# Freshwater inflows and blue crabs: the influence of salinity on selective tidal stream transport

**Kimberly M. Bittler\*, Lindsay P. Scheef, Edward J. Buskey**

*Department of Marine Science, University of Texas at Austin, Marine Science Institute, 750 Channel View Drive,*
*Port Aransas, Texas 78373, USA*

ABSTRACT: Freshwater inflows are crucial for the function of estuaries but can become limited in the Mission-Aransas Estuary, Texas, USA, due to drought and human use. Several studies have linked blue crab *Callinectes sapidus* populations to freshwater inflows, suggesting that inflows may be critical for an early life-history event such as megalopae recruitment through selective tidal stream transport. This study examined whether the salinity response behaviors of *C. sapidus* megalopae that regulate selective tidal stream transport provide a plausible link between blue crab populations and freshwater inflows in the Mission-Aransas Estuary. The results of behavior experiments indicate that Texas megalopae have more variable responses to rates of salinity change than megalopae in a North Carolina, USA, estuary with higher freshwater inflow and that this variance lies between cohorts. Also, the rate of salinity increase that elicited the maximum behavioral response is half the rate reported in the North Carolina study. A simple behavior-driven flux model shows that this higher sensitivity is adaptive for transport into the Mission-Aransas Estuary when an estuarine gradient is present but not during drought conditions, as export out of the estuary is possible due to increasing salinity on the ebb tide. Transport of megalopae out of the estuary during drought may lead to recruitment failures in the absence of other recruitment mechanisms.

KEY WORDS: Selective tidal stream transport · Blue crabs · Freshwater inflows · Recruitment · Salinity · Drought · *Callinectes sapidus* · Crustacea · Crustacean larvae

## INTRODUCTION

Freshwater inflows supply nutrients to estuaries and maintain low salinity nursery areas that are critical to the productivity and function of estuarine systems (Longley 1994, Powell et al. 2002). Despite the importance of maintaining flows into estuaries, freshwater resources are becoming limited as coastal development and the demand for water increase. The decrease in freshwater reaching estuaries is projected to continue and to be exacerbated by the effects of an increasingly arid climate in Texas, USA (Montagna et al. 2011). Changes in freshwater inflows may have the greatest consequences for Texas estuarine ecosystems during drought years when salini-

ties can approach or even rise above the salinity of seawater (CDMO 2013). Understanding the ecological effects of salinity fluctuations and variable freshwater inflows is crucial to the effective management of estuaries in changing environments.

Blue crabs *Callinectes sapidus* are an important species in Gulf of Mexico estuaries, both as a commercial fishery and because of their trophic role in estuarine ecosystems (Hoeinghaus & Davis 2007). Several studies have linked blue crab abundance to freshwater inflows in the US states of Florida (Wilber 1993), North Carolina (Posey et al. 2005), Louisiana (Guillory 2000, Sanchez-Rubio et al. 2011) and Texas (More 1969, Longley 1994, Hamlin 2005) across both space and time. These relationships generally indi-

cate that peak abundances of blue crabs occur in low to intermediate salinities (Longley 1994, Hamlin 2005, Posey et al. 2005) or show a positive correlation of crab abundance with freshwater inflows (Wilber 1993, Guillory 2000). Blue crabs have a complex life cycle, broad salinity tolerances, and the ability to move large distances within estuaries throughout their life (Aguilar et al. 2005). These factors make studying the specific mechanisms that regulate the connection between salinity, freshwater inflows, and abundance difficult.

Spawning females migrate to higher salinities at the mouths of estuaries (Carr et al. 2004, Aguilar et al. 2005) to release multiple clutches of larvae known as zoea, which require full ocean salinity to develop (Darnell et al. 2009). The conditions at the mouth of the estuary are favorable for transport into the coastal ocean, where larval development occurs (Epifanio & Garvine 2001). The planktonic zoea larvae develop near the surface of the coastal ocean for 31 to 49 d before molting into a postlarval megalopae stage (Costlow & Bookhout 1959, Epifanio 2007). The megalopae must move into an estuary in order to molt into juvenile crabs but can postpone metamorphosis if estuarine chemical cues are not present (Wolcott & DeVries 1994). The megalopae are advected towards estuary mouths by wind-driven currents and barotropic forcing (Goodrich et al. 1989, Epifanio 1995, Ogburn et al. 2012) and move farther up the estuary with behavioral adaptations that take advantage of hydrologic movements, such as tides (Forward et al. 2003b). These behavioral responses are triggered by physical factors, such as increasing salinity and turbulence during selective tidal-stream transport (Welch & Forward 2001, Tankersley & Forward 2007), and by chemical cues associated with estuaries that cause the megalopae to become negatively phototactic (Forward & Rittschof 1994) and accelerate metamorphosis (Forward et al. 1997, 2001). 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.

Once within an estuary, megalopae settle on vegetation, then quickly molt into first instar juveniles (Lipcius 2007) and develop and move into low salinity areas of the estuary (Hines 2007). Juvenile crabs are an important food source for many estuarine species, such as red drum (Scharf & Schlicht 2000) and the endangered whooping cranes (Chavez-Ramirez 1996). Juvenile crabs are found in higher densities in salt marshes and other vegetated habitats that may provide better refuge from predation relative to unvegetated bottom (Minello et al. 2003). In estuaries where vegetated habitats are limited, low salinity areas may provide an alternate nursery habitat, with lower predation risk and enhanced food supply (Posey et al. 2005). Therefore, there are 2 life stages when freshwater inflows are likely to be particularly important to blue crabs: when recruiting back to estuaries as megalopae (Welch & Forward 2001) and when susceptible to heavy predation as juveniles (Posey et al. 2005).

This study focused on the recruitment stage, specifically the behavioral responses of megalopae that ensure transport into estuaries. The behaviors that govern transport via tides are well understood from studies performed on the US Atlantic coast. Transport is generally limited to the night, as the chemical signature of estuarine waters induces photoinhibition of megalopae activity during daylight (Forward & Rittschof 1994), and megalopae only actively swim at night when in the estuarine plume. Welch & Forward (2001) experimentally demonstrated a mechanism for the transport of blue crab megalopae into Atlantic coast estuaries known as selective tidal-stream transport (abbreviated STST; later reviewed by Forward et al. 2003b). Their model proposed that megalopae utilize nocturnal flood tides to move up estuaries and avoid being transported back out to sea on the ebb tide through a series of responses to changes in salinity and turbulence, whereby recruiting megalopae rise on the flood tide with increasing salinity, remain swimming due to turbulence, and settle at slack tide due to decreasing turbulence. Megalopae are inhibited from being transported out of the estuary on the ebb tide by decreasing salinity and remain on the bottom.

While this model is plausible for estuaries on the Atlantic coast that have more consistent freshwater inflows, the Mission-Aransas and other Texas estuaries experience more extreme drought conditions than estuaries on the Atlantic Coast and can become hypersaline in dry years. During these conditions, the increases in salinity that cue upward swimming in blue crab megalopae would occur during the ebb tide rather than the flood tide, potentially transporting the megalopae away from estuarine nursery habitats. Tankersley et al. (1995) performed experiments that explored the effect of different rates of salinity change on the vertical distribution of North Carolina blue crab megalopae *Callinectes sapidus*. The megalopae and water used in their study were collected from a typical Atlantic estuary with reliable

freshwater inflows and a strong salinity gradient. The conditions in the Mission-Aransas Estuary make it an ideal location to test whether salinity's role in STST varies between regions with differing estuarine characteristics. This study examined the salinity response of megalopae collected from the Mission-Aransas Estuary and the effects that these behaviors may have on recruitment with the following hypotheses: (1) megalopae swim upward in response to changes in salinity when held in offshore water (independent of any ambient estuarine chemical signal), and this behavior does not vary between cohorts, and (2) megalopae swim upwards in response to the same rates of salinity change as the megalopae tested by Tankersley et al. (1995) when held in ambient flood tide water (and are exposed to any estuarine chemical cues present in this flood tide water).

## MATERIALS AND METHODS

### Study site

The Mission-Aransas Estuary is a bar-built estuary in South Texas (Fig. 1) that has relatively low impacts from development (NOAA 2006). The small watershed is predominantly influenced by coastal weather patterns, with freshwater inputs from the Mission and Aransas Rivers. These 2 rivers flow into Copano Bay, which is connected to Aransas Bay. Aransas Bay exchanges water with San Antonio Bay to the north through the Gulf Intracoastal Waterway and with the Gulf of Mexico to the south through the Port Aransas Ship Channel. The Ship Channel is the primary inlet not only for the Mission-Aransas Estuary but also for Corpus Christi Bay and the hypersaline Laguna Madre, resulting in complex salinity patterns.

The Mission-Aransas Estuary is within the Mission-Aransas National Estuarine Research Reserve (MANERR). The MANERR maintains 5 long-term System-Wide Monitoring Program (SWMP) stations throughout the estuary that continuously monitor temperature, salinity, dissolved oxygen, pH, turbidity, and water level at 15 min intervals with YSI 6600 EDS data sondes suspended 0.5 m from the bottom of the water column, which is typically 2 to 3 m deep (site locations in Fig. 1). We used the data collected at these stations to calculate the range of rates of salinity change that megalopae recruiting into the estuary might experience during both normal and drought years. These rates were compared to those that Tankersley et al. (1995) found to induce upward swimming behavior in megalopae in North Carolina.
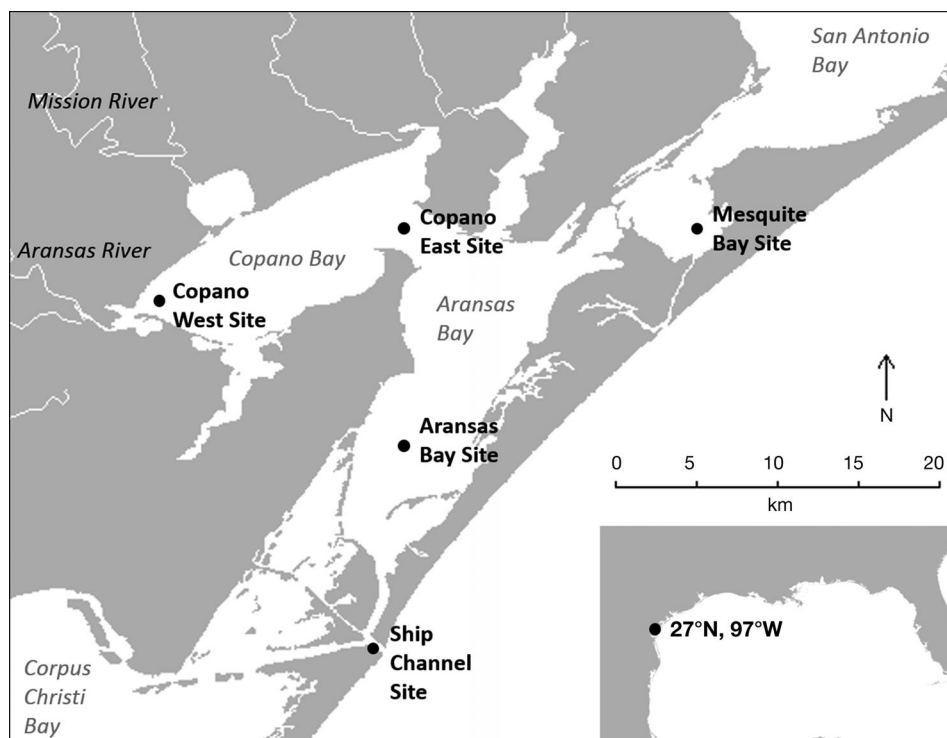


Fig. 1. Mission-Aransas Estuary and System-Wide Monitoring Program (SWMP) station sites. Inset shows location of Mission-Aransas Estuary within the Gulf of Mexico

### Behavior experiments

Two behavior experiments were conducted in the same apparatus to explore the behavioral responses of locally caught blue crab megalopae to changes in salinity. Following Tankersley et al. (1995), we built a vertical 18 × 6 × 6 cm Lucite flow-through chamber in which the ambient salinity could be gradually changed by pumping in water through a small opening at the base. To confine the megalopae to the center portion of the chamber where their response could be video recorded (Cohu 6500 camera with Sony Digital 8 Video Walkman), the column was divided into 3 sections by removable 83 μm mesh secured onto 2 PVC pipes (6 cm diameter) at the top and bottom of the chamber. A circular acetate sheath of 6 cm diameter was wrapped around the PVC to keep megalopae out of the corners of the chamber while reducing the effects of parallax when filming. All experiments were conducted in a dark room illuminated by dim red light, and the experimental chamber was back-illuminated with infrared LEDs (wavelength > 850 nm), which larval crustaceans cannot perceive (Cronin & Forward 1988). All megalopae were allowed to dark adapt for at least 10 min before any experiment began and were allowed to adjust to the experimental chamber for 3 to 5 min before video recording. Experiments were conducted at room temperature (~24°C).

Salinity within the chamber was manipulated by pumping water into the bottom section with a multispeed peristaltic pump. A stir bar at the bottom of the chamber ensured mixing of the inflowing water and uniform flow, and displaced water was allowed to spill through a drain at the top of the chamber to ensure water pressure would remain constant. Rates of change in salinity were calibrated according to the chamber dimensions and pump speed and were verified by measuring both the initial and final salinities in the chamber.

Megalopae were collected from the University of Texas research pier in the Port Aransas Ship Channel (27°50′N, 97°3′W) during nocturnal rising tides with a 0.5 m diameter, 500 μm mesh bongo net. Megalopae were identified as *Callinectes sapidus* (Ogburn et al. 2011), sorted into 10 cm diameter glass bowls, fed freshly hatched *Artemia franciscana* nauplii, and held at 20°C under a 12 h light:12 h dark cycle until experiments were conducted. All experiments were conducted within 3 d of collection, and only intermolt megalopae (i.e.

megalopae not showing signs of preparing to molt) were used. Megalopae were tested in groups of 25 to 30 and were transferred into water used for experiments at least 1 h before experiments were conducted. Megalopae were used for 1 treatment only and were not re-used in other treatments.

The first experiment was designed to assess the innate variability in behaviors between megalopae cohorts, as our second experiment would require us to use flood tide water and megalopae collected on different dates. Megalopae collected on dates at least 1 wk apart were considered to be independent cohorts. We subjected megalopae from 3 different cohorts to a change in salinity when held in ocean water (33.3 ppt, collected in the Gulf of Mexico, 30 miles [~50 km] offshore). Since megalopae behavior may be altered by chemical signals (Forward & Rittschof 1994), salinity was manipulated using deionized water and salt extracted from the offshore seawater by evaporation, and the same offshore water was used for each cohort. Salinity was either increased or decreased by 0.6 ppt at a rate of $1.0 \times 10^{-3}$ ppt s$^{-1}$, a rate within the range observed at the study site (see Table 1) and known to stimulate the maximum upward swimming response in megalopae from North Carolina (Tankersley et al. 1995). The response was measured as the number of megalopae actively swimming in the top two-thirds of the viewing chamber post-stimulus compared to the number of megalopae actively swimming before the stimulus was applied, following the procedure of Tankersley et al. (1995). Controls were conducted on each group of megalopae by circulating water at the same flow rate (0.12 ml s$^{-1}$) without any changes in salinity, and allowing the megalopae to recover without any stimulus for 3 to 4 min before the experimental stimulus was applied. For each of the 3 cohorts (Table 2), the experiment was conducted with 3 replicates per treatment. Data were arctan-

Table 1. Average rates of salinity changes (ppt s$^{-1}$) observed across Mission-Aransas National Estuarine Research Reserve (MANERR) System-Wide Monitoring Program (SWMP) sites from June 2010 to December 2012. Only increases in salinity are compared between drought (August 2011–January 2012) and normal (August 2010–January 2011) conditions

| Site | Increase | Decrease | Drought | Normal |
|---|---|---|---|---|
| Ship Channel | $2.89 \times 10^{-4}$ | $-2.92 \times 10^{-4}$ | $2.14 \times 10^{-4}$ | $4.11 \times 10^{-4}$ |
| Aransas Bay | $2.98 \times 10^{-4}$ | $-3.05 \times 10^{-4}$ | $2.79 \times 10^{-4}$ | $3.29 \times 10^{-4}$ |
| Copano East | $2.78 \times 10^{-4}$ | $-2.82 \times 10^{-4}$ | $2.10 \times 10^{-4}$ | $3.17 \times 10^{-4}$ |
| Copano West | $3.48 \times 10^{-4}$ | $-3.47 \times 10^{-4}$ | $4.59 \times 10^{-4}$ | $2.58 \times 10^{-4}$ |
| Overall | $3.07 \times 10^{-4}$ | $-3.10 \times 10^{-4}$ | $2.65 \times 10^{-4}$ | $2.91 \times 10^{-4}$ |

Table 2. Field conditions when megalopae *Callinectes sapidus* were captured during flood tide for the ocean salinity experiment, measured by the System-Wide Monitoring Program (SWMP) at the Ship Channel station. d$S$/d$t$: the rate of salinity change over time

| Date (mo/d/yr) | Time (h) | Salinity (ppt) | d$S$/d$t$ (ppt s$^{-1}$) |
|---|---|---|---|
| 3/27/2013 | 06:15 | 33 | 0 |
| 6/4/2013 | 22:00 | 28.4 | $-1 \times 10^{-3}$ |
| 6/21/2013 | 12:00 | 28.8 | $5.6 \times 10^{-4}$ |

gent transformed to produce normal distribution, and were analyzed with an ANOVA mixed model, which estimates variation due to cohort, treatment (increase or decrease in salinity), and treatment within a cohort. All data analyses and statistics were conducted in R (R Core Team 2013).

The second experiment examined the response of megalopae to a set of positive rates of salinity change: 0 (control), $2.5 \times 10^{-4}$, $5 \times 10^{-4}$, $1 \times 10^{-3}$, $2 \times 10^{-3}$, and $4 \times 10^{-3}$ ppt s$^{-1}$. These rates encompass the range observed during flood tide at the study site and are within the range tested by Tankersley et al. (1995). Megalopae were tested in the same flood tide water from which they were collected to capture the water chemistry conditions experienced by the megalopae as they were actively recruiting into the estuary through the Ship Channel. Salinity was increased by adding salt extracted by evaporation from offshore seawater (collected in the Gulf of Mexico, 30 miles [~50 km] offshore) to the water collected on the flood tide. During each collection date, every rate including the control was tested once without replicates following a complete block design. Five separate cohorts were tested, collected at least 1 wk apart (Table 3), in order to accurately capture the variability present in independent cohorts. The mean net response to each rate of salinity increase was compared to the mean net response of the control treatment using Dunnet's *t*-test.

## Field modeling component

We developed a simple qualitative model to explore how the proportions of megalopae positively responding to changes in salinity in the above experiments might affect the recruitment of megalopae into the Mission-Aransas Estuary. This model was designed to predict what proportion of megalopae present on a given night could be transported into or out of the estuary (positive or negative $P_{flux}$, respectively) based on the maximum proportion that would respond to the range of salinity changes on the flood tide that night ($P_{flood}$) minus the maximum proportion that would respond on the ebb tide that night ($P_{ebb}$), such that:

$$P_{flux} = P_{flood} - P_{ebb}$$

This approach is appropriate since megalopae respond to short-term salinity changes and once in the water column do not experience further changes in salinity but keep swimming due to turbulence (Welch & Forward 2001).

Actual rates of salinity change in the field were estimated based on data from the Ship Channel SWMP station, which is the major pass that megalopae developing offshore must be transported through to reach the estuary. Salinity has been measured every 15 min at this station since August 2007. The rate of salinity change was calculated per 15 min interval and smoothed within an hour window. All rates over $2.22 \times 10^{-3}$ ppt s$^{-1}$ were excluded from the analysis following standard quality control protocols of the National Estuarine Research Reserve System (NERRS 2013). The responses of megalopae to rates of salinity change measured in this study and from the Tankersley et al. (1995) study were estimated for each rate of salinity increase in the field with linear interpolation. Values measured during the day (between sunrise and sunset) were excluded from the model, as most transport of megalopae occurs at night (Forward et al. 2003b)

Table 3. Field conditions during megalopae *Callinectes sapidus* capture and water collection for the flood tide rate experiment, taken from the System-Wide Monitoring Program (SWMP) at the Ship Channel station. d$S$/d$t$: the rate of salinity change over time; Turb: turbidity, measured in nephelometric turbidity units (NTU)

| Date (mo/d/yr) | Megalopae | | | Water | | | |
|---|---|---|---|---|---|---|---|
| | Time (h) | Salinity (ppt) | d$S$/d$t$ (ppt s$^{-1}$) | Time (h) | Salinity (ppt) | Turb (NTU) | pH |
| 5/21/2013 | 05:00 | 33.1 | 0 | 24:00 | 34.7 | 5 | 8.1 |
| 6/5/2013 | 12:00 | 28.4 | $-1 \times 10^{-3}$ | 11:00 | 25 | 2 | 8.1 |
| 6/20/2013 | 12:00 | 35.8 | $-5.6 \times 10^{-4}$ | 11:40 | 35.8 | 8 | 7.9 |
| 7/9/2013 | 06:00 | 36.4 | 0 | 11:10 | 35.9 | 4 | 7.8 |
| 8/17/2013 | 02:15 | 36.6 | 0 | 11:00 | 36.7 | 1 | 8 |

The direction of the tide at the Ship Channel site was determined using velocity data collected by a Nortek Aquadopp Acoustic Current Profiler at 0 to 2 m depth from June 2010 to December 2012. Estimates of $P_{flux}$ were integrated from June 2010 to December 2012 to estimate net flux of megalopae over the time period for which current velocity estimates were available. For overall mean net flux estimates, 95% confidence intervals were calculated by bootstrapping (1000 iterations).

## RESULTS

### Rates of salinity change in the Mission-Aransas Estuary

The average rate of salinity increase across all SWMP sites in the NERR during the study period was $3.07 \times 10^{-4}$ ppt s$^{-1}$, with mean rates of increase ranging from $3.48 \times 10^{-4}$ ppt s$^{-1}$ at Copano West to $2.78 \times 10^{-4}$ ppt s$^{-1}$ at Copano East. The overall average increase was slightly below the minimum rate of change that Tankersley et al. (1995) found sufficient to stimulate a significant response ($5.53 \times 10^{-4}$ ppt s$^{-1}$) and results in a suitable absolute change to stimulate megalopae response over 15 min (0.4 ppt per 15 min). During wet periods, such as the summer of 2010, the mean change in salinity is higher at most sites compared to drought periods, such as the summer of 2011 (Table 1).

### Behavior experiments

In the first behavioral experiment there was high variability in the salinity responses of the 3 different cohorts in the experiment conducted in offshore water, despite holding experimental conditions, including water chemistry, constant (Fig. 2). The first cohort had almost no response to either an increase or decrease in salinity, while the second cohort appeared to have a non-significant positive response to decreasing salinity (rather than the expected positive response to increasing salinity). Only the third cohort showed the expected response to salinity, with a positive response to increasing salinity and a slightly negative response to decreasing salinity. There were non-significant main effects of cohort ($F = 2.32$, df = 2, p = 0.14) and of changing salinity ($F = 0.60$, df = 1, p = 0.45); however, changing salinity nested within cohort was significant ($F = 7.88$, df = 2, p = 0.01). There was a significant behavioral differ-
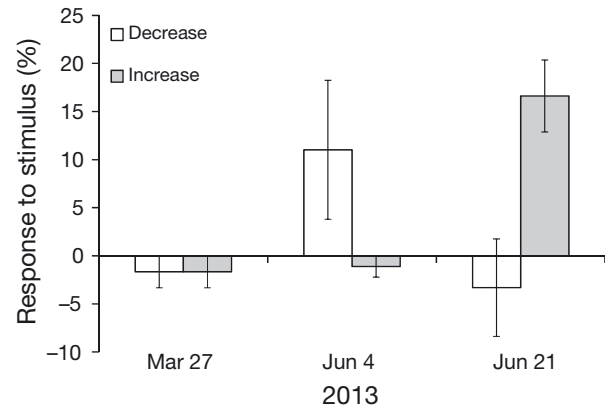


Fig. 2. Net response of *Callinectes sapidus* megalopae to an increase or decrease in salinity ($1.0 \times 10^{-3}$ ppt s$^{-1}$) when tested in offshore water with no estuarine cues across 3 cohorts (±SE, n = 3). A positive response indicates an increase in the proportion of megalopae in the upper two-thirds of the experimental chamber, while a negative response indicates a decrease. There were significant differences in response to the 2 stimuli nested by cohort (p = 0.0065), with only the cohort collected on June 21, 2013 displaying a significantly different response between an increase or decrease in salinity (p = 0.043)
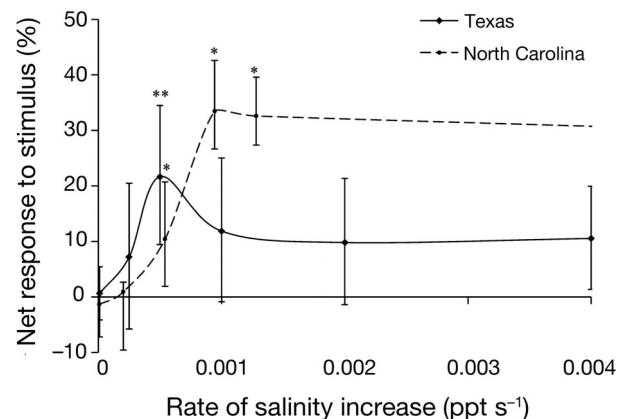


Fig. 3. Mean net response of *Callinectes sapidus* megalopae (±95% confidence interval, n = 5) to various rates of salinity increase in flood tide water in the present study (Texas, solid line) and in a study conducted in North Carolina (dashed line; data from Tankersley et al. 1995). *Net response significantly greater than control for North Carolina megalopae; **maximum response in Texas megalopae measured in this study

ence between the increase and decrease in salinity for the third cohort only (Tukey's honestly significant difference test p = 0.043).

In the second experiment, the responses to salinity increase were tested in 5 separate cohorts of megalopae in flood tide water (Table 3) across a geometric series of rates from $2.5 \times 10^{-4}$ to $4.0 \times 10^{-3}$ ppt s$^{-1}$. Both the magnitude of the response and the rate of
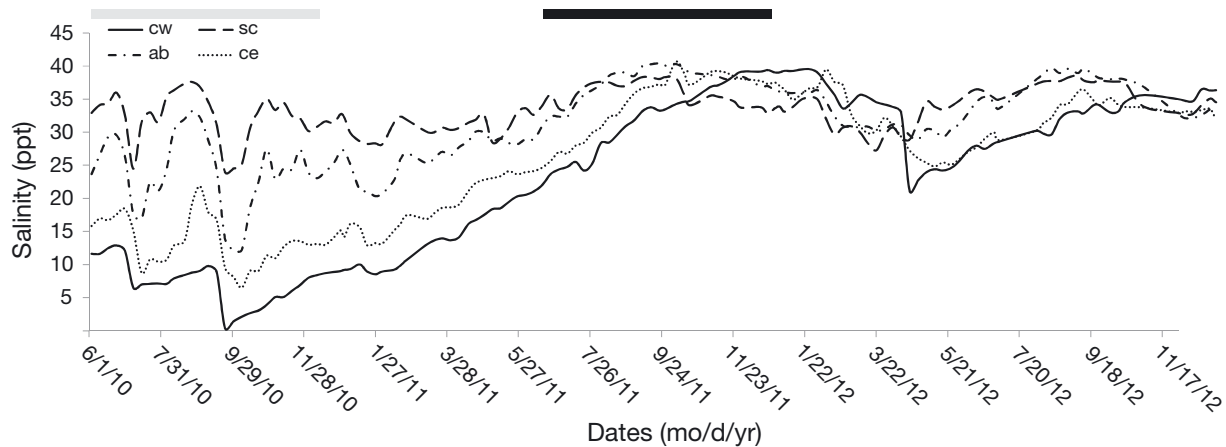
Fig. 4. Average weekly salinity across all sites at the MANERR from June 2010 to December 2012. Black bar: drought period used in model; gray bar: wet period used in model. Sites are Ship Channel (sc), Aransas Bay (ab), Copano East (ce), Copano West (cw), listed in order of closest to the Gulf of Mexico to farthest up the estuary. For site locations, see Fig. 1

salinity change that elicited the maximum response varied between cohorts, so none of the treatments were significantly different from the control when significance thresholds were adjusted for multiple comparisons (alpha = 0.05, p = 0.08). However, there was a distinct maximum net response of 21.6% (95% confidence interval: 9.5 to 34.5%) at $5 \times 10^{-4}$ ppt $s^{-1}$ (Fig. 3). This response was approximately 30 times higher than the mean response in the control treatment (0.67%, 95% confidence interval: −4.0 to 4.67%, p = 0.02), which was not significantly different from zero (p = 0.8). The response to more rapid rates of salinity change was dampened and not notably above zero or the controls.

## Model results

Average daily salinity at the Ship Channel ranged from 20.4 to 38.9 ppt over the 2 yr study period, generally increasing during drought and decreasing during wet years (Fig. 4). Positive rates of salinity change occurred more frequently on the ebb tide than on the flood tide (Fig. 5), but the rate of salinity change that elicited the greatest vertical response ($5 \times 10^{-4}$ ppt $s^{-1}$) occurred more frequently on the flood tide than on the ebb tide.

The model using rates of change of salinity measured in the Ship Channel and the response curves from the experiment series (rates in flood tide water) predicted an overall slight negative flux of Texas megalopae out of the estuary over the 2.5 yr time series (−0.84% $d^{-1}$, 95% confidence interval: −1.22, −0.45). However, positive net flux of megalopae into
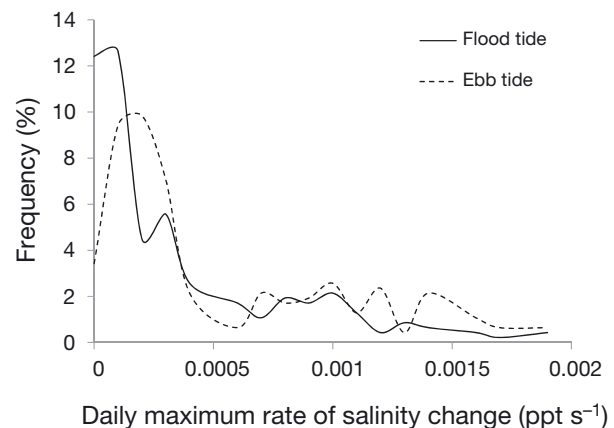


Fig. 5. Percent frequency of daily maximum rate of salinity change observed at the Ship Channel MANERR site on ebb and flood tides from June 2010 to December 2012. Only increases in salinity are shown

the estuary using salinity signals was substantial when a strong estuarine gradient was present such as the fall of 2010 (4.11% $d^{-1}$; Fig. 6), as was the flux out of the estuary during drought conditions (4.5% $d^{-1}$).

When the model was constructed at the Ship Channel using the rate-response curve of megalopae tested from North Carolina by Tankersley et al. (1995), fewer megalopae were expected to be transported out of the estuary over the whole time series (−0.22% $d^{-1}$, 95% confidence interval: −0.45, −0.01). When the model results were separated into dates when negative $P_{flux}$ was predicted and positive $P_{flux}$ was predicted, it became apparent that the lesser
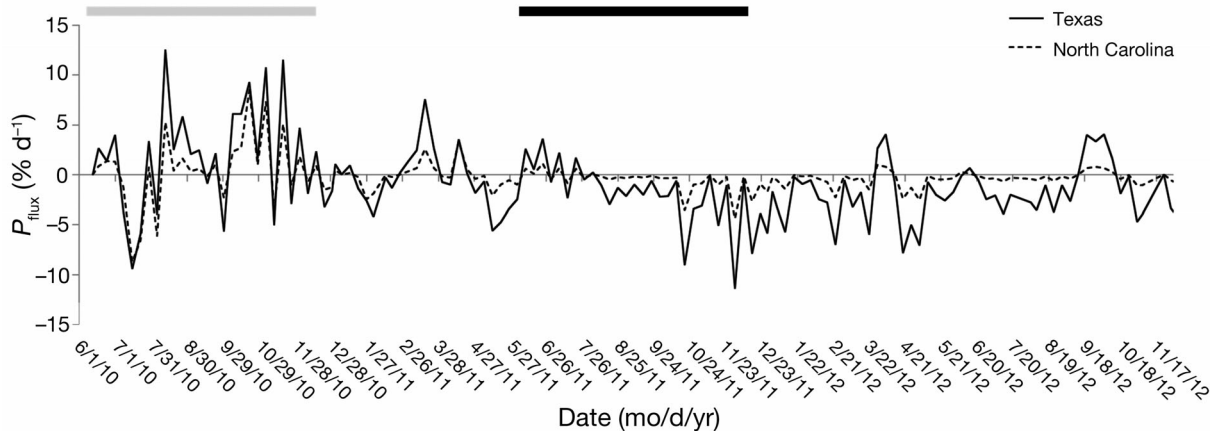
Fig. 6. Results of the behavioral transport model, predicting net flux as percent per day, averaged by week from June 2010 to December 2012 through the Aransas Ship Channel. Positive $P_{flux}$ indicates import of megalopae *Callinectes sapidus* into the estuary, while a negative value indicates export of megalopae out of the estuary. Gray bar: a wet time period; black bar: a period of drought. Texas dataset (present study); North Carolina dataset (Tankersley et al. 1995)
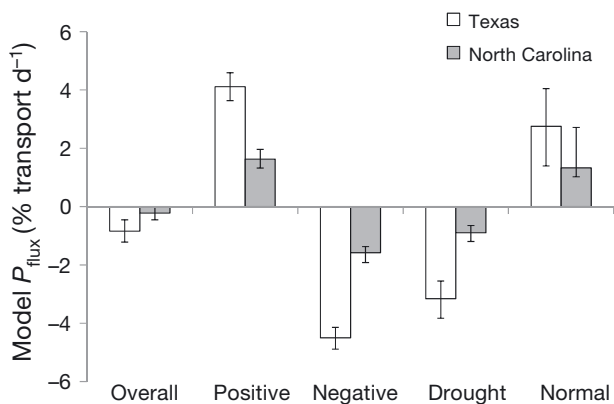


Fig. 7. Comparison of summarized model results ($P_{flux}$) for the transport of megalopae *Callinectes sapidus* through the Port Aransas Ship Channel; 95% confidence intervals for model results were obtained by bootstrapping (n = 1000). Texas dataset (present study); North Carolina dataset (Tankersley et al. 1995 applied to Texas estuary in present study)

export of the Tankersley-based North Carolina model is due to an overall lower response (Fig. 7). This lower response was due to the higher threshold rate of salinity change needed for North Carolina megalopae that is rarely reached in the Mission-Aransas Estuary. Twice as many Texas megalopae were transported into the estuary (4.11% $d^{-1}$, 95% confidence interval: 3.63, 4.59) compared to those in the North Carolina model (1.62% $d^{-1}$, 95% confidence interval: 1.32, 1.96). $P_{flux}$ follows climate trends, with the differences in model performance still favoring transport of the Texas megalopae over

those of North Carolina in a wet period from July 2010 to January 2011. Any advantage gained by the Texas model compared to the North Carolina model was lost during dry periods, such as July 2011 to January 2012, when over twice as many megalopae were predicted to be exported out of the estuary in the Texas model compared to the North Carolina model. Most of the 2.5 yr study period was during a severe drought (2009, 2011 to present), so the advantage of the Texas model during normal conditions was not reflected in the overall $P_{flux}$ averages that contained a range of salinity gradients over the tested time period, although it could feasibly exist on a longer time interval.

## DISCUSSION

### Experimental results

Our behavioral experiments have shown that *Callinectes sapidus* megalopae collected from the entrance to the Mission-Aransas Estuary, Texas, have a different response to various rates of salinity increase compared to megalopae in an experiment conducted in North Carolina (Tankersley et al. 1995;, Fig. 3). In the flood tide water experiment, the variance in net response to a given increase in salinity was much larger than the variance observed in megalopae collected in North Carolina by Tankersley et al. (1995), despite an experimental design with equal power in replicates (n = 5) and precision (25 to 30 megalopae trial$^{-1}$). Additionally, the rate of salinity increase that elicited the peak response was half that reported by

Tankersley et al. (1995) (Fig. 3). This heightened sensitivity to lower rates of salinity increase may be adaptive to transport into the Mission-Aransas Estuary, especially since the rate of salinity change that elicited the greatest net response of locally collected megalopae (Fig. 3; $5 \times 10^{-4}$ ppt s$^{-1}$) occurs more frequently on the flood tide than on the ebb tide (Fig. 5). The specific rates of salinity increase that megalopae collected in Texas responded to may reflect biological selection that favors recruitment over time to these historically prevalent conditions. As climate change continues, Texas estuaries are expected to become more freshwater limited (Ward 2011), and the rate of salinity change that is adaptive for megalopae recruitment may change at a rate that blue crabs are unable to match.

The variability of the behavioral response to salinity change reported in this study was much higher than the variability reported by Tankersley et al. (1995), despite using an equally powerful experimental design (Fig. 3). Potential sources of variability lie in: (1) experimental error and within-cohort behavior variability, (2) conditions of the water in which megalopae were tested, and (3) intrinsic behaviors in megalopae that vary between cohorts. Experimental error and within-cohort variability are unlikely to contribute large variability to our results, as the variance within treatments of the ocean salinity experiment (Fig. 2) was low. Additionally, 30 megalopae were used in each treatment of each experiment, increasing the precision of proportion estimates to 3–4%. This leaves 2 remaining sources of variation: the properties of the water in which the megalopae were tested and the intrinsic behaviors of megalopae that varied between cohorts.

The potential variance introduced by differences in flood tide water properties, such as chemical cues (Table 3), collected on different dates was not tested as part of this study. There is some evidence that chemical cues associated with estuaries have a role in transport. Refractory chemical cues in estuarine water induce negative phototaxis in megalopae, which limits transport to the night (Forward & Rittschof 1994), and megalopae orient towards chemical cues associated with optimal habitats in flow (Forward et al. 2003a) and on settlement substrates (Welch et al. 1997). Also, Forward & Rittschof (1994) reported that megalopae are more active in offshore water than in estuarine water. Differences in the chemistry of the flood tide water collected on different dates could plausibly have contributed to the variance noted in the flood tide experiment but vary as a function of drought, salinity, mixing, and other

processes that could not be adequately quantified in this study.

The results of the ocean salinity experiment isolated differences in intrinsic behaviors between cohorts as a source of variance, as experimental water chemistry was controlled for and within-cohort error was explicitly estimated using replicates (Fig. 2). In the ocean water experiment, there were strong differences between cohorts, supporting the assumption that there are intrinsic differences in behavior that vary widely between cohorts.

These 'intrinsic' differences may have a genetic basis or may be due to the influence of environmental conditions that modified their behavior before collection, such as the chemistry of the water the megalopae had experienced in the field. Wolcott & DeVries (1994) noted that megalopae in premolt (i.e. molting into a juvenile crab was imminent) tended to be collected within the estuarine plume compared to megalopae collected further offshore which tended to be in intermolt (i.e. had recently molted from the zoea stage), and Forward et al. (1997) showed that estuarine cues indeed did accelerate metamorphosis experimentally. While the water chemistry in which the megalopae developed could have contributed to some of the variability noted in this study, the selection of only intermolt megalopae for use in the experiments minimized this potential effect.

The Mission-Aransas estuary lies at a major climatic gradient, as estuaries farther north and east along the Texas coast have stronger freshwater inflows and salinity gradients, while estuaries farther south tend to be hypersaline (e.g. Laguna Madre). The high variability measured here may be a result of offshore currents mixing populations of megalopae from different estuaries in variable proportions and supports our hypothesis that the variability in the experiments was likely due, at least partially, to differences between cohorts.

### Model results

#### Flood and drought cycles

The model predicting $P_{flux}$ is a useful tool for understanding the potential ecological implications and adaptive function of laboratory-measured behaviors. The most notable model result is the strong role of wet and dry climate cycles on selective tidal stream transport behaviors (Fig. 6) and, potentially, on recruitment success. While strong transport into the estuary was possible during wet or normal condi-

tions, equally strong transport out of the estuary was predicted during drought conditions, suggesting that the response of locally collected megalopae to increasing salinity is not adaptive for entry into the Mission-Aransas Estuary during drought (Fig. 7). Previous studies have linked blue crab commercial landings or abundance to lagged freshwater inflows in estuaries (More 1969, Wilber 1993, Guillory 2000, Posey et al. 2005), but few have shown where the link lies. With the $P_{flux}$ model, we have provided a plausible link for the long-term correlations between freshwater inflows and blue crab abundance during selective tidal stream transport recruitment.

Even during drought conditions, when our model indicates a net flux of megalopae out of the estuary (Fig. 7), some flux of megalopae into the Mission-Aransas Estuary may still result from a variety of mechanisms (other than a salinity response in selective tidal stream transport) that may retain megalopae within estuaries after random transport. For example, Ogburn et al. (2012) showed that barotropic forcing processes drove a significant amount of the variation in megalopae recruitment within a given year using a modeling approach. Forward et al. (2003a) showed that blue crab megalopae, especially megalopae that were close to metamorphosis into crab stages, can orient towards the scent of suitable settlement habitats such as seagrass beds if they are transported close enough to them. Megalopae also become less active in estuarine water (Forward & Rittschof 1994), and this difference in kinesis could also function in transport, as demonstrated by Kingsford et al. (2002) in Japanese flounder *Paralichthys olivaceus*. Furthermore, metamorphosis is accelerated in the presence of estuarine cues (Forward et al. 2001). If megalopae are more likely to settle and molt within the estuary than outside of it, then a megalopae entering the estuary is less likely to leave as it will have molted into a juvenile crab. Although net flux may continue in the absence of effective salinity cues, transport is certainly enhanced by strong salinity cues as our models show.

### Comparison of North Carolina and Texas models

The model fitted using the North Carolina rate-response curve (from Tankersley et al. 1995) predicted less overall export of megalopae from the estuary compared to the model created using the rate-response curve for Texas megalopae from this study, although an additional study to assess the behaviors and abundance of megalopae caught on the ebb tide is needed. Further analysis of the models revealed that the difference in export was due to the overall weaker responses of North Carolina model megalopae to the rates of salinity change experienced at the Ship Channel compared to the responses of Texas megalopae (Fig. 7). The difference in transport—either into or out of the estuary—shows that Texas megalopae are adapted to respond to the lower rates of salinity change in Texas. However, the strong transport of Texas model megalopae out of the estuary during dry periods shows that the salinity response of Texas megalopae is not drought adaptive. The Texas megalopae are just as likely to be transported out of the estuary during drought as they are to be transported into the estuary during wet periods.

### Adaptive function of behaviors

The behavioral adaptations to salinity changes reported in this study are not unique to *Callinectes sapidus*. Salinity is a very important driver of selective transport in the meroplankton across a variety of taxa and geographic locations. In the green shore crab *Carcinus maenas* circatidal rhythms can be entrained with abrupt changes in salinity to enable selective tidal stream transport (Taylor & Naylor 1977), and accordingly, *C. maenas* megalopae are most abundant in high flood tide salinities in the field. Japanese flounder are more active in higher salinity waters, which facilitates transport on the flood tide (Kingsford et al. 2002), and models have suggested that salinity-cued STST results in more rapid and effective transport of brown shrimp *Crangon crangon* into estuaries in the North Sea (Daewel et al. 2011). Other modeling studies that were complemented with behavioral observations have shown that disparate behaviors can have huge consequences for dispersal. North et al. (2008) compared a simple behavior of oyster larvae encountering a halocline in 2 species: *Crassostrea virginica* and *C. ariakensis*. *C. virginica* swam up in response to a halocline, and *C. ariakensis* swam down in preliminary behavioral experiments. The simple difference in behavior resulted in huge differences in dispersal and was more influential in the dispersal model than hydrodynamic flows.

The geographically disparate salinity rate-response behaviors observed in this study within a single species appear to be adaptive for transport under different estuarine conditions. There is some genetic structuring of blue crab populations that

could feasibly support such local adaptation. While this structuring is significant, it does not appear to follow any geographic trend (Kordos & Burton 1993, McMillen-Jackson et al. 1994). In a study comparing population genetics of megalopae and adult blue crabs along the Texas coast, Kordos & Burton (1993) found that megalopae tended to have genetic structuring in the summer months. The temporal variability in megalopae genetics may be an underlying mechanism supporting the wide variance we observed in our rate-response curves measured in cohorts separated by time. The authors suggest that gene flow between populations is lower than the forces of random drift or natural selection, resulting in the genetic structuring observed. If natural selection is one of the structuring forces in blue crab populations, selection based on salinity-driven recruitment behaviors may allow for some local adaptation.

Local adaptation in marine and estuarine species is not unprecedented. Colin & Dam (2002) reported geographic gradients in the copepod *Acartia tonsa*'s tolerance to toxins of the dinoflagellate *Alexandrium* spp. However, locally adaptive behaviors are rarely studied in the marine environment, and the drivers of these behaviors are generally unknown.

## Implications for climate cycles and freshwater management

This study has provided evidence that net transport of blue crab megalopae out of the estuary on the ebb tide during periods of drought could be relatively substantial due to the rates of salinity change the megalopae experience on outgoing tides when salinities in the estuary are high (Fig. 6). Within a given estuary, rates of salinity change can be related to salinity gradients over the long term (Fig. 4). These salinity gradients are driven by the balance of freshwater inputs with evaporation and mixing processes and are linked to drought cycles and human alterations of freshwater inflows.

When freshwater inflows are lowered to a point where the rates of salinity change no longer reach the minimum threshold necessary to elicit a vertical response from megalopae during selective tidal stream transport, recruitment of blue crab larvae into the estuary will be impaired, as will movement farther up estuary to suitable nursery sites. Lower blue crab recruitment success to favorable nursery grounds may lead to declines in the population within the estuary. The established importance of selective tidal stream transport in recruitment of blue crabs and the results of this study lend support to reports of a link between blue crab populations and freshwater inflows reported in the literature.

LITERATURE CITED

Aguilar R, Hines AH, Wolcott TG, Wolcott DL, Kramer MA, Lipcius RN (2005) The timing and route of movement and migration of post-copulatory female blue crabs, *Callinectes sapidus* Rathbun, from the upper Chesapeake Bay. J Exp Mar Biol Ecol 319:117−128

Carr SD, Tankersley RA, Hench JL, Forward RB Jr, Luettich RA Jr (2004) Movement patterns and trajectories of ovigerous blue crabs *Callinectes sapidus* during the spawning migration. Estuar Coast Shelf Sci 60:567−579

Chavez-Ramirez F (1996) Food availability, foraging ecology, and energetics of whooping cranes wintering in Texas. PhD dissertation, Texas A&M University, College Station, TX

Colin SP, Dam HG (2002) Latitudinal differentiation in the effects of the toxic dinoflagellate *Alexandrium* spp. on the feeding and reproduction of populations of the copepod *Acartia hudsonica.* Harmful Algae 1:113−125

Costlow JD, Bookhout CG (1959) The larval development of *Callinectes sapidus* Rathbun reared in the laboratory. Biol Bull 116:373−396

Cronin TW, Forward RB (1988) The visual pigments of crabs. I. Spectral characteristics. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 162:463−478

Daewel U, Schrum C, Temming A (2011) Towards a more complete understanding of the life cycle of brown shrimp (*Crangon crangon*): modelling passive larvae and juvenile transport in combination with physically forced vertical juvenile migration. Fish Oceanogr 20:479−496

Darnell MZ, Rittschof D, Darnell KM, McDowell RE (2009) Lifetime reproductive potential of female blue crabs *Callinectes sapidus* in North Carolina, USA. Mar Ecol Prog Ser 394:153−163

Epifanio CE (1995) Transport of blue crab (*Callinectes sapidus*) larvae in the waters off mid-Atlantic states. Bull Mar Sci 57:713−725

Epifanio CE (2007) Biology of larvae, Chap 12. In: Kennedy V, Cronin L (eds) The blue crab: *Callinectes sapidus*. Maryland Sea Grant, College Park, MD

Epifanio CE, Garvine RW (2001) Larval transport on the Atlantic continental shelf of North America: a review. Estuar Coast Shelf Sci 52:51−77

Forward RB Jr, Rittschof D (1994) Photoresponses of crab megalopae in offshore and estuarine waters: implications for transport. J Exp Mar Biol Ecol 182:183−192

Forward RB Jr, Tankersley RA, Blondel D, Rittschof D (1997) Metamorphosis of the blue crab *Callinectes sapidus*: effects of humic acids and ammonium. Mar Ecol Prog Ser 157:277−286

Forward RB Jr, Tankersley RA, Rittschof D (2001) Cues for

metamorphosis of brachyuran crabs: an overview. Am Zool 41:1108–1122

Forward RB Jr, Tankersley RA, Smith KA, Welch JM (2003a) Effects of chemical cues on orientation of blue crab, *Callinectes sapidus*, megalopae in flow: implications for location of nursery areas. Mar Biol 142:747–756

Forward RB Jr, Tankersley RA, Welch JM (2003b) Selective tidal-stream transport of the blue crab *Callinectes sapidus*: an overview. Bull Mar Sci 72:347–365

Goodrich DM, van Montfrans J, Orth RJ (1989) Blue crab megalopal influx to the Chesapeake Bay: evidence for a wind-driven mechanism. Estuar Coast Shelf Sci 29: 247–260

Guillory V (2000) Relationship of blue crab abundance to river discharge and salinity. Proc Ann Conf SEAFWA. 54: 213–220

Hamlin L (2005) The abundance and spatial distribution of blue crabs (*Callinectes sapidus*) in the Guadalupe Estuary related to low freshwater inflow conditions. PhD dissertation, Texas State University, San Marcos, TX

Hines A (2007) Ecology of juvenile and adult blue crabs, Chap 12. In: Kennedy V, Cronin L (eds) The blue crab: *Callinectes sapidus*. Maryland Sea Grant, College Park, MD

Hoeinghaus DJ, Davis SE (2007) Size-based trophic shifts of saltmarsh dwelling blue crabs elucidated by dual stable C and N isotope analyses. Mar Ecol Prog Ser 334:199–204

Kingsford MJ, Leis JM, Shanks A, Lindeman KC, Morgan SG, Pineda J (2002) Sensory environments, larval abilities and local self-recruitment. Bull Mar Sci 70:309–340

Kordos LM, Burton RS (1993) Genetic differentiation of Texas Gulf Coast populations of the blue crab *Callinectes sapidus.* Mar Biol 117:227–233

Lipcius R (2007) Ecology of postlarval and young juvenile blue crabs, Chap 12. In: Kennedy V, Cronin L (eds) The blue crab: *Callinectes sapidus*. Maryland Sea Grant, College Park, MD

Longley WL (1994) Freshwater inflows to Texas bays and estuaries: ecological relationships and methods for determination of needs. Texas Water Development Board, Austin, TX

McMillen-Jackson AL, Bert TM, Steele P (1994) Population genetics of the blue crab *Callinectes sapidus*: modest population structuring in a background of high gene flow. Mar Biol 118:53–65

Minello TJ, Able KW, Weinstein MP, Hays CG (2003) Salt marshes as nurseries for nekton: testing hypotheses on density, growth and survival through meta-analysis. Mar Ecol Prog Ser 246:39–59

Montagna P, Brenner J, Gibeaut J, Morehead S (2011) Coastal impacts, Chap 4. In: Schmandt J, Clarkson J, North GR (eds) The impact of global warming on Texas. Texas Climate Initiative, University of Texas Press, Austin, TX, p 96–123

More W (1969) A contribution to the biology of the blue crab in Texas, with a description of the fishery. Tech Ser Texas Parks Wild Dept 1:1–31

NERRS (National Estuarine Research Reserve System) (2013). System-wide monitoring program. US Dept. of Commerce, NOAA, NOS, NERRS, Central Data Management Office, Washington, DC. Available at: cdmo.baruch.sc.edu (accessed 20 Dec 2013)

NOAA (National Oceanic and Atmospheric Administration) (2006) Final programmatic environmental impact statement: federal approval of the Texas national estuarine

research reserve and management plan, the Mission-Aransas Estuary. US Dept Commerce, NOAA, NOS, Silver Spring, MD

North EW, Schlag Z, Hood RR, Li M, Zhong L, Gross T, Kennedy VS (2008) Vertical swimming behavior influences the dispersal of simulated oyster larvae in a coupled particle-tracking and hydrodynamic model of Chesapeake Bay. Mar Ecol Prog Ser 359:99–115

Ogburn MB, Stuck KC, Heard RW, Wang SW, Forward RB (2011) Seasonal variability in morphology of blue crab, *Callinectes sapidus*, megalopae and early juvenile stage crabs, and distinguishing characteristics among co-occurring Portunidae. J Crustac Biol 31:106–113

Ogburn MB, Hall M, Forward RB (2012) Blue crab (*Callinectes sapidus*) larval settlement in North Carolina: environmental forcing, recruit–stock relationships, and numerical modeling. Fish Oceanogr 21:20–32

Posey MH, Alphin TD, Harwell H, Allen B (2005) Importance of low salinity areas for juvenile blue crabs, *Callinectus sapidus* Rathburn, in river-dominated estuaries of southeastern United States. J Exp Mar Biol Ecol 319: 81–100

Powell G, Matsumoto J, Brouck D (2002) Methods for determining minimum freshwater inflow needs for Texas bays and estuaries. Estuaries 25:1262–1274

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for statistical computing, Vienna. Available at: www.R-project.org/

Sanchez-Rubio G, Perry HM, Biesiot PM, Johnson DR, Lipcius RN (2011) Climate-related hydrological regimes and their effects on abundance of juvenile blue crabs (*Callinectes sapidus*) in the north central Gulf of Mexico. Fish Bull 109:139–146

Scharf FS, Schlicht KK (2000) Feeding habits of red drum in Galveston Bay, Texas: seasonal diet variation and predator–prey size relationships. Estuaries Coasts 23:128–139

Tankersley R, Forward R (2007) Environmental physiology, Chap 10. In: Kennedy V, Cronin L (eds) The blue crab: *Callinectes sapidus*. Maryland Sea Grant, College Park, MD

Tankersley RA, McKelvey LM, Forward RB (1995) Responses of estuarine crab megalopae to pressure, salinity and light: implications for flood-tide transport. Mar Biol 122:391–400

Taylor AC, Naylor E (1977) Entrainment of the locomotor rhythm of *Carcinus* by cycles of salinity change. J Mar Biol Assoc UK 57:273–277

Ward GH (2011) Water resources and water supply, Chap 3. In: Schmandt J, Clarkson J, North GR (eds) The impact of global warming on Texas, 2nd edn, University of Texas Press, Austin, TX, p 69–95

Welch JM, Forward RB (2001) Flood tide transport of blue crab, *Callinectes sapidus*, postlarvae: behavioral responses to salinity and turbulence. Mar Biol 139:911–918

Welch JM, Rittschof D, Bullock TM, Forward RB (1997) Effects of chemical cues on settlement behavior of blue crab *Callinectes sapidus* postlarvae. Mar Ecol Prog Ser 154:143–153

Wilber DH (1993) The influence of Apalachicola River flows on blue crab, *Callinectes sapidus*, in north Florida. Fish Bull 92:180–188

Wolcott DL, DeVries MC (1994) Offshore megalopae of *Callinectes sapidus*: depth of collection, molt stage and response to estuarine cues. Mar Ecol Prog Ser 109:157–163