The Evolution of Flight in Insects: Reconstruction and
Biomechanical Analysis of a Protopterygote Insect Ancestor

by

Ryan Joseph Chlebak

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Abstract

Powered flight in insects has allowed them to become one of the most successful groups of organisms on the planet. How they evolved to become flyers from non-flyers over 400 mya remains a mystery. Few published works have experimentally attempted to explore how flight may have arisen in insects and as such, the objective of this thesis is to attempt to define a possible protopterygote insect and its pathway to selection for powered flight. Here, I have employed a combination of behavioural analysis on an extant basal group insects and allometric scaling of morphology to define and construct a likely protopterygote insect. I have also enacted biomechanical methods such as force measurement and flow visualization on a physical wingless, static winged and a mechatronic (dynamic) flapping protopterygote model to explore potential elements for the selection of flight in insects. I have found that body shape (posture) is important to increasing drag as evidenced by the decreasing descent velocity and self righting ability of the Archaeognatha (Chapter 2), while their unique jumping behaviour can be useful to escape from the confines of standing water, likely present during the time of the protopterygote insect (Chapter 3). Biomechanical testing has revealed the presence of a low pressure zone behind the dorsum of a protopterygote model emulating the posture of falling Archaeognatha (Chapter 5) and that flapping can increase drag within the Reynolds regime of the putative protopterygote (Chapter 6) potentially setting the stage for thoracic wing selection. My thesis undertakes a novel approach, contributes empirical data and new insights to an emerging theory of the evolution of flapping flight in insects. This theory draws on previously defined jumping, parachuting and surface skimming hypotheses and attempts to combined elements from each while also providing support

for the likelihood of a falling, flapping protopterygote insect contributing to selection for powered flight in the Insecta.

Statement of Contributions

Dr. Jeff W. Dawson is co-author on all manuscripts expected to be published from this thesis due to his role as my graduate supervisor and mentor.

The data presented in Chapter 2 was collected by myself; however the pilot studies which led to finalizing of the methods were assisted by Lauren Ellis while completing her undergraduate thesis. Statistical tests for drop and vertical wind tunnel data was performed by myself with guidance from my supervisor, Dr. Dawson. Statistical tests on horizontal wind tunnel data were completed with assistance by Sarah Harrison and Ian Thompson.

Jumping trial and water drop experiments in Chapter 3 were designed by me. Statistical testing of the data in Chapter 3 was performed by myself with assistance from Dr. Dawson. Jumping data was collected by Brittany Lowden, Samantha Britton and myself. Shaughna McDonald assisted me with data collection from water drop trials.

The initial reconstruction of the protopterygote insect in Chapter 4 was provided by Dr. Jarmila Kukalová-Peck, while all other original drawings were created by myself and are indicated in the figure captions. Allometric scaling regressions were completed by me with the guidance of Dr. Dawson. Morphological data was collected by me and those who contributed to Chapter 2 and 3, listed previously. The digital protopterygote rendering described in Chapter 5 and 6 was completed in its entirety by myself as well as the physical model assemblies and associated custom hardware. Programming for the flapping model servos was completed by Dr. Dawson and myself. Mike Jutting assisted me with the servo wire harness assembly. Drag force data was collected by me; however,

the construction of the force meter is due to the efforts of Dr. Dawson and Mike Jutting.

Dye flow data was collected and analyzed by myself with assistance from Dr. Dawson.

All stereo PIV experiments were performed by myself, Dr. Dawson assisted with

interpretation. The writing and data analysis in this manuscript was completed by me.

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List of Symbols and Abbreviations

$\dagger$	Extinct
ϕ	Wing beat amplitude
f	Wing beat frequency
Γ	Circulation
ρ	Density of medium
Cl.	Class
dPIV	Digital Particle Image Velocimetry
CNS	Central Nervous System
F.	Family
fps	Frames Per Second
mya	Million Years Ago
O.	Order
R	Wing length
Re	Reynolds Number
S.C	Super Class
SEM	Standard Error of the Mean
SP.O	Super Order
sp.	Species
St	Strouhal Number
Tf	Time to fall
U	Velocity
ν	Kinematic viscosity
VD	Vertical drop specimen
Vf	Velocity of fall
V_t	Terminal Velocity
VT	Vertical wind tunnel specimen

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Chapter 1: General Introduction

Flight is arguably the single most important factor in the successful radiation and diversification of the insects. Flight renders new environments and unexploited resources attainable where they would otherwise be unavailable to terrestrially limited organisms. In general, this has led to the success of the Insecta as a whole, supporting numerous and diverse lineages within. The massive Meganisopterans (up to 71 cm wingspan) of the Carboniferous era, regularly referred to as the “giant dragonflies”, represent perhaps the most awe inspiring pinnacle of insect size during their successful adoption of aerial life (Carpenter, 1947). However, before such impressive fliers could exist there would have been a less aerially oriented early insect ancestor which could successfully survive amongst other flightless semi-aquatic invertebrates of the Ordovician to Silurian time periods. The successful survival of these organisms is likely attributed to the employment of specific morphology potentially used in escape and/or locomotion. Such behaviours involving an aerial component (jumping, falling etc.) become candidates for precursor behaviour towards aerial adaptation thus leading towards the evolution of powered flapping flight in the Insecta.

Modern insects fly with one or two pairs of wings controlled by an array of thoracic muscles and nerves. Functional insect wings and specialized wing musculature evolved in a terrestrial environment but stemmed from precursor morphology that was present in aquatic ancestors. These early pre-wing apparati were likely pre-adaptations and may have become useful for more terrestrially based survival, well before they were large enough to be used in flight. If they ceased to be useful and/or became a potential

burden, as in the groups that have adopted cryptic lifestyles (Archaeognatha, †Monura, †Cercopoda, Zygentoma), they would first become diminished and then be lost (Kukalová-Peck, 1998, 2008). Suggestions for how such structures could be selected for are plentiful; an example of a current concept that remains a potential avenue for the selection of thoracic wings for flapping flight is presented by Marden and Kramer (1994) and Marden (1995, 2003) *via* their observations of surface skimming. According to the authors, the initial beginnings of flapping flight would have been coupled to a need to maintain forward motion while supporting the body weight on the surface tension of a water body, possibly to escape predators. However, before even this state, the ancestors of †Cercopoda, Pterygota and Zygentoma bore crustacean-originated, abdominal twisted rope muscles for jumping and resembled the extant Archaeognatha (Manton, 1972, 1977; Kukalová-Peck, 1983, 1998). Since the polyramous arthropod limbs originated in the Proterozoic (before the Palaeozoic, Figure 1.1), the accompanying rami supported by the limbs (which evolved into various morphological features including wings (Kukalová-Peck, 1983, 2008; Averof and Cohen, 1997)) were already present when the Insecta diverged. The potential function of such rami is currently unknown and open for speculation.

Along with an overview of the literature available in regards to flight evolution in the Insecta, throughout this thesis I present supportive data and my interpretation of a possible pathway toward flight evolution in the insects through the examination of extant basal insects and their behaviours, published fossil record descriptions, and, direct

Eon	Era	Period	Mya
Phanerozoic	Mesozoic	Cretaceous	135
		Jurassic	205
		Triassic	250
	Palaeozoic	Permian	290
		Carboniferous	355
		Devonian	410
		Silurian	438
		Ordovician	510
		Cambrian	570

Figure 1.1. The timeline surrounding the evolution of the early insects and flight in insects. Wings and crude flight is expected to have occurred from the Ordovician period to the early Devonian period, diversifying into multiple flying lineages throughout the Carboniferous period. Timeline based on Long (2003).

biomechanical examination of a model of a putative pre-flight insect ancestor (a protopterygote insect). It is my intention to demonstrate that the simple act of transiting into and descending through the air column may yield additional insights than previously thought, and that my findings may play into and/or account for, current observation-based theories towards insect flight, such as jumping (Norberg, 1972; Kukalová-Peck, 1987, 1998, 2008) and surface skimming (Marden and Kramer, 1994; Marden et al. 2000; Marden, 1995, 2003).

1.1. Early Insects

The Carboniferous period (approximately 300 to 350 mya) is well known for the radiation of the insects and diversification of numerous flying insect lineages. Although continually debated, the extreme diversity and remarkable specialization for flight present in the fossil record strongly suggests that crude, early flying/flapping insects appeared sometime between the Silurian period (approximately 425 mya) to the early Devonian period (approximately 410 mya), while the precursors to these insects likely appeared as far back as the Ordovician period, approximately 440 mya (Figure 1.1) (Kukalová-Peck, 1987, 2008). Although the fossil record of the Carboniferous era is extensive, the Devonian and Silurian era fossil records are virtually non-existent. In fact, one of the precious few samples from the Early Devonian time period is a partial fossil of mouth parts interpreted as an early Archaeognatha (Labandeira et al., 1988). As a consequence of the lack of direct fossil evidence of the early insect lineages, palaeobiologists resort to painstaking efforts in preparing, examining, reconstructing and cross referencing

numerous fossil insect specimens so as to establish and systematically tease out the most basal conditions of various morphological features present across all insect lineages (synapomorphies). As insects are monophyletic (Figure 1.2) (Kukalová-Peck, 2008, 2009), it is only through such time-consuming methods that specific morphological features, such as legs and individual leg podites, wings and wing veins, cerci, reproductive organs, etc. can be homologised across all insects and the true most basal morphology (the insect ground plan) of an early insect ancestor can be described. Such a breakthrough would contribute to a greater clarification of the pathway the Insecta followed from their early origins to modern day and efforts are currently continuing to this end.

Figure 1.2 The phylogeny of the Insecta including the known extinct wingless groups, denoted by (†). Projected evolutionary steps towards the Pterygota are indicated. Based on Kukalová-Peck, 1987 and information from Bechly and Stockar, 2011.

1.2. Wings

It is currently accepted that wings are homologous and monophyletic (Forbes, 1943; Wigglesworth, 1963; Kukalova-Peck, 1978, 1983; Wootton 1986). There are two theories as to the appearance of wings, the static tergal projection or paranotal lobe theory (Müller, 1875 in Ellington, 1991; Snodgrass, 1935; Forbes, 1943; Hinton, 1963; Rasnitsyn, 1981; Willmann, 1997; Hörnschemeyer, 1998, 2002; Wheeler et al. 2001; Grimaldi and Engel, 2005) and the articulated theory (Oken, 1811 in Ellington, 1991; Wigglesworth, 1973, 1976; Kukalová-Peck, 1983, 1987, 1998, 2008). These ideas of wing formation are currently the most accepted yet are not mutually exclusive.

1.2.1. The Paranotal Lobe or Rigid Wing Theory

The rigid paranotal lobe theory, which was first suggested by Müller (1875) (as stated in Ellington, 1991), has foundations in the fact that many insect fossils from the Carboniferous era, as well as modern *Zygentoma* (silverfish), show small plate-like outgrowths that project laterally from the tergum of the prothorax (Šulc, 1927; Snodgrass, 1935; Manton, 1977). This observation lead to speculation that an earlier period may have produced insects with similar lobes on the mesothorax and metathorax with a body plan differentiated into head, thorax and abdomen (Snodgrass, 1935; Ellington, 1991). These cuticular projections from the terga of the pro, meso and metathorax are known as paranotal lobes and are described as primitively non-articulated. This theory suggests a possible use initially as protective coverings while crawling through leaf litter or for thermoregulation, but eventually being used for gliding while falling from tall vegetation

(Hinton, 1963; Flower, 1964; Grimaldi and Engel, 2005). This theory suggests that early insects capable of twisting their lobes evolved complex articulation and musculature and, with subsequent reduction of abdominal lobes, thoracic wings developed over time from static plates ultimately becoming flapping aerofoils that functioned in powered flight.

The hypothesis of gliding with paranotal lobe-like appendages as a possible precursor to flight has been a subject of past studies (Hinton, 1963; Flower, 1964; Kingsolver and Koehl, 1985, 1994; Wootton and Ellington, 1991; Hassenfuss, 2002). The resulting progression of flight evolution within this wing origin theory begins with gliding as an initial state and flapping flight as the end point.

The articulated winglet theory (See section 1.2.2) based on accessory appendages of leg podites, called exites Figure 1.3, places gliding *via* wings as a secondary adaptation in insects, as the necessary morphological adaptations required for an insect to glide appeared later in the evolutionary timeline. It may be the case that gliding animals only ever become better gliders, not powered flyers, as in the case of vertebrates such as the flying squirrel, arboreal lizards and snakes and even frogs (Emerson et al. 1990; Emerson and Koehl, 1990; Socha 2002, 2006, 2011; Bishop and Brim-Deforest, 2008). Modern insects have evolved mechanisms to stiffen and hold wings outstretched, such as fluting, thickening of veins and wing cuticle and cross veins, while modern Lepidoptera, hook their wings together to rigidify them and form a single wing that can be effective for lower wing loading, energy conservation and the occasional glide (Brodsky, 1994; Wootton, 1979, 1981, 1990, 1992, 1995, 1996; Wootton and Kukalová-Peck, 2000). Others have conformed their wings to allow gliding as well as flight, such as the Palaeoptera (i.e. Odonata: Anisoptera) which have fused their original wing articulation

Figure 1.3. The homology of the leg segments (1-11) from the head, thorax and abdomen of the Insecta. 1, epicoxa (ECX); 2, subcoxa; 3, coxa (CX); 4, trochanter (TR); 5, pre-femur; 6, femur; 7, patella; 8, tibia; 9, basitarsus; 10, tarsus; 11, posttarsus (primitively with 2 claws). Note the position of the exites and endites as well as the pleuron (Thorax – 1) surrounding the base of the wing exite below the Tergum (TE). From Kukalová-Peck, 1987.

components to the tergum by the eighth developmental instar. These components are unfused in the first instar (Bocharova-Messner, 1959; Kukalová-Peck: Personal communication). This fusion, along with other specializations not in the Neoptera, presumably evolved to conserve energy since extending and holding wings outstretched requires energy expenditure through the use of contracting muscles pulling on the wing base (Kukalová-Peck, 1983, 1985, 1991, 1998; Riek and Kukalová-Peck, 1984; Haas and Kukalová-Peck, 2001). Fossil evidence from Palaeozoic nymphs show articulated wings and winglets along the thorax and abdomen, utilizing musculature from leg podites, the ontogeny of which can be seen in the Odonata and in the Coleoptera supporting the articulated origin theory of wings (Kukalová-Peck, 1978, 1983; 1998; personal communication).

Studies of locusts, crickets, dragonflies and mayflies have demonstrated the existence of homologous flight interneurons in all three thoracic segments and the first three segments of the abdomen (Robertson et al., 1982; Dumont and Robertson, 1986; Roberston and Olberg, 1988). If these lateral tergal outgrowths became articulated following evolution from an immobile state, these interneurons involved in wing movements would not have formed in the abdominal segments of any modern, more derived, insect since no extant or even fossil flying insects support 'true' wings from the abdomen. Thus, the presence of abdominal interneurons in extant flying orders suggests an evolution from a common ancestor supporting mobile winglets or pre-winglet structures serially repeating and homologous within the thorax and adomen (Roberston and Olberg, 1988; Kukalová-Peck, 1983).

1.2.2. *The Articulated Origins Theory*

Wing articulation likely evolved from the first segment of the euarthropodan leg (the epicoxa) which, through evolutionary processes, became flattened and incorporated into the body wall forming a pleuron (above the subcoxa) (Figure 1.3) (Kukalová-Peck, 1983, 1987). The development of the pleuron and the modification of the musculature that accompanies it and its exite were essential to the development of the flapping insect wing, this concept is discussed in great detail by Kukalová-Peck (2008 and previous + co-authors) and is briefly described in the following section. Further to these publications, both developmental and classical genetics fields also provide evidence in support of the articulated appendage (or ramus) theory. Developmental work with *Drosophila* has discovered that initial embryonic formation of the wing (the wing disc) separates from the initial formation of the leg (the leg disc) rather than a tergal origin suggested by the paranotal theory (García-Beillido et al., 1979; Kukalová-Peck, 1983). Complementing this, tissue transplant experiments support this finding as well. Transplanting the wing disk to different areas of the tergum in Lepidoptera results in a wing, tegula, axillary sclerites and most of the wing processes developing at the transplant site, no tergal component is required (Stenzhorn, 1974 in Kukalová-Peck, 1983). More recently, Averof and Cohen (1997) using Crustacean fairy shrimp (*Artemia* sp.) as a study organism, have reported homology between insect wings and *Artemia* sp. gills, which formed from the same epicoxal leg exites. They found that wings, gills and legs originate in a common primordium in early insect embryos and share common regulatory pathways. Continuing from this work, Niwa et al. (2010) also found that the wings have evolved from limb structures and that the styli found on the abdominal segments of Archaeognatha, the most

basal order of Insecta (Figure 1.1), are of the same origin with the limb-wing base fused within the tergum. This concept of wing origins from leg components is important when considering the serially repeating limb-wing bud structures that are present from the thorax through the abdomen in the early Insecta and observed as remnants in Carboniferous era fossils.

Some of the most convincing evidence against the paranotal theory can be traced back to the very phylogenies produced by the post-ground plan way of evolutionary thinking. Limiting data to compiled characters only from extant insects and similarities found among them leads to unresolved phylogenies, endless corrections and varying results that have been published from the same data sets resulting in confusion and awkward suggestions to resolve them. Examples of unresolved phylogenies can be found in Kristensen (1991, 1995, 1997) Buetel and Pohl (2006) and Grimaldi and Engel (2005). In contrast to this, the Hennigian ground-plan approach regularly produces the well known Pterygota = (Palaeoptera + Neoptera) sister to the flightless Zygentoma (Kukalová-Peck, 2008, 2009; Hennig, 1969, 1981), continually supporting the monophyly of insect wings, wing articulation and folding as a primitive state and provides a solid basis to reconstruct the insect evolutionary pathway. Although still in their infancy, genetic studies are beginning to tease out the true phylogentic relationships (see Thomas et al. (2013)).

Crustaceans, evolving from the euarthropodan ancestor, possess limbs that are functionally biramus; they have two appendageal protrusions from the same point on the body wall, one becoming the leg or leg like appendage and the other an upper gill/paddle/wing like structure. Extant insect limbs are secondarily uniramous (derived

from the biramous condition), supporting only a leg from one specific point on the body wall (Kukalová-Peck, 1991, 1992, 2008).

All limb appendages, including wings, are serially homologous in the head, thorax and abdomen as they evolved from a single shared limb model (Sharov, 1966; Norling, 1982; Kukalová-Peck, 1983, 1985, 1991, 1998, 2008; Brodsky, 1994; Kingsolver and Koehl, 1994; Shubin et al., 1997). This homology comes from the hypothesized fusion of a leg segment, the epicoxa, to the body wall in the arthropodan ancestor. The epicoxa in the early ancestor was the basal segment to the body and supported an exite at its interconnecting membrane to the subcoxa, which was also incorporated into the body wall (Kukalová-Peck, 1983, 1985, 1991, 1998, 2008). Exites are protrusions that originally projected outward from between leg segments (podites); they are articulated by the musculature of the leg segments they sit between. Over time, the epicoxa became incorporated and fused to the body to form the horseshoe-shaped pleuron. The exite ‘migrated’ up through the epicoxa/pleuron, giving the appearance of the pleuron surrounding the exite, and eventually became a tracheated, flattened structure protruding from the middle of the pleuron (Figure 1.3). This exite would have retained its musculature from its corresponding leg segments (epicoxa and subcoxa) and maintained its movable characteristics as the pleuron formed sutures to accommodate articulation and muscle selection for structures such as the gills in aquatic insect nymphs and the protowings in terrestrial insect forms (Kukalová-Peck, 1983, 1998). This entire evolutionary process likely began in the Cambrian period (approximately 500 mya), and resulted in a flattened articulated structure in the Ordovician period (approximately 460 mya) at the latest.

The exite origin of wings is supported by the work of Dr. Jarmila Kukalová-Peck and co-authors through numerous publications. However, despite the abundance of detail and description, not all authors agree with the conclusions of these works. Other authors examining the same fossils, or representations of, report inability to locate the morphological structures described in Kukalová-Peck's publications (see Willmann (1999), Rasnitsyn and Novokshonov (1997), Bitsch and Nel (1999), Béthoux and Briggs (2008)). As well, the homologies proposed by Kukalová-Peck and her supporters have also been brought into question in other publications (see Jockusch and Nagy (1997), Deuve (2001), Boxshall (2004), Jockusch and Ober, (2004) and Béthoux et al. (2008)). At the very least, the literature exists in great abundance and can be difficult to wade through and isolate for any specific concern. Responses to criticisms, corrections, updates to information and re-evaluations should always be taken into account, such as those from Kukalová-Peck (2008), Kukalová-Peck and Beutel (2009), and Bechly and Stockar (2011). Interpretation and revaluation continues as new fossils are discovered and new analytical techniques are developed which invariably leads to new ideas and potential evolutionary pathways the insects may have followed. Whatever position one adopts, there will always be intense scrutiny and alternate scenarios, a fact simply due to the nature of the field of study.

Despite scrutiny, the most likely origin for complex articulated wings is from a moveable exite which used pre-existing musculature and was anchored within a, stable but modifiable structure (the pleuron). The continued articulation of the exite after the fusion of the euarthropodan epicoxa forming a pleuron was very important as it suggests that wings began as articulated rami (exites) of the epicoxa, then became flattened and

useful in some way (possibly as rowing oars or gill-like structures) and then became the protowings. Many crustaceans use swimming appendages, derived from coxal exites, and even epicoxal exite-appendages as in the case of fairy shrimp (Cl. Branchiopoda) (Averof and Cohen, 1997). The movement of the oar/gill structure and the retained musculature and innervation would have been an essential pre-adaption for the exite functioning later as a flapping aerofoil.

Considering the potential for an exite derived structure to have formed a gill it is of value to briefly explore this concept. A gill is a structure employed for gas exchange and can exist in many forms and from many morphological precursors. A stationary surface with a static layer of water, which becomes exhausted of oxygen very quickly, can be disrupted by even a small movement of the gill; the movement breaks the static layer of water (i.e. boundary layer) allowing fresh oxygen laden water to reach the surface (Streeter and Wylie, 1985; Vogel, 2003). Further, if wings evolved from gill-like structures then the venation pattern seen in modern pterygote insects should be homologous with the tracheation pattern in the gills of the protopterygote insect. The homology of the wing venation throughout all insect wings has been studied and reconstructed (Kukalová-Peck, 1983; Haas and Kukalová-Peck, 2001; Kukalová-Peck and Lawrence, 2004). When compared to the reduced remnants of protowings, known as side lobes, in the sister group to the pterygota, the Zygentoma, a similar internal tracheae branching pattern is observed to modern insect wings (Šulc, 1927). The potential for insect wings derived from epicoxal exite-originating gills is a possibility but requires more study to establish whether winglets functioned as gills first before being employed

as aerodynamic structures, but does not conflict with a plesiomorphic articulated origin of wings.

1.3. The Pterygota Precursors

The pterygotes began to split into all their main superordinal lineages when protowings still retained their initial, almost symmetrical, shape (Kukalová-Peck, 1983, 1987; Kukalová-Peck and Lawrence, 2004). Protowings from juvenile insects have been documented as having a ‘ground plan’ of eight veinal pairs within their structure. When this basic structure in the almost symmetrical protowing is compared to the more modern insect lineages, wings can be fully homologised (Kukalová-Peck and Richardson, 1983; Kukalová-Peck, 1983, 1985, 1991, 1992, 1998; Riek and Kukalová-Peck, 1984; Haas and Kukalová-Peck, 2001; Kukalová-Peck and Lawrence, 2004; Kukalová-Peck, et al., 2009). At the time of writing, there is currently a 55 million year span between the earliest hexapod fossil, a bristletail from 390 mya, and the first winged insects (Dudley, 2000) making direct observation of transitional forms from fossils a significant challenge. However, in order to better understand the evolution of the pterygotes we must look at the apterygotes. Examination of the “more primitive” wingless orders, Archaeognatha (bristletails, also known as the Microcoryphia), Zygentoma (silverfish and firebrats), †Monura and †Cercopoda can provide important information about the morphology of the pterygote ancestor.

When the Archaeognatha and Zygentoma are examined, numerous features are evident that may have been integral to the evolution of flight. Thoracic segments with

pleural apodemes (sutures on the lateral thorax) and muscles that appear to be homologous to the musculature in the pterygotes, as well as a unifying arthropod limb model, all reinforce the idea of not only a single insect ancestor but a single arthropod ancestor as well (Kukalová-Peck, 1983, 1992, 1998; Waagele 1993; Barlet, 1950, 1988; in Dudley, 2000). Coxal, prefemoral and femoral exites can still be found on Archaeognathan prothoracic legs as well as a solid (not fragmented around wing musculature) pleuron on the prothoracic segment, which is an important feature to the ramus theory of wing evolution (Kukalová-Peck, 1983, 1987, 1991, 1992, 1998).

The thoracic morphology of the Archaeognatha suggests that the selection for proto-wings was unlikely from an early evolutionary stage. They do not exhibit fragmented pleurons, required to support articulated winglets, as well as having completely lost any remnant of an exite structure within the body wall (Kukalová-Peck, 1983, 1998). This potentially led to the adoption of a cryptic lifestyle in the archaeognathan ancestor; choosing to hide and live in small confining spaces or detritus matter where wings would be a hindrance when moving through such an environment. The archaeognathans do retain the so called ‘rope muscles’ (see Sec. 1.6 below) within their abdomen which is a feature of their escape jumping ability (Shear and Kukalová-Peck, 1990; Manton, 1972, 1977).

The Zygentoma (silverfish) are thought to have evolved away from wing evolution at the proto-wing stage, likely for similar reasons to the Archaeognatha, but diverged from the pterygote ancestor after the archaeognathans. Silverfish do support side lobes with tracheation similar to the venation pattern of pterygota wings (Šulc, 1927). This has been suggested to represent a possible fusion between the epicoxal segment and

the protowing development (Kukalová-Peck, 1983, 1998) evolving into a unique arrangement of leg articulation that allows the silverfish to move at rapid speeds for escape purposes when disturbed (Manton, 1972, 1977). The Zygentoma have more complex mouth parts than the archaeognathans which are also believed to be derived from the leg components and appendages of an ancestor group (Manton, 1977; Kukalová-Peck, 1987). The archaeognathan mandible is very narrow and possesses only one articulation point (defining the group Monocondylia) while the Zygentoma mandible has a double articulation point (defining the group Dicondylia) and is broader (Kukalová-Peck, 1992), which is also found in all pterygote insects. This morphological layout places the Zygentoma in the phylogenetic tree as the sister taxon to the pterygotes (Figure 1.2).

Although little is known about the †Monura and †Cercopoda, the fossil evidence does appear to show a similar body form to the archaeognathans, however the †Cercopoda appear to share more evolutionary traits with Zygentoma and Pterygota overall (Figure 1.4). In spite of this however, their fossilized body positions suggest they used the same physiological escape mechanism as archaeognathans likely employing dorso-longitudinal rope muscles (Figure 1.5) (Shear and Kukalová-Peck, 1990; Manton, 1977). Numerous features shared by the †Cercopoda and Archaeognatha may be important to early pre-flight behaviour, one of which may be the large annulated telson (Figure 1.4), a trait that may prove to be important for flight and aerodynamic stability, previously considered but only briefly tested in previous studies (Flower, 1964; Kingsolver and Koehl, 1985; Wootton and Ellington, 1991).

Figure 1.4. A reconstruction of a †Cercopoda from Kukalová-Peck, 1987. Note the thoracic side lobes (circled area) which are reduced protowings fused with epicoxae in this figure, as well as the large annulated telson. †Cercopoda progressed from an early pterygote ancestor towards a terrestrial lifestyle and employed rope muscles for escape jumping, much like extant Archaeognatha.

Figure 1.5. Longitudinal section of a jumping Bristletail, *Petrobius brevistylis*, and its abdominal musculature. Note the twisted dorsal longitudinal ‘rope like’ muscles 1-5, and 8-11 used for the rapid abdominal jumping behaviour. From Sturm and Machida, (2001).

1.4. The Ancient Environment

The earth during the Silurian, Devonian and Carboniferous periods was very different from that which we know today. It was during these periods that the Insecta began to evolve wings and diversify into the flying forms we are most familiar with in the fossil record and in the living fauna. The Silurian period sustained relatively basal and minimally diversified terrestrial plant life. The beginning of the transition to vascular plants was occurring during this time, stick like plants such as *Cooksonia* sp. and *Salopella* sp. as well as rhyniophytes appeared in the latest stage of the Silurian (Behrensmeyer et al., 1992). These stick-like plants occurred in isolated patches in aquatic to semi-aquatic environments extending just above the water surface and were always very low in stature (Kukalová-Peck, 1987, 2008, 2009; Wilson, 2005). This low height would have been ideal for insect ancestors to quickly climb to avoid predation from other arthropods living around the live and decaying vegetative outcrops and may even have been a food source (Mamayev, 1971). As vascular plants invaded the land, these newly formed perches for the evolving terrestrial invertebrates were expected to have had many trophic relationships (the plants are described to have terminal sporangia, a potential food source) with the arthropods of the time, perhaps becoming very important for their survival some 412 million years ago (Behrensmeyer et al., 1992; Shcherbakov, 1999; Dudley, 2000;).

The Devonian period (following the Silurian, Figure 1.1) shows a continuing complexity and size of terrestrial vegetation (Figure 1.6). By the upper Devonian more familiar growths of primitive ferns, lycopods and horsetails were present. The upper Devonian also saw the emergence of gymnosperm (seed bearing with open cones),

Figure 1.6 – An artists conception of the terrestrial landscape of the Early Devonian period. From Špinar and Burian, (1972).

pteridosperm (extinct seed fern) and cycadophyte (evergreen) stem groups (Behrensmeyer et al., 1992; Hennig, 1981; Kukalová-Peck, 1987). Most recently the potential for the emergence of taller growing plant life was found in a fossil forest discovery (Stein et al., 2012), existing in the mid to late Devonian period. However, it is now believed that pterygotes originated prior to the emergence of tall trees complicating theories of the origin of flight involving gliding from high vegetation (e.g. Yanoviak et al., 2009). The early pterygota emerged and began to diversify when vegetation was still low to the ground, under a meter tall, which may not have been a sufficient height to encourage dropping or gliding as a mode of transportation towards new resources; dropping from this height is also unlikely to injure a small insect (Vogel, 2003; Ellington, 1991; personal observations). However this low, branching, vegetation is still important to a (potentially) jumping protopterygote that may have employed a crude ‘parachuting’ behaviour to descend to safety or cling to passing branches.

1.5. Current Theories Leading to Flight

The ability to take to the air has been proposed *via* three currently recognized potential pathways: the surface skimming theory (Marden and Kramer, 1994; Marden, 1995, 2003), the gliding theory (Grimaldi and Engel, 2005) and the flapping protowing theory in combination with climbing and jumping (Kukalová-Peck, 1983, 1987, 1998, 2008). Taking into account the information known about the physiology of insects and vegetation of the time, we can now examine these hypothetical pathways in more detail

In paragliding, winglets are thought to assist the protopterygote in controlled gliding from tall vegetation such as trees. This ‘trees down’ theory has been applied to vertebrate flight evolution (Bock, 1986; in Marden et al., 2000) as well as invertebrates. Under the articulated wing origin theory, gliding is secondary in insects and does not require wing articulation as previously discussed in response to the paranotal lobe theory of evolution (Kukalová-Peck, 1983, 1998, 2008; personal communication). This in combination with experimental studies with models that only glide when supplied with specific wings of an appropriately large size (Wootton and Ellington, 1991; Hasenfuss, 2002) and the potential for wingless insects to glide from tall heights using their body planform (Yanoviak, 2007; Yanoviak et al., 2005, 2009), is not conducive to a logical evolutionary progression. It is worthy of note that the concept of a gliding origin to flight in birds is also under scrutiny, as a flapping origin to flight is considered a viable possibility (Dial, 2003).

Surface sailing and surface skimming, has also been observed in modern Plecoptera and has recently been suggested by Marden as a pathway to true flight (Marden and Kramer, 1994; Marden, 1995, 2003). In surface skimming, an adult protopterygote would use its articulated, raised winglets as flapping “propellers”, beginning from the shoreline, and pushing itself along the surface of a pond or other water body using the surface tension to support the weight of the insect, thus allowing the aerodynamic propulsion to push it along. It is thought that when certain wing dimensions were reached in terms of area, provided they could break away from the surface tension of the water, the surface skimming protopterygotes would begin gaining altitude and staying aloft for longer periods of time utilizing the flapping motion (Marden and

Kramer, 1994; Marden, 1995, 2003). Plecopterans are relatively primitive insects however they have secondarily reduced wings, which is not congruent with this behavior being a precursor to flight evolution. Although Dr. James Marden continues to support surface skimming as a valid precursor behaviour to insect flight, it may also be explained as an offshoot behaviour from the flapping protopterygote (or even a true flying insect) in which powered flight was a pre-adaptation for this escape or rapid locomotion behaviour rather than result in flight. The surface skimming theory falls under the articulated theory of wing evolution and appears to be the only current theory that cannot be completely or entirely discounted with the evidence at hand. It remains at least a possible component of the pre-flight puzzle and must be considered.

1.6. Escape Plans, the Mechanisms for Escape

With invasion of the terrestrial environment came new opportunities for survival of the arthropods. Predation on other invertebrates was one method of surviving and as a result tactics to avoid predation must have evolved. The most obvious form of escape is simply running away. This is still common today, for example, modern silverfish will quickly scurry across the floor or wall when disturbed. A speedy getaway generally ensures survival provided there is somewhere to run and/or a means to hide. The evolution of long bristles sensitive to mechanical (surface vibration) disturbances and giant interneurons for rapidly sending sensory signals to the CNS and to muscles is important for predator detection and a running get away. Annulated cercal filaments for

detecting vibrations and air movement replaced the cercal legs of many of the Entognatha presumably for this reason (Kukalová-Peck, 1987, 1998).

Another ancient escape mechanism is to jump away from predators. This behaviour is common in the jumping bristletails (Archaeognatha: Machilidae) of today. The ability to fling into the air is achieved by internal dorsal-longitudinally arranged “rope muscles” (Figure 1.5), which are twisted along their length within the thorax and abdomen and act in conjunction with flexible tendons and elastic endoskeleton (Bartlet, 1948; Manton 1972, 1977). The contraction of these muscles is believed to be more powerful than those of a straight musculature and in combination with other internal muscles, translates into a fast flex of the abdomen and curl of the body (Sturm and Machida, 2001). These jumps could be over 10 cm high depending on species (Evans, 1975; Sturm and Machida, 2001), and would be very effective in rapidly evading the grasp of a predator (Shear and Kukalová-Peck, 1990). Fossil evidence suggests that the extinct monurans and cercopodans utilized the same escape mechanisms *via* rope muscles; this is due to the characteristic curled position of the fossil insects and their similarity to the body position of archaeognathans in death (Shear and Kukalová-Peck, 1990). Further, talitrid amphipods (beachhoppers) employ what appears to be a similar jump to that of the jumping bristletails (Kukalová-Peck: personal communication). Certain nymphs and naiads of species of Ephemeroptera and Megaloptera have also been suggested to support remnants of the rope muscles (E.L. Smith in Kukalová-Peck, 1987; E.L. Smith in Sturm and Machida, 2001) however no published data exists at this time. This musculature appears to have been carried over from aquatic ancestors and is relevant to the pancrustacean origin of the insects as the malacostraca (SP.O. Syncarida) support a

similar muscle type and are believed to be closely related to the Insecta (Figure 1.7) (Evans, 1975; Bartlet, 1978a; Shcherbarkov, 1999) and even Collembola (E.L. Smith in Sturm and Machida, 2001).

Figure 1.7. A comparison of a drawing of *Anaspides tasmania* (C. Crustacea, SP.O Syncarida) (Top – Taken with permission From The Freshwater and Marine Image Bank) and a photograph of *Trigoniphthalmus alternatus* (O. Archaeognatha) (Bottom). Aside from the strikingly similar appearance the Archaeognatha and syncarids share similar abdominal muscle morphology.

1.7. Climbing, Jumping, Flapping, Flying?

Perhaps the most unifying, parsimonious, theory of the origin of flight is published by Jarmila Kukalová-Peck (1987, 1998, and 2008) and falls under the leaping and flapping mode of evolution mentioned in section 1.5. Kukalová-Peck maintains that wings formed from the flattened gill/paddle-like structures of a semi-aquatic to terrestrial protopterygote that initially originated from articulated epicoxal exites. These articulated protowings would be able to create a power stroke in air much like they would in water, or at least be useful in orienting the protopterygote in both of these mediums. This protowing would be present on a protopterygote possessing rope muscles and an annulated telson (caudal/tail filament), among other key morphological features (such as double clawed limbs for climbing). These structures would therefore be exaptations for flight in the environment of the protopterygote because they would assist with becoming airborne and in stabilization. This hypothetical protopterygote may have been climbing the low-lying vegetation and even rock faces during its daily, or possibly nightly, routine. Encountering a predator during its foraging and climbing may have initiated an escape response such as jumping. This would have flung the creature into the air from its perch, clear of obstructions, where it could employ its articulated protowings to flap and possibly direct its descent through parachuting-like behaviours, thus beginning the transition from apterygote to pterygote insect.

1.8. The Goals of this Thesis

The intention of this doctoral work is to continue to uncover the progression of how a flightless insect lineage began to take to the air to eventually become the diverse, multi-lineaged group of aerial organisms we know today (the Pterygota). To accomplish this, the data and concepts explored in the following chapters will draw on multiple fields of study, including insect behaviour and paleontology as well as modern techniques in biomechanics, such as three dimensional digital modeling, rapid prototyping and particle image velocimetry (PIV), not previously attempted for aerodynamic evolution study in insects.

In this thesis I will present in a progressive fashion, data that will lend support towards a possible evolutionary avenue into flapping insect flight. In Chapter 2 I will test the hypothesis that a terrestrial, basal, wingless lineage of extant insects (the Archaeognatha) is capable of some form of aerial control while falling from heights reflective of pre-flying insect lineages surviving in the semi-aquatic to terrestrial environment of the Silurian to Early Devonian period. In Chapter 3 I will test the concept and hypothesis that the same basal extant insect lineage is capable of demonstrating an ability to cope with an environmental challenge (water) that was likely also present to protopterygote insects despite being adapted for a more terrestrial lifestyle.

In Chapter 4 I will review and describe the likely protopterygote insect morphology and potential accompanying behaviour from previously published work, data from previous chapters and new descriptions as well as discuss the implications of such an insect lineage in regards to habitat and possible progression towards flight. In Chapter

5 I will begin to explore the biomechanical aspects of the previously described propterygote insect in Chapter 4 when exhibiting similar behaviours to the Archaeognatha, brought to physicality through modern three dimensional computer design techniques and rapid prototyping (3D printing). Chapter 6 will continue to explore the biomechanics of the representative model as I propose the hypothesis that an array of flapping winglets will affect the drag and flow of air around a falling propterygote and could have been the initial step towards thoracic winglelet selection in insects. I will wrap up the overarching concepts I have presented in Chapter 7 and discuss the future of this field of biology as well as suggest avenues for continued exploration that would further tease out the concept of flight evolution in insects through flapping winglets.

Chapter 2: Aerial descent behaviour of two species of Archaeognatha in the context of insect flight evolution

2.1. Introduction

The evolution of the flying Insecta from their non-flying ancestral form is a topic of spirited debate and significant importance in the success of the insects overall.

Although fossils provide a glimpse at past morphology and even hints of past behaviour, the absence of a living specimen creates challenges necessary for understanding the morphology, physiology and behaviour surrounding how insects may have begun their transition to volant forms.

Since no living protopterygote insect exists, the best alternative is to turn to is the most appropriate extant basal orders that are available and to carefully examine and consider likely behaviours that may have existed in the pre-flight insect lineages (Shigeno, et al., 2007; Coates, 1994; Goudemand, 2011; Wroe et al., 2005; Van Valkenburgh, 1987). Jumping bristletails (O. Archaeognatha) appear in the fossil record some 390 MYA, and likely diverged when insects were restricted to the semi-aquatic environment of the Silurian-Devonian period (Labandeira et al., 1988; Shear and Kukalová-Peck, 1990). They have very simple single jointed mouth parts (monocondylous), relatively large complex eyes, long antennae, a telson (tail filament), and, possess remnants of legs, usually described as styli, along their abdominal segments. These features, among others in the order, help distinguish and place the order Archaeognatha nearest to the Insecta class division on the phylogenetic tree of arthropods (See Chapter 1, Figure 1.2).

Flight is a broad term that includes a variety of aerial behaviours that allow organisms to travel through the air. More specifically, passive flight such as gliding, soaring and parachuting, are activities that require only minimal, or even no, muscle activity and typically have very low energy input requirements once the animal is in the air (Alexander, 2002; Dudley et al., 2007). In vertebrates, these types of flight are usually accomplished with the presence of wings or wing-like structures or even the body itself. Many invertebrates such as spiders, ants and aerial plankton also use forms of passive flight for dispersal while concurrently conserving energy, sometimes even utilizing light breezes for transport. The most direct example of this is demonstrated *via* falling from trees or plants and using body or limb movements and body planform to guide and control a landing (Yanoviak et al., 2005, 2009; Ribak et al., 2013). Further exemplifying this concept, many animals that regularly ascend *via* climbing into tree canopies have evolved some form of passive flight. Records of flying frogs, lizards, snakes, opossum, squirrels, monkeys, dinosaurs and many forms of human operated gliding hardware provide clear and regular examples (see Dudley et al., (2007), for review).

Within the Archaeognatha, canopy dwelling species (F. Meinertellidae, *Meinertellus* sp., *Neomachilellus* sp., *Janetschekilis* sp.) were shown to be able to direct their descent to a safe landing on nearby tree trunks after falling from the lower branches of c. 15-20 m high tree crowns in the jungles of Peru, Panama and Gabon (Yanoviak et al., 2009). The authors of this study present this as the first evidence for complex aerial behaviours in a non-flying basal insect order. Although the results of Yanoviak et al., (2009) are intriguing and deserve attention when considering the evolution of the Insecta overall, they may not be as indicative of the evolution of insect flight as the authors

indicate. I do, however, agree with Yanoviak et al., (2009) and Dudley and Yanoviak (2011) that members of the Archaeognatha can contribute to our understanding of how flight in insects evolved, provided members supporting more plesiomorphic morphology (Figure 2.1, F. Machilidae) (Sturm and Machida, 2001) and existing in habitats reflective of early insects (non-arboreal) are employed (Kukalová-Peck, 1987, 1998, 2008).

Just as important as the specific method of moving through the air, the posture an animal may adopt while doing so is of particular importance. Whether increasing drag during descent or creating lift from the body surface itself, presenting specific morphological features in particular positions to an oncoming flow of air carries potential benefits and costs. In directed aerial descent studies from tree canopies, *C. atratus* ants regularly fall with the abdomen leading, ventrum down, and limbs splayed out and held above the lateral midpoint of the body (Yanoviak et al., 2005). Wingless pea aphids and first instar stick insects also demonstrate a similar behaviour, positioning long legs out and near dorsum level to initiate rotation and self righting while falling from vegetation (Ribak et al., 2013). In small invertebrates, rotation and orientation from inertia, as in vertebrates (Jusufi et al., 2010, 2011), is generally not available for exploitation so aerodynamic forces become the major hurdle to orienting and landing.

Canopy dwelling Archaeognatha are reported to fall head first and limbs splayed out (Yanoviak et al., 2009). Lepismatids dorsally curve their abdomen to initiate rotation to a right side up orientation and then straighten out with limbs extended as they descended, landing ventrum down (Hasenfuss, 2002). Attaining an appropriate attitude during a fall is essential before the various forms of flight may be initiated; consequently the continued exploration of such posturing and orienting is a point of interest for this

Figure 2.1. A basic phylogeny of the Archaeognatha including the 2 species used for behavioural studies (see Chapter 2 and 3). Based on information from Sturm and Machida (2001) and Bechly and Stockar (2011). Archaeognatha phylogenies may change as new species are described or current ones are reassessed. Numbers indicate species for the given genus.

thesis and will be important for further insect flight evolution studies (Yanoviak et al., 2009; Dudley and Yanoviak, 2011).

Conceding that it is not possible to fully account for behaviours derived over time, even within basal lineages (i.e. there is no such thing as a true living fossil); I have chosen to focus on the Archaeognatha: Machilidae. This group is considered to be similar, morphologically, to the expected pre-flight insect lineages while also surviving in environmental conditions that fit within the boundaries defined by the Silurian and Early Devonian eras. The Zygentoma (silverfish), despite being primitively wingless, are a more derived order in the class Insecta (Kukalová-Peck, 1983, 1998, 2008; Hennig, 1969, 1981) and therefore are less appropriate for investigation of pre-flight behavioural evolution studies. The Zygentoma appear to possess specialized leg articulation morphology unique in the insect lineages, likely a result of selection towards and specialization for life in a confined, cluttered terrestrial environment (Matsuda, 1970; Manton, 1972, 1977; Kukalová-Peck, 1983, 1998). The Archaeognatha retain much of the plesiomorphic morphology of crustacean insect ancestors and, along with their unique jumping ability, are very near to the ancient protopterygote insect described by Kukalová-Peck (1983, 1998, Personal Communication) and Wootton (1986), qualifying this group as the best extant insect example known for investigation of pre-flight behaviours in insects.

In this chapter I document and quantify the aerial behaviour of two species of a wingless (apterygote) extant basal order of the Insecta, the Archaeognatha: Machilidae. I will show that these behaviours function in decreasing the descent speed of the insect when falling from 1 m and I will discuss these behaviours in the context of the potential

precursor to the evolution of powered flight in insects. Given that Archaeognatha (F. Machilidae) are a flightless, basal insect group, it is likely similar behaviours existed earlier non-flying lineages and supports the hypothesis that increasing drag by postural changes is an important precursor in regard to insect flight. Furthermore, my findings may assist in defining the beginning of a pathway toward flapping insect flight that does not conflict with the numerous environmental, morphological and behavioural possibilities of likely early pterygote ancestors.

2.2. Methods

2.2.1. Insect Collection and Husbandry

Jumping bristletails, *Pedetontus saltator* and *Trigoniophthalmus alternatus* (Silvestri 1904), were identified *via* keys and descriptions provided by Wygodzinsky and Schmidt (1980). *P. saltator* specimens were collected along the rock wall shoreline of the Rideau River on the Carleton University Campus in Ottawa, Ontario, Canada (Latitude = 45.38293, Longitude = -75.69428) during the summer and fall months (June – November) of 2007, 2008 and 2009. Specimens were located under loose rocks and collected by manoeuvring them into 2.5 cm diameter plastic vials and kept alive in a colony in the lab consisting of a screen covered glass aquarium (61 cm long x 31 cm wide x 29 cm deep) with rocks collected from their original habitat. A Petri dish of pebbles was made available in the tank and kept filled with water to prevent desiccation of the collected live specimens. Rocks were sprinkled with tap water every few days and carrot slices were provided in the colony along with leaf litter and organic matter as a food source. Any other arthropods discovered, such as spiders and isopods, were removed from the colony as well as periodic cleaning and removal of mould generated from carrot slices. Some specimens were kept over many weeks and remained in good health as evidenced by periodic observation of behaviour and exuvia from moulting.

T. alternatus individuals were collected from Borer's Falls conservation area, Hamilton, Ontario, Canada (Latitude = 43.294942, Longitude = -79.931847) on two separate occasions during the summer of 2009. The collected *T. alternatus* specimens were kept in a small aquarium and cared for as described for *P. saltator*.

All individuals used in experiments were weighed using a Mettler AE163 Dual range digital balance, examined for physical damage as well as photographed and measured (± 0.1 mm) using a Zeiss SteREO Discovery V12 Stereomicroscope equipped with a Canon Power Shot A620 digital camera linked to Axiovision V. 4.6 software (Carl-Zeiss, Oberkochen, Germany). The microscope and imaging software were calibrated using a stage micrometer with increments of 0.5 mm; measurements are accurate to ± 10 μ m. Only specimens that were intact (no missing appendages etc.) and actively moving in a normal manner were used in experiments; living specimens found to exhibit physical damage (missing leg, antennae, telson etc.) were returned to the colony to allow continued survival and moulting which could result in rejuvenation and potential for use in experimentation in later trials.

2.2.2. Vertical Drop Tests

Individuals from both species were placed on a square acrylic peg (1x1x1 cm) mounted one meter from the base of a constructed three wall arena with a white painted background (Figure 2.2 A). Specimens were left on the peg until they walked or slipped off of the peg thus descending through the air until landing on the floor of the arena. The beginning of the fall and the landing event was filmed with paired high speed digital cameras (dual synchronized Motionscope 1000 S, HSI Inc.) with a 6mm Pentax TV lens for a wide camera view. Images were captured at a rate of 500 frames per second with a $1/500^{\text{th}}$ of a second shutter speed. Illumination was provided by infrared lighting ($\lambda = 850$ nm) as well as ambient room light (approximately <100 Lux).

Figure 2.2. Diagram of the vertical drop arena showing synchronized high-speed cameras imaging the initial drop and landing areas **(A)** and the vertical wind tunnel used to measure terminal velocity of falling bristletails **(B)**.

After live testing, individual bristletail specimens were caught and subsequently dispatched in kill jars with ethyl acetate. The dead bristletails were then placed on the square peg in the same arena and orientation into such a position as to mimic the method of departure when alive (e.g. ventral side down, head positioned to the appropriate edge of the peg). The peg was gently tapped from behind the mounting point until the specimen slipped off the peg head first as when live. The resulting initial descent and landing was filmed in the same way with high speed cinematography.

Recorded high speed video files were examined for body position, landing orientation, velocity and duration of fall using SkillSpector kinematics analysis software (Version 1.2.4.0, <http://video4coach.com/>), Midas Player (Version 5.0.0.3, <http://www.xcitex.com/>) and Redlake Motionscope® Media Player software (Version 2.21, HIS Inc., Mississauga, Ontario, Canada). Final descent velocity was calculated based on distance travelled between video frames. The duration of the fall for live and dead specimens was considered to be from the moment the thorax of the specimen passed the bottom face of the square peg to the moment where the specimen ceased all downward travel (landing). Any captured time during which the specimens bounced after the initial contact with the base of the enclosure, was not considered as part of the fall.

2.2.3. Vertical Wind Tunnel Trials

To analyse the drag, posture and orientation of falling *P. saltator* and *T. alternatus* specimens, a vertical wind tunnel was constructed from a round (2.5 cm diameter) rigid

clear plastic tube, with a height of 55.5 cm (Figure 2.2 B). Terminal velocity (V_T) is defined as the maximum velocity a given object can reach while falling under the influence of the Earth's gravity. Terminal velocity of live and dead specimens was determined when the upward air flow stopped the vertical descent of the specimen in the tunnel such that the specimen maintained a consistent position within 20 cm of the approximate center of the working area for more than two seconds without deviating more than approximately 2 cm. The upward flow of air was generated using a regulated compressed air cylinder. Wind speed in the tunnel was measured using a hotwire anemometer (VWR Scientific, Model 21800-024) with the probe positioned at the top (outflow) of the tunnel. Straws and vinyl screen were placed in the bottom of the tunnel to help straighten the flow of air.

A total of 20 specimens of various body lengths (3.61-11.57 mm) were tested from each species of jumping bristletail.

2.2.4. Low Speed Horizontal Wind Tunnel Tests

A low speed horizontal wind tunnel was used to test the falling behaviour of live and dead *P. saltator* specimens in a light wind. Air flow laminarity through the working section was confirmed *via* photographs of white smoke produced from a volatilized glycerol solution (See Figure 2.3 inset photograph). The wind tunnel working section is constructed from clear acrylic measuring 30.5 cm tall by 30.5 cm wide by 60 cm long. Specimens were dropped from the top of the working section and thus had a maximum drop distance of 30.5 cm (Figure 2.3). Wind speed was set at 0.8 (± 0.1) m/s. This speed

Figure 2.3. Diagram of the horizontal wind tunnel with a photograph (expanded region) of the working section. White smoke in the photograph illustrates the laminarity of air flow in the working section. Specimens are introduced to the wind tunnel during testing *via* a 4 cm hole in the centre of the top of the working section. A single high-speed camera was used to capture images of the specimens during descent. Blue arrows indicate the direction of air flow through the wind tunnel.

was the median of recorded wind speeds measured from all collecting sites for *P. saltator*. This measured wind speed is also consistent with previously published measurements of readings of expected continuous wind speeds recorded amongst vegetation on a calm day (Force 1 on the Beaufort scale of winds) (Flower, 1964).

Specimens (n=15) were tested with and without wind both alive and dead. For both wind and windless conditions, living specimens were gently placed in a narrow channel constructed of acrylic walls (2 cm tall) open at one end and closed at the other. The open end of the channel was located at the edge of an access hole (4 cm diameter) on the top of the working section of the wind tunnel that allowed the specimens to walk down the channel to the hole and drop into the working section. The channel was oriented so that the specimens would walk to the hole in the same direction as the air was flowing, enabling the specimens to drop into the working section head first and ventrum towards the airflow. Dead specimens were oriented in the same way at the edge of the access hole for both wind and windless conditions and gently nudged forward until they slipped over the edge and fell into the working section.

Descents were recorded with a single high speed camera (Motionscope 1000 S high speed camera, HSI Inc.) with a 6mm Pentax TV lens for a wide camera view. A single, wide, camera view was able to capture the entire fall. The recording rate was set at 500 frames per second with a $1/500^{\text{th}}$ of a second shutter speed. The camera was oriented perpendicular to the flow of air and decent path of the bristletails. White paper marked with horizontal and vertical 1 cm increments was used as a background while an infrared light ($\lambda = 850 \text{ nm}$) was used to increase the contrast of the images of limb and body movements of the bristletails against the background. The recorded digital video was

analyzed for total falling time, and horizontal displacement upon landing using the same methods as described above.

2.2.5. Statistical Analysis

Given that bristle tails are ametabolous (exhibit continuous growth with indeterminate numbers of moults), antennal length was expected to increase with body size. It is also not possible to know if an antenna experienced breakage prior to our testing (i.e. the antenna could have broken in its natural habitat prior to collecting, or, after collecting when in our colony). To be conservative, the longest of the left and right antennae was chosen as a measure of antennal length and was normalized by dividing by body length to control for age. Telson length was also normalized by dividing by body length for the same reason.

Morphological measurements between species were analyzed using a two tailed t-test for means assuming unequal variances. Velocities and duration of fall during vertical drop tests as well as terminal velocities attained in vertical wind tunnel tests were analyzed using a paired two tailed t-test for means. Landing orientation was analyzed using a binomial distribution test for means. All of the above tests were performed using the Data Analysis Tools Add-in Package in Microsoft Excel (2007).

For low speed horizontal wind tunnel testing, body weight and length measures were significantly positively correlated and thus were reduced to a single composite measure of body size using Principal Component Analysis (PCA) in JMP (v.10; SAS

Institute Inc., Cary, NC, USA). The PC1 axes explained 94.0% (Eigenvalue = 1.88) of the observed variation in weight and length, with both size measures loading heavily on the PC1 axes (Eigenvectors = 0.71). To determine whether each of the three measured variables are influenced by the presence or absence of wind or the state of being alive or dead, I used general linear mixed models (GLMMs) in JMP (v.8.0; SAS Institute Inc., Cary, NC, USA). The three models included fall time, horizontal displacement or stabilization time as the dependent variable, individual insect identity as a random effect, size PC1 as a covariate, and live/dead (L/D), wind/ no wind (W/NW), and the interaction term L/D*W/NW as fixed effects. The interaction term was non-significant in all three models and so was removed from the final analyses.

2.3. Results

Morphological and behavioural differences between both species were observed in the test specimens. For vertical drop trials (VD), despite not being significantly different in body length, *P. saltator* specimens exhibited significantly longer antennae than *T. alternatus* while for vertical wind tunnel trials (VT) *T. alternatus* supported a longer telson, shorter antennae and weighed less than *P. saltator* tested specimens (Table 2.1). *T. alternatus* appeared to be the more energetic of the two, jumping more readily when disturbed and exploring their surroundings more actively when placed in a Petri dish. In general, both species appeared very similar to each other, with similar shapes and scale colouring (Figure 2.4).

Table 2.1. Measured morphological values and statistical comparisons (t-test) results of specimens used in drop (VD) and vertical wind tunnel (VT) tests.

	<i>P. saltator</i>			<i>T. alternatus</i>			<i>t</i>	<i>P</i>
	$\bar{x}$	SEM	<i>n</i>	$\bar{x}$	SEM	<i>n</i>		
(VD) Normalized Antenna	1.13	0.048	17	0.86	0.022	20	5.1011	<0.001
(VD) Normalized Telson	1.09	0.052	18	1.15	0.099	20	-1.1915	>0.05
(VD) Body Length (mm)	7.25	0.669	20	7.74	0.620	20	-0.5368	>0.05
(VD) Weight (g)	0.0158	0.003	20	0.0163	0.003	20	-0.1195	>0.05
(VT) Normalized Antenna	1.09	0.052	19	0.89	0.021	40	3.5250	0.0017
(VT) Normalized Telson	1.00	0.045	20	1.13	0.037	19	-2.2372	0.0316
(VT) Body Length (mm)	8.94	0.471	20	7.87	0.390	20	1.7551	>0.05
(VT) Weight (g)	0.0232	0.003	20	0.0138	0.002	20	2.5911	0.0145

Figure 2.4. Photographs of *P. saltator* (Top) and *T. alternatus* (Bottom) jumping bristletails on rocks found within their collecting areas. *P. saltator* exhibits flagellated antennae and shoe-sole shaped ocelli, where *T. alternatus* exhibits scale covered antennae with triangular shaped ocelli as well as dark eye colour.

2.3.1. Vertical Drop Tests

Specimens of both species consistently explored (from approximately 5 seconds to 2 minutes) the acrylic peg until slipping off on their own without any external assistance. Specimens included for data analysis were those that did not cling to, or collide with, the wall of the drop arena during descent which would presumably have interfered with the velocity and descent time. A ventral landing was defined as initial impact of the ventral side of the individual upon landing while a non-ventral landing was considered to be an initial impact of the dorsal side or impacting vertically on their head or tail. All (20/20) specimens landed ventral side down while alive and dorsal side down when dead (two tailed binomial distribution test, *P. saltator*, $P < 0.01$, $n=20$; *T. alternatus*, $P < 0.01$, $n=20$).

Both *P. saltator* and *T. alternatus* required more time to fall one meter while alive than when compared to being dead (Paired t-test, *P. saltator*: $t=7.432$, $n=20$, $p<0.001$; *T. alternatus*, $t=7.928$, $n=20$, $P<0.001$). Similarly, both *P. saltator* and *T. alternatus* had a significantly lower velocity immediately before impact when alive as compared to when dead (Paired t-test, *P. saltator*: $t=-9.248$, $n=20$, $P<0.001$; *T. alternatus*: $t=-11.33$, $n=20$, $P<0.001$). *P. saltator* individuals ($n = 20$) took an average time of 523.7 msec (± 13.93 SEM) and reached an average speed of 2.59 m/s (± 0.10 SEM) immediately before landing from a fall of one meter when alive (Figure 2.5). When dead, *P. saltator* specimens averaged 479.7 msec (± 9.23 SEM) to fall one meter and reached an average speed of 3.08 m/s (± 0.08 SEM) (Fig. 2.5). *T. alternatus* individuals spent an average time of 502.4 msec (± 9.40 SEM) falling one meter alive and 458.5 msec (± 4.45 SEM) when dead (Figure 2.5). On average, *T. alternatus* achieved a speed of 2.84 m/s

Figure 2.5. Fall durations (descent time) of *P. saltator* (circles) and *T. alternatus* (triangles) falling from a 1 meter height in the vertical drop arena. Live specimens take significantly more time to fall when compared to dead (Paired t-test, *P. saltator*: $t=7.432$, $n=20$, $p<0.001$; *T. alternatus*, $t=7.928$, $n=20$, $P<0.001$). The average duration of fall (inset plot of averages $\pm$ SEM) for live (closed symbols) and dead (open symbols). Note: due to collecting times for *P. saltator* it was not possible to test specimens between 6 mm and 9 mm in length. As a result, specimens of *T. alternatus* of comparable sizes to *P. saltator* sizes were selected for testing to yield comparable results.

($\pm$ 0.09 SEM) when alive and 3.36 m/s ($\pm$ 0.07 SEM) when dead (Figure 2.6).

Remarkably, both species demonstrated nearly the same average time difference between falling while alive vs. dead: 44 msec for *P. saltator* and 43.9 msec for *T. alternatus* (t-test, $t=0.012$, $n=20, 20$, $P=0.990$). Both species reached a faster velocity immediately before landing when dead than while alive: *P. saltator* specimens were falling 0.48 m/s faster when dead than when alive and *T. alternatus* falls 0.51 m/s faster when dead than when alive immediately prior to landing. These changes are not significantly different (t-test, $t=0.503$, $n=20, 20$, $P=0.618$).

Figure 2.6. Velocities of two species of jumping bristletail just before landing from a one meter drop. Dead specimens reach a significantly higher velocity when compared to live. The average velocity at the end of a 1 meter drop is shown as a bar graph. Live (closed symbols) vs. dead (open symbols) for both species +/- SEM. (Paired t-test, *P. saltator*: $t=-9.248$, $n=20$, $P<0.001$; *T. alternatus*: $t=-11.33$, $n=20$, $P<0.001$).

2.3.2. Vertical Wind Tunnel Trials

Both *P. saltator* and *T. alternatus* specimens consistently demonstrated the ability to maintain a ventral side down (or right side up) body posture while at terminal velocity (Figure 2.7 B). A dorsal side down body position was observed in all (20/20) specimens when dead while in free fall as dead individuals do not actively arch their abdomens upwards to alter their centre of gravity (Figure 2.7 C).

Individuals tested in vertical wind tunnel experiments demonstrated the same body posture and behaviours observed of the *P. saltator* and *T. alternatus* specimens when in free fall from one meter. Average terminal velocity was 6.43 m/s (± 0.22 SEM) for all *P. saltator* specimens when alive. The same *P. saltator* specimens showed a significantly greater average terminal velocity of 9.16 m/s (± 0.32 SEM) (Paired t-test, *P. saltator*: $t = -17.816$, $n = 20$, $p < 0.001$) when tested immediately after they were killed with ethyl acetate (Figure 2.8). *P. saltator* specimens varied in length from 3.61 mm to 11.57 mm and in weight from 0.0010 g to 0.0446 g.

The terminal velocity of *T. alternatus* specimens also differed significantly (Paired t-test, *T. alternatus*, $t = -20.674$, $n = 20$, $P < 0.001$) between live and dead trials. This species demonstrated an average terminal velocity of 6.24 m/s (± 0.21 SEM) while alive and 9.21 m/s (± 0.28 SEM) after they were deceased. A total of 20 *T. alternatus* specimens were tested ranging from a length of 5.81 mm to 10.07 mm. Individual weights also varied between 0.0050 g and 0.0250 g.

Figure 2.7. A single high-speed camera image of a *P. saltator* specimen in mid descent filmed at 500 fps (A). Photograph of a live *P. saltator* (B) and a dead *P. saltator* (C) in the vertical wind tunnel at terminal velocity. The characteristic arched body posture was observed in both live *P. saltator* and *T. alternatus* specimens and is adopted whether specimens jump or fall from a given height. See Sturm (1955) for a discussion of shared postural behaviours.

Figure 2.8. Two species of jumping bristletail, alive and dead, at terminal velocity in a vertical wind tunnel. Live specimens (closed symbols) exhibit a significantly slower terminal velocity than dead specimens (open symbols) +/- SEM. (Paired t-test, *P. saltator*: $t = -17.816$, $n=20$, $p<0.001$), (Paired t-test, *T. alternatus*, $t = -20.674$, $n=20$, $P<0.001$).

The live-dead terminal velocity differences between species are not significantly different (t-test, $n=20, 20$, $t= -1.143$, $p= 0.260$).

2.3.3. Low Speed Horizontal Wind Tunnel Tests

Only *P. saltator* was used in low speed wind tunnel tests due to the lack of *T. alternatus* specimens available at the time of testing.

Live specimens regularly approached the edge of the access hole to the wind tunnel working section and slipped into a fall to the bottom of the section (a total distance of 30.5 cm). Live individuals upon leaving their perch would immediately adopt the previously described dorso-ventral body curvature and maintain this posture until landing. Live individuals would roll (Figure 2.9), pitch and yaw until their ventral side was directed downward during the first half of the drop height. The characteristic splayed posture of the limbs and antenna was also regularly observed.

Dead individuals would slip into a fall in the same way as when live but would roll and pitch less often but never yaw. Most often a primarily abdomen and dorsal side down posture was observed that resulted in the specimen landing on the dorsal side of its abdomen and telson when contact was made with the wind tunnel working section floor. Dead specimens adopted a straighter body posture with minor curvature of the thorax in the ventral direction (see Figure 2.7 C).

Live specimens required significantly more time to descend through the working section distance than when dead for both the wind and no wind conditions (Table 2.2).

Figure 2.9. *A. P. saltator* individual rotating in mid fall (approximately 8 cm below the square peg) to a ventral side down position while splaying out its antennae and legs. Scale bar is 5 mm.

With a 0.8 m/s (± 1.0 m/s) wind, live specimens took 4.8 msec longer to reach the floor of the working section (average) than when dead (Figure 2.10). A similar trend was observed when specimens were tested without wind; live specimens taking an extra 5.07 msec (average) to reach the working section floor (Table 2.2). When compared between wind and nowind conditions, live specimens falling without a wind source required more time to descend when compared to live and no airflow, windless falls taking an average 4.04 msec longer vs. wind conditions. A similar trend was again observed for dead specimen tests, resulting in an average 3.47 msec longer descent time for dead specimens in a windless drop vs. flowing air (Figure 2.10 A).

The time required for the specimens to stabilize during the fall, considered the point at which the specimen stopped rotating about its anterior-posterior axis and consistently exhibited a ventrum down position for living specimens or dorsum side down, falling abdomen first position for dead specimens. Live specimens in no wind conditions required more time to stabilize their rotation and posture vs. when dead; an average of 19.6 msec more time was required. Under wind conditions, living specimens required less time to stabilize, an average of 3.33 msec less, when compared to dead specimen tests (Figure 2.10 B). However, these times are not significantly different (Table 2.3).

Total horizontal displacement, displacement down the wind tunnel in the direction of air flow, was also measured. Live and dead specimens both travelled farther in the direction of the airflow on average under wind conditions with live specimens traveling 15.84 mm farther in wind tests and dead specimens traveling 12.68 mm farther (Figure

2.10 C). The wind source significantly altered the horizontal displacement for all tested specimens, live or dead (Table 2.4).

Figure 2.10. Average ($\pm$ SEM) time to descend 30.5 cm within the low speed wind tunnel (A) time to reach a stable posture during the fall (B) and horizontal displacement (C) from starting position of *P. saltator* specimens tested in a light wind (0.8 ± 1.0 m/s) and without. Living specimens took more time to descend for both wind and no wind (Table 2.2), stabilization times were not significantly different (Table 2.3) and wind significantly altered the displacement of all tested specimens, live or dead (Table 2.4).

Table 2.2. Statistical information for *P. saltator* descent time for low speed wind tunnel tests.

	DF	DFDen	F Ratio	Prob > F
Size PC1	1	13	1.2779	0.2787
Live/Dead	1	43	12.0149	0.0012
Wind/No Wind	1	43	6.3980	0.0152
Adjusted R Square = 0.61316				

Table 2.3. Statistical information for *P. saltator* stabilization time for low speed wind tunnel tests.

	DF	DFDen	F Ratio	Prob > F
Size PC1	1	13	0.0002	0.9882
Live/Dead	1	43	0.8782	0.3539
Wind/No Wind	1	43	0.0463	0.8307
Adjusted R Square = -0.18933				

Table 2.4. Statistical information for *P. saltator* horizontal displacement for low speed wind tunnel tests.

	DF	DFDen	F Ratio	Prob > F
Size PC1	1	13	0.0058	0.9402
Live/Dead	1	43	0.8766	0.3544
Wind/No Wind	1	43	67.2868	<0.0001
Adjusted R Square = 0.52663				

2.4. Discussion

2.4.1. Aerial Behaviours of *Archaeognatha*

The body posture and position of the limbs and extremities (Figures 2.7 and 2.9) was the most significant observation in regard to the aerial performance of both *P. saltator* and *T. anternatus*. The posture is presumably a consequence of the rope-like longitudinal muscles found in the thorax and abdomen; musculature unique to this group of extant ground dwelling *Archaeognatha* (Manton, 1972, 1977; Bartlet, 1948, 1978a; Sturm and Machida, 2001). Hasenfuss (2002) noted Silverfish briefly exhibit a similar dorsally oriented abdominal position at the beginning of their fall but quickly straighten out possibly because the rope muscles are reduced or lost in silverfish as well as their sister group, the *Pterygota* (Manton, 1972; Kukalová-Peck, 2008).

The functional significance of the dorsal flexion during a fall is twofold. First, this posture shifts the centre of gravity, located just behind the thorax of the bristletail (Evans, 1975), apex of the overall curvature. This shift in centre of gravity facilitates a stable, ventrum-down orientation during descent. Keeping the center of mass low is a common strategy for maintaining stability during parachuting and in flight. Deploying a parachute or constructing an aircraft with the wings above the body, as in Cessna planes, results in a natural pendulum stability while airborne as most of the mass of the apparatus is below the light weight aerofoil. Dorsal flexion and the resulting ventrum-down posture effectively present the thorax and first abdominal segment to the oncoming flow of air during descent. This is the largest part of the insect that, along with extended thoracic legs, abdominal styli and bristles, contributes to increasing drag, thus slowing the

individual down. The result of the increased drag is well exemplified through the decrease in terminal velocity. Terminal velocity was reduced by approximately 2.85 m/s (a 31% decrease) and velocity after one meter of descent decreased 0.5 m/s (an 18% decrease) (Figure 2.8 and 2.6). In contrast, a cork ball of comparable weight to a large bristle tail (0.03 g) showed a terminal velocity almost twice as much as the live adult specimens using this method.

The extended antennae and telson also play a role in drag production as increasing the overall surface area generally leads to increasing drag. The differences in antennae and telson size as well as individual weight (Table 2.1) may therefore explain the observed differences in landing velocity, duration of fall and terminal velocity between species. The similar trends when comparing living to dead specimens for both species render the morphological differences a function of the tested group of specimens and not entirely indicative of a true difference at the species level. This concept is supported based on the fact that the differences between live and dead falling times, landing velocity and terminal velocity are not significantly different between species (See results section 2.3.1 and 2.3.2). Further morphological measurement work on these species increasing sample size may yield different results than those found here.

The dorsal curvature of the body and splaying out of the thoracic limbs and antennae of the tested specimens also resulted in the individual presenting tactile apparatus in multiple planes during a fall (Figure 2.7 A and B; 2.9). Thus, if the individual were to contact a structure during its fall, it would increase the chance of using that structure for landing or altering its path of descent (Ribak et al., 2013; Gravish et al., 2013). To this effect, on occasion, specimens were observed landing on a vertical painted wood surface

from a fall or jump from up to one meter high, sometimes initially grasping and absorbing the force of landing with a single thoracic leg. This is important to an insect is escaping from a predator by jumping or intentionally falling from a branch or leaf in that it increases the chance of escape and survival by reducing the likelihood of injury during a fall.

Low lying vegetation not immediately surrounded by large structures or trees, is exposed to environmental elements such as steady wind speeds. Experiments on *P. saltator* in a steady wind reflective of average wind speeds collected during field collections (0.8-0.9 m/s) have resulted in the continuing trend of live specimens being able to significantly increase their overall time to descend (Figure 2.9 A, Table 2.2) despite falling a much shorter distance (30.5 cm) than previous drop work from one meter. This trend is observed whether wind is present or not however when a wind source is present both living and dead specimens take significantly less time to descend vs. no wind conditions (Figure 2.8 A, Table 2.2). The decreased duration of the fall may be due to the possibility that moving air simply provides less resistance and lower atmospheric pressure as the specimen passes through. Much like drafting in competitive auto racing and bicycle riding, the moving air is less viscous than still air, which becomes particularly important to small animals like insects. The *P. saltator* experiments presented in this chapter demonstrated that a low velocity wind (similar to a light breeze) can alter the horizontal displacement of an individual over a 30.5 cm drop (Figure 2.9 C) however there was no significant difference between live or dead individuals during drop trials (Table 2.4). Falls from higher than 30.5 cm with a wind source were not investigated but should be tested in future experiments. Such experimental work may help to establish if

living specimens can benefit from the wind if provided a longer drop distance, however one meter would likely be the highest height that should be considered as vegetation likely did not exceed this height in the time of the early pre-flight insects and potentially non-arboreal archaeognathan environmental heights.

The camera resolution and experimental setup during low speed horizontal wind tunnel trials allowed for a closer examination of the entire fall over the 30.5 cm working section distance (Figure 2.3). The time required for *P. saltator* to stabilize their body position was not significantly different between living and dead specimen trials (Figure 2.9 B, Table 2.3). This was true for both wind and windless conditions, however the specific posture living and dead specimens assumed when stabilized was different. Living specimens adopted the previously described ventrum down, dorsally curvature position (Figure 2.7 A and B) while dead specimens again fell dorsum down with the abdomen leading the fall to landing. This is consistent with the tendency of the Archaeognatha body in death to passively roll over and orient abdomen down when falling. The ability of living specimens to achieve the ventrum down and abdominal curvature both in wind and in no wind must involve an active modification of abdomen position to counteract the dorsum down natural tendency. This is similar to Ribak et al.'s (2013) study on pea aphids using leg positioning to ensure a ventral landing when slipping off vegetation.

Active manipulation of body components during flight and even an arched back position during a fall (or in microgravity) in vertebrates has been documented previously (Pellis et al., 1989; Wassersug, et al., 2005; Jusufi et al., 2010). A jumping bristletail utilizing its abdominal position while airborne to right itself and help maintain a stable descent is not unlike the ruddering ability of locusts while initiating a steering response to

a stimulus (Camhi, 1970). Both species tested here are capable of controlling their posture through the use of their inherent physiology and morphology. The inability of a dead Archaeognatha to evoke the dorso-ventral curvature during descent indicates that this behaviour is an actively initiated and maintained and suggests that they may be able to control their landing to some degree, despite not being true aerial insects.

The results presented here may help to better understand the success of the Archaeognatha overall, as existing within and around rocky environments (sometimes as tall piles or boulder walls) would invite the potential falls, or recovery for a misdirected escape jump from a predator resulting in a fall greater than the jump height. Individuals demonstrating the ability to orient right side down and land ventrally are likely to soften their landing through drag forces as well as increase the possibility of contacting a solid surface by way of an extended limb on the way down. Thus, they may be less likely to be injured and more likely make a fast escape upon landing (Ellington, 1991).

2.4.2. Evolutionary Aspects

I have shown that two species of Archaeognatha are capable of aerial righting behaviour while falling from heights reflective of Silurian and early Devonian vegetation. The self righting ability along with the characteristic dorso-ventral curved posture (Figure 2.7 A and B, 2.9) contributes to the slowing of the descent of the individual which may be significant when considering early pre-flight insect behaviour. Given that the early protopterygote insect had potentially a similar morphology and lifestyle to the Archaeognatha, the potential for such an animal to make use of the extra aerial hang time

during a fall is an important possibility to consider in regards to flight evolution in insects.

It is of particular importance to note that the ability to stabilize body posture while falling occurs without the use of wings or winglets (flapping or fixed), a concept previously suggested as a potential pathway for selection of wings from winglets (Hinton, 1963; Flower, 1964; Kingsolver and Koehl, 1985; and Shcherbakov, 1999). Given the monophyletic evolution of the Insecta and the morphology of the Archaeognatha, the abdominal behaviour to maintain attitude control may be an important reflection of the ancient protopterygote's behaviour.

Yanoviak et al., (2009) present their findings that canopy dwelling species of Archaeognatha can glide to safety when dropped from heights of twenty meters, this was first discovered in *Cephalotes* sp. ants living in the same canopies (Yanoviak et al., 2005). A height of twenty meters is more consistent with later Devonian vegetation well after the Archaeognatha evolved and the Pterygota diversified into divisions (Behrensmeyer et al., 1992; Dilcher et al., 2004; Kukalová-Peck, 2008; Stein et al., 2012). Yanoviak et al., (2009) themselves also note that understory jumping bristletails, and even the more derived silverfish (*Zygentoma*), do not glide, suggesting that gliding in canopy species of Archaeognatha and gliding *Cephalotes* ants are an excellent example of behavioural convergence within the Insecta of arboreal canopies and not a reflection of a precursor to flapping flight.

The ability to glide appears in many animal groups capable of aerial powered flapping flight (birds, bats, dinosauria and insects). In the Insecta, wing stiffing and

joining mechanisms like those in the Lepidoptera, as well as secondary fusions to keep their wings in a flight ready position, such as the Odonoptera and Ephemeroptera, are also employed to effectively glide. However, gliding from an energetics point of view is a very efficient process (Pennycuik, 1986; Alexander, 2002; Koehl et al., 2011). This efficiency is most obvious in animals like albatrosses and flying squirrels, both of which have specific morphological adaptations for performing such an act. Perhaps, if gliding was selected for before the appearance of the energetically demanding (and now highly refined) flapping flight it seems flapping should not have occurred at all and would have resulted in a selection and modification of morphology to further increase gliding and soaring abilities in various lineages. Effectively, gliding is “cheap” and seen in arthropods, amphibians, reptiles, mammals (including humans) and every flapping flyer within. Instead, all insects share flapping powered flight, with gliding as an energy saving option. Kukalová-Peck (2008, 2009) provides an overview of these concepts in relation to insect flight evolution as well as the previous work that addresses gliding as a precursor to flapping. Results from Wootton and Ellington (1991) and Ellington (1991) further support this concept *via* their observation of glide angles increasing readily along with control of a glide using the most posterior set of winglets on a protopterygote model. The further evolution of which could imply retention and refinement of abdominal winglets as gliding became more prevalent only to be lost as flapping flight spontaneously appeared.

Breezes can also alter the overall displacement of small animals as previous ‘floating’ theories on insect flight evolution have described (Wigglesworth, 1963, 1976; Norberg, 1972). Although floating theories apply to insects much smaller than archaeognathans the potential for displacement *via* a wind gust still remains. If light

winds cause increased descent times in general, the use of flapping winglets may become even more important in staying aloft or possibly using the wind as a source of aerodynamic boost *via* flapping winglets harnessing the airflow. It is probably unlikely that the early semi-aquatic vegetation of the Silurian period was regularly surrounded by still air; wind was likely a challenge to small arthropods climbing up stems for food or escape. If this is true, the possibility still remains for living protopterygotes to utilize the wind *via* flapping winglets, extending a fall's horizontal displacement where wingless lineages cannot.

The early Paleozoic environment was abundant with predatory arthropods likely feeding on insects (Shear and Kukalová-Peck, 1990; Rolfe, 1980). Fast escape or hiding in dense low lying (<1 m) vegetation were the two main modes of survival. Veinal adaptations to flight which identify modern pterygote higher taxa (divisions, lineages, superorders) all took place very early during the initial flight adaptation. All recorded fossil Pterygota (starting just before the end of the early Carboniferous) already belong to modern divisions, subdivisions and lineages (Kukalová-Peck, 2008, 2009; Grimaldi and Engel, 2005). As a result, the Silurian and early Devonian periods become of great importance for flight and phylogenetic understanding of modern insects as the emergence of flying insects likely occurred well before the tall trees and recognizable jungle-like canopies of the late Devonian period (Behrensmeyer et al., 1992; Dilcher et al., 2004; Stein et al., 2012).

The observed rotational righting and parachuting behaviours are likely to have existed in the semi-aquatic †Cercopoda-like protopterygote Insecta (Kukalová-Peck, 1998, 2008). When coupled with flapping winglets, the resulting behaviour could have

been the initial step towards surface skimming away from predators as they inadvertently fell on the water surface (Marden and Kramer, 1994; Marden, 1995, 2003) and flapping flight in insects. Or, in the case of the Archaeognatha, the early loss of limb-exite based structures due to an adoption of a more cryptic lifestyle within low lying vegetation and tight crevices while still relying on jumping for escape. This active attitude control with dorsal-ventral curvature and righting behaviour which I dub ‘active parachuting’, may have been the first step towards vertical and horizontal movement *via* lift and thrust from flapping limb-exite based winglets (Kukalová-Peck, 1978, 1983, 1991, 2008; Averof and Cohen, 1997).

My results describe a potential exaptation that is conducive towards flapping flight evolution. The ability of the Archaeognatha to consistently achieve a stable ventral side down body position while falling from a one meter height to significantly increase their falling time and decrease their falling velocity, provides an excellent setting to further increasing hang time *via* thrust from flapping winglets and even produce significant horizontal displacement that may not otherwise be possible. This would inherently present more time to prepare for an escape jump upon landing near predators or potentially increase distance from, and avoid landing on, predators in similar pre-flight insect ancestors. This characteristic behaviour seems to be a result of the individual’s ability to actively maintain attitude as shown by the comparison to the same individuals when deceased (Figure 2.7 C) and may be important when considering the overall survival and success of the jumping bristletails as a whole.

Chapter 3: Jumping in two species of Archaeognatha as a means of traversing and escaping water

3.1. Introduction

In modern insects, jumping to initiate flight is not an uncommon occurrence. Previous studies on stick insects, hemipteran shore insects, fruit flies and orthopterans have demonstrated keen abilities to jump using various leg pairs, and combinations thereof, to effectively launch the individual high enough to clear obstacles and to a sufficient velocity for the initiation of flapping (Burrows and Morris, 2002; Burrows, 2007, 2009; Card, 2012; Pond, 1972; Habib, 2013). In addition to locomotion for its own sake, jumping has served a well documented role in escape from predators (e.g. lemurs, lizards, hexapods) (Crompton and Sellars, 2007; Losos and Irschick, 1996; Manton, 1972) and in flight initiation (e.g. birds, gliding squirrels, snakes, insects) (Earls, 2000; Essner Jr., 2002; Socha, 2006; Burrows and Morris, 2002; Burrows, 2007). From a flight evolution perspective, jumping has been suggested as a viable mode of becoming airborne in early flightless insects as well (Norberg, 1972; Wootton and Ellington, 1991; Kukalová-Peck, 1987, 1998). Jumping bristletails (O. Archaeognatha, F. Machilidae) are the most basal (or least derived) order within the extant insect lineages and consequently, they possess morphology that may reflect the early, semi-aquatic to terrestrial, protopterygote insect. Part of this morphology includes a unique abdominal musculature that allows them to perform rapid, abrupt jumps from their perch into the air for predator evasion (Sturm, 1955; Manton, 1972, 1977; Evans, 1975; Bartlet, 1978b). Examining extant lineages for behavioural clues towards those of extinct ones (Janis, 1997; Van Valkenburgh, 1987; Wroe et al., 2005) may yield valuable information for determining if

jumping may have been a valid precursor to flapping flight. Jumping in the Archaeognatha, previously studied by Sturm (1955), Evans (1975) and Manton (1972, 1977), has been suggested to be directionally random and has been reported as a tactic to evade predators with a succession of rapid, consecutive vertical jumps when the insect is disturbed. The jump is powered by a unique set of twisted dorso-longitudinal “rope” muscles and tendons (See Chapter 1, Figure 1.5) that are derived from the early crustacean-like ancestor of the insects (Matsuda, 1957; Manton, 1972, 1977; Bartlet, 1978a, b). A variation of this musculature and subsequent abdominal flexion is still found in modern malacostracans (Matsuda, 1957; Evans, 1975; Manton, 1972; Bartlet, 1978) and is believed to have existed in the †Cercopoda and †Monura (Kukalová-Peck, 1987, 1998, 2008). Similarly, talitrid amphipods have been reported jumping from coastal waters and beaches in a similar fashion to Archaeognatha (Tshernyshev, 1997), evidence of jumping perhaps being useful at the air-water interface.

The jump response can be evoked by lightly contacting the insect with a small, fine bristle paint brush or even a puff of air directed on, or in the vicinity of, the insect (Evans, 1975). To initiate a jump, the bristletail will raise its abdomen into the vertical plane, placing the major point of flexion at the thoraco-abdominal segment, in preparation to jump. This appears to be the ‘winding up’ phase of the abdominal muscles. The energy stored in this cocked position is released over 2-8 msec and is directed in either a somersaulting vertical jump or a more forward jump which usually does not employ the somersault motion (Sturm, 1955; Evans, 1975). The jump behaviour is a very effective method of evading capture, as routinely observed by the author when trying to collect these insects for study.

The legs of the jumping bristletail do not appear to contribute a great deal to the jumping force but may function more in orienting the body into a stable position prior to jump initiation (Evans, 1975; personal observations). In this regard, the legs and tarsi are used to grip the substrate and even glass walls of their culture aquaria in some species (Sturm and Machida, 2001). This ability seems to be due to specialized curved setae in the distal ends of the tarsi called scopulae which may also help maintain the body position while the abdomen is flexed rapidly downward during a jump, although investigation is needed. As the force of abdominal impact with the substrate is transferred to the body of the insect, the legs release and the insect is thrown into the air. A jump most often results in the insect landing ventral side down but closer examination reveals their ability to rapidly self right if landing happens in any other orientation (Evans, 1975; personal observation). Considering these insects in their entirety, the rapid jump, self righting behaviour after a missed right side up landing, and ability to cling to various surface types work together to allow jumping bristletails to take advantage of their rocky and coarse textured environments to successfully survive and avoid predation.

Jumping to escape and locomote (Manton, 1972) is an important evolutionary aspect to consider in pre-flight insects because of the potential for increased risk of injury or predation upon landing in undesired locations. Past work on jumping in the Archaeognatha focused on the movement, and the distance and height of various jumps (Sturm, 1955; Evans, 1975) but investigation of the possibility of a directed jump for traversing an obstacle like a pool of water is lacking. Thus, the first objective of this chapter is to determine if jumping bristletails can execute directed jumps and to characterize the kinematics of the jump.

As the early protopterygote insect existed in a time of abundant swampy, semi-aquatic habitats (Mamayev, 1971; Kukalová-Peck, 1987, 1998; Wootton, 1990) it is plausible that interaction with water after landing from a jump or fall was a common scenario. Terrestrial insects landing on, and maintaining an interface with, the water surface creates a risk from predation as well as undesired displacement *via* wind gusts and water currents (Kramer and Marden, 1997; Bush and Hu, 2006). This is of particular importance to genera that survive around water laden areas like shore lines, marshes and even forest floors during the rainy season. Accompanying the benefits of manoeuvring while on the water surface to escape danger comes the possibility that early pre-flight insect ancestors with similar archaeognathan and/or †cercopodan morphology (Kukalová-Peck, 1998) potentially exhibited similar behaviour. This potential behaviour could link to or encourage the previously described aerial observations (Chapter 2), that may have coupled with the active flapping of multiple pairs of protowings, all in an effort to avoid the water surface or possibly escape its confines upon initial contact. Archaeognatha have been reported to be able to slide along and even jump from the water surface (Shcherbakov, 1999; Kukalová-Peck, 1987) but to my knowledge no quantitative study has been published to date. Such ability is hypothesized to be valuable to an early flying insect ancestor supporting similar jumping abilities as well as early protowings. Likewise, the ability of the Archaeognatha to interact with and escape a potentially life threatening event involving landing in a water body may further exemplify their continued success and adaptation to their environment as a group despite being primitively wingless and supporting much of the basal morphology known in the †Monura and †Cercopoda.

Jumping remains valid as one of the ancient behaviours still to be considered as a mechanism to become airborne in early pre-flight insects and still requires investigation.

In the previous chapter I explored the behaviour of two species of Archaeognatha while in mid-air, which provided clues to how a flightless basal insect may react when falling a short distance. In this chapter I examine the behaviour of two species of Archaeognatha (*Pedetonus saltator* and *Trigoniophthalmus alternatus*) and show that the unique jumping ability may be useful when presented with a natural obstacle, such as water and an exposed location. I also investigate the behaviours of both species when landing upon the water from two different drop heights and show that individuals from both species are capable of landing on, and extracting themselves from, the surface tension of standing water. These results are important in regards to considering the success of the Archaeognatha lineage as a whole as well as the potential environmental challenges expected during the time of the protopterygote insect.

3.2. Methods

3.2.1. Insect Collection and Husbandry

Jumping bristletails, *Pedetontus saltator* and *Trigoniophthalmus alternatus* (Silvestri, 1904), were identified *via* key's and descriptions provided by (Wygodzinsky and Schmidt, 1980). *Pedetontus saltator* specimens were collected along the rock wall shoreline of the Rideau River on the Carleton University Campus in Ottawa (Latitude = 45.38293, Longitude = -75.69428), The Old Quarry Trail in Kanata (Latitude = 45.30378, Longitude = -75.873376) and at the Borer's Falls conservations area, Hamilton (Latitude = 43.294942, Longitude = -79.931847), Ontario, Canada, during the summer and fall months (June – November) of 2010 and 2011. *T. alternatus* specimens were collected during the summer of 2011 from Borer's Falls conservation area, Hamilton, Ontario, Canada. Individuals were collected with plastic vials and kept alive in a colony in the lab consisting of a screen covered glass aquarium (61 cm long x 31 cm wide x 29 cm deep) with rocks from their natural habitat.

A shallow Petri dish filled with pebbles was made available in the tank and kept filled with water to prevent desiccation of the collected living specimens within the colony. Rocks were misted with tap water every few days and carrot slices were placed in the colony as a food source along with leaf litter from the collection sites. Any other arthropods discovered such as Arachnids and Isopoda were removed from the colony. Lighting within the room was on a sixteen hour ON eight hour OFF cycle with full spectrum fluorescent lights. Some specimens were kept over many weeks and remained in

good health as evidenced by their behaviours and periodic observations of exuvia from moulting.

Individual *P. saltator* and *T. alternatus* specimens were weighed on a Mettler AE163 Dual range digital balance before being used for trials. All specimens were also photographed (Nikon D60 SLR with a AF-S DX Zoom-Nikkor 18-55mm f/3.5-5.6G ED lens) and were measured using morphometric software (Image J, Version 1.4.1, <http://imagej.nih.gov/ij/index.html>; a calibration scale was included in each photograph). Some specimens were measured using a Zeiss SteREO Discovery V12 Stereomicroscope equipped with a Canon 1000D digital SLR camera linked to Axiovision software (Version 4.6, Carl-Zeiss, Oberkochen, Germany). Specimens that appeared injured or behaving uncharacteristically (i.e. not walking properly, unable to self right if lying on their side, etc.) were not used in trials.

3.2.2. *Water Obstacle Trials*

A shallow plastic tray (35.4 cm by 45.5 cm) was filled with water, to a depth of approximately 1 cm. The tray was enclosed vertically with white foam-core poster board (25 cm high) to eliminate visual stimuli and to provide a contrasting background for image analysis of high-speed video (see below) and photographs. A small opening (14 cm x 10.5 cm) was cut into the poster board to allow high-speed filming of the jump events (Figure 3.1).

A small collection of stones (1.5-3 cm diameter each) obtained from the natural habitat of the bristletail was centered at one end of the tray, providing a safe refuge

(hereafter referred to as the landing rocks). During trials, an individual bristletail was placed on a single rock (approximately 3 cm diameter) rock (hereafter referred to as the launching rock) at different distances (2, 4 and 6 cm) from the landing rocks (Figure 3.1). For illuminated trials, a 20 Watt halogen spot light at a distance of 40 cm, illuminated the launching rock only to provide a non-tactile, presumably irritating, stimulus, for the bristletail. For non-illuminated trials, the halogen lamp was not used allowing only the ambient room light to provide illumination (<100 lux). In both illuminated and non-illuminated trials, an infrared light was used to illuminate the surface of the landing rocks from above for better visualization with the high-speed camera.

For *P. saltator* trials, twenty individuals were tested using the halogen light to illuminate the launching rock. Trials were repeated three times per distance per individual when possible. For non-illuminated trials, four individuals were tested. For *T. alternatus* trials, 10 individuals were tested for both the illuminated and non-illuminated trials. Each individual was tested 3 times per distance when possible.

Figure 3.1. Top view of the arena used for jumping trials. For illuminated trials only the area around the launching rock was lit. The launching rock (L) was placed at 2, 4 and 6 cm distances from the landing rocks (LD). The red triangle denotes the area in which an individual would land when jumping towards the landing rocks. High speed videos were captured through the cut out in the arena sidewall (CO) and calibrated for distance using the calibration square (C). Note: The white disk is the reflection of the halogen lamp off of the water and was not present during trials.

3.2.3. Videography and Data Analysis for Water Obstacle Trials

Each trial was filmed using a digital video camera (Canon Optura 500 at 30 fps or a Toshiba Camileo SX900 HD at 30 fps) to ensure the bristletails were jumping directly to the landing rock and for timing and jump distance measurements. The trials were concurrently filmed with a high speed camera (Motionscope® 1000 S, HSI Inc) at 500fps with a 1/500th of a second shutter to record and later analyze the kinematics and body postures during jumping of each specimen. As well, images from low-speed Canon and Toshiba cameras ensured that jumps were within the plane perpendicular to the HS camera field of view to reduce perspective distortion in subsequent analyses.

Normal speed (30 fps) video was analyzed for exploration time, water behaviour and jump trajectory for the water obstacle trials. High speed (500 fps) video from jumping trials for *P. saltator* was digitized using Skillspector (V. 1.3.2, <http://video4coach.com/>), for jump height and distance as well as general body position in transit. Videos were calibrated in the x and y plane using the in frame calibration square (17 mm x 17 mm) located in the plane specimens jumped within, perpendicular to the camera lens.

Observations from trials were separated into 3 categories that described the mode of leaving the launching rock: jump, swim/crawl into the water and remaining on the rock. Individuals that jumped from the launching rock were further categorized as jumping toward the landing rocks (+45° and -45° from the launch rock midline with respect to the landing rocks) or away from the landing rocks (Figure 3.1). Success after a jump was considered as jumping and landing on the landing rocks or jumping and extricating themselves from the water onto the landing rocks, tray edge or launching rock.

Trials were stopped if an individual reached the landing rocks or tray edge or after 10 minutes regardless of whether the individual was floating on the water surface after jumping or if it was unable to extract itself from below the water surface.

All categorical data was analyzed using Log-likelihood ratio tests (G-tests) using MS Excel (2007) following procedures described in Zar (1984).

3.2.4. *Water Drop Trials*

A water drop apparatus was constructed based on previous drop methods (Chapter 2) to allow *Archaeognatha* individuals to fall into water. Individuals were placed on a square acrylic peg at 50 cm and 100 cm height from a white tray (20.4 cm x 34.5 cm) filled with tap water to a depth of 1.5 cm (Figure 3.2). Two rock groups were placed 7.6 cm to the left and right side of the drop location (measured as the position vertically below the acrylic peg) (Figure 3.2). The distances from the surface of the water to the top of the peg were 48.5 cm and 98.5 cm. For 48.5 cm drop heights, 19 *P. saltator* and 20 *T. alternatus* individuals were tested. For 98.5 cm drop heights, 21 *P. saltator* and 21 *T. alternatus* individuals were tested.

Figure 3.2. Drop trials were performed in a semi enclosed structure with peg heights of 48.5 and 98.5 cm above the water surface. A photo (inset) of the landing tray with dry rocks used as a “safe zone” for bristletails caught in the surface tension of the water after a fall. Specimens would actively maneuver along the surface tension of the water until encountering a solid surface such as the tray edge or rocks provided and climb out of the water.

3.2.5. Videography and Data Analysis for Water Drop Trials

Two synchronized high-speed cameras (Motionscope® 1000 S, HSI Inc) with 6 mm Pentax TV lenses for a wide camera view recording at 500 fps and 1/500th of a second shutter speed were used to capture the initial exit from the peg and landing on the water's surface as well as any immediate behaviours after landing. A digital high-definition camera (Toshiba Camileo SX900) recording at 30 fps was set up to capture the long term activity of the specimen after landing on the water surface.

Trials were limited to ten minutes in which the water surface activity of the specimen was recorded before they were removed from the experiment. Trials were ended if individuals succeeded in climbing onto either the landing rocks or the tray edge.

Normal speed video for water drop trials was quantified for behaviour type while bound to the surface tension of the water within the tray. Activity while floating on the water surface was categorized as abdominal jumping (J), abdominal jumping with forward movement (JF), walking or flailing their legs (W), abdominal jumping with backwards movement (JB), lateral undulation (U), turning (T), flailing legs forward or rapid leg movements propelling the specimen forward (WF) and combinations of therein. For further detailed descriptions of the behaviour categories, see Table 3.1. Total time spent active and the behaviours exhibited were recorded for each specimen as well as total time spent motionless. High speed video was qualitatively analyzed using Skillspector for method of leaving the peg to fall and for landing posture. T-tests were performed using the Data Analysis Toolbox add-in feature in Microsoft Excel (2007).

Table 3.1. Acronyms and descriptions of the activity individual *Archaeognatha* were observed performing while in contact with the surface tension of the water of the drop experiments tray.

Activity	Description
J	Abdominal Jump: Specimen rapidly moves its abdomen dorso-ventrally resembling the terrestrial jumping movement.
JF	Abdominal Jump Forward: Specimen rapidly initiates a dorso-ventral abdominal movement that results in displacement in the direction the specimen initially faces.
JB	Abdominal Jump Backward: Specimen rapidly initiates a dorso-ventral abdominal movement that results in a displacement in the opposite direction it initially faces.
U	Lateral Undulation: The specimen performs a lateral undulation in the surface tension of the water.
T	Turn: The specimen performs a lateral turning maneuver.
W	Walking Movement: The specimen actively moves its legs resembling walking while in the surface tension of the water with no displacement.
WF	Walking Forward: The specimen initiated limb movements resembling walking in the surface tension of the water but results in a displacement when complete in the direction it initially faces.
Activity Combination	
JF/W	The specimen performs W behaviour and ends the activity by performing a J or JF.
JF/U	The specimens performs U and ends the activity by initiating a JF.
JF/U	The specimen alternates between WF and U while in the surface tension of the water.
J/U	The specimen performs a U and ends the activity with a J.

3.3. Results

3.3.1. Jumping Trial Exploration Time

The average exploration time on the launching rock for those that left the launching rock (n=18 all distances) gradually decreased as launch rock distance increased in the illuminated trials for *P. saltator* (Figure 3.3). Overall, *P. saltator* spent less time on the launch rock before departing (Grand average= 106.8 ± 13.91 s, $\pm$ SEM) than *T. alternatus* across all launch rock distances (Grand average= 245.67 ± 200.37 s) for illuminated trials. For *P. saltator* in illuminated trials (Figure 3.3), exploration time was not correlated with launch rock distance (Linear regression, $F=0.998$, $n=54$, $P=0.322$) while exploration for *T. alternatus* in illuminated trials was also not correlated with launch rock distance (Linear regression, $F=0.222$, $n=3$, $P=0.720$).

This trend continued with non-illuminated trials, *P. saltator* grand average= 60.47 ± 14.56 s; *T. alternatus* grand average= 207.92 ± 60.99 s (Figure 3.3). No correlation with launch rock distance was found for either species during non-illuminated trials (*P. saltator*, Linear regression, $F=0.501$, $n=11$, $P=0.497$; *T. alternatus*, Linear regression, $F=0.437$, $n=8$, $P=0.533$). As the majority of *T. alternatus* specimens stayed on the launching rock during ten minute non-illuminated trials, the sample size is smaller for illuminated trials but similar to *P. saltator* for non-illuminated trials (Figure 3.3). *P. saltator* generally left the launching rock sooner than *T. alternatus* no matter which trial type was conducted (Figure 3.3).

Figure 3.3. Average time spent on launching rock before leaving *via* jumping, swimming or crawling into the water. Illuminated (top) data consist of n = 18/20 at 2 cm, n = 19/20 at 4 cm and n = 18/20 at 6 cm for all tested distances for *P. saltator*. *T. alternatus* tests produced n = 2/10 at 2 cm and 0/10 at 4 and 1/10 at 6 cm for *T. alternatus* tests. Non-illuminated trial (bottom) data consists of n = 4/4 at 2 and 4 cm and 3/3 for 6 cm for *P. saltator* tests while *T. alternatus* tests consist of n = 3/3 for 2 and 4 cm distances and n = 2/2 for 6 cm distance tests. Although trend lines indicate a decrease in exploration time as launch rock distance increased, regression analysis found this to be non-significant (see section 3.3.1). Hatched line = *P. saltator*, solid line = *T. alternatus*.

3.3.2. Jumping Behaviour

Pedetontus saltator were observed jumping 144 times from 215 trials overall (n=24) while *T. alternatus* jumped only 9 times out of 179 trials (n=20). The posture adopted by specimens during the aerial portion of the jump always appeared to be the same as the previously described dorso-ventrally curved with raised abdomen posture described in Chapter 2 (Figure 3.4). Specimens would perform the jump, leaving the launching rock and uncurling from the jump movement extending into the curved posture presenting the legs and ventrum down for landing for each of the observed jumps. Partial tumbling (pitching) and in yaw and roll was observed in mid-air at different points throughout the specimen's transit for each observed jump.

Fewer jumps overall were observed when the launch rock was not illuminated with the halogen light. In these trials, in addition to jumping both species were observed leaving the launch rock *via* a swimming behaviour (*P. saltator*= 4 observations, n=4, *T. alternatus*= 6 observations, n=3) which involved the individual walking off the rock onto the water's surface while continuing to move its legs while caught in the surface tension of the water. As well, both *P. saltator* and *T. alternatus* were observed (5 and 3 observations, n= 2 and 2 respectively for each species) crawling beneath the water surface from the launching rock and even walking along the bottom of the tray. Jumping was observed to be the most frequent exit behaviour for *P. saltator* during illuminated experiments (Figure 3.5). While staying on the launching rock was the next most likely outcome of the experiment.

T. alternatus regularly stayed on the launch rock during illuminated trials while rarely (3/10 individuals) jumping during the trial time (Figure 3.5). Non-illuminated trials for jumping were similar to illuminated trials resulting in only two observations of jumping behaviour (2/10 individuals) while no observations were made involving swimming or crawling into the water during *T. alternatus* illuminated trials. This absence of swimming/crawling off of the launch rock trend was not observed during non-illuminated trials as observations (5/10 individuals) were made at every launching rock distance of *T. alternatus* swimming or crawling into the water from the launch rock (Figure 3.5 B). In either trial type the majority of observations resulted in the individual not leaving the launch rock. Jumping observations gradually decreased as launch rock distance increased, conversely remaining on rock behaviour gradually increased as launch rock distance increased (Figure 3.5).

Log-likelihood ratio tests were performed to determine if the observed behaviours (jump, swing/crawl, stay) were contingent upon the distance between the launch and landing rocks (2, 4 or 6 cm). The behaviours observed in the illuminated trials were not contingent on the distance for either species (Log-likelihood ratio test, *P. saltator*: $G=4.251$, $df=4$, $P=0.373$; *T. alternatus*: $G=6.245$, $df=4$, $P=0.182$). The behaviours observed in dark trials were dependent on launch rock distance for *T. alternatus* (Log-likelihood ratio test, $G=9.651$, $df=4$, $P=0.046$) wherein remaining on the rock was the most frequently observed behaviour for all three distances. This was not the case for *P. saltator* that showed no dependence of behaviour on distance (Log-likelihood ratio test, $G=7.504$, $df=4$, $P=0.111$).

Figure 3.4. Successive single high speed video frames of a *P. saltator* specimen jumping towards the landing rocks from the launching rock and successfully landing (Arrows). Launching rock is 4 cm from the landing rock, the calibration square pictured is 10mm x 10mm.

Figure 3.5. Outcomes from all illuminated trials **A)** at 3 launch rock distances *P. saltator* (20 individuals) were observed jumping more than swimming/crawling or staying on the rock while *T. alternatus* (10 individuals) was observed staying on the rock most often. Despite these observations no one behaviour was significantly more prevalent than another for increasing launch rock distances (Log-likelihood ratio test, *P. saltator*: $G=4.251$, $df=4$, $P=0.373$; *T. alternatus*: $G=6.245$, $df=4$, $P=0.182$). Outcomes from non-illuminated **B)** trials at 3 different launch rock distances. Jumping from the launch rock was most common in *P. saltator* (4 individuals) while decreasing in count as launch rock distances increased. Staying on the launching rock during trial time was the most observed outcome for *T. alternatus* (10 individuals) at all distances. Swimming or crawling off the rock was observed at all launch rock distances for *T. alternatus*. Despite these observations only *T. alternatus* remaining on the launch rock was associated with launch rock distance (Log-likelihood ratio test, $G=9.651$, $df=4$, $P=0.0467$). *P. saltator* behaviour was not associated with launch rock distance (Log-likelihood ratio test, $G=7.504$, $df=4$, $P=0.112$).

3.3.3. *Jumping and Successful Water Exits*

Illuminated trials always produced more jumps towards the landing rocks than away from the landing rocks for *P. saltator* (Figure 3.6 A). The behaviours observed in the illuminated trials were not contingent on the distance for either species (Log-likelihood ratio test, *P. saltator*: $G=2.958$, $df=2$, $P=0.228$; *T. alternatus*: $G=1.1849$, $df=2$, $P=0.553$). Non-illuminated trials resulted in more away jumps as the launching rock was placed at 4 and 6 cm distances from the landing rocks (Figure 3.6 B). However, as in illuminated trials non-illuminated trials found no dependence of jump direction on launch rock distance for either species (Log-likelihood ratio test, *P. saltator*: $G=1.099$, $df=2$, $P=0.577$; *T. alternatus*: $G=5.3E-05$, $df=2$, $P=1.0$). *Trigoniophthalmus alternatus* exhibited jumps as exit behaviour rarely (see sec. 3.3.2), so directional trends may not be apparent during this study. Non-illuminated trials produced only towards landing rocks jumps, albeit only at a 2 cm distance of the launching rock for *T. alternatus* (Figure 3.6 B).

When *P. saltator* jumped towards the landing rocks they landed on, or near the rocks resulting in climbing onto them, more often than not (Figure 3.7 A). As well, *T. alternatus* was recorded to jump and land on two occasions at 2 and 6 cm, Figure (3.7 A). The behaviours observed in the illuminated trials were not contingent on the distance for either species (Log-likelihood ratio test, *P. saltator*: $G=2.614$, $df=2$, $P=0.271$; *T. alternatus*: $G=1.046$, $df=2$, $P=0.593$).

Non-illuminated trials resulted in more successful landings and rock extrications during toward jumps for *P. saltator* and *T. alternatus* at 2 cm launch rock distances as well as the 4 cm distance for *P. saltator* (Figure 3.7 B). Despite these observations,

Figure 3.6. A) Counted jumps towards (Black Bars) and away (Grey Bars) from landing rocks during illuminated trials. *P. saltator* jumped more frequently towards the landing rocks than away at all distances. *T. alternatus* jumped infrequently during trial time. *P. saltator*, 18 individuals at 2 cm, 17 at 4 cm and 17 at 6 cm. *T. alternatus*, 2 individuals at 2 cm, 0 at 4 cm and 1 at 6 cm. Jumping direction was not associated with launch rock distance for illuminated trials (Log-likelihood ratio test, *P. saltator*: $G=2.958$, $df=2$, $P=0.228$; *T. alternatus*: $G=1.185$, $df=2$, $P=0.553$). **B)** Counted jumps from non-illuminated trials towards (Black Bars) and away (Grey Bars) from the landing rocks. *P. saltator* jumped towards the landing rocks more often only at the 2 cm launching rock distance. *T. alternatus* jumped only at the 2 cm launch rock distance and only towards the landing rocks. *P. saltator*, 4 individuals for 2 cm and 4 cm and 3 at 6 cm. *T. alternatus* 2 individuals for 2 cm and 0 at 4 and 6 cm. Jumping direction was not associated with launch rock distance for non-illuminated trials (Log-likelihood ratio test, *P. saltator*: $G=1.099$, $df=2$, $P=0.577$; *T. alternatus*: $G=5e-05$, $df=2$, $P=1.0$).

behaviours observed in non-illuminated trials found no dependence of jump success on launch rock distance for either species (Log-likelihood ratio test, *P. saltator*: $G=2.003$, $df=2$, $P=0.367$; *T. alternatus*: $G=1.2 \times 10^{-6}$, $df=2$, $P=1.0$).

When considering the success of individuals after a jump in any direction during illuminated trials, *P. saltator* continues a similar trend only deviating at the 6 cm launch rock distance, where jumping concluding in a non-extrication from the water becomes more observed (Figure 3.8 A). The behaviours observed in the illuminated trials were not contingent on the distance for either species (Log-likelihood ratio test, *P. saltator*: $G=2.232$, $df=2$, $P=0.328$; *T. alternatus*: $G=2.231$, $df=2$, $P=0.328$). Non-illuminated trials produced more successful outcomes for both *P. saltator* and *T. alternatus* at 2 cm launch rock distances but both 4 and 6 cm distances resulted in mostly unsuccessful attempts or no jumps for both species (3.8 B).

As in illuminated trials, the behaviours observed in non-illuminated trials found no dependence of jump success on launch rock distance for either species (Log-likelihood ratio test, *P. saltator*: $G=5.307$, $df=2$, $P=0.0704$; *T. alternatus*: $G=1.15 \times 10^{-6}$, $df=2$, $P=1.0$).

Figure 3.7. Illuminated trials **A)** Counts of jumping towards the landing rocks with success (Black bars) or missing (Grey Bars) for both species. *P. saltator*, 15 individuals at 2 cm, 11 at 4 cm and 16 at 6 cm. *T. alternatus*, 1 individual at 2 cm, 0 at 4 cm and 1 at 6 cm. There is no association with behaviour for increasing launch rock distance (Log-likelihood ratio test, *P. saltator*: $G=2.614$, $df=2$, $P=0.271$; *T. alternatus*: $G=1.046$, $df=2$, $P=0.593$). Non-illuminated trials **B)** Counts of jumping towards the landing rocks with success (Black bars) or missing (Grey Bars) for both species. *P. saltator*, 3 individuals at 2 cm, 3 at 4 cm and 1 at 6 cm. *T. alternatus*, 2 individual at 2cm, 0 at 4 cm and 0 at 6 cm. There is no association of behaviour with increasing launch rock distance (Log-likelihood ratio test, *P. saltator*: $G=2.003$, $df=2$, $P=0.367$; *T. alternatus*: $G=1.2e-06$, $df=2$, $P=1.0$).

Figure 3.8. Illuminated trials **A)** Success of specimens jumping in any direction and reaching a solid surface (Black Bars) or remaining within the water (Grey Bars). *P. saltator*, 18 individuals at 2 cm, 17 at 4 cm and 17 at 6 cm. *T. alternatus*, 2 individuals at 2 cm, 0 at 4 cm and 1 at 6 cm. There is no association with behaviour for increasing launch rock distance (Log-likelihood ratio test, *P. saltator*: $G=2.232$, $df=2$, $P=0.328$; *T. alternatus*: $G=2.231$, $df=2$, $P=0.328$). Non-illuminated trials **B)** Success of specimens jumping in any direction and reaching a solid surface (Black Bars) or remaining within the water (Grey Bars). *P. saltator*, 4 individuals at 2 cm, 4 at 4 cm and 3 at 6 cm. *T. alternatus*, 2 individuals at 2 cm, 0 at 4 cm and 0 at 6 cm. There is no association with behaviour for increasing launch rock distance (Log-likelihood ratio test, *P. saltator*: $G= 5.307$, $df= 2$, $P= 0.0704$; *T. alternatus*: $G= 1.15E-06$, $df= 2$, $P= 1.0$).

3.3.4. *Jumping Distance and Height*

Trials resulting in a jump from *P. saltator* in a plane perpendicular to the high speed camera lens were digitized using SkillSpector (V. 1.3.2). The average jump height decreased from the 2 cm to 4 cm launch rock distance by 0.34 cm while only decreasing a further 0.04 cm from 4 to 6 cm launch rock distances (Figure 3.9 A). The highest jump height observed was 2.86 cm from one individual at the 4 cm launch rock distance while the lowest height was 0.13 cm from another individual at 6 cm launch rock distance. The average horizontal jump distance increased gradually as launch rock distance increased from landing rocks with the average distance increasing by 0.11 cm from 2 to 4 cm and 0.36 cm from 4 to 6 cm. All digitized jumps were between 2.18 cm and 7.59 cm during trials (Figure 3.9 B).

Regression analysis of jump height revealed no significant change as launch rock distance increased. Similarly, jump distance did not significantly increase as launch rock distance increased (Figure 3.9).

Figure 3.9. A) Average jump distances and **B)** heights for *P. saltator* specimens jumping perpendicular to the high speed camera lens during illuminated trials. At 2 cm $n = 8$ (11 observations), at 4 cm $n = 10$ (10 observations) and at 6 cm $n = 9$ (13 observations). Although the jump distance increases and jump height decreases with launch rock distance they are not statistically dependent on each other. (Distance: Linear regression, $F = 1.004$, $n = 27$, $P = 0.326$; Height: Linear regression, $F = 1.365$, $n = 27$, $P = 0.254$). Dashed lines are 95% confidence intervals.

3.3.5. Water Drop and Landing Trials

Trigoniophthalmus alternatus regularly landed and actively traversed the water surface through a series of energetic jumps until reaching the rocks placed at the tray ends or reaching the tray edge. On 13 occasions *T. alternatus* specimens dropped from 48.5 cm appeared to contact the water and immediately initiate a jumping movement (Figure 3.10) causing the individual to ‘skip’ along the water to a dry surface in ten seconds or less. When dropped from 98.5 cm, 8 individuals initiated the jump response to landing and exited the water in 10 seconds or less. Only one *P. saltator* specimen out of all specimens at any height was able to initiate a jumping response and exit the water in less than 10 seconds. As a result of this contrast, the average time to exit the water upon landing was lower for *T. alternatus* at both drop heights than *P. saltator* (Figure 3.11). Those that landed and remained on the water surface also used a tail flicking movement combined with a variety of other movements to move along the surface tension of the water until a solid surface was encountered or the trial ended.

Although exiting time for successful individuals landing on water differed for both species when dropping from both 48.5 cm and 98.5 cm, they were not significantly different (Figure 3.11) (*P. saltator*, n= 12, 7, t= 0.867, P= 0.402; *T. alternatus*, n= 16, 11, t= -0.880, P= 0.392). When comparing between species, *T. alternatus* escaped the water after landing significantly quicker than *P. saltator* at 48.5 cm (t-test, n= 12, 16, t= 2.607, P= 0.018), however this trend is not significant when dropped from 98.5 cm (t-test, n= 7, 11, t= 0.390, P= 0.703).

Figure 3.10. Single high speed video frames of a *P. saltator* performing a jump forward (JF) while on the surface of the water after a drop from 98.5 cm.

Figure 3.11. The average time required for all successfully exiting specimens to extract themselves *via* a solid surface. Although exiting time differed for both species when dropping from both 48.5 cm and 98.5 cm they were not significantly different (*P. saltator*, $n=12, 7$, $t=0.867$, $p=0.402$; *T. alternatus*, $n=16, 11$, $t=-0.880$, $p=0.392$). When comparing between species, *T. alternatus* escaped the water after landing significantly quicker than *P. saltator* at 48.5 cm (t-test, $n=12, 16$, $t=2.607$, $p=0.018$), however this trend is not significant when dropped from 98.5 cm (t-test, $n=7, 11$, $t=0.390$, $p=0.703$).

The proportion of time spent active while on the water surface was also analyzed for all tested specimens; those successful and those unsuccessful at escaping the water (Figure 3.12). Overall, *T. alternatus* was proportionally more active than *P. saltator* after a 48.5 cm drop (t-test, $n = 19, 21$, $t = -3.845$, $P = <0.001$). However, this trend is not present after a 98.5 cm drop (t-test, $n = 20, 21$, $t = -1.779$, $P = 0.086$). Successful specimens spent proportionally more time active while on the water surface after a drop from both 48.5 cm (*P. saltator*, t-test, $n = 12, 7$, $t = 4.492$, $P = <0.001$; *T. alternatus*, t-test, $n = 16, 5$, $t = 8.287$, $P = <0.001$) and 98.5 cm (*P. saltator*, t-test, $n = 7, 13$, $t = 4.046$, $P = 0.007$; *T. alternatus*, t-test, $n = 11, 10$, $t = 5.390$, $P = <0.001$) (Figure 3.12).

3.3.6. Behaviour on Water

The dominant observable behaviour between both species involved the dorso-ventral abdominal jumping movement usually employed as a terrestrial jumping mechanism. This abdominal movement would result in a displacement forward of the starting position (JF), movement backward (JB) or no obvious displacement (J). The jumping movement was also observed in combination with other movements such as leg movements/walking motions (W) and lateral undulations (U) (Table 3.1).

Figure 3.12. *T. alternatus* is significantly more active overall than *P. saltator* while on the water surface after a 48.5 cm drop (top). Successful specimens from both species spent significantly more time active than inactive while on the water surface. Successful *T. alternatus* were significantly more active than *P. saltator* from 48.5 cm drop heights, See sec (3.3.5) for statistics.

3.3.7. Success and Behaviour on the Water

Between successful and unsuccessful *P. saltator* specimens, the behavioural observations were generally dominated by an abdominal jump resulting in a forward movement (JF) as well as a combination of this movement with walking movements (W) (Figure 3.13). Un-successful specimens still employed these behaviour types but were also observed to combine the abdominal jump with a lateral undulation (U), sometimes moving forward (JF/U) and sometimes not (J/W) (Figure 3.13).

T. alternatus demonstrated less diversity in regard to behaviour types while on the surface of the water. Less time overall was spent jumping resulting in a forward movement for successful individuals from both drop heights, while unsuccessful specimens followed a similar trend to *P. saltator* exhibiting a lateral undulation-like movement (U) from the 48.5 cm drop height.

Figure 3.13. Average time spent for individual behaviours for all specimens successfully and unsuccessfully exiting the water after landing from a drop height of 48.5 cm (Black Bars) and 98.5 cm (Grey Bars). Abdominal jumping forward (JF) is still a dominant behaviour however more time is spent performing other movements for unsuccessful specimens. Table 3.1 defines acronyms for behaviour types.

3.4. Discussion

3.4.1. Jumping Trial Experiments

Despite identical treatment of both species for trials, *P. saltator* generally demonstrated more interest in exploring and leaving the launching rock compared with *T. alternatus*, as evidenced by the fact that during both illuminated and non-illuminated trials, all *P. saltator* individuals (20/20; 4/4) were recorded leaving the launch rock in some way at least once over the three tested launch rock distances while only 3/10 and 5/10 *T. alternatus* individuals did so (Figure 3.3). As well, the apparent discontinuity in the tendency to jump between the two species tested is intriguing considering their morphological similarity and lifestyle. Why this difference exists remains speculative, but unexpected when considering the more active nature of *T. alternatus* during field collecting. Further exploration of Archaeognatha species in similar behavioural trials to those presented here may help to shed light on the specifics of each species responses. Despite the non-significant result in regard to exploration time decreasing with increased launch rock distance, the regression line does exhibit a negative slope raising the possibility that there is a relationship between these two variables. The regressions performed (Figure 3.3) had powers of 0.097 (*P. saltator* non-illuminated), 0.165 (*P. saltator* illuminated), 0.086 (*T. alternatus* non-illuminated) and <0.001 (*T. alternatus* illuminated). Considering that the powers of the regression tests are low, a larger sample size is warranted to assess if the observed trend is significant. The challenge faced in this study was the availability of specimens due to seasonality and location, which limited the

data collection however the framework for further study now exists from these experiments.

The method of exiting the launch rock was also not contingent on launch rock distance for tested individuals, with the exception of *T. alternatus* that opted to remain on the launching rock for non-illuminated trials rather than opting to leave (Figure 3.5 B, Sec 3.3.2.). The absence of any significant relationship otherwise may simply be a reflection of the sample size and inherent variability of the data for these experiments. When considering the data for illuminated trials, *P. saltator* showed a decreasing tendency to jump as the launch rock distance increased while the choice to remain on the rock during the ten minute experimental time increases (Figure 3.5 A).

The ability of *P. saltator* and *T. alternatus*, or any other Archaeognatha, to breach the surface of the air-water interface and actively walk along a submerged rock face has not been recorded to my knowledge. Two *P. saltator* and two *T. alternatus* specimens were observed entering and exiting the water without any type of interference; surprisingly, one specimen even employed an underwater jump from the tray bottom through the water column to end up on the surface of the water. One *P. saltator* specimen (illuminated) and one *T. alternatus* (non-illuminated) individual actively used their limbs to “swim off” the launching rock on top of the water surface to move to the rock pile or remount the launching rock within the arena while two other *P. saltator* (illuminated) and two other *T. alternatus* (non-illuminated) individuals swam off but did not exit the water. Although a fascinating and somewhat puzzling behaviour, the evolutionary implications of a basal wingless insect group, supposedly entirely terrestrial, demonstrating the ability

to traverse and pass through the potential obstacle of the air to water interface may be of importance to evolutionary concepts.

Opting to use the forward jump *via* the dorso-ventral abdominal flexion mechanism possessed by the jumping bristletails to exit an environment surrounded by water, potentially from flooding or a fall, is also a novel observation for this group of insects (Figure 3.4). Although jumping is regularly documented as a predator evasion technique, to my knowledge this is the first study to potentially show some degree of control in regard to direction and overall success of a jump (Figure 3.6 A, 3.7 A and B). The ability to identify an unsatisfactory situation, in this case being exposed (open rock face) and surrounded by a non-desirable medium (standing water), identify a more desirable location (rock group with multiple hiding places), initiate an energy expending jump to travel through the air, land and acquire that desirable location, has great potential for the survival purposes of a flightless insect.

Specimens observed floating using the surface tension of the water were observed taking on a characteristic dorso-ventrally curved (abdomen up) body position prior to abdominal jumping behaviour. This stance appears to be the same as the pre-jump position which involves the raising of the abdomen or ‘cocking’ of the musculature for release as a jump (Manton, 1972, 1977; Evans 1975). The antennae and telson are also held up and off the surface of the water in this stance which, through brief experimentation with air gusts, appears to act like a sail, catching the air current and pushing the individual along the water surface under near frictionless conditions much like how fishing spiders, stoneflies and mayflies can sail with the wind (Deshefy, 1981; Marden et al., 2000). This stance, similar to Figure 3.10 at 20 ms, was only observed if

the individual was able to prevent submersion of the antenna and telson as well as maintain a stable ventral side down attitude on the water surface. This parallels the skimming behaviours previously reported in basal pterygote insects such as mayflies and stoneflies (Marden and Kramer, 1994, Marden et al. 2000; Marden, 1995, 2003), albeit at potentially less energetic costs as this type of transit could be considered more passive than active. Further investigation in both the lab and field sites is required to quantify this sailing possibility in Archaeognatha.

Both species used in this study survive in rocky, detritus laden areas that are exposed to rainfall which may form static pools within their habitat. The presence of river shorelines with rocky, slow flowing pools was also noted at collecting sites. The ability to traverse and extricate oneself after an inadvertent fall or flooding from rains would be invaluable to any invertebrate surviving in a terrestrial habitat. It is not entirely surprising that such behaviour is apparent in both species to their reported extents, however the basal stature of the order and morphology is of significance as opposed to more derived Orthoptera, Hemiptera, Coleoptera and Blattodea that use their legs primarily to achieve this goal (Franklin et al. 1977; Cocatre-Zilgien and Delcomya, 1990; Bush and Hu, 2006; Gardiner, 2009).

P. saltator specimens observed using high speed cinematography were evaluated for body position as well as jump distance and height when appropriate. Consistent with the observations of Chapter 2, bristletails adopted the dorso-ventral curvature when airborne (Figure 3.4). Forward jumping towards landing rocks resulted in varying jump distances, demonstrating a slight increase in distance and decrease in jump height overall, as the launching rock distance was increased (Figure 3.9). I suggest it is possible

Archaeognatha specimens can assess a distance over an obstacle and initiate a jump of the approximate force required to traverse the distance to the desired location of landing. The power of the jump distance and height regressions are once again low enough to suggest a larger sample size is required to potentially support or deny this possibility (Distance, power = 0.162; Height, power = 0.205) (Figure 3.9). If this trend holds true in further testing the potential for controlled jumping using the unique abdominal flexion behaviour used by jumping bristletails may further contribute to the understanding of the success of the modern Archaeognatha.

3.4.2. Drop and Landing Experiments

Although the lower 48.5 cm drop height resulted in significant differences in escape time between species tested (Figure 3.11), the specific reason why remains unclear. The 98.5 cm drop height did not result in the same trend for escape times between species, while comparing between drop heights for each species also did not result in a significant difference (Figure 3.11). Since *T. alternatus* was observed landing on the water and immediately initiating the abdominal jump response more-so than *P. saltator*, resulting in less overall time spent performing the behaviour (Figure 3.13), the proportion of time spent active on the water is significantly higher for *T. alternatus* overall. This trend is also observed for successfully escaping individuals in general from both species (Sec. 3.3.5) (Figure 3.12).

Bristletails also exhibited other behaviours while bound to the surface tension of the water after a fall. Along with the jumping behaviour, individuals were observed moving their legs in what appears to be a walking-like movement (W) as well as lateral undulations (U) and turns to the right or left (T) (Figure 3.13). From observing these behaviours it appeared that the individual would attempt to orient themselves in such a way so as to set up for an abdominal jump (J, JF, JB) to enable a change in displacement. Individuals that found themselves partially submerged and not completely ventral side down would appear to have difficulty exhibiting the jump behaviour to its best ability, specifically, moving forward. From my observations, it would seem that the jumping response (J, JF) is the key behaviour type to allow for an effective escape from the water surface tension, however, *T. alternatus* specimens tested here appear to be able to employ this behaviour more effectively to decrease their time spent floating on the water.

3.4.3. *Surviving Around Water*

Although adopting a terrestrial lifestyle, encountering water in the form of pools and streams is a likely aspect of these insects' lives. Adapting to the challenges presented by these water bodies is of importance for any insect that must survive and cannot take to wing and avoid the problem all together. The potential to enter and exit the water, seemingly at will, despite being adapted for a terrestrial lifestyle carries the benefits of a solution to dealing with flooding from rains and river overflows without serious detrimental harm. Likewise, if a wingless insect has no choice but to wait out a flood, at times it may be beneficial to do so underwater and prevent being dislodged by rain drops,

flows or detritus. At this time it is unclear how long an Archaeognatha may be able to stay submerged without succumbing to drowning, further investigation is warranted to discover if there are truly benefits and if they outweigh any costs in this insect group.

The use of a terrestrial escape and locomotory mechanism in an aquatic environment has occasionally been reported in the Insecta (Hymenoptera using wings to swim, skimming stone flies and mayflies, walking on water, swimming in orthopterans/locusts) (Bradley, 1902; Hirashima et al. 1999; Fursov and Øypcob, 2004; Mineo et al. 2010; Bush and Hu, 2006; Marden and Kramer, 1994, 1995; Gardiner, 2009; Jeff Dawson, personal communication). However, employing an abdominal jump from terrestrial applications is a novel method of locomotion on the water surface. Even more interesting is that this behaviour is present in the most basal group of the Insecta, which still exhibit much of morphology that is considered to be present within the early pre-flight protopterygote insect group (Kukalová-Peck, 1983, 1998, 2008).

Although the specific process and mechanism for how a bristletail can utilize the surface tension of the water to render an abdominal jump motion useful, it would not be unreasonable to speculate that the combination of the hydrophobic scales and other chitinous body parts are important. These components may create a type of plastron to deform and create a type of depression in the surface tension of the water, which provides structure to apply force to (Bush and Hu, 2006). This is similar to how water striders and spiders can deform the surface tension of the water without breaching it with their tarsal leg segments in order to walk on water (Thorpe and Crisp, 1947; Thorpe, 1950; Caponigro and Eriksen, 1976). Further exploration of this mechanism in Archaeognatha is required to confirm this possibility.

Other terrestrial, yet more derived, insects have been documented to be able to effectively swim along the surface tension of a water body, primarily using their legs as oars (grasshoppers, beetles) (Franklin et al., 1977; Betz, 1999) or the hydrophobic hairs on the tarsi of the legs (water striders) (Thorpe and Crisp, 1947; Thorpe, 1950) and even abdominal gills as oars (stoneflies, mayflies) (Marden et al., 2000; Marden, 2003).

Swimming along the water surface becomes a relevant behaviour when flying or terrestrial insects inadvertently fall onto water surfaces, sometimes from overhanging vegetation or misjudged aerial manoeuvres as in the case of mayflies during mating behaviours. The more time spent bound in the surface tension of the water the more likely the individual is to succumb to predators such as fish, waterfowl and other insects.

Further to this concept, the risk of drowning becomes greater when terrestrial insects are even partially submerged for extended periods of time. Subsequently, the ability to transit along the air/water interface rapidly upon contact is of great value for survival. The ability of *T. alternatus* to quickly initiate a jumping response and traverse a given distance over water may be a useful tool in the survival of the species around flooded forest areas.

Curiously, *P. saltator* (which also makes its home around rocky areas near water) does not appear to initiate a rapid water transit upon contact but can utilize the abdominal jumping behaviour to effectively propel along the water surface until a solid surface is contacted. This would suggest that *P. saltator* would be more exposed to predation *via* time spent bound to the surface tension of the water compared to *T. alternatus* when landing on a water body. Further testing involving predators or a predator substitute in an isolated environment may be advisable to confirm this possibility.

3.4.4. *Evolutionary Aspects in the context of a Preadaptation for Fight Initiation*

The unexpected utility of the jumping behaviour on and around the surface of a water body may be of importance to reconstructing the pre-flight behaviours of an early protopterygote insect, living in and around the semi-aquatic environment of the Silurian and early Devonian period. Jumping has been suggested in the past as a potential mechanism for early, protopterygote, insects to become airborne. Previous suggestions have involved the use of jumping, and jumping combined with flapping winglets, as a viable escape procedure from early Silurian and Devonian predators (Norberg, 1972; Shear and Kukalová-Peck, 1990; Kukalová-Peck, 1983, 1998, 2008). The results presented here may be considered with regard to the possibility of jumping also being used as a viable transport mechanism albeit undertaken with a certain degree of environmental pressure.

Utilizing an energetic dorso-abdominal flexion *via* specialized rope-muscles and other antagonist muscle groups is an effective survival mechanism for Archaeognatha (Manton, 1972, 1977); a terrestrial, wingless insect group. The musculature used for this type of jumping likely has crustacean-like anatomy, the malacostracan's in particular (Evans, 1975; Barlet, 1978a, b; Shcherbakov, 1999). As such, the potential for early Silurian insect ancestors using a rapid succession of jumps to either jump along, over or out of the water, to escape from predators or a directed leap to traverse an environmental obstacle becomes more tantalizing with the results presented here. Furthermore, the growth and increasing infiltration of plants from their aquatic origins to the terrestrial world would have provided low overhanging branches and shoots which insects may have climbed and jumped from, likely resulting in a fall onto water after a misdirected jump (as

seen here). A fall after a leap from a primitive low lying plant may have been aided by flapping winglets, resulting in the early beginnings of crude flight and/or surface skimming upon landing (Marden and Kramer, 1994; Marden, 1995, 2003). The potential for jumping protopterygote insects (perhaps ones with less refined abilities than the Archaeognatha) increasing their jumping distance after a directed jump though the use of flapping winglets is an avenue towards powered flight that should also be considered. Such behaviour may have been useful in propelling a jumping protopterygote over a larger water body and/or skimming to safety as a flapping individual leapt over or onto the water surface.

The ability of the terrestrially adapted Archaeognatha to crawl in and out of the water, seemingly un-phased after brief periods of submersion, suggest that adaptations to an aquatic environment from a terrestrial one, as in the case of early Hydropalaeoptera (Hennig, 1981; Rasnitsyn, 1981; Kukalová-Peck, 2009), or potentially the reverse for earliest insect lineages, may not have been as physically challenging as it may seem. The evolution of the Insecta from their crustacean-like ancestral group living in and around shallow pools of the Ordovician, Silurian and Devonian periods when selection for terrestrial survival was taking place, is perhaps better conceptualized through the behaviours presented here.

Chapter 4: Morphological and behavioural reconstruction of a putative Pterygote insect ancestor

4.1. Introduction

Exploiting the terrestrial-aerial regime for dispersal, reproduction, feeding and escape from predators has resulted in the remarkable evolutionary success of the insects as evidenced by their presence in nearly all terrestrial ecosystems. Early flying insects evolved over 400 million years ago from a single flightless ancestor (Kukalová-Peck, 1987, 2008). Their divergence from this single ancestor is extensive yet no remaining lineages of extant flying insects are morphologically similar to the likely ancestral protopterygote insect to provide a clear picture of the selective forces leading to flight as observed in modern insects. The clues we have in regards to the morphology of the protopterygote ancestor remain sparse, yet key, in the fossil record. Recent work (Chapter 2 and 3) and from other authors (Marden, 2013; Kukalová-Peck, 2008, 2009) have provided tantalizing clues to the basic behaviours and corresponding morphology a protopterygote insect may have exhibited. As the beginnings of flight began in flightless insects, reconstructing the past requires us to address the pterygote ‘ground plan’ in respect to morphology (Hennig, 1969, 1981; Kukalová-Peck, 1983, 2009). In this chapter I present a reconstruction of the flightless protopteryote insect ancestor using information from past publications, fossil reconstructions (Kukalová-Peck, unpublished) and data collected from previous chapters in this thesis. I will employ allometric scaling based on data collected from Archaeognatha specimens and present a range of morphological size parameters the putative protopterygote ancestor may have resided within. I also will discuss the potential behaviours of this insect in conjunction with the morphology

expected to have been exhibited by the protopterygote insect, and, I explore concepts important to the continuation of undertaking research into the evolution of flight in insects.

4.2. The Early Insect Environment

During the early Silurian period (425+ million years ago) plant life was in the early stages of diversification from the early, low growing, forms (Behrensmeyer et al., 1992; Kenrick and Crane, 1997; Diltcher et al., 2004), into forms more closely resembling forests by the mid to late Devonian (360+ million years ago) (Stein et al., 2012). This also coincides with when the ancient lineages within the Arthropoda were likely moving or had moved from their aquatic, pancrustacean, ancestry to their more semi-aquatic (Handlirsch, 1908; Mamayev, 1971, 1975) and terrestrially evolved forms (for review, see Shcherbakov, 1999; Almond, 1985). During this time, both the early insect forms and predatory arachnids were likely in abundance (Shear et al., 1984; Rolfe, 1985; Shear and Kukalová-Peck, 1990; Regier et al., 2010). Early insects were believed to have been closely associated with the progression of vegetation onto land as they were likely surviving and crawling along floating algal mats and ascending dense, stick-like, vegetation (< 1 m in height) along freshwater pools in swamps and river outlets, using double clawed appendages to gain access to the soft tissues of the branch tips for feeding and potentially reproductive purposes (Kukalová-Peck, 1983, 1987; Shcherbakov, 1999). Climbing in and along the elevated tangles of early fresh water and terrestrial vegetation presents potential danger by way of becoming confined or trapped by a pursuing predator

once atop a stem like growth. In this landscape, the protopterygote insect would likely be dealing with components of both an aquatic and terrestrial existence, effectively a semi-aquatic environment. This environment would have included all of the environmental, predatory and evolutionary challenges that come with such a lifestyle, presumably shaping the early insect pterygote lineage to a fashion leading in some, currently speculative, way towards flight (Kukalová-Peck, 2008).

4.3. Protopterygote Morphology

Dr. Robin Wootton originally proposed a possible protopterygote insect in his 1986 study (Figure 4.1A) (Wootton, 1986). Although portraying only minor details in the hypothesized insect, the overarching concept was sound. Dr. Wootton's reconstruction employed long slender thoracic legs, long cercal filaments and appears to have possessed a somewhat silverfish-like dorso-ventrally flattened body. The serially repeating winglets along the abdomen are perhaps the most noticeable feature in this reconstruction and are still believed to be the basal condition for such an insect.

Other work exploring the potential early Insecta has involved crude models that resemble insects only in principle and not with any significant amount of anatomical detail. Examples include Wootton and Ellington's (1991) constructions of cylindrical models with static winglets for use in gliding experiments (Figure 4.1 B) and Hassenfuss' (2002) model experiments with flat simple shaped designs in an attempt to mimic morphology of the *Zygentoma* (Thysanura). Kingsolver and Koehl (1985) also constructed models (Figure 4.1 C), which were more detailed than those of Wootton and

Figure 4.1. A) A drawing of a possible protopterygote (from Wootton, 1986). B) Simple geometric model diagrams (modified) of tested models used by Wootton and Ellington (1991). C) (Modified) Kingsolver and Koehl (1985) also tested models of potential pre-cursor insect shapes in their study.

Ellington (1991), employing a more organic body shape in cylindrical and flattened profiles. These models were used for drag and temperature experimentation including the installation of simple rigid wings along the thorax.

Although somewhat similar to the hypothetical insect in Figure 4.1 A, it is now believed the protopterygote insect likely resembled the morphology of the †Cercopoda (Originally described as a †Monura sp. in Kukalová-Peck, (1985) but revised in Kukalová-Peck (1998)) as well as aspects of the †Monura and extant Archaeognatha. The likely body shape of such a protopterygote insect and its appendages were very much like the semi-cylindrical bodied (D-Shaped) Archaeognatha but without ventrally extended tergal plates along the thorax and abdomen, much like the †Cercopoda (Kukalová-Peck, 1998, 2008). Like all Arthropoda, the layout of the protopterygote insect consisted of three tagmata (Minelli and Peruffo, 1991; Dudley, 2000); a head, a thorax supporting pro-, meso- and meta-thoracic segments, and abdomen with eleven segments (plus a long filament called the telson). Body surface details of such an organism are open to speculation but the presence of scales like the Archaeognatha or a densely bristled texture should be considered (Kukalová-Peck, 1987). Although past work from Kukalová-Peck has been under scrutiny, see Sec. 1.2.2, a recent publication from Bechly and Stockar (2011) has agreed with Kukalová-Peck's interpretations of the †Cercopoda.

In the following sections I will outline the most likely morphology and potential behaviours associated with the protopterygote insect based on previously published work, new unpublished drawings from Dr. Jarmila Kukalová-Peck and myself as well as behavioural and morphological data from Archaeognatha work presented in the previous chapters.

4.3.1. Protopterygote Morphology - The Head

The putative protopterygote insect was likely dicondylous (Shear and Kukalová-Peck, 1990), like all pterygote insects (+Zygentoma) today, possessing an obvious head with two large eyes located bilaterally on each side of the head (Figure 4.2). Most modern Machilids (Order Archaeognatha) generally have a head surrounded by the tergum of the prothorax and eyes located at the front of the head touching each other (See Chapter 2, Figure 2.4), possibly a product of their nocturnal nature and adaptations to see in low light. Archaeognatha are monocondylous, as they diverged from the ancestral lineage before the protopterygote ancestor had evolved. The protopterygote mandible is expected to be broadly triangular with an interior mandibular articulation (Kukalová-Peck, 1985). Like the Archaeognatha, the precursor condition of the mouthparts of a protopterygote insect likely included adaptations for scraping and sucking material from rocky surfaces (Manton, 1964) that would have been later modified into the sucking beak-like form described in Carboniferous pterygotes which may have been used to feed on plant ovules (Shcherbakov, 1999). Long maxillary palps like modern Archaeognatha are also hypothesized to have been present. Derived from leg precursors, supporting curved double claws and arched anteriorly, they may have been useful for pulling the insect forward as it climbed through vegetation (Kukalová-Peck, 2008).

Much like modern Archaeognatha, two long articulated and annulated antennae are believed to have been present, located just in front of the eyes as in the †Cercopoda (Figure 4.2 and 4.3). The antennae would possibly have been tapered and supported by a broad base. These antennae, as in many insects, would presumably have been used for

Figure 4.2. An interpretation of the protopterygote head from an oblique angle (left, modified from Kukalová-Peck, unpublished) and from a dorsal view (right, bottom, original drawing RJC based on Kukalová-Peck, unpublished). Maxillary, and mandibular palps are present and were likely used to assist mobility and feeding. Antennae were likely long with thick bases projecting out in front and below the eyes.

Figure 4.3. (Top) A dorsal view of a †Cercopoda (original drawing, RJC) based on Kukalová-Peck (1987, 1998, 2008) and body width to length ratios of Archaeognatha. (Bottom) A side view of †Cercopoda originally from Kukalová-Peck (1987).

tactile sensory cues, and possibly chemical cues, from its surroundings as well as aiding in control of descent while in free fall from the semi aquatic vegetation of the late Silurian period. Recent work on Archaeognatha have shown that their antennae possessing weak points which are meant to break upon excess force (Fröhlich and Lu, 2013), an advantage for any insect possessing long antennae for sensory purposes but not wishing to be caught and held by predators with clasping apparati.

4.3.2. Propterygote Morphology - The Thorax

Thoracic pleurae were present in the pro-, meso- and meta-thoracic segments. They consist of the primordial epicoxal and subcoxal leg segment incorporated into and making up much of the side wall of the thorax (Kukalová-Peck, 1983, 1987, 1998, 2008). The thorax (Figure 4.4) supported six legs, two per segment, made up of 10 leg segments; the subcoxa was incorporated into the body wall below the epicoxal exite structure creating the nine segments consistent with most modern insects today (Archaeognatha still have cylindrical subcoxa on the meso- and meta-thoracic legs, Kukalová-Peck, 1998). The incorporation of the epicoxa and the subcoxa into the body side wall of both the thorax and abdomen allowed the exite derived protowings to be articulated from the musculature of the epicoxal and subcoxal leg segments, but rooted within the body wall itself (Figure 4.5 A).

Previous reconstructive work of the carboniferous palaeoptera have provided what has been described as a near groundplan or basal winglet-like structure with venation that

Figure 4.4. An interpretation of the protopterygote thorax at an oblique angle and left side winglets removed (Left, modified from Kukalová-Peck, Unpublished), from a dorsal view with winglets (top Right, original drawing RJC based on Kukalová-Peck, unpublished) and from a dorsal view with winglets omitted (bottom right, original drawing RJC based on Kukalová-Peck, unpublished).

A)

B)

Figure 4.5. A) The arthropod leg and specific structures within in regard to exites and leg segments. The subcoxa is cylindrical in the meso- and metathorax of Archaeognatha but a flat pleuron in all other extant insect lineages (Modified from Kukalová-Peck, 2008). **B)** The abdominal arrangement of the exite of the subcoxa and surrounding morphology within the Insecta. The winglet is supported in the embayment created from the folding and fusing of the pleuron to the tergum, while the exite retains its musculature attached to its base. From Kukalová-Peck (unpublished).

has effectively been, and continues to be, homologized throughout numerous insect lineages (Kukalová-Peck, 1978, 1983, 1998, 2008, 2009; Haas and Kukalová-Peck, 2001; Kukalová-Peck and Lawrence, 2004, Kukalová Peck et al., 2009). It is the prothoracic winglets of these lineages that are described as the nearest to the groundplan level of an insect wing in terms of venation, shape and potentially size in relation to body (Kukalová-Peck and Lawrence, 2004; Kukalová-Peck, 2008). The prothoracic protowings of the protopterygote would likely be very similar to those found on fossil palaeodictyopterans (Kukalová-Peck and Richardson, 1983; Shear and Kukalová-Peck, 1990; Wootton and Kukalová-Peck, 2000; Kukalová-Peck and Lawrence, 2004; Grimaldi and Engel, 2005). These “heart shaped” prothoracic winglets present in the palaeodictyopterans (Figure 4.6 A) were more elevated than the forewings but still may have overlapped and supported articulation that had begun to fuse or reduce (Kukalová-Peck and Richardson, 1983). Prothoracic winglet function is unknown but if they were only partially moveable, or even fused, they may have acted as stabilizers or flow deflectors while in flight in the Palaeodictyoptera. The protopterygote prothoracic winglets are of interest as they likely did not contribute to powered flapping flight as evidenced by their lack of forward oriented morphology, general lack of rigidified leading edge and presence with potential fusions within the articulation. However, their presence in the Palaeodictyoptera, and others, did not negate the selection for thoracic wings, as evidenced by the fact that carboniferous flying lineages still had these winglets (Kukalová-Peck and Richardson, 1983; Shear and Kukalová-Peck, 1990; Wootton and Kukalová-Peck, 2000; Kukalová-Peck and Lawrence, 2004; Grimaldi and Engel, 2005).

Modern Insecta do not support wings on the pro-thorax, and potentially, the loss of winglets on this body segment over time was simply a function of ‘use it or lose it’. The flapping meso- and meta-thoracic winglets were likely powered by musculature from the pleural leg segments later diversifying into more advanced forms of articulation as well as direct and indirect musculature, as seen in the flying lineages today. They likely supported the early beginning of forward oriented morphology including the beginnings of a leading edge re-enforcement (Figure 4.6 B and C) (Kukalová-Peck and Lawrence, 2004). Selection for thoracic winglets remains a topic of great debate and curiosity. A model based study will be presented in Chapter 6 of this thesis that will begin to explore the potential of flapping thoracic and abdominal winglets in an attempt to better understand this mystery.

4.3.3. Protopterygote Morphology - The Abdomen

The abdomen is presumed to have terminated in a single long filament which is consistent with morphology observed in the Archaeognatha, †Cercopoda and †Monura. As in the Crustacea, this filament is sometimes referred to as the telson. Arched cercal legs with terminal double claws were present on the eleventh (terminal) abdominal segment like in †Cercopoda, these being the precursor to cerci in the flying orders (Figure 4.3, 4.7) (Kukalová-Peck, 1985, 1998). The ninth abdominal segment supported the gonangulum, likely derived from endites of the early pancrustacean leg segments as they were also incorporated into the body wall or lost, while all previous segments had articulated smaller legs, much smaller than those of the thorax. These legs were reduced

Figure 4.6. A schematic diagram of the prothoracic winglets of Palaeodictyoptera: *Stenodictya lobata* (A) Odonatoptera: †Geroptera, undescribed species (B) and an exploded view of a representation of the most plesiomorphic state in terms of veinal characters in the Pterygota, a potential protowing model (C). From Kukalová-Peck and Lawrence, 2004.

Figure 4.7. An interpretation of the protopterygote abdomen at an oblique angle and winglets on left side omitted (left, modified from Kukalová-Peck, unpublished) and from a dorsal view (right, original drawing RJC based on Kukalová-Peck, unpublished).

to the leglet remnants (styli) in the present day Archaeognatha (Niwa et al., 2010) when they diverged from the common insect ancestor (pre-protopterygote) and were also reduced or lost in the Zygentoma and flying orders. An example of this comes from the fossil records of the †Diaphanopterodea which show small leglet remnants on the abdomen (Kukalová-Peck and Brauckmann, 1990).

Archaeognatha abdominal leglets appear to be used to prop up the abdomen when at rest or are used as ‘sliding skis’ when walking (Smith, 1970). Although not experimentally tested, the potential for abdominal leglets to help increase drag when falling or possibly help cushion the ventral landing observed in Chapter 2 and 3 exists. Considering this, longer leglets may have contributed to increased drag in a falling protopterygote, assuming similar behaviours to those documented in Chapter 2 and 3 for the Archaeognatha. The effects of legs on the flow around a falling protopterygote-like insect will be explored in Chapter 5 of this thesis to better understand the drag effects.

Palaeozoic juvenile insects, as well as modern day mayfly and stonefly larvae, are regularly documented with articulated abdominal, likely exite-based, winglet or gill like structures (Kukalová-Peck, 1985, 2008; Grimaldi and Engel, 2005). The protopterygote abdomen likely also possessed laterally articulated winglets to the ninth segment (Figure 4.7), although winglets on the tenth and eleventh segment may have existed as well. The function of these abdominal winglets is unknown but they could have been used as gills, swimming paddles (Shear and Kukalová-Peck, 1990) or even some type of thermoregulatory mechanism (Whalley, 1979; Douglas, 1981; Kingsolver and Koehl, 1985) in the semi-aquatic to terrestrial ancestor and were retained for some currently unknown purpose until full flight was achieved. Modern mayfly nymphs support

abdominal gills that are considered to be homonomous to insect wings (Kukalová-Peck, 2008). They are believed to be derived from the same structure as wings however their mechanism of articulation is slightly different, as the epicoxal pleuron is folded and fused to the tergum as a paranotal lobe, while the corresponding exite (winglet) kept its musculature attached to its base but is located in the resulting embayment (Figure 4.5 B). This is in contrast to the thoracic winglets which evolved such that the musculature of the exite (winglet) base became incorporated into the pleuron itself, forming eight sclerite rows across three separate sclerite columns, the Proaxalaria, Axalaria and Fulcalaria, which also help to protect the blood channels beneath them (Arnold, 1964). The morphology of the ‘protowing’ and abdominal winglet (exite) was published by Kukalová-Peck (1978) and since has undergone extensive study and confirmation which has resulted in a revised abdominal model (Figure 4.5 B) and, at the time of writing, a detailed account and consolidation of publications of the exite-wing origin and wing homologization across the Insecta by Kukalová-Peck is expected in the near future.

The abdominal musculature was likely much like that of modern Archaeognatha, as well as the †Monura and †Cercopoda. Dorsal longitudinal musculature within the abdomen including the twisted rope-like muscles used in rapid abdominal flexion for vertical and forward jumping (Sturm, 1955; Evans, 1975) (See Chapter 1, Figure 1.5) were likely present in the protopterygote but reduced and lost in the Pterygota + Zygentoma (Thysanura). Evidence of this musculature is found in the dorsal-ventral curvature of Archaeognatha, †Monura and †Cercopoda in death (Figure 4.8). The ability to perform rapid abdominal flexions to produce a vertical or forward jump when startled is also similar to the escape response of many modern malacostracans (specifically

species in the Super Order Syncarida) (Evans, 1975; Manton, 1977; Shcherbakov, 1999). As insects began to specialize for aerial life the complex, abdominal jumping musculature may not have been used as frequently. Combined with potential extra energy likely needed to stay aloft while supporting this musculature, and the presumed expense of maintaining and powering such musculature, the reduction and loss with further flight specialization could logically follow. It is worthy to note that the Zygentoma do not share the jumping musculature as they likely diverged from the proteropterygote ancestor to adopt a more cryptic, terrestrial lifestyle and survival *via* utilizing their unique legs, leg articulation and thoracic musculature for running (Manton, 1972). Unconfirmed reports of abdominal rope-like musculature remnants have been reported in mayfly larvae, by E.L. Smith (Kukalová-Peck 1987; Sturm and Machida, 2001) but have yet to be verified.

Figure 4.8. Fossil †Cercopoda show a characteristic body curvature in death due to the specialized abdominal musculature, similar to Archaeognatha. Original photo, specimen courtesy of Jarmila Kukalová-Peck.

4.4. *The Protopterygote and Potential Morphometrics*

The result of the complete morphological assembly of the descriptions in the above sections is presented in Figure 4.9 and 4.10. These original drawings are presented in detail based upon the current knowledge available, but remain open to potential modification as new discoveries are made. Although a potential protopterygote insect has been outlined in the above sections, a method of characterising such an organism for comparative study must now be established. Defining the morphological parameters in terms of body size and expected biomechanical performance becomes a possibility using allometric scaling (Vogel, 2003). The potential size of the protopterygote insect is a topic of debate but it has been suggested that they were likely larger than the majority of insect lineages today (Wootton, 1990). The †Cercopoda are described as larger than their comparable, more basal, modern day flightless sister group (the Archaeognatha), at 35 mm in length (Kukalová-Peck, 1998, 2008). As they are considered to be nearest the protopterygote insect, their availability only as somewhat two dimensional fossils is not ideal for establishing what the animal's other morphometrics, such as weight and body width, were in life. Since the Archaeognatha are similar in morphology, albeit smaller than a †Cercopoda, they could be considered a sufficient proxy to predict the potential size range a †Cercopoda, and subsequently a †Cercopoda-like protopterygote insect, might occupy. From the collected morphometric data of two species of Archaeognatha, *Pedetontus saltator* and *Trigoniophthalmus alternatus*, employed in the experimental studies of Chapter 2 and 3, the possible range of weight, planform surface area, antenna and telson length, pro-thorax width, meta-thorax width and ninth abdominal segment width are extrapolated for a 35 mm protopterygote.

Figure 4.9. An interpretation of the putative protopterygote insect before flight evolved. Original drawing (Kukalová-Peck, unpublished). The winglets on the left side of the insect are not included to show the leg and body detail.

Figure 4.10. A dorsal view of a protopterygote insect drawn using Archaeognatha body width to length ratios and winglets with forward oriented morphology on thoracic and initial abdominal segments. Original drawing RJC based on Kukalová-Peck, unpublished.

The morphometric data collected from the two species of Archaeognatha used in behavioural trials (see Chapter 2 and 3) was pooled then tested for normality and equal variances. Since the majority of the data was normally distributed and homoskedastic, regression analyses were performed. Regression analyses also require independence in samples, and given data were pooled, this assumption may have arguably been violated. Considering the data as a whole, which represents the Archaeognatha as a group, was deemed more appropriate for establishing a more specific prediction for a potential protopterygote ancestor per variable. The regression results reveal an allometric relationship for weight in relation to body length ($b=0.0791$) (Figure 4.11). The presence of scales, bristles, naturally broken antennae and telson as well as the specialised abdominal musculature for jumping may explain this result, as this may cause more variation in mass in respect to length. The regression results also revealed that isometric scaling exists for surface area and body segment widths, although the meta-thorax width appears to increase allometrically ($b=1.0521$) at a greater rate than the prothorax or 9th abdominal segment with body length (Figure 4.11 and 4.12). This may be due to the accommodation of specialized internal musculature, used in jumping, which extends from the abdomen into the thorax of the Archaeognatha. Antenna and telson length do not appear to be isometrically scaled, however due to the potential for breakage of these structures due to natural causes the maximum biological length of these structures may not be represented within the data. Furthermore, a larger antennae and telson may contribute to altering the centre of mass of a jumping or falling insect and may even become detrimental if too large. Not surprisingly, all velocity and time variables scaled allometrically in respect to body length (Figure 4.13).

Figure 4.11. The weight and planform surface area as a function of body length derived from two Archaeognatha species, *P. saltator* and *T. alternatus*. Data from both species are pooled (closed circles). †Cercopoda/Protopterygote weight and surface area are predicted from allometric scaling (open circle), Table 4.1. Red lines are 95% prediction intervals and blue lines are 95% confidence intervals.

Figure 4.12. The average antenna length, telson length, pro-thorax, meta-thorax and ninth abdominal segment width as a function of body length derived from two Archaeognatha species, *P. saltator* and *T. alternatus*. Data from both species are pooled (closed circles). †Cercopoda/Protopterygote antennae length, telson length and body segment widths are predicted from allometric scaling (open circle). Red lines are 95% prediction intervals and blue lines are 95% confidence intervals.

Figure 4.13. The landing velocity at 1 m, terminal velocity, velocity 10 and 20 mm after stabilization orientation, time to achieve stabilization orientation and time to descend 1 m as a function of body length derived from two Archaeognatha species, *P. saltator* and *T. alternatus*. Data from both species are pooled (closed circles). †Cercopoda/Protopterygote velocities and time variables are predicted from allometric scaling (open circle). Red lines are 95% prediction intervals and blue lines are 95% confidence intervals.

From all of the presented regressions, a summary of the predicted range of values for the hypothesized protopterygote insect, including those of both Archaeognatha, species is presented in Table 4.1. These estimates are within 95% prediction intervals produced from exponential regressions from Archaeognatha measurements with the expectation that a †Cercopoda-like protopterygote with similar morphology would fall within these morphological ranges.

Table 4.1. A morphometric comparison of two species of Archaeognatha (Specimens from Chapter 2 and 3) and the expected corresponding values for a hypothesized †Cercopoda-like Protopterygote. The resulting expected values are useful towards biomechanical study of a †Cercopoda-like protopterygote insect. Data are averages ($\pm$ SEM). Predicted data based on allometric scaling.

	<i>P. saltator</i>	(n)	<i>T. alternatus</i>	(n)	Reconstructed †Cercopoda/ Protopterygote
Length (mm)	8.79 $\pm$ 0.75	136	7.72 $\pm$ 0.75	106	35 ⁽¹⁾
Weight (g)	0.0206 $\pm$ 0.002	136	0.0122 $\pm$ 0.001	106	0.724-1.058
Antenna Length (mm)	7.79 $\pm$ 0.46	40	6.55 $\pm$ 0.31	40	19.72-33.46
Telson Length (mm)	7.63 $\pm$ 0.39	47	8.82 $\pm$ 0.42	39	21.84-32.48
Planform ventral Surface Area (mm²)	24.7 $\pm$ 1.96	19	17.04 $\pm$ 1.73	19	296.95-376.77
Pro thorax Width (mm)	1.11 $\pm$ 0.10	29	1.28 $\pm$ 0.10	20	5.05-5.77
Meta Thorax Width (mm)	1.54 $\pm$ 1.11	29	1.87 $\pm$ 0.90	20	8.68-9.83
Ninth Abdominal Segment Width (mm)	0.70 $\pm$ 1.14	29	0.84 $\pm$ 0.94	20	3.24-3.62
Velocity at 1m (m/s)	2.59 $\pm$ 0.10	20	2.84 $\pm$ 0.09	20	4.43-5.53 ⁽²⁾
Velocity (m/s) at 10mm after stabilization	1.25 $\pm$ 0.08	17	1.33 $\pm$ 0.08	18	1.9-3.58
Velocity (m/s) at 20mm after stabilization.	1.32 $\pm$ 0.08	17	1.40 $\pm$ 0.08	18	2.0-3.62
Terminal Velocity (m/s)	6.43 $\pm$ 0.22	20	6.24 $\pm$ 0.21	20	10.38-15.54
1 m Descent Time (msec)	523.7 $\pm$ 13.93	20	502.4 $\pm$ 9.40	20	309.33-376.25 ⁽³⁾
Stabilization Time (msec)	87.9 $\pm$ 9.05	20	101.22 $\pm$ 5.26	18	163.59-447.39

(1) From fossil measurements

(2) Calculated maximum velocity exceeds that of object at no drag velocity (4.43 m/s)

(3) Exceeds the minimum theoretical time to fall 1 m without drag (319.44 msec)

4.5. Exploring Insect Flight Scenarios

Predatory pressures on proptterygote insects, possibly feeding on soft branch tissue at the tips of low growing vegetation, may have been a selective pressure for the development of ‘active parachuting’ (see Chapter 2). Archaeognathan-like jumping from branches and stems into open air and falling to the ground/water surface in response to predatory pressures, or possibly as a means of transport to new food sources and locales, seems a likely scenario (Figure 4.14). The proptterygote insect supporting a long terminal filament and antennae would likely have benefited by the aid of these structures in stabilizing the pitch of the insect during its parachuting descent from a one meter distance (Wootton and Ellington, 1991). The righting behaviour, previously shown in the Archaeognatha in Chapter 2 and 3, and by Hasenfuss (2002) in the somewhat more derived Zygentoma (sister group to the pterygota), would likely also have been beneficial to the ancestral insect in regards to landing undamaged and recovering quickly to initiate further escape behaviours. This righting behaviour is also possible without the use of winglets, flapping or fixed, suggesting their presence is not essential for sufficient control while descending, a concept previously discussed by Hinton (1963) Flower (1964) and Wootton and Ellington (1991). Dorso-ventral flattening, as represented in the Zygentoma, is also unnecessary for aerial righting behaviour while descending from a one meter height (and even less, see Chapter 2 and 3) and is likely a result specialization after selection for a terrestrial lifestyle in the Zygentoma (Manton, 1972; Kukalová-Peck, 2008).

Figure 4.14. A conceptual drawing of a likely Silurian to Early Devonian Scene exhibiting early vegetation and protopterygotes in the early stages of flight evolution, including active parachuting and skimming. Drawing by RJC.

Body posture is important to consider when performing a controlled descent from vegetation. As previously shown in Chapters 2 and 3 (see also Hasenfuss 2002), a dorso-ventral curvature, shifting the centre of gravity to a lower, more ventral, position relative to the body during descent, helps with righting and maintaining orientation. This also has the added benefit of presenting a flat ventral surface, and potentially the maximum surface area of winglets, to the overall flow of air. Extant Insecta only have wings on the thorax in one or two pairs for thrust and/or steering, while the potential protopterygote insect likely had articulated winglets along its abdomen. By consistently presenting the ventral thorax to the oncoming flow of air during a fall the thoracic winglets, which were likely near the center of mass of the Archaeognatha-like body of the protopterygote (Evans, 1975; Shcherbakov, 1999), could have been the winglet group that was most likely to interrupt the oncoming flow of air to create drag or possibly even minor displacement altering thrust. This concept will be explored further in Chapter 6 of this thesis.

The loss of abdominal winglets and the retention and selection of thoracic winglets in the Pterygota remains an important area of biomechanical investigations of the evolution of flight in insects. Identifying and establishing the possible uses and pitfalls of abdominal winglets in a terrestrial environment could be essential to defining a parsimonious pathway towards flight in insects. Whatever the cause of the reduction, the procurement of abdominal winglets was likely an aquatic or semi-aquatic adaptation that was lost somewhere within the transition to a terrestrial environment (Shear and Kukalová-Peck, 1990; Kukalová-Peck, 2008).

4.6. Establishing the Physical Boundaries of the Protopterygote

In this chapter I have described the potential protopterygote insect ancestor in detail as a whole and in parts. Here and in the preceding chapters, I have discussed how the posture during falling, the appendages, overall morphology, including winglet movement, could be important variables to consider when attempting to define the general nature of the protopterygote insect. Although I have presented data here (Table 4.1) that provides a starting point for models and experimentation, using the data still requires a cautious and informed approach. For example, the resulting range of landing velocities (V_f) from a 1 m drop presented in table 4.1 appears reasonable, but when cross checking with expected velocities of an object under no drag conditions (a vacuum) using the equation:

$$V_f = \sqrt{2gh}$$

Where h is the drop height and g is gravity ($9.81 \text{ m}\cdot\text{s}^{-2}$), the maximum velocity any object can achieve from a 1 meter fall is 4.43 m/s. This is the slowest extrapolated velocity of landing for a †Cercopoda-like protopterygote based upon Archaeognatha data (Table 4.1, Figure 4.13).

Likewise, when considering the Archaeognatha-based range of times predicted to fall (T_f) 1 m they again appear reasonable but when cross checked with predicted time to fall in no drag conditions using the equation:

$$T_f = \sqrt{\frac{h}{g}}$$

where h is the drop height and g is acceleration due to gravity ($9.81 \text{ m}\cdot\text{s}^{-2}$), the minimum time required to descend 1 m is 319.28 msec. Once again, referring to Table 4.1 and Figure 4.13, the predicted range extends below the minimum predicted time required.

Ensuring that potential issues like those outlined above are accounted for before undertaking physical model testing is essential to limiting the possibility of conflicting or erroneous conclusions. These conflicts can, however, be resolved in a number of ways. The predicted landing velocity range and theoretical maximum velocity do overlap which suggests that a wingless insect with †Cercopoda-like dimensions may not experience a sufficient amount of drag to slow down its descent from a 1 m drop height. In such a case, flapping winglets may have served to compensate for the drag deficit present if only the body planform and orientation are slowing descent. It has not escaped my attention that this is a potential selective force for the retention and possible enlargement of the winglets. The predicted 1 m descent time range overlaps with the theoretical minimum time required, however, it also extends to well over the minimum time, which may indicate that there is likely more to the drag experienced by such an insect than predictions show. Puzzles that appear from concepts such as these are best solved by either testing the actual animal in laboratory experiments; an obviously impossible task considering that there are no living †Cercopoda or protopterygote insects, or employing physical, articulated, appropriately scaled biomechanical models.

The reconstructions in this chapter rely heavily on the published work from Dr. Jarmila Kukalová-Peck and her co-authors. Although this work is peer reviewed, many authors disagree with the conclusions of her papers and the evolution of wings from exites (see Béthoux et al, 2008). The putative protopterygote insect described here is the

most likely candidate based upon the extant origins of wings and the suggested phylogenetic placement of the †Cercopoda. Barring the discovery of a fossil protopterygote, or insect nearest to such a stage in evolution, this reconstruction may be the best representation yet of the earliest flapping winglet insects.

Chapter 5: Flow visualization and drag measurements of a putative protopterygote insect body

5.1. Introduction

Using physical models to answer biological questions has been common practice for many decades especially when the living organism is not available because it is extinct, inaccessible, protected or is simply unfeasible for study (e.g. too small or large or requires unusual environmental controls). In this case and particularly for biomechanical analyses the construction of morphologically and behaviourally similar models is very useful. The usefulness of both physical and mathematical models, was succinctly explored in a recent publication by Koehl et al., (2011), where they emphasize the importance of the adjustability and customizability of a physical model of an extinct theropod dinosaur (*†Microraptor gui*) in their study of this animal's gliding ability. A physical model allows specific variables to be changed without altering others; something not always achievable with a generally unpredictable living organism (Rayner and Wootton, 1991; Koehl et al., 2011; Koehl 2003; Hutchenson and Allen, 2009). Physical models can also allow a large number of variables to be explored in a systematic way (Emerson et al., 1990) including the exaggeration of certain characteristics. This “customizability”, therefore, brings an important caution – that it is important the experimenter know the limitations present within the true biological operating realm so that appropriate conclusions can be drawn from their studies.

5.1.1. The Reynolds Number of an Extinct Protopterygote

A scaled model, operating at the same (or near) Reynolds number (Re) as the real organism, regardless of the medium (air or water), will be in the same flow regime, and therefore, will experience and produce the same flow patterns in the fluid within which it is immersed (Vogel, 2003). Reynolds number is a non-dimensional ratio expressing the relative effects of the inertial and viscous forces experienced by an object moving through a fluid. Reynolds number is calculated as:

$$Re = \frac{\rho UL}{\mu}$$

Where U is the velocity of the fluid in relation to the object or model, L is the characteristic length of the object or model, and ρ and μ are the density and dynamic viscosity of the fluid. Of these variables the characteristic length and velocity are of particular importance when it comes to reconstructing a fossil organism. The characteristic length is chosen as the linear dimension of the object moving tangentially to the fluid. The velocity can be that of the object moving through a stationary fluid or of the fluid moving over and around a stationary object. Biological organisms rarely behave as static objects, and as a result, the Reynolds number is usually presented as a range of values and the results of the behaviour of the fluid are usually a generalization of the flow within that Reynolds number range (Williamson, 1996). For this study, allometric scaling equations based on Archaeognatha morphology and behaviour (Chapter 4) were used to estimate Re for the hypothesized protopterygote insect model and velocities were derived from observed velocities of Archaeognatha specimens during 1 m falling trials (Chapter 2).

Kingsolver and Koehl (1985) reported a Reynolds range of 100-1000 (L =thorax width) using various fixed wing and wingless six-leg insect models supporting thoracic fixed wings in a wind tunnel. They found that in order to significantly increase drag in their straight body model, which would slow the descent of the insect falling through the air, it required fixed winglets 20% the size of the body length. However they did not report differences in body orientation or shape in their study. A parachuting organism generally tries to achieve a slower descent velocity, which carries benefits upon landing *via* reducing potential injury and allowing for faster recovery for escape (Ellington, 1991; Koehl, et al., 2011). In order to maintain a decelerated descent in a wingless insect the ability to maintain stability becomes a key component (Kingsolver and Koehl, 1994; Ellington, 1991; Koehl et al., 2011). A falling Archaeognatha can achieve a stable body position while falling or jumping a short distance (see Chapter 2 and 3) producing a right side up landing. The body orientation and posture during falling, therefore, appears to be a significant feature of these insects when airborne that may assist in their stability as the protopterygote ancestor was likely to exhibit the same musculature (Kukalová-Peck, 1998, 2008). The posture of the falling Archaeognatha may result in a fluid structure (i.e. aerodynamic flow) that may also have been similar to that of a parachuting †Cercopoda-like protopterygote. As stability is achieved in Archaeognatha without winglets, the resulting flow could also provide a benefit to a crudely flapping winglet, which, in turn, could be useful for manoeuvring, corrections or displacement *via* thrust production (Flower, 1964; Lighthill, 1975; Maynard Smith 1952; Saunders, 1977) if they are not primarily used for stability as previously suggested (Hinton, 1963; Kingsolver and Koehl, 1985; Wootton and Ellington, 1991). As described in Chapter 2 and 3, a falling

Archaeognatha adopts a dorso-ventral curvature of approximately 119° (between thorax and abdomen) while falling or while in aerial transit after a jump. As the early pre-flight insects likely possessed a similar internal musculature and body plan (Kukalová-Peck, 1998, 2008) this curvature should be incorporated into the protopterygote model system.

5.1.2. Measuring Drag on a Physical Model

Given the complex shape of biological organisms, especially an organism like the hypothesized protopterygote, drag force must be empirically determined; it cannot be predicted or estimated based on geometry. This is because many factors contribute to the net drag experienced by an object moving through a viscous fluid including velocity, body shape and size, and the properties of the fluid itself (density, viscosity, temperature, etc). Deformation of the object and shearing in the fluid layers moving around the object produce complex pressure variations around the body. As Fox et al., (2006) eloquently states, calculating the net drag force by mathematically integrating pressures or stresses on the body is exceptionally difficult for anything except a simple geometric shape. As a consequence, geometric objects (e.g. spheres and cylinders) are often the model design of choice for many biomechanical studies. Furthermore, even if calculations are achievable, Fox et al., (2006) notes a tendency for results to be “erroneous and confusing” as they cannot account for such phenomena as boundary layers and flow separation. The low Reynolds number expected for the proposed protopterygote indicates that these insects are operating in a flow regime that is not well understood in terms of fluid behaviour; it is a regime dominated by flow separation and vortex formation (Kingsolver and Koehl, 1985;

Vogel, 1981; Ellington, 1991). The flows observed are also expected to be significantly affected by even small changes in insect size and velocity which can result in large changes in measured drag. To this end, establishing a likely size for the protopterygote will always be of great importance to biomechanical investigations. As a result of these crucial realizations and difficulty involved in bringing substance to a mathematical model, the use of a custom build force measurement system *via* a load cell and bioamplifier is required to record direct drag force measurements of the dynamically scaled model while mimicking the parachuting behaviour observed in *P. saltator* and *T. alternatus* in Chapter 2 and 3.

Understanding aerodynamics, and the evolution of insect flight in general, has regularly been pursued through the use of constructed, physical models that have only partially mimicked the form of the hypothesized protopterygote insect, and, often these models are based on speculation of what a basal insect may have been from the literature at the time, see Flower (1964), Kingsolver and Koehl, (1985), Wootton and Ellington (1991) and Hasenfuss (2002). Many of these models were further limited by the available published material and construction techniques at hand. The constructed model presented in this chapter is the most up-to-date design based on morphological, behavioural and kinematic data available. Many of the more intricate and precise morphological details are achieved in physicality through the 3D rapid prototyping process on this new †Cercopoda-like model as well as postural and kinematic variables from living Archaeognatha specimens.

5.1.3. The Scope of this Chapter

In this chapter I explore the biomechanics of a variety of constructed models, from a simple cylinder to bluff body forms based on an extinct †Cercopoda-like protopterygote (Chapter 4) in two different anatomical positions, and a complete wingless putative protopterygote insect with appendages, antennae and telson. This chapter will define the differences between each model in regard to drag forces encountered when presented to oncoming flow in a similar fashion to a falling Archaeognatha (Chapter 2 and 3) and the fluid flow around the model using dye flow and PIV techniques. Through these methods, I will begin to uncover some of the aerodynamic mechanisms that may be present in the flow around both a falling Archaeognatha and around the likely flightless ancestor to the Pterygota as I present a likely Reynolds range for this early insect.

5.2. Methods

5.2.1. Model Design and Construction

Protopterygote models were rendered *via* 3D modeling and animation software (3D Studio Max® 8, Autodesk Inc., San Rafael, CA, U.S.A.) by defining and building each appendage and body component separately (Figure 5.1). A two dimensional drawing of a †Cercopoda from Kukalová-Peck (1987) was imported and used as the initial reference for the body and limb layout of the protopterygote insect. Double claws were modeled for the terminus of the palps of the protopterygote in freeform using leg claws from the †Cercopoda drawing as a reference. The three dimensional aspect of the insect within the digital environment was created and scaled using previously determined length to width ratios of Archaeognatha specimens (data from Chapter 2 and 3) while the dorso-ventral dimensions were based on the †Cercopoda drawing. Each body feature was created vertex by vertex using carefully placed insertions and joining procedures until a complete element was created in the digital three dimensional space (Figure 5.1). An exterior skin was then applied using a built in surface modifier and any gaps and surface detail was corrected/created *via* manual vertex adjustment and alteration. The final assembly of the complete †Cercopoda-like protopterygote models (in a straight and curved body configuration) is shown in Figure 5.2 and 5.3. Note: the physical model is built from individually created pieces of the insect, rather than one single large piece, to facilitate rapid prototyping (see below) and allow for minor customization without digital modification.

Figure 5.1. A screen shot of the working area in 3D Studio Max 8 depicting the antennal base modeled from the †Cercopoda drawing from Kukalová-Peck (1987). The outline was created from individual vertex insertions and where it was then made into a fully enclosed shell with surface modifiers.

Figure 5.2. Images of the hypothesized †Cercopoda-like protopterygote insect in a straight body configuration. Winglets, telson and antennae are not rendered.

Figure 5.3. Images of computer rendered model of the hypothesized †Cercopoda-like protopterygote insect with a curve body posture, approximately 119° between the thorax and abdomen. Winglets, telson and antennae are not rendered.

Rendered components were exported as Stereolithography files (.STL) and were rapid prototyped in ABS (acrylonitrile butadiene styrene) plastic using a Dimension SST 1200es rapid prototyping machine. The body of each model was created in two parts, an anterior and posterior half, due to limitations in the size of the printing chamber in the Dimension SST printer (Figure 5.4). The two halves of each model were joined with silicone to create the body of the model after removing a small amount of material to create clearance for an aluminum sub-structure that allowed rigid mounting of the model to a sting for flow visualization testing. This substructure included the connection of a 12.7 mm diameter aluminum mounting rod through a drilled hole in the dorsal abdominal segments of the models (Figure 5.4). Limbs for the complete body model were attached to the assembled body using hot glue; the limbs were carefully adjusted into a splayed out position characteristic of a falling Archaeognatha (Chapter 2 and 3) as well as in the appropriate origins portrayed from the †Cercopoda and protopterygote reconstructions (Chapter 4). For the fully assembled model (Figure 5.5, 5.6), the antennae and telson were created using 3.4 mm diameter insulated solid copper wire nested inside 6.9 mm Tygon® tubing near their respective bases and held together *via* heat-shrink tubing. They were mounted to the model in their appropriate locations by drilling and securing with hot glue. These components were not created *via* digital design and rapid prototyping due to their awkward profile, difficulty in 3D printing, and size. The model, mounting rod and block were painted with light absorbing, water resistant, black paint (Krylon black #44290) to decrease laser light scatter during stereo dPIV imaging.

A cylinder model (“tube-bug”) was constructed from ABS pipe as a comparable geometric insect model. The cylinder was 385 mm in length including end caps and had a

Figure 5.4. Model parts are rapid prototyped in ABS plastic as individual components. The model bodies are made in two halves (Top) and are assembled with mounting rod and block before painting (Bottom). The mounting rod was custom manufactured and disconnects from the mounting block (MB) so models can easily be changed in the tow-tank. The assembled model body length is 38.5 cm.

Figure 5.5. Images of the assembled curved body model with legs, palps, antenna bases, and gonangulum. The entire model, including mounting rod and block, are painted with light-absorbing paint for stereo dPIV imaging.

Figure 5.6. Images of the assembled curved body model painted black with all appendages including antennae and telson.

61 mm outer diameter. End cap diameter was 64.5 mm however all imaging trials were completed along the 61 mm pipe diameter. An aluminum mounting rod and sub structure was installed to stabilize the cylinder for test trials and to attach it to the gantry sting for testing.

5.2.2. Test Facility

All flow visualization and drag measurements were performed in a custom built towing tank located at Carleton University, Ottawa, Ontario, Canada. The towing tank measured 4 m X 0.8 m X 0.8 m and was constructed of 12.7 mm glass. The glass tank rests on a frame constructed from 10.16 cm square hollow structural steel members. The entire length of the tank is unobstructed by vertical support rails (Figure 5.7) allowing uninterrupted visualization during model transit

A large gantry straddles the tow tank and is responsible for moving the models and imaging equipment along the length of the tow tank. The gantry consists of an archway (178 X 93 X 94 cm) that is pulled along pair of ground stainless steel rails that float on rubber cushions (for vibration isolation) on the tow tank frame. The archway is drawn by paired toothed belts driven by a brushless servo motor (with gear head) (Macron Dynamics, Croydon, PA, U.S.A.). The archway position, velocity and acceleration are controlled *via* a Danaher motor controller with National Instruments (Austin, TX, U.S.A.) hardware and Measurement and Automation Explorer software (V. 4.5.1f1, Apache Software Foundation, www.apache.org). Models are attached by their mounting rod to an adjustable sting fixed to the gantry arch with an aluminum mounting

Figure 5.7. Photographs of the towing tank facility located at Carleton University, Ontario, Canada. Glass dimensions are 4m (L) by 0.8 m (W) by 0.8 m (H). The large glass planes allow for continuous viewing of the models in transit.

block (72 X 44.8 X 19.3 mm) (Figure 5.8). All steel components were painted matte black before assembly to reduce risk of laser light scatter from the tank structure during imaging. Additional light shields were constructed and installed on the gantry archway to further contain laser light while testing was underway.

Descriptions of the design and construction of additional custom components required to perform stereo dPIV imaging and dye-flow visualizations are presented in Appendix A.

5.2.3. Drag Force Measurement

Drag forces measured for all models used for this project were collected *via* a custom built force transducer system composed of a load cell and a custom built DC amplifier. The load cell was constructed from a (75 X 12 X 12 mm) aluminum block with material removed from the centre portion to create a thin-walled (1 mm) c. 15 mm long, channel. Loads applied to one end of the aluminum block therefore caused deformation of the thinned regions in the centre that were detected by thin-foil type resistive strain gauges (1 k Ω) bonded to adjacent surfaces of the block at the thinned regions. The paired strain gauges were connected in a Wheatstone bridge configuration. The amplifier unit supplied an excitation voltage to the bridge and changes in resistance of the strain gauges produced a change in voltage proportional to the load applied to the cell. A multimeter (Agilent True RMS Multimeter, Model U1233A) was used to display the amplified voltage changes from the load cell.

Figure 5.8. A schematic diagram of the tow tank gantry archway with PIV hardware end on (Top) and from above (Bottom). The tow tank itself is defined by the blue box within and under the archway.

To calibrate the force measurement system, the model, under water, was attached directly to the load cell which was attached to the sting of the gantry system. A light-weight cable (SubOZero, braided nylon tip-up ice-fishing line, 25 lb. test) was attached to the attachment block of the model that was routed *via* pulleys on a second sting to a location just outside of the tank. Ten separate masses ranging between 20 g and 960 g were then suspended from the cable to apply known forces to the model attached to the load cell. The amplifier output was zeroed with the model unloaded and removal of masses from the model produced a zero output from the amplifier. Because the calibrations weights loading the model hung under the influence gravity, force (Newtons) was calculated using:

$$F = mg$$

where m = the calibration mass in grams and g = the gravitational constant (9.81 m/s^2). Voltages read from the multimeter and the applied forces were then plotted and linear regression was performed to obtain a calibration equation. All calibrations were linear with R^2 always > 0.99). Calibrations were always repeated when models were removed and reattached from the sting. For force measurement trials, the calibration line and secondary sting were removed from the gantry and tow tank.

During towing, force increased as the model accelerated to constant velocity, therefore, the force recorded for a model was the maximum drag force detected during a run after the model reached constant velocity. This corresponded to at least 10 body diameters of forward displacement after acceleration to tow velocity was completed. The order of tow velocities tested was always varied wherein one datum for one speed was

always followed by a different tow speed. Force measurements for each tow speed are presented as an average of five runs of the maximal drag force reading, $\pm$ 95% confidence intervals.

5.2.4. Dye Flow Visualization - ABS Cylinder, Straight and Curved Body Models

All models were subjected to dye flow image experiments at 0.0025, 0.005, 0.01, 0.015, 0.020, 0.025 and 0.03 m/s tow speeds, corresponding to a Reynolds range of approximately 145-1930. For appropriate imaging, protopterygote models were fixed to the sting with their body parallel to the bottom of the tow tank and their head closest to the imaging camera. A digital SLR camera (Nikon D60 SLR with a AF-S DX Zoom-Nikkor 18-55mm f/3.5-5.6G ED lens) was focused on dye flowing from the ventral thoracic region with the movement of flow past the sidewalls of the model towards the dorsum. The ABS cylinder model (“tube-bug”) was mounted horizontally in the same fashion as the body models. The resulting flow behind the models was photographed over the distance of the tank as the model travelled at the given test speed and photographs were taken consecutively. Additional lighting was provided by two halogen lamps positioned above the model (no flash was used on the camera).

Malachite green dye (1.5 g mixed in 200 ml of tap water) was injected into the flow with a syringe attached to flexible tubing terminating with a 60 cm long rigid stainless steel tube (3.2 mm OD, 1.5 mm ID). Dye was injected into the flow in two different locations (separate for each run): into the water ahead of the model that

intercepted the dye bolus and into the boundary layer on the ventral side of the models. The tank was allowed to settle for at least 2 minutes after each test run.

5.2.5. Stereo Digital Particle Image Velocimetry

Quantitative flow visualization was achieved using stereo digital particle image velocimetry (stereo dPIV). A light sheet was generated from the output of a twin head pulsed Nd:YAG 220 mW laser (New-Wave Laser, Model YAG200-NWL) and focused into a c. 1 mm thick light sheet measuring c. 15 cm wide at the model. The light sheet was reflected off of a front coated mirror at an angle of 45° into the tow tank through the bottom of a half-submerged Lexan® boat (Appendix A) fixed to the gantry above the model under test. Water in the tow tank was seeded at a density of 4 mg·l⁻¹ (9 grams in 2240 l) with hollow glass spheres (TSI Inc. model 10089, diameter 8-12 µm). The seed particles remained suspended in the water (were effectively neutrally buoyant and particle sinking was negligible) for the duration of all experiments. Two synchronized Powerview™ Plus 4 MP cameras (Model 630159) with frame-straddling and Nikon AF Micro 60 mm f2.8 D lenses were used to capture stereo image pairs of the particles illuminated by the laser sheet. The laser light sheet (generated at 7.25 Hz) was synchronized with image acquisition from the cameras with a laser pulse synchronizer (TSI, Inc. Model 610035). Image acquisition, pre- and post-processing was performed with Insight 3G (TSI Inc., V.9.0.5) software running on a twin-Xeon processor based computer with Windows XP + Service Pack 3. The entire stereo dPIV system was supplied by TSI Inc. (Shoreview, MN, U.S.A.).

The model being imaged was attached to the gantry sting and the laser light sheet plane was positioned to such that it illuminated a c. 15 X 15 cm area of interest (Figure 5.8). The cameras were set at 23 cm apart (mounting base to mounting base) at an angle of 51° and were focused on the area of interest of the model. Given the particle seeding density cited above, the cameras could be focused and a uniform star-field like image could be viewed in real time upon illumination *via* the laser (Figure 5.9). Image pairs were captured with a ΔT sufficient to allow between 8 and 10 pixels of particle displacement between sequential images. Image pair ΔT was adjusted for each tow speed tested. Stereo dPIV images were analyzed for particle displacement by means of two-frame cross-correlation (FFT Correllator) using an interrogation window of 64 by 64 pixels. Erroneous vectors were filtered using the ‘local validation’ protocol within the Insight 3G software. Local validation compares vectors to their immediate neighbours to ensure the calculated path of motion is realistic for the specific volume of flow it resides within. This analysis yielded a 12.8 by 12.4 cm 3D flow field characterized by approximately 3200 water velocity vectors.

All models were allowed to travel at least 10 body diameters before imaging was initiated to ensure models had reached constant velocity after their start from an initial standing position. Analysis of the 3D vector fields was performed using MATLAB® Spatial and Time Series Toolbox (TSI Inc.) available within the Insight 3G software package and additional custom MATLAB software (MATLAB® V. 7.13.0.564) (see below).

Figure 5.9. Images from the left and right dPIV cameras captured simultaneously of the head and thorax area of the straight body model. The laser sheet is projected downwards 1 cm from the midline of the model in these images.

Stereo dPIV calibration was completed for each model tested and any time camera or light-sheet optics adjustments were made. Calibration was completed according to protocols specified in the Insight 3G software documentation. The Seteroautomapping protocol was applied to each calibration file (X2) to correct for disparity and to confirm calibration accuracy.

5.2.6. Confirming Expected Behaviour in the Flow Field

To confirm the function and accuracy of stereo dPIV measurements, test runs at different velocities were recorded with no model or sting in the tank and with only the sting in the tank but no model. These tests confirmed that the movement of the gantry at tow speeds of 1.0, 1.5, 2.0, 2.5 and 3.0 cm/s did not cause movement of the water in the tank and that the wake caused by the sting was small and did not influence flow where the model was positioned. Testing with a vertical flat plate also revealed expected behaviour in the flow field and confirmed that the processing and post processing methods employed do not artifactually alter the results.

5.2.7. Vector Field Post-Processing

The straight body and curve body models were imaged in four vertical zones (head to tail) with up to seven slices per zone (midline to 6 cm lateral of the model). Zone overlap was approximately 2 cm while the head zone for the straight model was horizontally displaced towards the ventrum of the model by approximately 3 cm to

capture more of the flow field occurring around the head as a whole (Figure 5.10).

Similarly, the head zone for the curve body model was horizontally displaced towards the dorsum by approximately 3 cm. Each model was adjusted *via* known physical dimensions vertically and horizontally using the previously described traverse setup to create the zone and slice for the location of interest. To reduce the amount of shadowing caused by the body intercepting the light sheet from above, the straight body model was mounted head up while the curve body model was mounted head down during testing. Each run consisted of ten captures and each capture creates a single three dimensional data file (.V3D format). Each capture is a replicate of the specific trial being run for a stationary model at constant speed, three runs were completed for each slice in each zone and the resulting producing thirty captures for each trial type. Processed image fields were analyzed individually for generalized trends however; 3D rendered figures presented in this chapter are generated from the 8th capture from the 2nd run of each tow velocity.

Figure 5.10. A rendering of the 4 imaging zones of the straight body model (top) at the midline from dPIV data. Each zone is represented by a different colour. (Middle and Bottom) A close up of the head region depicting vectors, both images are rendered using MATLAB®.

5.3. Results

5.3.1. Drag Force

The average drag forces throughout all the tested models ranged from 0.036 N for the straight body model at 1 cm/s to a maximum of 0.291 N for the legged model at 3 cm/s. Figure 5.11 and 5.12 show that, for all models tested including the cylinder, the largest amount of drag is generated when the models are at the fastest tow speeds (2.5 and 3 cm/s) but also that relative to the slowest tow speed (0.25 cm/s), there is a local peak around 0.5 to 1 cm/s. Also, the data show overall that the different models produced different amounts of drag.

At the faster tow speeds (2.5 and 3 cm/s) the models began to vibrate during transit down the tank. Subsequent testing (data not shown) found these vibrations to be related to vortex shedding from the models causing a resonant interaction between the movement of the model and the structure of the sting and gantry. As such, force data around 2.5-3.0 cm/s may not be entirely representative of the actual forces encountered by a towed model; methods to reduce and counteract the resonance are currently under review for this facility. For these reasons, the results presented at tow speeds over 2.0 cm/s will be discussed but will not be the main focus of this chapter.

The curve body model and cylinder produce the highest average drag forces at 0.25 cm/s, $Re = 160$, (0.0855 N and 0.0836 N) while the straight body model experienced lower average drag forces (0.0456 N) but not as low as the legged body model (0.038 N). When tow velocity increased to 0.5 cm/s, $Re = 320$, the legged model produced the highest average drag (0.1387 N) and the curved body (0.133 N), cylinder (0.1026 N) and

straight body (0.057 N) followed. From this velocity onwards the legged model consistently produced the highest average drag forces. The curved body model produced the second highest drag readings consistently while the cylinder and straight body model produced the third and fourth highest average drag forces for each subsequent tow speed (Figure 5.11 and 5.12).

The cylinder model produced significantly more drag than the comparable straight bodied model at tow speeds from 0.25-1.0 cm/s ($Re = 145-640$) (0.25 cm/s, t-test, $t = -5.774$, $P < 0.001$); 0.5 cm/s, t-test, $t = -8.232$, $P < 0.001$; 1.0 cm/s, t-test, $t = -8.05$, $P < 0.001$) and at 3.0 cm/s ($Re = 1735-1920$) (t-test, $t = -3.343$, $P = 0.010$) (Figure 5.11). The greatest contrast between model designs, and the most relevant to the concept of geometric objects as proxies for biological organisms, is the legged model vs. cylinder experiment (Figure 5.12). At tow velocities of 0.5, 1.0 and 1.5 cm/s ($Re = 320-960$) as well as 2.5 cm/s ($Re = 1600$) the legged model produced significantly more drag than the cylinder model (0.5 cm/s, t-test, $t = -6.532$, $P < 0.001$; 1.0 cm/s, t-test, $t = 7.730$, $P < 0.001$; 1.5 cm/s, t-test, $t = 7.839$, $P < 0.001$; 2.5 cm/s., t-test, $t = 2.820$, $P = 0.026$) (Figure 5.12). At the slowest tow velocity, 0.25 cm/s the cylinder experienced significantly more drag than the fully assembled model (0.25 cm/s, t-test, $t = -6.532$, $P < 0.001$).

The trend of the curved body shape vs. the straight body shape becomes apparent in Figure 5.11, as the average drag due to the curve body itself becomes significantly more at 0.5, 1.0, 2.5 and 3.0 cm/s (0.5 cm/s, t-test, $t = 6.761$, $P = 0.001$; 1.0 cm/s, t-test, $t = 11.159$, $P < 0.001$; 2.5 cm/s, t-test, $t = 4.274$, $P = 0.003$; 3.0 cm/s, t-test, $t = 2.487$, $P = 0.042$). The legged model also employing the curved body shape produces significantly more

Figure 5.11. Drag force measurements of the straight body model and a cylinder of comparable diameter (Top) result in higher forces produced by the cylinder. Curve body models also produce more drag force than the straight body models (Bottom). Values are averages $\pm$ 95% confidence.

Figure 5.12. Drag force on the legged curve body model is initially less than just the body alone but becomes greater than the body alone as tow velocity/Reynolds number increases (Top). Legged curve body models also produce more drag than a comparably sized cylinder at all but 0.25 cm/s (Bottom). Values are averages $\pm$ 95% confidence.

drag at 1.5 and 2.0 cm/s vs. the curve body alone (1.5 cm/s, t-test, $t = 4.0$, $P = 0.007$; 2.0 cm/s, t-test, $t = 4.743$, $P = 0.003$), (Figure 5.12).

5.3.2. *Dye Flow Experiments*

Observing the progression of malachite green dye around the cylinder, straight body model, curve body model and assembled legged curve body model provided insight into the overall fluid structure generated over and behind the models as they travelled down the tow tank. At the slow speed of 0.25 cm/s (0.0025 m/s, $Re = 145-160$), for all models, dye moved laminarily around the model and was shed from clearly defined separation points at the model posterior (Figure 5.13). Note the dye entrained in the boundary layer of the cylinder model that remains attached to the most posterior surface of the cylinder despite injection at the leading edge (Figure 5.13 A). Alternate shedding of vortices (a von-Kármán vortex street) was clearly seen for all models but especially so for the cylinder model (Figure 5.13 A). Vortex diameter, as expected, increased with distance from the model after shedding (downstream and out of frame in the figure). There was relatively little turbulence and mixing in the fluid regions behind the three models tested at this tow speed.

As tow speed increased to 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 cm/s, two observations were prominent. First, there was increased mixing (turbulence) in the area immediately behind the models (at the thorax-abdomen region), and second, the size of the wake behind the models increased, as evidenced in Figures 5.14, 5.15, 5.16, 5.17, 5.18 and 5.19. Flow around the legged model resulted in a larger cloud of turbulent dye being

Figure 5.13. Images of dye flow around the cylinder (A) straight body (B) and curve body (C) models at 0.25 cm/s (Re approximately 145-165). Dye streams in a laminar fashion off of all the models tested at this velocity. The saw tooth dye stream in C appears to be a result of gravitational effects on the dye clusters at this tow speed.

Figure 5.14. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 0.5 cm/s (Re approximately 280-325). Dye streams are mostly laminar with some mixing behind the cylinder model A.

Figure 5.15. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 1.0 cm/s (Re approximately 575-640). Dye streams within the flow become disrupted and mixed in a zone directly behind the dorsum or trailing face of the model.

Figure 5.16. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 1.5 cm/s (Re approximately 865-965).

Figure 5.17. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 2.0 cm/s (Re approximately 1155-1285). Legged body model images depict the model passing through a bolus of dye as opposed to dye being deposited on the ventrum of the model as seen in A-C.

Figure 5.18. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 2.5 cm/s (Re approximately 1445-1605). Legged body model images depict the model passing through a bolus of dye as opposed to dye being deposited on the ventrum of the model as seen in A-C.

Figure 5.19. Images of dye flow around the cylinder (A) straight body (B) curve body (C) and curve body with legs models (D) at 3.0 cm/s (Re approximately 1730-1925). Dye is caught in a mixed zone of circulation behind the dorsum of the model and is retained as the models are towed down the tank length.

Figure 5.20. Dye flow around the abdomen and thorax is drawn into the turbulent area behind the dorsum of the curved leg model. Dye collects and re-circulates in this area for the majority of the run. Tow velocity is 1.0 cm/s (Re is approximately 640) .

drawn in behind the model when compared to body models without legs. Dye was also observed being drawn along the dorsum from anterior to posterior in the curve body and legged model where it would be retained and become turbulent within the curvature of the legged model for much of the trial run (Figure 5.20).

5.3.3. Digital Particle Image Velocimetry (dPIV) of Body Models

Flow around the straight body model is strongest in the head region as much of the flow is near free stream velocity (Figure 5.21). The body creates a turbulent zone that extends from the dorsum out past the periphery of the imaged zone that is present at all tow velocities. The widest, and most influential part of the body on the flow, is the thorax and initial abdominal segments, as seen when contour plots are extended to the lateral edges of the body (Appendix B). When stream traces are inserted into the rendered flow field along the body at 1 and 3 cm from the midline, both near and away from the dorsum, they are drawn into a circulating flow pattern (Figure 5.22). The stream traces presented near the bulk flow region behind and beside the head, at 1.0 and 1.5 cm/s ($Re = 640-960$), travel into the free stream flow or become drawn down along the dorsum towards telson end of the model. Higher tow velocities, 2.0, 2.5 and 3.0 cm/s, result in stronger, more turbulent flow with less recognizable entrained flow structure (Figure 5.23, 5.24). This flow still appears to be drawn more posterior towards the minor dorsal curvature of the abdomen at the higher tow velocities when visualized with stream traces (Figure 5.24, 5.25). The turbulent change in stream trace flow pattern behind the model with increasing tow velocity is presented in Figure 5.25.

Figure 5.21. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure 5.22. A rendered image of the straight body model with the contour plot at 1 cm from the midline. The colour bar indicates the horizontal velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. Bulk Flow is moving at 0.015 m/s. Axis are in mm.

Figure 5.23. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03 m/s.

Figure 5.24. Rendered images of the straight body model with the contour plot at 1 cm from the midline. The colour bar indicates the horizontal velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. The bulk flow is moving at 0.03 m/s. Axis are in mm.

Figure 5.25. Rendered images of the straight body model with the contour plot at 1 cm from the midline. The red lines are stream traces 3 cm from the midline at **A)** 1.0 cm/s **B)** 2.0 cm/s **C)** 2.5 cm/s and **D)** 3.0 cm/s. Flow moves past the head as well as down towards the telson into the dorsal area of the abdomen. Stronger reversed flow is visible at lower speeds behind the thorax which is the widest part of the model. Axis are in mm.

The curved body model produces much of the same type of flow as was present in the straight body model, with zones of reverse or turbulent flow appearing behind the dorsum of the thorax and abdomen at all tow velocities. Flow is drawn out from the midline to the lateral edges as it circulates in the area behind the dorsum (Figure 5.26, 5.27). Stream traces originating near to the body result in caudal seeds being drawn out into the free stream flow. Rostral stream traces flow towards the dorsum of the thorax and out towards the free stream flow moving past the lateral edges of the model even at the 3.0 cm/s tow velocity (Figure, 5.29). Curve body models appear to maintain more of this reversed flow pattern from head to tail through the higher tow velocities, unlike the straight body model (Figure, 5.30). However, like the straight body model the flow is still more turbulent at the higher tow velocities, 2.5 and 3.0 cm/s. As imaging of the contour slice moves out laterally from the midline, the intensity of reverse flow decreases transitioning towards the free stream direction and velocity. Supplemental figures in Appendix B and C demonstrate this change through colour contour plots of the tested slices and extracted velocity data (Reverse flow is indicated by positive values in Appendix C figures).

Figure 5.26. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure 5.27. A rendered image of the curve body model with the contour plot at 1 cm from the midline. The colour bar at the side indicates the velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. The bulks flow is moving at 0.015 m/s. Axis are in mm.

Figure 5.28. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure 5.29. Rendered images of the curve body model with the contour plot at 1 cm from the midline. The colour bar at the side indicates the velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. Bulk flow is moving at 0.03 m/s. Axis are in mm.

Figure 5.30. Rendered images of the curve body model with the contour plot at 1 cm from the midline. The red lines are stream traces 3 cm from the midline at **A)** 1.0 cm/s **B)** 2.0 cm/s **C)** 2.5 cm/s and **D)** 3.0 cm/s. The reverse flow (towards the dorsum) is preserved with the curved body shape as tow velocities increase when compared to the straight body model. Axis are in mm.

Legged models exhibited similar flow overall to the curve body model, producing a zone of reversed flow extending out from the dorsum (Figure 5.31, 5.32) which is again seen up to 3.0 cm/s (Figure 5.33). Stream traces demonstrate the legged model's ability to retain the pattern of reversing flow to the highest tow velocity (Figure 5.34). The addition of legs results in a greater volume of reversed or turbulent flow when compared to the legless curved body, extending out as far as 6 cm from the midline (Appendix B, Figure B.106. and Appendix C, Figure C.21). The legs appear to "straighten" the flow allowing the stream traces to move out from the midline laterally to a greater degree, thus entraining the flow more-so, behind the body. The continued flow pattern is demonstrated through the progression of tow velocities in Figure 5.35. The flow above the tip of the abdomen and below the head is not imaged due to the constraints of the physical dimensions of the model within the tow tank.

Figure 5.31. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 0.015 m/s bulk flow.

Figure 5.32. Rendered images of the straight body model with the contour plot at 1 cm from the midline. The colour bar indicates the velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. Bulk flow is moving at 0.015 m/s. Axis are in mm.

Figure 5.33. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 0.03 m/s bulk flow.

Figure 5.34. Rendered images of the legged curve body model with the contour plot at 1 cm from the midline. The colour bar indicates the velocity for specified contour colours and the red lines are stream traces depicting the movement of the flow. Bulk flow is moving at 0.03 m/s. Axis are in mm.

Figure 5.35. Rendered images of the legged curve body model with the contour plot at 1 cm from the midline. The red lines are stream traces 3 cm from the midline at **A)** 1.0 cm/s **B)** 2.0 cm/s **C)** 2.5 cm/s and **D)** 3.0 cm/s. Axis are in mm.

5.4. Discussion

The results of this chapter can be summarized as follows. First, I have shown that a model of a protopterygote insect traversing through a fluid produces drag and that the amount of drag produced depends on body posture, the presence of limbs, antennae and a telson. I have also shown that a cylinder of equivalent size to the insect model produces more drag than the insect modeled with a straight body configuration (an unnatural posture when falling, see Chapter 2) but less drag than compared with an insect modeled with a curved body configuration (as shown to be biologically relevant – Chapter 2, 3). Second, dye flow visualization suggests that low tow speeds ($Re = 145-960$) are dominated by laminar flow, vortex shedding and a non-turbulent wake but as tow speed increases ($Re = 960-1925$) that the wake becomes much larger and more turbulent. Dye flow visualization also showed that a splayed limb posture increases the size of the wake and affects the flow of fluid over the body. Third, quantitative stereo dPIV data confirmed that at Reynolds numbers estimated to be biologically relevant for the protopterygote, that the fluid flowing around the insect body decreases in velocity and reverses direction along the dorsal thoracic and abdominal region. Splayed limbs do not abolish this feature of the fluid flow and possibly preserve it at higher Reynolds numbers. These data collectively suggest that increasing drag to slow descent through the air by adopting a curved body posture and splayed limbs may produce a pattern of fluid flow/fluid structure that facilitated the evolution of thoracic/abdominal wings.

5.4.1. *The Protopterygote Reynolds Number*

The previously presented morphometric and velocity data can be interpreted in different ways to calculate a Reynolds regime for the Archaeognatha and the protopterygote insect. Traditionally, calculating Reynolds number for a flying insect involves using the chord length of the wing as a length parameter, since the wing chord is usually representative of the path of air flow that results in force production. Since the Archaeognatha and the models tested in this chapter do not have wings a different length parameter must be considered. One possible way to calculate Re is to use body length, however this is usually associated with gliding studies in which air flows along the length of the animal's body (Ellington, 1991). A falling, or actively parachuting insect, presents its ventrum to the oncoming flow of air and effectively experiences airflow from ventrum to dorsum (i.e. around the body rather than along the body), suggesting that an appropriate characteristic length measurement is the depth of the insect body or diameter of a cylindrical body shape. Considering this, I have presented in Table 5.1 Re calculations based on the body length and meta-thorax width (Data from Table 4.1) of the Archaeognatha species tested in the previous chapters and the predicted protopterygote insect for comparative purposes to previous studies. As well, I present potential Re ranges based on the meta-thorax depth (approximately 6.1 mm) of the reconstructed †Cercopoda from Kukalová-Peck (1987, 1998) in Table 5.2 which are somewhat less than those calculated using the meta-thorax predicted size range from allometric scaling for the †Cercopoda-like protopterygote. Despite the differences much of the predicted Re range overlaps in terms of the thorax width and depth estimates, and I suggest the most accurate

Table 5.1. A comparison of Reynolds numbers based on Archaeognatha velocities (See table 4.1, Chapter 4) and the expected corresponding values for a †Cercopoda-like protopterygote insect. Re is calculated using the kinematic viscosity of air at 20° C.

	<i>P. saltator</i>	<i>T. alternatus</i>	Reconstructed †Cercopoda/Protopterygote
Terminal Velocity Re based on body length	3743	3190	24060-36020
Landing velocity (1 m) based on Body length	1508	1452	10268 ⁽¹⁾
Re 20 mm after stabilization based on body length	768	716	4636-8391
Re 10 mm after stabilization based on body length	728	680	4404-8298
Terminal velocity Re based on metathorax width	656	773	5967-10116
Landing velocity (1m) Re based on metathorax width	264	352	2547-5650 ⁽¹⁾
Re 20 mm after stabilization based on metathorax width	135	173	1150-2357
Re 10 mm after stabilization based on metathorax width	127	165	1092-2331

(1) Based on maximum theoretical velocity under no drag conditions (4.43 m/s)

Table 5.2. Potential Reynolds numbers for a †Cercopoda-like protopterygote insect. Re is calculated using the kinematic viscosity of air at 20°C and the metathorax depth of the †Cercopoda, 6.1 mm.

	Reconstructed †Cercopoda/Protopterygote
Terminal velocity Re based on metathorax depth	4193-6278
Landing velocity (1m) Re based on metathorax depth	1790 ⁽¹⁾
Re 20 mm after stabilization based on metathorax depth	808-1462
Re 10 mm after stabilization based on metathorax depth	768-1446

(1) Based on maximum theoretical velocity under no drag conditions (4.43 m/s)

protopterygote Re range may span to the highest value predicted for the metathoracic calculations based on a 1 m drop height ($Re = 5650$), since terminal velocity was likely not achievable in the absence of tree-like vegetation during the Silurian period (See general introduction and Chapter 4). Amongst the predicted Re ranges, perhaps the most likely Re range the protopterygote may have operated within, as the selection for flight was beginning, is the range predicted from velocities achieved when stabilization occurred during descent (Table 5.1 and 5.2). If the putative protopterygote insect was able to stabilize its orientation, as the Archaeognatha do with body posture and limb splaying (Chapter 2), it seems that the ability to create drag to decrease descent velocity would occur in this range as well. Considering this, the tested velocities in this chapter for the protopterygote models predict a Re of up to approximately 2000, which makes the results applicable to both Archaeognatha at lower tow speeds (0.25-1.0 cm/s, $Re = 145-640$) (compare with Table 5.1) and the potential falling protopterygote insect at both the lower and higher tow speeds (1.0-3.0 cm/s, $Re = 640-1920$) (compare with Table 5.1 and 5.2).

Several previous studies have predicted the Re range of a likely protopterygote insect. Flower (1964) predicted a Re range of 1-10,000 based on body length of a gliding insect while Wootton and Ellington (1991) predicated a Re of 1000-7500 using body diameter from experimental trials using models with static wings. Kingsolver and Koehl (1985, 1994), testing models with static wings oriented 90° to the oncoming air flow in a wind tunnel, estimated a Re range of 100-10,000 based on body diameter. Ellington (1991) provides a detailed analysis of these past studies and the potential for Re to be calculated but focuses mostly of the lift to drag ratio and the gliding Reynolds range, using winglet chord as in flying insects as the main length measurement. As such he

suggests a Re of 10,000 or higher based on the likely protopterygote winglets, and a Re of 100-10,000 based on body length, as the realm in which a gliding protopterygote may have existed. As my experimental methods mimic a falling wingless insect that does not glide, my Re regimes are based upon both actual data from a basal insect group falling a 1 m distance and allometric extrapolation of a potential protopterygote insect. To my knowledge, no other study has attempted to calculate a Reynolds regime for a protopterygote in this manner. My results agree somewhat with the calculations by Kingsolver and Koehl (1985, 1994) however, I suggest that a Re over 5500 (using body depth and width as a length variable, Table 5.1 and 5.2) may be somewhat high considering the likely drop heights available to the early pre-flight insects and the potential †Cercopoda-like size of the likely protopterygote. Using body diameter to establish the Re regime of the non-flying protopterygote insects is perhaps the best way to attempt to define early aerial behaviours

5.4.2. Drag on the Protopterygote and Cylinder Models

Past studies on parachuting and gliding insects have employed the use of cylinders and cylinder-like insect models in an attempt to understand the aerodynamics of an insect (Flower, 1964; Kingsolver and Koehl, 1985; Wootton and Ellington, 1991). The drag experienced by a cylinder of comparable size to the protopterygote model was measured (Figure 5.11, 5.12) and was found to be different from the straight bodied model and the curved body model. The straight body model could be considered nearest to a cylinder in shape out of all the protopterygote models. Although the straight body model is nearest to

a cylinder in shape it still exhibits detailed segmentation within its body, a head and a tapered anterior to posterior profile which may result in less surface area overall being presented tangent to the oncoming flow. These features may contribute to why the resulting drag forces exhibited by the straight body model are less than the cylinder. Skin friction may also play a role in this outcome, as the protopterygote models have a slightly more sandy texture due to the method of construction and painting. Much like how a golf ball may fly farther due to the dimpled pattern altering the separation point of the flow around it (Streeter and Wylie, 1985; Vogel, 2003), the model may also be changing the flow in this way. When the cylinder model is compared to a more realistic insect model, the fully assembled protopterygote (Figure 5.12), it experiences significantly less drag than the assembled model at tow speeds of 0.5, 1.0, 1.5 and 2.5 cm/s ($Re = 290-960$), while at the slowest tow speed (0.25 cm/s) the cylinder experiences significantly higher drag forces (Sec. 5.3.1). The increase in surface area of the legged model vs. the cylinder is likely a major component of drag contribution as well as the curved body posture (see below). Although Reynolds number can be sensitive to a variety of factors, these results put into question the use of a cylinder as a proxy for an insect for biomechanical study within the specified Reynolds regime. If this result holds true through further verification, the use of models with biologically relevant detail in their construction, such as those employed here, will be essential for future biomechanical experimentation with insect models.

An Archaeognatha is stable while falling because it adopts a dorsal-ventral curvature; this study shows that this curvature has an effect on drag, falling velocity and descent time. Drag forces were significantly greater for the curve body model at lower

tow speeds (0.5-1.0 cm/s, $Re = 320-640$) when compared to the straight body model (Figure 5.16) (Sec. 5.3.1). This Re range is within the capability of the Archaeognatha at landing velocity, Table 5.2, and subsequently is reflective of the tested species ability to slow their falling velocity when alive (Chapter 2). Similarly, at a tow speed of 2.5 and 3.0 cm/s ($Re = 1600-1930$) the curve body model again experiences significantly more drag than the straight body model, however the drag forces at 1.5, and 2.0 cm/s are not significantly different for these models (Sec. 5.3.1). Why drag significantly differs at these higher tow speeds is unclear but may indicate that the separation of flow has become less laminar than that of lower tow velocities, resulting in more turbulent wakes.

Insects possess legs and other appendages such as antennae, cerci and other outward projecting structures. Such morphological features would presumably also affect the behaviour of air flow, and thus drag, while falling (Vogel, 1967; Ellington, 1991). When drag forces are compared between the curve body model and the full assembled curve body model (legs, antennae, telson included), the resulting drag forces are significantly higher for the assembled model at tow speeds of 1.5 and 2.0 cm/s ($Re = 960-1280$). The array of appendages present on the assembled model appears to increase drag exhibited on the model in its entirety at all tested tow speeds with the exception of 0.25 cm/s (Figure 5.12). However, it would appear the curvature of the body is the main morphological component of drag initiation, with the appendages further increasing this drag significantly at a Re of approximately 960 to 1280. Based on this data, Archaeognatha would seem to benefit more in terms of drag production from the curved body posture than from their appendages during a fall, particularly since the abdominal leglets are much smaller than the abdominal legs exhibited on the protopterygote model.

These drag results also suggests that once a falling protopterygote approaches, and surpasses, this Re appendages supported on the body may not make any real difference to its falling velocity. Further studies with Archaeognatha and limb ablations, as well as various model configurations are needed to test this hypothesis.

5.4.3. Dye Flow on the Wingless Protopterygote and Cylinder Models.

Dye flow experimentation helps qualitatively portray the general movement of the flow around a body. By identifying laminar and turbulent flow as well as reversal of flow direction, the mechanisms of drag as a body moves through the air (and water) can better be defined. When considering the cylinder models compared to the protopterygote models, the surface texture may be an important factor in explaining the results seen here. The cylinder clearly showed von Karman vortex streets (Figure 5.13 A-5.19 A), which are expected at Re 100-1000 (Van Dyke, 1982), while the insect bodies show more laminar boundary layers with evidence of some turbulence in the wake at higher tow velocities. Attached flow around the cylinder indicates a large friction component (fluid at low Re is highly viscous) to the drag produced, while the insect bodies are dominated by a pressure drag component. The total drag experienced by an object is the sum of the viscous/friction and pressure components, although each will contribute at different magnitudes for a given object in a specified Re range (Ellington, 1991).

When a boundary layer transitions from a laminar to a turbulent state, the drag decreases. This is regularly observed in plots of drag coefficients over increasing Reynolds regimes as the curve drops somewhat suddenly after initially rising with

increasing Re . Evidence of this phenomena is seen in the drag measurements (Figure 5.11 and 5.12) as a drop (or at least stalled increase) in drag force occurs for all models after reaching the 0.5 cm/s ($Re= 290-320$). Curve body models with and without legs may be exhibiting the friction component of the drag they experience more than the straight and cylinder models. By curving the body more surface area is presented for longer to the passing flow.

Legs and appendages are much like numerous small cylinders, each intercepting the oncoming flow and contributing to a large wake around the body. Figure 5.18 D demonstrates how a bolus of dye is caught in the turbulent flow generated by the legs and disperses into the flow behind the model as it continues down the tank. In Figure 5.15 D, dye deposited onto the ventrum of the model stays near the body and becomes entrained in the wake structure caused by the body itself. This suggests that the legs do not greatly interfere with the drag causing mechanism generated by the curve body alone but do contribute somewhat to drag on their own. Splaying out the legs and antennae, as Archaeognatha do (Chapter 2), is important since holding the legs in close to the body may interfere with the flow around the body itself as well as remove the drag contributing component of the legs encountering the flow lateral of the body. The possibility of legs being held close to the body altering the center of mass, and thus stability is also something to be considered, however further investigation is needed to confirm this possibility.

5.4.4. Stereo dPIV Experiments on the Wingless Protopterygote Models

The most significant observation from this work is the reversal of flow observed behind the model near the thorax and initial abdominal segments. This is a result of the fluid flowing around the top and sides of the model as it descends through the air (travels through the water). This flow pattern is not a transient one because 1) it was consistently observed at several tow velocities (Reynolds numbers) that overlap with the predicted Reynolds range for the putative protopterygote insect; 2) the pattern occurred in both the straight and curved body models and 3) was not destroyed by the presence of appendages. When flow re-circulates within a volume, it creates a low pressure region, in this case behind the insect at the thorax and abdomen (i.e. lift). Lift is proportional to circulation, expressed through the equation:

$$L = l\rho v\Gamma$$

where l is the length over which the vortex is formed (body diameter here), ρ is density of air/water, v is the velocity (falling/towing speed), and Γ is the circulation in the vortex. The possibility of more lift (more drag) can be created by increasing the width of the thorax.

Quantitative data from PIV work demonstrates how the area of reverse flow (the vortex) is isolated to between the midline and lateral periphery of the thorax on models (Appendix C). Legs help somewhat in extending this vortex zone outwards but, potentially, the most effective solution would be a larger thorax or extension of the thorax. The potential for selection of insect size also exists, as evidence of the loss or

decreasing of strength of the reversing flow appears at higher tow velocities, as seen in the green area of free stream direction flow behind the thorax in Figure 5.33.

The curvature of the body of the curve body models helps to contain the re-circulating vortex. The natural taper of the abdomen to the posterior limits the amount of flow that can be kept in circulation behind this area of the falling insect. A similar effect occurs at the head region, as the head is narrower in diameter than the thorax. The benefit of such a profile may be that the inability of a large re-circulating vortex zone to form around these areas prevents the insect from pitching due to the lift/drag that would be produced in either direction. The antennae and telson also contribute to stability and preventing pitching moments and are sometimes very long in Carboniferous insect lineages (Grimaldi and Engel, 2005). It may be possible that antennae and the telson help to counteract aerodynamic forces, either from the body and legs as shown here or from a set of flapping winglets during parachuting behaviours.

Shadowing effects from the curve body imaging methods prevented the complete analysis of flow immediately behind the dorsum at some slices, as opposed to the straight body model (Appendix B). In spite of this, the reversed flow is still evident, as observed using the dye flow technique, and remains through all tested tow speeds of curve body models where as in the straight bodies model it is essentially lost after 2.0 cm/s. The question of where this re-circulating vortex zone is present through an entire descent or whether it is transient (as in vortex shedding) remains. The results presented from dPIV work suggests it is continuously present or the volumetric reconstructions from multiple runs and stream traces would not have yielded these results.

5.4.5. Overarching Concepts

Flower (1964) found that cylinders glide best at an angle of attack of 30° or less when dropped from 15 m and that cerci and antennae help to stabilize a cylinder while attempting to glide. Hasenfuss (2002) found that a limbless apterygote insect model glides the best at a length around 8 mm and suggests various ways for a glide to be initiated from a fall including the complete loss of limbs for optimum gliding performance. As there are no records of non-arboreal Archaeognatha showing the ability to glide or direct their descent (Yanoviak et al., 2009) nor do any of the Zygentoma show gliding ability (Hasenfuss, 2002), the results of this study in regards to parachuting behaviour become more significant when accepting that neither the available vegetative heights nor the likely insect fauna of the Ordovician to early Devonian time periods cater to a glide being initiated by a falling protopterygote insect. The increase in drag forces as a result of the curvature of the body as well as the further increase in drag with the addition of legs and other appendages does, however, bring potential benefits. Maintaining this posture, while also holding a ventral side down position, results in lower falling velocities and increased hang time (as demonstrated in Chapter 2) and a greater likelihood of contacting a solid structure, like a branch, with a limb on the way down from a fall (Gravish et al., 2013; Ribak, et al., 2013). With the body and limbs contributing to drag to slow a descent, the addition of an articulated group of winglets may provide an additional benefit and potentially help slow a descent even further; the potential for drag increases *via* flapping winglets is explored in Chapter 6.

The dye and Stereo dPIV trials in this chapter involve a stationary, non-flexible and non-articulated model. Both the dye streams and particle flow about the tested models

are somewhat variable, particularly at the higher tow velocities/Re range. The figures provided in this chapter are essentially a snap shot of the flow occurring around the model within the given tow velocity well past the acceleration phase. Due the nature of flow about an object at the Re range tested, some variation is expected, as represented *via* confidence intervals in the extracted data figures for each trial. The colour rendered figures provide a glimpse of a single trial for each zone for each slice, they should not be considered as an expected exact flow for the specific trial, only an image of a likely regime with potential for some variation. However, it is the variation within the turbulent zone that contributes to the resulting pressure drag by lowering the pressure in relation to the surrounding fluid/air, thus creating the push-pull effects normally associated with lift on a wing.

5.4.6. Parachuting in the Past

As the †Cercopoda are an extinct group of wingless insects known primarily from the Carboniferous, but thought to have emerged from the same ancestor to the flying insects (+ Zygentoma) around the Silurian Period, it is possible their extinction may have been due to their evolution towards a non-volant lifestyle. Being larger than Archaeognatha, their parachuting ability may have been limited given the heights of early vegetation and trees of the Carboniferous. They may have been susceptible to damage if they were to fall even from moderate heights. The fact that the Archaeognatha continued despite having similar morphology and (probably) similar lifestyles could mean their size and other adaptations gave them an advantage. Although the †Cercopoda and

protopterygote insect likely could jump using an abdominal flexion like the Archaeognatha (Kukalová-Peck, 1987, 1998), it begs the question whether this type of jump was as successful and specialized as that which is exhibited in the Archaeognatha. It is possible the smaller size and potentially more refined jumping capability of the Archaeognatha, is what has allowed them to survive where the extinct groups were unsuccessful. Similarly, the Zygentoma survive *via* a specialized leg articulation likely a result of selection towards a more cryptic, terrestrial existence including a dorso-ventrally flattened profile with an ability to run very quickly (Manton, 1972, 1977). Of course, one avenue that trumps all of these life histories is the evolution of flight *via* selection for winglets; winglets that may have been able to sufficiently increase the drag, possibly by building off of the aerodynamic lift and drag mechanisms created by the body of a falling protopterygote. This is the subject of the following chapter.

Chapter 6: Functional significance of winglets on a pterygote insect ancestor

6.1. Introduction

Powered flight in nature is only possible if certain criteria are met. To fly under power an organism must possess 1) a wing: a lift generating aerodynamic structure that consists of a broad surface, is flat or somewhat flattened overall and exhibits a camber within its profile, 2) the means to move this wing in numerous degrees of freedom (articulation) and 3) the appropriate kinematics (control of movement) to harness and direct aerodynamic forces towards a useful end (forward movement, hovering etc). The oldest flying group of organisms, insects, possess these features and have adapted and modified their powered flight abilities in numerous ways. Just how highly controlled flapping wings were before they were selected for from relatively small, likely crudely flapping, winglets may be answered through model based biomechanical studies.

The high-lift generating aerodynamic mechanisms currently known to be employed by flapping insects include: the clap-fling technique (Weis-Fogh, 1973), the leading edge vortex (LEV) (Ellington, 1984, 1997, 1999), wing rotation and wake capture (Dickinson, 1994; Dickinson et al., 1999). The clap-fling mechanism is primarily present in insects with wings long enough that can extend over their thorax, such as hawk moths, encarsia wasps, locusts etc. (Weis-Fogh, 1973; Cooter and Baxer, 1977) on the upstroke to the point where they meet at the tips. The rapid initiation of the down stroke from this point allows the wings to peel apart causing a low pressure zone to occur effectively pulling the insect upwards during hovering flight. The LEV is essentially a rotating mass

of fluid formed along the leading edge of the wing and is considered to be responsible for 60-70% of the lift generated during the downstroke (Ellington, 1984, 1997, 1999). It is produced when air meets the sharp leading edge of the wing and abruptly separates into a spiral (vortex). The rotating mass of air creates a pressure difference (low pressure above at the LEV) that contributes to lift. As well, axial flow from the wing base to the tip as the wing is depressed is thought to stabilize the vortex. The LEV prevents or delays stall of the wing at high angles of attack and may also contribute during the upstroke when the stroke plane is horizontal and the wing structure is appropriate. The lift generated from rotation and wake capture occurs in lineages with faster wing beat frequencies (flies and bees) at the point where the downstroke is transitioning to the upstroke, as the wing is rotating about its longitudinal axis. The change of angle of attack and translation of the motion of the wing back through the wake of the previous stroke can produce lift through unsteady state mechanisms acting about the wing. Vortices generated by the downstroke can be captured by the wing as it rotates and moves into the upstroke, generating lift (Dickinson, 1994). These lift generating mechanisms are the four currently known and best described in regard to insect flight. However, the precursor wing behaviours to these mechanisms are not known nor are the means by which they arose in the early protopterygote insect. Biomechanical models and experimental testing, such as those presented in this chapter, may result in discoveries that could help to explain the origin and ultimate selection of such mechanisms

Also of significant importance to understanding how lift (and thrust) is produced is accounting for the form, structural basis and makeup of the wings. Examination of extant insect wings reveals a variety of shapes, sizes and 3 dimensional features that may

contribute to the overall aerodynamic performance of the insect in flight (Wootton, 1992; Dudley, 2000). Surface structure may include hairs, scales and other waxy microsculptures and are present in many forms on many extant pterygote lineages (Gorb et al., 2000). These features should be considered for further investigation of the aerodynamic performance of insect wings in flight. However, whether such structures existed during the time of the protopterygote insect currently remains a mystery. How a given wing behaves aerodynamically likely depends on how it is presented to the flow of air. Flying lineages present the leading edge of the fore and hind wing first, characteristic dorsal and ventral sides up and down and enact aerodynamic mechanisms in doing so, however the potential for a falling protopterygote insect to present its winglets in this manner may not be as easily accomplished if it did not exhibit the ability to self right (ventrum down), as demonstrated in Chapter 2.

The discovery of winglets on the prothorax of Palaeozoic insects that are described as essentially a “groundplan” in terms of veinal structure across all wings was reported by Kukalová-Peck and Lawrence (2004). This discovery, described as a “Eureka moment” as it corresponded with the past reconstruction of a potential groundplan state of the insect wing by Kukalová-Peck (1978, 1983), lent support and have helped validate the applied reconstructive methods and rules defined by the author. The insect wing and its articulation can be homologized across lineages further supporting the monophyly of insect wings and likely the only structure that can unite all the flying insect lineages phylogenetically (See Kukalová-Peck, 2008, 2009). An eight basal vein structure was present that begins to branch after leaving the basivenale (blood sinus), fusions and alterations take over as diversification began as discussed and supported by Kukalová-

Peck (1985, 2009). The first winglet structures likely present on the protopterygote supported the initial beginnings of a rigidified leading edge, potentially a function of prior use in a more aquatic ancestor, but other known structure within the winglets is somewhat speculative. From this initial heart shape ground plan structure the beginning of a more “forward” oriented morphology was adopted (Chapter 4, Figure 4.6), which potentially may have been useful for rudimentary lift or drag production.

The use of a flapping protowings by the protopterygote insect remains a topic of great interest to entomologists and evolutionary biologists alike, however little to no experimental work has been done to understand its functional significance. A number of possible hypotheses have been formulated on how a small flattened and articulated appendage may be used during a time of mostly freshwater swamps and low lying vegetation as habitat for early insects, Kingsolver and Koehl (1994) briefly describe some of these potential origins. As this appendage was likely possessed in the insect lineage as it transitioned from an aquatic lifestyle to a terrestrial one, the potential for a winglet to initially be used as a respiratory device is suggested (Edmunds and Traver, 1954; Wigglesworth, 1963b, 1976). By breaking the stagnant boundary layer surrounding the protowing-like structure *via* muscle powered movements, oxygen laden water is replenished for gas exchange through the tracheated winglet effectively forming a gill, much like some modern mayfly nymphs. With the evolution of trachea during the transition to dry land the diffusive properties of the gill may have no longer been essential resulting in the protowing being retained for another purpose. Courtship display has also been suggested as a possible use for an articulated array of protowings (Alexander and Brown, 1963), leading to selection for larger and stronger articulated wings until an

aerodynamic benefit took over. Thermoregulation is yet another possible function for a small protowing in a semi-aquatic to terrestrial environment. Whalley (1979) and Douglas (1981) discuss how radiation may be absorbed through an array of protowings which would help contribute to a longer period of locomotory activity or more vigorous movements. Kingsolver and Koehl (1985) explored this possibility with model protopterygotes in a wind tunnel and found that shorter wings on small insects generally do help increase body temperature, but not longer wings. As this scenario may help in allowing for vigorous movements of already established winglets *via* metabolic increases, it likely does not require extreme (or any) initial articulation of a protowing, as sessile insects are entirely capable of orienting their entire body to and within a sunny locale.

Further speculation of a terrestrial aerodynamic use involves the articulated protowings employment for locomotive or escape reasons. Marden and Kramer (1994) and Marden (1995, 2003) suggested surface skimming as a potential use for small winglets, providing enough thrust to allow the Paleozoic pre-flight insects to traverse the semi-aquatic landscape while still maintaining surface contact as well as escape from predators. This possibility stems from their observations of skimming mayflies with artificially shortened wings to 0.33 the length of the body. Kukalová-Peck (1987, 2008) maintains that running, crawling and jumping over and around the tangle of low and semi aquatic vegetation was assisted by flapping winglets, a behaviour observed in the mecoptera (Kukalová-Peck, 1987), which could be folded backwards and out of the way while moving through tight awkward spaces. These scenarios are all logical and potential uses for an articulated short protowing before selection began for aerodynamic purposes. It may not be unrealistic that the initial function of the protowing structure may have been

a combination of some or all of these possibilities, with selection for the increasing size of thoracic wings taking place as the flapping insects became more proficient at producing lift and thrust.

Previous work exploring the potential for small wings or winglets to contribute aerodynamically on a proposed protopterygote insect has primarily focussed on models with stationary wings. Much of the foundation for this work was aimed at achieving gliding from the models as it was believed gliding was an initial first step towards powered flight at the time (Ellington, 1991). Other factors that have limited potential experiments with flapping winglets include the difficulty in constructing an acceptable wing and articulation mechanism, a challenge suited to a more engineering based approach. Studies such as Kingsolver and Koehl (1985) have found that stationary winglets must be a length of at least 20% the body's length, to generate sufficient drag while falling to be of any use in slowing a descent and potentially reduce the possibility of damage upon landing (Ellington, 1991; Flower, 1964). Wootton and Ellington (1991) suggest that smaller insects (25 mm) similar to that which this current work is based around ($\dagger$ Cercopoda= 35 mm) may find benefit in short winglets by stabilizing a drop while parachuting.

Both Kingsolver and Koehl (1985) and Wootton and Ellington (1991) conclude that gliding is only realistically possible if the early protowinged insects were initially very large (>10 cm), even if outfitted with multiple short winglets along the entire body. Wootton and Ellington (1991) also found that caudal filaments were required to initiate a glide when a cylindrical body of various lengths was equipped with stationary small winglets along its length. The winglets were found to decrease the gliding sinking speeds,

from a 6.5 m drop adopting what was described as a “mushy” stall. The largest models, 80 and 160 mm in length, performed the best under these conditions, requiring trailing filaments to stabilize the pitch if posterior winglets were removed. Adjusting the winglet to various inclinations produced better glide angles in these models. The recurring theme from these past works appears to favour larger insects (>80mm) with small protowings for both an effective initiation of glide and to generate a high enough lift to drag ratio (L/D) to glide sufficiently. This may explain why some arboreal Archaeognatha and Hymenoptera (F. Formicidae) can direct their descent towards a tree base using their body profile only as short, rigid, winglet-like tergal structures would likely provide no real benefit (Yanoviak, et al., 2009). However, further exploration may be necessary to verify this concept as presented by Ellington, (1991).

At the time of writing, I am not aware of any study that has attempted to apply flapping winglets to a potential protopterygote model in regard to exploring potential avenues for flight origins in insects or any other reason for that matter. In this chapter I present qualitative and quantitative results that demonstrate the potential utility of a simple flapping array of winglets attached to a protopterygote insect model. I continue to employ novel modern model construction techniques, as presented in Chapter 5, to experimentally test the possibility that flapping winglets may provide aerodynamic benefits to a falling putative protopterygote insect. I measure drag forces on the flapping and fixed winglet protopterygote model as well as aerodynamic structures in the flow around the model that may yield important clues for thoracic wing selection and the evolution of flight. This scenario assumes a flapping insect has achieved the condition of

becoming airborne *via* a jump or fall from a structure such as a simple, low growing plant of no more than one meter in height.

6.2. Methods

A new model was constructed from the 119° thorax-abdomen curve body model design from Chapter 5. Flapping trials of protopterygote models consisted of a rapid prototyped body in a 119° thorax-abdomen angle, dorso-ventral curved body position created using the same 3D Studio Max 8 (Autodesk Inc., San Rafael, CA, U.S.A.) method as described in Chapter 5. For appropriate support and mounting of flapping hardware the protopterygote body was printed in a higher density (80% solid) ABS plastic format using the same Dimension SST 1200es, Rapid Prototyping machine available through the Carleton University Engineering Department, Ottawa, Canada.

6.2.1. *Construction of the Flapping Model*

The body of the protopterygote was printed in two sections, anterior and posterior, as in Chapter 5. The head and thorax half was modified *via* the use of a mill to remove material to allow the installation of an aluminum mid support rod mounted lengthwise internally intersecting and threaded into a 15.9 mm diameter aluminum mounting rod protruding through the dorsum (Figure 6.1). The dorsal mounting rod is slid into a solid aluminum mounting block and is secured by grub screws when testing was underway. The block and model assembly was mounted to a support sting used in the Particle Image Velocimetry (PIV) facility after complete assembly, as previously described in Chapter 5.

Figure 6.1. Modifications to the model. The anterior half of the rapid prototyped ABS plastic body set for material removal for servo installation (Top) and the modification for internal support rod in the posterior half (Bottom).

Prior to body half assembly and sting mount, the head/thorax half was milled to allow installation of waterproof, low voltage, digital micro servos (HiTec Inc., Model HS-5086WP). Four slots were milled on each side of the thorax half of the propterygote body mirroring their position on each side (Figure 6.1, 6.2). The slots were made slightly larger than the thickness, depth and length of the servos (approximately 16 mm (W) 35mm (D) and 50 mm (L)). The slots were milled out in an orientation of approximately 45° from the dorsal surface to allow for clearance of the servo bodies within the model to each other and as well as account for a wing stroke plane and angle of attack possible in winged insects. Servos were press fitted into the milled slots using thin foam rubber shims between the servo body casings and the slot edges to hold the servos in place during testing. A through hole was drilled through the base of the head from the ventrum to the dorsum of the “neck” area to allow for the wire routing from the servo array to pass through and limit any interference within the flow during testing (Figure 6.3).

After body model testing, the previously designed and printed rapid prototyped limbs were glued to the assembled body model using hot modeling glue to the ventral surface of the model, as previously described in Chapter 5, for fully limbed model tests. The limbs and other appendage structures were printed using the low density (30% solid) format for rapid prototyping. The antenna and telson bases were included but the flexible (flagellated) antenna and telson components were omitted to allow for unobstructed

Figure 6.2. The assembled body. The anterior half solid body model with internal support rod and dorsal mounting rod installed (Top) and the completed body model in a mounting block (Bottom)

Figure 6.3. The assembled body with servos installed and the wire harness secured *via* a through hole in the head to limit flow disturbance during testing.

imaging using stereo PIV techniques. Insulated solid copper wire and heat shrink tubing was used to construct the antennae and telson for drag force trials as in Chapter 5.

6.2.2. Winglet Construction and Mounting

Winglets for flapping trials were based on previously published planforms (Figure 6.4) and constructed of clear vinyl film (0.19 mm thickness) folded over at the leading edge of the winglet over two acrylic support rods (1.7 mm diameter) adhered to the film *via* glue applied through a heated glue gun (Figure 6.5). The rod nearest the leading edge of the winglet spanned the winglet base to the tip and was curved to fit the leading edge profile when adhered to the film to partially support the lower trailing edge vinyl through tension. A small second acrylic rod was adhered to the wing base area 40° from the first rod base to stabilize the winglet base area. The acrylic rods were fitted into a custom machined aluminum servo horn (6 mm D x 16 mm L) with milled and drilled faces at 20° angles, opposite to each other and secured with a small dab of hot glue. The aluminum servo horn fitting was attached to the waterproof servo *via* the supplied machine screw and lock washer *via* a milled flat face and drilled through hole (Figure 6.6). Winglets mounted to the servos resulted in a simple up and down flapping motion (stroke plane set at 45° to dorsal surface of each body segment). Servos were set at the 0 position before the installation of winglets, resulting in winglets projecting laterally from the model at the 0 position (Figure 6.7). Prothoracic winglets were non-articulated and were installed directly to the prothorax of the model *via* a machined aluminum base cylinder to project

Figure 6.4. (Top) A diagram of a prothoracic winglet found on some Palaeodictyoptera, modified from Kukalová-Peck and Lawrence (2004), the arrow indicates a rigidified area equivalent to the leading edge of a wing. (Bottom) A planform of a winglet supporting the initial beginning of forward morphology, the arrow indicates the area that is folded back over the wing covering an acrylic rod for support creating a thickened leading edge to the winglet model.

Figure 6.5. Images of the thoracic and abdominal winglet design (Top) and the pro-thoracic winglet (Bottom) with custom fabricated aluminum mounting pins and acrylic internal support rods.

Figure 6.6. An image of the anterior body model half with servos installed (Top) and the fabricated aluminum mounting pins that secure to the servos near the dorsum (Bottom).

Figure 6.7. The body model with installed servos, thoracic and abdominal winglets set at the 0 position and 45° from the dorsal plane.

dorso-laterally at an angle of approximately 45° from the dorsum as depicted in the protopterygote reconstruction in Chapter 4.

Due to the overall size of the servos, available control hardware, and protopterygote body model, only four pairs of servos with associated winglets could be installed and flapped for these trials. The prothoracic basal winglet was included as a stationary, dorsally oriented feature that represents the corresponding winglet in the early protopterygote. I have assumed this wing had no function in flapping in early flight evolution due to a likely partial fusion of articulation within the wing base (Kukalová-Peck and Richardson, 1983).

6.2.3. Flap Control and Parameters

Servo control was achieved *via* an eight channel Arduino motor controller board utilizing custom software (V. 1.0., <http://arduino.cc/en/>). The programmed flap software is adjustable for flapping frequency, phase and amplitude however; experiments were limited to single set of variables for this chapter. The range of motion for the servo powered winglets was set to $\pm 70^\circ$ from the zero point (lateral to the body), to allow for the maximum range of flapping movement without contacting the model body itself during the flapping stroke. Flap frequency was limited by servo constraints to 0.03 Hz in order to achieve a smooth movement in wing stroke. Wing phase for all winglets was set to 0.03, with the most posterior winglet leading during the flap cycle. Winglets were constructed to allow trailing edge flexion to mimic true insect wings and so winglets could deform upon the event of contact with each other or objects in general.

6.2.4. Flap Parameter Selection

Flapping frequency was calculated based on previous work with scaled flapping models by Van Den Berg and Ellington (1997). Flapping frequency was limited to the capabilities of the servos themselves which limits the lowest Reynolds range possible using the winglets constructed for these flapping trials. Reynolds number for flapping frequency was calculated as:

$$Re = \frac{\phi f R^2}{\nu}$$

Where ϕ is the wingbeat amplitude, f is the wingbeat frequency, R is the wing length and ν is the kinematic viscosity. The operating Re during flapping trials was approximately 550 for the 8.5 – 9.0 cm flapping winglets. Similarly, Strouhal number for flapping flight was calculated as:

$$St = \frac{f \cdot A}{U}$$

Where f is wingbeat frequency in Hz, A is the arc length stroke amplitude in m/stroke and U is the velocity of the model in m/s. The Strouhal number calculated for the reconstructed protopterygote at stabilization velocity was 0.395, which was similar to the Strouhal number of 0.329 that was calculated for the model at a tow speed of 2 cm/s. The subsequent motion of the array of winglets when in motion resembled a posterior to anterior progressing sine wave.

6.2.5. Dye Flow Trials

The assembled flapping legless model was subject to dye flow experiments using a mixture of malachite green powder and water previously described in Chapter 5. The model was towed at speeds of 0.25, 0.5, 1.0, 1.5 and 2.0 cm/s in the tow tank facility, previously described in Chapter 5, and digital photographs were captured of dye flow around the flapping model using a digital SLR camera (Nikon D60 SLR with a AF-S DX Zoom-Nikkor 18-55mm f/3.5-5.6G ED). Flapping was symmetrical on each side of the model during flapping trials as well as stationary trials as winglets were held out laterally at the 0 point during testing. Subsequent captured images were analyzed qualitatively for overall bulk flow effects within the flow.

6.2.6. Digital Particle Image Velocimetry (dPIV) on Flapping Winglets

PIV testing was completed in the same fashion as described in Chapter 5. Models undergoing PIV testing were allowed to complete at least one flap cycle before image capture began. Trials were repeated three times with the image plane positioned 10 cm from the model midline, which corresponds to approximately $2/3$ the winglet length. Each trial consisted of a series of twenty images capturing almost the entire flap cycle and were completed for tow speeds of 1.0, 1.5, 2.0, 2.5, and 3.0 cm/s for the legless model with flapping winglets and the assembled limbed model with flapping winglets. For the assembled legged flapping model, two trials were completed per tow speed at 4 cm from the midline to image the flow around the wing base and comparison to non-winged flow as presented in Chapter 5.

PIV trials were also completed on the model with stationary winglets set at the 0 position, extending laterally at approximately 90° from the body. Stationary winglets were imaged in two trails for each tow speed at both the 10 cm and 4 cm from midline offsets. Each trial consisted of ten images as the winglets were not altering position during testing resulting in ten compiled images of the same test scenario.

Rendered image files were analyzed in MATLAB® (V. 7.13.0.564, Mathworks Inc., Natick, MA, U.S.A.) for horizontal velocity and magnitude in general across the vertical mid-point of the field for each image of the mid-upstroke, mid-downstroke and full-downstroke and qualitatively assessed as a whole using MATLAB® functions, as in Chapter 5.

6.3. Results

6.3.1. Drag Forces on the Winglet Models

Tests on the legless model with static and flapping winglets resulted in the general trend of flapping producing more drag than the model with both static winglets or no winglets at all for all tow speeds. Static winglets produced significantly more drag than the wingless model at 0.5, 1.5, 2.0, and 2.5 cm/s (Figure 6.8) (0.5 cm/s, t-test, $t = -2.954$, $P = 0.032$; 1.5 cm/s, t-test, $t = -4.234$, $P = 0.003$; 2.0 cm/s, t-test, $t = -2.939$, $P = 0.019$; 2.5 cm/s, t-test, $t = -4.25$, $P = 0.003$). Flapping winglets also produced significantly more drag than the same model tested with no winglets at all tow speeds except 1.0 cm/s ($Re = 640$) (Figure 6.8), (0.25 cm/s, t-test, $t = -2.777$, $P = 0.024$; 0.5 cm/s, t-test, $t = -11.98$, $P < 0.001$; 1.5 cm/s, t-test, $t = -7.733$, $P < 0.001$; 2.0 cm/s, t-test, $t = -6.532$, $P < 0.001$; 2.5 cm/s, t-test, $t = -6.033$, $P < 0.001$; 3.0 cm/s, t-test, $t = -3.833$, $P = 0.009$). Flapping winglets created significantly more drag than static winglets only at 1.5 cm/s tow speeds on the legless model (paired t-test, $t = -6.325$, $P = 0.003$) (Figure 6.9).

In winged models with legs, testing revealed that static winglets increased drag forces at all but the slowest tested velocity of 0.25 cm/s, (Figure 6.10 A). Drag forces increased significantly with static winglets at 0.5, 2.0, 2.5 and 3.0 cm/s (Figure 6.10 A) (0.5 cm/s, t-test, $t = -2.994$, $P = 0.020$; 2.0 cm/s, t-test, $t = -2.649$, $P = 0.033$; 2.5 cm/s, t-test, $t = -2.321$, $P = 0.049$; 3.0 cm/s, t-test, $t = -2.652$, $P = 0.033$). Somewhat unexpectedly, the drag forces significantly decreased on the static winglet model vs. the wingless model at 0.25 cm/s (Figure 6.10 A) (t-test, $t = 3.919$, $P = 0.004$).

Flapping winglets on the legged model produced significantly more drag over the wingless model at all but the 0.25 cm/s tow speed (Figure 6.10 B) (0.5 cm/s, t-test, $t = -5.807$, $P < 0.001$; 1.0 cm/s, t-test, $t = -3.780$, $P = 0.005$; 1.5 cm/s, t-test, $t = 2.917$, $P = 0.022$; 2.0 cm/s, t-test, $t = -4.35$, $P = 0.003$; 2.5 cm/s, t-test, $t = -3.372$, $P = 0.012$; 3.0 cm/s, t-test, $t = -3.936$, $P = 0.004$).

Flapping winglets on the legged model produced significantly more drag than the static wing condition at 0.25 cm/s, $Re = 160$ (paired t-test, $t = -4.996$, $P = 0.008$) (Figure 6.11).

Figure 6.8. Static winglets (**A**) and flapping winglets (**B**) generally produce more drag force when present on legless insect models. Flapping winglets produce greater drags forces than static winglets at all tow velocities. Data points are an average of 5 values +/- 95% confidence.

Figure 6.9. Flapping winglets on a legless protopterygote model produce more drag at all two speeds than static winglets held at 90° from the tergal body wall. Data points are an average of 5 runs +/- 95% confidence. Flapping winglets produced significantly more drag at 1.5 cm/s than static winglets (Sec .6.3.1).

Figure 6.10. Static winglets (A) and flapping winglets (B) generally produce more drag force when present on insect models equipped with legs, antennae and telson. Flapping winglets produce greater drags forces than static winglets at all tow velocities. Data points are average values +/- 95% confidence.

Figure 6.11. Flapping winglets on a fully assembled legged propterygote model produce more drag at all two speeds than static winglets held at 90° from the tergal body wall. Data points are average values +/- 95% confidence.

6.3.2. *Dye Flow*

Dye flow visualization using Malachite green dye was performed on the legless model with flapping winglets at tow speeds of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 cm/s. At the lower tow speeds, 0.25-1.0 cm/s, dye initially deposited near the dorsal surface of the most anterior winglets was observed being entrained by the winglets as they progressed through the downstroke. The dye would begin to disperse and spread outwards along the winglet from the base to the tip throughout the downstroke as well as progress along the dorsal surface of the winglets, posterior to anterior (Figure 6.12-6.14). At the upper tow velocities (1.5-2.5 cm/s) the dye deposited between the anterior winglets would be drawn down with the downstroke but quickly move out and into the direction of the free stream flow. The dye would curl around and become entrained in a recirculating zone of flow above the winglets, perpendicular to the dorsal side of the winglet array at 0°, (Figure 6.15-6.17) until the upstroke sequence began where the dye would then be lost to the free stream bulk flow.

Figure 6.12. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 0.25 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke.

Figure 6.13. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 0.5 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke.

Figure 6.14. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 1.0 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke.

Figure 6.15. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 1.5 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke. The dye spreads out and is entrained in a re-circulating zone of flow well above the dorsum of the winglets.

Figure 6.16. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 2.0 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke. The dye spreads out and is entrained in a recirculating zone of flow well above the dorsum of the winglets.

Figure 6.17. From left to right and top to bottom, photographs of dye flow during the downstroke around the body only model with flapping winglets at a tow speed of 2.5 cm/s. The arrows indicate a dye structure drawn between the winglets and along the dorsal surface of the winglets during the downstroke. The dye spreads out and is dispersed along the winglets and through the wake produced.

6.3.3. Stereo dPIV and the Flapping Models

The general structure of the flow about the winglets of the legless and legged model is not noticeably different. At the midpoint of the downstroke a zone of reverse flow begins to form behind the dorsal side of the array of flapping winglets. This pocket of rotating and reverse flow continues to build as the winglets continue through to the maximal down stroke position. As the winglets begin to reverse to the upstroke, the zone of turbulent reverse flow persists until the winglets pass the midpoint again and end up dissipating the turbulent zone as it is lost in the free stream flow moving past the models, whereupon the cycle begins again with the downstroke.

The legless model regularly exhibited flow moving downwards towards the head region of the model during the downstroke across all tow tested tow speeds. As the midpoint of the downstroke was reached by the winglet array, the flow began to move downwards with the winglets and towards the head of the model (Figure 6.18). This downward movement of fluid continued as the downstroke progressed. At the end of the downstroke, a zone of turbulent and reverse flow remained despite the winglets being at the furthest point from this zone (Figure 6.19, 6.20). Fluid movement in this zone occasionally appeared as a counter-clockwise mass of rotating fluid in this, as seen in Figure 6.20. As the upstroke began the circulating zone was observed to become less obvious until the winglets reached the midpoint of the upstroke, where free stream velocity fluid dominated the flow pattern as flow slipped between the winglets at all tow velocities (Figure 6.21). Fluid more posterior of the most abdominal winglet appeared to be most affected by free stream flow, as evidenced by the direction of vectors in this region.

Figure 6.18. Rendered images of the curve body model with flapping winglets at mid-downstroke. Flow above the winglets is drawn towards the head and down as the winglets progress through the stroke. Image plane is 10 cm from the midline, tow speed was 2.0 cm/s, axis are in mm.

Figure 6.19. Rendered images of the legless body model with flapping winglets at the end of the downstroke. Flow is drawn towards the head and down with the motion of the winglets. The image plane is 10 cm from the midline, tow speed was 2.0 cm/s, axis are in mm.

Figure 6.20. Rendered images of the legless model with flapping winglets at the end of the downstroke. Flow is drawn towards the head and down with the motion of the winglets. A counter clockwise zone of rotation above the winglets is present in these images above the winglets. The image plane is 10 cm from the midline, tow speed was 1.5 cm/s, axis are in mm.

Figure 6.21. Rendered image of the curve body model with flapping winglets at mid-upstroke. Oncoming flow mostly slips between the thoracic winglets and is partially disturbed by the abdominal winglets. The image plane is 10 cm from the midline, tow speed was 3.0 cm/s.

Legged models produced much of the same flow patterns as legless models. The same downward movement (posterior to anterior) of fluid was observed at all tested velocities for the flapping legged model (Figure, 6.22, 6.23). The legs did not appear to influence the flow pattern in any obvious way using the current methods, with the possible exception of the large re-circulating zone of flow created by the winglets maintaining its presence for more of the stroke cycle. Similar to the legless model, counter clockwise rotating fluid was occasionally observed.

Fluid flow near the wing base, 4 cm from the midline, produced a similar pattern to that which was presented in Chapter 5, with reverse flow moving towards and along the dorsum of the model (Figure 6.24). This flow did not appear to change as the stroke cycle progressed on flapping models.

Static winglet model tests revealed the same flow pattern at all test speeds. Fluid slips between the winglets that are extended laterally out from the model and appears similar to the mid-upstroke pattern observed during flapping trials (Figure 6.25). The most posterior winglets are presented more broadside to the oncoming flow and cause some turbulence in the resulting wake.

Figure 6.22. Rendered image of the curve body model with legs and flapping winglets at the end of the downstroke. Flow is drawn down towards the head with the motion of the winglets. The image plane is 10 cm from the midline, tow speed was 1.0 cm/s.

Figure 6.23. Rendered image of the curve body model with legs and flapping winglets at mid-downstroke. Flow begins to be drawn down towards the head with the motion of the winglets. The image plane is 10 cm from the midline, tow speed was 1.5 cm/s.

Figure 6.24. Rendered image of the curve body model with legs and flapping winglets at mid-downstroke (top) and the end of the downstroke (bottom). Flow near the wing base is drawn along the dorsum as it circulates in the zone behind model. The image plane is 4 cm from the midline, tow speed was 1.0 cm/s.

Figure 6.25. Rendered images of the curve body model with legs and static winglets at 1.0 cm/s (top) and 2.0 cm/s (bottom). Flow slips through the thoracic winglets but is partially disturbed by abdominal winglets. The image plane is 10 cm from the midline, axis are in mm.

6.4. Discussion

The results presented in this chapter are, admittedly, the product of a degree of simplicity when considering the complexity of a flapping insect wing. However, this study was not meant to be a thorough and exhaustive study, rather it attempts to shed light on the basic idea that a flapping winglet may have been aerodynamically important to a falling pterygote insect ancestor. What is remarkable is that despite the relative unsophistication in the control mechanism incorporated into the protopterygote robot, the end result is that a set of flapping winglets can indeed increase drag. Furthermore, the observed aerodynamic structure in the flow behind the winglets and model itself could potentially be important for early selective pressures leading to wings and powered flight and deserve investigation.

6.4.1. Drag Force on the Models

The most interesting force measurement results are those pertaining to the flapping winglets *via* the servo mechanisms. To date, no previous studies have attempted to test physical models that exhibit flapping wings or winglets while parachuting. Drag forces increase across almost all tow speeds, and are significantly more than the wingless body alone at all but 1.0 and 0.25 cm/s for the legless and legged models respectively (Sec, 6.3.1, Figure 6.8 B, Figure 6.10 B). The realization that flapping winglets are better producers of drag than stationary winglets further supports the articulated winglet as the pleisiomorphic state for insect wings (see Kukalová-Peck, 2008, 2009). If a set of flapping winglets functions better to slow a descent and possibly exhibit directional

control the adoption of stationary winglets seems unnecessary. Likewise, if winglets were not initially mobile the likelihood of selection and enhancement, *via* increased size, with the burden of the cumbersome nature of supporting such structures (i.e. while climbing through low, dense and branching vegetation), only to somehow incorporate articulation after selection for wings, does not fit with parsimony.

Drag forces generally followed the trend of increasing with progressing model complexity. Drag forces were higher for the model with legs vs. without, similar to result from Chapter 5. On legless models the static wings were effective at increasing drag significantly (Sec 6.3.1) at fewer Reynolds regimes than flapping winglets (Figure 6.8). This result emphasizes the ability of both increasing drag through the addition of wings in a passive way and further demonstrates the usefulness of actively employing the same structures by way of flapping. From the results of Chapter 5 the likely main component to the drag experienced by the curve body model is due to the curvature of the body to the oncoming flow. Flapping winglets appear to add to this drag at nearly all the Reynolds regimes, the stand out tow speed of 1.0 cm/s ($Re = 640$) produced a wide range of drag force measurements as evidenced by the larger SEM presented in Figure 6.8. The somewhat unexpected drop in drag force when static winglets are added to the body model may be explained through analysis of flow around the body. The potential for the winglets to act as flow straighteners, similar to the legs (Chapter 5) should not be dismissed. If winglets are presented more broadside to the oncoming flow, testing may yield different results than those found here which rely on the winglets orientation of approximately 45° from the dorsal surface of their respective body segment as a result of servo placement and the required wing stroke path.

The legged curve body model emulates the most realistic form of putative protopterygote insect from these experiments. The arrays of thoracic and abdominal legs are the initial structures encountering the oncoming flow to the model. Static winglets tested on the legged model do not significantly increase drag force at 1.5 cm/s, which suggests the addition of legs at this flow regime may alter the flow enough to destroy the drag otherwise seen in the legless model at this tow speed ($Re = 960$). When these winglets are tested flapping, they significantly increase drag (Sec 6.3.1) over the wingless condition at all velocities except the slowest tow speed where viscous forces are likely the most extreme out of all the tested regimes here (Figure 6.10 B) (Ellington, 1991).

Despite the fixed angle of attack and stroke path for each winglet, flapping produces significantly more drag at more Reynolds regimes vs. simply holding out winglets while falling. It would seem that if a falling protopterygote insect were to benefit from increased drag while falling a short distance it may slow its fall even further simply by flapping its wings and in potentially simple flapping motions.

The winglets were constructed to a length that was approximately the same as the width for each thoracic segment they were supported by, based on measurements of prothoracic winglets from fossil Palaeozoic insect reconstructions (Kukalová-Peck, 1970, 2008; Wootton and Kukalová-Peck, 2000; Grimaldi and Engel, 2005). Kingsolver and Koehl, (1985, 1994) found that winglets with lengths of 20% or less of the body length did not produce significantly different drag forces from wingless models. Their tests were performed on a straight body model oriented at 90° to the oncoming flow in a wind tunnel with stationary winglets presented broad side to the wind. The results presented here agree with Kingsolver and Koehl (1985), as the largest winglets among the four pairs

tested are approximately 20-23% the length of the body. Even with the addition of two extra pairs of static winglets, albeit arranged at various angle of attacks as a result of the servo orientation of 45° from the dorsal plane, the resulting drag forces were not significantly different than the wingless body alone at certain Re 's (tow speeds) for the legless and legged models (Sec 6.3.1., Figure 6.8 A, 6.10 A).

Flapping winglets created significantly more drag than static winglets at 1.5 cm/s for the legless model and 0.25 cm/s for the legged model (Figure 6.9 and 6.11). Although only two tow speeds produced this result the overall trend of flapping winglets consistently increasing drag is still apparent. Why drag is not significantly different at other tow speeds is not entirely clear but may be clarified with further statistical analysis. Furthermore, the installation of the remaining expected abdominal winglets as well as continued advancement of winglet design towards a more realistic, 3 dimensional, insect wing-like profile may result in a greater separation between static and flapping drag forces. Continued experimental testing as well as further investigation into flapping is required to better understand the sensitivity of drag creating mechanisms in this Re range.

The force measurements achieved in this study were completed to investigate the changes, if any, in maximum force generated over increasing model complexity culminating in crudely flapping winglets. Comparing drag forces of a wingless but fully limbed †Cercopoda-like insect to itself equipped with both flapping and non-flapping winglets provides a glimpse at the potential benefit of flapping while adopting the active parachuting behaviour observed in Chapter 2 and 3.

6.4.2. *Flow Visualization on the Models*

Flow visualization techniques such as dye flow and digital particle image velocimetry provide a new and novel way of exploring such concepts as those presented here. Fluid flow around the models has provided a glimpse at possible aerodynamic structure that may lead to more clues about the initial stages of the putative protopterygote's aerial behaviour. The consistent observation of reverse flow back towards the dorsum in Chapter 5, and again here with imaging near the wing base (Figure 6.24) (Appendix D), continues to present the intriguing possibility that the early protopterygotes may have adopted this curved posture. The rotation of fluid generated from and located behind the winglets may act as an extension of this reversed flow and low pressure zone behind the falling insect, facilitating the increased drag observed here. By extending this mass of moving fluid out over an articulated, light weight, surface the potential for drag (and thus lift) to begin to contribute more overall to the falling insect becomes significant. In Appendix D I present extracted horizontal velocity and magnitude data of the reversed and turbulent flow empirically at different parts of the stroke cycle across the tested tow velocities.

Investigation of the downstroke in this study yields, perhaps, the most curious clues within the flapping trials. Tests on the flapping model would periodically reveal a potential leading edge vortex (LEV) (Ellington, 1984, 1997, 1999) forming on the dorsal side of the winglet, particularly in dye flow trials. The orientation of the model, presenting the trailing edge of the winglet first to the oncoming flow, may be of significance to the possibility of a trailing edge vortex forming on the leading edge of the wing, as the flow separation occurs around the winglets. If a body curvature was

maintained in early falling, winglet supporting, insects the possibility of the posterior edge of the winglets being presented initially to the oncoming flow, even with a wide range of articulation, would be a likely scenario. Further investigation is required however this remains an intriguing possibility.

6.4.3. Evolutionary Concepts from Model Construction

Undertaking any kind of accurate reconstruction of fossil or fossil based organisms comes with difficult and lofty challenges. From those challenges usually come technological and mechanical barriers that can be somewhat revealing when establishing the likely morphology and behaviour of the animal of interest. The protopterygote insect presented here is an attempt to incorporate all known and reconstructable morphology that is believed to have existed within the pre-flight insect lineage (see Chapter 4). Considering this, the support of multiple articulated winglets on this creature comes with important challenges to make note of. This insect would have likely flapped its winglets from posterior to anterior at some given phase, possibly similar to stoneflies and mayflies while surface skimming (Marden and Kramer, 1994; Marden, 1995, 2003) or mayfly gill movements in water (Sensenig et al., 2009, 2010). These undulating winglet movements would have required a very free range of movement but controlled in such a way as to avoid contact with one another during their stroke cycle, preventing damage and potentially loss of function due to flow interruption. These points came to light as the construction of the flapping model with single stroke plane servos was attempted. To avoid wing damage and potential binding during stroke winglets were constructed with a

pliable trailing edge extending up to near the midline (extending out from base to tip) of the winglet. The moderately flexible nature of the winglet construction is more similar to a real insect wing than a winglet constructed of hard flat plastic. This free, pliable membrane like area of the wing allowed the neighbouring winglets to bend upon contact with each other without physical harm. However, it is worthy to note that natural insect wings operating in this fashion would quickly become frayed and broken rendering them less effective for aerodynamic purposes unless maintaining a phase lag to allow adequate winglet clearance during movement.

Although the inclusion of durable vinyl material was appropriate for the experiments at hand it is unreasonable to assume that a wing with the same engineered durability would occur in nature. These experiments enabling a flapping motion upon the propterygote body model revealed some key concepts and points of interest. Flapping multiple winglets, with lengths equivalent to their corresponding body segment widths and winglet widths proportional to the near basal groundplan-like wing from fossils, revealed that a phase difference of more than -0.03 would result in the winglets colliding with each other and binding in the current dorso-ventral body curvature model setup. This was particularly apparent within the abdominal winglets as the apex of the body curvature was located in this area. Even though the tested model was only representative of a simple one plane motion of wing stroke it is likely that the binding and crashing together of winglets would occur (or possibly be amplified) with more planes of motion. This concept essentially restricts the phase difference between winglets, as well as the potential size of the winglets, if abdominal winglets were to have been maintained in the flying Insecta.

The selection for thoracic winglets for insect flight continues to be debated. These results could potentially begin to demonstrate the mechanical factors for thoracic wing selection in an early multi-winglet protopterygote insect. The dorso-ventral curvature of the descending insects from one meter or less may be essential to the selection of thoracic winglets or winglet reduction and/or loss overall, as in the †Cercopoda, Zygentoma, †Monura and Archaeognatha.

Mechanical constraints are not absent from the test setup as well. Due to the specified working range of the employed micro-servos the presence of minor stepping, chatter-like movements is unavoidable at some points within the flap cycle. The results presented here are based on the lowest achievable flap frequency of the servos and correspond to a true frequency of around 49 Hz, with respect to the model winglet size. The existence of such a rapid flapping frequency from pre-flight insect ancestors may be possible but from past research on skimming stoneflies and mayflies the least derived of the extant flying insects morphologically and physiologically, the tested flap frequency here is slightly higher than the faster frequencies reported (≤ 45 Hz) (Marden and Kramer, 1994; Marden et al., 2000). Further modification to the current model design may allow for testing within the stonefly and mayfly flapping frequencies in regards to thoracic winglets with the current aquatic servo technology. In this way, various flapping frequencies can be attempted and further explore what frequency may be most likely to result in higher drag or propulsion for forward movement.

6.4.4. *Flapping Makes Sense*

Flapping vigorously to make use of aerodynamic mechanisms to enable a delayed fall and the potential for more air time, in which further manipulation of aerodynamic mechanisms can be employed, seems entirely plausible. Previous work with stationary winglets has demonstrated that they can be useful for aerodynamic purposes only if they are a certain size, specifically greater than 20% of the body length in model tests (Kingsolver and Koehl, 1985). My findings are in agreement with Kingsolver and Koehl (1985, 1994), however here I have shown that crude flapping winglets, oriented in such a way that they are neither optimal for forward thrust nor for maximum surface area encountering flow, create more drag on a falling insect than if it were falling without winglets as well as if the flapping winglets were held stationary. This is also in spite of a lack of abdominal winglets on segments 3-9 which are expected to behave in continuing synchrony with the previous articulated winglets that are present. If these winglets were present it is likely the trend of increased drag could continue and potentially be amplified to even greater levels over the wingless model. It is unclear if abdominal winglets would have produced any imbalance or potential forward or backward thrust during flapping in a fall due to the orientation of the body curvature. The likelihood of decreasing sized abdominal winglets providing any significant beneficial alteration to the attitude or displacement of the falling protopterygote seems minimal, particularly since they may act in an opposing motion (pitching or tipping) to the thoracic ones due to the body curvature. The contribution of the abdominal winglets must have been negligible enough during the progression of powered flight that they would be reduced and lost giving way to thoracic wing selection as flight was adopted. Possibly, the initial abdominal curvature and how

abdominal winglets are presented to oncoming flow is why insects never evolved to fly with a greater array of wings along the body, further testing may provide more clues to this concept.

The presence of legs along the protopterygote insect body is not a trivial morphological state when considering a parachuting insect. Falling insects generally hold their legs out for stabilization, steering and potentially to increase their chances of contacting a solid surface. Subsequently, the secondary effects of airflow past the legs may have been beneficial to a flapping winglet within that flow. The addition of legs to a flapping protopterygote model may result in more reverse flow during the down stroke of the winglet array vs. the legless flapping model. Previous Stereo dPIV work in Chapter 5 showed that thoracic legs can create streams of lower velocity flow as they pass through the oncoming bulk flow. This creation of turbulent slowing flow below the winglets may aid in allowing the winglets to essentially harness more of the stagnating fluid and project it down or backwards increasing the drag during descent. As the thoracic legs are larger than the abdominal ones, they may have provided more turbulence below the thoracic winglets than the abdominal equivalents. This may be a possible precursor condition to thoracic winglets, which would have been more refined through articulation and rotational mechanisms than those tested here. The end result (powered flight) would require the winglets becoming more efficient at harnessing this air flow to the point where an increase in thoracic wing size and strengthening of articulation and musculature could become useful for thrust pushing the insect forward through the air.

6.4.5. Concluding Remarks

In this chapter, I have presented results that empirically support a potential pathway towards flight evolution in insects. Flapping winglets can produce more drag than winglets held stationary and even when constrained (one stroke plane, angle of attack) appear to influence the flow of fluid (and thus air) trailing behind in such a way as to potentially increase the zone of re-circulating and mixing flow caused by the body and limbs themselves during a fall (see Chapter 5). This in turn, may contribute to pressure drag by extending the zone of low pressure which acts on the falling insect in its entirety slowing a descent even further than a comparable wingless insect under the same circumstances.

My work is relevant for purposes of understanding the potential evolution of flapping flight in insects in many ways. Assembling an experimental system to test flapping winglets while simultaneously ensuring the parameters of the model employed remain within the biological realm, as far as the technology of such a model can support, is essential to producing useable and relevant data. The results presented in this chapter have built on those of the previous chapters in this thesis and have combined into what could be a very good candidate for the true protopterygote insect and the behaviour it may have exhibited towards selection for powered flight.

Chapter 7: General Discussion

7.1. The Path to Insect Flight

Throughout this thesis I have suggested and presented various components for a path towards flight in the insects. In this chapter, I present my interpretation of a likely progression of pre-flight to flight behaviours and the required morphological components known in the fossil record and/or possessed by extant insects. I have also incorporated the known modes of aerial descent and propulsion presented in the scientific record to date (Table 7.1). The discovery of an Ordovician, Silurian and/or early Devonian fossil of an undisputed pterygote ancestor may be the only real, truly convincing evidence that can confirm such a progression as the one I present in Table 7.1. Although the likelihood of such a fossil may be minimal, due to the very specific conditions required for fossilization of the soft-tissues typical of a semi-aquatic organism, or any arthropod surviving within areas subject to regular erosion (See Shcherbakov, 1999), I, and the majority of the entomological world, eagerly await the day when such a discovery happens.

Table 7.1. An interpretation of the events that may have occurred in association with important morphological features a protopterygote insect may have exhibited.

<i>Major Progressive Event</i>	<i>Important Morphological Feature</i>
1. Emergence and adaptation to semi-aquatic lifestyle progressing to more terrestrial lifestyle. Rolfe, 1980; Shear et al., 1984.	Spiracles, scaled or desiccation resistant exoskeleton.
2. Exploitation of vegetation not more than 1 meter in height for nutrients, predator evasion, mating?	Clawed thoracic and abdominal appendages for climbing (Kukalová-Peck, 1983, 1987).
3. Falling from vegetation <i>via</i> jumping from stems, shoots by choice or startle response or falling <i>via</i> losing purchase from vegetation, rocky outcrops. Jumping and climbing possibly assisted by articulated winglets (Edwards, 1992; Kukalová-Peck, 1987)	Abdominal rope musculature retained and modified from crustacean –like ancestry (Manton, 1972; Kukalová-Peck, 1998) Retained exite derived flattened gill/paddle structures which would become winglets (Kukalová-Peck, 1983; 1985; 1987).
4. Aerial righting and maintained ventral side down posture <i>via</i> actively induced dorsal curvature. Active parachuting in early Insecta. Flapping winglets helping to increase drag and allow fluttering while falling to assist landing and avoid undesirable landscape and predators.	Abdominal musculature, including rope muscles, ‘cocked’ arching body position, for escape jumping upon landing in danger from a fall (Fig 2.4).
5. Divergence of powered flying insects from winglet fluttering ancestor, surface skimming insects (Marden and Kramer, 1994; Marden, 1995, 2003) from fluttering ancestor landing on water surface.	Selection for thoracic wings, possibly from dorsally curved body position presenting thoracic wings to air flow.
6. Continued divergence of flying insect lineages, Divergence of gliding insects within flying lineages using thoracic wings, Divergence of gliding and directed aerial descent, and parachuting wingless arboreal insects (multiple times to present day) with the emergence of tall forest canopies (Yanoviak et al., 2005, 2009).	Loss or deactivation of rope muscles in wingless and winged insects allowing for varying body positions for directed aerial descent and gliding. Fusion of wing articulation, rigidified wing venation, braces and cross venation (multiple times, various ways) allowing for gliding via outstretched wings (Wootton and Kukalová-Peck, 2000; Kukalová-Peck, 1983, 1985).

7.2. Contributions from this Thesis

I have presented the results and concepts from my work across the six previous chapters in this document. My introductory chapter reviewed the literature surrounding the field of insect flight evolution and insect evolution in general. I discussed the potential environment the protopterygote insect was expected to have survived within and described the current theories of wing evolution and flight evolution that are accepted today. I presented potential avenues towards flight which I continued to allude to throughout the following chapters as I endeavoured to tailor my thesis toward providing evidence that may cater to an origin of flight that involves, jumping and/or falling as well as contribute to a somewhat limited publication database of the behaviours of the Archaeognatha.

In Chapter 2, I presented the results of experiments that focused on the behaviour of the basal insect lineage, the Archaeognatha, while falling from heights reflective of the vegetation of the later Silurian to early Devonian periods. My results showed that, although wingless and not adapted to powered flight in any way, two species of Archaeognatha can influence their overall drag while descending a 1 m distance by actively adopting a dorso-ventrally curved body position which contributes to their ability to orient right side up while falling. I also investigated the terminal velocity of these species in a custom constructed vertical wind tunnel and found that the achieved velocity while alive was significantly lower than when dead. The curved body posture was also observed during the vertical wind tunnel trials, which further contributed to our understanding of posture and orientation in jumping and falling. This body posture was only observed in living specimens and resulted in landing on their ventrum each time they

were dropped, suggesting the Archaeognatha actively control their posture and thus some aspect of their descent. *Pedetontus saltator* specimens were also tested in a low speed wind tunnel and continued to demonstrate the ability to decrease velocity and increase the duration of their fall in a wind source or not. Although wind did not alter the displacement significantly when compared to being dead, it did result in an increase in horizontal displacement for both living and dead specimens when compared to no wind conditions. The size and body shape of the Archaeognatha likely are why the wind can cause such a displacement change, and implications for such an outcome could be detrimental if the landing area is undesirable (predators, water etc.). It may be possible that the addition of flapping wings to counteract the effect of the wind could be beneficial to insects climbing and falling, such as the potential early pre-flight Insecta.

The potential ability of the two species of Archaeognatha to cope with an obstacle potentially present in their natural habitat, namely standing water, was investigated in Chapter 3. Water may have been a significant obstacle for early pre-flight insects during the Silurian and Devonian period. My results demonstrated that Archaeognatha are capable of jumping from a launch rock surrounded by water to a larger, potentially safer, group of rocks within an isolated area. Although no significant difference was observed for any specific outcome, both species were observed jumping onto the rocks or onto the water surface and extricating themselves onto a dry surface. The very observation of this behaviour suggests a larger data set with various conditions (i.e. launch rock tested further away, a more natural test arena setup, multiple specimens placed within an experimental area for longer amounts of time) may yield significant results. Further study could identify if the jumping ability is truly random or if there is some degree of control

associated with it. Such control of a jump could explain the occasional observations I have made during field and colony collecting of individuals performing jumps from sticks and rocks to traverse gaps without any outside stimulus. The implications for this possibility not only include a better understanding of why jumping bristletails are successful as a group but also the possibility that such behaviour was employed by a jumping protopterygote to purposefully jump from vegetation. This chapter also investigated what may happen when an Archaeognatha falls onto the water surface and how they may escape it once landed. I presented results that demonstrate the capability of both species of Archaeognatha to escape the water surface and primarily use their unique, more terrestrially adapted, jumping mechanism to do so while floating on the surface tension of the water. I found that successfully escaping individuals were active for a significantly higher proportion of the time while in the water, suggesting that avoiding potentially fatal confines of the water requires a more efficient use of escape-oriented movements. The results I have presented demonstrate another way of traversing the surface tension of the water, surface skimming, sailing and swimming/walking being the others. A protopterygote falling on the water surface after a jump or fall from vegetation may have used the jumping behaviour in association with flapping winglets to traverse the water, thus employing an active method of escape. If thoracic winglets were helpful to extend the jump or escape from the surface tension of the water after a jump was initiated, there may have been selective pressure to enlarge them.

The fourth chapter presented the morphology of a putative protopterygote insect, including original drawn figures of each body tagmata and the fully assembled insect. I employed the use of allometric scaling, with the morphometric and experimental data

from Chapter 2 and 3, to define a potential size range and operating range in regard to landing velocity after a 1 m fall, terminal velocity, time to fall and time to stabilize orientation during a fall for a †Cercopoda-like protopterygote insect. I presented a potential scenario for the early beginnings of flight evolution through description and an original drawing based on knowledge gained from numerous publications and avenues that have not been disproven. I presented in Chapter 4 a table of potential morphological and experimental ranges extrapolated from data from Archaeognatha data (Table 4.1) that a protopterygote may operate within and discussed the possible problems with some of the estimates and suggested likely solutions to variables such as falling velocity. This chapter was novel in that it involves the extrapolation of a likely protopterygote size from data from living basal relatives and fossil evidence. It was also crucial to the development of a physical model (Chapter 5 and 6) and the range of variables a protopterygote insect (and thus the model) should be tested within.

In Chapter 5, using data from Chapter 4, I described the construction of a physical model of a protopterygote based on the descriptions and extrapolated values from two species of Archaeognatha. I presented drag measurement results and the resulting flow of fluid around two bluff body models in a straight and a biologically relevant curved posture, as well as a cylinder of similar diameter through dye flow and Particle Image Velocimetry techniques. I also presented results of a curve body model with legs, antennae and a telson which emulates both Archaeognatha and the expected †Cercopoda-like protopterygote during a fall. These protopterygote models were designed to be as realistic as possible but still be useful in an aquatic testing environment. As such, they are somewhat more rigid and less detailed in surface texture than that of a living insect. The

models did produce very different results than the cylinder “tube bug” model, which brings into question the use of geometrically simple objects, like cylinders, as proxies for insects when modern model building methods now exist. Although I tested one mounting position for each model, further investigation with different pitched mounts (forward and backward) may be of interest and could simulate the flow of a forward jumping Archaeognatha or protopterygote insect. Such variables deserve future attention but comparison to the results in this chapter would be of great interest for further investigation of specific behaviours and morphology. Through flow visualization, I established the presence of a low pressure zone of fluid circulation directly behind the dorsum of the curve body model. I proposed that this low pressure zone may be acting as pressure drag on the insect and that the curved body position helps to increase drag through this mechanism. I suggested that this resulting increase in drag and manipulation of the flow around the body may have implications towards how a flapping array of winglets may perform as potential drag-increasing morphology. The fact that this phenomenon was observed across multiple models and experimental conditions means that this is not an artifact of the experimental design and that it is a product of the morphology and posture of a falling basal insect.

In Chapter 6 I tested the protopterygote model in the curve body form with a set of eight flapping winglets projecting out from the thorax and first two abdominal segments. Despite the somewhat limited plane of movement, I found that drag increases with a flapping array of winglets while falling. I showed that fluid flow is affected by these winglets, as they appear to extend the zone of mixed circulating fluid outwards from the body midline effectively increasing the drag creating a low pressure zone behind the

dorsum of the falling insect. I suggested that the ability to influence drag with flapping winglets may be the initial start to creating lift through unsteady state aerodynamics on the dorsal surface of the thoracic winglets leading to a selection for larger wings and thus, flying insects using thoracic wings. Had a full set of flapping winglets been possible to test in the model, I expect the flow pattern would likely not have dramatically changed. However, I would expect the abdominal winglets to be less effective at creating drag than the thoracic ones since they would be oriented more leading edge first to the oncoming flow of air/water as they progressed up the curvature of the abdomen. This orientation would allow oncoming flow to slip through the winglets rather than flow around the ventral face of them. Flapping of such winglets may even serve to destabilize a falling insect by producing a pitching moment. The study presented in Chapter 6, to my knowledge, is the first that involves flapping winglets tested on a protopterygote model and may be a valuable contribution towards discovering the likely progression from flightless to powered flight in insects.

7.3. The Folly of Extant Insects

It is the very reason that the known insect orders are so diverse and derived that we must be exceptionally careful at what we accept as a valuable behavioral and morphological observation. Only through the painstaking efforts of trained insect morphologists and palaeontologists can the true nature of modification and adaptation be established. Only when basal morphological states are verified across numerous lineages, such as that of the insect wing and its articulation, through several publications like those

from Kukalová-Peck (1978, 1983, 1985, 1987, 1998, 2008, 2009) Shear and Kukalova-Peck (1990), Kukalová-Peck and Lawrence (2004), Wootton and Kukalova-Peck, (2000), Kukalová-Peck and Brauckman (1990), Kukalová-Peck and Richardson (1983), Wootton (1986), Niwa et al., (2010), Averof and Cohen (1997), Bechly and Stokar (2011) can the pieces of a primitive ancestor be defined.

No extant insect lineages are a perfect substitute for a pre-flight insect ancestor. Specific and feasible behaviours must be confirmed through appropriate ‘cross checking’ with other fields of scientific investigation. Specifically, I employed the Archaeognatha (F. Machilidae), which are generally agreed upon in Entomology to be the oldest extant insect lineage. This group has a body shape and plan that is the closest to an early pre-flight insect out of all extant lineages and as such, behavioural studies relevant to environmental conditions expected for protopterygote insects are justified. However, even within the Archaeognatha, only certain species may be of use when considering the environmental challenges defined in the fossil record during the rise of the flying insects. Namely, the height, location and environmental makeup of vegetation eliminates canopy dwelling extant Archaeognatha to remove any possibility of later aerial specialization for surviving at tree top heights (Yanoviak, et al., 2009). Unidentified bias can never be removed from a system but it is short-sighted not to at least try to account for what is known.

It is only by accounting for and defining the limitations of the morphological and environmental variables that behavioural experiments can be made relevant. Only through these efforts can the results of which provide clues towards the behavioural origin of insect flight under evolutionarily feasible circumstances. It is our inherent tendencies

towards engineering and organization that cause us to naturally gravitate towards reconstructing evolutionary processes by beginning with a concept that appears morphologically simple (generally with minimal overall costs) that progresses to a complex but still refined and efficient process. Unfortunately, numerous observations and morphological states are regularly mistaken as “basic” or plesiomorphic, when in actuality they may be the result of significant specialization and adaptation. It is this tendency that is the stumbling block that continually produces theories of winged gliding flight as a step towards flapping flight (Dudley, et al., 2007; Bradley, et al., 2009).

7.4. Further Scenarios for the Path towards Insect Flight

I have described and presented data throughout this thesis for a potential path towards flight in the insects based upon the mainly semi-aquatic to terrestrial habitat expected of the protopterygote insect. However there may be numerous other scenarios that one might consider based on current and currently unknown information within the fossil record. The Devonian period is known as the time of the fishes, labeled as such for the abundance of marine fossils showing a great diversity in the morphology, size and even behaviour of multiple pre-historic fish lineages. I have not considered that potential for predatory pressures on insect flight evolution from below the water surface by way of insectivorous fish species potentially living around the semi-aquatic landscapes the early pre-flight insects likely inhabited. One only needs to spend a warm summer evening fishing on the lake to realize that modern flying insects are regularly preyed upon by fish, both in their larval and adult winged forms, through various predatory behaviours from

multiple fish species. As the ‘time of the fishes’ partially overlaps with the expected time of insect flight selection, it stands to reason that fish may have been a significant selective pressure on the Insecta becoming adapted to a more aerial existence. Considering that surface skimming and sailing behaviours can occur across bodies of water (Marden and Kramer, 1994; Marden, 1995, 2003) and that basal wingless insects are capable of propelling themselves along the water surface tension (shown in this thesis) the ability to lift off from the water surface may have been essential for the selection of wings and flight overall. Laboratory and field studies involving fish predation on surface tension bound, or potentially sailing, Archaeognatha may yield expected survival times for such insects and potentially help define the likelihood of survival for insects performing these behaviours. Such experimentation may quantify the benefits of possessing the ability to lift off of or avoid the water, potentially *via* powered flight.

Abdominal legs become somewhat of an aerodynamic hindrance upon the selection for forward flight and lift-based mechanisms through flapping insect wings alone. Extra appendages would likely add more mass and surface area creating drag in an undesirable way as well as become an energetically expensive morphological feature to maintain. As I have shown in this thesis, legs may be useful for their initial drag initiating ability upon a fall. However, as flapping thoracic winglets became larger and more effective than the body at producing lift/drag to continue to decelerate falling velocity, it seems reasonable that selection for powered flight was likely accompanied by a reduction and eventual loss of abdominal legs, as evidenced by both fossil and extant pterygote insect lineages.

There are likely numerous potential ideas about specific behaviours that may arise from the results and concepts I have presented throughout this thesis. Such ideas are the lifeblood of the investigative process required to continue to solve the puzzle of how insects evolved into flyers. I suggest that concepts arising from the knowledge available and still to be discovered about the time of the protopterygote insects may be complimented by the work I have completed here.

7.5. The Science of Insect Flight

Tackling the puzzle of how powered flight evolved in insects is perhaps one of the greatest challenges to entomologists. Trying to piece together what likely occurred 400 + million years in the past, over 5 major biological extinctions and an unknowable number of significant planetary environmental changes of varying intensities, is more than a tall task. Indeed, we may never know what truly occurred over the millennia to give rise to the insect diversity that exists today, but when it comes to scientific discovery one can never really say never. Ingenuity and a realistic approach in regard to evolutionary processes can be the most important factors when attempting to further expand the pool of scientific contributions. During my Ph.D at Carleton University, I have attempted to approach the major theme and subsequent questions regarding flight evolution in insects with scrutiny and a willingness to look past the superficial in an attempt to find the likely answers for each potential avenue a flightless insect may have available in the right context. I endeavor to continue to seek out the truth and adopt the concept that experimental designs can always be tweaked when new and more fitting ideas and

hypotheses make themselves known. However, the ability to accept new contributions cannot be allowed to overpower the previous more fundamental concepts that make up the foundation of evolutionary theory as we understand it. The field of insect flight evolution is no different from any other science, it can be riddled with inaccuracies, misinterpreted reading as well as writing and simple subconscious bias. All of which are a direct consequence of our ability to ask “why?” and the unwillingness to except the answer “because”. I have tried to incorporate what I believe are significant clues to insect flight evolution through multiple disciplines, in an attempt to show that no one question can ever be entirely answered with one type of science. I argue that the concepts and observations I have put forth in this thesis are perhaps the best starting point towards discovering the details of how insects adopted flight and became so prevalent.

7.6. Recommendations for Future Work in Insect Flight Evolution Research

I have provided data based on actual experimental work in the field of insect flight origins in my thesis. This type of data is valuable when attempting to construct mathematical models, such as those presented by Ellington (1991). I suggest that a natural next step from the work I have presented in this thesis may be to define a mathematical model for a flapping parachuting insect which could be useful in future experiments and predictions upon the evolution of insect flight. Such a task would require thorough empirical observation of fluid structure to confirm prediction from models (mathematical and CFD).

The methods I have adopted here in regard to the design and construction of a protopterygote model are meant to be open to further modification. As new and updated concepts of early insect morphology are published, the current computer generated design (Figure 7.1) can be modified to reflect these changes. With the ever increasing march of technology and increased accessibility, the alteration and creation of such models like the one I have created becomes easier. The recent availability of submersible electronic servos in the used in Chapter 6 through the retail market provided a sufficient solution to the problem of wing movement on such a model in aquatic conditions, a problem through which the design and implementation of more realistic wing articulation may be an entire Ph.D thesis in itself.

At the time of writing, the potential next experimental steps from the work I have presented include the creation and testing of various models each more modified towards more traditional flying insects than the last. Some of the initial first steps include, installing and characterizing winglets along the remainder of the abdomen, and continuing to seek out more advanced, preferably smaller, aquatic servos or equivalent technology to continue to explore the effects of flapping wings. Future work orienting the model in such a way as to begin to establish the required wing morphology and body position to begin to adopt forward flight is also of great interest to me. It has recently been expressed to me by Dr. Jarmila Kukalová-Peck that the juvenile of the gigantic mayfly, *Bojophlebia prokopi* (Figure 7.2), may be the closest, morphologically, known fossil insect to the earliest flying lineage of the Insecta. The progression of the current protopterygote model towards morphology similar to this insect may be advisable for

future biomechanical study and may yet uncover more of the mystery of how insects evolved to fly.

Figure 7.1. A three dimensionally rendered protopterygote insect.

Figure 7.2. A reconstruction of Paleozoic gigantic mayfly, *Bojophlebia prokopi*, nymph that may be the nearest insect to the first flying insect. From Kukalová-Peck, 1985.

Appendix A: Tow Tank Facility Custom Fabricated Hardware

A.1. Introduction

The tow tank facility located at Carleton University in Ottawa Ontario required a substantial amount of customization and accessory hardware to allow the presented experiments to be feasible. The significant custom hardware associated procedures that were required to render the facility operational for propterygote model testing purposes is outlined in the following sections.

When assessed as a whole, the individual components create an ergonomic and usable traversing system with a wide range of possible model positions within the boundary of the tow tank volume. Consisting of both pre-made and custom fabricated parts, this assembly is not only usable for the purpose of this project but for future model testing and construction. Attention to detail from the initial stages of model setup to final data analysis is crucial when considering the ramifications of artifactual and undesired structures in the flow field upon model testing, issues that could potentially prove significant if left unchecked.

A.2. Adjustable Traverse and Sting Mount

Due to the size and area of interest of the models for testing an adjustable traverse and sting mount assembly was required to accurately position each model within the

image field. Components used to allow for a lengthwise adjustment in respect to the tow tank include two 91.44 cm long beams of 4x8 cm 80-20 aluminum, one sliding unbearing (16x14.2 cm), one custom 90 degree bracket with custom tension handle and various mounting bolts and brackets to secure the beams to the gantry arch. A 72.3 cm long 4x8 cm beam of 80-20 was secured to the unbearing on a 4 cm face side perpendicular to the length of the tank for increased rigidity and surface area mounting for the model sting assembly. The entire sliding assembly is secured via the manual tension handle on the 90° custom bracket and break knob on the unbearing when positioned appropriately (Figure A.1).

One 104.14 cm length of 4x4 cm 80-20 aluminum with flat right and left side faces was used as the sting which models would be mounted to. The sting is mounted to a 7.5x14.2 cm unbearing which slides onto and off of the perpendicular 80-20 edge mounted traverse beam previously described. A second 80-20 stabilizer beam (4x4 cm profile) was rigidly mounted to the sting and secure to the archway structure to prevent vibration during all imaging experiments. The sting assembly and traverse can be fine tuned in mm increments in the length and width directions of the tank, defined by a plastic ruler mounted to the open side of the perpendicular 80-20 beam, to position the model or calibration grid beneath the mirror mount assembly and within plane of the laser sheet. The overall result of this assembly is a rigid but adjustable model and calibration mount secured to the automated gantry system.

A.3. Adjustable Mirror Assembly

Due to the mount and position of the laser head, an adjustable mirror was required to fine tune the projection of the laser field to establish vertical illumination down onto the model to be tested. The mirror assembly was created from a plate of 2.2 mm thick aluminum (9x20 cm) bent on a break to create a mirror mounting face of approximately 45° from the horizontal. A front coated mirror (12.7 cm x 5.715 cm) was siliconed front coating out to a backer plate of 1.2 mm thick aluminum with flanged top and bottom edges to help prevent reflection from laser light into the room. The backer plate and mirror was siliconed to the 45° plate face which was then mounted via a ¼-20 zinc plated bolt, washer and aluminum pedestal component to a sliding carriage of aluminum, and PTE material supported by drill rod within a 40.64 cm length of 4x8 cm 80-20 aluminum. A 12 volt DC motor was connected via rubber belt drive to a pulley in the drill rod which allowed the entire mirror assembly to be moved in increments of 1 mm *via* battery power to the DC motor. A stainless steel ruler was mounted to the side of the 80-20 beam as a reference for mirror advancement and retraction. A manually adjustable tension knob was installed on the 45° plate via threaded aluminum block and spacer washers to allow for fine tuning of the mirror angle to accurately align the laser field with the calibration grid as well as ensure a vertical laser field projecting downward to the desired area of the model (Figure A.1).

Figure A.1. The adjustable traverse where the model sting is attached to behind the mirror assembly (MA) and in the path of the laser emitted from the laser head (LH).

A.4. Clear Lexan® Boat

The construction and assembly of a clear Lexan® boat, (35.56 cm (L) X 7.87 cm (W) X 2.29 cm side wall height), made from 0.635 cm thick Lexan® to eliminate shadows and water surface disturbances in the tow tank that could otherwise diffract and skew the laser field projected downward into the tank from above while the gantry was in motion. The boat was created from cut Lexan® pieces, one per face, and fused together with chloroform (Figure A.2). Both the bow and stern faces were set at 30° from the horizontal to help prevent wake formation while still avoiding the potential from water intrusion over the bow or stern. The boat was rigidly mounted to the mirror traverse assembly, hanging directly below the adjustable mirror assembly. The installation of the boat was such that only the bottom surface (approximately 3 mm depth) was in contact with the water to prevent any wake from being created through excess contact with the bow of the boat. The leading edge of the boat was mounted such that it was slightly higher than the stern to allow for less resistance on the water contacting and sliding under the bottom surface but not so much as to allow air bubbles to pass under. The boat is adjusted manually to ensure clearance for the model sting and for alignment to the laser field. Storage and removal of the boat after used required cleaning and care to avoid scratching the bottom surface which could allow undesired reflection or diffraction of the laser field in future trials.

Figure A.2. The clear Lexan® boat designed to allow an uninterrupted laser sheet to reach the model after deflecting downwards off of the mirror assembly.

**Appendix B: Supplementary rendered dPIV images of the wingless
protopterygote models.**

Figure B.1. A MATLAB® compiled flow field image of the straight body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.2. A MATLAB® compiled flow field image of the straight body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.3. A MATLAB® compiled flow field image of the straight body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.4. A MATLAB® compiled flow field image of the straight body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.5. A MATLAB® compiled flow field image of the straight body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.6. A MATLAB® compiled flow field image of the curve body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.7. A MATLAB® compiled flow field image of the curve body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.8. A MATLAB® compiled flow field image of the curve body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.9. A MATLAB® compiled flow field image of the curve body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.10. A MATLAB® compiled flow field image of the curve body model at the midline slice through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03m/s.

Figure B.11. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.12. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure B.13. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.14. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.15. A MATLAB® compiled flow field image of the straight body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03m/s.

Figure B.16. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.17. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.18. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.19. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.20. A MATLAB® compiled flow field image of the curve body model at 1 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.21. A MATLAB® compiled flow field image of the straight body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.22. A MATLAB® compiled flow field image of the straight body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure B.23. A MATLAB® compiled flow field image of the straight body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.24. A MATLAB® compiled flow field image of the straight body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.25. A MATLAB® compiled flow field image of the straight body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03m/s.

Figure B.26. A MATLAB® compiled flow field image of the curve body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.27. A MATLAB® compiled flow field image of the curve body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.28. A MATLAB® compiled flow field image of the curve body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.29. A MATLAB® compiled flow field image of the curve body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.30. A MATLAB® compiled flow field image of the curve body model at 2 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.31. A MATLAB® compiled flow field image of the straight body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.32. A MATLAB® compiled flow field image of the straight body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure B.33. A MATLAB® compiled flow field image of the straight body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.34. A MATLAB® compiled flow field image of the straight body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.35. A MATLAB® compiled flow field image of the straight body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03m/s.

Figure B.36. A MATLAB® compiled flow field image of the curve body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.37. A MATLAB® compiled flow field image of the curve body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.38. A MATLAB® compiled flow field image of the curve body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.39. A MATLAB® compiled flow field image of the curve body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.40. A MATLAB® compiled flow field image of the curve body model at 3 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.41. A MATLAB® compiled flow field image of the straight body model at 4 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.42. A MATLAB® compiled flow field image of the straight body model at 4 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicated flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure B.43. A MATLAB® compiled flow field image of the straight body model at 4 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.44. A MATLAB® compiled flow field image of the straight body model at 4 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.45. A MATLAB® compiled flow field image of the straight body model at 4 cm from the midline through 4 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03m/s.

Figure B.46. A MATLAB® compiled flow field image of the curve body model at 4 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.47. A MATLAB® compiled flow field image of the curve body model at 4 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.48. A MATLAB® compiled flow field image of the curve body model at 4 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.49. A MATLAB® compiled flow field image of the curve body model at 4 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.50. A MATLAB® compiled flow field image of the curve body model at 4 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.51. A MATLAB® compiled flow field image of the straight body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.52. A MATLAB® compiled flow field image of the straight body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

Figure B.53. A MATLAB® compiled flow field image of the straight body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.54. A MATLAB® compiled flow field image of the straight body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.56. A MATLAB® compiled flow field image of the straight body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03 m/s.

Figure 5.93. A MATLAB® compiled flow field image of the curve body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.57. A MATLAB® compiled flow field image of the curve body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.58. A MATLAB® compiled flow field image of the curve body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.59. A MATLAB® compiled flow field image of the curve body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.60. A MATLAB® compiled flow field image of the curve body model at 5 cm from the midline through 3 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.61. A MATLAB® compiled flow field image of the straight body model at 6 cm from the midline through 2 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.01 m/s.

Figure B.62. A MATLAB® compiled flow field image of the straight body model at 6 cm from the midline through 2 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.015 m/s.

FigureB.63. A MATLAB® compiled flow field image of the straight body model at 6 cm from the midline through 2 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.02 m/s.

Figure B.64. A MATLAB® compiled flow field image of the straight body model at 6 cm from the midline through 2 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.025 m/s.

Figure B.65. A MATLAB® compiled flow field image of the straight body model at 6 cm from the midline through 2 vertical zones of images. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow, which is moving at 0.03 m/s.

Figure B.66. A MATLAB® compiled flow field image of the curve body model at 6 cm from the midline through 1 vertical zone. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.01 m/s.

Figure B.67. A MATLAB® compiled flow field image of the curve body model at 6 cm from the midline through 1 vertical zone. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.015 m/s.

Figure B.68. A MATLAB® compiled flow field image of the curve body model at 6 cm from the midline through 1 vertical zone. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.02 m/s.

Figure B.69. A MATLAB® compiled flow field image of the curve body model at 6 cm from the midline through 1 vertical zone. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.025 m/s.

Figure B.70. A MATLAB® compiled flow field image of the curve body model at 6 cm from the midline through 1 vertical zone. Flow velocity is depicted in the horizontal direction (mU) in m/s. Red colouration indicates flow in the reverse direction of the oncoming bulk flow which is moving at 0.03 m/s.

Figure B.71. Fluid flow at the midline of the curve body model with legs across 1 vertical zone at 1 cm/s (0.01 m/s).

Figure B.72. Fluid flow at the midline of the curve body model with legs across 1 vertical zone at 1.5 cm/s (0.015 m/s).

Figure B.73. Fluid flow at the midline of the curve body model with legs across 1 vertical zone at 2 cm/s (0.02 m/s).

Figure B.74. Fluid flow at the midline of the curve body model with legs across 1 vertical zone at 2.5 cm/s (0.025m/s).

Figure B.75. Fluid flow at the midline of the curve body model with legs across 1 vertical zone at 3 cm/s (0.03 m/s).

Figure B.76. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.77. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.78. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.79. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.80. Fluid flow at 1 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.81. Fluid flow at 2 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.82. Fluid flow at 2 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.83. Fluid flow at 2 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.84. Fluid flow at 2 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.85. Fluid flow at 2 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.86. Fluid flow at 3 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.87. Fluid flow at 3 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.88. Fluid flow at 3 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.89. Fluid flow at 3 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.90. Fluid flow at 3 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.91. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.92. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.93. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.94. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.95. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.96. Fluid flow at 4 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.97. Fluid flow at 5 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.98. Fluid flow at 5 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.99. Fluid flow at 5 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.100. Fluid flow at 5 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.101. Fluid flow at 5 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.102. Fluid flow at 6 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.103. Fluid flow at 6 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.104. Fluid flow at 6 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.105. Fluid flow at 6 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.106. Fluid flow at 6 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.107. Fluid flow at 7 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.108. Fluid flow at 7 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.109. Fluid flow at 7 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.110. Fluid flow at 7 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.111. Fluid flow at 7 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.112. Fluid flow at 8 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.113. Fluid flow at 8 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.114. Fluid flow at 8 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.115. Fluid flow at 8 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.116. Fluid flow at 8 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.117. Fluid flow at 9 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.118. Fluid flow at 9 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.119. Fluid flow at 9 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.120. Fluid flow at 9 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.121. Fluid flow at 9 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Figure B.122. Fluid flow at 10 cm from the midline of the curve body model with legs across 2 vertical zones at 1 cm/s (0.01m/s).

Figure B.123. Fluid flow at 10 cm from the midline of the curve body model with legs across 2 vertical zones at 1.5 cm/s (0.015m/s).

Figure B.124. Fluid flow at 10 cm from the midline of the curve body model with legs across 2 vertical zones at 2 cm/s (0.02 m/s).

Figure B.125. Fluid flow at 10 cm from the midline of the curve body model with legs across 2 vertical zones at 2.5 cm/s (0.025 m/s).

Figure B.126. Fluid flow at 10 cm from the midline of the curve body model with legs across 2 vertical zones at 3 cm/s (0.03 m/s).

Appendix C: Extracted velocity data from dPIV experiments with wingless models.

Figure C.1. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the Midline slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.1. con't

Figure C.2. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the Midline slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.2. con't

Figure C.3.. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 2 cm slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.3. con't

Figure C.4. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 3 cm slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.4. con't

Figure C.5. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 4 cm slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.5. con't

Figure C.6. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 5 cm slice of the straight body model. Data is average +/- 95% confidence.

Figure C.6. con't

Figure C.7. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 6 cm slice of the straight body model. Data is average $\pm$ 95% confidence.

Figure C.7. con't

Figure C.8. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the Midline slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.8. con't

Figure C.9. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 1cm slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.9. con't

Figure C.10. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 2 cm slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.10. con't

Figure C.11. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 3 cm slice of the curve body model. Data is average +/- 95% confidence.

Figure C.11. con't

Figure C.12. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 4 cm slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.12. con't

Figure C.13. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 5 cm slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.13. con't

Figure C.14. Extracted horizontal velocities from the vertical mid point of each zone across the horizontal distance of each zone at the 6 cm slice of the curve body model. Data is average $\pm$ 95% confidence.

Figure C.14. con't

Figure C.15. Horizontal velocities at the vertical mid point of each zone for each Re. Only a partial area of the abdomen is imaged overall due to shadowing. Data is average $\pm$ 95% confidence.

Figure C.16. Horizontal velocities at the vertical mid point of each zone for each Re. Only a partial area of both zones is imaged due to shadowing. Data is average $\pm$ 95% confidence.

Figure C.17. Horizontal velocities at the vertical mid point of each zone for each Re. Only a partial area of both zones is imaged due to shadowing. Data is average $\pm$ 95% confidence.

Figure C.18. Horizontal velocities at the vertical mid point of each zone for each Re. Only a partial area of the thorax is imaged due to shadowing. Data is average $\pm$ 95% confidence.

Figure C.19. Horizontal velocities at the vertical mid point of each zone for each Re. Only a partial area of the thorax is imaged due to shadowing. Data is average $\pm$ 95% confidence.

Figure C.20. Horizontal velocities at the vertical mid point of each zone for each Re. Data is average $\pm$ 95% confidence.

Figure C.21. Horizontal velocities at the vertical mid point of each zone for each Re. Data is average $\pm$ 95% confidence.

Figure C.22. Horizontal velocities at the vertical mid point of each zone for each Re . Data is average $\pm$ 95% confidence.

Figure C.23. Horizontal velocities at the vertical mid point of each zone for each Re . Data is average $\pm 95\%$ confidence.

Figure C.24. Horizontal velocities at the vertical mid point of each zone for each Re. Data is average $\pm$ 95% confidence.

Figure C.25. Horizontal velocities at the vertical mid point of each zone for each Re . Data is average $\pm$ 95% confidence.

Appendix D: Extracted velocity and magnitude data from dPIV trials on flapping models.

Figure D.1. Velocity and magnitude profiles from an individual trial of the body with flapping winglets and no legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length. 1 cm/s, Run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.2. Velocity and magnitude profiles from an individual trial of the body with flapping winglets and no legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length. 1.5 cm/s, run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.3. Velocity and magnitude profiles from an individual trial of the body with flapping winglets and no legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length. 2 cm/s, run 3. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.4. Velocity and magnitude profiles from an individual trial of the body with flapping winglets and no legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length. 2.5 cm/s, run 3.

Figure D.5. Velocity and magnitude profiles from an individual trial of the body with flapping winglets and no legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length. 3 cm/s, run 3. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.6. Velocity (A, B) and magnitude (C, D) profiles from an individual trial of the body with flapping winglets and legs. The image field (A) and (C) is approximately 10 cm from the midline at 2/3 the winglet length while the image field for (B) and (D) is at 4 cm from the midline of the model showing flow near the wing base. 1 cm/s, run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.7. Velocity (A, B) and magnitude (C, D) profiles from an individual trial of the body with flapping winglets and legs. The image field (A) and (C) is approximately 10 cm from the midline at 2/3 the winglet length while the image field for (B) and (D) is at 4 cm from the midline of the model showing flow near the wing base. 1.5 cm/s, Run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.8. Velocity (A, B) and magnitude (C, D) profiles from an individual trial of the body with flapping winglets and legs. The image field (A) and (C) is approximately 10 cm from the midline at 2/3 the winglet length while the image field for (B) and (D) is at 4 cm from the midline of the model showing flow near the wing base. 2 cm/s, Run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.9. Velocity (A, B) and magnitude (C, D) profiles from an individual trial of the body with flapping winglets and legs. The image field (A) and (C) is approximately 10 cm from the midline at 2/3 the winglet length while the image field for (B) and (D) is at 4 cm from the midline of the model showing flow near the wing base. 2.5 cm/s, Run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.10. Velocity (A, B) and magnitude (C, D) profiles from an individual trial of the body with flapping winglets and legs. The image field (A) and (C) is approximately 10 cm from the midline at 2/3 the winglet length while the image field for (B) and (D) is at 4 cm from the midline of the model showing flow near the wing base. 3 cm/s, Run 2. MDS = Mid down stroke, DS = full down stroke, MUS = Mid up stroke.

Figure D.11. Velocity (A) and magnitude (B) profiles from an individual trial of the body with static winglets and legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length while the image field near the wing base is at 4 cm from the midline of the model. 1 cm/s, 95% conf.

Figure D.12. Velocity (A) and magnitude (B) profiles from an individual trial of the body with static winglets and legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length while the image field near the wing base is at 4 cm from the midline of the model. 1.5 cm/s, 95% conf.

Figure D.13. Velocity (A) and magnitude (B) profiles from an individual trial of the body with static winglets and legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length while the image field near the wing base is at 4 cm from the midline of the model. 2 cm/s, 95% conf.

Figure D.14. Velocity (A) and magnitude (B) profiles from an individual trial of the body with static winglets and legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length while the image field near the wing base is at 4 cm from the midline of the model. 2.5 cm/s, 95% conf.

Figure D.15. Velocity (A) and magnitude (B) profiles from an individual trial of the body with static winglets and legs. The image field is approximately 10 cm from the midline at 2/3 the winglet length while the image field near the wing base is at 4 cm from the midline of the model. 3 cm/s, 95% conf.

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