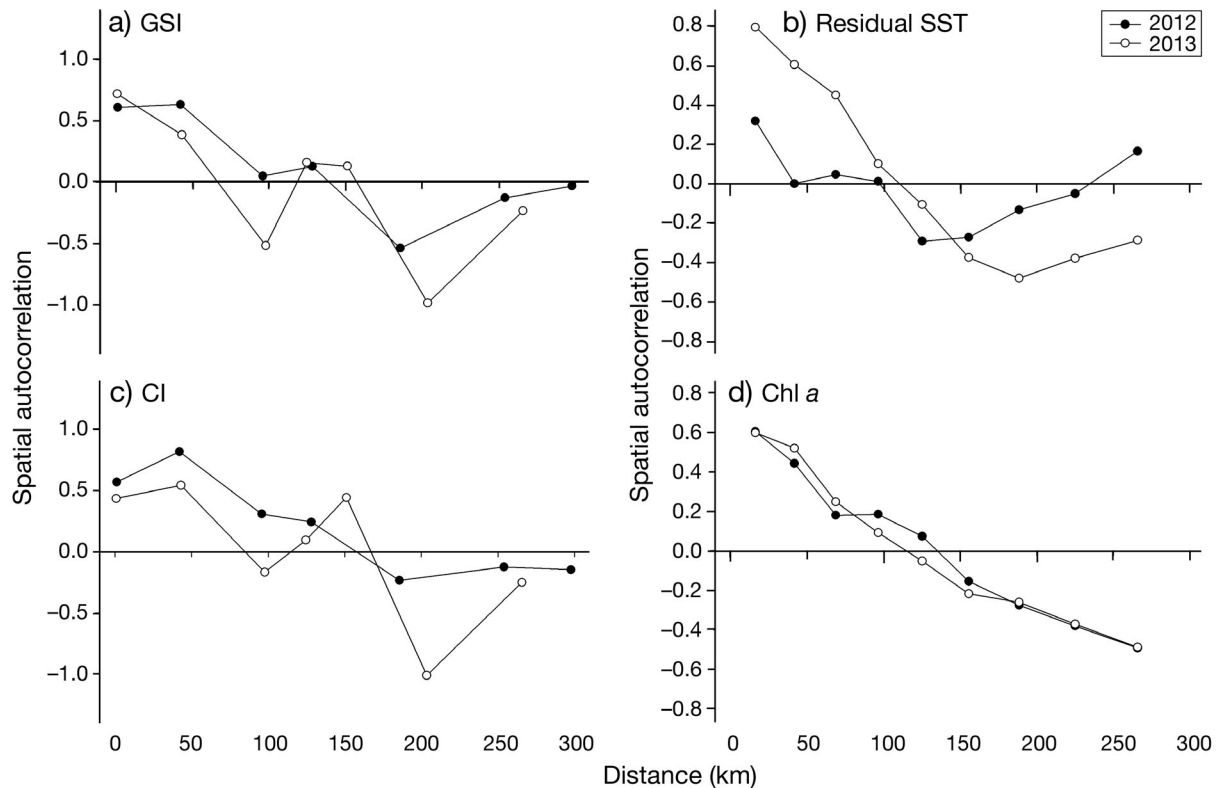


Fig. 6. Spatial correlograms (Moran's I autocorrelation index as a function of the distance separating platforms) of the average ranking of (a) gonadosomatic index (GSI) and (c) condition index (CI) values of *Perumytilus purpuratus*. Spatial correlograms for satellite (Moran's I index) (b) residual sea surface temperature (SST) and (d) chl a measurements along the coast

closely followed that of GSI across the study region (Fig. 9 and see Fig. S5), with the exception of Temblador. The relationships between CI and environmental variables (SST, chl a , UI) were therefore quite similar to those observed between GSI and these variables (Figs. S6 & S7), but with a notable exception. This was the significant positive relationship between observed values of chl a and CI for both years, suggesting that greater CI values are related to increased food availability (Fig. S6b).

DISCUSSION

Our results here show that in the intertidal mussel *Perumytilus purpuratus*, GSI and individual condition have very characteristic scales of variability, with the main scales of variation found among individuals centimeters to several meters apart, within platforms, and among localities separated by 10s and up to about 100 km. Intermediate scales contribute much less to variation in reproduction and condition. Importantly, the spatial structure of variance and geographic patterns were highly pre-

served among months and between years, which is of great significance for site-based management. While the spatial structure of variation in GSI suggests the influence of mesoscale oceanographic processes associated with upwelling variability, single environmental variables that correlate with upwelling (SST, chl a , UI) were not associated with GSI in any year. Below we discuss potential processes underlying spatial-temporal patterns of variation in mussel reproductive output and condition, suggest that they are consistent with the hypothesis of persistent multivariate mosaics of environmental conditions, and highlight the consequences for metapopulation dynamics and management.

Spatial scales of variability in reproductive investment

Our spatially intensive study on reproductive patterns of *P. purpuratus* along the coast of central Chile shows that there are no latitudinal gradients or geographic breaks across the study region, but there are characteristic spatial scales that account

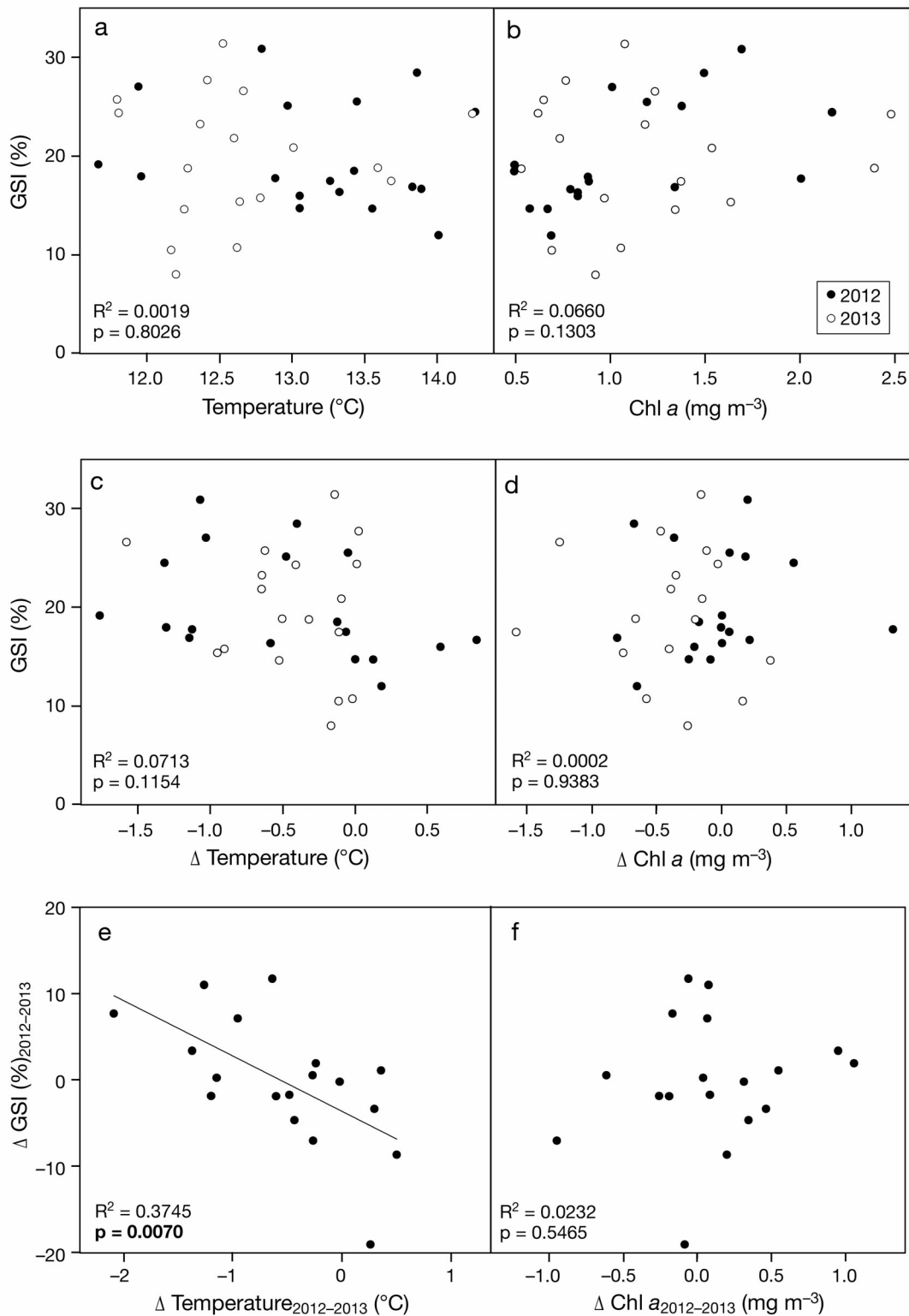


Fig. 7. Regressions of the environmental variables (a,c,e) sea surface temperature (SST) and (b,d,f) chl a in relation to gonadosomatic index (GSI) of *Perumytilus purpuratus* at the level of locality. The current state of the environment per month was compared to (a,b) mean GSI values and (c,d) the environmental change between months. (e,f) Interannual relationships were determined by calculating the difference of monthly GSI, SST, and chl a values between 2012 and 2013. The **bold** p-value in (e) represents a significant regression at $\alpha = 0.05$, visualized with a regression line

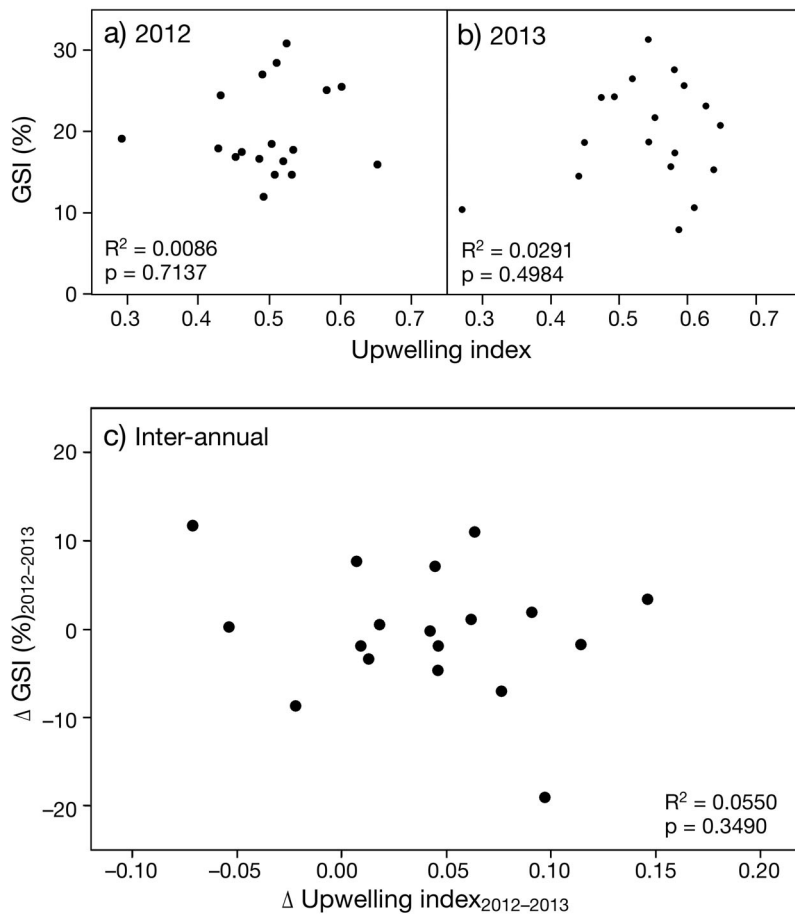


Fig. 8. Relationship between the gonadosomatic index (GSI) of *Perumytilus purpuratus* and the upwelling index (Pfaff et al. 2011) for (a,b) each year and (c) the interannual change

for most of the variability. First, a large fraction of variance in GSI and CI was observed among localities located 10s of kilometers apart, which coincided well with correlogram analyses, showing positively correlated patterns up to about 100 km. Variability over these scales is likely related to variation in coastal morphology (bathymetry, orientation, elevation) and the interaction with wind-driven upwelling of subsurface waters, which is generally intensified around capes and headlines and strongly modifies patterns of coastal circulation (Figueroa & Moffat 2000, Strub et al. 1998) as well as SST variability, nutrient delivery, and surface chlorophyll (Wieters et al. 2003, Lagos et al. 2005, Correa-Ramirez et al. 2007, Tapia et al. 2009). These environmental conditions, especially temperature and chlorophyll, modulate the reproductive output of several mussel species (Jaramillo & Navarro 1995, Thorarinsdóttir & Gunnarsson 2003, Zardi et al. 2007, Fearman & Moltschaniewskyj 2010, Oyarzún et al. 2018). The

fact that the spatial structure of variability was persistent not only within the reproductive season, but also between years that differed in mean SST and overall upwelling intensity again suggests that coastal topography at scales of 10s of kilometers generates persistent oceanographic features that modulate the performance of benthic filter-feeders on the shore. Indeed, this is characteristic of the physical oceanographic profile of the physical oceanographic profile found in coastal systems and particularly within the coastal boundary layer, which, in contrast with offshore waters, is controlled by the strong modulation of flows by bottom topography and coastal heterogeneity (Lentz & Fewings 2012, Nickols et al. 2015), leaving persistent signals in coastal communities (Menge et al. 1997, 2003, Wieters et al. 2009, Pfaff et al. 2011). Thus, while we did not find a direct correlation between the calculated UI and biological indices, we did find that the dominant spatial scales of variability in coastal hydrodynamics, associated with upwelling processes (Narváez et al. 2004, 2006, Lagos et al. 2005, Wieters 2005), coincide well with the dominant scales of variation in GSI of *P. purpuratus* within our study region.

Second, a large fraction of variation in GSI and CI was also attributed to variability over scales a few centimeters to several meters within a comparatively homogeneous wave-exposed rocky platform. Environmental and biological factors associated with an individual's microhabitat have been shown to affect its performance (e.g. growth, size, shape) and alter behavior and energy allocation to different individual needs. For instance, mussel location within the mussel bed can affect physiological development, including mussel geometry, shell thickness, and reproduction (Briones & Guíñez 2005, Petes et al. 2008, Briones et al. 2014). Other studies in intertidal mussels show large inter-individual variation in reproductive investment and spawning cycles. For instance, Petes et al. (2008) found that the mussel *Mytilus californianus* on the coast of Oregon, USA, displayed varying physiological trade-offs within the same mussel bed that differed with respect to mussel placement. High-edge mussels showed less repro-

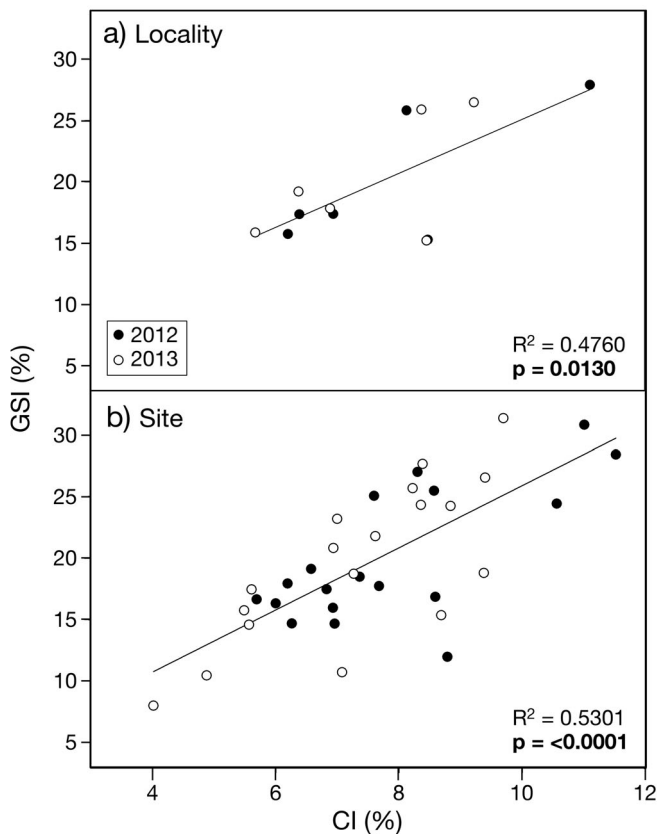


Fig. 9. Linear regression between mean gonadosomatic index (GSI) and condition index (CI) values of *Perumytilus purpuratus* among months at the scale of (a) locality and (b) site (see Fig. 1)

ductive investment with a singular spawning event, whereas low-edge mussels showed continuous spawning throughout the year. Similarly, Phillips (2007a) reported that variation of the maternal investment in egg volume and egg energy content of *M. californianus* in California was primarily due to the individual female rather than other spatial or temporal factors. Although we attempted to standardize the position that collected mussels occupied within the bed (towards the center and surface layers), our results showed large variation among individuals that were in close proximity. Thus, it is possible that a combination of the previously described environmental and biological microenvironment, individual hormonal condition, and genetic makeup can introduce large variation in reproductive investment and individual condition in this dominant intertidal mussel.

Intermediate spatial scales, among platforms 100s of meters apart and among sites 1–5 km apart, accounted for much smaller fractions of variance in GSI and CI of *P. purpuratus*. This suggests that environmental conditions affecting mussel GSI among

selected platforms and sites were relatively more homogeneous across the study region than conditions encountered within a platform or in different localities. A notable exception occurred in June 2013, when variance due to site surpassed the contribution due to the scale of locality (Fig. 4). Such results are due in part to incomplete sampling for that month, but it may also reflect a temporary change in environmental conditions which maximized differences among sites placed 1–2 km apart. Unfortunately, we do not have *in situ* SST records of all platforms and sites, nor the available wave data to evaluate this high-resolution environmental variability across such scales. Satellite-based data also lacked the resolution to compare environmental variables at all of our spatial scales.

Historically, hierarchical sampling designs have been used in marine ecology to identify spatial patterns of species density/cover and community composition (e.g. Underwood & Chapman 1996, Fraschetti et al. 2001), several of which show an emerging generality of large variation at the smallest and the largest of scales, similar to our own results. However, these results are dependent on the range of spatial scales chosen by the authors and the variables measured, rendering direct comparisons difficult at best. Moreover, few studies have used hierarchical sampling to examine patterns of reproduction of invertebrates across multiple spatial scales (e.g. Hughes et al. 2000), so it is difficult to compare hierarchical spatial structure of reproduction with other mussel or invertebrate studies, which are typically concentrated in a single scale (e.g. across sites). It is possible that the same factors that affect the abundance and density of organisms may also affect their individual performance and reproductive success, thus linking patterns of abundance and reproductive investment at certain scales with resulting influences on total larval output (reproductive output) and recruitment. However, a similar pattern of mesoscale variability in reproduction and absence of latitudinal trends was observed in the sea urchin *Strongylocentrotus purpuratus* by Lester et al. (2007) along the upwelling ecosystem shoreline from central to Baja California, which the authors attributed to upwelling modulation of food supply and local conditions. Thus, the pattern documented for *P. purpuratus* may not be restricted to filter-feeders but may be general to many invertebrates in upwelling-influenced shores and could play a key role in recruitment variation along the coast (Hughes et al. 2000).

Although there were no latitudinal trends or clear regional-scale differences in GSI, as could have been

expected given the well documented latitudinal gradient in SST (Broitman et al. 2001) and regional differences in oceanographic regimes (Navarrete et al. 2005, Tapia et al. 2014), we did observe that the localities straddling the reported discontinuity in oceanographic regimes (Guanaqueros and Punta Talca) showed comparatively greater GSI than other sites. Similar results of increased reproductive investment around a major oceanographic regime were also observed by Phillips (2007b) in the intertidal mussel *Mytilus californianus* around Point Conception in California, suggesting that the coastal morphology and oceanographic changes encountered at both of these breaks could generate ideal conditions for reproduction of benthic filter-feeders (i.e. a combination of low temperatures and high phytoplankton biomass).

Determinants of among-site variability in GSI and CI and persistent environmental mosaics

Neither *in situ* measured SST nor a thermal index of UI explained among-site variation in *P. purpuratus* GSI. As previously noted, temperature seems to play an important role in the initiation of gametogenesis and acts as a cue for spawning in many intertidal mussels (Suchanek 1985, Gosling 2003). Indeed, Oyarzún et al. (2018) reported a link between SST and GSI of *P. purpuratus* across sites spanning over 2000 km of the Chilean coastline, with increased GSI values at sites with colder mean SST. They also noted that spawning tended to occur when SST increased by about 1°C, which was witnessed in our results as well (July 2013 at Punta Talca). The lack of congruence concerning temperature correlations between studies is probably due to the large differences in geographic scope and the magnitude of the temperature gradient observed between extremes of the study regions. We showed that over a region of 600 km in central Chile, variability in mean SST is not a simple predictor of GSI among sites. Moreover, although the dominant scale of spatial variability in GSI coincides well with variability in mesoscale processes associated with upwelling (Lagos et al. 2005, Navarrete et al. 2008), thermal indices of upwelling, such as the one presented here (UI, Pfaff et al. 2011), or the MUZIC UI proposed by Tapia et al. (2009, results not shown), did not explain among-site variation in GSI or CI. We interpret this apparently contradictory result as an indication of the multiple environmental determinants and stressors that control individual condition and, in particular, reproductive

investment. Indeed, while the thermal field is usually used as an indicator of upwelling intensity (Wieters 2005, Tapia et al. 2009, Pfaff et al. 2011), SST variability is only one of the many variables that are altered by the phenomenon of upwelling and that can influence an individual's energy allocation. Changes in circulation, dissolved oxygen, trace elements, nutrients, pH, particulate organic matter (POM), chl *a*, zooplankton abundance, and even atmospheric conditions (e.g. winds and fog) are only some of the many variables that could be modulated by mesoscale oceanographic processes (Strub et al. 1998, Menge et al. 2003, Wieters et al. 2003, Broitman & Kinlan 2006). Many of these variables can have direct and indirect effects on individual performance and/or shifts in energy allocation.

Since intertidal mussels rely on the influx of phytoplankton and POM for survival, growth, and reproduction, the expectation is that there are good correlations between reproductive investment and satellite-measured surface chl *a*, and earlier studies have shown this (Jaramillo & Navarro 1995, Narváez et al. 2008). While we did observe a weak but significant direct correlation between chl *a* concentration and the individual CI across the study region, suggesting that increased food availability supports increased growth of soft tissue within the mussel shell, we did not observe any relationship with GSI. Again, this points to the rather complex relationship between upwelling intensity, food availability, and food quality for benthic filter-feeders (Blanchette et al. 2007, Puccinelli et al. 2016), which can interact with other environmental variables to determine an individual's energy budget. Ultimately, the amount of food consumed by mussels will be controlled by particle selection, absorption efficiency, gut capacity, and the ingestion rate of each mussel (Gosling 2003). While both the quantity and quality of the diet will affect the available energy, temperature plays a role in the rates of each physiological process and stimulates key neurohormonal mechanisms that regulate gamete development (Zwann & Mathieu 1992). Thus, at the microscale, there exists 'a complex interplay between neuroendocrine and environmental factors' (Gosling 2003, p. 140).

Interestingly, interannual variation in reproductive investment was small but significant at all localities, and was significantly and inversely associated with observed interannual changes in SST at each site and month of the year. This is consistent with the hypothesis that SST plays an important role in determining mussel GSI, but that it is not the only factor modulating spatial variability. After examining the

reproductive investment of this bioengineer, we found the highest CI values in Coquimbo Bay (Temblador and Guanaqueros) where chl *a* values were highest. GSI values were greatest around the break at Punta Talca, Guanaqueros, and Pichilemu in 2013 when temperatures were lowest and cooling rates were greatest, indicative of strong upwelling. In the case of Guanaqueros, it is worth noting that these conditions (low temperature, high chl *a*) seem to be responsible for a slightly more allometric rather than linear relationship between body and mantle weight, suggesting that mussels may 'opportunistically' take advantage of pockets of favorable environmental conditions (Hawkins et al. 1985), perhaps altering patterns of energy allocation.

CONCLUSIONS

Our results also support the idea that mussel development and the resulting reproductive output is 'context-dependent' and driven by multiple factors that may nonetheless form persistent environmental mosaics (Kroeker et al. 2016), which in this case occur at scales of 10s and up to 100 km. Results found on *P. purpuratus* may not be too different, in general, from other mussel species, many of which are commercially harvested. Indeed, the identification of such dynamics for a community of species across a region is an important ecological consideration for the spatial planning of marine protected areas (Crowder & Norse 2008) and understanding connectivity among populations (Carson et al. 2010). The existence of temporally persistent patterns of reproduction provides grounds for spatial management designed to protect sources of larvae for the metapopulation. The fact that such a persistent pattern occurs over scales of 10s of kilometers may also facilitate implementation of management or conservation measures. Spatial patterns over scales too small (e.g. 100s of meters or less) make it impossible to enforce effective regulation, or require larger areas to achieve similar results. Moreover, since the scales of variability probably are within the same order of the scales of larval dispersal, the spatial pattern of reproduction and therefore expected larval output along the shore may have important consequences on metapopulation dynamics and structure. Lack of simple correlations with environmental variables illustrates how site-specific information on reproductive investment is required to make decisions and shows that general assumptions based on upwelling, SST, or chl *a* are not useful for anticipating mussel

reproductive investment and total larval output across localities. This is the result of the multifactorial nature of the environment, and studies should therefore consider these multiple stressors and determinants of individual performance across multiple spatial scales when designing experiments.

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