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Factors influencing the pedal laceration frequency of a subtropical anemone

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FACTORS INFLUENCING THE PEDAL LACERATION FREQUENCY OF A
SUBTROPICAL ANEMONE

A Thesis
Presented to
the Faculty of Moss Landing Marine Laboratories
San Jose State University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

by
Chad Eric King
December 2003

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ABSTRACT

FACTORS INFLUENCING THE PEDAL LACERATION FREQUENCY OF A SUBTROPICAL ANEMONE

by Chad Eric King

Adaptation of sea anemones to changing environmental parameters such as temperature, irradiance, and disturbance can manifest itself in the modification of individual size and frequency of asexual reproduction. The purpose of this study was to investigate, through manipulative field experiments and observational data, the effects that light, nutrition, and disturbance have on size and asexual reproduction of *Aiptasia californica*, a subtropical zooxanthellate anemone that uses non-geniculate coralline algae as a common substrate within Bahía Concepción, Mexico. Diversity of the symbiont (*Symbiodinium* sp.) was also investigated using restriction fragment length polymorphisms of partial 18S ribosomal DNA.

Results indicate that rhodolith instability is the primary determinant of pedal laceration frequency and biomass of *A. californica*. Biomass increases with depth, where water motion and disturbance decrease. The effects of irradiance and nutrition remain uncertain. Symbiosis occurs with one of five previously described clades of *Symbiodinium*. The adaptive significance of monomorphic symbiosis and worldwide symbioses are discussed.

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more than the money I saved. Their continual support and interest in my work maintained my illusion that the genetics and reproductive behavior of a Mexican cnidarian were actually interesting to anyone else. However, I would like to dedicate this three-year project to my wonderful wife-to-be, Trish. Without her unconditional support, enduring love, and complete understanding during the many times I left her without companionship on those lonely Friday and Saturday nights, I would have never finished. Thank you.

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INTRODUCTION

Sea anemones are a successful group of animals well adapted to changing environmental conditions through various modes of behavioral and physiological responses. Part of their success is related to the occurrence of both sexual and asexual modes of reproduction in many species (Shick et al. 1979). Rapid colonization and propagation of a successful genotype are postulated to be the benefits of asexual reproduction (Hunter 1984).

Sexual reproduction has rarely or never been seen in some species of sea anemones. Thus, population growth within these species has been traditionally thought to be solely controlled by asexual reproduction (Shick and Lamb 1977), although recent literature cast doubt on these observations (Ting and Geller 2000). Differences in asexual reproductive rates and body size could be genetically predetermined (Fautin et al. 1989). However, Shick et al. (1979) suggest that genotypic differences may interact with environmental factors to give the observed effect on body size and reproductive rates. Many studies have examined the effect of environmental factors on the rates of asexual reproduction, including food (Smith and Lenhoff 1976; Hunter 1984; Cook et al. 1992), temperature (Johnson and Shick 1977), the rate of water flow (Shick and

Hoffmann 1980; Anthony and Svane 1994), predation (Shick et al. 1979), burial (Pineda and Escofet 1989) and substratum instability (Anthony and Svane 1995).

Rhodoliths, free-living nongeniculate coralline algae, provide one substratum for the asexual anemone, *Aiptasia californica* in Bahía Concepción, Baja California Sûr, Mexico. Rhodoliths form large aggregations or beds, which are found in temperate, tropical and polar continental shelves throughout the world (Bosence 1983). This anemone is very abundant in rhodolith beds, with densities as high as 300 individuals per m² recorded at Isla El Requésón in April 1990 (Steller 1993). *Aiptasia californica* primarily attaches to hard substrate and is absent in sand, but has been observed on plants such as *Sargassum* sp. and *Caulerpa sertularoides* and shell debris in sandy areas.

Water motion inside the Gulf of California and Bahía Concepción follows a predictable pattern related to seasonal wind strength and direction. During the summer, a high pressure system resides over the southwestern United States, driving winds down the Gulf in a south-eastern direction (Badan-Dangon et al. 1991). A reversal and increase in intensity of the winds occurs during the winter months as a low-pressure system resides over the Sonoran desert, which favors advection of tropical air masses up the gulf. The central region of the Gulf (26 - 28° N), where this study took place, has the highest average surface wind

velocity from November through May (Merrifield et al. 1987; Pares-Sierra et al. 2003) (Figure 1). This correlates well with the seasonality of rhodolith movement frequency (Marrack 1997).

Wind-propagated water motion overturns the rhodoliths, causing them to be highly branched and sometimes spherical (Bosence 1983; Steller and Foster 1995). Rhodoliths occur in a zone of mild water motion because the occasional turning prevents burial by sediment, which would inhibit photosynthesis (Foster et al. 1997). When water motion reaches a certain threshold, the rhodoliths will break up. Therefore, a distinctive zone or bed is formed between the two extremes. The shallow edge of the bed is marked by a band of coralline sand made up of rhodolith fragments, while the deep end of the rhodolith bed gives way to a band of fine sediment and mud. *A. californica* is zooxanthellate, thus burial beneath overturned rhodoliths would inhibit photosynthesis and therefore metabolic input from its symbiotic algae. Destruction of rhodoliths at a threshold of water motion would eliminate substrate.

Food and light availability can be strong factors in shaping an anemone's size and reproductive output, but their individual effects on anemones can vary widely. Light is not required for survival in zooxanthellate anemones, but most live in photic areas, and the mutually beneficial host-symbiont relationship may

be partly responsible for their present success. Zooxanthellate anemones have even been kept in the dark for over 4 years and have been able to survive when food is available, although they become aposymbiotic (Steen 1986).

Photosynthetic byproducts are a part of the zooxanthellate anemones' nutritional input, and it is possible that a change in this portion of the energy budget may affect an anemone's behavior in the same way as a change in its feeding regime (Shick 1991). During periods of food limitation *Aiptasia pellucidus* increases its rate of pedal laceration, creating more ramets per clone and a subsequent smaller biomass. This may increase feeding efficiency, allowing more food to be caught per unit of biomass (Smith and Lenhoff 1976). A contradictory, but common response to a high influx of nutrients is rapid growth to a certain size, followed by asexual reproduction (Minasian 1982). A possible mechanism explaining this latter observation is one of optimal size. Some anemones will grow to a size where the maximum difference between energy intake and metabolic cost occurs (Sebens 1980). Asexual reproduction will then occur to hold the current anemone at the optimum size, and the new daughter anemones will grow again to a similar weight. Starved *Aiptasia pulchella* were found to increase their number of zooxanthellae and decrease their protein biomass with an increase in culture irradiance, but this increase in zooxanthellae

productivity did not result in a proportional increase in sea anemone biomass (Muller-Parker 1985). Fed *A. pulchella* increased their biomass as irradiance increased, but did not increase zooxanthellae density. Muller-Parker (1985) suggested that high irradiance and productivity of zooxanthellae does not necessarily cause increased biomass of the anemone.

Unlike studies on light and food availability, studies on physical disturbance clearly demonstrate an effect on anemone size and reproduction. Numerous secondary substratum studies have looked at mussels and the anemone *Metridium senile*. Mussels and other benthic bivalves are generally stationary, but offer a less stable substratum than bedrock. Clonal *M. senile* living on mussels had a much higher rate of pedal laceration compared to their counterparts on more stable substrata, which explained their smaller size (Anthony and Svane 1995). These higher rates of asexual reproduction were thought to be a response to the small-scale physical disturbance of mussel movement, which may crush or bury part of the epifauna (Anthony and Svane 1995). Large *M. senile* are also more likely to be damaged by moving mussels, which may induce pedal locomotion and laceration, thus suppressing anemone size (Anthony and Svane 1994; Anthony and Svane 1995). As an individual moves across the substrate, a portion of the pedal disc will tear off and grow into

a fully functioning individual. Pedal locomotion and laceration likely require more energy from individual anemones, thereby reducing the amount of energy that can be put into individual ramet growth (Anthony and Svane 1995). Other studies have revealed similar inverse relationships between individual biomass and number of individuals per clone (Johnson and Shick 1977; Shick et al. 1979; Harris 1991). Larger clones of *A. californica* are also likely to be damaged between the moving rhodoliths and may escape mechanical damage and increase survivorship like *M. senile*. Therefore, individuals of *A. californica* that experience fewer disturbances in deeper parts of the rhodolith bed may attain a larger size. Indeed, there may be a direct parallel between the sizes of *M. senile* on rock and mussel and the sizes of *A. californica* on rock and rhodolith.

Symbioses with dinoflagellates (zooxanthellae) are a common occurrence in cnidarian and other marine hosts. Many anemones from a wide range of environments and geographies express this ability to host (Muller-Parker et al. 1996; Saunders and Muller-Parker 1997; Stochaj and Grossman 1997; Swanson and Hoegh-Guldberg 1998; Banaszak et al. 2000; LaJeunesse and Trench 2000; Secord and Augustine 2000; Davy and Cook 2001b; Perez et al. 2001; Sawyer and Muscatine 2001; Verde and McCloskey 2001; Dunn et al. 2002). *Aiptasia californica* was assumed to have such a relationship, as it has a characteristic green-brown

coloration to its tentacles and body. This mutualistic relationship provides nutrients for the host in the form of photosynthate carbon released as a byproduct of photosynthesis, while the symbiont receives inorganic nutrients collected from the host that are crucial to its survival (Wang and Douglas 1998; Goodson et al. 2001; Leggat et al. 2002).

Symbiodinium microadriaticum was traditionally identified by morphology, and until recently, most zooxanthellae were considered to be classified as this one species (Rowan 1991). Recent molecular data suggest that there are at least five distinctive clades (A, B, C, D, E) of *Symbiodinium* (Rowan and Powers 1991b; Baillie et al. 2000; Loh et al. 2001; Rodriguez-Lanetty et al. 2001; Yang 2001; Santos et al. 2002).

Although no correlation between zooxanthellae clade and photosynthetic efficiency has been found (Bythell et al. 1997), different clades of zooxanthellae have been shown to exhibit different algal densities within their host, possibly affecting nutrient translocation (Perez et al. 2001). Other studies have shown that host physiology is affected by the type of symbiont (Fitt 1985). This includes growth and sexual reproduction in *Aiptasia pulchella* (Kinzie and Chee 1979). In addition, the photobiology of *Symbiodinium* sp. in culture shows how different species variably cope with different intensities of light due to dissimilar amounts

of photoprotective pigments (Banaszak and Trench 2001; Kuffner 2002). If a particular strain of zooxanthellae cannot acclimatize to changing conditions (temperature, irradiance, etc.), it may seek a more suitable host, or the host may expel the symbiont to acquire one that is better-adapted; a process within corals known as coral bleaching (Buddemeier and Fautin 1993; Rowan et al. 1997; Dunn et al. 2002; Yentsch et al. 2002).

Coral bleaching is thought to be a complex response by host, symbiont or both to environmental stress or change, including temperature and light intensity. Traditional views of coral symbiosis have assumed that a population of a particular host harbors one species of symbiont. However, a few recent studies have revealed habitat-specific zooxanthellae species that are optimized to the light regime of that habitat (Baker and Rowan 1997; Rowan et al. 1997). For example, the coral species *Montastraea annularis* and *M. faveolata* were found to associate with at least three taxa of *Symbiodinium*. Zooxanthellae were identified by restriction fragment length polymorphism (RFLP) in small ribosomal unit DNA (ssDNA) genes, and grouped into clades A, B, and C. Clade(s) A and/or B were symbionts in shallow water corals (high irradiance), while deep water corals (low irradiance) hosted clade C. Symbionts reestablished the same pattern of zonation in a subsequent transplantation experiment. Spatial heterogeneity in

coral bleaching events most likely occurs due to genetically diverse symbionts (Perez et al. 2001). Polymorphic symbiosis occurs in at least one temperate cnidarian, the anemone *Anthopleura elegantissima*. It has been shown to host two distinct symbionts, *Symbiodinium* (clade B) and unicellular green algae, called zoochlorellae (LaJeunesse and Trench 2000; Secord and Augustine 2000; Verde and McCloskey 2001). Although polymorphic symbiosis is not common in actinarians, this possibility was investigated in *A. californica*, with respect to zooxanthellae.

The objective of this study was to determine the factors that modify the asexual reproductive rate and subsequent biomass of *A. californica* in Bahía Concepción, Baja California Sur, Mexico. The specific questions were: 1) Do fish prey upon *A. californica*? 2) Is there a difference in the rate of pedal laceration in *A. californica* when exposed to varying irradiance and food? 3) Does *A. californica* lacerate more frequently when its vertical orientation is changed often or when exposed to simulated substratum instability? 4) Does the biomass of *A. californica* change with depth? 5) Is there a difference in biomass between individuals of *A. californica* living on primary (rocks, boulders) versus secondary substratum (rhodoliths)? 6) Is there a seasonal difference in the biomass of *A. californica*, related to water motion? and 7) Are there polymorphic symbioses occurring

between zooxanthellae (*Symbiodinium* sp.) and *A. californica* in Bahía Concepción, Baja California Sûr, Mexico?

My hypotheses were that substratum disturbance stimulates pedal laceration and that biomass will increase along a depth gradient in the rhodolith bed from shallow to deep. This hypothesis implicitly states that differences in substratum instability can primarily explain the difference in biomass between *A. californica* living in the deep and shallow portions of rhodolith beds, those occurring on primary and secondary substratum, and a difference in biomass between the winter and summer months in Bahía Concepción, Baja California Sûr, Mexico. Ancillary hypotheses are that increased light intensity will increase pedal laceration rates and that zooxanthellae harbored within *A. californica* in Bahía Concepción will be of the same clade of *Symbiodinium* sp.

METHODS AND MATERIALS

Study Sites

All study sites were located in Bahía Concepción, a large, shallow bay along the eastern side of Baja California Sûr, Mexico (Figure 2). The bay is 36 km long, 3-5 km wide, and oriented in a north/south direction. Water depths range from 10 m at the northern end to 40 m in basins at the southern end. Rhodolith beds up to 1 km long exist on the west side of the bay (Steller and Foster 1995). The deep end of rhodolith beds are characterized by a transition of live rhodoliths to fine silt. The shallow end, where greater wave energy occurs, is characterized by a transition from live rhodoliths to rhodolith and shell fragments. Most of the beach material in the area is comprised of carbonate sand originating from rhodoliths. Only depths from 0 to 12 m were included in this study, and no attempt was made to quantify the area comprised of live rhodoliths around all study sites.

Isla El Coyote and Isla Cueva

Isla El Coyote and Isla Cueva are true islands residing in Bahía Coyote, a bay within Bahía Concepción. (Figure 2). Isla El Coyote (26°43.21' N, 111°52.8' W) is the larger of the two islands, and is closer to the Baja peninsula than Isla

Cueva (26°44.56' N, 111°52.56' W). The bottom surrounding both islands is predominantly rhodolith beds, although the primary distribution of these beds are more abundant on the wave-exposed sides (Steller and Foster 1995).

Isla El Requésón

Isla El Requésón (26°38.66' N, 111°50.07' W) is a kidney-shaped island that is connected to the peninsula via a tombolo, a sand bar periodically covered by high tide. This forms two shallow lagoons on the north and south side of the tombolo. Boats were launched from either lagoon for transportation to the two study sites on opposite sides of the island. One site on the north side of the island was used for the predator-exclusion experiment and one of two transects where *A. californica* size was sampled. The second transect ran on the south-east side of the island. Food and irradiance limitation experiment were done in the lagoon on the north side of the tombolo.

Scallop Aquaculture Site

Some anemones were collected from a rhodolith bed at a former scallop aquaculture site (26°35.39' N, 111°47.4' W) for characterization of zooxanthellae DNA. This site was approximately 0.5 km north of the southern end of Bahía Concepción and is characterized by a very gentle slope and sparse rhodoliths, atypical from the other sites.

Hydrothermal Vent Site

A shallow slip fault is exposed at the surface approximately five miles south ($26^{\circ}41.26' \text{ N}$, $111^{\circ}51.59' \text{ W}$) of Isla Coyote (Ledesma-Vazquez and Johnson 2001). Super-heated water (90°C) and gases from a shallow magmatic source escape through channels in the sediment at depths of 3 to 15 m (Forrest unpub.). Temperature seems to be an important factor in the occurrence of a particular strain of *Symbiodinium* in some hosts (Perez et al. 2001), and the localized increase in ambient water temperature may have an effect on which clade may be best adapted for photosynthesis within *A. californica* residing near vents.

Experimental Studies

Collection of Anemones for Manipulative Experiments

To collect anemones for all experimental treatments, various artificial substrata (PVC pipes and plates) were placed haphazardly in a 5 to 8 m deep rhodolith bed at each experiment's respective location. Although anemones on artificial substrate may not be a random collection, the method was consistent for all treatments. Enumeration and measurements were also more easily made on a less complex substrate as anemones can be difficult to measure inside the

complex thalli of a rhodolith. Satisfactory migration onto the PVC pipes and plates always occurred within two days.

Predator-Exclusion

Three replicates in each of three treatments were constructed to test whether *A. californica* is preyed upon by fish. All three treatments consisted of an acrylic square plate (30.5 cm²) attached to a 20 cm PVC pipe (Figure 3). The PVC pipe attached to the bottom of each treatment raised the plate off of the rhodolith bed substrate to avoid contamination by migrant anemones onto the test rhodoliths. Three anemone-encrusted rhodoliths of similar size were placed in the center of the plate of each treatment and the number of anemones on each rhodolith were counted and recorded.

Effect treatments were bare acrylic plates, so as to completely expose the anemones to predation by fish. Cages were constructed by drilling a series of holes around each plate and using cable ties to attach commercial rain gutter mesh guard around and above the plate. This guard completely protected the anemones from any source of predation. Cage controls were constructed to account for hydrodynamic flow variation caused by the walls of the cage, while allowing predation to occur from above. The controls were constructed with a

perimeter of rain gutter mesh attached to and surrounding the plate. This perimeter wall was approximately 10 cm high.

Each treatment was placed randomly within 5 m of a buoy marker at Isla el Requesón at depths ranging from approximately 6.7 to 7.3 m for five days, beginning on March 21, 2000. Anemones were counted again on March 25, 2000.

Food and Irradiance Limitation

To verify the presence of zooxanthellae, a small portion of a tentacle was excised and placed between a glass slide and cover slip, pressing down to compress the tentacle to a single layer of cells. Examination of the tentacle squash through a fluorescence filter under ultraviolet light in an epifluorescence microscope was used to confirm the presence of zooxanthellae, as chlorophyll fluoresces red (Shick 1991).

To determine the effects of food and irradiance on the asexual reproduction of *A. californica*, three anemone-encrusted rhodoliths of similar size were placed in 20 Gladlock™ transparent plastic containers and were divided into five replicates of four treatments. These treatments exposed anemones to different levels of irradiance and food: 1) light with food; 2) light and starved; 3) dark with food; 4) dark and starved. Anemones were fed anemone food (Micro-Food Brand®, Ocean Star International, Inc., Snowville, UT 84336, USA), a

combination of fish meal, whole egg solids, dried yeast, and fish oils for suspension and filter feeders. Each food treatment was fed once a day according to the manufacturer's suggestions (one scoop; approximately 1 g). Food was not added to the starved treatments. Each of the light treatments were randomly placed in one of two goodie bags (five in each), and dark treatments were placed in plastic garbage bags and tied shut before being placed randomly in each of two other goodie bags. All four goodie bags were placed in the northern lagoon at Isla el Requesón to keep the temperature constant. The water in each treatment was changed daily (raw seawater) as to avoid excessive waste buildup and possible anoxic conditions. The experiment was run from March 21 to 26, 2000. Individuals present in each replicate were counted before and after the experiment.

Zooplankton in raw seawater might have been a food source for the anemones in the starved experiments. Zooplankton would have also metabolized oxygen, possibly causing anoxic conditions. To minimize this possible effect, the first experiment was duplicated from March 27 to 29, 2000, using filtered sea water changed daily. Raw sea water was filtered through a 300 μm plankton net and collected in a bucket for immediate use. Data were

analyzed with separate two-factor ANOVAs on each of the experiments with light and food as the two factors.

Basic parametric assumptions were tested before all data analyses. Homoscedasticity was tested using either Cochran's test of variance or a visual check of the residuals of the data on a scatterplot (Winer 1971; Zar 1999). Normality was tested by running the general linear model and subsequently testing the residuals with a one-sample Kolmogorov-Smirnov test. These assumptions were tested on subsequent data analyses. Any significant differences found in general linear models throughout this study were analyzed with Tukey pairwise comparisons (Zar 1999). Alpha level for all analyses was set at 0.05. Computer statistical software (SYSTAT® Version 10, Systat Software Inc., Richmond, CA 94804, USA) was used for most statistical analyses.

Relationship Between Irradiance and Pedal Laceration

To determine the effects of shading on pedal locomotion and laceration, 3 replicates of 4 shade treatments (heavy shade, light shade, no shade and control) were placed over anemones that had recruited onto 10 cm² PVC panels. To prevent continued anemone immigration and emigration, a mixture of 34 ml of latex paint and 12 cc of copper powder was painted around the edge of each panel. In a trial, the copper paint was deemed to be effective, as not one

anemone migrated onto copper-treated PVC plates, while dozens of adults and lacerates had attached to all other non-treated PVC plates. Each treatment consisted of a 20 cm³ PVC cube structure with a frame on one end (Figure 4). Each of the 4 PVC legs (12.7 mm diameter) driven 5 cm into an area cleared of rhodoliths so that the frame was approximately 15 cm from the substrate and PVC plate. The light shade treatment, which reduced ambient light by approximately 50%, had one layer of black patio shading (4 mm² mesh) stretched across the 20 cm² frame. The dark shade treatment, which reduced ambient light by approximately 75%, had 4 layers of patio shading. A cage with no shading exposed anemones to ambient irradiance, and the cage control compared the effects of no cage and no shading. All treatments were separated by 2 m and randomly interspersed in a line parallel to shore. All lacerates (≤ 1 mm basal disc diameter) were removed from the PVC panels at the beginning of the experiment and number of adult (> 1 mm basal disc diameter) anemones noted. *In situ* treatment irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$) was measured with a spherical underwater quantum sensor (model LI-193SA, LI-COR, Inc., Lincoln, NE 68504 USA) and recorded on a data logger (model LI-1400). Ten readings separated by 10 seconds were made under every replicate between 1100 and 1145 local time on April 1, 2001, a day with no cloud cover. These irradiance measures were

systematically interspersed among treatments throughout the time interval so as to avoid any bias with light conditions through the 45 minute period. Replicates of mean light intensities were nested under treatment and analyzed by a nested analysis of variance. Mean laceration rates were compared with a non-parametric, one-sample Kruskal-Wallis analysis of variance. This experiment ran from March 31 to April 4, 2001.

Relationship Between Overturning and Pedal Laceration

To determine the effects of substratum instability on pedal locomotion and laceration, anemones were recruited onto 24 sets of two 20 cm PVC pipes (12.7 mm diameter) that intersected in the middle to form a cross (Figure 5). All lacerates were removed and the number of starting adult anemones was recorded. These 24 replicates were subject to two different treatments of substratum instability. One treatment was turned vertically 180 degrees every day while the other treatment was not. Each replicate was attached to a 7 cm PVC pipe (12.7 mm diameter) with a PVC coupling joint. This pipe extension was pounded approximately 5 cm into a 10 cm² area cleared of rhodoliths, so that the coupling joint was 1 to 2 cm above the substrate. To prevent anemone immigration and emigration, a mixture of copper powder and white latex paint was applied to the joint. Before each replicate was overturned, the numbers of

adult anemones and lacerates on each PVC bar were recorded and then lacerates were removed. All replicates were randomly interspersed in a line running parallel to shore at a depth of 7 m in a rhodolith bed on the southwest corner of Isla Coyote. Each horizontal bar was oriented in the same direction to reduce the bias of a flow gradient. The numbers of adult anemones and lacerates were recorded after two and five days. Due to potential bias in lacerate mortality and parent anemone migration, mean daily rates of laceration were calculated based on intervals between monitoring rather than being based upon a census before and after the experiment (Anthony and Svane 1994):

$$\text{Laceration rate} = \frac{\Delta N_{lac}}{N_{par}\Delta t}$$

where ΔN_{lac} = number of lacerations produced between days of monitoring, N_{par} = mean number of parents on the panels during that period and Δt = number of days between monitoring. Mean laceration rates were analyzed by a repeated-measures analysis of variance (Zar 1999). This experiment ran from June 19 to June 23, 2001.

Measuring Water Motion

Dissolution rates of plaster blocks (clod cards) as a dimensionless index of water motion are an inexpensive yet reliable way to quantify relative water

motion between sites (Doty 1971). However, traditional clod card mixtures consisted primarily of Plaster-of-Paris and would dissolve in a matter of 24 to 48 hours in the field. The use of slower dissolving materials does not change relative dissolution rates and allows for longer exposure time, which was desired in this type of experiment (Jokiel and Morrissey 1993).

Clod cards were prepared according to the general instructions of Jokiel and Morrissey (1993). These clods were formed from a mixture of powdered plastic resin glue (Weldwood® Brand, DAP, Inc. Baltimore, MD 21224, USA) and commercial wall patching compound (Fixall® Brand, Custom Building Products, Seal Beach, CA 90740, USA). Only one batch was made to control for density variation between different batches. The volume ratios of each compound were as follows: 10.5 parts wall patching compound, 5.1 parts water and 1.2 parts powdered plastic resin glue. Dry ingredients were mixed thoroughly prior to the addition of water, then mixed thoroughly again for several minutes. The resulting mixture was poured into rectangular ice cube trays that served as molds. The volume of each individual mold was approximately 21 ml. The ice cube trays were held on a laboratory vortex mixer and tapped forcefully against the table top to cause any trapped air bubbles to rise to the surface. This vortexing and tapping process was repeated until bubbles no longer surfaced.

The molds were dried at approximately 20°C for 24 hours. Each clod was then removed from its ice cube tray and oven dried at 68°C for 48 hours. Clods were then sanded to bring all of them to a weight of 38.1 ± 2.8 g. Clods were then glued onto waterproof/tearproof paper with waterproof contact cement (Fastbond® 10 Brand Contact Cement, 3M Company, St. Paul, MN 55101, USA). A standard paper hole-puncher was used to punch holes in each of the 4 corners of the water proof paper under each clod. Each clod card was then marked with a unique identification number directly on the clod and the card.

Humidity can affect the weight of each clod card, so all clod cards were equilibrated for another 48 hours in an air-conditioned (low humidity) environment at Moss Landing Marine Laboratories, Moss Landing, California. Each clod card was weighed to the nearest 0.1 g and this weight was written on each paper base. Sixty-six (66) clod cards were randomly selected and assigned to 22 groups of 3. Each group of 3 clod cards was attached to separate pieces (40 cm x 15 cm) of $\frac{1}{4}$ in² plastic mesh (BirdBlock® Brand) at equally-spaced intervals (15 cm) with cable ties through each of the four holes punched through the paper bases (hereto referred as a "clod group").

To calculate relative water motion, three calibration clod cards from the same batch were dissolved for 41 days in 40 l of filtered sea water at 24°C. After

sufficient dissolution, calibration and field clod cards were rinsed with fresh water and air-dried for a minimum of 7 days before measurement on an electronic balance. Water motion and weight loss of a clod card is linear until weight loss exceeds 70% (Jokiel and Morrissey 1993). Any clod cards exceeding this limit were excluded from analyses. Mean daily weight loss of each individual clod card that dissolved in the field was divided by the mean daily weight loss of the three calibration cards to obtain a diffusion factor, which was used to calculate relative water motion between sites. A higher diffusion factor reflects higher water motion.

Manipulative Substrate Instability

To address natural substrate instability and its affect on the rate of pedal laceration, rhodoliths were haphazardly collected and placed into nine, 1.5 m² plots in a 3 x 3 Latin square arrangement with each plot separated by 2 m. The destabilized treatment (n = 3) was manually disturbed with a diver's hand for 60 seconds every other day for 14 days, ensuring complete turning of the plot. Stabilized treatments were covered with 6 mm² plastic mesh (BirdBlock® Brand Protective Mesh Covering, Easy Gardener Inc., Waco, TX 76702, USA) and secured with eight, 22 cm plastic tent stakes. The purpose of the mesh was to prevent rhodoliths from moving. Control plots were left unaltered. The entire

arrangement ranged in depth from 6 m to 8 m on the wave-exposed side of Isla Cueva, and ran from January 7 to January 21, 2002, the time of year when winds are typically strongest. Ten rhodoliths were randomly collected from each plot and placed in containers of 55% ethanol to ensure complete collection of all attached anemones. Wet and ash dry weights of each anemone were recorded at Moss Landing Marine Laboratories. To obtain wet and ash-dry weights, each anemone was rinsed for a minimum of five minutes in deionized water before being blotted three times with Kimwipes® and weighed to 0.001 g with an electronic balance. All anemones were dried at 67°C for 72 hours before being weighed to 0.0001 g. Replicate means were compared by a three factor ANOVA, including within cell and residual error. A test of the hypothesis that all interactions are negligible were tested by dividing the residual mean square error by the within cell mean square error (Winer 1971). The resulting value was compared against the critical value of the *F* distribution for significance.

To account for variable water motion throughout the experiment, three clod cards were randomly placed within a 1.5 m² area at the center of each of the four 'sides' of the Latin square (*N* = 12). Mean diffusion factor (*n* = 4) was compared with a one-way ANOVA.

Descriptive Studies

Relationship Between Depth, Season, and Anemone Biomass

To ascertain a possible relationship between depth, season, and anemone biomass, three 10 m transects were run parallel to shore, using SCUBA, at each of three haphazardly-chosen sites around Isla Coyote in June 2001. One transect was 2 m below the upper margin of the rhodolith bed, one 2 m above the lower margin and one at the middle depth of the bed. Transects were run in the same manner at Isla Coyote and Isla Cueva in January 2002, with an additional transect run along the shallow subtidal rocks (< 2m). The largest anemone within 10 cm of 10 random points along each transect was collected and preserved in 55% ethanol for biomass measurements. In rocky areas, a hammer and chisel were used to remove a portion of volcanic rock on which an individual anemone resided, and then preserved in 55% ethanol.

Mean ash-dry weights from Isla Coyote were log-transformed and compared by depth (rock treatment excluded) and season (January versus June) by a three-factor hierarchical ANOVA (Winer 1971). Location ($n = 6$) was nested under season ($n = 2$), and depth ($n = 3$) was crossed with location. Log-transformed mean ash-dry weights from Isla Coyote and Isla Cueva in January 2002 were compared with a randomized block ANOVA (6 blocks, $n = 4$).

Anemones were collected from unique substrates from within Bahía Concepción to compare qualitative (color, morphology) and quantitative (biomass) traits to individuals collected from rhodoliths and shallow subtidal primary substrate. These substrates were primarily rocks and mangrove tree roots. A pooled regression of wet weight to dry weight from all anemones was made to determine if there was a general, significant relationship between size and weight. Comparison of anemone biomass according to different primary substrata was made with a pooled t-test.

Relationship Between Anemone Biomass and Water Motion

To compare anemone biomass with relative water motion, clod groups were placed at 3 depths each at 3 randomly chosen locations around Isla Cueva and Isla Coyote. Since each of the 6 locations varies in slope and exposure to swells, actual depths of each clod group were not fixed; rather they were standardized to the edges of the rhodolith bed as a relative representation of water motion. Shallow locations were defined as 2 m inside the shallow edge of rhodolith bed (the transition area from rhodoliths to sand and non-living rhodolith fragments). Deep locations were defined as 2 m within the deep edge of the rhodolith bed (the transition area from living rhodoliths to dead rhodoliths covered by mud and silt). Actual depths were recorded for shallow and deep

placements of clod groups. Middle rhodolith bed depths were calculated by subtracting the shallow depth from the deeper and dividing by two.

Ten anemones were randomly collected around clod groups. The largest anemone within 10 cm of 10 random points along each transect was collected and preserved in 55% ethanol for biomass measurements. A simple, linear regression was calculated between the mean diffusion factor of each clod group and individual biomass of anemones associated with each clod group. Clod cards were placed in the field ranging from January 11 to January 24, 2002. The average exposure time for each clod group was 8.8 days $\pm$ 0.19 S.E.

Intraspecific Diversity in Cnidarian-Algal Symbiosis

Collection

To assess the diversity of zooxanthellae species within *A. californica*, 10 anemones were haphazardly collected from eight locations within Bahía Concepción. Anemones were collected from habitats with variable substrate, photic exposure, and water motion (Table 1). Within each location, anemones were separated by a minimum of 5 m.

DNA extraction

Within 12 hours of collection, several tentacles (approximately 25 mg wet weight) were cut from each anemone and placed in a labeled 1.5 ml

microcentrifuge tube containing 0.5 ml genomic DNA isolation reagent (DNAzol® Brand, Molecular Research Center, Inc., Cincinnati, OH 45212 USA) and approximately 0.05g 106 µm acid-washed glass beads (Sigma-Aldrich, Inc., St. Louis, MO 63103 USA). Tentacles were then manually homogenized for 10 to 60 seconds with disposable polypropylene pestles that conformed to the microcentrifuge tube. After transport to Moss Landing Marine Laboratories, samples were slowly rotated for approximately 90 minutes and centrifuged at 11,000 RPM in a microcentrifuge for 10 minutes. Supernatant was transferred to labeled, fresh 1.5 ml microcentrifuge tubes. Next, 0.5 ml of 100% ethanol was added and the tube inverted several times to precipitate DNA, which was then spooled with melted Pasteur pipette tips and transferred to labeled, new 1.5 ml microcentrifuge tubes. For samples without visible strands of DNA, supernatant was transferred instead and centrifuged to pellet precipitate. DNA precipitate or pellets were washed twice with 0.8 ml of 75% ethanol. To resuspend DNA, 0.2 ml 1x TE buffer (1 M Tris-HCl, pH 8, with 100 mM EDTA) was added to each tube and placed in a 55°C water bath for one hour. Genomic DNA was further purified with a kit (DNEasy Tissue Kit, QIAGEN®, Valencia, CA 91355 USA) according to the manufacturer's instructions. Purified genomic DNA (5 µl) was visualized by electrophoresis on a 0.8% agarose gel in 1x TAE stained with

ethidium bromide and compared with 5 µl Lambda/*EcoRI* + *HindIII* marker (Invitrogen Life Technologies, Inc., Carlsbad, CA 92008 USA) for size estimation.

DNA Amplification

Small subunit ribosomal RNA (ssRNA) genes were amplified from extracted genomic DNA using either 'universal eukaryotic' (Rowan 1991; Rowan and Powers 1991a) or 'zooxanthellate-specific' polymerase chain reaction (PCR) primers, designed by Rowan (1991). The universal eukaryotic primers ss5 (5' – GGTGATCCTGCCAGT-AGTCATATGCTTG- 3') and ss3 (5' – GATCCTTCCGCAGGTTCACCTACGGAA-ACC- 3') were used for amplification of host DNA and in separate reactions, ss5 was used in combination with the zooxanthellate-specific primers ss3Z (5' –AGCACTGC- GTCAGTCCG- AATAATTCACCGG- 3') or p3Z (5' –TGCKKCAGTCYRAATWA-TTCACC- 3'). These specific primers are internal to the universal primers and allowed selective amplification of zooxanthellae DNA in the mixture of both organisms' DNA (Figure 6). DNA amplifications were performed using *Taq* DNA polymerase (HotStarTaq® Brand, QIAGEN, Valencia, CA 91355, USA) and a programmable thermal cycler (model # PTC-100®, MJ Research, Inc., South San Francisco, CA 94080 USA). Amplifications (volume = 25 µl) included 1 µl genomic DNA and began with an initial denaturing step of 15 minutes at 94°C, followed by 30 cycles

of the following thermal profile: 1 min at 94°C; 1 min at 57°C; 2 min at 72°C.

Final extension occurred for 10 minutes at 72°C. Amplified DNA (5 µl) was visualized by electrophoresis on a 0.8% agarose gel in 1x TAE stained with ethidium bromide and compared with 5 µl Lambda/*EcoRI* + *HindIII* marker for size estimation.

Characterization of DNA

Amplified DNA was characterized by restriction fragment length polymorphism (RFLP). Ten µl of each sample was digested by 10 units of the restriction endonuclease *TaqI* (New England BioLabs, Inc., Beverly, MA 01915 USA), the enzyme that produced the most restriction fragments in six zooxanthellae genotypes (Rowan and Powers 1991b) The restriction digest mixture also contained 2.85 µl nanopure H₂O, 1.5 µl 10x NEB *TaqI* Buffer, and 0.15 µl 100x mg/ml BSA and was incubated in a 65°C water bath for three hours. A subsequent 10 minute, 80°C water bath stopped the reaction. Digested products (5 µl) were visualized by electrophoresis on a 1.6% agarose in 1x TAE stained with ethidium bromide with 5 µl Lambda/*EcoRI* + *HindIII* and 2.5 µl DNA Ladder I (GeneChoice© Brand, Frederick, MD 21703 USA) as markers for size comparison. Anemone genomic DNA and zooxanthellae genomic DNA and

undigested PCR products were run alongside digested products for further comparison.

RESULTS

Experimental Studies

Predator-Exclusion

The number of anemones in all but one replicate were unchanged, or had increased due to asexual reproduction. The rhodolith placed on one of the effect plates was missing when the experiment ended, presumed to have been pushed off by currents as some of the rhodoliths had moved slightly off center on some of the other plates. It is possible that they suffered piscivorous predation, but this is unlikely since some of the caged rhodoliths had moved as well. Perhaps these rhodoliths should have been secured to their respective plates to prevent loss of subject and data. Regardless of this oversight, only one cage control replicate had a reduction in anemones, and it was only by one (Table 2).

Food and Irradiance Limitation

Preliminary identification of zooxanthellae was made in the field under a 40x microscope and was confirmed by Geller (Moss Landing Marine Laboratories). Unfiltered magnification revealed golden-brown cells indicative of symbiotic algal cells. These structures fluoresced red when excited with ultraviolet light, confirming that they contained chlorophyll (Figure 7).

There was a clear difference in relative change in numbers of anemones between treatments exposed to light versus dark in both trials 1 and 2 (Figure 8). There was a significant difference between light and dark treatments in trial 1 ($p = 0.005$), while food was not significant ($p = 0.69$) with no interaction ($p = 0.54$) (Table 3a). Trial 2 again showed a significant difference in asexual reproduction between light and dark treatments ($p = 0.006$), food was not significant ($p = 0.14$) with no interaction ($p = 0.79$) (Table 3b). Many of the anemones in the dark treatments lost their golden-brown color, and had become a dark gray or purple throughout their body column and tentacles. Anemones also appeared to be more flaccid, much like a partially deflated balloon.

Relationship Between Irradiance and Pedal Laceration

Due to high variability of the mean laceration rates of the shading experiment, there was no detectable difference in laceration rate between treatments ($p = 0.385$, power = 0.10) (Figure 9). Approximately 60 samples per treatment would have been required to meet a desired power of 80%. Irradiance levels were significantly different between all treatments ($p < 0.001$). However, each replicate exhibited a narrow range of light intensities, creating significantly different means between all replicates ($p < 0.001$), with the exception of two pairs

from the control and none from the shade treatments (Figure 10). Eleven of 12 replicates showed a reduction of adult anemones (Table 4).

Relationship Between Overturning and Pedal Laceration

Anemones subject to daily vertical rotation showed a significantly higher rate of pedal laceration ($p = 0.029$) (Figure 11, Table 5). This was the case for each of the two dates when data were recorded, and there was no difference between days within treatments. In fact, each treatment showed a remarkably similar laceration rate between the two dates. There were unequal numbers of adult anemones between replicates at the beginning of this experiment because of low recruitment on several replicates. However, both treatments had a similar total number of beginning adult anemones (turned = 153, stationary = 161). The number of beginning adult anemones ranged from 1 to 28 within each replicate (Table 6).

Manipulative Substrate Instability

Water motion or bioturbation was strong enough to move rhodoliths during this experiment, as rhodoliths from the control plot had spread outside of the plot boundaries after 14 days. The mesh seemed to secure rhodoliths and prevent significant movement within stabilized plots. Rhodoliths were also observed to have rolled back and forth over an approximate 2 cm span during

several dives (pers obs). Water motion, characterized by diffusion factor, was fairly homogeneous throughout the Latin square arrangement ($p = 0.09$, power = 0.21) (Table 7, Figure 12).

Due to unequal subsample sizes, biomass values were randomly chosen from 8 of 9 replicates to equal that of the lowest common subsample size ($n = 15$). Row ($F = 0.20$, $F_{0.05(1) 2,126} = 3.06$, $p > 0.05$) and column Row ($F = 0.53$, $F_{0.05(1) 2,126} = 3.06$, $p > 0.05$) effects were negligible, while treatment had a significant effect Row ($F = 14.8$, $F_{0.05(1) 2,126} = 3.06$, $p < 0.05$) (Table 8a). A partial test of the hypothesis that all interactions are negligible is given by the residual mean square error divided by the within cell mean square error (Winer 1971). This value is 0.18, which is less than the critical $F_{0.05(1) 2,126} = 3.06$, therefore no interactions are present. Post-hoc pairwise comparisons reveal a significantly lower mean biomass in the destabilized treatment (Figure 13, Table 8b).

Descriptive Studies

Relationship Between Depth, Season and Anemone Biomass

Anemone biomass was significantly different between depth ($p < 0.001$), season ($F = 8.35$, $F_{0.05(1) 1,4} = 7.71$, $p < 0.05$) and location (nested within season, $p = 0.04$) (Figure 14, Table 9). However, there was a significant interaction between

depth and location ($p = 0.009$, Figure 15). Separating analyses for each factor, anemones from one location in the January 2002 collection was significantly smaller than the other 5 (one-factor ANOVA $p < 0.001$; Tukey pairwise comparison). Anemones from shallow depths were significantly smaller than deep anemones (one-factor ANOVA $p < 0.001$; Tukey pairwise comparison).

Anemone biomass increased with depth at three locations each around Isla Coyote and Isla Cueva ($p < 0.05$) (Figure 16, Table 10). Multiple comparisons revealed that anemones collected from the intertidal rocks and deep rhodolith beds have a greater biomass (Table 11). Differences between locations were assumed in the randomized block design; therefore no interaction term was calculated.

The regression between wet weight and dry weight was significant ($p < 0.001$), and the relationship was strong ($r^2 = 0.90$) despite the inclusion of several outliers (Table 12, Figure 17). This relationship could be used for future laboratory or field work and enable the repeated measuring of anemones without destroying them.

Comparison of log-transformed anemone biomass from different primary substrata, including rocks and mangrove roots, reveals no significance ($p = 0.388$, power = 0.47) (Figure 18).

Relationship Between Anemone Biomass and Water Motion

Only 15 of the 27 clod cards that were placed around Isla Coyote and 21 of the 27 from Isla Cueva were collected (Table 13). Three clod cards from Isla Cueva had less than 30% of their original mass remaining and were thus omitted from the analysis. Possible reasons for clod card loss include poor visibility, poor underwater navigation, and possible destruction or removal due to trawling and hookah diving by local fisherman (pers obs).

Mean diffusion rates differed little within location, and were thus used as the fixed, independent factor compared to the 10 anemones collected from their respective locations (Figure 19). Regression analysis of variance of the relationship between diffusion factor and dry weight reveals a positive correlation ($p = 0.022$) (Table 14). However, precision of this relationship was very low, with an r^2 of 0.043.

Intraspecific Diversity in Cnidarian-Algal Symbiosis

Locations sampled included common rhodolith beds in northern and southern Bahía Concepción, a rhodolith bed residing in an area of actively venting superheated seawater, shallow *Caulerpa* beds and a clear lagoon experiencing no water motion and high UV exposure. It is important to note, however, that the ambient water temperature surrounding individuals collected

near the shallow water venting site were not significantly higher than non-venting sites. Temperature quickly drops within a meter of localized venting, and no anemones were observed within 10 m of such a vent.

The number of samples collected at each of the eight locations ranged from 8 to 10 for a total of 76. Amplification of zooxanthellae DNA in amounts sufficient for restriction digests occurred in 56 of 76 (74%) samples, and was approximately 1600 to 1650 base pairs, the expected size. Restriction digests were successful in 53 of 56 (95%) samples (some bands cannot be seen due to assumed very weak PCR products) and all appear to have been cut at the same restriction site, producing two bands of approximately 500 and 1030 base pairs, the sum of which corresponds to the size of the undigested PCR product (Figure 20). The 1030 bp fragment is approximately 140 bp longer than the analogous 890 bp fragment indicative of clade B (Rowan and Powers 1991b). The discrepancy arises from the use of the universal primer ss5 in conjunction with the specific primer ss3z. Rowan had used two internal primers, ss5z being 140 bp internal to ss5. Universal amplification produced a product of approximately 1800 base pairs, which *TaqI* cut into two products of approximately 620 and 820 base pairs. Amplification with universal primers followed by *TaqI* digestion produces two fragments of 620 and 820 bp, which can be attributed to anemone

18S rDNA. The sum of these fragments do not equal 1800 bp probably due to a partial digest expressed as a faint bar just below 2000 bp in size. A smaller piece (<200 bp) was probably lost due to inadequate staining or lost in the visual noise of the gel image (Geller, pers com).

DISCUSSION

Predation

Predation upon *A. californica* seems unlikely from experimental evidence and direct observations. Anecdotally, the Spotted sandbass (*Paralbrax maculofasciatus*) and Pacific porgy (*Calamus brachysomus*) were observed to momentarily ingest *A. californica* when offered by SCUBA divers before expelling them. Given the limited observations and experimental evidence, it is still assumed that predation has little effect on anemone locomotion and asexual reproduction. However, passive predation could alter anemone locomotion. The rose urchin (*Toxopneustes roseus*) consumes rhodoliths (James 2000), and this predatory act would probably elicit avoidance behavior by the anemone. Density of *T. roseus* is low (0.1 - 1 individual per 10m²) but concentrated in depths ranging between 7.5 - 12 m (Marrack 1997; James 2000) and it is unlikely that it could radically alter the movement patterns of a significant amount of anemones.

Food and Irradiance

Food, at least in the short term, does not seem to have an effect on asexual reproduction. The food and irradiance limitation experiment suggests that light

was the primary factor causing the observed effect, but the enclosed environment of the experiment could have played a confounding role (Figure 8). This is not to completely rule out food limitation as an ancillary factor, as experiments have shown that anemones cannot rely solely upon the nutritional input from their photosynthetic symbionts (Muller-Parker 1985; Cook et al. 1988; Muller-Parker et al. 1990; Acuna and Zamponi 1995). Other long-term studies have demonstrated that as freshly isolated zooxanthellae become more nutrient deficient, they release a greater percentage of photosynthate to their host (Davy and Cook 2001a). However, this may only be a scenario in oligotrophic waters near the equator. Perhaps an experiment with a longer duration and exclusive focus on food limitation (as opposed to possible confounding with an overwhelming factor such as irradiance) would answer the question more clearly, at least as it pertains to *A. californica*.

Supporting modifiers that may affect the influence of irradiance on asexual reproduction can be quite complex. Differences in the structure and arrangement of zooxanthellae as well as host-symbiont interactions could significantly alter the amount of nutrition translocated from symbiont to host. Algae from *Aiptasia pulchella* appear to adapt to different photic environments by changing photosynthetic unit size, and the amount of chlorophyll within each

species were different between species (Chang et al. 1983). This phenomena may not be ubiquitous because of contrasting studies (Muller-Parker 1984; Muller-Parker 1985). Mycosporine-like amino acids (MAAs) are substances found in most marine organisms, including sea anemones, and are a primary factor in protecting marine organisms from the damaging effects of ultraviolet radiation (Dunlap and Shick 1998; Kuffner 2002). Studies have shown that MAA concentration corresponds with UV levels along a depth gradient in corals collected from the field (Dunlap and Chalker 1986; Banaszak et al. 1998). MAA levels have also been enhanced by increasing the dose of UV radiation for sea anemones (Banaszak and Trench 1995) and dinoflagellates (Lesser 1996).

Although irradiance levels were modified to differ significantly in the food and irradiance limitation experiment, the "dark" treatment was placed in a black garbage bag, which assumedly lowered irradiance levels much below that of the "dark" rhodolith bed plots. Therefore, light levels of the "dark" rhodolith bed plots might not have been reduced to such a point as to affect laceration rates. However, a "threshold" level of irradiance at which the anemones would lacerate more often, would not be valid because individuals exposed to the highest irradiance should be lacerating the most often and subsequently be

smaller. These areas, such the shallow subtidal rocks, and lagoons showed a ten-fold increase in biomass over anemones occurring in the rhodolith beds.

Modifying *in situ* irradiance levels in the irradiance and pedal laceration experiment did not seem to affect laceration rates; rather the structure seemed to be the modifying factor. Although the "dark," "partial shade," and control treatments differed significantly in their measured irradiance, lacerations rates were approximately equal (Figure 9). However, the "no shade" treatment, with light intensity level matching that of the control, had a substantially higher, though not significant laceration rate. With no structure to baffle currents, the 'no shade' plots may have been exposed to higher water velocities, eliciting a slight increase in laceration rate, possibly due to increased photosynthetic rates.

Substrate Instability

Substrate instability, manipulatively administered or inferred from observational data, seems to elicit an increase in pedal laceration and subsequently reduced biomass. However, questions about some of the data should be addressed. In the artificial substrate instability experiment, anemones were left to recruit haphazardly onto experimental units that were randomly distributed within a portion of a rhodolith bed, which resulted in a wide variety

of initial numbers of adult anemones (Table 6). This may have masked an unbiased assessment of the anemone's true behavior under the conditions of substrate instability. However, if many anemones had to be removed from the more populated replicates, a significant amount of data would have been discarded, and the experiment would not have been as robust. Each replicate was normalized by the rate of laceration, but there may have been other valid approaches to this situation. Competition for space could have played a role in modifying laceration rates. While *Anthopleura elegantissima* and *Haliplanella luciae* are known to compete for space with conspecifics (Shick 1991), it is not known if *A. californica* will inter- or intraspecifically compete, nor is there any data on the range and dispersal of different clones throughout the bed. I assume that one or two clones populated the PVC pipes, and aggressive competition for space was unlikely. However, a higher density of anemones will mean a higher chance of contact between individuals, and it is unknown whether this contact will alter movement and/or pedal laceration.

The relatively thin diffusive boundary of zooxanthellae in corals, which allows increased diffusion of waste and nutrients, also explains why water motion has been shown to increase photosynthesis and MAA production (Dennison and Barnes 1988; Jokiel et al. 1997; Kuffner 2002). Zooxanthellae

become nitrogen- and/or carbon-limited when water motion decreases, but when water motion increases, photosynthate production increases, possibly causing an increase in zooxanthellar density and/or growth of the host (Kuffner 2002).

Again, this may only be a supporting factor, as anemones from two habitats (intertidal rocks and mangrove lagoon) are approximately the same size, despite similar irradiance levels and dissimilar water motion, substratum instability seems to be the distinguishing feature between anemones of disparate sizes.

Differences in anemone biomass and the inferred frequency of pedal laceration follow a depth gradient that is replicated among sites and season (Figure 15). This model fits the main working hypothesis that anemones subject to more frequent and intense episodes of substratum motion, which occurs in the shallow rhodolith beds, will lacerate more frequently. Since more energy is put into laceration, lacerating adults will be smaller than those that do not lacerate as often. The data by seasonality fits this hypothesis too, as anemones sampled during the winter, when longer and more intense wind events occur, were smaller than their summer counterparts.

Observational data collected from both Isla Cueva and Isla Coyote in January re-exhibit this correlation between depth and anemone biomass (Figure 16). In addition, anemones residing upon primary substrate (rocks) were an

order of magnitude larger than rhodolith bed anemones. It is the stable substrata that allow anemones to reach a larger size because of a reduced risk of mortality due to mechanical disturbance or dislodgment.

Factors that may change the threshold velocity need to overturn a rhodolith include variation in size, shape, surface area to volume ratio, density, and fouling of rhodoliths, and the substratum interface. Higher water velocities are required to overturn lumpy or laminar rhodoliths (Bosence 1983; Marrack 1997). Although rhodolith morphology was not recorded during this study, Steller and Foster (1995) found that weight, longest dimension and branch density of rhodoliths followed a depth gradient at Isla Coyote, but this was controlled by water motion itself. Thus, the apparent lack of water motion is a reinforcing cycle as it allows rhodoliths in the deep bed margin to grow larger and thus resist episodic water motion further. Rhodoliths packed into the sediment will have a higher threshold velocity as it is more difficult for fluid to force an object out of a densely packed surface layer (Dyer 1986). Rhodoliths can also fit together like jacks as their thalli may interconnect. This can form a large network or patch of rhodoliths that creates a significantly higher threshold velocity (Steller 1993; Marrack 1997). Marrack (1997) also states how sponges, tunicates, foliose algae (2-5 cm) and anemones and other epiphytes not only can

increase drag, but also create a meshwork, joining rhodoliths together, again increasing the movement velocity threshold. The alga *Caulerpa sertularoides* was growing in thick mats on some rhodolith beds during the summer of 2001 and 2002 and in some locations almost completely obscured rhodoliths from view. This algal meshwork, intertwining rhodoliths together, was apparent as many rhodoliths were lifted when a diver would attempt to remove an individual rhodolith from the bed. Marrack (1997) observed this same entrapment of rhodoliths by *C. sertularoides* in another bed within the Gulf of California and comments on its inhibition of rhodolith movement.

Bioturbation can greatly modify vast areas of substrate, and this could have contributed to disturbance frequency. Rays observed during this study included the round stingray (*Urolophus halleri*) and bulls eye stingray (*U. concentricus*) and are a prevalent bioturbators throughout the rhodolith beds in Bahía Concepción (Steller 1993, Steller and Foster 1995). Many ray pits were routinely seen during all SCUBA dives, and this burrowing action may significantly contribute to rhodolith disturbance. Ray pits are created by either the feeding action of jetting water downwards through the mouth and/or gill clefts, and/or the frequently-observed behavior of self-burial (Gregory et al. 1979). Although the frequency of rhodolith bed disturbance by rays was not

estimated, one study estimated that eagle rays (*Myliobatis tenuicaudatus*) effectively disturb any given area every 70 days (Thrush et al. 1991). Given the anecdotal frequency and depth of ray pits observed during this study, there may be a significance that should be further studied. Other fish may be responsible for bioturbation, as Spotted sandbass (*Paralbrax maculatofasciatus*), Pacific porgy (*Calamus brachysomus*) and bullseye puffers (*Sphoeroides annulatus*) have all been observed picking and lifting rhodoliths as they foraged for invertebrates living within the thalli of the rhodoliths, and a stone scorpionfish (*Scorpaena mystes*) was observed making a burrow within a rhodolith bed (Marrack 1997). Sea cucumbers (Steller and Foster 1995), the rose urchin (*Toxopneustes roseus*) (James 2000), seastars and crabs (Marrack 1997) are other possible bioturbators. Marrack (1997) quantified bioturbation events, although the majority of events comprised of a rhodolith momentarily lifting up before settling back down in the same place and orientation.

Routine and episodic anthropogenic events could add to the disturbance rhodolith beds. Trawling is a destructive fishing method, as fish are collected by a towed beam trawl that rakes the seafloor (pers obs). Furthermore, numerous vessels belonging to local fisherman, tourists and scientists drop anchor quite often, causing a singular disturbance, but is quite apparent in popular bays, such

as Bahía Coyote. However, estimation of the frequency and duration of anthropogenic disturbances was not attempted, nor is it assumed that these events are the primary force behind substrate instability or rhodolith bed disturbance within Bahía Concepción.

While substrate instability may reduce biomass by increasing pedal laceration, wave-driven substrate instability is variable. Only significant storms produce the necessary water motion to turn rhodoliths, and these occur primarily in the winter (Merrifield et al. 1987; Pares-Sierra et al. 2003). While a short three or four months of intermittent storm activity may have little direct effect on laceration and body size during the calm portion of the year, the effect may be strong enough to last throughout the year, lasting before individuals in the shallow can grow to a similar size of anemones in the deep. Seasonal experiments were not replicated, but do support this hypothesis.

Symbiosis

Monomorphic symbiosis does not variably interfere with the growth and reproduction in *A. californica* within Bahía Concepción, Mexico. A literature review of 143 occurrences of clade characterization worldwide revealed that actinarians harbor almost exclusively clade B (85%, $n = 13$), with the exception of

Aiptasia pallida (Florida) and *Anemonia viridis* (United Kingdom) which have been found to host clades A and B simultaneously. Of the genus *Aiptasia*, all studies (n = 9) showed a host preference for clade B, with clade A co-existing in one Florida study. Polymorphic symbioses are more common within scleractinian corals, and have exhibited symbiosis with all 5 clades. These studies were dominated by scleractinian corals (73%), most likely due to the contemporary importance of coral bleaching (Figure 21a). Other studies show a correlation between algal genotype and depth in corals (Iglesias-Prieto and Trench 1994; Rowan and Knowlton 1995). Clades A and B are preferentially acquired in shallow corals, plausibly because these clades are better adapted for high irradiance (Baker and Rowan 1997; Rowan et al. 1997). Clade C is found most often in corals residing in deeper waters, but has shown non-specific depths in clams (Baillie et al. 2000) and gorgonians (Coffroth et al. 2001). However, clade B has been preferentially sequestered in gorgonians after initial infection of three free-living clades (Coffroth et al. 2001).

Since all major clades of *Symbiodinium* have been found in all the major oceans, it seems quite likely that *A. californica* either expels all clades except for B after infection, or preferentially acquires clade B. The process of infection in *A. californica* is not known, and the biochemical process is not clear for all hosts

overall. However, other asexually reproducing actinarians have been shown to acquire their zooxanthellae through vertical transmission from parent to daughter (Shick 1991; Loh et al. 2001). What little is known regarding specific symbiont acquisition includes glycoprotein recognition. Glycoproteins covering the algal cell walls are recognized by their host, and modifying these glycoproteins changes the rate at which a host will acquire them (Lin et al. 2000). It is possible that symbiont variability coincides with glycoprotein variability, plausibly controlling how a host "chooses" its clade.

Other species of actinarians have shown the capability to acquire clades A, B and C, with preferential expulsion based upon environmental conditions such as temperature (Perez et al. 2001). Furthermore, clade C has the lowest temperature of photosynthetic inhibition at 32°C (Perez et al. 2001). Clades A and B continued to photosynthesize until 34°C. The highest temperature recorded in Bahía Concepción was 31.5°C (Bahía Coyote, June 2001). It seems plausible that temperatures approach or have exceeded the inhibitive temperature, considering that the warmest temperatures of the year do not occur until August-September (Mitchell et al. 2002). Consequently, harboring clade C might not be the most advantageous. Although this model may fit for the warm summer in Bahía Concepción, it does not explain the lack of clade C observed in

actiniarians in temperate areas. A seasonal transition to another clade also cannot be ruled out.

Latitudinal variation in symbiont specificity is common, but not ubiquitous (Loh et al. 2001; Rodriguez-Lanetty et al. 2001; Verde and McCloskey 2001; Rodriguez-Lanetty and Hoegh-Guldberg 2002). This is not a function of clade availability, but rather host preference. Symbiosis with clade B does not seem to express any particular latitudinal variation (Figure 21b), although it has been suggested in particular host groups and as being better adapted for low-light, low-temperature environments (Rodriguez-Lanetty et al. 2001).

Further confounding the importance of specific clades is the resolution at which they are characterized. RFLP is a rather gross determination of symbiont type, but cannot resolve intra-genotypic variation. Recent random amplified polymorphic DNA (RAPD) analyses have shown several genotypes within clades A and C (Carlos et al. 1999; Yu 2000; Yang 2001). Documentation on intraspecific variation of clade B has not been found, but any possible variability could influence reproduction and growth in *A. californica*.

Intrinsic effects on body size may exist, but it is unlikely that genetic differences of the host caused the observed size differential between the shallow subtidal and throughout the rhodolith bed. More heterozygous *Metridium senile*

tend to be larger (Shick et al. 1979; Shick and Dowse 1985). However, these observations were based on one PGI locus instead of a random analysis of many loci. If this is the case, how heterozygosity manifests itself in a larger body size is unknown (Shick 1991). One theory suggests that higher heterozygosity allows a higher tolerance of changing environmental conditions. To fit this model, the anemones would be mostly heterozygous in the shallow subtidal followed by a gradient of decreasing heterozygosity from the deep to the shallow rhodolith bed.

Analysis of the adaptive significance of living in the shallow portion of the rhodolith bed is difficult. Two species of barnacle and one species of limpet were found to have lower mortality and higher recruitment rates on *Mytilus californianus* than that on primary substratum (Lohse 1993). This pattern may have been caused by the superior recruitment of these three invertebrate species over other sessile invertebrates. The enhanced clonal growth on unstable substrata may render *A. californica* superior to sessile colonial animals in terms of recolonizing small patches on the rhodoliths. A mutualistic relationship between *A. californica* and rhodoliths has not been established, but there are examples of secondary substratum and epibiont mutualism. Mussels provide *Metridium senile* with secondary substratum, while *M. senile* may protect mussels from sea

star predation (Kaplan 1984). *Toxopneustes roseus* preys upon rhodoliths (James 2000), and it is possible that *A. californica* may provide some protection.

Regardless, *A. californica* shows great plasticity and success in habitat choice.

The results from this study suggest that experimental substrate instability and/or mechanical disturbance stimulates *A. californica* to lacerate at higher rates. Therefore, this concept may be applied to the nature of rhodolith beds and how the shallow depth of the bed is subject to greater water motion. This disturbance modifies clonal and individual growth, helping to determine the structure of population of *A. californica* in rhodolith beds. This argument is supported by the significant increase in biomass from shallow to deep and increased laceration rates of anemones from experimentally disturbed substrata. Food and irradiance may have a supporting role in modifying reproduction and growth, but to what extent is not fully resolved. Using restriction fragment length polymorphisms of partial 18S ribosomal DNA, it was also discovered that *A. californica* hosts *Symbiodinium* clade B within Bahía Concepción, although the adaptive significance of this symbiont preference could not be ascertained. Reproduction and growth should not be variably influenced by this monomorphic symbiosis.

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TABLES

Table 1. Collection sites for restriction fragment length polymorphism.

n	Latitude 26° N	Longitude 111° W	Location	Depth (m)	Shade (y/n)	Substrate
10	43.210'	53.229'	South Isla Coyote	2-3	n	rhodolith
9	43.210'	53.229'	South Isla Coyote	1	n	volcanic rock
10	43.210'	53.229'	South Isla Coyote	7-8	n	rhodolith
10	44.494'	52.502'	South Isla Cueva	15-16	n	rhodolith
10	35.390'	47.400'	Aquaculture site	4-5	n	rhodolith
10	45.750'	53.520'	Santispac lagoon	≤1	y	mangrove root
8	45.750'	53.520'	Santispac lagoon	≤1	n	variable (not mangrove root)
9	44.556'	51.590'	Vent	1-2	n	rhodolith and volcanic rock

Table 2. Results for the predator exclusion experiment. March 21 cells show the number of anemones on each rhodolith at the beginning of the experiment in that treatment. March 25 cells show the number of anemones on each rhodolith at the end of experiment in each treatment. Effect treatments exposed anemones to predation without any cage structure on an open platform; Cage treatment prevented predation; and Cage Control exposed anemones to predation, controlling for a possible cage effect with 4 walls surrounding anemone-encrusted rhodoliths. The columns labeled "Change" show the number of anemones gained or lost during the experiment. n = 3 for each treatment. ND = No data.

Effect			Cage			Cage Control		
Mar. 21	Mar. 25	Change	Mar. 21	Mar. 25	Change	Mar. 21	Mar. 25	Change
5	ND	ND	6	6	0	8	7	-1
6	9	+3	3	6	+3	2	2	0
12	12	0	3	3	0	9	12	+3

Table 3. Results of food and light limitation experiment that tested effects of food and light on the frequency of anemone pedal laceration. a) Trial 1 with raw seawater, changed daily. b) Trial 2 with filtered seawater, changed daily.

a

Source	SS	df	MS	F	p
Light	2.074	1	2.074	10.72	0.005
Food	0.031	1	0.031	0.162	0.693
Light*Food	0.074	1	0.074	0.385	0.544
Error	3.095	16			

b

Source	SS	df	MS	F	p
Light	2.713	1	2.713	9.81	0.006
Food	0.671	1	0.671	2.42	0.139
Light*Food	0.021	1	0.021	0.077	0.785
Error	4.424	16			

Table 4. Raw data from irradiance and pedal laceration experiment that tested the effect of overturning on the frequency of anemone pedal laceration. Counts of adult anemones (AA) and lacerates (Lac) from the shading experiment. AAI = Initial number of adult anemones, AA2 = number of adult anemones after 2 days, AA4 = number of adult anemones after 4 days, Lac2 = number of lacerates after 2 days, Lac4 = number of lacerates after 4 days. Note the reduction of adult anemones in 11 out of 12 replicates.

Treatment	Replicate	AAI	AA2	AA4	Lac2	Lac4
Dark	1	6	3	2	1	0
	2	7	4	5	1	5
	3	9	5	4	1	5
Partial	1	7	7	6	3	1
	2	5	3	4	3	2
	3	4	1	0	1	0
No Shade	1	2	0	1	1	3
	2	2	0	0	0	0
	3	4	2	1	0	2
Control	1	2	1	1	0	0
	2	4	2	2	4	0
	3	4	1	1	2	1

Table 5. Univariate repeated measures analysis of variance examining difference in laceration rate between treatments (turned and stationary) and duration (2nd and 5th days of observation).

Between Subjects

Source	SS	df	MS	F	p
Treatment	0.018	1	0.018	5.473	0.029
Error	0.072	22	0.003		

Within Subjects

Source	SS	df	MS	F	P
Duration	0	1	0	0.145	0.707
Duration x Treatment	0	1	0	0.024	0.877
Error	0.04	22	0.002		

Table 6. Raw data from simulated substrate instability experiment that tested the effect of overturning on the frequency of anemone pedal laceration. Total number of adult anemones (AA), lacerates (# lacerates), duration of experiment to recording data (# days) and laceration rate (Lac/AA/D = total lacerates divided by average AA divided by number of days).

Turned Replicates	Start	2 Days				5 Days			
	AA	# days	AA	# lacerates	Lac/AA/D	AA	# days	# lacerates	Lac/AA/D
T1	1	2	1	0	0.000	1	3	0	0.000
T2	22	2	16	6	0.079	16	3	2	0.041
T3	10	2	9	2	0.053	7	3	2	0.094
T4	20	2	15	4	0.057	11	3	3	0.089
T5	17	2	13	2	0.033	13	3	1	0.025
T6	8	2	7	2	0.067	6	3	0	0.000
T7	5	2	5	0	0.000	3	3	0	0.000
T8	13	2	13	10	0.192	13	3	5	0.120
T9	16	2	16	4	0.063	12	3	1	0.027
T10	14	2	17	7	0.113	7	3	1	0.046
T11	7	2	6	2	0.077	4	3	3	0.244
T12	20	2	15	7	0.100	14	3	3	0.069
Total Mean	153	2	133	46	0.069	107	3	21	0.063
Stationary Replicates	Start	2 Days				5 Days			
	AA	# days	AA	# lacerates	Lac/AA/D	AA	# days	# lacerates	Lac/AA/D
N1	7	2	7	0	0.000	2	3	0	0.000
N2	16	2	9	1	0.020	11	3	3	0.090
N3	13	2	11	1	0.021	7	3	3	0.142
N4	8	2	6	0	0.000	3	3	0	0.000
N5	11	2	7	1	0.028	4	3	0	0.000
N6	7	2	6	1	0.038	7	3	0	0.000
N7	3	2	3	1	0.083	1	3	0	0.000
N8	17	2	11	2	0.036	8	3	0	0.000
N9	28	2	27	6	0.055	27	3	4	0.049
N10	18	2	14	1	0.016	20	3	0	0.000
N11	19	2	17	2	0.028	14	3	2	0.047
N12	14	2	9	1	0.022	4	3	0	0.000
Total Mean	161		127	17	0.029	118		12	0.027

Table 7. Analysis of variance of mean diffusion factor (water motion) between the four sides of the substrate instability Latin square arrangement (n = 4). This experiment tested the effect of simulated substratum instability on the frequency of anemone pedal laceration

Source	SS	df	MS	F	p
Location	43.569	3	14.523	3.082	0.090
Error	37.691	8	4.711		

Table 8. a) Analysis of variance of mean anemone biomass exposed to three disturbance levels (stabilized, destabilized, control) in the Latin square substrate instability experiment. 15 subsamples were nested within each plot (M = 135). This experiment tested the effect of simulated substratum instability on the frequency of anemone pedal laceration. Within cell variation accounts for subsample variation. Critical F-value for row, column, treatment and residual is $F_{0.05(1) 2,126} = 3.06$. b) P values of Tukey pairwise comparisons of disturbance treatments.

a

Source	SS	df	MS	F	p
Row (A)	0.218	2	0.109	0.198	> 0.05
Column (B)	0.584	2	0.292	0.531	> 0.05
Treatment (C)	16.29	2	8.148	14.80	<< 0.001
Residual	0.197	2	0.098	0.179	
Within cell	69.35	126	0.550		
Total	86.65	134	0.646		

b

Treatment	control	destabilized	stabilized
control	1.00		
destabilized	<< 0.001	1.00	
stabilized	0.76	<< 0.001	1.00

Table 9. Hierarchical, nested three-way analysis of variance of mean anemone biomass season, depth and location (M = 175). This experiment tested the effect of simulated substratum instability on the frequency of anemone pedal laceration.

Source	SS	df	MS	F	p
Season	11.309	1	11.308	8.35	< 0.05
Depth	10.827	2	5.413	10.256	< 0.001
Season x Depth	0.570	2	0.285	0.540	0.583
Location(Season)	5.416	4	1.354	2.565	0.040
Depth x (Location(Season))	11.202	8	1.400	2.652	0.009
Error	82.871	157	0.527		

Table 10. Randomized block design analysis of variance of mean anemone biomass by depth (intertidal rocks, shallow, middle and deep) at 6 locations collected in January 2002 (M = 236). This analysis looked to correlate depth, location and season with anemone biomass.

Source	SS	df	MS	F	p
Location	22.096	5	4.419	7.142	<<0.001
Depth	183.455	3	61.151	13.84	<.05
Error	140.446	227	0.618		

Table 11. Tukey multiple comparisons (P values) of mean biomass among four depths collected in January 2002. Mean biomass of anemones collected from the intertidal rocks and deep rhodolith beds are significantly different. This analysis looked to correlate depth with anemone biomass.

Depth	deep	middle	rocks	shallow
deep	1.000			
middle	0.027	1.000		
rocks	< 0.001	< 0.001	1.000	
shallow	< 0.001	0.117	< 0.001	1.000

Table 12. Regression analysis of variance of wet weight versus ash-dry weight for all 1036 anemones collected for all manipulative experiments measuring biomass.

Source	SS	Df	MS	F	p
Regression	117014.2	1	117014.2	9080.2	<< 0.001
Residual	13312.0	1033	12.89		

Table 13. Clod cards pre and post-dissolution rates. 39 cards placed in the field were recovered and 3 of these (in italics) exceeded the 70% weight loss threshold and were excluded from analyses. Three calibration clod cards are shown at the bottom of this table. DF = diffusion factor.

ID	Island	Site no	Bed depth	Depth (m)	Date in (2000)	Days out	Initial weight (g)	Final weight (g)	Weight loss per day (g)	DF
49b	Coyote	1	middle	6.1	1/16	8	37.60	23.70	0.05	41.81
63b	Coyote	1	middle	6.1	1/16	8	41.00	29.00	0.04	36.10
45b	Coyote	1	middle	6.1	1/16	8	40.00	29.00	0.03	33.09
37b	Coyote	1	shallow	2.1	1/16	8	37.40	18.70	0.06	56.25
61b	Coyote	1	shallow	2.1	1/16	8	40.10	22.40	0.06	53.24
53b	Coyote	1	shallow	2.1	1/16	8	38.50	24.30	0.05	42.71
47b	Coyote	2	middle	5.5	1/15	9	40.80	26.80	0.04	37.43
33b	Coyote	2	middle	5.5	1/15	9	39.50	26.70	0.04	34.22
56b	Coyote	2	middle	5.5	1/15	9	37.00	24.50	0.04	33.42
50b	Coyote	2	shallow	2.1	1/15	9	36.70	16.40	0.06	54.28
58b	Coyote	2	shallow	2.1	1/15	9	38.60	19.50	0.05	51.07
43b	Coyote	2	shallow	2.1	1/15	9	40.10	21.40	0.05	50.00
1b	Coyote	3	shallow	2.1	1/16	8	42.10	25.30	0.05	50.53
9b	Coyote	3	shallow	2.1	1/16	8	38.80	22.20	0.05	49.93
25b	Coyote	3	shallow	2.1	1/16	8	40.40	25.90	0.04	43.62
2b	Cueva	1	deep	12.8	1/17	7	40.70	28.00	0.04	43.66
26b	Cueva	1	deep	12.8	1/17	7	40.60	29.60	0.04	37.81
32b	Cueva	1	deep	12.8	1/17	7	42.10	31.20	0.04	37.47
2b	Cueva	1	middle	6.1	1/17	7	39.80	25.40	0.05	49.50
57b	Cueva	1	middle	6.1	1/17	7	37.00	24.00	0.05	44.69
59b	Cueva	1	middle	6.1	1/17	7	38.30	26.50	0.04	40.56
39b	Cueva	2	deep	12.5	1/11	10	39.70	22.10	0.04	42.35
28b	Cueva	2	deep	12.5	1/11	10	40.50	23.50	0.04	40.91
7b	Cueva	2	deep	12.5	1/11	10	38.50	21.60	0.04	40.67
16b	Cueva	2	middle	6.1	1/11	10	39.00	24.20	0.04	35.61
21b	Cueva	2	middle	6.1	1/11	10	41.90	27.80	0.03	33.93
40b	Cueva	2	middle	6.1	1/11	10	37.80	24.70	0.03	31.52
14b	Cueva	2	shallow	2.4	1/11	10	40.00	19.40	0.05	49.57
15b	Cueva	2	shallow	2.4	1/11	10	39.30	21.00	0.05	44.04
35b	Cueva	2	shallow	2.4	1/11	10	36.70	20.70	0.04	38.50

ID	Island	Site no	Bed depth	Depth (m)	Date in (2000)	Days out	Initial weight (g)	Final weight (g)	Weight loss per day (g)	DF
36b	Cueva	3	deep	9.8	1/11	10	40.10	20.40	0.05	47.41
4b	Cueva	3	deep	9.8	1/11	10	40.20	21.90	0.05	44.04
34b	Cueva	3	deep	9.8	1/11	10	39.40	23.00	0.04	39.46
27b	Cueva	3	shallow	2.4	1/11	10	42.00	22.00	0.05	48.13
8b	Cueva	3	shallow	2.4	1/11	10	38.90	19.30	0.05	47.16
42b	Cueva	3	shallow	2.4	1/11	10	38.80	22.00	0.04	40.43
12b	Cueva	1	shallow	2.1	1/17	7	39.10	7.60	0.12	108.3
60b	Cueva	1	shallow	2.1	1/17	7	39.20	9.00	0.11	103.8
38b	Cueva	1	shallow	2.1	1/17	7	37.50	8.10	0.11	101.1

Calibration clod cards:

ID	Days out	Initial weight (g)	Final weight (g)	Weight loss per day (g)
24b	41	41.5	25.9	1.60
44b	41	38.9	22.1	1.76
19b	41	41.8	23.9	1.75
			Mean	1.70

Table 14. Regression of water motion (diffusion factor) versus anemone biomass (M = 118).

Source	SS	df	MS	F	p
Regression	2.989	1	2.989	5.330	0.022
Residual	65.051	116	0.561		

FIGURES

Figure 1. Monthly average surface wind velocity for the central (26 - 28°N) region of the Gulf of California from September 1996 to September 1999. Average magnitude for winter months (December – February) was 4.93 ms^{-1} and the average direction was 333.5° , reflected by the orientation of the lines. Reproduced from Pares-Sierra et al. (2003).

Figure 2. Locations of experimental and descriptive studies in Bahía Concepción, Baja California Sur, Mexico.

Figure 3. Treatments used for the predator-exclusion manipulative experiment.
 $n = 3$ for each treatment.

Figure 4. Shading treatments and recruitment plates used to study the relationship between irradiance and pedal laceration. Treatments varied in thickness of material stretched across the top. Control plate was not exposed to shading. N = 3 for each treatment

Figure 5. Experimental units used in the substratum orientation experiment. One treatment experienced 180° rotation daily for five days.

Figure 6. Internal and universal primer ss3Z

Figure 7. Chlorophyll in zooxanthellae excited by ultraviolet light.

Figure 8. Percent change in number of individuals relative to light and food ($\bar{x}$ - SE; $n = 5$). Trial 1 used raw seawater, changed daily; Trial 2 used filtered seawater, changed daily.

Figure 9. Mean laceration rate ($\times 100$) ($\bar{x} - \text{SE}$; $n = 3$) plotted against irradiance levels for respective treatment

Figure 10. Mean light intensity ($\bar{x} \pm \text{SE}$; $n = 12$) by replicate from irradiance and pedal laceration experiment ($n = 12$). Mean values differed significantly between all replicates, with the exception of C1 with N1 and C2 with N3. C = control treatment, D = dark or shaded treatment, P = partial shade treatment, N = no shade treatment. Error bars represent standard deviation.

Figure 11. Substrate orientation experiment. Mean number of lacerates per adult anemones (AA) per day ($\bar{x}$ - SE; $n = 12$).

Figure 12. Mean diffusion factor calculated from 'clod cards' between the four sides of the substrate instability experiment ($\bar{x}$ - SE; $n = 3$).

a

b

Figure 13. a) Two-dimensional representation of mean biomass of anemones subjected to three treatments of disturbance ($\bar{x}$ - SE; $M = 135$). Groups underlined at the same level are not significantly different by the Tukey comparison. b) Three-dimensional representation of the same data in its spatial context of the Latin square. White columns represent control plots, grey columns represent stabilized plots, and cross-hatched columns represent destabilized plots. Error bars not shown.

Figure 14. Mean biomass of *Aiptasia californica* by rhodolith bed depth and by season ($\bar{x}$ - SE; M = 175). The treatment effect was significant by depth and by season. Depths underlined at the same level are not significantly different by the Tukey comparison.

Figure 15. Mean biomass of *Aiptasia californica* by rhodolith bed depth and location ($\bar{x}$ - SE; $n = 175$). The significant interaction between location and season originates from the middle bed depth from locations 4 and 6. Solid lines represent June 2001 samples; dotted lines represent January 2002 samples. Numbers in legend represent 6 locations.

Figure 16. Mean log-transformed biomass by depth from 6 locations within Bahía Coyote ($\bar{x}$ - SE; M = 236). Rocks and deep are significantly different. Groups underlined at the same level are not significantly different by the Tukey comparison.

Figure 17. Regression of anemone wet weight to dry weight (pooled, $M = 1035$). Regression is linear and slope is not zero ($p \ll 0.001$).

Figure 18. Comparison of anemone biomass by primary substrate type ($\bar{x}$ - SE). Mangrove roots, n = 44; rocks, n = 59.

Figure 19. Regression of dry weight subsamples (within diffusion factor). Each sample is represented by open circles, while the mean of these values ($n = 3$) at each diffusion factor is represented by triangles. Square-root transformed regression equation is $Y = 3.24 - 0.286x$, $r^2 = 0.043$.

Figure 20. Comparison of zooxanthellae and host DNA using ssRNA gene RFLP. Zooxanthellae genes were amplified using a combination of universal and specific primers and digested with the restriction enzyme *TaqI*. Unmarked lanes are the samples showing one distinct genotype. Genechoice marker (G); restriction digest of anemone PCR product (D); zooxanthallae PCR product (Z); anemone PCR product (A), *EcoRI* marker (E); negative control (N); blank (B); Lambda/*EcoRI* + *HindIII* marker (E).

Figure 21. a) Map of literature review representing the variety of hosts surveyed. Scleractinian corals comprised 73% of this literature survey. b) Map of clades characterized worldwide (n = 143) from literature review. Clade C is dominant, making up 49% of the clades surveyed.

APPENDICES

Appendix A. Literature review of worldwide Symbiodinium sp. clades and their hosts.

Host	Location	Clade	Reference
Phylum Cnidaria Class Anthozoa Order Actinaria			
<i>Aiptasia californica</i>	Bahía Concepción, Mexico	B	This publication
<i>Aiptasia tagetes</i>	Puerto Rico	B	(Banaszak et al. 2000)
<i>Aiptasia pallida</i>	Key Largo, Fl Bermuda Florida Keys Bermuda	B B A B	(Hill and Wilcox 1998) (Rowan and Powers 1991a) Coffroth, unpub. (Rowan and Powers 1992)
<i>Aiptasia pulchella</i>	Hawaii Oahu, Hawaii Taiwan	B B B	(Banaszak et al. 2000) (Rowan and Powers 1991a) (Yang 2001)
<i>Aiptasia tagetes</i>	Puerto Rico	B	(Banaszak et al. 2000)
<i>Anemonia viridis</i>	Cornwall, UK	A	(Bythell et al. 1997)
<i>Anthopleura elegantissima</i>	Pacific Grove, CA	B	(Rowan and Powers 1991a)
<i>Anthopleura xanthogrammica</i>	Pacific Grove, CA	B	(Rowan and Powers 1991a)
Class Anthozoa Order Alcyonacea			
<i>Sinularia lochmodes</i>	Okinawa, Japan	C	(Yang 2001)
Class Anthozoa Order Gorgonacea			
<i>Plexaura kuna</i>	San Blas, Panama	B	(Coffroth et al. 2001)
<i>Pseudoplexaura porosa</i>	San Blas, Panama	B	(Coffroth et al. 2001)
Class Anthozoa			

Host	Location	Clade	Reference
Order Heliporacea			
<i>Heliopora coerulea</i>	Southern Taiwan	C	(Yang 2001)
Class Anthozoa Order Scleractinia			
<i>Acropora cervicornis</i>	Caribbean	A,C A	(Baker and Rowan 1997) (Rowan and Powers 1991a)
<i>A. digitifera</i>	Southern Taiwan	C	(Yang 2001)
<i>A. formosa</i>	Tahiti	A	(Darius et al. 2000)
<i>A. intermedia</i>	Southern Taiwan	C	(Yang 2001)
<i>A. latistella</i>	Southern Taiwan	C	(Yang 2001)
<i>A. longicyathus</i>	Australia Malaysia Japan	A,C C C	(Loh et al. 2001)
<i>A. palifera</i>	Southern Taiwan	C,E	(Yang 2001)
<i>A. palmata</i>	Caribbean	A A	(Baker and Rowan 1997) (Rowan and Powers 1991a)
<i>Agaricia agaricites</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Agaricia danae</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Agaricia fragilis</i>	Caribbean	C	(Hill and Wilcox 1998)
<i>Agaricia lamarcki</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Agaricia tenuifolia</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Astrangia danae</i>	Caribbean	B	(Rowan and Powers 1991a)
<i>Astreopora myriophthalma</i>	Southern Taiwan	C	(Yang 2001)
<i>Cyphastrea ocellina</i>	Hawaii	C	(Rowan and Powers 1991a)
<i>Diploria clivosa</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Diploria labyrinthiformis</i>	Caribbean	B,C	(Baker and Rowan 1997)
<i>Diploria strigosa</i>	Caribbean	B	(Baker and Rowan 1997)

Host	Location	Clade	Reference
<i>Eusmilia fastigiata</i>	Caribbean	B	(Baker and Rowan 1997)
<i>Favia fragum</i>	Caribbean	B B	(Baker and Rowan 1997) (Rowan and Powers 1991a)
<i>Fungia paumotensis</i>	Tahiti	C	(Darius et al. 2000)
<i>F. scutaria</i>	Tahiti	A,C	(Darius et al. 2000)
<i>Galaxea fascicularis</i>	Red Sea	A	(Darius et al. 2000)
<i>Gardineroseris planulata</i>	Panama	C	(Baker and Rowan 1997)
<i>Goniastrea aspera</i>	Thailand	E	(Brown et al. 2000)
<i>Isophyllastrea rigida</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Leptastrea transversa</i>	Tahiti	C	(Darius et al. 2000)
<i>Leptoseris cucullata</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Madracis decactis</i>	Curaçao Panama	B B	(Diekmann et al. 2001) (Baker and Rowan 1997)
<i>Madracis formosa</i>	Curaçao	B	(Diekmann et al. 2001)
<i>Madracis mirabilis</i>	Curaçao Caribbean Caribbean	B B B	(Diekmann et al. 2001) (Baker and Rowan 1997) (Rowan and Powers 1991a)
<i>Madracis pharensis</i>	Curaçao	B	(Diekmann et al. 2001)
<i>Madracis senaria</i>	Curaçao	B	(Diekmann et al. 2001)
<i>Meandrina meandrites</i>	Jamaica Caribbean	A B	(McNally et al. 1994) (Baker and Rowan 1997)
<i>Montastraea annularis</i>	Curaçao Panama San Blas, Panama	C A,B,C A,B	(Diekmann et al. 2001) (Rowan and Knowlton 1995) (Rowan et al. 1997)

Host	Location	Clade	Reference
<i>Montastraea cavernosa</i>	Bermuda	C	(Billinghurst et al. 1997)
<i>Montastraea curta</i>	Tahiti	B	(Darius et al. 2000)
<i>Montastraea faveolata</i>	Panama	A,B,C A,B	(Rowan and Knowlton 1995) (Rowan et al. 1997)
<i>Montastraea franksii</i>	Panama Florida	C C	(Rowan and Knowlton 1995) (Hill and Wilcox 1998)
<i>Montipora cactus</i>	Penghu Islands	C,E	(Yang 2001)
<i>M. digitata</i>	Southern Taiwan	C	(Yang 2001)
<i>Montipora patula</i>	Hawaii	C	(Rowan and Powers 1991a)
<i>Montipora verrucosa</i>	Hawaii Hawaii	C C	(Rowan and Powers 1991a) (Carlos et al. 1999)
<i>Montipora</i> sp.	Southern Taiwan	C	(Yang 2001)
<i>Mycetophyllisa ferox</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Oulastrea crispata</i>	Penghu Islands	E	(Yang 2001)
<i>Pavona cactus</i>	Tahiti	C	(Rowan and Powers 1991a)
<i>Pavona clavus</i>	Panama	C	(Baker and Rowan 1997)
<i>Pavona duerdeni</i>	Hawaii	C	(Rowan and Powers 1991a)
<i>Pavona gigantea</i>	Panama	C	(Baker and Rowan 1997)
<i>Pavona varians</i>	Panama	C	(Baker and Rowan 1997)
<i>Plesiastrea versipora</i>	Port Jackson, Australia Batemans Bay, Australia	B C	(Rodriguez-Lanetty et al. 2001) (Rodriguez-Lanetty et al. 2001)
<i>Pocillopora damicornis</i>	Panama Penghu Islands	C C	(Baker and Rowan 1997) (Yang 2001)
<i>Pocillopora elegans</i>	Panama	C	(Rowan and Powers 1991a)
<i>Pocillopora</i>	Panama	C	(Baker and Rowan 1997)

Host	Location	Clade	Reference
<i>eydouxii</i>			
<i>Pocillopora verrucosa</i>	Tahiti	C	(Darius et al. 2000)
<i>Porites astreoides</i>	Caribbean	A,C	(Baker and Rowan 1997)
<i>Porites colonensis</i>	Caribbean	A	(Baker and Rowan 1997)
<i>Porites compressa</i>	Hawaii	C	Baker and Rowan 1997
<i>Porites divaricata</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Porites furcata</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Porites lobata</i>	Panama	C	(Baker and Rowan 1997)
<i>P. lutea</i>	Southern Taiwan	C	(Yang 2001)
<i>Porites panamensis</i>	Panama	C	(Baker and Rowan 1997)
<i>Porites porites</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Psammocora stellata</i>	Panama	C	(Baker and Rowan 1997)
<i>Psammocora superficialis</i>	Panama	C	(Baker and Rowan 1997)
<i>Seriatopora hystrix</i>	Australia Malaysia Japan	C D C	(Loh et al. 2001)
<i>Siderastrea sidereal</i>	Caribbean	C	(Baker and Rowan 1997)
<i>Stephanocoenia michelinii</i>	Caribbean	C A,C	(Diekmann et al. 2001) (Baker and Rowan 1997)
Class Anthozoa Order Zoanthiniaria			
<i>Zoanthus sociatus</i>	Jamaica Jamaica	A A	(Sadler et al. 1992) (Rowan and Powers 1992)
<i>Zoanthus sp.</i>	Australia	A	(Carlos et al 1999)
Class Hydrozoa Order Milleporina			

Host	Location	Clade	Reference
<i>Millepora</i> sp.	Southern Taiwan	C	(Yang 2001)
Class Scyphozoa Order Rhizostomeae			
<i>Cassiopea xamachana</i>	Jamaica	A	(Rowan and Powers 1992)
<i>Cassiopea</i> sp.	Australia	B	(Carlos et al. 1999)
Phylum Mollusca Class Bivalvia Order Heteroconchia			
<i>Corculum cardissa</i>	Palau	A	(McNally et al. 1994)
	Palau	A	(Carlos et al. 1999)
<i>Fragum fragum</i>	Palau	C	(Carlos et al. 1999)
<i>F. unedo</i>	Palau	A,C	(Carlos et al. 1999)
<i>Hippopus hippopus</i>	Phillippines	A	(Carlos et al. 1999)
<i>Sarcophyton glaucum</i>	Australia	A	(Carlos et al. 1999)
<i>Tridacna crocea</i>	Phillippines	A	(Carlos et al. 1999)
<i>T. gigas</i>	Palau	A,C	(Carlos et al. 1999)
Phylum Protozoa Class Granuloreticulosea Order Foraminifera			
<i>Amphisorus hemprichii</i>	Red Sea	A	(Lee et al. 1995)
	Palau	A	(Carlos et al. 1999)
<i>Marginopora kudakajimensis</i>	Japan	C	(Lee et al. 1995)
<i>Sorites orbiculus</i>	Polynesia	C	(Langer and Lipps 1994)
Phylum Porifera Class Demospongiae Order Haplosclerida			

Host	Location	Clade	Reference
<i>Haliclona koremella</i>	Palau	E	(Carlos et al. 1999)
Free living	Hawaii	A	(Carlos et al. 1999)

Appendix B. Summary of clades surveyed by host Order. Scleractinian corals comprised 73% of the 143 samples in this literature survey.

Phylum	Class	Order	Clade					Grand Total
			A	B	C	D	E	
Cnidaria	Anthozoa	Actinaria	2	11				13
		Alcyonacea			1			1
		Gorgonacea		2				2
		Heliporacea			1			1
		Scleractinia	16	22	62	1	4	105
		Zoanthiniaria	3					3
	Anthozoa Total		21	35	64	1	4	125
	Hydrozoa	Milleporina			1			1
	Scyphozoa	Rhizostomeae	1	1				2
Cnidaria Total			22	36	65	1	4	128
Mollusca	Bivalvia	Heteroconchia	7		3			10
Porifera	Demospongiae	Haplosclerida					1	1
Protozoa	Granuloreticulosea	Foraminifera	2		2			4
Grand Total			31	36	70	1	5	143