Sublethal effects on reproduction in native fauna: are females more vulnerable to biological invasion?

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Abstract: Although invasive species are a major threat to survivorship of native species, we know little about their sublethal effects. In soft-sediment marine systems, mat-forming invasive species often have positive effects, facilitating recruitment and enhancing the diversity and abundance of native invertebrates. However, because mat-forming invasive species change the habitat in which they invade, and benthic invertebrates are sensitive to environmental disturbance, important sublethal effects on native species may exist. Using a model marine system we show that the widespread mat-forming invasive alga *Caulerpa taxifolia* (Vahl) C. Agardh has strong negative effects on the reproductive traits of a native bivalve *Anadara trapezia* (Deshayes, 1840) (e.g. timing of reproductive development and spawning, and follicle and gamete production) even though the invader has positive effects on recruitment. Moreover, gender specific responses occurred and indicated that females were more susceptible to invasion than males. Our results indicate that sublethal effects of an invasive species on reproductive traits will have severe consequences for fitness of the native species.

Keywords: *Caulerpa taxifolia*, invasion biology, life history, reproductive traits, sex ratios.

Introduction

Invasive species have a range of direct and/or indirect lethal effects on native species. For example, predation and competition by invaders or changes in the disturbance regime following invasion can all cause high mortality of native species (reviewed in Mack et al. 2000). In contrast to what is known about the lethal effects of invasive species, much less is known about their sublethal effects on the life-history traits of native species (but see review in Levine et al. 2002). Sublethal effects may be more important than currently recognized and particularly important in the context of invasion by mat-forming plants, algae or sessile invertebrates as these species can form dense aggregations (e.g. D'Antonio and Mahall 1991; Equihua and Usher 1993; Crooks 1998; Wright 2005) and modify the habitat to such an extent that they can be considered habitat-forming or foundation species (*sensu* Bruno and Bertness 2001). Because sessile native plants and animals that occur in these modified habitats are unable to escape the invaders, sublethal effects on reproductive traits (e.g. gametogenic development, the timing of gamete release, investment in reproduction vs.

growth, and fecundity) may have important implications for the maintenance of these populations.

Marine ecosystems colonized by invasive mat-forming species often have higher diversity, recruitment, and survivorship of native species compared to nearby uninvaded habitats. These positive effects are linked to the provision of additional habitat complexity and/or a refuge from predation for native organisms (e.g. Crooks 1998; Gribben and Wright In Press). However, because invasive species that form dense aggregations have the potential to alter sediment structure, sediment chemistry, water flow and food supply (Crooks 1998; Crooks 2002; Chisholm and Moulin 2003), they represent a major perturbation to the ecosystems in which they invade and may also cause strong negative effects on native species. Life-history traits of marine invertebrates, such as growth and reproduction, are sensitive to changes in the benthic environment (e.g. Keck et al. 1975; Carmichael et al. 2004) and therefore, mat-forming invasive species may inflict more pervasive sub-lethal effects. These effects may be pronounced in sessile species (such as soft-sediment bivalves) that suffer long term exposure to changes in environmental conditions caused by invading species because they cannot escape an invaded environment.

Stressful environmental conditions can alter energy allocation decisions, potentially compromising the amount of energy allocated to reproduction and therefore fitness (e.g. Bayne et al. 1978; Brody et al. 1983; Elkin and Reid 2005). Because of the suggested higher energetic requirements of being female (Ghislen 1969), females may be expected to be more sensitive to environmental stress than males. However, for free-spawning invertebrates, zygote production is partly constrained by fertilization success (Levitan 1995), which is sensitive to changes in reproductive output, such as ambient sperm concentration

(Pennington 1985; Levitan 1991; Oliver and Babcock 1992). Therefore, effects on males may be equally important and investigation of the effects of mat-forming invasive marine species on reproduction in free-spawning invertebrates needs to consider both sexes. However, we know very little about how *in situ* changes in environmental conditions (particularly those induced by mat-forming invasive species) affect the allocation of resources, and the response of reproductive traits of native species.

In this study we used histology to examine changes in the reproductive traits and allocation of resources to reproduction in both males and females of the Sydney ark shell, *Anadara trapezia* (Deshayes, 1840), when invaded by the green alga *Caulerpa taxifolia* (Vahl) C. Agardh. *C. taxifolia* is perceived as a threat to temperate soft-sediment marine ecosystems. It has successfully invaded four temperate regions (Jousson et al. 2000; Creese et al. 2004), where it reaches high abundance (Meinesz et al. 1995, Wright 2005) and invades seagrass beds (Ceccherelli and Cinelli 1999). Fish foraging behaviour and population structure (Levi and Francour 2004; Longepierre et al. 2005), and the abundance of native invertebrates on hard substrata (Bellan-Santini et al. 1996) also appear negatively affected. We have previously documented high levels of recruitment of *A. trapezia* in *C. taxifolia* compared to nearby unvegetated substrata, possibly due to reduced predation within *C. taxifolia* beds (Gribben and Wright In Press). Because adult *A. trapezia* occur in *C. taxifolia* beds (Gribben and Wright In Press), *A. trapezia* is a useful model to investigate how mat-forming invasive species affect the reproductive traits of native marine invertebrates. Specifically, we examined whether there were differences between *A. trapezia* in unvegetated bare sediments and those from sediments invaded by *C. taxifolia* in the gametogenic development and timing of spawning events, sex ratios, the allocation of energetic resources between somatic and reproductive tissue, gamete output and size, and whether responses were gender specific.

Material and Methods

Study sites and collection and processing of samples

We investigated the effect of *C. taxifolia* on the reproductive traits of *A. trapezia* using both qualitative and quantitative analyses of histologically prepared slides of the visceral mass (with associated gonad, gut and attached foot). *A. trapezia* were collected from Sponge Bay, Lake Conjola (35°15'44.3" S, 150°26'47.8" E). Lake Conjola is a temperate barrier estuary heavily invaded by *C. taxifolia*. *C. taxifolia* was first discovered in Lake Conjola in April 2000 but may have invaded Lake Conjola between 1987 and 1995 (Creese et al. 2004). It is not known when *C. taxifolia* invaded Sponge Bay, although it has been in the bay since at least 2002 (Wright pers. obs.). The invasive alga is spreading in Lake Conjola and it now covers greater than 150 hectares of the lake floor (Creese et al. 2004, Wright 2005). Sponge Bay (approx. 5000m^2) contains a large population of *A. trapezia* and has extensive areas of the benthos covered by thick beds of *C. taxifolia,* intermingled with patches of bare sediment. Monthly samples of 30 *A. trapezia* were collected from both bare unvegetated sediments (hereafter referred to as bare habitat) and sediments containing dense stands of *C. taxifolia* (hereafter referred to as *Caulerpa* habitat) from August 2004 (winter) to July 2005 (winter). Samples were not collected during September 2004 and May 2005. On each sampling date *A. trapezia* were collected haphazardly in 1.5-2 m water depth from several intermingled patches of *Caulerpa* and bare habitat throughout the bay to avoid any confounding effects. Once collected, shell length (anterior-posterior axis of the right valve) was measured to the nearest millimeter using vernier calipers. The shell length of males from *Caulerpa* habitat ranged from 33-73 mm and females from 31-67 mm. The shell length of males collected from bare habitat ranged from 34-68 mm in shell length females from 38-68 mm, although the majority of clams were >50 mm (95% for both sexes in both habitats). The visceral mass was then excised, and fixed in Bouin's solution for a period of 24 hrs. All *A. trapezia* were processed within one hour of collection. Following fixation, samples were dehydrated using a graded ethanol series, blocked in paraffin wax and sectioned at 7 μm. One longitudinal section was taken along a standard position down the centre of the viscera (along the anterior-posterior axis) for each *A. trapezia*. All sections were stained with Haematoxylin and counterstained with Eosin. The histologically prepared slides were examined using a compound microscope at x4, x10 and x20 magnification.

Reproductive cycle of *A. trapezia*

Because the reproductive cycle of soft-sediment bivalves generally follow an annual pattern involving a period of gametogenic development, followed by gamete maturation, spawning and post-spawning recovery (e.g. Gribben *et al* 2004), individuals are generally placed into one of six reproductive stages based on the reproductive state of the majority of follicles on the histologically prepared slides. The six stages are generally categorised as: 1) primary development (beginning of gametogenic development; many oogonia and small oocytes present in follicles), 2) secondary development (many maturing oocytes present), 3) mature (ready to spawn with follicles full of large, round ova), 4) partially spawned (some spawning of ova, many oocytes still present), 5) spawned (few ova or oocytes remaining), and 6) indeterminate (resorbtion of all residual unspent gametes making sexing of clams difficult). For more detailed descriptions of the stages see Hadfield and Anderson (1988) and Gribben et al. (2004). Hadfield and Anderson (1988) used gross gonad appearance and quantitative measures of oocytes for female *A. trapezia*, and histological classification of male *A. trapezia,* to show that *A. trapezia* spawns during summer with gonad regeneration commencing during winter and spring following a resting stage. We qualitatively assessed whether *Caulerpa* habitat affected the annual reproductive cycle of *A. trapezia* (including the timing of gametogenic development, maturation of gametes, spawning and post-spawning recovery) compared to bare habitat. The gonadal state of individual *A. trapezia* was described as one of the six stages based on the most dominant stage present in 10 haphazardly selected follicles for each individual.

Sex Ratios of *A. trapezia*

The ratio of male to female *A. trapezia* in each habitat was determined from clams deemed sexually mature; i.e. if gametes were present. Pearson's chi-square (X^2) test was used to test whether the sex-ratios of *A. trapezia* were independent of habitat type.

Ratio of Reproductive to Somatic Tissue

Partitioning of energetic resources between somatic and reproductive tissue in males and females from bare and *Caulerpa* habitat was determined using video image analysis (Abramoff et al. 2004) for each individual. Typically, partitioning of resources between reproductive and somatic tissue is examined by separating reproductive tissue and/or products from the remaining soft-tissue parts (e.g. Bayne et al. 1983; Harvey and Vincent 1989). However, as for most bivalves, in *A. trapezia* the gonad is incorporated into the viscera and it is impossible to accurately separate them. Therefore we used an alternative approach. We quantified the ratio of area of each section occupied by follicles to total area of each section and determined whether there were differences in the ratio between habitats and sexes using a 3-factor ANCOVA with factors time (random), habitat and sex (both fixed) and size (shell length) as a covariate.

Size and Abundance of Eggs

Fecundity in soft-sediment bivalves is often measured by counting the number of gametes spawned by sexually mature adults or using Gonad Somatic Indices (GSIs) (see review in see Eversole 1989). However, it is often difficult to induce adults to spawn and GSIs are generally only good indicators of fecundity when the gonad can be easily separated from the rest of the viscera (e.g. mussels). We compared the number of oocytes/female and the mean diameter of oocytes/female between habitats from the histological sections. For each female, the number of oocytes/female was estimated by pooling the number of oocytes across six haphazardly selected follicles, because follicles are convoluted and sectioning results in inconsistently sectioned follicles. However, we assumed that the variability in follicle sectioning was consistent between individuals. Mean oocyte diameter for each female was determined from the pooled oocytes. All oocytes in follicles were counted but only oocytes with visible nuclei were measured, as measuring oocytes with no visible nuclei would underestimate oocyte diameters as these have not been sectioned across the centre of the egg. Differences in the abundance and mean diameter of oocytes were analysed using a 2-factor ANCOVA with factors time (random) and habitat (fixed) with size as a covariate.

Results

Reproductive cycle of A. trapezia

The annual reproductive cycle of male and female *A. trapezia* appeared synchronous with the proportion of stages present within each month similar between males and females within each habitat (Figs. 1A-D). Therefore, the results were pooled across sexes within habitats and we only discuss these pooled results for the reproductive cycle (Figs. 1C and

F). Qualitatively, *Caulerpa* habitat had a negative effect on the timing of gametogenic development, the proportion of *A. trapezia* that were fully mature and post-spawning recovery of *A. trapezia*. Nearly all *A. trapezia* collected from bare habitat in August 2004 (winter) had begun gametogenic development (97% of were in a primary and secondary stage of development), whereas, 69% of *A. trapezia* from *Caulerpa* habitat were in a primary and secondary stage of development (Fig. 1). Delayed reproductive development was even more evident in 2005: 36% of *A. trapezia* from bare habitat began gametogenic development as early as June (winter), whereas nearly all *A. trapezia* from *Caulerpa* habitat remained in a spawned or indeterminate stage through June and July. The qualitative stagings suggested that there were two spawning periods and that spawning was synchronous between sexes within habitats. The first spawning period occurred during spring (October 2004) and, although the second spawning period started in late summer (February), the majority of spawning appeared to occur in early autumn (from March). Despite synchrony in spawning within habitats, a smaller proportion of *A. trapezia* in *Caulerpa* habitat matured compared to bare habitat prior to both spawning events (October 2004 and February 2005). Between spawning events (October 2004 to January 2005), a higher percentage of *A. trapezia* in *Caulerpa* habitat were in the partially spent or spawned stages suggesting they did not recover as well compared to the bare habitat where *A. trapezia* maintained a higher level of condition (most were either in a secondary development or mature stage). Post-spawning (during April 2005) clams from both habitats went into a resting phase in which there was very little gametogenic activity. Gametogenic development had resumed by winter (June/July) 2005, although it may have begun as early as May as we did not collect samples then. Importantly, nearly all clams from bare habitat had gametes present year round, which did not occur in *Caulerpa* habitat. Over the entire study, a higher percent of *A. trapezia* in *Caulerpa* habitat (12%) compared to bare habitat

(1%) could not be sexed (i.e. were indeterminate). This was particularly evident post-spawning 2005, when 23-40% of *A. trapezia* from *Caulerpa* habitat from April to July could not be sexed. This was due to the complete resorbtion of residual gametes, and was not related to size (i.e. *A. trapezia* with no gametes being immature) as only two of 25 indeterminate *A. trapezia* collected from *Caulerpa* habitat during this period were <50 mm in shell length.

Sex Ratios

The ratio of total number of males to females sampled in *Caulerpa* habitat (ratio 1:1) and bare habitats (ratio 1:1.4) was significantly different (χ^2, 1 *df*, *p*=0.036). These differences in sex ratio were due to lower numbers of females in *Caulerpa* habitat (131) compared to bare habitats (173). Because the total number of males sampled from each habitat was similar (132 and 122 from *Caulerpa* and bare, respectively), the large number of unsexed clams in *Caulerpa* habitat (37) compared to bare habitat (5) were most likely females that had not developed gametes.

Ratio of Reproductive Tissue to Somatic Tissue

Overall, females had a significantly higher ratio of follicle area to total tissue area than males (Fig. 2; Table 1). For most months (7 out of 10 samping occasions), both males and females had a significantly lower ratio of follicle area to total tissue area in *Caulerpa* vs. bare habitats (Fig. 2; Table 1). The temporal patterns in follicle area to total tissue area for males and females in both habitats were generally consistent with those observed in the qualitative stagings. In both habitats, ratios of follicle area to total tissue area increased from August 2004 and peaked in October prior to spawning and fell as spawning progressed. We can confirm that the drop in ratio of follicle area to total tissue area was due to the start of the spawning season as we observed a large spawning event *in situ* between collecting the October and November 2004 samples. The ratio of follicle area to total tissue area increased again from January 2005. However, in this second spawning event, the follicle area to total tissue area ratio for both males and females in *Caulerpa* habitat peaked a month after those in the bare habitat (February vs. January), indicating delayed spawning in *Caulerpa* habitat (Fig. 2)

Size and abundance of oocytes

The temporal patterns of oocyte abundance and size were very similar between habitats and closely follow the patterns observed in the qualitative stagings. However, females from bare habitat had significantly more oocytes compared to *Caulerpa* habitat (Fig. 3A; Table 2). This was particularly evident prior to and just after the first spawning event (August to December 2004). However, from January 2005, oocyte abundance was very similar between habitats. *Caulerpa* habitat did not have an effect on oocyte size (Fig. 3B; Table 3). Unlike the temporal patterns in the ratio of follicle area to total tissue area, no difference occurred in oocyte number and diameter between habitats around the second spawning period. An increase in oocyte diameter values in July 2005 suggested gametogenesis had begun again in both habitats. However, this is not supported by the number of oocyte/female (Fig. 3A) or the ratio of follicle area to total tissue (Fig. 2) and is probably a result of the development of any previously unspent oocyte.

Discussion

Invasive species that form dense aggregations can increase the diversity and abundance of native species (e.g. Crooks 1998; Gribben and Wright In Press). Here, we have demonstrated that even though *A. trapezia* recruits into *C. taxifolia* (Gribben and Wright In Press) where adult populations also exist, important reproductive traits of *A. trapezia* are strongly compromised with delayed gametogenic development, changes in the timing of spawning events, less reproductive tissue and fewer oocytes and sperm produced. Moreover, there appear gender specific effects with *Caulerpa* habitat having proportionally fewer females than bare habitat. This is likely due to reduced gametogenic development and suggests that females may be more vulnerable to invasion than males.

Although the energetic costs of females are purported to be higher than those for males (Ghislen 1969; Wright 1988), no studies have previously demonstrated gender specific effects of invasive species on the life-history traits of native species or moreover, gender specific susceptibility of reproductive traits in bivalves to habitat change. Combining undifferentiated and females *A. trapezia* in *Caulerpa* habitat gives the same male:female ratio (1:1.4) as in bare habitat suggesting the gender specific effects on female *A. trapezia* are sublethal. The large number of undifferentiated clams in *Caulerpa* habitat occurred mainly during the period of gamete resorbtion post-spawning when females from *Caulerpa* appear to resorb all remaining gametes as quickly as possible. In contrast, this level of resorbtion does not occur in males or females in bare habitat where almost all *A. trapezia* had gametes year round. Earlier resorbtion of gametes and the later start to the reproductive cycle in 2005 suggests that the energetic requirements are female *A. trapezia* in *Caulerpa* habitat are not being met. It is also possible that *Caulerpa* habitat affects the sexual development and/or size at sexual maturity of *A. trapezia* but small size classes (< 35 mm) are largely absent from both habitats, particularly bare, we could not assess this.

Temporal patterns in the ratio of follicle area to total tissue area showed that the timing of the second spawning period for both male and female *A. trapezia* was delayed in *Caulerpa* habitat. Spawning during periods of optimal temperature and food supply enhances the survivorship of planktotrophic larvae (Mackie 1984). Thus, if spawning in *C. taxilfolia* is decoupled from periods of optimal food supply or temperature, then larval development, and therefore fitness, may be severally compromised. Sub-optimal environmental conditions can have negative effects on planktotrophic larvae, such as increasing developmental times, and reducing metamorphic and post-settlement success (Mackie 1984). Furthermore, there can be a relationship between larval supply and recruitment (see review in Lenihan and Micheli 2001), but we have no information regarding the relative contributions of the spring and autumn spawning periods to recruitment. Within habitats, follicle (for males and females) and oocyte production peaked at similar levels prior to each spawning event. However, females in bare habitat had a slightly higher follicle area to total tissue area ratio prior to the autumn spawning suggesting this period may be more important in terms of egg and zygote production. If this is the case, our findings of a temporal shift in the autumn spawning period resulting from *C. taxifolia* invasion may indeed have implications for population maintenance.

The reduction in the ratio of follicle area to total tissue area in both males and females in *Caulerpa* habitat suggested that both sexes maintain somatic tissue at the expense of gamete production. This strategy may maximize long term reproductive success at the expense of short-term gains if investment in current reproductive activity is detrimental to future growth and/or survivorship (Brody et al. 1983; Stearns 1992). Moreover, although follicle production by males and females appeared to be similarly affected by being in *Caulerpa* habitat (absence of a significant Habitat x Sex interaction; Table 1), the differences in sex ratios between habitats suggest that the affects on females were greater. Not only did females in *Caulerpa* habitat allocate fewer resources to reproduction but there also appeared to be changes in how resources were allocated between follicle and oocyte production between spawning events. Prior to the first spawning event, females in *Caulerpa* habitat had fewer oocytes and a lower follicle area to total tissue than those in bare habitat, but the timing of spawning was maintained. In contrast, prior to the second spawning period, females in *Caulerpa* habitat produced more oocytes relative to the first spawning event, but follicle production was slower and spawning was delayed. It appears that a certain level of follicular production may have to be obtained before spawning commences, although this is reduced in *Caulerpa* habitat compared bare habitat.

Females in *Caulerpa* habitat produced significantly fewer eggs than females in bare habitat but there was no difference in egg size. Theory predicts that under different environmental conditions an optimal egg size is produced that optimizes the trade-offs between egg size and egg number (Smith and Fretwell 1974). Faced with increasing environmental uncertainty, females often produce fewer, larger eggs (e.g. George et al. 1991; Koops et al. 2003 and references therein). A larger egg size confers increased fitness (Moran and Emlet 2001) through increased survivorship of offspring in poor environmental conditions, or through increased dispersive ability (via increased energy reserves) with which to ‘escape’ the poor habitat (Koops et al. 2003; Marshall and Keough, 2003). For free-spawning marine invertebrates, however, this response is not fixed and a reduction in number but no change in egg size, and a reduction in egg size and number have been observed (Bayne et al. 1978; Thompson 1982; Bayne et al. 1983). Adjustments in offspring size in response to environmental variation should occur if conditions faced by the mother are accurate predictors of the circumstances young will encounter (McGinley et at. 1987), as may occur with species that produce lecithotrophic larvae with limited dispersive ability. Many free-spawning marine invertebrates produce planktotrophic larvae derived from eggs with limited maternal investment that spend extended periods in the water column (in the order to weeks). Consequently, the site of juvenile settlement may be very distant from the parental environment (in the order of kilometers). In this case, females cannot predict the environment their off-spring will encounter so changing egg size to maximize fitness would appear unnecessary. For free-spawning invertebrates, it may be more likely that there are changes in egg number than egg size when mothers are faced with environmental uncertainty, as was found in this study.

The significant reduction in both sperm and egg production in *A. trapezia* from *Caulerpa* habitat may have consequences for fertilisation success and zygote production. Several *in situ* studies have demonstrated low fertilization success, even over short distances, due to gamete dilution (e.g. Pennington 1985; Levitan 1991). If population size is constrained by gamete production then variations in fertilization success will have consequences for population maintenance (Levitan 1995). Studies have demonstrated a relationship between larval supply and population density for free-spawning invertebrates (e.g. Peterson et al. 1996). However, as Levitan (1995) notes, the influence of fertilization success in producing variation in settlement success in species with planktonic larvae is uncertain. Even though we did not find any evidence of a difference in mean egg diameters between habitats, egg size may be a poor indicator of egg quality. Thompson (1982) found that adult green urchins, *Strongylocentrotus droebachiensis*, fed a high quality food ration produced eggs with a higher energetic content (increased lipid reserves) than eggs from adults fed a lower quality diet, although egg sizes were similar between treatments. Similar results were also obtained for the blue mussel, *Mytilus edulis* (Bayne et al. 1978). Any effects on the quality of gametes produced between habitats and their effects on fertilization success and larval development remain to be explored. However, if *Caulerpa* generally invades habitats in which *A. trapezia* occurs then reduced individual fitness of *A. trapezia* has implications not only for the sustainability of local populations, but populations on a regional scale.

Factors regulating reproductive responses of marine invertebrates within habitats (e.g. change in gamete production or gamete size) remain obscure (Jaeckle 1995). For free-spawning marine invertebrates, studies that have investigated resource allocation and reproductive trade-offs have focused on the effect of food ration in controlled laboratory conditions (e.g. Bayne et al. 1983; Thompson 1982). Food limitation in *Caulerpa* beds resulting from hydrodynamic damping of advective flows into *Caulerpa* beds may also be responsible for the observed effects on the reproductive traits of *A. trapezia*. In a reverse scenario, Allen and Williams (2003) found that food-limitation reduced the growth, reproduction and survivorship of the invasive Asian mussel, *Musculista senhousia*, that settled in native eelgrass, *Zostera marina*. However, we have no information on whether changes in hydrodynamic regimes result in differences in food supply inside and outside *Caulerpa* beds. Changes in sediment structure and composition may also contribute to the observed effects on reproductive traits. High densities of vegetation in soft sediments can result in anoxic or hypoxic conditions and a build up of toxic by-products (e.g. ammonia and sulphide) (Diaz and Rosenberg 1995; Bolam and Fernandes 2002) due to the decomposition of organic material. Indeed, sediments containing dead macrophyte material turn black once penetrated by *C. taxifolia* rhizoids due to the presence of sulphides produced by the nitrogen fixing bacteria associated with the rhizoids (Chisholm and Moulin 2003). Furthermore, we did not monitor densities of adults over time and do not know whether there are differences in natural mortality between habitats. However, *A. trapezia* and *Caulerpa* have co-existed in Sponge Bay and a number of other sites in Lake Conjola for at least 4 yr (Wright pers. obs) indicating that sublethal effects will be important.

The majority of studies on invasive species that demonstrate positive effects on native species have been conducted at the community level (e.g. Posey 1988; Crooks 1998; Neira et al. 2005; Wonham et al. 2005). That is, they solely describe differences in the abundance of individual species, or changes in diversity between invaded and non-invaded sites. However, studies focusing on a single aspect such as community structure could lead to erroneous conclusions about the overall influence of the invader. Despite enhanced recruitment of *A. trapezia* in *Caulerpa* habitat compared to nearby unvegetated substrata (Gribben and Wright In Press), it appears likely that, instead of enhancing population abundance through increasing recruitment, *Caulerpa* may actually reduce the fitness of *A. trapezia* — enhanced recruitment is of little consequence if the reproductive traits are compromised. Therefore, *Caulerpa* may in fact represent an 'ecological trap' for *A. trapezia* if larvae preferentially choose to settle in habitats modified by *Caulerpa* (i.e. poor quality habitat) vs. bare habitat (i.e. good quality habitat). Although a growing number of studies indicate that invasive species may function as ecological traps (e.g. Jones and Bock 2005; Remes 2003; Schlaepfer et al. 2005), the role of invasive species as 'trap' habitats is yet to be empirically tested. The challenge remains to determine whether *Caulerpa* does represent an ecological trap or whether native species have the ability to recover or adapt to habitat modification by invasive species.

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Table 1. 3-Factor ANCOVA examining the effects of Sex, Habitat, and Time on follicle production with Size as the co-variate. Significant values ($p<0.05$) shown in bold.

Source of Variation	df	MS	*F*	p
Size	1	78.134	3.436	0.064
Sex	1	141.268	12.442	**0.006**
Habitat	1	1016.327	6.036	**0.036**
Time	9	582.667	25.622	< **0.001**
Sex x Habitat	9	7.128	0.477	0.507
Sex x Time	9	11.354	0.499	0.875
Time x Habitat	9	168.373	7.404	< **0.001**
Sex x Habitat x Time	9	14.949	0.657	0.748
Error	516	22.741		

Table 2. 2-Factor ANCOVA examining the effects of Time and Habitat on the number of oocytes/female with Size as the co-variate. Significant values ($p<0.05$) shown in bold.

Source of Variation	df	MS	*F*	p
Size	1	2310.071	0.940	0.333
Time	9	97944.145	39.866	< **0.001**
Habitat	1	26534.419	6.542	**0.031**
Time x Habitat	9	4043.588	1.646	0.102
Error	277	2456.861		

Table 3. 2-Factor ANCOVA examining the effects of Time and Habitat on oocyte diameters with Size as the co-variate. Significant values ($p<0.05$) shown in bold.

Source of Variation	df	MS	*F*	p
Size	1	82.138	0.765	0.382
Time	9	56075.959	522.181	**<0.001**
Habitat	1	879.885	0.420	0.533
Time x Habitat	9	2094.663	19.503	**<0.001**
Error	20771	107.388		

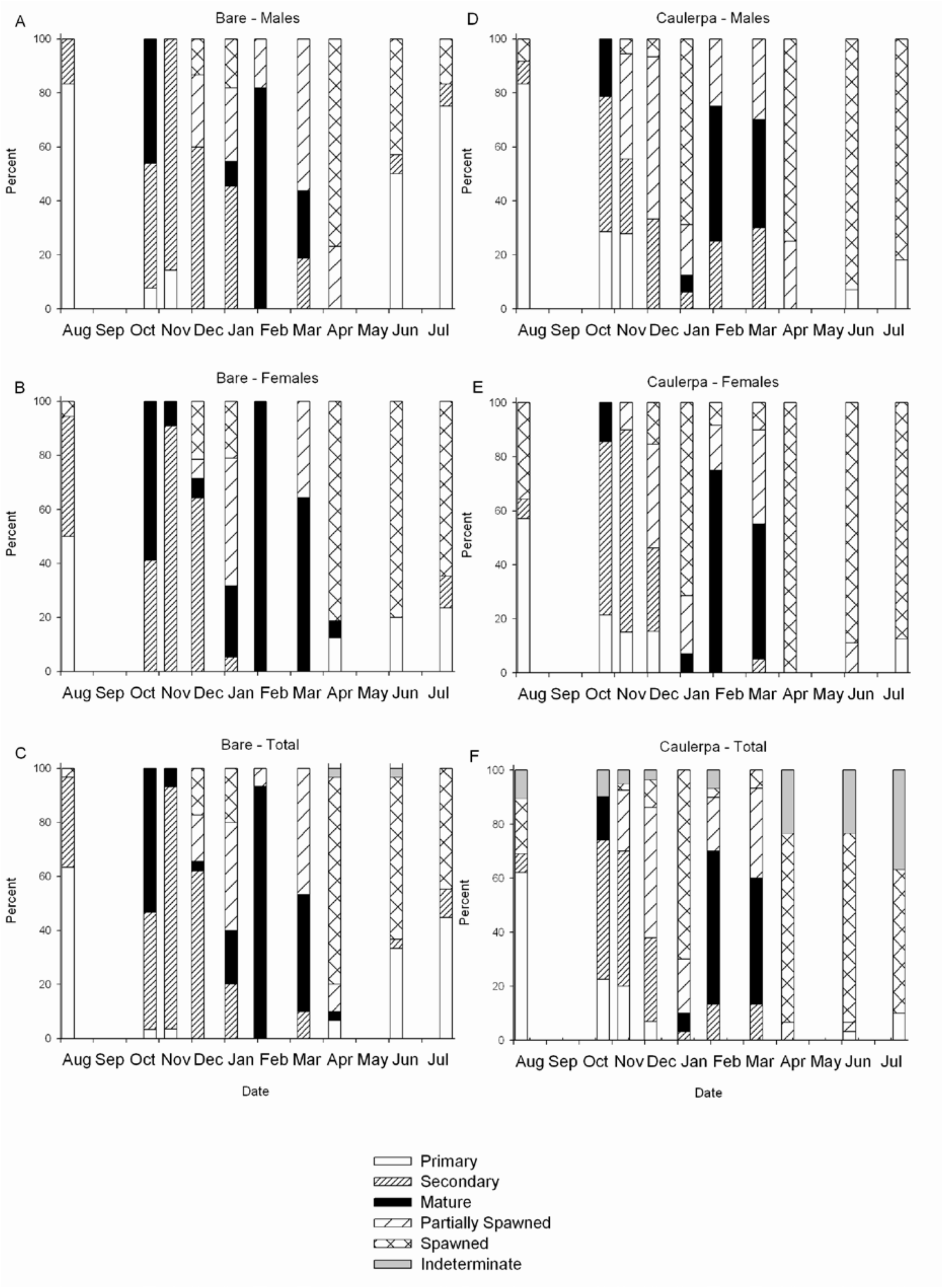
A
Bare - Males
D
Caulerpa - Males
B
Bare - Females
E
Caulerpa - Females
C
Bare - Total
F
Caulerpa - Total
Percent
0
20
40
60
80
100
Aug Sep Oct Nov Dec Jan Feb Mar Apr May Jun Jul
Date
Primary
Secondary
Mature
Partially Spawned
Spawned
Indeterminate

Figure 1

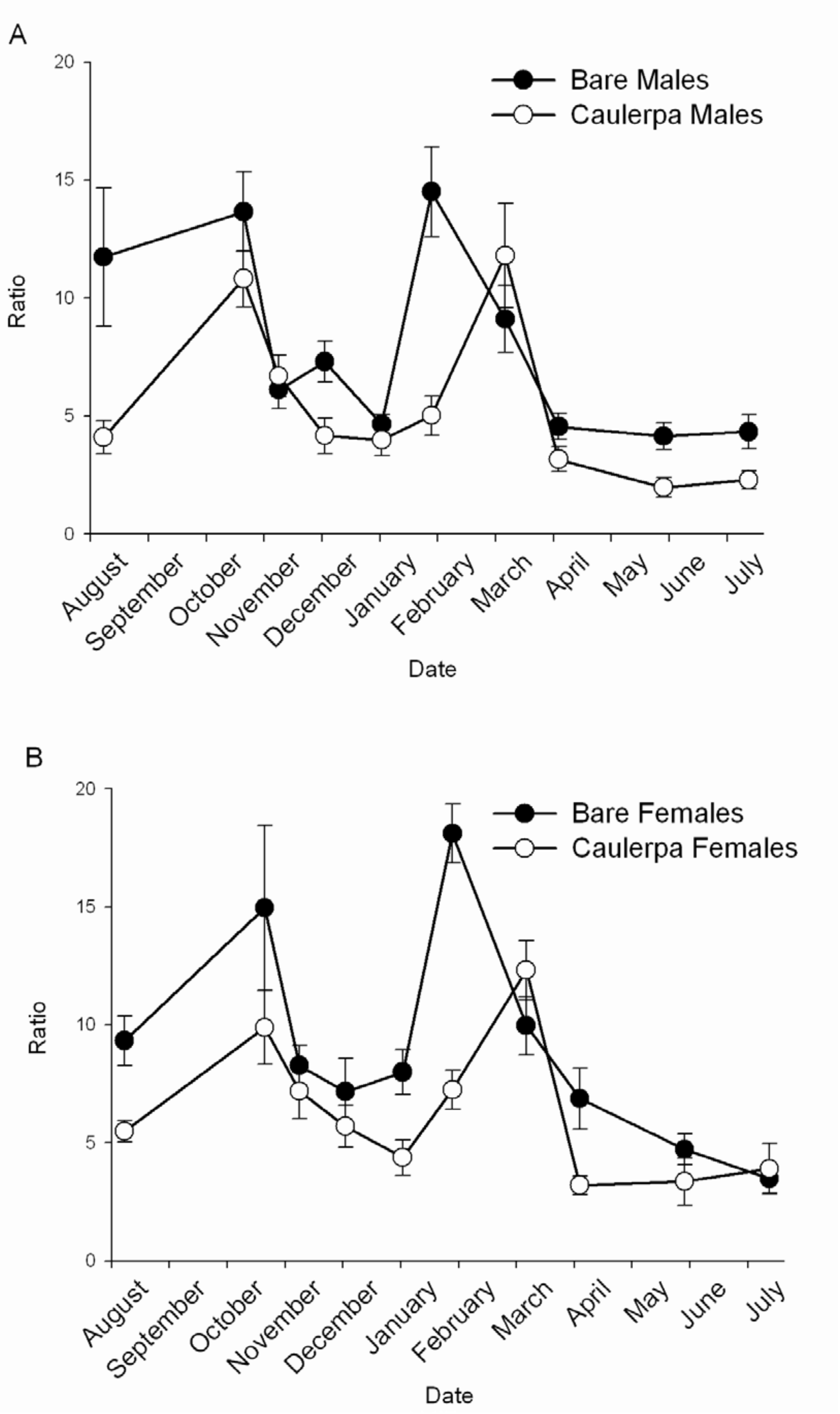
A
20
15
10
5
0
Ratio
Bare Males
Caulerpa Males
August
September
October
November
December
January
February
March
April
May
June
July
Date
B
20
15
10
5
0
Ratio
Bare Females
Caulerpa Females
August
September
October
November
December
January
February
March
April
May
June
July
Date

Figure 2

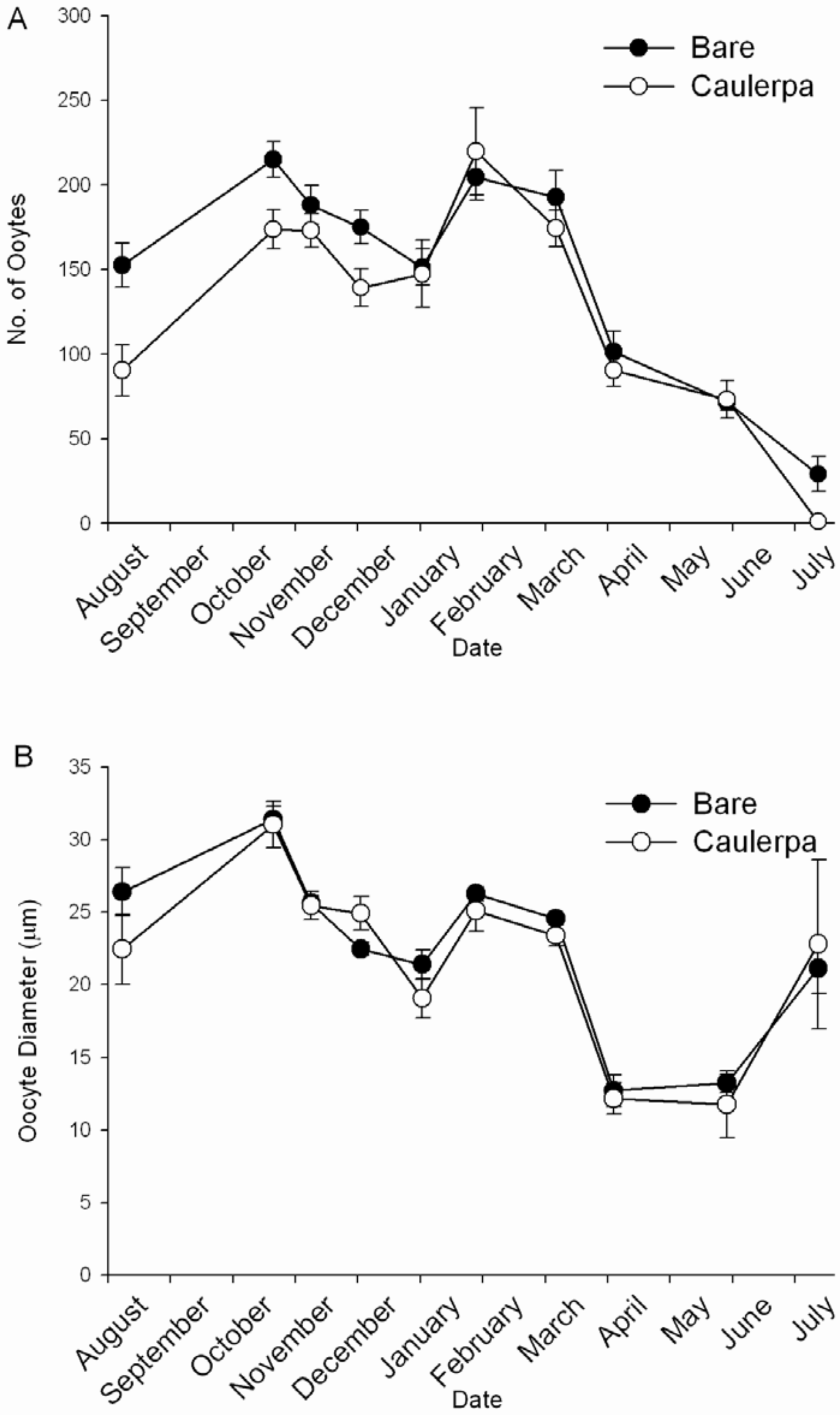
A
No. of Ooytes
0
50
100
150
200
250
300
Bare
Caulerpa
August
September
October
November
December
January
February
March
April
May
June
July
Date
B
Oocyte Diameter (µm)
0
5
10
15
20
25
30
35
Bare
Caulerpa
August
September
October
November
December
January
February
March
April
May
June
July
Date

Figure 3

Captions to Figures.

Figure 1. Histograms showing the gametogenic cycle of *A. trapezia* in *Caulerpa* and bare habitats sampled from August 1994 to July 2005 determined from histological sections: A) males in bare habitat, B) females in bare habitat, C) combined sexes for bare habitat, D) males in *Caulerpa* habitat, E) males in *Caulerpa* habitat and, F) combined sexes for *Caulerpa* habitat.

Figure 2. Temporal patterns in the ratio of follicle area to total tissue are for (A) male *A. trapezia* collected from bare and *Caulerpa* habitats and B) female *A. trapezia* collected from bare and *Caulerpa* habitats.

Figure 3. Temporal patterns in (A) the total number of eggs and (B) and mean diameter of oocytes in six pooled follicles for each female collected from bare and *Caulerpa* habitats.