Photosynthetic plasticity of the genus *Asparagopsis* (Bonnemaisoniales, Rhodophyta) in response to temperature: implications for invasiveness

M. Zanolla · M. Altamirano · R. Carmona · J. De La Rosa · A. Sherwood · N. Andreakis

Abstract Invasive species display remarkable levels of ecophysiological plasticity, which supports colonization, population establishment and fitness across their introduction range. The red seaweed genus *Asparagopsis* comprises genetically homogeneous invasive species (*A. armata*) and cryptic species complexes (*A. taxiformis* sensu lato) consisting of invasive mitochondrial lineages introduced worldwide. The photosynthetic plasticity of Australian, Mediterranean and Hawaiian Falkenbergia stages (i.e. the tetrasporophytic stage) of *A. taxiformis* lineages 2, 3 and 4 and Mediterranean isolates of *A. armata* was assessed by challenging their photosynthetic performance at five different temperatures (12–26 °C). Our aim is to portray the photosynthetic profiles in relation to temperature for each of the aforementioned *Asparagopsis* OTUs. We additionally test the physiological response of *A. taxiformis* lineage 2 sampled within its invasive (Mediterranean Sea) and native range (Australia) to identify physiological features associated with invasive strains. Based on photosynthesis optima, *Asparagopsis* isolates were recovered into a tropical (NL2 and L4) and a temperate (AA, Il2 and L3) group that presented no differences in most photosynthetic parameters at the experimental temperatures, thus indicating a greater physiological plasticity. On the other hand, low I_c values together with an apparent lack of sensitivity in the photosynthetic response to changing temperatures were revealed for the Mediterranean lineage 2, indicative of adaptive benefits that likely support its invasive success compared to the rest of the genus. Our results represent a valuable resource to predict distributional shifts in some of the lineages and to anticipate control programs for lineage 3, potentially invasive.

Keywords *Asparagopsis* · Climate Change · Invasive · Plasticity · Photosynthesis · Temperature

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Introduction

Introductions of non-indigenous marine seaweeds represent a direct consequence of global climate change and habitat degradation yet a major challenge

for conservation and management of local marine communities (Thresher 1999; Nyberg and Wallentinus 2005; Schaffelke et al. 2006; Andreakis and Schaffelke 2012). In the near future, global biodiversity hot-spots such as the Mediterranean Sea are expected to be the world's most severely compromised areas due to multiple species introductions, many of which are considered invasive and pests (Galil 2000; Boudouresque and Verlaque 2002, 2010; Zenetos et al. 2010).

Besides its potential involvement in biodiscovery and as a biofilter in aquaculture (Salvador et al. 2007; Genovese et al. 2009; Manilal et al. 2010; Mata et al. 2010), the red seaweed genus *Asparagopsis* Montagne (Bonnemaisoniales, Rhodophyta) is notorious for its invasiveness in the western Mediterranean Sea and worldwide (Cebrián and Ballesteros 2004; Altamirano et al. 2008). *Asparagopsis* comprises well-established morphospecies and cryptic species complexes, i.e. the cold-temperate *A. armata* Harvey and the tropical to warm temperate *A. taxiformis* (Delile) Trevisan de Saint-Léon (Guiry and Guiry 2011). Whereas *A. armata* is a genetically homogeneous species, *A. taxiformis* consists of at least four cryptic mitochondrial lineages (L1–L4) either dispersed or introduced in multiple regions (Andreakis et al. 2004, 2007a; Ní Chualáin et al. 2004; Sherwood 2008). For instance, the invasive Indo-Pacific L2 sensu Verlaque and Boudouresque (2004), considered native to Australia, has invaded the Mediterranean sea through the Suez Canal and has been recently found on the western coasts of the Mediterranean Sea (Andreakis et al. 2004, 2007b; Ní Chualáin et al. 2004; Altamirano et al. 2008). The Atlantic L3, on the other hand, occurs sympatrically in South Africa with L2, where both lineages are considered invasive and the latter recently introduced (Bolton et al. 2011). For sake of clarity, we will refer to *A. armata* and L1–L4 of *A. taxiformis* as operational taxonomic units (OTUs).

Rapid micro-evolutionary changes triggered by founder events, population bottlenecks or high rates of hybridization among genealogically related taxa drive speciation in marine organisms (Knowlton 1993). However, although genetic divergence has often been associated with reproductive incompatibility, morphological and ecophysiological differentiation may not necessarily complement full lineage sorting, leading to the formation of cryptic species complexes (Kooistra et al. 2002; Zuccarello and West 2002, 2003;

De Clerck et al. 2005; Andreakis and Schaffelke 2012). In invasive species with identical genetic backgrounds between native and introduced strains, phenotypic or ecophysiological plasticity induced by the novel environmental conditions may at times be adaptive (Eggert 2012). This will lead to superior fitness associated with distinct ecophysiological and/or morphological profiles of the introduced individuals, compared to the donor population in the species native range (Andreakis and Schaffelke 2012). Therefore, adaptive plasticity confers evolutionary advantages to invasive species by optimizing acclimation mechanisms to the new environmental conditions (Davidson et al. 2011).

Local temperature regimes are known to govern photosynthetic response, survival and fitness in seaweeds (Breeman 1988; Padilla-Gamiño and Carpenter 2007) since the genome traits and associated enzymes involved in these processes are sensible to temperature fluctuations (Webster et al. 2013). Different thermal regimes or temperature gradients are therefore expected to influence drastically the expression profile of these genes resulting in the establishment of local ecotypes and in macroecological scale, to new lineages and species (Andreakis and Schaffelke 2012; Eggert 2012). Following introduction, Falkenbergia, the resilient stage in the triphasic life cycle of *Asparagopsis*, is expected to face unusual temperature regimes. In the present study we assess the photosynthetic profiles and physiological tolerance of tetrasporophytes of *A. armata* and *A. taxiformis* L2, L3 and L4, by means of their photosynthetic performance at different temperatures. In addition, for the invasive L2, we compare photosynthetic performance of isolates collected from the lineages' native and introduced ranges to assess the extent of physiological plasticity for that lineage and evaluate its contribution in support of invasiveness of *A. taxiformis* L2 in the Mediterranean Sea. Our hypothesis is that Mediterranean isolates of the invasive L2 (IL2) show a broader physiological plasticity compared to any other *Asparagopsis* OTU and they are physiologically distinct from isolates of L2 native to Australia (NL2). Since the photosynthetic response of each of the OTUs analysed will likely be influenced by local thermal regimes, the information gathered from this study represents a useful tool to infer OTU-specific distribution ranges and predict potential range expansions.

Materials and methods

Algal material and culture conditions

Falkenbergia stages of *Asparagopsis armata* (AA) and *A. taxiformis* lineages 2 (L2), 3 (L3) and 4 (L4) were collected by snorkeling and/or SCUBA diving from a depth range of 0–1.5 m (Table 1), gently cleaned of epiphytes and maintained in culture in 2L beakers with aeration for at least six months in the University of Málaga, in sterilized seawater enriched with Provasoli (PES) medium Provasoli (1968). Culture medium was changed weekly for all isolates at the same time. All cultures were kept, as described previously (Guiry and Dawes 1992; Paul 2006), at 18 °C, 12:12 (L:D), 20–30 μmol photons m^{-2} s^{-1} of white light provided by fluorescent Sylvania grolux lamps, except for L4 that was cultured at 28 °C, 12:12 (L:D), 20–30 μmol photons m^{-2} s^{-1} because it failed to survive at 18 °C.

DNA extraction, PCR amplification and genetic identification of isolates

For species and lineage identification, DNA extraction and sequencing of the *cox* 2–3 IGS was performed as described previously (Andreakis et al. 2004;

Sherwood 2008). Sequences were compared to the following *cox* 2–3 sequences available in GenBank: EU146198, EU146197, EU146196 (for L2 of *A. taxiformis*), AY589524 (for L3 of *A. taxiformis*), EU146220, EU146221, EU146222 (for L4 of *A. taxiformis*), and AY589522, AY589523 (for *A. armata*). Sequences were aligned by eye in Bioedit v. 4.8.5 (Hall 1999); hierarchical likelihood ratio tests (hLRTs) were performed in Modeltest v3.06 (Posada and Crandall 1998) to find the best-fitting parameters for maximum likelihood (ML) analysis given the alignment and ML phylogenies were inferred in PAUP* for Windows 4.0b10 (Swofford 2002). Computations were performed using heuristic searches and ten random sequence additions to find the highest likelihood tree. Bootstrap support for individual clades was calculated with 1,000 replicates using the same options and constraints as used in the tree-inferences. More detailed information of the samples used in this study is given in Table 1.

Photosynthetic measurements

We used photosynthesis-irradiance (P-E) curves tested at different temperatures to assess the photosynthetic tolerance of the Falkenbergia isolates from each OTU

Table 1 Collection and accession information for *Asparagopsis* samples

Taxon	Collection site	Collector	*Cox 2–3* GenBank accession
A. armata	Calaburra Beach, Málaga (Spain, 36°30′N, 4°38′W)	J. De La Rosa	JF820070[a]
A. taxiformis Invasive lineage 2 (IL2)	Marina del Este, Granada (Spain, 36°43′N, 3°43′W)	M. Zanolla	AY589537[a]
A. taxiformis Native lineage 2 (NL2)	Kissing Point Northward, Townsville (Australia, 19°14′S, 146°48′E)	M. Altamirano	AY589537[a]
A. taxiformis Lineage 3 (L3)	El Hierro, Canary Islands (Spain, 27°47′N, 17°59′O)	J. De La Rosa	KJ584611
A. Taxiformis Lineage 4 (L4)	Makai Pier, Oahu, Hawaii (USA, 21°19′N, 157°40′W)	M. Zanolla[a]	EU146224

Lineages of *A. taxiformis* are designated as in Andreakis et al. (2007b)

[a] Identical to the #sequence

used in this study. Four replicates, ca. 0.04 g FW, each belonging to a single individual, were pre-incubated at 12, 15, 18, 22 and 26 °C, for 45 min in 50 mL Falcon tubes with sterile seawater (Padilla-Gamiño and Carpenter 2007). Temperature regime was chosen on the basis of the thermal range to which OTUs are exposed in their natural environment. Specimens were then transferred to the reaction chamber of a Clark-type oxygen electrode system (Hansatech Instruments, Norfolk, England), in 2 mL sterile seawater at the experimental temperature to determine photosynthetic performance.

Changes in dissolved oxygen concentration were used to estimate the respiration and photosynthetic rates of OTUs at each temperature under increasing irradiances ranging from 0 to 600 μmol photons m^{-2} s^{-1}. Different irradiances were achieved by setting neutral density filters between the light source (KL 1500 Compact, Schott) and the reaction chamber and they were measured with a quantum photon meter (LICOR, LI-185B, Lincoln, NE, USA) inside the reaction chamber. Algal samples were initially exposed to darkness for 15 min to estimate the dark respiration rates (DR), and then exposed for 5 min at each irradiance value for the assessment of photosynthetic rates. P-E curves were fitted to the Edwards and Walker equation (1983) using Kaleidagraph (Synergy Software, Version 4.0) to obtain the maximum net photosynthetic rate (NPR_{max}) and the light compensation point (I_c). The photosynthetic efficiency (α) was estimated from the linear portion of each P-E curve as the slope of the linear model. The light saturation parameter (I_k) was obtained from the ratio NPR_{max}/α (Henley 1993). The photoinhibition rate (β) was estimated as the slope of the linear regression of the last four values of the P-E curve. All photosynthetic parameters were expressed on a fresh weight (FW) basis.

Photosynthetic pigment analysis

Samples used for P-E curves were weighed following removal of the surface water and frozen at −20 °C for pigment concentration analysis due to its role in the photosynthetic performance. Chlorophyll *a* and total carotenoids were extracted in DMF (*N,N*-dimethyl-formamide) at 4 °C in darkness overnight. Chlorophyll *a* and carotenoid concentration were estimated spectrophotometrically (Jenway 6500 Scanning spectrophotometer, Keison Products, UK) according to the

equations of Wellburn (1994). Values of pigment concentration were also expressed on FW basis.

Data analysis

Comparison of photosynthetic parameters was performed by a two-way (temperature and OTU) analysis of variance (ANOVA). Homogeneity of variances was assessed by the F_{max} test. When significance was found, a Holm–Sidak post hoc test was applied ($p < 0.005$) to separate means. Pigment data were compared using a one-way (lineages/species) analysis of variance (ANOVA) followed by a Holm–Sidak post hoc test ($p < 0.001$), since there were no significant differences in the pigment content among temperatures within species or lineage. All tests were performed using the SIGMAPLOT software (Systat Software Inc., version 11.0).

Results

Differences in photosynthetic profiles among OTUs given a specific temperature

Photosynthetic performance varied significantly among the *Asparagopsis* OTUs and was highly influenced by temperature (Fig. 1).

No significant differences in NPR_{max} were observed in the temperature range of 12–15 °C for L3, NL2, IL2 and AA, whereas L4 displayed negative values (Fig. 2a). The response of AA and L3 at 18 °C was higher compared to the rest of the OTUs and was followed by similar values of IL2 and NL2 (Fig. 2a; Table 2). L4 and L3 behaved similarly at 22 °C, showing significantly lower NPR_{max} values than NL2; AA and IL2 presented similar rates than the other taxa studied at that temperature. At 26 °C, AA displayed the lowest values compared to the rest of the OTUs, which showed no differences at that temperature. DR was not significantly different among OTUs or between IL2 and the rest of the OTUs at any temperature tested. However, AA showed 60 % lower respiration rates compared to tropical L4 and NL2 at all temperatures. L4 showed twofold higher DR values than L3 at all temperatures. Finally, all OTUs displayed the lowest rates of DR at the lower temperature range (12–15 °C) (Fig. 2b; Table 2). The highest values for α were recorded for AA at 18 °C
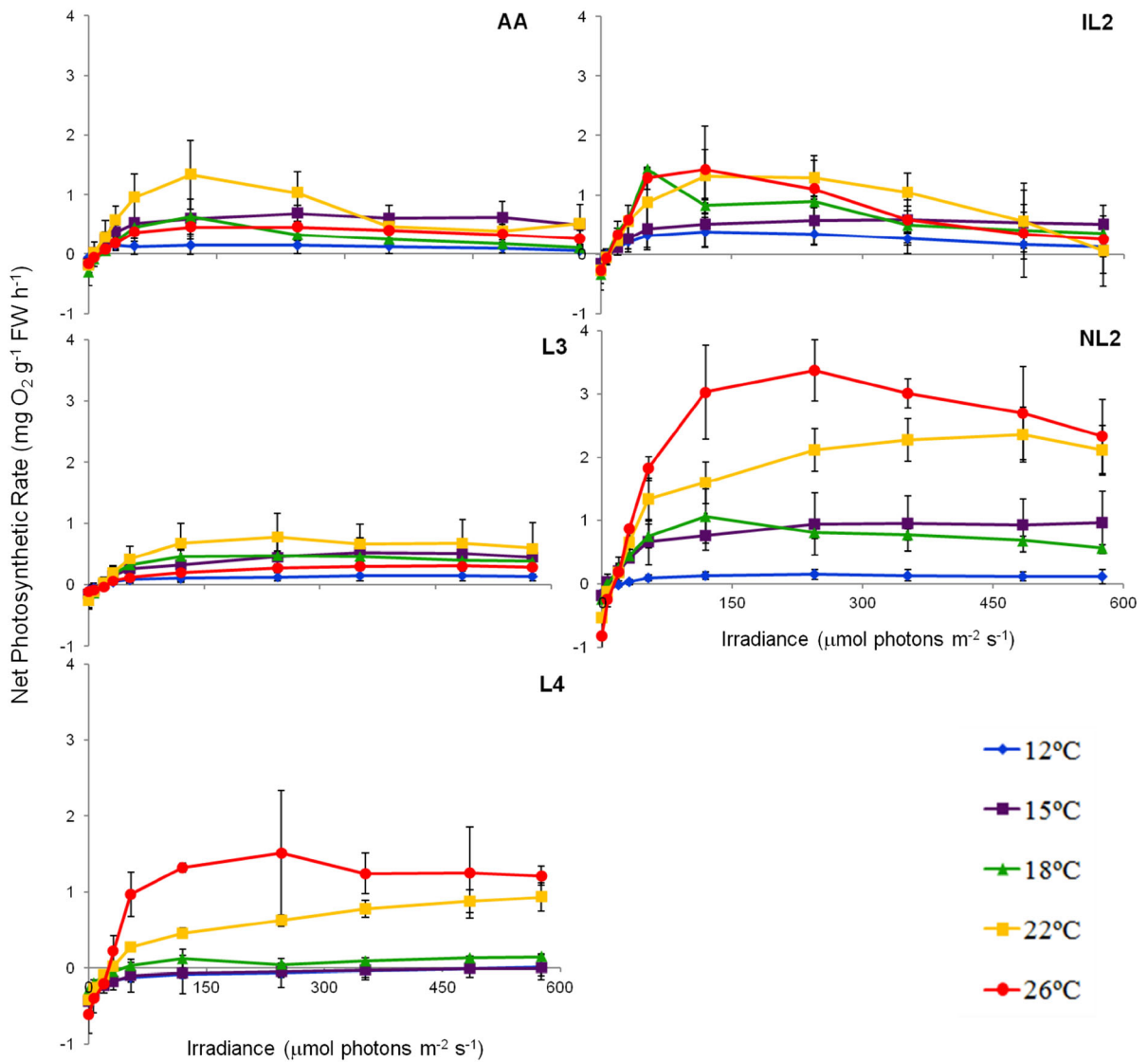
Fig. 1 P–E curves of *A. armata* and the lineages of *A. taxiformis* at different temperatures. Vertical bars represent SD of the mean values (n = 4). AA: *Asparagopsis armata*; IL2: invasive lineage 2 of *A. taxiformis*; NL2: native lineage 2 of *A. taxiformis*; L3: lineage 3 of *A. taxiformis*; L4: lineage 4 of *A. taxiformis*

$(0.094 \pm 0.012$ mg O_2 g^{-1} FW h^{-1}/μmol photons m^{-2} s^{-1}), almost fourfold higher than the ones observed for L3 and L4 at the same temperature (Fig. 2c). We found no differences among OTUs at lower temperatures (12–15 °C) and 22 °C. NL2 showed higher α values at 26 °C $(0.050 \pm 0.027$ mg O_2 g^{-1} FW h^{-1}/μmol photons m^{-2} s^{-1}) than AA and L3, yet statically similar values with IL2 and L4. The values of α obtained for L4 at 12 and 15 °C are here excluded from the comparisons since photosynthetic rates were negative at those temperatures (Fig. 1). Photoinhibition rates were

generally low, ranging from 3×10^{-4} to 2.8×10^{-3} mg O_2 g^{-1} FW h^{-1}/μmol photons m^{-2} s^{-1} for *A. armata* and 6.2×10^{-5} to 6.6×10^{-3} mg O_2 g^{-1} FW h^{-1}/μmol photons m^{-2} s^{-1} for the *A. taxiformis* lineages. L4 was the only one to show photoinhibition at 26 °C (Fig. 2d; Table 2). Yet, no consistent differences in β among OTUs at any tested temperature could be observed.

Both *A. armata* and IL2 did not show significant differences in the light compensation point (I$_c$) at all temperatures tested, showing values 80 % smaller than L4 and L3. The latter presented intermediate
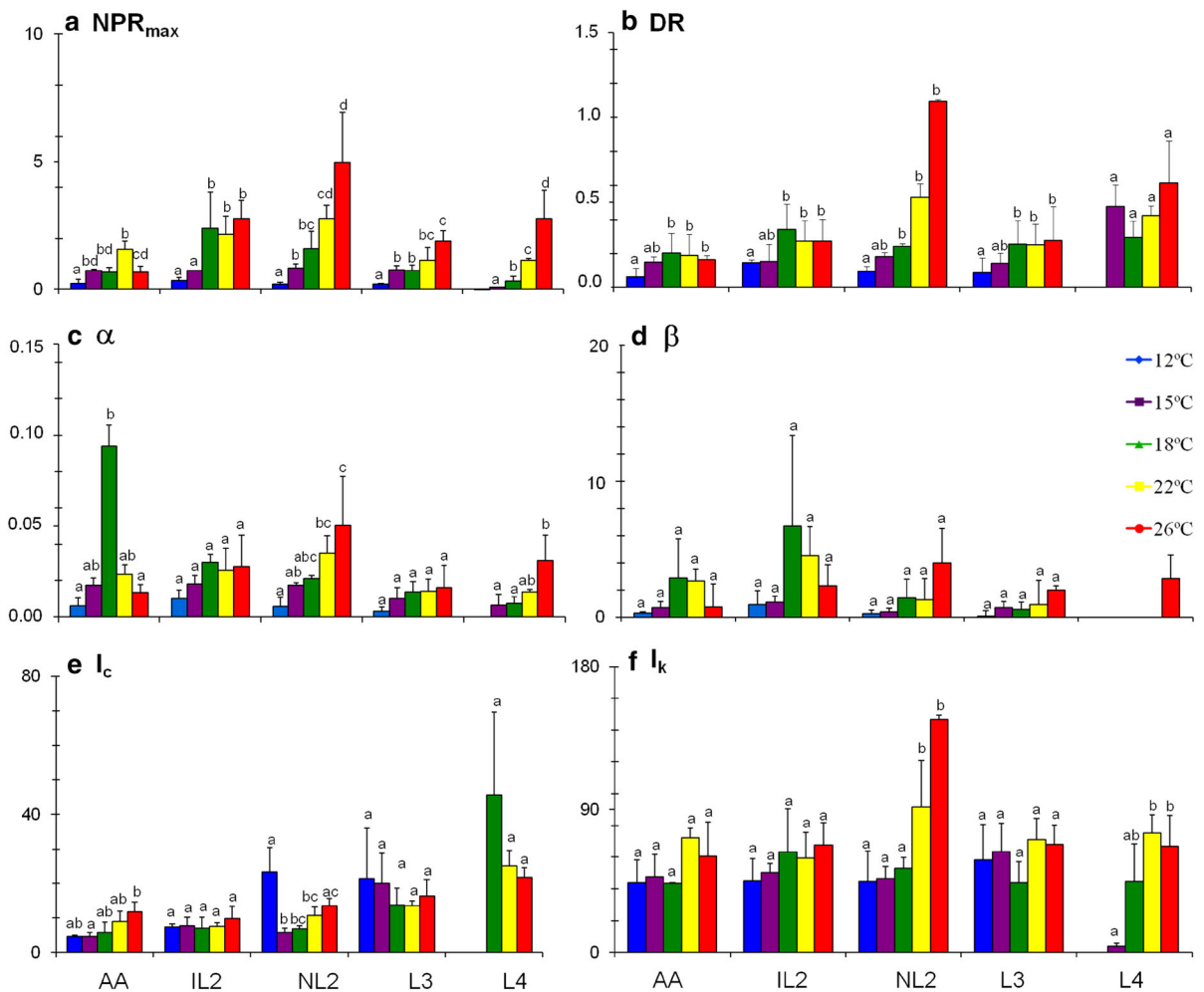
Fig. 2 Photosynthetic parameters of *Asparagopsis* OTUs estimated from P–E curves (see "Materials and methods") at different temperatures. Vertical bars represent SD of the mean values (n = 4). Different letters represent significant differences ($p < 0.001$) among temperatures within an OTU. **a** Maximum net photosynthetic rate (NPR_{max}) and **b** dark respiration (DR) are expressed on FW basis (mg O_2 g^{-1} FW h^{-1}); **c** α is expressed as mg O_2 g FW^{-1} µmol photons m^{-2} s^{-1}, and **d** β as mg O_2 g FW^{-1} h^{-1}/mmol photons m^{-2} s^{-1}; **e** light compensation point (I_c) and **f** light saturation parameter (I_k) are expressed as µmol photons m^{-2} s^{-1}. AA: *Asparagopsis armata*; IL2: invasive lineage 2 of *A. taxiformis*; NL2: native lineage 2 of *A. taxiformis*; L3: lineage 3 of *A. taxiformis*; L4: lineage 4 of *A. taxiformis*

values than L4, and the group formed by AA and IL2 at most temperatures, except at 22 °C that was recovered similarly for all of the OTUs analysed (Fig. 2e; Table 2). Finally, L4 scored the highest values of I_c at all temperatures tested. However, the values measured at 12 and 15 °C will not be considered in the comparison since they represent artefacts obtained from the fitting procedure of the P–E curves (see "Materials and methods"; Fig. 1).

Differences in I_k were not statistically significant for IL2 in the temperature range analysed. However, significant differences for this parameter were

observed at extreme temperatures with NL2 showing the highest values at 26 °C (Fig. 2f; Table 2).

Differences in photosynthetic profiles
among temperatures within an OTU

Similar trends and a correlation of NPR_{max} with temperature were found for IL2 and L3. However, none of them presented consistent differences in the remainder of the parameters studied at all temperatures (Fig. 2). The values of NPR_{max} and I_c were lower for *A. armata* at temperatures ranging from 15 to

Table 2 Two-way (temperature and OTUs) ANOVA for the estimated photosynthetic parameters

Differences among temperatures within OTUs are represented in Fig. 2

F values with an asterisk represent significant differences ($p < 0.05$) in the studied parameter; ns: not significant. L: lineage; T: temperature. AA: *Asparagopsis armata*; IL2: invasive lineage 2 of *A. taxiformis*; NL2: native lineage 2 of *A. taxiformis*; L3: lineage 3 of *A. taxiformis*; L4: lineage 4 of *A. taxiformis*. When lineage/species are not indicated, no significant differences were found in relation to the other OTUs

Parameter		*F* value	*p* level	Differences among lineages/species within temperatures
NPR_{max}	L	58.8*	<0.001	12 °C: L4 < L3 = NL2 = IL2 = AA
	T	140.5*	<0.001	15 °C: L4 < L3 = NL2 = IL2 = AA
	L × T	12.2*	<0.001	18 °C: L4 < IL2 = NL2 < L3 = AA
				22 °C: L4 = L3 < NL2
				26 °C: AA < L4 = L3 = NL2 = IL2
I_c	L	97.8*	<0.001	12 °C: NL2 = L3 > AA = IL2
	T	15.4*	<0.001	15 °C: L3 > IL2 = AA = NL2
	L × T	9.72*	<0.001	18 °C: L4 > L3 > IL2 = NL2 = AA
				22 °C: L4 > IL2 = NL2 = AA; L3 = L4
				26 °C: L4 > IL2
I_k	L	7.7*	<0.001	12 °C: no differences among lineages/species
	T	18.0*	<0.001	15 °C: no differences among lineages/species
	L × T	3.1*	<0.001	18 °C: no differences among lineages/species
				22 °C: no differences among lineages/species
				26 °C: L4 = IL2 = L3 = AA < NL2
	L	6.3ns	<0.001	12 °C: no differences among lineages/species.
	T	13.6ns	<0.001	15 °C: no differences among lineages/species
	L × T	2.0*	0.026	18 °C: L4 = L3 < AA
				22 °C: no differences among lineages/species.
				26 °C: AA = L3 < NL2
DR	L	7.1*	<0.001	All temperatures: L4 = NL2 > AA; L4 > L3
	T	4.9*	0.002	
	L × T	1.1ns	0.41	
	L	1.5ns	0.2	
	T	1.1ns	0.4	
	L × T	0.9ns	0.6	

26 °C although I_c values at 26 °C were 14 % higher than at 12 and 15 °C (Fig. 2a, e). On the other hand, I_k did not show differences among all temperatures tested. The greatest value of α was recorded at 18 °C, almost 84 % higher than at the rest of the temperatures (Fig. 2c). L4 showed statically higher values of NPR_{max} and α at 26 °C, 76 and 66 %, respectively higher compared to intermediate temperatures of 18–22 °C. The greater value of I_c was recorded at 18 °C and I_k values were also increased at higher temperatures. Finally, NL2 showed optimum values for NPR_{max}, I_c and I_k at the highest temperatures (22–26 °C), while α was positively correlated with temperature (Fig. 2a, e, f).

Pigment content analysis

Inconsistent differences were observed in either chlorophyll *a* or carotenoids content among IL2, L3 and *A. armata* isolates (Fig. 3a, b). However, NL2 presented the highest concentration of chlorophyll *a* (1.40 ± 0.28 mg g^{-1} FW) and carotenoids (0.45 ± 0.09 mg g^{-1} FW), up to fourfold higher compared to the rest of the OTUs analysed. On the other hand, L4 presented intermediate values of chlorophyll *a* (0.12 ± 0.03 mg g^{-1} FW) between NL2 and L3/IL2/*A. armata* (Fig. 3a, b).

Discussion

We report distinct patterns of photosynthetic performance among the *Asparagopsis* OTUs analysed in this study. These patterns reflect their genetic divergence and the observed differences in their distribution range. We further describe remarkable contrasts in photosynthetic profiles among genetically identical L2 isolates collected from Australia and the
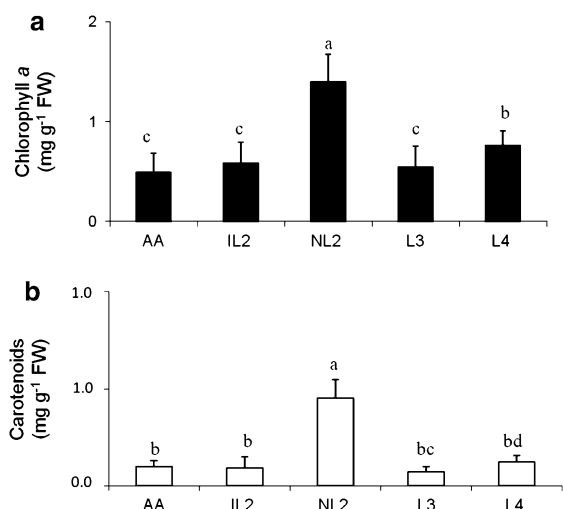
Fig. 3 Photosynthetic pigment content (**a** Chlorophyll *a*, **b** Carotenoids) of *A. armata* and the different lineages of *A. taxiformis*. Values for each OUT are means of all temperatures assayed since there were no significant differences among them. Vertical bars represent SD of the mean values (n = 4). Different letters represent significant differences ($p < 0.001$). AA: *Asparagopsis armata*; IL2: invasive lineage 2 of *A. taxiformis*; NL2: native lineage 2 of *A. taxiformis*; L3: lineage 3 of *A. taxiformis*; L4: lineage 4 of *A. taxiformis*

Mediterranean Sea. We argue that the extent of ecophysiological plasticity observed in *Asparagopsis* lineage 2 affects its fitness differently within its native and introduced range. The capacity of this strain to turn plastic physiological responses into adaptive benefits makes L2 a successful invader.

Independently of genomic architecture, phenotypic plasticity and physiological tolerance have been accepted as specific responses to novel environmental factors or a particular environmental cue (Stearns 1989; Raniello et al. 2004; Bradshaw and Holzapfel 2006). Defining the range of morphological and physiological variation within cryptic species complexes has significant ecological and evolutionary consequences and has often challenged classical taxonomy in several marine groups (Stearns 1989; Raniello et al. 2004; Flagella et al. 2010; Schmidt-Roach et al. 2013). Phenotypic plasticity has been identified as a character supporting invasiveness (Davidson et al. 2011), yet, its role in the success of many marine biological invasions has been largely overlooked, and it may explain why some organisms behave as invaders whereas others do not (Bradshaw 1965; Rejmánek and Richardson 1996; Bradshaw and Holzapfel 2006; Richards et al. 2006; Smith 2009). In

non-indigenous marine invasive species, understanding the development of specific morphological and physiological traits useful for species survival and fitness is essential for the interpretation of the invasion process itself (Schaffelke et al. 2006; Theoharides and Dukes 2007).

Temperate versus tropical response to increased temperature

Two groups of *Asparagopsis* OTUs are herein recognised on the basis of their geographical distribution and photosynthetic response to the tested temperatures: (a) a temperate group consisting of invasive lineages L2, L3 and AA, characterized in general, by homogeneous photosynthetic behaviour and therefore higher physiological tolerance; (b) a tropical group of native lineages L2 and L4, characterized by photosynthetic optima displaced towards higher temperatures, corresponding to a limited capacity of acclimation to the experimental temperatures other than those similarly found in their habitat (Figs. 1, 2).

Based on multiple lines of evidence, Ní Chualáin et al. (2004) successfully delineated *A. armata* tetrasporophytes collected worldwide from uncharacterized (sensu Andreakis et al. 2007b) *A. taxiformis* tetrasporophytes. The latter set of isolates formed a Caribbean/Atlantic (Canary Islands, Florida, México and Puerto Rico) and a Pacific/Italian (Mediterranean Sea, Australia and Hawaii) group. Despite Caribbean/Atlantic isolates showing survival at higher temperatures, the Pacific/Italian group presented intermediate survival temperatures between *A. armata* and the Caribbean/Atlantic group. The latter was therefore proposed as an *Asparagopsis* thermal ecotype; the former suggested as a different species (Ní Chualáin et al. 2004). It was later assessed that these two groups of isolates correspond to *A. taxiformis* L2 and L3 (Andreakis et al. 2007b). These occur sympatrically with *A. armata* in the Mediterranean Sea. Whereas L3 is confined to few spots of the Almerian (Spain) and Lebanese coasts, the invasive lineage 2 is ubiquitous and replaces *A. armata* in locations where the two OTUs overlap (Altamirano et al. 2008, Zanolla et al. in preparation).

The photosynthetic findings from Australian native L2 and L3 isolates analysed in this study do not match the physiological profiles described for the Pacific/Hawaiian group nor the Caribbean/Atlantic group (Ní

Chualáin et al. 2004), despite their previous genetic placement into *A. taxiformis* L2 and L3 respectively (Andreakis et al. 2007b). In our study, Atlantic L3 showed a temperate profile. Sister lineage L4 on the other hand, could represent a tropical clade due to its optima at higher temperature and the lack of photosynthetic activity at lower temperatures (Figs. 1, 2). Furthermore, our physiological assessment of *A. armata* and Mediterranean isolates of invasive L2 [the only lineage collected from Italy in Ní Chualáin et al. (2004)] fits the one reported by these authors for *A. armata* and the Italian isolates of *A. taxiformis*, showing lower survival temperatures than the tropical isolates of *A. taxiformis*.

Asparagopsis armata showed unique features in our study, such as photosynthetic efficiency (α) displaced towards lower temperatures than any lineage of *A. taxiformis* (Fig. 2c), lowest NPR_{max} at lower temperatures (Table 2) and in general, low I_c values similar to the ones recorded for IL2, which corresponds to the native environment of these species (Table 2). For the latter parameter, it is worth mentioning that despite the similarities in I_c values between *A. armata* and IL2 Falkenbergia isolates, gametophytes of the latter occur in deeper habitats than *A. armata* when these two OTUs are found in sympatry (Altamirano et al. 2008). Differences in I_c values between gametophytes of these OTUs can account for their distinct depth preferences. For *A. Armata* they were set as 20 μmol photons s^{-1} m^{-2} (Flores-Moya et al. 1996) whereas for *A. taxiformis* they were 62 % lower (personal observation). Although from a methodological perspective the bathymetric range of the tetrasporophytes for each OUT is difficult to establish, the assessment of I_c values alone may suggests whether *A. armata* and *A. taxiformis* IL2 tetrasporophytes are able to survive at similar depths, whereas their respective gametophytes do not.

Finally, the ecophysiological profile inferred in this study for L4 agrees with the high survival rates in high temperatures reported for the Hawaiian isolates by Ní Chualáin et al. (2004) and with the short temperature range of photosynthetic performance scored in isolates sampled from the same location (Padilla-Gamiño and Carpenter 2007). However, without molecular work for lineage assessment and taking into account that lineages 1, 2 and 4 are sympatric on the windward coast of Oahu (personal observation), where the algal

material from the aforementioned study was collected, the identity of the isolates cannot be accurately assessed. In addition, the study by Padilla-Gamiño and Carpenter (2007) used the gametophyte stage of *Asparagopsis* and not the tetrasporophytes (as in this study). It is now well understood that species characterized by triphasic life strategies present different temperature tolerance depending on the life stage considered. It has been proposed that studies of temperature tolerance and physiological performance should be performed with the most resistant stage of the life cycle, which is often the microscopic phase in many seaweeds (Breeman 1988; Dieck 1993). Additionally, these statements are supported by the fact that L4 had to be cultured at 28 °C because at 18 °C the pompom-like structures broke apart and turned into yellow–orange colour, not being able to recover from this state. These previous results were then confirmed by the photosynthetic performance of L4 and also account for its present distributional range.

Dark respiration rates were uninformative in discriminating *Asparagopsis* OTUs, although temperate strains showed lower values than the tropical ones (Table 2). All OTUs on the other hand showed low DRs at 12 °C confirming that a rise in temperature can be translated into an increase in DR for most seaweed (Lüning 1990; Necchi 2004). In addition, despite photoinhibition being known to be temperature dependent (Hanelt 1996), photoinhibition rates observed in our study did not show any significant variation among the temperatures tested for any of the OTUs. L4 only showed photoinhibition at 26 °C, reflecting its inability to activate this defence mechanism at sub-optimal temperatures compared to temperatures that resemble its natural thermal range (Osmond 1994). Similarly, no significant differences in photoinhibition were reported between tropical and temperate isolates of *Valonia utricularis* (Roth) C. Agardh (Eggert et al. 2006). However, whereas the temperate isolates recovered from sub- and supra-optimal temperatures for photosynthesis, the warm temperate isolates did not, showing chronic photodamage (Eggert et al. 2006).

Genetic differentiation in A. *taxiformis* meets lineage-specific ecophysiological profiles

Our study shows for first time solid physiological differences among the *Asparagopsis* OTUs examined,

as well as contrasting profiles found between the invasive and native range of *A. taxiformis* lineage 2. Distinct physiological features were expected to correlate well with the four mitochondrial lineages of *A. taxiformis* described previously (Andreakis et al. 2007b). However, striking differences between invasive L2 and native L2 can be observed not only in the physiological response to the temperature but also by their distinct morphologies (Zanolla et al. 2014) despite the absence of genetic differentiation between Australian (native) and Mediterranean (invasive) isolates of L2. Invasive Mediterranean strains are characterized by high population genetic diversity compared to native strains of the same lineage (Andreakis et al. 2009). In addition, differences in ploidy levels for the invasive L2 have been suggested by microsatellite analyses of Mediterranean isolates (Andreakis et al. 2007a), a condition associated with invasiveness in higher plants (Pandit et al. 2011). Similar patterns have been observed in another invasive species, *Undaria pinnatifida* (Harvey) Suringar, whose introduced populations are more variable compared to the native ones (Uwai et al. 2006). Several physiological innovations leading to adaptive advantages have been related to highly variable genetic pools, such as quantitative and qualitative changes in enzymatic activity via the use of isoenzymes or enzymatic modulation in order to minimize the temperature effects and/or the activation of several recovery mechanisms (Hochachka and Somero 2002; Eggert 2012). Invasive Mediterranean L2 is therefore better equipped to cope with thermal stress associated with greater levels of phenotypic plasticity in response to temperature changes and physiological advantages (i.e. very low I_c) that allow the invasive lineage to colonize deeper habitats (Altamirano et al. 2008).

Isolates of NL2 showed the higher photosynthetic rates within *A. taxiformis* sensu lato expressed by fresh weight rather than pigment content (Fig. 1), which was also the highest in this lineage. If the photosynthesis rate is expressed as Chlorophyll *a* (data not shown), the obtained values match the ones found in the other *A. taxiformis* lineages and *A. armata*. Taking into account that all isolates were grown under the same environmental conditions, the differences observed in the pigment content are likely due to the aggregation level of the filaments composing the tetrasporophyte structure, which is more compact in

NL2 and to lineage intrinsic characteristics. The lack of differences in the pigment content of the replicates within an OTU exposed to the experimental temperatures (<2 h, considering the DR acclimation plus the P–E curve) reflects that the synthesis of Chl*a* and carotenoids require more time in longer exposure periods (Davison 1991). We suggest that a longer term experiment should be appropriate to asses this type of physiological responses.

Our results confirm an Indo–Pacific origin for IL2 given the similarities in higher and lower photosynthetic rates at temperatures ranging from 22–26 to 12–15 °C respectively (Ní Chualáin et al. 2004). However, the differences found between NL2 and IL2 (i.e. higher values of I_k, I_c, NPR_{max}, at higher temperatures for NL2) likely suggest a distinct *A. taxiformis* thermal ecotype adapted to the Mediterranean marine environment (Ní Chualáin et al. 2004).

Temperature, combined with photoperiod, are important determinants of seaweed distribution (Breeman 1988). It is expected that seaweeds native to habitats with broad annual temperature variations display a wider photosynthetic response compared to species from habitats with more stable seasonal regimes (Eggert 2012). This has been previously demonstrated for lineages of the green algal genus *Cladophora* Kützing collected from different biogeographical regions (Breeman et al. 2002). We were able to test this effect in *Asparagopsis* using long-term cultured isolates (see "Materials and methods" section), to assure the same physiological background and that the observed photosynthetic differences were due to the genetic differentiation among the OTUs studied. Isolates of IL2, L3 and *A. armata* in the Mediterranean Sea are distributed in areas with wide thermal ranges. The same thermal tolerance is therefore expected for the aforementioned OTUs although phylogenetic constraints may eventually bypass adaptive responses (Breeman 1988). The photosynthetic optimum for *A. armata* was observed at a lower temperature (18 °C) than any lineage of *A. taxiformis* tested, reflecting the temperate distribution of the latter (Bonin and Hawkes 1987). On the other hand, L3 is exposed to the same thermal range, yet no ecophysiological differences were found between this lineage and IL2. Despite their phylogenetic divergence, L2 and L3 do not present major differences, mainly due to the same thermal range they are exposed to and their adaptive potential.

Conclusions

Invasive species and lineages of *Asparagopsis* represent a major threat to the integrity of marine ecosystems in the Mediterranean Sea and worldwide due to explosive range expansions and competition with local biota (Cebrián and Ballesteros 2004; Altamirano et al. 2008). When invasive species characterized by broader physiological tolerance compete against local residents for the same thermal habitat, it is likely that increased temperatures due to climate change will favour invaders (Zerebecki and Sorte 2011). Our results confirm the existence of thermal ecotypes within the *A. taxiformis* species complex, which is notoriously invasive. Under this caveat, *A. taxiformis* sensu lato represents an ideal model system for understanding the evolutionary and ecological pressure of the environmental changes in promoting invasiveness in marine seaweeds. As previously suggested (Ní Chualáin et al. 2004; Boudouresque and Verlaque 2010), in light of solid genetic and ecophysiological differences among *Asparagopsis* OTUs, formal taxonomic recognition of *A. taxiformis* mitochondrial lineages is urged to facilitate local management of the *Asparagopsis* invasive lineages (Andreakis et al. 2007b; Zanolla et al. 2014).

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