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Resisting flow – laboratory study of rheotaxis of the estuarine copepod *Pseudodiaptomus annandalei*

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Rheotaxis is a ubiquitous phenomenon among aquatic animals and thought to be an adaptation to maintain populations in flowing waters. While many estuarine copepods can retain their populations in estuaries with net seaward flow, rheotaxis of individual copepods has not been reported before. In this study, the behavior of a calanoid copepod *Pseudodiaptomus annandalei* in flow was examined in a recirculating laboratory flume. This estuarine copepod displayed different responses to ambient flow fields while swimming in the water column or attaching to the flume bed (walls). Copepods in the water column showed vigorous countercurrent swimming by occasional bounding when flow velocity was increased up to $2.1\,\mathrm{cm\,s^{-1}}$, but none of the individuals in the water column were retained in the flume when flow speeds were higher than $4\,\mathrm{cm\,s^{-1}}$. This indicates *P. annandalei* profits little from rheotaxis to withstand flow when they were swimming in the water column. Instead, more individuals attempted sinking downwards to the slow flow region near the flume bed (walls) and showed active substrate attachment to avoid being flushed out by the high-velocity channel flow. The results suggest that *P. annandalei* benefits from rheotaxis and association with the substrate which allows them to hold position well at ambient flow velocities up to $3\,\mathrm{cm\,s^{-1}}$. These adaptive responses might be important for population maintenance.

Keywords: rheotaxis; estuarine copepod; pseudodiaptomus; flume experiment; positional behavior; ballast water; species invasions

Introduction

Fluid motion is a characteristic property of the aquatic environment. In streams, rivers and estuaries, the unidirectional flow of water sets limitations on upstream movement and might cause downstream displacement of organisms (Vogel 1981). Many aquatic organisms perform upstream migrations at some stage in their life cycle as a compensatory mechanism for downstream losses by drift (Elliott 1971; Williams and Williams 1993), particularly of eggs and larvae. Rheotaxis, a behavioral orientation towards the origin of flow, has been found ubiquitously in several aquatic animals such as fish (Montgomery et al. 1997), crabs (Ryan and Choy 1990), shrimps (Hancock and Bunn 1999) and larvae of benthic invertebrates (Abelson 1997). This is generally understood as an important mechanism to maintain populations in flowing waters.

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Copepods make up the largest part of zooplankton (Castro and Huber 2003). A common perception of zooplankton is that it drifts with the ambient fluid. Although planktonic copepods can migrate a long distance vertically within a day (Steele and Henderson 1998), they drift in advection due to their weak locomotory capabilities, which results in directional transport by ocean currents or loss immediately after being released into rivers downstream of reservoirs (Akopian et al. 1999). Many copepods can maintain populations in estuaries with net seaward flow by vertical migration to make use of stratified flows or lateral movement to areas of decreased flushing such as in channel margins (Cronin et al. 1962; Hough and Naylor 1991; Morgan et al. 1997), but they remain planktonic at all times. Although *Pseudodiaptomus* (Jacobs 1961) and *Gladioferens* (Kennedy 1978; Rippingale 1994) hold on to firm surfaces above sediments to allow them to withstand considerable currents, it is not known whether they exhibit rheotaxis. Light induced rheotactic behavior has been mentioned in other plankters such as cladocerans (Roozen and Lürling 2001), and some copepod species can swim against horizontal (Buskey et al. 1996) and vertical flow (Genin et al. 2005) by swarming.

As a dominant estuarine copepod, *Pseudodiaptomus* is believed to be mainly epibenthic and shows typical diel vertical migration (Jacobs 1961; Hart and Allanson 1976; Walter 1987; Kouassi et al. 2001). These copepods exhibit outstanding abilities to resist water currents, maintain their position in the estuary against the net seaward flow and often become the predominant population there. They even avoid being swept away by ballast water exchange and this has resulted in at least three species of *Pseudodiaptomus* (*P. forbesi*, *P. inopinus* and *P. marinus*) being introduced to estuaries of North America since the 1980s (Orsi and Water 1991; Cordell et al. 1992). Understanding the basic biology and ecology of these invasive copepods is essential for managing introductions but few studies examine relevant behavioral mechanisms, for example, their behavior has been particularly poorly studied (Bollens et al. 2002). The behavioral response of copepods to ambient currents has not been demonstrated *in situ* as yet. This is largely due to a lack of technology that can track the motions of these small, nearly transparent organisms in a large volume of water (Genin et al. 2005). On the other hand, flume experiments have been applied widely in behavioral studies of aquatic animals to examine behavior in boundary layers (Jonsson et al. 1991; Weissburg and Zimmer-Faust 1993; Welch et al. 1999). Since *Pseudodiaptomus* is considered to be a demersal copepod with clear substratum preferences, examining their behavior on and above the bottom seems to be important to reveal how they respond to ambient water currents in estuaries and a laboratory stream, formed in a flume channel, would be a feasible approach.

In this study, we examined the behavior of *P. annandalei* in a laboratory flume to test whether this common estuarine copepod, from the southeast coast of China (Shen 1979), benefits from rheotaxis like other estuarine animals.

Materials and methods

Individuals of *P. annandalei* were collected by horizontal hauls of a plankton net (50 cm mouth diameter, 2 m length, 160 μm mesh aperture) during the nighttime high tide in November 2004, from the brackish region in the head of the Jiulongjiang estuary. Live copepods were brought to the laboratory in ambient seawater within 2 h. Adults of *P. annandalei* were picked up by a pipette under a dissecting microscope for the flume experiments.

The swimming of *P. annandalei* in response to water current was examined in a recirculating laboratory flume consisting of a horizontal open channel (100 cm length × 10 cm width × 10 cm depth, with 8 cm water depth at operation) between two tanks. Two honeycomb baffles (10 cm length × 10 cm width × 10 cm height) were used to even the flow. Each consisted of parallel tubelets (0.3 cm diameter) and one was placed at the upstream end of the bed section, and the other at the downstream end. Nets (100 μm mesh aperture) were positioned at either end of the channel to prevent copepods from swimming beyond this region. The flow was induced gravitationally by water fed from a tank upstream, and a pump returned water to the upstream tank. This system provided smooth flow that was easily controlled by regulating the pump output. The water flow produced velocities from 0 to approximately 10 cm s^{-1} in the middle of the channel.

The vertical velocity gradient through the boundary layer in the center of the flume was measured by recording neutrally buoyant particles (debris composed of dead cells of *Chlorella* sp.) with dissecting microscope equipment with CCD video camera. The time taken for the debris to pass over a certain length (2.0 cm) at six known heights (0.3, 0.6, 0.9, 1.2, 1.5, 2.0 cm) above the bottom, was noted by analyzing the digital video on a computer, a technique similar to that used by Ertman and Jumars (1988). Video recordings were sampled at 30 frames and 10–15 particle velocities were measured at each height. The boundary shear velocity ($u*$) of flow at each speed setting was calculated from the slopes of the logarithmic velocity profiles:

$$U(z) = \left(\frac{u*}{k}\right) \ln\left(\frac{z}{z_0}\right)$$

where $U(z)$ is the mean velocity at height z above the bottom and k is von Karman's constant (0.41). The roughness height (z_0) was determined as the y intercept of the equation regressing log height above the bottom against the measured flow velocity. The distance from swimming leg to dorsum of *P. annandalei* when they attached to the flume bed was 0.441 ± 0.022 mm (mean ± 1 SD, $N = 50$). The velocity at this height 0.044 cm above the bottom was measured as the fastest flow experienced by the copepod attaching to the bed.

The behavior of *P. annandalei* in the flume flow was determined in three experiments and recorded by the dissecting microscope equipment with CCD video camera. In treatment A we simulated a flow field where the current velocity increased gradually, similar to the flow condition that copepods face in the reversal between flood and ebb tide *in situ*. Copepods were released at the midpoint of the flume first, then the flow was started and increased to the experimental speed in 1–3 min, according to the maximum flow velocity. To examine behavior in an established constant flow, the flume was started before the copepods were placed in the flow in treatments B and C. Copepods were released from a pipette at the head of the current. The releasing spot in treatment B was in the mid-layer (approximately 4 cm water depth) and in treatment C, near to the flume bed. As treatment A could not determine whether *P. annandalei* exhibited actively sinking or bed attaching in the water column, comparison of the results from treatments B and C would test this tendency.

Rheotactic responses were measured as the percentage of copepods (started with 10 individuals and replicated five times) remaining in the flume facing within 45° of the upstream direction. The copepods attached to the mesh net at either end of the flume were not counted as remaining individuals. Five observations were taken at 30 s intervals 3 min after the flow was established in treatment A, or just after the copepods were released

in treatment B and C. Swimming behavior in the flow was examined by observing whether the copepods (started with 1 individual and replicated twenty times) moved upstream during a 1 min duration after the ambient flow reached different initialized velocity (treatment A) or after copepods were released in flow (treatment B, C). Countercurrent movement included observations in which a copepod tried to swim upstream (positive rheotaxis) but was carried downstream because the current speed was faster than its swimming speed. The ability to resist water current was determined as the mean number of individuals (started with 20 individuals and replicated five times) sustained in the channel 10 min after the flow was initiated (treatment A) or the copepods were added (treatment B, C). Because the duration of determining the ability to resist water current (10 min) was longer than measurement of rheotactic responses or swimming behavior, more copepods were flushed out and as a result fewer individuals remained in the channel. A different group of copepods was used in each trial. As only a few copepods could maintain their position in the water column after the flow was established, and most of the attaching individuals exhibit significant rheotactic behavior under high flow speed, the states of copepods in the channel could be recorded promptly by visual observation. The rheotactic responses of the individuals sticking to the flume bed (walls) or swimming in the water column were counted separately to examine the effect of hydrodynamic conditions in and out of the boundary layer.

Results

Hydrodynamic characterizations

Velocity profiles for flow at each speed setting are shown in Figure 1. Regressions were used to calculate Z_0 and therefore shear velocities accounted for more than 95% of the variation in the data in each case. The hydrodynamic characterization of the boundary layer in the center of the channel under different flow conditions is shown in Table 1.
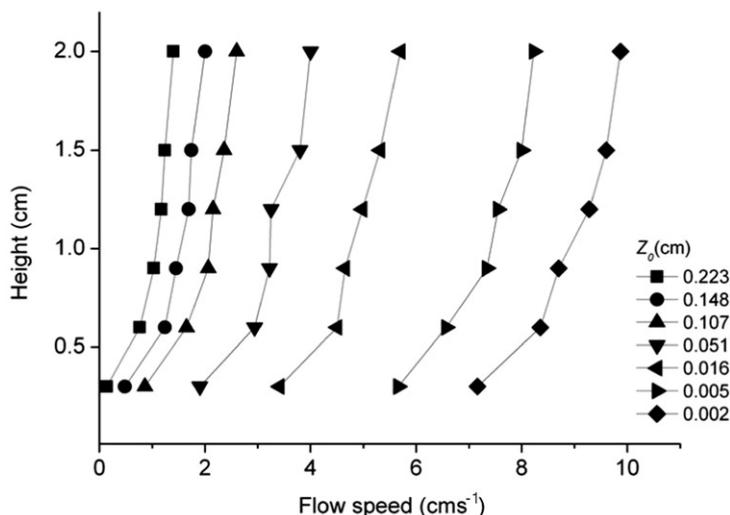

Figure 1. Velocity profiles for flowing water at free-stream velocity used in the experiments. z_0 is the roughness height which was determined by the y intercept of the equation regressing log height above the bottom against the measured flow velocity.

Behavior of Pseudodiaptomus annandalei *in an increasing flow*

Pseudodiaptomus annandalei showed significant rheotaxis in flow fields, especially the individuals attaching to the flume bed (Figure 2(a)) and lateral wall (Figure 2b). *Pseudodiaptomus annandalei* swimming in the water column drifted passively at flow speeds slower than $1.4 \, \text{cm s}^{-1}$. Countercurrent movement increased dramatically at flow speeds of $2.1 \, \text{cm s}^{-1}$ even though the proportion of individuals oriented upstream was constant under this flow condition (Figures 3(a) and 4(a)), indicating that the threshold for a rheotactic response of *P. annandalei* swimming in the water column was $<2 \, \text{cm s}^{-1}$. This threshold velocity, however, is much higher than the speed of forage swimming of *P. annandalei*. Hence, animals attempted to resist the flow by swimming upstream using occasional bounding. This was presented as swimming with legs beating in a very fast metachronal rhythm. This bounding behavior was unable to allow the copepods to orient upstream continuously. Upstream orientation increased somewhat as flow accelerated, but the larger deviation in orientation suggests that the animals experienced a more unstable state than they faced in the slow current. While the copepods continued to swim (bound) upstream as the flow velocity was increased, few individuals in the water column could hold upstream orientation and therefore fewer individuals remained in the channel (Figure 5). None of the individuals swimming in the water column were able to stay there at flow speeds higher than $2.8 \, \text{cm s}^{-1}$, and no rheotactic behavior was observed out of the slow flow region near to the flume bed (walls) during those trials.

In contrast to the rheotactic behavior of individuals swimming in the water column, those that attached to the flume bed (walls) showed a significant upstream orientation. The mean flow velocity threshold for rheotaxis was $2.8 \, \text{cm s}^{-1}$ (Figure 3(b)), somewhat higher than that for copepods swimming in the water column. In such a flow field, the exact flow speed experienced by the animals (0.044 cm above the flume bed) was $0.11 \, \text{cm s}^{-1}$. No upstream movement was observed until the velocity 0.044 cm above the bottom reached $0.27 \, \text{cm s}^{-1}$ ($U_\infty = 4.0 \, \text{cm s}^{-1}$) (Figure 4(b)), indicating only behavioral orientation of *P. annandalei* in near-bed slow flow in this situation. Nearly 70% of the individuals exhibited upstream movements when the flow experienced by copepods attaching to the flume bed increased to $1.18 \, \text{cm s}^{-1}$ ($U_\infty = 5.7 \, \text{cm s}^{-1}$) (Figure 4(b)). The normal foraging swimming did not allow the copepods to resist the flow as velocity increased, and they used bounding to move upstream. Bounding behavior affected the balance of the body by increasing the cross-sectional area, then increasing the risk of being flushed away downstream. Only one fourth of individuals still remained in the ambient flow up to

Table 1. Hydrodynamic characterization of the boundary layer in the middle of the flume under different flow conditions.

U_∞ (cm s^{-1})	$u*$ (cm s^{-1})	$u_{0.044}$ (cm s^{-1})
1.4	0.27	0.04
2.1	0.33	0.07
2.8	0.38	0.11
4.0	0.46	0.27
5.7	0.49	1.18
8.4	0.57	2.95
10.1	0.59	4.43

Notes: U_∞: free-stream velocity; $u*$: boundary shear velocity; $u_{0.044}$: velocity 0.044 cm above the bottom.
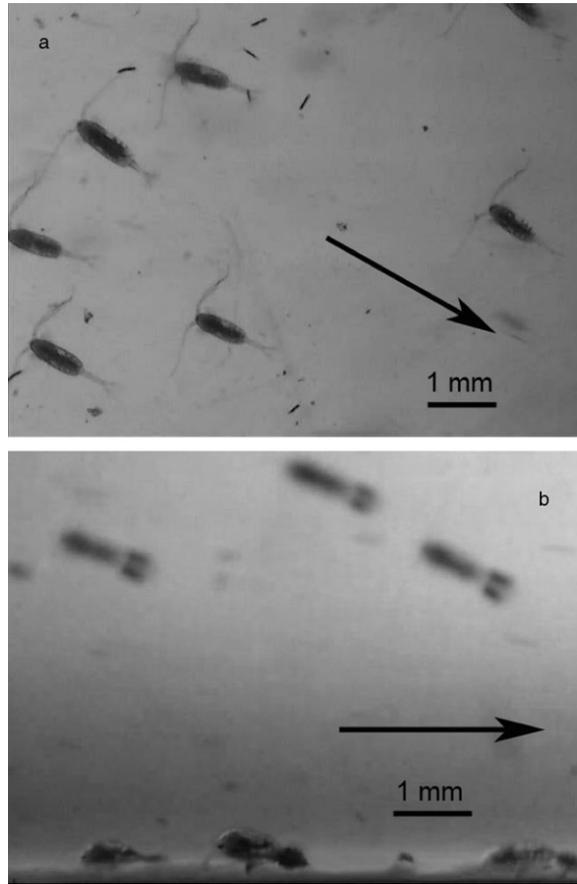
Figure 2. *Pseudodiaptomus annandalei* oriented upstream in flow. a: top view; b: side view. Black arrow shows flow direction.
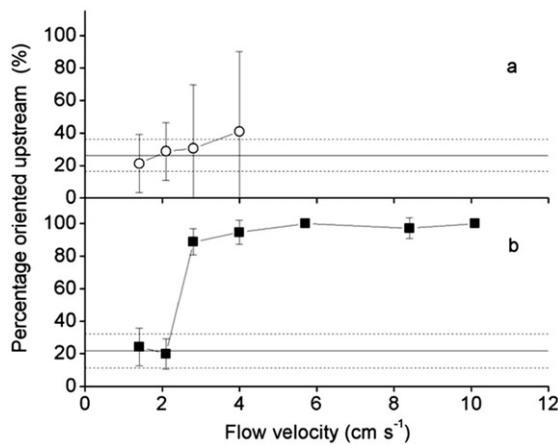

Figure 3. Rheotactic responses in treatment A. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* oriented upstream under different flow conditions. The horizontal solid and dashed lines indicate the mean ± 95% confidence intervals of the orientation response in the absence of current; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).
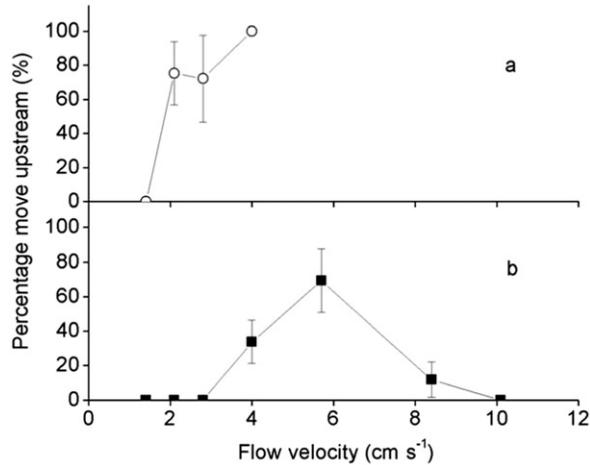
Figure 4. Swimming behavior in treatment A. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* that moved upstream under different flow conditions. (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).
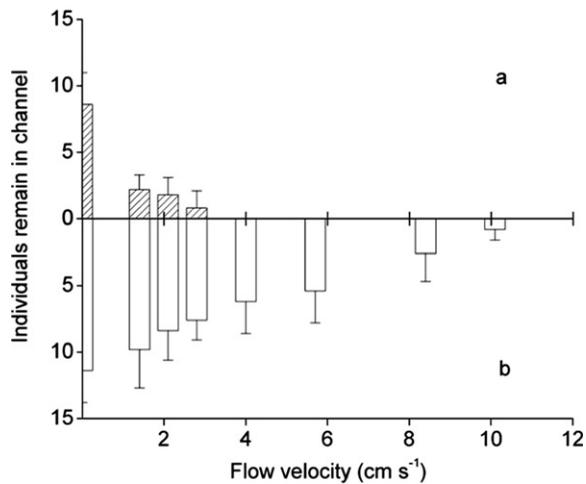

Figure 5. The ability to resist flow in treatment A. The number (mean + 1SD) of *Pseudodiaptomus annandalei* that maintained position in the channel under different flow conditions; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).

$2.95\,\mathrm{cm\,s^{-1}}$ $(U_\infty = 8.4\,\mathrm{cm\,s^{-1}})$ (Figure 5(b)). This disadvantage also reduced the upstream bounding attempt of attaching individuals in higher speed flow (Figure 4(b)). When the flow velocity 0.044 cm above the bottom reached $4.43\,\mathrm{cm\,s^{-1}}$ $(U_\infty = 10.1\,\mathrm{cm\,s^{-1}})$, the number of *P. annandalei* remaining in the channel decreased to less than 6% 10 min after the flow was established (Figure 5).

Behavior of Pseudodiaptomus annandalei *encountering flow*

Similar to individuals in a steadily increasing flow field, individuals of *P. annandalei* encountering the established flow also exhibited strong rheotactic behavior, whether they

were released in the mid-layer water column or boundary layer near to the flume bed. While the proportion of copepods orientated upstream did not change markedly after being released in mid-layer waters (Figure 6(a)), the effort of swimming against the current increased remarkably at low flow velocity (Figure 7(a)), as implied by vigorous upstream attempts to keep position in the flow field. As the number of swimming copepods remaining in the channel decreased with increasing flow speed (Figure 8(a)), upstream swimming of individuals in the water column was insufficient to maintain their populations under flow even as slow as $4\,cm\,s^{-1}$. On the other hand, some individuals
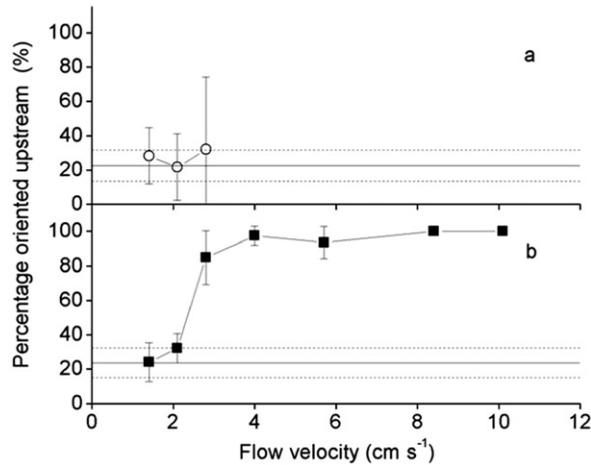

Figure 6. Rheotactic responses in treatment B. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* oriented upstream under different flow conditions. The horizontal solid and dashed lines indicate the mean ± 95% confidence intervals of the orientation response in the absence of current; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).
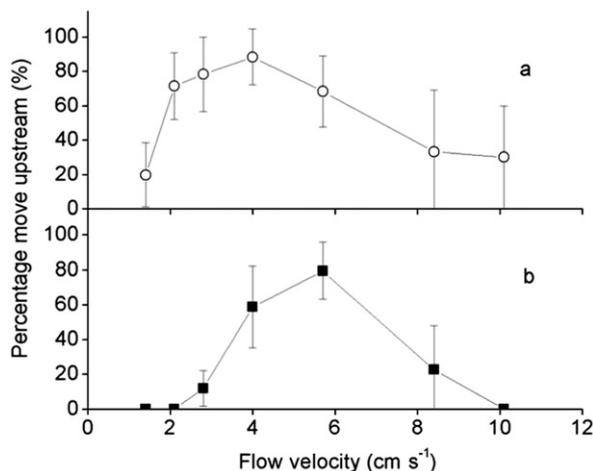

Figure 7. Swimming behavior in treatment B. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* that moved upstream under different flow conditions; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).

of *P. annandalei* sank to the flume bed after being released in the flow. Staying near the bed boundary layer prevented them from being flushed away by an even faster flow velocity (Figure 8(b)), as shown by active selection of downward swimming (or just passive sinking) to escape from the high-flow speed region. The downward tendency increased along with increasing flow speed, but the number of attached copepods declined when flow speed reached $4 \, \mathrm{cm \, s^{-1}}$, reflecting that more individuals in the water column could not sink to the bottom before being flushed out of the channel. Moreover, the individuals that sank to the flume bed did not show strong rheotaxis under low flow velocity ($<4 \, \mathrm{cm \, s^{-1}}$) (Figures 6(b) and 7(b)). This implies that although copepods swimming in the water column sensed the ambient current and reacted to it, low velocity near the bed (walls) flow could not induce a rheotactic response from individuals that moved into the boundary layer.

Despite that *P. annandalei* swimming in the water column exhibited active bed (wall) attachment to withstand the flow, not all individuals released in the near bed boundary layer took this opportunity to attach to the flume bed and some copepods were still present in the water column under low velocity flow conditions (Figure 11). Copepods did not show rheotactic behavior after being released near the bed under low flow velocity ($<2.1 \, \mathrm{cm^{-1}}$) (Figures 9(b) and 10(b)) and they swam into the water column. On the contrary, they exhibited strong rheotactic behavior and occasionally bounded upstream in high velocity flow. They then appeared in the water column as a result of being swept away from the boundary layer. In general, the closer the individuals were to the low flow speed region near the bottom (or walls) initially, the more they could stay in flume. The live observation and rheotaxis measurements are summarized in Table 2 with a schematic diagram of general behavioral patterns in Figure 12.

Discussion

A study on *Eurytemora affinis*, a common and dominant species in most estuaries of the northern hemisphere, showed that it cannot sustain its position in a current with a speed of $\geq 2 \, \mathrm{cm \, s^{-1}}$ (Castel and Veiga 1990). Light-induced swarming behavior
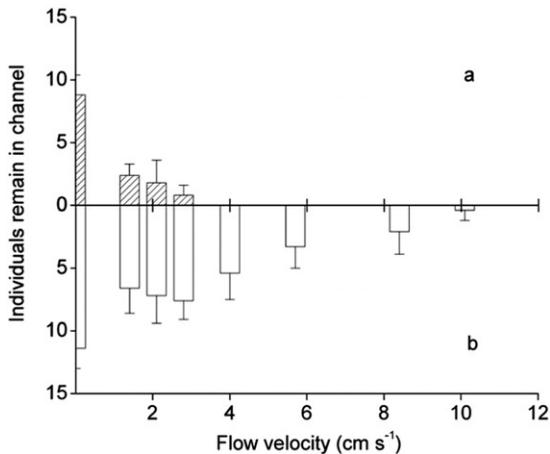

Figure 8. The ability to resist flow in treatment B. The number (mean + 1SD) of *Pseudodiaptomus annandalei* that maintained position in the channel under different flow conditions; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).
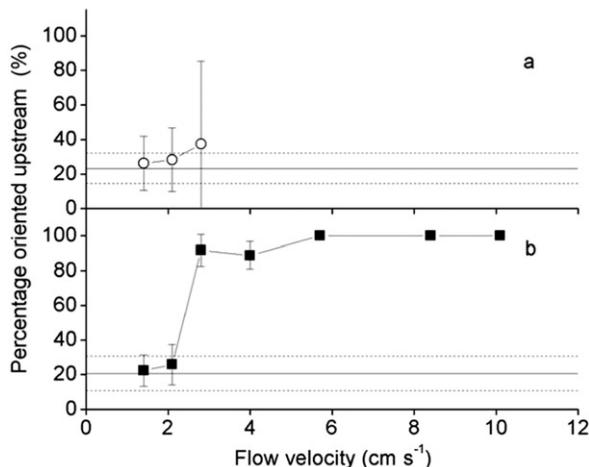
Figure 9. Rheotactic responses in treatment C. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* that oriented upstream under different flow conditions. The horizontal solid and dashed lines indicate the mean ± 95% confidence intervals of the orientation response in the absence of current; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).
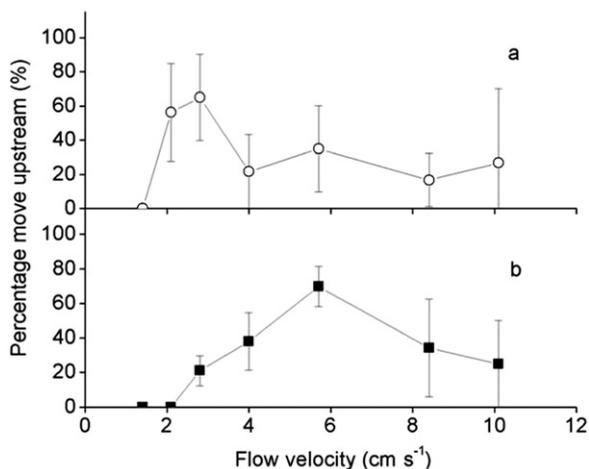

Figure 10. Swimming behavior in treatment C. Percentage (mean ± 1SD) of *Pseudodiaptomus annandalei* that moved upstream under different flow conditions; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).

allows *Dioithona oculata* to maintain their position despite currents of up to $2 \, \text{cm} \, \text{s}^{-1}$, but they are unable to orient into the currents and keep their position without swarming in the dark (Buskey et al. 1996). Similar mechanisms have been proposed as important for maintaining depth by actively swimming against vertical currents (Hardy and Bainbridge 1954; Franks 1992; Mackas et al. 1997; Genin 2004; Genin et al. 2005), but there are few observations on the behavior of individual copepods. It also remains unclear whether copepods exhibit upstream orientation to resist currents as many other animals do in flow fields. As common copepods of estuarine
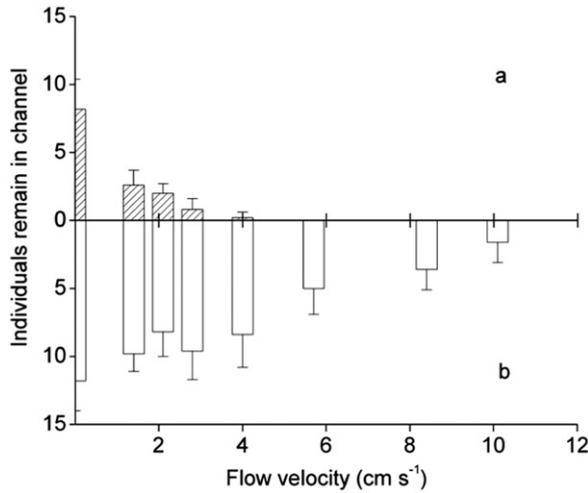
Figure 11. The ability to resist flow in treatment C. The number (mean + 1SD) of *Pseudodiaptomus annandalei* that maintained position in the channel under different flow conditions; (a) copepods swimming in the water column; (b) copepods attaching to the flume bed (walls).

Table 2. Summary of the states of *Pseudodiaptomus annandalei* in different flow speed.

Flow speed (cm s^{-1})	In the water column	Phase switch	On the substrate
1.4	Random swimming; transfer to downstream slowly	←→	Random 'walking'
2.1	Upstream bounding actively; hard to keep orientation; transfer to downstream slowly	←→	Random 'walking'
2.8	Upstream bounding actively; hard to keep orientation; sweep out quickly	—→	Upstream orientating mostly; upstream moving begin; transfer to downstream slowly
4.0	Upstream bounding actively; hard to keep orientation; only few retain	—→	Upstream orientating mostly; upstream moving partially; sweep out begin
5.7	None remains		Upstream orientating mostly; upstream moving vigorously; sweep out more
8.4	None remains		Upstream orientating mostly; upstream moving partially; sweep out quickly
10.1	None remains		Upstream orientating mostly; seldom upstream moving; only few retain

Notes: The arrows in the list of 'Phase switch' represent the active migration between individuals in the water column and those on the substrate.

fauna in Australia, *Gladioferens* are similar to *Eurytemora* of the northern hemisphere and occupy similar niches. These copepods display temporary attachment to underwater surfaces to hold their position in moving water using fine hair sensillae on the dorsal surface of the prosome (Sheehy and Greenwood 1989; Rippingale 1994). However, whether this type of 'holding' is accompanied by rheotaxis, and whether copepods show a tendency for active attachment induced by flow, is not known. Our results demonstrate that the estuarine copepod *P. annandalei* performs pronounced rheotaxis in flow. This behavior assists these animals, especially those in the slow flow region near the bed (walls) of the flume channel, to maintain their position in near bottom flows ($u_{0.044}$) up to $3\,\mathrm{cm\,s}^{-1}$ when free-current velocity (U_∞) reaches $8.4\,\mathrm{cm\,s}^{-1}$. Copepods swimming in the water column can hardly benefit from upstream swimming, but they exhibit flow induced passive sinking in high flow velocity layers and active attachment to the flume bed when they sink to the low flow velocity layer near to the bottom (Figure 12). In contrast to *Gladioferens* which 'hold' to surfaces with their dorsal body sides (Sheehy and Greenwood 1989; Rippingale 1994), *P. annandalei* attaches to underwater surfaces ventrally and exhibits forage swimming to move upstream or sustain position.

Swimming velocity of copepods averaged 1.2–21mm s^{-1} (Buskey et al. 1993). The burst velocity of copepods can reach over $1000\,\mathrm{mm\,s}^{-1}$ in some species (Yen and Strickler 1996). The amazing burst of swimming speed enables the copepods to escape predators effectively, but are not suitable for sustained swimming against currents. Similarly, upstream bounding behavior could not help *P. annandalei* withstand the currents in our experiment. Although bounding may result in a sudden upstream movement, it increases the risk of losing balance and being flushed away downstream. To the contrary, the much slower but continuous swimming behavior of foraging seems important for *P. annandalei* to withstand flow. The forces produced by this motion were small relative to the mean channel flow speed and therefore were unable to keep the copepods swimming in the water column.
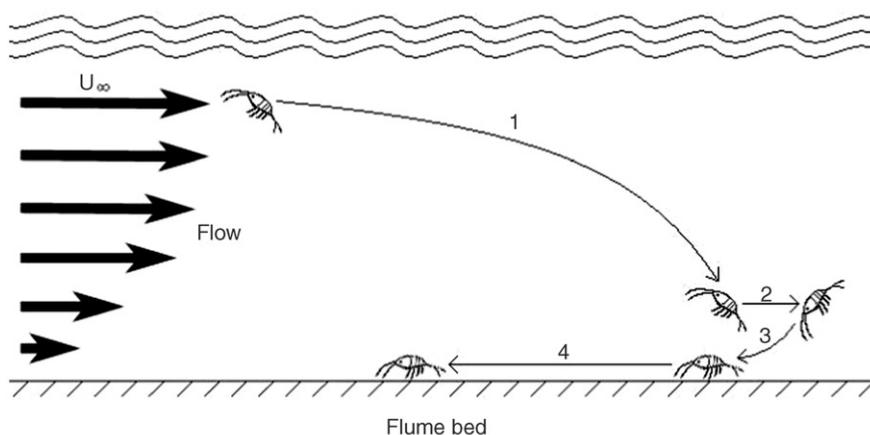

Figure 12. Schematic diagram of behavior of *Pseudodiaptomus annandalei* in flume flow. Bold arrows represent the flow characteristics over the flume bed. Arrow length indicates relative flow velocity; non-bold arrows represent tracks of *P. annandalei* in flow field: 1 – passive sinking; 2 – pose adjustment; 3 – active attachment; 4 – upstream foraging swim.

Both horizontal and vertical orientations affect the cross sectional area and therefore impact on resistance to flow. For individuals swimming in the water column, the difficulty of keeping their vertical orientation makes rheotactic behavior intermittent and eventually results in animals being swept away. This disadvantage resulted in only a few copepods in the water column bounding upstream at relatively high flow velocity (Figures 7 and 10). More of them chose to sink and attach to the flume bed. Vertical orientation was much easier for animals that were attached to the channel bottom. Being parallel to the flume bed allowed them to keep streamline in the current to minimize flow forces, just as other animals living under flow conditions do (Vogel 1981).

As a brackish water copepod that is widely distributed in estuarine and coastal regions, *Pseudodiaptomus* usually exhibit outstanding retention ability and often become one of the few predominant copepods in these waters with net seaward flow. They often reach tremendous population densities and hence play important roles in estuarine ecosystems (Jerling and Wooldridge 1991; Wooldridge 1999). A common explanation for the retention mechanism of copepods in estuaries is tidally-oriented vertical migration to make use of stratified flows. However, how can these copepods maintain their position without stratification? An investigation on the distribution of brackish-water zooplankton in the Chikugo river estuary during a heavy flood caused by rainfall and discharge from an upstream dam indicated that the zooplankton was almost completely swept out from the water column, but the examination of water trapped by a sediment grab sampler revealed that at least part of the adult population of *P. inopinus* was aggregated in a very thin layer above or clinging onto the bottom of the submerged channel outside the river mouth. After the flood, these settled animals returned into the river foremost (Ueda et al. 2004). This flow-induced substrate attachment enabled *P. inopinus* to survive floods and the same mechanism might contribute to the return after riverine flooding of a pioneer copepod, *P. hessei*, another member of *Pseudodiaptomus* (Wooldridge and Melville-Smith 1979). Does this mechanism only work during floods? In our study, *P. annandalei* can attach to the flume bed (walls) actively and orientate upstream against the flow. This may imply that maintaining position by rheotaxis is a widespread behavior among estuarine copepods. Furthermore, in the course of our samplings, the density of *P. annandalei* in the water column was much lower during ebb tide than during flood tide, and their abundance increased immediately after low tide (unpublished data). This indicates that the returned individuals could not be recruited from the downstream region. Their 'emergence' from bottom sediments *in situ* seemed to be a more reasonable explanation.

Besides being used to retain populations in estuaries, rheotaxis of *Pseudodiaptomus* may also provide a key mechanism for successful colonization. Three Asian representatives of *Pseudodiaptomus*, namely *P. forbesi*, *P. inopinus*, and *P. marinus*, invaded North America from the 1980s, settled down successfully and became predominant species there. Introductions of these copepods were apparently mediated by the discharge of ballast water (Cordell et al. 1992; Cohen and Carlton 1998). Mid-ocean exchange is thought to be an effective treatment to prevent aquatic invasions and some widely used methods, such as empty-refill and continuous flow-through, have demonstrated that these treatments are capable of replacing >95% of the original ballast water (Rigby 2001). The efficacy of the removal of organisms, however, is usually much lower than the proportion of water replaced (Bills et al. 2003). As for *Pseudodiaptomus*, they would be able to actively approach and cling to any solid surface inside ballast water tanks to resist the flow of the pumped out water. Moreover, as one of the basic biological characteristics of *Pseudodiaptomus*, rheotaxis might assist them to colonize new estuarine habitats. Due to strong flow resistance, these individuals could reside in river channels without being

dispersed into the sea outside of estuaries. Maintain positions closely in estuarine environment might also increase mating probabilities and hence could rapidly expand the population.

In conclusion, our experiments on the behavior of *P. annandalei* in flow indicated that current-induced rheotaxis is present in this species. This may also hold for other congeners and maybe some other copepod taxa that have to confront constant losses caused by unilateral flow transport. The rheotactic behavior, along with substrate attachment, not only enable these copepods to maintain their population in native estuaries, but also facilitates both their introduction into new habitats by ballast water and their subsequent colonization. Such behaviors raise issues for ballast water procedures and the control of aquatic invasions.

Acknowledgments

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