Larval settlement preference of a native bivalve: the influence of an invasive alga versus native substrata

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ABSTRACT: Recruitment patterns of marine invertebrates are strongly influenced by the habitat preference of larvae as larvae can choose to settle (or not) in response to positive or negative cues. High abundances of recruits of the native infaunal bivalve *Anadara trapezia* occur in the invasive alga *Caulerpa taxifolia* compared to native habitats. Using controlled laboratory experiments, *A. trapezia* larval habitat preference was investigated through exposure to available native (adult *A. trapezia*, *Zostera capricorni* and sediment from unvegetated areas) and invasive (*C. taxifolia* and sediments from *C. taxifolia*) substrata that co-occur in estuaries invaded by *C. taxifolia* in New South Wales, Australia. When exposed to all substrata, larval settlement was significantly higher on adults compared to all other substrata except *Z. capricorni*. Although settlement to *C. taxifolia* was low, larvae did not reject it as settlement surface. In pairwise comparisons, larval settlement was always higher on adults compared to all other substrata, although differences were only significant compared to *C. taxifolia* and unvegetated sediments. There was no difference in settlement when larvae were exposed to *Z. capricorni* and *C. taxifolia*. When offered a single substratum, larval settlement was significantly higher on adults and *Z. capricorni* compared to all remaining substrata. Manipulations of shells of adults indicated that larvae may be responding positively to biofilms on the surface of shells. The data indicate that *A. trapezia* larvae prefer to settle on adults and, while they do not prefer *C. taxifolia*, they do not reject it as a settlement surface. Therefore, *C. taxifolia* may serve as a sink habitat for *A. trapezia*.

KEY WORDS: *Anadara trapezia* · Bivalve · *Caulerpa taxifolia* · Habitat choice · Habitat-forming invasive species · Invasion biology · Larval settlement

INTRODUCTION

In the marine environment, recruitment is strongly influenced by the habitat preference of larvae, and there is a rich history of studies, particularly for invertebrates, that demonstrate larvae settlement choice in response to positive or negative cues. Marine invertebrate larvae settle in response to a range of chemical and physical factors (see Burke 1986, Butman 1987, Pawlik 1992, Steinberg et al. 2001 for reviews). Natural cues from biofilms (Keough & Raimondi 1996, Harder et al. 2002, Huggett et al. 2006), macroalgae

(Morse & Morse 1984, Walters et al. 1996, Swanson et al. 2004) and conspecifics (Jensen & Morse 1990, Zimmer-Faust & Tamburri 1994) can induce settlement of invertebrates. Acceptance or rejection of a surface has also been correlated to the surface profile (roughness), including microtexture (Berntsson et al. 2000, Scardino & de Nys 2004, Schumacher et al. 2007) or the number of adhesion points an organism can make on a surface (Callow et al. 2002, Scardino et al. 2008). Although habitat selection by larvae should be adaptive, studies demonstrate that larvae can make poor or maladaptive settlement choices that reduce individual fitness or key performance traits (Connell 1961, Williamson et al. 2004, Gribben et al. 2009a).

Marine soft-sediments with invasive habitat-forming mussels (Crooks 1998) and macrophytes (Posey 1988, Neira et al. 2005, Gribben & Wright 2006a) often have higher invertebrate recruitment, species diversity and abundance compared to nearby unvegetated sediments. Provision of a refuge from predation and added habitat complexity are important mechanisms driving facilitation by habitat-forming species (Crooks 1998, 2002, Gribben & Wright 2006a). However, active habitat selection by larvae may also play a role in the facilitation of native fauna by habitat-forming invasive species. From an evolutionary perspective, recruitment to structured surfaces per se may be considered adaptive because they offer a refuge from predation; larvae may preferentially settle in structured habitats for this reason. Whether the positive effects of habitat-forming invasive species on the recruitment of native species can be attributed to larval settlement preference has not been explored.

The invasive green alga *Caulerpa taxifolia* has invaded several temperate regions worldwide where it covers large areas of soft-sediment habitat and forms high density beds (Meinesz et al. 2001, Anderson 2005, Wright 2005), modifying chemical and physical sediment properties (Chisholm & Moulin 2003, Gribben et al. 2009a). For example, sediments invaded by *C. taxifolia* are higher in sulphides, are more anoxic and have lower redox potential than those in nearby unvegetated sediments (Gribben et al. 2009a, McKinnon et al. 2009). In southeastern Australia, *C. taxifolia* has invaded 14 estuaries since it was first reported in 2004 and many of these areas contain populations of the soft-sediment bivalve *Anadara trapezia*, commonly known as the Sydney cockle (Wright et al. 2007, McKinnon et al. 2009). *A. trapezia* are generally estuarine, occurring from the intertidal to shallow subtidal (0 to 2 m water depth) in sheltered mud, sand and seagrass habitats. *A. trapezia* are epifaunal as recruits and infaunal as adults (Gribben & Wright 2006a, Wright et al. 2007).

Although the reported effects of *Caulerpa taxifolia* on native biota (e.g. seagrass, infaunal bivalves, fish and epibiota on hard substrata) are largely negative (Bellan-Santini et al. 1996, Levi & Francour 2004, Longepierre et al. 2005, Gribben & Wright 2006b, York et al. 2006, Wright et al. 2007), *C. taxifolia* has positive effects on the recruitment of *Anadara trapezia* (Gribben & Wright 2006a), epibionts on the shells of adult *A. trapezia* (Gribben et al. 2009b) and epifauna in general (McKinnon et al. 2009). In 2005, high abundances of post-settlement juveniles (recruits) of *A. trapezia* were observed in *C. taxifolia* (Gribben & Wright 2006a) compared to unvegetated sediments, adult *A. trapezia* and the native seagrass *Zostera capricorni* in Lake Conjola, New South Wales. Although *C. taxifolia* offered a refuge from predation by fish (Gribben & Wright 2006a), recruit abundance may also reflect preferential settlement of *A. trapezia* larvae onto *C. taxifolia.* Alternatively, sediment properties influence the settlement of soft-sediment invertebrates including bivalves (Butman 1987, Woodin 1991, Snelgrove et al. 1998). Because *C. taxifolia* has negative effects on sediment quality and on the life-history traits of juvenile and adult *A. trapezia* (Wright & Gribben 2008, Gribben et al. 2009a, McKinnon et al. 2009), larvae may avoid invaded sediments and recruit to *C. taxifolia* itself.

In order to understand recruitment processes determining high abundances of native organisms in habitat-forming invasive species, it is necessary to understand the role of larval habitat preference in determining these patterns. In the present study we employed a series of controlled laboratory experiments to investigate: (1) whether *Anadara trapezia* larvae show a general preference for *Caulerpa taxifolia* over other habitats, (2) whether individual substrata induce settlement of larvae and (3) possible mechanisms for the observed positive response of larvae to adult *A. trapezia*.

MATERIALS AND METHODS

Study organisms and sites. Larval habitat choice of *Anadara trapezia* was investigated under laboratory conditions at the bivalve hatchery of the New South Wales Department of Primary Industries (NSW DPI) Port Stephens Fisheries Institute during November 2005. Although field studies testing our hypotheses would be preferable, habitats that differ in water flow did not explain differences in epibiont abundance on the shells of adult *A. trapezia* in *C. taxifolia* or unvegetated sediments (Gribben et al. 2009b). We therefore consider static controlled trials appropriate for assessing the settlement preference of *A. trapezia* larvae. All settlement substrata used in the trials were collected

from Lake Conjola (35.268° S, 150.508° E), NSW, except adult *A. trapezia* which were collected from Lake Macquarie (33.086° S, 151.662° E).

Anadara trapezia broodstock were transported to the hatchery and held in recirculating seawater systems at 21°C for 1 wk before spawning. Spawning induction and larval rearing techniques were similar to those described by Nell et al. (1994). Spawning commenced when water temperature was raised 4°C over a 1 h period. Clams which had begun spawning were removed from the table and placed in individual 500° ml plastic containers of fresh seawater. Eggs were pooled, then fertilised before embryos were stocked into 1000 l aerated polyethylene tanks of seawater at 24°C. All seawater (34 g l^{-1} salinity) used was filtered using 1 μm (nominal) cartridge filters. Seawater was exchanged thrice weekly with the larvae being retained on polyester mesh netting cylindrical sieves. Larvae were fed twice daily with a mixture of 3 algal species: *Pavlova lutheri*, Tahitian *Isochrysis* aff. *galbana* and *Chaetoceros calcitrans* as described by O'Connor & Heasman (1997). Larvae were deemed ready to settle once they had reached the pediveliger stage (i.e. they had developed a foot) and had begun searching behaviour (after ca. 12 d). All experiments described below were conducted concurrently from a single batch of larvae.

General trial methods. Larvae were offered different combinations of substrata consisting of sediment from *Caulerpa taxifolia*-invaded areas, *C. taxifolia*, the seagrass *Zostera capricorni*, sediment from unvegetated areas and adult *Anadara trapezia.* These habitats represent the major components of the benthos available for settlement and reflect actual habitat choices that larvae would encounter in Lake Conjola (Gribben & Wright 2006a). Although *Z. capricorni* is sparse and only found fringing bays in Lake Conjola, we included it in our trials as recruits are found in this habitat and seagrass can be an important habitat for bivalves. However, we did not include sediments from this habitat as a treatment. *Caulerpa taxifolia* and unvegetated sediments were included as separate habitats, as bivalve larvae can settle in response to complex structures and sediment cues and we considered it important to test the response of larvae to sediments from both invaded and unvegetated areas.

All *Caulerpa taxifolia*, *Zostera capricorni* and adult *Anadara trapezia* added to the experimental aquaria had standard surface areas of 42 cm². All clams were 4.7 cm in shell length and we added the equivalent amount of each macrophyte (i.e. equivalent to an area of 42 cm²) based on known wet weight:area relationships: surface area (cm²) of *A. trapezia* = 9.8 × shell length (cm) (W. O'Connor unpubl. data), *C. taxifolia* = 224.602 × wet weight *C. taxifolia* (g) + 2.946 (J. T.

Wright unpubl. data) and *Z. capricorni* = 53.019 × wet weight *Z. capricorni* (g) + 2.3992 (P. E. Gribben unpubl. data). Sediment treatments were added to the experimental aquaria in Petri dishes with a surface area of 63 cm² (5 mm deep). Containers of an equivalent surface area to those of the clams and macrophytes were not available, therefore the number of larvae settling on the sediment surface of each Petri dish was standarised to the same area (42 cm²) once larvae had been counted (see below). All substrata were placed equidistant (approx. 3 cm apart) on the bottom of the aquaria and anchored to the bottom of the aquaria using a 0.5 cm² piece of inert plasticine. We did not run plasticine controls as all surfaces were treated equally.

All trials were conducted in 10 l aquaria (24 cm diameter × 23 cm height) containing approximately 8 l (i.e. filled to a height of 18 cm) of 1 μm filtered seawater. Once the appropriate habitat treatments were added to the aquaria, they were stocked with ca. 0.10 pediveligers ml^{-1}. We used high larval densities to increase precision in our experiments. That said, we know little about the *in situ* densities of *Anadara trapezia* larvae, although these densities, while high, may still be realistic. Glass pipettes delivering air were lowered into each aquarium to a depth at which enough gentle circulation was achieved to maintain larvae in suspension and yet mixing did not disturb the settlement surfaces. Pediveligers were maintained in the settlement aquaria for 10 d. Although most of the larvae were at the pediveliger stage when the experiment started, many larvae were still at earlier developmental stages, so we ran the experiment for a sufficient amount of time to give most larvae the opportunity to settle. Water within each aquarium was changed every second day and replaced with 1 um filtered, temperature-equilibrated seawater. Throughout the trial, larvae were fed a mixture of *Chaetoceros muelleri*, *Pavlova lutheri* and *Isochrysis galbana* at a rate of 10 000 cells larva^{-1} d^{-1}. These feeding are rates typical of those used to maintain healthy larvae under culture conditions (O'Connor & Heasman 1997).

At the end of each trial, all substrata with attached post-settlement juveniles (recruits) were removed from the experimental aquaria, placed in individual containers and fixed with formalin. Following this, any unsettled larvae were sieved and fixed. Finally, the surfaces of the aquaria were carefully scraped to remove any recruits that had settled directly onto the aquaria. These larvae were also fixed for later counting. Trials containing sediments were treated as above, except the Petri dishes with sediment were first removed, the sediment then rinsed into a container and rose bengal added to stain any recruits in the sediments prior to fixing to assist in later sorting and counting of recruits. The number of recruits on adult

Anadara trapezia, *Caulerpa taxifolia*, *Zostera capricorni* and the surfaces of the aquaria and the number of unsettled larvae were counted under a dissecting microscope (1 to 4× magnification). Because of the difficulty in counting recruits in the sediment samples, recruits were separated from sediments in a high density sucrose solution using the methods outlined in Munroe et al. (2004). Sediment samples were placed in 100 ml test tubes containing 60 ml high-density sucrose solution. The samples were then shaken for 2 min and the particles left to settle out by gravity for 24 h. Because recruits sink more slowly than the higher density mineral components of the samples, they settle on the top layer of the sediment and can be easily pipetted off. To ensure that all recruits had been removed, 3 successive layers were removed from the surface of each sample and counted under a dissecting microscope. In total, the top ~5 mm of sediment in each sediment sample was sampled for recruits.

Habitat choice trials. *What is the general habitat preference of larvae?* General habitat preferences of *Anadara trapezia* larvae were explored by offering larvae a choice of all 5 substrata (*Caulerpa taxifolia*, adult *A. trapezia*, *Zostera capricorni*, *C. taxifolia* sediments and unvegetated sediments). A single unit of each of the 5 substrata was randomly added to replicate aquaria (n = 8) prior to the addition of pediveligers (see Appendix 1, Table A1).

On a smaller scale, larvae are unlikely to encounter all 5 substrata at once. Therefore, we also investigated whether the general pattern of habitat choice found in the 5-way experiment was maintained when larvae were offered fewer substrata (see Table A2). Larvae in the above experiment exhibited a strong preference for adults over the other substrata. In a series of pairwise comparisons, we tested whether larvae show a preference for a particular macrophyte in the absence of adults (i.e. *Caulerpa taxifolia* vs. *Zostera capricorni*) and whether the strength of larval response to adults is maintained if one of the macrophytes is absent (i.e. *C. taxifolia* vs. adults and *Z. capricorni* vs. adults). In addition, we conducted 2 further pairwise trials (*C. taxifolia* sediments vs. adults and unvegetated sediments vs. adults) to specifically investigate the effects of sediment on settlement in the presence of a positive cue. We only included comparisons with adults as they had a strong positive effect on larval settlement in the first trial, and we wished to test whether changes in sediment quality altered the strength of the response of larvae to positive cues (i.e. adults). Pairs of substrata were randomly added to replicate aquaria (n = 8 pairs for each trial).

Do individual substrata induce settlement of larvae? Here we tested whether individual substrata induced higher levels of settlement. We tested the effect of indi-

vidual substrata on recruitment to those substrata and on total settlement (i.e. settlement on substratum + settlement on the sides of the aquaria) (see Table A3). Larvae were offered a single substratum consisting of *Caulerpa taxifolia*, adult *Anadara trapezia*, *Zostera capricorni*, *C. taxifolia* sediments or unvegetated sediments. Replicates (n = 8) of each single substratum were randomly assigned to aquaria (n = 40 aquaria in total).

Do larvae respond to chemical or physical cues of adults? Higher levels of settlement were recorded on adults compared to other treatment substrata in all trials (see 'Results'). In a preliminary assessment of the nature of settlement cues, we tested whether larvae were responding to potential chemical cues (surface associated and waterborne) or the structure (shell topography) of adults (*Anadara trapezia* have thick radiating ribs approximately 1 to 2 mm wide and 1 mm deep in adults (P. E. Gribben pers. obs.). Three adult treatments were created: (1) live intact adults (live adults), (2) inert dead adults (dead–surface) and (3) dead adults with the external surface intact (dead+surface). Dead–surface adults were created by shucking clams and thoroughly cleaning the valves before autoclaving them. Immediately prior to the experiments, the dead+surface adults were created by shucking live adults in a bucket of filtered seawater and thoroughly cleaning the surface of the inside of the shell with a scouring pad, being careful not to disturb the outside surface. The resulting shells were then rinsed several times in filtered seawater and kept in a bucket of filtered seawater for 2 h until use in the trials. Although the valves of the dead clams had to be separated in order to shuck them, we did not need to apply any adhesive to keep valves together when placed in experimental aquaria. We conducted 3 pairwise trials (see Table A4). Larvae were given a choice between a live adult and dead+surface adult, a live adult and dead–surface adult and a dead+surface adult and dead–surface adult. Because the surface treatments of the live adult and dead+surface adult trial were the same (i.e. the external surfaces were left intact), this trial investigated whether larvae respond to potential waterborne internal cues (such as waste products) from live adults or cues associated with the adults' external shell surface. The second trial, between live adults and dead–surface adults, investigated larval preference for potential waterborne cues over physical surfaces. The last trial, between dead+surface adults and dead–surface adults, investigated larval preference between external surface-associated cues and physical structure. All 3 trials were run concurrently with pairs of substrata randomly added to replicate aquaria (n = 4 pairs for each trial; n = 12 aquaria in total).

Statistical analyses. Because of non-independence issues related to multi-choice preference trials, for the 5-way settlement trial, differences in larval settlement were analysed using Pearson's chi-square test on total number of larvae on each settlement substratum pooled across aquaria. All pairwise trials were analysed using paired *t*-tests. One-way ANOVA was used to determine percent settlement on individual substrata (fixed). For both the pairwise trials and 1-way ANOVA, percent settlement was estimated as the total number of larvae settled on each treatment divided by total number of larvae recovered in each of those treatments (total larvae settled + larvae remaining swimming in a treatment) (see Tables A1 & A2 for raw data). Tukey's pairwise comparisons were used to explore differences between treatments. Effects of individual substrata on settlement were analysed as a percentage of total settlement within a treatment due to the potential filtering of larvae by live adults in aquaria that contained adults; aquaria containing adults had consistently lower numbers of larvae remaining at the end of the trials (see 'Results', Tables A3–A4 and for raw data). All data are expressed graphically as mean percent settlement (±SE) for ease of comparison among experiments. For all analyses, assumptions were checked by examining distributions of residuals and plots of residuals versus means (Quinn & Keough 2002).

Fig. 1. *Anadara trapezia*. Mean (±SE) percent settlement of larvae on *Zostera capricorni* (Zostera), *Caulerpa taxifolia* (Caulerpa), unvegetated sediment (Bsed), *Caulerpa* sediment (Csed) and adult *A. trapezia* (Adult) in a 5-way settlement experiment. Data are expressed as percent settlement of total larvae averaged over replicate aquaria, each containing a single representative of all substrata (n = 8 aquaria)

RESULTS

Total larval abundance at the end of the trials varied among aquaria within trials and between trials (see Appendix 1, Tables A1–A4 for total number of larvae settled on all test substrata, on the sides and walls of aquaria, total number of larvae settled and total number of larvae recovered for each aquarium in all trials). Because all trials were run concurrently from a single batch of larvae, these differences could not be attributed to batch-to-batch variation in larval viability.

What is the general habitat preference of larvae?

For the 5-way preference experiment, there was a significant difference in settlement to treatment substrata ($\chi^2 = 33.64$, df = 3, p < 0.001). Percent settlement was highest on live adults (13.6 ± 2.4 %) and lowest on *Caulerpa taxifolia* (0.8 ± 0.4 %) (Fig. 1). Settlement on live adults was more than twice that of *Zostera capricorni*, the next highest surface (5.7 ± 2.2 %). Settlement was very low on both sediment treatments.

For the pairwise comparisons, settlement was higher on *Zostera capricorni* compared to *Caulerpa taxifolia*,
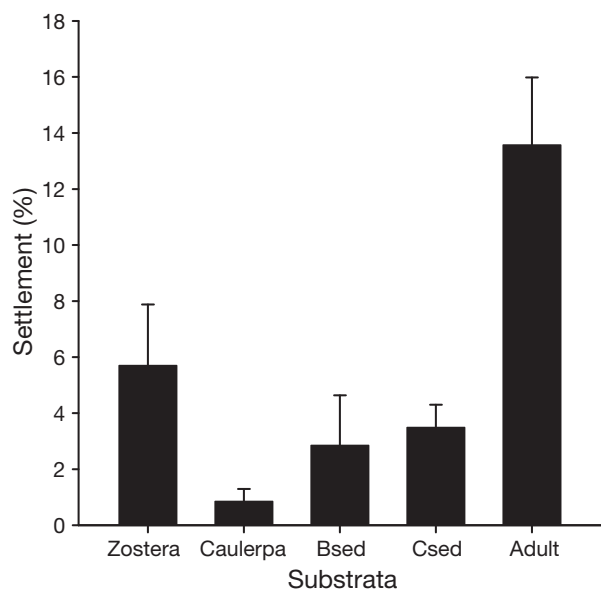
although differences were non-significant ($t_7 = 1.784$, p = 0.118) (Fig. 2A). Settlement was higher on adults compared with all other surfaces (Fig. 2B–E), although differences were only significant compared to *C. taxifolia* ($t_7 = -3.523$, p = 0.010) and unvegetated sediments ($t_7 = -3.286$, p = 0.013). Settlement on adults compared to *Z. capricorni* ($t_7 = -1.914$, p = 0.097) and *C. taxifolia* sediment ($t_7 = -0.878$, p = 0.409) were not significantly different.

Do individual substrata induce settlement of larvae?

For the individual substrata experiments, there were significant differences in percent settlement among treatments ($F_{4,35} = 5.140$, p = 0.002) (Fig. 3A). Settlement was higher on adults compared to all other surfaces (Tukey's tests, p < 0.021) although differences between adults and *Zostera capricorni* were non-significant (p = 0.410). There were no differences in settlement between any other substrata (all p > 0.263). There was no difference in total settlement between treatments ($F_{4,35} = 0.822$, p = 0.520), although total settlement was highest in aquaria containing adults (67.1 ± 11.3 %) and lowest in aquaria containing *Caulerpa taxifolia* (47.7 ± 6.6 %) (Fig. 3B). The total number of larvae recovered was >3 times lower in aquaria con-
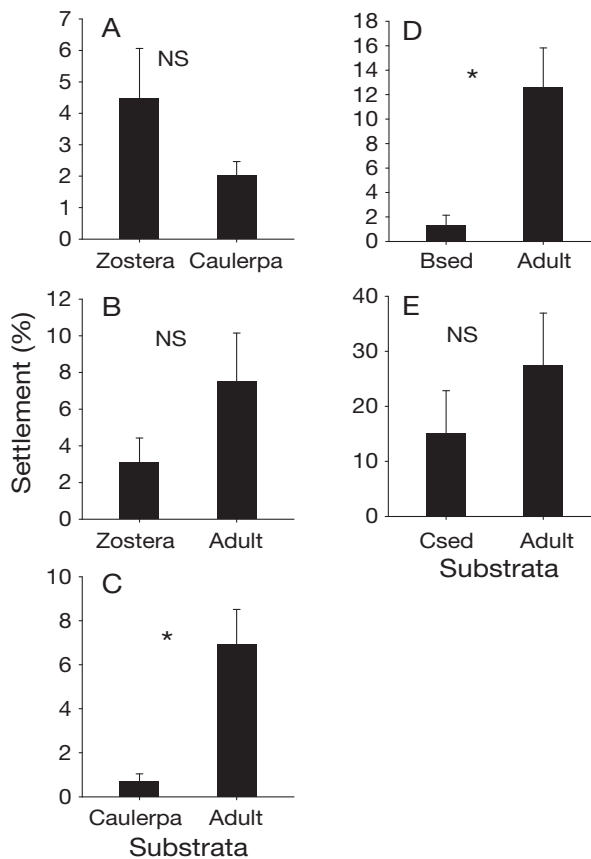
Fig. 2. *Anadara trapezia*. Mean (±SE) percent settlement of larvae on paired substrata added to aquaria (n = 8 aquaria per pair). (A) *Zostera capricorni* (Zostera) vs. *Caulerpa taxifolia* (Caulerpa); (B) Zostera vs. adult *A. trapezia* (Adult); (C) Caulerpa vs. Adult; (D) unvegetated sediment (Bsed) vs. Adult; and (E) Caulerpa sediment (Csed) vs. Adult. Data are expressed as percent settlement of total larvae in each aquarium averaged over replicate aquaria. NS: no significant difference between treatments; *: significant at α = 0.05

taining live adults (151.5 ± 39.0%) compared to all other substrata (range from 497 ± 64.8 to 593.5 ± 41.7%; 1-way ANOVA, $F_{4,35}$ = 11.350, p < 0.001; Tukey's tests, all p < 0.001). There was no difference in the total number of larvae recovered between other substrata (Tukey's tests, p > 0.665).

Do larvae respond to chemical or physical cues of adults?

Settlement was significantly higher on the dead+surface adults compared with live adults (t_3 = –3.673, p = 0.035) (Fig. 4A). There was no difference in settlement between live adults and the dead–surface adult treatment (Fig. 4B). However, settlement was higher on dead+surface adults compared to dead–surface adults, although differences were not significant (t_3 = 1.664,
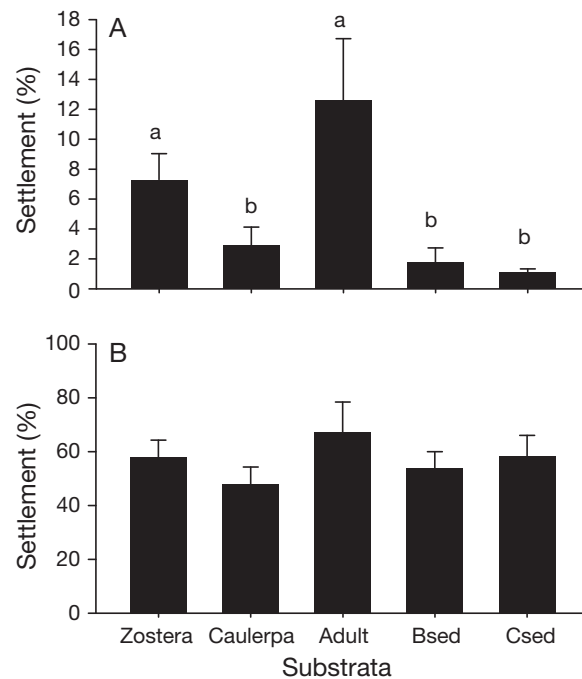

Fig. 3. *Anadara trapezia*. (A) Mean (±SE) percent settlement of larvae on substrata added to separate test aquaria (n = 8 aquaria per treatment) and (B) mean (±SE) total settlement for each treatment. Substrata are as in Fig. 1. Data are expressed as percent of total settlement in each aquarium averaged over replicate aquaria. Treatments with a common letter did not differ significantly

p = 0.195) (Fig. 4C). Total larvae recovered from the dead+surface and dead–surface adult trial (527.5 ± 70.6%) was >1.5 times higher than for both the dead–surface adult and live adult (335.5 ± 79.5%) and dead+surface adult and live adult (313.5 ± 78.4%) trials. However, differences in total number of larvae were not significantly different ($F_{2,9}$ = 11.350, p = 0.148).

DISCUSSION

Despite observed high abundances of *Anadara trapezia* recruits on *Caulerpa taxifolia* compared to other native habitats in the field, larvae in our controlled laboratory experiments did not show a strong preference for *C. taxifolia*. Importantly, however, they did not reject *C. taxifolia* as a settlement surface. Larvae did, however, exhibit a consistent positive response to adult *A. trapezia*. In the absence of adults, settlement was higher on *Zostera capricorni* compared to the remaining substrata, although generally larvae exhibited little preference or avoidance of native or invasive substrata.

Given the lack of a strong settlement response of *Anadara trapezia* larvae to *Caulerpa taxifolia* but a positive response to adults, other mechanisms may
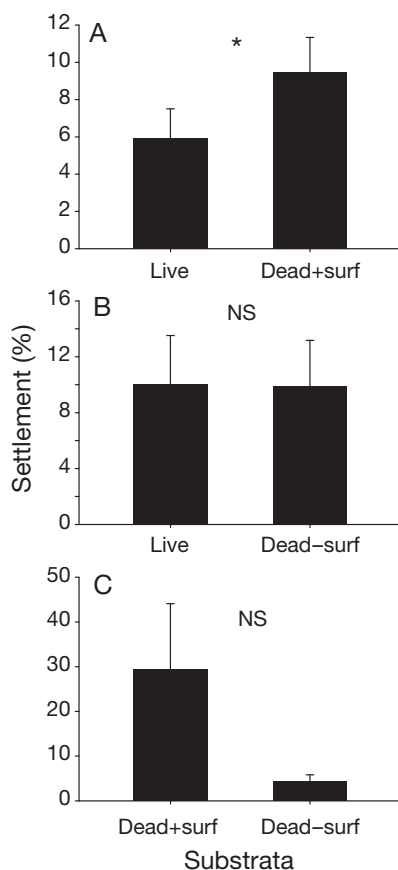
Fig. 4. *Anadara trapezia*. Mean (±SE) percent settlement of larvae on paired substrata including (A) live adults (Live) vs. dead adults with surface intact (Dead+surf), (B) Live vs. dead adults with surface cleaned (Dead−surf) and (C) Dead+surf and Dead−surf (n = 8 replicate aquaria for each trial). Data are expressed as percent settlement of total larvae in each aquarium averaged over replicate aquaria. NS: no significant difference between treatments; *: significant at α = 0.05

explain the high recruitment to *C. taxifolia* in the field. First, recruits may be responding to chemical cues from adults and settle onto *C. taxifolia* because it is a complex habitat and they subsequently cannot find the adults. However, Gribben & Wright (2006a) also found recruits attached to *C. taxifolia* in high abundances at sites where adults were absent. Second, alteration of hydrodynamic regimes or baffling of larvae by *C. taxifolia* may increase the supply of larvae to this habitat as has been shown to occur in seagrasses (see Williams & Heck 2001 for review). Although water flow is reduced in very dense *C. taxifolia* compared to unvegetated sediments (Gribben et al. 2009a), water flow is generally low in Lake Conjola as flows are tidally driven and tidal exchange is low (20% of adjacent coastal areas, MHL 2003). Moreover, the recruitment of epibionts to shells of adult clams is similar between unvegetated and *C. taxifolia*-invaded habitats (Gribben et al. 2009b), suggesting that entrainment of larvae is not

responsible for high recruitment to *C. taxifolia*. Third, our trials indicated that in the absence of adults, recruits settle onto all other substrata that we tested. Because *C. taxifolia* is the most abundant macrophyte in Lake Conjola and now covers >150 ha of the seafloor (Creese et al. 2004, Wright 2005), recruits may not be able to find adults and simply settle on any substrata. *C. taxifolia* may subsequently provide a refuge from predation (Gribben & Wright 2006a). We believe that the latter is the most likely explanation for high abundances of recruits in *C. taxifolia*.

Colonisation of *Caulerpa taxifolia* by *Anadara trapezia* larvae appears to have a predation risk/fitness trade-off. In unvegetated habitats, fitness of surviving recruits is high (Gribben et al. 2009a) but predation risk is very high (Gribben & Wright 2006a), although some recruits must survive to maintain populations. In *C. taxifolia*, predation risk is low but fitness and survivorship are also very low (Gribben & Wright 2006a, Gribben et al. 2009). In fact, 1 yr after a large recruitment event of *A. trapezia* to *C. taxifolia*, almost all recruits had disappeared and those that remained grew very little (Gribben & Wright 2006a). Moreover, there was no evidence of secondary settlement of recruits into higher quality habitats from *C. taxifolia* (Gribben et al. 2009a). Therefore, larvae that settle into *C. taxifolia* are unlikely to contribute to population maintenance. In fact, populations of *A. trapezia* are much lower in *C. taxifolia* compared to unvegetated sediments (Wright et al. 2007, McKinnon et al. 2009) and continue to decline (P. E. Gribben & J. T. Wright unpubl. data). Given that *C. taxifolia* is the dominant surface available for colonistion — >95% of the benthos in bays in which *A. trapezia* populations occur in Lake Conjola are covered in poor quality *C. taxifolia* (P. E. Gribben & J. T. Wright pers. obs.) — and larvae are also recruiting to *C. taxifolia* where adult populations do not exist, then *C. taxifolia* may represent a sink habitat (Dias 1996) for *A. trapezia*.

When exposed to similar substrata as larvae in this study, *Anadara trapezia* post-settlement juveniles (recruits approx. 6 mo old) exhibited a strong gregarious response to adults (Gribben & Wright 2006a). However, larval response to adults in the present study was not as strong as those of recruits. Although this may represent an ontogenetic shift in the response level of larvae and recruits to adults, it may alternatively be an artifact of our methodology. Whereas the trials in Gribben & Wright (2006a) only ran for 24 h and were not aerated, the trials in the present study ran for 10 d and the different settlement surfaces may have become contaminated with cues from other surfaces. However, *A. trapezia* larvae did exhibit a consistent positive settlement response to adults when they were present.

The adult preference trials suggest that larvae responded to cues from the surface of the adult shells, not just to the structure of the shell. Steinberg et al. (2002) argue that settlement cues for marine invertebrates are more likely to be waterborne, and the larvae of several marine invertebrates show a strong settlement response to specific waterborne cues (Williamson et al. 2000, Swanson et al. 2004). Although we could not determine whether larval cues from adults were waterborne or surface-associated, the adult preference trials suggest that larvae are responding to cues generated on the external surface of the shells. Such cues may be generated by surface-associated biofilms. Components of biofilms such as bacteria and microalgae (e.g. diatoms) found on the surface of host organisms have demonstrated positive effects on the recruitment of marine invertebrates (Keough & Raimondi 1996, Huggett et al. 2006). However, further research is needed to isolate the source of potential settlement cues.

Filtration of conspecific larvae by adults has been demonstrated for bivalves (André et al. 1993). The reduced total number of larvae recovered from our single choice trial suggests that settlement on conspecifics may carry a filtration and/or predation risk. This may also explain the lack of difference in settlement between live adults (which also had a +surface treatment) and dead–surface adults. Interestingly, for the settlement inducement trial (i.e. trials with individual substrata) there was no difference in total settlement among treatments (Fig. 3B). This suggests that adult *Anadara trapezia* may be filtering larvae that do not settle or are not ready to settle, increasing their chance of filtration by adults.

We found little evidence for active habitat selection for *Caulerpa taxifolia* by larval *Anadara trapezia*. However, in the absence of adults, larvae will recruit to most available surfaces — including *C. taxifolia*. We suggest that because *C. taxifolia* is the dominant habitat available for colonization by *A. trapezia* larvae in many invaded estuaries, if larvae cannot find their preferred habitat (adults in unvegetated settlements), *C. taxifolia* may still represent a severe sink habitat for this native bivalve.

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Appendix 1. Five-way experiment (choice of substrata)

Table A1. *Anadara trapezia*. Number of larvae settling on *Zostera capricorni* (Zostera), *Caulerpa taxifolia* (Caulerpa), bare sediment (Bsed), *Caulerpa* sediment (Csed), live adult (Adult), as well as on the walls and bottom of the aquarium (sides), and number of larvae remaining in the water column (water) in replicate containers (n = 8)

Repl.	Zostera	Caulerpa	Bsed	Csed	Adult	Sides	Water	Total settlement	Total larvae
1	3	1	2	3	10	86	8	105	113
2	9	0	0	7	10	79	10	105	115
2	8	2	1	4	25	146	10	186	196
3	4	1	0	3	7	18	3	33	36
4	5	0	7	14	11	85	14	122	136
5	1	0	0	13	21	119	79	154	233
7	0	1	4	2	8	25	12	40	52
8	10	0	13	2	16	4	27	45	72

Appendix 2. Pairwise experiments

Table A2. *Anadara trapezia*. Five pairwise experiments investigating preference of larvae (number of larvae settling) for sub-strata in the absence of adults (Zostera vs. Caulerpa), absence of one of the macrophytes (Zostera vs. Adult; Caulerpa vs. Adult), and effect of sediment on the presence of adults (Bsed vs. Adult; Csed vs. Adult) (n = 8). See Table A1 for abbreviations

Replicate	Zostera	Caulerpa	Sides	Water	Total settlement	Total larvae
1	2	7	171	97	180	277
2	48	11	331	222	390	612
3	44	12	174	82	230	312
4	15	5	299	70	319	389
5	15	3	378	279	396	675
6	11	7	492	427	510	937
7	23	24	265	332	312	644
8	16	12	321	332	349	681

Replicate	Zostera	Adult	Sides	Water	Total settlement	Total larvae
1	0	1	94	137	95	232
2	3	7	167	54	177	231
3	1	7	50	48	58	106
4	2	5	107	117	114	231
5	6	8	38	1	52	53
6	6	7	61	32	74	106
7	7	11	138	112	156	268
8	5	51	107	67	163	230

Replicate	Caulerpa	Adult	Sides	Water	Total settlement	Total larvae
1	0	7	113	31	120	151
2	2	4	85	25	91	116
3	1	17	128	106	146	252
4	0	9	53	135	62	197
5	1	11	87	19	99	118
6	0	22	92	16	114	130
7	5	7	145	55	157	212
8	2	26	241	136	269	405

Replicate	Bsed	Adult	Sides	Water	Total settlement	Total larvae
1	10	9	75	5	94	99
2	0	2	86	5	88	93
3	3	7	99	8	109	117
4	2	26	127	5	155	160
5	0	12	6	183	18	201
6	0	12	109	17	121	138
7	2	41	103	5	146	151
8	1	26	75	4	102	106

Replicate	Csed	Adult	Sides	Water	Total settlement	Total larvae
1	13	3	2	3	18	21
2	4	6	81	1	91	92
3	9	25	60	21	94	115
4	45	3	30	5	78	83
5	1	11	21	52	33	85
6	1	26	75	4	102	106
7	3	18	2	16	23	39
8	4	56	3	4	63	67

Table A3. Anadara trapezia. Effect of five individual substrata (Zostera, Caulerpa, Adult, Bsed, Csed) on recruitment of larvae (number of larvae settling) to those substrata and on total settlement (i.e. settlement of substratum and on sides) in replicate containers (n = 8). See Table A1 for abbreviations

Replicate	Zostera	Sides	Water	Total settlement	Total larvae
1	56	184	187	240	427
2	36	416	170	452	622
3	18	234	248	252	500
4	14	235	315	249	564
5	50	377	377	427	804
6	72	223	192	295	487
7	39	306	30	345	375
8	11	181	352	192	544

Replicate	Caulerpa	Sides	Water	Total settlement	Total larvae
1	7	316	246	323	569
2	21	230	230	251	481
3	63	432	80	495	575
4	5	148	395	153	548
5	8	253	346	261	607
6	6	173	362	179	541
7	5	174	376	179	555
8	21	416	435	437	872

Replicate	Adult	Sides	Water	Total settlement	Total larvae
1	10	46	4	56	60
2	19	139	49	158	207
3	7	156	24	163	187
4	19	215	134	234	368
5	3	7	53	10	63
6	11	16	5	27	32
7	4	34	152	38	190
8	26	75	4	101	105

Replicate	Bsed	Sides	Water	Total settlement	Total larvae
1	4	235	284	239	523
2	6	128	96	134	230
3	16	224	226	240	466
4	6	348	146	354	500
5	2	321	343	323	666
6	3	187	395	190	585
7	1	306	449	307	756
8	30	188	36	218	254

Replicate	Csed	Sides	Water	Total settlement	Total larvae
1	4	288	133	292	425
2	7	237	175	244	419
3	8	259	18	267	285
4	10	291	135	301	436
5	2	117	338	119	457
6	15	174	421	189	610
7	11	489	367	500	867
8	8	287	165	295	460

Table A4. *Anadara trapezia.* Pairwise comparisons of adult shell surface investigating preference of larvae (number of larvae settling) given a choice of live adults vs. dead adults with intact surface (dead+surf), live adults vs. dead adults with cleaned surface (dead−surf), and dead adults with intact vs. cleaned surface in replicate aquaria (n = 8). See Table A1 for abbreviations

Repl.	Live	Dead +surf	Sides	Water	Total settlement	Total larvae
1	7	20	252	88	279	367
2	26	49	311	107	386	493
3	9	10	100	2	119	121
4	25	39	165	44	229	273

Repl.	Live	Dead −surf	Sides	Water	Total settlement	Total larvae
1	56	18	179	31	253	284
2	26	72	235	239	333	572
3	13	42	161	21	216	237
4	26	7	111	105	144	249

Repl.	Dead +surf	Dead −surf	Sides	Water	Total settlement	Total larvae
1	23	11	188	281	222	503
2	240	13	44	41	297	338
3	185	22	285	158	492	650
4	83	52	221	263	356	619