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Adult density affects larval recruitment in the calyptraeid gastropod *Crepidula fornicata*

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ABSTRACT

Like many other marine invertebrate larvae, those of *Crepidula fornicata* metamorphose in response to a waterborne cue or cues from conspecific adults. However, the relationship between adult density and larval metamorphosis has not been quantified. Around Long Island, New York, U.S.A., *C. fornicata* occurs patchily, sometimes at very high densities. The density of adult *C. fornicata* was experimentally manipulated in the field and larval recruitment was measured. Recruitment increased with increasing adult density, consistent with previous laboratory results demonstrating that larvae settle in response to waterborne cues from conspecific adults. Larvae showed a threshold response to adult cues on the scale of meters, and select adult conspecifics over a control substrate at smaller scales. These results indicate that recruitment in populations of *C. fornicata* with densities below the threshold of detection may be limited by the ability of larvae to find conspecific adults, which in turn may affect population dynamics at range edges by limiting the species' rate of expansion.

1. Introduction

For those invertebrate species with sessile, benthic adults that produce planktonic larvae, larval dispersal is the crucial first step in determining the distribution of adults (reviewed in Cowen and Sponaugle, 2009). Larvae disperse in the water column for a period of hours to years (Shanks, 2009; Strathmann and Strathmann, 2007), and then must shift from a planktonic habitat to the benthic habitat that they will occupy as an adult. A series of developmental and behavioral steps leads the planktonic larvae to the proximity of a suitable place for the adult to eventually grow and reproduce. If the larva does reach an appropriate habitat, a final transition, known as settlement, involves small-scale movements to a final location, and metamorphosis from a planktonic to a juvenile body plan (Pawlik, 1992).

The cues that invertebrates use to initiate metamorphosis and settlement are diverse and often poorly characterized (reviewed by Hadfield and Paul, 2001; Pawlik, 1992). However, many species settle in response to cues associated with adult conspecifics (e.g., the barnacle *Semibalanus balanoides*, Gabbott and Larman, 1987; the polychaete *Phragmatopoma californica*, Jensen and Morse, 1984; the oyster *Crassostrea virginica*, Zimmer-Faust and Tamburri, 1994).

Larvae of the calptraeid gastropod *Crepidula fornicata* (Linnaeus, 1758) have a 2–4 week planktonic phase (Ament, 1979; Collin, 2003), metamorphose, and then spend the rest of their life as sedentary, suspension-feeding adults (Collin, 1995). Although newly-settled juveniles are mobile, adults of this species live in semi-permanent aggregations of multiple individuals, often referred to as stacks (Collin, 1995). *C. fornicata* is a protandrous species, meaning that all settling individuals must go through a male phase before reproducing as a female. Once settled, adults rarely move among stacks (Collin, 1995) and mate within the stacks (Dupont et al., 2006; Le Cam et al., 2009; Proestou et al., 2008) via internal fertilization (Collin, 1995). The sessile and gregarious lifestyle of *C. fornicata* means that the ability of a larva to locate a group of conspecifics should increase its potential fitness after metamorphosis, since an individual that is unable to locate a stack of conspecifics is dependent on subsequent settlers for reproduction.

Larvae of *Crepidula* species settle in response to a water-borne cue or cues from adult conspecifics (McGee and Targett, 1989; Pechenik and Gee, 1993; Pechenik and Heyman, 1987; Zhao and Qian, 2002), as well as congeners (McGee and Targett, 1989). Larvae also respond to other environmental cues, including biofilms (Pechenik and Gee, 1993; Zhao and Qian, 2002), dibromomethane (a compound made by coralline algae; Taris et al., 2010), and cues from other molluscan shells (e.g., *Busycon*; McGee and Targett, 1989). In laboratory studies that test multiple natural cues, settlement is associated with adult conspecifics (Bohn et al., 2013b; McGee and Targett, 1989), though similar patterns are not always observed in the field (Bohn et al., 2013a,2013b).

The number of adults or the amount of cue or cues required to elicit a response is not known. Although *C. fornicata* can reach very high densities (>1000 individuals m^{-2}) in its introduced European range (Ehrhold et al.,

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1998) as well as its native range (Hoch and Cahill, 2012), densities can vary across two or three orders of magnitude both within and among sites (from fewer than 10 ind. m^{-2} to more than 1000 ind. m^{-2}; Hoch and Cahill, 2012). The effect of patchy adult distributions on the ability of *C. fornicata* larvae to locate conspecific adults is an important factor in understanding the ecology of this species, particularly population dynamics in both its native and introduced ranges.

Here, a manipulative field study was used to test the hypothesis that adult density influenced larval recruitment in *C. fornicata*. Based on the fact that adult conspecifics produce a waterborne settlement cue or cues, increasing adult density was predicted to increase larval recruitment.

2. Methods

2.1. Experimental design

Adult *C. fornicata* were collected from Crab Meadow Beach (Northport, New York, USA: 40°55′46″N, 73°19′38″W) 1–2 weeks before the experiments were deployed. The animals were returned to the lab, where they were held in a recirculating seawater system (salinity = 30, temperature = 21 °C) to be prepared for transfer to the field. During this time the animals were fed 1 l of Shellfish Diet (Reed Mariculture, San Jose, California; concentration = 10 million cells/ml) every day.

To test the effect of adult density on settlement, settlement arrays with 5 different densities of adult *C. fornicata* were used (Table 1). Each of the five treatments contained a different mass of living *C. fornicata* adults in multiple stacks of two to seven individuals each, attached to empty conspecific shells (i.e., the bottom member of each stack was attached to the empty shell of a dead *C. fornicata*). To control for area available for settlement, each bag also contained artificial substrate in the form of plastic ping-pong balls (37 mm in diameter), which were sanded to create a rough surface and placed in the seawater system for approximately one week to develop a biofilm. Although using an artificial substrate confounded all possible effects associated with adult snails (i.e., texture, biofilm, waterborne or contact cues), manipulating adult density in this manner is ecologically relevant. *C. fornicata* habitat in New York contains small cobbles, an alternative, non-living substrate for settlement. Using ping-pong balls instead of the cobbles themselves standardized the area and texture of the alternative settlement substrate.

Settlement arrays were prepared using mesh bags constructed from plastic hardware net (30 cm × 30 cm, mesh size 1 cm^2). Stacks were left intact (i.e., were not reassembled from field-collected individuals). Mass was measured as wet tissue mass + shell mass, and large epibionts were removed from shells using a wire brush before weighing. Each ball was approximately equivalent to the surface area (SA) of 25 g of adult snails calculated as a semi-ellipsoid (ping-pong ball SA = 4300 mm^2; average SA of 25 g snails = 5100 mm^2, SD = 598 mm^2). The number of balls per bag varied inversely in proportion to the snail mass in the bag (Fig. 1, Table 1). Since all bags were the same size, varying adult snail mass was equivalent to varying adult density. Balls and snails were not fixed in place, but loose in the bags.

The five treatments are shown in Fig. 1 and a summary of the mass and number of adult snails in each of these treatments is shown in Table 1. The treatments contained 0 g snails (no snails present + 40 balls), 25 g snails (39 balls), 100 g snails (36 balls), 500 g snails (20 balls), and 1000 g snails (0 balls). All snails <5 mm in length were removed from stacks to avoid mistaking outplanted individuals for new recruits at the end of the experiment.

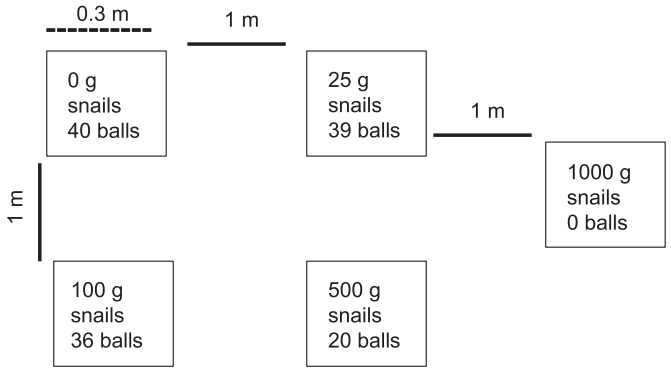

Fig. 1. Schematic of settlement arrays. Spatial array of bags containing different masses of snails and different numbers of ping-pong balls in a field experiment. Bags were made of plastic hardware cloth. Five replicated blocks of this set of five treatments were deployed on three different dates, and the placement of treatments within the array was randomized for each block.

Three replicates of the experiment were deployed at the Southampton Marine Park in Shinnecock Bay, Southampton, New York, U.S.A. (40°50′25″N, 72°29′56″W) on 1 August 2012, 20 June 2013, and 18 July 2013. In New York, *C. fornicata* begins brooding larvae in late April to mid-May, and although females can be found with eggs as late as October, most recruitment happens in June–August (pers. obs.). The site was in an area known to have large *C. fornicata* populations (Hoch and Cahill, 2012) and therefore sources of larvae for the experiment. The immediate vicinity of the arrays did not have large numbers of *C. fornicata*: the nearest population of adults was found approximately 0.5 km away. The experimental arrays were thus the only source of *C. fornicata* cue at the study site. The presence of *C. fornicata* larvae near the arrays was confirmed with a plankton tow each time the arrays were deployed, but the density of larvae was not quantified.

At each start date, five blocks (25 mesh bags) were deployed in a randomized blocked design (Fig. 1). Each bag was attached to two cement bricks (23 cm × 11.5 cm × 6.5 cm) using cable ties. The bags were in contact with the bricks, but were not in contact with the substrate. They were placed at a tidal height of ca. −0.5 m (measured from mean low water). Spring low tide in the summer at the site is approximately −0.1 m relative to mean low water, so the experimental animals were never exposed to the air. Within a block, bags were spaced 1 m apart (Fig. 1). Blocks were placed 5 m apart on a transect parallel to the shore. The substrate is sandy at the study site, making it unlikely that small recruits crawled among bags once they had metamorphosed. Bags were retrieved after two weeks following each deployment, individually wrapped to prevent movement among treatments during transport, and returned to the lab. All settled individuals (<5 mm) were then located by eye or with a dissecting microscope, removed from the substrate, and counted. The stacks and balls were not heavily fouled from their two weeks in the field, and settled individuals were easily visible.

2.2. Statistical analyses

Ordinary least squares regressions were used to test for an increase in settlement with increasing adult mass (density), with adult mass as

Table 1
Summary of treatments. The mass of adult snails (in grams), the number of adult snails, and the number of ping-pong balls for each of the five treatments in the experiment. Treatments are identified by the ratio of snails:balls according to the surface area of the two substrates within each bag.

Ratio of snails:balls by surface area	0:40	1:39	1:9	1:1	40:0
Mass of snails (range)	0 g	25 g (22.84–30.09)	100 g (95.01–108.3)	500 g (489.69–510.13)	1000 g (993.05–1028.84)
Mean number of snails (range)	0	8 (7–11)	28 (20–44)	134 (115–160)	257 (190–289)
Number of ping-pong balls	40	39	36	20	0