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Kinematics and Hydrodynamics of Cephalopod Turning Performance in Routine Swimming and Predatory Attacks

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KINEMATICS AND HYDRODYNAMICS OF CEPHALOPOD TURNING
PERFORMANCE IN ROUTINE SWIMMING AND PREDATORY ATTACKS

by

Rachel Anne Jastrebsky
B.S. December 2008, University of Rhode Island

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ABSTRACT

KINEMATICS AND HYDRODYNAMICS OF CEPHALOPOD TURNING PERFORMANCE IN ROUTINE SWIMMING AND PREDATORY ATTACKS

Rachel Jastrebsky
Old Dominion University, 2015
Director: Dr. Ian Bartol

Steady rectilinear swimming has received considerable attention in aquatic animal locomotion studies. Unsteady swimming movements, however, represent a large portion of many aquatic animals’ locomotive repertoire and have not been examined extensively. This study incorporates kinematic analyses of routine turning performance of brief squid Lolliguncula brevis and dwarf cuttlefish Sepia bandensis (Chapter 2), 3D velocimetry techniques to examine hydrodynamic turning performance of L. brevis (Chapter 3) and kinematic analyses of turning performance of L. brevis during predatory attacks on shrimp and fish prey (Chapter 4).

Both L. brevis and S. bandensis demonstrated high maneuverability, having the lowest measures of length-specific turning radii reported to date for any aquatic taxa. Lolliguncula brevis was more agile than S. bandensis, i.e., L. brevis exhibited higher angular velocities during turning. In L. brevis, jet flows were the principle driver of angular velocity. Asymmetric fin motions played a reduced role in turning, and arm wrapping increased turning performance to varying degrees depending on the species.

Flow patterns and relative torque contributions from the fins and jet varied with the speed of oncoming flow and orientation of the squid. Four turning categories were identified: (1) short tail-first turns, (2) long tail-first turns, (3) vertically oriented turns and (4) arms-first turns. The jet generally contributed more to turning torque than the fins in short tail-first, long tail-first and vertical turns. However, the fins produced a wider repertoire of flows, including isolated vortex rings, linked vortices and regions of elongated tubular vorticity, and were more important than the jet for turning torque generation during arms-first turns. Both the jet and fins produced torque contributing to roll and pitch, but the relative importance of these flows differed by turning category, with jet roll/pitch stabilization being critical for short tail-first turns and fin roll/pitch stabilization being integral to arms-first and vertical turns.

Squid attack sequences involved three phases: (1) approach, (2) strike and (3) recoil. Lolliguncula brevis employed different attack strategies for fish and shrimp and turning performance played a significant role during predatory encounters. The squid exhibited high
agility during the approach for both prey types. However, positioning, maneuverability and synchronized fin motions were more important for attacks on shrimp than fish. For attacks on fish, squid favored maximizing linear attack speeds over high maneuverability. Squid controlled their translational velocity and tentacle extension velocity during the strike, and demonstrated considerable rotational control during the recoil phase despite prey escape attempts.

This study represents the most comprehensive quantitative turning performance study of cephalopods to date and demonstrates that the unique body architecture of these taxa provides exceptional advantages for maneuvering in the marine environment.
This dissertation is dedicated to all of the people in my life that have helped me to
develop and grow into the person I am today.
ACKNOWLEDGEMENTS

There are many people who have contributed to the completion of this dissertation. This process especially would not have been possible without all of the support, input, patience and copious amounts of red ink from my major advisor. Dr. Ian Bartol -- thank you for not only conducting research with me and helping me to put together the words to bring that research to the world, but also for being a friend and mentor. I would like to thank my dissertation committee for their collaboration, support, patience and guidance. Dr. Paul Krueger deserves special recognition for all of his assistance with code, as well as for patiently answering my many fluid mechanics questions. My lab mate Carly -- chatting with you across our desk partition for the last five years has been an absolute pleasure. I will miss our trawling trips together and thank you for all of your help, tips and advice. My lab mates Kristy and Maggie -- you made this fun and enjoyable and I am glad to have made great, lasting friendships with you in the process. My family and friends -- thank you for the unending love and support; it has not gone unnoticed, and this work would have not been possible without it. Lastly, but certainly not least, thank you to my husband, Brian, for trawling trips, presentation run throughs, listening to me “geek out” over my results, lab construction checks, de-stressing workouts together, and all of the many many other ways that you have supported me through this process.

“This way of living that we once took for granted isn’t necessarily a ‘natural’ process at all. It’s not like water flowing down to the sea, not like aging. It takes effort, determination, conviction. But mostly it takes will. It takes a conscious decision to follow one difficult uphill path, and then the will to stay with it and not waver, to not give up.”

~John L. Parker Jr. in Again to Carthage
NOMENCLATURE

DML dorsal mantle length
COR center of rotation
$L$ total body length
$R$ radius of the center of rotation
$(R/L)_{\text{mean}}$ mean length specific turning radius
$(R/L)_{\text{min}}$ minimum length specific turning radius using a 90% cut-off
$(R/L)_{\text{amin}}$ absolute minimum length specific turning radius using a 90% cut-off
$\omega_{\text{avg}}$ mean angular velocity
$\omega_{\text{max}}$ maximum angular velocity
$\omega_{\text{amax}}$ absolute maximum angular velocity
Max $D$ maximum distance in cm between the COR at any two instances during the turn
$\Theta_v$ ventral angle between the arms and mantle
$\Theta_{v\text{min}}$ minimum ventral angle between the arms and mantle
$\Theta_{v\text{mean}}$ mean ventral angle between the arms and mantle
$\Theta_{\text{lam}}$ lateral angle between the arms and mantle
$\Theta_{\text{lmh}}$ lateral angle between the mantle and the horizontal
$\Theta_{\text{total}}$ total angular displacement
$T_y$ net torque acting about the yaw axis
$T_{xz}$ torque acting about the roll and pitch axes
$I$ hydrodynamic impulse
$I_A$ angular impulse

$T$ period of flow generation

$A_{\text{avg}}$ mean acceleration

$A_{\text{peak}}$ peak acceleration

$V_{\text{avg}}$ mean swimming velocity

$V_{\text{peak}}$ peak swimming velocity
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CHAPTER 1

INTRODUCTION

_Cephalopods and Swimming_

The class Cephalopoda, of the phylum Mollusca, is a diverse group of organisms living in habitats across the global marine spectrum. The class Cephalopoda contains two subclasses: Nautiloidea and Coleoidea. My research focused on the subclass Coleoidea, which includes the octopods, squids and cuttlefishes. All squid, octopus and cuttlefish species employ a pulsed jet to some degree for locomotion and ventilation of the gills (Boyle and Rodhouse, 2005), with a close coupling occurring between jet volume flux and oxygen extraction rates (O’Dor and Webber, 1991). The evolution of the jet in cephalopods may be related to animals pulling their head and body into their shell as a means of avoiding predators, resulting in the displacement of water and production of a crude jet (Hoar et al., 1994). This early use of jet propulsion probably involved little control of the jet stream, but nonetheless may have allowed early cephalopods to jettison themselves off the bottom into the water column and escape more benthically-constrained predators (Hoar et al., 1994). The subsequent development of a chambered shell in early cephalopods enabled them to achieve near-neutral buoyancy, helping early cephalopods maintain their position in the water column (Hoar et al., 1994). The ability to be neutrally buoyant coupled with a pulsed jet allowed cephalopods to dominate other species ecologically. This domination lasted until 530 million years ago during the Cambrian explosion when fishes appeared and began to out-compete some of the cephalopod species (Hoar et al., 1994; O’Dor and Webber, 1991). Today, many cephalopods still employ a pulsed jet that can be directed within a hemisphere below the body, providing them with high flexibility in movement.

Most cephalopods do not utilize the chambered shell, but some cephalopods use versions of this chambered shell for buoyancy, including nautilus, spirulidae squid, and cuttlefish. In addition to the chambered shell, cephalopods achieve buoyancy by several
other methods. For example, some oceanic octopods have a gelatinous body with very little muscle, and substitute sulphate ions for lighter chloride ions within the body. One family of squids (Gonatidae) stores large volumes of low-density fats for buoyancy. The majority of oceanic squid species have exchanged sodium for ammonium ions, creating a low-density fluid that allows them to achieve neutral buoyancy (Clarke et al., 1979; Seibel et al., 2004). Most squids lack an internal chambered shell and are either negatively or neutrally buoyant (Clarke et al., 1979). These two different strategies correlate closely with lifestyle, with more negatively buoyant squids tending to be more active swimmers, relying heavily on dynamic lift, and neutrally buoyant species are less active swimmers and reside deeper in the water column where visual predators are less abundant.

In addition to a pulsed jet, cuttlefish and squid both have fins that are used in swimming. These fins vary in form and function across species (Hoar et al., 1994). Fast-moving squid tend to have shorter, triangular fins that are beneficial for stability adjustments and drag reduction at high speeds but are less effective for slower undulatory swimming (O’Dor and Webber, 1991; Webber and O’Dor, 1985; Webber and O’Dor, 1986). In contrast, coastal squids and cuttlefishes generally have larger fins that, for some squid species and all cuttlefish species, extend along the length of the mantle. These larger fins are more suited for slower swimming as well as for maneuvering and stability (Anderson and DeMont, 2000; Bartol et al., 2001a). The fins are muscular hydrostats, consisting of a tightly packed three-dimensional array of musculature with no bony elements or fluid-filled cavities (Kier et al., 1989). The musculature provides the force and support for movement of the fins, and connective tissue provides support and elastic energy storage (Kier et al., 1989).

The muscular engines for locomotion

Squids and cuttlefishes employ a dual mode system for propulsion, involving a pulsed jet and paired fins. The pulsed jet is produced using mantle muscle fibers arranged in two different orientations (circumferentially and radially) and three networks of intramuscular collagen fibers (Thompson and Kier, 2001a). There are two phases of the
pulsed jet, an inhalant and exhalant phase. The inhalant phase of the jet is characterized by radial expansion of the mantle that causes an inflow of water into the mantle cavity through the anterior intakes (Bartol et al., 2001a; Bartol et al., 2008a; Bartol et al., 2009a; O’Dor, 1988). During this expansion of the mantle, the radial muscle fibers contract, thinning the mantle wall and increasing the mantle circumference (Gosline and Shadwick, 1983; Gosline et al., 1983; Thompson and Kier, 2001a; Thompson and Kier, 2001b). During the exhalant phase, contraction of circular muscles in the mantle decreases mantle circumference and increases the pressure in the mantle cavity. This increase in pressure closes the valves on the intake slots so that the water is driven out of the mantle cavity through the funnel, producing a thrust force that propels the cephalopod (Anderson and DeMont, 2000; Bartol et al., 2008a; Bartol et al., 2009a; Thompson and Kier, 2001b). The funnel is flexible and can be rotated within a hemisphere below the body, allowing the animal to move backwards, forwards, upwards, and side to side depending on the trajectory of the jet (Boyle and Rodhouse, 2005).

Intramuscular collagen fibers are arranged in three networks that originate and insert on the inner and outer tunics of the mantle (Thompson and Kier, 2001a; Thompson and Kier, 2001b). These fibers control changes in the mantle shape when the muscles contract during locomotion. The low fiber angles (10° to 15°) for the first network of intramuscular collagen fibers are thought to limit the elongation and thickening of the mantle during the exhalant phase of the jet. The second network of intramuscular collagen fibers are associated with the radial muscle bands and are arranged at a higher angle (55°) to the mantle surface. Both the second and first networks of fibers may resist increases in mantle thickness and restore mantle shape. The third network of intramuscular collagen fibers are arranged parallel to the circumferential muscle fibers. The three intramuscular fiber systems are thought to store elastic energy during the exhalant phase of the jet and help restore mantle shape to allow for refilling during the inhalant phase of jetting, augmenting or, in some cases, even replacing muscle action (Thompson and Kier, 2001a; Thompson and Kier, 2001b).

The fins of squid and cuttlefish move in complex patterns ranging from undulatory waves to pronounced flaps to produce thrust, maintain stability, and provide
lift (Bartol et al., 2001a; Hoar et al., 1994; Kier et al., 1989; Stewart et al., 2010). The complex motions are achievable using the muscular hydrostatic system (Kier et al., 1989). However, no cephalopods use fins as an exclusive means of locomotion, suggesting that the combination of the fins and jet is more effective than the fins alone (Hoar et al., 1994). Squid fin use changes with swimming speed. At low speeds the fins generally undulate to produce thrust and lift, whereas at intermediate speeds, both undulatory and flap-like motions have been observed (Bartol et al., 2001a; Hoar et al., 1994). At high speeds the fins roll up tightly against the mantle to reduce drag in many species (Hoar et al., 1994; O’Dor, 1988). Squid fins can operate either synchronously or asynchronously (see Chapter 2), which is useful for maneuverability and stability adjustments. Cuttlefish generally rely more extensively on their fins for locomotion compared to squid and can produce undulatory fin waves in opposite directions on each side of the body for turning (Hoar et al., 1994).

The complex motions of squid and cuttlefish fins are achieved using a similar muscular hydrostatic mechanism that involves sequential dorsal and ventral bending. Three muscle orientations must operate simultaneously to accomplish ventral and dorsal bending: (1) the dorsal transverse muscle bundles reduce the width of the dorsal portion relative to the ventral portion; (2) dorso-ventral muscles resist increases in the thickness of the fin; and (3) longitudinal muscles resist increases in the length of the fin (Kier et al., 1989). The dorsal bending of the fin is accomplished by simultaneously contracting the dorsal transverse muscle bundles and ventral dorso-ventral muscle bundles. Ventral bending of the fin is accomplished by simultaneously contracting the ventral transverse muscles and dorsal dorso-ventral muscles. The longitudinal dimension of the fin is controlled by contraction of the longitudinal muscles (Kier et al., 1989).

Hydrodynamics of the jet

Cephalopods produce a pulsatile jet for propulsion. Other animals that use this mode of locomotion include jellyfish and salps. The locomotor system in a hydromedusae, or jellyfish, is simplistic when compared to that of cephalopods and is powered by the contraction of circular muscles in the bell. Contraction of these circular
muscles reduces the diameter of the bell and expels water from the bell cavity, propelling the animal in the opposite direction (Demont and Gosline, 1988). The re-expansion of the bell is a passive process involving elastic recoil without muscle input. Both the squid mantle and bell of the hydromedusae are constant volume systems with negligible changes in length during contraction of the circular muscles. The elastic energy storage system involves elastic fibers embedded in the mantle/bell that store strain energy when the muscles’ mechanical output is not used to generate thrust in the jet cycle (Gosline and Shadwick, 1983). The volume of fluid expelled is proportional to the change in the radius squared (Demont and Gosline, 1988). The locomotor system in salps is similar to that of the squid mantle and the hydromedusae bell. Fluid enters the hollow body of the salp through the anterior oral siphon. The oral siphon then closes, circular muscles contract, the volume of the body chamber decreases and the fluid is expelled through the posterial atrial siphon (Madin, 1990; Sutherland and Madin, 2010). Salps differ from squid and hydromedusae by having incurrent and excurrent siphons on opposite ends of their bodies, allowing for unidirectional flow of fluid (Madin, 1990; Sutherland and Madin, 2010). This unidirectional flow strategy allows the salp to filter food during water intake (Madin, 1990).

For pulsed jets produced in stationary water, the rapid acceleration of water during the initiation of jet flow causes the leading part of the jet to roll up into a toroidal fluid mass known as a vortex ring (Dabiri and Gharib, 2005; Krueger and Gharib, 2003). Though there has been some debate over whether such leading edge vortices form in the wakes of biological jetters, as the jet is issued with a co-flow component around the aperture exit (Anderson and Grosenbaugh, 2005), several studies have shown that vortex rings are common features of jet wakes in squid, jellyfish, and salps (Bartol et al., 2001a; Bartol et al., 2008a; Bartol et al., 2009a; Bartol et al., 2009b; Bartol et al., in press; Dabiri et al., 2005; Dabiri et al., 2006; Dabiri et al., 2010; Katija et al., 2015; Madin, 1990; Sutherland and Madin, 2010). Based on mechanical jet studies, a pulsed jet with a leading edge vortex ring produces greater impulse per pulse than an equivalent steady jet of fluid (Dabiri and Gharib, 2005; Krueger and Gharib, 2003). This added impulse effect derives from jet ‘overpressure’ i.e., fluid pressure above the local ambient pressure during jet
ejection (Krueger and Gharib, 2003). This overpressure is produced when ambient fluid is accelerated by entrainment and added mass effects (Bartol et al., 2009a; Krueger and Gharib, 2003) and is one of two important components contributing to thrust production during pulsed jetting. The other component is the impulse per pulse supplied by the jet momentum flux (Bartol et al., 2009a; Krueger and Gharib, 2003). In steady subsonic jets the overpressure component is zero, but in pulsed jets with leading edge vortex rings, overpressure may contribute as much as 42% to the total impulse of the jet (Krueger, 2001; Krueger and Gharib, 2003; Krueger and Gharib, 2005). This thrust augmentation effect known to accompany short jets in tethered mechanical jet studies (Krueger and Gharib, 2003; Krueger and Gharib, 2005) can also lead to enhanced propulsive efficiency in freely swimming squid (Bartol et al., 2009a).

Stroke ratio, defined as the ratio of the length of the ejected fluid (L) to the diameter of the jet aperture (D), also plays an important role in pulsed jetting. Based on work by Gharib et al. (1998), there is a specific stroke ratio where vortex rings stop forming midway through the pulse and pinch-off from the trailing jet. This specific stroke ratio where pinch-off occurs is called the formation number (F) (Gharib et al., 1998). Once F is reached, the remainder of the jet downstream of pinch-off contributes no overpressure and behaves much like a steady jet. Thus, to maximize propulsive efficiency, it would seem reasonable to expect jetters to produce short jets near F. In squid, jetting behavior related to F is complex. Bartol et al. (2009a) observed two distinct jet modes in juvenile and adult brief squid Lolliguncula brevis. In the first mode, ejected fluid rolls up into an isolated vortex ring. This first mode is associated with greater propulsive efficiency, lower slip and higher frequency of fin activity. In the second mode the ejected fluid develops into a leading vortex ring that pinches off from a long trailing jet. The second mode is associated with greater time-averaged thrust and lift forces and is used more heavily than the first jet mode. Jets that are near F can produce more thrust per amount of expelled fluid than longer duration jets (Bartol et al., 2009a; Krueger and Gharib, 2003) and Bartol et al. (2009a) found that jets near F can also lead to higher propulsive efficiency.
For paralarvae, Bartol et al. (2009b) observed no clear pinch-off (i.e., no clear F), only elongated vortex rings, and suggested this may be a product of either (1) viscous diffusion blurring the separation between the ring and jet or (2) vortex rings whose formation has been preempted by viscous diffusion such that a vortical tail remains behind the ring. In adult squid, pinch-off does occur (i.e., F is observable) and squid have the ability to form jets near F (Bartol et al., 2009a; Bartol et al., 2009b). However, despite the ability of squid to form jets near F, they do not always do so, which may be a product of their dual mode system, i.e., jets near F may be used when the fins are active and less jet thrust is required but jets exceeding F may be unavoidable and used when the fins contribute minimal thrust.

Though a number of studies investigating steady jet-propelled swimming in cephalopods have been performed (Anderson and Grosenbaugh, 2005; Bartol et al., 2001a; Bartol et al., 2001b; Bartol et al., 2008a; Bartol et al., 2009a; Bartol et al., 2009b; O’Dor, 1988; Stewart et al., 2010; Wells and O’Dor, 1991), no studies on jet hydrodynamics during maneuvering have been conducted to date.

Hydrodynamics of the fins

Squid and cuttlefish swim using a combination of jet pulses and fin undulations (O’Dor, 1988; Stewart et al., 2010). Not only do squid employ multiple jet modes during swimming as described earlier, there is evidence that they produce different fin modes or gaits as well. At lower speeds, the opalescent squid *Loligo opalescens* exhibit two fin flaps for each mantle contraction. At intermediate speeds, only one fin flap is produced for each mantle contraction, and at the highest speeds, the fins roll tightly against the mantle (O’Dor, 1988). These changes in mode/gait are most characteristic of pelagic, fast-moving squid that have high-aspect ratio, triangular fins (O’Dor and Webber, 1991). Bartol et al. (2001) found that in *Lolliguncula brevis* the fins are important for both vertical lift and horizontal thrust production over a broad range of swimming speeds. Fin activity is thought to be more economical than jetting (Hoar et al., 1994) and cannot be characterized as strictly drag or lift-based propulsion. Lift-based propulsion is observed more frequently at intermediate to high speeds when the fins move parallel to the
dorsoventral axis, such that positive thrust can be achieved during both the upstroke and downstroke (Bartol et al., 2001a; Vogel, 1994). In this way, fin propulsion generates continuous lift and thrust throughout the fin cycle and affects a relatively large volume of water with each fin stroke (Bartol et al., 2001a). Anderson and DeMont (2005) found that *Loligo pealeii* transitioned to lift-based thrust production when swimming speed exceeded the speed of fin waves, and fin flapping was more pronounced. Drag-based propulsion is more effective at lower speeds when the fin moves backwards faster than the animal moves forwards (Bartol et al., 2001a). Although lift based fin propulsion is favored at higher speeds, fin activity also decreases with increased swimming speed and ultimately stops at the highest swimming speeds because of force and support limitations (Bartol et al., 2001a; Kier et al., 1989). In general, fin activity is more frequent during mantle contraction, but fin motions also have been observed during mantle refilling, which helps mitigate abrupt deceleration during the jet cycle (Bartol et al., 2001a; Anderson and DeMont, 2000). This coordination of the fins and jet has the potential to enhance propulsive efficiency as a powerful fin flap at the start of jetting can reduce jet thrust requirements for a given speed. Both Bartol et al. (2001a) and Anderson and Demont (2000) found that this coordination was most prominent at low/intermediate swimming speeds.

Stewart et al. (2010) were the first to directly quantify force production in the fins of a cephalopod using bulk properties in the fin wake. Using digital particle image velocimetry (DPIV), Stewart et al. (2010) identified four fin modes in the brief squid *Lolliguncula brevis*, all of which occur during tail-first swimming and two of which occur during arms-first swimming. The first fin mode, which occurs at low speeds, involves the shedding of one vortex during each downstroke of the fin, with little vorticity being associated with the upstroke. The absence of pronounced upward-directed fin flows during the upstroke is beneficial for maintaining vertical position in the water column, as brief squid are negatively buoyant. In the second fin mode, a continuous linked chain of vortices is produced by undulating the fins, resulting in the production of significant lift but limited thrust. Stewart et al. (2010) proposed that the pulsed jet plays a greater role in thrust production for this fin mode. The third fin mode involves the
generation of one vortex ring for each downstroke and one vortex ring for each upstroke of the fin. In the fourth fin mode, a discontinuous chain of linked vortices is shed. Both the third and fourth fin modes generate more thrust than the other modes. In the tail-first swimming orientation, the fins act as stabilizers at low speeds and switch to propulsors at higher speeds, with dynamic lift increasing with swimming speed. In the arms-first swimming orientation, where only modes two and three above are employed, the fins generally provide lift with reduced thrust production. Arms-first swimming is used primarily at low and intermediate speeds for maneuvering and investigating prey items, whereas tail-first swimming is used for sustained swimming over a wide range of speeds and escape responses (Bartol et al., 2001a; Stewart et al., 2010).

Squid and cuttlefish both have lateral fins that aid in maintaining and controlling orientation while swimming (Boyle and Rodhouse, 2005). However, the fins of most cuttlefish species extend along the entire length of the mantle, a feature that distinguishes them from many squids (Boyle and Rodhouse, 2005), and these long marginal fins are capable of producing multiple propulsive waves through undulatory movements (Hoar et al., 1994). Cuttlefish rely primarily on these undulatory motions of the fins for locomotory translation and hovering, with the jet being used for escape responses, and possibly turning maneuvers (Kier et al., 1989; O’Dor and Webber, 1991). The undulatory fin waves can travel either direction along the length of the fin depending on the direction the animal is swimming (Kier et al., 1989). During hovering or resting, the undulatory waves are small in amplitude, but during feeding, rapid locomotion and maneuvering, fin motions increase in frequency and amplitude (Kier et al., 1989). The speeds that these animals can achieve using undulatory motions of the fins may be limited by the shortening speeds of the obliquely striated muscles in the muscular hydrostatic fin system (Kier et al., 1989). The control and coordination of fin-beat amplitude, force and direction is complex and likely involves feedback from mechanoreceptors found along the length of the fin. This sensory feedback may be especially important during maneuvering (Kier et al., 1985; Kier et al., 1989). Though a few studies have investigated fin hydrodynamics during steady swimming (Bartol et al., in press; Bartol et al., 2001a;
Bartol et al., 2008; Stewart et al., 2010), there have been no studies of fin hydrodynamics during turning in any cephalopod.

*Buoyancy*

Cuttlefish differ from squids and octopods in that they regulate their buoyancy using gas and fluid retained within a cuttlebone. The compartments of a cuttlebone are similar to those in a Nautilus shell except that they are more flattened and laminated (Denton and Gilpin-Brown, 1961; Denton and Gilpin-Brown, 1973). The cuttlebone consists primarily of calcified chitin and accounts for about 9.3% of the animal’s volume (Denton and Gilpin-Brown, 1961; Denton and Gilpin-Brown, 1973). The cuttlefish can change its density and position in the water column by varying the amount of liquid in the chambers of the cuttlebone. This liquid is added or removed though the siphuncular wall of the cuttlebone (Denton and Gilpin-Brown, 1961; Denton and Gilpin-Brown, 1973). The spaces between the laminae of the cuttlebone are partially filled with a nitrogen rich gas, and the control of the volume of the gas space is regulated by pumping salts out of the fluid in the cuttlebone (Denton and Gilpin-Brown, 1961; Denton and Gilpin-Brown, 1973). This pumping action ensures that the osmotic pressure across the membrane is in balance with the hydrostatic pressure that tends to force water into the shell (Denton and Gilpin-Brown, 1973), thus preventing water from entering the cuttlebone as the animal dives to deeper depths (Denton and Gilpin-Brown, 1961). A major advantage of this cuttlebone system is that it allows the cuttlefish to achieve neutral buoyancy without having to contend with volume changes of the gas chamber itself, as is the case with swimbladders in many fishes. The impact of buoyancy on turning performance is not well known in cephalopods, but comparisons between negatively buoyant squids and neutrally buoyant cuttlefishes can potentially provide useful insights into buoyancy’s role in turning performance.

*Kinematics: Maneuverability and Agility*

Turning in aquatic taxa is an unsteady motion that is less well studied than steady rectilinear locomotion, which only constitutes a small proportion of an animal’s
locomotive repertoire (Drucker and Lauder, 2001b). Unsteady movements that involve changes in heading, speed and acceleration are commonly observed in many species of aquatic taxa and are integral to their ecology, as these motions are used for predator avoidance, prey attacks, and navigation of complex habitats. Two important parameters for assessing unsteady motions are maneuverability and agility. Maneuverability is defined as the ability to turn in a confined space, and is measured as the length-specific minimum radius of the turning path ($r_{\text{path}}/L$, where $L$ is total body length) (Walker, 2000). Agility is defined as the rate of turning or the translation of a vertical axis across a horizontal plane, measured as the average and maximum angular velocity ($\omega_{\text{max}}$) during turning (Walker, 2000). Exceptional turning performance is characterized by a swimmer’s ability to exhibit both high agility and high maneuverability (Rivera et al., 2006).

Most of the aquatic turning performance work to date has focused on vertebrates, namely freshwater turtles, marine mammals and fishes. Maneuvering research on fishes has primarily focused on the importance of body flexibility in turning performance (Blake et al., 1995; Domenici and Blake, 1991; Gray, 1933; Webb, 1983; Weihs, 1972) and acceleration during fast-starts (Domenici and Blake, 1991; Domenici and Blake, 1997; Harper and Blake, 1990; Kasapi et al., 1993; Webb, 1994). However, the roles of propulsors and control surfaces in turning performance have not been investigated extensively. Drucker and Lauder, (2001b) investigated the asymmetrical forces generated by the pectoral fins of the bluegill sunfish during turning. The strong side fin generates a vortex with a larger laterally directed force than during steady swimming and effectively acts to rotate the body away and change the fish’s heading. In contrast, the weak side fin creates anteriorly directed forces, moves slower, produces minimal lateral force and has a primary role in body translation. These results indicate that turning involves asymmetrical movements of the fins and fluid forces that are different in both magnitude and direction from those produced during steady swimming (Drucker and Lauder, 2001b). This study was one of the first to use DPIV (Digital Particle Image Velocimetry) to examine hydrodynamic properties of unsteady maneuvers in nektonic animals and the fluid forces produced by control surfaces such as the fins.
Aquatic taxa considered in turning performance studies traditionally fall under three classifications: (1) flexible-bodied, (2) stiff-bodied and (3) rigid-bodied. Flexible-bodied animals include many species of ray-finned fishes, sharks and some marine mammals, such as sea lions and dolphins (Fish, 2002; Fish et al., 2003; Maresh et al., 2004). Stiff-bodied animals include large cetaceans and the thick-skinned tuna (Blake et al., 1995; Fish, 2002). Having a stiff body, as in the yellowfin tuna, tends to limit maneuverability as the body is not as bendable, limiting the animal’s ability to complete turns in a confined space (Blake et al., 1995; Fish, 2002). More flexible bodied animals, such as the angelfish, sea lion and knifefish, tend to achieve not only greater maneuverability than stiff-bodied animals but greater agility as well (Domenici and Blake, 1997). Rigid-bodied animals, which are not able to bend their body axis due to an exoskeleton, hard carapace or internal shell, should also exhibit more limited maneuverability and agility relative to more flexible-bodied nekton. This is because the inability to bend the body axis precludes the animal from effectively turning in tight spaces (limiting agility) and reduces the body’s second moment of area about the dorsoventral rotational axis, resulting in high inertial resistance to rotation (limiting maneuverability) (Walker, 2000).

Though it seems reasonable to conclude that more rigid-bodied nekton have more limited maneuverability and agility than flexible-bodied nekton, current data does not always support this presumption. Boxfishes, which have 2/3 – 3/4 of their bodies encased in a rigid carapace, are considered rigid-bodied and are highly maneuverable relative to flexible-bodied animals, but not very agile (Blake, 1977; Walker, 2000). The high level of maneuverability derives from their ability to rotate along a tight vertical axis. The whirligig beetle is another rigid-bodied swimmer. In contrast to boxfish, however, it is highly agile, even in comparison to flexible-bodied animals, but not very maneuverable (Fish and Nicastro, 2003). Both of these animals use propulsors to aid their turning performance (Fish and Nicastro, 2003; Walker, 2000). Boxfish use oscillating and undulating movements of the pectoral, dorsal and anal fins during turning maneuvers (Blake, 1977). Whirligig beetles use asymmetrical paddling motions of the outboard legs to turn as well as abduction of the inboard elytra (a modified, hardened forewing) and
sculling of the wing (Fish and Nicastro, 2003). Rivera et al., (2006) investigated turning performance in the painted turtle, Chrysemys picta, another rigid-bodied swimmer, and found that it is relatively similar to boxfish in terms of maneuverability. However the turtle’s shell morphology and limb positioning facilitates greater agility than that observed in boxfish.

Cuttlefish and squid are unique in that they do not fall neatly in any of the three body categories described above. Both groups have internal structures (cuttlebone or chitinous pen) that limit appreciable longitudinal length changes and bending along the mantle, much like the carapace of a rigid-bodied boxfish does. On the other hand, the arms are highly flexible, even to a higher degree than the bodies of flexible-bodied nekton. Moreover, cuttlefish and squids use two fundamentally distinct propulsors for turning (fins and jet) and have a number of control surfaces (fins and keeled arms). Thus, squids and cuttlefishes represent a unique group for comparison with previously studied nekton. To date, only Foyle and O’Dor (1988) have included data on turning performance in a cephalopod, indicating that squid have a length specific turning radius of about 0.5 body lengths. This study investigated predatory behavior and feeding strategies. Documenting the acceleration of the approach was the primary objective of the study; all other kinematic variables were estimated and only considered briefly (Foyle and O’Dor, 1988). Thus, more quantitative research is needed to determine how maneuverable and agile cephalopods are, especially considering the wide suite of behaviors that involve turning.

In addition to Foyle and O’Dor’s (1988) study of squid, some other studies have examined cephalopod behaviors that have relevance for turning. Cuttlefish live in complex habitats and engage in predator-prey interactions that require them to change direction frequently (Hanlon and Messenger, 1996). Cuttlefish are ambush predators, camouflaging themselves and lying still in wait of prey (Hanlon and Messenger, 1996; Messenger, 1968), while visually orienting to their prey (Messenger, 1968). As the prey approaches, the cuttlefish will frequently reposition itself with its fins to maintain the appropriate attack distance and orientation, requiring quick, sharp turning (Hanlon and Messenger, 1996; Messenger, 1968). Squid orient to their prey in a similar manner and
must be able to make rapid positional adjustments to capture prey (Hanlon and Messenger, 1996). Some species have even been observed swimming backwards (tail-first) towards prey and then turning quickly to seize the prey with the tentacles and arms in an arms-first orientation (Hanlon and Messenger, 1996; Squires, 1966). Cuttlefish and squid undergo similar turning behaviors when evading predators and navigating through complex habitats. Quantification of agility and maneuverability in cuttlefish and squid will provide important data on the limits of turning performance and critical insight into how cephalopods make complex movements in their habitats.

**Cuttlefish and Squid Predatory Attack**

Squid and cuttlefish are equipped with eight arms and a pair of tentacles. The tentacles are longer than the arms and although the arms have two rows of suckers along their entire length, the suckers on the tentacles are limited to four rows on the expanded club (Kier, 1982). The tentacles lack bony elements and operate as muscular hydrostats, rapidly elongating during prey capture (Kier, 1982; Van Leeuwen et al., 2000). The arms are used to hold and manipulate the prey once it has been captured (Kier, 1982). This rapid elongation by the tentacles is accomplished by contraction of extensor muscles that have very short sarcomeres. Short sarcomeres can only generate a small amount of force but have high strain rates for a given sliding velocity (Van Leeuwen et al., 2000). This lengthening is coupled with a decrease in radius. The tentacles are then retracted by contracting the longitudinal muscles (Kier, 1982). In this retracted state, the tentacles have a low length to width ratio which provides the system with mechanical amplification similar to a lever system (Kier and Smith, 1985). Any decrease in length of the tentacle is accompanied by a lesser increase in diameter allowing the structure to operate over a greater range of extension and contraction (Kier and Smith, 1985).

Messenger (1968) described cuttlefish, *Sepia officinalis*, prey strikes as occurring in three parts. The first part, known as attention, is characterized by the cuttlefish reacting to the presence of a prey item by movement of the eyes and color changes to the arms, head and mantle. The head and body will turn toward the prey, and sometimes the cuttlefish will raise its first pair of arms in an S-shape and wave them from side to side
The second part of the attack, known as positioning, occurs after the cuttlefish has turned so that it is now facing the prey, i.e., oriented arms-first relative to the prey. The cuttlefish may then stay where it is or move towards or away from the prey along the prey-body axis (Messenger, 1968). The cuttlefish usually positions itself so that the arms are about one mantle length away from the prey. The first pair of arms may still be extended upwards (Messenger, 1968). If the prey has moved more than 2 to 3 cm during the positioning phase, the cuttlefish will withdraw the tentacles (if it has started to extend them) and reposition, returning to the attention phase if necessary. The third part of the attack, known as seizure, is an all or none phenomenon. At the beginning of this phase, the tentacles may be partly extended, the first arm pair is erect and the rate of fin beat increases (Messenger, 1968). The cuttlefish will then swim forward quickly about 2 cm and rapidly extend and retract the tentacles. The ends of the tentacles strike the prey and buckle to some degree during impact before being retracted. Although Messenger’s (1968) study provides valuable qualitative observations of cuttlefish prey attacks, high-speed kinematic analysis of these movements is necessary for a more quantitative assessment of these encounters. This analysis could include measurements of tentacle trajectories, body positioning during approach and attack, fin movements, swimming velocity and acceleration, and response latencies like those calculated by Weihs and Webb (1984) for fish predators.

Limited kinematic data on prey attacks in squid are also available. Kier and Van Leeuwen (1997) investigated tentacle striking for *Loligo pealei*. A prey strike includes the approach of the animal, initial elongation of the tentacles followed by slight tentacle shortening, and then the tentacle strike. The tentacular strike is an explosive, very rapid elongation. The arms are flared before the strike is initiated, which may provide stability and alignment to the tentacle stalks. In some prey strikes the tentacles were not extended. Instead the animal swam forward, flared the arms and enclosed the prey in the arms without tentacle movements (Kier and Van Leeuwen, 1997). This study reported a strike swimming velocity ranging from 0.7 to 1.2 m s\(^{-1}\). The tentacles had a maximum extension velocity of >2 m s\(^{-1}\) with peak accelerations >250 m s\(^{-2}\). Buckling was observed after the tentacular clubs contacted the prey (Kier and Van Leeuwen, 1997).
The primary objective of this study was to document movements of the head and tentacles during a prey strike. Neither the kinematics of the approach to the prey nor the fin motions were examined. Moreover, only one camera, positioned in a dorsal perspective, was used, thus the angles of the mantle, arms and funnel were not quantified.

As mentioned above, Foyle and O’Dor (1988) conducted a study on predatory strategies of the squid *Illex illecebrosus* and made observations of the approach as well as measured the acceleration rate during the prey strike. Two distinct feeding patterns were observed. Rapid head-first attacks occurred with smaller prey, whereas a long tracking phase was present for larger prey (Foyle and O’Dor, 1988). The turning radius and rotation rate were estimated at 0.5 body lengths and 90° s\(^{-1}\) during these encounters. However, the researchers measured movements at a low frame rate (30 frames s\(^{-1}\)) and did not record detailed kinematic measurements of the interactions, suggesting that their performance measurements are not accurate representations of squid performance capabilities.

*Project Objectives*

Most of the literature concerning swimming behaviors in aquatic taxa has focused on rectilinear swimming. However, unsteady maneuvers represent a large proportion of an aquatic animal’s locomotive repertoire and should be considered for a comprehensive assessment of swimming performance. Investigations of turning performance have focused on selected fishes (Blake et al., 1995; Domenici and Blake, 1997; Domenici et al., 2004; Kajiura et al., 2003; Parson et al., 2011; Walker, 2000; Webb and Fairchild, 2001; Webb and Keyes, 1981), marine mammals (Fish, 2002; Fish et al., 2003; Maresh et al., 2004), one species of aquatic beetle (Fish and Nicastro, 2003), and one species of aquatic turtle (Rivera et al., 2006). Ray-finned fishes and many marine mammals are considered flexible bodied, allowing them to curve their bodies and presumably exhibit high levels of agility and maneuverability. On the other hand, rigid-bodied animals cannot bend their body axis, requiring them to use propulsors and control surfaces to accomplish unsteady maneuvers such as turning. Although the assumption is that rigid-bodied locomotors have lower agility and maneuverability than flexible-bodied
swimmers, the limited studies performed on rigid-bodied swimmers to date suggest that this assumption may be incorrect.

Squids and cuttlefishes are unique in that they do not fit neatly in either the flexible-bodied or rigid-bodied camp. They lack a hard exterior and have flexible arms and tentacles, but they are constrained by a mantle of relatively fixed length as a result of their chitinous pen or cuttlebone. The flexible arms add to the total body length but can be manipulated in various ways relative to the mantle, which can potentially minimize the length-specific radius of the turn. Therefore, squid and cuttlefish represent an interesting group for comparison to nekton studied to date.

For my doctoral research I investigated turning performance using three separate but complementary lines of investigation: (1) kinematic analysis of body and appendage movements during routine turns (Chapter 2), (2) 3D hydrodynamic analysis of turns (Chapter 3), and (3) kinematic analysis of the approach and strike of prey (Chapter 4). For Chapter 2, high-speed video was used to track mantle, fin, and funnel body features and Matlab routines were used to measure the radius of the center of rotation (a metric for maneuverability) and angular velocity of turns (a metric for agility). The goal was to document how the body and appendages are used to achieve turns and quantitatively measure squid and cuttlefish turning performance. The hydrodynamic analysis (Chapter 3) involved the use of DDPTV (Defocusing Digital Particle Tracking Velocimetry) to quantitatively measure force and torque contributions and vortical flow patterns produced by the jet and fins during turns. The advantage of the DDPTV approach is that it allows for quantification of true 3D flow fields from both the fins and jet, which is required to understand the complex asymmetrical vortex-wake flows associated with turns. Finally, in Chapter 4 high-speed video and Matlab routines were used to quantify measures of turning performance, the speed of approach and tentacle strikes, body positioning, tentacle positioning, arm positioning and fin movements during predator-prey encounters with fish and shrimp. Considered collectively, these three lines of investigation provided a comprehensive quantitative picture of turning performance during routine swimming in squid and cuttlefish and during predatory attacks in squid.
CHAPTER 2

TURNING PERFORMANCE IN SQUID AND CUTTLEFISH: UNIQUE DUAL MODE, MUSCULAR HYDROSTATIC SYSTEMS

Introduction

Many studies on aquatic locomotion have focused on steady rectilinear swimming of fishes (Bartol et al., 2008b; Blake et al., 1995; Domenici and Blake, 1991; Domenici and Blake, 1997; Drucker and Lauder, 1999; Drucker and Lauder, 2000; Gray, 1933; Harper and Blake, 1990; Kasapi et al., 1993; Liao et al., 2003; Maia and Wilga, 2013; Webb, 1975; Webb, 1978a; Webb, 1983; Wilga and Lauder, 2000), cephalopods (Anderson and Grosenbaugh, 2005; Bartol et al., 2001a; Bartol et al., 2001b; Bartol et al., 2008a; Bartol et al., 2009a; Bartol et al., 2009b; O'Dor, 1988; Stewart et al., 2010; Wells and O'Dor, 1991), and marine mammals (Fish, 1993; Fish, 1994; Fish et al., 2008).

Although these studies have provided valuable information on swimming performance, much less is known about unsteady and intermittent swimming movements. Unsteady mechanisms comprise a significant portion of the locomotive repertoire for most aquatic taxa and are ecologically important for capturing prey, eluding predators, and navigating through complex habitats (Webb, 1983; Weihs, 1972; Weihs, 1993).

Two important parameters for assessing unsteady motions are maneuverability and agility. Maneuverability is the ability to turn in a confined space, and is defined as the length-specific radius of the turning path ($R/L$), where $R$ is the radius of the turning path and $L$ is total body length (Walker, 2000). Agility is the rate of turning, and defined as the average and maximum angular velocity, $\omega_{\text{avg}}$ and $\omega_{\text{max}}$, during turning (Norberg and Rayner, 1978; Webb, 1994). Exceptional turning performance is characterized by a swimmer’s ability to exhibit both high agility and high maneuverability (Norberg and Rayner, 1978; Webb, 1994).

The role of body flexibility in turning performance has been considered in a variety of aquatic taxa and aquatic animals are often placed in three general classifications: (1) flexible-bodied, (2) stiff-bodied and (3) rigid-bodied. These
classifications derive from Webb (1984) and relate to transient and sustained swimming preferences. Flexible-bodied animals include many species of ray-finned fishes, some smaller sharks and some marine mammals, such as sea lions (Fish, 2002; Fish et al., 2003; Maresh et al., 2004; Webb, 1984). Paired fins/appendages are generally present and help to control turns in these flexible-bodied nekton (Webb, 1984). Stiff-bodied animals, including large cetaceans and thick-skinned tuna, tend to be streamlined and have a stiff body that is deepest about halfway between the head and tail, and often possess a deep, narrow caudal fin. This body form maximizes thrust while reducing drag (Blake et al., 1995; Fish, 2002; Webb, 1984). Finally, rigid-bodied animals are not able to bend their body axis due to an exoskeleton, hard carapace or internal shell, and include animals, such as boxfish, aquatic beetles, and aquatic turtles. Highly flexible-bodied animals, such as sea lions, spiny dogfish, and knifefish, tend to achieve not only greater maneuverability than stiff-bodied and rigid-bodied animals but greater agility as well (Domenici and Blake, 1997; Domenici et al., 2004; Fish et al., 2003). This is not surprising given that more rigid bodies limit body axis bending, precluding turning effectively in tight spaces (limiting maneuverability) and reducing the body’s second moment of inertia about the dorsoventral rotational axis, resulting in high inertial resistance to rotation (limiting agility) (Walker, 2000). Rigid bodies also result in relatively high pressure drag resisting rotation since the angle of attack of the body and local flow is close to 90° along the length of the body (Walker, 2000).

Though it seems reasonable to conclude that more rigid-bodied nekton have limited maneuverability and agility relative to flexible-bodied nekton as stated above, certain studies reveal this is not always the case. For example, boxfishes, which have 2/3-3/4 of their bodies encased in a rigid carapace, are highly maneuverable relative to flexible-bodied animals, but not very agile (Blake, 1977; Walker, 2000). The high level of maneuverability derives from their ability to rotate along a tight vertical axis using oscillating and undulating movements of the pectoral, dorsal and anal fins, while the caudal fin acts as a rudder (Blake, 1977; Walker, 2000). The whirligig beetle is another rigid-bodied swimmer, but unlike boxfish, is highly agile with limited maneuverability. Whirligig beetles use asymmetrical paddling motions of the outboard legs to turn as well
as abduction of the inboard elytra (a modified, hardened forewing) and sculling of the wing (Fish and Nicastro, 2003). Rivera et al. (2006) investigated turning performance in the painted turtle, Chrysemys picta, another rigid-bodied swimmer, and found that it is relatively similar to the boxfish in terms of maneuverability. However, the turtle’s shell morphology and limb positioning facilitates greater agility than that observed in boxfish.

Cuttlefishes and squids are unique in that they do not fall neatly in any of the three body categories described above. Squids and cuttlefishes possess structures (cuttlebone or chitinous pen) that limit appreciable longitudinal length changes and bending along the mantle, much like the carapace of a rigid-bodied boxfish. However, the arms, which extend outward from the head, are highly flexible, even to a higher degree than the bodies of flexible-bodied nekton.

An additional distinction is that cuttlefishes and squids use two fundamentally distinct propulsors for turning (fins and jet) and have a number of control surfaces (fins and keeled arms). The dual mode system of a pulsed jet and paired fins is powered by muscular hydrostats, or tightly packed, three-dimensional muscular arrays that lack hardened skeletal support elements (Kier et al., 1989). The pulsed jet is generated in two phases, an inhalant and exhalant phase. During the inhalant phase, radial expansion of the mantle causes an inflow of water into the mantle cavity through intakes located at the anterior portion of the mantle (O’Dor, 1988). During the exhalant phase, circular muscles in the mantle contract, decreasing mantle circumference and increasing the pressure in the mantle cavity. The increase in pressure closes the slots at the anterior intakes so that the water in the mantle cavity is forced out through the funnel, producing a thrust force that propels the cephalopod (Anderson and DeMont, 2000; Bartol et al., 2008a; Bartol et al., 2009a; Thompson and Kier, 2001b). The funnel is flexible and can be rotated within a hemisphere below the body, allowing the animal to move backwards, forwards, upwards, and sideways depending on the trajectory of the jet (Boyle and Rodhouse, 2005). The fins of squid and cuttlefish move in complex patterns ranging from undulatory waves to pronounced flaps to produce thrust, maintain stability, and provide lift (Bartol et al., 2001a; Bartol et al., 2001b; Hoar et al., 1994; Kier et al., 1989; Stewart et al., 2010). Cuttlefish can produce undulatory fin waves in opposite directions on each side of the
body and generally rely more heavily on their fins for locomotion compared to squid (Hoar et al., 1994). The cuttlebone of cuttlefishes allows them to regulate and achieve desired buoyancy levels (Denton and Gilpin-Brown, 1973), a mechanism that is absent in most squids. In fact, many inshore, coastal squid species, such as *Lolliguncula brevis*, are negatively buoyant and must expend energy swimming to remain at a position in the water column (Bartol et al., 2001a; Bartol et al., 2001b). Given their distinct body flexibility and propulsion system characteristics, squids and cuttlefishes represent a unique group for comparison with previously studied nekton.

This study investigated turning performance of two cephalopod species with very different morphologies and locomotory strategies than previously studied aquatic animals. The two species considered here were the coastal inshore squid species, *Lolliguncula brevis*, and the tropical coastal cuttlefish species, *Sepia bandensis*, which differ morphologically and physiologically. The paired fins of *L. brevis* are relatively short and rounded, and the fins of *S. bandensis* extend along the length of the mantle, though they are not especially broad in span. *Lolliguncula brevis* is negatively buoyant and must expend considerable energy maintaining position in the water column (Bartol et al., 2001a; Bartol et al., 2001b), whereas *S. bandensis* uses an internal cuttlebone to maintain neutral buoyancy, which reduces energetic costs associated with vertical positioning (Denton and Gilpin-Brown, 1973). Moreover, *S. bandensis* relies more extensively on their fins for locomotion than *L. brevis*. Given that *S. bandensis* has longer fins than *L. brevis* and is neutrally buoyant, characteristics that presumably favor turning control, I expect *S. bandensis* will have higher maneuverability than *L. brevis*. However, the more powerful jets of squid relative to cuttlefish should translate to an advantage in agility for *L. brevis* compared to *S. bandensis*. Given the unique flexibility of the propulsors and control surfaces employed by these two cephalopods, I expect both *S. bandensis* and *L. brevis* will exhibit higher turning performance, i.e., agility and maneuverability, than more classical rigid-bodied swimmers.
Methods

The animals used for this study were the dwarf cuttlefish, *Sepia bandensis*, and the brief squid, *Lolliguncula brevis*. All subsequent means will be reported as mean ± s.e.m. The dorsal mantle length (DML) of *L. brevis* individuals ranged from 3.2 cm to 7.4 cm (mean = 5.5±0.3 cm). The total length, including the arms, ranged from 4.8 cm to 11.9 cm (mean = 9.03±0.5 cm). The mantle, on average, made up 61.5±0.8 % of the total body length. The DML of *S. bandensis* individuals ranged from 2.5 cm to 3.8 cm (mean = 3.1±0.2 cm). The total length ranged from 4.5 cm to 6.9 cm (mean = 5.7±0.4 cm). The mantle, on average, made up 55.5±0.8 % of the total body length. *Sepia bandensis* were purchased from a commercial supplier (Consistent Sea Inc., California, USA) and were kept individually in submerged plastic buckets (36 cm deep and 30 cm wide) with drilled 6 cm diameter holes and mesh liners for water circulation. The buckets floated freely in a recirculating 450-gallon seawater system at a salinity of 33-35 ppt, temperature of 24-25°C, and pH of 8.0-8.2. Ammonia levels were kept below 0.2 ppm. *Lolliguncula brevis* were caught by trawl net at the Virginia Institute of Marine Science Eastern Shore Marine Lab, Wachapreague, VA, USA and were transported back to Old Dominion University in aerated livewells. The adults were maintained in a 450-gallon recirculating seawater system (separate from the cuttlefish system) at salinity of 25-30 ppt, temperature of 15-21°C, and pH of 8.0-8.2. Ammonia levels again were maintained below 0.2 ppm. A moderate current was maintained in the *L. brevis* holding tank to facilitate active swimming. Both species were fed a diet of live grass shrimp *Palaemonetes pugio*.

A Plexiglass viewing chamber measuring 30.5 cm by 30.5 cm by 25.4 cm was placed on a stand that allowed unobstructed viewing from both lateral and ventral perspectives. The chamber was filled with seawater of the same salinity and temperature as the holding tanks. The water in the chamber was aerated overnight prior to conducting trials. The chamber was illuminated with five 500 W lights outfitted with color gel #27 filters (transmits wavelengths > 600 nm), as red light tended to reduce stress on the animals compared to full spectrum illumination. For each trial, the cuttlefish or squid was placed in the chamber and allowed to acclimate for at least 5 minutes prior to recording. Turns either occurred naturally without any experimental intervention or, in
cases where the cephalopods would not turn consistently, were elicited by simulating predatory behavior. Simulating predatory behavior was accomplished by tying a grass shrimp to a piece of tubing and moving the tubing in gentle circular motions in the chamber. Trials were terminated if the animal became unresponsive or caught the shrimp. Data from 5 *S. bandensis* (2.5 – 3.8 cm DML (mean = 3.1±0.2 cm DML)) and 14 *L. brevis* (3.2 - 7.4 cm DML (mean = 5.5±0.3 cm DML)) were collected, with 3-15 turning sequences per animal being considered for further analyses.

The turns were recorded using two synchronized high-speed Dalsa Falcon video cameras (1400 x 1200 pixel resolution; DALSA Corp., Waterloo, ON, Canada) positioned ventrally and laterally to the viewing chamber. The ventral camera was fitted with a 25 mm lens and the lateral camera was fitted with a 35 mm lens (Fujinon TV Lens, Fujinon Corporation, China). The high-speed cameras were triggered by the onboard counter on two CLSAS capture cards (IO Industries Inc., London, ON, Canada) to capture video at 100 frames per second. Video frames from the DALSA cameras were transferred to hard disk in real time using the two CLSAS capture cards and Streams 5 software (IO Industries Inc., London, ON, Canada).

Frame-by-frame position tracking of the cephalopod body features was accomplished using image tracking software (Hedrick, 2008). Seven points were tracked in the ventral view: (1) tail tip, (2) funnel base, (3) arm tip, (4) mantle right side (midway along length of mantle), (5) mantle left side (midway along length of mantle), (6) right fin tip (at maximum chord point), and (7) left fin tip (at maximum chord point) (Fig. 1A). Six points were tracked in the lateral view and were: (1) tail tip, (2) eye, (3) arm tip, (4) proximal funnel opening, (5) distal funnel opening, and (6) fin tip (maximum chord point) (Fig 1B). The tracked points in the ventral view were used to determine (1) the center of rotation (COR), (2) angular velocity, (3) total angular displacement (θ<sub>total</sub>), (4) direction of the turn, (5) time to execute the turn, (6) angle between the mantle and arms (θ<sub>s</sub>), (7) frequency of fin beats, and (8) mantle diameter. θ<sub>s</sub> is defined as the angle between the arms and the mantle in the ventral perspective; I report a mean angle throughout the turn, θ<sub>vmean</sub>, and an absolute minimum angle during the turn, θ<sub>vmin</sub> (Fig. 2),
Figure 1. Points tracked in the lateral (A) and ventral (B) views; only *L. brevis* is pictured for simplicity. A: Points tracked in the lateral view for *L. brevis*: (1) tail tip, (2) eye, (3) arm tip, (4) proximal funnel opening, (5) distal funnel opening, (6) fin tip. B: Points tracked in the ventral view for *L. brevis*: (1) tail tip, (2) funnel base, (3) arm tip, (4) mantle right, (5) mantle left, (6) fin tip right, (7) fin tip left.
Figure 2. *L. brevis* turning, demonstrating the wrapping of the arms close to the mantle to decrease the ventral angle between the mantle and arms. The dotted line illustrates how the ventral angle between the arms and mantle was determined.
both averaged over all turning sequences. The mantle diameter was determined for *L. brevis* only, as the cuttlebone in *S. bandensis* limits visible changes in the ventral view.

The data were smoothed using Cross-Validation Criterion (CVC). This smoothing method uses smoothed splines where the level of smoothing is determined such that the root-mean-squared error of the splines determined with points from the data individually excluded is minimized (Walker, 1998). In the present implementation, the minimization is determined to within 0.1% of the actual minimum to speed convergence of the method. The COR was the point in the ventral view that moved the least during the turn. Finding the COR was performed using an in-house Matlab code that either used the line segment connecting the tail tip to the funnel base, or used a two segment approach with the lines connecting the tail tip to the funnel base and then the funnel base to the arm tip. The code was generalized so that the COR did not actually have to fall directly on these line segments. Rather, it could lie along a line at a fixed angle \( \alpha \) with respect to the tracked body segment where \( \alpha \) and the position of the COR along the line at this angle were selected such that the movement of the COR during the turn was minimized.

The radius \( (R) \) of the turning path is the radius of curvature of the COR. This was computed from analytical geometry using

\[
\frac{1}{R} = \frac{y''}{\left(1 + (y')^2\right)^{3/2}}
\]

where \( y' = dy/dx \), \( x \) and \( y \) are the coordinates of the COR in the ventral view, and the derivatives were evaluated using fourth order accurate finite difference equations. For each turn sequence, both the mean radius of the turning path and the minimum radius of the turning path were determined using in-house Matlab routines. To compare my data with previous studies, the minimum and mean radius of the turning path \( (R) \) was normalized (divided by the length of the animal) to get a length-specific turning radius \( (R/L) \). \( (R/L)_\text{mean} \) is the average of all center of rotation (COR) radii comprising the turning path, divided by the length of the animal. All of the turning radii values for each sequence were ranked smallest to largest and the 90th percentile value was taken as the minimum \( ((R/L)_{\text{min}}) \). The absolute minimum \( ((R/L)_{\text{min}}) \) was the lowest 90th percentile minimum from all turn sequences. \( \omega_{\text{avg}} \) is the mean angular velocity throughout the turn. \( \omega_{\text{max}} \) is
the maximum angular velocity found during the turn, averaged over all turning sequences. $\omega_{\text{amax}}$ is the absolute maximum angular velocity of all turning sequences. Translation was defined as the maximum distance in cm (Max D) between the COR at any two instances during the turning sequence, divided by total body length. All reported values, other than absolute values, are from individual averages rather than individual turning sequences.

Two different methods were used to calculate turning performance parameters. The first method considered only the mantle segment, using the tail tip point to the funnel base point. The second method considered the entire length of the squid or cuttlefish. In this case, two connected lines were drawn, one from the tail tip point to the funnel base point, and another from the funnel base point to the arm tip point. A nested one-way MANOVA with individual nested within method was performed for each species to determine any differences between the two methods for each parameter (SPSS, Version 18, SPSS Inc., Chicago, IL, USA). There were no significant differences between the two methods for $(R/L)_{\text{min}}$ or $(R/L)_{\text{mean}}$, $\omega_{\text{max}}$, $\omega_{\text{avg}}$ and $\Theta_{\text{total}}$ ($L.\ brevis$ MANOVA: F=0.6, df=5,40, P=0.7; $S.\ bandensis$ MANOVA: F=2.1, df=5,98, P=0.07). Therefore, only the second method, using the entire length, will be discussed.

Mantle diameter and angular velocity were smoothed using a fourth order Butterworth filter and cutoff frequency of 4 Hz. Mantle contraction rate and angular acceleration were calculated from the smoothed data for each sequence by evaluating the derivatives using fourth order finite difference equations. Jet pulses were identified as periods where the mantle contraction rate was negative, indicating that mantle diameter was decreasing, and jet pulses shorter than 0.15 sec were excluded from analysis. The mantle contraction rate and angular acceleration for jet pulses greater than 0.15 sec were analyzed using Pearson correlations.

The tracked points in the lateral view were used to determine (1) the mantle angle with respect to the horizontal ($\Theta_{\text{lmh}}$) (Fig 3C), (2) arm angle with respect to the mantle ($\Theta_{\text{lam}}$) (Fig 3D), (3) fin beat amplitude and (4) funnel diameter. These parameters were calculated using Matlab routines developed in-house. The funnel diameter was not
Figure 3. The four different postures displayed by *L. brevis* and *S. bandensis*; only *L. brevis* is pictured for simplicity. A. Tail up arms up. B. Tail up arms down. C. Tail down arms up. D. Tail down arms down. The dotted lines in C demonstrate how the acute angle between the arms and mantle was determined in lateral views. The dotted lines in D demonstrate how the acute angle between the mantle and horizontal was determined in lateral views.
always visible for the entire duration of some turns, thus mantle diameter measured in the ventral view was used to compute jet pulse frequency. Only one fin was consistently visible in lateral views, and therefore fin points in ventral views were used for fin-beat analyses in this study. Unfortunately, fin beat frequency was not determined for *S. bandensis* as the fins were too small to resolve consistently in either the lateral or ventral perspectives.

Values that were compared between the two species included \((R/L)_{\text{mean}}, (R/L)_{\text{min}}, \omega_{\text{avg}}, \omega_{\text{max}}, \theta_{\text{total}}, \theta_{\text{lmh}}, \theta_{\text{am}}, \theta_{\text{vmin}}, \theta_{\text{vmean}}\) and translation. A nested two-way mixed model MANOVA, with individual nested in species, was used to determine if there was a statistical difference for \((R/L)_{\text{mean}}, (R/L)_{\text{min}}, \omega_{\text{avg}}, \omega_{\text{max}}, \theta_{\text{total}}, \theta_{\text{vmin}}, \theta_{\text{vmean}}\), and translation between the two species (SPSS, IBM, New York, USA). A nested two-way mixed model MANOVA with individual nested in posture category was used to determine differences in \(\theta_{\text{lmh}}\) and \(\theta_{\text{am}}\) for *L. brevis* and *S. bandensis*. A Log10 transformation was used to meet assumptions of normality. The Wilks’ Lambda test was used for all multivariate analyses. To determine if there was a difference in fin beat frequency for the outboard and inboard fins of *L. brevis*, a paired two-tailed t-test was performed (SPSS).

**Results**

A total of 36 turns from 14 individuals was analyzed for *L. brevis* and 56 turns from 5 individuals were analyzed for *S. bandensis*. All subsequent mean values are reported as mean ± s.e.m. Total angular displacement of the turns ranged from 58° to 345° (mean angular displacement = 117.2±18.7°) for *L. brevis* and from 72° to 150° (mean angular displacement = 98.3±14.0°) for *S. bandensis*.

*Lolliguncula brevis* turning performance

The fin beat frequency was determined for each fin for *L. brevis*, and the fins were characterized as either inboard (fin located in interior of turn) or outboard (fin located at periphery of turn) in relation to the turning direction. The outboard fin beat frequency (mean = 3.5±0.2 beats s\(^{-1}\)) was significantly higher than the inboard fin beat
frequency (mean = 2.9±0.2 beats s⁻¹) during turning maneuvers (paired t-test: t=2.8, df₁₃, P<0.05). The few turns that involved similar fin beat frequencies on each side, or that had a higher inboard frequency than outboard frequency, often were not synchronized, or there was a phase shift between the inboard and outboard sides.

(R/L)ᵟₘᵢₙ was 3.4x10⁻³±5.9x10⁻⁴, with (R/L)ᵟₐₘᵢₙ = 4.2x10⁻⁴. (R/L)ᵩₐₘᵢₙ, was 8.8x10⁻³±3.9x10⁻³ (Table 1). The range in (R/L)ᵩₐₘᵢₙ values was 0.0004 to 0.05. ωᵩₐᵥₑₙ was 110.3±14.6° s⁻¹ and ωₐₘᵢₙ was 268.4±32.9° s⁻¹. ωₐₘᵢₙ was 725.8° s⁻¹ (Table 1). The trend between ωₐₘᵢₙ and (R/L)ᵟₘᵢₙ is illustrated in Fig. 4 along with S. bandensis, however only the S. bandensis relationship was significant. As ωₐₘᵢₙ increased, the Θₐᵟᵞᵦₐₘᵢₙ decreased (Pearson correlation: r=0.7, df₁₃, P=0.005), and as ωᵩₐᵥₑₙ increased, the Θᵩₐᵥₑₙ decreased (Pearson correlation: r=0.7, df₁₃, P=0.01) (Fig. 5). The 36 turns for L. brevis were divided into four different orientations: (1) tail and arms up, (2) tail up and arms down, (3) tail down and arms up, and (4) tail and arms down (Fig. 3). The most commonly observed orientation was tail and arms up (orientation 1, 19 turns). Tail up arms down (orientation 2) and tail down arms up (orientation 3) were observed in 6 and 8 turns, respectively, and lastly tail and arms down (orientation 4, 3 turns) was the least common. There were no significant differences among any turning parameters or body angles among the different orientations even if turns were pooled into tail up versus tail down and arms up versus arms down orientations.

Mantle contraction rate generally correlated with angular acceleration, indicating a relationship between the jet pulse and angular velocity, with some sequences correlating very strongly (Pearson correlation: r>0.6, P<0.005). Specifically, angular acceleration increased with increased mantle contraction rate (Fig. 6). Generally, the highest angular velocity occurred during mid mantle contraction (Fig. 7). During turns, multiple fin beats were employed during each mantle contraction. The fin beats on the outboard and inboard sides were usually synchronized for the majority of the turn, and often became asynchronous towards the middle to end of the turn (Fig. 8). The difference in average beat frequency was driven by one or two main periods of fin asymmetry during a single turn sequence. Asymmetric fin motions often occurred at the same time as the more dominant jet, making it difficult to evaluate clear fin-related impacts on angular
Table 1. Kinematic variables for *L. brevis* and *S. bandensis*

<table>
<thead>
<tr>
<th></th>
<th>((R/L)_{\text{min}})</th>
<th>((R/L)_{\text{mean}})</th>
<th>(\omega_{\text{max}}) (\text{(^\circ \text{sec}^{-1})})</th>
<th>(\omega_{\text{avg}}) (\text{(^\circ \text{sec}^{-1})})</th>
<th>(\Theta_{v\text{min}}) (\text{(^\circ)})</th>
<th>(\Theta_{v\text{mean}}) (\text{(^\circ)})</th>
<th>Mantle length percentage (%)</th>
</tr>
</thead>
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<tr>
<td><em>L. brevis</em></td>
<td></td>
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<td></td>
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<tr>
<td>Minimum</td>
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<td>3.6x10^{-4}</td>
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<td>41.7</td>
<td>81.2</td>
<td>128.5</td>
<td>53.9</td>
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<tr>
<td>Maximum</td>
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<td>725.8</td>
<td>390.2</td>
<td>171.02</td>
<td>176.5</td>
<td>66.6</td>
</tr>
<tr>
<td>Mean</td>
<td>3.4x10^{-3}</td>
<td>8.8x10^{-3}</td>
<td>268.4</td>
<td>110.3</td>
<td>138.9</td>
<td>161.8</td>
<td>61.5</td>
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<tr>
<td></td>
<td>(5.9x10^{-4})</td>
<td>(3.9x10^{-3})</td>
<td>(32.9)</td>
<td>(14.6)</td>
<td>(5.9)</td>
<td>(3.3)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><em>S. bandensis</em></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum</td>
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<td>16.3</td>
<td>125.6</td>
<td>146.3</td>
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<td>485.0</td>
<td>109.7</td>
<td>172.9</td>
<td>177.8</td>
<td>64.3</td>
</tr>
<tr>
<td>Mean</td>
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<td>9.5x10^{-2}</td>
<td>160.2</td>
<td>54.8</td>
<td>156.4</td>
<td>167.9</td>
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<tr>
<td></td>
<td>(4.7x10^{-4})</td>
<td>(3.2x10^{-2})</td>
<td>(19.7)</td>
<td>(8.4)</td>
<td>(2.6)</td>
<td>(1.9)</td>
<td>(0.8)</td>
</tr>
</tbody>
</table>

Values in parentheses are standard error of the mean.
R, minimum radius of the turning path; L total body length; R/L, length specific minimum radius of the turning path; \(\omega_{\text{avg}}\), average angular velocity of the turn; \(\omega_{\text{max}}\), maximum instantaneous angular velocity of the turn. Minimum and maximum values are absolute minimums and maximums for all turning sequences.
Figure 4. The relationship between the length-specific minimum radius of the turn \((R/L)_{\text{min}}\) and maximum angular velocity \((\omega_{\text{max}})\) for *L. brevis* (open circles, N=14) and *S. bandensis* (filled circles, N=5, solid best-fit line). The points are averages of sequences by each individual. Only the *S. bandensis* relationship is significant (see text for correlation statistics).
Figure 5. **Relationship between the ventral angle between the arms and mantle and angular velocity for *L. brevis***. A. Relationship between the minimum ventral arm/mantle angle ($\Theta_{vmin}$) and the maximum angular velocity ($\omega_{max}$), for *L. brevis* (N=14). The relationship is significant (see text for correlation statistics). B. Relationship between the mean ventral arm/mantle angle ($\Theta_{vmean}$) and the mean angular velocity ($\omega_{avg}$), for *L. brevis* (N=14). The relationship is significant (see text for correlation statistics).
Figure 6. **Angular acceleration plotted with mantle contraction rate for three separate jet pulses.** The jet pulses are from three different turning sequences. Positive values for angular acceleration are indicative of increasing speed while negative values for angular acceleration are indicative of decreasing speed. The Pearson correlation coefficients for these particular pulses are $r^2 = -0.8$ (black filled circles), -0.7 (open circles), -0.8 (gray circles) and with all P values being $<0.001$. 


Figure 7. Mantle diameter (filled circles) plotted with angular velocity (open circles) for six different *L. brevis* turning sequences. Mantle diameter and angular velocity data were smoothed using a fourth order Butterworth filter and cutoff frequency of 4 Hz. Decreasing mantle diameter is indicative of contraction resulting in a pulsed jet. Peak angular velocity (broken arrow) generally follows shortly after the mid-point of the mantle contraction (black arrow). Angular velocity appears to be driven primarily by the jet pulse with angular velocity beginning to increase shortly after the mantle begins contracting.
Figure 8. Fin amplitude plotted for three different *L. brevis* turning sequences, with the outboard fin (open circles) and the inboard fin (filled circles). Generally, the fin beats are synchronized through the majority of the turn with one or two main periods of asymmetry occurring late in the turn (black arrow).
velocity. Though fin action generally did not influence angular velocity as substantially as the jet, some forceful fin beats did contribute to obvious spikes in angular velocity, which were superimposed onto the dominant jet-driven angular velocity patterns.

*Sepia bandensis* turning performance

\[(R/L)_{\text{min}} = 1.2 \times 10^{-3} \pm 4.7 \times 10^{-4}, \text{ with } (R/L)_{\text{amin}} = 1.3 \times 10^{-4}, (R/L)_{\text{mean}} = 9.5 \times 10^{-2} \pm 3.2 \times 10^{-2} \text{ (Table 1).}\] The range of \((R/L)_{\text{mean}}\) values was 0.04 to 0.2. \(\omega_{\text{avg}}\) was 54.8 \pm 8.4° s\(^{-1}\) and mean \(\omega_{\text{max}}\) was 160.2 \pm 19.7° s\(^{-1}\) (Table 1). \(\omega_{\text{amax}}\) was 485.0° s\(^{-1}\). As \((R/L)_{\text{min}}\) increased, \(\omega_{\text{max}}\) also increased (Pearson correlation: \(r=0.9, \text{ df}, P=0.04\) (Fig. 4). There was no correlation between \((R/L)\) and \(\Theta\).

As was the case for squid, the 56 turns for *S. bandensis* could be classified according to four different orientations: (1) tail and arms up, (2) tail up and arms down, (3) tail and arms down, and (4) tail down and arms up. The most commonly observed orientation was tail up arms down (orientation 2, 20 turns). Tail and arms down (orientation 3) and tail and arms up (orientation 1) and tail down and arms up (orientation 4) were observed in 15, 11 and 10 of the turns, respectively. There were no significant differences for turning parameters or body angles among the four orientations even if turns were pooled into either tail up versus tail down or arms up versus arms down groupings. The only significant difference found for body orientation was that the arm angle relative to the mantle was significantly steeper for the arms down orientation (mean = 21.4±3.8°) than the arms up orientation (mean = 10.2±1.8°) (MANOVA: \(F=4.9, \text{ df}, 19, P=0.04\)).

*Species comparison*

*Sepia bandensis* \((R/L)_{\text{min}}\) (mean = 1.2 \times 10^{-3} \pm 4.7 \times 10^{-4}) was significantly lower than that for *L. brevis* (mean = 3.4 \times 10^{-3} \pm 5.9 \times 10^{-4}) (MANOVA: \(F=6.6, \text{ df}, 8,66, 16.7; P=0.01\)). However, \((R/L)_{\text{mean}}\) was not significantly different for *L. brevis* (mean = 8.8 \times 10^{-3} \pm 3.9 \times 10^{-3}) and *S. bandensis* (mean = 9.5 \times 10^{-2} \pm 3.2 \times 10^{-2}) (MANOVA: \(F=6.6, \text{ df}, 8,66, P=0.09\)). \(\omega_{\text{max}}\) was significantly greater for *L. brevis* (mean = 268.4±32.9° s\(^{-1}\)) than for *S. bandensis* (mean = 160.2±19.7° s\(^{-1}\)) (MANOVA: \(F=8.008, \text{ df}, 8,66, P=0.006\), and \(\omega_{\text{avg}}\) was
also significantly greater for *L. brevis* (mean = 110.3±14.6° s\(^{-1}\)) than for *S. bandensis* (mean = 54.8±8.4° s\(^{-1}\)) (MANOVA: F=22.5, df\(_{8,66}\), P<0.001). There was no significant difference in Θ\(_{\text{total}}\) between the two species (MANOVA: F=0.4, df\(_{8,66}\), P=0.5). The Θ\(_{\text{vmin}}\) was significantly less for *L. brevis* (mean = 138.9±5.9°) than for *S. bandensis* (mean = 156.4±2.6°) (MANOVA: F=6.8, df\(_{8,66}\), P=0.01) during turns. The Θ\(_{\text{vmean}}\) was also significantly lower for *L. brevis* (mean = 161.8±3.3°) than for *S. bandensis* (mean = 167.9±1.9°) (MANOVA: F=5.2, df\(_{8,66}\), P=0.03). Translation was defined as the maximum distance in cm (Max D) between the COR at any two frames, divided by total body length, during the turn. There was no significant difference in translation between *L. brevis* turning maneuvers (mean = 0.2±0.02) and *S. bandensis* turning maneuvers (mean = 0.1±0.01) (MANOVA: F=1.2, df\(_{8,66}\), P=0.3). An example of this difference in translation is illustrated in Fig. 9. The only other body orientation parameter that differed between the species was the angle of the arms with the mantle, which was significantly steeper for *L. brevis* in the arms down orientation (mean = 18.3±5.8°) than *S. bandensis* in the arms up orientation (mean = 10.2±1.8°) (MANOVA: F=1.8, df\(_{3,28.7}\), P=0.02).

**Discussion**

Squid and cuttlefish represent a unique group of aquatic animals, relying on two dissimilar propulsors (jet and fins) that are powered by obliquely striated muscles in a hydrostatic arrangement. Both squid and cuttlefish swim using a combination of paired fin movements and a pulsed jet that can be directed in any direction within a hemisphere below the body. Using this dual mode system, squids and cuttlefishes are capable of a wide repertoire of unsteady turning motions. This study represents the first quantitative study of turning performance in any cephalopod. Both species of cephalopods considered in this study were found to be highly maneuverable with absolute length-specific minimum radii of their turns approaching zero, i.e., 4.2x10\(^{-4}\) for *L. brevis* and 1.2x10\(^{-4}\) for *S. bandensis*. In addition, *L. brevis* had greater agility (Ω\(_{\text{amax}}\) = 725° s\(^{-1}\)) than *S. bandensis* (Ω\(_{\text{amax}}\) = 485° s\(^{-1}\)), though *S. bandensis* exhibited the capacity for more controlled turns with many examples of tight grouping of the center of rotation. During turns for *L. brevis*,...
Figure 9. Turning path trajectories from one turning sequence for *S. bandensis* (top) and *L. brevis* (bottom). The black circles are the center of rotation path throughout the turn, light gray circles are the tail tip point, and the dark gray circles are the arm tip point. The data were smoothed using a CVC filter (see text).
angular velocity was driven, to a large extent, by the pulsed jet with the fins playing a more subordinate role.

The level of flexibility in aquatic swimmers can impact turning performance. Flexibility is thought to improve turning performance by decreasing the rotational inertia, and allowing the animal to turn with a small radius of curvature (Fish and Nicastro, 2003; Parson et al., 2011; Rivera et al., 2006; Walker, 2000) and this has been observed in flexible bodied animals, such as the sea lion and spiny dogfish (Domenici et al., 2004; Fish et al., 2003). There is some evidence that suggests this flexibility and increased turning performance results in decreased stability, or that the increase in turning performance is a consequence of decreased stability (Fish et al., 2003). Squid do have some characteristics of a stable body, however the flexibility of the propulsors (jet and fins) combined with flexible control surfaces (fins and arms) allow squid to easily override static stability when necessary to achieve high maneuverability. Squid employ propulsors (fins) and control surfaces (fins and arms) that are located distant from their center of mass. Having these propulsors and control surfaces so far from the center of mass allows these animals to produce corrective moments that are capable of enhancing both stability and maneuverability.

Several studies investigating turning performance of rigid bodied aquatic taxa have demonstrated that a rigid body does not necessarily limit turning performance due to the contribution of propulsors and control surfaces. It has been commonly thought that rigid-bodied aquatic animals demonstrate either high agility or high maneuverability, but not both (Fish, 2002; Fish and Nicastro, 2003; Rivera et al., 2006; Walker, 2000). This is observed in boxfish Ostracion meleagris, which exhibits high maneuverability but low agility (Walker, 2000) and can also be seen in the whirligig beetle, Dineutes horni, which exhibits high agility but low maneuverability (Fish and Nicastro, 2003). However, Rivera et al. (2006), examined turning performance in the painted turtle, Chrysemys picta, and did not find a pronounced trade-off between agility and maneuverability, reporting that turtles exhibit intermediate values for both maneuverability and agility.
Squid and cuttlefish have a chitinous pen and cuttlebone, respectively, that restrict bending and length changes in the mantle, although their arms are flexible. Thus cephalopods do not fit neatly into the flexible, stiff, and rigid-bodied categories described earlier. Nonetheless comparisons between cephalopods and other nekton that do fit within these categories are instructive. Previously, the rigid-bodied spotted boxfish, *O. meleagris*, was considered the most maneuverable aquatic animal, with a mean length-specific minimum radius of the turn of $3.3 \times 10^{-2}$ and an absolute minimum of $5.0 \times 10^{-4}$ (Walker, 2000). These values were based on one individual performing 12 turning sequences. Comparable values for *S. bandensis* and *L. brevis* in this study are $1.2 \times 10^{-3}$ and $3.4 \times 10^{-3}$, respectively, for $(R/L)_{min}$, which are orders of magnitude below boxfish, and $1.3 \times 10^{-4}$ and $4.2 \times 10^{-4}$, respectively, for $(R/L)_{amin}$, which are also lower than boxfish. The values given here for $(R/L)_{min}$ are conservative, as the 90th percentile $(R/L)$ value was used for each turning sequence, instead of the absolute minimum for each sequence. This ensured that any extreme values that could be due to digitization error were accounted for.

Taking minimum values from the 90th percentile of each turning sequence, averaging these minimum values per individual and then taking an average of all the individual minima to calculate $(R/L)_{min}$ gives a much more representative and conservative estimate of maximum maneuvering capability. The mean, $(R/L)_{mean}$, was also low for both cephalopods with values of $9.5 \times 10^{-2}$ for *S. bandensis* and $8.8 \times 10^{-3}$ for *L. brevis* (Table 1). Given the lower mean $(R/L)_{min}$ values for *S. bandensis*, and their capacity for low translation (see Fig. 9), we expected $(R/L)_{mean}$ to be lower for *S. bandensis*. However, this was not observed, with $(R/L)_{mean}$ values being lower for *L. brevis*. This finding likely reflects behavioral variability. Although *S. bandensis* is capable of achieving a very low $(R/L)_{min}$, it does not always turn at this performance extreme. Instead it uses a wide range of turning behavior, which is reflected in the observed greater $(R/L)_{mean}$ range for *S. bandensis* relative to *L. brevis*, and similar values for length specific translation. When $(R/L)_{mean}$ is considered, *L. brevis* still ranks as the most maneuverable aquatic animal measured to date. Though *S. bandensis* did demonstrate the capability of tighter turns, as seen in the $(R/L)_{min}$, *S. bandensis* is closer
to pike, *Esox lucius*, (Domenici and Blake, 1997) and dolphins, *Coryphaena hippurus* (Webb and Keyes, 1981), in terms of maneuverability when the \((R/L)_{\text{mean}}\) is considered.

For *S. bandensis*, \((R/L)_{\text{min}}\) correlated with \(\omega_{\text{max}}\). As \(\omega_{\text{max}}\) increased, \((R/L)_{\text{min}}\) also increased. This finding was expected and indicative of faster turns also being wider turns. As turns become faster and inertia increases, it becomes more difficult to control the tightness of the turn, resulting in higher length specific turning radii. Turning speed, measured as angular velocity \((\omega)\), was also quite high for *L. brevis* and *S. bandensis*. The observed values of \(\omega_{\text{amax}} = 485.0^\circ \text{s}^{-1}\) (mean \(\omega_{\text{max}} = 160.2^\circ \text{s}^{-1}\)) for *S. bandensis* and \(\omega_{\text{amax}} = 725.8^\circ \text{s}^{-1}\) (mean \(\omega_{\text{max}} = 268.4^\circ \text{s}^{-1}\)) for *L. brevis* are higher than peak turning speeds for spotted boxfish \(\omega_{\text{amax}} = 218^\circ \text{s}^{-1}\) and, for *L. brevis*, higher than painted turtles \((501.8^\circ \text{s}^{-1})\) (Rivera et al., 2006; Walker, 2000). Indeed the values for *S. bandensis* are comparable to those reported for yellowfin tuna \(\omega_{\text{amax}} = 426^\circ \text{s}^{-1}\) (Blake et al., 1995) and painted turtles, and the values for *L. brevis* exceed those of more flexible-bodied taxa, such as sea lions \(\omega_{\text{amax}} = 690^\circ \text{s}^{-1}\) (Fish et al., 2003).

Since agility is size dependent, with smaller animals generally achieving greater levels of agility, comparisons using angular velocity divided by body length are useful (Alexander, 1967). When these comparisons are made, *L. brevis* is comparable to similar sized painted turtles \((L. brevis): 113.4^\circ \text{s}^{-1} \text{L}^{-1}; C. picta: 105.4^\circ \text{s}^{-1} \text{L}^{-1}\) but still displays far greater agility than the rigid bodied spotted boxfish \((O. meleagris: 18.8^\circ \text{s}^{-1} \text{L}^{-1})\) and dwarf cuttlefish \((S. bandensis: 86.6^\circ \text{s}^{-1} \text{L}^{-1})\) (Rivera et al., 2006; Walker, 2000). Larger flexible and stiff bodied animals such as the sea lion, spiny dogfish and yellowfin tuna have lower angular velocities than squid and cuttlefish when angular velocities are normalized to body lengths, reflecting the greater amount of drag that a larger animal must overcome while rotating \((Zalophus californianus: 4.01^\circ \text{s}^{-1} \text{L}^{-1}; Squalas acanthias: 17.5^\circ \text{s}^{-1} \text{L}^{-1}; Thunnus albacares: 14.2^\circ \text{s}^{-1} \text{L}^{-1})\) (Blake et al., 1995; Domenici et al., 2004; Fish et al., 2003). When *L. brevis* and *S. bandensis* are compared to length specific measures of agility for all aquatic taxa measured to date, they fall along a line separating flexible bodied and rigid bodied taxa (see Fig. 7 in Fish and Nicastro, 2003). This finding reflects
the hybrid body architecture of squid and cuttlefish, which consists of both rigid and flexible components.

Flexible-bodied animals can bend their body axis to minimize the length of the body creating drag during the turn. Rigid-bodied animals cannot bend in this way, so the entire rigid portion of the body will resist rotation, often leading to lower turning speeds. The dorsal region of *L. brevis*’ mantle is inflexible as a result of the chitinous pen, but it can compensate for this inflexible component by wrapping its arms close to the mantle. Despite having a more restrictive cuttlebone in its mantle and relatively longer head and arms than *L. brevis* (44.6 ± 0.8% total body length versus 38.5 ± 0.8% in *L. brevis*), *S. bandensis* did not display this arm wrapping behavior as prominently as *L. brevis* (based on higher ventral arm/mantle angles). Thus, greater arm drag could help explain the lower observed \( \omega_{\text{max}} \) for *S. bandensis*.

The arm positioning relative to the body impacted turning performance for *L. brevis*. As the mean and minimum angle between the arms and mantle decreased in *L. brevis*, \( \omega_{\text{mean}} \) and \( \omega_{\text{max}} \) increased, respectively. This is expected, as bending any part of the body axis reduces the body’s moment of inertia about the dorsoventral rotational axis, resulting in lower inertial resistance to rotation and lower hydrodynamic rotational resistance (Walker, 2000). Therefore, *L. brevis* wraps its arms towards the mantle to achieve faster turns. Though *S. bandensis* was also capable of wrapping its arms to the mantle, it generally did so at higher minimum and mean ventral angles than *L. brevis*. Moreover, the ventral angle between the arms and mantle was not correlated with angular velocity or the minimum radius for *S. bandensis*. These differences may derive from how the turns were performed. Turns for *S. bandensis* were often prompted by moving a prey item around the experimental chamber, which was not necessary for *L. brevis*. Since cephalopods orient arms-first towards prey items, *S. bandensis* may have been tracking the prey with its arms rather than bending them close to the body to increase angular velocity.

The interplay between the jet and fins plays an important role in turning performance in cephalopods. Although fin motions were not quantified for *S. bandensis* because of the small size and translucency of the fins, they were clearly active during
turns and likely aided turning as was the case for *L. brevis*. In *L. brevis*, the outboard fin on the far side of the turn beat significantly faster than the inboard fin on the near side of the turn, and the turning sequence with the highest $\omega_{\text{max}}$ corresponded with the greatest difference in fin beats on the far and near side of the turn. However, there were also some turning sequences where angular velocity was high and/or $(R/L)_{\text{min}}$ was low, without a large difference in fin beat frequency between the fins. In these sequences, the timing of the fin beats seemingly was more important than mean frequency and/or the jet played a larger role in these turns. To help determine the role the jet played in turns, the mantle contraction, angular velocity and fin amplitudes were tracked throughout turning sequences. In general, the highest angular velocity throughout turning sequences closely followed strong mantle contractions and mantle contraction rate correlated with angular acceleration, suggesting that the jet contributes more to agility, i.e., the speed of the turn, than the fins. The fins appeared to be synchronized throughout most of the turn but became asynchronous for several fin strokes midway through the turn, or towards the end of the turn. During these instances, the outboard and inboard fin exhibited different flapping frequencies. Though this asymmetry may have contributed to small increases in angular velocity, the impact of the fins on turning velocity were often masked by the jet and difficult to fully evaluate. However, in some sequences, forceful fin flaps did produce angular velocity spikes superimposed on the larger jet-driven velocity patterns, indicating that fins can indeed impact angular velocity patterns, albeit to a lesser extent than the jet. Although not examined specifically in this paper, the fins are likely important for controlling the stability of the turn, and in minimizing the length specific radius of the turn. The fins could offset the ventral position of the funnel to improve stability. Though the same four postures were observed in both *L. brevis* and *S. bandensis*, different postures were more prevalent in each species. However, posture does not appear to influence turning performance to a significant extent.

When the points for the arm tip, tail tip and COR for both animals were visualized, *S. bandensis* had many turns where the COR was very tightly grouped, while *L. brevis* had more turning sequences where the COR path exhibited long arms before and after an area of tight grouping. When translation during the turn was normalized
using total body length, no difference was observed between *S. bandensis* and *L. brevis*, but it appears that *S. bandensis* is at least capable of turns with very little translation since the long arms of the COR turning path were not a prominent feature of turning sequences. *Sepia bandensis* possesses an internal cuttlebone that allows it to maintain neutral buoyancy, which is not achievable in *L. brevis*. With neutral buoyancy, cuttlefish do not need to constantly direct flows downward for lift production, either through jetting or fin movements. In *L. brevis*, downward jet and/or fin-derived forces are required at all times for lift production, even when thrust is not required, and this constant fin/jet vectoring likely leads to greater drift during turns. Having fins that extend along the entire mantle, like *S. bandensis*, also provides more longitudinal control surfaces that can potentially limit translational movements. *Sepia bandensis* has fins with a longer chord length but smaller span than *L. brevis*, which may facilitate finer force control.

**Concluding Thoughts**

*Lolliguncula brevis* and *S. bandensis* are highly maneuverable, with (R/L) values that are the lowest reported to date for any aquatic animal, and are quite agile, with ω that are either comparable to flexible-bodied species (in the case of *L. brevis*) or highly agile rigid-bodied and stiff-bodied species (in the case of *S. bandensis*). Although moving the flexible elements of the body, such as the arms, do appear to impact turning performance to some degree, the fins and jet are the primary drivers of turning performance, as both are extremely active during turns. The shallow coastal sandy and reef habitats in which these cephalopods reside in require a high level of turning performance. Mobile inhabitants of these environments must be able to effectively navigate in and around complex structures, and hide in small crevices and openings to avoid predators, thus high maneuverability is important. Tail-first swimming is more economical than arms-first swimming (Bartol et al., 2001a), but squid and cuttlefish always orient arms-first to attack prey items (Foyle and O’Dor, 1988; Kier and Van Leeuwen, 1997; Messenger, 1968), making arms-first turning integral for their survival. Not surprisingly, squid and cuttlefish are both effective at turning in the arms-first mode. Given that squid and cuttlefish are also effective predators as well as common prey targets, it is important for
them to exhibit moderate to high levels of agility, as high turning speeds are essential for both capturing prey and escaping predators. Thus evolutionary pressures may have contributed to increased maneuverability and agility in terms of extreme turning limits, control of turns, and overall turning flexibility, all of which are reflected in the performance findings of this study.

Although this study provides quantitative kinematic data of turns in two cephalopods, more research is needed on a wider range of species to develop a more comprehensive picture of unsteady capabilities of cephalopods. Moreover, to fully understand the flow patterns and force contributions of the fins and the jet to turning performance, flow quantification studies involving 3D velocimetry are required and represent the next logical step in understanding turning performance in cephalopods.
CHAPTER 3

EXAMINATION OF TURNING HYDRODYNAMICS OF BRIEF SQUID

LOLLIGUNCULA BREVIS USING VOLUMETRIC (3D) VELOCIMETRY

Introduction

Although many studies have focused on swimming performance of aquatic animals during steady rectilinear swimming (Bartol et al., 2001a; Bartol et al., 2003; Bartol et al., 2009a; Drucker and Lauder, 2002; Fish and Lauder, 2006; Liao, 2007; Liao and Lauder, 2000; Liao et al., 2003; Tytell and Lauder, 2004; Videler and Weihs, 1982; Webb, 1988; Webb, 1994), animals rarely travel in a straight trajectory for long periods and this type of swimming represents only a small fraction of the daily activities of most aquatic nekton (Webb, 1978a; Webb, 1983). Unsteady locomotion, such as turning, is a crucial yet understudied component of locomotion (Weihs, 1972; Weihs, 2002). Unsteady swimming is comprised of any time-dependent variation in heading, speed, or acceleration and is important for navigation of complex habitats, obstacle avoidance, predator evasion, predatory attacks and corrections due to external perturbations (Webb, 1983; Weihs, 1972). Traditionally, unsteady swimming performance of aquatic animals has been assessed by recording animals conducting turns and measuring relevant kinematic parameters, such as maneuverability (tightness of the turn) and agility (speed of the turn) (Fish, 2003b; Kasapi et al., 1993; Norberg and Rayner, 2015; Rivera et al., 2006; Walker, 2000; Webb, 1983).

An area of particular interest in kinematic aquatic turning studies is how body flexibility impacts turning performance. Generally, more flexible bodied animals are capable of increased maneuverability and agility since flexibility permits the animal to turn in a relatively small space and allows for less rotational resistance during the turn (Fish and Nicastro, 2003; Walker, 2000). Stiff and rigid-bodied animals are often thought to have decreased turning performance due to the inability of the body to bend, which would reduce the second moment of inertia about the dorsoventral axis. This inability to bend likely creates high pressure drag, thereby resisting rotation and limiting agility.
(Walker, 2000). However, hydrodynamic input from various propulsors and control surfaces (i.e. fins, paddles, legs, etc.) can improve turning performance in rigid-bodied animals. In fact, some rigid-bodied animals use their propulsors and control surfaces to achieve very high measures of maneuverability and agility, even relative to flexible bodied animals (Fish and Nicastro, 2003; Rivera et al., 2006; Walker, 2000).

Squid and cuttlefish are not easily categorized as rigid, stiff or flexible bodied and utilize very different propulsors and control surfaces than the other nektom considered in prior kinematic turning studies. Both groups possess structures (cuttlebone or chitinous pen) that limit appreciable longitudinal length changes and bending along the mantle, much like conditions in rigid-bodied animals. However, the arms, which extend outward from the head and comprise a significant portion of the total length, are highly flexible, even to a higher degree than the bodies of flexible-bodied nektom. Thus, squids and cuttlefishes fall somewhere in between the rigid and flexible body extremes, representing an unexplored body architecture. Squids and cuttlefishes also employ a unique dual mode propulsion system consisting of paired fins and a pulsed jet that can be directed in any direction within a hemisphere below the body via a flexible funnel (Bartol et al., 2001a; O’Dor and Webber, 1991; Wells and O’Dor, 1991). Coordination between these two systems affords squids and cuttlefishes remarkable hydrodynamic versatility, including the ability to swim forward, backward, sideways, and vertically (Bartol et al., 2001a; Foyle and O’Dor, 1988; Hanlon and Messenger, 1996; O’Dor and Webber, 1991). The interplay between these propulsive systems facilitates high turning performance. In fact, recent kinematic analyses have revealed that brief squid Lolliguncula brevis and cuttlefish Sepia bandensis are highly agile and maneuverable relative to other aquatic animals (Chapter 2). Indeed L. brevis are comparable to the more flexible bodied sea lion in terms of agility and both L. brevis and S. bandensis are among the most maneuverable aquatic animals measured to date.

The kinematic analyses described above, which focus primarily on measures of agility, maneuverability, and appendage motions, have played important roles in highlighting turning performance parameters in animals with different body rigidity and propulsive mechanisms. However, measurements of flow forces, moments, and their
respective directions are essential for understanding the relative contributions of propulsors and body surfaces to maneuverability. Although collecting these hydrodynamic measurements is critical for a comprehensive understanding of maneuvering, quantifying flows around turning aquatic animals with multiple propulsors, such as squid, is not trivial. Conventional flow quantification techniques like DPIV (digital particle image velocimetry) image flows in a planar laser sheet (Willert and Gharib, 1991), with the assumption that flow structures are largely symmetric when calculating impulse. However, during turns, asymmetric flows rotating into and out of the laser sheet are common, and thus DPIV-derived impulse calculations are problematic. Furthermore, it is very difficult, if not impossible, to study flow hydrodynamics of multiple propulsors and control surfaces that fall out-of-plane with conventional DPIV. This limitation certainly applies to squids, which produce complex, 3D asymmetric flows from multiple propulsors (Bartol et al., in press).

Given their distinctive body architecture and unique coordinated dual-mode propulsive system, squids are a compelling choice for investigation of the hydrodynamics of turning. Because the squid’s propulsive systems, i.e., pulsed jet and fins, produce asymmetric flows that fall outside a 2D plane, I used a volumetric (3D) velocimetry technique known as DDPTV (defocusing digital particle tracking velocimetry) to study global hydrodynamic features during turns. I also collected simultaneous high-speed video of body motions. My primary interests were (1) documenting different hydrodynamic turning approaches and kinematics, (2) measuring the relative force/momentum contributions of the fins and jet to various turns, and (3) determining if background flow plays a role in turning performance.

Methods

Animal husbandry

The target animal for this study was the brief squid, *Lolliguncula brevis* Blainville. Brief squid (N = 14; mean dorsal mantle length (DML) = 4.6±0.1 cm s.e.m.) were collected by otter trawl at the Virginia Institute of Marine Science Eastern Shore Lab, in Wachapregue, VA. *Lolliguncula brevis* were then transported to the marine
aquatics facility at Old Dominion University, Norfolk, VA in live wells (Aquatic Eco-
Systems, Inc., Apopka, FL, USA) filled with aerated filtered seawater. Animals were
maintained in two 450 gallon recirculating seawater systems at a salinity of 30 ppt,
temperature of 15-21°C and pH of 8.0-8.2. Ammonia levels were kept below 0.2 ppm. A
moderate current was maintained in the holding tank to facilitate active swimming and
reduce wall abrasions. Animals were fed a diet of grass shrimp, *Palaemonetes pugio*. The
squid were allowed to acclimate in the holding tanks for at least 24 h before trials.

**Kinematics**

A 250 liter flume (Model 502 {S}, Engineering Laboratory Design, Inc., Lake
City, MN, USA) with a 15 cm × 15 cm × 43 cm working section was used for this
experiment. Three high-speed synchronized DALSA Falcon video cameras (1400 × 1200
pixels) were used to record squid swimming behavior (DALSA Corp., Waterloo, ON,
Canada). Two of the cameras were positioned ventrally, with the third camera positioned
laterally to the working section. The lateral camera was outfitted with a 25 mm lens and
the two ventral cameras were outfitted with 35 mm lenses (Fujinon TV Lens, Fujinon
Corporation, China). The high-speed cameras were triggered by the onboard counter on
two CLSAS capture cards (IO Industries Inc., London, ON, Canada) to capture video at
100 frames per second. Video frames from the DALSA cameras were transferred to hard
disk, in real time, using the two CLSAS capture cards and Streams 5 software (IO
Industries Inc., London, ON, Canada).

The high-speed cameras were illuminated using two 500W lights outfitted with
color gel #27 filters (transmits wavelengths > 600 W). Illuminating the flume with red
light minimizes stress on the animal and provides different wavelengths than those used
for particle illumination, allowing for spectral filters to be used to prevent cross-
illumination. The flume was filled with aerated, artificial seawater of similar salinity and
temperature to that of the holding tanks. One squid was placed in the flume at a time, and
was acclimated to the flume at low speeds of 1-4 cm s⁻¹ for 5-10 minutes. The flow
velocity of the flume was kept low (0-5 cm s⁻¹) for many of the turning sequences, as
turning sequences in low flow conditions were of greatest interest. However, turning
behaviors were also captured at higher speeds, i.e., speeds = 7-12 cm s\(^{-1}\) to investigate turning hydrodynamics at more moderate flows. Having some flow in the swim tunnel was beneficial because it facilitated active swimming and turning behaviors and allowed for a consistent refreshment of reflective particles, which reduced background flow noise. Turning behaviors were spontaneous and not stimulated by external factors other than the flume flow.

**Hydrodynamics**

TSI’s V3V system (TSI, Inc. Shoreview, MN, USA) was used to collect volumetric hydrodynamic data around the squid during turns. The V3V system is based on the DDPTV technique (also referred to as DDPIV) (Pereira and Gharib, 2002; Pereira et al., 2000; Pereira et al., 2006). The flume was seeded with neutrally buoyant 50 µm polyamide light-reflective seeding particles (Dantec Dynamics, Skovlunde, Denmark). Two pulsed Nd:YAG lasers (wavelength=532 nm, power rating 350 mJ per pulse; LaBest Optronics Co. Ltd., Beijing, China) outfitted with optics to produce a cone of light were used to illuminate the particles in the working section of the flume. The camera probe (V3V-8000, TSI, Inc.), which consists of three cameras (2048 × 2048 pixels each), was positioned orthogonally to the working section and was synchronized with the laser pulses, allowing for the collection of paired DDPTV images (\(\Delta t = 0.5-6.0\) ms) at 7 Hz. Synchronization of the lasers and V3V probe was achieved by sending a master 7 Hz TTL signal generated with a PCI NI-6602 timing board, BNC-2121 breakout box, and NI timing software (National Instruments, Austin, TX, USA) to both a TSI synchronizer, which sent control signals to the V3V-8000 camera probe, and a BNC-565 pulse generator (Berkeley Nucleonics, San Rafael, CA), which sent control signals to the Nd:YAG lasers. During DDPTV image capture, images from the three Falcon high-speed cameras (1400 × 1200 pixels, 100 fps) were also collected simultaneously using a manual start trigger. The high-speed cameras were fitted with notch filters to block the 532 nm wavelengths and prevent overexposure from laser light. The V3V probe was also fitted with optical filters that transmit wavelengths of 532±5 nm so that only the light from the lasers illuminated the V3V CCD sensors.
The hydrodynamic data were processed using V3V 4G software (TSI, Inc., Shoreview, MN, USA) (TSI, 2012). The first step was to find particle images in each of the six images comprising the pairs (3 images for laser pulse A, 3 images for laser pulse B). Particle image identification was accomplished using an advanced 2D Gaussian fitting algorithm. The algorithm is capable of obtaining highly accurate subpixel positions of particle images and can identify overlapped particles, even under high particle seeding conditions (TSI, 2012). Approximately 100,000 particles were identified in each image. The second step involved matching particles among the three images for each laser pulse, i.e., finding triplets. The triplet formed by the three matched particles was used to derive the 3D position of the particle in the camera coordinate system (TSI, 2012). Typically, 50,000 to 60,000 triplets were found. Step three involved matching particles in the first paired image (frame A) with those in the second paired image (frame B), measuring the displacement between the particles (in 3D), and calculating the volumetric velocity fields. This process involved a particle tracking relaxation algorithm to obtain the 3D velocity vectors (Pereira et al., 2006). Particle vector outliers were removed using a modified universal median filter (finds outliers by comparing vectors with the median vector length in a neighborhood surrounding the vectors), which was set to a threshold magnitude of 6, and a global range filter (step four). Generally 18,000 to 25,000 particle vectors were obtained. Lastly, particle vectors were interpolated onto a regular grid. The vectors obtained from the particle tracking algorithm are located at the particles, which are randomly distributed inside the 3D measurement volume. Therefore, a Gaussian weighting interpolation was used to obtain velocity data on a regular grid. A voxel size of 8 mm with a 95% overlap and a smoothing factor of 1.5 was used. Prior to processing, the body of the squid was masked, i.e., subtracted out of the image, to eliminate any vectors that might be a result of body movement. Velocity vector and vorticity data were examined in V3V 4G software during analysis (TSI, 2012), but all velocity vector and vorticity isosurfaces were generated in Tecplot 360 (Tecplot, Bellevue, WA, USA).

One to two frames with fully developed vorticity structures from each turning sequence were selected to calculate impulse and torque. This approach is reasonable given that these images contain the cumulative history of the flow generation and
calculation of impulse and angular impulse (see below) are integral methods. The hydrodynamic impulse (I) and angular impulse (I_A) were calculated using custom Matlab routines developed in-house. The impulse was computed from

\[ I/\rho = \frac{1}{2} \int \mathbf{x} \times \mathbf{\omega} dV \]

where \( \mathbf{x} \) is the position vector, \( \mathbf{\omega} \) is the vorticity vector (computed from the measured velocity field, \( \mathbf{u} \), according to \( \mathbf{\omega} = \nabla \times \mathbf{u} \) using central differences), \( \rho \) is the fluid density, and the integral is computed over the volume of the vortex (Saffman, 1992). Impulse is physically determined by the time integral of the force vector that generated the flow. Hence, the average thrust/drag vector (magnitude and direction) associated with measured vortices was calculated by dividing I by the period of the flow generation (T). Impulse computed from the above equation is the impulse required to generate the flow. From Newton’s third law, the thrust (force) on the squid is in the opposite direction as the impulse vector. Similarly, I_A was computed from

\[ \frac{I_A}{\rho} = -\frac{1}{2} \int |\mathbf{x}|^2 \mathbf{\omega} dV \]

which is physically determined by the time integral of the torque/moment vector required to generate the flow and can be used to determine the (average) fluid dynamic moments applied to the animal upon division by the flow generation period T (Wu et al., 2006). Similar to linear impulse, the torque on the animal will be in the direction opposite to that determined from the above equation. By default, the torque associated with a vortex selected for analysis was computed with respect to the centroid of the vorticity magnitude of the vortex using the above equation. Torque about the center of mass of the squid was then computed by shifting the origin of the calculated impulse to the squid center of mass using the distance between the center of gravity of the squid (as the origin of the vortex) and the centroid of the vortex (determined from the custom Matlab routines) in accordance with the equation above under the added assumption that

\[ \int \mathbf{\omega} dV = 0 \]

which is true for a vortex contained entirely in the integration volume. Torque was categorized into the axis of rotation the impulse was acting about, i.e., yaw, roll or pitch
(Fig. 10). The yaw axis was defined as the major axis of the turn since the squid were primarily rotating in the X-Z plane. The sign convention used in the following considers negative torque about the yaw axis as resisting the motion of the turn and positive torque about the yaw axis as producing the turning motion of the squid. Net overall torque about the yaw axis (net overall $T_y$) was computed by summing all positive and negative torques contributing to yaw. Sequences with negative overall net $T_y$ were not considered for descriptive torque analyses since only positive net overall $T_y$ will result in a turning motion. Net fin torque (net fin $T_y$) and net jet torque (net jet $T_y$) were also computed by summing all positive and negative torque about the yaw axis created by the fins and jet, respectively. Rotation of the squid in Z-Y and X-Y planes were considered secondary, i.e., these rotations did not occur about the main turning axis, and contributed to torque about the roll and pitch axes, respectively. The overall torque magnitude acting about the roll and pitch axes (overall $T_{xz}$) was computed

$$T_{xz} = \sqrt{(T_{x}^2 + T_{z}^2)}$$

Fin torque (fin $T_{xz}$) and jet torque (jet $T_{xz}$) about the roll and pitch axes were also computed by summing values of positive and negative torque contributions and evaluating the equation above for either the fins or jet, respectively. The ratio of jet to fin torque was computed by dividing the mean absolute values of overall $T_y$ or $T_{xz}$ for the jet by the mean absolute values of overall $T_y$ or $T_{xz}$ for the fins. A ratio of the mean jet/fin $T_y$ or mean jet/fin $T_{xz}$ was used rather than averaging the ratio of each sequence because some sequences had zero net fin or jet torque.

Kinematic variables were determined from high-speed data following similar protocols as those described in Chapter 2. Frame-by-frame position tracking of the cephalopod body features was accomplished using image analysis software (Hedrick, 2008). Seven points were tracked in the ventral view: (1) tail tip, (2) funnel base, (3) arm tip, (4) right fin tip (at maximum chord point), and (5) left fin tip (at maximum chord point). The tracked points in the ventral view were used to determine (1) the center of rotation (COR), (2) angular velocity, (3) total angular displacement, (4) direction of the turn, (5) time to execute the turn, (6) frequency of fin beats, and (7) translation.
Figure 10. The three axes of rotation about the center of gravity (yellow dot) of brief squid *Lolliguncula brevis*. When the squid is oriented relative to oncoming flow as depicted above, rotation about the Y-axis results in yaw, rotation about the X-axis results in roll and rotation about the Z-axis results in pitch.
The data were smoothed using the Cross-Validation Criterion (CVC). This smoothing method uses smoothed splines where the level of smoothing is determined such that the root-mean-squared error of the splines determined with points from the data individually excluded is minimized (Walker, 1998). In the present implementation, the minimization is determined to within 0.1% of the actual minimum to speed convergence of the method.

The COR was the point relative to the squid in the ventral view that moved the least during the turn. Finding the COR was performed using an in-house Matlab code that used a two segment approach with the lines connecting the tail tip to the funnel base and then the funnel base to the arm tip. The code was generalized so that the COR did not actually have to fall directly on these line segments. Rather, it could lie along a line at a fixed angle $\alpha$ with respect to the tracked body segment, where $\alpha$ and the position of the COR along the line at this angle were selected such that the movement of the COR during the turn was minimized.

The radius ($R$) of the turning path is the radius of curvature of the COR. This was computed from analytical geometry using

$$\frac{1}{R} = \frac{y''}{\left(1 + (y')^2\right)^{3/2}}$$

where $y' = dy/dx$, $x$ and $y$ are the coordinates of the COR in the ventral view, and the derivatives were evaluated using fourth order accurate finite difference equations. For each turn sequence, both the mean radius of the turning path and the minimum radius of the turning path were determined using in-house Matlab routines. To compare the present data with previous studies, the minimum and mean radius of the turning path ($R$) was normalized (divided by the length of the animal) to get a length-specific turning radius ($R/L$). The mean length specific minimum radius of the turn, $(R/L)_{\text{mean}}$, was the average of all the radii throughout the turn divided by the total length of the animal. The minimum length-specific minimum radius of the turn, $(R/L)_{\text{min}}$, was the absolute minimum radius of each turn divided by the total animal length. The 90th percentile value for $(R/L)_{\text{min}}$ was used to be conservative and account for digitization error. $\omega_{\text{avg}}$ was the mean angular velocity throughout the turn. $\omega_{\text{max}}$ was the maximum angular velocity found during the
turn. $\Theta_{\text{total}}$ was the total angular displacement for each turn. Translation was defined as the maximum distance (Max D) between the COR at any two instances during the turn, divided by the total length of the animal.

A total of 25 turning sequences from 14 animals exposed to varying flow speeds (range = 1.0-11.4 cm s$^{-1}$) were analyzed for this study. Turns were categorized into four groupings: (1) short tail-first turns, (2) long tail-first turns, (3) vertical tail-first turns and (4) arms-first turns. All vertical turns occurred while the animal was moving with the flow as opposed to actively swimming against the flow. The arms-first or tail-first orientations described above refer to the orientation of the squid at the beginning of the turns and flow speed was the tunnel free-stream velocity.

The following kinematic variables were compared among the four categories, as well as with turning data collected in stationary water from Chapter 2: $\omega_{\text{max}}$, $\omega_{\text{mean}}$, $(R/L)_{\text{min}}$, $(R/L)_{\text{mean}}$, $\Theta_{\text{total}}$, translation, tunnel flow speed, inboard fin frequency and outboard fin frequency. If data did not meet assumptions of normality a Log10 transformation was used. A nested MANOVA with individual nested within category was used to determine if there was a statistical difference between $\omega_{\text{max}}$, $\omega_{\text{mean}}$, $(R/L)_{\text{min}}$, $(R/L)_{\text{mean}}$, total angular displacement, translation and tunnel flow speed (SPSS, V.22, IBM, New York USA). A LSD post hoc test was used for multiple comparisons (SPSS). Outboard and inboard fin frequencies for each category were compared using paired t-tests (SPSS, V. 22, IBM, New York USA).

**Results**

Four patterns of turns were identified in brief squid *L. brevis*: (1) short tail-first turns, (2) long tail-first turns, (3) vertical tail-first turns and (4) arms-first turns (Fig. 11). A number of qualitative differences in flow patterns, torque magnitude and flow speed were observed among these different turning categories. Moreover, contributions from the fins and jet differed with each type of the turn. These differences are described in the paragraphs that follow.

*Patterns of turning*
Short tail-first turns were observed in 28% of the turning sequences and occurred at intermediate flow speeds (range = 3.4-7.7 cm s⁻¹; mean = 5.9± 0.7 cm s⁻¹) (all means are reported as the mean ± s.e.m.). Short tail-first turns were characterized by two strong jet vortex ring pulses and minimal fin flow (Fig. 12). The initial jet pulse (inset 3 in Fig. 12A and inset 2 in Fig. 12B) was often the dominant jet, producing greater torque than the secondary pulse, and was typically directed away from the outboard side and perpendicular to the longitudinal axis of the squid body. The secondary jet pulse (inset 2 in Fig. 12A and inset 1 in Fig. 12B) created lower torque magnitudes. The jet created a greater degree of net $T_y$ than the fins (jet $T_y = 4.1x10^{-5} ±1.4x10^{-5}$ N m; fin $T_y = -7.0 x10^{-6} ±2.4x10^{-5}$ N m) with a jet to fin ratio of 5.9 (Table 2). Short tail-first turns had lower overall net $T_y$ (3.4x10⁻⁵±1.3x10⁻⁵ N m) and net jet $T_y$ than other categories. The jet also created more torque about the roll and pitch axes ($T_{xz}$) than the fins (jet $T_{xz} = 2.4x10^{-4} ±1.6x10^{-4}$ N m; fin $T_{xz} = 3.9x10^{-5}±1.7x10^{-5}$ N m) with a jet/fin ratio of 6.2. Thus, the jet was the major effector of these turns, producing most of the torque contributing to rotation about the main turning axis (yaw axis) and producing a greater degree of torque resulting in roll and pitch than the fins. Relative to long tail-first turns, short tail-first turns involved similar overall $T_{xz}$ (short tail-first turns: 2.8x10⁻⁴±1.5x10⁻⁴ N m; long tail-first turns: 2.4x10⁻⁴±2.3x10⁻⁴ N m). During these short tail-first turning sequences, stronger flow fields were observed when turning occurred against as opposed to with oncoming tunnel flow.

Long tail-first turns were the least common turning behavior observed, occurring in only 12% of the recorded turns. All three of these turns involved one individual squid. These turns occurred at low flow speeds (range = 1.5-5.3 cm s⁻¹; mean = 3.1± cm s⁻¹) and were longer in duration (1.7±0.2 s) compared to the other three categories (short tail-first: 1.1±0.1 s; vertical: 0.6±0.2 s; arms-first: 1.3±0.2 s). These turns involved directed jet flows and an extended outboard fin, whereby the speed of the squid generally exceeded the free-stream flow. The vorticity field for these turns included one or two elongated regions of vorticity extending from the fins (Fig. 13). The jet was used to effect the turn while the fins generated negative net $T_y$ via drag forces that opposed the direction of the turn. The jet generated greater net $T_y$ than the fins (net jet $T_y = 1.9x10^{-4}±1.9x10^{-4}$ N m;
Figure 11. **The four categories of turning.** A. Short tail-first turns. B. Long tail-first turns. C. Vertical turns. D. Arms-first turns. The pink region is the vorticity isosurface. Water tunnel flow was moving from right to left, but mean stream velocity was subtracted out of the images for clarity. The background is an X-Y plane slice of the velocity flow field.
Figure 12. **Two examples of short tail-first turns.** A. Inset 1 is fin flow velocity from a slice in the Z plane. Inset 2 is a velocity slice in the X-Y plane of the vortex ring associated with a secondary jet pulse. Inset 3 is a velocity slice in the X-Y plane for the dominant (lower) jet pulse. B. Inset 1 is a velocity slice in the X-Y plane showing fin flows (top arrow) and the secondary jet pulse (middle arrow). Inset 2 is a velocity slice in the X-Y plane of the dominant (lower) jet pulse. The thumbnails on the right are a series of images from the turn (0.14 s apart), with the image that corresponds to the flow field highlighted in yellow. The pink region is the vorticity isosurface. Water tunnel flow was moving from right to left, but mean stream velocity was subtracted out of the images for clarity.
Table 2. **Net torque about the yaw axis (Ty) and total torque about the combined roll and pitch axes (Txz).** Overall, jet and fin contributions are included for both torque categories along with ratios of jet to fin torque.

<table>
<thead>
<tr>
<th>Category</th>
<th>Net Overall $T_y$ (N m)</th>
<th>Net Jet $T_y$ (N m)</th>
<th>Net Fin $T_y$ (N m)</th>
<th>Net Jet/Fin $T_y$ Ratio</th>
<th>Net Overall $T_{xz}$ (N m)</th>
<th>Net Jet $T_{xz}$ (N m)</th>
<th>Net Fin $T_{xz}$ (N m)</th>
<th>Net Jet/Fin $T_{xz}$ Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short tail-first</td>
<td>3.4x10^-5 (1.3x10^-5)</td>
<td>4.1x10^-5 (1.4x10^-5)</td>
<td>-7.0x10^-5 (2.4x10^-5)</td>
<td>5.9</td>
<td>2.8x10^-4 (1.5x10^-4)</td>
<td>2.4x10^-4 (1.6x10^-4)</td>
<td>3.9x10^-5 (1.7x10^-5)</td>
<td>6.2</td>
</tr>
<tr>
<td>Long tail-first</td>
<td>1.5x10^-4 (1.2x10^-4)</td>
<td>1.9x10^-4 (1.9x10^-4)</td>
<td>-4.6x10^-5 (7.2x10^-5)</td>
<td>4.2</td>
<td>2.4x10^-4 (2.3x10^-4)</td>
<td>8.8x10^-5 (8.8x10^-5)</td>
<td>1.5x10^-4 (1.4x10^-4)</td>
<td>0.6</td>
</tr>
<tr>
<td>Vertical</td>
<td>1.5x10^-4 (5.0x10^-5)</td>
<td>9.8x10^-5 (9.8x10^-5)</td>
<td>4.8x10^-5 (4.8x10^-5)</td>
<td>2.0</td>
<td>5.9x10^-4 (3.2x10^-4)</td>
<td>1.3x10^-4 (1.3x10^-4)</td>
<td>4.5x10^-4 (4.5x10^-4)</td>
<td>0.3</td>
</tr>
<tr>
<td>Arms-first</td>
<td>7.8x10^-4 (2.3x10^-4)</td>
<td>1.5x10^-4 (8.2x10^-5)</td>
<td>6.3x10^-4 (1.5x10^-4)</td>
<td>0.2</td>
<td>1.8x10^-4 (6.8x10^-4)</td>
<td>2.6x10^-4 (1.5x10^-4)</td>
<td>1.5x10^-3 (8.0x10^-4)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

All values are means for all sequences within the category (values in parentheses are standard error of the mean)
Figure 13. **An example of a long tail-first turn.** Inset 1 is a velocity slice in the X-Z plane through the elongated region of vorticity. Inset 2 is a velocity slice in the X-Y plane midway through the elongated region of vorticity. The thumbnails on the right are a series of images from the turn (0.14 s apart), with the image that corresponds to the flow field highlighted in yellow. These turns were longer in duration than other categories. The pink region is the vorticity isosurface. Water tunnel flow was moving from right to left, but mean stream velocity was subtracted out of the images for clarity.
net fin $T_y = -4.6 \times 10^{-5} \pm 7.2 \times 10^{-5}$ N m) with a jet to fin ratio of 4.2 (Table 2). The overall net $T_y$ for this category ($1.5 \times 10^{-4} \pm 1.2 \times 10^{-4}$ N m) was similar to vertical turns. The fins contributed more to torque acting about the roll and pitch axes than the jet ($T_{xz} = 8.8 \times 10^{-5} \pm 8.8 \times 10^{-5}$ N m; $T_{xz} = 1.5 \times 10^{-4} \pm 1.4 \times 10^{-4}$ N m) (Table 2) resulting in a jet to fin ratio of 0.6 (Table 2). Fin $T_{xz}$ and jet $T_{xz}$ for long tail-first turns were lower than these measurements in the other categories, but overall $T_{yz}$ for long tail-first turns was similar to that of short tail-first turns.

Vertical turns were always performed tail first while translating with the flow. These turns occurred in higher speed flows (range = 8.9-10.6 cm s$^{-1}$; mean = 9.7± cm s$^{-1}$) and were only observed in 20% of the turning sequences. The jet often produced linked vortex rings (Fig. 14B), with the fins also producing vortex rings, though they were generally not as prominent (Fig. 14A). Given that net jet $T_y$ ($9.8 \times 10^{-5} \pm 9.8 \times 10^{-5}$ N m) was greater than net fin $T_y$ ($4.8 \times 10^{-5} \pm 4.8 \times 10^{-5}$ N m) and the jet to fin $T_y$ ratio was 2.0, the jet contributed more to the turning motion. The jet and fin flows generated high torque about the roll and pitch axes (secondary axes) with only arms-first turns having higher overall net $T_{xz}$. The fins contributed to a greater extent than the jet to roll and pitch ($T_{xz} = 1.3 \times 10^{-4} \pm 1.3 \times 10^{-4}$ N m; $T_{xz} = 4.5 \times 10^{-4} \pm 4.5 \times 10^{-4}$ N m) with a jet to fin ratio of 0.3 (Table 2).

Arms-first turns occurred in 40% of the sequences and over a wider range of tunnel background flow speeds (range = 0.7-11.4 cm s$^{-1}$; mean = 6.0± cm s$^{-1}$) than other turns. Nine of these sequences occurred while squid swam against the flow and one sequence occurred while squid translated with the flow. Arms-first turns generally involved more prominent vorticity from the fins compared to short tail-first and vertical turns, and the fin wakes often consisted of linked vortices (Fig. 15). Although the tail-first turns generally had clear delineations between fin and jet pulses, arms-first turns often included significant jet/fin or outboard fin/inboard fin flow interactions (Fig. 15B). Many of these turns began with a quick jet with the fins becoming more active later in the turn. Arms-first turns had the highest mean overall net $T_y$ ($7.8 \times 10^{-4} \pm 2.3 \times 10^{-4}$ N m) relative to the other turning categories, driven primarily by high fin net $T_y$ ($6.3 \times 10^{-4} \pm 1.5 \times 10^{-4}$ N m). The high $T_y$ for the fins indicates that the fins are more important for
Figure 14. **Two examples of vertical turns.** A. A vertical turn with prominent flows from the fins (the jet was in a previous frame). Inset 1 is a velocity slice in the X-Y plane illustrating stabilizing flow from the fin. Inset 2 is a velocity slice in the X-Y plane illustrating fin flow in the yaw plane and stabilizing moments in the pitch plane. B. A vertical turn with linked vortices from the jet. Inset 1 is a velocity slice in the X-Y plane through the linked rings towards the back of the working section. Inset 2 is a velocity slice in the X-Y plane through the linked rings towards the front of the working section. The thumbnails on the right are a series of images from the turn (0.14 s apart), with the image that corresponds to the flow field highlighted in yellow. The vorticity isosurface is in pink. Water tunnel flow was moving from right to left, but mean stream velocity was subtracted out of the images for clarity.
Figure 15. **Two examples of arms-first turns.** A. An example of an arms-first turn where fin flows and the jet pulse (lower inset) were spatially separate. Inset 1 is a velocity slice in the X-Y plane of vortex rings produced by the inboard fin (upper arrow) and the outboard fin (lower arrow). Inset 2 is a velocity slice in the X-Y plane of the jet pulse. B. An example of an arms-first turn where fin flows were interacting with jet flows. Inset 1 is a velocity slice in the X-Y plane of linked vorticity rings from the fins. Inset 2 is a velocity slice in the X-Y plane of the jet pulse. The pink region is the vorticity isosurface. The thumbnails on the right are a series of images from the turn (0.14 s apart), with the image that corresponds to the flow field highlighted in yellow. Water tunnel flow was moving from right to left, but mean stream velocity was subtracted out of the images for clarity.
producing rotation in this category of turning than the jet and are capable of generating substantial force. The jet resulted in positive net $T_y$ \((1.5 \times 10^{-4} \pm 8.2 \times 10^{-5} \text{ N m})\) and the jet to fin $T_y$ ratio was 0.2 (Table 2). Arms-first turns had higher overall $T_{xz}$ than all other turn categories \((1.8 \times 10^{-3} \pm 6.8 \times 10^{-4} \text{ N m})\), and the overall $T_{xz}$ was driven primarily by a high fin $T_{xz}$ \((1.5 \times 10^{-3} \pm 8.0 \times 10^{-4} \text{ N m})\). Jet $T_{xz}$ was \(2.6 \times 10^{-3} \pm 1.5 \times 10^{-4} \text{ N m}\) and the jet to fin $T_{xz}$ ratio was 0.2 (Table 2). Both fin $T_{xz}$ and jet $T_{xz}$ were greater for arms-first turns than all other categories.

**Kinematics**

There were no statistical differences in beat frequency between the inboard and outboard fins (Table 3). $\omega_{\text{max}}$ was significantly higher for vertical turns than arms-first turns, short tail-first turns, long tail-first turns and turns in stationary water (Chapter 2) (MANOVA: $F=6.4, \text{df}_{21,64}, P<0.001$) and $\omega_{\text{avg}}$ was significantly higher for vertical turns than long tail-first turns (MANOVA: $F=5.8, \text{df}_{21,64}, P<0.03$) (Table 3). $\theta_{\text{total}}$ was significantly higher in vertical turns than in short tail-first turns, arms-first turns and turns in stationary water (MANOVA: $F=5.8, \text{df}_{21,64}, P=0.006, 0.03, 0.02$, respectively) (Table 3). $(R/L)_{\text{min}}$ was significantly lower for turns in stationary water than short tail-first turns (MANOVA: $F=5.8, \text{df}_{21,64}, P=0.007$) (Table 3). $(R/L)_{\text{mean}}$ was significantly lower for turns recorded in stationary water than those recorded in short tail-first turns, vertical turns and arms-first turns in the current study (MANOVA: $F=5.8, \text{df}_{21,64}, P=0.001, P=0.001, P<0.001$, respectively). Both short tail-first turns and vertical turns had significantly greater $(R/L)_{\text{mean}}$ than long tail-first turns (MANOVA: $F=5.8, \text{df}_{21,64}, P<0.05$) (Table 3). There was no correlation between tunnel flow speed and $(R/L)_{\text{min}}$ or tunnel flow speed and $(R/L)_{\text{mean}}$. All four turning categories had greater translation than turns in stationary water (MANOVA: $F=5.8, \text{df}_{21,64}, P<0.02$) (Table 3). Vertical turns also had greater translation than arms-first turns (MANOVA: $F=5.8, \text{df}_{21,64}, P=0.01$). No difference in background flow speed was detected for short tail-first turns and arms-first turns. However, all four with-flow categories were performed at significantly greater flow speeds than turns in stationary water (MANOVA: $F=5.8, \text{df}_{21,64}, P<0.001$). Long tail-first turns were performed at significantly slower flow speeds than the other turning categories.
Table 3. Kinematic variables for turning categories.

<table>
<thead>
<tr>
<th></th>
<th>Short Tail-First</th>
<th>Long Tail-First</th>
<th>Vertical</th>
<th>Arms-First</th>
<th>Stationary</th>
</tr>
</thead>
<tbody>
<tr>
<td>$(R/L)_{\text{min}}$</td>
<td>0.03 (0.03)</td>
<td>0.004 (0.001)</td>
<td>0.01 (0.007)</td>
<td>0.01 (0.003)</td>
<td>0.003 (0.0005)</td>
</tr>
<tr>
<td>$(R/L)_{\text{mean}}$</td>
<td>0.3 (0.08)</td>
<td>0.2 (0.005)</td>
<td>0.3 (0.09)</td>
<td>0.3 (0.06)</td>
<td>0.008 (0.003)</td>
</tr>
<tr>
<td>$\omega_{\text{max}}$ (deg s$^{-1}$)</td>
<td>270.1 (39.0)</td>
<td>411.5 (229.5)</td>
<td>950.3 (462.5)</td>
<td>319.2 (74.7)</td>
<td>268.4 (32.9)</td>
</tr>
<tr>
<td>$\omega_{\text{avg}}$ (deg s$^{-1}$)</td>
<td>101.8 (16.7)</td>
<td>69.4 (6.1)</td>
<td>148.1 (26.6)</td>
<td>103.7 (14.5)</td>
<td>110.3 (14.6)</td>
</tr>
<tr>
<td>$\Theta_{\text{total}}$ (°)</td>
<td>101.4 (9.8)</td>
<td>117.7 (24.4)</td>
<td>137.6 (17.6)</td>
<td>113.6 (8.2)</td>
<td>117.2 (18.6)</td>
</tr>
<tr>
<td>Translation</td>
<td>0.4 (0.1)</td>
<td>0.4 (0.1)</td>
<td>0.6 (0.1)</td>
<td>0.4 (0.05)</td>
<td>0.2 (0.02)</td>
</tr>
<tr>
<td>Mean Tunnel Speed (cm s$^{-1}$)</td>
<td>5.7 (0.6)</td>
<td>3.1 (1.1)</td>
<td>9.8 (0.3)</td>
<td>6.0 (1.0)</td>
<td>--</td>
</tr>
<tr>
<td>Inboard fin freq. (beats s$^{-1}$)</td>
<td>1.8 (0.3)</td>
<td>2.7 (1.5)</td>
<td>2.1 (1.1)</td>
<td>2.6 (0.5)</td>
<td>2.9 (0.2)</td>
</tr>
<tr>
<td>Outboard fin freq. (beats/s$^{-1}$)</td>
<td>2.8 (0.7)</td>
<td>1.5 (1.2)</td>
<td>2.9 (1.4)</td>
<td>2.3 (0.4)</td>
<td>3.6 (0.2)</td>
</tr>
</tbody>
</table>

All values in parentheses are standard error of the mean. Stationary column derives from data in Chapter 2.
categories (MANOVA: F=5.8, df<sub>21,64</sub>, P<0.001), and vertical turns were performed at significantly higher flow speeds than the other turning categories (MANOVA: F=5.8, df<sub>21,64</sub>, P<0.001). As the tunnel flow speed increased, ω<sub>mean</sub> increased (Pearson correlation: r=0.5, df<sub>18</sub>, P=0.02) (Fig. 16).

**Discussion**

Maneuverability involves the creation and amplification of disturbances, whereas hydrodynamic stability involves the prevention and correction of disturbances (Weihs, 1993). Stable configurations decrease the cost of rectilinear locomotion, but can result in decreased maneuverability (Weihs, 2002). Many nektonic animals have flexible control surfaces and propulsors to override dynamic stability when necessary, which increases turning performance (Fish, 2002). This is the case for squid, as they have a pulsed jet, flexible fins, and flexible arms to override dynamic stability, allowing them to achieve high levels of turning performance (Chapter 2). Using coordinated jet and fin motions, *L. brevis* in the present study produced four categories of turning behaviors, each with unique hydrodynamic characteristics. The high variability in flow patterns produced during these four different types of turning behaviors indicates that the dual-mode propulsion system in squid affords them enormous flexibility for unsteady swimming maneuvers.

**Turning patterns**

Long tail-first turns were the rarest turns observed and involved the most unusual flow field patterns, i.e., long trains of concentrated fin vorticity. During these turns, the squid spun its body using a directed jet while extending its outboard fin. The extended fin produced drag that resulted in negative net fin $T_y$, which effectively slowed and presumably controlled the turning motion. In this category the turning motion was largely driven by the jet, evident by the observed high jet to fin net $T_y$ ratio. The overall torque contributing to roll and pitch was fairly low compared to all the other categories, with the fins playing a greater role in pitch/roll control than the jet. This finding suggests that stabilization in pitch and roll are less important for long tail-first turning than in other
Figure 16. The relationship between the mean angular velocity ($\omega_{avg}$) and the tunnel flow speed for pooled turns for *L. brevis* (N=19). The relationship is significant (see text for correlation statistics).
modes of turning, and may be a product of greater fin control for primary axis turning. In these turns the squid typically jetted at a slight angle into the turn, banking slightly inward and extending the outboard fin. By banking and extending the outboard fin, the body and fin are canted at a higher angle incident to the flow, which provides a greater projected area facing the axis of the turn (Parson et al., 2011). This is common in animals that lack a dorsal keel and has been shown to aid turning performance in other nekton (Fish et al., 2003; Parson et al., 2011; Weihs, 1993). During banking in other nekton, the pectoral fins generate lift that can be divided into vertical and horizontal vectors. The vertical component counteracts negative buoyancy and the horizontal vector is directed towards the center of rotation, creating centripetal force to power the turn (Fish et al., 2003).

Vertical turns were also not especially common and occurred at the highest flow speeds considered in this study. In these turns, the jet was responsible for the majority of the turning motion, and produced prominent linked vorticity structures. The fins produced some torque for turning to assist the jet, but were especially important for generating torque about the roll and pitch axes, likely to keep the squid positioned in a vertical orientation relative to oncoming flow. In fact, rotation about the roll and pitch axes was more important in this category than both short tail-first and long tail-first turns. For vertical turns, the squid essentially spun on their longitudinal axis while orienting vertically to oncoming flow with the aid of stabilizing torque in pitch and roll. Vertical turns had the highest $\omega_{\text{max}}$, $\omega_{\text{avg}}$ and translation of all turning categories (including turns in stationary water). The high level of translation for this category is not surprising given these turns were associated with the highest background flows. The high values of $\omega_{\text{max}}$ and $\omega_{\text{avg}}$ also seem reasonable given a combination of rotation and translational movements can promote increased agility, as demonstrated in batoids (Parson et al., 2011), and spinning about the longitudinal axis reduces resistance to rotation (and subsequent pressure drag). Spinning longitudinally not only aids agility but it also can potentially lower $(R/L)$, since it lessens the impact of the body length on the turning radius. However, low $(R/L)_{\text{mean}}$ and $(R/L)_{\text{min}}$ were not observed for vertical turns in the present study probably because high translation outweighed this advantage. Although this
vertical orientation was not observed in turns in stationary water, it has been observed in *L. brevis* in holding tanks with current flow (pers. observations) and in deep-sea cephalopods moving with the current (Denton and Gilpin-Brown, 1973; McSweeney, 1978; Vecchione and Roper, 1991; Robison 2015). Currents and regions of turbulent and high flow are common in inshore environments, and thus *L. brevis*’ ability to turn while in a vertical orientation is likely an effective and cost-effective strategy in natural marine habitats.

Short tail-first turns were commonly observed in this study, and are similar to routine turns observed in stationary water. The jet was the major effector of turns in this category, contributing to the highest jet to fin $T_y$ ratio for the turning categories. The ratio of jet to fin $T_{xz}$ was also high relative to other categories, indicating that the jet is important for stabilization, i.e., torque about pitch and roll axes, during short tail-first turns. Similar to long tail-first turns, the net $T_y$ from fin flows was negative, indicating that the fins slowed and controlled the turn, though the fin negative torque contributions were low overall relative to the positive torque contributions of the jet. The jet pulses in this category resulted in short, rolled up vorticity structures that are associated with lower impulse, but greater propulsive efficiency than longer pulses (Bartol et al., in press; Bartol et al., 2009). These short jet pulses are likely more effective in turning given that smaller corrective motions facilitate greater control throughout the turn as the angle of the body relative to flow is constantly changing. Longer, high force pulses result in greater deviation from the turning trajectory, less control and likely greater translation by increasing momentum in the jet direction, which requires more effort to steer into a turn. The jet can cause the head and arms of the squid to pitch upwards, thus jet and fin torque about the roll and pitch axes can play an important role in counteracting the upward movement of the head and arms. While fin flows were lower in magnitude along all axes than jet flows, the flows were directed downwards (inset 1 of Fig. 12A and inset 1 of Fig. 12B) and were likely employed to counteract slight upward pitching moments of the head and arms. Therefore, short tail-first turning, which involves short, efficient jet pulses and some stabilizing jet and fin flows, facilitates controlled maneuvering and is likely a good
strategy for overcoming minor flow perturbations, making adjustments in local flow, and navigating complex environments.

During arms-first turning the fins drove the turning motion, with the lowest ratio of jet to fin $T_y$ being observed in this turning category. The jet also produced some $T_y$ contributing to the turning motion, but the fin torque contributions to the turning motion were consistently higher. This finding indicates that the fins are capable of producing high forces for turning, and although the fins did not contribute substantially to the turning motion for other categories, they are crucial components of arms-first turning. Based on the high $T_{xc}$ fin magnitude and low jet to fin ratio of $T_{xc}$, the fins are also important for roll and pitch stability control during the turns. In fact torque about the roll and pitch axes was more important in arms-first turns than in any other turn category. As discussed above, rolling, or banking, is an efficient means of accomplishing turning maneuvers by providing a greater projected area facing the axis of the turn, so that the body and fins are positioned at higher angles incident to the flow, facilitating centripetal forces that are beneficial for turns (Fish et al., 2003; Parson et al., 2011). Though the muscular hydrostatic fins of squid are different structurally from the pectoral fins of rays, both squid and rays lack median fins, i.e., mid body keels, and exhibit high levels of banking for effective turning (Parson et al., 2011).

The presence of linked fin vortices, high fin torque resulting in turning motion, and high stabilization in pitch and roll by the fins during arms-first turns suggest that $L. brevis$ uses complex wave motions during arms-first turns. Indeed undulatory and oscillatory (flapping) motions have been observed in $L. brevis$ during arms-first rectilinear swimming (Bartol et al., 2001a; Stewart et al., 2010) and recent studies have shown that linked vortex flows are common in the wakes of $L. brevis$ fins during steady swimming (Bartol et al., in press; Stewart et al., 2010). Furthermore, proper orthogonal decomposition (POD) analyses of fin motions in $L. brevis$ have revealed that arms-first fin motions involve both flapping and prominent wave components of different amplitudes and frequencies (Bartol et al., in review). This differs from tail-first swimming where fin motion is less complex, with flapping dominating the motions and wave components playing a more reduced role. The large contributions of fin $T_{xc}$ to arms-
first turning observed here is consistent with previous studies that emphasize the importance of fins for stabilization of motions during arms-first steady swimming (Zuev, 1966; Hoar et al., 1994; Bartol et al., 2001a, b; Stewart et al. 2010; Bartol et al., in press).

The substantial net fin $T_y$ and high relative reliance on the fins for torque adjustments in the roll and pitch axes may be related to (1) high arm drag, (2) funnel bending, and (3) the fin location. During arms-first swimming, it may be difficult for the leading edge arms to remain streamlined during turns and thus any abrupt arm movements may elevate pressure drag on the animal, requiring more fin activity to counteract these motions. Additionally, the funnel has to bend backwards towards the tail to create a jet during arms-first swimming and turning. Bending places more strain on the muscles that control funnel rotation and aperture (Kier and Thompson, 2003; Thompson and Kier, 2001b), which can potentially limit the jet, requiring more fin input to produce and control the turn. Finally, during arms-first swimming the jet is directed along an axis farther from the center of mass than in tail-first swimming, resulting in greater pitching during arms-first swimming (Stewart et al., 2010). The distant location of the fins from the center of mass makes them effective control surfaces for producing corrective pitching moments.

\textit{Jet contributions to turning}

The jet flow exits through the funnel, which is located relatively close to the squid’s center of gravity. The jet velocity can be high, subsequently moving the flow away from the body and generating a large amount of thrust. In this way, even if the moment arm (distance of the jet flow from the body) is small, the resultant torque can still be high and create high levels of body rotation. The effectiveness of a force (and moment of the force) to turning is a product of magnitude and perpendicular distance from the line of action to the axis of rotation (Vogel, 2003). Squid are capable of producing higher magnitude forces at greater perpendicular distance with their jets than their fins (Bartol et al., 2008; Bartol et al., 2009a; Stewart et al., 2010; Bartol et al., in press), and thus it is not surprising that jet $T_y$ played such a significant role in producing rotation in short tail-first, long tail-first and vertical turns. The flow from the jet was
directed predominantly downwards and perpendicular to the outboard side of the squid (outer side of squid during the turn) for most turns. These directional flows rotated the body in the yaw axis while providing upward forces to counteract negative buoyancy. Upward force generation is especially important when these animals swim and turn at low speeds and dynamic lift forces are minimal (Bartol et al., 2001a; Bartol et al., 2009a). As swimming speed increases, the vertical component of the jet decreases due to a quadratic increase in dynamic lift with speed (Bartol et al., 2001a; Bartol et al., 2009a).

Although the jet primarily acted as the driver for turning motions for tail-first and vertical turns, it also contributed to roll and pitch in short tail-first turns as described earlier. This is not surprising given that the funnel can quickly redirect flows, allowing for rapid turning and positional adjustments when necessary.

Directed jet pulses often resulted in vortex rings. Vortex rings were clearly observable during short tail-first turns and arms-first turns, though they were often linked with fin flows during arms-first turning. The observed vortex rings are similar to the jet mode I pattern observed for _L. brevis_ steady swimming during 2D DPIV (Bartol et al., 2009a) and 3D DDPTV (Bartol et al., in press) experiments. Jet mode I is characterized by fluid rolled up into an isolated vortex ring, and has low overall time-averaged force production but high propulsive efficiency. Although this pattern is observed most frequently at low swimming speeds, it also occurs at intermediate and high speeds (Bartol et al., 2009a; Bartol et al., in press). Given the high propulsive efficiency associated with isolated vortex rings, it is not surprising that short pulses were employed for controlled turning maneuvers. As the angle of the body axis relative to flow constantly changes during the turn, short pulsed flows are an effective mechanism for generating precise turning movements, as evident by the frequent detection of multiple jet-derived vortex rings during turns in the present study. Longer jets with high force production, e.g., jet mode II (Bartol et al., 2009), would offer less control during the turn, increasing turning radius and energy requirements. Although vortex rings were more prominent in the most common turning modes (short tail-first and arms-first turns), linked rings and elongated rings were also observed in vertical and long tail-first turns, suggesting that vortex ring
formation and its derivatives are important hydrodynamic structures for a wide range of turns in squid.

**Fin contributions to turning**

The flexibility of the fins and their ability to undulate and oscillate independently of each other allows for the creation of complex flows (Kier and Thompson, 2003; Stewart et al., 2010). In fact, during turns this flexibility often allowed one fin to contribute to $T_y$ while the other fin generated $T_{xc}$. Asymmetric production of forces was common in the present study and has been observed in some fishes (Drucker and Lauder, 2001a; Drucker and Lauder, 2001b). The fins are clearly important for both torque generation and stability control. In this study, the fins produced most of the torque for effecting turns in the arms-first orientation and provided substantial stabilization about the roll and pitch axes in vertical, long tail-first and arms-first turns. Furthermore, during long tail-first and short tail-first turns, the fins produced negative net $T_y$, suggesting that the fins are important not only for stabilizing the body in pitch and roll but also for providing stability and speed control along the primary turning axis (yaw) for certain turning behaviors. Because the fins are positioned a significant distance from the center of gravity, they can provide large correcting moments, as is the case with certain fins in fish (Fish, 2002; Parson et al., 2011). Positioning control surfaces behind the center of gravity, relative to the direction of motion, further enhances their ability to influence stability (Fish, 2002; Weihs, 1993) and is likely why the fins are much more active during arms-first turns. Like batoids that lack dorsal fins, *L. brevis* do not have a dorsal keel and thus their flexible paired fins must act as both propulsors and control surfaces, as evident in the present study. The ability to vary the dihedral angles of the fins with the body, as in the highly flexible squid fins, is especially effective for maneuvering because it facilitates banking and subsequent stabilization in the roll plane (Fish et al., 2003; Parson et al., 2011; Weihs, 1993). Control surfaces lose their effectiveness at lower swimming speeds, as forces generated by the control surfaces are often less than inertial forces (Webb, 1993). Though this is assuredly true, I found that the fin stabilizing flows,
as active control surfaces, and torque resisting the turning motion could nonetheless be effective for tight, controlled turning, even at low speeds.

In short tail-first turns flow from the fins was directed downwards and horizontally as the result of a fin downstroke. There was generally only a single vortex ring or region of flow with each downstroke, similar to *fin mode I*, a fin flow pattern observed in tail-first rectilinear swimming (Stewart et al. 2010). In steady tail-first swimming, this mode is actually rare relative to other fin modes, and does not involve flow interactions between fin strokes (Stewart et al., 2010), much like the fin flows observed here for short tail-first turns. In contrast, arms-first turns resulted in linked fin vorticity, and often linked fin and jet vorticity. The linked fin vortices in arms-first turns most closely resemble *fin mode II* in Stewart et al. (2010) in terms of vortex wake structure. However, flows during turning sequences in the present study were not completely downward in nature like those for *fin mode II*, and often had a horizontal component more similar to *fin mode III*. The horizontal component observed here could be related to the need to direct force towards the center of rotation for turning, creating the centripetal force necessary to complete the turn (Fish et al., 2003; Parson et al., 2011). Undulating fin motions are generally responsible for linked vorticity and may offer squid a higher degree of control and flexibility than flapping motions. The prevalence of linked vortices and fin wave motions recorded here for arms-first turns is consistent with fin wake and kinematic findings for steady arms-first swimming (Bartol et al., in press; Bartol et al., 2001b; Bartol et al., 2009a; Stewart et al., 2010).

Fin flows differed among the various turning categories. For short tail-first turns fin flows did not result in high $T_y$ or $T_{xz}$. In general, the fin wakes for this category did not involve well-developed vorticity structures, with flows producing low magnitude negative net $T_y$ and/or low magnitude net $T_{xz}$. Thus, the fins played only minor roles in controlling the turning motion and stabilizing pitch and roll perturbations. In long tail-first turns the fins produced unique paired elongated regions of vorticity that have not been observed previously in steady swimming studies (Bartol et al., in press; Bartol et al., 2009a). These flows resulted in drag that opposed the direction of the turn presumably to slow and control the turning motion. The fins were important for $T_{xz}$ in vertical turns and
likely assisted in maintaining the vertical orientation and controlling for pitch and roll instabilities. During arms-first turns, the fins were the major effectors of the turns and were important stabilizers of the turn, producing substantial torque about the roll and pitch axes.

\textit{Impact of background flow}

Background flow speed impacted turning behavior, with higher angular turning velocities occurring at higher flow speeds and turning categories correlating with background flow conditions. The influence of flow conditions on steady swimming has been examined (Bartol et al., in press; Bartol et al., 2009a; Drucker and Lauder, 2002; Liao, 2007) but these effects have not been explored extensively for aquatic animal turning. Arms-first turns were observed over the widest range of background speeds, long tail-first turns occurred in the lowest background flows, and vertical turns occurred at the highest flow speeds. Arms-first and short tail-first turns, which occurred at similar mean flow speeds and were the most frequently observed turning behaviors in the present study, were also the most frequent turning behaviors observed during kinematic trials performed in stationary water (Chapter 2). Long tail-first turns and vertical turns, which were recorded less frequently in the present study, were not observed in stationary water kinematic trials. As described earlier, in long tail-first turns, the squid jetted while extending its outboard fin to create drag to help control the turning trajectory. Although free-stream flow was low (<5.5 cm s\(^{-1}\)) for these turns and the squid swimming speed (and trajectory) likely impacted the flow field to a greater extent based on the observed diagonal vorticity patterns (see Fig. 13), oncoming flow striking the extended fin presumably aided the turn during at least a portion of the turn cycle. During vertical turns, the squid clearly translated with the flow while spinning vertically, suggesting that the flows also likely contributed to these turning motions. Given that natural currents are ubiquitous, strategies and turning behaviors that use ambient flow like those described above are probably important for squid in their natural environments. The observed higher turning radii for the turning behaviors in this study relative to those performed under stationary water conditions derive from body displacement from the background
flows, which has been observed in other water tunnel studies (Liao, 2007). High translation and high radii during turns are likely common in many marine environments, as background flows are rarely negligible in natural conditions.

The suite of observed speed-specific tail-first turning behaviors together with the wide speed range of arms-first turns suggest that *L. brevis* is well-equipped to turn effectively over a diverse range of flow conditions. For rectilinear swimming, the tail-first orientation is used over a wider range of speeds relative to arms-first swimming and is generally the preferred swimming mode (O’Dor, 1988; Bartol et al., 2001; Bartol et al., 2008; Bartol et al., in press). The multiple tail-first turning strategies observed here, which encompass a broad range of background flow conditions (e.g., long tail-first (mean flow - 3.1 cm s\(^{-1}\)), short tail-first (mean flow - 5.7 cm s\(^{-1}\)), and vertical (mean flow - 9.8 cm s\(^{-1}\))), indicates that *L. brevis* can match appropriate turning behaviors with their flow environment while swimming in a tail-first orientation. Given the wide background flow range observed for arms-first turning (0.7-11.4 cm s\(^{-1}\)), *L. brevis* is also clearly capable of employing arms-first turning in different flow regimes. This flexibility in arms-first turning performance is likely critical for successful prey attack strategies (Messenger, 1968). Squids seize stationary prey using their arms, and mobile prey are captured using tentacular extension (Kier and Van Leeuwen, 1997; Nicol and O’Dor, 1985), both of which are performed in an arms-first orientation relative to the prey. Clearly, having the ability to effectively maneuver and self-correct when tracking prey in various flow regimes is important. Not only is maneuvering while in an arms-first orientation essential during an attack, it is also critical for the squid to be able to effectively rotate into an arms-first orientation prior to an attack under various environmental conditions (Foyle and O’Dor, 1988; Nicol and O’Dor, 1985; Squires, 1966). These adaptations can only be achieved with a wide repertoire of both tail-first and arms-first turning capabilities.

**Comparison to other taxa**

There have been several studies on aquatic turning performance in fish, marine mammals and turtles (Blake et al., 1995; Domenici and Blake, 1991; Domenici et al., 2004; Fish, 2002; Fish and Nicastro, 2003; Fish et al., 2003; Kasapi et al., 1993; Maresh
et al., 2004; Parson et al., 2011; Rivera et al., 2006; Walker, 2000; Webb, 1978a; Weihs, 1972). These studies have largely focused on kinematic turning performance variables, such as the minimum radius of the turn and angular velocity, and very few involve hydrodynamic analyses. Instead, most hydrodynamic investigations to date have focused on steady rectilinear swimming (Bartol et al., in press; Bartol et al., 2008a; Bartol et al., 2009a; Drucker and Lauder, 2002; Lauder and Drucker, 2004; Stewart et al., 2010).

The studies that do include hydrodynamic flow measurements of turning behavior focus on the role of pectoral and dorsal fins in fish during turns. Drucker and Lauder (2001) observed that turning in bluegill sunfish requires asymmetric fin motions that are significantly shorter in stroke duration than those used during steady swimming. During steady swimming, the vortex ring jets resulting from pectoral fin flows are directed 45° from the body on average. During turning the same patterns of vortex ring jets are directed perpendicular and parallel to the longitudinal axis of the body by the strong side (outboard) and weak side (inboard) fins, respectively (Drucker and Lauder, 2001b). The force produced by the fins for bluegill sunfish during turning is also much greater in magnitude than in steady swimming. The jet in squid, which produced the greatest torque contributing to the turning motion for three of four categories, was also directed primarily perpendicular to the longitudinal axis of the squid during the turn. When the fins were the major effector of the turn (arms-first turns), they also produced flows directed primarily perpendicular to the longitudinal axis. However, unlike the sunfish pectoral fin, the jet also had a downward component. This downward component counteracts negative buoyancy and is also observed during steady swimming (Bartol et al., 2001a; Bartol et al., 2008a; Bartol et al., 2009a). Bluegill sunfish are neutrally buoyant, so a downward component is not essential for maintaining vertical position in the water column (Webb, 1993).

Fish that specialize in maneuvering employ multiple systems to achieve turning, using median and paired fins for low-speed routine activity and body and caudal fin motion for fast starts and turns (Blake and Chan, 2006). Drucker and Lauder (2001a) examined the hydrodynamic role of the dorsal fin during turning and found that it produces vortices that counteracted torque produced by the pectoral fins to reduce the
translational component of the turn. In both squid and sunfish, the propulsor contributing a high amount of torque about the yaw axis (the jet and outboard pectoral fin, respectively) is located close to the center of mass. The squid creates flows using its high velocity directed jet that are much farther from the body and can be large in magnitude. Based strictly on the distant position of the fins from the center of mass, similar to the dorsal fin of the sunfish, it would seem that the fins are best suited as stabilizers rather than creating rotation. However, even though the fins act as important stabilizers and likely reduce translation for many turning behaviors, they are also capable of driving rotation during arms-first turns. Turns in sunfish were only investigated at a single flow speed (Drucker and Lauder, 2001a; Drucker and Lauder, 2001b). If turning dynamics of sunfish were studied over a speed range, it is conceivable that different speed-related turning patterns would emerge, much like the results presented here. Nonetheless, the dual mode system of squids (jet and fins) represents an interesting parallel to fishes with multi-propulsor systems. Clearly, both taxa are effective at turning with their multi-propulsor systems, but squid achieve their turns using propulsors and control surfaces that are more hydrodynamically and morphologically distinct than those found in fishes and many other nekton. The results of this study indicate that the pulsed jet and fins coordinate effectively to produce a wide repertoire of turns and squid can use ambient flow conditions to their advantage while maneuvering.

**Concluding thoughts**

This study includes the first volumetric 3D flow quantification dataset of turning for any aquatic animal. The results of this study, which focus on brief squid *Loliguncula brevis*, indicate that both the jet and fins contribute to turning with their relative roles depending on the type of turn employed. For this study, four categories of turning behaviors were identified: (1) short tail-first turns, (2) arms-first turns, (3) long tail-first turns, and (4) vertical turns. Short tail-first, long tail-first and vertical turns were driven predominantly by jet $T_y$ contributions, and arms-first turns were driven primarily by fin $T_y$ contributions. The jet and fins also contributed to roll and pitch control during the turn, with jet and fin stabilization being especially important for vertically oriented turns and
arms-first turns, where the jet to fin $T_{x z}$ ratio was 0.3 and 0.2, respectively. Many of the turns involved short, efficient, isolated vortex ring jet pulses, which provided significant trajectory control during the turn, and turning behaviors were often selected according to background flow conditions. Vertical and long tail-first turns, which have not been detected previously in stationary flow trials, are likely employed frequently in nature, where background flow is common, as both turning categories involve interactions with the free-stream flow. Arms-first turns, the most common pattern recorded, are important for prey capture as squid are visual predators and the eyes, arms and tentacles need to be projected anteriorly toward the prey for successful capture. Brief squid, *L. brevis*, inhabit an inshore environment where flow speeds vary depending on currents, wave action and tidal cycles. Having different turning strategies to exploit these varying flow conditions and maneuver in complex shallow water habitats, pursue prey, escape predators, and even guard mates is important for a squid’s ecological success.

The turning categories presented here, which are based on 3D hydrodynamic flow patterns and squid turning behavior, represent an important first step in understanding turning behavior in cephalopods. Clearly, 3D velocimetry is a powerful tool for evaluating turning performance in multi-propulsor systems as it allows for the visualization and quantification of asymmetric, out-of-plane flows from multiple propulsors and calculation of forces from 3D bulk wake properties. The next challenge is to develop mathematical approaches for quantitatively grouping similar and distinguishing different 3D hydrodynamic wakes of turns to move the field beyond largely qualitative descriptions of turning patterns and toward more quantitative evaluations of wake features. Although development of these approaches is not trivial, the mathematical toolkit will allow for unprecedented evaluations of complex wakes, such as those observed during turning maneuvers.
CHAPTER 4

TURNING PERFORMANCE OF BRIEF SQUID, LOLLIGUNCULA BREVIS,
DURING ATTACKS ON SHRIMP AND FISH

Introduction

Many squid need to swim continuously using a combination of jet propulsion and fin movements to oxygenate their gills and offset negative buoyancy (Bartol et al., 2001b; O’Dor and Webber, 1991). This can be energetically costly, and coastal, inshore squids have very limited energy reserves, requiring them to feed frequently (O’Dor and Webber, 1991). Squid are active visual predators, and feed on small crustaceans and fishes (Messenger, 1968). Attacks on mobile prey are always performed in an arms-first orientation, with the tentacles being the primary means of capture (Kier and Van Leeuwen, 1997; Messenger, 1968; Nicol and O’Dor, 1985). The arms and tentacles of squid lack hardened elements and operate as muscular hydrostats, with the musculature of the arms and tentacles acting as both the effectors of movement and the support system (Kier, 1982; Kier and Smith, 1985). The muscular hydrostatic architecture in the tentacles allows for significant bending, elongation, shortening and torsion (Kier and Smith, 1985), motions that aid cephalopods during prey strikes.

In addition to the arms and tentacles, the paired fins and a pulsed jet, which comprise the locomotory system of cephalopods, are also powered and supported by muscular hydrostats (Kier et al., 1989). This muscular hydrostat system is very flexible, with fins that are capable of undulating and oscillating independently of each other, and a pulsed jet that is produced by mantle contraction and ejection of fluid through a flexible funnel that can be rotated in any direction within a hemisphere below the body. This allows squid to have enormous flexibility in locomotion. They are capable of swimming backwards, forwards and vertically. Squid typically swim in two primary orientations, arms-first and tail-first (Anderson and DeMont, 2000; Bartol et al., 2001a; Foyle and O’Dor, 1988; O’Dor and Webber, 1991). Tail-first swimming appears to be the preferred
orientation for sustained, economical locomotion over a wide range of speeds (Bartol et al.; Bartol et al., 2001a). However, squid always orient arms-first for attacks on prey. Arms-first attacks by squid and cuttlefish on prey have been well-documented (Foyle and O’Dor, 1988; Kier and Van Leeuwen, 1997; Messenger, 1968; Nicol and O’Dor, 1985) and allow the squid to position the tentacles and arms toward the prey so that they can be used to strike, manipulate, and deliver prey to the mouth (Foyle and O’Dor, 1988; Messenger, 1968; Nicol and O’Dor, 1985).

Several studies have examined tentacle strikes during prey attacks in cuttlefish Sepia officinalis (Messenger, 1968) and squid Illex illecebrosus (Kier, 1982; Kier and Van Leeuwen, 1997). Working with cuttlefish, Messenger (1968) described tentacle strikes as an all or none response, indicating that there is limited neuromuscular variability in the strikes. When the ends of the tentacles are 4-6 cm from the prey, squid, I. illecebrosus, lunge forward, the arms separate outward from the tentacles and the tentacles are rapidly elongated, extending in a straight trajectory toward the prey (Kier, 1982; Kier and Van Leeuwen, 1997; Messenger, 1968). Maximum extension velocities of the tentacles of I. illecebrosus are >2 m s\(^{-1}\) with peak accelerations of approximately 250 m s\(^{-2}\) (Kier and Van Leeuwen, 1997). After the tentacles strike the prey, the tentacular stalk of I. illecebrosus buckles from compression forces and the prey is displaced slightly. The buckling dissipates as the tentacles shorten and the prey is brought into the arms, which subdue and manipulate the prey into an appropriate position for delivery to the mouth (Kier, 1982; Kier and Van Leeuwen, 1997).

A number of studies have also examined cephalopod movements during prey attacks. Messenger (1968) described prey attacks by cuttlefish Sepia officinalis as falling into three phases: attention, positioning and strike. The attention phase starts with the cuttlefish tracking the prey with its eyes and turning its head so that the arms and tentacles are also projected towards the target. During this attention phase, the cuttlefish turns so that the angle between the body axis and the prey axis is near or at zero (Messenger, 1968), and some brief coloration changes may also occur (Kier, 1982). During positioning, the cuttlefish swims either forwards or backwards to create the optimal attacking distance, and the arms and tentacles are extended outward, pointing
towards the prey (Messenger, 1968). The arms are held in a tight cone shape enclosing the tentacles, with just the tip of the tentacles protruding at the end of the cone made by the arms. If the prey moves, the arms and tentacles also move tracking the prey, along with the head and body after a slight time lag (Messenger, 1968). Sometimes, the first arm pair is extended vertically, which may serve as a lure or distraction (Messenger, 1968). Squid and cuttlefish differ in strategies employed for prey capture across ontogeny. Cuttlefish Sepia pharaonis utilize the tentacular, lunging style of attack as both hatchlings and adults. In contrast, squid Sepia lessoniana paralarvae/early juveniles capture prey using solely their arms, and do not use the tentacles during strikes until they reach 30 days of age (Sugimoto and Ikeda, 2013).

Foyle and O’Dor (1988) studied attack behavior of squid I. illecebrosus on large and small fish and divided attacks into similar phases as those reported by Messenger (1968): (1) rotation, where the squid changes from tail-first to arms-first swimming; (2) approach, which involves rapid acceleration towards the prey; (3) tracking, where the squid slowly follows the prey; and (4) capture, where the prey is subdued. For larger fish prey, I. illecebrosus employs a prolonged tracking phase at low speed before the strike. This longer tracking phase is thought to be important for more extensive behavioral assessment of faster prey (Foyle and O’Dor, 1988). Maximum arms-first body acceleration rates for I. illecebrosus during these experiments were 12 m s\(^{-2}\) (Foyle and O’Dor, 1988), which is low relative to fish that exhibit maximum acceleration rates of 30-40 m s\(^{-2}\) (Webb, 1978b). In addition to relatively low body accelerations, low rotation rates (\(~90^\circ \text{s}^{-1}\)) and large turning radii (0.5 body lengths) were also observed, though these may be underestimates given that a detailed kinematic analysis was not performed.

In Chapter 2, I found that squid L. brevis are capable of both high agility (turning rates) and high maneuverability (low turning radius), even relative to flexible bodied fish species, suggesting that some squid may be well-equipped in terms of turning capabilities for attacks on highly mobile prey. By wrapping the arms close to the mantle and using rapid jet pulses L. brevis can achieve high angular velocity during turns (725° s\(^{-1}\)) and can employ asymmetric motions of the fins and short jet pulses for high maneuverability (length specific turning radius = 4.2×10\(^{-4}\)) (see Chapter 2). The jet is an important driver
of short tail-first and arms-first turns, by creating torque primarily about the yaw axis and using short directed isolated vortex ring pulses for controlled trajectory adjustments (see Chapter 3). The fins are important for controlling and stabilizing turns, especially in arms-first turns, by producing torque resisting the turning motion and torque about the roll and pitch axes, respectively (Chapter 3).

Although the studies above have provided valuable insight into attack behavior in cephalopods, few studies have examined kinematic parameters of attacks in detail, particularly with regard to quantitative turning performance metrics. Clearly, the tentacles are effective high-velocity tools for capturing prey, but it is unclear how important turning is during prey encounters. Therefore, in this study, we focus on measuring the kinematics of approach and attack behavior of brief squid *L. brevis* during encounters with both shrimp and fish prey. The objectives are: (1) document turning behaviors during prey encounters, (2) determine if squid exhibit high levels of maneuverability and agility during prey encounters, and (3) determine if turning performance in squid changes with prey type.

**Methods**

*Lolliguncula brevis* (2.8 – 6.3 cm dorsal mantle length (DML)), the squid predator used in this study, were caught by trawl net at the Virginia Institute of Marine Science Eastern Shore Marine Lab, Wachapreague, VA, USA and were transported back to Old Dominion University’s Marine Aquatic Facility in aerated livewells. The adults were maintained in a 450-gallon recirculating seawater system (salinity=25-30 ppt, temperature = 15-21°C, pH = 8.0-8.2, ammonia <0.2 ppm). A moderate current was maintained in the holding tank to facilitate active swimming of squid, and squid were fed a diet of live grass shrimp *Palaemonetes pugio* and small fish *Pimephales promelas*. This study was conducted in accordance with Old Dominion University’s IACUC protocol 692436-2. The *P. pugio* that were used for this study (mean total length = 2.3±0.1 cm) were also collected at the Virginia Institute of Marine Science Eastern Shore Marine Lab (Wachapreague, VA, USA) by dip net. They were maintained in aerated aquaria filled with seawater matching the conditions described above and fed fish flake food. The *P.*
*promelas* (mean total length = 3.4±0.1 cm) were purchased at Animal Adventure (Chesapeake, VA, USA) and maintained in separate aerated aquaria at ambient temperature (19-22°C).

A Plexiglass chamber (30.5 cm × 30.5 cm × 25.4 cm) filled with seawater matching conditions of the holding tank was placed on a stand that allowed unobstructed viewing from both lateral and ventral perspectives. Prior to trials, the water in the chamber was aerated and maintained at temperatures consistent with the holding tanks. The chamber was illuminated with five 500 W lights outfitted with color gel #27 filters (transmits wavelengths > 600 nm), as red light tended to reduce stress on the animals compared to full spectrum illumination. For each trial, squid were placed in the chamber and allowed to acclimate for at least 5 minutes prior to recording. Several squid were often placed in the chamber at one time as attacks occurred more readily when multiple squid were present in the chamber. *P. pugio* and *P. promelas* were dropped into the chamber at random locations, and the squid only had the choice of one type of prey at a time. The fish or shrimp were placed in the experimental chamber for approximately 30 seconds and trials were terminated if the fish or squid were unresponsive during that time period. A total of 58 attacks from 49 *L. brevis* individuals were recorded, with 40 attacks by 34 individuals for attacks on shrimp and 18 attacks by 15 individuals for attacks on fish (*L. brevis* mean size (shrimp trials) = 4.1±0.2 cm DML, *L. brevis* mean size (fish trials) = 3.5±0.8 cm DML). 

Attack behavior during trials was recorded using two synchronized high-speed Dalsa Falcon video cameras (1400 x 1200 pixel resolution; DALSA Corp., Waterloo, ON, Canada) positioned ventrally and laterally to the viewing chamber. The ventral camera was fitted with a 25 mm lens and the lateral camera was fitted with a 35 mm lens (Fujinon TV Lens, Fujinon Corporation, China). The high-speed cameras were triggered using the onboard counter on two CLSAS capture cards to capture video at 100 frames per second. Video frames from the DALSA cameras were transferred to hard disk in real time using the two CLSAS capture cards and Streams 5 software (IO Industries Inc., London, ON, Canada).
Successful attack sequences were classified into three phases: approach, strike and recoil. The approach was defined as the interval from when the squid head began to rotate toward the prey to when the arms started to spread in preparation for the tentacle strike; the strike was the interval from when the arms started to spread to when contact was made with the prey; and the recoil was the interval from tentacle contact with the prey to when the squid exhibited control of the prey, i.e. the prey was wrapped in the arms close to the mouth (determined visually). Unsuccessful attack sequences were separated into two categories: approach without a strike, where the squid rapidly approached the prey but did not extend the tentacles, and approach with a strike but where the strike missed the prey.

Frame-by-frame position tracking of cephalopod body features was accomplished using image tracking software (Hedrick, 2008). Ten points were tracked in the ventral view: (1) tail tip, (2) base of the funnel, (3) tip of arm pair V, (4) right side of the mantle midway down the length, (5) left side of the mantle midway down the length, (6) right fin tip (max chord), (7) left fin tip (max chord), (8) prey (just behind the head for fish, and midway along the body for shrimp), (9) tentacle one tip, and (10) tentacle two tip (Fig. 17A). Seven points were tracked in the lateral view: (1) tail tip, (2) eye, (3) tip of ventral arm pair, (4) fin tip, (5) midpoint along prey body, (6) tentacle tip one, and (7) tentacle tip two (Fig. 17B). The tracked points in the ventral view were used to determine (1) center of rotation (COR) radius during the approach, (2) angular velocity during the approach, (3) total angular displacement, (4) fin beat frequency, and (5) mantle contraction. The tracked points in the lateral view were used to determine (1) angle of the mantle during approach and recoil and (2) angle of the arms relative to the mantle during approach and recoil. Other parameters examined using both lateral and ventral views included (1) squid swimming velocity, (2) squid acceleration, (3) prey swimming velocity, (4) prey acceleration, (4) distance traveled during the approach by both squid and prey, (5) distance to the prey at the start of the approach and strike, (5) tentacle extension velocity during the strike and (6) distance traveled during the strike by the squid. Angles and distances at the start of the approach and strike were measured using
Figure 17. Points that were digitized in the ventral (A) and lateral (B) views.
ImageJ software (National Institute of Health, USA, available at http://imagej.nih.gov/ij/index.html) including (1) ventral distance between the predator (between the eyes) and prey (midway along the body axis) at the start of approach and start of strike, (2) ventral angle between the predator and prey at the start of approach, \( \Theta_{vs} \), and start of strike, \( \Theta_{ss} \) (see Fig. 18), (3) lateral distance between the predator and prey at the start of approach and start of strike, (4) lateral angle between the predator and prey at the start of approach and start of strike (see Fig. 19). The minimum radius of the turn, angular velocity, total angular displacement, acceleration, mantle contraction rate, and fin beat frequency were not determined for the strike as that portion of the sequence was too short to use proper smoothing methods. Average and peak velocities of the squid and tentacles were examined for the strike. Values for both tentacles were averaged for each sequence. All kinematic parameters were calculated using individual averages.

The data for turning parameters (minimum radius of the turn and angular velocity) were smoothed using Cross-Validation Criterion (CVC). This smoothing method uses smoothed splines where the level of smoothing is determined such that the root-mean-squared error of the splines determined with points from the data individually excluded is minimized (Walker, 1998). In the present implementation, the minimization is determined to within 0.1% of the actual minimum to speed convergence of the method. The squid swimming velocity, prey swimming velocity and mantle diameter data were smoothed using a fourth order Butterworth filter. Mantle contraction rate and squid acceleration were calculated from the smoothed data for each sequence by evaluating the derivatives using fourth order finite difference equations.

The COR was the point relative to the squid in the ventral view that moved the least during the turn. Finding the COR was performed using an in-house Matlab code that used a two segment approach with the lines connecting the tail tip to the funnel base and then the funnel base to the arm tip, each line defining one of the two segments. The COR was found with respect to each segment, resulting in two CORs, so that different behaviors and functions of each segment could be characterized during the turn. The code was generalized so that the COR did not actually have to fall directly on these line segments. Rather, it could lie along a line at a fixed angle \( \alpha \) with respect to the tracked
Figure 18. **How the ventral angle was measured between the squid and the prey.** A. Illustration of how the ventral angle was measured between the squid (midway between the eyes) and the prey (midway along the body) at the start of the approach. B. Illustration of how the ventral angle was measured between the squid and the prey at the start of the strike.
Figure 19. How the lateral angle was measured between the squid and the prey. A. Illustration of how the lateral angle was measured between the squid and prey at the start of the approach. B. Illustration of how the lateral angle was measured between the squid and prey at the start of the strike.
body segment where $\alpha$ and the position of the COR along the line at this angle were selected such that the movement of the COR during the turn was minimized.

The radius ($R$) of the turning path is the radius of curvature of the COR. This was computed from analytical geometry using

$$\frac{1}{R} = \frac{y''}{(1 + (y')^2)^{3/2}}$$

where $y' = dy/dx$, $x$ and $y$ are the coordinates of the COR in the ventral view, and the derivatives were evaluated using fourth order accurate finite difference equations. For each turn sequence, both the mean radius of the turning path and the minimum radius of the turning path were determined using in-house Matlab routines. The minimum and mean radius of the turning path ($R$) were normalized (divided by the length of the animal) to get a length-specific turning radius ($R/L$). ($R/L)_{\text{mean}}$ is the average of all the center of rotation radii throughout a turn divided by the length of the animal. ($R/L)_{\text{min}}$ is the minimum center of rotation turning radius during a turn, using a 90th percentile cut-off value divided by animal length. $\omega_{\text{avg}}$ is the mean angular velocity throughout a turn. $\Theta_{\text{total}}$ is total angular displacement during a maneuver. $\omega_{\text{max}}$ is the maximum angular velocity found during a turn. $V_{\text{avg}}$ and $V_{\text{peak}}$ are mean and peak velocity, respectively, during a maneuver. $A_{\text{avg}}$ and $A_{\text{peak}}$ are mean and peak acceleration during a maneuver.

For each phase, i.e., approach, strike, and recoil, differences in kinematic variables for attacks on shrimp versus fish were determined using one-way MANOVAs (SPSS, IBM, New York, USA). Turning parameter differences in approach toward shrimp, recoil from shrimp and non-predatory turns were determined using a one-way MANOVA; turning parameter differences in approach toward fish, recoil from fish and non-predatory turns were also determined using a MANOVA (SPSS). Data for non-predatory turns derive from Chapter 2 and were recorded using identical approaches to those described here. The LSD Post-hoc test was used for multiple comparisons. The ventral angles and distances between predator and prey from the start of the approach and start of the strike were compared using paired t-tests (SPSS). The inboard and outboard fin beat frequencies for each of the attack segments were compared using paired t-tests.
(SPSS). Percentages for postures and the position of the prey item were evaluated using a Kruskal-Wallis one-way ANOVA (SPSS). The mantle and arm angles for posture were compared using one-way ANOVAs (SPSS). The average and peak velocity of the squid between the approach, strike and recoil for attacks on shrimp and fish were examined using repeated measures ANOVAs (SPSS). If the data did not meet assumptions for normality, either a Log10 or square root transformation was performed.

**Results**

High-speed video of brief squid *L. brevis* attacking both shrimp and fish was collected to study the kinematics of attack behaviors. Attack sequences were divided into three phases: (1) approach, (2) strike and (3) recoil. In contrast to previous studies, distinct sub-phases of approach behavior were not observed and thus behaviors prior to the strike were all considered part of the approach phase.

**Shrimp attacks**

Squid \((R/L)_{\text{min}}\) was greater for the approach (mean approach = 0.007±0.001 s.e.m.) than for non-predatory turns (mean non-predatory turn = 0.004±0.0006), but no difference in \((R/L)_{\text{min}}\) between the approach and recoil phases was present (mean recoil = 0.007±0.001) (MANOVA: F=2.02, df\(_{10,138}\), P=0.1). \((R/L)_{\text{mean}}\) was greater for both the approach (mean = 0.3±0.04) and recoil (mean = 0.3±0.07) than in non-predatory turns (mean = 0.009±0.004) (MANOVA: F=7.0, df\(_{10,138}\), P<0.005). The \(\omega_{\text{max}}\) was significantly higher for the recoil (mean = 425.6±39.6° s\(^{-1}\)) than for the approach (mean = 288.3±33.4° s\(^{-1}\)) and non-predatory turns (mean = 268.4° s\(^{-1}\)) (MANOVA: F=4.4, df\(_{10,138}\), P<0.02). \(\omega_{\text{mean}}\) was significantly lower for both the approach (mean = 36.2±3.8° s\(^{-1}\)) and recoil (mean = 60.0±9.3° s\(^{-1}\)) than in non-predatory turns (mean = 110.3±14.6° s\(^{-1}\)) (MANOVA: F=13.5, df\(_{10,138}\), P<0.002) and \(\omega_{\text{mean}}\) was significantly greater in the recoil than in the approach (MANOVA: F=13.5, df\(_{10,138}\), P<0.05). \(\Theta_{\text{total}}\) was significantly lower for both the approach (mean = 46.9±6.6°) and recoil (mean = 26.9±3.9°) than in non-predatory turns (mean = 117.2±18.6°) (MANOVA: F=23.0, df\(_{10,138}\), P<0.001) and \(\Theta_{\text{total}}\) was significantly greater for the approach than the recoil (MANOVA: F=23.0, df\(_{10,138}\), P<0.05). The ventral
angle, ventral distance and three-dimensional distance (using both lateral and ventral views) between the squid and the shrimp significantly decreased from the start of the approach (mean ventral angle = 52.3±7.6°, mean ventral distance = 9.3±0.9 cm and mean distance = 14.9±2.0 cm) to the start of the strike (mean ventral angle = 5.7±0.6°, mean ventral radius = 3.6±0.3 cm and mean distance = 4.4±0.4 cm) (paired t-test (ventral angle): t=9.0, df33, P<0.001; paired t-test (ventral radius): t=8.8, df33, P<0.001; paired t-test (distance): t=8.9, df32, P<0.001) (Fig. 20). The squid $V_{avg}$ was significantly lower during the approach than during the recoil and strike (ANOVA: F=13.6, df2,29, P<0.001) and squid $V_{peak}$ was significantly lower during the approach than during the strike (ANOVA: F=4.4, df2,29, P=0.006) (Fig. 21). Out of 40 attacks, there were 33 successful captures, 4 strikes with no capture and 3 approaches with no strike. Therefore, capture success rate when strikes were initiated was 89.2%.

**Fish attacks**

Squid $(R/L)_{min}$ was significantly greater for both the approach (mean = 0.01±0.002) and recoil (mean = 0.01±0.002) than for non-predatory turns (mean = 0.004±0.0006) (MANOVA: F=8.3, df10,70, P=0.001). $(R/L)_{mean}$ was significantly greater during the recoil (mean = 1.0±0.4) than in non-predatory turns (mean = 0.009±0.004) (MANOVA: F=4.8, df10,70, P=0.004) but there was no difference between the approach (mean = 0.6±0.1) and recoil. The $\omega_{max}$ in the recoil (mean = 444.0±55.6° s$^{-1}$) was significantly higher than both the approach (mean = 302.6±50.7° s$^{-1}$) and non-predatory turns (mean = 268.4 s$^{-1}$) (MANOVA: F=3.7, df10,70, P<0.05 and P<0.02, respectively). The $\omega_{mean}$ was significantly lower in both the approach (mean = 50.3±12.8° s$^{-1}$) and recoil (mean = 71.2±12.9° s$^{-1}$) than in non-predatory turns (mean = 110.3±14.6° s$^{-1}$) (MANOVA: F=5.3, df10,70, P<0.005 and P=0.05, respectively). $\Theta_{total}$ was significantly higher in non-predatory turns (mean = 117.2±18.6°) than both the approach (mean = 44.9±9.6°) and recoil (mean = 26.4±3.8°) (MANOVA: F=14.8, df10,70, P<0.001). The ventral angle, ventral radius and distance between the squid and the fish significantly decreased from the start of the approach (mean = 42.1±9.4°, 14.0±3.1 cm and 24.4±6.3 cm) to the start of the strike (mean = 3.3±0.4°, 4.6±0.6 cm and 5.3±0.6 cm) (paired t-test
Figure 20. Mean ventral angle between the squid and shrimp (dark gray) and squid and fish (light gray) at the start of the approach and the start of the strike. Different letters indicate significant differences; same letters indicate no significant difference. Error bars = s.e.m. See text for statistics.
Figure 21. The average (solid fill) and peak (diagonal line fill) squid swimming velocity during the approach, strike and recoil for attacks on shrimp (dark gray: A, B) and fish (light gray: C, D). Same letters indicate no significant difference; different letters indicate a significant difference. Error bars = s.e.m. (see text for statistics).
(ventral angle): t=8.1, df_{14}, P<0.001; paired t-test (ventral radius): t=3.9, df_{14}, P=0.002; and paired t-test (distance): t=4.05, df_{13}, P=0.001, respectively) (Fig. 20). The squid $V_{\text{avg}}$ was significantly lower for both the approach and recoil than the strike (ANOVA: $F=9.7$, df$_{2,12}$, $P=0.001$ and 0.01, respectively) and squid $V_{\text{peak}}$ was also significantly lower during both the approach and recoil than during the strike (ANOVA: $F=6.9$, df$_{2,12}$, $P=0.045$ and $P=0.01$, respectively) (Fig. 21). Out of 18 attacks there were 13 successful captures, 3 strikes with no capture and 2 approaches with no strike. Thus, capture success rate when strikes were initiated was 81.3%.

**Approach kinematics**

The time from the start of the approach to the start of the attack was greater for attacks on shrimp than on fish (MANOVA: $F=4.03$, df$_{20,28}$, $P=0.05$). The distance traveled by the squid during the approach did not significantly differ between attacks on shrimp and fish (MANOVA: $F=0.9$, df$_{20,28}$, $P=0.3$). The squid approach $V_{\text{avg}}$ and $V_{\text{peak}}$ were significantly higher for attacks on fish (mean $V_{\text{avg}} = 3.7\pm0.4$ DML s$^{-1}$ and $V_{\text{peak}} = 8.5\pm1.1$ DML s$^{-1}$) than for attacks on shrimp (mean $V_{\text{avg}} = 2.1\pm0.2$ DML s$^{-1}$ and $V_{\text{peak}} = 4.1\pm0.3$ DML s$^{-1}$) (MANOVA ($V_{\text{avg}}$): $F=20.6$, df$_{20,28}$, $P<0.001$; MANOVA ($V_{\text{peak}}$): $F=27.9$, df$_{20,28}$, $P<0.001$). The squid $A_{\text{avg}}$ and $A_{\text{peak}}$ were also significantly higher for attacks on fish (mean $A_{\text{avg}} = 12.7\pm1.3$ DML s$^{-2}$ and mean $A_{\text{peak}} = 44.3\pm5.0$ DML s$^{-2}$) than for attacks on shrimp (mean $A_{\text{avg}} = 6.1\pm0.5$ DML s$^{-2}$ and mean $A_{\text{peak}} = 24.5\pm2.6$ DML s$^{-2}$) (MANOVA ($A_{\text{avg}}$): $F=33.8$, df$_{20,28}$, $P<0.001$; MANOVA ($A_{\text{peak}}$): $F=15.7$, df$_{20,28}$, $P<0.001$). The distance traveled by the prey and the $V_{\text{avg}}$ of the prey during the squid approach did differ significantly (MANOVA (distance traveled by prey): $F=8.2$, df$_{20,28}$, $P=0.006$; MANOVA ($V_{\text{avg}}$): $F=18.5$, df$_{20,28}$, $P<0.001$), with fish travelling farther and faster than shrimp (mean travel for fish = 10.5±2.4 cm; mean travel for shrimp 8.4±1.0 cm; mean $V_{\text{avg}}$ for fish = 8.9±1.5 cm s$^{-1}$; mean $V_{\text{avg}}$ for shrimp = 3.5±0.5 cm s$^{-1}$, respectively). Fish also displayed significantly higher $A_{\text{avg}}$ (mean = 43.5±7.3cm s$^{-2}$) than shrimp (mean = 25.9±3.7 cm s$^{-2}$, respectively) (MANOVA: $F=5.8$, df$_{20,28}$, $P=0.02$). The squid $A_{\text{peak}}$ was also significantly higher for fish than shrimp during the approach (MANOVA: $F=15.7$, df$_{20,28}$, $P<0.001$). The squid travelled significantly faster than the prey on average during
the approach for attacks on shrimp (t-test: \( t=4.5, \) df\(_{33}, \) P\(<0.001 \)) but not for attacks on fish (t-test: \( t=0.8, \) df\(_{14}, \) P\(=0.4 \)).

As prey \( V_{avg} \) for both shrimp and fish increased during the approach, so did squid \( V_{avg} \) (Pearson correlation (shrimp): \( r=0.5, \) df\(_{34}, \) P\(=0.002 \) and Pearson correlation (fish): \( r=0.7, \) df\(_{15}, \) P\(=0.006 \)) (Fig. 22). The distance to the prey at the start of the approach did not differ significantly between shrimp and fish encounters. The approach \( V_{avg} \) of the squid increased the farther away the fish was at the start of the approach (Pearson correlation: \( r=0.73, \) df\(_{15}, \) P\(=0.002 \)), but no such correlation was found for squid/shrimp encounters (Fig. 23).

Both \((R/L)_{min}\) and \((R/L)_{mean}\) were significantly lower for attacks on shrimp (mean \((R/L)_{min}\)= 0.007±0.0009 and mean \((R/L)_{mean}\)= 0.3±0.04, respectively) than attacks on fish (mean \((R/L)_{min}\)= 0.01±0.002 and mean \((R/L)_{mean}\)= 0.6±0.1, respectively) (MANOVA \((R/L)_{min}\)): \( F=7.8, \) df\(_{20,28}, \) P\(=0.007 \); MANOVA \((R/L)_{mean}\): \( F=6.4, \) df\(_{20,28}, \) P\(<0.02 \)). No significant differences in \( \omega_{max} \) or \( \omega_{mean} \) were found for squid approaching shrimp or fish (\( \omega_{max} \) MANOVA: \( F=0.06, \) df\(_{20,28}, \) P\(=0.8 \); \( \omega_{mean} \) MANOVA: \( F=2.02, \) df\(_{20,28}, \) P\(=0.2 \)). The \( \Theta_{total} \) did not differ significantly for squid approaching shrimp (mean = \( 46.9\pm6.6^\circ \)) and fish (mean = \( 44.9\pm9.6^\circ \)) (MANOVA: \( F=0.03, \) df\(_{20,28}, \) P\(=0.9 \)). As \( \Theta_{total} \) during the attack increased, \( \omega_{max} \) and \( \omega_{mean} \) also increased for attacks on both shrimp and fish (Pearson correlation (\( \omega_{max} \) shrimp): \( r=0.5, \) df\(_{34}, \) P\(=0.001 \); Pearson correlation (\( \omega_{max} \) fish): \( r=0.7, \) df\(_{15}, \) P\(=0.003 \); Pearson correlation (\( \omega_{mean} \) shrimp): \( r=0.9, \) df\(_{34}, \) P\(<0.001 \); Pearson correlation (\( \omega_{mean} \) fish): \( r=0.8, \) df\(_{15}, \) P\(<0.001 \)) (Fig. 24).

Higher \( \Theta_{va} \) also resulted in higher \( \Theta_{total} \) for attacks on shrimp (Pearson correlation: \( r=0.8, \) df\(_{34}, \) P\(<0.001 \)) (Fig. 25). No significant difference in \( \Theta_{va} \) for attacks on shrimp and fish was found. Higher \( \Theta_{va} \) were associated with higher \( \omega_{max} \) for both shrimp and fish (Pearson correlation (shrimp): \( r=0.7, \) df\(_{34}, \) P\(<0.001 \); Pearson correlation (fish): \( r=0.6, \) df\(_{15}, \) P\(=0.02 \)) and higher \( \omega_{mean} \) for shrimp (Pearson correlation: \( r=0.6, \) df\(_{34}, \) P\(<0.001 \)) (Fig. 26).

There was no significant difference between inboard and outboard fin frequency during squid approaches toward shrimp (paired t-test: \( t=-0.5, \) df\(_{22}, \) P\(=0.6 \)), but the outboard fin frequency was significantly higher than inboard fin frequency for
Figure 22. Mean squid swimming velocity plotted against mean prey swimming velocity during the approach. Mean squid approach velocity increased with increased mean shrimp velocity (filled circles, solid regression line) and mean fish velocity (open circles, broken regression line). See text for correlation statistics.
Figure 23. Mean squid approach velocity plotted against the distance of prey at the beginning of the squid approach. Squid mean approach velocity increased with greater distances to the prey (fish: open circles, broken regression line and shrimp: filled circles, solid regression). Only the fish relationship is significant. See text for correlation statistics.
Figure 24. Maximum (A, C) and mean (B, D) angular velocity plotted as a function of total angular displacement for squid during the approach (A, B) and recoil (C, D) phases of attacks on shrimp (filled circles) and fish (open circles). As total angular displacement increased, both maximum and mean angular velocity also increased for attacks on both shrimp (solid line) and fish (broken line) during the approach and recoil. All relationships are significant, see text for correlation statistics.
Figure 25. Total angular displacement of squid plotted with the ventral angle between squid and prey at start of approach for squid/shrimp encounters (filled circles) and squid/fish encounters (open circles). Total angular displacement increased with increased ventral start angle for squid/shrimp encounters, but a significant correlational relationship was not detected for squid/fish encounters.
Figure 26. Maximum (A) and mean (B) angular velocity plotted against the ventral angle between the squid and prey (shrimp = filled circles, fish = open circles) at the start of the approach. If the prey item was at a greater ventral angle at the start of the approach, the angular velocity of the squid during the approach increased. Both relationships are significant for maximum angular velocity. Only the shrimp (filled circles, solid regression line) relationship is significant for mean angular velocity. See text for correlation statistics.
approaches toward fish \((t\)-test: \(t=-2.4, \text{df}_{12}, P=0.03\)). Moreover, the outboard and inboard fins were not as synchronized for attacks on fish as they were for attacks on shrimp (Fig. 27). The approach often ended with both fins in a downstroke, corresponding with a mantle contraction. Mantle contraction rate largely correlated well with acceleration, indicating that the pulsed jet had a large impact on speed during the approach. Generally, there were two peaks in velocity during the approach, with the first peak occurring at the beginning of the approach and the second occurring at the end of the approach, just before the strike was initiated (Fig. 28). The first peak in linear velocity corresponded with a peak in angular velocity, and the second peak in linear velocity was during the final arms-first lunge towards the prey, right before the strike.

*Strike kinematics*

During the strike phase, the squid \(V_{\text{avg}}\) and \(V_{\text{peak}}\) were significantly higher for fish encounters (mean \(V_{\text{avg}}=6.8\pm0.8\ \text{DML s}^{-1}\); mean \(V_{\text{peak}}=10.35\pm1.1\ \text{DML s}^{-1}\)) than shrimp encounters (mean \(V_{\text{avg}}=3.3\pm0.3\ \text{DML s}^{-1}\); mean \(V_{\text{peak}}=5.1\pm0.4\ \text{DML s}^{-1}\)) (MANOVA (\(V_{\text{avg}}\)): \(F=27.7, \text{df}_{10,36}, P<0.001\); MANOVA (\(V_{\text{peak}}\)): \(F=30.9, \text{df}_{10,36}, P<0.001\)). The highest individual squid swimming velocity was 47.5 cm s\(^{-1}\) (8.2 DML s\(^{-1}\)) for attacks on shrimp and 56.4 cm s\(^{-1}\) (16.5 DML s\(^{-1}\)) for attacks on fish. The \(V_{\text{avg}}\) and \(V_{\text{peak}}\) of the tentacles did not differ significantly for attacks on shrimp (mean \(V_{\text{avg}}=21.6\pm1.7\ \text{cm s}^{-1}\) and 5.4±0.4 DML s\(^{-1}\); mean \(V_{\text{peak}}=59.7\pm5.8\ \text{cm s}^{-1}\) and 14.6±1.4 DML s\(^{-1}\)) and fish (mean \(V_{\text{avg}}=23.9\pm3.1\ \text{cm s}^{-1}\) and 6.9±0.9 DML s\(^{-1}\); mean \(V_{\text{peak}}=52.5\pm7.1\ \text{cm s}^{-1}\) and 15.1±2.0 DML s\(^{-1}\)) (\(V_{\text{avg}}\) MANOVA: \(F=0.5\) and 3.0, \(\text{df}_{10,36}, P=0.5\) and 0.09; \(V_{\text{peak}}\) MANOVA: \(F=0.5\) and 0.03; \(\text{df}_{10,36}, P=0.5\) and 0.9). The fastest tentacle \(V_{\text{peak}}\) (averaged from both tentacles) observed from an individual was 123.7 cm s\(^{-1}\) for shrimp and 102.4 cm s\(^{-1}\) for fish. The tentacle \(V_{\text{avg}}\) during the strike was significantly faster than both the shrimp \(V_{\text{avg}}\) (\(t\)-test: \(t=10.4, \text{df}_{32}, P<0.001\)) and fish \(V_{\text{avg}}\) (\(t\)-test: \(t=4.3, \text{df}_{13}, P=0.001\)). No significant differences in squid/prey angles or distances at the start of the strikes were detected for attacks on shrimp and fish. As squid/prey distance at the start of the strike increased, squid \(V_{\text{peak}}\) and tentacle \(V_{\text{peak}}\) increased for attacks on shrimp and fish (Pearson correlation
Figure 27. **Fin beat amplitude of the inboard (filled circles) and outboard (open circles) fins for the approach in 6 squid attacks.** The plots on the left (A, C, E) are for attacks on shrimp and the plots on the right (B, D, F) are for attacks on fish. Inboard and outboard fins were generally synchronized for the approach in attacks on shrimp, but periods of asynchrony between the inboard and outboard fins (denoted with a black arrow) occurred for the approach in attacks on fish. See text for statistics.
Figure 28. **Mantle diameter** (filled circles) and **swimming velocity** (open circles) for squid during 6 approach sequences. The plots on the left (A, C, E) are for attacks on shrimp and the plots on the right (B, D, F) are for attacks on fish. Note the peak in velocity at the beginning of the approach (solid black arrow) and near end of approach, before the strike phase (dotted black arrow) generally correlated with mantle contraction.
For attacks on shrimp, both squid \( V_{\text{avg}} \) and \( V_{\text{peak}} \) significantly correlated with tentacle \( V_{\text{avg}} \) and \( V_{\text{peak}} \), respectively (Pearson correlation \( (V_{\text{avg}}) \): \( r=0.5, \text{df} \, 15, \text{P}=0.001 \); Pearson correlation \( (V_{\text{peak}}) \): \( r=0.4, \text{df} \, 15, \text{P}=0.01 \)). However, no such relationship was detected for attacks on fish. Accurate mantle contraction rates and accelerations could not be determined for the strikes because of the short temporal window associated with high-velocity strikes; however the mantle diameter did consistently decrease during most strikes. There was no significant difference in capture success for attacks on shrimp versus attacks on fish.

**Recoil kinematics**

During the recoil phase, the squid switched from arms-first swimming to tail-first swimming and reversed swimming direction from the approach and strike. Squid \( V_{\text{avg}} \) and squid \( V_{\text{peak}} \) were significantly higher for attacks on fish than shrimp (mean squid \( V_{\text{avg}} \) (fish) = 4.4±0.4 DML s\(^{-1}\); mean squid \( V_{\text{peak}} \) (fish) = 7.4±0.9 DML s\(^{-1}\)); mean squid \( V_{\text{avg}} \) (shrimp) = 2.9±0.2 DML s\(^{-1}\); mean \( V_{\text{peak}} \) (shrimp) = 4.5±0.3 DML s\(^{-1}\)) (MANOVA \( (V_{\text{avg}}) \): \( F=43.2, \text{df} \, 15,29, \text{P}<0.001 \); MANOVA \( (V_{\text{peak}}) \): \( F=11.8, \text{df} \, 15,29, \text{P}=0.001 \)). The squid \( A_{\text{avg}} \) and \( A_{\text{peak}} \) were also significantly higher for attacks on fish (mean \( A_{\text{avg}} \) = 22.2±3.6 DML s\(^{-2}\); mean \( A_{\text{peak}} \) = 52.0±0.9 DML s\(^{-2}\)) than shrimp (mean \( A_{\text{avg}} \) = 12.0±1.0 DML s\(^{-2}\); mean \( A_{\text{peak}} \) = 30.0±2.9 DML s\(^{-2}\)) (MANOVA \( (A_{\text{avg}}) \): \( F=10.3, \text{df} \, 15,29, \text{P}=0.002 \); MANOVA \( (A_{\text{peak}}) \): \( F=6.2, \text{df} \, 15,29, \text{P}<0.02 \)).\(^{(R/L)_{\text{min}}} \) and \( (R/L)_{\text{mean}} \) were both significantly lower for shrimp recoils (mean \( (R/L)_{\text{min}} \) =0.007±0.001; mean \( (R/L)_{\text{mean}} \) = 0.3±0.07) than for fish recoils (mean \( (R/L)_{\text{min}} \) =0.01±0.002; mean \( (R/L)_{\text{mean}} \) = 1.0±0.4) (MANOVA \( ((R/L)_{\text{min}}) \): \( F=7.6, \text{df} \, 15,29, \text{P}=0.009 \); MANOVA \( ((R/L)_{\text{mean}}) \): \( F=6.0, \text{df} \, 15,29, \text{P}<0.02 \)). There were no significant differences in \( \omega_{\text{max}} \), \( \omega_{\text{mean}} \) or \( \Theta_{\text{total}} \) among shrimp and fish recoils (\( \omega_{\text{max}} \) MANOVA:

\( F=0.06, \text{df} \, 15,29, \text{P}=0.8 \); \( \omega_{\text{mean}} \) MANOVA: \( F=0.4; \text{df} \, 15,29, \text{P}=0.5 \); \( \Theta_{\text{total}} \) MANOVA: \( F=0.004; \text{df} \, 15,29, \text{P}=0.9 \)). There was no significant difference between inboard and outboard fin frequency for either shrimp or fish recoils (shrimp paired t-test: \( t=0.3, \text{df} \, 21, \text{P}=0.8 \); fish paired t-test: \( t=-1.3, \text{df} \, 10, \text{P}=0.2 \)). \( \omega_{\text{mean}} \) increased with greater \( \Theta_{\text{total}} \) for attacks...
Figure 29. Peak squid swimming velocity (A) and tentacle velocity (B) plotted as a function of distance to prey at the start of the strike phase. As the distance to the prey at the start of the strike increased, both peak squid velocity and peak tentacle velocity also increased for attacks on shrimp (filled circles, solid regression line) and fish (open circles, broken regression line). See text for correlation statistics.
on both shrimp and fish (Pearson correlation (shrimp): $r=0.9$, $df=32$, $P<0.001$; Pearson correlation (fish): $r=0.9$, $df=13$, $P<0.001$). $\omega_{\text{max}}$ also increased with greater $\Theta_{\text{total}}$ for attacks on both shrimp and fish (Pearson correlation (shrimp): $r=0.5$, $df=32$, $P=0.002$; Pearson correlation (fish): $r=0.6$, $df=13$, $P=0.03$) (Fig. 24). $A_{\text{peak}}$ generally occurred during mantle contraction in the recoil phase, indicating that the pulsed jet was integral to recoil behavior (Fig. 30). Angular velocity plots for the recoil phase did not reveal extensive changes in direction that would indicate the prey are driving the turning motions (Fig. 31), though filtering the data may have smoothed smaller changes in direction.

**Predator-Prey Angles**

At the beginning of the squid approach, shrimp prey were positioned equally above and below the squid. However, at the beginning of the strike, significantly more shrimp were located below than above the squid (ANOVA: $F=11.6$, $df=3,156$, $P=0.02$) (Fig. 32A). This was also the case for fish/squid interactions (ANOVA: $F=4.3$, $df=3,68$, $P=0.03$) (Fig. 32B).

**Postures**

Mantle orientation played a role during shrimp attacks, with the percentage of tail up strikes (mean = 41.3±6.0 %) being significantly greater than tail down strikes (mean = 6.3±2.7 %) and tail down approaches (mean = 21.3±5.0 %) (ANOVA: $F=10.1$, $df=3,316$, $P<0.001$ and $P=0.01$, respectively) (Fig. 33A). There was also a significantly greater percentage of tail up approaches than tail down strikes (ANOVA: $F=10.1$, $df=3,316$, $P=0.004$) (Fig. 33A). In contrast, there were no differences in percentage of attacks in tail up or down orientations for attacks on fish (Fig 34B). Arm angle did not play a major role for either type of attack (i.e., shrimp or fish attacks), with the only difference being that the arms were up more often for shrimp approaches (ANOVA: $F=3.0$, $df=3,316$, $P=0.02$) (Fig. 34). There were no significant differences in the angle of the arms relative to the mantle and angle of the mantle relative to the horizontal in approaches or strikes for attacks on shrimp (approach: mean = 19.2±3.0° (arm) and 18.4±3.5° (mantle); and strike: mean = 15.2±2.1° (arm) and 13.2±2.2° (mantle)) and fish (approach: mean =
Figure 30. Mantle diameter (filled circles) and squid swimming velocity (open circles) for the recoil phase for 6 different attack sequences. A, C, E are for attacks on shrimp and B, D, and F are for attacks on fish.
Figure 31. **The corresponding angular velocity plots to the recoil sequences in Fig. 30.** The plots on the left (A, C, E) are for recoils for attacks on shrimp. The plots on the right (B, D, F) are for recoils for attacks on fish. The angular velocity plots for the recoil were relatively smooth, exhibiting few dramatic changes in direction that would indicate struggling prey are driving the turning motions. Positive angular velocity indicates rotation in the clockwise direction; negative angular velocity indicates rotation in the counter-clockwise direction from the ventral perspective.
Figure 32. The percentage of attacks with the prey (A: shrimp, B: fish) above (dark gray) or below (light gray) the squid at the start of the approach and at the start of strike. Same letters indicate no significant difference; different letters indicate a significant difference. Error bars = s.e.m.
Figure 33. The percentage of attacks (A: shrimp; B: fish) with the squid in a tail down (dark gray) or tail up (light gray) orientation at the start of the approach and start of the strike. Same letters indicate no significant difference; different letters indicate a significant difference. Error bars = s.e.m.
Figure 34. The percentage of attacks (A: shrimp; B: fish) with the arms down (dark gray) or up (light gray) at the start of the approach and at the start of the strike. Same letters indicate no significant difference; different letters indicate a significant difference. Error bars = s.e.m.
20.2±4.9° (arm) and 18.3±5.8° (mantle); and strike: mean = 10.0±2.3° (arm) and 13.5±3.6° (mantle)). The actual angle of the mantle relative to the horizontal did not differ between tail up or tail down orientations for approaches and strikes for attacks on shrimp. The mantle angle relative to the horizontal for attacks on fish was significantly steeper for tail up strikes (mean = 44.7±9.1°) than for both tail down strikes (mean = 11.7±2.6°) and tail down approaches (mean = 16.7±5.2°) (ANOVA: F=5.1, df=3,29, P=0.006 and 0.03, respectively) (Fig. 35).

**Discussion**

This study represents the first comprehensive quantitative kinematic analysis of squid/prey encounters. Squid are capable of high maneuverability and agility during non-predatory behavior (Chapter 2), and the results presented here demonstrate that high turning performance is also important during prey encounters. During fish and shrimp prey interactions, squid exhibited values of $\omega_{\text{max}}$ for the approach and recoil phases comparable to those observed at the performance extremes of non-predatory turning. During shrimp encounters, squid exhibited $(R/L)$ values more similar to those reported for non-predatory turning than in fish encounters, and $(R/L)$ values for approaches on shrimp were lower than those for approaches on fish. However, when squid pursued faster moving fish, they traded-off maneuverability for faster swimming trajectories. Therefore, different attack strategies were used for dissimilar prey. These strategies seem to be very effective given the high capture success rates observed for both fish and shrimp prey in the present study.

Like many nekton, squid located prey positioned laterally to their bodies, turned, and attacked head-first (and eyes-first). However, unlike most other nekton, squid also pointed their arms toward the prey and attacked using an extensible pair of tentacles. Having the eyes oriented toward the prey allows for ocular convergence, which can be as high as 75-86° overlap in other cephalopods, such as cuttlefish *Sepia officinalis* and *S. esculenta* (Messenger, 1968; Watanuki et al., 2000). The eyes of cuttlefish differ from squid in that they have a W-shaped pupil, and thus there are likely differences in the level of ocular convergence between cuttlefish and squid, a topic that was not addressed in the
Figure 35. The mean mantle angle with the horizontal during attacks (A: shrimp; B: fish) in tail down (dark gray) and tail up (light gray) postures for the approach and strike. Same letters indicate no significant difference; different letters indicate a significant difference. Error bars = s.e.m.
present study. Nonetheless, the greatest ventral angle that an individual *L. brevis* noticed prey in this study was 156.1°, indicating that *L. brevis* is capable of detecting prey at high angles. Squid have well developed eyes, demonstrate excellent visual acuity even in low light environments, and are capable of complex visual behavior including convergent eye movements for binocular vision (Budelmann, 1995; McCormick and Cohen, 2012; Sweeney et al., 2007; Watanuki et al., 2000; Young, 1963). Although the visual field in these cephalopods appears to be extensive and prey can be detected at high angles, the prey needs to be directly in front of the squid’s arms and head before the tentacles are deployed and the prey is captured. In this arms-first orientation, binocular vision becomes especially important for focusing on the prey (Budelmann, 1995; Messenger, 1968).

Irrespective of prey type, squid reduced their ventral angle relative to the prey from the beginning of the approach, when the target was first identified, to the beginning of the strike. This change in angle is important so that the tentacles can be aligned with the prey in preparation for the strike. During approaches, it is not only important to reduce ventral angles to achieve tentacle alignment but it is also important to make these angular adjustments quickly. This is supported by higher squid angular velocities correlating with larger ventral angles and larger total angular displacements during the approach for both shrimp and fish prey encounters. Though these relationships may seem obvious, there was no relationship between angular velocity and total angular displacement for non-predatory turns where prey strikes were not the end point (Chapter 2), suggesting that squid actively elevate agility, i.e., turning speed, when targeting prey positioned at high ventral angles. Since prey items can be detected at high angles, increased agility is clearly beneficial during the initiation of an attack.

The maximum angular velocity during the approach phase for squid encounters with shrimp and fish was comparable to non-predatory turns, but mean angular velocity was actually less than in non-predatory turns. The lower mean angular velocity for the approach phase in the present study is most likely due to the approach phase not being entirely rotational, i.e., when tracking a prey, translation is required to keep pace with the prey as opposed to exclusive turning. Though squid clearly are capable of exhibiting high agility (turning speed) during the approach, agility did not consistently increase for faster
prey, suggesting that other factors besides prey speed impact agility performance during the approach toward the prey. The peak angular velocity during the approach usually occurred shortly after the squid noticed the prey. Thus, squid initiate a rapid turn toward the prey early during prey encounters irrespective of the type of prey and then approach the prey in a more or less straight trajectory (Fig. 36). In cases where the prey was moving quickly during the approach, additional corrective turns were often necessary to realign with the prey (Fig. 36B and 36D). However, the initial turn toward the prey was still associated with the greatest angular velocities. Shrimp tended to move more slowly than fish, so on average, squid corrective movements tended to be faster for fish encounters than shrimp encounters.

Clear differences were observed in how squid behave in encounters with shrimp versus fish. Fish prey swam and accelerated significantly faster than the shrimp prey during the squid approach, and the fast swimming fish also elicited higher squid approach velocities and accelerations. Moreover, the farther away the fish was at the start of the approach, the faster the squid approached the fish, but this relationship was not observed in shrimp/squid interactions. Fish likely had a greater capacity for escape than shrimp during the approach due to faster swimming speeds and the presence of a lateral line for sensing predator wakes, and thus the squid may have increased its approach speed to limit the opportunity for the fish to initiate a fast escape. Fish prey that are approached by a fish predator moving at a higher speed respond and escape at greater distances than fish prey approached by predators moving at slower speeds (Domenici and Blake, 1991; Domenici and Blake, 1997; Stewart et al., 2013; Stewart et al., 2014). The improved escape response at higher predator approach speeds is due to the formation of larger bow waves from faster predators, which can be more readily sensed by the lateral line system of fish prey (Stewart et al., 2014). Though squid also produce bow waves as predators, the correlation between approach speed and prey escape success was not obvious in this study. This may be simply a product of limited successful prey escape sequences (i.e., more sequences may bear this relationship out), or perhaps, it is related to the production of smaller bow wakes by squid. Unlike fish that strike prey targets with their jaws, often with an associated suction wake (Stewart et al., 2013, 2014), squid impact their prey
Figure 36. Approach trajectories of the squid (black circles) and prey (gray circles; A,B: shrimp; C,D: fish). The squid trajectory was tracked using the arm point. The approach was complete when the arms began to splay to initiate the strike. Arrows indicate the direction of motion during the approach.
targets with streamlined, fast moving tentacles that likely have a reduced bow wake. This intriguing hypothesis merits further study.

Based on the lower \((R/L)_{\text{min}}\) and \((R/L)_{\text{mean}}\) observed in this study for squid approaching shrimp versus fish, squid exhibited a higher degree of maneuverability when targeting shrimp. Since shrimp move much slower than the fish prey, the squid likely had more time during the approach to perform controlled tight turns to get into an optimal position for striking. Squid presumably did not have the luxury of precise turning during encounters with faster swimming fishes. Instead, the squid relied more heavily on fast swimming speed and acceleration to approach the fish.

Asymmetrical motions of the fins are typically employed during non-predatory turns, with the outboard fin beating faster than the inboard fin, which assists the jet in turns (Chapter 2). Similar asymmetry in fin beats was observed during the approach for attacks on fish in the present study, especially during the end of the approach. However, during attacks on shrimp, the inboard and outboard fins were generally synchronized. Higher fin synchronization during shrimp attacks may be a result of decreased squid velocity during these encounters. As the squid approached shrimp prey, it often swam slowly and then hovered near the prey within striking distance, as if waiting for an optimal attack opportunity. In contrast, the squid rapidly approached fish prey and struck quickly, with no pause to hover and position. At these high approach speeds, squid likely needed to make rapid last-minute adjustments to align with the fast swimming fish, which may be the reason for the greater fin asymmetry late in the approach. The posterior positioning of the fins coupled with their large distance from the center of gravity during arms-first approaches allows for large corrective moments that are beneficial for rapid stability and trajectory adjustments. Keeping the head stable during the approach is especially important for sensory processing and accurate strikes as is the case in other animals (Land, 1999).

The arms-first approaches toward fish in the present study were performed at relatively high speeds, reaching average velocities of up to 5 body lengths \(s^{-1}\). This result differs from observations in other squids. Foyle and O’Dor (1988) found that \textit{I. illecebrosus} slowly stalk faster and larger fish prey. This strategy was thought to be due
to swimming limitations in the arms-first orientation (Foyle and O’Dor, 1988). Though there are funnel aperture constraints during arms-first swimming that potentially limit steady rectilinear swimming speeds (Bartol et al., 2001a), the high arms-first speeds recorded here coupled with recent recorded *L. brevis* swimming speeds of 8 body lengths s⁻¹ in the arms-first orientation (Bartol et al., in press) indicate that *L. brevis* is certainly capable of overcoming these constraints and producing short bursts of high-speed arms-first swimming when pursuing prey.

Several consistent behavioral patterns were observed for squid encounters with both fish and shrimp. For both prey, the squid approach began with a peak in angular velocity after a strong mantle contraction, indicating that the initial speed of the squid is mostly rotational. A second peak in linear velocity occurred at the end of the approach after a mantle contraction, moving into the strike. Thus, two jets are important for prey attacks: (1) an initial powerful jet to rotate the squid and orient it to the prey and (2) a secondary jet to accelerate the squid toward the prey for the attack. Interestingly, for both types of attacks, a synchronized downstroke occurred during the secondary jet, presumably to augment thrust forces as the squid accelerates toward the prey target.

The arms-first attack orientation observed in the present study has been welldocumented in squid (Foyle and O’Dor, 1988; Nicol and O’Dor, 1985; Squires, 1966). The arms and tentacles are integral to prey capture, thus it is obviously important for squid to position themselves anteriorly toward the prey when performing attacks. Somewhat unexpected, however, was the finding that squid were capable of controlling not only their own peak swimming velocity but also the tentacle peak extension velocity, depending on the squid/prey distance at the start of the strike. For both shrimp and fish encounters, the peak velocity of the squid and the tentacles increased with increased squid/prey distance at the start of the strike. This strategy probably improves strike success, as the squid needs to close the predator/prey gap more quickly with greater distances to reduce prey reaction times. The average and peak squid swimming velocity during the strike was higher for attacks on fish than shrimp, most likely due to fish having a higher swimming velocity and therefore greater escape capacity. However, the average and peak tentacle extension velocity was no different for shrimp or squid prey,
indicating that squid opt to increase swimming speed and not tentacle strike velocity when attacking faster prey.

Sugimoto and Ikeda (2013) determined that the squid *S. lessoniana* only increased maximum swimming speed with greater distances to prey for the strike, while cuttlefish *S. pharaonis* only increased the maximum speed of the tentacles for greater distances to prey for the strike. These results suggest that there are two strategies involved in striking prey: (1) increase swimming speed to secure prey, which is used by squid, and (2) increase tentacle speed to secure prey, which is employed by cuttlefish. Findings from the present study indicate that squid *L. brevis* use a combination of both approaches, increasing both swimming speed and tentacle speed when necessary. The peak speed of the squid and tentacles, expressed in cm s\(^{-1}\), was lower in *L. brevis* (*squid* \(V_{\text{peak}} = 63\) cm s\(^{-1}\); *tentacle* \(V_{\text{peak}} = 124\) cm s\(^{-1}\)) than values recorded for *Loligo pealei* and *Illex illecebrosus* (*L. pealei* squid \(V_{\text{peak}} = 120\) cm s\(^{-1}\); *L. pealei* tentacle \(V_{\text{peak}} = 250\) cm s\(^{-1}\); *I. illecebrosus* squid \(V_{\text{peak}} = 390\) cm s\(^{-1}\)) (Foyle and O’Dor, 1988; Kier and Van Leeuwen, 1997). These findings are not surprising given the smaller size of *L. brevis* relative to *L. pealei* and *I. illecebrosus*. Larger animals are generally capable of reaching faster overall swimming speeds. Unfortunately, normalized speeds expressed in body lengths s\(^{-1}\) could not be determined in the other studies for comparison purposes because squid lengths were not reported. Although squid swam faster during the strike for fish targets than shrimp targets, they still swam slower than the escaping fish. In contrast, squid swam faster than shrimp during the strike. However, tentacle speed was much faster than shrimp/fish swimming speeds. Thus clearly a combination of swimming and tentacle extension is critical for strike success, i.e., squid cannot capture faster moving fish based on their swimming speed alone - they require fast tentacular extensions as well.

Vertical positioning of the prey played a role in squid attack sequences. At the beginning of the approach phase, the shrimp or fish was equally likely to be above or below the squid. However, at the start of the strike both shrimp and fish were more likely to be positioned below the squid, as the squid preferentially repositioned itself so that it was above the prey before initiating the strike. From this higher vantage point, the tentacles generally moved down and outward towards the prey during the strike. The base
of the tentacles is located ventrally within the arm web (Young and Vecchione, 1996), and thus squid may select higher positions in the water column to facilitate easier tentacle elongation and more accurate strikes. Positioning of the mantle and arms played a more important role in attack behavior for shrimp prey than fish prey, with tail up orientations being preferred during shrimp strikes. As is the case for other cephalopods, squid likely have significant anterior ocular convergence and the arms likely obscure the anterior visual field to some degree during prey strikes since they are often in-line with the prey strike zone. Thus, a tail up posture during attacks, which elevates the eyes slightly above the arms, should provide a less obstructed view of the prey and allows the body to be more in line with the prey item as it is usually positioned below the squid for the strike. This tail up posturing was more prevalent during shrimp than fish encounters possibly because slower swimming shrimp allowed for more postural adjustments by the squid prior to attacks. Although the arms were more likely to be up relative to the mantle at the start of shrimp approaches when the eyes were likely focused laterally, this was not the case for the strike. The observed lower arm angles during strikes likely occurred to allow the arms to be more in line with the prey below and to improve their line of attack to the prey.

During the recoil phase, the squid switched from swimming arms-first to swimming tail-first. This reversed motion, combined with simultaneous retraction of the tentacles which adhere to the prey via suckers on the club, mitigates prey escape success. The prey escape attempts are slowed by retraction forces created by the squid swimming in reverse and tentacle recoil. To escape, fish typically use C-starts that project the fish at a heading approximately 90° from their original trajectory in the direction opposite the side with initial lateral line hair cell stimulation (Domenici and Blake, 1997; Eaton and Emberley, 1991; Eaton et al., 1977; Gazzola et al., 2012). Shrimp are also capable of rapid escape responses using tail flips (Nicol and O’Dor, 1985). In the present study, tail-flipping escape behavior was not usually initiated until after the tentacles made contact with the shrimp, when capture success was 89%. Interestingly, in those few instances where the shrimp did initiate a tail-flip response prior to tentacular contact, the squid either abandoned the approach and did not deploy the tentacles, or deployed the tentacles
but was unsuccessful at capturing the shrimp. In contrast, unsuccessful fish captures were not characterized by successful C-start escapes, but rather inaccurate tentacle strikes, which often induced the fish to initiate a C-start escape response following the full deployment of the tentacles. In successful fish captures, there was no evidence of C-start behavior before the strike, indicating that the fish had likely not sensed the approach of the squid. The observation that escapes were generally not initiated until full deployment of the tentacles provides support for the idea that squid are capable of keeping their body, fin, and jet wakes away from the sensing structures of the prey (Arnott et al., 1998; Denton and Gray, 1989; Stewart et al., 2013; Wine, 1984) and maintaining a low tentacle bow wake signature, which is certainly reasonable given the streamlined shape and smaller cross-sectional area of the tentacles.

For attacks on shrimp, mean angular velocity was higher for the recoil than for the approach, but for both attack types recoil angular velocities were not greater than mean angular velocities detected during non-predatory turns without prey stimuli. During both the recoil and approach, squid exhibited agility measures (peak angular velocities) greater than or similar to the performance extremes of turns when translation is not required to track prey targets. Although the observed angular velocities during the recoil phases may be driven, at least partially, by the escape attempts of the prey items, it is unlikely that prey movements are playing a major role in the observed high angular velocity values. No unexpected and erratic turning behaviors were observed in squid during recoil that would suggest prey were driving turning motions as the angular velocity plots were relatively smooth and unidirectional (see Fig. 37). Moreover, even during attacks where fish attempted multiple escape maneuvers during the recoil, the squid was still capable of keeping the variability in angular velocity relatively low. Peak angular velocity was higher during the recoil than during the approach and non-predatory turns, indicating that turning speed may play a critical role in helping to obtain control over prey. The main consequence of interactions with highly active prey was longer time durations to control the prey within the arms, which would contribute to lower as opposed to higher angular velocity measurements. No instances of prey escape were observed once the tentacles made contact with the prey. During the recoil phase, the speed and acceleration of the
squid were higher for attacks on fish, while the minimum radius of the turn was lower for attacks on shrimp. This finding indicates that squid rely more heavily on swimming speed than tight turning to obtain control of faster, stronger fish prey. Shrimp were slower and smaller than the fish used in this study and thus squid did not need to swim as powerfully in reverse to subdue them. Enhanced control of their target allowed for more precise turning. Despite the escape attempts of the prey, the results of this study indicate that squid are able to secure their prey while maintaining control of their turning performance during the recoil, making them formidable predators.

Concluding thoughts

The results of this study clearly demonstrate that turning performance is central to prey capture in squid, with both the tightness (maneuverability) and speed (agility) of turning playing important roles throughout attack sequences. Agility parameters were consistently high during encounters with both shrimp and fish, and high maneuverability was especially important for attacks on shrimp. Though maneuverability was also important for fish encounters, *L. brevis* relied more on maximizing linear attack velocities than minimizing turning radii when attacking fast swimming fish. Squid are seemingly capable of determining the optimal swimming speed to employ during the strike, increasing swimming speed with greater distance from their prey target. Moreover, when encountering fast moving prey, such as fish, small squid like *L. brevis* opt to increase their own speed rather than the speed of the tentacles for greater attack success.

Capture success rate in squid was high and did not differ significantly for shrimp (89%) or fish (81%), despite the ability of fish to swim much faster than shrimp. This result indicates that squid are very effective predators, irrespective of the prey target. This does not come as a surprise given their unique ability to swim readily in multiple orientations, their high maneuverability and agility (Chapter 2), their ability to produce complex interactive fin and jet flows (Chapter 3), their capacity to rapidly extend their tentacles and muscular arms (Kier and Van Leeuwen, 1997), and their high visual acuity (McCormick and Cohen, 2012; Watanuki et al., 2000). Indeed squid are voracious,
opportunistic, formidable predators capable of attacking and subduing a wide variety of prey.

A logical next step for study is to examine flow fields during predator/prey attacks. It is possible that squid are capable of directing fin and jet flows away from the sensory detection field of the prey, which would help explain their high capture success rate. The tentacles are also very streamlined with small cross-sections and likely have a smaller wake signature than the bow wave created by the head of other predators, making them harder to detect. This would certainly explain why an escape reaction from the prey is typically initiated only after the tentacle tip has made contact. Given the flow complexity of these predator/prey interactions, a full 3D velocimetry and videography platform would likely be required. Although such an approach would involve significant technological challenges, it promises to yield unprecedented insights into how squid achieve such high prey attack performance.
CHAPTER 5

CONCLUSIONS

Unsteady swimming movements make up a large portion of an aquatic animal’s locomotive repertoire, yet have not been studied extensively. Turning, in particular, is an unsteady movement that is required for navigation in complex habitats, predator avoidance and predatory attacks. Turning performance is typically quantified by measures of maneuverability and agility. Maneuverability is the ability to turn in a confined space, and is measured as the length-specific radius of the turn. Agility is the rate of turning, measured as average and maximum angular velocity. Another method of studying turning performance is to examine vorticity and velocity hydrodynamic profiles during turns. This type of analysis is useful for determining which propulsor is producing the greatest torque, or turning force, during various turning maneuvers, and the relative importance of torque acting about the roll and pitch axes.

Squid and cuttlefish are unique relative to other aquatic taxa in that they have a muscular hydrostat driven, dual-mode propulsive system consisting of flexible paired fins and a pulsed jet. Previously, aquatic turning performance has been assessed in the context of body flexibility. In general, more flexible bodied animals are thought to exhibit higher agility and maneuverability. Most rigid-bodied animals exhibit a tradeoff and are either very agile or very maneuverable, but not both. This type of categorization does not work well for squid and cuttlefish since they have both prominent rigid components (pen and cuttlebone) and flexible components (arms, funnel, fins) and thus to do not fall neatly within classical body rigidity categories.

Both squid and cuttlefish were found to be highly maneuverable with absolute minimum length specific turning radii of 0.0004 for *Lolliguncula brevis* and 0.0001 for *Sepia bandensis*. The mean and minimum length specific radii for *S. bandensis* and *L. brevis* place them as the most maneuverable aquatic animals measured to date, more so even than the highly maneuverable spotted boxfish, *Octracion meleagris* (Walker, 2000). Although *S. bandensis* is certainly capable of very tight, controlled turns with low
translation, it exhibited a wider range of turning behaviors than *L. brevis*, resulting in a greater mean length specific turning radii. *Lolliguncula brevis* demonstrated greater agility than *S. bandensis* with an absolute maximum angular velocity measuring $725.8^\circ \text{s}^{-1}$ compared to $485.0^\circ \text{s}^{-1}$ for *S. bandensis*. As expected, an increase in angular velocity corresponded with greater turning radii. Faster turns generally result in wider turns; as inertia during the turn increases, it can become more difficult to control the tightness of the turn. *Lolliguncula brevis* bent the arms closer to the body axis during turns, which increased angular velocity. This behavior may help compensate for the inability of the mantle cavity to bend, thereby minimizing the length of the body that is contributing to drag during the turn. Bending of any part of the body axis reduces the body’s second moment of inertia about the dorsoventral rotational axis, resulting in lower inertial and hydrodynamic resistance to rotation (Walker, 2000).

For routine turns in stationary water and for turns made during the approach and recoil phases of prey attacks, periods of high angular velocity correlated with strong mantle contractions, indicating that jet pulses were the primary contributor to increased agility. Hydrodynamic analyses also provided support for the jet being the principle driver of rotation for short tail-first, long tail-first, and vertical turning categories. The jet generally produced pulsed vortex rings that were directed predominantly downwards and perpendicular to the longitudinal axis of the squid at the midway point in the turn. The downward directed component of the jet counteracts the negative buoyancy of the squid. The jet also proved to be very important during attacks with two peaks in velocity being observed, both of which corresponded to decreases in mantle diameter. The first peak occurred shortly after the approach was initiated and corresponded with a peak in angular velocity. The second peak was at the end of the approach, moving into the strike, when the squid was lunging towards the prey.

Based on DDPTV data of squid maneuvering in a water tunnel against flow (Chapter 3), four main categories of squid turning were identified: (1) short tail-first turns, (2) long tail-first turns, (3) vertical turns and (4) arms-first turns. Arms-first turns were observed over the widest range of background flow speeds. Short tail-first turns occurred at intermediate background flow speeds, while long tail-first and vertical turns
occurred at low and high flow speeds, respectively. Short tail-first turns and arms-first turns were the most common turns in both hydrodynamic 3D velocimetry studies conducted in flowing water and kinematic studies performed in stationary water.

Vertical and long tail-first turns were only observed in hydrodynamic flow quantification trials. During vertical turns, the jet contributed more of the torque for turning about the primary axis than the fins. The jet and fins both contributed to production of torque acting about the roll and pitch axes, presumably to keep the squid in the vertical orientation as it spun and translated with free-stream flow. During long tail-first turns, the jet was used to effect the turn while the fins produced significant drag to slow and control the turning motion. These long tail-first turns involved a degree of banking. Banking, or rolling, is an efficient means of turning because it increases the projected body surface area to oncoming flow, allowing for more directed force towards the center of rotation and production of greater centripetal forces (Parson et al., 2011). Both vertical and long tail-first turns demonstrate that squid can harness ambient flow to aid in turning. Moreover, the elongated regions of concentrated vorticity and linked vortex ring structures observed in long tail-first turns and vertical turns, respectively, indicate that squid are able to produce a wide range of complex vortex-wake flows using their highly flexible dual-mode system.

Short tail-first and arms-first turns, which were the most common turns observed in the DDPTV studies, were also observed in the kinematic studies (Chapter 2) conducted in stationary water. Short tail-first turns were characterized by efficient vortex ring jet pulses that are important for maintaining control and conserving energy throughout the turn. Fin flow was minimal in short tail-first turns. Rather, the jet was the major effector of these turns, producing most of the torque contributing to rotation about the main turning axis (yaw axis), and producing a greater degree of torque resulting in roll and pitch than the fins. Arms-first turns occurred over a wide range of flow speeds relative to the other categories. This type of turning behavior is important for predatory attacks since prey are seized in an arms-first orientation. In contrast to short tail-first turning, the fins, not the jet, were the major effector for arms-first turning, and were important for producing torque stabilization about the roll and pitch axes. Overall, the fins contributed
more relative $T_y$ and $T_{xz}$ for arms-first turning compared to the other turning categories. The fins created linked vortices that often interacted with vorticity from the jet during arms-first turns. The linked vorticity in this category is likely due to complex fin wave motions that tend to be more prevalent in the arms-first orientation based on previous studies. The high reliance on the fins for turning and stabilization during arms-first turning may be related to high arm drag and funnel bending constraints, which necessitate higher fin contributions, and more optimal placement of the fins.

Maneuverability and agility measures differed in stationary water versus flowing water. Both the mean and minimum length specific turning radii of squid were greater when turning in background flow than in stationary water. This difference is most likely driven by increased translation, as background flow can cause an animal to deviate from its intended trajectory (Liao, 2007). Even at low flow speeds, squid performing long tail-first turns had greater translation and turning radii than squid performing turns in stationary water. The highest translation was observed in vertical and short tail-first turns. As the background flow speed increased for all turn categories, so did mean angular velocity of the turn, suggesting that higher ambient flow can promote faster turns. Squid performing vertical turns had the greatest angular velocity, or agility, compared to squid performing other categories of turning, which is not surprising given the lower resistance to rotation from the vertical orientation and that high flow speeds were associated with vertical turns. However, spinning on the longitudinal axis, as *L. brevis* were observed doing in this category, can result in less rotational resistance from the body during the turn as well, leading to decreased pressure drag. Since tail-first swimming is used over an extensive range of speeds and *L. brevis* inhabits environments with variable flow conditions, having the ability to turn in multiple ways in the tail-first mode (short tail-first, vertical, long tail-first) is an important adaption.

Asymmetric fin motions were observed for *L. brevis* during routine turns in stationary water, with the outboard fin beating significantly faster than the inboard fin. Typically, the fins were synchronized for the majority of the turn, with one or two main periods of asymmetry mid to late in the turn that contributed to these observed frequency differences. Fin beats on the outboard side were also greater in frequency during the
approach for attacks on fish, but were synchronized for attacks on shrimp. The increased speed during the approach in attacks on fish likely resulted in a greater need for corrective movements. Consistent differences in fin beat frequency were not observed across the turning categories observed in background flow. However, the fins were integral to the turns. The fins were important stabilizers for controlling the turn, and in the case of arms-first turns, were also important effectors of the turns.

Turning performance becomes very important during prey capture (Chapter 4). Steadily swimming squid in the tail-first orientation generally have their eyes focused laterally. Shrimp and fish prey are detected at mean angles of 52.3° and 42.1°, respectively, but the strike is initiated at more acute angles (5.7° and 3.3°, respectively), indicating that the squid must turn significantly during the approach to orient itself appropriately. Although squid often swim in a tail-first orientation, they approach and attack their prey in an arms-first orientation so that the arms and tentacles are directed towards the prey.

The greater the ventral angle relative to the prey at the start of the approach, the greater and faster the squid turned during the approach, suggesting that high agility is important for prey capture. In fact, quick turning at the very start of the approach, resulting in a peak in angular velocity, was employed regardless of the prey type or distance to the prey. However, overall approach speed by the squid was higher during encounters with faster fish prey than encounters with slower shrimp prey. The squid also increased its approach speed when the fish was farther away, but this was not the case for shrimp. During encounters with shrimp, squid moved more slowly and exhibited high maneuverability, i.e., tight turning, following the initial rapid turn. Since shrimp move slower than fish, the squid likely has more time to get into an optimal strike position than is possible with fish.

Positioning of the body relative to the prey also played a larger role for attacks on shrimp than for attacks on fish. Although posture did not appear to be important at the start of the approach, the squid generally maintained a tail-up posture at the start of the strike for attacks on shrimp. This posture likely elevates the eyes over the arms (the arms were lowered slightly) to obtain a better vantage point for the strike. Positioning in this
way was only used for attacks on shrimp, probably because slower moving shrimp allowed the squid to have more time for corrective movements to get into an optimal strike position. At the start of the approach, the prey was just as likely to be positioned above or below the squid. However, at the start of the strike, the prey was much more likely to be below the squid, indicating the squid preferred to attack prey from above. This preference in positioning could be due to higher above-prey strike proficiency since the tentacles are more ventrally located in the arm web.

Squid favored increasing swimming speed over increasing tentacle speed when attacking faster fish prey. The increased swimming speed during the strike was initiated at the end of the approach before deployment of the tentacles, as indicated by a peak in angular velocity. There is likely some control of the speed of the tentacles during the strike because prey at larger distances at the start of the strike triggered higher peak tentacle velocities. The use of highly streamlined thin tentacles projected at high speed probably limits wake detection by the prey, thereby increasing the probability of success for the squid.

During the recoil phase of prey attacks, the squid reversed direction, switching from an arms-first orientation to a tail-first swimming orientation. This reversal in direction, combined with a powerful mantle contraction and retraction of the tentacles, reduces the ability of the prey to escape. Angular velocity was high during the recoil, but this was not driven by escape attempts of the prey, since the turning behavior was generally smooth and unidirectional. Shrimp most often produced a tail-flipping behavior shortly after the tentacles made contact. However, sometimes the shrimp initiated a tail-flip before the squid deployed its tentacles. In these cases, the squid either abandoned the approach or deployed the tentacles but failed to make contact. Fish generally attempted an escape after the squid tentacles made contact using a C-start response. Interestingly, successful evasion by the fish was not the result of successful C-start responses by the fish, but rather the product of inaccurate tentacle strikes by the squid. Successful prey evasion was rare, however, given the very high capture success rates exhibited by squid for both fish and shrimp prey.
Unsteady swimming performance is a critical component of locomotion for squids. The unique and highly adaptable locomotive system of cephalopods affords them enormous flexibility to accomplish a variety of turning behaviors. Flexibility in locomotive performance is essential for predatory attacks, predator avoidance, navigation in complex habitats, and handling perturbations that significantly alter swimming trajectories. This study revealed that squid have the ability to perform powered turns in both arms-first and tail-first swimming orientations using coordinated jet and fin motions, as well as the ability to use ambient flow to control the turning motion. The coordinated movements produced complex 3D vortex-wake flows with associated turning forces, which were quantified for the first time in any maneuvering cephalopod in this study using a novel 3D velocimetry approach. Clearly the high degree of agility and maneuverability reported in this study indicates that squid and cuttlefish are not typical rigid-bodied swimmers, as suggested in previous studies. Rather, they represent an interesting hybrid body type with the ability to manipulate their flexible components (arms, fins, funnel) to overcome some of the inherent limitations of their rigid elements (pen, cuttlebone). Indeed squids are exceptional predators equipped not only with acute sensory systems, but also high turning capabilities. These turning abilities coupled with their flexible dual-mode locomotory system have contributed significantly to their ecological and evolutionary success.
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