Title: **Spatial and temporal settlement patterns of blue crab (*Callinectes sapidus* and *Callinectes similis*) megalopae in a drought-prone Texas estuary**

Running page head: Texas estuary blue crab megalopae settlement

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Abstract:

The Mission-Aransas Estuary is the wintering ground for the only sustained wild population of the endangered whooping crane (*Grus americana*), and blue crabs (*Callinectes sapidus*) are an important component of their diet as well as being a major food source for important sport fishes such as the red and black drum. Blue crabs also support a commercial crabbing industry, and fisheries data indicate that blue crab populations have been declining since the 1980s. Possible factors leading to decline in blue crab populations include overfishing, increased populations and predation by regulated sport fishes, reduced freshwater inflows into estuaries, and reduced larval recruitment. Little is known about blue crab recruitment dynamics in this region, but restricted passes between coastal estuaries and the Gulf of Mexico along with extended periods of drought that often lead to hypersaline conditions in coastal bays may limit larval recruitment from the Gulf into the bays. To investigate blue crab larval recruitment patterns, citizen scientist volunteers used hogshair settlement collectors to sample five monitoring sites over a four year period. Results show that large numbers of blue crab megalopae are common in nearshore waters of the Gulf of Mexico, but only a small fraction (~ 1%) recruit into the estuary. Peak periods of ingress into the estuary occur during fall and winter months, with *C. sapidus* primarily contributing to the fall peak and *C. similis* dominating the winter peak. Increased salinity in the estuary during droughts may reduce the ability of blue crab larvae to detect and enter passes into the estuary.

Keywords: *Callinectes sapidus*; blue crab megalopae; recruitment patterns; larval settlement; citizen science; hogshair collector

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1. Introduction

Blue crabs (*Callinectes sapidus* and *C. similis*) are an important food source for the migratory endangered whooping crane (*Gus americana*, Linnaeus, 1758) population which overwinters in or near the Aransas National Wildlife Refuge in Texas (Westwood & Chavez-Ramirez 2005). It is also a major food source for sport finfishes such as black drum (*Pogonias cromis*, Linnaeus, 1766), red drum (*Sciaenops ocellatus*, Linnaeus, 1766), and spotted seatrout (*Cynoscion nebulosus,* Cuvier in Cuvier & Valenciennes, 1830) in Texas bays and estuaries (Scharf & Schlicht 2000, Vanderkooy 2013). The Atlantic blue crab (*C. sapidus*) is also regarded as an important commercial fishery throughout its range including Texas (Sutton & Wagner 2007). Picariello & Rosenberg (2015) reported that 1.9 million pounds of blue crab, valued at 2.3 million dollars, were landed in Texas in 2013. However, the Texas Parks and Wildlife Department (TPWD 2007) has reported declining commercial landings of Atlantic blue crab in Texas waters since 1987. Many factors could be contributing to the downward population trends such as limited freshwater inflow into the estuarine system (Guillory et al. 2001: Picariello & Rosenberg 2015), habitat alteration and/or loss (Guillory et al. 2001), reduced larval recruitment (Longley 1994), and increased predation by regulated sportfishes (Guillory & Prejean 2001, Picariello & Rosenberg 2015).

Interest in blue crab population dynamics in South Texas has increased due to their importance in the diet of the endangered whooping crane (Nelson et al., 1996). The whooping crane is the tallest bird in North America and nearly went extinct in the middle of the 20th Century (Urbanek and Lewis, 2015). In 2008, after years of steady population increases, 28 birds died in the winter of 2008-2009 and it was suggested that these deaths were due in part to reduced blue crab populations that resulted from drought conditions and diversions of freshwater from the Guadalupe and San Antonio Rivers (Gulley 2014).

Atlantic blue crabs undergo a complex life cycle as they transition from larval to adult stages and utilize a variety of habitats including the lower, middle, and upper estuary as well as adjacent nearshore coastal waters of the Gulf of Mexico (Perry & McIlwain 1986). Zoeae (first larval stage) hatch in the higher salinity waters of the Gulf of Mexico and drift among other plankton for several months undergoing 5-7 zoeal stages until metamorphosing into the megalopae postlarval stage (Epifanio 2007). Megalopae are then transported into the estuary by nearshore currents, flood tides, and wind driven processes (Tilburg et al. 2009, Epifanio & Garvine 2001) where they settle into a primarily benthic existence and metamorphose a final time into the juvenile crab stage (Lipcius et al. 1990). As juvenile blue crabs grow and molt to maturity, they tend to utilize less saline shallow waters of the estuary, occupying areas of structured habitats such as seagrass beds, salt marshes, and oyster reefs as well as soft muddy and sandy non-structured substrates (Lipcius et al. 2005). As adults, males prefer less saline waters of the upper estuary whereas female crabs usually occupy the middle to lower estuary with higher salinities. Mating usually occurs in the lower saline waters of the upper estuary, then female blue crabs migrate to the higher saline waters of the lower estuary and adjacent coastal waters of the Gulf of Mexico when ready to release their larvae (Perry & McIlwain 1986).

Although reduced recruitment of blue crab at the megalopae stage may be a factor contributing to their declining populations in the Mission-Aransas Estuary, very little is known about their recruitment patterns in the area. Larval recruitment may be an especially important component of blue crab population dynamics on the South Texas coast, since connections between local estuaries and the Gulf of Mexico are limited by nearly continuous barrier islands with widely separated narrow passes. The behavioral adaptation that allows weakly swimming planktonic blue crab larvae to be transported from the coastal ocean to estuaries is known as selective tidal-stream transport (Forward et al., 2003). By responding to environmental variables including light, changes in salinity, and turbulence, blue crab larvae move into the estuary by swimming up into the water column during nocturnal flood tides of increasing salinity and remain on the bottom during ebb tides with decreasing salinity. Freshwater inflows into South Texas estuaries are often reduced due to extended periods of drought, increased demand for freshwater by agriculture and municipal purposes, and capture of water in reservoirs (Montagna and Kalke 1992). These factors lead to increased salinity in South Texas estuaries, and experimental and modeling studies indicate that increased salinity can lead to reduced transport of blue crab larvae by selective tidal-stream transport (Bittler et al., 2014).

A simple but labor-intensive method for estimating the recruitment of blue crab larvae involves the deployment of standardized settlement collectors constructed of an artificial substrate (air-conditioning filter) in a cylindrical design over a 24 hour period (Metcalf et al., 1995). A citizen science larval blue crab monitoring project was started in 2012 to better understand the potential role of larval recruitment in the population dynamics of blue crabs in the winter feeding grounds of the whooping crane, and to investigate whether reduced freshwater inflows and resulting hypersalinity in estuaries of south Texas affected larval recruitment. Using settlement collectors this study gained insight into the proportion of blue crab larvae that recruit into the estuary from the Gulf of Mexico, how far these larvae travel into the estuary before metamorphosing into juveniles, and the seasonal pattern of larval recruitment in subtropical south Texas.

2. Methods

2.1 Study Area

The Mission-Aransas National Estuarine Research Reserve (NERR), located along the south-central coast of Texas, encompasses 751.5 sq. km of terrestrial, wetland and marine habitats characteristic of western Gulf of Mexico estuaries (Diener 1975, Mission-Aransas NERR 2015) and includes the Aransas National Wildlife Refuge, winter home to the last wild whooping crane flock. The extensive shallow bays within the reserve boundaries are diverse with an array of complex habitats such as seagrass meadows, oyster reefs, mangroves, and wind driven tidal flats, as well as tidal marshes that provide essential habitat for the endangered whooping crane. Hydrology is primarily influenced by freshwater inflow from the Mission and Aransas Rivers, and to a lesser extent by inflow of the Guadalupe and San Antonio Rivers into San Antonio Bay to the northeast. Exchange with the Gulf of Mexico occurs via the Aransas Ship Channel (southern extent of the Reserve) and, to a lesser degree, at Cedar Bayou at the Reserve's northern boundary (Mission-Aransas NERR 2015).

2.2 Sample collection

Blue crab megalopae tend to have very patchy spatial and temporal distributions, and they are rarely collected in samples using plankton nets, especially when nets are deployed during the day (unpublished data). Instead, settlement collectors designed to retain settling megalopae are commonly used to investigate crab megalopae recruitment (Lipcius et al. 1990, van Montfrans et al. 1990, Metcalf et al. 1995). Settlement collectors are deployed over 24 hour periods, allowing for sampling over extended periods during day and night and during various phases of the tidal cycle. Each sampling site in the present study was supplied with three replicate settlement collectors, which consisted of a piece of synthetic "hogshair" air conditioner filter sleeve (45 x 25 x 1.0 cm) wrapped around a weighted 10 cm diameter, 25 cm long PVC pipe. Each collector was suspended just below mean low tide level for ~24 hours per day. Trained citizen scientist volunteers collected crab megalopae samples daily from the collectors and, at the same time, deployed replacements. Collector sleeves were removed from each trap and rinsed with freshwater into a 19-liter bucket to remove all accumulated crab megalopae. The sample was then filtered through a 16 cm diameter 105 μm mesh sieve, and material retained in the sieve was rinsed into a vial and preserved in ethanol.

2.3 Sampling sites and dates

To investigate larval blue crab settlement and distribution patterns, *Callinectes* spp. megalopae were collected by citizen scientist volunteers approximately daily from a total of 4 sites within the Mission-Aransas estuary and 1 site just outside the estuary on Mustang Island (Figure 1, Table 1). These study sites were selected to represent the upper (R1/R2), middle (AP), and lower (LH and UT) reaches of the Mission-Aransas estuary and the adjacent coastal waters of the Gulf of Mexico (HC). The entire monitoring period spanned approximately 4 years, from April 26, 2012 to January 12, 2016, although the temporal coverage and number of samples collected varied between sites (Table 1, Figure 2).

Three sampling sites were originally established during the spring of 2012: an upper estuary site in Aransas Bay near the town of Rockport (R1), a site within the Aransas Ship Channel on the University of Texas Marine Science Institute pier (UT), and a coastal Gulf site just outside the Aransas Ship Channel on the Horace Caldwell pier (HC). In 2013, the location of the Rockport site was moved lower in the estuary (R2) due to low megalopae abundance despite daily volunteer sampling efforts, and two additional sites were added: a mid-estuary site at Conn

Brown Harbor in the town of Aransas Pass (AP) and a lower-estuary site at the Lydia Ann Lighthouse (LH) off the Lydia Ann Channel. These three new stations were only sampled for a short period of time due, in part, to limited volunteer availability (AP and LH) and low numbers of megalopae at all three sites. The coastal station HC was terminated after 20 months of sampling because of its high vulnerability to tampering and the physical demands associated with sample retrieval on that tall pier. The UT site was selected for continued sampling over the entire 4 year monitoring period. Its location within the pass connecting the Mission-Aransas Estuary to the Gulf of Mexico made it the most appropriate site at which monitor megalopae ingress into the estuary. Megalopae individuals were also consistently present in samples at this site, and accessibility and sampling conditions were optimal for citizen scientist volunteers.

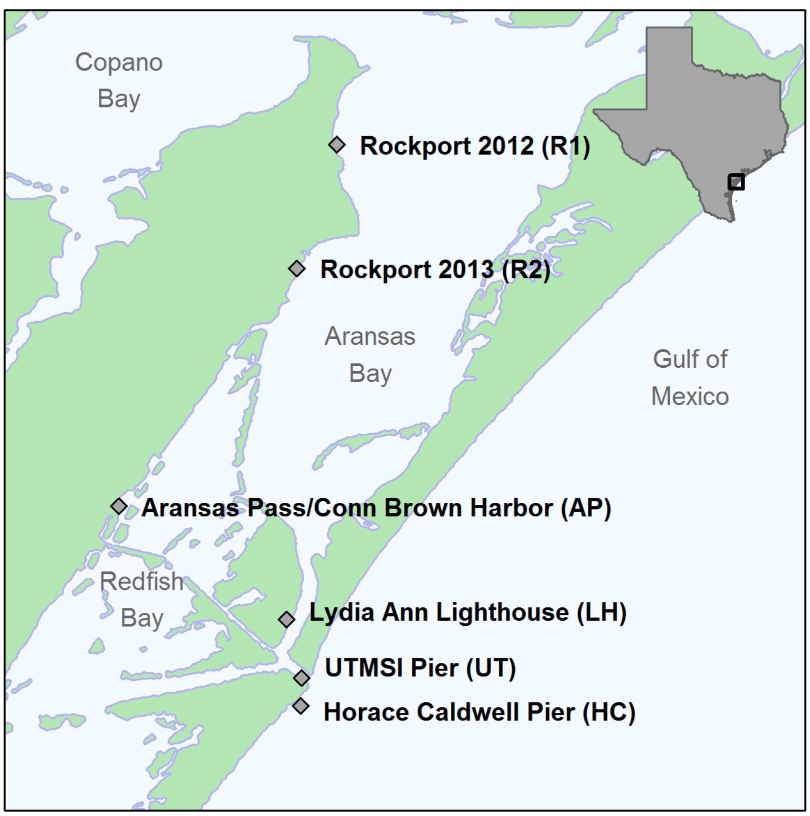

Figure 1. Map showing the locations of the megalopae settlement monitoring sites

Table 1. Names, locations, temporal sampling coverage, and total number of samples collected at each of the monitoring sites

Site Name	Location	Dates sampled	Total # samples
Horace Caldwell Pier (HC)	Gulf of Mexico coastline, Mustang Island, TX	6/12//2012–11/22/2013	246
University of Texas Marine Science Institute Pier (UT)	Aransas Ship Channel	04/26/2012–01/12/2016	791
Lydia Ann Lighthouse (LH)	Lydia Ann Channel	03/27/2013–05/29/2013	34
Aransas Pass (AP)	Conn Brown Pier, Redfish Bay	03/28/2013–11/21/2013	92
Rockport 2012 (R1)	Heron's Roost Private Pier, Aransas Bay	04/26/2012–10/30/2012	169
Rockport 2013 (R2)	Hunt's Castle Hotel Pier, Aransas Bay	03/06/2013–12/03/2013	197

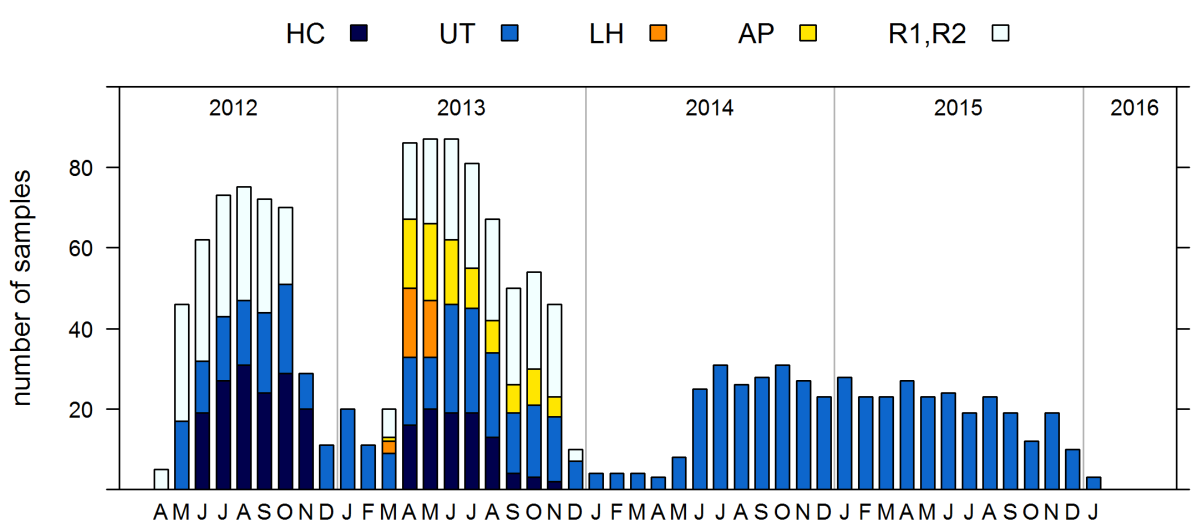

Figure 2. Number of samples collected each month at each of the monitoring sites

2.4 Sample processing

In the lab, *Callinectes* spp. megalopae were sorted from all other organisms using a stereomicroscope. Samples were split using a 0.5 l Folsom plankton splitter if the number of megalopae in the sample appeared to be greater than 200. Once all megalopae were identified, counted, and recorded, up to 60 were then randomly selected for identification to species (*Callinectes sapidus* and *Callinectes similis*) following Ogburn et al. (2011).

2.5 Data analysis

For data analysis, the 2 upper estuary sampling locations in Aransas Bay at Rockport were considered as a single site since they were relatively close in proximity and their sampling periods did not overlap (see Figures 1 and 2). This resulted in a total of 5 sites for spatial comparisons: 1 coastal Gulf site (HC), 2 lower estuary sites (UT and LH), 1 mid-estuary site (AP), and 1 upper estuary site (R). All data analyses described below were performed using R statistical software (R Core Team 2015).

To compare settlement among these 5 sites, basic settlement statistics (e.g., means, standard errors, frequency of zero values) were calculated. However, these values could not be directly compared to assess spatial patterns in settlement because the sampling periods differed between sites. Restricting the analysis to months during which all 5 stations were sampled resulted in only 3 months to average for site comparisons. Similarly, restricting the analysis to dates where sampling occurred at all 5 stations resulted in three days to use for site comparisons. Therefore, rather than making direct comparisons between the sites, settlement at each of the 4 estuary sites was individually compared to settlement at the HC Gulf site for dates on which both sites were sampled, and the proportions of estuary settlement to Gulf settlement were used to assess spatial patterns in megalopae settlement.

For analysis of temporal trends in megalopae settlement, the UT site time-series was used since it contained the longest and most complete sampling record relative to the other sites. Although the sampling frequency varied from month to month (Figure 2), ultimately, an average of 4 samples per week were taken at UT over the entire 4-year monitoring period (Table 1). The UT daily megalopae settlement time-series was aggregated into monthly means and a Kruskal-Wallis test was applied to assess whether there were seasonal patterns in monthly settlement that were consistent between years.

The UT time-series was also used to examine whether there were seasonal trends in the proportion of *C. sapidus* relative to *C. similis* settling on the collectors. Proportions were calculated for each month of each year by dividing the number of *C. sapidus* or *C. similis* identified within that month by the total number of *C. sapidus* + *C. similis* identified within that month.

3. Results

3.1 Data summary

The complete daily time-series of megalopae settlement for each of the 5 sites analyzed in this study differed greatly in length and temporal coverage (Figure 3; see also Methods). There were also distinct differences in megalopae settlement between sites (Figure 3, Table 2). Overall, the average number of megalopae settling on collectors in the open Gulf of Mexico at HC was anywhere from 3 to 5 orders of magnitude higher than the average settlement at the estuary sites. Also, the relative proportion of 0 values in the HC time-series was less than one fourth that for any of the estuary site time-series.

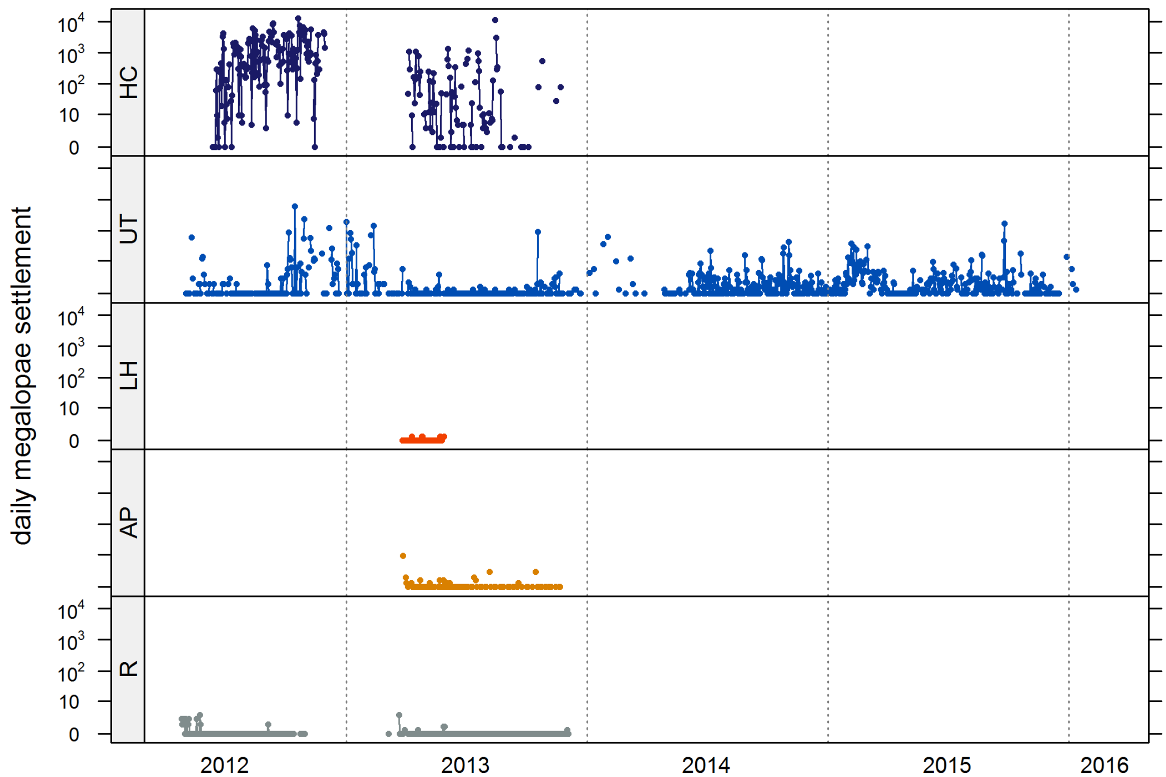

Figure 3. Time-series of abundance of megalopae (log scale) on settlement collectors at each of the 5 monitoring sites

Table 2. The total number of samples taken, the proportion of samples containing zero megalopae, and the mean number of megalopae per sample for each of the 5 monitoring sites

Site	# samples	0 values	Mean # megalopae	
			all samples	non-zero samples
HC	246	13%	1060	1217
UT	791	52%	4	9
LH	34	85%	0.05	0.3
AP	92	84%	0.2	1.3
R	366	95%	0.07	1.4

3.2 Comparison between sampling sites

Dramatically higher settlement was seen at the HC Gulf site relative to the sites within the estuary, suggesting that megalopae settling at the estuary sites originated from the Gulf. The average number of megalopae settling at an estuary site relative to the average number of megalopae settling at the HC site for a shared set of sampling dates was therefore considered to be the proportion of megalopae able to migrate from the Gulf to the estuary site area before settling, molting, or dying. These estimates for the estuary sites are given in Table 3 as percent settlement relative to HC and are mapped in Figure 4 as the number of megalopae reaching each site out of 10,000 at HC. Less than 1% of the settlement seen at the HC site was seen at any of the estuary sites, but settlement at UT relative to HC was still 1-4 orders of magnitude higher than at the sites further up the estuary. Settlement at R, the uppermost estuary site, was the lowest of the 4 estuary sites. Although the number of samples available to calculate the relative settlement for LH and AP were small compared to those for UT and R, the resulting estimates are in line with what would be expected based on the overall mean numbers of megalopae seen at those sites (Table 2).

Table 3. Percent megalopae settlement at each estuary site relative to settlement at the HC site outside the estuary and the number of shared sample dates used to calculate the percentage for each site

Site	**% HC Settlement**	**n**
UT	0.699	147
LH	0.001	19
AP	0.052	34
R	0.0001	193

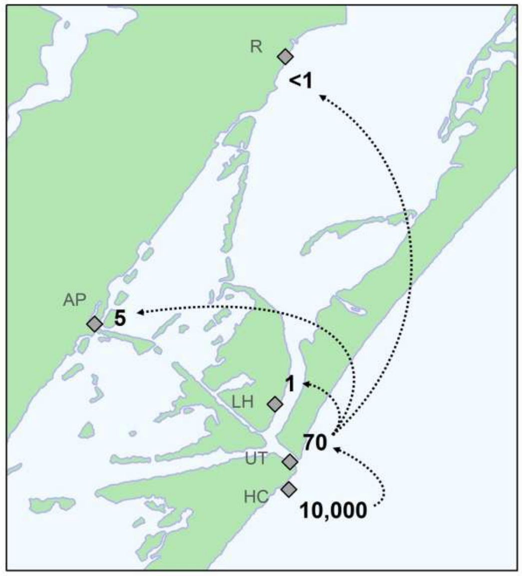

Figure 4. Theoretical number of megalopae expected to reach each site given 10,000 megalopae at the HC site. Calculations based on % HC settlement values given in Table 3

3.3 Seasonal trends at UT site (Aransas Ship Channel)

The time-series of monthly mean megalopae settlement at the UT site is plotted in Figure 5. Although there was considerable variability in settlement within each month between years, a significant seasonal trend was apparent (X^2=20.4, p=0.04), with peaks in settlement occurring in late winter (February) and early fall (October) and a minimum occurring in spring (April).

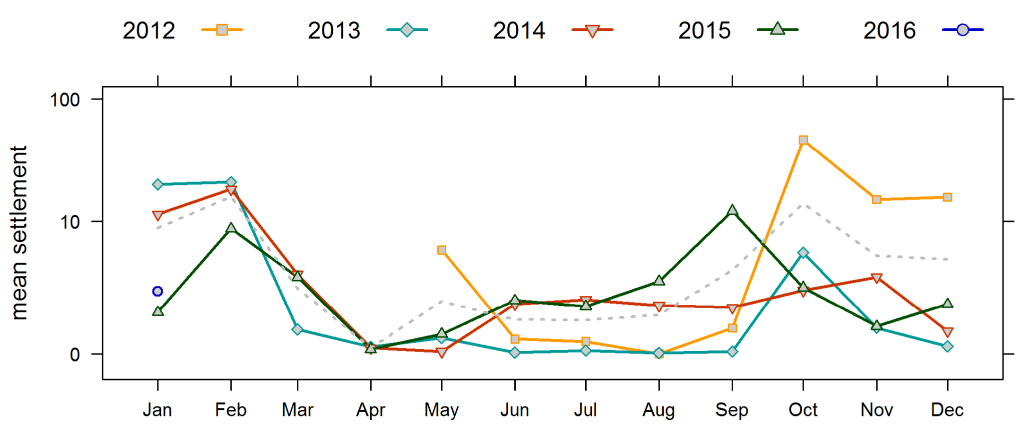

Figure 5. Monthly megalopae settlement means (log scale) by year for the UT site. The overall trend in monthly mean settlement is represented by a dotted line

Trends in species composition were examined by multiplying the monthly mean megalopae settlement time-series by the proportions of megalopae identified as *C. sapidus* or *C. similis* for each respective month. When the resulting values are grouped by month and compared to the overall seasonal settlement trend (Figure 6), it is apparent that the late winter settlement peak is due to an increase in the number of *C. similis* settling and that the early fall peak is primarily due to an increase in *C. sapidus* numbers.

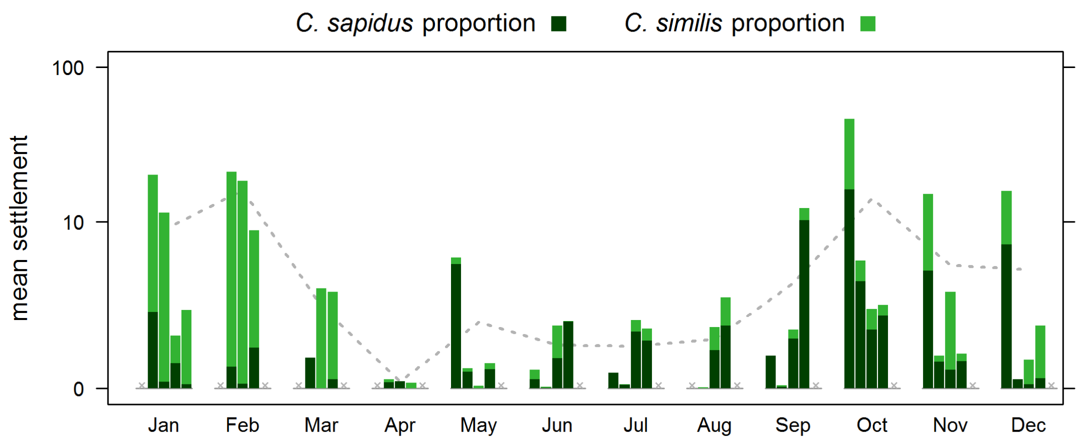

Figure 6. Proportions *C. sapidus* and *C. similis* composing mean settlement (log scale) for each month at the UT site. Each bar represents the settlement for the respective month during a different year (2012-2016), with × representing unsampled months. The colored portions of each bar correspond to the proportions of each species composing the total mean settlement for the month (e.g., 50% of a bar represents a 50% proportion). The overall trend in monthly mean settlement is represented by a dotted line

4. Discussion

One goal of this study was to gain insight into the recruitment dynamics of blue crab larvae in arid South Texas, examining a hypothesized relationship between periods of drought, high salinity in estuaries and the decline in adult and juvenile blue crab populations, which in turn might reduce food supply to winter flocks of whooping cranes (Gully 2014). Due to an extended period of drought during this study and the high variability in crab larvae collected a clear relationship between freshwater inflows, salinity and blue crab recruitment could not be documented. Other more tangible goals included investigating the seasonal abundance of blue crab megalopae larvae in the nearshore Gulf of Mexico, and the abundance of larvae within the estuary at varying distances from the Gulf of Mexico. Settlement data for blue crab megalopae using standardized settlement collectors produces data with high levels of short term variability, making it challenging to determine spatial and temporal patterns of larval blue crab recruitment and the factors affecting this variability. However, this is the most comprehensive study of recruitment of blue crab larvae to Texas estuaries carried out to date, and previously published settlement studies have rarely covered more than 3 consecutive years, sampled during winter months, or evaluated both *Callinectes sapidus* and *Callinectes similis* settlement patterns. This study compared settlement of megalopae between 4 sites in the Mission-Aransas Estuary and a nearby coastal site, to determine the proportion of larvae entering the estuary, and sampled year-round over the course of 4 years at one site to determine seasonal patterns in *C. sapidus* and *C. similis* settlement.

4.1 Daily Settlement

The blue crab megalopae settlement time-series for the stations sampled during this study all exhibited high short-term variability (Figure 3). The number of megalopae settling at a station could sometimes fluctuate by multiple orders of magnitude between consecutive sampling days, and zero values within the time-series were common (Table 2). This pattern of continuous low levels of settlement episodically punctuated by dramatic peaks is typical of settlement collector studies both in the Gulf of Mexico (e.g., Rabalais et al. 1995, Grey et al. 2015) and on the Atlantic Coast (e.g., Shanks 1998, Forward et al. 2004).

Multiple factors potentially contributing to variability in megalopae settlement have been previously investigated, but the successful detection of their influences on settlement trends has been highly inconsistent between years, sites, and studies. Wind direction and speed, temperature, salinity, rate of salinity change, and sea water levels have been implicated as having influences on blue crab megalopae settlement (Ogburn et al. 2012, see Grey et al. 2015 for review). Tidal patterns in association with lunar cycle and lunar declination have also been associated with patterns of blue crab megalopae settlement in multiple studies (e.g., Mense et al. 1995, Rabalais et al. 1995, Hasek & Rabalais 2001). High salinities within the estuary during the monitoring period may have reduced the number of megalopae transported into the estuary. Blue crab megalopae use a mechanism referred to as selective tidal stream transport (STST) to enhance their ingress into estuaries (Forward et al. 2003). During STST, megalopae exhibit adaptations in their swimming behavior that allow them to take advantage of incoming tidal currents to move up-estuary. Megalopae increase swimming activity in response to increasing salinity and turbulence on the incoming tide, remain swimming within the water column due to continued turbulence, and settle to the bottom in response to decreasing turbulence with the approach of slack tide. Decreasing salinity during ebb tide inhibits this turbulence-induced swimming behavior (Welch & Forward 2001), so megalopae remain on the bottom and avoid being carried back out of the estuary. During the first three years of this study there was a period of extended drought in South Texas and salinities in Aransas Bay and the Ship Channel were very similar on average, often differing by only a few parts per thousand (Figure 7) with the direction of the salinity gradient often differing over short time scales (CDMO 2018). During the last year of this study, salinities in Aransas Bay were reduced when more normal rainfall levels returned leading to a salinity gradient that should be more conducive to STST behavior of blue crab larvae. Despite various analyses done to determine the effects of lower salinity and rates of salinity change during incoming tides on megalopae recruitment, the natural variability in the data was too high to detect any patterns. Additional sampling during non-drought conditions is needed to establish whether recruitment is enhanced by the presence of a salinity gradient.

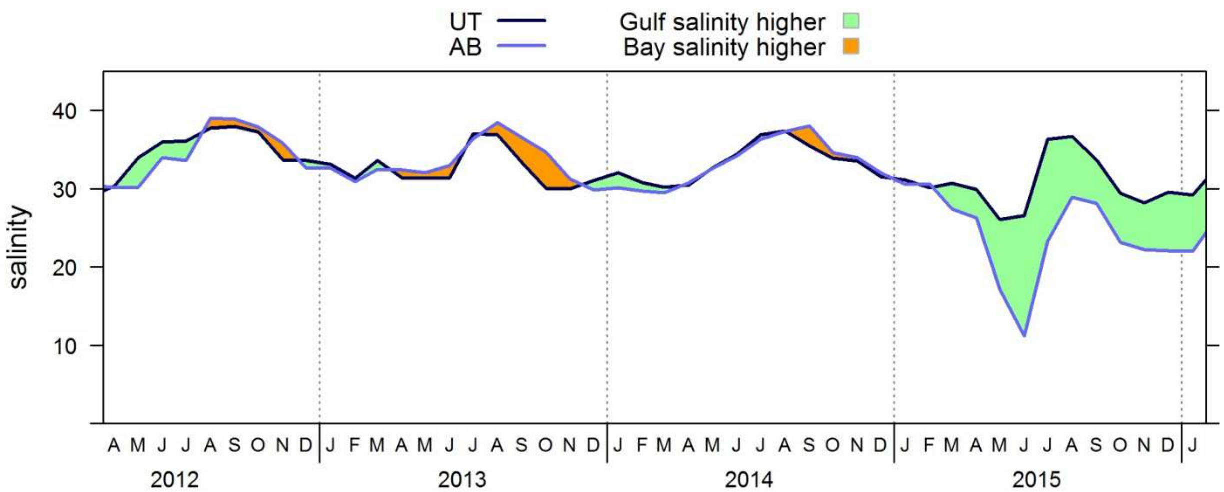

Figure 7. Monthly mean salinity values over the period of this study at two monitoring stations of the Mission-Aransas National Estuarine Research Reserve; one in Aransas Bay (AB) near the larval crab sampling sites in Rockport (R1, R2) and one on the UTMSI Pier in the Ship Channel (larval crab sampling site UT).

For our settlement collectors in the Ship Channel (UT) tidal patterns have a strong influence on current speed in the narrow channel between the Gulf of Mexico and the coastal estuaries, ranging from 0 to 1.5 m s^{-1} (Bittler 2013). In experiments making direct comparisons between the number of blue crab megalopae collected at night during short collections at the same depth with plankton nets or settlement collectors, higher current rates lead to larger numbers of blue crab megalopae captured in the plankton nets (larger volume of water sampled) but highest numbers of larvae were captured with settlement collectors at low current speeds of ca. 0.2 m s^{-1} (Bittler 2013). In a laboratory based flume experiment, highest rates of settlement were observed at intermediate current speeds of ~ 0.2 m s^{-1} (range tested: 0.035 – 0.313 m s^{-1}).

The likely explanation is that faster current speeds lead to more encounters with the collectors, but the ability to attach and remain attached to the collectors decreased at the higher current speeds.

In addition to these factors that may have contributed to short-term variability in the number of megalopae settling on the collectors in the present study, there are also factors that may have affected the number of megalopae retained on the collectors up to the time they were sampled the following day. Tankersley et al. (2002) found that the number of megalopae remaining on collectors that were sampled the morning after being submerged all night was lower than the number of megalopae observed on collectors that were sampled at hourly intervals during the night. They hypothesized that some megalopae may leave the collectors to avoid light at sunrise or that predation may reduce the number of megalopae remaining on collectors submerged for longer periods of time. On multiple occasions during the present study, small fish and crabs were directly observed feeding on organisms inhabiting the collectors, suggesting that predation may have also contributed to short term variability.

4.2 Spatial Settlement Patterns

Substantially higher settlement was observed at the Gulf site (HC) than at any of the estuary sites, including UT less than 2 km away, and UT had notably higher settlement than sites further up-estuary (Table 3, Figure 4). Substantially higher settlement at a coastal site just outside an estuary relative to a nearby estuarine site has previously been observed in the Newport River estuary on the Atlantic Coast (Ogburn et al. 2009), and decreasing settlement with increasing distance up-estuary is consistent with findings in Mobile Bay in the northern Gulf of Mexico (Morgan et al. 1996). Lower settlement at estuary sites relative to the coastal site in the present study is indicative of the restricted water exchange through the pass creating a bottleneck for megalopae entering the estuary from the Gulf of Mexico. However, there are other factors that could have contributed to the spatial settlement patterns observed, including high salinities within the estuary, the molting of megalopae into juveniles, and differing behavior of megalopae between estuarine and offshore water.

During the present study, salinities within the Mission-Aransas Estuary ranged from 30-40 ppt for the majority (>65%) of the entire monitoring period and for nearly all (>90%) of the monitoring period prior to 2014, during which the 3 stations furthest up-estuary were sampled. These high salinities within the estuary can lead to salinity decreases during flood tides rather than ebb tides, which could potentially decrease or reverse the flux of megalopae into the estuary (Bittler et al. 2014). The lack of blue crab megalopae at the up-estuary collectors during the period of drought and high estuarine salinities and the absence of blue crab larvae in monthly plankton samples taken within the estuary (CDMO 2018) suggest that female blue crabs do not remain within the estuary and release their larvae. This indicates that factors in addition to salinity are responsible for the migration of gravid adult female blue crabs out of the estuary to release their larvae in the open Gulf of Mexico.

Individuals molting from the post-larvae stage into juvenile crabs is another factor that may have contributed to lower megalopae settlement at up-estuary sites. Chemical cues in estuarine water and the presence of submerged vegetation such as seagrass can accelerate the metamorphosis of megalopae relative to individuals in offshore water (Forward et al. 1994, Forward et al. 1996). Megalopae within the estuary would have been exposed to these cues longer than megalopae at the ship channel or Gulf sites, where estuarine water is diluted by nearshore water, and may have been more likely to molt prior to reaching the collector sites. Accelerated metamorphosis of megalopae into juveniles prior to or after settling on collectors within the estuary could therefore have partially contributed to the low settlement counts at those sites.

Chemical cues in estuarine water also induce changes in the swimming behavior of megalopae that might reduce their exposure to settlement collectors within the estuary relative to collectors outside the estuary. In offshore water, megalopae actively swim during the day in response to light and become less active at night (Forward & Rittschof 1994, Forward et al. 1997). This pattern reverses in estuarine water, where megalopae swimming activity is suppressed by light during the day and is relatively higher at night (Forward & Rittschof 1994). A reversal of this photoresponse on its own would not change the exposure of megalopae to collectors between estuarine and offshore surface waters, but megalopae also maintain an endogenous rhythm in swimming activity independent of light conditions in which they are more active during daytime hours regardless of whether they are in offshore or estuarine water (Tankersley & Forward 1994, Forward et al. 1997). Since the period of greatest endogenous swimming activity is aligned with the positive swimming response to light in offshore waters, megalopae in the water column would accumulate at the surface during the day (Forward et al. 1997) and come into contact with the Gulf site collectors. At the estuarine sites, where light inhibition restricts swimming to the period of lowest endogenous activity at night, megalopae may be more evenly distributed in the water column or closer to the bottom and have a lower probability of interacting with the collectors near the surface.

4.3 Seasonal Settlement Patterns

Previous studies reporting seasonal patterns in megalopae settlement have primarily focused on the commercially important *C. sapidus*. On the Atlantic Coast of the US, the settlement season for *C. sapidus* megalopae is considered to be late July or early August to

November and various studies have reported peaks in settlement during that time of year (Goodrich et al. 1989, van Montfrans et al. 1990, Olmi 1995, van Montfrans et al. 1995, Forward et al. 2004, Ogburn et al. 2009). On the northern Gulf Coast, peaks in megalopae settlement have primarily been observed to occur within a similar July-November timeframe at various sites (Rabalais et al. 1995, Grey et al. 2015), including Mobile Bay (Morgan et al. 1996, Spitzer et al. 2003), Mississippi Sound (Perry et al. 1995, Morgan et al. 1996), Terrebonne Bay (Hasek & Rabalais 2001), and Galveston Bay (Rabalais et al. 1995, Grey et al. 2015). The September-November fall peak in *C. sapidus* settlement observed in the present study falls within the range reported in these previous studies.

Most Atlantic and Gulf coast settlement monitoring projects have focused their sampling efforts on times of year when the highest settlement of *C. sapidus* megalopae is expected; therefore winter and spring months (December-May) are typically not covered. The year-round sampling scheme employed in the present study allowed us to identify an additional peak in megalopae settlement that consistently occurred during January-February across the 4 year monitoring period (Figure 5). Unlike the fall settlement peak that was primarily composed of *C. sapidus*, the winter peak was largely dominated by *C. similis* megalopae (Figure 6). This seasonal trend in *C. similis* settlement is congruent with the winter peak of *C. similis* megalopae that has previously been reported in the Gulf of Mexico. In the northern Gulf, Perry (1975) and Adkins (1972) reported February peaks of megalopae in Mississippi Sound and Whiskey Pass, Louisiana, respectively. Subsequent examination of samples taken during winter in Mississippi Sound and of the samples taken in the Louisiana study identified the megalopae as *C. similis* (Stuck & Perry 1981). Stuck & Perry (1981) also observed a February-March peak in *C. similis* megalopae abundance in Mississippi coastal waters. Along the Texas Gulf Coast in the present study area, More (1969) and King (1971) noted a winter peak in *Callinectes* spp. megalopae from February-March in Aransas Pass and Cedar Bayou, respectively. Although these two studies did not distinguish between *C. sapidus* and *C. similis* megalopae, the findings from the northern Gulf studies as well as the present study point to the observed winter peaks being attributable to *C. similis*.

4.4 Conclusions

The success of this study relied on the efforts of citizen scientist volunteers. The potential and value of citizen science is becoming increasingly recognized in the field of ecology (Dickinson et al. 2010, Theobald et al. 2015). Volunteer contributions make it possible for scientists to collect data that time and resource limitations would otherwise prevent. Marine environments present a unique set of challenges to implementing citizen science projects (Cigliano et al. 2015), such as the difficulties with site accessibility that were experienced during the present study. This study required daily sampling of up to 5 sites covering a distance of ~20 km and spanned a 4 year period to ensure that spatial and seasonal trends in the data could be identified. Over 60 citizen scientist volunteers contributed approximately 2000 hours of service over the course of this project—an effort value equivalent to one year of salary for a full-time research assistant.

This study was undertaken to provide insight into the population dynamics of blue crabs in the Mission-Aransas Estuary by examining the hypothesis that low larval recruitment might be contributing to the decline in blue crab populations. Continued decline of blue crab populations in South Texas estuaries may pose a threat to the survival of the last remaining wild population of whooping cranes that rely on this species as a major source of protein before their long migration to their summer breeding grounds in northern Canada. Based on the results from standardized settlement collectors, it appears that the number of larvae that enter the narrow channel connecting the open Gulf of Mexico to adjacent estuaries are less than 1% of the blue crab larvae available to recruit in nearshore waters. Estuaries in South Texas may pose especially challenging conditions for the recruitment of blue crab larvae; nearly continuous barrier islands separately only by narrow passes provide only limited ingress to the estuary. This coupled with low freshwater inflows and high estuarine salinities may reduce the effectiveness of selective tidal stream transport of larvae back into estuaries

Hogshair collector sampling is labor-intensive, but the clear seasonal settlement pattern revealed by this year-round study will allow us to focus future sampling efforts on the peak period of settlement for *C. sapidus* (September, November). Future studies could focus on two stations (HC and UT) and target years of drought and years with high freshwater inflow to better understand the importance of freshwater inflow to the recruitment of blue crab larvae to the estuary. Furthermore, additional larval recruitment data could be analyzed in conjunction with the blue crab abundance data taken by the Texas Parks and Wildlife Department Coastal Fisheries monitoring program to determine the link between larval recruitment and cohorts of juvenile and adult blue crabs in the Mission-Aransas estuary.

Declarations of interest: none

Author contribution statement

T.F.W. carried out identification and analysis of crab larvae, assisted with data collection, and contributed to writing and editing the manuscript. L.P.S. performed data analysis and contributed to study design, writing and editing the manuscript. E.J.B. conceived and designed this study, supervised data collection, assisted with data analysis, and contributed to writing and editing the manuscript.

Role of the funding source

The funding sources had no role in study design, collection, analysis and interpretation of data; in the writing of the manuscript or the decision to submit the manuscript for publication.

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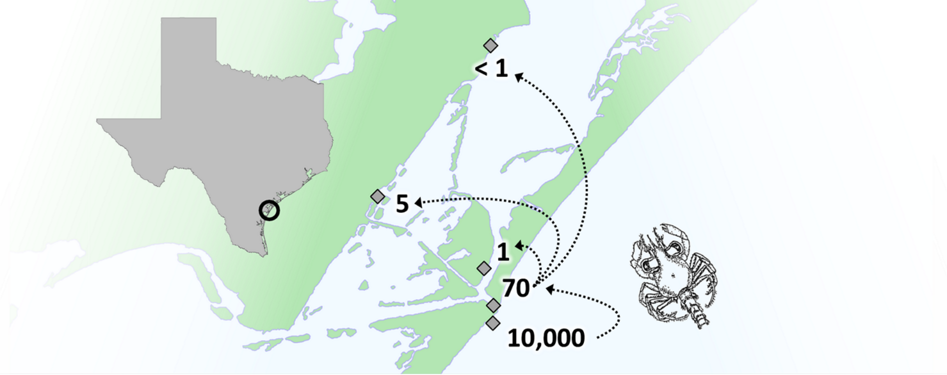

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