# Production, respiration, and photophysiology of the mangrove jellyfish *Cassiopea xamachana* symbiotic with zooxanthellae: effect of jellyfish size and season

E. Alan Verde[1,*], L. R. McCloskey[2]

[1]Oregon State University, Department of Zoology, 3029 Cordley Hall, Corvallis, Oregon 97331, USA
[2]La Sierra University, Department of Biology, 4700 Pierce Street, Riverside, California 92515, USA

ABSTRACT: The association between the symbiont *Symbiodinium microadriaticum* (zooxanthellae) and its host jellyfish, *Cassiopea xamachana*, was investigated as a function of jellyfish size and season. Symbiont cell diameter and volume were higher during January than September. Although zooxanthella-specific chlorophyll was independent of jellyfish size, both chlorophyll *a* and *c* were higher during January. Regardless of season, algal density and jellyfish size were inversely related. The diel mitotic index (MI) of zooxanthellae was phased, with a peak of 0.25% occurring between 09:00 and 12:00 h. September photosynthetic rates were always higher than January rates and reflected the seasonal light and temperature regimes at the latitude of the Florida Keys (USA). Photosynthesis, when normalized to either zooxanthella density or protein, displayed an inverse relationship with jellyfish size. Medusan respiration rates also showed an inverse relationship with jellyfish size, with September metabolism being higher than that of January. The carbon budgets calculated for these medusae indicate that the carbon photosynthetically fixed by the zooxanthellae, and subsequently translocated to the host, is capable of satisfying about 169% of the host's metabolic demand (CZAR) and is independent of both jellyfish size and season. These seasonally influenced physiological effects underscore the necessity for seasonal examinations of algal-cnidarian symbioses in order to understand the photophysiology of the association on an annual basis.

KEY WORDS: *Cassiopea xamachana* · Zooxanthellae · Jellyfish · Photosynthesis · Respiration · Photophysiology · Carbon budgets · CZAR

## INTRODUCTION

The relationship between marine unialgal symbionts (zooxanthellae) and cnidarian hosts has been more widely studied for benthic cnidarians (reviews by Muscatine 1990, Battey 1992, Davies 1992, Trench 1993) than for motile ones. Early studies on the scyphozoan *Cassiopea* sp. showed that endosymbiotic zooxanthellae (*Symbiodinium*) are capable of $CO_2$ uptake and fixation (Balderston & Claus 1969, Drew 1972) and subsequent carbon translocation to host tissues (Balderston & Claus 1969). Cates (1975) suggested that these photosynthesis-respiration interactions enhance the ecological efficiency of *Cassiopea* sp. due to the presence

of zooxanthellae. Differences in the excretion rates of ammonia between aposymbiotic and symbiotic *Cassiopea* sp. suggest that the zooxanthellae remove host-produced ammonia and aid in the recycling of nitrogen (Cates & McLaughlin 1976). Hofmann & Kremer (1981) and Hofmann et al. (1996) investigated the importance of zooxanthellae to strobilation in *Cassiopea andromeda* and reported that the presence of algae was indispensable although algal photosynthesis or photosynthate release was not essential for this process. Apparently, the symbiotic zooxanthellae produce and supply a 'factor' which promotes the elevated metabolism required for proceeding through developmental stages (Rahat & Hofmann 1987).

The photosynthetic carbon budget of the epipelagic coronate medusa *Linuche unguiculata* has been inves-

tigated by Kremer et al. (1990); zooxanthellae from these medusae are capable of contributing carbon products towards host respiratory requirements (CZAR) of approximately 160%. A similar study on the swimming rhizostomid medusa *Mastigias* sp. was conducted by McCloskey et al. (1994); CZAR estimates averaged 97 and 176% for lake and lagoon ecotypes, respectively. In contrast to both *L. unguiculata* and *Mastigias* sp., *Cassiopea xamachana* is more sedentary, spending most of its time with the bell's exumbrella in direct contact with the substrate and its tentacles elevated towards the surface (Blanquet & Phelan 1987). Therefore, we wanted to investigate whether or not these semi-sedentary medusae rely as much on symbiont-derived organic products for metabolism as do *L. unguiculata* and *Mastigias* sp.

To our knowledge, no study estimating the photosynthetic capabilities and resultant carbon budgets of scyphozoans has investigated these parameters as a function of seasonal environmental changes. Most photosynthesis-respiration estimates are based on a single-case time period, as is the case for *Linuche unguiculata* (Kremer et al. 1990), *Mastigias* sp. (McCloskey et al. 1994), and *Cassiopea xamachana* (Vodenichar 1995). The few seasonal-based studies on scyphozoans have only dealt with jellyfish population dynamics or distributions (Brewer & Feingold 1991, Olesen et al. 1994) or growth (Olesen et al. 1994). With respect to medusan size, 2 studies on *Mastigias* sp. are the only investigations that have documented the influence of jellyfish size on photosynthesis, respiration, and carbon budgets (McCloskey et al. 1994) and algal symbiont population density and regulation (Muscatine et al. 1986). Consequently, we also wanted to determine if medusan size or seasonal environmental change affects the carbon budgets of *C. xamachana* symbiotic with *Symbiodinium microadriaticum* (Freudenthal 1962, Trench & Blank 1987).

## MATERIALS AND METHODS

**Collection and processing of jellyfish.** Different-sized specimens of *Cassiopea xamachana* were collected daily from a shallow, mangrove-lined lagoon at Little Conch Key (mile marker 62.3) in the Florida Keys (USA). All production and respiration experiments were conducted during September 1992 and January 1993. After each experiment, bell diameter, wet weight (ww), and displacement volume of each jellyfish were measured. The jellyfish were individually homogenized in filtered seawater (Whatman no. 5 filter), the homogenate volume determined, and sub-samples taken for various assays. Each jellyfish was assigned to 1 of 3 size classes based on bell diameters: small, medium, or large.

**Algal parameters.** *Chlorophylls:* After homogenization of each jellyfish, the algae were obtained by 3 centrifugations, each at 3 to 4 min at top speed in a clinical centrifuge, with the algal pellet resuspended each time in filtered seawater. Three replicate aliquots (3.0 ml each) of resuspended algae were processed as described by Verde & McCloskey (1996). Between 15 and 20 hemacytometer grids were counted to obtain algal density (cells $ml^{-1}$) of the suspension which was used for chlorophyll assays. The chlorophyll content was calculated using the equations of Jeffrey & Humphrey (1975) as modified by Parsons et al. (1984).

*Density, size, and biomass:* Algal numbers from 20 replicate samples from each homogenate were counted with a hemacytometer and converted to total standing stock of algae per jellyfish. Concurrently, the diameters of 20 randomly chosen zooxanthellae were measured from each jellyfish homogenate. For biomass studies, freshly collected jellyfish (n = 4, January and September) were individually homogenized in filtered seawater. Aliquots of the algal suspension were centrifuged for 3 to 4 min in a clinical centrifuge and the jellyfish tissue layer above the algal pellet was discarded. Jellyfish contamination of the algal suspension was periodically checked by hemacytometer counts; after the algal:nematocyst ratio exceeded 95%, 20 hemacytometer grids were counted to obtain algal density (cells $ml^{-1}$).

Samples for C:N analysis were prepared for isolated algae as follows. Either 2.0 ml or 3.0 ml of the jellyfish-free algal cell suspension was filtered, under gentle vacuum, through a precombusted (450°C for 12 h) GF/C filter (25 mm Whatman). Each filter was subsequently rinsed with 0.5 ml of distilled water, to remove salts, and frozen. Four seawater-filter blanks were included in all sample sets as controls. Prior to C:N analysis, the filters were dried (45°C for 12 h) and the carbon and nitrogen masses quantified using a Control Equipment Corporation Elemental Analyzer (Model 240X). After accounting for seawater blanks, the algal carbon and nitrogen masses (pg $cell^{-1}$) were calculated. The nitrogen mass was multiplied by 6.25 to obtain protein per algal cell (Muscatine et al. 1986).

*Mitotic index and growth:* Tentacle snips chosen at random from freshly collected and individually marked medusae (January and September) were collected every hour for 24 h and frozen. Each sample was homogenized and the dividing algal cells counted in a hemacytometer. The mitotic index (MI) was calculated as described by Wilkerson et al. (1983). The phased-division formulae of Vaulot (1992) was used to calculate the algal-specific growth ($\mu$):

$$\mu = \ln[(1 + f_{max})(1 + f_{min})^{-1}] \tag{1}$$

where $f_{max}$ and $f_{min}$ are the maximum and minimum daily fraction of dividing cells, respectively.

**Jellyfish and algal biomass.** Frozen aliquots of the jellyfish homogenate were thawed and the protein content of the medusan fraction estimated by the BCA Protein Assay (Pierce Chemical) using bovine serum albumen (fraction V) as the standard. Total jellyfish biomass was determined as medusan and algal protein fractions combined. Individual medusan and algal biomasses, expressed as fractions of total protein, are referred to as β and 1–β, respectively (Muscatine et al. 1981).

**Photosynthesis and respiration parameters.** The diel oxygen fluxes of intact jellyfish were measured with a self-contained underwater respirometer (McCloskey et al. 1985) at a depth of $2.0 \pm 0.5$ m. Simultaneously, ambient light intensity was measured with a Li-Cor quantum sensor (Model LI-192S). From data obtained from each 24 h record of oxygen flux, the integrated total daily jellyfish respiration and gross photosynthetic rate were determined as outlined by McCloskey et al. (1994) and Verde & McCloskey (1996). Morning photosynthesis and irradiance (P-I) response curves were generated from diel oxygen flux measurements. For P-I analysis, hourly net photosynthesis was normalized to algal density and the curves were iteratively fit via the hyperbolic tangent function (Jassby & Platt 1976).

**Carbon budgets.** The carbon budget parameters of *Cassiopea xamachana* were modelled using the nomenclature and equations of McCloskey et al. (1994). The contribution of carbon from zooxanthellae to animal respiration (CZAR) was calculated as described by McCloskey et al. (1994) and Verde & McCloskey (1996).

**Statistical analysis.** Prior to analysis, all data sets were tested for compliance with the assumptions of each statistical test. If assumptions were violated, the data were transformed and retested, or non-parametric tests were utilized. All percentage data (i.e. the MI data) were arcsine transformed before analysis (Sokal & Rohlf 1995, Zar 1996). The software packages Statistica\W® (Statsoft, Inc.) and SlideWrite Plus (Advanced Graphics Software) were used to conduct statistical and graphics analysis, respectively.

## RESULTS

### Environmental parameters

Average ($\pm$ SD) daylength during January ($11.2 \pm 0.1$ h, n = 14) was significantly shorter (*t*-test, $p < 0.001$) than during September ($12.8 \pm 0.1$ h, n = 10). Average daily integrated irradiance during January ($31.0 \pm 9.6$ E m$^{-2}$ d$^{-1}$) was significantly lower (*t*-test, $p < 0.001$) than during September ($46.0 \pm 9.2$ E m$^{-2}$ d$^{-1}$). Likewise, water temperature during January was significantly lower than during September. Average ($\pm$ SD) January water temperature ($24.2 \pm 1.4$°C, n = 14) during the night (when respiration was measured) was significantly lower (*t*-test, $p < 0.001$) than for September ($29.9 \pm 0.5$°C, n = 10).

### Algal parameters

**Cell size.** Since no significant differences (ANOVA, $p > 0.05$) in algal diameters as a function of jellyfish size during either January or September occurred, the cell diameters from the 3 jellyfish sizes were pooled together. The average ($\pm$ SD) algal cell diameter for January was 9.08 µm ($\pm 0.87$, n = 540) and was significantly higher (*t*-test, $p < 0.001$) than the average of 8.64 µm ($\pm 0.81$, n = 820) for algal cells during September. It follows that the average cell volume for algae in January of 400.6 µm$^3$ ($\pm 106.8$, n = 540) was significantly higher (*t*-test, $p < 0.001$) than the average volume of 347.7 µm$^3$ ($\pm 103.8$, n = 820) for algae in September.

**Chlorophyll.** Zooxanthella chlorophylls *a* and *c* (chl *a* and chl *c*) were significantly higher during January than September (Table 1, Fig. 1A, B). However, there was no significant difference in symbiont chlorophyll per cell as a function of jellyfish size. The chl *a*:chl *c* ratio was not significantly different regardless of season or jellyfish size (Table 1, Fig. 1C).

**Density and biomass.** Since no significant differences between algal densities occurred during January and September, the data were pooled together

Table 1. *Symbiodinium microadriaticum*. Mean ($\pm$ SD) chlorophyll content (chl; pg cell$^{-1}$) and chl *a*:chl *c* ratio of zooxanthellae isolated from the jellyfish *Cassiopea xamachana* during September 1992 (n = 27) and January 1993 (n = 40). The Student's *t*-test was used to determine significance between January and September values. ns: not significant ($p > 0.05$), •••$p < 0.001$

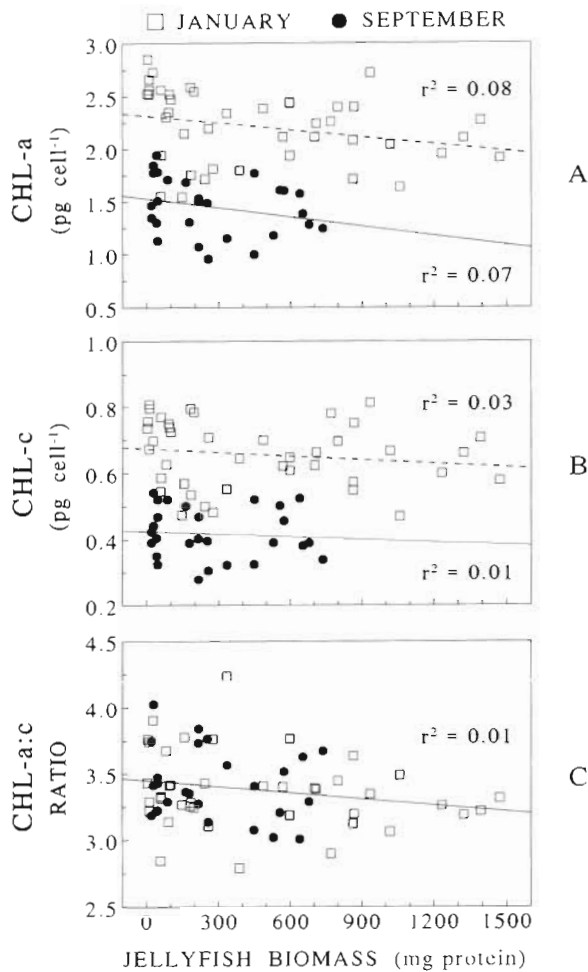| Chlorophyll | January | September | Significance | Jan:Sep ratio |
| --- | --- | --- | --- | --- |
| Chl *a* | $2.21 \pm 0.35$ | $1.45 \pm 0.27$ | ••• | 1.52 |
| Chl *c* | $0.66 \pm 0.10$ | $0.42 \pm 0.08$ | ••• | 1.57 |
| Chl *a* + *c* | $2.87 \pm 0.43$ | $1.87 \pm 0.34$ | ••• | 1.53 |
| Chl *a*:chl *c* ratio | $3.37 \pm 0.29$ | $3.49 \pm 0.49$ | ns | 0.96 |

Fig. 1. *Symbiodinium microadriaticum* and *Cassiopea xamachana*. Chlorophyll content of zooxanthellae from *C. xamachana* as a function of jellyfish size and season. Best-fit curve for the relationship between chlorophyll and host biomass is linear regression. (A) Chl *a*, January $r^2$ = 0.08, September $r^2$ = 0.07; (B) chl *c*, January $r^2$ = 0.03, September $r^2$ = 0.01; (C) chl *a*:chl *c* ratio, combined $r^2$ = 0.01. The slopes from each figure are not significantly different (p > 0.05) from zero and only the $r^2$ value is listed. Sample sizes for January and September were 41 and 27, respectively

**Mitotic index and growth.** An average (±SD) of 74.5% (±8.3, n = 20) of the algal symbionts resided solely within the tentacles, with the remaining 25.5% (±8.3, n = 20) inhabiting the bell. The average symbiont MI from the tentacles of *Cassiopea xamachana* was 0.047% (±0.068, n = 20) and from the bell was 0.084% (±0.095, n = 20), values which were not significantly different (paired *t*-test, p > 0.05). The MI values were significantly different (ANOVA, p < 0.05) throughout a diel period regardless of season and jellyfish size (Fig. 3). The near midday MI peak was significantly higher than the rest of the day (Tukey, p < 0.05) and we interpret this as phased mitosis. The maximum peaks of the MI, regardless of season or jellyfish size, were not significantly different (ANOVA, p > 0.05) and the grand mean (±SD) was 0.25% (±0.20, n = 72). Likewise, the MI minimums were not significantly different (ANOVA, p > 0.05) and the grand mean was 0.02% (±0.03, n = 72). Consequently, the algal-specific
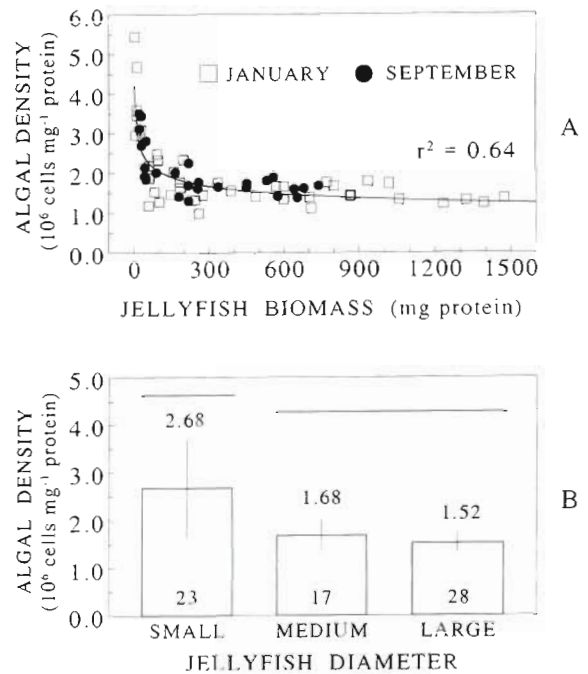


Fig. 2. *Symbiodinium microadriaticum* and *Cassiopea xamachana*. Density of zooxanthellae within *C. xamachana* as a function of jellyfish size and season. Best-fit curve for the relationship between algal density and host biomass (A) is a power function: Density = 4.82 (Biomass)$^{-0.18}$, $r^2$ = 0.64. For the relationship between algal density and host bell diameter (B) horizontal lines connect algal densities that are not significantly different (Tukey, p > 0.05); lines at different levels signify densities that are significantly different (Tukey, p < 0.05). Values above error bars are the mean (density) for each bell size class and values within bars represent sample size. Error bars show ± SD. Bell diameter (cm, $\bar{x}$ ± SD) of each size class, regardless of season, was: Small, 4.0 ± 1.1; Medium, 8.0 ± 0.8; Large, 13.0 ± 2.2

(Fig. 2A). Jellyfish bell diameter and algal density were inversely related, with smaller jellyfish containing significantly higher algal cell densities than larger jellyfish (Fig. 2B). With regard to algal biomass, both the carbon content and C:N ratio of zooxanthellae were significantly higher during January than September (Table 2), but the nitrogen and resultant protein masses were not significantly different regardless of season. The pooled averages (±SD) for January and September nitrogen and protein values were 15.23 pg cell$^{-1}$ (±1.63, n = 8) and 95.16 pg cell$^{-1}$ (±10.20, n = 8), respectively.

Table 2. *Symbiodinium microadriaticum.* Mean (±SD) carbon, nitrogen, and protein contents (pg cell$^{-1}$) and C:N ratio of zooxanthellae isolated from the jellyfish *Cassiopea xamachana* during September 1992 (n = 4) and January 1993 (n = 4). The Mann-Whitney *U*-test was used to determine significance between January and September values. ns: not significant (p > 0.05), $^*$p < 0.05

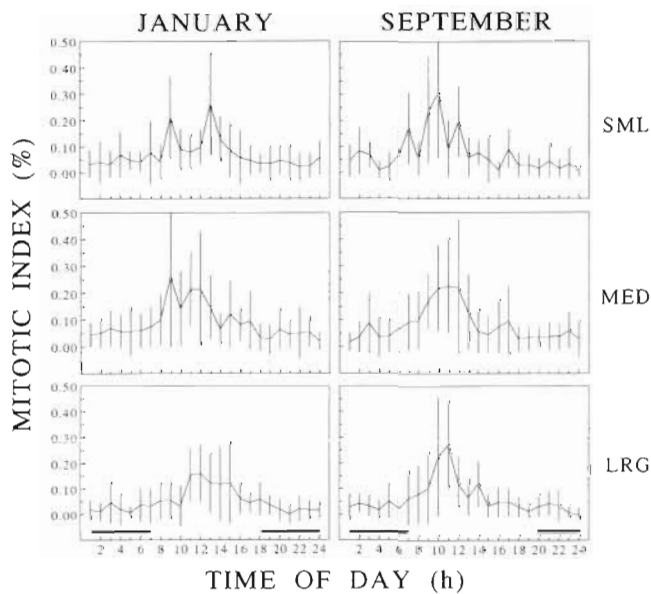| Algal parameter | January | September | Significance | Jan:Sep ratio |
|---|---|---|---|---|
| Carbon | 114.88 ± 9.40 | 85.29 ± 9.27 | $^*$ | 1.34 |
| Nitrogen | 15.89 ± 1.83 | 14.56 ± 1.30 | ns | 1.09 |
| Protein | 99.34 ± 11.43 | 90.98 ± 8.11 | ns | 1.09 |
| C:N ratio | 7.30 ± 1.05 | 5.87 ± 0.49 | $^*$ | 1.24 |



Fig. 3. *Symbiodinium microadriaticum* and *Cassiopea xamachana.* Diel mitotic index of zooxanthellae within *C. xamachana* as a function of jellyfish size (small: SML, medium: MED, and large: LRG) and season. Sample sizes for each hour, regardless of season, for SML, MED, and LRG were 10, 16, and 10, respectively. Error bars show ± SD. Bell diameter (cm, $\bar{x}$ ± SD) of each size class, regardless of season, was: Small, 4.0 ± 1.1; Medium, 8.0 ± 0.8; Large, 13.0 ± 2.2. Horizontal lines denote occurrence of night
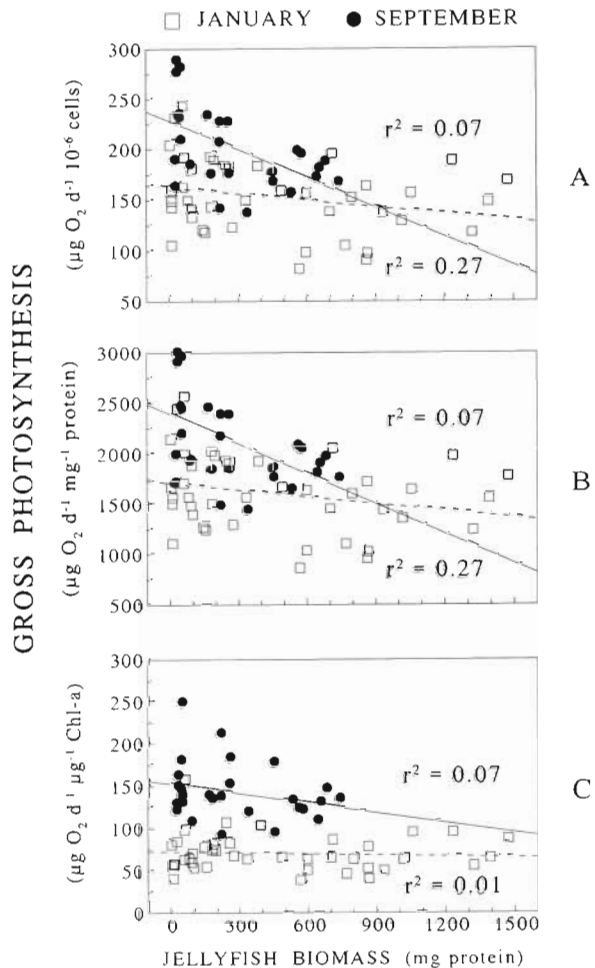


Fig. 4. *Symbiodinium microadriaticum* and *Cassiopea xamachana.* Gross photosynthesis ($P_g$) of zooxanthellae within *C. xamachana* as a function of jellyfish size and season. Best-fit curve for the relationship between gross photosynthesis and host biomass is linear regression. (A) Density; for January $r^2$ = 0.07, for September $P_g$ = –0.10 (Biomass) + 228.41, $r^2$ = 0.27. (B) Protein; for January $r^2$ = 0.07, for September $P_g$ = –0.99 (Biomass) + 2400.26, $r^2$ = 0.27. (C) Chl *a*; for January $r^2$ = 0.01, for September $r^2$ = 0.07. Regression profiles where regression equations are given display slopes significantly different from zero. Sample sizes for January and September were 41 and 27, respectively
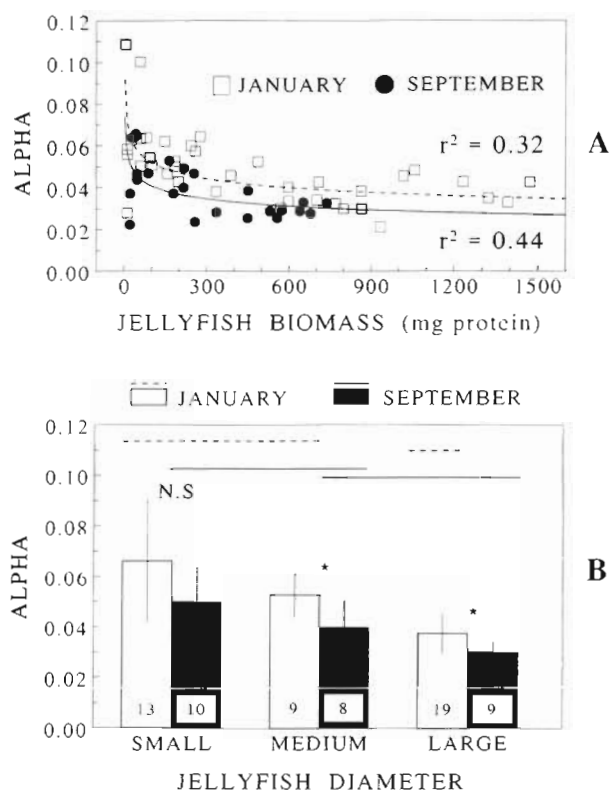
growth rate (μ), regardless of season and jellyfish size, was 0.0023 d$^{-1}$.

**Photosynthesis.** Daily gross photosynthesis, normalized to cell density, protein, and chl *a*, is shown in Fig. 4. Cell- and protein-specific photosynthesis during September were significantly higher than during January (Fig. 4A, B) and showed a significant host-size-specific relationship, with the photosynthesis of smaller jellyfish being significantly higher than that of larger individuals. The lower January rates, in contrast, exhibited no significant host size relationship (regression, p > 0.05) and averaged (±SD, n = 27)

Fig. 5. *Symbiodinium microadriaticum* and *Cassiopea xam-achana*. Photosynthetic efficiencies ($\alpha$) of zooxanthellae within *C. xamachana* as a function of jellyfish size and season. Best-fit curve for the relationship between $\alpha$ and host biomass (A) is a power function: January $\alpha = 0.08(\text{Biomass})^{-0.13}$, $r^2 = 0.32$; September $\alpha = 0.10(\text{Biomass})^{-0.15}$, $r^2 = 0.44$. For the relationship between $\alpha$ and host bell diameter (B) horizontal lines connect $\alpha$ values that are not significantly different (Tukey, p > 0.05), and lines at different levels signify values that are significantly different (Tukey, p < 0.05). Student's *t*-test: ns, not significant; *p < 0.05. Error bars show ± SD. Values within bars represent sample size. Bell diameter (cm, $\bar{x}$ ± SD) of each size class, regardless of season, was: Small, 4.0 ± 1.1; Medium, 8.0 ± 0.8; Large, 13.0 ± 2.2
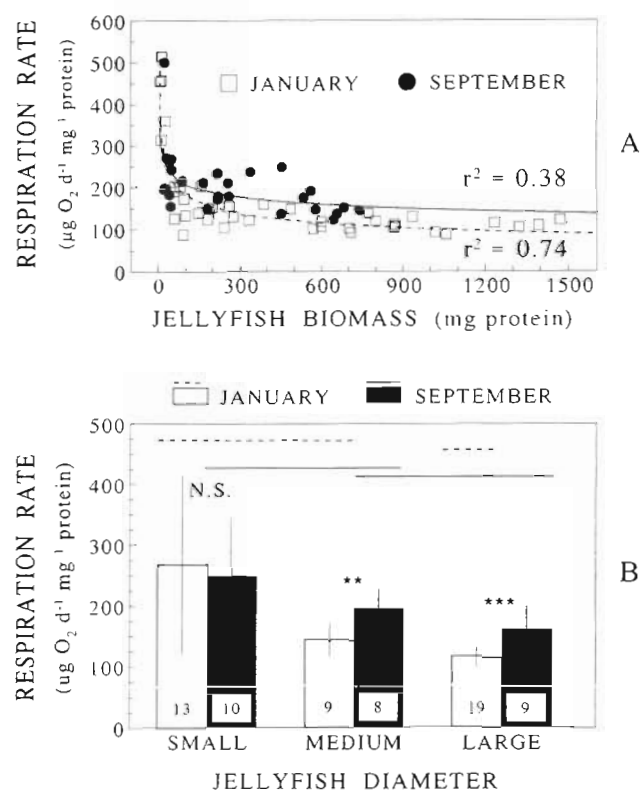
Fig. 6. *Cassiopea xamachana*. Respiration rate of *C. xam-achana* as a function of jellyfish size and season. Best-fit curve for the relationship between jellyfish respiration and biomass (A) is a power function: January resp. $= 607.91 (\text{Biomass})^{-0.26}$, $r^2 = 0.74$; September resp. $= 409.75 (\text{Biomass})^{-0.15}$, $r^2 = 0.38$. For the relationship between respiration and host bell diame-ter (B) horizontal lines connect respiration rates that are not significantly different (Tukey, p > 0.05), and lines at different levels signify rates that are significantly different (Tukey, p < 0.05). Student's *t*-test: ns, not significant; **p < 0.01; ***p < 0.001. Error bars show ± SD. Values within bars represent sample size. Bell diameter (cm, $\bar{x}$ ± SD) of each size class, regardless of season, was: Small, 4.0 ± 1.1; Medium, 8.0 ± 0.8; Large, 13.0 ± 2.2

152.64 ± 36.57 µg $O_2$ $d^{-1} \times 10^{-6}$ cells and 1604.01 ± 384.26 µg $O_2$ $d^{-1}$ $mg^{-1}$ protein, respectively. Chloro-phyll-specific photosynthesis during September was also significantly higher than photosynthesis during January (Fig. 4C). However, neither January nor September photosynthetic rates related to jellyfish size. The average (±SD) January and September photosynthetic rates were 70.69 (±21.93, n = 27) and 142.35 (±34.43, n = 40) µg $O_2$ $d^{-1}$ $\mu g^{-1}$ chl a, respec-tively.

January-acclimated jellyfish displayed significantly higher photosynthetic efficiencies ($\alpha$) (ANCOVA, p < 0.01) than their counterparts during September (Fig. 5A). Jellyfish size-specific $\alpha$ displayed both sea-sonal and size dependent differences (Fig. 5B). In all cases, average January $\alpha$ were higher than September $\alpha$ although they were only significantly higher for medium and large jellyfish. Regardless of season, small jellyfish showed significantly higher $\alpha$ than large jellyfish.

### Jellyfish respiration

Jellyfish respiration rates during September were significantly higher (ANCOVA, p < 0.01) than during January (Fig. 6A), particularly for medium and large jellyfish (Fig. 6B). Regardless of season, smaller jelly-fish displayed significantly higher respiration rates than larger individuals.
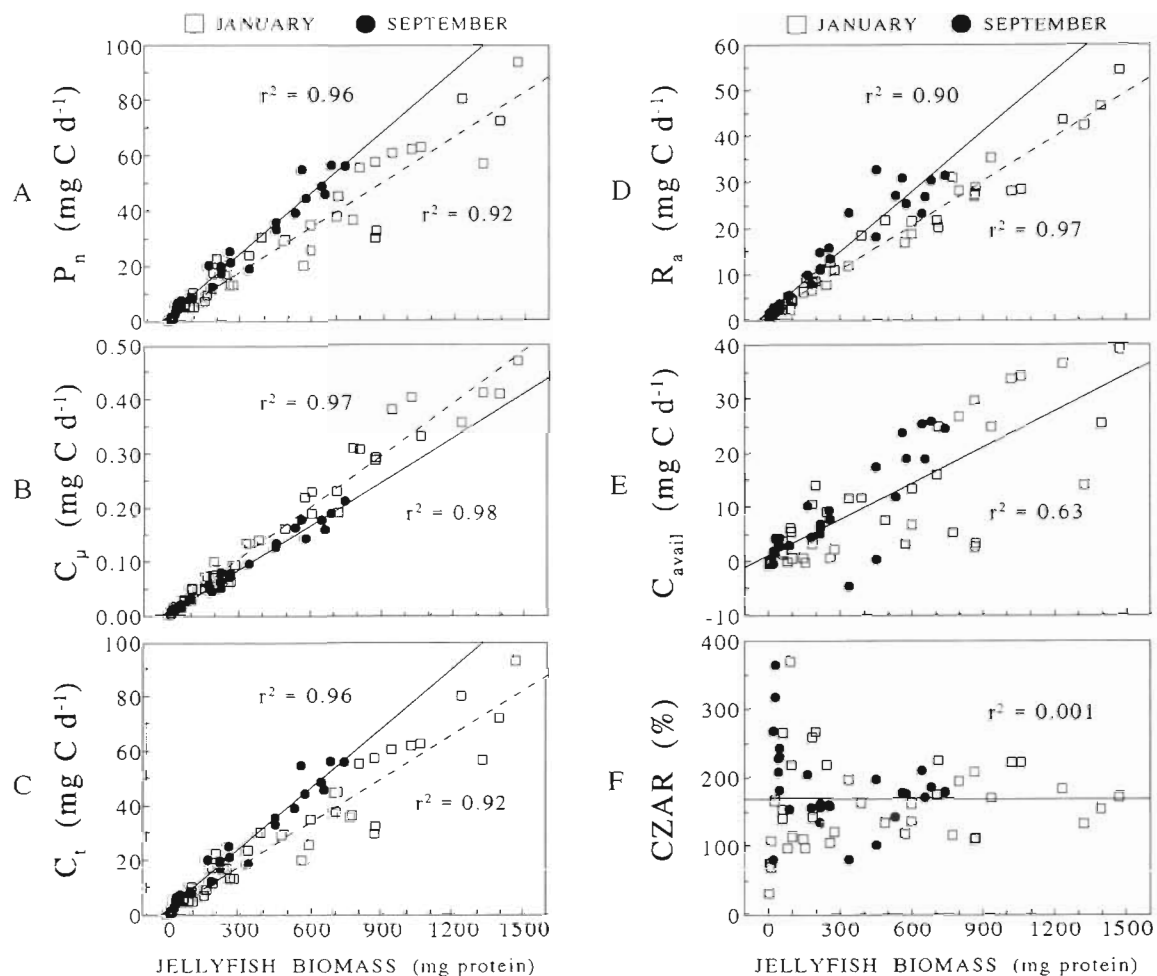
Fig. 7 *Cassiopea xamachana*. Daily carbon budget parameters of *C. xamachana* as a function of jellyfish size and season. Best-fit curves for the relationship between carbon budget parameter and jellyfish biomass are linear regression. (A) Net photosynthesis ($P_n$): January $P_n$ = 0.054 (Biomass) + 1.64, $r^2$ = 0.92; September $P_n$ = 0.074 (Biomass) + 2.49, $r^2$ = 0.96. (B) Algal carbon-specific growth rate ($C_\mu$): January $C_\mu$ = 0.00032 (Biomass) + 0.011, $r^2$ = 0.97; September $C_\mu$ = 0.00027 (Biomass) + 0.0047, $r^2$ = 0.98. (C) Potentially translocated carbon ($C_t$): January $C_t$ = 0.054 (Biomass) + 1.63, $r^2$ = 0.92; September $C_t$ = 0.073 (Biomass) + 2.49, $r^2$ = 0.96. (D) Animal respiration ($R_a$): January $R_a$ = 0.032 (Biomass) + 1.38, $r^2$ = 0.97; September $R_a$ = 0.043 (Biomass) + 1.94, $r^2$ = 0.90. (E) Available translocated carbon in excess of that utilized for animal respiration ($C_{avail}$): $C_{avail}$ = 0.022 (Biomass) + 1.09, $r^2$ = 0.63. (F) Potential carbon contribution of zooxanthellae towards the animal's respiratory requirements (CZAR): CZAR = 0.0050 (Biomass) + 171.19, $r^2$ = 0.001. Sample sizes for January and September were 41 and 27, respectively

## Carbon budgets

Daily net photosynthesis ($P_n$), algal carbon-specific growth rate ($C_\mu$), translocated carbon ($C_t$), animal respiration ($R_a$), carbon available for host growth ($C_{avail}$), and CZAR are presented in Fig. 7. Algal $P_n$ per jellyfish showed a direct relationship (ANCOVA, p < 0.001) with jellyfish biomass (Fig 7A), with September rates significantly higher (ANCOVA, p < 0.001) than in January (Fig. 7A). $C_\mu$ also displayed a direct relationship (ANCOVA, p < 0.001) with jellyfish biomass (Fig. 7B). However, January $C_\mu$ was significantly higher (ANCOVA, p < 0.05) than September $C_\mu$. The calculated $C_t$ (i.e. $P_n - C_\mu$) also showed a direct relationship

(ANCOVA, p < 0.001) with jellyfish size (Fig. 7C) and, as for $P_n$, September $C_t$ rates were significantly higher (ANCOVA, p < 0.05) than January $C_t$ rates.

Host $R_a$ was directly related (ANCOVA, p < 0.05) to biomass (Fig. 7D) with $R_a$ during September being significantly higher (ANCOVA, p < 0.01) than in January. Like the other parameters, $C_{avail}$ was also directly related (ANCOVA, p < 0.05) to jellyfish biomass (Fig. 7E); however, there was no significant difference (ANCOVA, p > 0.05) between January and September values. In contrast to the other carbon budget parameters, CZAR did not show a significant trend (ANCOVA, p > 0.05) with either season or jellyfish size (Fig. 7F). The average (± SD) CZAR for all jellyfish was 169.2% (± 65.2, n = 68).

The distribution of carbon for an average *Cassiopea* medusa (≤ 800 mg protein) acclimated to January or September conditions is shown in Fig. 8A, B, respectively. With the exception of $C_\mu$, carbon budget parameters (either estimated or directly measured) were between 39 and 48 % higher for September- than January-acclimated jellyfish. In contrast, January $C_\mu$ estimates were 20 % higher than those in September.

## DISCUSSION
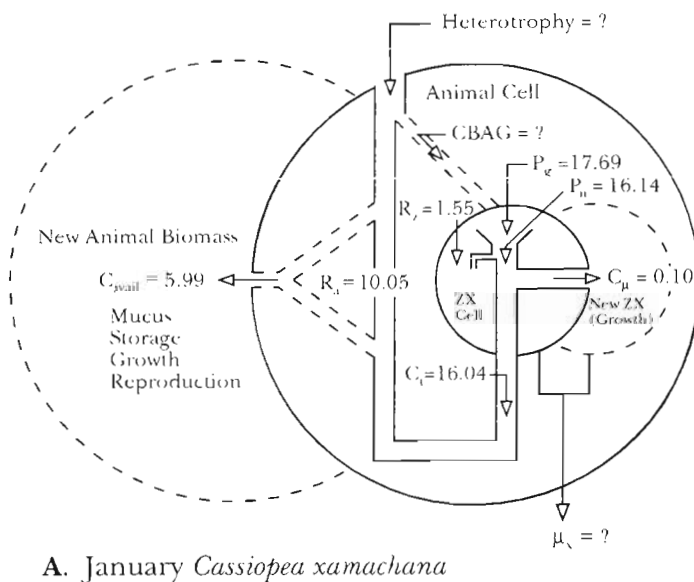
The principal conclusion of this study concerns the definitive influence of seasonal environmental parameters on both the photobiology and basic physiological aspects of algal-cnidarian symbioses. Both the photosynthetic (and resultant carbon budgets) and metabolic rates of *Cassiopea xamachana* are seasonally dependent, with higher rates occurring during September (summer) than January (winter), and are contingent on both light and thermal regimes. Winter-acclimated symbionts photoadapt to reduced light conditions by increasing their internal pools of chlorophyll and becoming photosynthetically more efficient. However, these compensatory mechanisms are not enough to enhance photosynthetic rates to the levels observed during the summer. Such seasonally dependent physiological effects emphasize the requirement for seasonal investigations of algal-cnidarian symbioses in order to comprehend the ecological physiology and photobiology of the symbiotic association on an annual cycle.
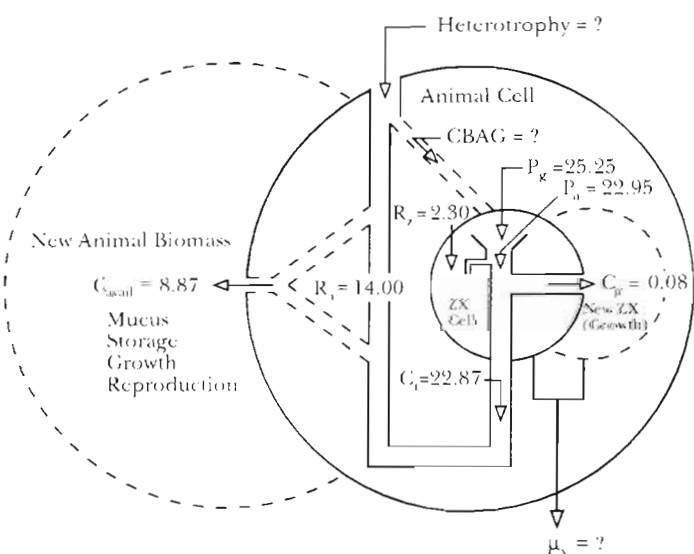
### Algal parameters

**Cell size.** A shift in algal cell size occurred as a function of season for *Cassiopea xamachana*, with medusae acclimated to 'winter' environmental conditions in the subtropical Florida Keys harboring larger-sized zooxanthellae (9.08 μm diameter) than jellyfish acclimated to summer conditions (8.64 μm diameter). Whether this phenomenon is an adaptation to environmental conditions or is directly regulated by the jellyfish host is not known, but it seems reasonable to posit that larger algal size may increase light-gathering efficiency.

Muscatine et al. (1986) reported an average cell diameter for zooxanthellae from small and large *Mastigias* sp. of 9.85 and 8.25 μm, respectively, while Kremer et al. (1990) reported a diameter of 8.9 μm from *Linuche unguiculata*. These values are similar to those we measured for zooxanthellae from *Cassiopea xamachana*.



**A.** January *Cassiopea xamachana*



**B.** September *Cassiopea xamachana*

Fig. 8. *Symbiodinium microadriaticum* and *Cassiopea xamachana*. Average carbon fluxes for zooxanthellae from *C. xamachana* during (A) January and (B) September. Values are given as mg C d$^{-1}$ for an average sized jellyfish which is ≤ 800 mg protein. $P_g$ and $P_n$ are gross and net photosynthesis, respectively; $R_z$ and $R_a$ are algal and animal respiration, respectively; $C_\mu$ is the carbon-specific growth rate; $C_t$ is the carbon translocated to the host; $\mu_z$ is expelled algae; $C_{avail}$ is the carbon available to the animal for mucus production, storage products, growth, and reproduction; CBAG is carbon back-translocated from the animal to the algae. Sample sizes for January and September were 31 and 27, respectively

**Chlorophyll.** Vodenichar (1995) reported an average symbiont chl a content of approximately 1.0 pg cell$^{-1}$ during summer conditions, which is lower than our values of 1.45 pg cell$^{-1}$ for September zooxanthellae. The average algal chl a of zooxanthellae (during January) from *Cassiopea xamachana* (2.21 pg cell$^{-1}$) was similar to the 2.1 and 2.0 pg cell$^{-1}$ reported by Kremer et al. (1990) and Wilkerson & Kremer (1992) for *Linuche unguiculata*, and the 2.0 pg cell$^{-1}$ for *Mastigias* sp. reported by McCloskey et al. (1994). Likewise, the mean chl a:chl c ratio for algae in *C. xamachana* (3.37) was similar to the average ratios for algae from *L. unguiculata* (3.23).

In *Cassiopea xamachana*, the zooxanthella chlorophyll content during January was 1.5 times higher than for algae during September. Since both the daylength and light intensities were significantly lower during January than in September, these differences in irradiance levels may be the reason winter-acclimated zooxanthellae photoactively increase their internal pools of chlorophyll, as described for both symbiotic and free-living dinoflagellates (Richardson et al. 1983, Prezelin 1987). Alternatively, there may just be an increase in symbiont chlorophyll content concomitant with larger cell volumes.

**Density.** Vodenichar (1995) showed an average algal density of approximately $4.5 \times 10^6$ cells g$^{-1}$ ww for *Cassiopea xamachana* ranging in size from 1 to 10 cm bell diameter. When the cell densities from jellyfish in our study were normalized to g ww, the average value, regardless of season, was $9.6 \times 10^6$ cells g$^{-1}$ ww, which is double the estimates of Vodenichar (1995). Why the medusae in our study contained twice the amount of algal symbionts contained in the jellyfish in Vodenichar's (1995) work remains a mystery. Perhaps experimental protocol or microhabitat differences between the 2 study sites are responsible, or possibly different algal taxa are involved. The jellyfish from the Vodenichar (1995) study were collected between June and September at Buttonwood Sound, Key Largo (FL, USA), whereas the medusae in this study were collected from Little Conch Key, Marathon, approximately 103 km further south. Vodenichar (1995) also brought the medusae into the laboratory for experimental manipulation, and the resultant handling may have caused algal expulsion, whereas medusae in our study were maintained *in situ* with minimal handling prior to estimation of algal densities.

In contrast to *Cassiopea xamachana*, the average algal density of *Linuche unguiculata* was $4.5 \times 10^6$ cells mg$^{-1}$ protein (Kremer et al. 1990), which is 1.7 times greater than the algal density of $2.68 \times 10^6$ cells mg$^{-1}$ protein for small-sized *C. xamachana*. Muscatine et al. (1986) reported that *Mastigias* sp. algal density was not a function of jellyfish size and calculated an average

value of $2.8 \times 10^6$ cells mg$^{-1}$ protein. This is almost identical to the algal density of small-sized *C. xamachana* but is 1.7 times higher than that for medium-sized medusae. Clearly there are significant differences as to how different species of symbiotic jellyfish maintain their complement of zooxanthella symbionts with respect to body size, but there are no obvious patterns or trends yet evident among the 3 species for which we have comparative data.

Regulation of algal symbionts by *Cassiopea xamachana* seems to occur primarily by host digestion of algae and by expulsion into the environment. Fitt & Trench (1983) showed that heat-killed algae which underwent phagocytosis were susceptible to lysosomal attack by acid phosphatase. This suggests that endosymbiotic algae that become senescent, for whatever reason, may be consumed by the host through phagocytocis. Individual jellyfish, maintained in buckets with seawater, secreted mucus bands containing many zooxanthellae (authors' pers. obs.), although the expelled zooxanthella numbers were not quantified. Whether *C. xamachana* expels zooxanthellae continuously, at times when internal pools of zooxanthellae become too high or when the animal host is stressed, is not currently known. Like *C. xamachana*, large-sized *Mastigias* sp. seems to regulate algal populations (Muscatine et al. 1986) by expulsion or digestion. However, in smaller-sized *Mastigias* sp. (Muscatine et al. 1986), algal populations may increase by transiently increasing the algal-specific growth rate ($\mu$). Whether zooxanthellae within small-sized *C. xamachana* employ such methods for regulating algal population is not known.

**Biomass: carbon, nitrogen, and protein.** When biomass-related parameters of various jellyfish zooxanthellae are compared, *Cassiopea xamachana* zooxanthellae are similar to most others, with some notable exceptions. The carbon content of zooxanthellae from *C. xamachana* (85.3 to 114.9 pg cell$^{-1}$), regardless of season, is higher than values previously reported for other jellyfish. Kremer et al. (1990) and Muscatine et al. (1986) reported values of 58.0 and 56.1 pg C cell$^{-1}$ for zooxanthellae from *Linuche unguiculata* and *Mastigias* sp., respectively. This carbon content difference between *C. xamachana*, *L. unguiculata*, and *Mastigias* sp. may be a by-product of the methods utilized to obtain cellular carbon. Algal carbon in our work was obtained from empirical C:N measurements of isolated algae, whereas the values of Muscatine et al. (1986) and Kremer et al. (1990) were derived from algal volumes applied to the regression equation of Strathmann (1967). If the cell volumes of zooxanthellae from *C. xamachana* are used to estimate carbon content via the equation of Strathmann (1967), the values obtained are 55.0 and 62.2 pg C cell$^{-1}$ and bracket the

numbers reported by Muscatine et al. (1986) and Kremer et al. (1990).

Kremer et al. (1990) and Wilkerson & Kremer (1992) reported that algae from *Linuche unguiculata* have a nitrogen content of 13.3 to 15.7 pg N cell$^{-1}$, which is very similar to values reported in this study. The protein content of zooxanthellae from *Cassiopea xamachana* is 2.5 times higher than that for algae from *Mastigias* sp., which have an average of 36.3 pg protein cell$^{-1}$ (Muscatine et al. 1986). Muscatine et al. (1986) and Kremer et al. (1990) reported C:N values of 9.7 and 6.2, respectively, which are also similar to the C:N ratio of zooxanthellae from *C. xamachana*. In summary, the biomass parameters of *Symbiodinium microadriaticum* within *C. xamachana* are nearly identical to those of zooxanthellae from other jellyfish.

**Mitotic index and algal growth.** Wilkerson et al. (1983) reported that zooxanthellae from *Mastigias* sp. display a phased, diel cell division profile (*sensu* McDuff & Chisholm 1982). Wilkerson et al. (1983) attributed such a phased MI to exposure of the algal symbionts to a 2 h pulse of ammonium at night. Other cnidarians exhibiting phased symbiont division include the corals *Seriatopora hystrix* (Hoegh-Guldberg & Smith 1989), *Stylophora pistillata*, *Fungia repanda*, and *Pocillopora damicornis* (Smith & Hoegh-Guldberg 1987). Clifford & Blanquet (1991) also reported that zooxanthellae extracted from *Cassiopea xamachana* and cultured with a 14 h light:10 h dark cycle display a diel pattern of cell motility, with phased division occurring during the latter part of the dark period.

As we have shown, zooxanthellae in *Cassiopea xamachana* also appear to display a phased algal MI. In contrast to the findings of Clifford & Blanquet (1991), the zooxanthellae within *C. xamachana* demonstrated a daytime phased division pattern between 09:00 and 12:00 h. Why *in vitro* phased division occurs in the dark and *in hospite* phased division occurs during daytime is not known, but one possibility may be that some host-derived fraction regulates algal cell division. Precedence for such regulation is provided by Carroll & Blanquet (1984a, b) and Blanquet et al. (1988) for alanine uptake and by McDermott & Blanquet (1991) for glucose uptake by zooxanthellae via inhibitory fractions derived from host homogenates.

Unlike *Mastigias* sp., *Cassiopea xamachana* does not exhibit diel migration but remains stationary on the sediment-water interface. It may be that carbon and nitrogen from bacterial metabolism at the sediment surface or tentacles (Schiller & Herndl 1989) or from mangrove litter decomposition (Lugo & Snedaker 1974, Fell et al. 1975, Newell et al. 1984, Robertson 1988, Mackey & Smail 1996) are transepidermally absorbed by *C. xamachana* as are various amino acids

in other soft-bodied invertebrates (Schlichter 1984, DeFreese & Clark 1991, Preston 1993) and are subsequently utilized by the algae to support phased division. Such uptake of carbon from decomposing mangrove litter is also expected to be higher during the summer than winter due to higher decomposition rates that occur during the summer season (Mackey & Smail 1996). Alternatively, inorganic nitrogen in the form of ammonium may also be directly absorbed transepidermally from the water, much as for *Mastigias* sp. (Muscatine & Marian 1982) or *Linuche unguiculata* (Wilkerson & Kremer 1992), and from the sediment, since the concentrations of these nutrients are higher inshore than offshore (Szmant & Forrester 1996). Similarly, dissolved organic matter may also be absorbed and used as a food source, as is the case for *Heteroxenia fuscescens* (Schlichter 1982a, b, Schlichter et al. 1983, 1984).

Alternatively, high levels of predation by the host on zooplankton could support phased algal division. *Cassiopea xamachana* is a rapacious predator of *Artemia* nauplii during feeding studies in aquaria (E.A.V pers. obs.). Support for prey-enhanced algal symbiont growth is provided by McAuley & Cook (1994), who reported that zooxanthellae in starved colonies of the hydroid *Myrionema amboinense* show an MI of 3.9%, whereas in colonies fed *Artemia* nauplii, the MI increased by a factor of 2.6 to 10.08%. McAuley & Cook (1994) also provide evidence that external nitrogen sources directly influence zooxanthella division rates. Other studies correlating increased zooxanthellae cell division with host feeding include Fitt & Cook (1990) on *M. amboinense* and Cook et al. (1988) on the anemone *Aiptasia pallida*.

From the algal-specific *in hospite* growth rate value ($\mu$) of 0.0023 d$^{-1}$ for zooxanthellae in *Cassiopea xamachana*, one can compute a doubling time for the symbiont population of 301 d using the equation of Wilkerson et al. [1983; $D_t = (\ln 2)\mu^{-1}$]. With such low growth rates and long doubling times, it is unlikely overgrowth by the symbionts poses a threat to the host. The average $\mu$ of zooxanthellae from *Mastigias* sp. and *Linuche unguiculata* is 0.10 d$^{-1}$ and 0.02 to 0.04 d$^{-1}$, respectively (Wilkerson et al. 1983, Kremer et al. 1990, McCloskey et al. 1994). However, these relatively high growth rates are based on a duration of cytokinesis of 11.0 h (Wilkerson et al. 1983) whereas the growth rate estimate of zooxanthellae from *C. xamachana* does not utilize this number since the diel division rates are phased. The $\mu$ of 0.0023 d$^{-1}$ for symbionts from *C. xamachana* is closer to those measured for zooxanthellae from the corals *Pocillopora eydouxi* (0.0017 d$^{-1}$, Davies 1984) and *Porites porites* (0.0023 to 0.0028 d$^{-1}$, Edmunds & Davies 1986, 1989) and the temperate anemone *Anthopleura elegantissima* (0.0029 d$^{-1}$, Verde & McCloskey 1996).

Table 3. Mean photosynthesis versus irradiance (P-I) parameters of 3 jellyfish, *Cassiopea xamachana*, *Mastigias* sp. and *Linuche unguiculata*. The P-I data of *Mastigias* sp. and *L. unguiculata* were obtained from McCloskey et al. (1994) and Kremer et al. (1990), respectively. The *C. xamachana* data were obtained from small and medium sized jellyfish from both January and September months which corresponded to the sizes of *Mastigias* sp. and *L. unguiculata*. C:M = *C. xamachana*:*Mastigias* sp. ratio. C:L = *C. xamachana*:*L. unguiculata* ratio. na: not available

| P-I[a] | Cassiopea | Mastigias | Linuche | C:M | C:L |
|---|---|---|---|---|---|
| $\alpha$ | 0.054 | 0.140 | 0.189[b] | 0.39 | 0.29 |
| $I_k$ | 413.9 | 133.0 | 200–300 | 3.11 | 1.4–2.1 |
| $I_{opt}$ | 1497.2 | 307.0 | na | 4.88 | – |
| $P_n^\circ$ max | 20.8 | 16.9 | 9.5[c] | 1.23 | 2.19 |

[a] P-I parameters:
$\alpha$ is a measure of photosynthetic efficiency $[(\mu g\ O_2\ h^{-1} \times 10^{-6}\ cells)\ (\mu E\ m^{-2}\ s^{-1})^{-1}]$
$I_k$ is the intersection of $\alpha$ and $P_n^\circ$ max tangents $(\mu E\ m^{-2}\ s^{-1})$
$I_{opt}$ is the optimum irradiance during $P_n^\circ$ max $(\mu E\ m^{-2}\ s^{-1})$
$P_n^\circ$ max is the maximum net photosynthesis $(\mu g\ O_2\ h^{-1} \times 10^{-6}\ cells)$

[b] Where $\alpha = 0.09\ mg\ O_2\ h^{-1}\ mg^{-1}$ chl *a* and chl *a* cell$^{-1}$ = 2.1 pg (1.0 mg chl *a* = 476.2 × 10$^6$ cells). Therefore, $(0.09\ mg\ O_2\ h^{-1}\ mg^{-1}$ chl *a*$)\ (476.2 \times 10^6\ cells)^{-1} = 0.189\ [(\mu g\ O_2\ h^{-1} \times 10^{-6}\ cells)\ (\mu E\ m^{-2}\ s^{-1})^{-1}]$

[c] Where $P_n^\circ$ max = 4.5 mg $O_2\ h^{-1}\ mg^{-1}$ chl *a* and chl *a* cell$^{-1}$ = 2.1 pg (1.0 mg chl *a* = 476.2 × 10$^6$ cells). Therefore, $(4.5\ mg\ O_2\ h^{-1}\ mg^{-1}$ chl *a*$)\ (476.2 \times 10^6\ cells)^{-1} = 9.5\ (\mu g\ O_2\ h^{-1} \times 10^{-6}\ cells)$

The determination of the MI only from symbionts specifically located within the tentacles may be justifiably questioned, since the algal MI from the tentacles of some cnidarians is elevated compared to the rest of the organism. Muller-Parker (1987) reported an algal MI from the tentacles of *Aiptasia pulchella* to be 1.5 times higher than the MI from the anemone column. In contrast, a tentacle-based MI for either zooxanthellae or zoochlorellae from *Anthopleura elegantissima* is similar to the MI of symbionts from the whole anemone (E.A.V. unpubl. data). For *Cassiopea xamachana*, we deemed it appropriate to determine algal growth rates from tentacle-derived symbionts because the majority of algae (75%) were located within the tentacles and this value was similar to the 70% reported for zooxanthellae in *Mastigias* sp. (Muscatine & Marian 1982). These tentacle snips also permitted time-course evaluations of MI changes in single jellyfish. For these reasons, we have used the more conveniently-obtained MI values from tentacle snips.

**Photosynthesis.** Although their photosynthesis-irradiance parameters differ (Table 3) the photosynthetic rates of *Cassiopea xamachana* and other symbiotic jellyfish, such as *Mastigias* sp., *Linuche unguiculata*, and *Cassiopea andromeda*, are very similar (Table 4). Not surprisingly, a strong seasonal influence on photosynthetic rates takes place. Since light intensity, light duration, and water temperature are significantly greater during September than during January, higher photosynthetic rates would be expected during September. If summer measurements were conducted during June (i.e. summer solstice), the summer photosynthetic rates (and resultant carbon flux values) would be expected to be even higher than reported here for September. On the other hand, January-acclimated symbionts have significantly higher $\alpha$ and chlorophyll content, suggesting that these algae adapt to these lower light and temperature regimes by being more efficient at capturing incident light. Similar seasonal photosynthetic rates were reported by Barnes (1988) on the Great Barrier Reef (Davis Reef, Australia), where gross photosynthesis was more than 30% higher during August compared to December. When a 10% reduction in respiration during December was considered, the resultant net photosynthesis was higher by 45% (Barnes 1988). The conclusions of Barnes (1988) and those reported in this study underscore the necessity for seasonal studies on algal-cnidarian symbioses.

When photosynthesis is examined as a function of jellyfish size, the highest rates occurred in smaller-sized individuals. This scaling of photosynthetic rates is due to the significantly higher algal densities and metabolic rates of smaller jellyfish, both of which promote elevated photosynthesis. Similar results were obtained by Fisher et al. (1985) from the giant clam *Tridacna gigas* and Vodenichar (1995) in *Cassiopea xamachana* in which both photosynthesis and respiration displayed an inverse relationship with animal size.

The reduction of photosynthetic rates with increasing jellyfish size during September may be due to algal shading, either from increased algal self-shading or from medusan pigmentation. The idea that algal self-shading plays a greater role in reducing photosynthesis than host pigmentation in *Cassiopea xamachana* is

Table 4. Comparison of the average maximum gross photosynthetic rates of 4 tropical symbiotic jellyfish: *Mastigias* sp., *Linuche unguiculata, Cassiopea andromeda,* and *Cassiopea xamachana.* na: not available

| Jellyfish | $P_g^{\circ}$ max ($\mu g\ O_2\ h^{-1} \times 10^{-6}$ cells) | $P_g^{\circ}$ max ($\mu g\ O_2\ h^{-1}\ \mu g^{-1}$ chl a) | Source |
|---|---|---|---|
| *Mastigias* sp. | | | |
| Lake | 17.6 | 8.8 | McCloskey et al. (1994)[a] |
| Lagoon | 29.6 | 14.8 | |
| *Linuche unguiculata* | 12.6 | 6.0 | Kremer et al. (1990)[a] |
| *Cassiopea andromeda* | na | 3.7–4.2 | Hofmann & Kremer (1981)[b] |
| *Cassiopea xamachana* | | | |
| January | 13.6 | 6.3 | Present study |
| September | 15.8[c] | 11.1 | Present study |

[a]From Table 7 of McCloskey et al. (1994)
[b]From Table 4 of Kremer et al. (1990)
[c]Based on a grand mean regardless of jellyfish size

supported by the work of Blanquet & Phelan (1987). They found that the unique mesogleal polymeric glycoprotein pigment 'Cassio Blue' in *C. xamachana* attenuated harmful solar radiation while permitting the penetration of photosynthetically active radiation. Consequently, in this instance, this particular host pigment does not significantly reduce algal photosynthesis.

Since *Cassiopea* sp. are generally found in shallow depths (Drew 1972) ranging from 0.1 m (seagrass and mangrove beds) to 10 m (coral borrow pits, see Clark & DeFreese 1987) in Florida, they are exposed to high light intensities and ultraviolet (UV) radiation. The symbiont photosynthetic rates are concomitantly higher without showing any photoinhibition effects (authors' unpubl. data) and these jellyfish may protect themselves from $O_2$ toxicity by producing both superoxide dismutase (SOD) and catalase (Dykens 1984, Lesser 1989, Lesser & Shick 1989a, b). Similarly, they would be expected to maintain elevated quantities of UV-absorbing compounds similar to those found within the mucus of *Fungia fungites* (Drollet et al. 1993) or mycosporine-like amino acids (MAAs) (Shick et al. 1992, 1995, Stochaj et al. 1994). Banaszak & Trench (1995a, b) described the effects of UV on *C. xamachana* and its symbionts. They showed that *Symbiodinium microadriaticum* from *C. xamachana* synthesizes 3 MAAs (mycosporine-glycine, shinorine, and porphyra-334). These MAAs are subsequently translocated to the host and are used by the host to minimize deleterious UV effects. It is these environmentally driven physiological adaptations which have permitted this symbiotic association to thrive at high densities in shallow areas of the tropical and subtropical subtidal zone.

## Jellyfish respiration

It is difficult to directly compare the respiration rates of *Cassiopea xamachana* with other jellyfish metabolic rates (Kremer et al. 1990, McCloskey et al. 1994) due to the problem of normalizing units and size variation. Small-sized jellyfish display higher mass-specific metabolic rates than larger individuals and this inverse scaling between animal size and metabolism is a well-known biological phenomenon. Similar size-specific metabolic rates were reported by Vodenichar (1995) for *C. xamachana.* September metabolism is much higher than that of January, and this is probably a simple consequence of elevated temperature effects since the average water temperature during September (29.9°C) was significantly higher than for January (24.2°C).

## Carbon budgets

The individual carbon budget components usually displayed a direct relationship with jellyfish biomass, and the 3 components $P_n$, $C_t$, and $R_a$ are significantly higher during September due to higher water temperatures, light intensities, longer daylength, or a combination of all three. In contrast, January $C_\mu$ is significantly higher than the $C_\mu$ during September, which we believe is a direct consequence of the higher carbon per algal cell during January. The $C_{avail}$ is the quantity of carbon available to the animal host for reproduction, storage, growth, mucus production, or the synthesis of a suite of important metabolites (such as SOD, catalase, MAA's, and peroxidase).

The potential contribution of algal carbon products to animal respiration (CZAR) showed no relationship

with either season or jellyfish biomass. This is counter-intuitive because $P_n$, $C_\mu$, and $C_t$ showed such a strong relationship with biomass. The explanation is that, since $R_a$ is also directly related to biomass, CZAR values, regardless of season, are not correlated with jellyfish size. CZAR is an estimate of the integrated $P{:}R$ ratio; if net photosynthesis (minus algal carbon-specific growth) is greater than host respiration, then CZAR is greater than 1 (i.e. 100%).

An average CZAR of 169% suggests that the zooxanthellae are potentially capable of providing all of the carbon necessary to support the respiration of the medusa, and *Cassiopea xamachana* may not require any external carbon sources. This value is similar to that reported for *Linuche unguiculata* (160%, Kremer et al. 1990) and for lagoon *Mastigias* sp. (143%, McCloskey et al. 1994). A comparatively lower CZAR of 97% for lake *Mastigias* sp. was attributed to elevated host metabolism resulting from this morph's diel migration patterns (McCloskey et al. 1994). A CZAR of 169% for *C. xamachana* may actually be a conservative estimate due to likely presence of a resident microbial fauna associated with the tentacular region of the medusa (*sensu* Schiller & Herndl 1989, Hansson & Norrman 1995). The combined bacterial respiration was not factored out of the calculation of the jellyfish respiration, which would overestimate host metabolism, and consequently, CZAR values would be reduced in magnitude commensurate with the extent of microbial metabolism.

Vodenichar (1995) reported CZAR values of approximately 106% in medusae with a bell diameter greater than 6 cm, whereas smaller-sized jellyfish showed CZAR values of approximately 73%. Vodenichar (1995) suggested that these smaller medusae as well as symbiotic larvae and scyphistomae may require external carbon sources to meet their respiratory carbon requirements. In contrast to the CZAR estimates of Vodenichar (1995), we calculate an average CZAR ($\pm$SD) value of 171% ($\pm$88, n = 22) for medusae with a bell diameter less than 6 cm. Because these 2 studies utilized decidedly different methods and equipment for measuring photosynthesis (with differing algal densities) and respiration, these differences undoubtedly contributed to the respective dissimilarities in CZAR estimates. Vodenichar (1995) measured both photosynthesis and respiration in a laboratory environment by taking $O_2$ readings every 5 min for a total of 30 min at ambient light intensities above 800 $\mu$E m$^{-2}$ s$^{-1}$ and calculated CZAR assuming 8 h of saturated photosynthesis. We believe that our CZAR estimates of 171% are more accurate and realistic for medusae of this size since our measurements of both photosynthesis and respiration took place *in situ* over the course of 24 h at the site where abundant densities of *Cassiopea xamachana* were located.

Four parameters were not estimated or measured in this study of *Cassiopea xamachana* carbon budgets: CBAG, new animal growth, $\mu_x$, and heterotrophy. The potential for carbon to be back-translocated from the animal to the algae (CBAG, see Verde & McCloskey 1996) is a remote possibility. Jellyfish growth rates are also an unknown variable and remain to be estimated. The carbon expelled from the association in the form of intact algal cells, $\mu_x$ (Hoegh-Guldberg et al. 1987, McCloskey et al. 1996), is also an unknown for *C. xamachana*. Heterotrophy represents the amount of carbon obtained from captured prey or dissolved organic carbon (DOC) directly absorbed by both the ectoderm and endoderm as previously discussed.

Another source of carbon loss from this association is the quantity of mucus that can be released by *Cassiopea xamachana*. Between 40 and 60% of the daily carbon fixed by photosynthesis in the coral *Acropora acuminata* and *Acropora formosa* was released as mucus or DOC (Crossland 1980, Crossland et al. 1980a, b). Recently, Hansson & Norrman (1995) estimated that an average of 1.2 mg C d$^{-1}$ is released as DOC in the temperate jellyfish *Aurelia aurita*. Consequently, mucus production and loss from *C. xamachana* could be a significant carbon sink for this association. This excreted mucus is likely used to foster bacterial growth on the medusan tentacles (Hansson & Norrman 1995), and this microbial population could be used directly or indirectly as a carbon resource as described by Schiller & Herndl (1989) for several coral species.

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