

Web construction behaviour
and ecological preferences of
the Eurasian diving bell spider
Argyroneta aquatica

Bryce Tidswell, BSc (Hons)

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Abstract

The diving bell spider (*Argyroneta aquatica*) is the only known species of spider to live a wholly aquatic lifestyle. It constructs a three-dimensional web, dubbed the diving bell, which it fills with air collected from the water's surface. The behaviours associated with the construction of such three-dimensional webs are largely understudied, in part because of their difficulty to dissect into discrete steps. There has been recent progress made in understanding the function of the diving bell as a gas exchange surface, and yet within that, the construction behaviours associated with this have seen little in the way of characterisation.

It was hypothesised that the webs of these spiders must contain some emergent properties ensuring their functionality across each instance they were built. This study has demonstrated that the process can be divided up into four key steps. Certain aspects of the process remain more consistent than others across the species, regardless of geographic location and despite genetic analysis revealing a high level of variation both within and between populations. Some features of construction are shown to differ based on ontogeny and sex, females are more repeatable than males in most regards – especially in the time taken to construct a web - and show a preference for web reinforcement. Females appear to follow a more rigid time frame in terms of construction, with repeatability increasing when analysed over a shorter period. Though web construction varied within individuals, it does seem that the process in females is more repeatable when the physical condition of the individual and the environmental conditions they are building in are more consistent. Some features related to optimisation of the process were noted. Body size was shown to determine the number of air bubbles collected to fill the web in a presumed trade-off between size and diving ability. Ultimately, the process appears highly plastic, being dependent upon the dynamic form of the pneumatic surface, and the volatile environment they inhabit.

Habitat preference data suggested the spiders rely far more on the vegetative substrate on which they build their web than on any factor of water quality as may have been assumed in some of the literature. This seems logical given the essential role of the diving bell in survival, and the necessary cover from predators the vegetation affords.

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Introduction

1.1.1. Modern spiders and their evolutionary history

The discovery of the fossilised spigots of an *Attercopus fimbriunguis* individual documents the first use of silk by animals (Selden *et al.* 2008). The oldest known spinnerets, and therefore the first true spiders (Family Araneae), are understood to have originated in the Middle Devonian ~385 million years ago (Decae, 1984; Shear *et al.* 1989), but there is compelling evidence to suggest that the group originated earlier, in the late Silurian or early Devonian around 420 million years ago (Coddington & Levi, 1991).

Discovery of fossilised spiders of this age is rare, most (~90%) fossil spiders are found in Cenozoic ambers which are less than 65 million years old (Selden *et al.* 2009). These ambers typically preserve representatives of almost all the 109 modern spider families (Selden *et al.* 2009). Today there exist around 45,000 species of spider with many species in Africa, Australia, and South America likely undiscovered (Decae, 1984; Coddington & Levi, 1991; Platnick, 2014).

Spiders within the infraorders Araneomorphae and Mygalomorphae are grouped based on their chelicerae position. The former have fangs that slope towards each other and cross, whereas the latter have fangs that point straight down. Spiders within the infraorder Araneomorphae, such as *A. aquatica*, are further subcategorised based on their silk spinning apparatus. They are placed in to one of two groups; ecribellate or cribellate (Blackledge *et al.* 2009). The presence of a silk spinning organ called the cribellum – which gives the cribellate spiders their name - and a comb of specially structured hairs on the metatarsus of the fourth pair of legs called the calamistrum (Joel *et al.* 2016) produce structurally sticky silk. This silks stickiness relies heavily on a large sum of van-der-Waals forces provided by a huge surface area (Römer & Scheibel, 2008). Ecribellate spiders on the other hand lack this organ and instead have an anterior median spinneret that produces glycoprotein based gluey silk (Coddington & Levi, 1991; Vollrath, 2006). The aggregate gland produces an aqueous layer that lines and plasticises flagelliform silk to produce the sticky capture threads (Römer & Scheibel, 2008).

1.2.1. Aquatic and semi-aquatic spiders

There are many species of spider found at the land-water interface who rely on the aquatic environment to hunt or for habitat, these species are considered to be semi-aquatic. Aquatic and semi-aquatic spiders include, but are not limited to, species of the genera; *Argyroneta*, *Dolomedes*, *Desis*, *Nilus* and *Pirata*. There are very few features that are shared by all aquatic spider genera, one fairly consistent adaptation being the presence of a plastron or some kind of trapped film of air on underwater hunters (Flynn *et al.* 2008) to allow air breathing while submerged. Many species of these genera actively hunt for fish (*Dolomedes*, *Nilus* & *Desis*) or supplement their diets (*Argyroneta*) with these kinds of prey (Nyffeler & Pusey, 2014). There are also instances of fish predation under experimental conditions by lycosids, in this case by individuals of the genus *Pardosa* (Nyffeler & Pusey, 2014). Little is known about the diet of these spiders in the wild, so it cannot be stated whether fish predation is a significant part of their feeding ecology (Nyffeler & Pusey, 2014). Most of the genera considered here are predatory hunters in that they do not build webs. Species of the genera *Dolomedes* are some of the most prolific aquatic hunters (Nyffeler & Pusey, 2014). With a leg span of 6-9 cm and a weight of ~0.5-2g they sit on the water's surface feeling for vibrations caused by moving prey below, much like the water were a web (Platnick, 2001). Species of the genus *Desis*, which hunt for aquatic prey at low tide, build silk nests within which they store air, so they can retreat into them during high tide (McQueen & McLay, 1983).

This large amount of variation in adaptation to the same ecological setting highlights the ability of spiders to inhabit a range of environments with various ecological niches (De Bakker *et al.* 2006). There is perhaps no better species for demonstrating this point than the Eurasian diving bell spider *Argyroneta aquatica* (Clerck 1757). The only known species to have adapted to live an entirely aquatic lifestyle (Seymour & Hetz, 2011). The unique context in which this species is found make it of particular interest in terms of its ecology and behavioural adaptations.

1.3.1. Distribution & Phylogeny of *Argyroneta aquatica*

A. aquatica is a Palearctic species with a distribution found to extend over a large proportion of Europe as well as Siberia and East Asia (specifically Japan, Korea & China) (Komnenov *et al.* 2011). Interestingly, there is a fairly recent record in Nigeria which enlarges the distribution of this species into the Ethiopian region (Olomukoro, & Dirisu, 2014; Komnenov *et al.* 2011).

The genus *Argyroneta* was originally placed in the subfamily Argyronetinae by Thorell (1870) which was then elevated to family by (Menge 1871). This was then expanded upon by Simon (1898) who recognised Argyroneteae as a tribe within Cybaeinae (Agelenidae). Some time later, Dahl (1937) concluded that Argyronetidae should be elevated to family status. Disagreements continued about its placement as Cybaeidae was elevated from subfamily to family status (Platnick, 2001) and it moved to the newly appointed family Cybaeidae (Selden, 2002). There is still debate over the systematic position of *A. aquatica* after several studies noted the karyotype of the sex chromosomes of *A. aquatica* (haploid) was different from most other members of Cybaeidae (diploid) (De Bakker *et al.* 2006).

A recent paper by (Wheeler *et al.* 2017) moved *A. aquatica* again from Cybaeidae to Dictynidae which is where it is considered to belong in the following study (See Fig. 2). There is one recognised sub species of *Argyroneta aquatica* denoted as *A. aquatica japonica* due to its presence in some Japanese localities (Masumoto *et al.* 1998a). The European and Japanese species are markedly similar, with this subspecies being classified as such due to the difference in size and shape of male palps between European and Japanese specimens (Ono, 2002).

The collection of behavioural data when determining the systematic position of different species can be useful and yet frequently it is rarely used. A review of 882 published phylogenies found that fewer than 4% relied on behaviour as a classification tool (Sanderson, *et al.* 1993). Similarly, in a review of 291 published phylogenies only 6% utilised behavioural data for classification (Sanderson *et al.* 1993).

It has been suggested that this may be because relevant data from behavioural studies is not always easily accessible, or in some instances even available (Benjamin & Zschokke, 2004). On top of this, any behavioural studies that may be required can be costly in terms of time and money when trying to determine classification (Benjamin & Zschokke, 2004).

1.4.1. Habitat, ecology & biology of *Argyroneta aquatica*

There are few instances in which air breathing animals have adopted an aquatic lifestyle, these being restricted almost solely to aquatic insects (Krivosheina, 2005) and marine mammals (Kramer, 1988). This study considers the only known spider to live its entire life underwater. With regards to *A. aquatica* specifically, it was noted by Mammola *et al.* (2016) that the complexity of vegetation structure was the most important factor affecting its presence in a location. This is likely due to the spider's necessity for an anchoring substrate. *A. aquatica* anchor their diving bells on aquatic plants for stability, and in turn the vegetation also provides some level of protection and cover from predators (Bristowe, 1971).

A. aquatica are typically found in wetland environments such as bog ponds, marshes, fens and swamps, as well as small lakes and ponds, all of which are ordinarily either oligotrophic or dystrophic (Mammola *et al.* 2016). In the UK, it is also known to settle and propagate in man made waterways such as canals. However, given their preferred habitat is that of wetlands, it is perhaps pertinent to address the issue of wetland habitat loss in the UK when considering their ecology and distribution. A paper by Davidson (2014) - that looked largely at wetland habitat loss in Asia, Europe, and North America - suggests that global wetland conversion and loss in the 20th and early 21st centuries has accelerated so much so that it is now happening 3.7 times faster than at any time period prior. Noting that 20th century losses of wetland worldwide were as high as 64-71%, considerably higher than the previously accepted 50% loss (Davidson, 2014). As well as this, the study being discussed considered the changes in area covered by wetland, rather than the state of habitat that remains i.e. it is still unclear to what extent the diminishing wetland that remains is suitable for species such as *A. aquatica*.

In established habitat where *A. aquatica* are found they have been shown to have further preferences in terms of their position in the water column, and in overall depth of the water where they construct their webs. According to Bristowe (1971), shallow areas of the water body are generally preferred by *A. aquatica*, where they construct their bells in the top 5-10cm (Seymour & Hetz, 2011). On warmer days - when the oxygen content of the water drops - *A. aquatica* may also prefer more shaded areas (Mammola, 2016). Although it has been noted by Seymour & Hetz (2011) that even under increased temperatures (22-31°C) the diving bells are still able to exchange enough O₂ from the water for spiders to persist. An extensive

paper by Seymour and Hetz (2011) concluded that if the water is reasonably oxygenated then the only limit to gas gill function is the longevity of the diving bell.

The feeding ecology of this species is largely unknown, though it is typical for spiders to be both predators of, and prey to, other species in their community food webs. A study by Perevozkin *et al.* (2004) examined the foraging behaviour of four genera of spiders and found that *A. aquatica* was a regulator of insect larvae numbers e.g. mosquito larvae in aquatic environments. Furthermore, a study by Mammola *et al.* (2016) found that habitat choice of the species was heavily based upon prey availability and predator abundance. The typical habitats of this species highlights a trade-off between the need to maximise energy intake but also limit any predatory pressures.

Possible predators of *A. aquatica* are thought to be fishes, frogs, and newts living in oxygenated waters of a neutral pH. However, Masumoto *et al.* (1998a) noted that fishes were not able to live in the low dissolved oxygen and lower pH (acidic) waters that *A. aquatica* could tolerate (DO ~3mg/l). These environmental conditions are thought to be due to the presence of sphagnum mosses which also provide the supporting structures on which the bell is anchored (Kadono, 1981). Along with environmental conditions limiting predation risk, some behavioural adaptations have also been noted. It was pointed out by Schutz & Taborsky (2005) that reduced activity of sub-adults and adult females during the day may aid in reducing the risk of predation. When the effects of predation are mitigated by habitat conditions and behavioural adaptations, it calls into question why *A. aquatica* typically has such a low density within aquatic habitats where it is present (Katsura & Nishikawa, 1982; Mammola *et al.* 2016). One suggestion is that the dispersal ability of the water spider is thought to be lower than other spiders because of its assumed lack of ballooning behaviour (Masumoto *et al.* 1998a).

It has been shown that toleration of variation in the environment is typical of many spider species and has led them to have been considered one of the most successful groups in terms of evolutionary radiation and ecological adaptability (Turnbull, 1973; Aakra & Dolmen, 2003). Given the limited data on *A. aquatica* it is easier to consider other species as more well studied examples of the potential for spider plasticity. Species of the genus *Troglohyphantes* have been shown to exploit small variations within largely environmentally consistent areas of caves (Mammola *et al.* 2018). This divides the community into microhabitats that can be

exploited given individual adaptive characteristics (Turnbull, 1973). A paper by Mammola *et al.* (2018) studied the recolonization of these spiders in a cave system that floods annually. They showed that natural variation within species of *Troglohyphantes* allows for rapid colonisation of new and different niches within the cave. As well as examples of plasticity across closely related species there are many examples of plasticity in individuals within a single species. One such example is *Agelenopsis aperta*, a spider which inhabits desert grasslands and is able to endure low temperatures over winter months through behavioural adaptations (Lee & Baust, 1985). *A. aperta* is hypothesised to over-winter in microhabitats that obviate the need for seasonal mechanisms such as the costly production of cryoprotectants (Lee & Baust, 1985). They are plastic in their ability to tolerate a wide spectrum of temperatures, much like *A. aquatica*. A study by Bromhall (1988) examined the over-wintering behaviour and super-cooling point (SCP) of *A. aquatica* and found that the mean SCP of all the spiders used in the study was 9.2°C, with no significant difference being found between maturity or size. As well as this, all the spiders collected from beneath the ice were capable of movement at temperatures slightly above 0°C (Bromhall, 1988). In the case of *A. aquatica*, this high level of ecological/adaptive plasticity is presumably afforded by the production of some undescribed cryoprotectants (Bromhall, 1988; Sømme, 1982). Moreover, other factors have also been shown to influence SCP including gut contents and dehydration levels (Sømme, 1982).

It is important to understand the biology of *Argyroneta aquatica* when trying to address its ecology and within that, its behavioural adaptations. There have been multiple studies that have addressed the biology of the *A. aquatica* and how it has evolved to cope with an aquatic lifestyle (Levi, 1967; Perevozkin, 2004; De Bakker *et al.* 2006; Seymour & Hetz, 2011; Neumann & Kureck, 2013; Neumann & Woerman, 2013). Levi (1967) noted that *A. aquatica* has one of the most elaborate tracheal systems of all spiders to compensate for a set of book lungs in which the number of lamellae are reduced to around 20 (Schutz & Taborsky, 2003). Wesenberg-Lund (1943) suggested this alteration in structure may be due to the effect of a localised gas exchange system on an animal's centre of gravity. A localised gas exchange system may make underwater movement more difficult, therefore, the evolution of an altered, more elaborate respiratory system was necessary for underwater locomotion.

1.4.2. The Plastron

The spiders cannot rely solely on the air in the diving bell for respiration and as such they carry a small pocket of air around when moving through their aquatic habitat, this air bubble is known as a plastron (Krivosheina, 2005). A plastron is produced by species specific surface morphology; morphological components include hydrophobic cuticle formations, bristles, plates, and hydrophobic hairs (Krivosheina, 2005). These components produce a rough surface that discourages fluid-solid contact and works to retain a film of air over the respiratory system of the individual (Krivosheina, 2005; Flynn, 2008). Water tolerance varies greatly between different species of spider due to variation in surface morphology, for example, the pisaurids are nearly 50 times more resistant to wetting than the pholcids (Suter *et al.* 2004). When these morphological components are at low density, the adhesion energy of the spider's cuticle alone can aid in the production of a plastron (Suter *et al.* 2004). When the film of air is present, the system is said to be in a Cassie state (Cassie & Baxter, 1944). The plastron, rather than the diving bell, can then function as an oxygen-carbon dioxide exchange surface (Atherton *et al.* 2014). In *A. aquatica* this gas layer typically covers the venter of the prosoma and the venter and dorsum of the opisthosoma (Levi, 1967).

In certain instances, O₂ in the plastron must be replenished and individuals need to surface or ventilate the plastron in some way (Krivosheina, 2005). One option is active ventilation of the plastron through leg beating, as seen in some insects. This can lead to high gill volume losses but reduced risk of predation from surfacing (Matthews & Seymour, 2010). *A. aquatica* are known to surface to replenish their plastrons as it is unable to satisfy their metabolic demands (See Fig. 1). The spider will move upwards in the water column, i.e. decreasing its diving depth, as it approaches the water's surface it performs a 180° turn that breaks through the surface exposing its abdomen, and with it the plastron, to the outer atmosphere where O₂ is then replenished (Neumann & Woermann, 2013). Surfacing to replenish the plastron O₂ supply exposes them to a high predation risk which is a significant selection pressure. Hence, they generally have an efficient system that maintains sufficient O₂ levels by removing most of the O₂ from the air bubble and allowing some to diffuse in (Levi, 1967). This can compensate for the low O₂ levels at night and allow for the longest possible submersion time (Levi, 1967).

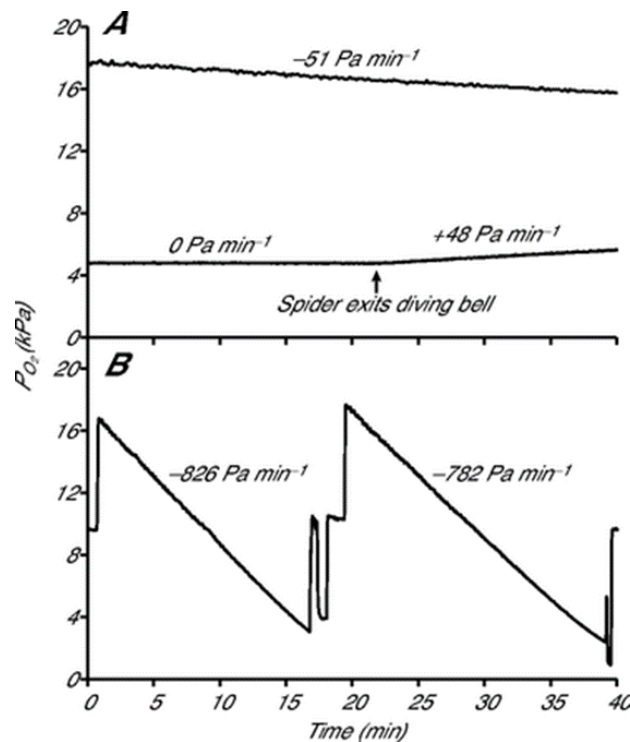


Figure 1. (A) Upper line: decrease in P_{O_2} in an occupied diving bell. Lower line: increase in P_{O_2} as spider leaves diving bell. (B) P_{O_2} measured in the body surface film of a spider away from its diving bell after two trips to the surface to replenish the air. Graph taken from (Seymour & Matthews, 2010).

1.5.1. Web construction

Silk production is common to all spiders, but the use of silk varies greatly between species. Typically, spider webs have exceptional structural and functional plasticity across different environments (Herberstein and Tso, 2011; Blamires *et al.* 2018), although, in the case of *A. aquatica* the silk seems to function most effectively in an aquatic environment. Their silk has been shown to become brittle and weak when removed from water (Neumann & Kureck, 2013).

This study is primarily concerned with plasticity in *A. aquatica* web construction. Spider webs are often discussed as extended phenotypes of the species in question (Schutz *et al.* 2007), with different studies demonstrating that behaviours related to web construction and decoration can be modified in response to changes in the external environment e.g. changes in abiotic factors such as temperature and gas composition (Schutz *et al.* 2007; Gan *et al.* 2010). A study by Seymour & Hetz (2011) tested the capacity of the diving bell of *A. aquatica*

to function as a gill by measuring the rate of oxygen transfer across the bell's surface (Seymour & Hetz, 2011).

They concluded that bells in severely hypoxic environments and with small surface areas had to be replenished frequently, though a typical bell will satisfy the oxygen requirements of a spider at rest (Seymour & Hetz, 2011). The capability of *A. aquatica* to alter their bell size and shape to fit dissolved oxygen concentrations and metabolic demand speaks to the complexity of their bell building behaviours (Seymour & Hetz, 2011).

Studies related to web building behaviour are common as the process is largely innate and these behaviours can be analysed and compared in a relatively easy way (Eberhard, 1982; Coddington, 1986). As well as this, terrestrial webs such as two-dimensional orb webs are systematic and predictable in their construction, even if the material properties of the silk may differ (Craig, 1987; Benjamin & Zschokke, 2004). Species that build these webs will always construct them in the same way making comparisons between individuals easier. Most webs can be referred to as two-dimensional as they are only constructed across two planes (Blackledge *et al.* 2003), though there are also multiple instances of terrestrial three-dimensional web construction. As stated previously, there is only one instance of aquatic web building in spiders - the diving bell - which is one of those that can be categorised as three-dimensional (De Bakker *et al.* 2006).

1.5.2. Three-dimensional webs

Three-dimensional webs are rarer than two-dimensional webs due to their increased demand for materials, energy, and time from the individuals constructing them (Blamires *et al.* 2013). Many three-dimensional webs exist due to additions to existing two-dimensional structures (Blamires *et al.* 2013; Bountry & Blamires, 2013). It has been shown that some araneid and nephilid orb web spiders will place stiff major ampullate silk threads around their two-dimensional webs to produce a barrier-like structure. (Blackledge *et al.* 2009; Blamires *et al.* 2012). There are also some examples of semi-aquatic spiders that produce three-dimensional webs that function similarly to *A. aquatica*. Members of the genus *Desis* are inhabitants of rocky shore intertidal zones where they are periodically submerged by high tide (McQueen & McLay, 1983). Because of this, they developed an interesting ecological-physiological solution in the form of silk nests that trap and store air, so they may take refuge when submerged

(Owen *et al.* 2018). A study by McQueen & Mclay (1983) found that the air space in and around the silk nest is sufficient for survival over periods of submersion greater than 24-hours (the typical survival period afforded by the plastron). Interestingly, they also seem to share similar behaviours in relation to mating. When *Desis marina* are joined by a mate of similar size the nest must be expanded in order to copulate and construct the egg cocoon – much like with *A. aquatica* (Mcqueen & McLay, 1983; Schutz & Taborsky, 2005). Throughout numerous studies related to web construction (Blackledge *et al.* 2009; Blamires *et al.* 2012 & 2013; Sue *et al.* 2018) in which spiders are made to replicate web building behaviours, it becomes clear that it is invariably more difficult for them to maintain a consistent shape in three-dimensional webs given the increased complexity of the structures (Blackledge *et al.* 2003; Blamires *et al.* 2013; Su *et al.* 2018). From this it can be suggested that diving bell construction is also likely to vary greatly.

There are clear benefits to this kind of plasticity in web construction. Biotic and abiotic factors such as prey availability, light, temperature, and oxygen are rarely uniform in aquatic environments (Downing, 1991). Therefore, *A. aquatica* must be able to alter their webs based on internal factors such as metabolic requirements and food intake, as well as external factors such as the substrate they choose to anchor the web on. However, plasticity in an extended phenotype is also known to incur fitness consequences (Blamires *et al.* 2018). In orb-web weaving species it has been shown that certain species will increase production of glycoproteins for glue droplets under nutrient deprivation (Blamires *et al.* 2018). This is presumably to increase web retention of prey (Blamires *et al.* 2018), but also increases the risk of individual mortality, if, for example, prey numbers in the area have dropped below requisite numbers to sustain the individual.

1.5.3. What is conserved across webs?

Even though three-dimensional webs are rarely consistent in shape, it stands to reason that there must still be some structural basis that underpins their assembly, certain behaviours that are conserved across all instances in which the web is built. Behaviours that are typical within an individual species. However, it is notable that building behaviours have been shown to differ between males and females of *Argyroneta aquatica*. A study by De Bakker *et al.* (2006) suggested that males build larger bells than females due to them being physically bigger, but that they use fewer threads than the female's use for their smaller bells. On the

other hand, a study by Schutz & Taborsky (2003) suggested it was the females that produced larger bells that are also positively correlated with their size. Females are ultimately restricted in their bell size, if a female grows to big it is too energetically costly to build a bell of the same size (Schutz & Taborsky, 2005). Females tended to use more anchor threads than males to secure their bells, whereas, males produce more walking threads (De Bakker *et al.* 2006). It is unknown whether the larger number of walking threads is due to the increased need for mobility in the males i.e. searching for females and food (De Bakker *et al.* 2006).

It is clearly apparent that there are major sex differences in construction behaviours within the species, though there are a multitude of factors that may play a part in the building process. Some internal parameters such as; age, silk supply, body size, or satiation are likely to be involved (Eberhard, 1988; Mammola *et al.* 2016), as well as external parameters such as; temperature, pH, dissolved oxygen (DO), turbidity, water depth and various aspects of vegetation height i.e. percentage of sub-merged, free-floating, and emerging plants (Mammola *et al.* 2016).

Given that this study is attempting to ascertain any consistency in the features of *A. aquatica* web construction, it was presumed that adults would be a best reflection of the consolidation of these behaviours (Bell et al. 2009). Conversely, Bell et al. (2009) noted that behaviours had appeared more repeatable in juveniles of certain ectotherms. In practice it is difficult to work with sub-adult spiders in the lab due to them typically being less bold than adults i.e. will not accept food when offered. As well as this, sub-adult spiders spin webs more quickly than adults due to them being correlated with size (Zschokke, 1997; Heiling & Herberstein, 1998; Schütz & Taborsky, 2003), making video analysis more difficult.

The results of a study carried out by Risch (1977) compared the webs of four species of three different genera and concluded that web design can actually be influenced by age, body size, weight, and sex, noting that differences in web structure between conspecifics was very variable across different ages. Other studies, such as work by Witt *et al.* (1972), found similar results, with differences in web construction between mature adults and sub-adults of the same species. Although all of these spiders were terrestrial orb-weavers, making links between the effects of age on web building between them and *A. aquatic* tenuous.

Even though bell building is seemingly highly variable across individuals, there are certain construction elements that remain the same. There are three kinds of threads that are produced by both males and females of *A. aquatica*, and one that is strictly produced by females (De Bakker *et al.* 2006). Both males and females will produce anchor threads, walking threads, and the diving bell threads themselves, with females also producing the threads necessary for egg-cocoons (Barghout *et al.* 1999; De Bakker *et al.* 2006). There is some information available on the web building behaviours and habitat ecology of *A. aquatica*, but as far as we are aware, there seems to be no comprehensive documentation in the literature of the behaviours associated with the construction of the diving bell, or with the properties of its aquatic habitat in England.

1.6.1. Ecological indicators

Ecological indicators, typically referred to in the context of indicator species, are used to understand, monitor and highlight ecological changes (Siddig *et al.* 2016). Indicator species are chosen for their known ecological preferences which provide a benchmark for understanding a habitat i.e. if a particular species is present then certain abiotic or biotic factors must be as such (McIver *et al.* 1992; Neet, 1996). Because of this, these indicators can also be useful in assessing the efficacy of environmental management (Siddig *et al.* 2016). There are two levels on which indicator species can be used to gather environmental data; a narrow approach looks at the known ecological preferences of an individual species (McIver *et al.* 1992), where as a broader approach gathers data on the species richness of communities (Allred & Allred, 1975; Neet, 1996).

Indicator species are frequently used in the assessment of water quality (Parker, 1979; Sládeček, 1983; Barik *et al.* 2017), with organisms such as fish, periphyton and benthic macroinvertebrates being used most commonly (Barbour *et al.* 1999). The use of specified biota in producing water quality indices are essential for ensuring that the water industry and conservation organisations can make quantifiable evaluations and objective decisions (Parker, 1979). Although the use of invertebrates as indicator species is common there seems to be little documented use of spiders, either terrestrial or aquatic. Because of this, very few studies have critically examined the limitations in the use of spiders as ecological indicators (Neet, 1996).

There are some examples in the literature of spiders being used as bioindicators at both levels, for example, a study by McIver *et al.* (1992) found that there was a potential for the use of certain species of spider as indicators of anthropogenic heavy metal pollution. In terms of measures of species richness, a study by Scott *et al.* (2006) looked at the spider assemblages of 11 peat bogs as a synthetic indicator of habitat quality (Neet, 1996). Their results also suggested a potential use for spiders as ecological indicators, at least in peat bogs, as they satisfied most of the criteria suggested by McGeogh (1998).

This study posits that they could be used as indicators of water quality due to the specific conditions in which they are found naturally (Seyyar & Demir, 2009; Mammola *et al.*, 2016). As well as this, they are largely consistent in numbers regardless of season making sampling possible at any point in the year (Bromhall, 1988). Although, they are typically in low numbers even in areas where they are considered continuously present and so may require a higher sampling effort than some other indicator species (Mammola *et al.* 2016). Spiders have been shown to be sensitive to different types of pollution at varying levels. It has been noted that spiders are particularly sensitive to certain insecticides, more so than insects (Royauté *et al.* 2015), and in one study it was found that hunting spiders in particular are more sensitive to insecticides than web building spiders (Bostanian *et al.* 1984). Being as *A. aquatica* spend their whole lives under water and both hunt and build webs it is unclear how they may respond to increased levels of different pollutants. On the other hand, there are studies that have examined the effects of heavy metal pollution on spiders that live semi-aquatic lifestyles such as *Pirata piraticus* (Tojal *et al.* 2002). Preferential selection of soil insect prey by *P. piraticus* rather than aquatic or flying prey lead to a higher accumulation of heavy metals on average in its tissues than other tested local species i.e. *C. phragmitis* that foraged on reed plants (Tojal *et al.* 2002). Increased heavy metal concentrations have been shown to have seemingly negligible effects on the survivability of many spider species (Migula & Babczyńska, 2013), moreover, heavy metals accumulating mostly in soils are likely to have little effect on *A. aquatica* due to its choice of prey and entirely aquatic lifestyle. Heavy metal solubility has been shown to increase as pH decreases, given that *A. aquatica* commonly inhabit acidic waters, accumulation of toxic levels of heavy metals – most likely anthropogenically - may be more pertinent to this species (Li *et al.* 2013).

An *Argyroneta aquatica* assay for environmental quality will only be useful when sufficient data on the effects of different levels of pollution or varied environmental conditions on this specific species have been gathered. There have been some studies that have gathered data on the ecological preferences of *A. aquatica* (Aakra & Dolmen, 2003; Seyyar & Demir, 2009; Mammola *et al.* 2016), and one study that has looked at the effects of dissolved oxygen on the size of the diving bell (Shutz *et al.* 2007). Though not cemented, it is now understood that this species is known to prefer slightly acidic, colder waters with little turbidity. However, it has also been shown that this species can survive across a large spectrum of conditions (Aakra & Dolmen, 2003; Olomukoro, & Eloghosa, 2009; Seymour & Hertz, 2011). Even though the species has been shown to have high ecological adaptability, it can still be assumed that when found in the wild it will exist in conditions that are optimal for its survival i.e. cold, acidic etc. Therefore, it does appear that once sufficient data are gathered, *A. aquatica* has the potential for use as an indicator of habitat quality.

1.7.1 Aims

This research aims to address the following questions:

- What sequence of behaviours is necessary for *Argyroneta aquatica* web construction?
- How does web construction differ within and among individuals, and across geographically distinct populations?
- Can *Argyroneta aquatica* be a useful predictor of water quality?
- How genetically diverse are *Argyroneta aquatica* populations from one another?

Materials & Methods

2.1.1 Pilot studies

It was originally thought that the spiders need cool, dark rooms in order to induce bell building more readily given their typical time budget and ecological preferences *in situ* (Masumoto, 1998b; Mammola *et al.* 2016). However, pilot studies in our laboratory revealed that they would build at similar rates in warm, well-lit conditions. This is likely due to the extended habituation period that moving the spiders between filming locations incurred. Spiders were left to construct bells in their own time as well as being stimulated to build through the provision of food. If left to construct without a stimulus, spiders could take hours before they would begin, because of this, food was presented to shorten wait times between building. Experiments were carried out in four different conditions, cooler than the housing lab and in light, cooler and in darkness, warmer and in light, warmer and in darkness. In dark trials all lights were turned off in the room and a lamp with a red bulb was used to visualise the spiders in their tank. This was used under the assumption that they were not able to perceive the red light (Zurek *et al.* 2015). Across all these pilot studies there was no observable difference in the propensity of the spiders to build once offered food. Given this, the trials were conducted in the housing lab (24°C) in light and with food used as a stimulus.

2.2.1. Collection of spiders

Study sites were visited between January 2019 and July 2019 at which spiders were collected. Four spiders were already present in the lab that had been collected from the Czech Republic (the same location as the new spiders, see Fig. 3) in May 2018. *Argyroneta* collected from English sites were done so using the British Arachnological Society (BAS) spider recording scheme (BAS, 2019b). Locations were selected based on the recorded presence of *A. aquatica* from 1992 onwards, with most sightings having been recorded between 1992 – 2000. 11 sites were visited where between one and a half and two and a half hours was spent looking for *A. aquatica*, covering around a three-metre stretch along the bank. Search time varied based on habitat geography (i.e. ease of access to water body), weather, and time restrictions. Searching was carried out both close to the bank of the water body and around one metre in towards the water's centre. In instances where specific locations were not given, a local water body was selected based on visual inspection in which water clarity and vegetation complexity

were considered. Because wetlands are more ephemeral than other lentic ecosystems, and given the very dry and warm summer of 2018, water bodies in these areas were only selected when they showed established aquatic vegetation and a depth of 10cm or greater at their centre (Seymour & Hetz, 2011).

Sitting on the banks of the water body and using two hands a large section of the bed was disturbed by pulling it up gently and allowing it to settle. Any reeds were then pulled up from the base along with the soil they grew in and placed in a white tray where it was separated by hand. Any fauna would then begin to move around the tray where it could be spotted and moved to a small plastic tube, if necessary, to be identified. If a spider was found that was thought to be *A. aquatica* it would be placed in a tube with some water to observe it swimming. If the spider did not swim then the water was quickly removed, and the spider returned to the appropriate habitat.

Spiders were collected from three sites: Thursley nature reserve (coordinates: 51.1678332, -0.7065321), Potteric Carr nature reserve (coordinates: 53.4988916, -1.1132371), and a small pond near lake Mácha, Czechia (coordinates: 50.571137, 14.651605) (See Fig.3). ~20 spiders were collected from the two U.K sites, and ~40 from the Czech site, with adults being preferentially collected. This was done so that web building behaviours could be compared at the same life-stage. All captured spiders were returned to the lab and selected preferentially by size to be used in behavioural trials. It wasn't possible to collect a sample of strictly adult spiders. It was suggested by Bell *et al.* (2009) that juvenile ectotherms are typically just as repeatable, if not more repeatable than adults, and so some juveniles were included in the trials.

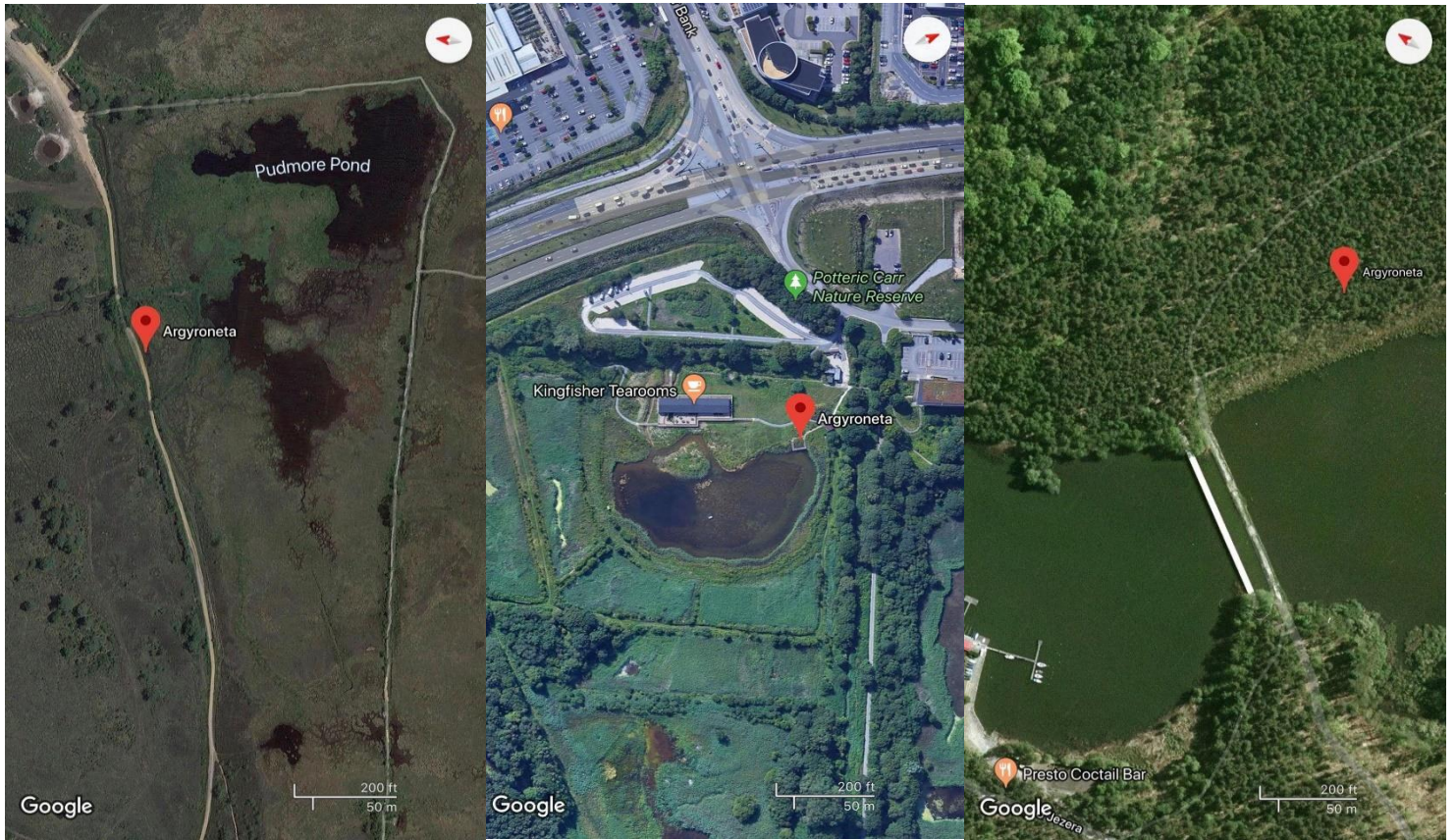


Figure 3. Aerial view of the three collection sites taken from google maps (ordered as listed above) (Google Maps, 2019), the red pin indicates the specific water body from which they were collected.

2.3.1. Housing of the spiders

The group of spiders were housed at the University of Nottingham, University Park Campus. The spiders were kept in a temperature-controlled room (24°C) in either small plastic tanks (dimensions: H: 13cm, W: 19.5cm, D: 12cm), or large plastic cups (H: 15cm) both half/three quarters filled with treated tap water and the bases lined with ~3cm of pebbles. To produce the treated water, a large tank (dimensions: L: 29cm, H: 20cm, D: 19cm) was filled with tap water and half a cap of aqua safe water treatment solution was added. This was then left overnight and used in the filming tank, as well as being added to storage tanks/cups as and when necessary. Air was supplied to the tanks via an air pump and a single plastic tube that fed into each tank. The plastic tubing was plugged at the ends to mitigate the disturbance caused by large air bubbles. Air flow could be controlled by taps that connected the tubing from the air pump to the tanks. Plastic cups were placed into larger trays (dimensions varied) that were half filled with treated water and contained an aerator tube. Slits were cut into the

bases of the plastic cups to allow the oxygenated water to flow between the tray and the cups. This allowed for mass storage of spiders in a small area. The tanks/cups also contained plastic pond reeds (~8cm) and small pieces of polystyrene at varying sizes that mimicked rocks. These were provided both for cover and to supply surfaces on which their webs could be built. Food was provided periodically and as necessary, with the spiders typically being fed twice a week at varying times of the day. At least two days were left between each feeding. Food supplied when not conducting trials varied between brine shrimp (*Artemia salina*) (typically 3 or 4 per feeding), glassworm (Genus *Chaoborus*) (typically 2 or 3), and small crickets of an unknown species (1 per feeding) (Zschokke & Herberstein, 2005; Lichenstein *et al.* 2016).

2.4.1. Taking measures of size

Measures cephalothorax width (mm) & leg length (mm) were taken so that the relationship between morphological parameters and web construction behaviours could be tested. Leg length measures were taken by removing the spiders from their tanks one at a time and placing them in a clear plastic tub with a 15cm ruler underneath. Once the spiders had habituated to their surroundings, they would extend out their legs and the tub could be orientated so that the front left leg and the back-right leg were lined up with the ruler. From there a measure was taken in millimetres from the tip of the front left leg to the tip of the back-right leg. In order to gather cephalothorax width of the spiders a light microscope was used. A petri-dish was placed under the microscope lens with the spider inside. A ruler was placed under the lens and above the spider where it was aligned with the widest part of the cephalothorax (the point at which the 2nd pair of legs is joined) (Hodge & Marshall, 2017), a measure was then taken by eye. The spiders were removed from their tanks one at a time using a 25ml plastic tube, placed on the petri-dish, and measured. Five minutes was given for the spiders to habituate i.e. extend their legs and expose their cephalothorax, after being removed from the water and before being measured.

2.5.1. Web construction trials

All construction studies were carried out in the same temperature-controlled room (24°C) within which the spiders were housed. This was done between 9am and 5pm from February 2019 to July 2019. All the spiders were filmed in the same tank in the lab (dimensions: H:

13cm, W: 19.5cm, D: 12cm). During filming the tank was always three quarters filled with treated water and contained one plastic pond reed placed at the bottom front left of the tank. This provided cover and a bell building surface. The tank was prepped the day prior to filming and allowed to acclimate to the temperature of the heated room overnight. The spiders were removed from their home tanks or from cups using a plastic pond reed and a small plastic tube and were placed in the filming tank. Individuals were given five minutes once in the tank to habituate to their new surroundings before being fed.

The polystyrene 'rocks' and other plastic pond reeds were not used as they shielded the spider from the cameras view and made it difficult to capture the web construction process. The tanks were placed in front of a white background to increase the contrast between the spider and its surroundings and make analysis of the videos easier (See Fig. 4). Pilot studies revealed that bell building could be stimulated through the provision of food. Given this, any diving bells in the tank were removed and the spiders were fed a single brine shrimp. The spiders were then watched consistently from that point. Typically taking around 10 to 30 minutes for the spider to begin building a new web. The camera would then be set to begin filming, the spiders were checked periodically – noting down more inconspicuous behaviours in a notebook to aid in later analysis - until the web was constructed and the filming would be stopped.

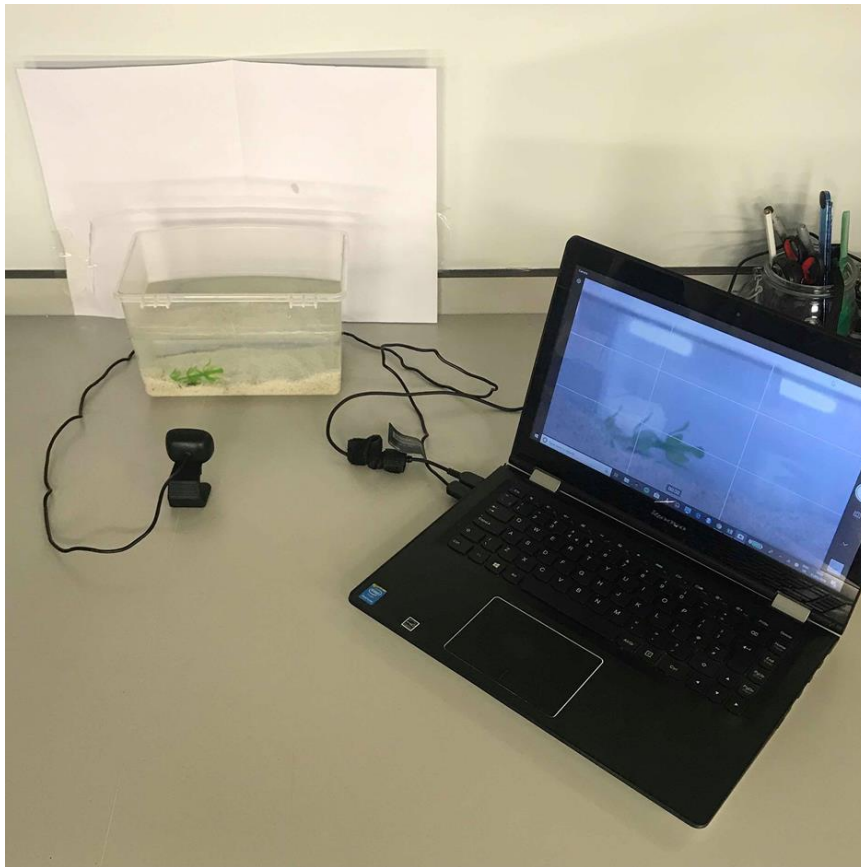


Figure 4. Photo of set up for web construction studies.

Five repeats were carried out for each individual following the synthesis of protocols described in a meta-analysis of behavioural repeatability that looked at 114 studies (Bell *et al.* 2009). Different numbers of individuals from 3 different populations (48 total spiders) produced 240 web construction videos. These videos were recorded using a Microsoft LifeCam HD-3000 webcam and were stored on a Seagate expansion portable drive for later analysis using BORIS behavioural software (Friard & Gamba, 2016). Using the BORIS software an ethogram was produced that characterised each of the behaviours exhibited in the pilot study videos and those noted in the literature that were relevant (Masumoto *et al.* 1998b; De Bakker *et al.* 2006). The videos were imported into and then played in Boris, with behaviours being marked using pre-selected keys. The data were then tallied into the total amount of time (in seconds) the spider spent exhibiting a particular behaviour across the entire process using the inbuilt time budget analysis function. This value could then be converted into the percentage of time spent exhibiting that behaviour out of the whole length of time taken to build the web, and then inputted into an excel spreadsheet. As well as these percentages, the

number of times a behaviour was exhibited was also noted. Other behaviours seen were also noted in order to calculate the frequency at which they occurred.

2.6.1. Measures of water quality taken in lab conditions

A YSI pro plus quatro multiparameter water probe (YSI incorporated, 2009) was used to gather data on the water conditions of the spiders kept in the housing lab. The water conditions of each spider from each of the three populations that were used in the construction studies were monitored for the first three months of housing. They were stored in either a tank or a plastic cup, with primary lab conditions including method of storage and delivery of food being kept consistent. Each tank was assessed for multiple measures of water quality once a week in the first month of trials. Given that these factors remained consistent over this period these checks were moved to monthly from then on. Spiders were stored in a temperature-controlled room where water temperature remained consistent (water temp between 20.0°C – 21.0°C).

The probe was left in the first tank/cup for 30 minutes and set to take measures every five minutes. The probe took measures of dissolved oxygen (DO) (ppm), conductivity ($\mu\text{S}/\text{cm}$), temperature ($^{\circ}\text{C}$), pH, oxidation-reduction potential (ORP) (mV), and salinity (ppt). These were to ensure the tanks remained habitable for the spiders and to track consistency in all areas. The probe would often take around 10 minutes to adjust to new water conditions in the first instance, therefore, only the final three values of the first tank were reported when taking an average of the water conditions i.e. last 15 minutes of data recorded. Each consecutive tank/cup after this would only require around 15 mins to gather data which was representative of the water conditions.

A similar process was used for the filming tank. In this case the water was checked on random days once a week for four weeks having been left to stand overnight (The filming tank used always contained water that had been prepped the previous day and then left overnight). The water in the film tank lacked consistency in DO however all other factors stayed largely consistent. pH consistently read between 7.0 – 8.0 (typically ~ 7.7). After the initial four weeks the water in the filming tank was checked randomly each month during the period over which behavioural trials were conducted to ensure the relevant properties of the tap water were consistent.

2.7.1. Water quality in the field

At each of the sites visited in search of the spiders a YSI pro plus multiparameter water probe was used to gather data on the conductivity ($\mu\text{S}/\text{cm}$), dissolved oxygen (DO) (ppm), temperature ($^{\circ}\text{C}$), pH, ORP (mV), and salinity (ppt) of the water at field sites. Numbers discovered were also recorded for other species of note such as *Pirata sp.*, *Dolomedes sp.* and great crested newts (*Triturus cristatus*). As well as this, numbers of possible prey items of *A. aquatica* were also recorded based solely on visual inspection and using a similar protocol to that in Mammola *et al.* (2016). This was done so comparisons could be made between biotic and abiotic measures of sites with and without *A. aquatica* present. Waters increase in temperature towards the middle of the year and there are studies that have shown increases in insect abundance along with this (Stark & Phillips, 2009). Although this is largely accepted, graphs were still plotted to ensure these trends were exhibited within this fairly contained study. The probe used was calibrated in January before field work began. The probe was placed in the water body at a point parallel to the search area and left to collect readings at 15-minute intervals for the duration of the search. The values reported in the final hour of searching were used to calculate averages for each measure i.e. the final 4 values.

A 100ml sample of the water was taken in falcon tubes for inspection of possible prey items of *A. aquatica* in the water column, and for further analysis of the water in the lab if necessary. Alongside the water sample, the number of different potential prey items – as could be distinguished based on morphology alone, and only those that were found on the white tray - was noted over the course of searching. The water samples were taken before searching commenced to avoid collection that would not truly reflect species/debris in the water column. The samples were then covered in parafilm to avoid evaporation.

Both Thursley nature reserve and Potteric Carr nature reserve were visited for a second time to gather more data on local distributions of the spiders and variation in water quality across the site. Initial visits and secondary visits were carried out on 24/03/2019-24/05/2019 and 18/04/2019-10/05/2019 respectively. A 5-metre belt transect was placed laterally across the centre of the water body. A quadrat was aligned at 1-meter intervals along the transect where in a YSI pro plus multiparameter water probe was placed to gather data on conductivity ($\mu\text{S}/\text{cm}$), DO (ppm), temperature ($^{\circ}\text{C}$), pH, ORP (mV), and salinity (ppt) of the water. Within each quadrat observations were also made on % submerged vegetation cover, % emerged

vegetation cover, and % free-floating vegetation cover through visual inspection. Depth at the centre (cm) of each quadrat was also measured using a 45cm ruler (Mammola *et al.* 2016).

Each of the 5 quadrats was searched in the same manner as explained in the 'collecting spiders' portion of the methods, with numbers of *A. aquatica* found being noted for each quadrat. In this instance search time was narrowed down to 15 minutes per quadrat with the water probe being set to collect data every 5 minutes so that an average of the three measures could be taken for that quadrat.

2.7.2. Testing for nutrient concentrations *in situ*

Across the period of site scouting and collection the Thursley site began to dry up and eutrophicate (See Fig. 5 & 6). One paper by Aakra and Dolemn (2003) suggested the spiders could be found in eutrophic lakes and ponds, this is corroborated through periodic visits to the Thursley site in which *A. aquatica* continued to be found in similar numbers. It was thought pertinent to test the waters to understand what nutrients had caused the process. Measurements of nutrient concentrations were taken *in situ* for the Thursley site (Moore & Locke, 2013). These measures were gathered in July, spiders were found during sampling but not collected, tests were carried out using three different types of test strips; King British 6 in 1 water testing strips for nitrite and nitrate, King British ammonia testing strips, and Hatch Phosphate testing strips. Nutrient concentrations have been shown to vary greatly throughout the day based on temperature, photosynthesis, and respiration levels (Wurts, 2003; Weihrauch *et al.* 2018), given this, the site was visited in July and was tested in the afternoon around ~1pm.

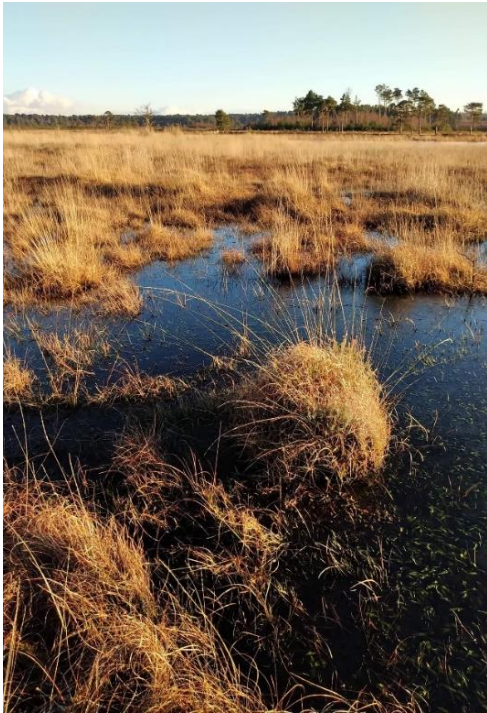


Figure 5. Thursley nature reserve in March 2019 upon initial visit scouting for collection sites.



Figure 6. Thursley nature reserve in July 2019 when water nutrient concentrations were tested.

2.8.1. DNA extraction

Genetic data was gathered to explore the level of diversity both within and between populations, as was done with the behavioural data. This was carried out in the hopes that it may better frame any variation seen within the behavioural data gathered. DNA was extracted from 2 individuals from each of the three populations that had been preserved in ethanol (2 males from Surrey, 2 females from Doncaster, and 2 females from Czechia). All individuals were adults at time of extraction. Extraction was completed using the GenElute™ Mammalian Genomic DNA Miniprep kit, which is based upon tissue lysis in proteinase K, DNA precipitation and binding to a column before eluting in an aqueous buffer solution. The protocol as listed in the booklet provided was followed exactly aside from the initial lysis step wherein the tissue sample was left in proteinase K overnight in a heat block set to 74°C. Immediately post extraction, DNA samples were placed in a freezer to prevent degradation.

2.8.2. PCR protocol

Initial Polymerase chain reactions (PCR) assessed the viability of universal spider primers collected from the literature (LCO1, CO1, 28S, Hedin, and H3) (Hedin & Maddison, 2001; Su & Smith, 2014) (See Table 1). The results of these initial reactions were visualised on a gel via gel electrophoresis (See Fig. 7). Primer CO1 only showed some viability with the two males taken from Surrey whereas all other primers readily bound to the DNA and produced large bands on the gels. Given that primers CO1/LCO and primers 28S/Hedin bind to two complementary sections of the same gene, more PCR reactions proceeded using the same primers in attempt to gather the sequences for all primers from all individuals. This was done with the intention of combining the corresponding sequences to increase the total length of DNA sequenced. In the second round of PCR reactions six individuals were used, 2 from each population, primers for CO1 continued to be unusable and so were dropped from other PCR reactions. PCR protocol was carried out in the same manner in each instance, using 50µl reaction volumes. Alongside the samples was a negative control. In each 50µl reaction was: 25µl of biomix, 10µl of sterile distilled water, 5µl of forward and reverse primer and 5µl of DNA. The PCR itself involved three minutes at 93°C for initial denaturation, then 35 cycles at the following temperatures: 94°C for 30 seconds, 54°C at 30 seconds, 72°C for one minute, and then a final extension at 72°C for five minutes.

Table 1. Primers used for genetic analysis along with the references for the corresponding papers from which the primers were taken.

R primer code	GGTTCGATTAGTCT TTCGCC	TAAACTTCAGGGTG ACCAAAAAAATCA	TCCTGCTAAATTTG CAAAATACAGCCCC	ATATCCTTRGGCAT RATRGTTGA	GAAACTGCTCAAAG GTAAACGG
R primer name	Hedin C	HCO-N-2198	COL-r	H3R	28S _o
F primer code	GAAACTGCTCAAAG GTAAACGG	GGTCAACAAATCAT AAAGATATTGG	GGGCTCCTGACATA GCCCTTCTCTCGAA	ATGGCTCGTACCAA GCAGACVGC	GGTTCGATTAGTCT TTCGCC
F primer name	Hedin O	LCO-J-1490	COL-F	H3F	28S _c
Gene target	D2–D3 region 28S nuclear ribosomal RNA	COL mitochondrion	COL mitochondrion	Histone H3	D2 – D3 region 28S nuclear ribosomal RNA
Reference	Hedin & Maddison, 2001	Su & Smith, 2014	Su & Smith, 2014	Su & Smith, 2014	Su & Smith, 2014

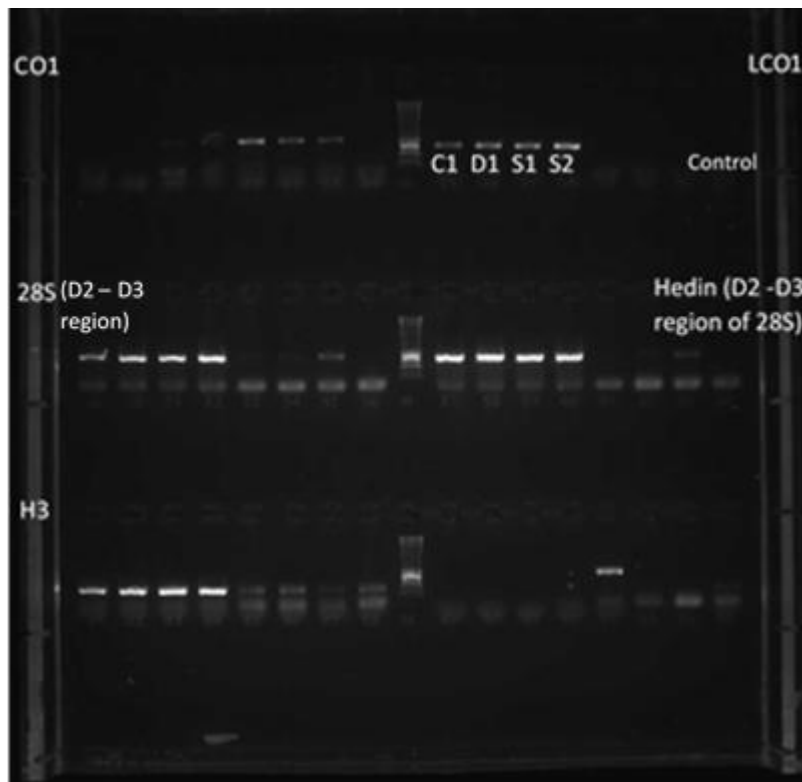


Figure 7. Results of preliminary PCR to assess the viability of universal primers taken from literature then visualised on a Gel. No DNA was sequenced from this round of PCR. Five primers were used with DNA taken from four individuals from the three populations (one from Czech, one from Doncaster and two from Surrey). The samples were placed in the first four wells from left to right as shown above (C1 = Czech individual one, D1 = Doncaster individual 1 etc.), with the negative control i.e. no DNA sample, placed in the final well. Ladder was placed in the centre of each row.

PCR reactions were carried out for a second time at higher volumes in order to gather a high enough concentration of DNA for sequencing. Gel electrophoresis was used again to conclude that the PCR reactions had worked and to determine which samples should be sent off for sequencing. Figure 8 is an example of one of the gels produced after a round of PCR. The primer for CO1 failed to successfully bind to the DNA samples again and so was not used in genetic comparisons.

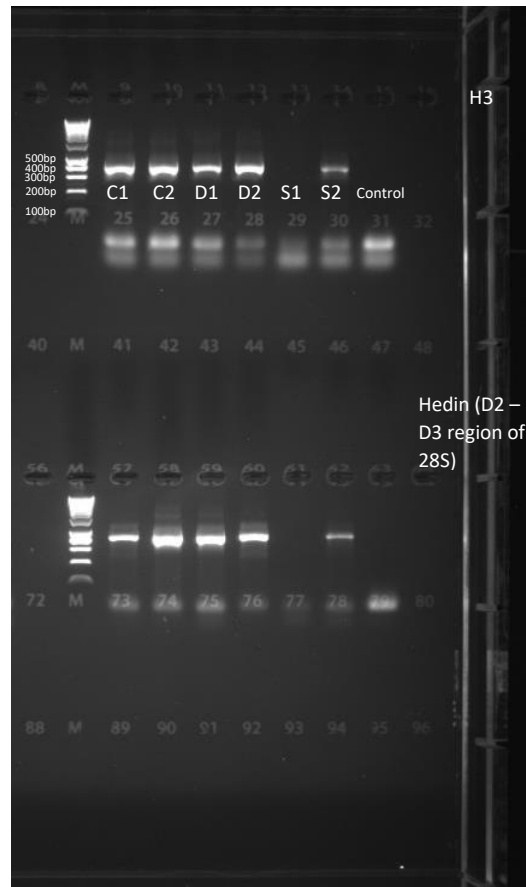


Figure 8. Results of an example gel from a PCR in which six individuals were used (C1 = Czech individual one, D1 = Doncaster individual 1 etc.) and a negative control i.e. no DNA sample.

Though bands were produced on the gel, the sequences returned for the Hedin primer were of poor quality. Ultimately, three genes were used for comparison between the populations. LCO1, 28S, and H3.

2.9.1. Statistical analysis

2.9.2. Behavioural data

- Linear regression analysis was used to compare time budget measures to each other (as proxy for behavioural decisions), as well as to compare morphological parameters (cephalothorax width (mm)) with time budget measures.
- Repeatability, also referred to as the intra-class correlation coefficient ICC, is a measure of individual consistency with regards to a specified trait. This was calculated via a one-way ANOVA for each behaviour that was repeated by all individuals across all trials i.e. construction, rest, reinforce, and air bubble. Equation shown below (Wolak, 2012). Where S^2_A is the variance among groups and S^2_W is the variance within groups.

$$ICC = \frac{S^2_A}{S^2_A + S^2_W}$$

- Multiple testing on the data was corrected in attempt to avoid type I errors. As such the Holm-Bonferroni sequential correction was applied to all behavioural and water quality data. An excel calculator was taken from Gaetano (2013).
- The non-parametric Kruskal-Wallis test was used to compare differences between total time taken in males and females, and between individual females.
- Fisher's exact test was used to compare the number of failed and successful attempts at gathering an air bubble between adults and sub-adults.
- Other behaviours were not repeated by all individuals across all trials and so repeatability could not be measured. Instead these behaviours were simply reported as frequency of times exhibited.

2.9.3. Water quality/field data

- The VIF and cor functions in R were used to determine multi-collinearity between predictors. A generalised linear model (GLM) was fitted to determine what predicts the presence of *A. aquatica* across the 11 sites visited. The best model was assessed through sequential deletion of models based on AIC (Akaike information criterion)

values (Mammola *et al.* 2016). AIC weights were produced using the Weights function in the MuMIn package in R (Bartoń, 2019; Wagenmakers & Farrell, 2004).

- Kruskal-Wallis was used again to compare 10 different measures (e.g. pH, DO, % submerged vegetation cover etc.) taken at the two sites where *A. aquatica* were present.

Results

3.1.1. Description of web construction

Before behavioural analysis could occur, the necessary steps had to be determined. Through observation of over 240 web construction videos, the behaviours observed were defined and tallied to draw conclusions on those which were necessary for the process – with necessity being defined as the presence of a behaviour in every video. From these observations it was concluded that web construction relied on the following 4 discrete behaviours. All figures show stills taken from the same video.

1. Construction - The spiders always began by laying down silk (See Fig. 9). They attached numerous anchor points to the substrate in no clear pattern, making them difficult to count for comparison. This process is the laying down of the framework of the diving bell, a sheet web under which an air bubble will be placed. This is performed across an axis of 360° (Doerstelmann *et al.* 2015), where they sequentially lay fibres in a hierarchical arrangement (De Bakker *et al.* 2006). Although most of the construction occurs with the abdomen physically above the cephalothorax, it can also occur in the opposite orientation, as shown in Figure 9.
2. Air bubble collection - The gathering of the air bubble from the surface follows a standard pattern as the spider swims to the surface, performs a 180° turn and forces its abdomen out of the water. As it re-submerges the abdomen and its 4th pair of legs are lined up parallel as to trap a large air bubble between the hairs on the abdomen and the legs (See Fig.10).
3. Reinforcement - this typically occurs across an axis of 180°. The spider selectively reinforces the pocket of air from within, laying silk more locally in order to stabilise the pneumatic habitat. Laying silk on only one side allows for an opening at the base of the diving bell where the spider can enter, as well as giving it the characteristic shape (See Fig. 11).
4. Rest – here it is defined as complete motionless, wherein the spider and its spinnerets are still (See Fig. 12).

By definition, the collection of an airbubble follows the initial construction of the sheet web and reinforcement follows the collection of the air bubble. However, rest can occur at any point in the process, though it also occurs frequently just before collection of the air bubble. Construction can also occur after an air bubble has been collected and reinforced, though this typically involves the fixing of more anchor points rather than any external addition of silk to the now trapped air bubble.

1. Construction



Figure 9. Still taken from video of *A. aquatica* constructing its diving bell, spinnerets are apart indicating the spider is spinning silk.

2. Collection of air bubble



Figure 10. The spider has collected an air bubble from the surface.

3. Reinforcement



Figure 11. The spider's spinnerets are apart indicating the spinning of silk but in this case on the inside of the newly collected air bubble.

4. Rest



Figure 12. The spider is at rest, in this instance just before surfacing to collect an air bubble.

3.2.1. Summary of web construction data

A summary of the data gathered from 44 spiders is shown below.

Table 2. The minimum, median, mean and maximum values for each of the main components of web construction. Data is taken for each web constructed from the newly collected spiders only (n=44) i.e. 220 videos, five for each individual. Total time is in seconds, No. of surfaces only includes successful attempts, all other measures are in percentages of time spent.

Total time		Construction		Rest		Reinforce	
Min	105.6	Min	35.00	Min	1.000	Min	1.000
Median	327.3	Median	69.00	Median	8.000	Median	13.00
Mean	340.2	Mean	68.02	Mean	9.809	Mean	14.98
Max	706.2	Max	89.00	Max	50.000	Max	40.00

Table 2. Continued

Air bubble		No. of surfaces		Spinning silk	
Min	1.000	Min	1.0	Min	45.00
Median	5.000	Median	2.0	Median	85.00
Mean	6.077	Mean	1.9	Mean	83.00
Max	22.00	Max	5.0	Max	98.00

Overall the spiders showed a difference of 600.6 seconds (~10 minutes) between the fastest and the slowest time taken to build a bell, with the mean sitting at around half the longest time (See table 2). Construction, rest, reinforce, and airbubble also showed a wide range in percentages of time spent, with all but construction showing spiders that spent as low as 1 percent of their time carrying out these behaviours at least once. The percentage of time spent spinning silk (the sum of time spent constructing and time spent reinforcing) revealed a large difference between the maximum and the minimum. With at least one individual spending 53% more-time spinning silk in at least one instance of web construction. However, the mean proportion of time spent spinning silk is high at 83% suggesting on average the spiders spent most of their time spinning silk.

3.3.1. Repeatability and the intra-class correlation coefficient (ICC)

Given that the data gathered was that of proportions it had to be transformed to fit the normality assumption of a one-way ANOVA. As it was proportion data an arcsine transformation was therefore performed in Microsoft Excel (2016) using the following formula, =ASIN(SQRT('relevant cell')) (McDonald, 2009). Once transformed the necessary assumptions were checked again using R (R core team, 2016). The data that then fit the assumptions were used.

Estimates of intra-class correlation coefficient (ICC) were gathered using the ICCest function in the ICC package (Wolak, 2012, 2016) using R statistical analysis software. This calculated the ICC from the components of a one-way ANOVA as well as reporting the corresponding confidence intervals (CI) for the estimate. The ICC was estimated for all the spiders from which videos were collected (n=48) and then only the newly collected spiders (n=44). The ICC was estimated for total time taken, the four core behaviours described in the methods, and the total amount of time spent spinning silk (this is a summation of the time spent constructing and reinforcing). Number of surfaces for an air bubble (Shapiro Wilk test for normality: $p < 2.2e-16$), proportion of time resting ($p < 5.942e-10$), and total silk span ($p < 4.117e-06$) were not normal, even after their corresponding transformations. These data gathered for these measures violated the assumptions of the necessary statistical tests, and so they were not included in the graphs displaying repeatability analyses. Although, rest and total silk span did appear similarly repeatable compared to other aspects of the construction process when initially tested (ICC = 0.18 and 0.15 respectively). Given the violation in both normality and equality of variances the ICC estimates of these behaviours are not robust. Even though other behaviours also violated assumptions, ANOVA's aren't particularly sensitive to normality (Blanca *et al.* 2017) and so the ICC estimates of all but those two factors remain reliable. Only those behaviours which fulfilled assumptions were analysed for comparison between populations and sexes.

Comparisons between the ICC estimates of older and more recently collected spiders show a clear decline (0.06) (See Figures 13 and 14) in the most repeatable aspect i.e. total time. These spiders were collected almost a year before this study began, therefore, only data from newly collected spiders was used in the analysis after this point.

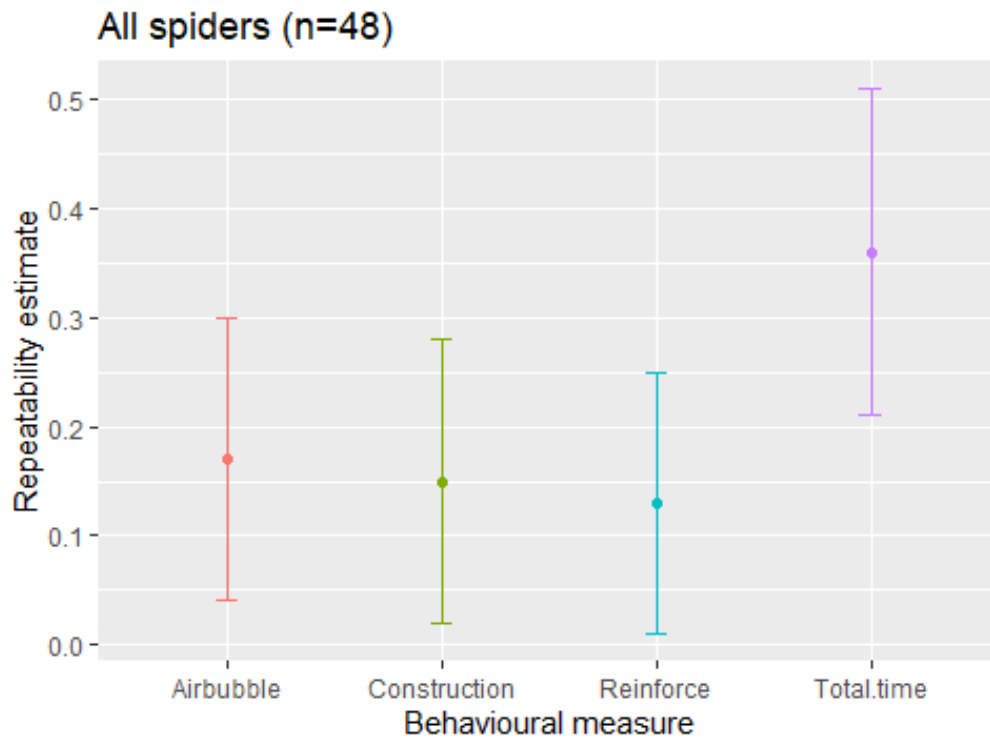


Figure 13. Repeatability estimates with their 95% CI's for total time taken, and three of the core behaviours for all spiders (n=48).

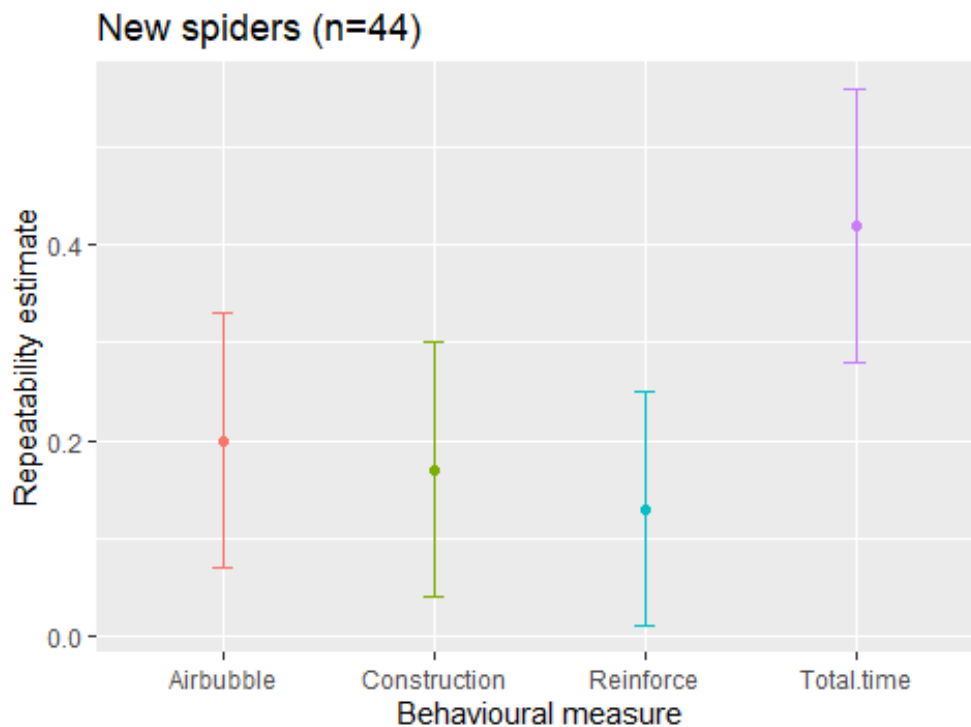


Figure 14. Repeatability estimates with their 95% CI's for total time taken, and three of the core behaviours for all newly collected spider (n=44).

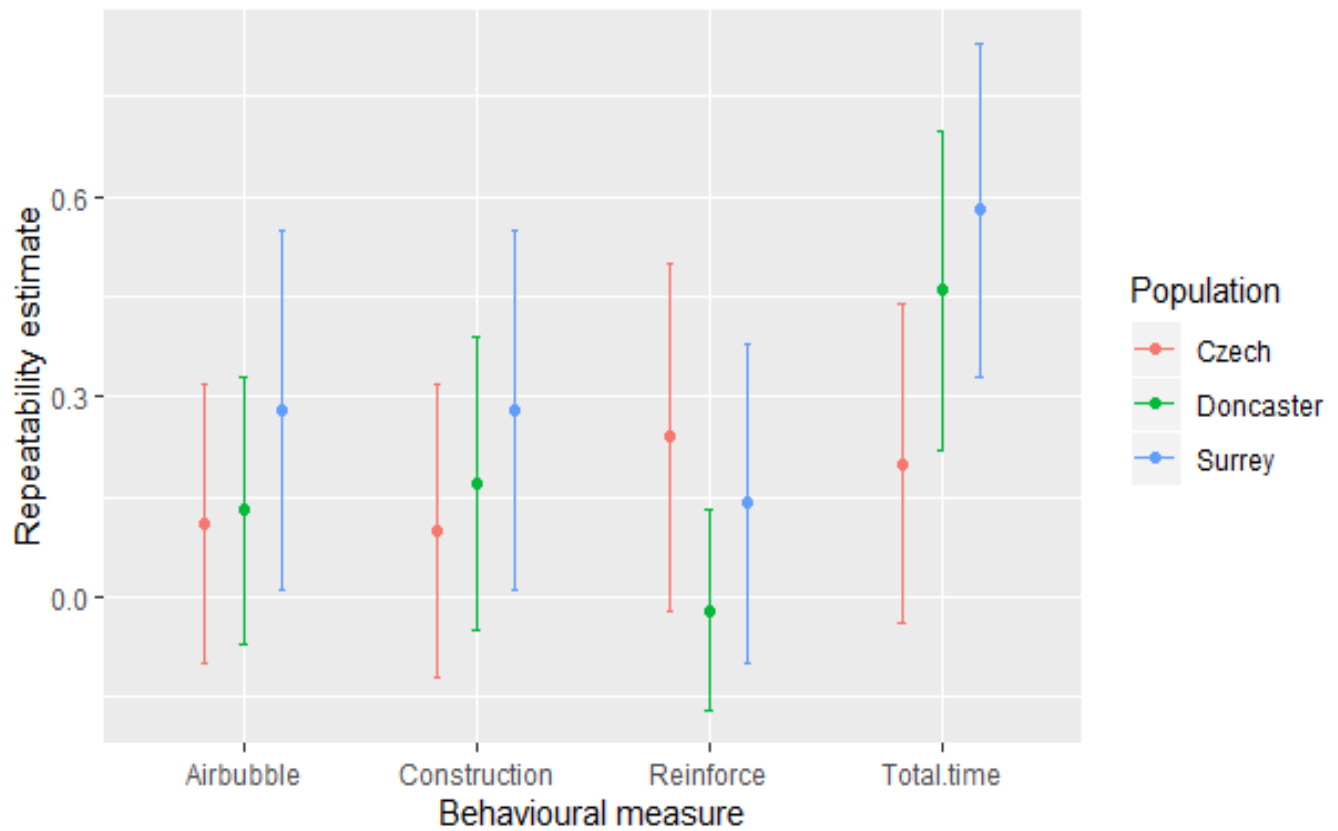


Figure 15. Repeatability estimates with their 95% CI's for total time taken and three of the core behaviours for the spiders collected from the three populations separately: Czech population ($n=14$, $F=7$, $M=4$, Sub adults = 3), Doncaster population ($n=17$, $F=11$, $M=5$, Sub adults = 1), and the Surrey population ($n=13$, $F=7$, $M=5$, Sub adults = 1).

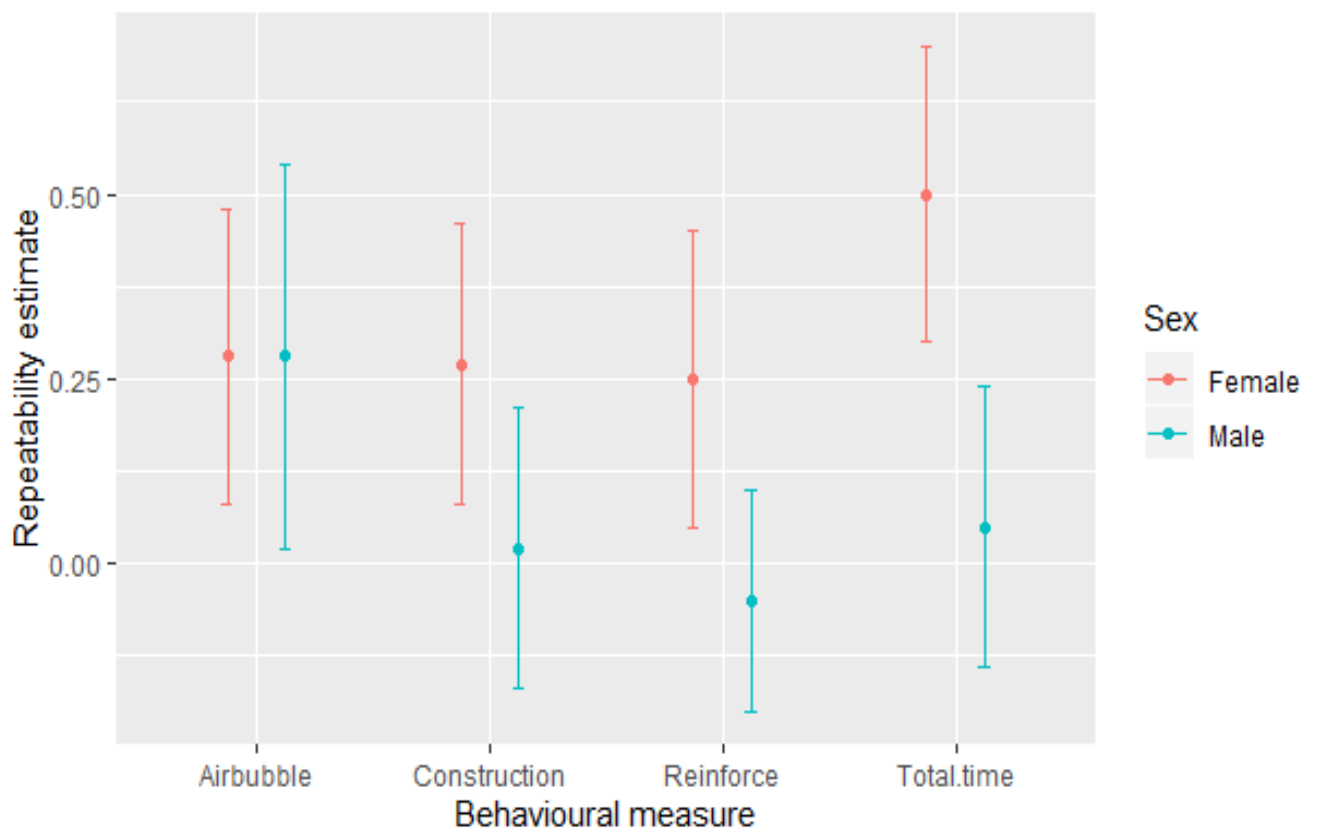


Figure 16. Repeatability estimates with their 95% CI's for total time taken and three of the core behaviours for male (n=14) and female spiders respectively (n=25). Females show higher repeatability in every measure except for air bubble where the two sexes are the same.

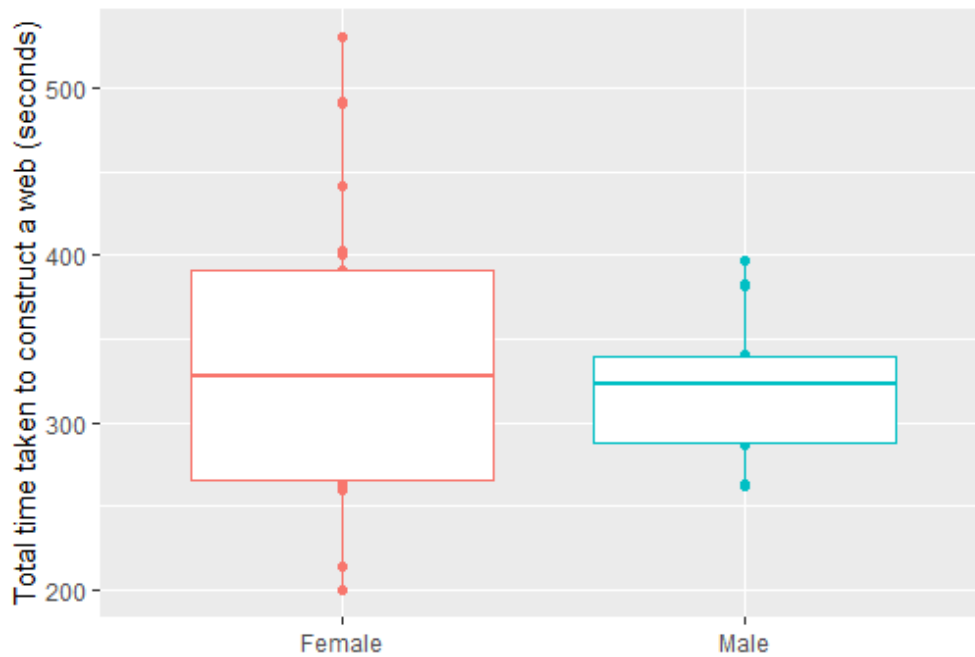


Figure 17. box plot showing the average total time taken (across five trials) for individual males ($n=14$) and females ($n=25$) to construct a diving bell. There is no significant difference in the median total time taken between sexes ($p = 0.58$), but there is a significant difference between individual females ($p = 3.129\text{e-}07$).

ICC estimates vary (defined as differences > 0.05) between all populations for almost all traits (except for air bubble between Doncaster and Czech). The most typically repeatable trait (total time taken) varies considerably between populations (See Fig. 15), and hugely between the sexes. The Czech population show little difference in the behavioural traits measured, with all estimates sitting between 0.10 and 0.25. When the data are split by sex we can see clear differences in estimates (See Fig. 16). The total time estimate for females is 0.45 higher than the males (See Fig. 16), with females also showing greater repeatability in construction and reinforcement time, both factors related to web architecture. Males and females show the same repeatability in time spent collecting air bubbles. Figure 17 demonstrates the wide span of total times exhibited by the considerably more repeatable females compared to the more compact male times. The median of both sexes sits almost equally, with the male times almost fitting between just the first and third quartile of the females. In all instances sample size is small and therefore confidence intervals wide ($\sim 0.3\text{--}0.4$), making inferences about differences between populations and sexes weak. However, CI's for total time between

females and males do not overlap, and so it can be suggested that females are still more repeatable than males in total time taken.

Once the data on ICC estimates had been gathered, the nest function in the ICC package was used to estimate the number of individuals needed to produce a desired CI width when $k = 5$ (k = number of individual repeats). For a CI width of 0.3, 42 individuals are needed, Whereas CI widths of <0.25 required 60+ spiders. This level of data collection seemed unlikely given the restraints placed on both travel for physical collection of specimens and the amount of time this would take. Therefore, analysis continued with the data gathered from 44 individuals.

The data were explored to understand in what respect the proportion of time at rest and the proportion of time spinning silk were not normally distributed (See Fig. 18). This demonstrates that individuals on average spend as little time as possible at rest, largely between 5-10% of their web building time. In contrast, and unsurprisingly, the proportion of time spinning silk takes up the majority of the web construction process (~75-90%).

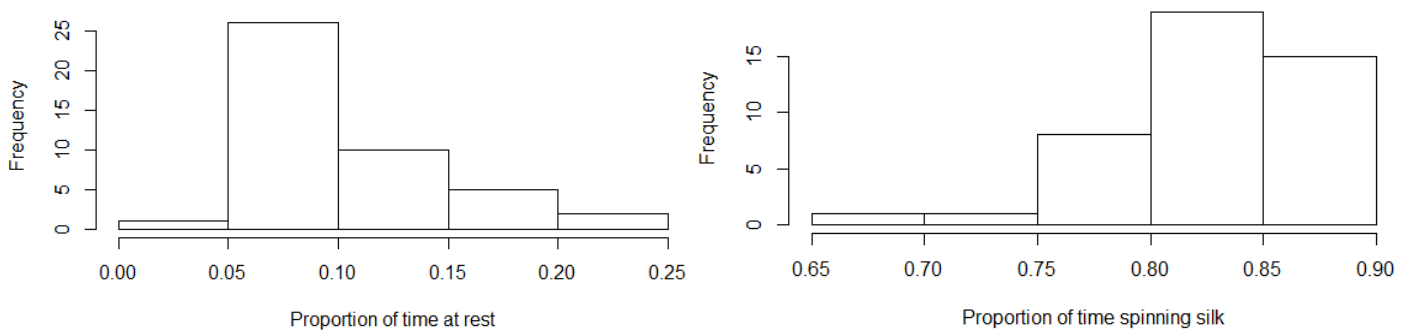


Figure 18. Histograms showing the average proportion of time spent at rest (left) and the average proportion of time spent spinning silk (right) for each individual ($n=44$).

3.3.2. Increased short-term repeatability

This study compared web construction over successive days, therefore, short term repeatability was considered as only the first two webs constructed.

When running repeatability analysis on data taken from the first two repeats per individual ($k=2$) (data taken 24 hours apart), we see an increase in the repeatability of all four considered measures (See Fig. 19). Most notably an increase of 0.22 in the ICC estimate of total time when compared to the same analysis run on five repeats.

When the short-term data is split by sex (See Fig. 20) we see a difference in ICC estimates for every measure, with the smallest difference (0.08) occurring between proportion of time spent constructing for each sex. Repeatability in total time taken results in huge differences as males produce a negative estimate. This means that when k was reduced to two, there was more variation within groups i.e. individual male spiders, than among their means. This may be due to the small sample size used for male ICC estimates ($n=14$). Although, again, confidence intervals for all measures of males and females separately are quite large due to the small sample size. However, even the upper CI for male total time does not overlap with the lower CI for female total time. We can still conclude that females are more repeatable than males in some aspects of web construction.

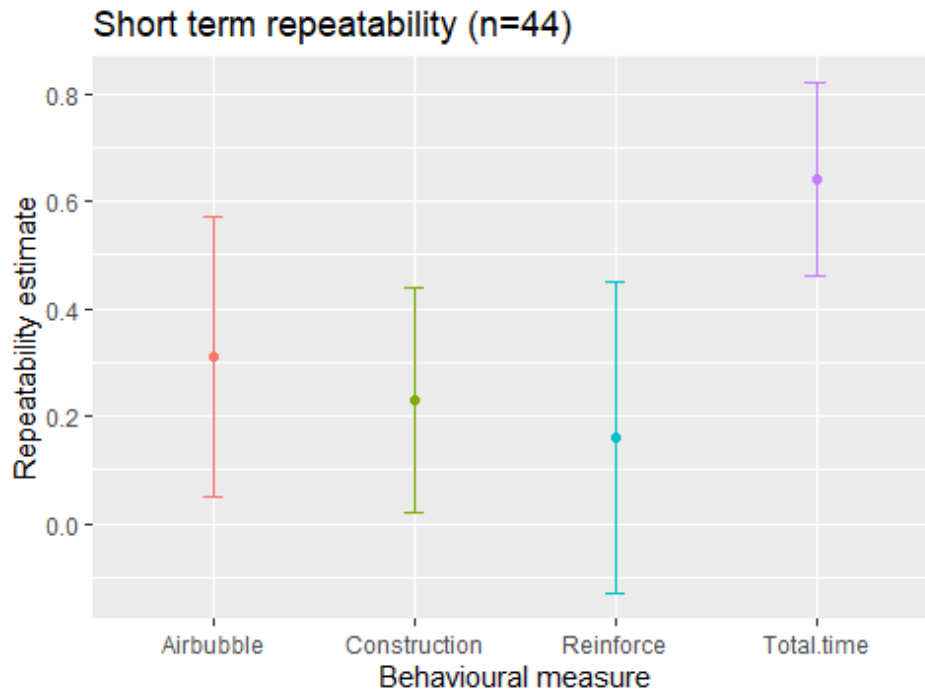


Figure 19. Repeatability estimates with their 95% CI's for total time taken, and three of the core behaviours for all newly collected spider (n=44). Only data from the first two web construction attempts are represented (k=2).

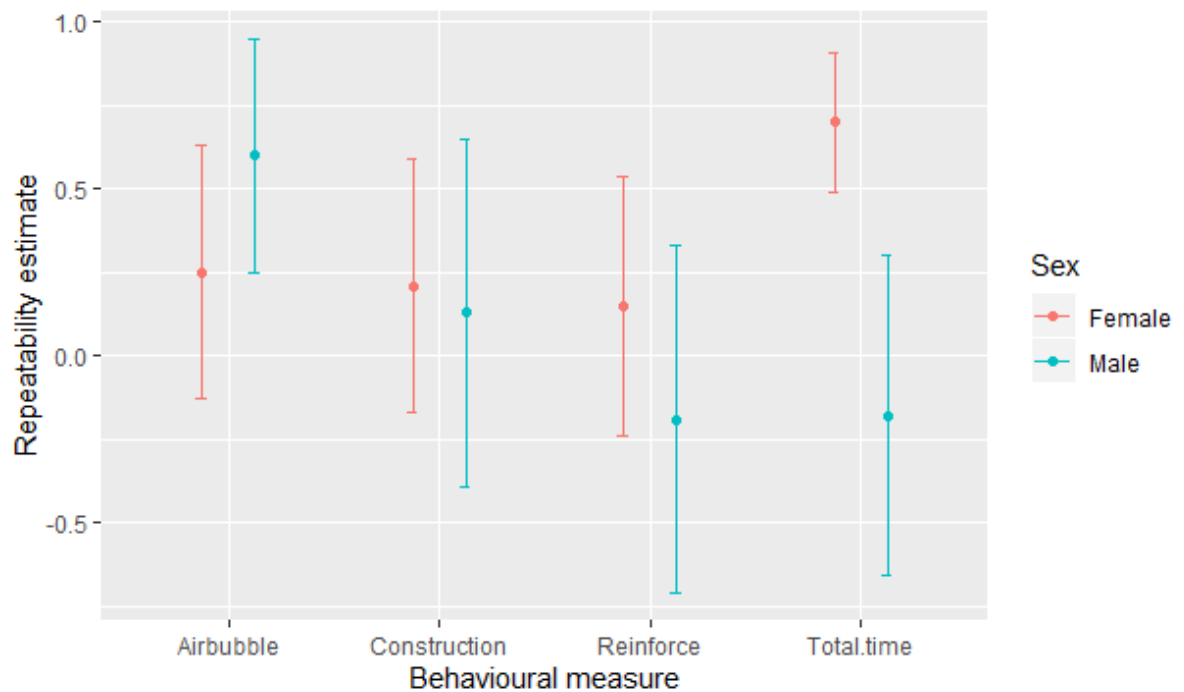


Figure 20. Repeatability estimates with their 95% CI's for total time taken, and three of the core behaviours for males (n=14) and females (n=25). Only data from the first two web construction attempts are represented (k=2).

3.4.1. Web construction time budget regressions

Table 3. Significant regressions before and after Holm-Bonferroni correction between behavioural aspects of web construction. All measures in average proportion of time spent per individual except for average number of surfaces.

Regression	P Value Before	P Value After	R ²	Equation of the line
Construct – Reinforce	7.21e-06	7.21e-06	0.476	$y = -0.497x + 0.487$
Construct – rest	0.0001	0.0009	0.303	$y = -0.4224x + 0.3854$
Construct – No. surfaces	0.001	0.008	0.86	$y = -0.2473x + 2.0648$
Rest – airbubble	0.02	0.1	0.122	$y = -0.1596x + 0.0764$
Rest – No. surfaces	0.004	0.024	0.182	$y = -5.1695x + 2.4037$
Airbubble – No. surfaces	0.002	0.014	0.208	$y = 12.111x + 1.1606$

Regressions compared the proportions of time carrying out different behaviours (for non-significant regressions see appendix 5). Many significant regressions between time budget measures taken during web construction were found to be significant (See table 3). However almost all these regression are simply artefacts in the data i.e. proportion of time spent collecting an airbubble is likely to correlate with number of surfaces as they are essentially the same measure, or, for example, the more air bubbles you collect (No. surfaces) the more the silk you will have to lay down to support the bigger structure (Construct). Other regressions are significant in that if the spider spends a large proportion of its time doing one thing i.e. constructing, then it will likely spend less time doing others i.e. resting, the spider has to be doing only one of four things making correlations between them likely. The only regression of real interest is the highly significant regression between constructing and reinforcing. These behaviours seem to provide similar yet distinct functions, making patterns between the two likely to be decision based.

Assumptions of the only relevant significant linear regression were checked using the gvlma package in R (Pena & Slate, 2006). The proportion of time spent constructing and proportion of time spent reinforcing were significantly negatively correlated ($y = -0.497x + 0.487$, $R^2 = 0.476$, $F = 35.47$, $p = 7.21e-07$) (See Fig. 21). Males confined themselves to always spending

on average between 60% - 80% of their time constructing, whereas female opted for a wider span of around 50% - 80% of their time constructing. Males never spent more than 18% of their time reinforcing, whereas two females are shown to spend more than 25% of their time reinforcing.

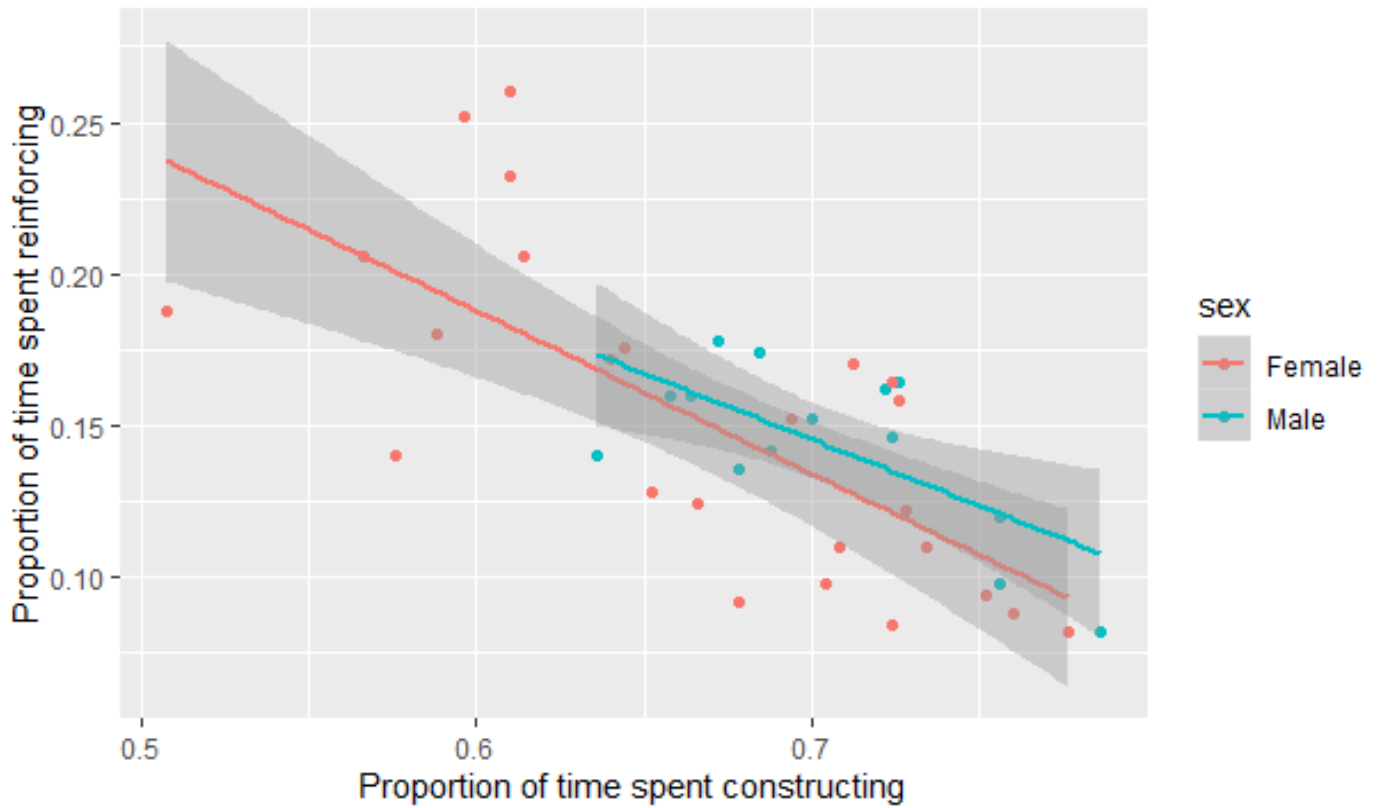


Figure 21. Aspects of laboratory-based time budget experiments as a proxy for the decision rules that govern web building. Only adults are considered to allow for comparison between known males and females ($n=39$). The more time spent constructing the less time is spent reinforcing the inside of the diving bell. Grey area shows the standard error around the line for its corresponding sex.

Regressions were carried out on the same data but for males and females individually to ensure that the wholly more repeatable females were not carrying the correlation. Regressions were significant for both males ($y = -0.4368x + 45.118$, $R^2 = 0.4598$, $F = 10.21$, $p = 0.008$), and females ($y = -0.5373x + 51.021$, $R^2 = 508$, $F = 23.75$, $p = 6.389e-05$).

3.5.1. Cephalothorax width and leg span

Both cephalothorax width and leg span varied amongst the populations (See Figs. 22 & 23). Two sets of Czech individuals were collected in different years. For data regarding the life stage of individuals and the dates they were collected see appendices 1 to 4. A regression of cephalothorax width and leg span was significantly correlated ($y = 7.4919x + 3.8502$, $R^2 = 0.4254$, $F = 31.101$, $p = 1.61e-06$) in the 44 spiders and thus cephalothorax width only is used for investigating the interactions between size and behavioural measures.

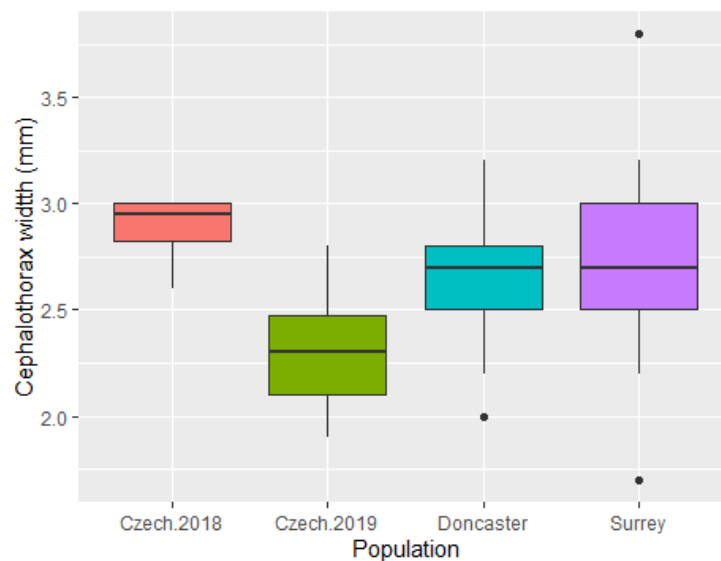


Figure 22. Distribution of cephalothorax widths for each population. Size of populations varied: Czech 2018 = 4, Czech 2019 = 14, Doncaster = 17, Surrey = 13.

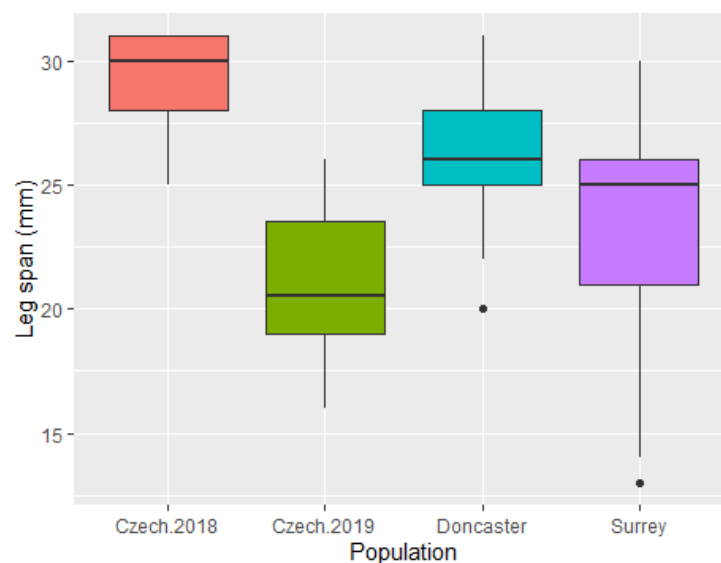


Figure 23. Distribution of leg spans for each population. Size of populations varied: Czech 2018 = 4, Czech 2019 = 14, Doncaster = 17, Surrey = 13.

3.5.2 Morphological vs behavioural regressions

Cephalothorax width was not a significant predictor of any behavioural time allotment. The sole significant regression between cephalothorax width and average no. of surfaces across five repeats per individual is visualised in Figure 24 ($Y = -0.5417x + 3.2828$, $R^2 = 0.1493$, $F = 8.742$, $p = 0.005$) (See table 4). For non-significant regressions see appendix 6. The data had to be log transformed (base 10) to meet the assumptions of the regression. The smaller the spider the more surfacing events they undertake. A Fisher's exact test compared the number of failed and successful attempts to collect an air bubble between adults and sub-adults, this was to garner whether sub-adults were less efficient bell builders. Two-tailed Fishers exact showed no significant difference between the ability of the two groups ($p = 0.6193$) and so only the average number of successful surfaces (air bubbles that were fixed to the web) per construction attempt were plotted. Figure 24 shows data for all the spiders ($n=44$) whereas figure 25 shows the same data but for known adult males and females only. Cephalothorax width proved difficult to discriminate to 0.1 of a millimetre and with the number of surfaces occurring generally between 1 and 3, some overlap in both cephalothorax width and average number of surfaces occurred i.e. two males had both a 2.5mm cephalothorax width and averaged 1.8 surfaces across five trials making the points indiscriminate on the graphs.

Table 4. Significant regression before and after Holm-Bonferroni correction comparing morphological parameters (cephalothorax width (mm)) to behavioural measures (average number of surfaces for an air bubble per individual).

Regression	P Value Before	P Value After	R2	Equation of the line
Ceph - No. of surfaces	0.005	0.03	0.1493	$Y = -0.5417x + 3.2828$

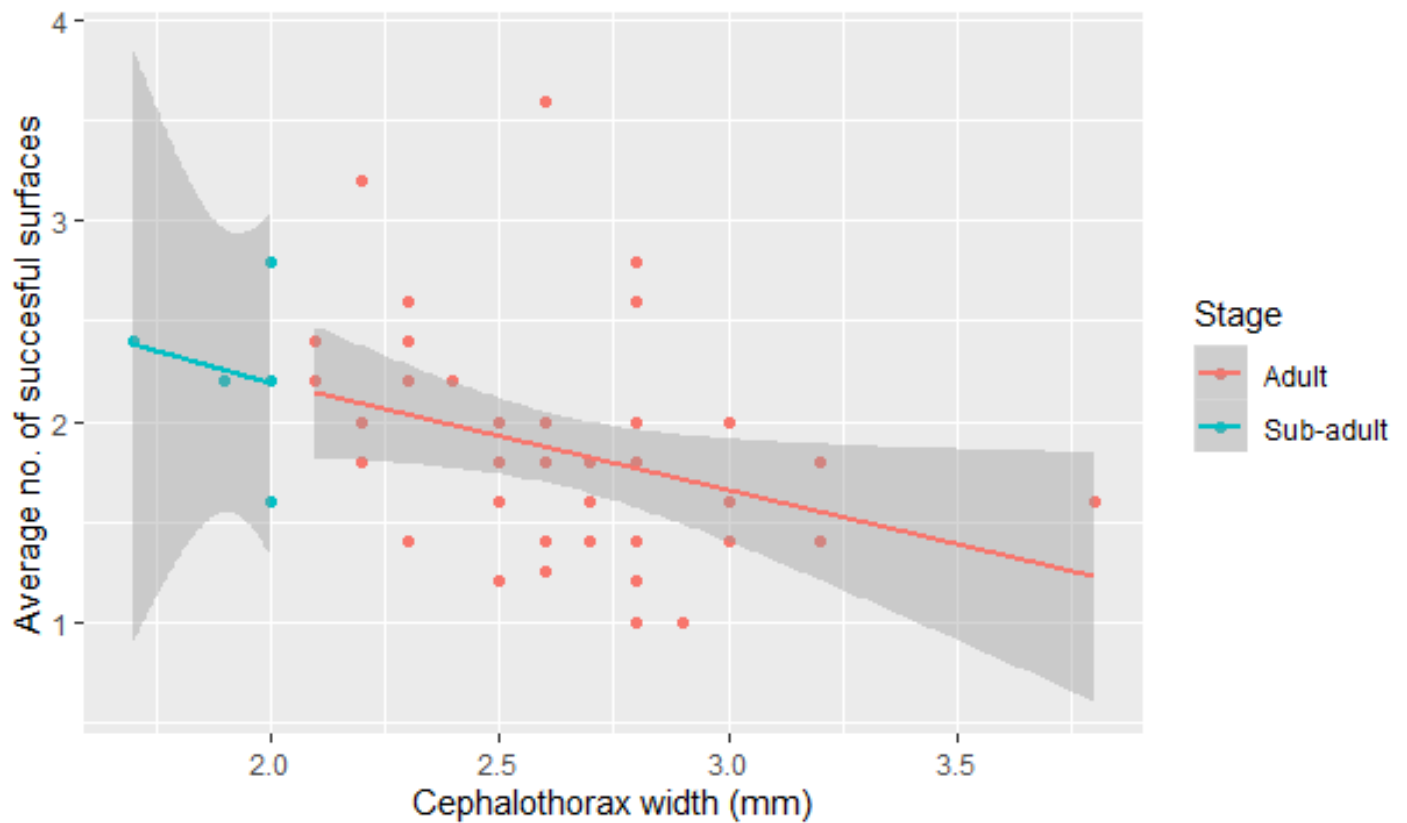


Figure 24. An increase in cephalothorax width negatively predicts the average number of surfaces for an air bubble (across five trials) an individual will carry out when constructing its web (n=44). Grey area shows the standard error around its corresponding line. Sub-adults appear to surface on average more times than adults.

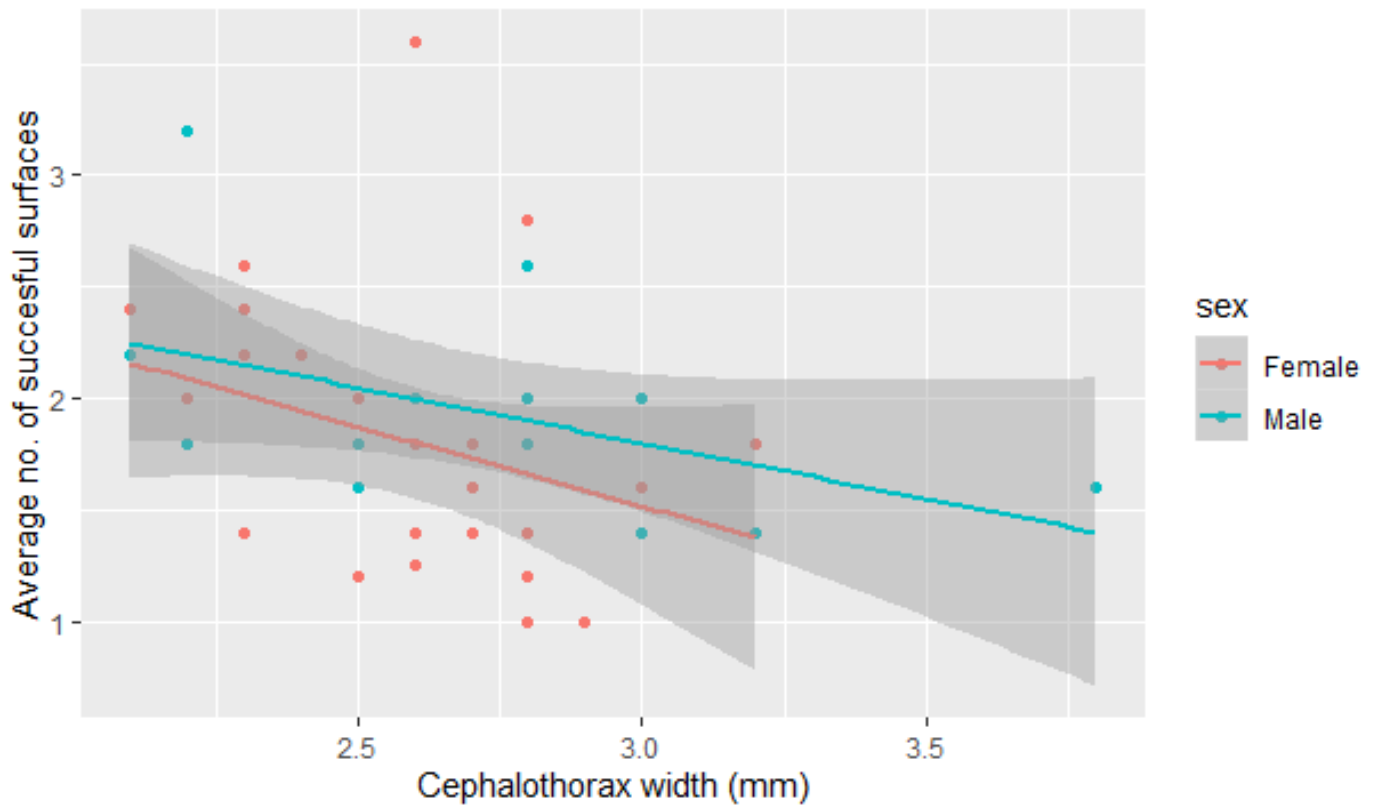


Figure 25. An increase in cephalothorax width negatively predicts the average number of surfaces for an air bubble (across five trials) an individual will carry out when constructing its web ($Y = -0.5423x + 3.2848$, $R^2 = 0.1031$, $F = 5.37$, $p = 0.03$) ($n=39$). Grey area shows the standard error around its corresponding line. Males and females show no clear differences overall in the average number of air bubbles collected.

3.6.1. Notes on other behaviours

There were two other behaviours noted during data collection that do not seem to have been noted in the literature thus far. In some instances, the spiders were seen ejecting some kind of misty white liquid into the water that would slowly dissipate (See Fig. 26). There does not seem to be any discernible reason for this, i.e. it was not only when disturbed or offered food. This occurred across ~31% of individuals and across ~8% of recordings, it was recorded 18 times across 15 individuals, seven males (once each) and eight females (11 times varying in number between individuals). This occurred with spiders from all three populations.

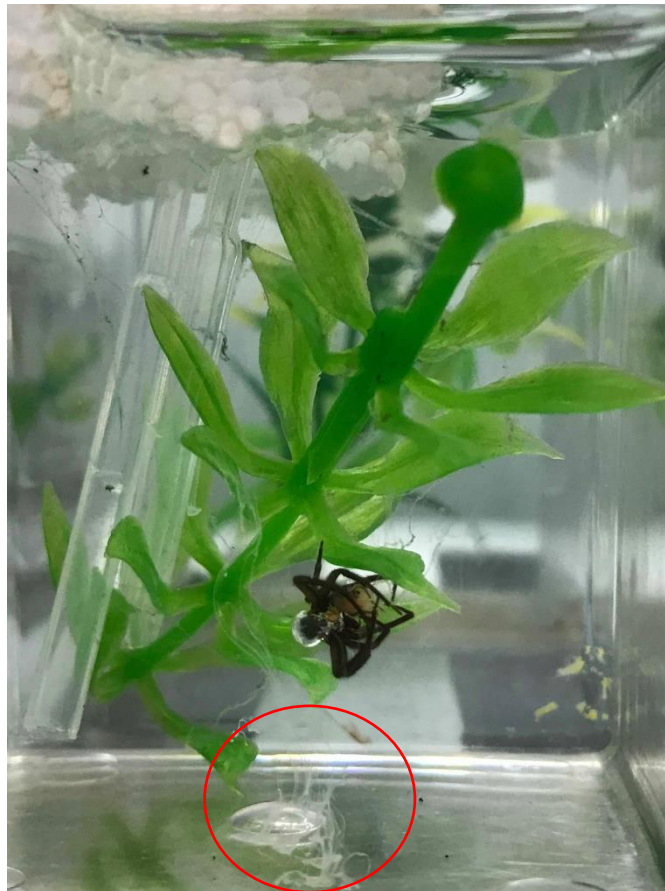


Figure 26. There is a white liquid beneath the spider that appeared to emerge from the spinnerets. The image shows the liquid dissipating in the water (Not taken from a video used in analysis). In this case the spider had just begun feeding.

As well as the behaviour described above, the spiders were also seen fixing their prey to the web. They would lay down some silk, < 1 min of building time, and then attach the shrimp prey. The diving bell would then be constructed adjacent to the food and the spider would return and collect the shrimp, forcibly pulling it from the silk once finished. All individuals seen carrying out this behaviour were from the Czech population except for one which was from

Doncaster. It occurred across ~21% of individuals and across 10% of recordings. It was described 24 times between 10 individuals, six males (13 times) and 4 females (11 times). With one female fixing the prey to the web in every instance the bell was constructed.

3.7.1. Phylogenetic trees

Trees were initially produced for each of the three genes individually (28S, H3, and LCO1) and then for a concatenated gene (28s and H3). This was done based on the maximum-likelihood principle as described in Guindon *et al.* (2010) using the PhyML online software (Guindon *et al.* 2010). Each tree was subject to a 100 bootstrap analysis which yielded generally low results, any values over 50% are shown on the branches (See Figs 27 to 30). The CO1 gene of a Chinese *A. aquatica* individual (See Fig. 29) was retrieved from GenBank (August 2019), it was isolated from the full mitochondrial genome in Liu *et al.* (2014). Sequences for an *Argenna patula* outgroup – also sourced from GenBank (August 2019) - was used to root the trees in all instances. GenBank accession numbers are provided in appendix 8.

Few clear distinctions can be made between individuals of a given population across the two nuclear genes studied. Only in one instance – the 28S gene – did the two Doncaster individuals share the same most recent branch point, and in one other instance - the mitochondrial CO1 gene - the two Czech individuals exhibited the same haplotype (See Fig. 29). However, the two U.K. populations remained fairly consistent across genes, *Argenna patula* was always rooted at the base of the trees while the *Argyroneta* typically occupied two divergent clades (See Figs. 27 to 29). Bootstrapping analysis frequently yielded low values indicating different individuals across all three genes could not be reliably placed on the tree. Take, for example, Czech individual 2 in figures 27 & 30 that is placed as a basal group, far more distant from the other individuals of the same species. There was no bootstrap value reported for this placement meaning the branch is not supported. It seems Czech individual 2 is likely to continue to be booted in and out of the main grouping of *Argyroneta*. Take also Doncaster individual 1 and the Chinese *Argyroneta* in figure 29, they are removed from the others of the same species and yet share the same haplotype.

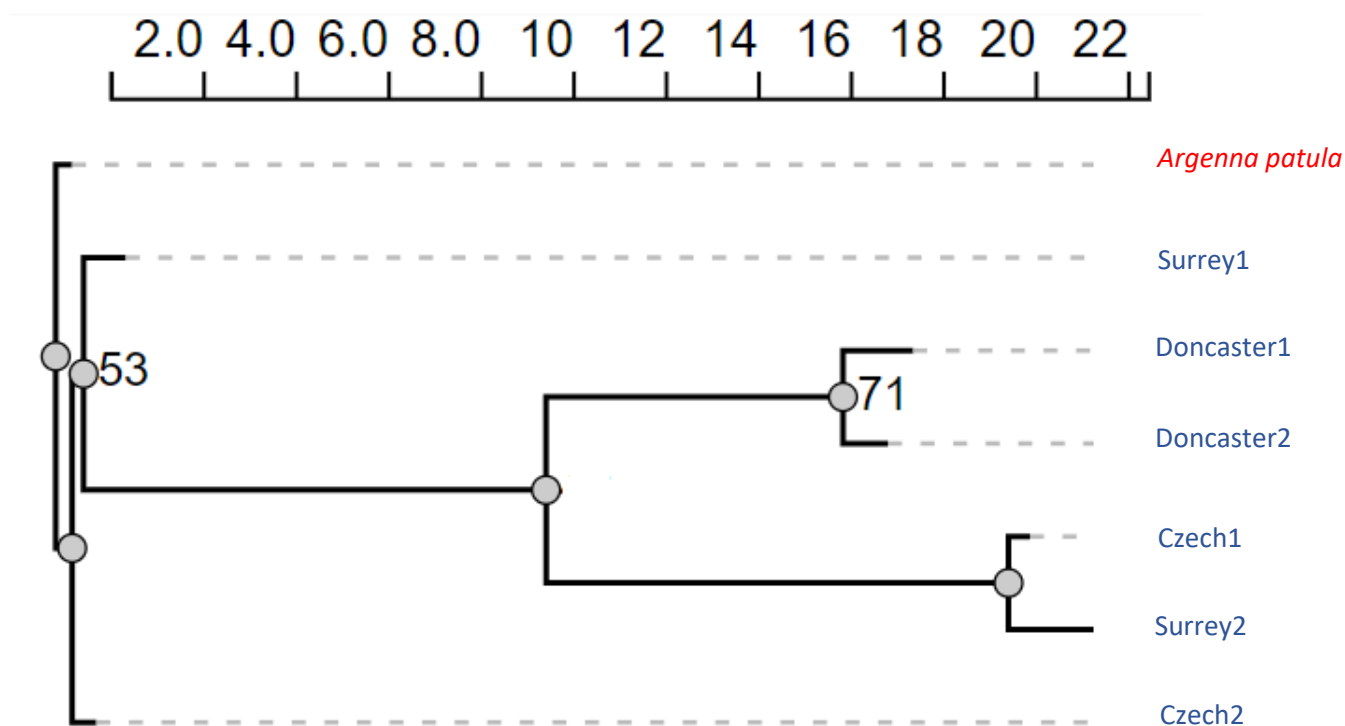


Figure 27. The relationships between six *A. aquatica* individuals and a dictynid outgroup based on 28S nuclear ribosomal RNA (D2 – D3 region) (. Genetic variation both within and between populations is clear. Results show generally low bootstrap support, only those over 50% are shown.

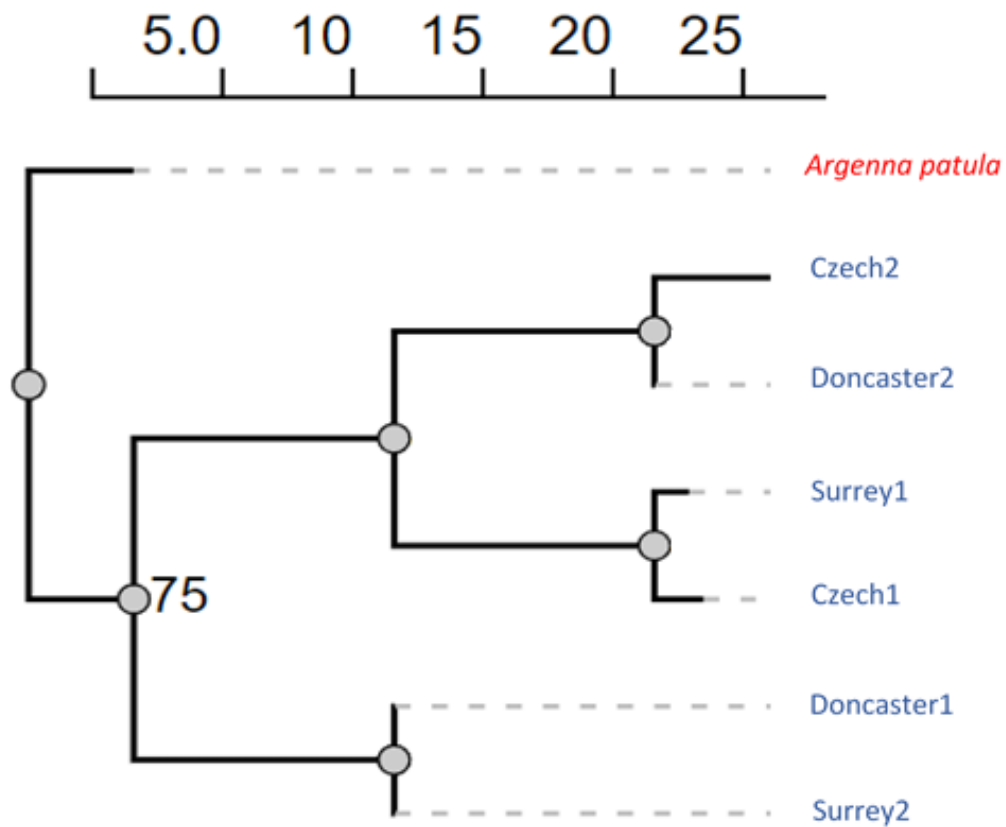


Figure 28. The relationships between six *A. aquatica* individuals and a dictynid outgroup based on the histone H3 gene. Genetic variation both within and between populations is clear. Surrey individual 2 and Doncaster individual 1 share the most recent common ancestor and the most similar sequence, suggesting some level of mixing amongst the U.K. populations from the north and the south. Results show generally low bootstrap support, only those over 50% are shown.

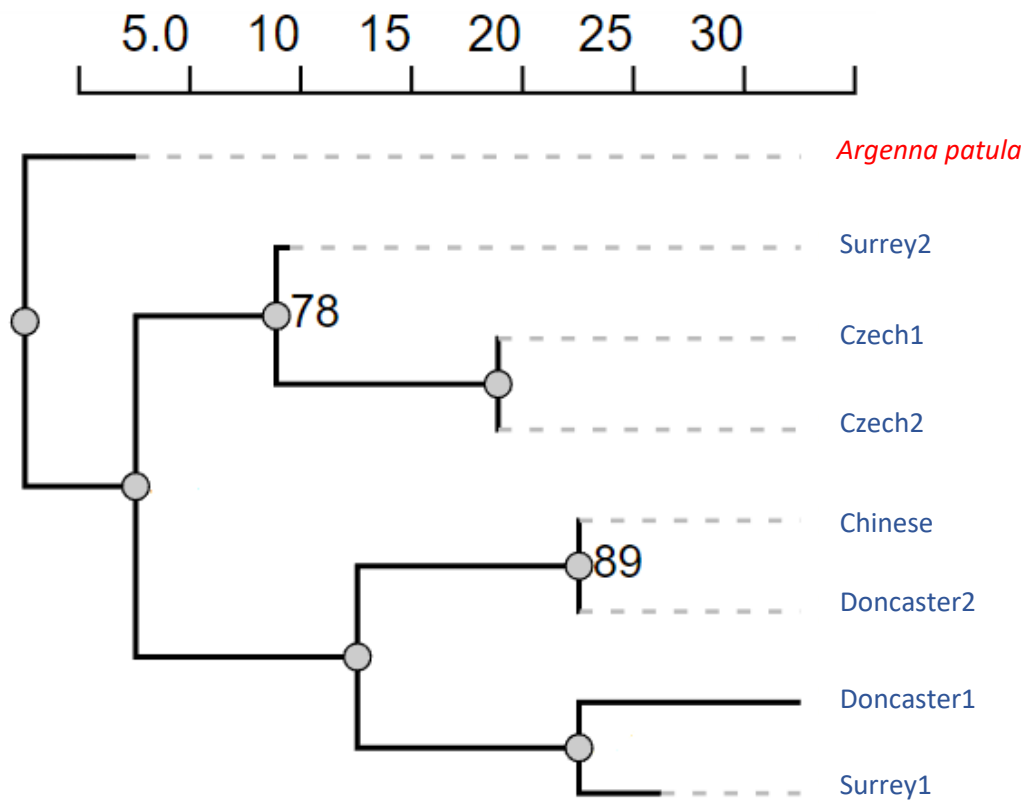


Figure 29. The relationships between seven *A. aquatica* individuals and a dictynid outgroup based on the cytochrome oxidase subunit 1 gene (LCO1). Results suggest variation in mitochondrial types both within and between populations, with the only exception being the two Czech individuals who appear to share the same haplotype. The Chinese individual, though geographically more distant from the U.K. than Czechia, shares the same haplotype as Doncaster individual 2. Results show generally low bootstrap support, only those over 50% are shown.

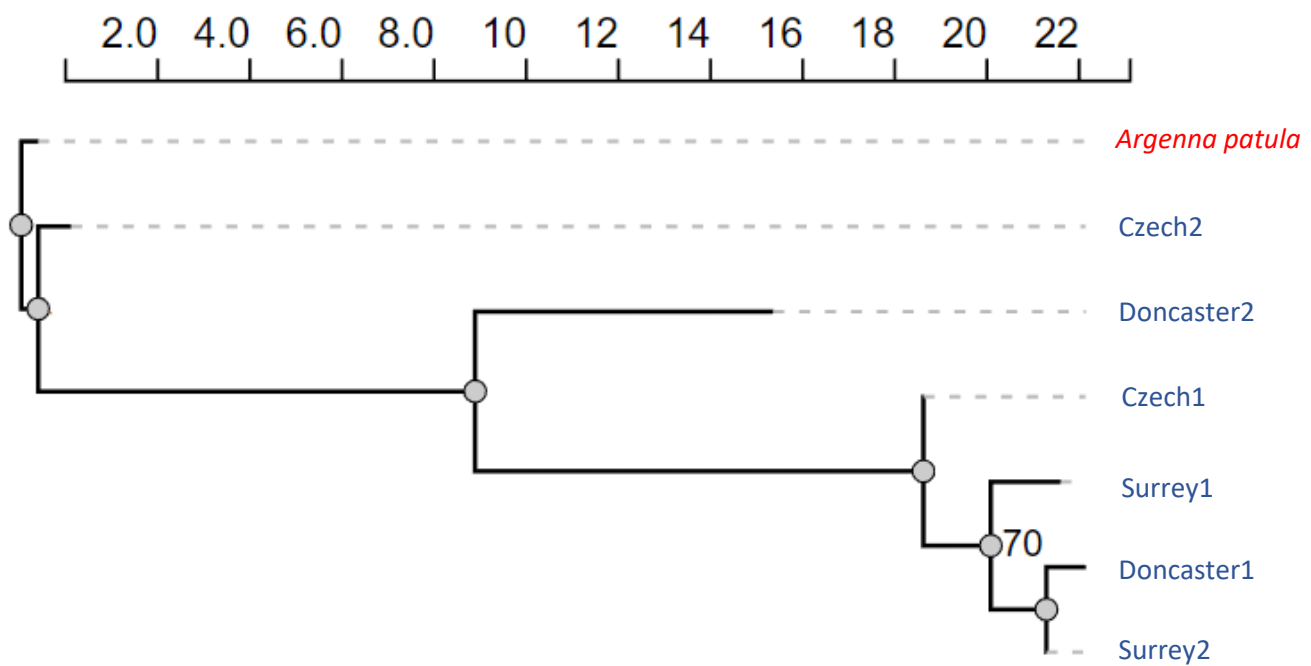


Figure 30. The relationships between six *A. aquatica* individuals and a dictynid outgroup based on a concatenated gene including 28S ribosomal RNA (28S) and histone H3. Genetic variation both within and between populations is clear. Surrey individual 2 and Doncaster individual 1 continue to show the highest similarity as seen in Figure 26. Results show generally low bootstrap support, only those over 50% are shown.

3.8.1. Ecological indicators and water quality

All the water quality measures gathered from 11 different sites were regressed against one another. Initially, pH significantly predicted ORP, as would be expected (James *et al.* 2004), though after correction returned a non-significant result. Temperature significantly predicted number of possible prey and almost significantly predicted number of newts. Number of potential prey also significantly predicted the presence of newts. Day of the year i.e. a value out of 365, very significantly predicted temperature and very nearly significantly predicted the number of possible prey. For non-significant regressions see appendix 7.

Table 5. Significant regressions before and after Holm-Bonferroni correction comparing water quality and biological measures taken from 11 sites.

Regression	P Value Before	P Value After	R ²	Equation of the line
pH – ORP	0.02	0.14	0.4908	$y = -77.052x + 681$
Temp – No. prey	0.003	0.03	0.602	$y = 0.6764x - 1.0575$
Temp – Newt	0.006	0.05	0.586	$Y = 0.0714x - 0.352$
No. prey – Newt	0.002	0.02	0.657	$Y = 0.0896x - 0.1764$
Day of the year – Temp	0.0002	0.002	0.8136	$y = 0.1033x + 1.4837$

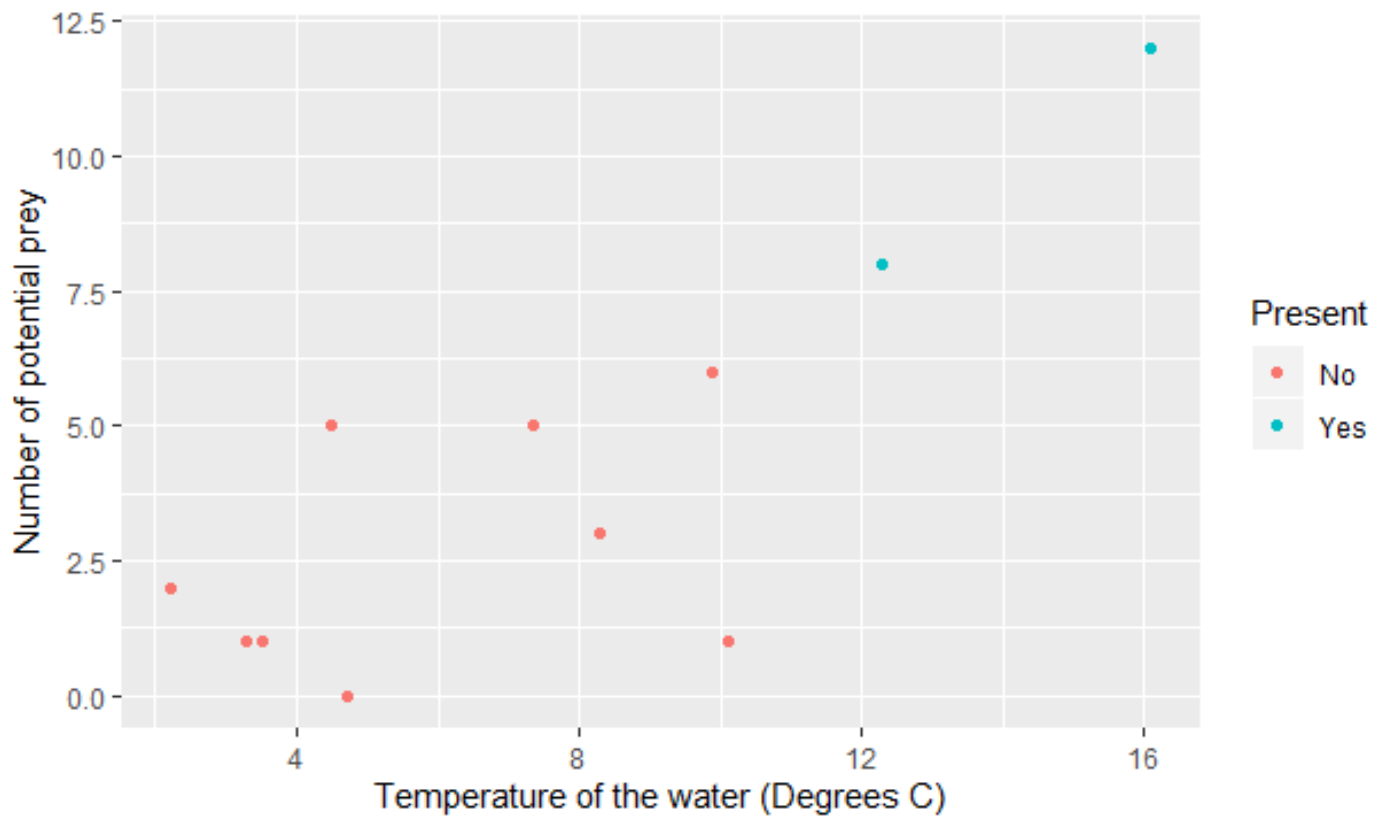


Figure 31. Water temperature (°C) significantly predicts the number of potential prey found in the water body. Points are coloured based on presence and absence of *Argyroneta aquatica* at the site. *A. aquatica* only appeared at the two warmest sites with the highest numbers of potential prey.

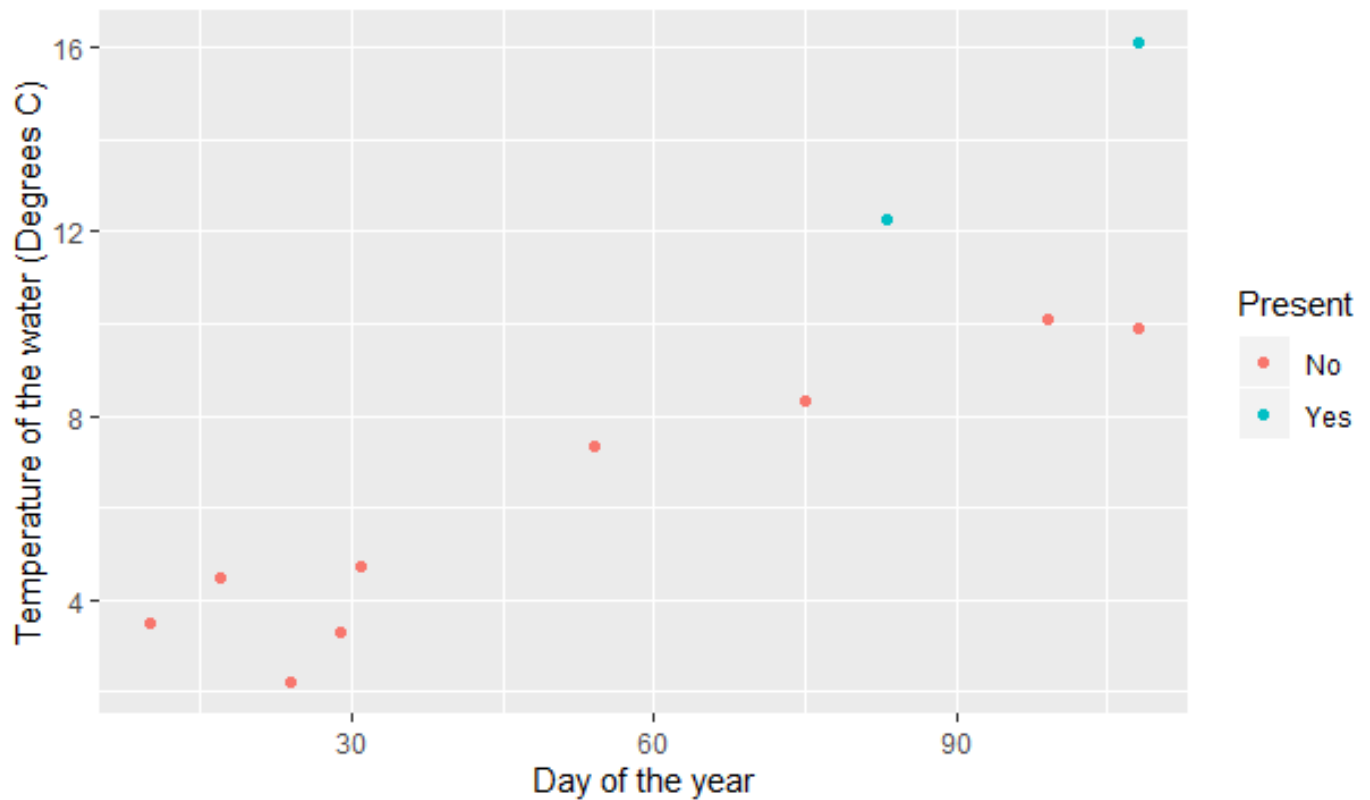


Figure 32. These points cover months January to April over which searching was carried out. As expected, the closer to summer you get in the year, the higher the temperature of the water body. Day of the year didn't seem to predict *A. aquatica*, they were in the two sites with the highest temperature.

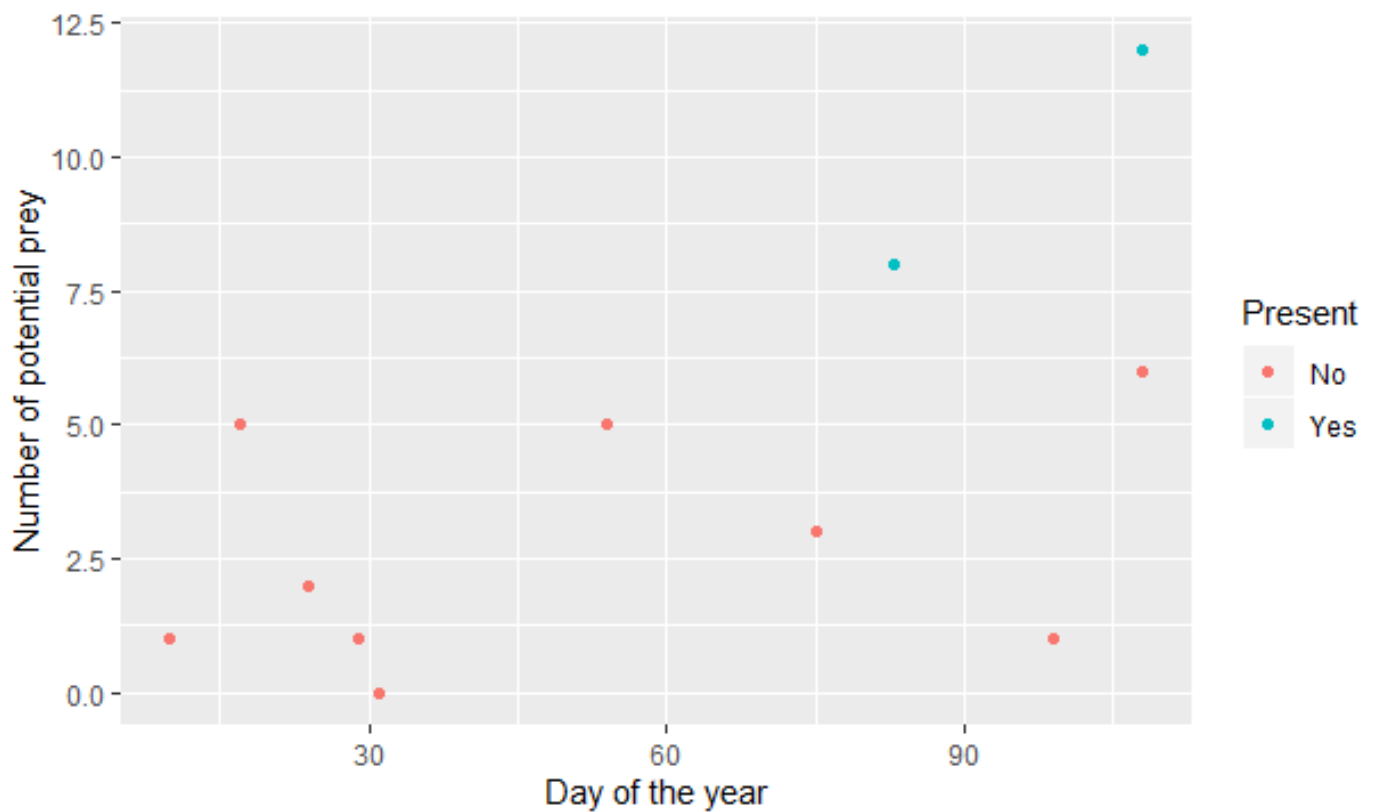


Figure 33. Regression bordering significance ($p = 0.05$) between day of the year (Jan – April) and the no. of potential prey. Again, day of the year did not seem to predict their presence because they were found in the two sites with the highest numbers of potential prey.

The later in the year the higher the water temperature (See Fig. 32). Higher temperatures normally predict higher productivity (See Fig. 31) and therefore more available prey for *A. aquaticus* (Stark & Phillips, 2009). The later in the year the more prey is available and thus you are more likely to find *A. aquaticus* which are active in the water column. The presence of great crested newts significantly positively correlated with temperature and number of potential prey, occurring only in sites where *A. aquaticus* were also present.

3.8.2. Comparisons between sites where *A. aquatica* is present

Five-metre belt transects were carried out at the two sites where *A. aquatica* were found. This allowed for five values to be collected from five points laterally across the widest point of the two water bodies. Given that only five values from each site were collected per measure the data did not fit any of the assumptions of the one-way ANOVA, sites were therefore compared through Kruskal-Wallis one-way analysis of variance (See table 6). The only measures which were consistent across the two sites were physical parameters. Comparisons between two measures of vegetation complexity at the two sites (% submerged cover and % emerged cover) and one of habitat geography (water depth at centre) were non-significant. Before correction all measures of water quality were significantly different between the two sites suggesting these factors have little effect on *A. aquatica* site choice (Mammola *et al.* 2016). However, after correction all results were found to be non-significant, suggesting a similarity across all factors measured. This is likely due low the statistical power of the results given that only two sites were compared.

Table 6. Kruskal-Wallis comparisons between data taken for 10 measures from two sites where *A. aquatica* are present before and after Holm-Bonferroni correction.

Measure	P Value Before	P Value After
Temperature	0.009	0.081
pH	0.009	0.081
Conductivity	0.009	0.081
Salinity	0.005	0.05
DO	0.009	0.081
% submerged cover	0.21	0.42
% emerged cover	0.11	0.33
% free floating cover	0.03	0.12
% shaded cover	0.01	0.081
Depth at centre (cm)	0.45	0.45

3.8.3. What predicts the presence of *A. aquatica*?

Across the survey (From January 10th – April 18th), the 11 different sites were characterised by water temperatures ranging from 3.3 to 12.3°C (mean = 7.5°C), DO ranging from 2 to 11 mg/l (mean = 4.93), pH ranging from 5 to 8 (mean = 7), conductivity ranging from 52 to 818 µs/cm (mean = 775.98), ORP ranging from -13 to 485 (mean = 133.98), and number of potential prey for *A. aquatica* ranging from 0 to 12 (mean= 4). There were 19 *A. aquatica* recorded across the 11 sites, four in the Surrey site (March) and 15 in the Doncaster site (April) upon first visit (both across a 2-hour search period).

To assess the data set, multi-collinearity was investigated via the cor function and variance inflation factors (VIF > 3) in R (Zuur *et al.* 2009, 2010). The following variables were found to be correlated; temperature of the water and number of potential prey, day of the year, and the presence of newts, number of potential prey and the presence of newts, and pH and ORP. ORP, day of the year, temperature of the water, and number of potential prey were dropped from the list of potential models after VIF analysis. VIF's were then produced for newt, DO, pH, and conductivity (See table 7).

Table 7. Table depicting VIF's for the four possible predictor variables, all VIF's are less than three and so are viable models for the GLM.

Predictor	VIF
Newt	1.264
DO	1.255
pH	1.504
Conductivity	1.918

These four variables were included in the regression model. Backwards model selection based on AIC's (Johnson & Omland, 2004) suggested the best predictor of the presence of *A. aquatica* was the presence of newts (the results hold low statistical power with only 11 sites being considered of which *A. aquatica* were only found at two) (See table 8).

Table 8. Results of a general linear model in which the presence of newts is the only significant predictor of *A. aquatica* presence across 11 sites.

Model	SE	T	Pr(> t)	AIC	AIC weights
Newt	2.027e+00	4.687	0.001	55.969	0.996
DO	0.654	-0.085	0.934	69.553	0.001
pH	1.082	0.446	0.666	69.322	0.001
Conductivity	0.003	-0.680	0.514	69.011	0.001

3.8.4. Nutrient concentrations taken at Thursley nature reserve

Table 9. Nutrient concentrations taken at Thursley nature reserve in July 2019. High levels of orthophosphates indicate the reason for eutrophication (Correll, 1998).

Measure	Result (ppm)
Orthophosphates	100
Ammonia	1
Nitrates	10
Nitrites	0.5

Discussion

Web and behaviour are so closely linked that it is possible to deconstruct web structure not only to provide a continuous record of the visible steps taken by the spider, but also to infer from this the guidelines involved (Gotts & Vollrath 1992; Krink & Vollrath 1997, 1998). By partitioning the process into its key steps and then analysing these results we can attempt to elucidate the decision rules that govern the web construction process.

The time budget of web construction provides a framework from which repeatable measures can be taken, and then with that, some measure of heritability could typically be drawn (Jacobs, 1981). When considered as a species overall, *A. aquatica* show lower than average repeatability's across all measured aspects of web construction (Wolak, 2012) (See Fig. 16), with the highest estimate of R being 0.42 for total time taken. It is worth noting, that from an evolutionary perspective, individual repeatability R, the proportion of variance attributable to among-individual differences, typically sets an upper bound for heritability (Wilson, 2018); for a full list of exceptions see Dohm (2002). Analysis of this data returned low repeatability estimates, and so there is perhaps one exception to this maxim that best explains the results. Dohm (2002) notes that repeatability may underestimate heritability if there is significant interaction between genotype and environment. Data from within individuals, though moderately repeatable in one aspect i.e. total time, show generally low repeatability across almost all other measures. Wolak (2012) compared 174 estimates of behavioural traits across 46 studies and found a mean and median repeatability of 0.48. Though this should be considered tentatively as behavioural traits are likely to vary greatly in their repeatability as reliant on their ecological relevance and evolutionary significance (Dohm, 2002).

Findings of low repeatability's have often led authors to suggest that the measured trait may lack the previously stated ecological relevance and evolutionary significance. Given the vital role of the diving bell in both the survival and reproduction of the spiders (Shutz & Taborsky, 2005; Seymour & Hetz, 2011) we can assume such construction behaviours are not inconsequential. As well as this, the evidence is strong that web-building decision rules are generally inherited for both three-dimensional and two-dimensional webs (Reed *et al.* 1970). Therefore, the results of this study suggest there is a large component of temporally adapted structural details that emerge from a high level of behavioural plasticity within this species (Eberhard 1981; Krink & Vollrath 1998).

If webs were constructed in the same pattern each time then we would expect the time-budget associated with this to be consistently repeatable within individuals, or at least more repeatable than is shown in this study. Even though it is more difficult to maintain a consistent shape in a three-dimensional web we would expect a higher repeatability for what is an extended phenotype that's serves similar, if not the same, functions every time it is constructed (Blackledge *et al.* 2003; Blamires *et al.* 2013; Su *et al.* 2018). This high level of plasticity becomes apparent when any small aspect of heterogeneity is seen in the environment. Even though experimental conditions were set identically in each trial and measures were taken by a single observer (Wolak, 2012), there were factors that proved difficult to control tightly in the laboratory. Temperature and dissolved oxygen (DO) levels of the water varied slightly between days. Although the room had a set, consistent temperature, high levels of foot traffic in and out of the room may have affected this on different days. Preparation of the filming tank was carried out identically between trials, but this cannot reliably reproduce the amount of DO in the water, DO varies in relation to a multitude of factors, temperature being just one example (Moore & Townsend, 1998). It has already been documented that *A. aquatica* can alter web size in accordance with DO concentrations (Seymour & Hetz, 2013), therefore it is not unlikely that these factors affected measures of repeatability as individuals altered web building accordingly. Placement and size of the pond reed on which the bell was constructed was kept consistent, but factors such as orientation of leaves could not always be relied upon to be identical. These small changes do seem likely to alter the shape of the web and by extension the associated time-budget. An individual's choice in terms of placement of the bell is also likely to affect the time-budget. In general, the repeatability of a trait is lower the more variable the influential environment is (Jacobs, 1981). This alterability in web shape, that maintains the same vital functions, makes living in environmentally heterogenous aquatic habitats possible (Downing, 1991).

Some have suggested that experimentally controlling for and removing natural environmental heterogeneity induces downward bias by removing an ecologically relevant source of among-individual variance within the natural population, and therefore the trait being measured (Wilson, 2018). This among-individual variance becomes altered in, for example, a territorial species with competitive outcomes determined by heritable traits such as web construction i.e. *A. aquatica*. But, any measure of the process underpinning such

understudied behaviours as *A. aquatica* web construction will remain strongly assumption-laden (Wilson, 2018). Therefore, such behaviours are best initially understood across a standardised condition. Moreover, there doesn't seem to be any clear removal of within and between individual variation as the spiders continued to show high variability across the measured traits.

These lower estimates of repeatability suggest webs are heavily dependent on the available substrate. Although superficially the shape of each bell is similar, the method by which this is achieved varies both within and between individuals. There could be however, repeatable aspects related to the construction that couldn't be observed during this study, such as the laying of the hydrogel on the silk itself, or the number of anchor points on the substrate (De Bakker *et al.* 2006).

4.1.1. Differences between the sexes

It is difficult to say whether variation both within and between individuals can be attributed to variation in physical condition, minute differences in the experimental environment, age, or sex. Though it is most likely some combination of all these factors that results in the final diving bell (Lichtenstein *et al.* 2016). The data gathered in this study does however suggest that a considerable predictor of repeatability in *A. aquatica* web construction is sex. Females were far more predictable than males in every aspect of web construction except for time spent collecting an air bubble in which they were the same (See Fig. 18).

The diving bell of the female must allow for resting, eating, mating, and laying the cocoon sac (within that the egg sac); two functions the males does not. Females lay their cocoon sac in the upper portion of their diving bell having allowed the male to mate with them inside their bell (Schütz & Taborsky, 2005). This accompanied with their known propensity to be far more sedentary than males (Schütz & Taborsky, 2003) suggests their requirements for a bell, or at least, a bell which is more robust in order to protect them and their egg sac (Nyffeler & Pusey, 2014), is greater than males. We would therefore expect the process associated with bell construction to be more consistent within females as demonstrated. Though still plastic, their steps are more well defined temporally than males, presumably to ensure a diving bell fit for mating and housing their developing young.

Female total time remained the most repeatable aspect of the whole process, carrying the total time repeatability estimates at whole species and population level. There is no significant difference in the average total time taken between the sexes ($p = 0.58$), but there is a significant difference in time taken between individual females ($p = 3.129\text{e-}07$). The whole species can complete their webs across a confined time frame i.e. between 2 and 12 minutes, individual females are just more consistent in the particular time they take.

It has already been discussed how vital the diving bell is to individual survival and species propagation, with females needing to invest more in air bell construction and maintenance under water (Schütz & Taborsky, 2003). It is appropriate therefore that females would follow a more rigid individual time frame. Repeatability analysis across both shorter and longer time frames suggests that for a given environmental condition, and/or physical condition of an individual female, they will follow a more similar construction process compared to males (Bell *et al.* 2009). Though they may differ in their time budgets as they make trade-offs between constructing or reinforcing etc., the end point of female web construction remains largely repeatable.

4.1.2. Differences between the populations

There is a clear difference in repeatability of total time taken between the populations (See Fig. 17). Less so between Surrey and Doncaster (0.12) but quite considerably between the Czech and Surrey populations and the Czech and Doncaster populations (0.38 and 0.26 respectively). The Czech population overall appears less repeatable than the other two populations, except for in reinforcement where it is the most repeatable. This difference could be related to the habitats from which they were taken. Three dimensional webs are so closely related to the substrate on which they are constructed (Blackledge *et al.* 2003) that the structure of the vegetation in the Czech habitat may have been far from that used in the study. The Czech spiders were taken from a reedy pond as opposed to the other two populations which were collected from water bodies wherein there were blankets of macrophytes or mosses.

The costs associated with relocation once a site has been found are high, especially in a species that is not hydrodynamically designed for swimming (Schmidt-Nielsen, 1972). Some species of orb-weavers have been shown to accept a structurally suboptimal site where they

will build a web which has been altered to fit the structural support available (Vollrath *et al.* 1997; Hesselberg, 2014), foregoing its traditional web shape. The structure of the vegetation may have differed in comparison to their typical structures to such an extent that the Czech spiders weren't able to assimilate their web construction process as efficiently on the plastic pond reeds used in the study (Vollrath *et al.* 1997), making the whole process less efficient and by extension less repeatable. This does, however, further highlight their ability to be behaviourally plastic in novel environments (Hesselberg, 2014). Though less repeatable than the other two populations the process by which their webs were constructed appeared identical (with the inclusion of the placing down of the prey). The spiders performed the same behaviours, following the same process, and were ultimately able to construct functional diving bells in every attempt. From this it could be assumed that there is no specific sensitive phase where in a single environmental application is imprinted on an individual at a particular stage in its ontogeny. Though this is not to say that the typical web construction process within the conditions of the habitat from which they were collected would not reduce the level of error around the measures taken in these lab conditions.

4.2.1. Short-term repeatability

There are few examples of studies into the repeatability of spider behaviour (Rivero *et al.* 2000; Bonte *et al.* 2003; Johnson & Sih 2007; Hoefler *et al.* 2009; Rodriguez & Gloudeman, 2011; Pruitt and Riechert 2012; McGinley *et al.* 2013). With Wignall *et al.* (2014) being the only example found that explored the notion of short-term repeatability in arachnid behaviour. Repeatability meta-analysis has suggested that repeatability estimates do not depend on the number of times individuals are measured (Bell *et al.* 2009). However, this study has shown that web construction behaviours that are so closely linked to their environment are much more repeatable in the short term than when the same behaviour was measured across a longer time frame. The results of this study analysed repeatability of five occurrences of web building across five days and produced low to moderate estimates. When analysing only the first two webs produced, we can see sizeable increases in repeatability (See Figs. 21 & 22). This may allow for a clearer insight into the factors that govern web construction. The previously most repeatable measure, total time taken, increased from 0.42 to 0.64, female total time increased from 0.50 to 0.70 whereas male total time decreased

from 0.05 to -0.18. The females of the species are largely more repeatable than the males, as was suggested by Bell *et al.* (2009).

If all individuals responded to the environment in the same way, then even environmentally altered behaviour can be repeatable (Bell *et al.* 2009). It seems apparent from analysis of the full data set that each spider responded differently to the environment in which they were building. Be that alterations based on chemical properties of the water, physical condition of the individual, or – perhaps most likely – the substrate. Conditions of the experimental set up and body condition are presumably more similar across shorter time scales. The increased short-term repeatability in every measure to some extent (See Fig. 21) could suggest that this species allot more time towards certain aspects of web construction when set under particular environmental and physiological constraints. Ultimately, the process does not appear to rely solely on the substrate.

4.3.1. Optimisation of the process

When the web is observed in micro detail, as in the SEM images described in De Bakker (2006) and Doerstelmann (2015), it becomes apparent that there is no discernible pattern in the fibres. The only clear structural inferences are that the fibres differ in both thickness and hierarchical placement (Doerstelmann, 2015). The initial construction of the sheet web followed by the selective reinforcement of the bell internally as described in the methods section explain why this would occur. There seems to be a consensus that physically these spiders appear to perform the same behaviours (Schütz *et al.* 2007; Seymour & Hetz, 2011; Doerstelmann, 2015). However, it seems that when web construction is considered temporally, we can begin to understand some of the decisions that govern the process.

Reinforcement is not repeatable across individuals ($n=44$, $R = 0.17$), there is no consistent amount of time a spider will spend ensuring the bell is reinforced to a set standard each time. It does however, base the need for reinforcement upon its time spent constructing, with time spent constructing and time spent reinforcing being very significantly negatively correlated ($p = 7.21e-07$) (See Fig. 23). The more time a spider spends on the initial sheet web under which an air bubble will be placed, the less time they spend reinforcing after the air bubble is collected. Alongside this, when the data is divided by sex, we can see a clear difference in

their choice of time allotment (See Fig. 23). Females show more of a preference for reinforcing than males do, with a wider span in time allotment compared to the more compact males.

There are perhaps three reasons that may explain this; the amount of effort that goes into the initial sheet web, the need for a stronger bell, and the placement of the bell. The females may have lay down a sheet web that was not sturdy enough to hold the air bubble and so it had to be reinforced more than the males. Although this seems unlikely given the females propensity to be repeatable in their web construction. This process may be better framed as an exercise in cost-benefit analysis, a branch of optimality theory (Parker & Smith, 1990). The diving bell of females has increased functionality compared to males i.e. housing the egg sac as discussed. Therefore, we could suggest that females have optimised their silk laying process to produce a sturdier, longer standing web, with this increased time spent reinforcing. Systematically placing more layers of silk threads in sparser areas to produce more robust diving bells (Benjamin & Zschokke, 2002). One factor that should be considered but proved difficult to characterise in this study is the choice involved in placement of the bell (Blackledge *et al.* 2003). Though purely from personal observation, bells constructed in areas which provided less cover i.e. fewer points on which to anchor the web, took longer to construct. From this it could be assumed that these webs required more reinforcing to hold the air bubble. Ten more females were analysed than males, though unlikely, it may be this included a selection of females that built in areas which were more exposed.

One factor that wasn't included in the repeatability analysis was rest. This presents its own peculiarity in that it was so far from normal in its distribution even post transformation. The data were left skewed (See Fig. 20) as all the spiders reduced their time spent resting to a minimum – over 50% of spider spent between five and ten percent of their time at rest - and maximised their time spent spinning silk. Perhaps there is an optimum amount of time spent at rest wherein the spiders do not exhaust the air supply in their plastron (Atherton *et al.* 2014), but do not spend longer than necessary outside of a diving bell carrying food. Average total construction time was around six minutes, with the longest taking ~12 minutes and the quickest taking less than two minutes. Across this process they must find a balance between constraining factors such as the need to feed, to rest, and the need for cover. Though taking breaks seems necessary during such an energy intensive activity as web construction (Tanaka, 1989), it is frequently not prioritised over other behaviours. This minimisation of rest during

web construction is likely to function as a method of predator avoidance in of itself (Vollrath, 1985; Nyffeler & Pusey, 2014). Resting poses risks for many animals (Semeniuk & Dill, 2005), and in the case of these spiders, resting out of diving bell puts them at a high risk of being spotted by predators and subsequently removed from the gene pool. Sitting in the diving bell on the other hand has been suggested as a form defence against predators (Nyffeler & Pusey, 2014), via either a physical barrier or relative cover. Even though they become more conspicuous to predators during the construction process, the relative fitness benefit obtained from protective cover and energy intake from the prey induces construction as quickly as possible to allow for retreating into and feeding inside the diving bell. These benefits must outweigh the increased risk of either being seen, or the increased risk of capture due to the reduced flee speed associated with carrying prey (Drakeley *et al.* 2015).

4.3.2. Ontogeny and behaviour

Studies have often considered the correlation between some aspect of species morphology and the behaviours of the animal (Risch, 1977; Johnson & Sih, 2007), for example, web design is known to be affected by body size and weight in some species of orb weavers (Risch, 1977). Across this study it was assessed whether body size plays any role in web construction through comparisons between cephalothorax width (mm) and different behavioural measures taken from web construction analysis. Cephalothorax width was shown to negatively correlate with the average number of successful surfaces for an air bubble ($p = 0.005$). There were instances in which individuals failed to either gather an air bubble or secure one to the web. This data was initially plotted where in sub-adults were shown to carry out the most surfaces for an air bubble, whether they were successful or not. A Fisher's exact compared the propensity of adults and sub-adults to fail in this regard and neither adults nor sub-adults were found to be more efficient at web construction than the other ($p = 0.62$). This meant only successful attempts were plotted (See Fig. 24). The data still showed that sub adults occupied the upper end of no. of air bubbles collected i.e. sub-adults do not have to surface more purely because they are more inexperienced at construct their diving bells.

There are multiple studies that have suggested that web size is not allometric with body size specifically (Schütz & Taborsky 2003; Gregoric *et al.* 2013). It is documented that females of *A. aquatica* build larger webs that correlate with their size, whereas males tend to build webs which are slightly smaller and that show no correlation with size (Schütz & Taborsky 2003).

Whether the spiders were male or female seems to have had no effect on the number of air bubbles collected, suggesting number of surfaces may not predict bell size (See Fig. 25). However, the results gathered in this study suggest that ontogeny plays some role in the web building process (Barrantes *et al.* 2017). The smallest spiders in this study collected more air bubbles on average than the larger spiders. This seems to suggest again that the number of surfaces does not predict bell size, except for maybe when making comparisons between spiders of the same size. The smaller spiders are selectively choosing to collect more air bubbles at the cost of the energy expended by swimming to collect them (Schmidt-Nielsen, 1972).

Firstly, this could be due to a lack in construction ability, perhaps they haven't fully consolidated the behaviours associated with web construction (Nogueira & Ades, 2012). There may be some level of trial and error learning involved in the process as the spiders hone the most effective mechanism of air bubble capture and fixation to the web (Nogueira & Ades, 2012). Although, this would likely predict more failures in sub-adult web construction which the data has already shown to be untrue. It is more likely that this tendency for the smallest spiders to surface more frequently is due to a trade-off between the size of the air bubble they gather and their ability to flee (Schütz & Taborsky, 2005). This further expands on the notion of optimisation of the construction process (Parker & Smith, 1990). The collection of an air bubble from the water's surface – or be it the replenishment of the plastron - can be a significant selective pressure as it places the spider at a high risk of predation (Neumann & Woermann, 2013). It is perhaps the most vital behaviour the spider exhibits and so this process should be optimised to allow for air bubble collection and a quick return to the safety of submerged vegetation. Schütz & Taborsky (2003) demonstrated that the diving ability of the spiders didn't correlate with body size. However, they considered diving ability only when the spiders had to compensate for plastron buoyancy, and only spiders with a cephalothorax width $\geq 2.5\text{mm}$. It might be hypothesised that the smaller spiders in this study had to surface more frequently as they could only carry air bubbles that allowed for this trade-off between speed of diving bell construction and increased buoyancy. It has been shown that optimal foraging can predict spider web allometry in orb weavers (Gregoric *et al.* 2013). Therefore, it stands to reason that the increased predation risk of surfacing has optimised the construction

process to ensure as few surfaces as possible whilst still allowing for efficient escape of the spider in terms of its diving ability.

Overall, this data still holds clear the idea that the environmental heterogeneity of aquatic habitats relies on the web building process being adaptable across both males and females and over an individual's ontogeny. It must be plastic depending upon the dynamic form of the pneumatic surface, and the volatile environment in which the nest is constructed (Doerstelmann, 2015). Simple rules are known to be more robust than complicated rules (Axelrod & Hamilton, 1981); The emergent properties of *A. aquatica* web construction i.e. the steps, processes and patterns, may be as basic as the behaviours outlined across this study, altered accordingly to a given context. Yet still, it does seem that they are so heavily linked to the substrate that they become difficult to extract from the video analysis employed here (Krink & Vollrath, 1998).

4.4.1. Genetic differences

CO1 data suggests there are historic mitochondrial lineages that rapidly colonised out towards Britain after the last glacial maximum (LGM) and were maintained in the population, both in the U.K and China. At no point did the dispersing Chinese individuals carrying that haplotype become removed from the gene pool, they were able to maintain sufficient numbers and travel great distances, suggesting an abundance of suitable habitat at the time. It also seems clear that there was mixing within U.K. *Argyroneta* from physically distant northern and southern populations. Individuals from Surrey and Doncaster have shown to be more closely related to each other than individuals from the same water body. Generally, Czech2 and Doncaster2 represent some level of diversity that isn't seen across the other 4 individuals. There does seem to be greater diversity amongst populations than might be expected given their presumed limited dispersal ability i.e. they don't balloon (Masumoto *et al.* 1998a).

Despite the evident genetic differences amongst individuals, both within and between populations, the behavioural data still highlights some level of consistency in the web construction process. The data holds that there are some conserved steps and patterns to the process regardless of genotype. This diversity within the species could explain the lack of overall repeatability in many of the steps i.e. a significant interaction between genotype and

environment (Dohm, 2002). There are studies that have documented intragenotypic variability, where in individuals with the same genotype which are reared in the same environment express different trait values (Stamps *et al.* 2013). Even from a single genotype stochastic variability can arise. Therefore, the genetic variation within the species seems to suggest there could be an array of building methods from an array of genotypes. Individuals may share some of the steps and patterns described, but the behaviours become difficult to characterise into a single uniform process across the species.

4.5.1. Water quality and their potential for use as an ecological indicator

Spiders have seen little use as indicator species despite their prolific abundance and increased sensitivity to certain anthropogenic pollutants (Neet, 1996; Royauté *et al.* 2015). Aquatic and semi-aquatic spiders in particular are considered in only a handful of papers regarding their potential for use as indicators. These studies largely consider only their ability to predict the abundance of other species rather than abiotic environmental factors. For example, the abundance of web building spiders that occupy riparian zones have been shown to fluctuate in response to aquatic insect flux (Kato *et al.* 2003). The data gathered in this study, though limited, seems to suggest similar possible applications for *A. aquatica*. To begin, it should be noted that although this species has been categorised as widespread by the BAS (BAS, 2019b), they were not found in 9 out of the 11 sites at which they had been previously recorded. Whether this was down to sampling methods, time of the year, or an actual loss of the species in that locality is difficult to establish conclusively. However, this propensity for the species to occupy very sparse and specific localities has been previously noted in the literature. Katsura & Nishikawa (1982) reported that the water spider was collected at only three out of one hundred ponds and wetlands in the Aomori prefecture. Possible disappearance of the species at multiple locations could be down to increased levels of certain pollutants (Royauté *et al.* 2015), or perhaps the introduction of predators such as fish into the water body. Katsura & Nishikawa (1981) have previously noted that the water spider seemed to live at ponds where no fish were observed due to their presumed predation on the spiders.

The spiders were only found at sites with the highest water temperatures and the highest numbers of potential prey, with warmer waters typically predicting species-rich habitats (Gustafson *et al.* 2009) (See Fig. 31). Sampling in warmer months increases the chances of

finding spiders which would be otherwise difficult to find in the colder months when they over-winter. Given both the more sessile nature of females and the active hunting of males (Schütz & Taborsky, 2003), areas of high potential prey are likely necessary to ensure high enough contact with prey to avoid individuals becoming calorie restricted. Studies have shown that it is possible to collect these spiders during the winter as they hibernate in their diving bells along the bed of water bodies (Bromhall, 1988). However, this data suggests the best period for sample collection is spring/summer. Day of the year doesn't appear to predict the presence of spiders alone, but it was significantly correlated with temperature suggesting searching during warmer months does increase the likelihood of finding the spiders (See Fig. 33)

Across the 11 sites sampled only two housed the water spider. Comparisons were made between some of the chemical and physical properties of these two sites specifically. The data presented suggests that these spiders rely far more on vegetation structure and complexity than on any factor related to water quality in choosing their habitat (Mammola *et al.* 2016) (See table 6). Furthermore, the results of the GLM suggest the best model for predicting *A. aquatica* was the presence of great crested newts (*Triturus cristatus*) in the water body (AIC weight = 1) (See table 8). But again, this data is limited given only two sites that housed *A. aquatica* were discovered.

These newts inhabit freshwater habitats, mainly permanent fish-free ponds, during their breeding season in spring and early summer (Gustafson *et al.* 2009). They are not permanent residence of freshwater systems meaning their use as indicators for the spiders is entirely dependent on the time of year, but their aquatic habitat ecology is much more well studied than the water spider. Their presence in a water body indicates a habitat with high plant diversity and low nutrient levels (Gustafson *et al.* 2006; Gustafson *et al.* 2009), factors that have been shown to predict the presence of *A. aquatica* (Aakra & Dolmen, 2003; Mammola *et al.* 2016). Water quality can be a significant determinant of great crested newts' presence and yet the results of this study suggest the spiders are unaffected by any of these measures directly (Mammola *et al.* 2016). It is well documented that habitats of great crested newt are of a high quality (Gustafson *et al.* 2009). Biotic variables such as a high amount of available prey, and a lack of predators are typical of *T. cristatus* habitat (Gustafson *et al.* 2006;

Gustafson *et al.* 2009), with these factors also being principally required by *A. aquatica* (Mammola *et al.* 2016). All three sites in which the spiders were collected (including the Czech site) were on protected and well-maintained nature reserves. This combination of biotic factors is only likely to occur in high quality habitat such as that found on reserves, limiting new areas of colonisation by this species (Gustafson *et al.* 2009). It is worth noting that after revisiting the Thursley reserve site the water body had become eutrophic due to a build-up of orthophosphates (See table 9), however, the spiders were still found in similar numbers. The spiders may only colonise desirable, high quality water bodies, but are able to subsist in presumably sub-optimal water bodies with sufficient vegetation for web construction and cover (Aakra & Dolmen, 2003). Acidification of water bodies, such as that seen at the Thursley site, has been known to prohibit many amphibian species from reproducing (Gustafson *et al.* 2009). It could be that their ability to exist in acidic waters, with or without a low dissolved oxygen content (Masumoto *et al.* 1998b), also functions as predator avoidance.

As described in other papers, the biggest pre-requisite for their locality in England appears to be an abundance of suitable vegetation on which they can spin their webs and the males can actively hunt (Aakra and Dolmen, 2003; Mammola *et al.* 2016). It was also noted by Aakra & Dolmen (2003) that they can utilise basically any standing water body in Norway, even eutrophic ones, as long as there is sufficient vegetation present. If the essential component of their habitat is vegetation, then it stands to reason that this structure will have the largest effect on their extended phenotype as is already suggested. Overall, these results do seem to indicate that the diving bell spider may be a useful indicator species for abundance/species richness of plants in ponds and wetlands (Gustafson *et al.* 2006), rather than an indicator of any abiotic measure of water quality. There could be implications here for their use in environmental monitoring in wetland landscapes.

5.1.1. Future work

Although this study does begin to detail the process of web construction in *A. aquatica* it would initially benefit from an expansion in data collection for both behavioural and genetic data. More individuals would be measured across a greater number of populations throughout the U.K. and mainland Europe so levels of variation could be measured across the species and across different environmental scales. *A. aquatica* genetic data is limited, increased understanding of genetic profiles of different populations may make it possible to address questions related to their dispersal mechanisms. Though limited, the observations related to habitat preferences seem consistent with published data, further data collection is likely to be surplus (Aakra & Dolmen, 2003; Mammola *et al.* 2016). Analysis of web building via the methods exploited in this study had some shortcomings. Analysis was carried out manually by a single observer making it more prone to observer bias. Studies that examine web construction behaviour often measure the process through a recording camera and an associated tracking software, allowing for the capture and analysis of more minute detail (Benjamin & Zschokke, 2002); however, this method may prove difficult to carry out on a moving target underwater.

This study attempted to describe the building process from its initial step to the point at which a functional bell had been constructed. In order to understand bell construction in its entirety, it would be pertinent to extend observation time to elucidate how maintenance or expansion of the bell continues after completion. Do these behaviours follow patterns like those already observed? Or, would these demonstrate a greater level of variation than is already seen?

Though difficult given the size of the study species and their chosen habitat, it would also be beneficial to gather behavioural data *in situ* so that this may be compared to data gathered from lab based studies. Possible further study should work to establish whether the observations gathered in the lab are a true reflection of web construction in the wild. This is necessary so that a baseline for intraspecific variation can be set.

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Appendices

Appendix 1. The date arrived, cephalothorax width in mm, leg span in mm, life stage, and sex of all spiders shipped from the Czech Republic (small pond near lake mácha) and used in the study.

Spider name	Date arrived	Cephalothorax width (mm)	Leg span (mm)	Life stage	Sex
Gimli	31/05/18	3.0	29	A	M
Frodo	31/05/18	2.9	31	A	M
Bilbo	31/05/18	3.0	31	A	M
Pippin	31/05/18	2.6	25	A	F

Appendix 2. The date collected, cephalothorax width in mm, leg span in mm, life stage, and sex of the 14 spiders physically collected from the Czech Republic (small pond near lake Mácha) and used in the study.

Spider name	Date collected	Cephalothorax width (mm)	Leg span (mm)	Life stage	Sex
Aragorn	17/05/2019	2.8	24	A	M
Boromir	17/05/2019	2.0	17	SA	N/A
Elrond	17/05/2019	2.5	21	A	F
Sauron	17/05/2019	2.8	26	A	F
Shelob	17/05/2019	2.4	24	A	F
Thranduil	17/05/2019	2.0	16	SA	N/A
Shadowfax	17/05/2019	2.3	22	A	F
Saruman	17/05/2019	2.6	25	A	M
Tauriel	17/05/2019	2.3	22	A	F
Treebeard	17/05/2019	2.1	19	A	M
Eowyn	17/05/2019	1.9	17	SA	N/A
Elwing	17/05/2019	2.1	20	A	F
Faramir	17/05/2019	2.2	20	A	M
Rosie	17/05/2019	2.3	19	A	F

Appendix 3. The date collected, cephalothorax width in mm, leg span in mm, life stage, and sex of the 13 spiders taken from Thursley nature reserve in Surrey and used in the study.

Spider name	Date collected	Cephalothorax width (mm)	Leg span (mm)	Life stage	Sex
Jolene	25/05/2019	2.8	28	A	F
Mary	25/05/2019	2.2	13	A	F
Mandy	25/05/2019	3.0	23	A	F
Angelina	25/05/2019	3.2	27	A	F
Archie	25/05/2019	2.5	21	A	M
Ziggy	25/05/2019	2.5	26	A	M
Eileen	25/05/2019	2.7	25	A	F
Ruby	25/05/2019	1.7	14	SA	N/A
Brandy	25/05/2019	2.6	25	A	F
Ginger	25/05/2019	2.3	13	A	F
Grant	25/05/2019	3.8	30	A	M
Stormy	25/05/2019	2.8	24	A	M
Ernest	25/05/2019	3.0	26	A	M

Appendix 4. The date collected, cephalothorax width in mm, leg span in mm, life stage, and sex of the 17 spiders taken from Potteric Carr nature reserve in Doncaster and used in the study.

Spider name	Date collected	Cephalothorax width (mm)	Leg span (mm)	Life stage	Sex
Sal	18/04/2019	3	28	A	M
Elizabeth	18/04/2019	2.7	26	A	F
Daisy	18/04/2019	2.8	28	A	F
Persephone	18/04/2019	2.8	27	A	F
Lorelei	18/04/2019	2.6	26	A	F
Enola	18/04/2019	2.5	26	A	F
Aphasia	04/05/2019	2.3	22	S	F
Otterley	04/05/2019	3.2	31	A	M
Alec	04/05/2019	2.2	23	A	M
Magnolia	04/05/2019	3	28	A	F

Appendix 4. Continued

Jimbo	04/05/2019	2.5	24	A	M
Pandora	04/05/2019	2.9	28	A	F
Elvira	04/05/2019	2.0	20	SA	N/A
Alvin	04/05/2019	2.8	27	A	M
Sadie	04/05/2019	2.6	27	A	F
Eleanor	04/05/2019	2.7	25	A	F
Jane	04/05/2019	2.6	25	A	F

Appendix 5. Non-significant regression between behavioural aspects of web-construction

Regression	P Value	R ²	Equation of the line
Construct – airbubble	1.00	0.017	$y = -0.0457x + 0.0919$
Rest – reinforce	1.00	0.012	$y = -0.1045x + 0.1601$
Reinforce – airbubble	1.00	0.004	$y = 0.0298x + 0.0563$
Reinforce – No. surfaces	0.28	0.078	$y = 3.5825x + 1.3599$

Appendix 6. Non-significant regressions between cephalothorax width and behavioural measures.

Regression	P Value	R ²	Equation of the line
Ceph – total time	0.96	0.014	$y = 24.827x + 276.65$
Ceph – constructing	0.96	1.00E-04	$y = -0.1604x + 68.429$
Ceph – rest	0.96	0.032	$y = 2.0802x + 4.4856$
Ceph – reinforce	0.96	0.031	$y = -1.9118x + 19.874$
Ceph – airbubble	0.95	0.041	$y = -1.0732x + 8.8238$

Appendix 7. Non-significant regressions between water quality measures.

Regression	P Value	R ²	Equation of the line
pH – DO	1.00	0.028	$y = 0.2656x + 3.1833$
pH – Temp	1.00	0.058	$y = 0.9871x + 1.6272$
pH – Conductivity	0.78	0.224	$y = 174.66x - 420.25$
pH – No. pirata	0.78	0.265	$y = -4.5775x + 45.226$
pH – No. prey	1.00	0.098	$y = 0.08074x - 1.1548$
pH – Newt	1.00	0.015	$Y = 0.0352x - 0.4226$
DO – ORP	1.00	0.119	$y = 21.721x + 32.714$

Appendix 7. Continued

DO – temp	0.62	0.028	$y = -0.4293x + 10.574$
DO – Conductivity	0.4	0.080	$y = -65.062x + 1108.3$
DO – No. pirata	0.19	0.090	$y = 1.568x + 63003$
DO – No. prey	0.86	0.004	$Y = 0.0966x + 3.811$
DO – Newt	0.96	<0.000	$Y = 0.0028x + 0.1957$
ORP – Temp	0.88	0.003	$y = -0.0022x + 8.7284$
ORP – Conductivity	0.2	0.172	$y = -1.5227x + 997.77$
ORP – No. pirata	0.8	0.009	$y = 0.0081x + 13.157$
ORP – No. prey	0.62	0.032	$y = -0.0044x + 4.9161$
ORP – Newt	0.88	0.003	$Y = 0.0001x + 0.1622$
Temp – Conductivity	0.53	0.046	$y = -19.407x + 945.67$
Temp – No. pirata	0.9	0.002	$y = 0.1209x + 13.428$
Conductivity – No. pirata	0.5	0.064	$y = -0.0058x + 18.877$
Conductivity – prey	0.56	0.045	$y = -0.0014x + 5.4176$
Conductivity - Newt	0.21	0.172	$Y = -0.0003x + 0.4275$
Day of the year – No. prey	0.05	0.352	$y = 0.0574x + 0.6711$
Day of the year – pH	0.75	0.012	$y = 0.004x + 6.6025$
Day of the year – DO	0.15	0.218	$y = -0.0286x + 6.5926$
Day of the year – ORP	0.42	0.073	$y = -1.041x + 194.35$
Day of the year – Conductivity	0.74	0.013	$y = -1.6008x + 868.82$
Day of the year – No. pirata	0.98	6E-05	$y = -0.0027x + 13.158$
Day of the year - Newt	0.13	0.240	$Y = 0.0052x - 0.1219$

Appendix 8. Genbank accession numbers for all the sequences gathered.

Species	Sequence	Accession no.
<i>Argyroneta aquatica</i>	CO1	KJ907736.1
<i>Argenna patula</i>	28S	KR074028.1
<i>Argenna patula</i>	H3	KR074080.1
<i>Argenna patula</i>	CO1	KR074054.1