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Seasonal acclimatization of *Asparagopsis taxiformis* (Rhodophyta) from different biogeographic regions

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Abstract

We studied the photosynthetic acclimatization of two populations of *Asparagopsis taxiformis* from two biogeographic regions (Santa Catalina Island, California, and Kaneohe Bay, Hawaii) in two seasons. We compared thermal variability between locations and estimated the environmental optima and tolerance limits of photosynthesis to understand the relationship between phenotypic plasticity and thermal variability at each location. Photosynthetic performance was assessed using oxygen evolution and pulsed amplitude modulated fluorometry to quantify responses to thermal variation. *A. taxiformis* from both locations had similar high temperature tolerances for maximum net photosynthesis ($P\text{net}_{max}$) and maximum relative electron transport rate $rETR_{max}$ (~30°C), but different cold temperature tolerances. Respiration rates from both locations (summer and winter) always increased above 25°C. Chlorophyll fluorescence yield from dark-adapted samples from both locations decreased at warmer temperatures in the winter and at colder temperatures during the summer. Populations exposed to greater thermal variability (across both short and long time scales) displayed greater capacity for photosynthetic acclimatization. *A. taxiformis* from California showed seasonal changes in photosynthetic performance within the environmental temperature range (14–21°C), whereas individuals from Hawaii did not. The narrower temperature tolerance (short-term response) of subtropical *Asparagopsis* may make it more susceptible than temperate populations to the predicted increases in global sea surface temperatures. Acclimatization of photosynthesis may play an important role in enabling *A. taxiformis* to respond to a variable environment and to persist in different biogeographic regions.

Marine ecosystems are influenced strongly by water temperature. From the tropics to the poles, marine organisms are exposed to fluctuating seawater temperatures that affect their physiology (Hochachka and Somero 2002), reproduction, dispersal (Pineda 1991), settlement (Pineda 2002), growth (Lonsdale and Levinton 1985), mortality (Hughes 2003), and distribution (Breeman 1988). Temperature changes are known to cause latitudinal shifts in species distribution (Walther et al. 2002), changes in community composition (Barry et al. 1995), and alter species interactions (Sanford 1999).

Temperature variations can influence marine algae at different spatial and temporal scales, including across latitudinal gradients (Pakker et al. 1996), between seasons (Cote 1983), El Niño events (Zimmerman and Robertson 1985), tidal immersion/emersion cycles (Helmuth 2002), upwelling events (Menge et al. 2003), and internal waves (Zimmerman and Kremer 1984).

The success of marine algae is dependent, in part, on adaptive responses to these long- and short-term fluctuations (Gerard and Du Bois 1988). Algae able to acclimatize or respond rapidly (or both) to changes in temperature (eurythermal) may live across a wide range of temperatures, whereas species with less phenotypic plasticity (stenothermal) may be more restricted (Lüning 1990). As climate changes because of global warming, temperature shifts may be one of the most important factors that enable a non-indigenous species to become established in new locations. Understanding the physiological mechanisms of acclimatization can be essential for predicting changes in the biogeographic distributions (along latitudinal gradients) of marine algae in response to climate change.

Algal responses to temperature include phenotypic changes in photosynthesis, respiration, pigment composition (Davison 1991; Morris and Kromkamp 2003), and morphology (Kübler and Dudgeon 1996). Temperature effects on photosynthetic metabolism can be reflected in light use characteristics (Davison et al. 1991; Kübler and Davison 1995), enzyme activity and concentration (Davison 1987; Descolas-Gross and de Billy 1987), damage to photosystem II (Morris and Kromkamp 2003), electron transport (Kübler and Davison 1993; Eggert et al. 2003; Morris and Kromkamp 2003), and the solubility of thylakoid membranes (Lynch and Thompson 1984).

Survival under extreme temperature regimes may depend primarily on phenotypic plasticity, in which case populations from different latitudes exhibit similar temperature

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responses. However, ecotypic differentiation (local adaptation) may also be important for temperature tolerance, especially in species with a wide geographic range. In this case, populations adapted to different temperature regimes exhibit different temperature responses after acclimation to similar conditions (Gerard 1988; Gerard and Du Bois 1988).

The present study examines phenotypic plasticity in temperature responses of the red alga *Asparagopsis taxiformis* (Delile) Trevisan. The hypothesis that phenotypic plasticity in response to temperature is related to environmental temperature variability was tested using populations of *A. taxiformis* from different locations (California and Hawaii). *A. taxiformis* is a cosmopolitan species that is widely distributed in warm temperate and tropical seas throughout the Pacific Ocean, Atlantic Ocean, and Mediterranean Sea (Abbott and Hollenberg 1976; Abbott 1999). The studied populations of *A. taxiformis* experienced temperature fluctuations at different time scales associated with annual ranges and varying degrees of short-term fluctuations throughout the year. Specifically, this study was designed to quantify seasonal acclimatization (individual changes in response to a change in seasonal conditions in the field) using short-term temperature responses. By comparing photosynthetic characteristics between *A. taxiformis* exposed to different thermal regimes, we addressed the following questions: (1) Is there a difference in the short-term response of photosynthetic performance between *A. taxiformis* from different locations? (2) Does this response differ between seasons? (3) Is there a relationship between phenotypic plasticity and environmental thermal variability?

Photosynthetic performance was assessed using two different techniques: oxygen evolution and pulsed amplitude modulated (PAM) fluorometry to obtain a complete picture of the photosynthetic response and to compare the phenotypic changes in different characteristics of the photosynthetic process. Oxygen evolution is an integrated measure of the light-dependent reactions of photosynthesis. PAM fluorometry can be used to indicate the efficiency of photosystem II (PSII) photochemistry, the relative electron transport rate, and the maximum quantum efficiency of PSII (dark-adapted yield), which is a sensitive indicator of photosynthetic performance (Bruhn and Gerard, 1996).

Methods

Study sites—Experiments were performed at Santa Catalina Island (California), located in the warm temperate northeast Pacific region (33°27′N, 118°30′W) with an annual seawater temperature range of 15–20°C (Zimmerman and Kremer 1984), and Kaneohe Bay (Oahu, Hawaii,) located in the tropical Central Pacific region (19°43′N, 155°4′W) with an annual seawater temperature range of 24–27°C (Bathen 1968). These locations were chosen to represent sites with different thermal variability where *A. taxiformis* can be found throughout the year.

Santa Catalina Island represents the northern distribution limit of this species in the Pacific Ocean (Miller pers. comm.). Specimens from this location were collected from Big Fisherman's Cove and Bird Rock, a small bedrock island located north of Big Fisherman's Cove. Specimens from Kaneohe Bay were collected at the bay entrance near Mokoli'i Island and on the Kaneohe Bay barrier reef flat.

Gametophytes of *A. taxiformis* were collected manually from a depth of 2–3 m in both locations during the summer 2003 and winter 2004. The gametophyte phase (haploid, macroscopic thalli) was used for this study because it was more common and more easily identified than the tetrasporophyte phase (diploid, microscopic stage) in the field at both locations.

To characterize the temperature fluctuations more accurately at the collection sites, temperature was measured at 5-min intervals (in California) and 10-min intervals (in Hawaii) using StowAway Tidbits (Onset Computer), accurate to ±0.2°C. Temperature data were collected in California (at Bird Rock) from June 2002 to September 2004, and in Hawaii (at Kanoehe Bay barrier reef flat) for 7 months from January to July 2004.

Photosynthetic performance—Photosynthetic performance of *A. taxiformis* was estimated during the summer (2003) and winter (2004) when field temperatures were close to the minimum and maximum values within the seasonal range (August and September during summer and January and February during winter) (Bathen 1968; Zimmerman and Kremer 1984). Photosynthesis was estimated at six different temperatures (10°C, 15°C, 20°C, 25°C, 30°C, and 35°C) to investigate the response of photosynthesis to temperature. These temperatures spanned and exceeded the range of normal environmental temperatures that the algae experience. Thalli were cut in 1-cm long sections at least 12 h before measuring photosynthesis and returned to sea tables to allow wound-healing. Preliminary experiments showed that this size was appropriate to estimate photosynthetic performance with respect to the chamber size, the flow inside the chamber, and oxygen evolution rates. The timing of wound-healing was assessed by measuring respiration rates after excision in preliminary experiments. Respiration was highly variable immediately after wounding, but relatively consistent after 4 h.

Independent thallus sections (n = 5–10 per temperature treatment) were exposed to the experimental temperature in a darkened, temperature-controlled water bath for 1h. Preliminary experiments indicated that 30–45 min was enough time to achieve dark acclimation. After exposure, algae were transferred to a light-shielded Clark-type oxygen electrode system (Rank Brothers) at the experimental temperature, and changes in dissolved oxygen (for 5 min) were used to estimate dark respiration. Then, measurements of dark-adapted yield (Fv/Fm) were obtained using a PAM fluorometer (model 210, Walz), followed by estimates of photosynthesis versus irradiance (P-E_d) at seven E_d levels (10, 20, 50, 100, 200, 500, and 1,000 μmol photons s^{-1} m^{-2}). E_d was measured as the downward irradiance over the spectral range of photosynthetically available radiation (PAR). An actinic lamp was used as a light source for the P-E_d curves, and a fiber-optic quantum sensor of an underwater fluorometer (diving PAM, Walz) was used to measure the E_d inside the

incubation chamber after being calibrated with a LiCor quantum sensor. Oxygen concentration with time was measured for 5 min using an analog to digital converter card (Cyber Research) at a sampling rate of 0.2 Hz. Once the P-E_d curve was recorded, the algal sample was transferred to a dark container (at the experimental temperature) for 5 min, then transferred to the incubation chamber (Clark-type oxygen electrode system) to obtain the electron transport rate (ETR) at 11 different E_d levels (1-min duration at each E_d) using the PAM fluorometer. The seawater was changed inside the incubation chamber between these two steps. Because the thalli are highly branched with branches <0.5 mm in diameter, determination of light absorption by the thalli was impractical, so ETR was expressed as the relative electron transport rate ($rETR$ = Yield × PAR) when yield (Φ_{PSII}) > 0.1 (Beer and Axelsson 2004). All photosynthetic measurements (oxygen electrode and PAM fluorescence) were obtained under the same conditions using filtered seawater (filter size: 1–5 micron, General Electric), with flow inside the chamber provided by a magnetic stirrer. Control incubations were performed between samples to correct for bacterial respiration and electrode oxygen consumption. Thalli were dried in an oven at 80°C, and respiration and maximum net photosynthesis ($P\text{net}_{max}$) rates were expressed as mg O_2 g dry mass^{-1} h^{-1} (dry mass was used because it was less variable than wet mass).

Photosynthetic pigment composition—Photosynthetic pigment concentrations were estimated from freshly collected algal samples from the two locations in both seasons (n = 10–30 for each site and season). Two samples from each thallus (1–2-cm long) were used to extract chlorophyll *a* (Chl *a*) and accessory pigments. Chlorophyll samples were ground using a mortar and pestle with 90% acetone and a magnesium carbonate solution. Samples for phycobiliproteins were ground with a cold phosphate buffer (Beer and Eschel 1985). Photosynthetic pigments were extracted in the dark for 24 h at 4°C and then estimated spectrophotometrically according to the equations of Jeffrey and Humphrey (1975) for chlorophyll and Beer and Eschel (1985) for phycobiliproteins.

Data analysis—Temperature time series analysis was performed using Matlab (version 6.5). Monthly means, standard deviations, and ranges (minimum–maximum) were calculated for each site. Thermal variability was quantified by calculating differences between mean and maximum values (every 12 h) and monthly coefficients of variation (standard deviation expressed as a percentage of the mean) from both sites.

$P\text{net}_{max}$ and photosynthetic efficiency at low light intensities (α) were estimated from the P-E_d curves. $P\text{net}_{max}$ (mg O_2 g dry mass^{-1} h^{-1}) was the value obtained at saturating E_d ($\geq$500 μmol photons s^{-1}m^{-2}). Photosynthetic efficiency [mgO_2 g dry mass^{-1} h^{-1}/(μmol photons s^{-1} m^{-2})], defined as the initial slope of the P-E_d curve, was estimated by fitting a linear regression to the initial values of the P-E_d curve. Estimates of maximum relative electron transport rate ($rETR_{max}$) were obtained by fitting a hyperbolic, tangent, nonlinear regression (Jassby and Platt 1976).

A two-way analysis of variance was used to evaluate the effects of season (fixed factor) and temperature (fixed factor) on $P\text{net}_{max}$, respiration, a, dark-adapted yield, and $rETR_{max}$. Pigment data were log-transformed, and a t-test was used to analyze differences in Chl *a* and accessory pigment content between seasons at each site.

Results

Thermal variability—Santa Catalina Island showed a broader temperature range (~14–21°C) than that for the Kaneohe Bay barrier reef (~24–28°C) (Fig. 1). Thermal variability at Santa Catalina Island occurred on several temporal scales. On a longer time scale (monthly and seasonal fluctuations), maximum temperatures (20–21°C) occurred in the months of July and August, and minimum temperatures (14–15°C) occurred between December and March (Table 1). On a shorter time scale, temperature was most variable during the months of September and November, when maximum coefficients of variation (C.V.) were found (Fig. 1a,b; Table 1). During September, the daily mean water temperature dropped approximately 3°C in 3 d, and short-term decreases of 3°C every 4 h also were observed (Fig. 1b). Minimum and maximum temperatures on the Kaneohe Bay barrier reef flat were observed during January and July, respectively (Fig. 1c,d). At this site, daily temperatures did not fluctuate more than one degree, and no shorter-term variability (hours) in temperature was recorded. C.V. of the monthly means were higher for Santa Catalina Island than Kaneohe Bay (t = 2.61, df = 6, p = 0.04, Table 1). For Hawaii, temperature data were compared to data collected from a National Oceanic and Atmospheric Administration buoy located near the collection site. This comparison showed that values obtained at the collection sites (maximum, minimum, and C.V.) were representative of temperatures measured throughout the year.

Differences between mean and maximum values (every 12 h) showed that California had larger differences between mean and minimum values (t-test, $p < 0.001$); the opposite was true for Hawaii data, where the larger differences were found between the mean and minimum and maximum values of temperature (every 12 h) (t-test, $p < 0.001$).

Oxygen evolution—Both populations of *A. taxiformis* showed a characteristic photosynthesis-temperature response. Values of $P\text{net}_{max}$ increased gradually at lower temperatures, remained constant between 15–25°C (California) or 25–30°C (Hawaii) and then decreased rapidly at 35°C (Fig. 2 a,b). In general, populations from California had a broader temperature range within which maximum values of $P\text{net}_{max}$ were found (15–25°C) than populations from Hawaii (25–30°C). The relationship between $P\text{net}_{max}$ and temperature varied between seasons for individuals from California (Temperature × Season, $F_{5,91}$ = 6.25, $p <$ 0.01; Table 2) but did not vary for individuals from Hawaii (Temperature × Season, $F_{5,102}$ = 2.12, $p > 0.05$; Table 2).

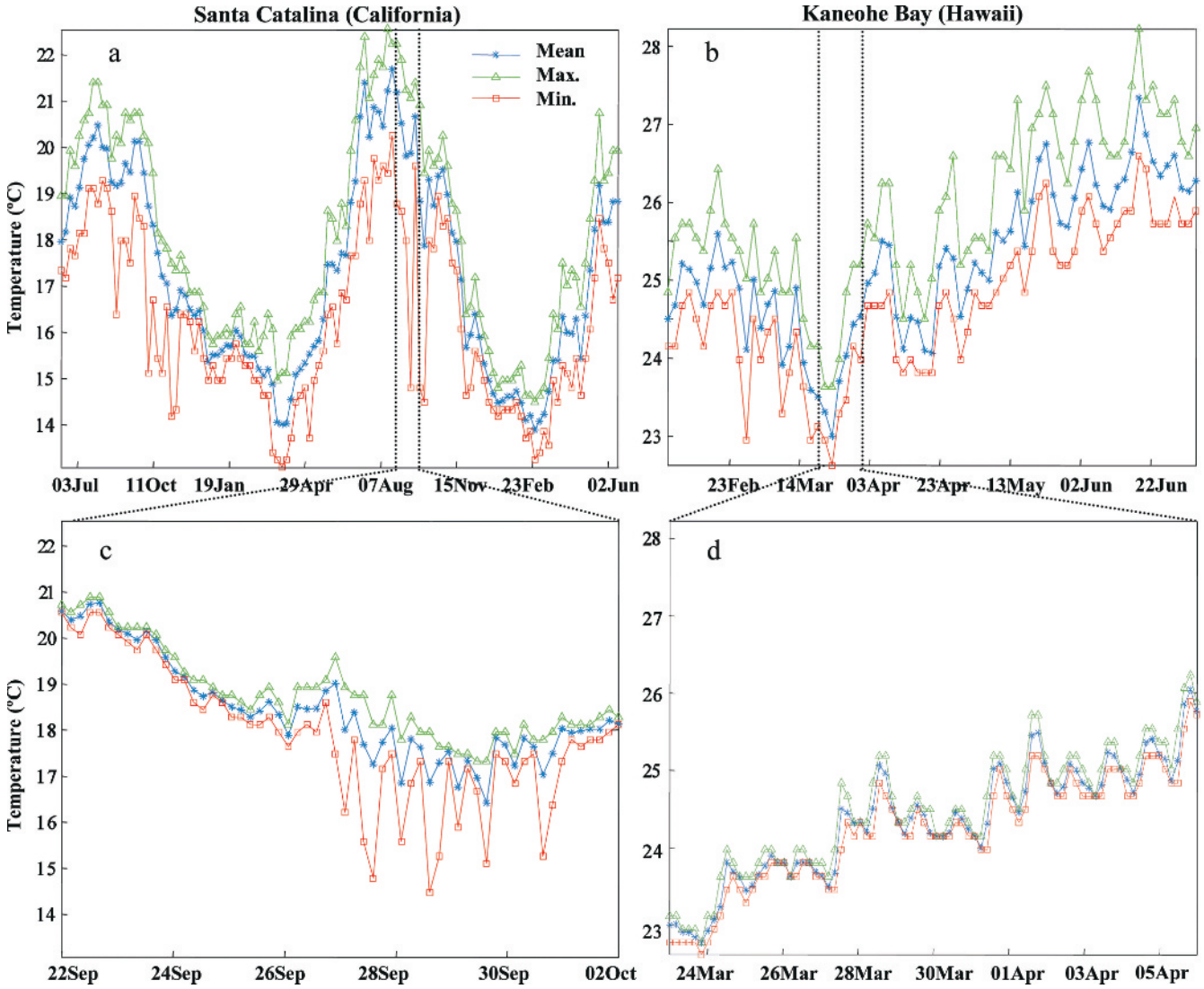

Fig. 1. (a) Time series of the temperature data from California (Bird Rock, Santa Catalina Is.) at 2–3 m depth, values every 6 d from 11 Jun 2002 to 14 Jun 2004. (b) Data from California during a period of high thermal variation (high C.V.); values every 4 h from 20 Sep to 02 Oct 2003, temperature was quantified at 5-min intervals. (c) Time series of the temperature data from Hawaii (Kaneohe Bay barrier reef) at 2-m depth, values every 2 d from 05 Feb to 05 July 2004. (d) Data from Hawaii during a period of high thermal variation (high C.V.); values every 4 h from 26 Mar to 06 Apr 2004, temperature was quantified at 10-min intervals.

Within the normal environmental temperature range, $P\text{net}_{max}$ values of *A. taxiformis* from California changed between seasons; higher values were found in winter than in summer. There was no change in values of $P\text{net}_{max}$ within the temperature range that *A. taxiformis* from Hawaii generally experienced (24–28°C).

The relationship between photosynthetic efficiency (α) and temperature (Fig. 2c,d) did not vary between seasons (Temperature × Season, $F_{5,92} = 0.5$, $p = 0.77$ and Temperature × Season, $F_{5,102} = 1.49$, $p = 0.20$ for California and Hawaii, respectively; Table 2). The independent effects of temperature and season on α were not significant for *A. taxiformis* from Santa Catalina Island, whereas the photosynthetic efficiency of *A. taxiformis* from Hawaii was affected significantly by season ($F_{1,102} = 3.66$, $p = 0.05$; Table 2) and temperature ($F_{5,102} = 8.67$, df = 5, $p < 0.001$; Table 2); α values increased as temperature increased (Fig. 2d).

The relationship between respiration and temperature did not differ significantly between seasons (Fig. 3) for *A. taxiformis* from either location. However, there was a significant effect of temperature on respiration rates ($F_{5,89} = 32.23$, $p < 0.001$, California; $F_{5,105} = 12.75$, $p < 0.001$, Hawaii; Table 2). The greatest changes in respiration were found at extreme temperatures, especially at the higher temperatures (30–35°C) where respiration rates dramatically increased by ~200%.

Chlorophyll fluorescence—Patterns of dark-adapted yield (*Fv*/*Fm*) and relative electron transport rate ($rETR_{max}$)

Table 1. Monthly temperature data from Bird Rock, Santa Catalina Island (California) and Kaneohe Bay barrier reef (Hawaii).

California					
Month	Mean	SD	Max	Min	C.V.
Sep 2003	19.6	1.2	21.4	14.5	6.1
Oct	19.1	0.5	20.2	17.6	2.6
Nov	17.2	1.1	19.3	14.6	6.6
Dec	15.5	0.7	17.2	14.3	4.2
Jan 2004	14.4	0.3	15.1	13.2	2.3
Feb	14.3	0.2	15.3	13.7	1.6
Mar	15.1	0.7	17.5	13.5	5.2
Apr	15.9	0.5	17.5	14.6	3.1
May	18.3	0.7	20.7	16.1	4.0
Jun	18.9	0.5	20.4	16.7	2.7
Jul	20.4	0.9	22.4	18.1	4.7
Aug	20.6	0.5	21.5	18.9	2.6
Hawaii					
Month	Mean	SD	Max	Min	C.V.
Jan 2004	24.3	0.3	25.2	23.3	1.4
Feb	24.9	0.5	26.4	23.0	2.0
Mar	24.1	0.6	25.7	22.6	2.7
Apr	24.8	0.6	26.6	23.8	2.4
May	25.8	0.6	27.5	24.7	2.4
Jun	26.4	0.5	28.2	25.4	1.9
Jul	26.6	0.5	28.0	25.7	2.0

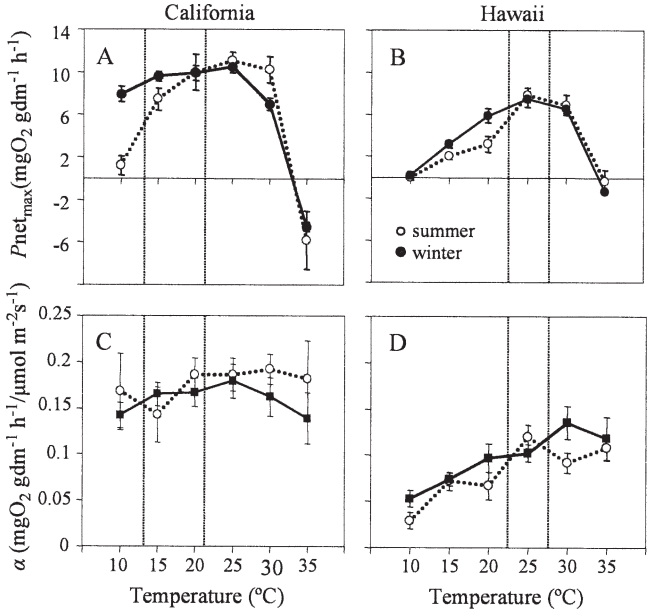

Fig. 2. Short-term temperature response of maximum net photosynthesis $P\text{net}_{max}$ (mg O_2 g dry mass^{-1} h^{-1}) and photosynthetic efficiency, α (mg O_2 m^{2}s μmol^{-1} g dry mass^{-1} h^{-1}) of *A. taxiformis* from Santa Catalina Island (California) and Kaneohe Bay (Hawaii) during summer and winter. Error bars indicate ±1 SE from the mean (n = 5–10 at each temperature). The areas between the vertical lines represent annual temperature ranges at the collection sites.

across temperatures were similar to that of $P\text{net}_{max}$ (Fig. 4). There was evidence of acclimatization for both populations (Temperature × Season, $F_{5,92} = 20.05$, $p < 0.001$ and $F_{5,110} = 5.68$, $p < 0.001$ for Hawaii and California, respectively; Table 3), however changes in *Fv*/*Fm* between seasons were not observed within the range of temperatures measured at both locations. Values of *Fv*/*Fm* from both locations decreased at warmer temperatures in the winter and at colder temperatures during the summer (Fig. 4a, b).

The relationship between maximum $rETR_{max}$ and temperature for *A. taxiformis* in Santa Catalina Island (Fig. 4c) varied significantly between seasons (Temperature × Season, $F_{5,81} = 10.51$, $p < 0.001$; Table 3). Values of $rETR_{max}$ from California decreased drastically above 25°C during the winter and above 30°C during the summer. The $rETR_{max}$–temperature curves from Hawaii individuals strongly resembled the pattern of the $P\text{net}_{max}$–temperature curves (Figs. 2b, 4d). Values of $rETR_{max}$ gradually increased between 10°C and 20°C, remained constant between 25°C and 30°C, and then decreased rapidly at 35°C. The relationship between $rETR_{max}$ and temperature in individuals from Hawaii (Fig. 4d) did not vary between seasons (Table 3).

Photosynthetic pigment composition—Concentrations of Chl *a* and phycobiliproteins of *A. taxiformis* from California did not differ significantly between summer and winter ($p > 0.05$; Table 4). Photosynthetic pigment concentrations of populations from Hawaii significantly differed between seasons ($p < 0.01$; Table 4); concentrations of Chl *a* were higher in summer than in winter. Phycobiliproteins showed an inverse pattern, with concentrations higher during winter.

Discussion

Thermal variability—Thermal variability was greater at Santa Catalina Island than for the Kaneohe Bay barrier reef for both long (seasons, months) and short (hours) time scales. The greatest variability between average and minimum daily temperatures was found at the end of the summer; in this period the daily mean water temperature dropped almost 3°C in 3 d and short-term decreases of 3°C every 4 h were recorded. These large decreases in temperature may be associated with internal waves that have been observed often during the summer along the coast of Southern California (Pineda 1991). The breaking of internal waves can generate internal tidal bores that transport cool, subsurface water into shallower habitats (Pineda 1991). The energy of internal tidal motion is dependent on water column stratification and wave amplitude (Pineda and Lopez 2002). During the period of maximum variability in this study (late summer, beginning of fall), temperature decreases occurred every 12 hours, suggesting that these fluctuations may be tidally related (semidiurnal tidal cycles are characteristic of Santa Catalina Island). Similar results were found by Zimmerman and Kremer (1984) during 1983 at Santa Catalina Island, in which periodic movements of cold water were linked to the

Table 2. Results of a two-way ANOVA of maximum net photosynthesis ($P\text{net}_{max}$; mg O_2 g dry mass^{-1} h^{-1}), photosynthetic efficiency [α; mg O_2 g dry mass^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$] and respiration (Resp.; mg O_2 g dry mass^{-1} h^{-1}) of *A. taxiformis* from Santa Catalina Island (California) and Kaneohe Bay (Hawaii) during summer and winter.

	California					Hawaii				
	Source	df	MS	*F* ratio	*p*	Source	df	MS	*F* ratio	*p*
$P\text{net}_{max}$	Temperature	5	485.523	58.872	<0.01	Temperature	5	210.972	50.068	<0.01
	Season	1	26.039	3.21	0.076	Season	1	4.033	0.957	0.33
	Temp × season	5	50.702	6.25	<0.01	Temp × season	5	8.931	2.119	0.06
	Error	91	8.109			Error	102	4.214		
α	Temperature	5	0.002	0.371	0.86	Temperature	5	0.014	8.675	<0.01
	Season	1	0.005	0.856	0.35	Season	1	0.006	3.664	0.05
	Temp × season	5	0.003	0.503	0.77	Temp × season	5	0.002	1.491	0.19
	Error	92	0.006			Error	102	0.002		
Resp.	Temperature	5	47.279	32.229	<0.01	Temperature	5	10.473	12.751	<0.01
	Season	1	1.045	0.712	0.4	Season	1	2.702	3.29	0.07
	Temp × season	5	3.236	2.206	0.06	Temp × season	5	0.734	0.893	0.48
	Error	89	1.467			Error	105	0.821		

semidiurnal internal tides during all seasons. In their study, larger amplitudes of these periodic motions were found during summer (when thermal stratification was greatest) than during winter.

During late summer and early fall, internal bores result in cold water pulses that can provide nutrients necessary to the kelp forest community when surface waters are nutrient-depleted (Zimmerman and Kremer 1984). At this site, surface waters with temperatures higher than 15°C essentially are nutrient-depleted, with nitrate concentrations <0.5 μmol L^{-1} (Zimmerman and Kremer 1984). Nutrient availability may influence synthesis and turnover of enzymes and therefore influence photosynthesis and marine algae growth (Lobban and Harris 1994). In contrast, Hawaii had a narrower temperature range than Santa Catalina Island, and no strong short-term fluctuations (time scale of ≤6 h) were observed. Because water typically flows from the ocean across the reef flat (Bathen 1968), water across the Kaneohe Bay barrier reef generally is nutrient-depleted and exhibits similar characteristics to oceanic conditions; in this location tides have a limited range (±30 cm) (Hearn 1999).

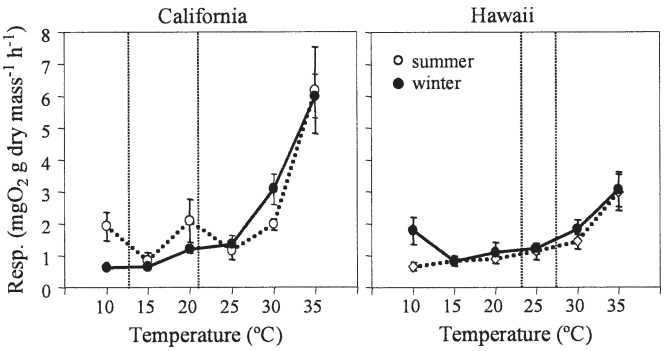

Fig. 3. Short-term temperature response of respiration (mg O_2 g dry mass^{-1} h^{-1}) of *A. taxiformis* from Santa Catalina Island (California) and Kaneohe Bay (Hawaii) during summer and winter. Error bars indicate ±1 SE from the mean (n = 5–10 at each temperature). Areas between the vertical lines represent annual temperature ranges at the collection sites.

Short-term temperature response—The short-term response of photosynthetic parameters ($P\text{net}_{max}$, respiration, α, *Fv/Fm,* and $rETR_{max}$) enabled us to quantify how individuals respond to sudden changes in temperature and elucidate some of the mechanisms involved in the phenotypic plasticity of the two populations across a short-time scale.

Although maximum values of $P\text{net}_{max}$ at each location did not vary between seasons, temperature ranges in which

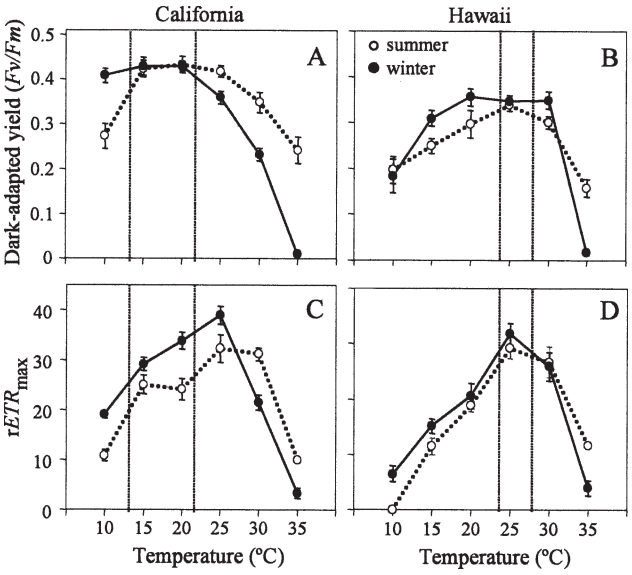

Fig. 4. Short-term temperature response of dark-adapted yield (*Fv/Fm*) and relative electron transport rate ($rETR_{max}$) of *A. taxiformis* from Santa Catalina Island (California) and Kaneohe Bay (Hawaii) during summer and winter. Error bars indicate ±1 SE from the mean (n = 5–10 at each temperature). Areas between the vertical lines represent annual temperature ranges at the collection sites.

Table 3. Two-way ANOVA of dark-adapted yield (*Fv*/*Fm*) and relative electron transport rate ($rETR_{max}$) of *A. taxiformis* from Santa Catalina Island (California) and Kaneohe Bay (Hawaii) during summer and winter.

	California					Hawaii				
	Source	df	MS	*F* ratio	*p*	Source	df	MS	*F* ratio	*p*
Fv/*Fm*	Temperature	5	0.168	55.14	<0.001	Temperature	5	0.158	39.682	<0.001
	Season	1	0.048	15.82	<0.001	Season	1	0	0.084	0.773
	Temp × Season	5	0.061	20.051	<0.001	Temp × Season	5	0.023	5.683	<0.001
	Error	92	0.003			Error	110	0.004		
$rETR_{max}$	Temperature	5	921.311	42.468	<0.001	Temperature	3	1,043.19	24.689	<0.001
	Season	1	62.043	2.86	0.095	Season	1	58.665	1.388	0.243
	Temp × Season	5	228.028	10.511	<0.001	Temp × Season	3	15.742	0.373	0.773
	Error	81	21.694			Error	69	42.254		

maximum values of $P\text{net}_{max}$ were measured varied between seasons for *A. taxiformis* from California. High-temperature inhibition occurred in both populations above 30°C; this response has been shown also for other taxa (Iglesias-Prieto et al. 1992; Kübler and Davison 1993; Morris and Kromkamp 2003). The decline in $P\text{net}_{max}$ of *A. taxiformis* (from both locations) with high temperatures (above 30°C) was due to higher respiration rates. Low-temperature inhibition of $P\text{net}_{max}$ was more evident during the summer at the two sites between 10°C and 15°C in California and 15°C and 20°C in Hawaii. Different mechanisms, such as a high concentration of photosynthetic enzymes or the production or activation of new enzymes with modified properties (Descolas-Gros and de Billy 1987), may be required to compensate for the adverse effects of low temperature on chemical reactions (Lüning 1990).

The light harvesting efficiency (α) of individuals from California was independent of temperature in both seasons (Fig. 2c). A similar result was found by Morris (2003) with the marine benthic diatom *Cylindrotheca closterium*. However, α values from Hawaii populations increased with temperature during both seasons (Fig. 2d). Other studies (Davison 1987; Gerard and Dubois 1988; Kübler and Davison 1995) using *Laminaria saccharina* and *Chondrus crispus* also have shown that the effect of temperature on α depends on the growth temperature (5°C, 15°C, and 20°C). In these studies, α decreased as temperature increased for high-temperature acclimated algae. Davison (1991) and Gerard (1988) found that the increase in α values in high-temperature-grown algae were directly proportional to chlorophyll content (PSU [photosynthetic unit] number or size). Our results do not show this pattern; *Asparagopsis* from California did not show a significant difference in pigment content between seasons, and *A. taxiformis* from Hawaii had higher concentrations of accessory pigments during the winter and less chlorophyll during the summer (Table 4). This suggests a covariation of temperature and other factors, such as photoacclimation to different irradiance, day length, and nutrient availability, also may have been important in determining pigment composition in both locations.

The state of photosystem II (*Fv*/*Fm*) did not differ between seasons within the environmental temperature range for either population, however, outside of this temperature range the *Fv*/*Fm* values of individuals from California were very sensitive to thermal stress. In contrast to $P\text{net}_{max}$, *Fv*/*Fm* values for individuals from California rapidly decreased above 20°C during the winter, suggesting damage to the photosynthetic apparatus in response to higher temperatures (Bruhn and Gerard 1996). In the summer, however, *Fv*/*Fm* decreased above 25°C and below 15°C, suggesting acclimatization to warmer temperatures and a loss of cold tolerance. *Fv*/*Fm* values of the Hawaii individuals decreased above 30°C and were not affected greatly by lower temperatures (especially during the winter).

In California, higher relative electron transport rates were found during the winter (between 15°C and 25°C), but $rETR_{max}$ decreased above 25°C, whereas $rETR_{max}$ in summer did not decrease until 30°C. During the winter, $rETR_{max}$ decreased above 25°C in both locations, suggesting acclimatization to colder temperatures and a loss of heat tolerance. Surprisingly, $rETR_{max}$ during the winter for California algae did not reach maximum values at low

Table 4. *t*-test results from California and Hawaii during summer and winter. Values are means ± SE. Chl *a*: chlorophyll *a* (μg g^{-1} dry mass); PC: phycocyanin (mg g^{-1} dry mass); APC: allophycocyanin (mg g^{-1} dry mass); PE: phycoerythrin (mg g^{-1} dry mass). Different superscripts represent significantly different pigment concentrations between seasons ($p < 0.05$).

	California		Hawaii	
Pigments	Summer (n=10)	Winter (n=16)	Summer (n=30)	Winter (n=20)
Chl *a*	516.935±84.024^{ns}	741.229±90.246^{ns}	342.337±35.064^{a}	271.186±66.638^{b}
PC	5.992±0.475^{ns}	5.595±0.495^{ns}	3.114±0.2^{a}	4.195±0.276^{b}
APC	4.187±0.465^{ns}	3.397±0.322^{ns}	2.174±0.152^{a}	2.951±0.21^{b}
PE	7.906±0.615^{ns}	8.704±0.487^{ns}	3.096±0.205^{a}	4.164±0.22^{b}

temperatures, which disagrees with the higher values of dark-adapted yield and maximum net photosynthesis. This suggests that the process of acclimatization of photosynthesis likely is occurring not only within the light reactions, but also in the enzyme-controlled dark reactions.

These results show that there are different short-term responses to temperature at different levels of the photosynthetic process and suggest that different parameters may be more important at different time scales during the acclimatization process between the two locations.

Phenotypic plasticity and thermal variability—Physiological parameters ($P\text{net}_{\max}$, respiration, α, *Fv*/*Fm,* and $rETR_{\max}$) estimated using two different techniques (oxygen evolution and PAM fluorescence) had different seasonal acclimatization patterns. Seasonal acclimatization of *A. taxiformis* within the environmental temperature ranges (14–21°C for California and 24–28°C for Hawaii) had similar trends for $P\text{net}_{\max}$ and $rETR_{\max}$; however, individuals from Santa Catalina Island showed different $P\text{net}_{\max}$ and $rETR_{\max}$ rates between seasons, whereas Hawaii individuals did not. This demonstrates that there is likely a threshold of environmental variability necessary to initiate an acclimation response. The weaker acclimatization between seasons for Hawaii individuals may reflect a more general purpose genotype (Lynch 1984) for the local conditions characterized by a narrower temperature range and a lack of environmental stimuli. Data from near-tolerance limits suggests that acclimatization occurs in Hawaii populations, but environmental characteristics (i.e., nutrient limitation) may constrain their response within the ambient temperature range.

Light harvesting efficiency (α) and dark-adapted yield (*Fv*/*Fm*) of both populations did not differ between seasons within the range of temperatures in the natural environment. This suggests that seasonal acclimatization enables these algae to maximize photosynthesis under different conditions or that environmental stimuli were insufficient to cause an acclimatization response in these two parameters.

In this study, *A. taxiformis* from both locations had similar high-temperature tolerances for $P\text{net}_{\max}$ and $rETR_{\max}$ (~30°C) and extremely different cold-temperature tolerances between locations, suggesting that the ability to photosynthetically acclimatize to cold temperatures in California occurs because (1) this population represents an ecotype of *A. taxiformis* or (2) populations from Hawaii can acclimatize as well as California populations if exposed to the same environmental conditions. However, from the results of our experiments we cannot distinguish between these two alternatives.

Work by Ní Chualáin et al. (2004) on tetrasporophyte strains of *A. taxiformis* (*Falkenbergia*) collected worldwide demonstrated that this species can be subdivided in two clades: a Pacific/Italian clade and a Caribbean/Canary Islands clade. Furthermore, this work suggests the presence of temperature ecotypes within the Pacific clade due to slightly different temperature responses for growth, survival, and tetrasporogenesis. Because the occurrence of phenotypic plasticity can correlate with the occurrence of ecotypes (Gerard 1990) and ecotypic differentiation may be consistent between sporophytes and gametophytes (Gerard 1990), further studies of gametophytes of *A. taxiformis* also should investigate the phylogeny, genetic regulation, and allelic sensitivity of this species to study the evolution of thermal traits and to detect varying degrees of adaptation to local temperature regimes.

Tolerance to high or low temperatures is a derived character that can be lost or retained because of local temperature conditions (Pakker 1994). For example, the acquisition of cold adaptation and the potential for temperature acclimation has enabled the expansion of *Valonia utricularis* (a species with a tropical origin) into warm-temperate regions (Eggert et al. 2003). Breeman et al. (2002) suggest that in the *Cladophora vagabunda* complex (ancestral lineage of *Cladophora albida/sericea* complex), major differentiation was found in cold tolerance but not in heat tolerance. In contrast, the derived *C. albida/sericea* complex differed mostly in heat tolerances, suggesting that during adaptation to colder climates acquisition of cold tolerance preceded the loss of heat tolerance.

For species in the Bonnemaisoniales, different life stages possess noticeably different temperature tolerance limits for growth, survival, and reproduction, and for *A. taxiformis,* the tetrasporophyte probably is the most resilient phase (Breeman 1988). In this study we found gametophytes of *A. taxiformis* with and without reproductive structures at the two sites during the two seasons (Padilla-Gamiño and Carpenter pers. obs.), which suggests that the presence of this species throughout the year is not restricted by reproduction limits in either location. However, in California many recruits (gametophyte phase) were seen during fall and *A. taxiformis* was more abundant in summer, which suggests a seasonality in the abundance of the tetrasphorophyte phase.

We have shown that the short-term photosynthetic physiology of populations from two locations respond differently to environmental conditions typical of summer and winter. However, short-term responses of photosynthesis do not necessarily infer long-term growth and survival. Other metabolic processes such as light absorption and nutrient uptake and assimilation also influence changes in growth (Davison 1991). To relate growth and recovery from thermal stress for *A. taxiformis*, long-term field experiments need to be conducted, especially during periods when internal wave events are most frequent. Nevertheless, short-term responses characterize phenotypic changes in photosynthesis and other key aspects of metabolism that may contribute to longer-term ecological performance.

Temperature changes in the field do not occur alone, and fluctuations in other parameters also occur simultaneously (Cote 1983). In fact, interaction of these factors (nutrient concentration, light, water flow), especially on a shorter time scale (upwelling, internal waves), also may influence the physiology of thermal tolerance. Using an experimental design based on in situ environmental conditions, we were able to quantify the short-term response of two populations of *A. taxiformis* grown in their natural environments.

A. taxiformis from Hawaii had narrower thermal tolerance limits and a lower degree of acclimatization potential than *A. taxiformis* from California, which could make it more susceptible than temperate populations to the predicted increases in global sea surface temperatures. In addition, our results suggest that short- and long-term physiological responses of *A. taxiformis* may be influenced by temperature variability at each location. Although both populations made physiological adjustments that affected their tolerance limits. Individuals exposed to higher temperature variability (short and long time scale) demonstrated greater photosynthetic acclimatization. This demonstrates that there is likely a threshold of environmental variability necessary to initiate an acclimatization response. Thus, depending on the thermal variation associated with the local conditions, acclimatization of photosynthetic performance may be a factor in allowing *A. taxiformis* to respond to a variable environment and successfully persist in different habitats.

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