Environmental tolerance of the two invasive species *Ciona intestinalis* and *Codium fragile*: their invasion potential along a temperate coast

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Abstract *Ciona intestinalis* and *Codium fragile* are among the most successful invasive species in marine systems worldwide, and they are currently in the process of expanding their distributional ranges along the Chilean coast. Herein we evaluated whether their tolerance to a wide range of environmental conditions contributes to the invasion potential of these two species. To examine the environmental tolerance and performance (e.g., growth) of these non-indigenous species, unifactorial experiments (8–10 days) were conducted with different environmental factors (solar radiation, salinity and temperature). Based on the results, the potential risk of invasion along the Chilean coast was evaluated for both species using a mechanistic niche modeling approach. Both species can tolerate extensive ranges of the abiotic factors salinity and temperature, with *C. intestinalis* being more tolerant to cold-temperate waters. Also, *C. intestinalis* was more susceptible to high light intensities than *C. fragile*. These results confirm those of other experiments, and the outcome of the niche modeling shows that both species can potentially invade most regions of the Chilean coast with the exception of the Magellan region. The results suggest that physiological capacity to tolerate and perform in a wide range of physical conditions is a pre-requisite for successful invasions by littoral biota, but predation and possibly competitive exclusion can slow down the invasion success of *C. intestinalis*, which in contrast to *C. fragile*, is consumed by many benthic predators. Sexual and asexual reproduction as well as buoyancy of its thalli further contribute to the dispersal and colonization success of *C. fragile*. Based on these considerations, it is concluded that the invasion risk of *C. fragile* along the Chilean coast is substantially higher than that of *C. intestinalis*.

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Introduction

The number of species that have successfully established and continue to invade new habitats is

increasing (Bax et al. 2003; Schaffelke et al. 2006; Occhipinti-Ambrogi et al. 2011). In many cases, these species are able to colonize and dominate invaded habitats due to functional traits of high performance under diverse environmental conditions (Lodge 1993; Osovitz and Hofmann 2007). They are considered to be physiologically or genetically pre-adapted to adjust to new habitats (Piola and Johnston 2006; Prentis et al. 2008; Bacigalupe 2009).

Non-indigenous species become invaders once they have been able to establish and spread (Lockwood et al. 2005; Novak 2007). Many non-indigenous species appear to be highly tolerant to stressful environmental conditions (Hoffmann and Hercus 2000), which is considered an important trait of successful invaders (Lenz et al. 2011). Generally, it is assumed that a broad tolerance to environmental factors, high reproductive rates and a wide physiological plasticity enhance the invasive potential of a non-indigenous species (Alpert et al. 2000; Stachowicz and Byrnes 2006; Roman and Darling 2007). In addition, species can adapt to new environments over the course of generations, which could explain the differences in time between introduction, establishment and spread (Mack et al. 2000; Mooney and Cleland 2001). Therefore, the physiological stress tolerance can be an important trait for non-indigenous species to establish in novel habitats.

The high variability of marine systems and the influence of multiple environmental factors suggest that coastal areas are typically subjected to a variety of stressors that control the performance of non-indigenous species. The invasion and expansion risk of non-indigenous species is highest in those areas where environmental conditions are within the tolerance range of these species. Consequently, the combination of species' traits and environmental properties are predictors of new invasions or of range expansions of non-indigenous species (Compton et al. 2010; de Rivera et al. 2011). Along coasts with widely varying environmental conditions it can be expected that certain areas face a high invasion risk of common invaders, while other areas might be relatively immune against invasions. For the SE Pacific coast, it has been postulated that environmental conditions restrict the establishment of non-indigenous species (Castilla et al. 2005). In particular, abiotic factors such as light, salinity, temperature and oxygen availability exhibit strong latitudinal variations along the SE Pacific coasts with extreme conditions towards

equatorial latitudes (high temperatures, high light intensities, low oxygen concentrations) and in the fjord region (low salinities).

Ciona intestinalis (Linnaeus 1767) (Ascidiacea) and *Codium fragile* ssp. *fragile* (Provan et al. 2008) (Chlorophyta) have established in northern-central Chile and are currently in the process of expanding their distributional ranges along the Chilean coast (Neill et al. 2006; Castilla and Neill 2009). *C. intestinalis*, with an unresolved native range, and *C. fragile*, originally from Japan, are among the most successful invasive species reported in marine systems worldwide (Trowbridge 1998; Provan et al. 2005; Castilla et al. 2005; Neill et al. 2006; Blum et al. 2007; Therriault and Herborg 2008; Rius et al. 2014a, b; Roman and Darling 2007). Wherever present, they are recognized as a serious and damaging problem, driving ecological changes in invaded habitats (Schaffelke et al. 2006; Blum et al. 2007; but see Cordell et al. 2013). The introduction and spread of these species have been facilitated by aquaculture activities and artificial structures (e.g., buoys, lines, nets and cages), and the continuous transfer of seed and materials between aquaculture centers (26°S–32°S) (Castilla and Neill 2009).

Both *C. intestinalis* and *C. fragile* have a number of different traits that support their ability to invade new habitats (Trowbridge 1998; Mathieson et al. 2003; Carver et al. 2006; Therriault and Herborg 2008). For example, they both have wide tolerance ranges to environmental conditions, encompassing salinities from ∼12 to 40 psu and temperatures from ∼0 to 30 °C (Dybern 1965, 1967; Hanisak 1979; Marin et al. 1987; Carver et al. 2006; Vercaemer et al. 2011; Rius et al. 2014b). Their sensitivity to solar radiation differs, with *C. intestinalis* living preferentially in dim-light habitats and the photoautotrophic *C. fragile* tolerating high irradiance levels (Dybern 1963; Ramus 1978; Hanisak 1979). The extensive geographic distribution and the range of habitats occupied by these invasive species suggest that their wide physiological tolerance might facilitate their invasion success and range expansion along the Chilean coast.

Species distribution modeling has been used to determine the potential risk of an invasive species successfully spreading into new geographic regions (Peterson 2003; Elith and Leathwick 2009; Jiménez-Valverde et al. 2011). Predictions have been based mostly on correlational models, where the spatial distribution of species in the native range is correlated

to environmental variables and then projected onto other regions (Herborg et al. 2007; Therriault and Herborg 2008; Carrasco and Barón 2010; Compton et al. 2010; de Rivera et al. 2011), but without explicit consideration of the underlying processes shaping the spatial distribution (Dormann et al. 2012). In contrast, mechanistic models explicitly consider processes underlying survival and reproduction to predict geographic distribution (Kearney and Porter 2009; Buckley et al. 2010; Dormann et al. 2012), and there is a growing number of studies using this approach (Kearney et al. 2010; Buckley et al. 2011; Dormann et al. 2012; Higgins et al. 2012). While mechanistic models are thought to provide more realistic and interpretable projections of species distributions (Kearney and Porter 2009; Buckley et al. 2010; Dormann et al. 2012), the need for extensive biological data of variables affecting species performance (i.e. species-specific physiological tolerance) precludes its application to many potential invading species.

Knowledge of their ecological traits and tolerance ranges under extreme abiotic conditions is thus necessary to predict the expansion potential of *C. intestinalis* and *C. fragile* along the Chilean coast. A series of unifactorial experiments was conducted to assess their tolerance to three important abiotic factors light, salinity and temperature. The specific objectives of this study were to compare and determine (1) survival and growth of *C. intestinalis*, and (2) photosynthetic efficiency and growth of *C. fragile* exposed to variations of the abiotic factors mentioned above. Using the information from these experiments and from the literature, the final objective was to (3) assess the performance and model the expansion potential of these two invaders along the Chilean coast.

Materials and methods

Collection and maintenance of organisms

The organisms used in this study were collected during austral spring and summer (September–March) of 2012 and 2013 on the northern-central coast of Chile (Coquimbo) where abundant populations of *C. intestinalis* and *C. fragile* are present on artificial structures related to aquaculture and harbor activities (Dumont et al. 2011).

Ciona intestinalis was collected in Bahía La Herradura (29°57′S, 71°28′W) (Fig. 1), which is an enclosed

bay with extensive anthropogenic activities, such as recreational boating, artisanal fishery ports, cultures of ascidians (*Pyura chilensis*) and algae, and commercial shipping (iron ore loading pier). Here, *C. intestinalis* is commonly found as a fouling organism on ropes, buoys and small fishing boats. It occurs only on artificial structures where it is protected from direct sunlight and from benthic predators (Dumont et al. 2011). Ascidians were carefully scraped from the hull of small boats and placed in monofilament mesh bags. To avoid air bubbles being caught within the mantle cavity of the ascidians, these were carefully transferred to large containers (2,000 L) with seawater, from where they were selected and placed in the corresponding experiments in the outdoor seawater laboratory of Universidad Católica del Norte, Coquimbo (UCN). Ascidians were immediately cleaned (separated from epibionts) in an outdoor-tank, selected and maintained overnight in plastic containers with running seawater.

Codium fragile was collected in Puerto Aldea (30°17′S, 71°36′W) (Fig. 1), which is a small fishing harbor at the southern edge of the major scallop culture zone of Chile in Tongoy Bay. Algae were collected from the pillars of the harbor pier (2 m depth) via snorkeling and placed in monofilament mesh bags. All algae were placed in a large cooler (100 L) and transported to UCN. In the laboratory, algae were carefully cleaned (from epiphytes and epibionts), and selected by size and weight. Algal thalli were cut into ∼10 cm long segments. The selected thalli were maintained in a container (80 L) with seawater and air supply until the next day.

General experimental design and setup

For each of the three environmental factors studied herein (light, salinity, temperature), the experimental organisms were exposed to three treatment levels (high, intermediate, low) (Table 1; Appendix Table S1). The effects of these factors on the performance (survival, photosynthetic efficiency and growth) of the study organisms were examined in unifactorial experiments. Variations of light intensities, salinity and temperature were achieved with different experimental manipulations. These levels were chosen according to the environmental conditions present at different water depths and sites (e.g., La Herradura and Totoralillo bays) from northern-central Chile (Appendix Table S1) and according to the tolerance ranges
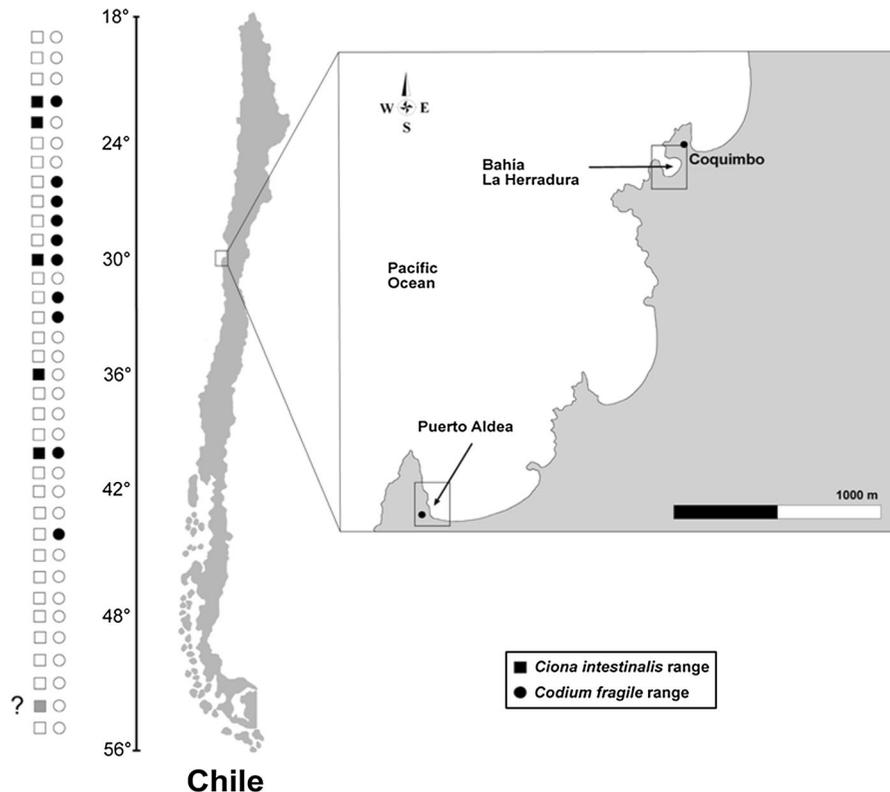
Fig. 1 Map of Chile with the areas from where individuals of *C. intestinalis* and *C. fragile* were collected for this study (*right side*), and the range of distribution along the Chilean coast (*left side*). *Filled* and *open dots* and *squares* indicate presence or absence, respectively. Information of present range distributions of both species were taken from status studies of marine non-indigenous species, aquaculture reports, biodiversity collections, fouling control and ecological studies (Uribe and Etchepare 2002; Neill et al. 2006; Zapata et al. 2007; Castilla and Neill 2009; Almanza et al. 2012; Erasmo Macaya, personal comment; Martin Thiel, personal observations). According to

the criteria identified by Sato et al. (2012) the populations of *C. intestinalis* in northern-central Chile correspond to the phenotype A (Xavier Turon, personal comment). The grey square with the interrogation symbol indicates a doubtful report of *C. intestinalis* in the Magellan region, which was based on a comment by Traustedt (1885) that has been questioned by later workers (e.g., Monniot and Monniot 1983); extensive recent surveys on natural and artificial substrata in the Magellan region did not produce any findings of *C. intestinalis* (Xavier Turon, personal comment)

reported for different populations of *C. intestinalis* and *C. fragile* worldwide (Appendix Table S2).

During the study period, environmental data were registered using in situ data loggers (HOBO Pendant Temperature/Light, Model 64K-UA-002-64) that were placed at four different locations: (1) in air at sea surface level in Bahia La Herradura, (2) 50 cm below the sea surface, attached on a rope in Bahia La Herradura, (3) 2 m below sea surface, attached to a weight on the seafloor in Bahia Totoralillo, and (4) 6 m below the sea surface, attached to a weight on the seafloor in Bahia La Herradura. At water depths where *C. intestinalis* and *C. fragile* form dense populations in northern-central Chile (0–2 m), light intensities in air at 0 m during spring and summer are

$1{,}257 \pm 271$ and $1{,}620 \pm 240$ µmol photon m^{-2} s^{-1}, respectively, and at 2 m water depth vary between 181 ± 66 and 231 ± 50 µmol photon m^{-2} s^{-1}, respectively, i.e. within the range of the experimental conditions used herein (Appendix Table S1). Typical salinities along the Chilean coast range from 20 to 35 psu (Tyberghein et al. 2011) and experimental salinities were 15, 25, 35 and 45 psu. Temperatures at 50 cm water depth during spring and summer are 15.4 ± 1.4 and 17.7 ± 0.8 °C, respectively, and at 2 m depth they vary between 15.6 ± 1.4 and 17.9 ± 1.0 °C, respectively (Appendix Table S1).

During the experiments, the environmental conditions in the different treatments were monitored with handheld instruments. For the light treatments

Table 1 Daily average of light (μmol photon m^{-2} s^{-1}), salinity (psu) and temperature (°C) variations during experiments of *C. intestinalis* (10 days) and *C. fragile* (8 days): data represent mean ± SD for the entire experimental duration

Experiment	Factor	Treatments	Conditions	Experimental date
I	Light			
			35 psu 18.6 ± 0.3 °C	
	C. intestinalis	Low	97 ± 33.0	
		Medium	353.6 ± 91.9	March 2012
		High	854.1 ± 232.1	
			35 psu 18 ± 0.3 °C	
	C. fragile	Low	121.9 ± 29.0	
		Medium	396.4 ± 58.3	February 2012
		High	1,144.4 ± 119.2	
II	Salinity			
			132 ± 44 μmol m^{-2} s^{-1} 14 ± 0.2 °C	
	C. intestinalis	Lower	15	
		Low	25	October 2012
		Medium	35	
		High	45	
			64 ± 48 μmol m^{-2} s^{-1} 14 ± 0.6 °C	
	C. fragile	Lower	15	
		Low	25	September 2012
		Medium	35	
		High	45	
III	Temperature			
			249 ± 57 μmol m^{-2} s^{-1} 35 psu	
	C. intestinalis	Cool	10.3 ± 0.1	
		Medium	17.2 ± 0.3	March 2013
		Warm	25.2 ± 0.1	
			251 ± 94 μmol m^{-2} s^{-1} 35 psu	
	C. fragile	Cool	10.3 ± 0.1	
		Medium	18.3 ± 0.5	February 2013
		Warm	29.5 ± 0.1	

(experiment I), we used a quantum sensor (Model MQ-200, Apogee Instruments, USA; measuring PAR between 400 and 700 nm, accuracy ±5 %). Salinity variations (experiment II) were monitored using a refractometer (Model S/Mill-E, Atago CO., Ltd, Japan; accuracy ±0.1), and temperatures (experiment III) were registered with a digital thermometer (Model HI93510, HANNA Instruments, USA; accuracy ±0.4 °C). Measurements were taken four times daily at 9:00, 12:00, 15:00 and 18:00, and the daily average was calculated (Table 1).

Only ascidians that were actively filtering (siphons extended) were used in the experiments. Ascidians were selected based on their wet weight (ww); individuals used in this study weighed between 11 and 18 g. To determine the ww, actively filtering ascidians were touched by hand on the extended siphons until they contracted. Measurements were taken from ascidians with completely contracted siphons on a digital balance (precision ±0.1 g).

During this study, the ascidians were maintained in still seawater, because for certain experiments (salinity, temperature) it was logistically unfeasible to produce large volumes of conditioned seawater for the more extreme treatments. Preliminary tests revealed that after 24 h the NH_4 concentration reached

0.075 mg L^{-1} in the experimental units (EU), and in order to avoid toxic levels of NH_4 the seawater in each EU was changed twice per day for the duration of the different experiments (10 days). Ascidians were fed daily by adding ~150 mL solution (algal cell concentration ~10^6 cells mL^{-1}, 6–8 µm) of the microalga *Chaetoceros* sp. to each EU.

In *C. fragile*, each selected thallus was weighed with a digital balance (precision ±0.1 g). Individuals used in this study weighed between 9 and 12 g ww and the photosynthetic efficiency (see explication) was recorded before transferring them to the EUs. During the experiments, the thalli were maintained in still seawater. The seawater in each EU was changed once per day throughout the duration of the different experiments (8 days).

The experimental units (EU) were distributed among three large outdoor tanks (2,000 L). Each of these three large tanks had an independent supply of running seawater. The EUs from the different treatments (low, medium, high) of the light experiment (Exp. I) were randomly distributed between the three large tanks. EUs from the different treatments of experiments II (salinity) and experiment III (temperature) were assigned to individual tanks (i.e. all replicates of one treatment were in the same tank) due to logistic requirements. The preparation of large volumes of conditioned water and the daily seawater exchange in the EUs (salinity experiment, Exp. II) required that the different salinity treatments (15, 25, 35 and 45 psu) were relatively close together. Therefore the EUs of the different treatments were not randomly distributed over the tanks. This should not have affected the outcome of the experiment, because the three large tanks were adjacent to each other with identical environmental conditions (e.g., air temperature, light). Similarly, for the temperature experiment (Exp. III), the EUs of each treatment were all in one tank, because each of the three large tanks maintained the desired water temperature during the experiment. In all experiments there was no water exchange between individual EUs for both species.

The experiments lasted 10 days for *C. intestinalis* and 8 days for *C. fragile*. These durations were established according to the responses (survival and maximal quantum yield F_v/F_m, respectively) of the experimental organisms to stress conditions in preliminary studies. In the light and salinity experiments, the organisms were exposed to experimental conditions without previous acclimation, because in their

natural environments (coastal waters) they also face strong and abrupt changes of these conditions. For the temperature experiment, the ascidians were acclimated for 1 day. Specifically, ascidians from the high temperature treatment were acclimated at 24 °C, and at the following day, the temperature was increased by 2 °C to reach the indicated experimental temperature (26 °C). This acclimation day was necessary because preliminary experiments had shown that *C. intestinalis* suffered high mortality when abruptly exposed to the high temperature (26 °C) treatment.

Experiment I: Light effect

To determine whether direct solar radiation affects the survival of *C. intestinalis* and the physiological performance of *C. fragile*, the experimental organisms were exposed to three light treatments: (1) 100 % light, representing high solar radiation, (2) 50 % light, representing medium solar radiation, and (3) 10 % light, which represented low light intensity (Table 1). These light intensities are also representative of natural light gradients in the water column or under rock overhangs. The experimental light levels were selected according to the natural and experimental conditions reported for populations of *C. intestinalis* and *C. fragile* (Dybern 1963; Ramus 1978; Hanisak 1979). Different light intensities were obtained by placing layers of grey mesh (aperture size 2 mm) over each EU. For the 10 % light level, we used three layers of mesh, for the 50 % light level, one layer, and to represent 100 % light level, the EU was open without any cover.

Experiment II: Salinity effect

To test for the effects of salinity on the survival of *C. intestinalis* and the physiological performance of *C. fragile*, individuals were exposed to different salinities: (1) 15 psu, representing the very low salinity treatment, (2) 25 psu for low salinity, (3) 35 psu for ambient salinity, and (4) 45 psu, which represented the high salinity treatment. These salinities were selected according to the range of salinity tolerance reported for different populations of *C. intestinalis* and *C. fragile* (Hanisak 1979; Marin et al. 1987; Trowbridge 1998; Carver et al. 2006; Vercaemer et al. 2011).

Salinities below that of natural seawater (35 psu) were obtained by diluting the filtered seawater with deionized (demineralized) water. For the higher salinities, natural

seawater was concentrated to the desired level by adding artificial sea salt (NaCl) (Choi et al. 2010). Salinity concentrations were newly prepared every second day in tanks of 200 L. To obtain a concentration of 15 psu, ~80 L natural seawater was diluted with ~120 L deionized water. For a concentration of 25 psu, ~140 L natural seawater was diluted with ~60 L deionized water. Lastly, for the high salinity concentration of 45 psu, an amount of ~1,300 g of artificial sea salt was dissolved in 200 L of natural seawater. For the experiments with *C. fragile* we added to each solution (including ambient conditions) approximately 0.014 g fertilizer L^{-1} seawater as a source of balanced nutrients (16 % N, 4 % P, 12 % K + microelements: Fe, Mg, S, Ca, Anasac Ambiental S.A., Chile).

Experiment III: Temperature effect

To test for the effects of temperature on the survival of *C. intestinalis* and the physiological performance of *C. fragile*, individuals were exposed to three different temperatures: (1) low temperatures (cool), (2) ambient conditions (medium), and (3) high temperatures (warm) (Table 1). These temperatures were defined following the maximum and minimum range from *C. intestinalis* and *C. fragile* populations reported in the available literature (Dybern 1965, 1967; Hanisak 1979; Marin et al. 1987; Trowbridge 1998; Neill et al. 2006; Carver et al. 2003; Vercaemer et al. 2011). For the minimum temperature, herein we decided on a temperature of 10 °C because populations from the northern hemisphere are commonly found at these or even lower temperatures (Dybern 1965; Carver et al. 2003, 2006).

In order to generate the indicated temperatures, seawater was pumped through three independent large outdoor tanks (2,000 L). Two of these tanks were equipped with a heat-exchanger to raise or reduce water temperatures as needed for the experimental treatments. The third tank was used with incoming seawater to reflect the ambient temperature defined as the medium treatment.

Species-specific setup and measurements

Ciona intestinalis

Experimental ascidians were maintained in plastic containers (EUs = transparent plastic aquaria, 29 × 29 × 12 cm, 7 L volume). As direct solar radiation affects the survival of *C. intestinalis*, EUs from salinity and temperature experiments were protected from direct sunlight, to exclude light as a potentially confounding factor. The lateral walls of the EUs were covered with a black plastic to exclude lateral light. For the salinity and temperature experiments (II and III), the top of each EU was covered with three layers of a grey mesh (allowing passage of ~10 % of the incoming light). In the experiments I (light) and III (temperature), 14 EUs were used per treatment, but due to logistic reasons (available water volumes) in experiment II (salinity) only 10 EUs were used for each treatment. Each EU contained 10 individual ascidians, which were separated from each other by plastic mesh. For each EU, ascidians with an average weight of 14.9 ± 0.4 g ww were used (Table 2).

Mortality was checked daily in each EU. Death was indicated when the ascidians *C. intestinalis* no longer contracted upon touching their siphons. Dead individuals were replaced to maintain the original biomass within the EUs. Survival of *C. intestinalis* was assessed with the Kaplan–Meier survival estimator curve (Efron 1988). This curve estimates the probability of death for an individual over time. Herein the total area of this curve for the 10-day experiment was calculated per EU based on the sum of the daily survival probability for all individuals within each EU.

Additionally, growth rates of ascidians were measured as biomass change (BC) as an estimator of overall physiological condition. All ascidians were weighed at the beginning (day 0) and only the survivors were weighed at the end of the experiment (day 10). Biomass change was calculated with the following equation: BC = (FW − IW)/T, where FW and IW are the final and initial ww and T is the duration of the experiment in days (Rothäusler et al. 2011). Changes in biomass were expressed as percent biomass change per day.

Codium fragile

Individuals were maintained in plastic containers (EUs = plastic aquaria, 25 × 18 × 16 cm, 3.5 L volume). A total of 14 EUs per treatment were used in experiments I and III. Due to logistic restrictions (preparation of large volumes of conditioned water) for the salinity experiment (Exp. II) only 10 EUs were used. For each EU, one algal thallus with an average weight of 10.9 ± 0.8 g ww was used (Table 2).

To assess the physiological performance of *C. fragile*, chlorophyll *a* fluorescence of PSII was

Table 2 Initial wet weight (g, ww) measurements per treatment of *C. intestinalis* y *C. fragile* species

Experiment	Factor	Treatment	Individual weight (range g, ww)	Mean ± SD	Statistic comparison
I	Light				
	C. intestinalis	Low	12.7–15.8	14.4 ± 0.7	
		Medium	13.7–15.2	14.3 ± 0.4	$F_{(2,39)} = 0.199$; $p = 0.819$
		High	13.5–15.8	14.5 ± 0.6	
	C. fragile	Low	9.9–11.8	11.2 ± 0.5	
		Medium	9.3–11.8	10.6 ± 0.8	$F_{(2,33)} = 3.385$; $p = 0.05$
		High	9.0–11.7	10.5 ± 0.8	
II	Salinity				
	C. intestinalis	Lowest	13.0–17.9	16.0 ± 1.4	
		Low	13.0–17.3	14.8 ± 1.5	$F_{(3,44)} = 1.599$; $p = 0.202$
		Ambient	13.5–17.5	15.8 ± 1.0	
		High	13.0–18.0	15.7 ± 1.5	
	C. fragile	Lowest	9.5–11.7	10.9 ± 0.7	
		Low	9.5–12.0	10.9 ± 0.8	$F_{(3,36)} = 0.108$; $p = 0.954$
		Ambient	9.3–12.0	10.9 ± 0.8	
		High	9.8–11.7	10.8 ± 0.6	
III	Temperature				
	C. intestinalis	Cool	12.0–18.3	15.0 ± 1.6	
		Ambient	12.0–18.3	14.9 ± 1.6	$F_{(2,39)} = 0.323$; $p = 0.725$
		Warm	12.1–18.3	14.9 ± 1.6	
		Cool	9.8–12.3	11.3 ± 0.9	
	C. fragile	Ambient	9.4–12.3	11.2 ± 0.8	$F_{(2,33)} = 0.458$; $p = 0.636$
		Warm	9.5–12.3	10.9 ± 0.9	

measured as maximal quantum yield (F_v/F_m), using a computer-aided portable pulse amplitude-modulated fluorometer (PAM 2500, Walz, Effeltrich, Germany). Measurements were carried out daily over a period of 8 days following the approach by Gómez et al. (2005). Each piece of alga was measured in three different sections to estimate the physiological condition of the entire thallus. The mean of these three measurements represented the photosynthetic performance for each experimental thallus. Decrease in the maximal quantum yield of algae after exposure to different experiments was expressed as percent inhibition of disturbed initial conditions as: $\mathrm{IN} = [\mathrm{I}_{F_v/F_m} - (\mathrm{I}_{F_v/F_m} - \mathrm{T}_{F_v/F_m})]/\mathrm{I}_{F_v/F_m}$, where T_{F_v/F_m} is the maximal quantum yield of final conditions and I_{F_v/F_m} is the corresponding maximal quantum yield of initial conditions. In addition, the biomass change per day (BC%) was calculated as a proxy for algal growth as explained above.

Mechanistic niche modeling

Reported values for the ecological performance of *C. intestinalis* and *C. fragile* (including cryptogenic and invaded distributions) were obtained from the literature. For *C. intestinalis*, responses of mortality (Marin et al. 1987; Vercaemer et al. 2011), filtration rate (Shumway 1978; Petersen and Riisgard 1992; Daigle and Herbinger 2009), and particle retention efficiency (Randlov and Riisgard 1979) were used from different experimental studies, ensuring to only use data from experiments that were conducted at temperatures and salinities at which this species performs well. For *C. fragile*, responses of maximal quantum yield (F_v/F_m) (Kim and Garbary 2007), growth (Hanisak 1979) and net photosynthesis (Fralick and Mathieson 1973; Arnold and Murray 1980) to experimental conditions of light, salinity and temperatures were used. Due to the scarcity of performance data in response to light

and the limited contribution of this factor in a preliminary classification analysis for *C. intestinalis*, it was decided not to include light as a predicting factor for the niche model of *C. intestinalis*.

Two performance categories were considered for the analyses of both species: (1) low performance response, and (2) high performance response under different environmental factors of light, salinity and temperature. To estimate both categories, maximum responses (the highest performance values) from each experimental study were taken as a 100 % value. Then values from 0 to 50 % were considered as low performance and values above 50 % as high performance. In the specific case of *C. intestinalis*, values of mortality responses from different studies (including this study) were converted to percent mortality per day; values ≤ 4 % mortality day^{-1} were considered as high performance and values >4 % day^{-1} as low performance (Fig. 4; Appendix Table S2). The threshold of 4 % mortality day^{-1} was chosen because it would have resulted in total mortality within one lunar cycle (~ 28 days).

The environmental predictors (light, salinity and temperature) used in this analysis were extracted from a global dataset with a spatial resolution of ca. 9.2 km (Tyberghein et al. 2011). Pre-processed remotely sensed data from ocean-observing satellite sensors and in situ measured oceanographic data of temporal mean (sea surface temperature, °C) and maximum (light data PAR, photosynthetically available radiation) from monthly climatologies were used. For comparability with the data from the experimental studies, light data extracted from the global database were transformed (from Einstein m^{-2} day^{-1} to µmol photon m^{-2} s^{-1}). Finally, the mean salinity data from an interpolation of in situ measurements were used.

Data analyses

Prior to analyses, all data were tested for normal distribution using Kolmogorov–Smirnow and Shapiro–Wilk's Test. Homogeneity of variances was tested with the F test. In case of more than two variances the Fligner–Killeen Test was applied. One-way ANOVAs were used to confirm that there were no significant differences in the initial ww of selected individuals at the beginning of the experiment (Table 2). Treatment effects on biomass changes of ascidians and algae

were also examined with one-way ANOVAs. When the ANOVAs revealed significant differences, a post hoc Tukey HDS was applied. In the light experiment, a *t* test was conducted to examine the mean differences in biomass change between light treatments with surviving ascidians (10 and 50 % light).

Survival curves of *C. intestinalis* in different experiments were analyzed separately. One-way ANOVA tests were used to compare the total area of survival between different temperature treatments (Exp. III). Using the function lme (as implemented in the R package "nlme", R Development Core Team 2013) to analyze the total area of survival between light intensities (Exp I), a mixed effects model was employed (Zuur et al. 2009). A specific non-linear function was used, where the parameters of the non-linear function are assumed to be random effects (Crawley 2005). Thus, the model was defined as: Survival-model $<-$lme (Surv-area–light-treatment), and the random intercept model was defined as: Tank ($\sim 1|$ Tank, data $=$ light-treatments). As variances of salinity experiment (Exp. II) could not be homogenized, a non-parametric Kruskal–Wallis test was used to compare the total area of survival between the different salinity treatments.

Differences in the maximal quantum yield of *C. fragile* were analyzed with repeated measure analysis of variance (ANOVA) separately for each abiotic factor, with the within-subject factor time (day) and the between-subject factor treatments (levels). If the assumption of sphericity (Mauchly test) was not met, the univariate approach with Greenhouse–Geisser adjusted degrees of freedom for the F-test was applied. When the analysis revealed significant differences, a post hoc Tukey HDS was applied.

A simple mechanistic niche model to predict the probability (high or low) of invasion for *C. intestinalis* and *C. fragile* along the Chilean coast was implemented using a random forest analysis (Cutler et al. 2007). The models relate the performance of each species (high/low; *C. intestinalis* n $=$ 71; *C. fragile* n $=$ 110) with experimental conditions, growing 1,000 trees to ensure output stability. The accuracy of each model was evaluated as the pseudo-R^2 $=$ 1 $-$ [out-of-bag error]. The models were projected onto 614 polygons using oceanographic variables encompassing the entire continental coast of Chile, from 18°S to 55°S.

Results

Experiment I: Light effect

Direct solar radiation had a significant effect on the survival of *C. intestinalis* [$t_{(37)} = 22.652$; $p < 0.001$]. At the end of the 10-day experiment, there was a mortality of 100 % in the high light treatment, followed by 66 % in the medium light treatment (Fig. 2). In the treatment with the lowest light intensity the accumulated mortality after 10 days only reached 4 % (Fig. 2). Surviving individuals from the low and medium light treatments lost biomass during the experiment (Fig. 3), but there were no significant differences between these two treatments [$t_{(1,181)} = 1.304$; $p = 0.195$].

In *C. fragile*, the maximal quantum yield (F_v/F_m) varied considerably based on light treatments and duration of exposure. There were significant differences between treatments [$F_{(2,33)} = 89.338$; $p < 0.001$] (Table 3). After
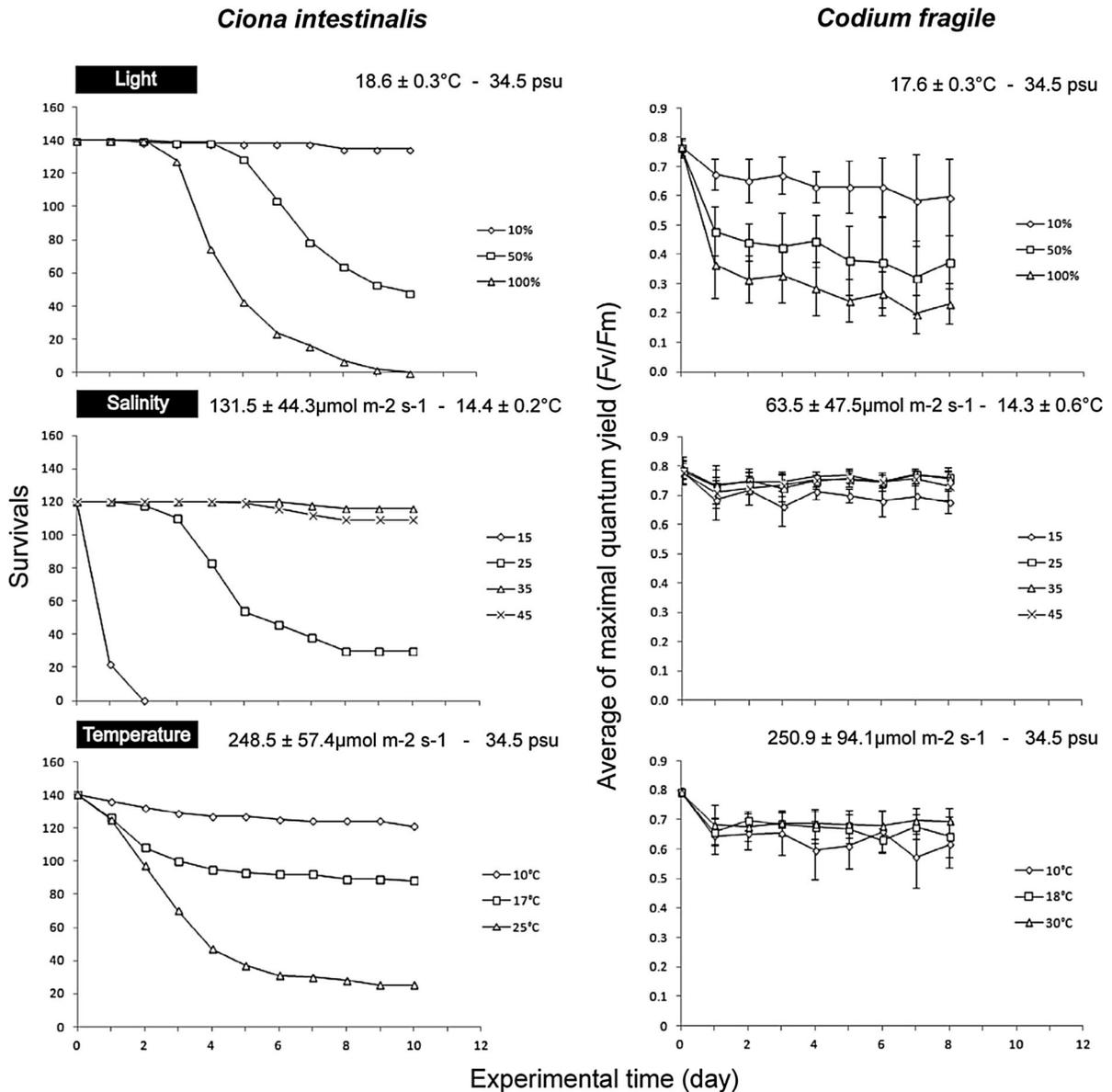
Fig. 2 Survival (%) of *C. intestinalis* and maximal quantum yield (F_v/F_m; mean ± SD) of *C. fragile* exposed to different abiotic factors (light, salinity and temperature). Experiments lasted 10 days (*C. intestinalis*) and 8 days (*C. fragile*)

Fig. 3 Biomass change (ww, % day^{-1}) of *C. intestinalis* and *C. fragile* in the different unifactorial experiments after 10 and 8 days, respectively. *Different letters* indicate significant differences ($p < 0.001$). *ND* no data, because all experimental individuals died during the experiments
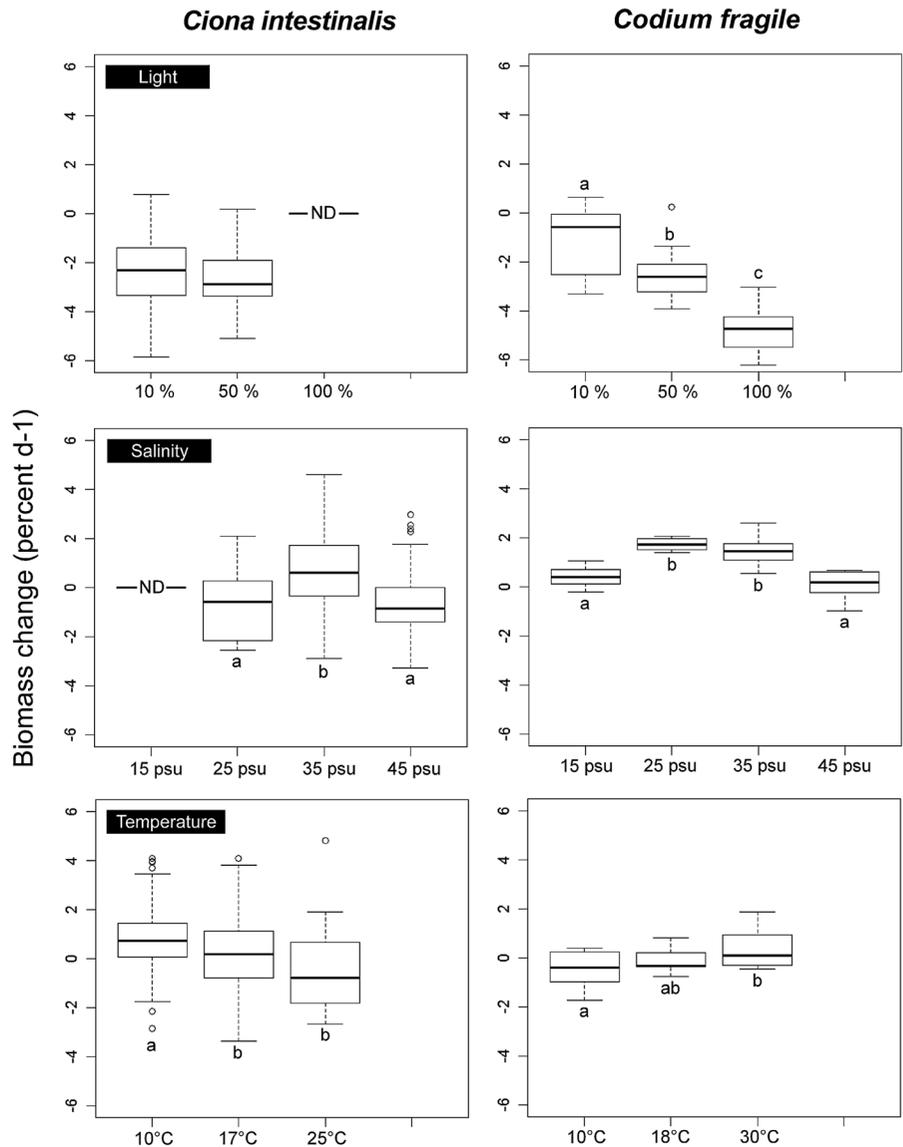
the 8 days of the experiment, thalli exposed to low light levels (10 % of ambient light levels) had a higher photosynthetic efficiency than the thalli from the other treatments, even though the efficiency decreased during the experiment (reduction from 0.76 to 0.59 F_v/F_m). At medium light levels, the quantum yield declined continually during the experiment varying from 0.76 to 0.37 F_v/F_m, i.e. an inhibition of 50 % (Fig. 2). At the highest radiation (100 %), there was a significant reduction of quantum yield from 0.76 to 0.36 F_v/F_m after the first day, showing an inhibition of ~70 % at the end of the experiment.

Light treatments had a significant effect [$F_{(2,33)} = 30.753$; $p < 0.001$] on the biomass change

of *C. fragile*. Biomass losses reflected the results of the photosynthetic efficiency (Figs. 2, 3; Table 4). Thalli of *C. fragile* lost more biomass when exposed to high light treatments than to the low light treatments (Tukey HSD test, Fig. 3).

Experiment II: Salinity effect

Low salinity had a drastic effect on the survival of *C. intestinalis* [$H_{(3)} = 42.098$; $p < 0.001$]. In the lowest salinity treatment (15 psu), all individuals died within 2 days. At moderately low salinity (25 psu), there was a 75 % mortality after 10 days, but at high salinity

Table 3 Results of the statistics analysis from the maximal quantum yield (F_v/F_m) of *C. fragile*, for each experiment using repeated measure analysis of variance (ANOVA), with the within-subject factor time (day) and the between-subject factor treatment (levels of abiotic factor exposed)

Experiment	Source	df	MS	F ratio	P value
I	Between-subject				
	Light	2	0.387	89.338	<0.001
	Error	33			
	Within-subject				
	Day	7	0.074	12.936	<0.001
	Day × light	14	0.004	0.702	0.772
	Error	231			
II	Between-subject				
	Salinity	3	0.008	17.877	<0.001
	Error	36			
	Within-subject				
	Day	4.088$_{GG}$	0.01	4.511$_{GG}$	0.002
	Day × salinity	12.265$_{GG}$	0.002	1.006$_{GG}$	0.447
	Error	252			
III	Between-subject				
	Temperature	2	0.012	7.816	0.002
	Error	33	0.001		
	Within-subject				
	Day	5.182$_{GG}$	0.005	1.575$_{GG}$	0.167
	Day × temperature	10.363$_{GG}$	0.01	2.904$_{GG}$	0.002
	Error	231			

GG Greenhouse–Geisser adjusted degrees of freedom

Table 4 Results of the statistical analysis of biomass change (BC%) of *C. intestinalis* and *C. fragile*

Experiment	Species	Source	df	MS	F ratio	P value
I	*C. intestinalis*	Light	1	t-value =	1.304	0.195*
	C. fragile	Light	2	41.908	30.753	<0.001
		Residuals	33	1.363		
II	*C. intestinalis*	Salinity	2	59.672	17.693	<0.001
		Residuals	234	3.373		
	C. fragile	Salinity	3	6.512	29.972	<0.001
		Residuals	36	0.217		
III	*C. intestinalis*	Temperature	2	19.918	10.277	<0.001
		Residuals	230	1.938		
	C. fragile	Temperature	2	1.871	4.226	0.023
		Residuals	33	0.442		

* A *t* test was done for the light experiment with *C. intestinalis* because in one treatment mortality was 100 %

(45 psu) most ascidians (91 %) survived until the end of the experiment (Fig. 2). Among the ascidians from the three treatments with survivors (25, 35, 45 psu) there were significant differences in biomass [$F_{(2,234)} = 17.693$, $p < 0.001$; Table 4], with the ascidians at typical seawater salinities (35 psu) gaining biomass, while those from the other two surviving treatments on average lost biomass (Fig. 3).

Although there were significant differences between salinity treatments [$F_{(3,36)} = 17.877$; $p < 0.001$] (Table 3), thalli of *C. fragile* exposed to extreme salinity conditions maintained a high photosynthesis quantum yield (F_v/F_m) after the 8-day experiment (Fig. 2). In all experimental conditions, algae had a constant quantum yield of 0.75 to 0.67 F_v/F_m, with an inhibition of ~ 14 % at very low salinity and ~ 4 % in the other treatments. This is also reflected in the fact that thalli gained biomass in all salinity treatments (Fig. 3). However, there were significant differences between treatments [$F_{(3,36)} = 29.972$; $p < 0.001$], and biomass gain was lower in the most extreme treatments than at ambient and moderately low salinities (Fig. 3; Table 4).

Experiment III: Temperature effect

Temperature had a significant effect on the survival of *C. intestinalis* [$F_{(2,39)} = 24.908$; $p < 0.001$] after 10 days (Fig. 2). There were significant differences between temperature treatments (Tukey HSD). Highest mortality (~ 82 %) was observed in the high temperature treatment compared to the ambient (mortality ~ 37 %) and cool (mortality ~ 14 %) temperature conditions. The biomass change of surviving ascidians differed significantly [$F_{(2,230)} = 10.277$; $p < 0.001$] (Fig. 3; Table 4). Ascidians from the cool temperature treatment gained more biomass, in contrast to the ambient and warm temperature conditions (Tukey HSD, Fig. 3).

Temperature had a significant effect on the photosynthetic performance of *C. fragile* [$F_{(2,33)} = 7.816$; $p = 0.002$] (Fig. 2; Table 3). At the end of the experiment (day 8), the photosynthetic efficiency varied slightly between treatments (0.61–0.69 F_v/F_m), being lower than that of the algae from day 0 (0.8) (Fig. 2). Inhibition was lowest (12.3 %) in the cool treatment (10 °C). Biomass change was significantly affected by the temperature treatments [$F_{(2,33)} = 4.226$; $p = 0.023$] (Fig. 3; Table 4). At low temperatures, thalli lost biomass while those maintained in warm temperature conditions increased their biomass (Tukey HSD, Fig. 3).

Niche modeling

While there are few studies explicitly testing the effects of light, the results from our experiments confirmed that *C. intestinalis* performed better under dim light than under direct solar radiation. According to various studies (Shumway 1978; Randlov and Riisgard 1979; Marin

et al. 1987; Petersen and Riisgard 1992; Daigle and Herbinger 2009; Vercaemer et al. 2011), *C. intestinalis* has a tendency to perform better at high salinities (20–50 psu), while performance was compromised at low salinities (<20 psu). Our results confirmed higher performance at high salinities (35 and 45 psu) than at low ones (15 and 25 psu). For temperature, previous studies (Shumway 1978; Randlov and Riisgard 1979; Marin et al. 1987; Petersen and Riisgard 1992; Daigle and Herbinger 2009; Vercaemer et al. 2011) and our own results showed that the performance of *C. intestinalis* is relatively high between 10 and 20 °C, but at high temperatures (>25 °C) their performance decreases (Fig. 4).

Generally, *C. fragile* had relatively high performances for a wide range of intensities of the three factors surveyed (light, salinity and temperature). In a wide range of light intensities (20–1,950 μmol photon m^{-2} s^{-1}) *C. fragile* performed well, albeit in a number of studies (Fralick and Mathieson 1973; Hanisak 1979; Arnold and Murray 1980) they did poorly at low light intensities (Fig. 4). The performance of the individuals from the Chilean population decreased at high light intensities (1,144 μmol photon m^{-2} s^{-1}). Populations of *C. fragile* from Chile are tolerant to a wide range of salinities (8–42 psu). Although the salinity range of this alga is relatively wide, at both extremes of this salinity range (0 and 48 psu) performance reported in the literature (Hanisak 1979; Kim and Garbary 2007) tended to be low. Similarly, *C. fragile* from Chile performed well throughout the range of temperatures tested herein (10–30 °C), although one experiment resulted in low performance at temperatures >24 °C (Fig. 4).

The results of niche modeling for both species along the Chilean coast had a high accuracy ($r^2 = 0.80$ in both cases). According to the classification analyses, for *C. intestinalis* the most important predictor was temperature, while for *C. fragile* it was light followed by temperature (Table 5). Both species have similar expected distribution patterns with a high probability of colonization on the coasts between Arica in northern Chile (18°S) and the Golfo de Penas (~ 50°S) in the south. In contrast, in the southern fjords (50°S–55°S) the probability of invasion is comparatively low (Fig. 5).

Discussion

Unifactorial experiments confirmed that both *C. intestinalis* and *C. fragile* can tolerate extensive ranges of the

Fig. 4 Performance of *C. intestinalis* and *C. fragile* under a range of different environmental factors: light, salinity and temperature conditions. *Open circles* indicate high performance values and *solid circles* the low performance values for each species; *plus symbols* represent the results from the present study (see Appendix Table S2)
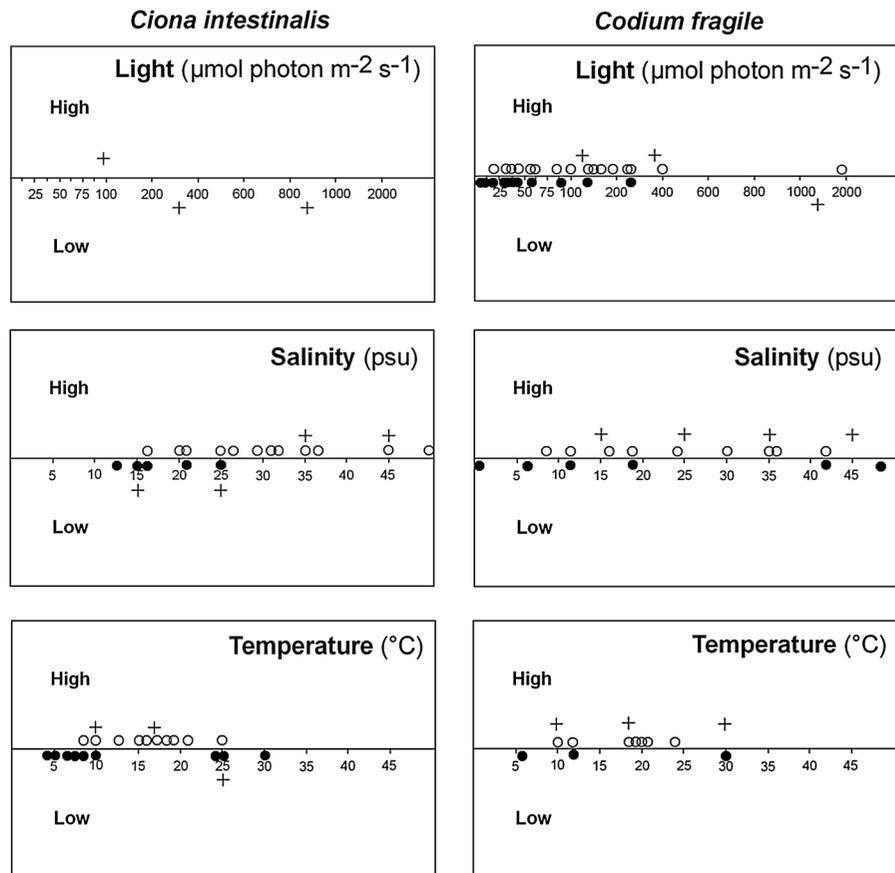
abiotic factors salinity and temperature, but *C. intestinalis* is more tolerant of cold-temperate conditions, while *C. fragile* is more tolerant of warmer conditions. Furthermore, the ascidian *C. intestinalis* is highly susceptible to intense direct light, while the photoautotrophic alga *C. fragile* survives well in response to solar radiation. The results of the mechanistic niche modeling show that the entire Chilean coast could be a suitable habitat for *C. intestinalis* and *C. fragile*, even though the main environmental factors determining the potential distribution differed between the two species.

**Physiological tolerance range and performance**

Physiological traits are known to be strongly affected by different environmental factors. Field observations of adult individuals and larval settlement have shown that *C. intestinalis* preferentially live in low-light habitats (Dybern 1963). Our results show higher survival and some growth in treatments with low

Table 5 Mean decrease of accuracy (MDA) from environmental variables of the random forest analysis of *C. intestinalis* and *C. fragile*

Variables	Species	
	C. intestinalis MDA	*C. fragile* MDA
PAR	ND	4.6
Temperature	5.2	4.4
Salinity	4.5	2.4

ND no data

compared to high light intensities. Probably the susceptibility of *C. intestinalis* to solar radiation is related to their transparency and the absence of UV-shielding compounds as had been reported for other littoral ascidians (Bingham and Reitzel 2000). This could also explain why *C. intestinalis* individuals prefer to live attached to the bottom of ship hulls or below other artificial structures, i.e. out of the reach of
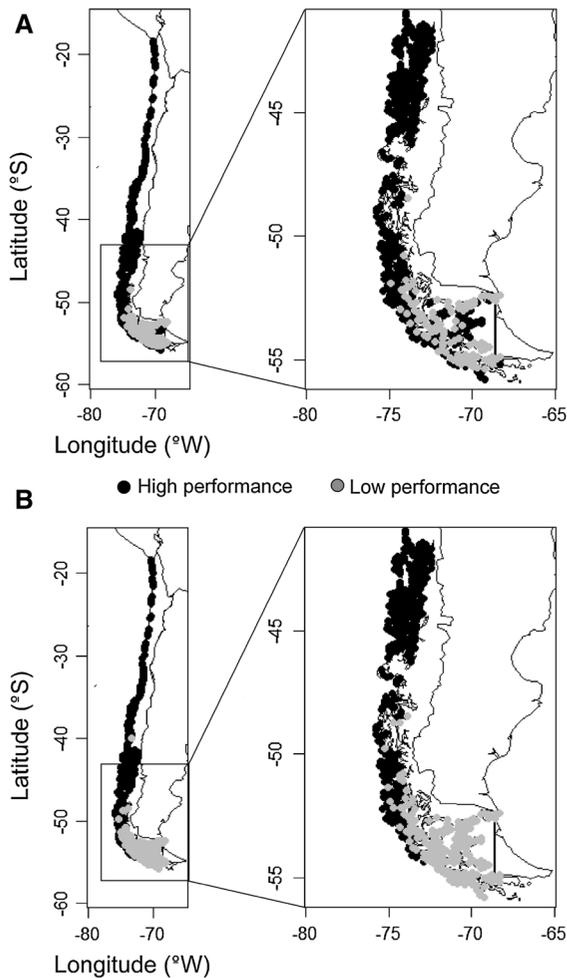
Fig. 5 Ecological niche model of **a** *C. intestinalis* and **b** *C. fragile* based on combined of light, salinity and temperature data used to identify potential environments of invasion, based on performance data from the literature and from the current study

direct solar radiation. Possibly, the risk of invasion by *C. intestinalis* is highest in areas with high turbidity or with many artificial structures.

The photoautotrophic macroalga *C. fragile* tolerates both direct and indirect exposure to light (Ramus 1978; Hanisak 1979), albeit the subtidal thalli tested herein did better under low light intensities than when exposed to high solar radiation. Hanisak (1979) demonstrated that thalli of *C. fragile* grow at different irradiances (14–140 μmol photon m^{-2} s^{-1}) and maximum growth was observed at intermediate intensities (56 and 86 μmol photon m^{-2} s^{-1}), which coincides with the results from the current study. In areas with eutrophication, where water transparency is reduced

and nutrient availability is enhanced (Cloern 2001), the adaptation to low light intensity environments and efficient nutrient uptake (Chapman 1999) might enhance the invasion success of *C. fragile*.

The study population of *C. fragile* has been relatively sensitive to strong solar radiation, which is most likely because the experimental individuals used herein came from subtidal habitats. While the anatomy of *C. fragile* is considered adaptive to low light intensity environments (Ramus 1978), this alga can also acclimate to high solar radiation over time (Ramus 1978; Arnold and Murray 1980; Michler et al. 2002). Indeed, despite a substantial reduction in photosynthetic yield and growth at high light intensities, no mortality was observed in our experiments. If experiments had lasted longer and experimental algae would have acclimated, the photosynthetic performance of *C. fragile* might have increased again. In general, the broad tolerance to a wide range of light regimes enhances the risk of spreading of *C. fragile* along temperate coastlines.

Our results indicate that physiological responses of *C. intestinalis* and *C. fragile* differ under the most extreme temperatures and salinities. Both species have similar reported tolerance ranges for salinity and temperature ($\sim$ 12–40 psu and $\sim$ −1 to 30 °C, respectively) (Dybern 1965, 1967; Hanisak 1979; Marin et al. 1987; Chapman 1999; Carver et al. 2006; Vercaemer et al. 2011). However, their tolerances and other physiological traits (e.g., growth, Fig. 3) towards these factors vary among different populations (Dybern 1965, 1967; Hanisak 1979; Trowbridge 1998; Carver et al. 2006; Vercaemer et al. 2011).

Ciona intestinalis tolerates a wide range of salinities (Dybern 1967), but some populations (e.g., Southern California harbors) are vulnerable to pulses of low salinity (Lambert and Lambert 1998). Experiments with Canadian and Scandinavian populations revealed that at 20 and 25 psu, and up to 41 psu adult ascidians are doing well, but at salinities below 15 psu their performance is compromised (Dybern 1967; Vercaemer et al. 2011), which is confirmed by the results of the current study. On the other hand, as a euryhaline species, *C. fragile* can tolerate low salinities (16 and 8 psu), and they can even survive for several hours at 0 psu (Kim and Garbary 2007). Thalli of *C. fragile* from NW Atlantic populations grow in a salinity range of 12–42 psu but their maximum growth was at 24 and 30 psu (Hanisak 1979). Our Chilean

population tolerated the same wide salinity range as reported for other populations, but we observed maximum growth at intermediate salinities.

Ciona intestinalis is considered a cold-water (or temperate) ascidian (Carver et al. 2006). The survival and growth of *C. intestinalis* directly depends on the temperature conditions. For Scandinavian populations for instance, Dybern (1965) pointed out that individuals tolerate temperatures from ~ 1 to 30 °C and grow faster at higher temperatures (~ 18 °C) than at low ones (~ 0 °C), even though high temperatures (>24 °C) may also constitute a thermal stress (Petersen and Riisgard 1992; Carver et al. 2003; Vercaemer et al. 2011). The experimental results of the present study confirm these temperature ranges for the population from northern Chile, which did well at the lower temperatures tested (10 and 18 °C) but had low survival and growth at high temperatures (25 °C). Also, early developmental stages of *C. intestinalis* performed better at intermediate temperatures (Rius et al. 2014b). *C. fragile*, in contrast, is considered a warm-temperate species (Fralick and Mathieson 1973; Trowbridge 1998), and optimal growth and reproduction (formation and release of gametes) is observed at maximum water temperatures (19–23 °C) even up to 24 °C, while minimal growth occurs at low temperatures (<5 °C) (Fralick and Mathieson 1973; Hanisak 1979; Villaseñor-Parada et al. 2013). The results of our experiments confirmed that the non-indigenous Chilean population of *C. fragile* grew at low and high temperatures (30 °C).

The adaptation to tolerate and perform in response to environmental factors (light, salinity and temperature) in different areas may be genotypically or phenotypically conditioned. The invasive populations of *C. intestinalis* and *C. fragile* from Chile had similar tolerances as populations from Canada, Denmark, Sweden, Italy, US and UK that included native, cryptogenic and invasive populations. In all those regions, *C. intestinalis* had a tendency to perform better in cold-temperate waters, while *C. fragile* did well in a wide range of temperatures.

Identifying potentially suitable habitats

The Chilean coast (from 18°S to 55°S) is characterized as a temperate coast with high variability in abiotic environmental factors, including PAR ranging from ~ 300 to ~ 800 µmol photon m^{-2} s^{-1}, salinity from 20 to 35 psu, and temperatures from ~ 5 to ~ 20 °C (Tyberghein et al. 2011; see also environmental data in Appendix Table S1). In southern Chile, water temperatures and salinities may even reach lower values, especially in the interior regions of the fjords.

The results of the model indicate that the high temperature and light tolerance of both studied species facilitates their high invasion potential. Thus, almost the entire Chilean coast presents a suitable habitat for both species with the exception of the interior fjords in southern Patagonia (Magellan region), where low salinities appear to limit the invasion potential of both species. Earlier reports of *C. intestinalis* from this region are questionable (Monniot and Monniot 1983) and could not be confirmed (Xavier Turon, personal comment). Similarly, the *C. fragile* population from Cape Horn is closely related to other populations within the circum-antarctic region, and differs from the invasive *C. fragile* ssp. *fragile* reported in northern Chile (Provan et al. 2008). While high latitudes present several conditions that reduce the invasion risk of non-native species, the opportunity to spread may be enhanced by increased warming of high-latitude waters (De Rivera et al. 2011; Rius et al. 2014b).

Temperature and salinity are considered important factors influencing the distribution, persistence and potential for range expansions of *C. intestinalis* populations (Therriault and Herborg 2008; Vercaemer et al. 2011; Rius et al. 2014b). While *C. fragile* has also invaded cold waters (Carlton and Scanlon 1985; Chapman 1999; Harris and Jones 2005; Neill et al. 2006; Lyons and Scheibling 2009), its spread has been mostly associated with warm waters, similar to other successful invaders such as *Crassostrea gigas*, *Styela clava*, *Littorina saxatilis*, *Amphibalanus improvisus* and *Carcinus maenas* (Compton et al. 2010; Carrasco and Barón 2010; De Rivera et al. 2011). Changing temperature and salinity conditions will likely alter the distribution patterns of both species. The wide range of water temperatures, especially associated with the El Niño Southern Oscillation (ENSO) events and climate change effects (Rius et al. 2014b), may also represent an opportunity for these widely adapted species to spread. According to Neill et al. (2006), a faster spreading of *C. fragile* populations is expected towards the northern rather than the southern parts of Chile.

Even though direct solar radiation had a significant impact on the performance of *C. fragile* tested herein,

this species is also reported within intertidal zones (Neill et al. 2006). This suggests that direct solar radiation it not an impediment to the spreading of *C. fragile*. In contrast, *C. intestinalis* appears to be highly vulnerable to light damage, which suggests that direct solar radiation might limit its distribution. Possibly, invasion could proceed via subtidal waters (several meters water depth), but there, these ascidians become more accessible to benthic predators, which should suppress their invasion potential (Dumont et al. 2011).

In the natural environment, organisms are exposed to a range of combinations of several environmental factors, and combined effects may be more stressful than extreme treatments in unifactorial experiments where the other factors were maintained at optimum conditions for the two tested species. In particular, in southern Chile low temperatures and low salinities often covary, and consequently, the expansion potential, in particular of *C. intestinalis*, could be even more limited than estimated herein. Furthermore, the niche model is based on average values, yet during spring–summer months the salinities in the inner fjords may be very low due to enhanced river run-off (Marín et al. 2013), which would further limit the expansion of species that are susceptible to low salinities. Future studies are needed to examine the invasion potential of species near their tolerance limits more thoroughly. Moreover, more complex physiological studies could provide the basis for implementing more sophisticated mechanistic models (i.e. biophysical, Buckley et al. 2010) that could be combined with more traditional correlational models into hybrid models (Buckley et al. 2010, 2011) in order to improve the precision of the predictions.

Potential risk on the Chilean coast

Since *C. fragile* can live over a wide intertidal and subtidal gradient of environmental conditions, and is consumed by few herbivores (Trowbridge 1993, 1995; Trowbridge and Todd 2002; Bégin and Scheibling 2003; Sumi and Scheibling 2005; Lyons et al. 2007; Lyons and Scheibling 2008; Scheibling et al. 2008), we conclude that its invasion potential along the Chilean coast is high. In contrast, the survival and growth of *C. intestinalis* appears to be limited at the sea surface, and since this species has many predators in subtidal waters (Gulliksen and Skaeveland 1973; Yamaguchi 1975; Petersen and Svane 1995; Carver

et al. 2003; Dumont et al. 2009, 2011; Rius et al. 2014a), limited larval dispersal and specific settlement requirements (Petersen and Svane 1995; Collin et al. 2013), and may be susceptible to competitive exclusion (Paetzold et al. 2012; Bullard et al. 2013; Rius et al. 2014a), its invasion potential is relatively limited, despite the wide physiological tolerance ranges reported herein. Also, other aspects of the biology of *C. fragile* such as sexual and asexual reproduction as well as buoyancy contribute to short and long-range dispersal, and the colonization success of this species (Fralick and Mathieson 1972; Chapman 1999; Prince and Trowbridge 2004; Watanabe et al. 2009; Gagnon et al. 2011; Villaseñor-Parada et al. 2013). Based on these considerations, we suggest that the invasion risk of *C. fragile* along the Chilean coast is substantially higher than that of *C. intestinalis*.

In Chile, the invading populations of *C. intestinalis* and *C. fragile* are already causing economic damage in the aquaculture industry (Uribe and Etchepare 2002; Neill et al. 2006; Castilla and Neill 2009). Ecological effects are not well known, even though *C. intestinalis* has been reported as one of the most abundant recruits in *Macrocystis pyrifera* kelp forests (Almanza et al. 2012). Once established, both invasive species can become the dominant species in new habitats, suppressing species richness and altering (positive and negative effects) community assembly processes (Harris and Jones 2005; Scheibling and Gagnon 2006; Blum et al. 2007; Thomsen et al. 2009; Drouin et al. 2011; Cordell et al. 2013). Considering that the entire Chilean coast constitutes a suitable habitat for these non-indigenous species, particular emphasis should be placed on preventing human-mediated dispersal, especially by intentional transfer of aquaculture species to other regions along the coast. Since floating trash (from aquaculture operations) and artificial marine structures can contribute to the spread of these species (Bulleri and Airoldi 2005; Astudillo et al. 2009), the deliberate or accidental disposal of these items in the sea should be regulated and fined.

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