E.A. Verde · L.R. McCloskey

A comparative analysis of the photobiology of zooxanthellae and zoochlorellae symbiotic with the temperate clonal anemone Anthopleura elegantissima (Brandt)

II. Effect of light intensity

Received: 12 April 2001 / Accepted: 22 February 2002 / Published online: 8 May 2002
© Springer-Verlag 2002

Abstract The temperate anemone Anthopleura elegantissima hosts two phylogenetically different symbiotic microalgae, a dinoflagellate Symbiodinium (zooxanthellae, ZX) and a chlorophyte (zoochlorellae, ZC), throughout certain regions of its latitudinal range. Because of the broad intertidal and geographic range of this anemone, we examined the role of irradiance to ascertain which specific symbiotic parameters are affected and whether light intensity governs the observed distributions of natural populations of ZX and ZC. Irradiance appears to be a key factor in regulating both the photophysiology and metabolism of this alga–cnidarian association. Regardless of light intensity, algal densities remained stable for anemones harboring ZX or ZC, whereas the mitotic indices of ZX and ZC both varied directly with light intensity. The chlorophyll content of ZX remained fairly constant regardless of irradiance; in contrast, ZC chlorophyll content was inversely proportional to light intensity. Regardless of irradiance, the carotenoid content of both symbionts was constant; however, ZX carotenoid levels were higher than those of ZC. Net photosynthesis was directly related to light intensity for both algal symbionts and ZX photosynthetic rates were consistently higher than those of ZC. Similarly, the potential carbon contribution of ZX and ZC to animal respiration (CZAR) displayed a direct relationship with light intensity, peaking at 800 μmol m⁻² s⁻¹, then subsequently declined. Lower ZX growth rates, coupled with higher photosynthetic rates and higher CZAR estimates, compared to ZC, suggest that the ZX should be the dominant symbiont as light intensity increases; this may explain the high densities of anemones in the field containing ZX where the levels of irradiance are naturally high. These results support the interpretation that irradiance is a significant environmental parameter that dictates the microhabitat and latitudinal distribution of the two symbiotic algal taxa. This is the second in a series of papers examining the physical parameters that influence the distribution of ZX- and ZC-bearing A. elegantissima.

Introduction

The sea anemone Anthopleura elegantissima is a temperate clonal cnidarian occurring abundantly in the intertidal zone of the west coast of North America (Hand 1955; Littler 1980; Fitt et al. 1982; McFadden et al. 1997). These anemones are capable of maintaining a stable association with two distinctly different algal taxa: Symbiodinium sp., a dinoflagellate (zooxanthellae, ZX), and a unicellular chlorophyte (zoochlorellae, ZC) initially described by Muscatine (1971). The dinoflagellates have been assigned to different taxa correlating with geographic locations: anemones with ZX in California contain Symbiodinium californium, whereas anemones with ZX in Oregon and Washington contain S. musca-ainei (Lajeunesse and Trench 2000). As intertidal residents, these anemones exhibit a wide array of physiological and behavioral adaptations to cope with a constantly changing environment (Shick 1991), compared to anemones found in relatively stable tropical environments (Muller-Parker and Davy 2001).

The ZX and ZC are separated at the microhabitat level in the intertidal zone and in their latitudinal ranges. At the microhabitat level, A. elegantissima with ZX can span the entire vertical range in the intertidal
habitats, but prefer the higher reaches of the intertidal zone (McCloskey et al. 1996). In contrast, *A. elegantissima* with ZC are primarily found lower in the intertidal or in areas not directly exposed to solar radiation (McCloskey et al. 1996). *A. elegantissima* with ZX are found from northern Washington, USA, to Baja California, Mexico (Secord and Augustine 2000). McCloskey (unpublished data) has observed *A. elegantissima* with ZX from the northern tip of Vancouver Island, Canada, and southeast Alaska, USA, but the northern range limits of ZX are still unknown; nor is the northernmost distribution range of ZC in *A. elegantissima*, although Bates (2000) reported ZC in *A. xanthogrammica* at ~49°N (Vancouver Island, Canada). The southernmost distribution of *A. elegantissima* containing ZC is at about 43°N (Oregon; Secord and Augustine 2000).

In the first of this series of papers, Verde and McCloskey (2001) compared the photosynthesis, respiration, and carbon budgets of ZX and ZC in *A. elegantissima* as a function of temperature. In anemones with ZC, both the algal density and the chlorophyll content declined at elevated temperatures (24°C), whereas in anemones containing ZX, both parameters remained unchanged. As temperature increased, ZX displayed higher photosynthesis (per algal cell) and carbon translocation rates than did ZC, implying a higher potential for ZX to contribute carbon to the host’s respiration. Based on these results, Verde and McCloskey (2001) suggested that, as temperature increases, ZX becomes the symbiont more likely to maintain a stable association. Verde and McCloskey (2001) discuss the potential for ZX and ZC to incur a cost to the host anemone, based on evidence from ZC and ZX in *Hydra* or *Aiptasia*. Such back-translocation from ZC to *A. elegantissima*, if occurring, may be exacerbated by elevated temperature, and could easily become a metabolic liability to the host anemone. Consequently, at elevated thermal regimes, the potentially higher cost of maintaining ZC may preclude them from becoming the dominant algal symbiont (Verde and McCloskey 2001).

Solar radiation of the intertidal zone has at least four separate components: temperature (via infrared), light intensity, light quality, and UV. With regard to symbiont–anemone biology, each of these variables has individual effects and, undoubtedly, interactions with each other. Verde and McCloskey (2001) investigated the effects of only temperature while holding light intensity constant. However, lack of available information on the differences in the physiology, densities, and growth rates of ZX and ZC in *A. elegantissima* as a function of only light intensity, prompted us to explore how this environmental parameter might influence the symbiotic association and explain the microhabitat and geographical distribution of ZX and ZC. In view of the limited number of direct comparisons of biological parameters of ZX and ZC within a single cnidarian species, our objective was to investigate the comparative photosynthetic and physiological capacities of ZX and ZC under varying light regimes. We asked the following:

1. As irradiance increases, does the symbiotic stability of either of the two algal symbionts change?
2. What specific aspects of the symbiotic association are affected and which remain unchanged as a function of light intensity?
3. Can irradiance levels alone explain the natural habitat distributions of the two algal–anemone associations?

Answers to these questions will begin to address the issue of whether irradiance directly influences which algal symbiont will be hosted by *A. elegantissima*.

**Materials and methods**

Most of the methods presented here are provided in detail by Verde and McCloskey (1996b, 2001), dealing with a series of comparative experiments on the ZX– and ZC–*Anthopleura elegantissima* symbiosis. Although the methods are identical, we summarize them here for convenience.

**Collection and maintenance**

*A. elegantissima* harboring exclusively ZX or ZC (McCloskey et al. 1996) were collected from various anemone aggregations on Swirl Rocks, Wash., USA, between March and July 1992. All anemones were collected at an intertidal height between 0.4 and 0.8 m above mean lower low water (0.0 m). The anemones were transported to the Walla Walla College Marine Station, Anacortes, Wash., where all experimental procedures were performed. All foreign material adhering to the anemones (shell hash and macroalgae bits) were removed within 12 h of collection and the anemones were maintained in a 760-L refrigerated aquarium containing natural seawater. Water-flow velocities through the maintenance and experimental tanks were maximized by several large submersible pumps. The temperature of the seawater was maintained at 12 ± 1°C within the aquarium, which approximated ambient water temperature at Swirl Rocks.

**Light regimes**

Within 24 h of collection, each group of anemones was exposed to a specific acclimation irradiance regime (12 h light:12 h dark cycle) for a minimum of 10 days using seven different light intensities: 25, 100, 200, 400, 800, 1,400, and 2,000 μmol m⁻² s⁻¹. A longer acclimation period was not possible, owing to other competing experiments. Nevertheless, we based our rate of chlorophyll turnover in marine algae on the results of Riper et al. (1979) who showed that chlorophyll-α turnover times ranged from 3 to 9 h (see also Prezelin and Matlick 1980). At each irradiance level, six anemones harboring either ZX (n = 6) or ZC (n = 6) were used, except at 1,400 μmol m⁻² s⁻¹ for which the sample size was three for ZX (n = 3) and ZC (n = 3) anemones. The artificial light system consisted of two separate industrial ballasts with GE Multi-Vapor R bulbs (metal halide, 400 W). Specific light intensities were produced by increasing or decreasing the distance between the light source and the anemones, or by utilizing neutral density filters (nylon screens). We conducted the acclimation regime in an increasing order, from 25 to 2,000 μmol m⁻² s⁻¹, which was designed to track the spring-to-summer increase in ambient sunlight. These short-term irradiance experiments were designed to directly compare the photophysiology of ZX and ZC in the host anemone.