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

Stella M Januario, Sergio A Estay, Fabio A Labra, Mauricio Lima

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 30º S of latitude, but no official reports exist for the presence of *C. intestinalis* in southern regions (above 40º 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 40º 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 30º and 40º S, the model for sp B classifies the areas around 45º 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.

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

4   **Abstract:** The tunicate *Ciona intestinalis* is an opportunistic invader with high potential for

5   causing economic losses in aquaculture centers. In Chile, the species has been observed around

6   30° S of latitude, but no official reports exist for the presence of *C. intestinalis* in southern regions

7   (above 40° S), where most of the mollusk aquaculture centers are located. Here, we used

8   occurrences from multiple invaded regions sourced from public databases, and extensive field

9   sampling to model and validate the environmental conditions that allow the species to persist and

10   to find the geographic areas with the most suitable environmental conditions for the spread of *C.*

11   *intestinalis* along the Chilean coast. Our results highlight that, by using portions of the habitat

12   that are apparently distinguishable, the species seem to be not only genetically distinct, but

13   ecologically distinct as well. The two regional models fitted for sp A and for sp B showed

14   disagreement on which sections of Chilean coastline are considered more suitable for these

15   species. While the model for sp A identifies moderately to highly suitable areas between 30° and

16   40° S, the model for sp B classifies the areas around 45° S as the most appropriate. Data from

17   field sampling show a positive linear relationship between density of *C. intestinalis* and the index

18   of suitability for sp A in aquaculture centers. Understanding the relationship of the distinct

19   species with the surrounding environment provided valuable insights about probable routes of

20   dispersion in Chile, especially into those areas considered suitable for aquaculture activities but

21   where the species has not yet been recorded.

22   Stella M. Januario, *Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia*

23   *Universidad Católica de Chile, Santiago, Chile. and Departamento de Ciencias Biológicas y*

24   *Químicas, Facultad de Ciencias, Universidad San Sebastián, Valdivia, Chile*

25   Sergio A. Estay, *Instituto de Ciencias Ambientales y Evolutivas, Facultad de Ciencias.*

26   *Universidad Austral de Chile, Valdivia, Chile. and Center of Applied Ecology & Sustainability*

27   *(CAPES), Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago,*

28   *Chile.*

29   Fabio A. Labra, *Centro de Investigación e Innovación para el Cambio Climático, Facultad de*

30   *Ciencias, Universidad Santo Tomas, Santiago, Chile.*

31   Mauricio Lima, *Departamento de Ecología, Pontificia Universidad Católica de Chile, Santiago,*

32   *Chilea and Center of Applied Ecology & Sustainability (CAPES), Facultad de Ciencias*

33   *Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile.*

34   **Corresponding author**

35   Stella M. Januario*, *Departamento de Ecología, Pontificia Universidad Católica de Chile,*

36   *Santiago, Chile. Phone 56962162006, stella.mjs@gmail.com*

37   *Current Address: *Departamento de Ciencias Biológicas y Químicas, Facultad de Ciencias,*

38   *Universidad San Sebastián, General Lagos 1163, Valdivia, Chile*

Introduction

39

40   Marine invertebrates are amongst the species with the highest potential for invasion and

41   damage (Capinha et al., 2012; Lee et al., 2008; Robinson et al., 2011). Most of the incursions of

42   invasive species in coastal areas are nowadays inevitable due to the worldwide traffic of marine

43   vessels (Ramsay et al., 2008; Ashelby et al., 2013), and the co-transference of organisms during

44   importation of commercially exploited species for stocking or aquaculture purposes (Locke &

45   Hanson, 2009). Many of these opportunist species take advantage of human activity to extend

46   their distributions, and are associated with aquaculture centers, causing large damage to both

47   cultures and natural environments. This is the case of the tunicate *Ciona intestinalis* (Karayucel,

48   1997, Hecht & Heasman, 1999, Uribe & Etchepare, 2002), a sessile filter feeder that lives in

49   dense aggregations in enclosed or semi-protected marine embayments (Carver et al., 2006). Many

50   of its life history traits make this species a successful invader, including its rapid growth rates

51   (20mm/month), early maturation (8-10 weeks) and high reproductive output (> 10000

52   eggs/individual). In addition, it exhibits wide environmental tolerance, especially temperature

53   (Carver et al., 2003). Across its native range (North Atlantic) it is considered a dominant

54   competitor in benthic communities, while in its exotic range it occurs as an opportunistic fouling

55   organism on artificial substrates in harbors or in association with aquaculture equipment (Carver

56   et al., 2006).

57   Recently, it has been discovered that the taxon *C. intestinalis* actually corresponds to a genetic

58   complex of 2 to 4 species (Suzuki et al., 2005; Iannelli et al., 2007; Zhan et al., 2010). Two of

59   them, the species A and B are the most common forms, with the widest geographic distribution

60   (Zhan et al., 2010). Both sp A and sp B are distributed worldwide: sp A has invaded the Pacific

61   Ocean, the Mediterranean Sea, Australia and South Africa, while sp B occupies Northern Europe,

62   including the British coastline, as well as the east coast of North America and Canada. The two

63   remaining species, C and D, are rare, and remain restricted to small areas in the Mediterranean

64   and Black Sea, respectively (Zhan et al., 2010). Recently, has been proposed that sp. A actually

65   correspond to *C. robusta* and sp. B to the original *C. intestinalis* (Brunetti et al., 2015; Pennati et

66   al. 2015). Although there have been efforts to use phenotypic traits such as body color,

67   pigmentation at the distal end of the siphons and the presence or absence of tubercles on the sides

68   of the siphons to facilitate the identification of these different species in the field (Sato et al.,

69   2012; Brunetti et al., 2015; Pennati et al. 2015), it is likely that available information regarding

70   the distribution of the species is a mix of records of the whole genetic complex. For this reason,

71   in this study we will maintain the sp. A and sp. B classification, but keeping in mind the new

72   proposal taxonomy.

73   Over the last few decades population outbreaks have been observed at many sites around the

74   world including South Africa (Hecht & Heasman, 1999), Scotland (Karayucel, 1997), and Chile

75   (Uribe & Etchepare, 2002). As a result, the species has become a serious biofouling problem to

76   the marine aquaculture industry (Edwards & Leung, 2009). In particular, few years ago the

77   invasion by *C. intestinalis* in Canada was considered to be at "crisis level", and the species has

78   been considered a major marine invasive issue for the Department of Fisheries and Oceans of

79   Canada (Edwards & Leung, 2009). Under these circumstances, understanding the ecological

80   niche of this particular species complex would provide valuable information about how they

81   manage to survive and establish dense populations in such distinct areas as the Mediterranean Sea

82   and the much colder North Atlantic Ocean.

83   Along the temperate South American ecoregion, the species has been recorded from around 30º S

84   Lat. in the Chilean coast, in the regions of Coquimbo and Antofagasta (Castilla et al., 2005),

85   where it has been reported as being responsible for economic losses caused by damage to

86   suspended cultures of *Argopecten purpuratus* (Uribe & Etchepare, 2002). According to Castilla &

87   Neill (2009) the introduction and spread of the species into this region has been facilitated by the

88   continuous transfer of propagules and materials between aquaculture centers. However, its wide

89   physiological tolerance, reflected in its extensive world distribution (Madariaga et al., 2014),

90   might facilitate the expansion of its range along the Chilean coast. Currently there are no official

91   reports for the presence of *C. intestinalis* in southern regions (above 40º S), where most of the

92   centers for the culture of molluscs are located (Norambuena & Gonzalez, 2005), and where small

93   and medium size aquaculture centers play a key role in the economy and social interaction of

94   local communities (Norambuena & Gonzalez, 2005).

95   A practical way to understand and ultimately predict range expansions of invaders is by
96   characterizing the environmental conditions that are currently suitable for the persistence of a
97   given species (Pearson, 2007), and then identifying those areas where such conditions are
98   distributed in the geographic space (Colwell & Rangel, 2009, Franklin, 2010). A group of
99   quantitative modeling approaches, known collectively as Ecological Niche Modeling (ENM)
100   have been widely used for this purpose in recent years (Soberón & Peterson, 2005; Peterson,
101   2006; Soberón & Nakamura, 2009; Elith & Leathwick, 2009; Zimmermann et al., 2010; Peterson
102   & Soberón, 2012). The central assumption of ENM is that the response functions estimated in
103   these models provide an effective representation of the spatial response of the species to different
104   environments (Cassini, 2011). In this sense, ENMs provide a quantitative and formal procedure to
105   establish better plans of management and prevention through the assessment of risk or likelihood
106   for potential or ongoing invasions (Locke & Hanson, 2009).

107   Despite their merits, the use of these methods in the management of invasive species requires two
108   important difficulties to be overcome. First, results from ENMs rely heavily on the assumption
109   that species are in equilibrium with the environment (Pearman et al., 2008; Colwell & Rangel,
110   2009; Peterson, 2011). To fulfill this main assumption it is important to take into account habitat
111   similarity between ranges, aiming to ensure that the ENM analysis remains restricted to those
112   areas that present similar environmental conditions (Randin et al., 2006). In this vein, analog
113   environments in an invaded range represent those habitats inside the range of values considered
114   to quantify the native range and so, correspond to environmental conditions that have been
115   experienced by the species before the invasion; otherwise, habitat are considered non-analog, and
116   conclusions about these environments must be taken with caution (Owens et al., 2013; Fitzpatrick
117   & Hargrove, 2009). Second, results of ecological niche models (ENMs) are usually expressed as
118   quantitative suitability indexes or as probability of presence, which are not necessarily linked to
119   population abundances, a key parameter for pest managers or conservation biologists
120   (VanDerWal et al., 2009). To overcome this second caveat, adequate modeling procedures and
121   field validation of the fitted ENMs are necessary.

122   In this study we combine ENM with extensive field sampling to provide valuable information
123   that might help the development of plans of control before these species become a significant

124   problem. In particular we try to answer the following questions: Will the complex *C. intestinalis*

125   continue to spread in the Chilean coast, or does it already occupy most of its potential range? If it

126   continues to spread, will the spread extend to regions containing high concentration of

127   aquaculture centers?. Finally, are niche models indexes reflecting population abundances at a

128   confidence level useful for pest managers and conservation biologists? The answers to these

129   questions will provide key information for an adequate planning of prevention and control task in

130   aquaculture centers, especially in southern Chile, where these centers represent a major economic

131   activity for local communities.

132   **Methods**

133   *Species occurrence data*

134   Confirmed records on the occurrence of *C. intestinalis* were obtained from the Global

135   Biodiversity Information Facility (GBIF – www.gbif.org). After removing duplicate records and a

136   few records that presented obvious errors of georeference, the final dataset consisted of 776 from

137   Northern Europe (considered here as the native range) as well as 107 presences registered in

138   Canada, 98 records from the Pacific coast of the United States, 212 from Southern Europe, and

139   24 records from Japan. Due to their morphological similarity, and the consequent difficulty

140   involved in the identification of each species in the field (but see Sato et al., 2012), we cannot

141   unambiguously attribute records from the public databases to the different species within the

142   genetc complex. Therefore, we decided to follow Zhan et al., (2010) and allocate the set of

143   occurrences to the species that dominates a specific area.Therefore, data from Canada and

144   Northern Europe were considered as the current distribution of sp B. The other areas represent

145   the distribution of sp A. We used occurrences from each area to calibrate single-models (hereafter

146   called "Canada model" or "Southern Europe model", etc.) and regional models (considering

147   occurrences from more than one region where each species dominates). These single and regional

148   models were then used to predict the potential distribution of *C. intestinalis* throughout the

149   Chilean coast. Original distributions were defined using a 20 km buffer around the reported

150   presence points.

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

152   To validate the results of our ENMs, we obtained confirmed presence of *C. intestinalis* by

153   surveys in aquacultures centers throughout the Chilean coast. We visited the three main regions

154   where aquacultures centers are located in northern, central and southern Chile according to the

155   information provided by regional agencies of the Sernapesca (National Fishery Service). Centers

156   producing oysters, mussels, abalones and scallops were visited. Fifteen localities were sampled

157   from 27 to 43° S. latitude. along the Chilean coast (spanning approximately 1,700 Kms) during

158   the summer seasons of the years 2013-2015. In each locality, all aquaculture centers and

159   infrastructure (docks and pilings) were visually inspected for presence of *C. intestinalis*.

160   Photographic records were taken and later were used to calculate the relative density of *C.*

161   *intestinalis* in each site. Considering that some aquaculture centers are located closer than the

162   resolution of oceanographic layers used in this study (see next section),we used average density

163   by locality in our analysis to avoid pseudo-replication. Density was expressed as the number of

164   individuals per 225 cm^2 (15 × 15 cm grid).

165   *Environmental variables*

166   We chose oceanographic layers representing various quantitative environmental predictors with a

167   recognized physiological and ecological relevance for *C. intestinalis* (Carver et al., 2006;

168   Madariaga et al., 2014). These were Sea Surface Temperature - *SST* (minimum, mean, maximum

169   and range), Photosynthetically Available Radiation - *PAR* (mean, maximum), Salinity (mean), pH

170   (mean), Dissolved Oxygen - *Dissox* (mean), Chlorophyll A – *Chlo* (maximum, mean, minimum).

171   All variables were obtained from BioOracle database (Tyberghein et al., 2012) with a spatial

172   resolution of 5 arcmin (c. 9km). Most of the grids contained monthly records for the period

173   between 2002 to 2009, except PARmax and PARmean, which encompassed records from 1997 to

174   2009. The environmental layers were processed with Quantum GIS 2.6.0 to fit the extent of each

175   zone.

176   *Statistical methods*

177   The dataset was separated into separate geographic areas to build single area models (East

178   Canada, West USA, Japan, Southern Europe, Northern Europe), and regional models that

179   grouped more than one area where each species dominates. For sp A we calibrated a model with

180   records from Japan, United States and Southern Europe, and for sp B a model with occurrences

181   from Northern Europe and Canada. We did not consider models for sp C and sp D, given their

182   lower frequencies in empirical records and more restricted geographic distributions. We then used

183   Niche Analyst (Qiao et al., 2013), to perform a Principal Component Analysis (PCA) on the

184   environmental variables and visualize the environmental space into transformed principal

185   component dimensions. The program uses a covariance-based approach to PCA calculation. We

186   used minimum volume ellipsoids around the points of occurrence to delimit, in the environmental

187   space, the conditions considered favorable for the persistence of the species. We later identified

188   the geographic areas in the Chilean Coast where those environmental conditions can be found.

189   Finally, we interpreted our results (suitability index) for those regions where the analog

190   environments (similar conditions between the area where model was calibrated and the area of

191   projection) were similar. This is relevant especially from a management perspective, because it

192   makes easier to recognize areas with novel environments where niche model algorithms tend to

193   extrapolate predictions. We also identified those areas most suitable for aquaculture in the

194   Chilean coast (courtesy of Subsecretaria de Pesca - Subpesca). This allowed us to visualize the

195   areas under higher risk of invasion and damage, and hence with more potential for economic

196   losses.

197   Ecological niche models were fitted using Maximum Entropy Species Distribution Modelling

198   software v. 2.3 (Maxent). This is a useful method for making predictions especially when

199   incomplete information about species distribution is available. By evaluating the climate data at

200   each location where the species of interest is present, Maxent calculates a probability function

201   that describes the chances of observing a presence giving the observed distribution of the species

202   and the environmental conditions across the study area (Phillips et al., 2004; 2006). The output of

203   Maxent is a continuous variable which indicates environmental suitability. For each individual

204   model, we used a 20-fold cross-validation scheme, except for the "Japan model", where we used

205   a 17-fold cross-validation scheme. The area under the curve (AUC) statistic for the Receiver

206   Operating Characteristic (ROC) was used to measure how well each model discriminates

207   presences more accurately than a random prediction (Phillips et al., 2006). Fitted models were

208   later projected over the Chilean coast, using the same environmental variables, to identify where

209   suitable environments for *C. intestinalis* are likely to occur. The importance of each

210   environmental variable for every model was assessed by a jackknife procedure, fitting a model

211   using each variable separately and ranking them according to the test gain.

212   Finally, we evaluate the relationship between suitability values with the observed density. To do

213   so, we follow VanDerWal et al., (2009) and use linear regression and quantile regression (90%

214   percentile) to determine if suitability indexes successfully predict the upper limit of local

215   abundances. Goodness of fit was assessed using simple R^2 values for linear regression, and

216   pseudo R^2 values (Koenker & Machado, 1999) for quantile regression.

217   **Results**

218   Principal component analysis of the pooled climatic data revealed three significant axes of

219   climatic variation. The first three principal components accounted for 82.02 % of the total

220   variation in the data. The first principal component (PC1) was mainly thermal (related to

221   SSTmean and SSTmin), whereas PC2 was related to SSTrange and the three Chlorophyll

222   measures. Finally, PC3 was mainly related to salinity and pH. Interestingly, the PCA split the

223   populations into two groups (Fig 1). The first cluster includes mainly populations from Japan,

224   Southern Europe and West USA. The other group embraces both, Northern Europe and

225   populations from Canada. The climatic separation into both groups reflects the distribution of the

226   two most common species that compose the genetic complex of *C. intestinalis*. Once the

227   environmental space was defined using the first three principal components, we estimated the

228   environmental niche by fitting a minimum volume ellipsoid around the presence points of each

229   species. All points inside the ellipsoid represent environments with favorable conditions for the

230   species in the original region. We projected the minimum volume ellipsoid in Chilean territory to

231   identify analog environments (habitats inside the range of values found in the original region).

232   Analog environments for sp.A are almost four times more common in Chile than those for sp.B

233   according to the projections from the minimum volume ellipsoid (Fig 2).

234   Models for original distributions were significantly better than random and performed well

235   according to AUC (table I). The lowest AUC was obtained for the model of Northern Europe

236   (AUC = 0.82), while the highest was obtained for the model of West USA (AUC = 0.94). For the

237   regional models, the observed AUC values were 0.87 and 0.80 for sp A and sp B, respectively.

238   Only regional models are shown. All the remaining models, obtained with single modelling areas

239   as well as the projections with non-analog environments may be found in Figures S1-2.

240   The two regional models (sp A and sp B) showed strong disagreement for portions of Chile that

241   are considered suitable for the establishment of *C. intestinalis*. Projections of the model of sp A

242   showed that central and southern regions (30°-40° S, Fig 2) seem more suitable for establishment

243   than the peripheral regions of Chile (below 25° and above 43° S). Only a few patches of suitable

244   habitats are found in the most extreme southern region of the continent, around the 53° S. The

245   model for the sp B revealed that the most suitable habitats are located around the 45° S (Fig 2).

246   Additional areas near 53° S were also classified with high suitability index, the same as observed

247   with the model of sp A.

248   Based on Jacknife analyses for regional models the distribution of sp A was most influenced by

249   mean Salinity and Chlorophyll A (both mean and minimum). For sp B, Sea Surface Temperature

250   (minimum) and Photosynthetically Available Radiation (PAR) (mean) were important

251   contributors (Table 1). The most important variables also varied in the single models, as in

252   Canada where mean values of Chlorophyll A correspond to the variable that contributed most to

253   the model, while in Southern Europe, Salinity and pH were the most important variables (Table

254   I).

255   Our field sampling detected *C. intestinalis* in most aquaculture centers examined in 15 localities

256   between 27° and 43°S latitude (Fig 2). To the best of our knowledge, this is the first report of *C.*

257   *intestinalis* infestations south of 30°S latitude on the temperate South American pacific coast,

258   specifically in mussel, oyster and abalone farms. Density of *C.intestinalis* showed a clear north-

259   south pattern, with higher densities in northern Chile and low densities in the South.

260   The relative density of the species in aquaculture centers revealed contrasting results. For sp A.

261   the plot showed a positive, linear relationship between observed density and the suitability index

262   (R^2=0.26, Fig 3), and a strong relationship at the 90% percentile (pseudo $R^2 = 0.55$, Fig 3). For sp

263   B, the relationship was completely absent, and for the upper limit was negative, which makes no

264   sense in this context (R^2=0.0, pseudo $R^2 = 0.08$, Fig 3).

265   **Discussion**

266   Our results showed that, when considering the distribution of each species of the genetic

267   complex of *C. intestinalis* (sp A and B), the species seem to be not only genetically distinct, but

268   also appear to be ecologically distinct. Our analyses separated the species into two main groups

269   based on their environmental preferences. Interestingly, the PCA analysis of environmental

270 conditions for the presence points was coherent with the genetic separation among the species

271 within the complex. In consequence, not considering these differences in environmental models

272 might lead to imprecise conclusions about the potential distribution of the species outside their

273 original range.

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

275 temperature. These variables grouped occurrences from Northern Europe and Canada where the

276 minimum sea temperature reach values close to 5° C and separated them from observations

277 obtained in areas like Japan, east USA and the Mediterranean Sea, where minimum values of

278 temperature are much higher (11.7° C, based on layers provided by BioOracle). Although the

279 taxon is recognized by its wide tolerance to temperature variation (Carver et al., 2006)), our

280 results suggest a distinct range of preferences at least for the two most common species. By

281 isolating the two groups, we could improve the power of our predictions by restricting the

282 projections of the models to the geographic areas where the environmental conditions are analog

283 to those where the models were calibrated. We took this precaution because some model

284 algorithms tend to extrapolate projections beyond the range of environmental values used to

285 calibrate the models and end up identifying high values of suitability even in conditions where

286 most of the species are unlikely to survive (Owens et al., 2013).

287 The two regional models (for sp A and for sp B) showed disagreement for portions of Chile that

288 are considered suitable for the maintenance of the species. Projections of the model of sp A

289 showed that climate in Chile is moderately to highly suitable, especially around the 30° and 40° S,

290 while for sp B, the areas around the 45° S are the most appropriate. Also, the model for sp B

291 predicted several suitable areas beyond the extent of the current invaded range of *C. intestinalis*

292 in the Chilean coast.

293 We interpreted projections in the Chilean coast only to those analog environments to those where

294 the models were calibrated. For the model of sp A, it resulted in a void in the projection layer

295 between the 34° – 37° S and between 46° – 51° S. These areas are known as strong upwelling

296 centers, and they also receive important influxes of freshwater (Atkinson et al., 2002; Dávila et

297 al., 2002). Both factors may generate particular conditions that are not shared by the other areas

298 where the species has been found. In any case, our survey confirmed the presence of *C.*

299 *intestinalis* in some aquaculture centers around the 36° S Lat. Initial colonization in this zone

300   started, most likely, with specimens that benefited from the exchange of equipment between

301   aquaculture centers from other parts of the country where the species has already established

302   dense populations (IV Región, around 30º S). For sp B, analog environments, identified by the

303   minimum volume ellipsoid, are just found southern 40º S, which means that only above this

304   latitude coastal environmental conditions are similar to those the species found in Canada and

305   Northern Europe.

306   The model for the sp A seems to capture quite well the current distribution of *C. intestinalis* in

307   Chile, especially in the area of Coquimbo (29º S 71º W) where the biofouling effect of the species

308   has been a major problem on the suspended cultures of scallops(Uribe & Etchepare, 2002).

309   Indeed, it is assumed the species first arrived in the area brought by Japanese boats which

310   transported the personal and equipment used in the implementation of the first centers for the

311   culture of scallop (Madariaga et al., 2014). The exchange of boats and equipment facilitated the

312   spread of the species northward, where it can be found in dense populations also associated with

313   cultures of scallops, especially during the summer. Later, the species has the potential to spread to

314   the area close to Puerto Montt (around 41º S), although in a much lower density. Although the

315   origin of the specimens found in the Chilean coast have been tentatively attributed to Japan,

316   which could correspond to the sp A, the single model adjusted with occurrences from Japan

317   identified no analog environments in the Chilean coast (Online Resource 1,2). This model is

318   probably biased by the few points of occurrence that we obtained from the public registry for the

319   area of Japan, and also to the proximity of the points. It means, our single model for Japan is

320   probability characterizing only a narrow portion of the possible niche for the species. The model

321   for sp B considered suitable areas situated outside the present distribution in Chile, and where

322   most of the aquaculture centers are located. In some areas, the species is already established but

323   still at low densities. Indeed, most of the mariculturalists that we could contact in southern

324   regions (above 40º S) did not recognize *C. intestinalis* as a real threat to their cultures, which

325   contrasted with the response from mariculturalists from the northern-central Chile, who could

326   readily identify *C. intestinalis* and view it as a real threat. Anecdotally, farm workers only

327   recognize *C. intestinalis* correctly in the northern region of Chile. In the farms located at southern

328   Chile, workers misidentify *C. intestinalis* with early stages of *Pyura chilensis*. Hence, southern

329   regions must be considered a priority in future plans of management and control, which should

330   include programs to provide adequate training to local mariculturalists to improve the changes of

331   early detection of new invasions and to prevent contamination due sharing equipment between

332   farms.

333   Madariaga et al., (2014) used information from unifactorial experiments to assess the tolerance to

334   light, salinity and temperature of individuals collected in the Region of Coquimbo (30° S). They

335   also compiled data from literature to associate performance (mixing several metrics as mortality,

336   filtration rate and particle retention efficiency) with salinity and temperature. These authors

337   suggest that the species is physiologically capable of tolerating the wide range of physical

338   conditions found in Chile, suggesting the whole Chilean coastline may be considered at high risk.

339   However, data compiled in this study was not separated into the different species within the

340   complex (i.e. sp A, B , C or D); therefore, the observed performance cannot be assigned to any

341   species in particular or be considered representative of the species already present in Chile. To the

342   best of our knowledge, there are no available studies that compare the physiological tolerance of

343   each of these four species independently. Nevertheless, our results suggest the two dominant

344   species of the genetic complex are probably using different portions of the environmental space.

345   The Jacknife analyses that included each variable alone, reinforced the separation of the two

346   species in the environmental space. For sp A, Salinity and Chlorophyll A (mean; minimum) are

347   the most important variables. For sp B, Sea Surface Temperature (minimum) and

348   Photosynthetically Available Radiation (PAR) were important contributors. Previous studies have

349   reported that temperature is an important cue for sexual maturation, spawning and recruitment in

350   *C. intestinalis* (Dybern, 1967; Marin et al., 1987; Carver et al., 2003; Howes et al., 2007). For

351   instance, in Scandinavian and subarctic populations, where temperature rarely exceeds 8° C, the

352   generational time is 2-3 years and individuals are reproductively mature at the first year (Dybern,

353   1965; Dybern, 1967). For Japan and the warmer Mediterranean where temperatures are always

354   above 10° C, the generational times vary between 3 and 6 months and sexual maturity is reached

355   after 1 to 2 months, depending on the season (Yamaguchi, 1975). The optimal salinity for

356   Mediterranean populations (35‰), is much higher than would normally experienced by northern

357   Atlantic coastal populations (Marin et al., 1987). Lambert and Lambert (1998) reported that *C.*

358   *intestinalis* populations on floating docks in southern California harbors were vulnerable to

359   pulses of low salinity. On the other hand, Dybern (1967) found that the lower salinity limit for

360   adults and developmental stages in Scandinavian populations was 11‰. Such differences might

361   emphasize the capability of the species to survive under a broad range of conditions, but can also

362   reinforce the implications from our results, that each species of the genetic complex might be

363   using different portions of the niche. This information is crucial, especially when using ENMs to

364   study how species colonize new environments (Sax et al., 2007) and whether they retain their

365   climatic niche in a new range (Pearman et al., 2008).

366   Here, we observed a linear relationship between relative density and suitability index for sp A,

367   especially in relation to the upper limit of local abundances (Fig 3, 90% quantile regressions).

368   VanDerWal et al., (2009) pointed out that suitability indexes reflect potential abundance, but

369   other factors may prevent the species attain this potential. In our case, the index explains 56% of

370   the variation on the upper limit of the population density among aquacultures centers, which

371   means that those centers located in areas classified as possessing a high suitability index could

372   sustain more abundant populations. For sp B, the same relationship was null. In our case, we can

373   speculate the reason why some localities do not show the expected higher abundance could be

374   associated to environmental variables outside the model or acting at a small scale such as

375   turbidity or management activities in aquaculture centers; however, finding the exact reason will

376   require new studies.

377   According to the National Fishery Service, the areas around the 42° S encompass most of the

378   centers for the culture of molluscs in Chile. Such areas must be of high priority for control plans.

379   Some areas above the 50° S may also serve as potential habitat for *C. intestinalis* from a strictly

380   climatic perspective. However, they are not considered suitable for aquaculture, so it is not clear

381   whether they could support wild population of the species. In fact, Dumont et al. (2009) suggest

382   that despite the well-established populations on artificial structures, the species appears unable to

383   colonize natural communities due to predation pressure from native benthic species, especially

384   the rock shrimp *Rhyncocinetes typus*. On the other hand, competition with the native ascidian

385   *Pyura chilensis* seems unimportant in natural benthic communities exposed to natural predation

386   (Dumont et al. 2009).

387   In the case of the few places where marine invaders have been successfully controlled, first

388   actions typically occurred in the early stages of invasion, right after establishment and initial

389   spread (Edwards & Leung, 2009). The higher a population size, the longer the species will

390   persist, and eradication will be no longer a management option (Lockwood et al., 2013). In this

391   context, our results might help the Chilean regulatory agencies to identify which areas must be

392   prioritized in eventual control plans. Considering the potential risk to southern Chile,

393   management of *C. intestinalis* invasions should concentrate on the reduction of the probability of

394   introduction due to contaminated individual vessels (Drake & Lodge, 2004), controlling the

395   number of potential invaders on transport or recreational boats. Controlling the exchange of

396   contaminated equipment between aquaculture centers might also reduce the fortuitous spread of

397   the species.

398   Risk maps are in worldwide demand for management purposes, however they are clearly

399   dependent on the type of occurrence data used (Therriault & Herborg, 2008). If it is possible to

400   link suitability and abundance, ENMs may turn into a very powerful tool in the management of

401   invasive species. Even if management measures have not been able to eliminate biological

402   invasions, slowing the rate of invasion or spread of an established species has considerable value

403   (Ruiz & Carlton, 2003). In our case, ENMs have allowed us to understand the relation of the

404   distinct species that conform the genetic complex known as *C. intestinalis* with their surrounding

405   environment providing valuable insights about probable routes of dispersion in Chile, especially

406   into those areas considered adequate for aquaculture activities and where the species has not been

407   recorded.

408   **Acknowledgments**

409   We thank Daniela Lopez, Melissa Pavez, Kennia Morales, Roger Sepúlveda for their valuable

410   help during field work, and to all anonymous mariculturalists that kindly received us and shared

411   their experience with the field team. This paper was much improved by the comments of Dr. AT

412   Peterson.

413

References

414

415 Ashelby CW, Johnson ML, De Grave S. 2013. The global invader *Palaemon macrodactylus*
416   (Decapoda, Palaemonidae): an interrogation of records and a synthesis of data. Crustaceana
417   86:594–624.

418 Atkinson LP, Valle-Levinson A, Figueroa D, De Pol-Holz R, Gallardo VA, Schneider W, Blanco
419   JL, Schmidt M. 2002. Oceanographic observations in Chilean coastal waters between
420   Valdivia and Concepción. J Geophys Res 107:1–14.

421 Brunetti R, Gissi C, Pennati R, Caicci R, Gasparini F, Manni L. 2015 Morphological evidence
422   that the molecularly determined Ciona intestinalis type A and type B are different species:
423   *Ciona robusta* and *Ciona intestinalis*. J Zoolog Syst Evol Res 53: 186-193

424 Capinha C, Anastácio P, Tenedório JA. 2012. Predicting the impact of climate change on the
425   invasive decapods of the Iberian inland waters: an assessment of reliability. Biol Invasions
426   14:1737–1751.

427 Carver C, Chisholm A, Mallet AL. 2003. Strategies to mitigate the impact of *Ciona intestinalis*
428   (L.) biofouling on shellfish production. J Shellfish Res 22:621–632.

429 Carver CE, Mallet AL, Vercaemer B. 2006. Biological synopsis of the solitary tunicate *Ciona*
430   *intestinalis*. 55.

431 Cassini MH. 2011. Ecological principles of species distribution models: the habitat matching
432   rule. J Biogeogr 38:2057–2065.

433 Castilla JC, Neill PE. 2009. Marine bioinvasions in the Southeastern Pacific : status , ecology ,
434   economic impacts, conservation and management. In: Rilov and Crooks (eds). Biological
435   invasions in marine ecosystems. Springer. p. 439-457.

436 Castilla JC, Uribe M, Bahamonde N, Clarke M, Desqueyroux-Faúndez R, Kong I, Moyano H,
437   Rozbaczylo N, Santelices B, Valdovinos C, Zavala P. 2005. Down under the southeastern

438   Pacific: marine non-indigenous species in Chile. Biol Invasions 7:213–232.

439   Colwell RK, Rangel TF. 2009. Hutchinson's duality: the once and future niche. Proc Natl Acad
440   Sci U S A 106 Suppl :19651–8.

441   Dávila PM, Figueroa D, Müller E. 2002. Freshwater input into the coastal ocean and its relation
442   with the salinity distribution off austral Chile (35-55°s). Cont Shelf Res 22:521–534.

443   Drake JM, Lodge DM. 2004. Global hot spots of biological invasions: evaluating options for
444   ballast-water management. Proc Biol Sci 271:575–80.

445   Dumont CP, Urriago JD, Abarca A, Gaymer CF, Thiel M. 2009. The native rock shrimp
446   *Rhynchocinetes typus* as a biological control of fouling in suspended scallop cultures.
447   Aquaculture 292:74–79.

448   Dybern BI. 1965. The life cycle of *Ciona intestinalis* (L.) f. *typica* in relation to the
449   environmental temperature. Oikos 16:109–131.

450   Dybern BI. 1967. The distribution and salinity tolerance of *Ciona intestinalis* (L.) F. *typica* with
451   special reference to the waters around Southern Scandinavia. Ophelia 4:207–226.

452   Edwards PK, Leung B. 2009. Re-evaluating eradication of nuisance species: invasion of the
453   tunicate *Ciona intestinalis*. Front Ecol Environ 7:326–332.

454   Elith J, Leathwick JR. 2009. Species Distribution Models: ecological explanation and prediction
455   across space and time. Annu Rev Ecol Evol Syst 40:677–697.

456   Fitzpatrick MC, Hargrove WW. 2009. The projection of species distribution models and the
457   problem of non-analog climate. Biodivers Conserv 18:2255–2261.

458   Franklin J. 2010. Mapping Species Distributions: Spatial Inference and Prediction. Cambridge
459   University Press

460   Hecht T, Heasman K. 1999. The culture of *Mytilus galloprovincialis* in South Africa and the
461   carrying capacity of mussel farming in Saldanha Bay. World Aquac Rouge 30:50–55.

462   Howes S, Herbinger CM, Darnell P, Vercaemer B. 2007. Spatial and temporal patterns of
463   recruitment of the tunicate *Ciona intestinalis* on a mussel farm in Nova Scotia, Canada. J
464   Exp Mar Bio Ecol 342:85–92.

465   Iannelli F, Pesole G, Sordino P, Gissi C. 2007. Mitogenomics reveals two cryptic species in
466   *Ciona intestinalis*. Trends Genet 23:419–22.

467   Koenker, R., & Machado, JA. 1999. Goodness of fit and related inference processes for quantile
468   regression. JASA 94:1296-1310.

469   Lambert CC, Lambert G. 1998. Non-indigenous ascidians in southern California harbors and
470   marinas. Mar Biol 130:675–688.

471   Lee H, Reusser DA, Olden JD, Smith SS, Graham J, Burkett V, Dukes JS, Piorkowski RJ,
472   McPhedran J. 2008. Integrated monitoring and information systems for managing aquatic
473   invasive species in a changing climate. Conserv Biol 22:575–84.

474   Locke A, Hanson JM. 2009. Rapid response to non-indigenous species. 1. Goals and history of
475   rapid response in the marine environment. Aquat Invasions 4:237–247.

476   Madariaga DJ, Rivadeneira MM, Tala F, Thiel M. 2014. Environmental tolerance of the two
477   invasive species *Ciona intestinalis* and *Codium fragile*: their invasion potential along a
478   temperate coast. Biol Invasions 16:2507–2527.

479   Marin MG, Bressan M, Beghi L, Brunetti R. 1987. Thermo-haline tolerance of *Ciona intestinalis*
480   (L. 1767) at different developmental stages. Cah Biol Mar 28:47–57.

481   Norambuena R, Gonzalez L. 2005. National aquaculture sector overview Fact Sheets - Chile.
482   FAO, Rome.

483   Owens HL, Campbell LP, Dornak LL, Saupe EE, Barve N, Soberón J, Ingeloff K, Lira-Noriega
484   A, Hensz CM, Myers CE, Peterson AT. 2013. Constraints on interpretation of ecological
485   niche models by limited environmental ranges on calibration areas. Ecol Modell 263:10–18.

486   Pearman PB, Guisan A, Broennimann O, Randin CF. 2008. Niche dynamics in space and time.
487   Trends Ecol Evol 23:149–58.

488   Pearson RG. 2007. Species Distribution Modeling for conservation educators and practitioners.
489   Lessons in Conservation 3:54-89

490   Pennati R, Ficetola GF, Brunetti R, Caicci F, Gasparini F, Griggio F, Sato A, Stach T, Kaul-
491   Strehlow S, Gissi C, Manni L. 2015 Morphological Differences between Larvae of the
492   *Ciona intestinalis* Species Complex: Hints for a Valid Taxonomic Definition of Distinct
493   Species. PlosONE e0122879.

494   Peterson AT, Soberón J. 2012. Species distribution modeling and ecological niche modeling:
495   Getting the Concepts Right. Nat a Conserv 10:102–107.

496   Peterson AT. 2006. Uses and requirements of ecological niche models and related distributional
497   models. Biodivers Informatics 3:59–72.

498   Peterson AT. 2011. Ecological niche conservatism: a time-structured review of evidence. J
499   Biogeogr 38:817–827.

500   Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic
501   distributions. Ecol Modell 190:231–259.

502   Phillips SJ, Dudík M, Schapire RE. 2004. A maximum entropy approach to species distribution
503   modeling. Twenty-first Int. Conf. Mach. Learn. - ICML '04. ACM Press, New York, New
504   York, USA, p 83

505   Qiao H, Escobar LE, Soberón J, Campbell L, Peterson AT. 2014. NicheA. Version 1.2 beta.
506   Available at: http://biodiversity- informatics-training.org/software-data-sources/nichea/
507   (accessed on December 2014).

508   Ramsay A, Davidson J, Landry T, Arsenault G. 2008. Process of invasiveness among exotic
509   tunicates in Prince Edward Island, Canada. Biol Invasions 10:1311–1316.

510   Randin CF, Dirnböck T, Dullinger S, Zimmermann NE, Zappa M, Guisan A. 2006. Are niche-
511   based species distribution models transferable in space? J Biogeogr 33:1689–1703.

512   Robinson LM, Elith J, Hobday AJ, Pearson RG, Kendall BE, Possingham HP, Richarson AJ.
513   2011. Pushing the limits in marine species distribution modelling: lessons from the land
514   present challenges and opportunities. Glob Ecol Biogeogr 20:789–802.

515   Ruiz GM, Carlton J. 2003. Invasive Species: Vectors And Management Strategies. Island Press.

516   Sato A, Satoh N, Bishop JDD. 2012. Field identification of "types" A and B of the ascidian *Ciona
517   intestinalis* in a region of sympatry. Mar Biol 159:1611–1619.

518   Sax DF, Stachowicz JJ, Brown JH, Bruno JF, Dawson MN, Gaines SD, Grosberg RK, Hastings
519   A, Holt RD, Mayfield MM et al. 2007. Ecological and evolutionary insights from species

520   invasions. Trends Ecol Evol 22:465–71.

521   Soberón J, Nakamura M. 2009. Niches and distributional areas : Concepts , methods , and
522   assumptions. PNAS 106:19644–19650.

523   Soberón J, Peterson AT. 2005. Interpretation of models of fundamental ecological niches and
524   species' distributional areas. Biodivers Informatics 2:1–10.

525   Therriault TW, Herborg L. 2008. A qualitative biological risk assessment for vase tunicate *Ciona*
526   *intestinalis* in Canadian waters : using expert knowledge. J Mar Sci 65:781–787.

527   Tyberghein L, Verbruggen H, Pauly K, Troupin C, Mineur F, De Clerck O. 2012. Bio-ORACLE:
528   a global environmental dataset for marine species distribution modelling. Glob Ecol
529   Biogeogr 21:272–281.

530   Uribe E, Etchepare I. 2002. Effects of biofouling by Ciona intestinalis on suspended culture of
531   Argopecten purpuratus. Bull. AAC. 3:93-95.

532   VanDerWal J, Shoo LP, Johnson CN, Williams SE. 2009. Abundance and the environmental
533   niche: environmental suitability estimated from niche models predicts the upper limit of
534   local abundance. Am Nat 174:282-291.

535   Yamaguchi M. 1975. Growth and reproductive cycles of the marine fouling ascidians *Ciona*
536   *intestinalis, Styela plicata, Botrylloides violaceus* and *Leptoclinum mitsukurii* at Aburatsubo-
537   Moroiso Inlet (central Japan). Mar Biol 29:253–259.

538   Zhan A, Macisaac HJ, Cristescu ME. 2010. Invasion genetics of the *Ciona intestinalis* species
539   complex: from regional endemism to global homogeneity. Mol Ecol 19:4678–94.

540   Zimmermann NE, Edwards TC, Graham CH, Pearman PB, Svenning J. 2010. New trends in

541   species distribution modelling. Ecography (Cop) 33:985–989.

542   **Figure captions**

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

547   **Figure 2**. Sampling points and projections of the potential distribution of *C. intestinalis* sp A and
548   sp B on Chilean coast. In the first map, red dots show the localities sampled into the three major
549   aquacultural regions of Chile. Non analog environments are projections on Chilean environments
550   that may be or may not be represented at locations used to fit the model. Analog environments are
551   projections only on those Chilean environments that are represented at locations used to fit the
552   model.

553   **Figure 3**. Regressions between suitability indices from ENMs and observed densities in the field

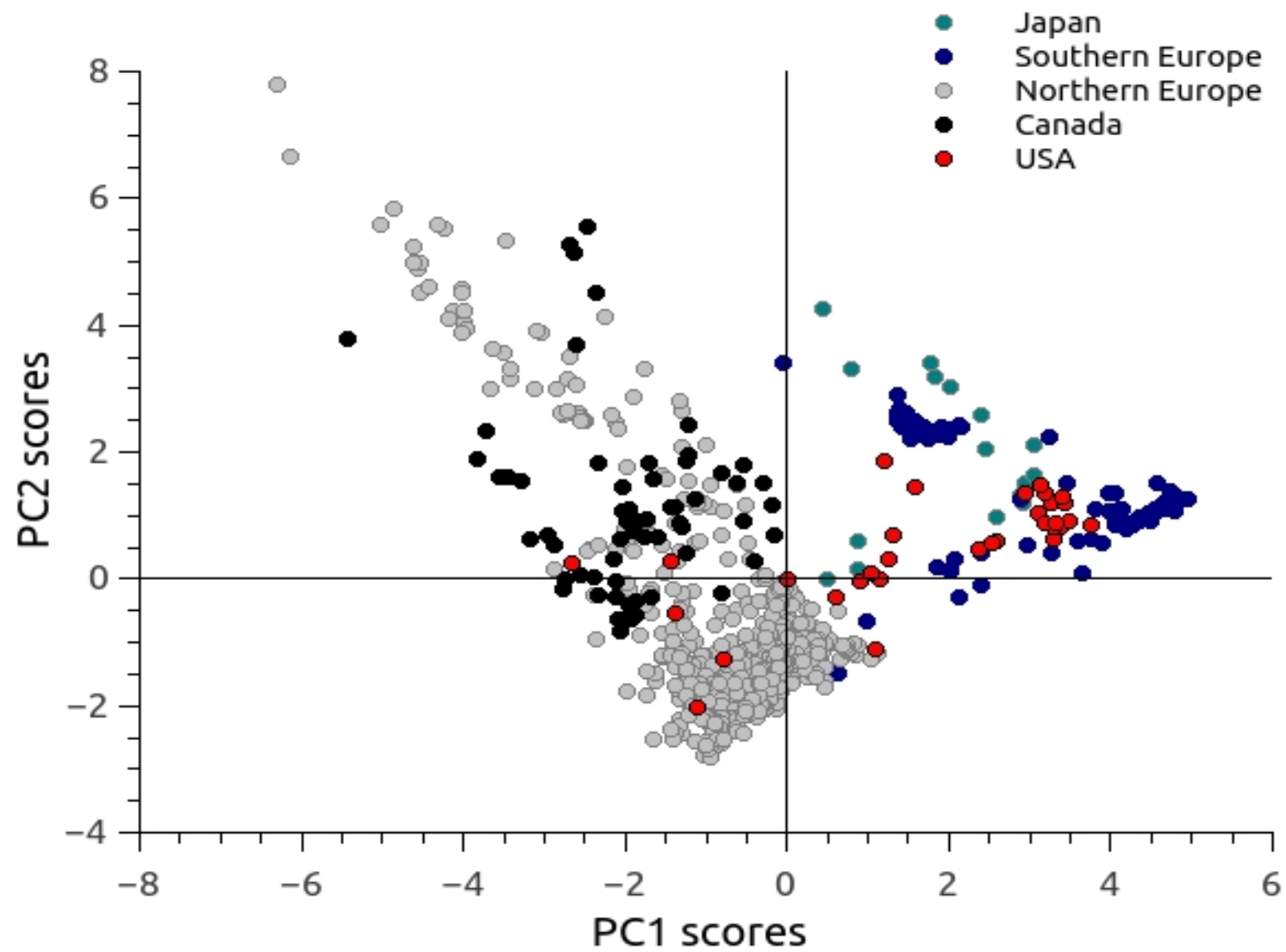
554   for sp A and sp B. Dark lines represent linear regressions, gray lines represent 90% quantile

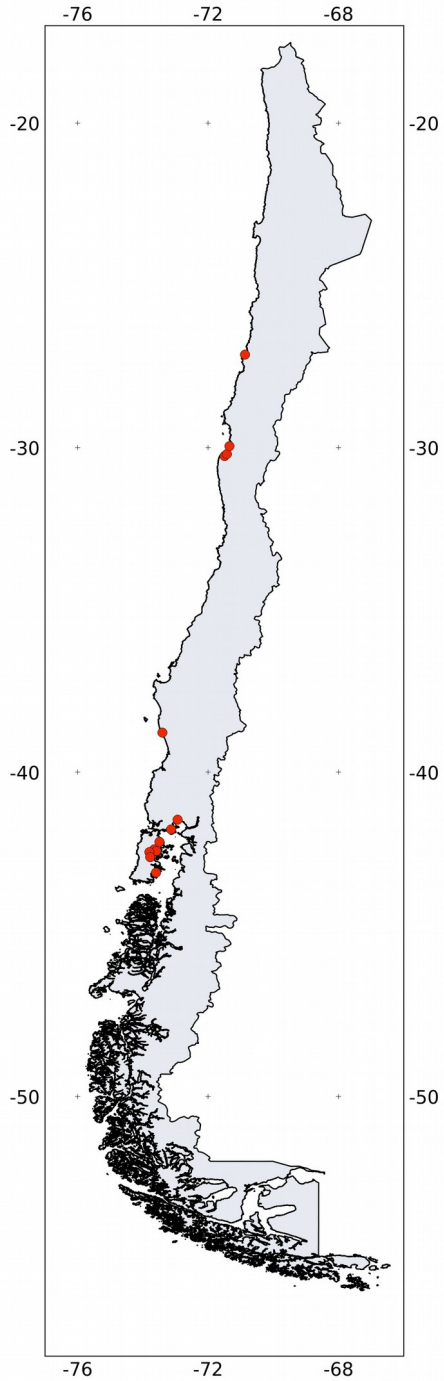
555   regressions.

Model's results

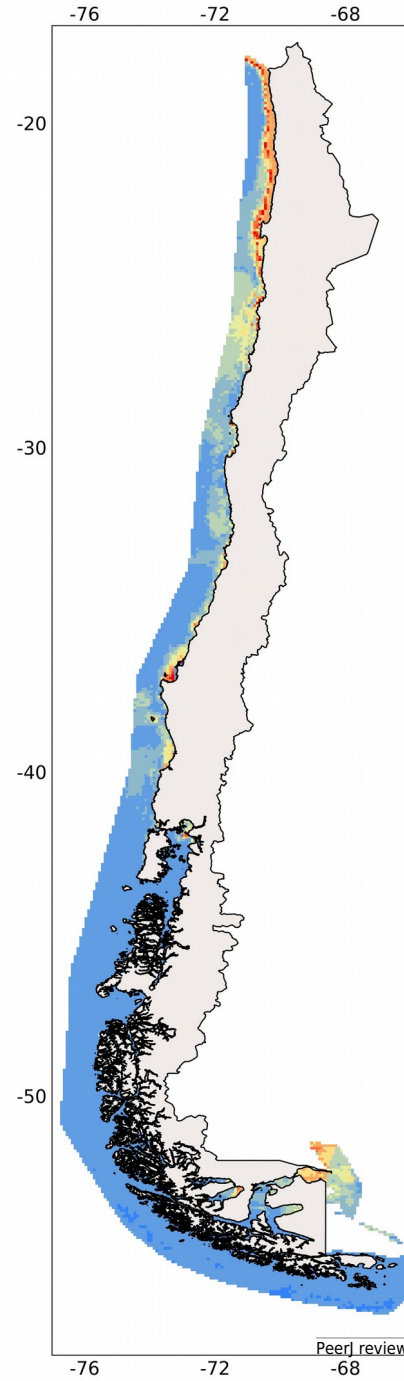
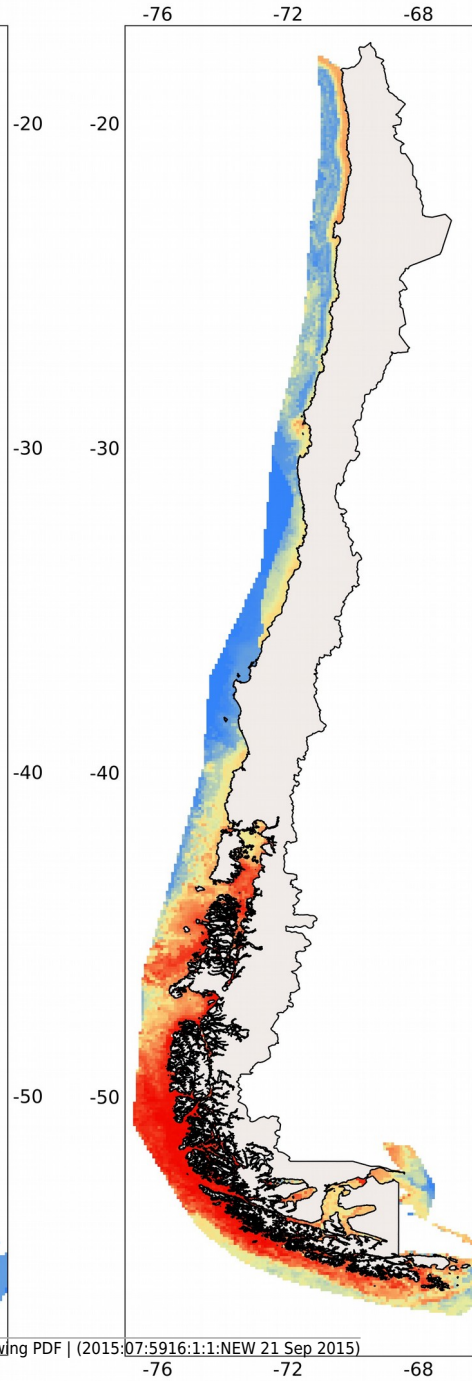
1   **Table I**. Values of the average test AUC for the replicate runs for each single and regional
2   model. Environmental variables with the highest gain when used in isolation are shown.

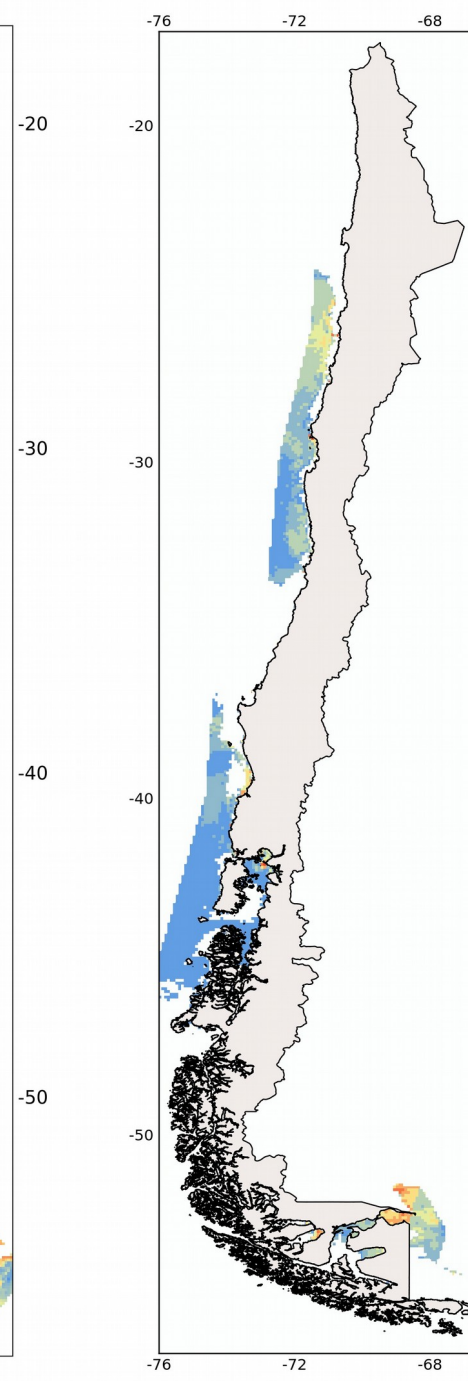
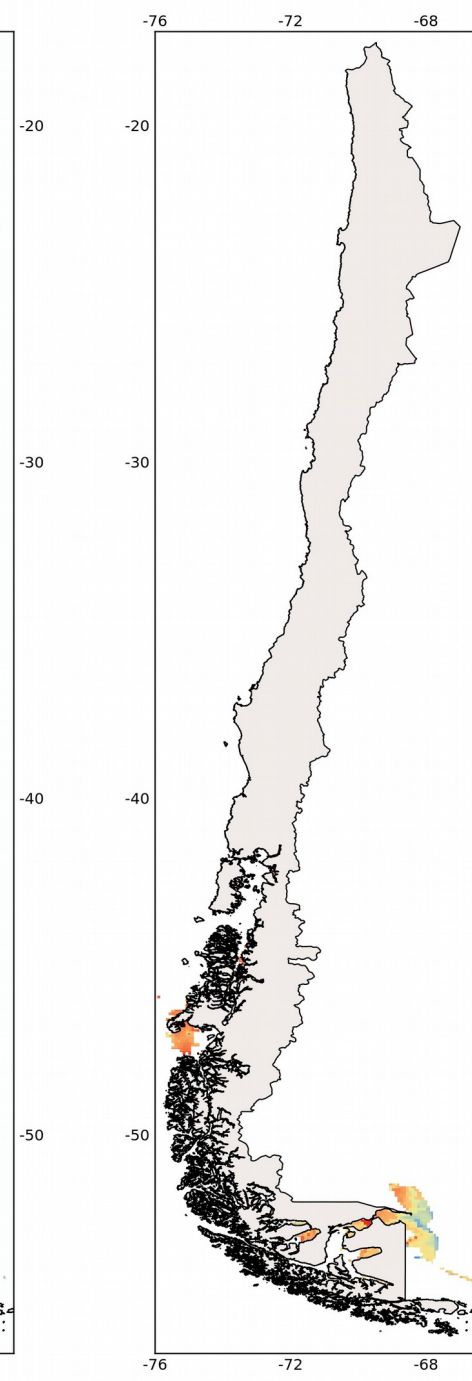
Model	AUC	Variable with the highest gain
Canada	0.834	Chlorophyll A (mean)
Japan	0.889	Chlorophyll A (mean; maximum)
West USA	0.939	Photosynthetically Available Radiation (PAR)(maximum)
Southern Europe	0.892	Salinity; Ph
Northern Europe	0.817	Sea Surface Temperature (minimum); PAR (mean)
sp A	0.873	Salinity;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

PCA plot

Maps

Sampling points

Non-Analog Environments

Sp A

Sp B

Analog Environments

Sp A

Sp B

Suitability Index

High: 1

Low: 0

regression plots