

# Combining environmental suitability and population abundances to evaluate the invasive potential of the tunicate *Ciona intestinalis* along the temperate South American coast

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The tunicate *Ciona intestinalis* is an opportunistic invader with high potential for causing economic losses in aquaculture centers. Recent phylogenetic and population genetic analysis support the existence of a genetic complex described as *C. intestinalis* with two ~~main~~ dominant species (sp A and B) occurring worldwide. In Chile, the species has been observed around 30° S of latitude, but no official reports exist for the presence of *C. intestinalis* in southern regions (above 40° S), where most of the mollusk aquaculture centers are located. Here, we used occurrences from multiple invaded regions and extensive field sampling to model and validate the environmental conditions that allow the species to persist and to find the geographic areas with the most suitable environmental conditions for the spread of *C. intestinalis* in the Chilean coast. By studying the potential expansion of *C. intestinalis* southward in the Chilean Coast, we aimed to provide valuable information that might help the development of control plans before the species becomes a significant problem, especially above 40° S. Our results highlight that, by using portions of the habitat that are apparently distinguishable, the species seem to be not only genetically distinct, but ecologically distinct as well. The two regional models fitted for sp A and for sp B showed disagreement on which sections of Chilean coastline are considered more suitable for these species. While the model for sp A identifies moderately to highly suitable areas between 30° and 40° S, the model for sp B classifies the areas around 45° S as the most appropriate. Data from field sampling show a positive linear relationship between density of *C. intestinalis* and the index of suitability for sp A in aquaculture centers. Understanding the relation of the distinct species with the surrounding environment provided valuable insights about probable routes of dispersion in Chile, especially into those areas considered suitable for aquaculture activities but where the species has not yet been recorded. We discuss the implications of our findings as a useful tool to anticipate the invasion of such harmful invasive species with regard to the most relevant environmental variables.

# Combining environmental suitability and population abundances to evaluate the invasive potential of the tunicate *Ciona intestinalis* along the temperate South American coast

**Abstract:** The tunicate *Ciona intestinalis* is an opportunistic invader with high potential for causing economic losses in aquaculture centers. Recent phylogenetic and population genetic analysis support the existence of a genetic complex described as *C. intestinalis* with two main dominant species (sp A and B) occurring worldwide. In Chile, the species has been observed around 30° S of latitude, but no official reports exist for the presence of *C. intestinalis* in southern regions (above 40° S), where most of the mollusk aquaculture centers are located. Here, we used occurrences from multiple invaded regions and extensive field sampling to model and validate the environmental conditions that allow the species to persist and to find the geographic areas with the most suitable environmental conditions for the spread of *C. intestinalis* in the Chilean coast. By studying the potential expansion of *C. intestinalis* southward in the Chilean Coast, we aimed to provide valuable information that might help the development of control plans before the species becomes a significant problem, especially above 40° S. Our results highlight that, by using portions of the habitat that are apparently distinguishable, the species seem to be not only genetically distinct, but ecologically distinct as well. The two regional models fitted for sp A and for sp B showed disagreement on which sections of Chilean coastline are considered more suitable for these species. While the model for sp A identifies moderately to highly suitable areas between 30° and 40° S, the model for sp B classifies the areas around 45° S as the most appropriate. Data from field sampling show a positive linear relationship between density of *C. intestinalis* and the index of suitability for sp A in aquaculture centers. Understanding the relation of the distinct species with the surrounding environment provided valuable insights about probable routes of dispersion in Chile, especially into those areas considered suitable for aquaculture activities but where the species has not yet been recorded. We discuss the implications of our findings as a useful tool to anticipate the invasion of such harmful invasive species with regard to the most relevant environmental variables.

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## Introduction

Marine invertebrates are among the species with the highest potential of invasion and damage (Capinha et al., 2012; Lee et al., 2008; Robinson et al., 2011). Most of the incursions of invasive species in coastal areas are nowadays inevitable due to the worldwide traffic of marine vessels (Ramsay et al., 2008), and the co-transference of organisms during importation of commercially exploited species for stocking or aquaculture purposes (Locke & Hanson, 2009). Many of these opportunist species take advantage of human activity to extend their distribution, and are associated to aquaculture centers, causing large damages in both cultures and natural environments. This is the case of the tunicate *Ciona intestinalis* (Karayucel, 1997, Hecht & Heasman, 1999, Uribe & Etchepare, 2002), a sessile filter feeder that lives in dense aggregations in enclosed or semi-protected marine embayments (Carver et al., 2006). Many of its life history traits make this species a successful invader, including its rapid growth rates (20mm/month), early maturation (8-10 weeks) and high reproductive output (> 10000 eggs/ind). In addition, it exhibits wide environmental tolerance (Carver et al., 2006). Across its native range (North Atlantic) it is considered a dominant competitor in benthic communities, while in its exotic range it occurs as an opportunistic fouling organism on artificial substrates in harbors or in association with aquaculture equipment (Carver et al., 2006).

Recently, it has been discovered that *C. intestinalis* actually corresponds to a genetic complex of 2 to 4 species (Suzuki et al., 2005; Iannelli et al., 2007; Zhan et al., 2010). Two of them, the species A and B are the most common forms, having the widest geographic distribution (Zhan et al., 2010). Both sp A and sp B are distributed worldwide: sp A has invaded the Pacific Ocean, the Mediterranean Sea, Australia and South Africa, while sp B occupies Northern Europe, including the British coastline, as well as the east coast of North America and Canada. The two remaining species, C and D, are rare, and remain restricted to small areas in the Mediterranean and Black Sea, respectively (Zhan et al., 2010). Although there have been efforts to use phenotypic traits such as body color, pigmentation at the distal end of the siphons and the presence or absence of tubercles on the sides of the siphons to facilitate the identification of these different species in the field (Sato et al., 2012), it is likely that available information regarding the distribution of the species is a mix of records of the whole genetic complex.

Over the last decades population outbreaks have been observed at multiple sites along the world including South Africa (Hecht & Heasman, 1999), Scotland (Karayucel, 1997), and Chile (Uribe & Etchepare, 2002). As a result, the species has become a real threat to the marine aquaculture



industry (Edwards & Leung, 2009) particular, few years ago the invasion by *C. intestinalis* in Canada was considered to be at "crisis level", and the species has been considered a major marine invasive issue for the Department of Fisheries and Oceans of Canada (Edwards & Leung, 2009). Under these circumstances, understanding the ecological niche of this particular species complex would provide valuable information about how they manage to survive and establish dense populations in such distinct areas as the Mediterranean Sea and the much colder North Atlantic Ocean.

Along the temperate South American ecoregion, the species has been observed around 30° S Lat. In the Chilean coast, in the regions of Coquimbo and Antofagasta (Castilla et al., 2005), where it has been reported as responsible for economic losses caused by damages on suspended cultures of *Argopecten purpuratus* (Uribe & Etchepare, 2002). According to Castilla & Neill (2009) the introduction and spread of the species into this region has been facilitated by the continuous transfer of seeds and materials between aquaculture centers. However, their wide physiological tolerance, reflected in its extensive world distribution (Madariaga et al., 2014), might facilitate the expansion of their range along the Chilean coast. Currently there are no official reports for the presence of *C. intestinalis* in southern regions (above 40° S), where most of the centers for the culture of mollusks are located (Norambuena & Gonzalez, 2005), and where these small and medium size aquaculture centers play a key role in the economy and social interaction of local communities (Norambuena & Gonzalez, 2005).

A practical way to understand and ultimately predict range expansions of invaders is by characterizing the environmental conditions that are currently suitable for the persistence of a given species (Pearson, 2007), and then identifying those areas where such conditions are distributed in the geographic space (Colwell & Rangel, 2009, Franklin, 2010). A group of quantitative modeling approaches, known collectively as Ecological Niche Modeling (ENM) have been widely used with this purpose in recent years (Soberón & Peterson, 2005; Peterson, 2006; Soberón & Nakamura, 2009; Elith & Leathwick, 2009; Zimmermann et al., 2010; Peterson & Soberón, 2012). The central assumption of ENM is that the response functions estimated in these models provide an effective representation of the spatial response of the species to different environments (Cassini, 2011). In this sense, ENMs provide a quantitative and formal procedure to establish better plans of management and prevention through the assessment of risk or likelihood for potential or ongoing invasions (Locke & Hanson, 2009).

Despite their merits, the use of these methods in the management of invasive species requires two

important difficulties to be overcome. First, results from ENMs rely heavily on the assumption that species are in equilibrium with the environment (Pearman et al., 2008; Colwell & Rangel, 2009; Peterson, 2011). To fulfill this main assumption it is important to take into account analog and non-analog conditions between ranges, aiming to ensure that the ENM analysis remains restricted to those areas that present similar environmental conditions (Randin et al., 2006). Non-analog environments in an invaded range represent those habitats outside the range of values considered to quantify the native range and so, correspond to environmental conditions that have not been experienced by the species before the invasion (Fitzpatrick & Hargrove, 2009). Therefore, conclusions about these areas must be taken with caution (Owens et al., 2013). Second, results of ecological niche models (ENMs) are usually expressed as quantitative suitability indexes or as probability of presence, which are not necessarily linked to population abundances, a key parameter for pest managers or conservation biologists (VanDerWal et al., 2009). To overcome this second caveat, adequate modeling procedures and field validation of the fitted ENMs are necessary.

In this study we combine ENM with extensive field sampling to provide valuable information that might help the development of plans of control before the species becomes a significant problem. In particular we try to answer the following questions: Will *C. intestinalis* continue to spread in the Chilean coast, or does it already occupy most of its potential range? If it continues to spread, will the spread extend to regions containing high concentration of aquaculture centers?, and finally, are niche models indexes reflecting population abundances at a confidence level useful for pest managers and conservation biologists? The answers to these questions will provide key information for an adequate planning of prevention and control task in aquaculture centers, especially in southern Chile, where these centers represent a major economic activity for local communities.

## Methods

### *Species occurrence data*

Confirmed records on the occurrence of *C. intestinalis* were obtained from the Global Biodiversity Information Facility (GBIF – data.gbif.org). After removing duplicate records and a few records that presented obvious errors of georeference, the final dataset consisted of 776 from Northern Europe (considered here as the native range) as well as 107 presences registered in Canada, 98 records from the Pacific coast of the United States, 212 from Southern Europe, and 24 records from Japan. Due to their morphological similarity, and the consequent difficulty that involves the identification of each species in the field (but see Sato et al., 2012), we cannot unambiguously attribute to neither of the species of the genetic complex the records that are available at the public



178 database. Therefore, we decided to follow Zhan et al., (2010) and allocate the set of occurrences to  
179 the species that dominates a specific area. In consequence, data from Canada and Northern Europe  
180 were considered as the current distribution of sp B. The other areas represent the distribution of sp  
181 A. We used occurrences from each area to calibrate single-models (hereafter called “Canada model”  
182 or “Southern Europe model”, etc.) and regional models (considering occurrences from more than  
183 one region where each species dominates). These single and regional models were then used to  
184 predict the potential distribution of *C. intestinalis* throughout the Chilean coast. Original  
185 distributions were defined using a 20 km buffer around the reported presence points.

186

### 187 *Occurrences and density of C. intestinalis in the Chilean coast*

188

189 To validate the results of our ENMs, we obtained confirmed records of *C. intestinalis* by surveys in  
190 aquacultures centers throughout the Chilean coast. We visited the three main regions where  
191 aquacultures centers are located in northern, central and southern Chile according to the information  
192 provided by regional agencies of the Sernapesca (National Fishery Service). Centers producing  
193 oysters, mussels, abalones and scallops were visited. Fifteen localities were sampled from 27 to 43°  
194 S. Lat along the Chilean coast (spanning approximately 1,700 Kms) during the summer seasons of  
195 the years 2013-2015. In each locality all aquaculture centers and infrastructure (docks and pilings)  
196 were visually inspected for presence of *C. intestinalis*. Photographic records were taken and later  
197 were used to calculate the relative density of *C. intestinalis* in each site. To avoid pseudo-replication  
198 we have used average density by locality in our analysis. Density was expressed as the number of  
199 individuals per 225 cm<sup>2</sup> (15 × 15 cm grid).

200

### 201 *Environmental variables*

202


203 We chose oceanographic layers representing various quantitative environmental predictors with a  
204 recognized physiological and ecological relevance for *C. intestinalis* (Carver et al., 2006;  
205 Madariaga et al., 2014). These were Sea Surface Temperature - SST (minimum, mean, maximum  
206 and range), Photosynthetically Available Radiation - PAR (mean, maximum), Salinity (mean), pH  
207 (mean), Dissolved Oxygen - Dissox (mean), Chlorophyll A – Chlo (maximum, mean, minimum).  
208 All variables were obtained from BioOracle database (Tyberghein et al., 2012) with a spatial  
209 resolution of 5 arcmin (c. 9km). Most of the grids contained monthly records for the period between  
210 2002 to 2009, except PARmax and PARmean, which encompassed records from 1997 to 2009. The  
211 environmental layers were processed with Quantum GIS 2.6.0 to fit the extent of each zone.

212



213 *Statistical methods*

214

215 The dataset was separated into separate geographic areas to build single area models (East Canada,  
216 West USA, Japan, Southern Europe, Northern Europe), and regional models that grouped more than  
217 one area where each species dominates. For sp A we calibrated a model with records from Japan,  
218 United States and Southern Europe, and for sp B a model with occurrences from Northern Europe  
219 and Canada. We did not consider models for sp C and sp D, given their lower frequencies in  
220 empirical records and more restricted geographic distributions. We then used Niche Analyst (Qiao  
221 et al., 2013), to perform a Principal Component Analysis (PCA) on the environmental variables and  
222 visualize the environmental space into transformed principal component dimensions. The program  
223 uses a covariance-based approach to PCA calculation. We used minimum volume ellipsoids around  
224 the points of occurrence to delimit, in the environmental space, the conditions considered favorable  
225 for the persistence of the species. We later identified the geographic areas in the Chilean Coast   
226 where those environmental conditions can be found. Finally, we interpreted the output of Maxent  
227 (suitability index) for those regions where the analog environments (similar conditions between the  
228 area where model was calibrated and the area of projection) were similar. This is relevant especially  
229 from a management perspective, because it makes easier to recognize areas with novel  
230 environments where niche model algorithms tend to extrapolate predictions. We also identified  
231 those areas most suitable for aquaculture in the Chilean coast (courtesy of Subsecretaria de Pesca -  
232 Subpesca). This allowed us to visualize the areas under higher risk of invasion and damage, and  
233 hence with more potential for economic losses.

234

235 Ecological niche models were fitted using Maximum Entropy Species Distribution Modelling  
236 software v. 2.3 (Maxent). This is a useful method for making predictions especially when  
237 incomplete information about species distribution is available. By evaluating the climate data at  
238 each location where the species of interest is present, Maxent calculates a probability function that  
239 describes the chances of observing a presence giving the observed distribution of the species and  
240 the environmental conditions across the study area (Phillips et al., 2004; 2006). The output of  
241 Maxent is a continuous variable which indicates environmental suitability. For each individual  
242 model, we used a 20-fold cross-validation scheme, except for the “Japan model”, where we used a  
243 17-fold cross-validation scheme. The area under the curve (AUC) statistic for the Receiver  
244 Operating Characteristic (ROC) was used to measure how well each model discriminates presences  
245 more accurately than a random prediction (Phillips et al., 2006). Fitted models were later projected  
246 over the Chilean coast, using the same environmental variables, to identify where suitable  
247 environments for *C. intestinalis* are likely to occur. The importance of each environmental variable



for every model was assessed by a jackknife procedure, fitting a model using each variable separately and ranking them according to the test gain.

Finally, we evaluate the relationship between suitability values with the observed density. To do so, we follow VanDerWal et al., (2009) and use linear regression and quantile regression (90% percentile) to determine if suitability indexes successfully predict the upper limit of local abundances. Goodness of fit was assessed using simple  $R^2$  values for linear regression, and pseudo  $R^2$  values (Koenker & Machado, 1999) for quantile regression.

## Results

Principal component analysis of the pooled climatic data revealed three significant axes of climatic variation. The first three principal components accounted for 82.02 % of the total variation in the data. The first principal component (PC1) was mainly thermal (related to SSTmean and SSTmin), whereas PC2 was related to SSTrange and the three Chlorophyll measures. Finally, PC3 was mainly related to salinity and pH. Interestingly, the PCA split the populations into two groups (Fig 1). The first cluster includes mainly populations from Japan, Southern Europe and West USA. The other group embraces both, Northern Europe and populations from Canada. The climatic separation into both groups reflects the distribution of the two most common species that compose the genetic complex of *C. intestinalis*. Analog environments for sp.A are almost four times more common in Chile than those for sp.B according to the projections from the minimum volume ellipsoid (Fig 2).



Models for original distributions were significantly better than random and performed well according to AUC (table I). The lowest AUC was obtained for the model of Northern Europe (AUC = 0.82), while the highest was obtained for the model of West USA (AUC = 0.94). For the regional models, the observed AUC values were 0.87 and 0.80 for sp A and sp B, respectively. Only regional models are shown. All the remaining models, obtained with single modelling areas as well as the projections with non-analog environments may be found in Figures S1-2.

The two regional models (sp A and sp B) showed strong disagreement for portions of Chile that are considered suitable for the establishment of *C. intestinalis*. Projections of the model of sp A showed that central and southern regions (30°-40° S, Fig 2) seem more suitable for the establishment for sp A than the extremes parts of Chile (below 25° and above 43° S). Only few patches of suitable habitats are found in the most extreme southern region of the continent, around the 53° S. The

283 model for the sp B revealed that the most suitable habitats are located around the 45° S (Fig 2).  
 284 Additional areas near ~~the~~ 53° S were also classified with high suitability index, the same as  
 285 observed with the model of sp A.

286

287 Based on Jackknife analyses for regional models the distribution of sp A was most influenced by  
 288 Salinity and Chlorophyll A (mean; minimum). For sp B, Sea Surface Temperature (minimum) and  
 289 Photosynthetically Available Radiation (PAR) (mean) were important contributors (Table 1). The  
 290 most important variables also varied in the single models, as in Canada where mean values of  
 291 Chlorophyll A correspond to the variable that contributed most to the model, while in Southern  
 292 Europe, Salinity and ~~Ph~~ were the most important variables (Table I).

293

294 Our field sampling detected *C. intestinalis* in most aquaculture centers examined in 15 localities  
 295 between 27° and 43°S ~~Lat~~. To the best of our knowledge, this is the first report of *C. intestinalis*  
 296 infestations south of 30°S ~~Lat~~, in the temperate South American pacific coast, specifically in  
 297 mussel, oyster and abalone farms. Density of *C.intestinalis* showed a clear north-south pattern, with  
 298 higher densities in northern Chile and low densities in the South.

299

300 The relative density of the species in aquaculture centers revealed contrasting results. For spA. the  
 301 plot showed a positive, linear relationship between observed density and the suitability index  
 302 ( $R^2=0.26$ , Fig 3), and a strong relationship at the 90% percentile (pseudo  $R^2 = 0.55$ , Fig 3). For sp B,  
 303 the relationship was completely absent, and for the upper limit was negative, which makes no sense  
 304 in this context ( $R^2=0.0$ , pseudo  $R^2 = 0.08$ , Fig 3).

305

## 306 Discussion

307

308 Our results showed that, when considering the distribution of each species of the genetic  
 309 complex of *C. intestinalis* (sp A and B), the species seem to be not only genetically distinct, but  
 310 appear to be ecologically distinct as well. Our analysis separated the species into two main groups  
 311 based on their environmental preferences. Interestingly, the PCA analysis of environmental  
 312 conditions for the presence points was coherent with the genetic separation among the species  
 313 within the complex. In consequence, not considering these differences into environmental models  
 314 might lead to imprecise conclusions about the potential distribution of the species outside their  
 315 original range.

316

317 The first principal component is mostly influenced by mean and minimum values of sea surface

318 temperature. These variables grouped occurrences from Northern Europe and Canada where the  
 319 minimum sea temperature reach values close to 5° C and separated them from observations obtained  
 320 in areas like Japan, east USA and the Mediterranean Sea, where minimum values of temperature are  
 321 much higher (11.7° C, based on layers provided by BioOracle). Although the species is recognized  
 322 by its wide tolerance to temperature variation (Carver et al., 2006)), our results suggest a distinct  
 323 range of preferences at least for the two most common species. By isolating the two groups, we  
 324 could improve the power of our predictions by restricting the projections of the models to the  
 325 geographic areas where the environmental conditions are analog to those where the models were  
 326 calibrated. We took this precaution because some model algorithms tend to extrapolate projections  
 327 beyond the range of environmental values used to calibrate the models and end up identifying high  
 328 values of suitability even in conditions where most of the species are unlikely to survive (Owens et  
 329 al., 2013).

330  
 331 The two regional models (for sp A and for sp B) showed disagreement for portions of Chile that are  
 332 considered suitable for the maintenance of the species. Projections of the model of sp A showed that  
 333 climate in Chile is moderately to highly suitable, especially around the 30° and 40° S, while for sp  
 334 B, the areas around the 45° S are the most appropriate. Also, the model for sp B predicted several  
 335 suitable areas beyond the extent of the current invaded range of *C. intestinalis* in the Chilean coast.



336  
 337 We interpreted projections in the Chilean coast only to those analog environments to those where  
 338 the models were calibrated. For the model of sp A, it resulted in a void in the projection layer  
 339 between the 34° – 37° S and between 46° – 51° S. These areas are known as strong upwelling  
 340 centers, and they also receive important influxes of freshwater (Atkinson et al., 2002; Dávila et al.,  
 341 2002). Both factors may generate particular conditions that are not shared by the other areas where  
 342 the species has been found. In any case, our survey confirmed the presence of *C. intestinalis* in  
 343 some aquaculture centers around the 36° S Lat. Initial colonization in this zone started, most likely,  
 344 with specimens that benefited from the exchange of equipment between aquaculture centers from  
 345 other parts of the country where the species has already established dense populations (IV Región,  
 346 around 30° S). For sp B, analog environments are just found southern 40° S, where sea surface  
 347 temperature emulates the conditions found in North Atlantic.

348  
 349 The model for the sp A seems to capture quite well the current distribution of *C. intestinalis* in  
 350 Chile, especially in the area of Coquimbo (29° S 71° W) where the species has been a major  
 351 problem for the culture of scallops (Uribe & Etchepare, 2002). Indeed, it is assumed the species first  
 352 arrived in the area brought by Japanese boats which transported the personal and equipment used in

the implementation of the first centers for the culture of scallop (Madariaga et al., 2014). The exchange of boats and equipment facilitated the spread of the species northward, where it can be found in dense population, also associated to cultures of scallops, especially during the summer. Later, the species could spread southward until reach the area close to Puerto Montt (around 41° S), although in a much lower density. Although the origin of the specimens found in the Chilean coast have been tentatively attributed to Japan, which could correspond to the sp A, the single model adjusted with occurrences from Japan identified no analog environments in the Chilean coast (Online Resource 1). This model is probably biased by the few points of occurrence that we obtained from the public registry for the area of Japan, and also to the proximity of the points. It means, our single model for Japan is probability characterizing only a narrow portion of the possible niche for the species. The model for sp B considered suitable areas situated outside the present distribution in Chile, and where most of the aquaculture centers are located. In some areas, the species is already established but still in low densities. Indeed, most of the fishermen that we could contact in southern regions (above 40° S) did not recognize *C. intestinalis* as a real threat to their cultures, which contrasted with the response from fishermen from the northern-central Chile, who could readily identify *C. intestinalis* and view it as a real threat to their cultures. Anecdotally, farm workers only recognize *C. intestinalis* correctly in the northern region of Chile. In the farms located at southern Chile, workers misidentify *C. intestinalis* with early stages of *Pyura chilensis*. Hence, southern regions must be considered a priority in future plans of management and control, which should include programs to provide adequate training to local fishermen.

Madariaga et al., (2014) used information from unifactorial experiments to assess the tolerance to light, salinity and temperature of individuals collected in the Region of Coquimbo (30° S). Besides, they compile data from literature to associate performance (mixing several metrics as mortality, filtration rate and particle retention efficiency) with salinity and temperature. These authors suggest that the species is physiologically capable to tolerate and perform highly well in a wide range of physical conditions found in Chile, concluding the whole Chilean coastline may be considered at high risk. However, data compiled in this study was not separated into the different species within the complex (i.e. sp A, B, C or D); therefore, the observed performance cannot be assigned to any species in particular or be considered representative of the species already present in Chile. To the best of our knowledge, there are no available studies that compare the physiological tolerance of each of these four species independently. Nevertheless, our results suggest the two dominant species of the genetic complex are probably using different portions of the environmental space. The analyses of Jackknife that included each variable alone, reinforced the separation of the two species in the environmental space, once each model were mostly influenced by particular

388 ~~variables~~. For sp A, Salinity and Chlorophyll A (mean; minimum) are the most important variables.  
 389 For sp B, Sea Surface Temperature (minimum) and Photosynthetically Available Radiation (PAR)  
 390 were important contributors. Previous studies have reported that temperature is an important cue for  
 391 sexual maturation, spawning and recruitment in *C. intestinalis* (Dybern, 1967; Marin et al., 1987;  
 392 Carver et al., 2003; Howes et al., 2007). For instance, in Scandinavian and subarctic populations,  
 393 where temperature rarely exceed ~~the~~ 8° C, the generational time is 2-3 years and the reproduction  
 394 beginning at the first year (Dybern, 1965; Dybern, 1967). For Japan and the warmer Mediterranean  
 395 where temperatures are always above 10° C, the generational times vary between 3 and 6 month and  
 396 sexual maturity is reached after 1 to 2 month, depending on the season (Yamaguchi, 1975). The  
 397 optimal salinity for Mediterranean populations (35‰), is much higher than would normally  
 398 experienced by northern Atlantic coastal populations (Marin et al., 1987). Lambert and Lambert  
 399 (1998) reported that *C. intestinalis* populations on floating docks in southern California harbors  
 400 were vulnerable to pulses of low salinity. On the other hand, Dybern (1967) found that the lower  
 401 salinity limit for adults and developmental stages in Scandinavian populations was 11‰. Such  
 402 differences might emphasize the capability of the species to survive under extremes conditions, but  
 403 can also reinforce the implications from our results, ~~such~~ that each species of the genetic complex  
 404 might be using different portions of the niche. This information is crucial, especially when using  
 405 ENMs to study how species colonize new environments (Sax et al., 2007) and whether they retain  
 406 their climatic niche in a new range (Pearman et al., 2008).

407  
 408 Here, we observed a linear relationship  between relative density and suitability index for sp A,  
 409 specially **considering the upper limit**. VanDerWal et al., (2009) pointed out that suitability indexes  
 410 reflects potential abundance, but other factors may prevent the species attain this potential. In our  
 411 case, the index explain 56% of the variation on the upper limit of the population density among  
 412 aquacultures centers, which means that aquaculture centers located at areas classified  with high  
 413 suitability index could sustain more abundant populations. For sp B, the same relationship was null.  
 414 This information is useful for the mussel industry, which is especially vulnerable to tunicates.

415  
 416 According to the National Fishery Service, the areas around the 42° S encompass most of the  
 417 centers for the culture of molusks in Chile. Such areas must be of high priority for control plans.  
 418 Some areas above the 50° S may also serve as potential habitat for *C. intestinalis* from a strictly  
 419 climatic perspective. However, they are not considered suitable for aquaculture, so it is not clear  
 420 whether they could support any wild population of the species. In fact, (Dumont et al., 2009)  
 421 suggest that despite the well-established populations on artificial structures, the species appears  
 422 unable to colonize natural communities due to predation pressure from native benthic species,

423 especially the rock shrimp *Rhyncocinetes typus*.

424

425 In the case of the few places where marine invaders have been successfully controlled, first actions  
426 typically occurred in the early stages of invasions, right after establishment and initial spread  
427 (Edwards & Leung, 2009). The higher a population size, the longer the species will persist, and  
428 eradication will be no longer considered a management option (Lockwood et al., 2013). In this  
429 context, our results might help the Chilean regulatory agencies to identify which areas must be  
430 prioritized in eventual control plans. Considering the potential risk of southern Chile, management  
431 of *C. intestinalis* invasions should concentrate on the reduction of the per-ship probability of  
432 introduction (Drake & Lodge, 2004), controlling the number of potential invaders on transport or  
433 recreational boaters. Controlling the exchange of contaminated equipment among aquaculture  
434 centers might also reduce the fortuitous spread of the species.

435

436 Risk maps are in worldwide demand for management purposes, however they are clearly dependent  
437 on the type of occurrence data used (Therriault & Herborg, 2008). If it is possible to link suitability  
438 and abundance, ENMs may turn into a very powerful tool in the management of invasive species.  
439 Even if management measures are not been able to eliminate biological invasions, slowing the rate  
440 of invasion or spread of an established species has considerable value (Ruiz & Carlton, 2003). In  
441 our case, ENMs have allowed us to understand the relation of the distinct species that conform the  
442 genetic complex known as *C. intestinalis* with their surrounding environment providing valuable  
443 insights about probable routes of dispersion in Chile, especially into those areas considered  
444 adequate for aquaculture activities and where the species has not been recorded.

445

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447

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458 **References**

- 459 Araújo MB, Peterson AT. 2012. Uses and misuses of bioclimatic envelope modeling. *Ecology*  
460 93:1527–1539.
- 461 Atkinson LP, Valle-Levinson A, Figueroa D, et al. 2002. Oceanographic observations in Chilean  
462 coastal waters between Valdivia and Concepción. *J Geophys Res* 107:1–14.
- 463 Capinha C, Anastácio P, Tenedório JA. 2012. Predicting the impact of climate change on the  
464 invasive decapods of the Iberian inland waters: an assessment of reliability. *Biol Invasions*  
465 14:1737–1751.
- 466 Carver C, Chisholm A, Mallet al. 2003. Strategies to mitigate the impact of *Ciona intestinalis* (L.)  
467 biofouling on shellfish production. *J Shellfish Res* 22:621–632.
- 468 Carver CE, Mallet AL, Vercaemer B. 2006. Biological synopsis of the solitary tunicate *Ciona*  
469 *intestinalis*. 55.
- 470 Cassini MH. 2011. Ecological principles of species distribution models: the habitat matching rule. *J*  
471 *Biogeogr* 38:2057–2065.
- 472 Castilla JC, Neill PE. 2009. Marine bioinvasions in the Southeastern Pacific : status , ecology ,  
473 economic impacts, conservation and management. In: Rilov and Crooks (eds). *Biological*  
474 *invasions in marine ecosystems*. Springer. p. 439-457.
- 475 Castilla JC, Uribe M, Bahamonde N, et al. 2005. Down under the southeastern Pacific: marine non-  
476 indigenous species in Chile. *Biol Invasions* 7:213–232.
- 477 Colwell RK, Rangel TF. 2009. Hutchinson’s duality: the once and future niche. *Proc Natl Acad Sci*  
478 *U S A* 106 Suppl :19651–8.
- 479 Dávila PM, Figueroa D, Müller E. 2002. Freshwater input into the coastal ocean and its relation  
480 with the salinity distribution off austral Chile (35–55°s). *Cont Shelf Res* 22:521–534.
- 481 Drake JM, Lodge DM. 2004. Global hot spots of biological invasions: evaluating options for  
482 ballast-water management. *Proc Biol Sci* 271:575–80.
- 483 Dumont CP, Urriago JD, Abarca a., et al. 2009. The native rock shrimp *Rhynchocinetes typus* as a  
484 biological control of fouling in suspended scallop cultures. *Aquaculture* 292:74–79.
- 485 Dybern BI. 1965. The life cycle of *Ciona intestinalis* (L.) f. *typica* in relation to the environmental  
486 temperature. *Oikos* 16:109–131.
- 487 Dybern BI. 1967. The distribution and salinity tolerance of *Ciona intestinalis* (L.) F. *typica* with  
488 special reference to the waters around Southern Scandinavia. *Ophelia* 4:207–226.
- 489 Edwards PK, Leung B. 2009. Re-evaluating eradication of nuisance species: invasion of the  
490 tunicate, *Ciona intestinalis*. *Front Ecol Environ* 7:326–332.
- 491 Elith J, Leathwick JR. 2009. Species Distribution Models: ecological explanation and prediction  
492 across space and time. *Annu Rev Ecol Evol Syst* 40:677–697.
- 493 Fitzpatrick MC, Hargrove WW. 2009. The projection of species distribution models and the  
494 problem of non-analog climate. *Biodivers Conserv* 18:2255–2261.
- 495 Franklin J. 2010. Mapping Species Distributions: Spatial Inference and Prediction. Cambridge  
496 University Press
- 497 Guisan A, Petitpierre B, Broennimann O, et al. 2014. Unifying niche shift studies: insights from  
498 biological invasions. *Trends Ecol Evol* 29:260–269.
- 499 Hecht T, Heasman K. 1999. The culture of *Mytilus galloprovincialis* in South Africa and the  
500 carrying capacity of mussel farming in Saldanha Bay. *World Aquac Rouge* 30:50–55.





- 501 Heinänen S, Rönkä M, von Numers M. 2008. Modelling the occurrence and abundance of a  
502 colonial species, the arctic tern *Sterna paradisaea* in the archipelago of SW Finland. *Ecography*  
503 (Cop) 31:601–611. 
- 504 Herborg L-M, Jerde CL, Lodge DM, et al. 2007. Predicting invasion risk using measures of  
505 introduction effort and environmental niche models. *Ecol Appl* 17:663–74.
- 506 Howes S, Herbing CM, Darnell P, Vercaemer B. 2007. Spatial and temporal patterns of  
507 recruitment of the tunicate *Ciona intestinalis* on a mussel farm in Nova Scotia, Canada. *J Exp*  
508 *Mar Bio Ecol* 342:85–92.
- 509 Iannelli F, Pesole G, Sordino P, Gissi C. 2007. Mitogenomics reveals two cryptic species in *Ciona*  
510 *intestinalis*. *Trends Genet* 23:419–22.
- 511 Koenker, R., & Machado, JA. 1999. Goodness of fit and related inference processes for quantile  
512 regression. *JASA* 94:1296–1310.
- 513 Lambert CC, Lambert G. 1998. Non-indigenous ascidians in southern California harbors and  
514 marinas. *Mar Biol* 130:675–688.
- 515 Lee H, Reusser DA, Olden JD, et al. 2008. Integrated monitoring and information systems for  
516 managing aquatic invasive species in a changing climate. *Conserv Biol* 22:575–84.
- 517 Locke A, Hanson JM. 2009. Rapid response to non-indigenous species. 1. Goals and history of  
518 rapid response in the marine environment. *Aquat Invasions* 4:237–247.
- 519 Madariaga DJ, Rivadeneira MM, Tala F, Thiel M. 2014. Environmental tolerance of the two  
520 invasive species *Ciona intestinalis* and *Codium fragile*: their invasion potential along a  
521 temperate coast. *Biol Invasions* 16:2507–2527.
- 522 Marin MG, Bressan M, Beghi L, Brunetti R. 1987. Thermo-haline tolerance of *Ciona intestinalis*  
523 (L., 1767) at different developmental stages. *Cah Biol Mar* 28:47–57.
- 524 Nielsen SE, Johnson CJ, Heard DC, Boyce MS. 2005. Can models of presence-absence be used to  
525 scale abundance? Two case studies considering extremes in life history. *Ecography (Cop)*  
526 28:197–208. 
- 527 Norambuena, R. and L. Gonzalez. 2005. National aquaculture sector overview Fact Sheets - Chile.  
528 FAO, Rome.
- 529 Owens HL, Campbell LP, Dornak LL, et al. 2013. Constraints on interpretation of ecological niche  
530 models by limited environmental ranges on calibration areas. *Ecol Modell* 263:10–18.
- 531 Parker IM, Simberloff D, Lonsdale WM, et al. 1999. Impact: Toward a Framework for  
532 Understanding the Ecological Effects of Invaders. *Biol Invasions* 1:3–19. 
- 533 Pearman PB, Guisan A, Broennimann O, Randin CF. 2008. Niche dynamics in space and time.  
534 *Trends Ecol Evol* 23:149–58.
- 535 Pearson RG. 2007. Species Distribution Modeling for conservation educators and practitioners.  
536 *Lessons in Conservation* 3:54–89
- 537 Peterson a. T, Soberón J. 2012. Species distribution modeling and ecological niche modeling:  
538 Getting the Concepts Right. *Nat a Conserv* 10:102–107.
- 539 Peterson AT. 2006. Uses and requirements of ecological niche models and related distributional  
540 models. *Biodivers Informatics* 3:59–72.
- 541 Peterson AT. 2011. Ecological niche conservatism: a time-structured review of evidence. *J Biogeogr*  
542 38:817–827.
- 543 Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic  
544 distributions. *Ecol Modell* 190:231–259.

- 545 Phillips SJ, Dudík M, Schapire RE. 2004. A maximum entropy approach to species distribution  
546 modeling. Twenty-first Int. Conf. Mach. Learn. - ICML '04. ACM Press, New York, New  
547 York, USA, p 83
- 548 Qiao H, Escobar LE, Soberón J, Campbell L, Peterson AT. 2014. NicheA. Version 1.2 beta.  
549 Available at: <http://biodiversity-informatics-training.org/software-data-sources/nichea/>  
550 (accessed on December 2014). 
- 551 Ramcharan CW, Padilla DK, Dodson SI. 2011. Models to Predict Potential Occurrence and Density  
552 of the Zebra Mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. 49: 2611–2620.
- 553 Ramsay A, Davidson J, Landry T, Arsenault G. 2008. Process of invasiveness among exotic  
554 tunicates in Prince Edward Island, Canada. Biol Invasions 10:1311–1316.
- 555 Randin CF, Dirnböck T, Dullinger S, et al. 2006. Are niche-based species distribution models  
556 transferable in space? J Biogeogr 33:1689–1703. 
- 557 Ricciardi A. 2003. Predicting the impacts of an introduced species from its invasion history: an  
558 empirical approach applied to zebra mussel invasions. Freshw Biol 48:972–981.
- 559 Robinson LM, Elith J, Hobday a. J, et al. 2011. Pushing the limits in marine species distribution  
560 modelling: lessons from the land present challenges and opportunities. Glob Ecol Biogeogr  
561 20:789–802.
- 562 Ruiz GM, Carlton J. 2003. Invasive Species: Vectors And Management Strategies. Island Press.
- 563 Sato A, Satoh N, Bishop JDD. 2012. Field identification of “types” A and B of the ascidian *Ciona*  
564 *intestinalis* in a region of sympatry. Mar Biol 159:1611–1619.
- 565 Sax DF, Stachowicz JJ, Brown JH, et al. 2007. Ecological and evolutionary insights from species  
566 invasions. Trends Ecol Evol 22:465–71.
- 567 Soberón J, Nakamura M. 2009. Niches and distributional areas : Concepts , methods , and  
568 assumptions. PNAS 106:19644–19650.
- 569 Soberón J, Peterson AT. 2005. Interpretation of models of fundamental ecological niches and  
570 species’ distributional areas. Biodivers Informatics 2:1–10.
- 571 Therriault TW, Herborg L. 2008. A qualitative biological risk assessment for vase tunicate *Ciona*  
572 *intestinalis* in Canadian waters : using expert knowledge. J Mar Sci 65:781–787.
- 573 Tyberghein L, Verbruggen H, Pauly K, et al. 2012. Bio-ORACLE: a global environmental dataset  
574 for marine species distribution modelling. Glob Ecol Biogeogr 21:272–281.
- 575 Uribe E, Etchepare I. 2002. Effects of biofouling by *Ciona intestinalis* on suspended culture of  
576 *Argopecten purpuratus*. Bull. AAC. 3:93-95.
- 577 VanDerWal, J., Shoo, L. P., Johnson, C. N., & Williams, S. E.. 2009. Abundance and the  
578 environmental niche: environmental suitability estimated from niche models predicts the upper  
579 limit of local abundance. Am Nat 174:282-291.
- 580 Venette RC, Kriticos DJ, Magarey RD, et al. 2010. Pest Risk Maps for Invasive Alien Species: A  
581 Roadmap for Improvement. Bioscience 60:349–362. 
- 582 Yamaguchi M. 1975. Growth and reproductive cycles of the marine fouling ascidians *Ciona*  
583 *intestinalis*, *Styela plicata*, *Botrylloides violaceus*, and *Leptoclinum mitsukurii* at Aburatsubo-  
584 Moroiso Inlet (central Japan). Mar Biol 29:253–259.
- 585 Zhan A, Macisaac HJ, Cristescu ME. 2010. Invasion genetics of the *Ciona intestinalis* species  
586 complex: from regional endemism to global homogeneity. Mol Ecol 19:4678–94.
- 587 Zimmermann NE, Edwards TC, Graham CH, et al. 2010. New trends in species distribution  
588 modelling. Ecography (Cop) 33:985–989

589 **Figure captions**

590

591 **Figure 1.** Principal component analysis for the environmental variables at the presence points. PC1  
592 and PC2 scores are shown. Colors represent different populations of *C. intestinalis*. Scores at PC1  
593 markedly separate distributions of sp A (Japan, Southern Europe and USA) and sp B (Northern  
594 Europe and Canada).

595

596 **Figure 2.** Projections of the potential distribution of *C. intestinalis* sp A and sp B on Chilean coast.  
597 Non analog environments are projections on Chilean environments that may be or may not be  
598 represented at locations used to fit the model. Analog environments are projections only on those  
599 Chilean environments that are represented at locations used to fit the model.

600

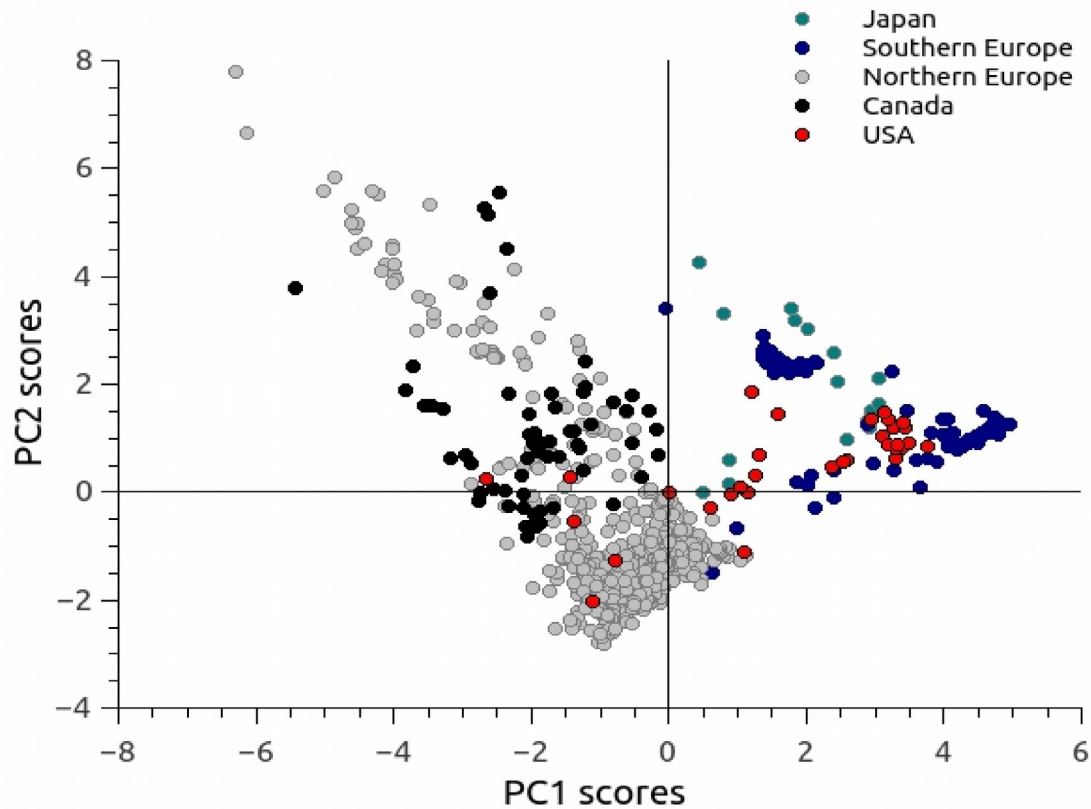
601 **Figure 3.** Regressions between suitability indices from ENMs and observed densities in the field  
602 for sp A and sp B. Dark lines represent linear regressions, gray lines represent 90% quantile  
603 regressions.

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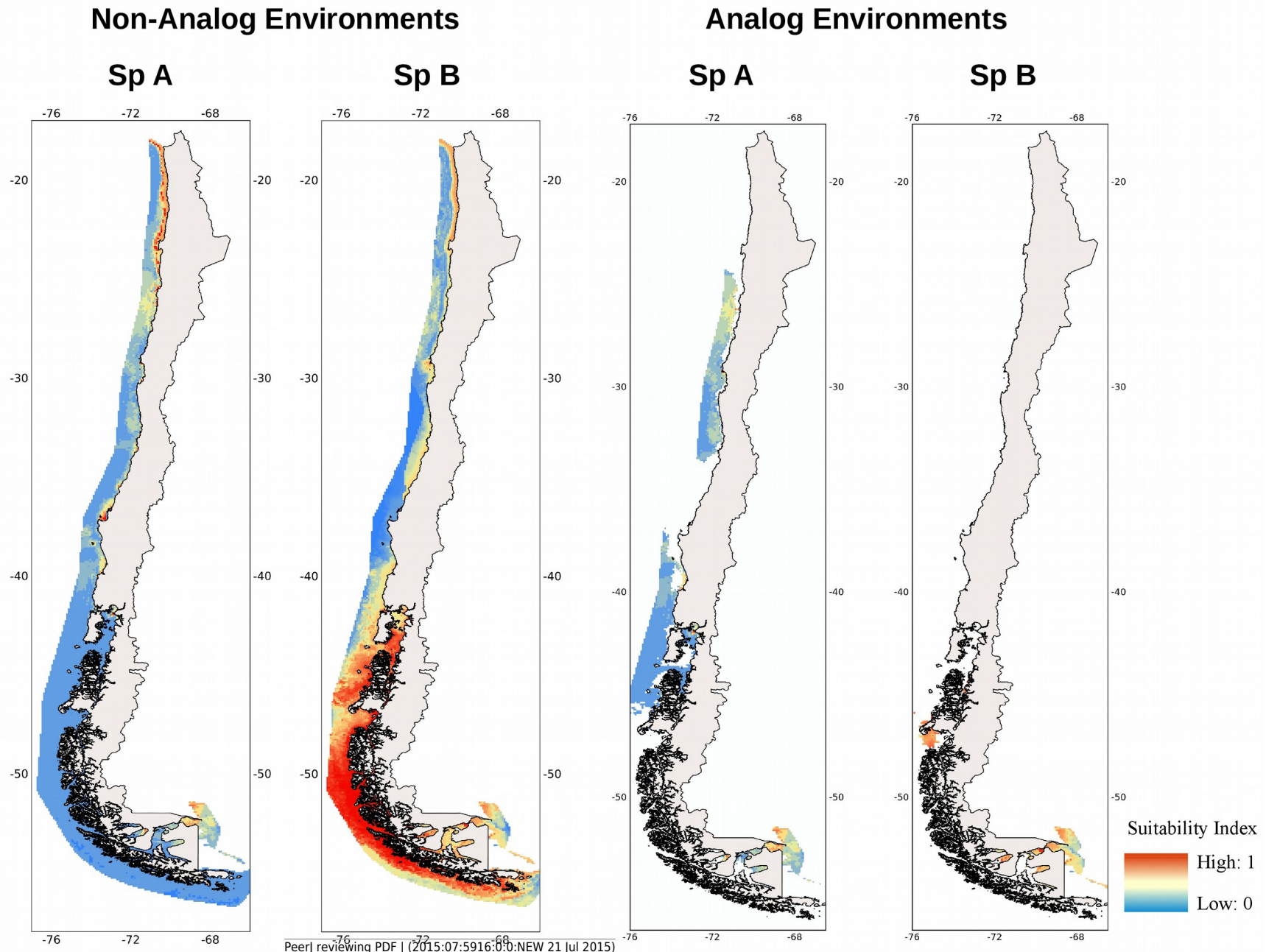
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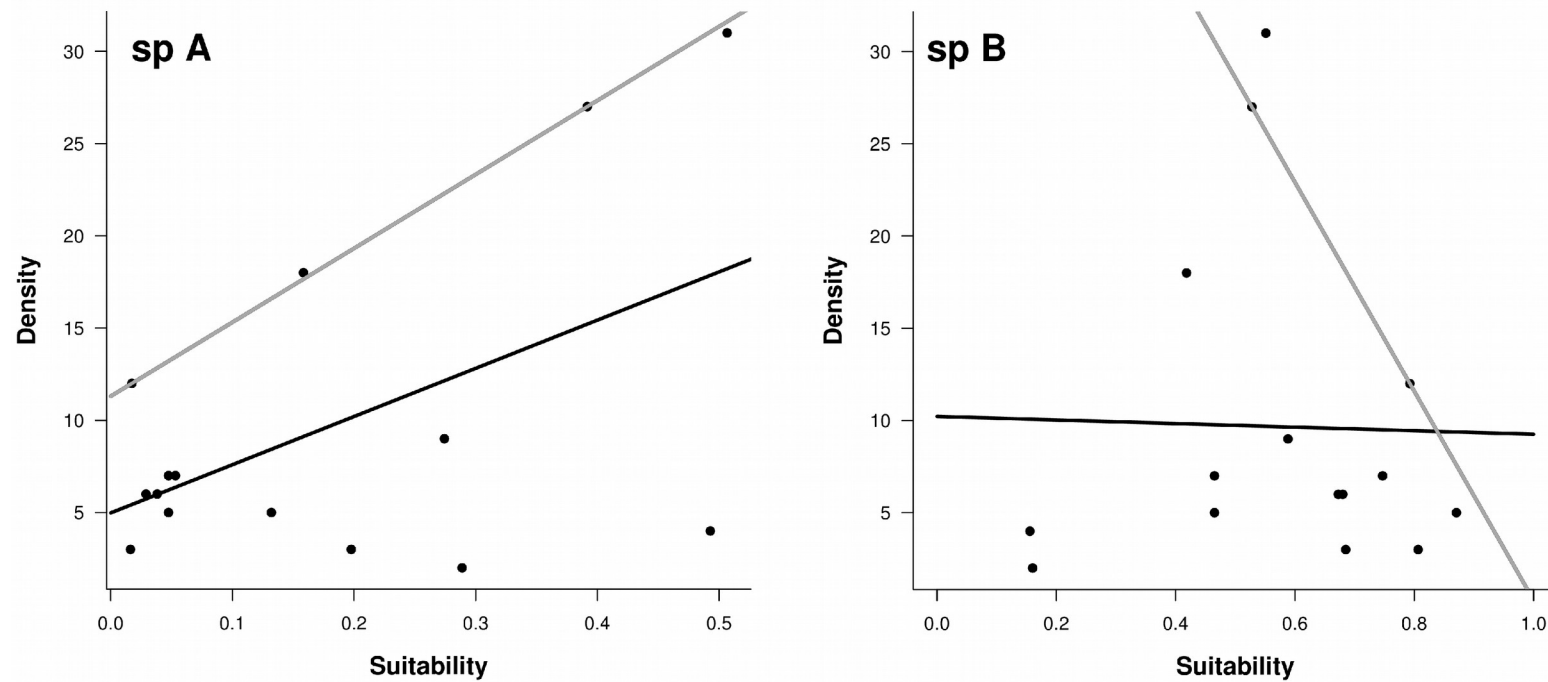
**Figure 1.** Principal component analysis for the environmental variables at the presence points. PC1 and PC2 scores are shown. Colors represent different populations of *C. intestinalis*. Scores at PC1 markedly separate distributions of sp A (Japan, Southern Europe and USA) and sp B (Northern Europe and Canada).



**Figure 2.** Projections of the potential distribution of *C. intestinalis* sp A and sp B on Chilean coast. Non analog environments are projections on Chilean environments that may be or may not be represented at locations used to fit the model. Analog environments are projections only on those Chilean environments that are represented at locations used to fit the model.



**Figure 3.** Regressions between suitability indices from ENMs and observed densities in the field for sp A and sp B. Dark lines represent linear regressions, gray lines represent 90% quantile regressions.



**Table I.** Values of the average test AUC for the replicate runs for each single and regional model. Environmental variables with the highest gain when used in isolation are shown.

Model	AUC	Variable with the highest gain
Canada	0.834	Chlorophyll A (mean)
Japan	0.889	Chlorophyll A (mean; maximum)
West USA	0.939	Photosynthetically Available Radiation (PAR)(maximum)
Southern Europe	0.892	Salinity; Ph
Northern Europe	0.817	Sea Surface Temperature (minimum); PAR (mean)
sp A	0.873	Salinity; Chlorophyll A (mean; minimum)
sp B	0.804	Sea Surface Temperature (minimum); PAR (mean)



Supplementary Figures 1, 2

# **Combining environmental suitability and population abundances to evaluate the invasive potential of the tunicate *Ciona intestinalis* along the temperate South American coast**

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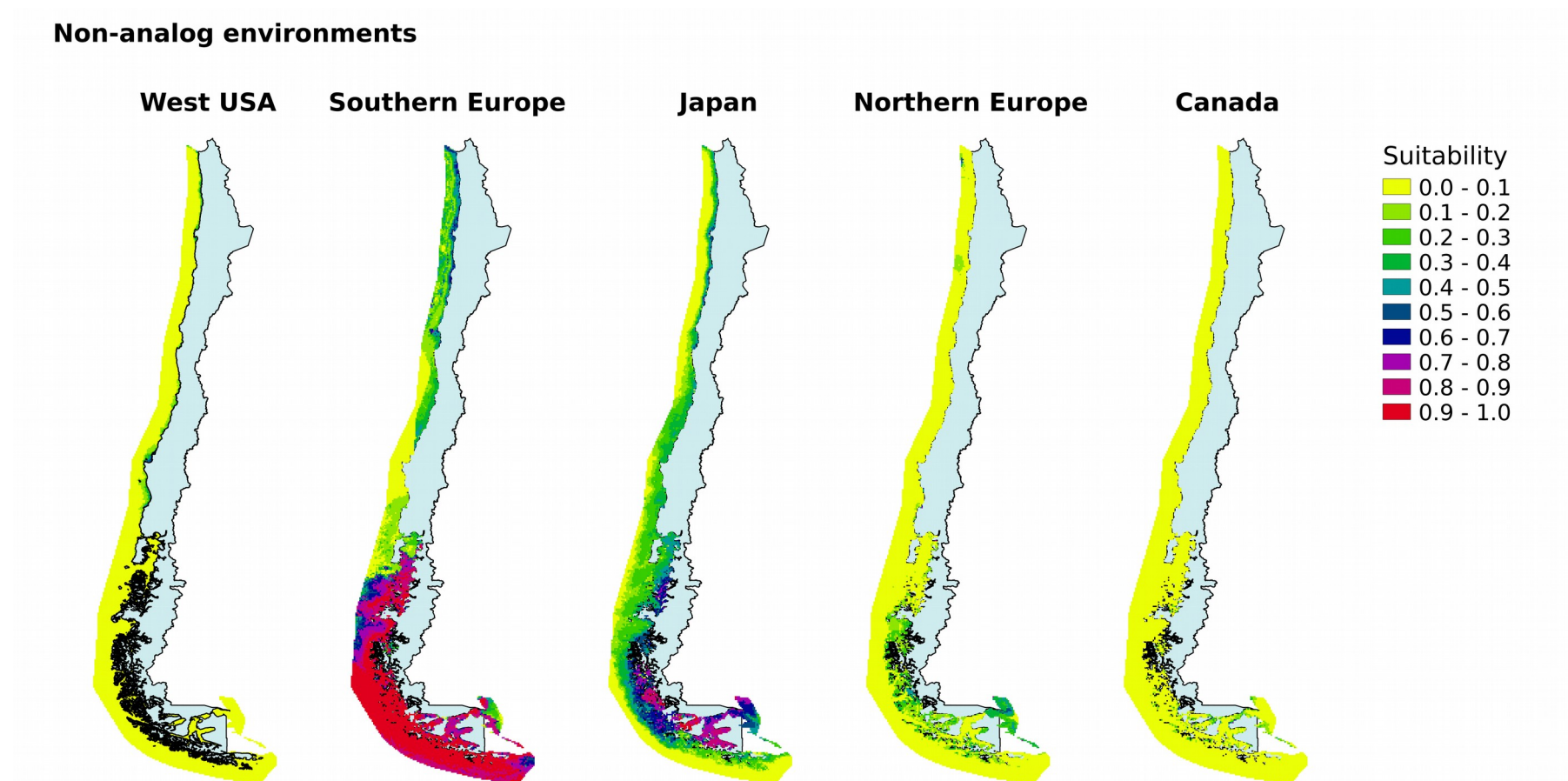
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# Supplementary figures

**Figure 1.** Projections of the potential distribution of *C. intestinalis* on Chilean coast using non analog environments for each of the five locations used in the analysis.



**Figure 2.** Projections of the potential distribution of *C. intestinalis* on Chilean coast using just analog environments for each of the five locations used in the analysis.

