

Effect of turbulence on the behavior of pink shrimp postlarvae and implications for selective tidal stream transport behavior

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ABSTRACT: Laboratory experiments were conducted to determine whether turbulence could be an environmental cue associated with the behavior of pink shrimp *Farfantepenaeus duorarum* postlarvae during their flood-tide migration to the nursery grounds. Postlarvae were exposed to variations of turbulent kinetic energy (TKE) generated by the flow speed of a water pump and variations of combined flow speed and vertical motions of an actuator-driven cylinder array. The position and swimming activity of the postlarvae were recorded using a video camera. Images of postlarvae were analyzed at 3 depths of the water column (upper half, bottom half, and floor of the experimental chamber). During trials varying flow speed alone, both TKE levels ($<0.009 \text{ cm}^2 \text{ s}^{-2}$) and the number of postlarvae in the upper half of the water column were low, suggesting that the TKE levels were below their stimulation threshold. In contrast, swimming activity increased substantially during trials incorporating additional TKE generated by the actuator. TKE levels varied between 1.1 and $3.5 \text{ cm}^2 \text{ s}^{-2}$, and postlarvae vertical position reflected increases and decreases in TKE. A statistically significant ($p < 0.05$) higher percentage of postlarvae in the upper half of the water column coincided with high TKE values. Increases in postlarval response relative to increases in TKE diminished after reaching TKE values of $2.5 \text{ cm}^2 \text{ s}^{-2}$, suggesting a dose-limited behavioral response. This trend was supported by a significant horizontally asymptotic regression of change in postlarval response against percent change in TKE relative to the previous time step. The results suggest turbulence involvement during flood-tidal migration behavior of this species. During most of the flood tide, TKE levels would be sufficiently high to stimulate postlarvae to swim in the water column and remain there while moving in the direction of settlement habitats. At the end of the flood tide, as the TKE decreases, postlarvae would descend to near the bottom.

KEY WORDS: Turbulence · Turbulent kinetic energy · TKE · Selective tidal transport · Pink shrimp · Postlarval behavior · Environmental cue · *Farfantepenaeus duorarum*

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INTRODUCTION

Many coastal and estuarine species of fishes and invertebrates perform vertical migrations synchronized with the phases of the tide to complete their ontogenetic migrations between oceanic and estuarine habitats. The coupling of these tidally timed vertical migrations with changes in horizontal water-

flow regime is a behavioral mechanism known as selective tidal stream transport (STST) (see Forward & Tankersley 2001 for review). Organisms that engage in STST require some kind of behavioral response and an associated triggering stimulus to synchronize vertical migrations with the proper tidal flow regime to achieve horizontal movement or, conversely, to be retained in specific areas. A flood-tide

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transport (FTT) occurs when organisms use the flood phase for shoreward transport and migration into estuaries (e.g. Tankersley et al. 1995, Morgan et al. 1996, Queiroga & Blanton 2004). An ebb-tide transport or ebb-phased migration is used for seaward transport and out-migration from estuaries (e.g. López-Duarte & Tankersley 2007, Lopez-Duarte et al. 2011, Ogburn et al. 2013). Among the organisms for which FTT has been reported are blue crab *Callinectes sapidus* megalopae (for review, see Forward et al. 2003), estuarine fish larvae (Rowe & Epifanio 1994), and penaeid shrimp postlarvae, including the pink shrimp *Farfantepenaeus duorarum* (Dall et al. 1990, Criales et al. 2011). The combinations of behaviors that comprise STST have been described in detail for only a few species, e.g. the blue crab (Forward et al. 2003).

The pink shrimp is one of the most economically and ecologically important species in south Florida and in Campeche Sound, southern Gulf of Mexico (Browder et al. 2002, Ramírez-Rodríguez et al. 2003). As with other penaeids, larvae develop offshore where they pass through a series of planktonic stages, requiring ~30 d to settle as benthic postlarvae on inshore nursery grounds (Ewald 1965, Munro et al. 1968). Larval migration from spawning to nursery grounds in Florida Bay is affected by tidal currents and winds and is facilitated by a series of specialized larval behaviors. Early protozoae exhibit a diel vertical migration (DVM) behavior (Jones et al. 1970, Criales et al. 2007, 2010); however, onshore transport resultant from this DVM behavior has not been demonstrated. During later ontogenetic stages, near the estuarine waters of Florida Bay, postlarvae perform rhythmic vertical migrations synchronized with the semidiurnal tide (Criales et al. 2007). Postlarvae enter Florida Bay almost exclusively during the nocturnal flood tide (Tabb et al. 1962, Roessler & Rehrer 1971, Criales et al. 2006, 2011), advancing between 3 and 5 km per night in a type of cumulative, or saltatory, process until they reach the appropriate seagrasses for settlement (Criales et al. 2010). Tides in Florida Bay are variable because of the interaction of Gulf of Mexico mixed semidiurnal tides and the primarily semidiurnal tides of the North Atlantic Ocean (Wang 1998, Smith 2000). The greatest tidal exchange occurs across the Bay's northwestern boundary with the Gulf of Mexico, where tidal amplitudes can reach 37 cm (Smith 1997). Pink shrimp postlarval access to the interior of Florida Bay is largely limited by tidal flow (Criales et al. 2010).

The upward and downward migration of organisms in the water column during the STST may be

cued by environmental factors that change predictably with the tide, such as hydrostatic pressure, temperature, salinity, and turbulence (Shanks 1995, Tankersley et al. 1995, Forward & Tankersley 2001). The STST behavior may also be controlled by an endogenous circatidal rhythm (López-Duarte & Tankersley 2007). Environmental variables may trigger the behavioral response and serve as phase synchronizers to amplify the endogenous tidal rhythm (Naylor 1988, Tankersley & Forward 1994). Different environmental cues may trigger differing behaviors at different phases of STST. For example, blue crab megalopae, despite exhibiting a circadian rhythm, react to changes in salinity and turbulence to achieve migration to nursery habitat. For this species, an increase in salinity triggers upward swimming near the beginning of the flood tide (Tankersley et al. 1995). Once a megalopae is positioned within a horizontally moving parcel of water, a different environmental cue is required to maintain vertical swimming since salinity is no longer changing relative to the megalopae's position. Turbulence within the water parcel has been demonstrated to stimulate maintenance of the vertical position and thus horizontal movement with the associated flood tide flow toward an estuary's interior (Welch et al. 1999). Declining turbulence associated with diminishing flood-tide flow cues descent toward the benthos during slackening flood tide and before the reverse-ebb flow increases, which would flush megalopae back toward the open ocean (Welch & Forward 2001). Thus, turbulence was identified as an important environmental factor influencing the swimming behavior of blue crab megalopae in order to achieve STST (Welch et al. 1999, Welch & Forward 2001). Turbulence is a predictable cue in STST because it consistently co-varies with current velocity, rising as the tidal current flows faster and decreasing to low levels at the slack tide stage (MacKenzie & Leggett 1991, Welch 1998).

Postlarval stages of some penaeid species seem to exhibit endogenous circatidal rhythm activity. Hughes (1969a) observed a circatidal activity rhythm in pink shrimp postlarvae, oriented with the current during the time of ebb tide and against the current during the time of flood tide. Interestingly, these orientations are opposite the current direction that would result in movement up-estuary during flood tide. However, the cues associated with the tidal behavior have not been well investigated. Hughes (1969b) proposed salinity as the environmental cue associated with the STST behavior that triggers the tidal rhythm. Under controlled laboratory conditions,

Hughes (1969b) and later Matthews et al. (1991) demonstrated that pink shrimp and brown shrimp *Farfantepenaeus aztecus* postlarvae were active in the water column when salinity increased and returned to the substrate when salinity decreased. Similarly, changes in water pressure have been tested as an environmental cue associated with STST behavior for both *Penaeus semisulcatus* and *P. esculentus* (Vance & Pendrey 1997). Postlarvae were active in the water column during periods of high water pressure and reduced their swimming activity as the water pressure decreased. Pink shrimp juveniles also reacted to changes in water levels from 10 to 40 cm (Wickham 1967). Despite the known importance of turbulence influencing STST behavior, this environmental cue has not yet been investigated for penaeid shrimp postlarvae.

In the present study, we investigated whether turbulence could act as an environmental cue associated with the behavior of pink shrimp postlarvae. Our hypothesis was that pink shrimp postlarvae react to increases in turbulence by ascending in the water column and conversely by descending near the bottom in response to decreases in turbulence. Determining the environmental cues that elicit behavioral changes in pink shrimp postlarvae during STST would improve our knowledge of ontogenetic migrations of this important species and could potentially be used to im-

prove biophysical modeling efforts. This information might help explain the recruitment variability of pink shrimp populations in south Florida in relation to water-management changes and climatic variations.

MATERIALS AND METHODS

Experimental flow tank

Turbulence experiments were conducted in the Air-Sea Interaction Saltwater Tank (ASIST), a tank (working section: 15 m \times 1 m \times 1 m) filled with natural seawater, located within a temperature- and light-controlled indoor setting at the University of Miami's Rosenstiel School of Marine and Atmospheric Sciences (RSMAS). Water movement within the ASIST was generated by recirculation of system seawater by a computer-controlled 60 HP pump located below the working section of the tank (Fig. 1). This pump system generates flow velocities throughout the vertical water column within the working section of the ASIST tank. The working section of the ASIST tank is constructed of flanged acrylic subsections; the transparency of this material allowed for transmission of light used to illuminate shrimp test subjects.

Turbulence was increased within the system by vertical motions of a horizontal rake of cylinders. The

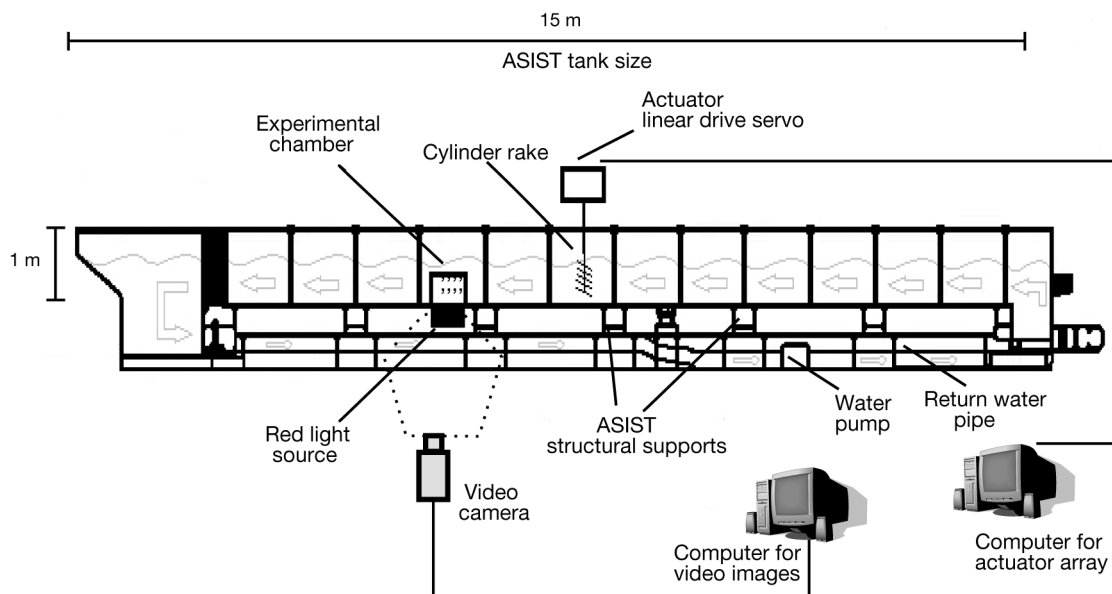


Fig. 1. Schematic diagram of the Air-Sea Interaction Saltwater Tank (ASIST), a stainless steel and acrylic tank (15 m \times 1 m \times 1 m) utilized to observe *Farfantepenaeus duorarum* postlarval behavioral response to differing turbulence levels. Water movement within the ASIST was generated by recirculation of seawater by a computer-controlled pump located below the working section. Turbulence was generated by vertical motions of a horizontal rake of cylinders mounted to a linear-drive servo actuator. Swimming activity of postlarvae within the experimental chamber (45 cm \times 41 cm \times 33 cm) was illuminated with a red light, which was positioned immediately below the chamber and recorded with a video camera. Components included in the diagram are not drawn to the same scale as the ASIST tank

rake consisted of 10 horizontally oriented 1.9 cm diameter PVC pipes spaced every 5 cm along a 5.1 cm diameter vertical support PVC pipe section. This design allowed for distribution of cylinders over the entire vertical water column. Thus, turbulence could be distributed more evenly over the water column by vertical motions of the cylinder rake. The vertical support pipe of the rake was mounted to a linear-drive servo actuator (Savelyev et al. 2011) suspended above the ASIST 1.5 m upstream of the experimental chamber. The movement parameters of the actuator, such as speed and range of motion, were controlled independently by Galil Motion Control computer software. Experimental sea water within the ASIST was pumped from surface waters in Bear Cut, Biscayne Bay, which is adjacent to RSMAS. Seawater was passed through a glass media filter to remove large particulate matter and then pumped through a cartridge filter (10 μm particle size filtration). ASIST filling occurred at least 1 d prior to experimental use to allow the water to equilibrate to room temperature ($\sim 27.0^\circ\text{C}$); temperature was controlled via air-conditioning control of the ASIST room. Seawater salinity in the tank varied between experimental trials from 32 to 35. Salinity variations between trials were caused by natural summer variation in Biscayne Bay. Salinity in the tank during each individual experimental trial was constant. Within the ASIST, seawater was never filled higher than ~ 5.1 cm from the upper surface of the experimental chamber in order to prevent escape of test subjects and eliminate creation of turbulence from water flow interaction with the uppermost sections of the experimental chamber walls.

Experimental chamber and postlarvae

Pink shrimp postlarvae were placed in an experimental chamber (45 cm \times 41 cm \times 33 cm) situated within the working section of the ASIST tank and secured against acrylic front wall (Fig. 1). The frame, back wall, and floor of the chamber were made of acrylic, while the lateral walls were constructed of 850 μm mesh screen attached to the acrylic frame. The mesh walls allowed continuous circulation of water through the experimental chamber while containing the test subject postlarval shrimps within the experimental section. The transparency of the experimental acrylic chamber allowed for transmission of light used to illuminate postlarvae. Experimental postlarvae were illuminated by a light source apparatus positioned within the ASIST structural supports

directly below the experimental chamber, creating a dark field image of the postlarvae within the chamber (Fig. 2A).

Each experimental trial utilized a group of 100 postlarvae with no previous exposure to experimental conditions. Postlarvae were collected during nocturnal flood tides as they migrated into Florida Bay (Criales et al. 2010). The total length of postlarvae ranged from 8.5 to 11.5 mm, and they weighed from 0.008 to 0.01 g. Before experimental use, postlarvae were maintained for at least 24 h in acclimation tanks (38 l) containing sea water from the same source as previously described. Water in the acclimation tanks was changed daily, and postlarvae were fed twice a day ad libitum on a mixture of fresh *Artemia* nauplii and commercial dry pellet food (PL RacewayPlus, Zeigler, www.zeiglerfeed.com).

Flow speed calibration and turbulence quantification

Water flow within the ASIST was controlled by the recirculating seawater system via a pump located in line with the return pipe below the working section of the ASIST. The pump speed was measured in Hz and controlled by an external electronic variable frequency drive (Danfoss VLT-100). Calibration of the flow speed within the experimental chamber to the pump speed was conducted with a Particle Image Velocimeter (PIV) (Dantec Dynamics, Flowmap) which measured current velocities in a 2-dimensional (2-D) plane oriented such that the y -axis was parallel to the current flow, and the z -axis was the vertical direction (Babanin & Haus 2009). A linear relationship between pump operating frequency (measured in Hz) and current speed (measured in cm s^{-1}) within the experimental chamber was established for pump frequencies greater than 1 Hz. The following final equation was derived from these measurements:

$$V = 0.38x - 0.334 \quad (1)$$

where V is the flow speed (cm s^{-1}) within the experimental chamber, and x is the pump speed (Hz). Thus, a 2 Hz pump speed corresponds to 0.43 cm s^{-1} water flow speed, and similarly, 10 Hz corresponds to 3.47 cm s^{-1} . By means of this relationship, we were able to directly equate water flow speed to pump speed settings programmed into the pump controller unit.

The system turbulence, whether produced by water flow alone or by the actuator, was measured by

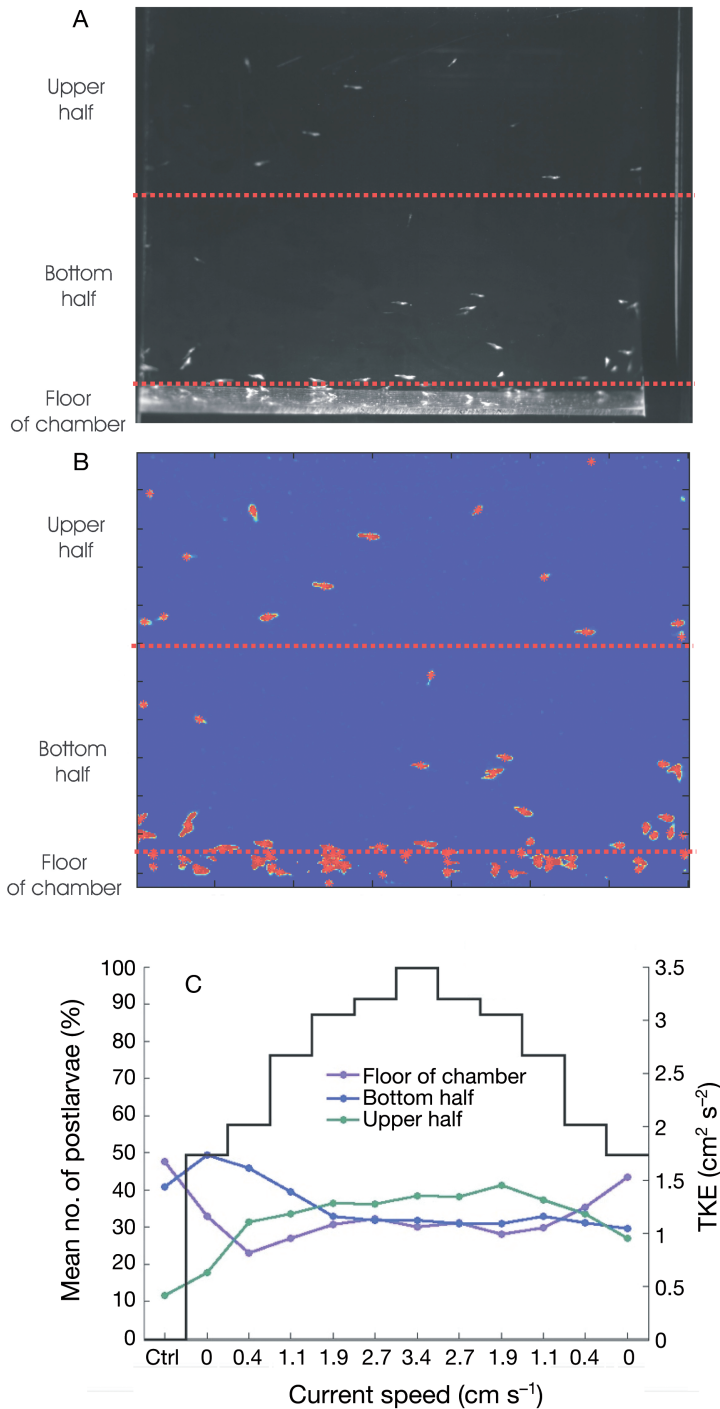


Fig. 2. *Farfantepenaeus duorarum*. Pictures of postlarvae swimming in the experimental chamber during the TC-2 trial depicting the 3 depths of the water column analyzed (upper half, bottom half, and floor of chamber): (A) original picture taken by the monochrome video camera; (B) picture analyzed for postlarvae counted as bright spots after image was converted in a 3-D matrix of lateral position, vertical position, and brightness and applying a 2-D median filter. The viewing size of the chamber was 34.5 cm \times 11.4 cm. (C) Mean percentage of postlarvae within the 3 analyzed depths analyzed from trial TC-2. The right y-axis indicates turbulence (measured as turbulent kinetic energy [TKE], cm² s⁻²), and the x-axis indicates the increases of current speed (cm s⁻¹). For more details, see 'Video and imaging analysis'

a hot film anemometer (TSI IFA 300). Thermal anemometers measure fluid velocity by sensing the changes in heat transfer from a small, electrically heated element exposed to the fluid. The electrical current required to keep the wire at a constant temperature is sampled at very short sampling intervals. In these experimental trials with postlarvae, a single-axis wire probe (1276-10AW) was oriented horizontally facing into the mean flow direction. The hot-film probe was mounted in such a manner so that measurements were taken from the center of the 3-D space enclosed by the experimental chamber. Measurements were taken separately from experimental trials under the same conditions utilized during the experimental trials; thus, the anemometer was not present to disturb shrimp swimming behavior.

Turbulence was quantified as turbulent kinetic energy (TKE, in cm² s⁻²) (Tennekes & Lumley 1997), defined as follows:

$$\text{TKE} = \frac{1}{2} [\overline{u'^2} + \overline{v'^2} + \overline{w'^2}] \quad (2)$$

where u'^2 , v'^2 , and w'^2 represent the variance of the flow velocities in the x , y , and z axes respectively, and the overbars denote the spatial averaging.

Assuming isotropy (i.e. $\overline{u'^2} = \overline{v'^2} = \overline{w'^2}$) and invoking the frozen turbulence assumption (time averaging = spatial averaging), the TKE was defined by the time average of the single-axis fluctuating velocity (u'^r) observed by the anemometer, as follows:

$$\text{TKE} = \frac{3}{2} [\overline{u'^2}] \quad (3)$$

Trials, acclimation, and control periods

Two sets of experiments were conducted, both including 5 trials, to investigate the behavior of pink shrimp postlarvae to different turbulence levels. The first set of trials investigated the effect of turbulence relative to the flow speed generated by the water pump (FS1-5). The second set of trials added higher turbulence levels produced by the vertical motion of the actuator (TC trials) grouped as TC1-2 (oscillation speed 2 cm s⁻¹) and TC3-5 (oscillation speed 2 cm s⁻¹). Preliminary trials demonstrated that postlarvae were not detectable by the video camera at current speeds higher than 3.47 cm s⁻¹. These observations may indicate that at higher flows, postlarvae may have been actively swimming with or unable to swim against the current flow, and they could not be cap-

tured by the camera. Therefore, the maximum flow speed used was 3.47 cm s^{-1} for all later trials.

To eliminate any possible effect of the circatidal rhythm activity of postlarvae during the experiments, all trials were conducted during ebb tide conditions in relation to the place of collection. For all trials, postlarvae were transferred to the experimental chamber ~30 min before the trial was initiated to allow them to adapt to the new experimental environment; during this period, overhead fluorescent lighting was maintained within the experiment room. Each trial was initiated with an acclimation period of 10 min followed by a 10 min control period. The acclimation period consisted of experimental conditions, including darkness illuminated by red light, but with no water flow and with no noise produced by the cooling fan used to cool the water pump. The control period consisted of activation of the tank pump at a minimal frequency of 0.01 Hz, which produced negligible water flow (0.0001 cm s^{-1}) but did produce the noise associated with the pump's cooling fan. The control periods allowed comparison of postlarval shrimp behavior to that recorded during the experimental periods that followed.

During all trials, each experimental period (control, increasing flow speed, and decreasing flow speed) was recorded as a separate block of images. A constant number of 180 images (the maximum capture number allowed by the camera acquisition software) was captured per experimental period. During the control period, images of postlarvae were taken for 10 min at an acquisition rate of 1 image every 3 s.

Trials without actuator varying flow speed only (FS1–5)

Five replicate trials were conducted during the first experiment. During these trials, the horizontal cylinder rake was not installed in position within the ASIST. The first trial was utilized to test the system setup and was not considered for further analysis ($n = 4$). During experimental periods, the flow speed was increased from 0 to 3.47 cm s^{-1} over 15 min for the first period and then decreased from 3.47 to 0 cm s^{-1} for another 15 min during a second period. The changes in flow speed were conducted at a constant rate of 0.0001 cm s^{-2} . For all experimental periods, initiation of image acquisition was timed with the initiation of that period (e.g. beginning of the control period or turning on of the water pump). For the experimental periods with either increasing or decreasing flow speed, image acquisition took place

every 4 s, which was the slowest camera setting allowed by the picture acquisition software. This resulted in a total picture capture period of 12 min for each of the increasing and decreasing periods with no pictures taken during the last 3 min of each period.

Trials adding actuator turbulence to flow speed (TC1–2 and TC3–5)

The second set of trials investigated the effect of higher turbulence levels on postlarval behavior by incorporating the vertical motion of the actuator, which acted independently of the water flow and turbulence related to the pump speed. A total of 5 trials were conducted. Within each trial, the vertical motion of the rake produced by the actuator was held constant, and flow speed was varied among treatments to produce different turbulence levels for each treatment. Actuator-based vertical speed was held constant under the assumption that differing flow speeds would produce differing levels of turbulence as water flowed past each cylinder within the rake, in accordance with principles of fluid dynamics over cylindrical bodies (Sobey 1982). Vertical back-and-forth movements of the rake were utilized to force turbulence at frequencies determined by the mean flow velocity, diameter of each cylinder (D) and the forced oscillation frequency (f) as given by the Strouhal number ($Sc = fD/V$). The forced oscillations served to distribute turbulence over the entire water column of the test chamber. This avoided the creation of alternating regions of high or low turbulence in the near-field before the von Kármán vortex street established by each individual cylinder (von Kármán 1963) merged downstream of the horizontal cylinders.

During the first 2 trials (TC1–2), the vertical motion of the actuator was set up with an oscillation speed of 2 cm s^{-1} ($n = 2$); the remaining 3 trials (TC3–5) utilized an oscillation speed of 4 cm s^{-1} ($n = 3$). Each individual trial was initiated with the 10 min control period. Actuator vertical motion was continuous throughout an entire trial once initiated during a 10 min at 0 cm s^{-1} pump speed step, marking the first experimental period. After the 0 cm s^{-1} speed step, pump speed was gradually increased ($n = 6$) in steps of 0.57 cm s^{-1} from 0 to 3.47 cm s^{-1} and then gradually decreased back to 0 ($n = 6$) in similar 0.57 cm s^{-1} steps. For each pump speed increment, the new speed was maintained for 10 min, marking periods at each speed. To complete the circuit of pump speeds, each trial lasted for ~2 h. Image acquisition was initiated at the beginning of each 0.57 cm s^{-1} pump speed

change and continued throughout each treatment period. Image acquisition was set at a rate of 1 image every 3 s.

Video and imaging analysis

The response of postlarvae to the turbulence stimulus was monitored and recorded using a closed-circuit video system. Filming was conducted in darkness illuminated with a red light with a transmission wavelength between 650 and 800 nm since this range falls outside the vision range of most crustaceans (Cronin & Forward 1988). A monochrome video camera (Dantec Dynamics HiSense), which was operated by a computer (equipped with Dantec Dynamics Particle Imaging software), recorded the position and swimming activity of postlarvae in the experimental chamber. The camera was positioned horizontal to the experimental chamber and within the same vertical plane as the chamber though separated from the wall of the ASIST tank by ~2 m (Fig. 1). The camera zoom and focus were adjusted in such a manner that the field of view of the images produced included only the view of the front face of the experimental chamber (Fig. 2A). A series of MATLAB routines was developed and scripted to determine the position of postlarvae within the 3 water depths analyzed (upper half of the water column, bottom half of the water column, and floor of the experimental chamber). Division of the water column into horizontal depths is commonly used in similar studies investigating this type of crustacean behavior (Tankersley et al. 1995, Welch et al. 1999).

Images were recorded as TIFF files, which were later uploaded into MATLAB and converted to 3-D matrices of lateral position, vertical position, and brightness. The mean background brightness of each group of images was subtracted from each individual image to eliminate false counts from factors, such as non-moving bright spots (e.g. moribund shrimp), brightness due to reflections off chamber walls, and turbidity of the water. After considerable trial and error, a threshold brightness was determined that optimally identified postlarvae and eliminated spurious targets. This pre-determined threshold was used for all runs because lighting conditions were normalized in the initial step. The MATLAB scripts identified and counted the positions of bright regions (contiguous regions with brightness above threshold) within each acquired image to obtain the number of postlarvae and their locations within the experimental chamber (Fig. 2). To correct for double counting of

shrimps, because head and tail regions sometimes appeared as 2 separate bright spots, a 2-D median filter was applied to 'smear' the data, allowing a more uniform brightness of each shrimp and resulting in only 1 positive count per shrimp. Finally, the centroid size of each shrimp was calculated, and centroid size was used to attain maximum and minimum bright particle size so that all other bright objects larger or smaller than these settings could be eliminated from counts. This step corrected for reflections of light off the air/water interface within the experimental chamber due to small surface waves varying the position of the reflected light and small bright particles that had settled on the experimental chamber floor; these features had not been removed during the background filtering step.

A primary MATLAB routine sorted the position of postlarvae within the 3 analyzed depths (upper and bottom halves of the water column, floor of the experimental chamber) and calculated the number of postlarvae in each depth (Fig. 2C). A secondary routine divided the collection of images into frame bins to allow comparison between FS1–5 and TC1–5 trials since differing image acquisition rates and rates of flow speed were utilized. For the flow speed trials (FS1–5), images were divided into 45-frame bins, while for the flow speed plus actuator trials (TC1–5), images were divided into 180-frame sets. This allowed for standardized analysis in discrete bins. The final data output from the MATLAB routines produced a total number of postlarvae in each of the 3 depths analyzed.

Postlarval behavioral response analysis

For all trials, the number of postlarvae in the 3 depths analyzed (upper half of the water column, bottom half of the water column, and floor of experimental chamber) was calculated to determine whether postlarvae were descending or ascending as a response to the TKE changes (Fig. 2). Similarly, this data investigation step allowed for observation of similar postlarval counts that were relatively constant between bins (i.e. time periods) within a trial. Statistical analysis was conducted on the percentage of the total number of postlarvae counted in the upper half of the water column within each bin (time period). Similar to related studies (Tankersley et al. 1995, Welch et al. 1999), the 'upper half' was considered as the criterion for a behavioral response of the postlarvae. All statistical analyses were conducted utilizing Statistica Ver. 7 (StatSoft) and Stata Release 10 (Stat-

aCorp). The percent data were arcsine transformed for parametric statistical analyses because percent data are binomially, not normally, distributed (Zar 2010). Significance levels for all statistical testing utilized an α level of $p < 0.05$. Data were tested for normality (Shapiro-Wilks) and homogeneity of variance (Brown-Forsythe) (Zar 2010). Two-way ANOVA with interactions were carried out separately for each of the 3 main trials (FS1–5, TC1–2, and TC3–5) to determine whether the percentage of postlarvae in the upper half of the water column was significantly affected by the different levels of turbulence observed within differing experimental trials.

To compare our results with those of previously published studies (Welch et al. 1999), a series of regression analyses was conducted. All data from TC1–5 were pooled since our previous analyses demonstrate a response of postlarvae to the stimuli presented. Initially, correlations were analyzed to investigate whether current speed or TKE would likely predict level of swimming response. Differing methods of representing both postlarval response and TKE data were utilized to explore several possible relationships between postlarval response and TKE. These comparisons were as follows:

(1) Absolute (observed) postlarval response (%) (R_i = response at time i) was analyzed in terms of absolute (observed) levels of TKE ($\text{cm}^2 \text{s}^{-2}$) (TKE_i = TKE at time i):

$$\%R_i \text{ vs. } \text{TKE}_i$$

(2) The distribution of absolute postlarval response (%) was investigated in relation to absolute changes in TKE ($\Delta\text{TKE} \text{ cm}^2 \text{ s}^{-2}$), i.e. the quantity of the difference between the present and the previous steps' TKE value, to determine whether comparison of postlarval response in terms of this metric improved observation of trends in response:

$$\%R_i \text{ vs. } (\text{TKE}_i - \text{TKE}_{i-1})$$

(3) Change in postlarval response ($\Delta\%$ response), i.e. change in percent of postlarvae responding in the present step ($\%R_i$) relative to the previous time step ($\%R_{i-1}$), was also considered in terms of change in TKE ($\Delta\text{TKE} \text{ cm}^2 \text{ s}^{-2}$):

$$(\%R_i - \%R_{i-1}) \text{ vs. } (\text{TKE}_i - \text{TKE}_{i-1})$$

(4) Postlarval response ($\%R_i$) was analyzed against relative change in TKE ($\% \Delta\text{TKE}$), i.e. the quantity of the difference between the present and the previous steps' TKE value as a percentage of the previous step's value:

$$\%R_i \text{ vs. } 100[(\text{TKE}_i - \text{TKE}_{i-1})/\text{TKE}_{i-1}]$$

(5) Change in postlarval response ($\Delta\%$ response) was analyzed against relative change in TKE ($\% \Delta\text{TKE}$):

$$(\%R_i - \%R_{i-1}) \text{ vs. } 100[(\text{TKE}_i - \text{TKE}_{i-1})/\text{TKE}_{i-1}]$$

Examination of plots of the above comparisons suggested that only linear and asymptotic regressions would logically fit the data distributions. Both of these regression forms were analyzed for each plot. Linear regression equations were developed as follows:

$$Y_i = a + bX_i \quad (4)$$

where a and b are constants representing intercept and slope, respectively, Y is the postlarval response, and X is the TKE value.

Asymptotic regression equations were developed as follows:

$$Y = b_1(1 - b_2^x) \quad (5)$$

where Y is the postlarval response, b_1 is coefficient 1, b_2 is coefficient 2, and x is the TKE value. Linear regressions were fit by least squares; asymptotic regressions were fit using nonlinear least squares. Initially, the parameters (b_1 and b_2) of the asymptotic response function were estimated by the maximum likelihood (least squares) method (Kutner et al. 2005). Resultant model residuals were analyzed for assumptions of parametric regression analysis, i.e. normality and homoscedasticity.

RESULTS

During trials varying flow speed only (FS1–5), the observed TKE levels varied between 6.9×10^{-3} and $9.1 \times 10^{-3} \text{ cm}^2 \text{ s}^{-2}$, and the rate of TKE change varied between 4.2×10^{-4} and $9.3 \times 10^{-4} \text{ cm}^2 \text{ s}^{-3}$. Postlarval response during these trials was relatively low, with a mean number of $14.9 \pm 1.3\%$ (mean \pm SD) swimming in the upper water column (Fig. 3). The response numbers did not substantially increase or decrease throughout the trial when subjected to the differing turbulence levels associated with changes in flow speed within the system (Fig. 3). This observation was confirmed by the lack of significant difference in the percentage of postlarvae swimming in the upper half of the water column during the different TKE levels (ANOVA $F_{3,5} = 1.73$, $p = 0.234$) (Table 1A).

Much higher TKE levels were observed during trials incorporating the use of the actuator. When the actuator oscillated the cylinder array at 2 cm s^{-1} (TC1–2), corresponding TKE levels were 1.7 to $3.5 \text{ cm}^2 \text{ s}^{-2}$, and the rates of change were 0.1 to $0.6 \text{ cm}^2 \text{ s}^{-3}$ (Figs. 3 & 4). Substantially higher levels of postlarval response were observed in conjunction

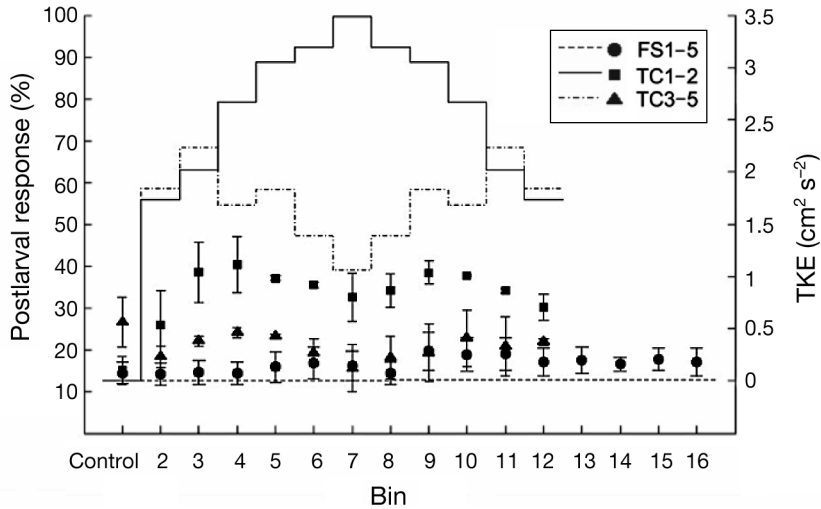


Fig. 3. *Farfantepenaeus duorarum*. (Symbols) Mean percentage (± 1 SD) of postlarvae in the upper water column in response to (lines) absolute levels of turbulence (measured as turbulent kinetic energy, TKE, $\text{cm}^2 \text{s}^{-2}$) during trials varying flow speed alone (FS1–5) or in conjunction with vertical motions of an actuator at an oscillation speed of 20 mm s^{-1} (TC1–2) or 40 mm s^{-1} (TC3–5). The x-axis indicates the controls and the different bins (i.e. time periods) at each trial

with the heightened TKE levels than in the trials of the effect of flow speed alone. Postlarval response averaged $34.9 \pm 4.1\%$ throughout the TC1–2 trials (Fig. 3). Furthermore, the percentage of postlarvae at the upper half of the water column increased as TKE increased, and as TKE decreased, the postlarvae descended lower within the water column or to the floor of the chamber (Fig. 2). These behavioral response observations were confirmed by determination of the TKE level as a significant factor affecting postlarval response (ANOVA, $F_{2,5} = 3.51$, $p = 0.04$) (Table 1B). The ascents in responses to rising TKE were not due to random movements since there was no net ascent in controls. These results suggested that postlarvae were stimulated to swim in the upper water column when TKE increased and descend when TKE decreased.

Increased TKE levels also were observed in trials TC3–5, which utilized an actuator speed of 4 cm s^{-1} . The associated TKE values varied between 1.1 and $2.2 \text{ cm}^2 \text{ s}^{-2}$ (Fig. 3), and the rates of change varied between 0.1 and $0.5 \text{ cm}^2 \text{ s}^{-3}$. Although the measured TKE values were greater than in the flow-only experiment, they were not greater than those observed in the TC1–2 trials. Comparison of TKE levels of TC1–2 and TC3–5 indicated that TKE did not increase proportionally with the increase in oscillation speed. The observed response of postlarvae to TKE in TC3–5 trials was lower ($20.8 \pm 2.2\%$) than in the TC1–2 trials

(Fig. 3), perhaps because of the lower levels of TKE. Postlarvae appear to respond to TKE changes by swimming up and down in the water column with the respective increases and decreases of TKE (Fig. 3). However, ANOVA demonstrated no significant differences in postlarval response and TKE level ($F_{2,5} = 3.51$, $p = 0.22$) in the TC3–5 trials (Table 1C).

The positive responses to TKE changes observed in all TC trials (TC1–5) reflected true behavioral response as postlarvae were observed to be competent swimmers in both the vertical (up and down within the chamber) and horizontal (with and against current flow) planes at the current speeds utilized in these trials. Furthermore, based upon observation of individual images collected during each trial, non-moving (e.g. moribund) postlarvae

were not passively advected into the water column. Thus, all observed vertical positions (upper or lower water column regions) were assumed to have

Table 1. *Farfantepenaeus duorarum*. Results of 2-way ANOVAs testing the effect of turbulence (measured as turbulent kinetic energy [TKE], $\text{cm}^2 \text{ s}^{-2}$) and test group (replicate) on the vertical position of postlarvae (percentage in the upper half of the water column, arcsine transformed) from experiments conducted under 3 sets of conditions: (A) with increases and decreases of flow speed alone (FS1–5) or flow speed increases and decreases in conjunction with vertical motions of an actuator at a speed of oscillation of (B) 20 mm s^{-1} (TC1–2) or (C) 40 mm s^{-1} (TC3–5). * $p \leq 0.05$

| Source | Partial SS | df | MS | F | p |
|-------------------------|------------|----|--------|--------|----------|
| (A) Trials FS1–5 | | | | | |
| TKE | 10.67 | 5 | 2.13 | 1.73 | 0.2340 |
| Group | 468.54 | 3 | 156.18 | 126.53 | <0.0001* |
| TKE \times Group | 49.09 | 15 | 3.27 | 2.65 | 0.0834 |
| Error | 9.87 | 8 | 1.23 | | |
| (B) Trials TC1–2 | | | | | |
| TKE | 120.76 | 5 | 24.15 | 3.507 | 0.0432* |
| Group | 3.32 | 1 | 3.32 | 0.482 | 0.5034 |
| TKE \times Group | 113.43 | 5 | 22.69 | 3.294 | 0.0513 |
| Error | 68.86 | 10 | 6.89 | | |
| (C) Trials TC3–5 | | | | | |
| TKE | 102.62 | 5 | 20.52 | 1.655 | 0.2256 |
| Group | 179.89 | 2 | 89.95 | 7.255 | 0.0098* |
| TKE \times Group | 144.26 | 10 | 14.43 | 1.164 | 0.4015 |
| Error | 136.38 | 11 | 12.40 | | |

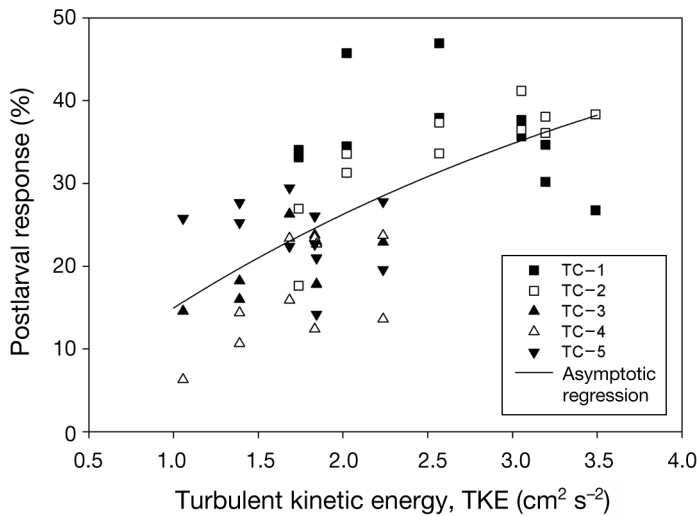


Fig. 4. *Farfantepenaeus duorarum*. Response of postlarvae (%) to absolute levels of turbulence (measured as turbulent kinetic energy, TKE, $\text{cm}^2 \text{s}^{-2}$) generated by the motions of an actuator at 2 different speeds of oscillations: 20 mm s^{-1} (TC1–2, squares), and 40 mm s^{-1} (TC3–5, triangles). The line represents the asymptotic regression of the postlarval responses (%) to absolute TKE values (for equation and parameters see ‘Postlarval behavioral response analysis’ and Table 2)

Table 2. *Farfantepenaeus duorarum*. Results of asymptotic logistic regression models developed for 3 differing metrics comparing postlarval response to turbulence (quantified as turbulent kinetic energy [TKE], $\text{cm}^2 \text{s}^{-2}$); Model 1: postlarval response (%) vs. absolute TKE ($\text{cm}^2 \text{s}^{-2}$), Model 2: change in postlarval response ($\Delta\%$) relative to previous time step vs. absolute change in TKE ($\Delta\text{cm}^2 \text{s}^{-2}$) relative to previous time step; and Model 3: change in postlarval response ($\Delta\%$) relative to previous time step vs. relative change in TKE ($\Delta\%$) relative to previous time step. Logistic regression equations were developed as $Y = b_1(1 - b_2^x)$; see explanation of variables in ‘Postlarval behavioral response analysis’. For Model 1, $b_1 = 61.2 \pm 21.18$ and $b_2 = 0.75 \pm 0.10$; for Model 2, $b_1 = 62.1 \pm 107.04$ and $b_2 = 0.85 \pm 0.25$; and for Model 3, $b_1 = 17.67 \pm 3.31$ and $b_2 = 0.99 \pm 0.003$. * $p \leq 0.05$

| Source | SS | df | MS | F | p | r ² |
|----------------|----------|----|----------|--------|---------|----------------|
| Model 1 | | | | | | |
| Model | 38132.00 | 2 | 19066.00 | 380.46 | 0.0000* | 0.94 |
| Residual | 2455.51 | 49 | 50.11 | | | |
| Total | 40587.50 | | | | | |
| Model 2 | | | | | | |
| Model | 1753.8 | 2 | 876.9 | 62.39 | 0.0000* | 0.72 |
| Residual | 688.7 | 49 | 14.05 | | | |
| Total | 2442.52 | | | | | |
| Model 3 | | | | | | |
| Model | 1714.1 | 2 | 857.03 | 62.39 | 0.0000* | 0.71 |
| Residual | 728.47 | 49 | 14.87 | | | |
| Total | 2442.52 | | | | | |

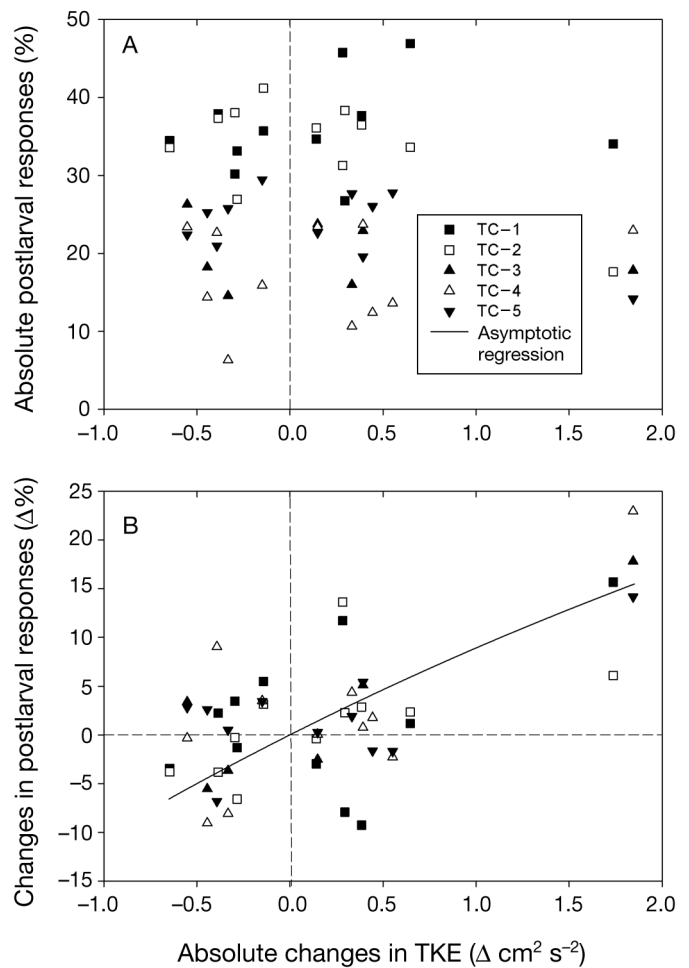


Fig. 5. *Farfantepenaeus duorarum*. (A) Absolute postlarval responses (%) to absolute changes in turbulence (turbulent kinetic energy, TKE, $\Delta\text{cm}^2 \text{s}^{-2}$), relative to the previous experimental timestep. (B) Changes in postlarval response ($\Delta\%$), relative to the previous experimental timestep, to absolute changes in turbulence ($\Delta\text{cm}^2 \text{s}^{-2}$), relative to the previous experimental timestep, with the respective asymptotic regression (for equation and parameters see ‘Postlarval behavioral response analysis’ and Table 2). Turbulence was generated by the motion of an actuator at 2 different speeds of oscillation: 20 mm s^{-1} (TC1–2, squares) and 40 mm s^{-1} (TC3–5, triangles)

been occupied by active, competent swimming postlarvae.

Data from TC trials were pooled for regression analysis to better compare the present results with those of Welch et al. (1999). Preliminary analysis detected a highly significant, positive correlation between percent postlarval response and TKE ($R = 0.6562$, $p < 0.0001$) and confirmed the observation of no trend in the relationship between current speed and postlarval response ($R = -0.0383$, $p = 0.7896$) within the TC1–5 dataset.

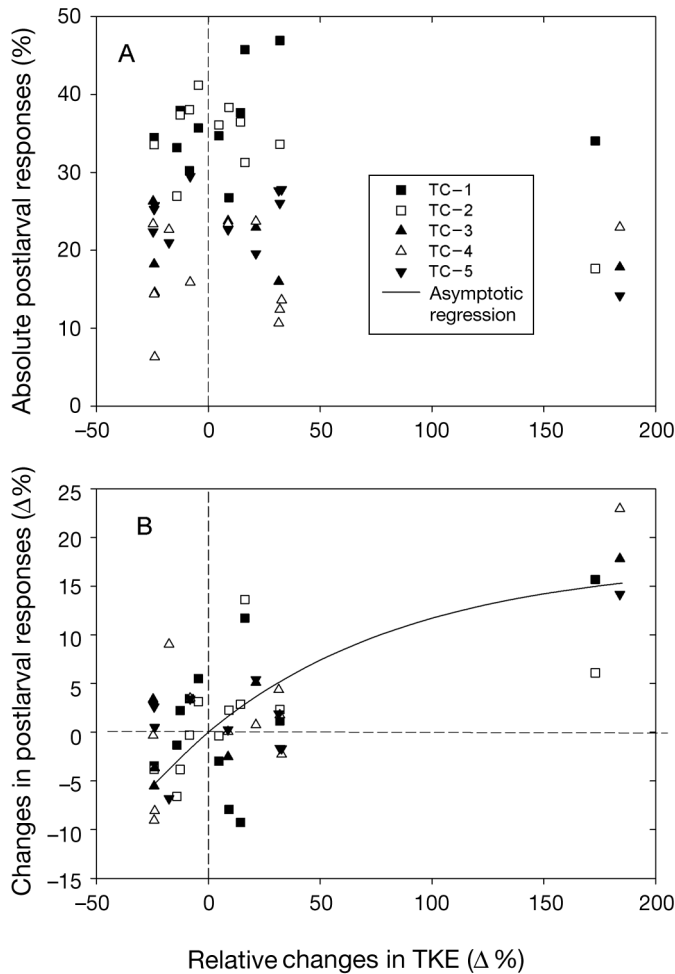


Fig. 6. *Farfantepenaeus duorarum*. (A) Absolute postlarval responses (%) to relative changes in turbulence ($\Delta\%$), relative to the previous experimental timestep. (B) Changes in postlarval response ($\Delta\%$), relative to the previous experimental timestep, to relative changes in turbulence ($\Delta\%$), relative to the previous experimental timestep, with the respective asymptotic regression (for equation and parameters see 'Postlarval behavioral response analysis' and Table 2). Turbulence was generated by the motion of an actuator at 2 different speeds of oscillation: 20 mm s^{-1} (TC1–2, squares) and 40 mm s^{-1} (TC3–5, triangles)

An increasing postlarval response in the TC1–5 trials coincided with increasing absolute TKE ($\text{cm}^2 \text{s}^{-2}$). However, this trend appeared to be dose limited because postlarval response leveled off with increasing TKE (Fig. 4). The threshold of the dose-limited trend occurred at values of $2.5 \text{ cm}^2 \text{s}^{-2}$ and was supported by a better fit of the data to an asymptotic regression ($F_{2,49} = 380.46$, $p < 0.001$, $R_a^2 = 0.94$) (Model 1: Table 2) than to a linear regression ($F_{2,49} = 37.06$, $p < 0.001$, $R_a^2 = 0.42$). Comparison of plots of

different representations of the TC1–5 data further supports the dose-limited response. No clear trend was discernible when the absolute percentage of postlarval response was analyzed in relation to absolute changes in TKE from the previous time step ($\Delta\text{TKE cm}^2 \text{s}^{-2}$) (Fig. 5A). However, the change in postlarval response from the previous time step ($\Delta\%$ response) in relation to absolute changes in TKE ($\Delta\text{TKE cm}^2 \text{s}^{-2}$) resulted in an asymptotic relationship (Fig. 5B). This response was confirmed by a better fit of the asymptotic model ($F_{2,49} = 62.39$, $p < 0.001$, $R_a^2 = 0.71$) (Model 2 in Table 2) than the linear model ($F_{2,49} = 33.72$, $p < 0.001$, $R_a^2 = 0.40$).

Postlarval response data analyzed relative to the percent change in TKE ($\% \Delta\text{TKE}$) indicated that, similar to previous results, absolute postlarval response ($\%$ response) plotted against relative change in TKE ($\% \Delta\text{TKE}$) did not present a clear trend (Fig. 6A). In contrast, change in postlarval response ($\Delta\%$ response) plotted against relative change in TKE ($\% \Delta\text{TKE}$) again suggested an asymptotic response (Fig. 6B). This was confirmed by the better fit of an asymptotic model ($F_{2,49} = 57.65$, $p < 0.001$, $R_a^2 = 0.69$) (Model 3: Table 2) than the linear model ($F_{2,49} = 45.99$, $p < 0.001$, $R_a^2 = 0.47$).

The asymptotic models (Table 2) conformed to parametric assumptions regarding normality and homoscedasticity of residuals (Brown & Rothery 1993, Kutner et al. 2005). Furthermore, rapid convergence of all 3 models (maximum iterations = 8) suggested that 'large sample theory' was satisfied, thus validating the use of F statistics to analyze these non-linear models (Kutner et al. 2005).

DISCUSSION

Results of the present trials confirm turbulence as a physical environmental cue stimulating the swimming activity of postlarval pink shrimp. Trials utilizing changes in flow speed alone did not stimulate this behavioral response in the postlarvae (Table 1, Fig. 3). Most likely, the highly laminar flow within this experimental system did not produce enough turbulence to stimulate swimming activity and was below the threshold necessary to produce a behavioral response. Indeed, the observed TKE values under these conditions, ranging from 6.9×10^{-3} to $9.1 \times 10^{-3} \text{ cm}^2 \text{s}^{-2}$, were very low compared to those that evoked postlarval response in later trials. Postlarvae exhibited an increase in swimming response during trials that incorporated use of the actuator (Fig. 3). This difference in experimental apparatus

allowed for production of TKE values (ranging from 1.1 and 3.5 $\text{cm}^2 \text{s}^{-2}$) 3 orders of magnitude greater than those produced in the FS1–5 trials. These substantial differences in TKE resulted in significant changes in postlarval behavioral response (Table 1, Fig. 3). In further contrast to results from the FS1–5 trials, postlarvae in trials TC1–5 responded to changes in TKE by ascending in the water column as TKE increased and descending to near the bottom as TKE decreased (Figs. 2C & 3). The present results accord well with the behavioral responses of blue crab megalopae to turbulence (Welch et al. 1999, Welch & Forward 2001). We rely extensively on the study conducted by Welch et al. (1999) in the discussion of our results due to the fact that, to the authors' knowledge, the stimulation of swimming behavior by turbulence has not yet been identified in penaeid shrimp species and due to the paucity of similar studies that demonstrate STST behavioral cues.

In the Welch et al. (1999, their Fig. 3) experiments, variation in flow speed alone induced the swimming response with blue crab megalopae; however, the experimental system in that study produced much higher levels of TKE (15 cm s^{-1} flow speed $\approx 1.5 \text{ cm}^2 \text{ s}^{-2}$ TKE; Welch et al. 1999, their Fig. 2). Within the present system, similar flow speeds produced TKE levels ~ 1 order of magnitude lower (14 cm s^{-1} $\approx 0.10 \text{ cm}^2 \text{ s}^{-2}$) than those reported by Welch et al. (1999). Perhaps this explains why we did not observe a behavioral response during FS1–5 trials. Irrespective of this difference, Welch et al. (1999) concluded that behavioral response was not directly related to flow speed alone because differing flow speeds produced different trends. In the present study, we arrive at a similar conclusion as, during the TC1–5 trials, no significant correlation of postlarval response and flow speed was observed. However, Welch et al. (1999) noted that when data were analyzed in terms of TKE, 1 simplistic trend in response was observed. Baseline activity near 0.0 $\text{cm}^2 \text{ s}^{-2}$ corresponded to $\sim 20\%$ megalopae response in that study (Welch et al. 1999, their Fig. 4). Similarly, we observed a baseline response of $14.9 \pm 1.3\%$ when TKE was negligible (Fig. 3).

Irrespective of flow speed, when differing relative TKE levels were produced within the present experimental systems, differing relative behavioral responses that reflected the changes in TKE levels were noted. Plotting absolute postlarval response to changes in TKE illustrates that vastly differing levels of response (ranging from $\sim 10\%$ to $\sim 45\%$) were exhibited while the organisms were experiencing similar absolute amounts of TKE (Fig. 5A). Similarly,

Figs. 5A & 6A also indicate broad ranges in response when TKE was displayed as relative change. These observations are similar to those presented in Figs. 5 & 6 of Welch et al. (1999). The response of postlarvae was different when data were analyzed as changes in postlarval responses (relative to the timepoint). An increase or decrease in TKE, whether absolute or relative, produced a proportional relative increase or decrease in the response of postlarvae (Figs. 5B & 6B). Thus, a positive or negative change in TKE results in a corresponding positive or negative change in the percent of shrimp responding. The changes in the percentage of shrimp responding to absolute and relative changes in TKE yield significant logistic regressions (Table 2). This conclusion is in accord with Welch et al. (1999), who noted that the percentage of responding blue crab megalopae could not be predicted by either relative or absolute change in TKE, but the changes in these measures could predict the change in their response behavior (Welch et al. 1999, their Fig. 8).

In the present study, the greatest behavioral response of postlarvae occurred at TKE levels of $\sim 2.5 \text{ cm}^2 \text{ s}^{-2}$ (Figs. 3 & 4). At higher TKE values, postlarval response diminished despite continued increases in TKE. This dose-limited response or saturation point was substantiated by fitting horizontally asymptotic regressions to both the postlarval response to absolute amounts of TKE (Fig. 4) and changes in postlarval responses ($\Delta\%$ response) to either absolute changes in TKE (ΔTKE) (Fig. 5B) or relative change in TKE ($\Delta\% \text{ TKE}$) (Fig. 6B). This suggests that postlarvae may have reached a response saturation point above which increases in TKE do not result in substantial increases in swimming behavior. Similarly, Welch et al. (1999) observed a stimulus-response saturation point; however, they report a threshold value of 1.1 $\text{cm}^2 \text{ s}^{-2}$ for crab megalopae in their studies, compared to the present observation of $\sim 2.5 \text{ cm}^2 \text{ s}^{-2}$ for pink shrimp postlarvae. Perhaps the difference in TKE response-saturation points in these 2 studies reflects species-specific differences or imprinting to different thresholds for different estuaries in response to the unique conditions each estuary would present. A better understanding of TKE regimes in different estuaries and more experimentation with other species are needed to clarify these differences between the present study and that of Welch et al. (1999).

Field observations in Florida Bay indicated that pink shrimp postlarvae were active in the water column almost exclusively during nocturnal flood tides, with the largest postlarval peaks in density occurring

at the highest current speed, between 40 and 80 cm s⁻¹ during new moon phases (Criales et al. 2011). Tides at the NW border of Florida Bay are mainly semidiurnal and characterized as strong (Smith 1997, Wang 1998). Investigations leading to calculation of TKE levels associated with the tidal cycle have not been undertaken for this location. However, in the Newport River Estuary, North Carolina, an estuary with tidal periodicity and depth similar to those of Florida Bay, Welch (1998) measured the TKE during several tidal cycles. Welch's (1998) data indicated that, at peak current velocities during flood tide, TKE averaged $3.4 \pm 1.7 \text{ cm}^2 \text{ s}^{-2}$, and at slack water after flood tide, TKE decreased to $0.65 \text{ cm}^2 \text{ s}^{-2}$. The TKE values generated by the actuator in our laboratory experiments varied between 1.0 and $3.5 \text{ cm}^2 \text{ s}^{-2}$, values comparable to those measured by Welch (1998) in the field during peak velocity currents of the flood tidal stage. Active swimming responses of postlarvae to changes in TKE in our experiments were observed at TKE values between 1.1 and $2.5 \text{ cm}^2 \text{ s}^{-2}$, which correspond to TKE values experienced with water velocity acceleration during the flood tide in the Newport River Estuary (Welch 1998).

The results of our experiments that utilized the actuator array suggest that an increase in actuator speed did not always reflect an increase in TKE. The TKE generated by the actuator's speed of 2 cm s⁻¹ varied between 1.7 and $3.5 \text{ cm}^2 \text{ s}^{-2}$, while the TKE generated by the actuator's speed of 4 cm s⁻¹ varied between 1.1 and $2.2 \text{ cm}^2 \text{ s}^{-2}$. This seemingly counter-intuitive decrease in TKE as the actuator speed increased may have been due to the size of the surface deflections generated by the cylinder rake on the surface of the water. In a test section of data from the actuator, a significant component of the fluctuating velocities was due to surface waves. As the speed increased, the rake interacted differently with the free surface (the top of the cylinder was not always in contact with the surface). This led to propagation of smaller-wavelength surface waves from the location of the actuator. These small eddies decayed more quickly with depth and led to the reduction in TKE production.

Turbulence in water flow is defined by eddies over a range of scales because the energy is transferred from large to successively smaller size eddies (Peters & Gross 1994, Tennekes & Lumley 1997). Penaeid postlarvae, as other crustacean decapods, seem to have different sensory capabilities for detecting turbulence at different scales depending on the size of eddies created by the turbulent flow. When large or intermediate eddies are created during the vertical

water displacement, postlarvae would be able to detect the associated changes in turbulence as hydrostatic pressure changes (Tankersley et al. 1995, Welch 1998) using mechanoreceptor organs, such as statocysts, located at the base of the antenna, or the sensory dorsal organ, which is considered a pressure detector organ (Laverack et al. 1996). On the other hand, smaller-scale eddies would generate fluid shear, which could be detected by mechano- and chemoreceptive sensors, such as 'aesthetascs,' hairs located in the antennules of decapods (Ache & Young 2005, Horner et al. 2004). Turbulent water flows usually disperse odors across aquatic habitats as filamentous, intermittent plumes, and some decapods, such as spiny lobster *Panulirus argus* and blue crab, detect odors by flicking the olfactory antennules, which contain rows of 'aesthetascs' (Reidenbach & Koehl 2011). Studies on the mechano- and chemoreceptive sensors of penaeid shrimp postlarvae are needed to determine how these organisms physically detect turbulence, which would allow them to undertake STST behavior.

Under constant laboratory conditions, Hughes (1969a) observed that pink shrimp postlarvae swam upstream during the flood tide and downstream during ebb tide over a 96 h period. The observed swimming behavior was hypothesized to result in movement up-estuary during flood tide despite the orientation in the opposite current direction. Recently, Ogburn et al. (2013) determined that the vertical swimming activity of pink shrimp postlarvae has a dominant period length of 12.7 h, but a 25 h period was also observed sporadically. Similarly, a circadian and a circatidal activity rhythm have been observed in brown shrimp postlarvae (Matthews et al. 1991) and in pink shrimp juveniles (Wickham 1967, Subrahmanyam 1976) and postlarvae (Criales et al. 2007). These studies suggest that the tidally synchronous activity of pink shrimp postlarvae may be controlled by a combination of 2 different rhythms, circatidal and circadian, rather than a fundamental circatidal period. In support of this behavioral activity pattern, pink shrimp postlarvae occurred in 2 large peaks at the surface layer both day and night in SW Florida shelf waters (Criales et al. 2007). Conversely, in the estuarine waters of Florida Bay, postlarvae occurred in the water column almost exclusively during the flood tide at night (e.g. Tabb et al. 1962, Criales et al. 2010, 2011). Once postlarvae approach estuarine waters, light most likely inhibits the behavioral responses to both environmental cues and endogenous rhythms. Similar behavior has been observed

in crab megalopae (Forward & Rittschof 1994, Tankersley et al. 1995), suggesting that organisms change behavior once they approach estuarine waters, possibly to avoid predation from visual planktivores, which feed during diurnal periods.

Although pink shrimp postlarvae may exhibit endogenous tidal and daily rhythms, exogenous factors are necessary to trigger behaviors and to synchronize the response to the endogenous tidal rhythm in order to achieve STST. The environmental variables associated with tidal currents that may serve as STST behavioral cues include pressure, temperature, salinity, and turbulence (Shanks 1995, Tankersley et al. 1995, Forward & Tankersley 2001). Salinity has been proposed as the environmental factor associated with the STST behavior that triggers the tidal rhythm in pink shrimp (Hughes 1969b). Postlarvae maintained in constant light conditions were active in the water column when salinity increased and returned to the substrate when salinity decreased (Hughes 1969b). Hughes (1969b) equated these increases and decreases in salinity to flood and ebb tides, respectively, and concluded that this cue acted as the mechanism that induces STST behavior. In Florida Bay, salinity changes gradually throughout a tidal cycle occurring in a range between 0.01 and 0.6 psu h⁻¹ (http://sofia.usgs.gov/exchange/zuckerwoods_patino/index.php). However, Hughes (1969b) used step changes in salinity of 1, which were highly exaggerated in comparison to the gradual changes typical of a tidal cycle under natural conditions. Further complicating this situation encountered by postlarval pink shrimp entering Florida Bay, salinity within Florida Bay is highly variable, and hypersaline conditions often occur during the summer (Lee et al. 2006, Kelble et al. 2007), coinciding with the greatest influxes of postlarvae arriving in Florida Bay (Criales et al. 2006). Hypersalinity creates a negative salinity gradient from the interior to the boundaries of Florida Bay (Criales et al. 2010), and under this condition, postlarvae would receive the wrong cue to enter the water column because salinity during flood tide may be lower than during ebb tide. Similar examples of a negative gradient may occur in other hypersaline estuaries that serve as important nursery grounds of penaeid shrimps, such as Laguna Madre (southern Texas and Mexico) (Pérez-Castañeda et al. 2010), Laguna Celestun (Mexico) (Pérez-Castañeda & Defeo 2001), Laguna Rio Lagratos (Yucatan, Mexico) (May-Kú & Ordóñez-López 2006), and Laguna La Restinga (Venezuela)

(Scelzo 2003). These considerations suggest that such a generic, heavy reliance on 1 cue (i.e. salinity) would not consistently trigger STST behavior in all natural settings experienced by a species and supports the notion that multiple or different cues are required to define and consistently produce a behavioral response that enhances forward movement on the flood tide across both different species and different natural systems.

The present experimental study demonstrates that pink shrimp postlarvae react to changes in TKE. Postlarvae ascended in the water column as TKE increased and descended near the bottom as TKE decreased. Water turbulence associated with the flood tidal current may induce sustained swimming during periods of high flood current to assist postlarvae during the STST. During most of the flood tide, TKE levels would be sufficiently high to stimulate postlarvae to swim and remain in the water column (Welch & Forward 2001). At the end of the flood tide, as the TKE decreases, the rate of decrease and the change in TKE relative to that recently experienced would cue decreased swimming, and postlarvae would descend to near the bottom. For this mechanism to be successful during STST, postlarvae would need some additional signal to enable them to differentiate the ebb from flood tide. In light of the salinity regime of Florida Bay and similar hypersaline estuaries, salinity may not be the best or most consistent environmental cue available for triggering the STST behavior of pink shrimp postlarvae. Under these conditions, a more probable choice is a chemical cue associated with the terrestrial or sea-grass environment. Postlarvae may recognize chemical cues by chemoreceptive capabilities, as has been demonstrated for other decapods (Reidenbach & Koehl 2011), which are able to discriminate odors from land and vegetation associated with the ebb versus oceanic waters associated with the flood tide. Further research is needed to test this hypothesis and to determine the mechano- and chemosensorial abilities of this species that allow it to successfully conduct STST behavior.

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