SEXUAL REPRODUCTION IN THE INVASIVE OCTOCORAL *CARIJOA RIISEI* IN HAWAII

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ABSTRACT

Since its initial discovery on Oahu in 1966, the azooxanthellate octocoral, *Carijoa riisei* (Duchassaing and Michelotti, 1860), has spread across the main Hawaiian Islands and proliferated in abundance. To help understand the substantial ecological success of *C. riisei* in Hawaii, its sexual reproduction was examined. *Carijoa riisei* is gonochoric with a male to female ratio of one. Gametogenesis is asynchronous, continuous, and does not exhibit seasonal or lunar periodicity. *Carijoa riisei* spawns negatively buoyant eggs which suggests external fertilization and possibly benthic larvae. Under favorable conditions, *C. riisei* exhibits high polyp fecundity. Asynchronous, continuous spawning of gametes is an unusual mode of reproduction which forgoes the advantages of concentrating gametes in space and time and requires dense aggregations of male and female colonies in close proximity to ensure fertilization success. Other life history traits such as fast growth, vegetative propagation, and superior competitive ability enable *C. riisei* to form dense, multicolony aggregations thereby facilitating sexual reproduction. Provided *C. riisei* can achieve a critical density, this unusual sexual reproductive strategy probably enables it to exploit the ephemeral availability of space across time with a high and continuous production of larvae.

Carijoa riisei (Duchassaing and Michelotti, 1860) is an azooxanthellate octocoral (Order Alcyonacea, Family Clavulariidae) originally described from the Caribbean and tropical Atlantic (Bayer, 1961). Since its initial discovery in Hawaii in 1966 (J. Hoover, Bishop Museum, pers. comm.), *C. riisei* has spread throughout the main Hawaiian Islands (Thomas, 1979; Coles and Eldredge, 2002) and caused significant ecological impact by smothering and displacing other benthic fauna (Kahng and Grigg, 2005; Kahng and Kelley, 2007). Because nonindigenous marine invertebrates on tropical coral reef ecosystems rarely proliferate to pest status (Coles and Eldredge, 2002; Hutchings et al., 2002), the ecological success of *C. riisei's* biological invasion in Hawaii is exceptional. Given the central role of reproduction in an organism's life history strategy, reproduction must be examined to understand an organism's ecology.

Alcyonacean octocorals are predominately modular colonial marine invertebrates that commonly reproduce both sexually and asexually (Lasker, 1988) and exhibit a diversity of sexual reproductive patterns (Benayahu, 1997) that support a range of life history strategies. Unlike scleractinian corals, which are predominately hermaphroditic spawners (Harrison and Wallace, 1990), alcyonacean corals are predominantly gonochoric brooders (Coma et al., 1995b; Benayahu, 1997). Brooding coral species typically exhibit extended or continuous periods of breeding (Harrison and Wallace, 1990; Benayahu, 1991). In contrast, spawning corals including many zooxanthellate alcyonaceans typically release gametes seasonally during short synchronized events (Alino and Coll, 1989; Benayahu et al., 1990) which are correlated to seasonal environmental cues such as temperature, lunar period, and solar insolation (Richmond and Hunter, 1990; Richmond, 1997; Penland et al., 2004).

We hypothesize that *C. riisei's* reproductive features contribute to its invasive success in Hawaii. The objectives of this study were to examine the reproductive features

of *C. riisei* in Hawaii, compare them with those of previously studied alcyonaceans, and identify possible characteristics which may provide insight into *C. riisei's* success in Hawaii.

Materials and Methods

Sexuality and Sex Ratio.—Colonies were sampled across seasons during 2003–2006 from a variety of study sites on Oahu (Fig. 1) and Kauai (Port Allen Harbor). At each site, samples were haphazardly collected from discontinuous aggregations at least 2 m apart to reduce the probability of sampling the same genet multiple times.

In order to determine the sex of a colony, gonads were analyzed using epi-fluorescent histology. The upper 8–12 cm of an axial polyp from each colony was preserved in seawater and 5% formalin for 24 hrs, rinsed in distilled water, and then transferred to phosphate buffer solution (1.86 mM NaH_2PO_4, 8.41 mM Na_2HPO_4, 175 mM NaCl, pH 7.4). For each sample, gonads and associated mesenteries were removed via dissection from lateral polyps with the aid of a Nikon PFX binocular scope. Gonads were stained with a mixture of 50% glycerol, 50% 1× phosphate buffer solution, and Hoechst 1 mg L^{-1} for 24 hrs at 5 °C. Gonads were then transferred to 70% glycerol and stored in the dark at 5 °C until they were mounted on microscope slides and viewed under an Olympus U-LH100HG epi-fluorescent binocular microscope. Sex ratios were calculated for each site as well as overall.

In conjunction with a time series analysis of reproductive cycle (see below), the same 10 gonochoric colonies were sexed at roughly 6 mo intervals for 2 yrs. For colonies which exhibited hermaphroditism, sex determination was repeated 5–10 times in subsequent months to investigate the nature of hermaphroditism.

Gametogenesis and Reproductive Cycle.—At two study sites on Oahu, YO-257 shipwreck and Hawaii Kai lagoon channel (Fig. 1), haphazardly selected colonies of *C. riisei* were sampled monthly and analyzed to characterize reproductive status. The study sites were selected for their large populations of *C. riisei* and contrasting habitats. At the YO-257 (YO) site, nine large colonies at 26 m depth were mapped and sampled monthly from July 2003 to September 2005. The YO site (21°15.633′N, 157°50.217′W) is a sunken ship located off Waikiki Beach. Colonies form dense aggregations on the shaded metal structures.

At the Hawaii Kai (HK) site, 10 large colonies at 0–2 m depth were mapped and sampled monthly from March to November 2003. The HK site (21°17.108′N, 157°43.120′W) is located under a bridge in a sheltered, turbid, lagoon channel. Colonies grow on a variety of hard substrata, are shaded from direct sunlight, and are subject to bidirectional, diurnal tidal currents. The time series was prematurely terminated due to an unusually large rain event (2 January 2004) that killed all colonies. Additional *C. riisei* samples from numerous locations on Oahu, Kauai, Maui, and Big Island were opportunistically collected and examined throughout 2003–2006.

Monthly samples consisting of the upper 8–12 cm of an axial polyp (July 2003 through July 2004 for YO-257; April–Nov 2003 for Hawaii Kai) for each colony were sampled by clipping and preserving it in a vial of 5% formalin. In the laboratory, six polyps from each axial polyp were haphazardly selected for dissection: two basal, two medial, one apical, and the terminal polyp forming the axial chamber (Fig. 2). For each polyp dissected, the diameter of the three largest gonads was measured and the number of large gonads (defined as > 250 μm in diameter) was determined using a Nikon PFX binocular microscope. This "large" size class coincides with gonad pigmentation and maturation. The average diameter of the three largest gonads in each polyp and percent of polyps with large gonads were plotted against time. The data were also analyzed for lunar periodicity by converting each sampling date to the number of days to the nearest full moon (–14 to +14) and plotting the data against the lunar period.

At the YO-257 study site, monthly histological analysis was also conducted on samples from nine mapped colonies and an additional three to six haphazardly selected colonies for 1 yr (September 2004–September 2005). Samples were fixed in 5% formalin for 24 hrs, rinsed

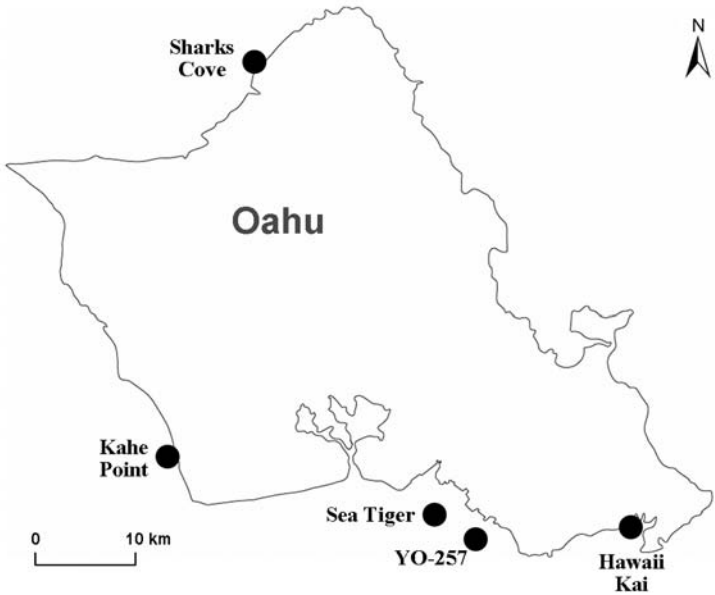

Figure 1. Map of primary study sites on the island of Oahu.

in fresh water, transferred to 95% ethanol, and decalcified using a mixture of equal volumes of 50% formic acid and 15% sodium citrate. Following procedures in Benayahu et al. (1989), samples were then stained, sectioned, mounted, examined under a compound microscope, and photographed. Oocytes and spermaries were examined in 4–6 polyps of each sample and classified into developmental stages using the criteria of Glynn et al. (1991). The frequency distribution for each developmental stage based on the total gonad measurements was plotted across time.

To determine the length of the gametogenic cycle, two female colonies were examined from July 2003 to March 2005. Each month, four lateral polyps (two basal and two medial) were dissected from an axial polyp. Within each lateral polyp, all gonads were counted and diameters measured (±10 μm) using a Nikon PFX binocular scope under 63× magnification. Volume of total gonad tissue for each gonad size class (in 100 μm increments) was calculated using a spherical volumetric approximation ($V = 4/3\pi r^3$). For each colony, the volume distribution by size class was plotted against time.

In order to investigate synchronization of gametogenesis, the size distribution of oocytes within an axial polyp was calculated and compared with other axial polyps. On each of four sample dates (April 14, 19, 25, and May 9 2005), three axial polyps (> 8 cm in length) were sampled from each of three large female colonies (3 × 3). For each axial polyp, four lateral polyps (two basal and two medial) were dissected, and all oocytes were counted and measured under a binocular microscope. For each axial polyp, the percentage of gonads within each size class was calculated. This size frequency distribution of oocytes was compared between axial polyps within the same colony (intra-colony synchronization) and between axial polyps of different colonies (inter-colony synchronization).

Fecundity for both males and females was measured in two ways: (1) the average number of large gonads per lateral polyp; and (2) the percentage of lateral polyps with mature gonads. Fecundity was tracked across time and compared between locations.

Mode of Reproduction.—To determine mode of reproduction, possible evidence (or lack thereof) for internal fertilization and/or brooding was sought during all polyp dissections and histological sections. To supplement this analysis, one large male and one large female *C. riisei* colony were cultured together in a closed aquarium with filtered seawater and monitored

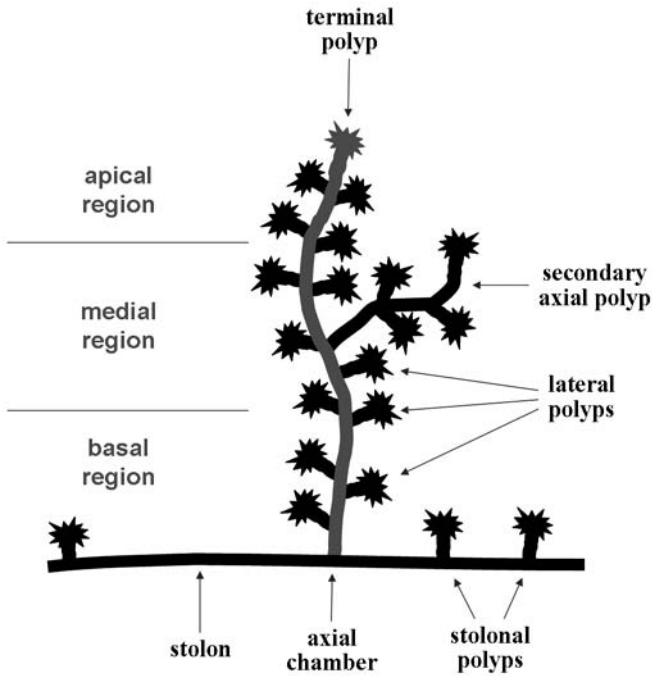

Figure 2. Diagram of a *Carijoa riisei* axial polyp.

across time. Two 4-wk trials were conducted: beginning on 14 Nov 2005 and 14 Feb 2006. Colonies were fed frozen, micro-crustaceans (Argent Cyclop-Eeze®) five times per week. Negatively buoyant particles were filtered through plankton mesh (200 µm) three to five times per week and examined for any released sexual propagules. Sexual propagules were fixed in 5% formalin, prepared for epi-fluorescent histology, and examined using epi-fluorescent light microscopy histology to determine their developmental status.

Onset of First Reproduction.—Age at first reproduction was inferred from average growth rates and minimum axial polyp size associated with production of gonads. The longest axial polyp (colony height) was sampled from well defined, small colonies. Small colonies of various heights were haphazardly sampled, dissected, and examined under a dissecting microscope to determine the presence of large gonads and the minimum axial polyp length associated with the onset of visible gonad development (at 63× magnification). Axial polyp growth rates from a parallel study (Kahng, 2006) were used to infer the age at first reproduction.

Results

Sexuality and Sex Ratio.—Oocytes were recognized by having a thick follicular cell layer enclosing an interior region containing a large, well defined nucleus, and only exhibiting diffuse fluorescence in the interior region. The nucleus within an oocyte was typically located adjacent to the site of attachment to the mesentery (Fig. 3A,B). Spermaries were identified by a thin layer surrounding gastrodermal cells enclosing numerous, small, well defined nuclei exhibiting marked DNA fluorescence (Fig. 3C,D). Simultaneous hermaphrodites were identified based on the presence of both male and female gonads within the same polyp cavity.

Carijoa riisei colonies were predominately gonochoric. Hermaphroditic colonies were uncommon: 1.3% of colonies examined had spermaries and oocytes occurring

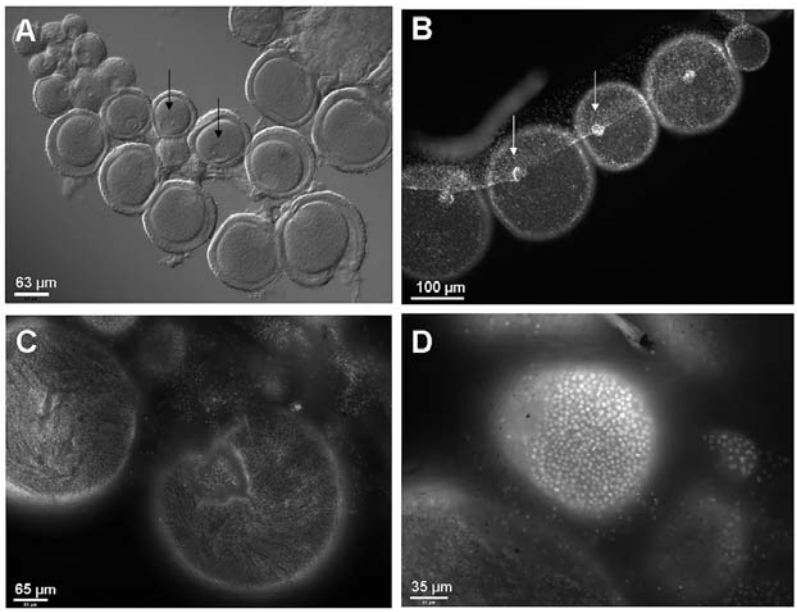

Figure 3. *Carijoa riisei* oocytes as viewed under (A) normal light microscopy with well-defined nucleoli (black arrows) and (B) epi-fluorescent microscopy with the nuclei (white arrows) visible adjacent to the mesentery. *Carijoa riisei* spermaries viewed under epi-fluorescent microscopy: (C) a mature spermary with a small portion the follicular layer torn away revealing nuclei of many sperm cells; (D) a close-up of an immature spermary with many well defined nuclei visible inside. Photo credit: W. Browne.

on the same mesentery within some of the polyps. Exclusively male polyps and exclusively female polyps were not observed co-occurring on the same colony on a given sampling date. The colony sex ratio of males to females was 1.16 and not significantly different from unity (normal distribution $P < 0.234$). Of the 255 haphazardly selected colonies, 128 were males, 110 were females, 3 were hermaphrodites, and 14 had no discernable gonads. The sex ratio varied considerably between field sites (data not shown).

Time series tracking of mapped colonies at the Hawaii Kai study site (not included in the sex ratio calculation) suggests that hermaphroditism may be ephemeral. One male colony exhibited hermaphroditic polyps for 2 mo but reverted back to male afterwards. Another male colony exhibited hermaphroditic polyps on two of nine sampling dates. Two female colonies exhibited hermaphroditic polyps on a single occasion but reverted back to female afterwards. No gonochoric colonies tracked across time reversed sex (i.e., from male to female or vice versa). Both small and large colonies were found of both sexes.

Gametogenesis and Reproductive Cycle.—Oocytes at all developmental stages were observed throughout the year (Fig. 4A). New oocytes < 100 µm in diameter were white or colorless and firmly attached to the mesenteries. As diameter increased, oocytes became peach in color. The onset of pigmentation generally occurred at ~250 µm but varied by colony. Oocytes (> 300 µm) were always pigmented and became darker with size. Large, pigmented oocytes were weakly attached or unattached to the mesenteries and negatively buoyant in seawater. Oocytes were observed up to 600 µm in diameter.

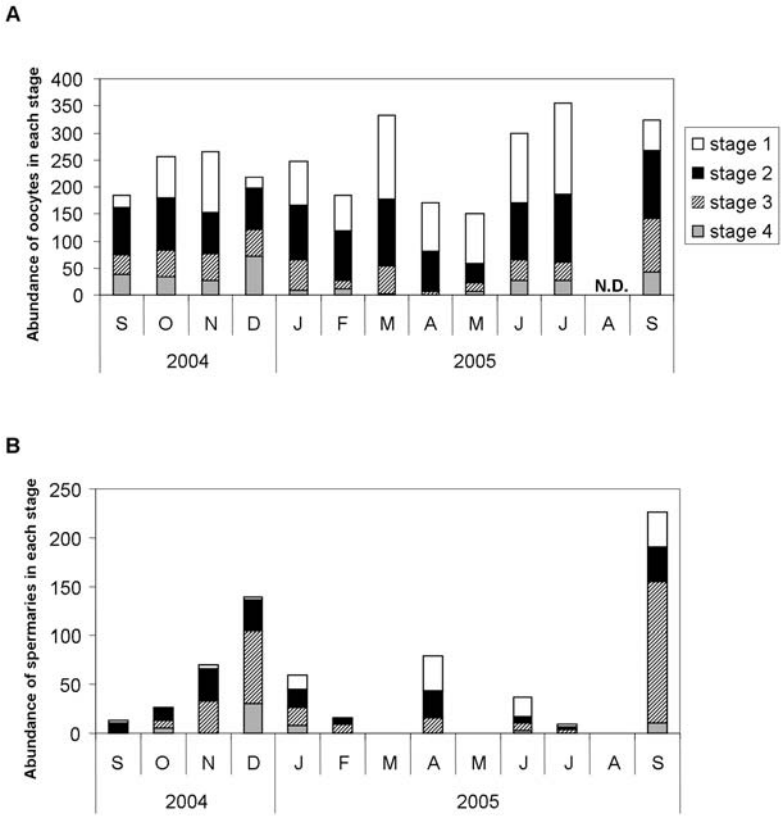

Figure 4. Frequency distribution of gonad developmental stages from the YO-257 study site (A) oocytes; (B) spermaries. N.D. = no data

The male reproductive cycle was similar to females. Spermaries at all developmental stages were observed throughout the year (Fig. 4B). Size range, onset of pigmentation, color, appearance, and buoyancy of spermaries were similar to oocytes. The gonads of both sexes were not distinguishable without histological analysis. On average, the largest spermaries (in each polyp) were ~85 μm smaller in diameter than the largest oocytes. The size difference was statistically significant (one-way ANOVA: $P < 0.001$), however, spermaries shared the same size maximum as oocytes.

One-way ANOVA tests of both maximum oocyte size and polyp fecundity indicated statistically significant differences between sampling dates (one-way ANOVA: $P < 0.001$) but no consistent pattern. The pattern of gonad development and polyp fecundity across time did not reveal any distinct seasonality (Figs. 4–6). Large gonads of both sexes appeared to be available in significant numbers throughout the year. Haphazard samples from other locations on Oahu, Kauai, Maui, and Big Island confirmed that mature gonads can be found at all locations throughout the year.

For female colonies, the level of intra-colony synchronization as measured by the size frequency distribution of oocytes between axial polyps within the same colony was often low (Fig. 7). The lack of intra-colony synchronicity and overlapping gametogenic cycles made it impossible to track a particular size class across time (Fig. 6). No inter-colony synchronicity was evident (Fig. 7).

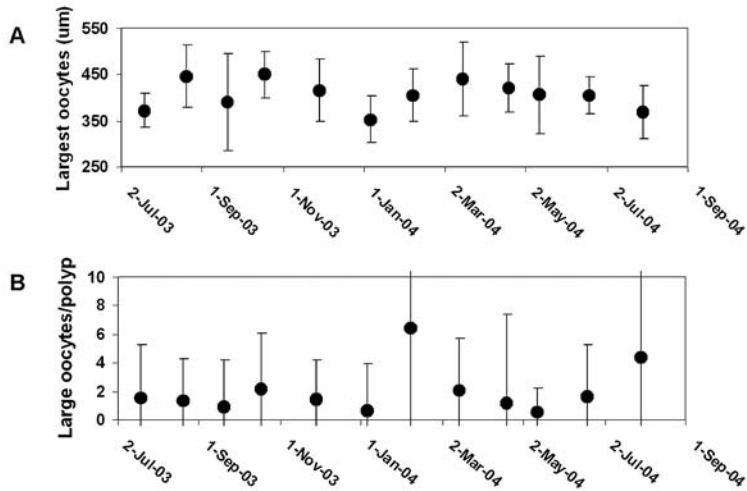

Figure 5. Time series reproductive analysis of seven female colonies from the YO-257 study site: (A) average size (diameter) of three largest oocytes per polyp; (B) number of large oocytes (> 250 µm) per polyp.

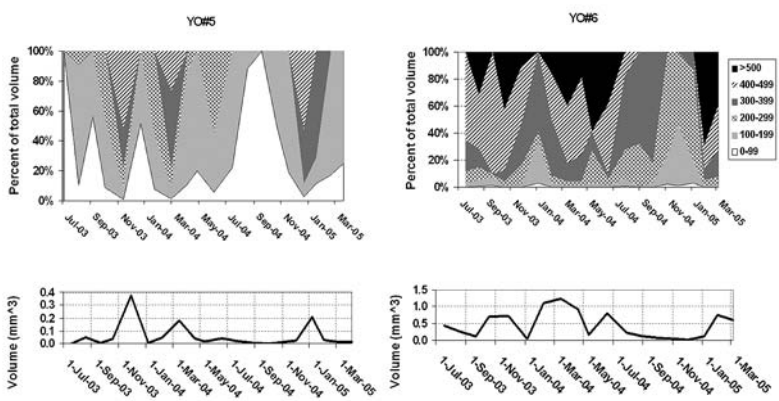

Figure 6. Time series of gametogenesis in two female *Carijoa riisei* colonies. Left panels = colony YO # 5; right panels = colony YO # 6. Size frequency distribution of oocytes and total oocyte volume across time.

Male and female fecundity (i.e., number of large gonads per lateral polyp and percent of lateral polyps containing large gonads) varied by location on the axial polyp, between colonies, and between study sites. The number of large gonads within a lateral polyp was highly variable. In some cases, gonads filled the entire polyp cavity to the point of causing surface deformations. The terminal polyp often had hundreds of large gonads filing the entire length of the axial chamber. Highly fecund colonies were found to be both male and female. At Hawaii Kai, male fecundity in terms of number of large gonads per lateral polyp tended to be several times higher than female fecundity (Fig. 8).

The colonies at the Hawaii Kai study site were more fecund than those at the YO-257 study site (Figs. 8–9). Over the course of the respective sampling periods, the

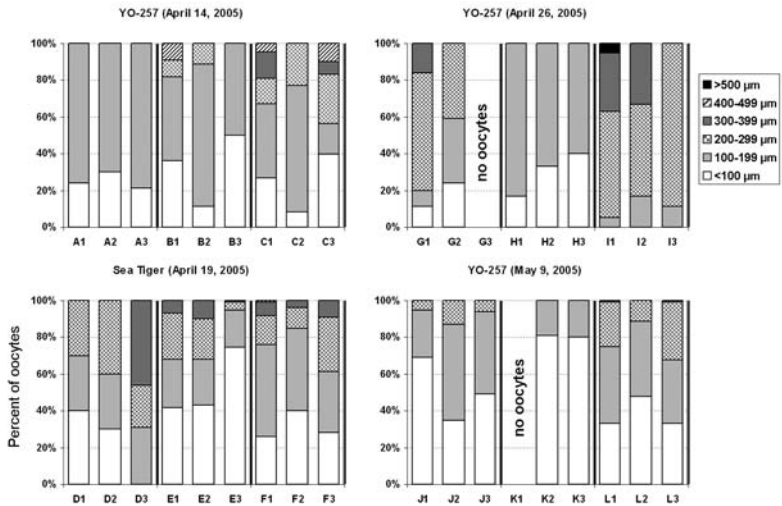

Figure 7. Intra-colony and inter-colony synchronicity for female *Carijoa riisei* colonies sampled from the YO-257 and Sea Tiger study sites. The size frequency distribution of oocytes are shown for three axial polyps (numbered 1, 2, 3) from each of three colonies (labeled A, B, C, etc.) on each of four sampling dates. Size is classified by oocite diameter.

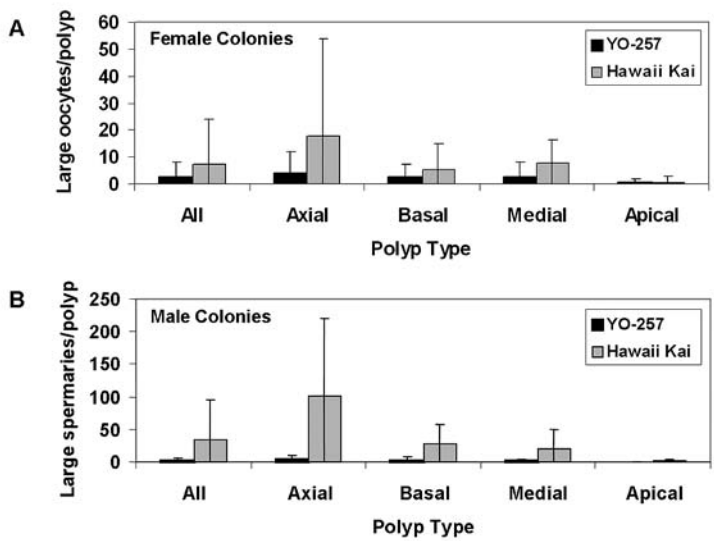

Figure 8. Comparison of *Carijoa riisei* polyp fecundity by polyp type between study sites: (A) number of large oocytes (> 250 µm) per polyp in female colonies; (B) number of large spermaries (> 250 µm) per polyp in male colonies.

mean number of large oocytes (> 250 µm in diameter) per lateral polyp was 7.5 at the Hawaii Kai study site (n = 222) and 2.2 at the YO-257 study site (n = 624). The mean number of large spermaries per lateral polyp was 32.7 at the Hawaii Kai study site (n = 158) and 1.2 at the YO-257 study site (n = 180). In general, periods of visible stress (e.g., parasitic sponge overgrowth) corresponded to cessation of gonad production.

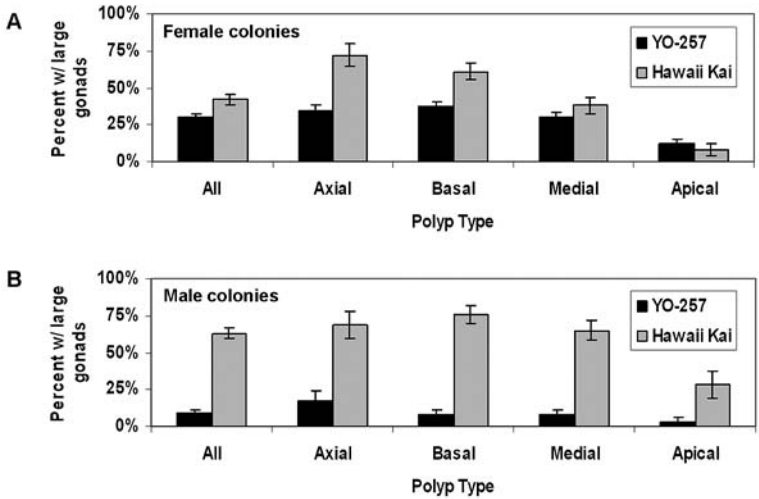

Figure 9. Comparison of *Carijoa riisei* polyp fecundity by polyp type between two study sites: (A) percent of polyps with large oocytes; and (B) percent of colonies with large spermaries.

Mode of Reproduction.—Evidence of internal fertilization or internal brooding of planulae was absent in over 2000 polyps dissected across all seasons and study sites. Furthermore, no evidence of external surface brooding was observed. Large, pigmented oocytes and spermaries dissected from live samples were negatively buoyant in seawater. In the aquarium, eggs were spawned continuously in low numbers with no correlation to lunar periodicity (Fig. 10). None underwent cleavage, and all disintegrated within several hours. Attempts to observe any planular behavior were unsuccessful. However, newly settled primary polyps were observed a few times within large aquarium cultures.

Onset of First Reproduction.—The smallest colony with spermaries was 2.5 cm in height while the smallest colony with oocytes was 5 cm in height. Many large, pigmented gonads were occasionally observed in colonies ≥ 8.0 cm in height (Fig. 11). It is important to note that these results reflect young colonies during their initial stages of growth and not short axial polyps from large, older colonies.

Discussion

Sexuality and Sex Ratio.—*Carijoa riisei* appears to follow the general pattern within octocorals where 87% of species studied to date (n = 133) are gonochoric (S. Kahng, University of Hawaii, unpubl. data). A very low incidence of hermaphroditism (<< 1%) has been reported for several gonochoric octocorals (Benayahu and Loya, 1984a; McFadden, 1991; Coma et al., 1995b; Kruger et al., 1998; McFadden, 2001). A regular incidence of hermaphroditism (> 1%) co-occurring with gonochoric colonies as found in *C. riisei* is rare. Such a "mixed" sexuality i.e., regular incidence of hermaphrodites and gonochoric colonies, has been previously reported in only two octocoral species, *Alcyonium coralloides* Pallas, 1766 in the temperate Atlantic and *Sarcophyton glaucum* Quoy and Gaimard, 1833 in South Africa (McFadden, 1999, 2001; Schleyer et al., 2004).

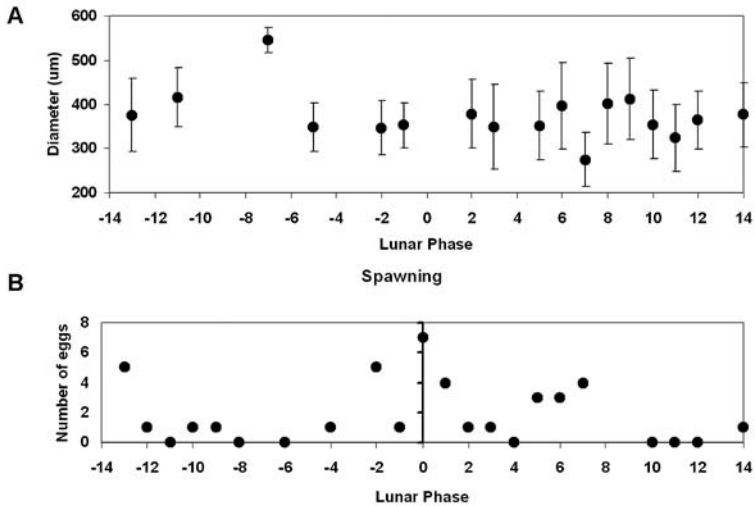

Figure 10. Lunar periodicity in female colonies. (A) Average diameter of the three largest oocytes per polyp in seven female colonies sampled approximately monthly from May 2003 to Feb 2005. (B) Total eggs released by a female colony of *Carijoa riisei* during two 4-wk aquarium trials (beginning on 14 Nov 2005 and 14 Feb 2006).

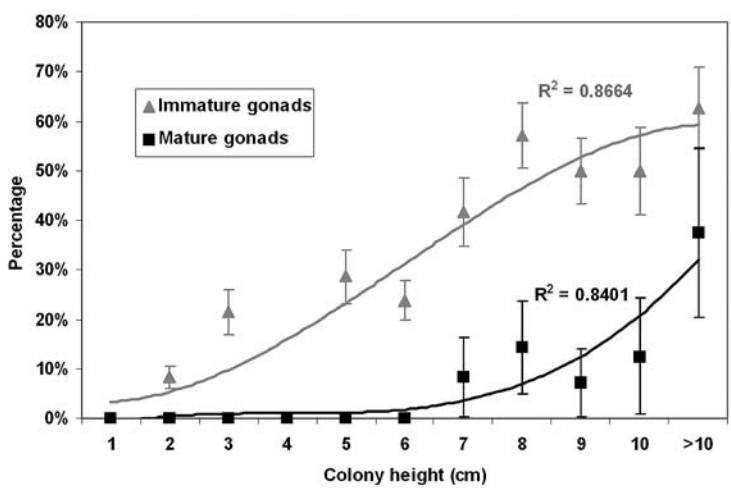

Figure 11. Minimum colony size associated with sexual reproduction in *Carijoa riisei* (n = 131). Colony size/height was indicated by its longest axial polyp. Large (> 250 μm) and pigmented gonads were classified as mature.

Hermaphroditism in *C. riisei* warrants additional research given its association with a specific population (all incidents but one were found at Hawaii Kai). It is possible that the Hawaii Kai *C. riisei* population exhibits a mixed sexuality while other *C. riisei* populations are gonochoric. *Sarcophyton glaucum* exhibits different sexuality by region, being mixed in South Africa but gonochoric in the Red Sea and Great

Barrier Reef (Benayahu and Loya, 1986; Alino and Coll, 1989). Species exhibiting different sexuality by region are rare and have only been confirmed in one additional species, *Heteroxenia elizabethae* Kölliker, 1874, which is hermaphroditic in the Red Sea but gonochoric in the Great Barrier Reef (Benayahu et al., 1990; Benayahu, 1991, 1997).

The seemingly ephemeral nature of hermaphroditism in *C. riisei* raises questions about the stability of colony sex over time. Due to colony morphology, it is difficult to confirm a single colony's boundary without following the history of the colony from original recruitment. Aside from the observations of hermaphroditic polyps, there was no clear evidence for changes in colony sex. At present for octocorals, complete reversals in colony sex (between male and female and vice versa) are unknown, and the same is true for scleractinian corals (Fadalallah, 1983; Harrison and Wallace, 1990; Richmond and Hunter, 1990).

Some hermaphroditic alcyonacean and scleractinian corals exhibit protandrous development where individuals change from male to simultaneous hermaphrodites after individuals reach a minimum size (Achituv and Benayahu, 1990; Richmond and Hunter, 1990) supporting the theory that oocyte development is more energetically expensive than spermary development (Charnov, 1982). For *C. riisei*, the observations of small female colonies and large, highly fecund male colonies are not consistent with protandrous development (Hall and Hughes, 1996). However, *C. riisei* does exhibit higher polyp fecundity in male versus female colonies (Fig. 8).

The male to female sex ratio in Hawaii is consistent with that reported for *C. riisei* in Puerto Rico by Bardales (1981). In Hawaii, the site specific sex ratios differed widely suggesting that vegetative propagation plays a significant role in local population growth. Vegetative propagation is common in other octocorals (Lasker, 1988, 1990) and sometimes dominates population structure over small spatial scales (McFadden, 1997). The results from this study indicate the need for caution when measuring the sex ratio of corals which propagate asexually.

Gametogenesis and Reproductive Cycle.—Fertile *C. riisei* colonies appear to have overlapping gametogenic cycles that continue year-round. Reproduction in *C. riisei* does not exhibit seasonal patterns or lunar periodicity which are common in corals (Grigg, 1970; Alino and Coll, 1989; Harrison and Wallace, 1990; Richmond and Hunter, 1990; Benayahu, 1997; Richmond, 1997; Penland et al., 2004). While the presence of mature gonads in one lateral polyp was typically accompanied by mature gonads in neighboring lateral polyps, this association likely reflects overall colony health rather than any periodicity in reproductive cycle.

Carijoa riisei appears to breed continuously with a potential for high fecundity throughout the year. Continuous breeding over an extended period of time has been reported in several shallow-water zooxanthellate, brooding alcyonaceans (*Sarcothelia edmondsoni* Verril, 1928, *Heteroxenia fuscescens* Ehrenberg, 1834, *Xenia macrospiculata* Gohar, 1940) (Davis, 1977; Benayahu and Loya, 1984a; Achituv and Benayahu, 1990; Dahan and Benayahu, 1997b) and the deep-water, azooxanthellate, brooding alcyonacean, *Anthomastus ritteri* Nutting, 1909 (Cordes et al., 2001). In the azooxanthellate, spawning alcyonacean *Dendronephthya hemprichi* Klunzinger, 1877, continuous year-round breeding occurs with fecundity correlated with seasonal availability of food (Dahan and Benayahu, 1997b).

Because of the continuum of gonad sizes within a given axial polyp, no dominant age/size class of oocyte or spermary was evident, and it was not possible to infer the

length of the oogenic or spermatogenic cycles. In alcyonaceans, concurrent development of multiple but distinct age/size classes of oocyctes has been associated with overlapping oogenic cycles which last more than one year (Benayahu, 1997). However, analysis of *C. riisei's* onset of reproduction suggests that oogenesis is shorter than one year (see below). Multiple overlapping oogenic cycles of *C. riisei* appears to result from continuous breeding associated with high fecundity and rapid oogenesis.

The consistent difference in fecundity between the two study sites is striking and may be caused by environmental differences and nutritional control of gametogenesis and fecundity. The Hawaii Kai site is characterized by turbid waters in close proximity to large coastal mud flats rich in organic matter and infaunal benthic organisms (pers. obs.). In contrast, the YO-257 study site is characterized by clear oceanic water with a sandy bottom.

During the sampling period, incidence of sublethal stress on specific colonies at the YO-257 was correlated to periods of reduced or zero fecundity. Stress was observed in the forms of mechanical injury and progressive overgrowth by the parasitic sponge, *Callyspongia* sp. Subsequent senescence of *Callyspongia* sp. overgrowth on colonies was associated with a return of gonad production. In Hawaii, other types of sponges are commonly found growing on *C. riisei* axial polyps but do not appear to affect fecundity in *C. riisei.*

Mode of Reproduction.—The collective evidence from this study suggests that *C. riisei* is an asynchronous, continuous spawner of negatively buoyant eggs. The release of unfertilized eggs in seawater tanks suggests that fertilization is external. Space limitation observed in highly fecund polyps may conflict with internal brooding. However, some evidence from other locations implicates internal fertilization in *C. riisei*. Bardales (1981) and Calcinai et al. (2004) each reported finding one planula larvae inside a *C. riisei* polyp during their respective studies. Given the large number of polyps examined from continuously breeding and highly fecund colonies, the lack of similar observations in Hawaii suggests that internal brooding, if it occurs, does not last for any significant length of time. However, both external fertilization and internal fertilization has been recorded in the same coral species from different geographical regions (Shlesinger et al., 1998; Vermeij et al., 2004).

Evidence from our study also indicates that *C. riisei* is not an external surface brooder. Unlike some octocorals which are known to produce antifouling chemicals (Coll, 1992; Sammarco and Coll, 1992), *C. riisei's* outer surface is typically heavily colonized with epifauna and thus is exceptional among Octocorallia. This fouling community is not consistent with surface brooding in other octocorals (Davis, 1977; Benayahu and Loya, 1983; Benayahu et al., 1990; Coma et al., 1995a; Benayahu, 1997).

Similar to *C. riisei*, *D. hemprichi* continuously spawns negatively buoyant eggs and spawning in one sex is not induced by the other (Dahan and Benayahu, 1997b). Compared with synchronized, mass spawning of buoyant gametes, this strategy reduces the probability of successful external fertilization and forgoes the advantage of predator satiation. The habitat of *C. riisei* is often characterized by high current flow and turbulence. In an isotropically turbulent hydrodynamic regime, the probability of successful fertilization of freely spawned gametes decreases exponentially with distance between male and female colonies and with time (Pennington, 1985; Denny and Shibata, 1989; Coma and Lasker, 1996). External fertilization success for *C. riisei* likely depends on dense aggregations of colonies in close proximity (Denny

and Shibata, 1989; Coma and Lasker, 1996; Lasker et al., 1996). Dahan and Benayahu (1997) suggested that the slow release of eggs by *D. hemprichi* during spawning may increase rates of fertilization in high flow environments. The prolonged availability of mature oocytes in spatially fixed locations might help counter the rapid dilution of freely spawned male gametes and increase the likelihood of contact (Denny and Shibata, 1989).

In contrast to synchronized spawning events, continuous release of sexual propagules across time probably enables *C. riisei* to effectively exploit ephemeral availability of substrata and favorable conditions for recruitment over time. Continuous breeding without prolonged internal brooding may facilitate high planula production over time and free polyp cavity space for oocytes development (Vermeij et al., 2004). Internal fertilization followed by immediate release of an early stage zygote (i.e., pseudo-brooding), which has been reported in several "spawning" scleractinians (Hagman et al., 1998a,b; Vermeij et al., 2004), remains a possibility for *C. riisei*. Such a strategy may overcome low population density and dilution effects by filtering low concentrations of sperm from seawater and fertilizing eggs throughout an extended period of time (Phillippi et al., 2004). Since unfertilized eggs may lyse after 6 hrs in seawater (Harrison and Wallace, 1990), this strategy may also extend the viable period of fertilization for eggs.

Onset of First Reproduction.—Because a *C. riisei* colony shares nutrition between axial polyps (Rees, 1969), minimum colony size including all interconnected axial polyps is relevant for the onset of sexual reproduction. The growth rate of the first (and longest) axial polyp can be used as a proxy for calculating minimum colony size (height) and the onset of sexual reproduction. The initial growth pattern for colonies typically involves 2–4 axial polyps extending into the water column from basal stolons. Axial polyps of *C. riisei* commonly sustain average growth rates of ~0.5 cm wk^{-1} for several months. Rates of axial polyp extension slow as they approach a determinate length (12–14 cm when growing upward and 16–20 cm when growing downward), and lateral colony expansion co-occurs as new axial polyps are generated (Kahng, 2006).

Carijoa riisei colonies appear to reach sexual maturity and begin gametogenesis within a few months. However, since growth rates were based on axial polyp extension from stolons and not from initial settlement of larvae, there is a pre-axial polyp colony stage of variable duration. Given the fast growth rates observed for axial polyps, it is likely that this pre-axial polyp colony stage is not very long under favorable conditions. Oogenesis in *C. riisei* appears to take < 6 mo and possibly much shorter in large, healthy colonies.

Conclusions

The reproductive features of *C. riisei* enable it to produce sexual propagules relatively quickly following colonization. *Carijoa riisei* appears to have an early age of sexual maturity relative to other alcyonacean octocorals which typically take ≥ 2 yrs to mature (Kinzie, 1970; Grigg, 1976, 1977; Benayahu and Loya, 1983, 1984a,b, 1986; Farrant, 1986; Gotelli, 1991; Coma et al., 1995a,b; Fitzsimmons-Sosa et al., 2004; Torrents et al., 2005). The only other octocoral known to have such an early age of sexual maturity is *D. hemprichi* which matures in 1.5 yrs (Dahan and Benayahu, 1997b). *Carijoa riisei's* fast growth and early age of reproductive maturity are exceptional

among alcyonacean octocorals. These characteristics likely contribute to its invasive success in Hawaii compared to native, azooxanthellate corals (e.g., *Antipathes dichotoma* Pallas, 1766, *Tubastraea coccinea* Lesson, 1829) that exhibit slower rates of growth and reproduction (Grigg, 1964, 1976; Fenner and Banks, 2004).

Carijoa riisei and *D. hemprichi* are the only alcyonaceans known to asynchronously spawn negatively buoyant gametes year-round. No scleractinian coral is known to exhibit this reproductive strategy (B. Richmond, University of Hawaii, pers. comm.; Harrison and Wallace, 1990; Richmond and Hunter, 1990). This unique strategy may contribute to successful colonization of artificial substrata, but also makes sexual reproductive success very density-dependent and likely requiring close proximity between male and female colonies. *Dendronephthya hemprichi* produces asexual propagules clonally, thereby reducing its dependence on sexual reproduction for dispersal (Dahan and Benayahu, 1997a). Analogously, *C. riisei* proliferates asexually via stolonization (Rees, 1969) which obviates sexual reproduction for local propagation. However, *C. riisei* must successfully produce planula larvae to enable dispersal. Fortunately for *C. riisei*, vegetative propagation and a superior ability to compete for space (Thomas, 1979) enable colonies to persist and expand through time—eventually forming the dense, multi-colony aggregations required for successful sexual reproduction.

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