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Proc. R. Soc. B 2014 **281**, 20140548, published 9 July 2014

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Cite this article: Bingham BL, Dimond JL, Muller-Parker G. 2014 Symbiotic state influences life-history strategy of a clonal cnidarian. *Proc. R. Soc. B* **281**: 20140548.
<http://dx.doi.org/10.1098/rspb.2014.0548>

Received: 7 March 2014

Accepted: 11 June 2014

Subject Areas:

ecology, environmental science

Keywords:

Anthopleura elegantissima, *Symbiodinium*, fitness, life history, symbiosis, reproduction

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2014.0548> or via <http://rspb.royalsocietypublishing.org>.



Royal Society Publishing

Symbiotic state influences life-history strategy of a clonal cnidarian

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Along the North American Pacific coast, the common intertidal sea anemone *Anthopleura elegantissima* engages in facultative, flexible symbioses with *Symbiodinium muscatinei* (a dinoflagellate) and *Elliptochloris marina* (a chlorophyte). Determining how symbiotic state affects host fitness is essential to understanding the ecological significance of engaging in such flexible relationships with diverse symbionts. Fitness consequences of hosting *S. muscatinei*, *E. marina* or negligible numbers of either symbiont (aposymbiosis) were investigated by measuring growth, cloning by fission and gonad development after 8.5–11 months of sustained exposure to high, moderate or low irradiance under seasonal environmental conditions. Both symbiotic state and irradiance affected host fitness, leading to divergent life-history strategies. Moderate and high irradiances led to a greater level of gonad development in individuals hosting *E. marina*, while high irradiance and high summer temperature promoted cloning in individuals hosting *S. muscatinei* and reduced fitness of aposymbiotic anemones. Associating with *S. muscatinei* may contribute to the success of *A. elegantissima* as a spatial competitor on the high shore: (i) by offsetting the costs of living under high temperature and irradiance conditions, and (ii) by promoting a high fission rate and clonal expansion. Our results suggest that basic life-history characteristics of a clonal cnidarian can be affected by the identity of the endosymbionts it hosts.

1. Introduction

Symbiotic relationships are fundamental to the biology and ecology of numerous archaea, bacteria, protists, fungi, plants and animals. In close associations of this kind, symbiont presence, identity and physiology can critically affect host fitness as measured by host growth, asexual replication and sexual reproduction. For example, removal of a bacterial symbiont from the fungus *Rhizopus microsporus* completely eliminates vegetative growth by preventing production of sporangia or spores [1]. Among plants, the presence and identity of mycorrhizal fungal symbionts can change relative rates of seed production and vegetative replication of the host [2–4], and numerous studies have demonstrated the effects of endosymbionts on basic reproductive processes in insects [5–9]. Evidence from symbiotic cnidarians reveals that loss of symbionts can reduce sexual reproduction [10,11], and a change in the symbiont complement can transform mutualistic partners to parasites [12]. In one coral species, the specific clade of the symbiont (*Symbiodinium* sp.) affects egg size at spawning [13].

On the northern Pacific coast of North America, the common intertidal sea anemones *Anthopleura elegantissima* and *Anthopleura xanthogrammica* host two taxonomically distinct phytosymbionts: the dinoflagellate *Symbiodinium muscatinei* (zooxanthellae [14,15]) and the chlorophyte *Elliptochloris marina* (zoochlorellae [16]). These symbionts, which can occur singly or together in individual anemones, have different physiologies, growth rates and biotrophic



Figure 1. *Anthopleura elegantissima* (a) at the collection site on Tatoosh Island, WA, USA, and (b) in the experimental tank at SPMC. Dashed lines indicate abrupt changes in the symbiotic complements of the anemones. (Online version in colour.)

relationships with their hosts [17–21]. Under high irradiance and temperature conditions, zooxanthellae will supplant zoochlorellae in *A. elegantissima*, and intense, ongoing stress can lead to complete bleaching (loss of symbionts) and hosts that are functionally aposymbiotic [22]. Zooxanthellate *A. elegantissima* are generally more productive than individuals hosting zoochlorellae: annual net productivity is estimated at 92 and 60 g C m⁻² y⁻¹, respectively, for zooxanthellate and zoochlorellate *A. elegantissima* in the San Juan Islands, WA, USA [19]. Thus, a change of symbiotic partners or complete loss of the symbionts may affect host nutritional balance leading to changes in growth or reproduction of the host.

The goal of our work was to determine how the presence and identity of symbionts affect growth and reproduction of *A. elegantissima*. We predicted that overall fitness would be highest in individuals hosting zooxanthellae, followed by those hosting zoochlorellae, and finally by those lacking symbionts altogether. *Anthopleura elegantissima* is the most abundant intertidal sea anemone on the west coast of North America [23], with densities reaching 500 individuals m⁻² [24] and with productivity rates that rival those of intertidal seaweeds [25]. Factors that substantially change anemone fitness, therefore, could cascade through this system, impacting the rocky shore community as a whole.

2. Material and methods

(a) Anemone experiment

On 3 September 2009, 180 *A. elegantissima* were collected from a rock outcrop on Tatoosh Island, WA, USA (48.392° N, 124.735° W) between +1 and +2 m mean lower low water (MLLW). Based on colour alone, 60 each of zooxanthellate (brown anemones hosting more than 90% *S. muscatinei*), zoochlorellate (green anemones hosting more than 90% *E. marina*) and aposymbiotic individuals (pale anemones hosting low densities of either symbiont) were collected from adjacent areas of the same rock surface (figure 1a). The anemones were transported on ice to Shannon Point Marine Center (SPMC) in Anacortes, WA, USA, where symbiotic state was verified for each anemone by clipping a single tentacle, compressing it on a microscope slide and determining the relative abundance of zooxanthellae and zoochlorellae. In this species, tentacle samples reasonably approximate symbiont composition, particularly if populations are dominated by a single symbiont [26], as they were in this case. Only four of 180 anemones had to be eliminated because they hosted mixed symbiont populations. The very low background populations of symbionts in aposymbiotic individuals were primarily zooxanthellae.

In the laboratory, the anemones were cleaned of debris, allowed to attach to pre-weighed slate tiles (4.7 × 4.7 × 0.8 cm), and held for one month prior to the start of the experiment in an indoor flow-through seawater system with natural sunlight

Table 1. Gonad indices of *A. elegantissima* held in the experimental tank. (Gonad index was evaluated by examining the dissected gonad of individuals sampled in June, August or September and determining the threshold magnification necessary to identify gonad tissue and determine sex of the anemone. Except for one zooxanthellate individual in the August sample (GI = 5), all anemones with gonads were female.)

| gonad index | gonad appearance | magnification required | | June | August | September |
|---|-----------------------|------------------------|-------------|-----------------------|--------|-----------|
| | | gonad visible | sex obvious | number of individuals | | |
| 0 | no identifiable gonad | — | — | 25 | 28 | 36 |
| 1 | bumps on mesenteries | 100× | 100× | 2 | 3 | 0 |
| 2 | swollen mesenteries | 10× | 100× | 3 | 1 | 0 |
| 3 | single-lobed masses | 10× | 10× | 3 | 1 | 0 |
| 4 | multi-lobed masses | 1× | 10× | 4 | 6 | 0 |
| 5 | multiple plump masses | 1× | 1× | 0 | 6 | 0 |
| total number of individuals examined each month | | | | 37 | 45 | 36 |

entering directly through large windows. Ten days after collection, each anemone on its tile was blotted dry and weighed, and initial body weight was determined by subtracting the known weight of the wet tile.

To determine how symbiotic state affects host growth and reproduction under different environmental conditions, the anemones were moved to an outside tank (3 m diameter, 0.9 m deep, 6400 l volume) and held there from October 2009 to September 2010, a period chosen to include an entire reproductive cycle and the full seasonal range of conditions experienced by these anemones. Locally, *A. elegantissima* begin developing gonads in January, spawn from August to October [27] and are exposed to the harshest physical stresses during mid-day summer low tides [28]. Shields were used to reduce natural irradiance in each of three treatment groups, approximating conditions that favour zooxanthellate, zoochlorellate and aposymbiotic anemones, respectively [20]: ultraviolet (UV)-transparent acrylic shield for 85% irradiance, UV-transparent acrylic shield plus a layer of window screen for 43% irradiance and opaque grey PVC shield for 2% irradiance. The shields were supported 14 cm above round, PVC platforms (60 cm diameter) covered with artificial turf to discourage anemones from leaving their tiles. Two platforms, each holding 10 zooxanthellate, 10 zoochlorellate and 10 aposymbiotic anemones, were randomly assigned to each irradiance treatment (figure 1b).

Ambient irradiance levels were obtained from a Padilla Bay National Estuarine Research Reserve database (D. Bulthuis, N. Burnett and H. Bohlmann 2011, unpublished data, Padilla Bay National Estuarine Research Reserve Monitoring Program; <http://cdmo.baruch.sc.edu/>). The monitoring instrument (located 15 km from SPMC) measured photosynthetically active radiation (400–700 nm) at sea level every 15 min throughout the study period. Irradiance in the treatments was determined by using a Biospherical Instruments QSL-100 4π quantum sensor to make repeated measurements under the shields during aerial exposures and calculating the average percentage reduction. Hobo WaterTemp Pro dataloggers on the platforms monitored temperatures at 5 min intervals.

Seawater was supplied to the tank at a rate more than or equal to 38 l min⁻¹, with overflow draining through an 85 cm tall PVC pipe. A Danner Mag Drive submersible pump (113 l min⁻¹) ran continuously, increasing water circulation. When the tank was full, simulating high tide, the irradiance shields were submerged and the anemones were under 16 cm of seawater. Low tides were created with a relay timer (Zelio SR2B121FU, Schneider Electric) programmed to open an electronic ball valve (Electromni Series 83, Asahi America) on a shorter drain pipe, dropping the water to the level of the platforms and completely exposing

the anemones. Timing and duration of exposure mimicked a +0.3 m MLLW tide based on predictions from nearby Burrows Bay, WA, USA. The tank, platforms and shields were cleaned regularly to remove sediment and fouling organisms. Because anemones in the tank had limited access to their natural prey [29], they were fed 0.1 g pellets of frozen squid every other week.

The anemones were maintained in the tank for 8.5–11 months, depending on their final processing date, which was varied to maximize the chance of capturing changes in reproductive condition. All anemones were reweighed on 10 February, 20 April 2010 and on their final processing date. On 22 June, 1 August and 13 September 2010, 5–12 randomly chosen individuals from each treatment combination were processed. If an anemone had divided, the clones were kept in the same treatment and their summed weight was used in calculations representing the original anemone. Processing involved weighing the anemones, opening their pedal discs and using increasing magnification (1×, 10×, 100×) as necessary to locate gonad tissue, identify gametes and assign a gonad index from 0 (no identifiable gonad) to 5 (fully ripe or recently spawned; table 1).

Only anemones that maintained their original symbiotic state (119 of 176 anemones = 67%) were used in the final analyses. The symbiont changes that occurred in anemones excluded from analyses and the effects of the treatments on the symbionts themselves are described elsewhere [22]. Final symbiotic state was assessed by homogenizing the anemones and using a haemocytometer to identify and count 80–100 symbiont cells in each of four replicate homogenate subsamples (or up to 16 haemocytometer chambers if densities were low). Symbiont counts were normalized to anemone protein content determined from replicate homogenate subsamples [30]. Anemones were considered aposymbiotic if symbiont densities were less than 8×10^4 cells mg protein⁻¹ (approx. 10% of a normal symbiont density for *A. elegantissima* in the nearby San Juan Islands, WA, USA [20]). All individual anemones with densities above this threshold and with less than 10% of the other symbiont were classified as either zooxanthellate or zoochlorellate and were included in the analysis.

(b) Field population

For comparison, we assessed the relationship of symbiotic state to anemone body size, fission and gonad development in a conveniently accessible field population of *A. elegantissima*. Anemones were collected from a southeast facing rock outcrop at Cattle Point, San Juan Island on 16 July and 11 November 2008 and on 6 February and 27 April 2009. On each date, the nearest anemone at 25 randomly chosen positions along a 25 m horizontal transect was

collected, blotted dry and weighed, and then examined with a dissecting microscope for the scar on the column that persists for approximately six weeks after a fission event (electronic supplementary material, figure S1) [31]. A +0.2 m MLLW tidal height was chosen for this sampling because zoochlorellate and zooxanthellate individuals were both found at this tidal height. Gonad indices were determined for anemones collected during the fertile period (July sample only). Symbiotic state of all sampled anemones was determined as described above and anemones with mixed symbiont complements were excluded from analyses.

(c) Statistical analysis

To ensure that body size did not confound tests for treatment effects, initial weights of the anemones in different symbiotic states were compared with one-way ANOVA following a Levene's test for equal variances. Per cent weight change per month (to account for different final processing dates) and gonad index at the end of the experiment were each analysed by two-way ANOVA with irradiance (85, 43 and 2%) and symbiotic state (aprosymbiotic, zooxanthellate and zoochlorellate) as fixed factors. Post hoc comparisons were carried out with Tukey tests. To correct for unequal variances, α was adjusted to 0.025 for these tests [32]. Numbers of fissions were insufficient to allow testing for combined effects of irradiance and symbiotic state, and those factors were tested individually with exact multinomial tests, followed by calculation of z-scores to determine where observed and expected values differed [33]. A discriminant analysis was used to determine whether combined measurements of per cent weight change, number of fission events and gonad index for each anemone could distinguish the nine treatment combinations (3 irradiance treatments \times 3 symbiotic states). Box's M test was used to verify homogeneity of the covariance matrices and Wilks' λ was used to test for significant discrimination of the groups.

To determine whether fission was related to symbiotic state in the field population of Cattle Point anemones, a 2×3 contingency analysis was carried out with fission scar (present and absent) and symbiotic state (aprosymbiotic, zoochlorellate and zooxanthellate) as the factors. The data from all four sampling periods were pooled to test for patterns across an entire annual cycle. Z-scores were calculated for each cell of the table to determine whether observed and expected values differed significantly.

3. Results

(a) Anemone experiment

Daily irradiance levels varied considerably during the seasonal progression of the experiment. Long days, high sun angle and clear skies increased irradiance in the tanks during the summer months (electronic supplementary material, figure S2a). Anemones in all irradiance treatments experienced similar mean, maximum and minimum temperatures from October through to February (electronic supplementary material, figure S2b–d) when ambient air temperatures are typically moderate during low tides, which occur during the night. However, beginning in March, and throughout the summer when low tides occur during the day, maximum temperatures in the 85% irradiance treatment were routinely 1–2°C and 2–4°C higher than in the 43% and 2% irradiance treatments, respectively. Measured effects of treatments on the anemones therefore reflect the combined effects of irradiance and maximum temperature, as would be the case in natural habitats.

Initial body weights were independent of symbiotic state ($F_{2,116} = 0.79$, $p = 0.45$), averaging 2.28 ± 1.22 g (mean \pm s.d.;

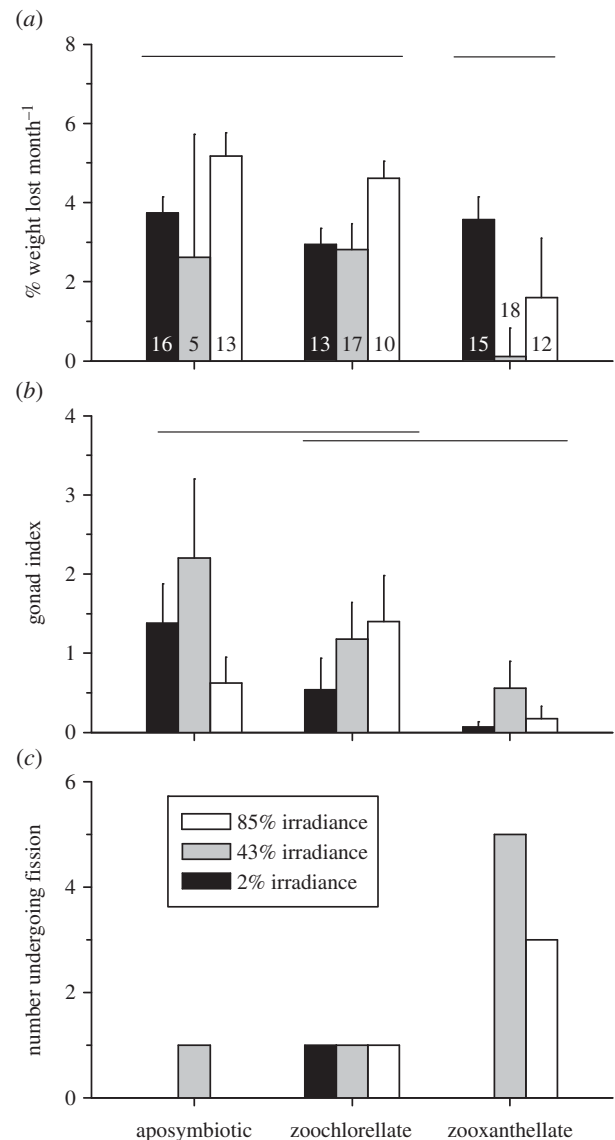


Figure 2. (a) Per cent weight loss per month (n for each treatment is indicated within the bars), (b) gonad index and (c) number of *A. elegantissima* undergoing fission events while held in the experimental tank for 8.5–11 months (standard errors are shown for (a,b)). Horizontal bars summarize results of Tukey tests comparing symbiotic states (groups not joined by a line are significantly different).

$n = 34$), 2.16 ± 0.91 g ($n = 40$) and 2.50 ± 1.55 g ($n = 45$) for aposymbiotic, zoochlorellate and zooxanthellate individuals, respectively. Most anemones lost weight during the experiment, decreasing from September to February, decreasing more gradually or increasing from February to April, then diverging with no clear pattern after April (electronic supplementary material, figure S3). The most obvious effects were a rapid initial weight loss in aposymbiotic anemones exposed to 85% or 43% irradiance, and an absence of weight loss among zooxanthellate anemones in the 43% irradiance treatment. Overall weight loss was significantly affected by symbiotic state ($F_{2,110} = 5.13$, $p = 0.007$), but not by irradiance ($F_{2,110} = 3.64$, $p = 0.029$). The interaction was also non-significant ($F_{4,110} = 1.93$, $p = 0.11$). On average, aposymbiotic anemones lost approximately $2.5\times$ as much weight as did zooxanthellate individuals (figure 2a).

Of the anemones sampled in June and August, 35% contained identifiable gonads (i.e. gonad indices from 1 to 5; table 1); no gonads were present in September samples. Of 29 individuals with gonads, 28 were female. The June sample

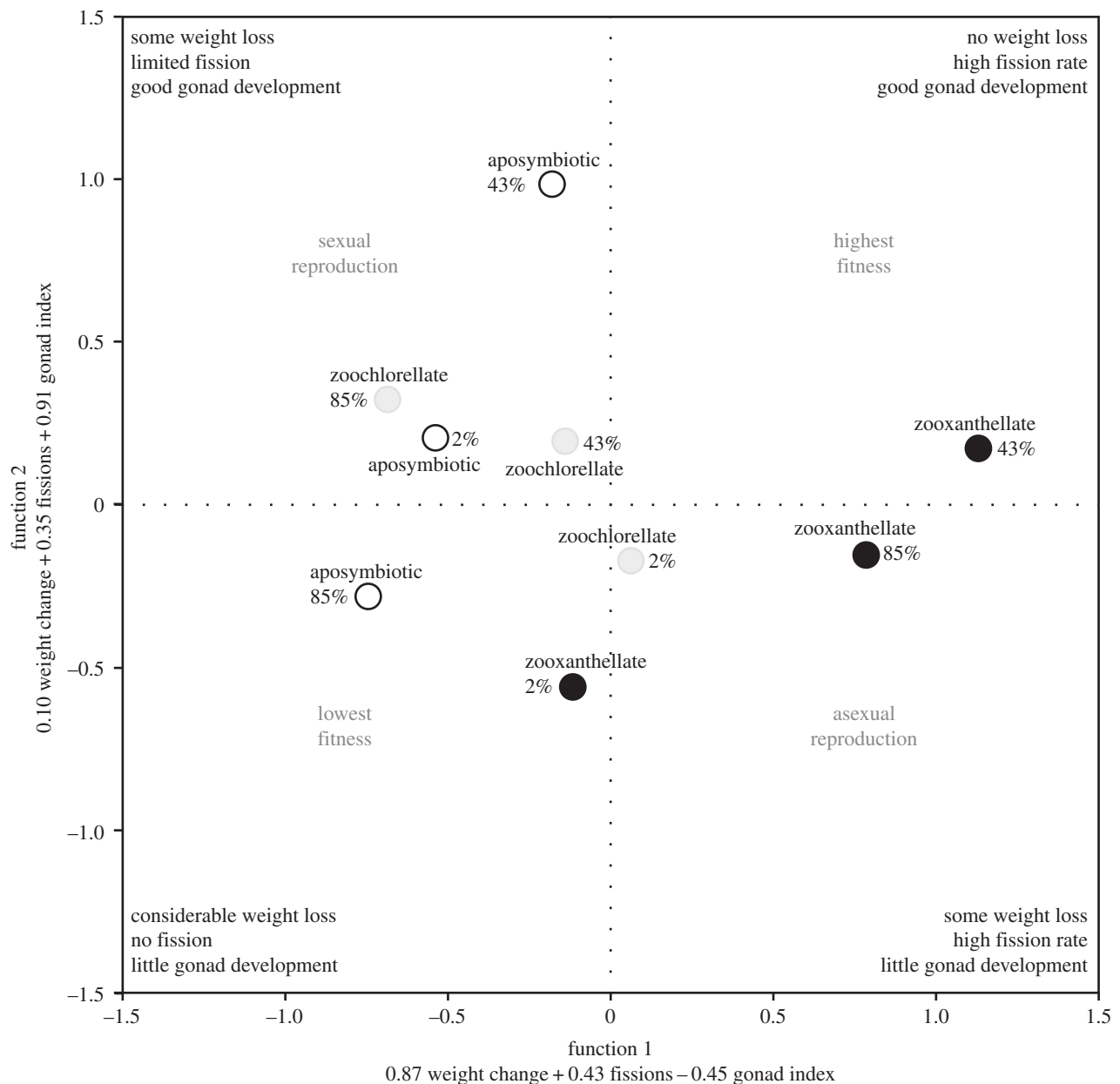


Figure 3. Discriminant analysis using number of fissions, per cent weight loss and gonad index measurements to maximally separate nine treatment groups (i.e. 3 irradiance treatments × 3 symbiotic states). Each labelled point is the centroid for that treatment combination (sample sizes shown in figure 2a). Discriminant function 1 explained 74.2% of the variance, and function 2 an additional 22.0%. Coefficients of the discriminant functions (shown on the axes) were used to separate the plot into quadrants representing different fitness strategies/outcomes for *A. elegantissima* in the experimental tank.

included individuals with gonad states from early development (GI = 1) to near spawning (GI = 4). The August sample captured the transition from pre- to post-spawning; nine of 12 reproductive individuals (GI = 4 or 5) had ripe gonads filled with gametes while the remainder had large gonads, but few gametes, suggesting that spawning had already occurred. Symbiotic state affected gonad index ($F_{2,110} = 5.22$, $p < 0.01$), with greater gonad development in aposymbiotic than in zooxanthellate anemones (figure 2b). There was no significant irradiance effect ($F_{2,110} = 1.76$, $p = 0.17$) and no significant interaction between irradiance and symbiotic state ($F_{4,110} = 1.17$, $p = 0.32$).

Twelve individuals underwent fission while in the experimental tank. Strong seasonality was evident: 67% of all fissions occurred in April, May or June. An exact multinomial goodness-of-fit test showed that the number of fissions was not significantly affected by irradiance alone ($p = 0.11$), but did differ among symbiotic states ($p = 0.05$; figure 2c).

Standardized residuals indicated significantly fewer divisions than expected among aposymbiotic ($z = -2.38$, $p < 0.05$) and more divisions than expected among zooxanthellate anemones ($z = 4.0$, $p < 0.01$).

The homogeneous variance assumption required for the discriminant analysis was violated (Box's $M = 97.7$, $p < 0.01$), but matrix scatterplots of the groups showed that the violation was not severe. Linear combinations of weight change, number of fissions and gonad index effectively discriminated the treatment groups (Wilks' $\lambda = 0.60$, $\chi^2 = 56.1$, $p < 0.001$). The first discriminant function was weighted most heavily by change in anemone weight, with smaller, and nearly equal but opposite contributions of fissions and gonad index (figure 3). The second discriminant function was heavily weighted by gonad index. The discriminant plot was divided into quadrants and labelled according to the coefficients of the functions (figure 3), producing regions qualitatively identified as sexual reproduction, highest fitness

Table 2. Presence or absence of fission scars of *A. elegantissima* collected from +0.2 m MLLW at Cattle Point, San Juan Island, WA, USA (pooled data for July and November 2008, and February and April 2009). (Numbers in parentheses are expected contingency table values.)

| | symbiotic state | | |
|---------|-----------------|----------------|-----------------------|
| | aprosymbiotic | zoochlorellate | zooxanthellate |
| scar | 0 (2.5) | 3 (6.5) | 14 (7.9) ^a |
| no scar | 13 (10.4) | 30 (26.5) | 26 (32.1) |

^aThe observed and expected values differ based on z-scores ($p < 0.05$).

(both sexual and asexual reproduction and no weight loss), lowest fitness (little reproduction and high weight loss) and asexual reproduction.

Zooxanthellate anemones in 43% irradiance fell in the highest fitness quadrant, maintaining their weight while showing a high degree of fission and sexual development. Aposymbiotic anemones in 85% irradiance and zooxanthellate individuals in 2% irradiance fell in the lowest fitness quadrant. Zoochlorellate anemones in 43% and 85% irradiance, and aposymbiotic anemones in 43% and 2% irradiance showed primarily sexual reproduction. Zooxanthellate individuals in 85% irradiance and zoochlorellate individuals in 2% irradiance showed the opposite pattern: more asexual reproduction and little gonad development.

(b) Field population

The presence of fission scars was related to symbiotic state in anemones collected from Cattle Point, ($\chi^2_2 = 11.43$, $p = 0.003$; table 2). Thirty-five per cent of zooxanthellate anemones had fission scars (nearly twice the number expected) versus 9% of the zoochlorellate and 0% of the aposymbiotic anemones. Gonad indices were also different among anemones in different symbiotic states. Of the zooxanthellate anemones, 54% were sexually mature (gonad indices of four or five), versus 20% of zoochlorellate and 0% of aposymbiotic individuals. Eighty per cent of the reproductive individuals were male (table 3). There were no significant differences in mean wet body weights of individuals in different symbiotic states ($F_{2,83} = 2.83$, $p = 0.06$), but the trend was towards larger size among aposymbiotic anemones: 2.4 ± 3.4 g (s.d.), 1.0 ± 1.3 g and 1.0 ± 1.6 g, respectively, for aposymbiotic, zooxanthellate and zoochlorellate individuals.

4. Discussion

Compared with tropical symbioses, cnidarian–algal associations in temperate seas are generally thought to have more limited benefits for the host, particularly during the cold, dark winter months [34]. Nonetheless, the symbionts can translocate substantial portions of their photosynthate to the host and have been hypothesized to augment the hosts' energy budget enough to boost reproductive output [34–36]. Our results are, to our knowledge, the first to directly support this hypothesis and further suggest that hosting different symbionts can fundamentally change the life-history strategy of the host. Rather than simply enhancing sexual reproduction, symbiosis with the more productive *S. muscatinei* may promote cloning by fission, a strategy that makes *A. elegantissima* a highly successful spatial dominant

Table 3. Gonad indices of *A. elegantissima* collected from +0.2 m MLLW at Cattle Point, San Juan Island, WA, USA (July 2008). (Gonad index values as in table 1.)

| gonad index | aprosymbiotic | zoochlorellate | zooxanthellate |
|-------------|-----------------------|----------------|----------------|
| | number of individuals | | |
| 0 | 1 | 3 | 5 |
| 1 | 0 | 0 | 0 |
| 2 | 1 (♂) | 0 | 0 |
| 3 | 1 (♂) | 1 (♀) | 0 |
| 4 | 0 | 1 (♀) | 3 (♂) |
| 5 | 0 | 0 | 3 (♂) |
| number | 3 | 5 | 11 |
| examined | | | |

in upper intertidal zones. Under bright, warm conditions, both in the tank experiment and at the Cattle Point field site, symbiosis with *S. muscatinei* was associated with more fission events, possibly at the expense of sexual reproduction. The reduced body sizes produced by cloning may be physiologically advantageous for *A. elegantissima* on the high shore, where a larger feeding surface area created by fission may compensate for reduced feeding opportunities resulting from shorter immersion times [27,37]. Cloning may also decrease the risk of extinction for individual genotypes in these high stress locations where clonal diversity is low and successful sexual reproduction may be infrequent [31,38]. Aggregation of cloned individuals can reduce the costs of a high surface to volume ratio associated with smaller body size by reducing air drying and causing individuals to warm more slowly during low tides [39], allowing the clone to extend outward from protected crevices onto open rock where conditions would be too harsh for isolated individuals [31]. By supporting a shift towards clonal replication, *S. muscatinei* may contribute critically to the abundance and persistence of *A. elegantissima*, and indeed itself, in upper shore habitats along much of the North American Pacific coast.

Anemones hosting *E. marina* generally had higher fitness than aposymbiotic individuals, suggesting that *E. marina* contributes to host success, possibly by facilitating sexual reproduction. This bears some similarity to *Hydra viridis* (green hydra), which produces female gonads only if the *Chlorella* algal symbiont is present [40]. Although *E. marina* is less tolerant of high temperature and irradiance [17–19] and consistently less productive than *S. muscatinei* on a per-cell basis [41], the growth rate of *E. marina* is higher [19,21], and in habitats where irradiance and temperature are low year round, *A. elegantissima* commonly hosts *E. marina* at up to twice the density of *S. muscatinei* [20,28]. Thus, zoochlorellate *A. elegantissima* may be nearly as productive as zooxanthellate individuals under some conditions [19,28], but appear to direct more energy to sexual reproduction. Although anemones held in our experiment for many months may have experienced decreased feeding or assimilation efficiency, some of the most pronounced weight loss actually occurred among aposymbiotic anemones during the first four to five months, before mid-day, summer low tides resulted in possible thermal stress. The comparatively low rate of weight loss in symbiotic as compared with aposymbiotic individuals suggests that both *S. muscatinei* and *E. marina*

contribute nutritionally to their hosts even under the low-light conditions of winter.

On sample dates falling within the reproductive period of *A. elegantissima*, 35% of the anemones in the tank produced gonad. This is lower than the 66% fertility reported for field populations of *A. elegantissima* at Tatoosh Island, but is close to the 39% fertility reported for *A. elegantissima* at Cattle Point by Sebens [27]. The degree of gonad development was significantly affected by symbiotic state, but not as expected. Independent of irradiance, aposymbiotic individuals in the tank produced the most gonad. However, freshly collected anemones from Cattle Point showed the reverse pattern, with the most fully developed gonad in zooxanthellate anemones. The pattern of gonad development in the field may be related to timing of gonad development among anemones in different symbiotic states, to differences in the microhabitats from which the anemones were collected or to differences in sex of the anemones (table 3). More fine-scale study of gonad development, with larger sample sizes, will be necessary to explain this result.

We obtained an average of 0.09 divisions individual⁻¹ yr⁻¹ among all anemones during the 8.5–11 months they were in the experimental tank. This is within the range of fissions reported for Tatoosh Island populations of *A. elegantissima* (0–0.3 divisions individual⁻¹ yr⁻¹ depending on tidal height and location) [37]. Exposure to direct sunlight increases *A. elegantissima* fission rate, with the most pronounced effect on zooxanthellate individuals [42]. Although this suggests that irradiance alone can affect fission, there is some interplay between irradiance and symbiotic state, as association with *S. muscatinei* enhanced asexual reproduction.

The comparatively low fitness of both the zooxanthellate anemones in 2% irradiance and the aposymbiotic anemones in 85% irradiance suggests a high host cost for symbiosis under low irradiance and for aposymbiosis under high irradiance. Under low irradiance, a large symbiont population can create significant respiratory demands not compensated by photosynthesis [43], and the symbionts, unable to meet their metabolic needs through photosynthesis, may instead consume host nutrients [44]. This would explain why, in our 2% irradiance treatment, anemones hosting *S. muscatinei*, which photosynthesizes poorly under low irradiance, showed lower fitness than anemones lacking symbionts altogether. At the other extreme, aposymbiotic anemones under high irradiance conditions were also at a disadvantage. Increased host respiration owing to elevated temperatures [17,18] may have created an energy deficit uncompensated by contributions

from the symbionts, leaving less energy for growth and reproduction. This suggests that, in addition to increasing fission rates, symbiosis with *S. muscatinei* may offset the high metabolic costs associated with living in open, sun-exposed microhabitats.

The intertidal distributions of *E. marina* and *S. muscatinei* populations are remarkably stable over annual cycles, undergoing only minor changes in density and relative abundance despite large daily and seasonal fluctuations in the physical environment [28]. However, persistent changes in irradiance or temperature can change symbiont composition [22] or, in extreme cases, lead to bleaching [20,28,45]. Thus, shifts in symbiont dominance are likely to occur in response to climate change [28], with the more heat-tolerant zooxanthellae replacing zoochlorellae where air and seawater temperatures increase. Over the short term, this could increase anemone productivity and carbon cycling where *A. elegantissima* is abundant. Over the longer term, changes in the relative importance of sexual versus asexual reproduction could impact abundance, distribution, dispersal, population dynamics and genomics of this important species.

Experimental infections of the tropical anemone *Aiptasia pulchella* with homologous or heterologous *Symbiodinium* types show the fundamental impact of symbiont identity on autotrophic potential, functionality and, presumably, fitness of that species [46], and work with multiple coral species demonstrates that photosymbiont density can significantly affect tissue biomass and levels of gamete production [10,11]. Our results indicate that, in *A. elegantissima*, the presence and identity of the symbionts interact with environmental conditions to alter the balance between growth, asexual cloning and sexual reproduction. Such basic life-history differences undoubtedly affect the fitness of this temperate anemone host. Working to understand the biochemical and energetic mechanism driving these divergent life histories and determining whether similar changes occur in other species, are important next steps, particularly in the context of a changing global climate that may drive changes in other symbiotic partnerships.

Acknowledgements. We thank Vince Cook, Environmental Division Manager for the Makah Nation, and Cathy Pfister and Tim Wootton for access to the collection site on Tatoosh Island. Angela Fletcher, Terra Hiebert, Mike Levine, Monica Ponce-McDermott, Gene McKeen, Eli Patmont, Zullaylee Ramos, Nate Schwarck and Alan Verde provided laboratory or field assistance. Lisbeth Francis provided editing and helpful discussion, and assisted with gonad measurements.

Funding statement. The work was funded by NSF grant nos. IOS-0822179, OCE-0741372 and OCE-0551898.

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