

The Role of Conspecific Damage-Released Alarm Cue and Plastic Pollution on Caddisfly Larvae Case Construction Behaviour

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By

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Abstract

Organisms interact with their environment using sensory systems to forage, communicate with other organisms, and detect predators. Prey can detect and assess predation risk using predator-related chemical cues, and this can influence their decision-making process. Antipredator responses are essential for the survival of an organism, and these responses can be displayed physiologically, morphologically, and behaviourally. While some prey rely on single antipredator responses, others combine several antipredator responses to increase their chances of survival. For instance, caddisfly larvae are known to display both morphological changes and behavioural responses when exposed to predation risk (i.e., their case construction behaviour). The portable case of an actively foraging caddisfly larva is generally understood to have evolved as a predator defence strategy. Larvae of many caddisfly species construct transportable cases of different sizes and shapes with various surrounding materials present using self-secreted silk to bind them together. This case construction behaviour can be influenced by predation risk and the surrounding sediments. Thus, this thesis examined the effect of these two factors on caddisfly larvae case construction behaviour. In the first experiment, denuded *Limnephilus* spp. larvae were exposed to conspecific damaged-released alarm cue, a predator-related chemical cue, twice within 48 hours during case reconstruction. Larvae exposed to alarm cue added more sticks to their cases than those exposed to dechlorinated tap water (control) after 24 hours of the first exposure. However, alarm cue had no effect on larval case length and the rate at which they constructed new cases when compared to larvae in the control group after 24 and 48 hours of exposure. In the second experiment, *Phryganea* spp. larval preference for different case construction materials was examined based on their surrounding materials. The experiment also investigated which case construction material offered more protection when the *Phryganea* spp. larvae were exposed to predatory crayfish. As an addition to the second experiment, larval recognition by predatory crayfish using empty larval cases was examined. Larvae were removed from their cases and provided with either their native construction materials (leaves), non- native case construction materials (plastics), or a mixture of both materials for case construction. Larvae were exposed to crayfish after 72 hours of case construction to determine which case type offers more protection against predation. Larvae constructed their cases using the materials provided, and these materials did not affect the proportion of larvae that constructed new cases in each group. However, larvae provided with both leaves and plastics preferred their native case construction materials (leaves).

Also, larval survival when exposed to crayfish was not affected by their case type, rather, larval survival depends on the predator's experience with case-building caddisfly larvae. Similarly, only those crayfish that had previously eaten larvae attacked the empty cases. These two studies also show that predation risk and surrounding materials could influence case construction behaviour. Larvae adjusted their behaviour based on the information perceived from their environment. In this study, *Limnephilus* spp. larvae were able to modify their case building behaviour to match the intensity of the perceived predation risk by adding more sticks to their cases. Although the protective function of *Phryganea* spp. larval cases based on material type could not be assessed in the second experiment, the result shows that prey recognition needed to be learned to initiate predatory attack. The assessment of larvae case construction behaviour can help understand larval interactions with their environments, such as predation risk and human activities (plastic pollution). Caddisfly larvae offer various ecosystem services in the freshwater habitat, and their case construction behaviour has been linked to their survival. Hence, natural (e.g., predation risk) and anthropogenic activities (e.g., plastic pollution) that can influence this construction behaviour needs to be investigated and monitored to ensure that their role within the ecosystem is not altered.

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Dedication

With a humble heart, I acknowledge your support, O GOD!

With sincere gratitude, I appreciate your grace, O GOD!

Were it not for your assistance, this work would not have been a reality,

It is dedicated to you, O GOD!

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Chapter 1: Introduction

1.1: General Introduction

Antipredator adaptations are essential for the survival of an organism; hence evolution plays an important role in predator-prey relationships (Evans and Schmidt, 1990). It is essential for prey to take defensive action against predators while minimizing costs, and this can be achieved through continuous evolution (Barbosa and Castellanos, 2005). Prey defence mechanisms have evolved to either avoid or escape predator attacks or hinder the predator's success (Lima and Dill, 1990). The transportable cases constructed by some caddisfly larvae have been described as a defensive mechanism that protect larvae from a predator's attack (Otto and Svensson, 1980; Wiggins, 2004).

Many caddisfly larvae species construct mobile cases of varying sizes and shapes using different materials present in their surrounding. The case materials are glued together using silk secreted by the larvae. Case construction in caddisfly larvae is a continual process, and larvae increase their case sizes as they grow (Anderson, 1974). However, predation threat affects caddisfly larvae morphologically and behaviourally, and this influences their case construction behaviour, case material selection, and antipredator behaviour (Boyero, 2011; Boyero *et al.*, 2006; Correa-Araneda *et al.*, 2017; Gall and Brodie Jr, 2009; Okano *et al.*, 2017).

1.2: Caddisfly Larvae and Their Environment

Aquatic insects constitute a major part of the freshwater fauna, and their interactions with the environments maintain the flow of energy and nutrients within their ecosystem (Wiggins, 1998). Caddisflies (Trichoptera) are one of the most diverse aquatic insect orders (Wiggins, 1998) whose life stages (egg, larva, and pupa) are exclusively aquatic with few exceptions whose larvae are terrestrial (Anderson, 1967; Flint 1958; Hickin, 1967). The evolution of the diverse biology and behavioural patterns in the aquatic larval stages of caddisflies enables them to inhabit various freshwater ecosystems, including springs, streams, rivers, lakes, marshes, to temporary pools (Wiggins and Currie, 2008). Caddisfly larvae are abundant and widespread in Saskatchewan, and they inhabit both the lentic and lotic habitat (Smith, 1984).

As an important constituent of freshwater habitat because of their larval abundance, caddisfly larvae play significant roles in the aquatic system trophic level as they serve as food for most aquatic organisms (Hickin, 1967; Wiggins, 1977) thereby sustaining the aquatic system. They serve as bioindicators for evaluating the effect of man-made activities on freshwater habitats (Sheffield *et al.*, 2019; Smith, 1984), and to determine the pollution level and disturbance in those habitats (Wiggins, 1977). They also play an important role in freshwater ecology as they decompose plant materials into fine organic particles through feeding (Correa-Araneda *et al.*, 2017; Wiggins, 1977) as well as using them for case construction (Hickin, 1967).

Caddisfly larvae construct a variety of structures, and these structures vary from transportable cases and dome-shaped cocoons adapted for foraging actively, to fixed tubes and retreats with silk nets used for filtering and gathering food particles carried by stream currents (Hickin, 1967; Wiggins, 2004). Retreat-makers (sub order Annulipalpia), also known as net-spinners, do not forage around like the portable case makers. They construct fixed retreats or tubes attached to rocks or logs in their habitat, and they remain within the retreat while gathering food that comes their way (Wiggins, 2004). Larvae are not the only life stage known to construct cases; pupae also construct characteristic cocoons and shelters (Wiggins, 2004) either from their respective larval cases, silk nets, fixed tubes, or they construct pupal cases as in the free-living species whose larvae do not construct either cases, cocoons, tubes, or retreats (Hickin, 1967). Caddisfly larvae construction behaviour influences their feeding behaviour, pupation efficiency, survival, and reproduction. The cases, cocoons, retreats, and nets constructed are used to access areas that other aquatic insects are not able to exploit fully (Wiggins, 2004). Retreat-makers are able to filter food from moving water while conserving the energy needed for active foraging and reducing the risk of predation (Wiggins, 2004). Cocoon-makers (sub order Spicipalpia) mostly feed on algae, and their structures provide protection while they scrape algae from the exposed rock surfaces. Similarly, the case of the portable case makers (sub order Integripalpia) also serves as protection and camouflage while the larvae forage (Wiggins, 2004).

The analysis of the feeding behaviours (e.g., shredders, collectors, scrapers, and predators) of three North American orders of aquatic insects, Ephemeroptera (Mayflies), Plecoptera (Stoneflies), and Trichoptera (Caddisflies) based on the type of food consumed and the foraging mode revealed that Ephemeroptera are mainly collectors and scrapers, while Plecoptera are

mainly shredders and predators (Wiggins, 2004). However, unlike the Ephemeroptera and Plecoptera, there is a greater diversity of foraging habits in Trichoptera; four of these feeding behaviours are greatly represented in this order (Wiggins, 2004). Shredders feed on dead decaying leaves which has been colonized by aquatic fungi and bacteria, collectors accumulate and feed on fine particulate organic matter less than 1mm in size, scrapers graze on periphytons and diatoms from rocks and plants, while the predators feed on other invertebrates (Wiggins, 2004). Thus, it has been suggested that silk secretion by caddisfly larvae is the main factor influencing trichopteran diversity in the environment (Wiggins and Mackay, 1978), because their cases, retreats and filter-nets characteristics enhance their ability to exploit food resources (Wiggins, 1998). The silk glands of caddisfly larvae secrete the silk, which is released from the tip of the labium; this silk thread is of great importance in case construction (Wiggins, 2004).

1.3: Caddisfly Larvae Case Construction Behaviour

Animals build structures for one of three reasons: for protection, to catch prey, and for communication within the same species; however, protection is the most common function of these structures (Hansell, 2005). Animal structures protect their builders against predation risk and extreme temperatures (Hansell, 2005). It has been proposed that case construction in caddisfly larvae does not have a single function. Larval cases protect the larvae from predator attack (Boyero *et al.*, 2006; Ferry *et al.*, 2013; Johansson, 1991; Otto, 2000; Otto and Svensson, 1980), and it also ensures efficient respiration (Wiggins, 2004; Williams *et al.*, 1987). The ability of some caddisfly larvae (e.g., family Limnephilidae) to secrete silk and construct tubular cases enhances the respiratory efficiency of these species, and this allows them to exploit lentic and warmer waters with less oxygen unlike most larvae that inhabit fast-flowing water with sufficient oxygen (Wiggins, 2004). Larval cases aid respiration through the undulation of the abdomen while concurrently enabling movement over a substrate (Wiggins, 2004; Williams *et al.*, 1987). The undulation of the abdomen allows a current of water to enter from the anterior opening of the larval case and move to the posterior opening thereby enabling ventilatory flow of water. This process ensures the flow of oxygenated water over the body and the gills on the abdominal segments, thus facilitating respiration through gaseous exchange (Wiggins, 2004).

Animals construct various structures using surrounding materials, and these materials are effectively manipulated to serve the desired function (Hansell, 2005). For example, larvae of many caddisfly species construct transportable cases (Otto, 2000) of different sizes and shapes with various materials (Kwong *et al.*, 2011; Okano *et al.*, 2012; Williams *et al.*, 1987) using self-secreted silk to bind them together (Wiggins, 2004; Williams *et al.*, 1987). Some of the materials used in case construction include mineral materials, e.g., sand grains and stones, or organic materials, such as leaves, tree bark, twigs, mollusc shells (Otto and Svensson, 1980) or in some instances, the empty cases of other caddisfly larvae (Boyero and Barnard, 2004). Although caddisfly larvae are usually referred to as “case-bearing,” not all caddisfly larvae construct transportable tube-like cases; some either live freely without a larval case, construct dome-shaped cocoons, make fixed tubes fastened to stationary objects like rocks, or construct bag-like nets with tube-like shelter (Hickin, 1967; Wiggins, 2004).

The case-making caddisflies (sub order Integripalpia) are primarily known for their ability to construct transportable tubular cases (Wiggins, 2004). During construction, larvae join construction materials together to form an irregular chain, which is sometimes referred to as a provisional case (Copeland and Crowell, 1937) or temporary case (Stuart and Currie, 2001). These provisional or temporary cases are loosely joined together and are the foundation on which the permanent case is constructed (Copeland and Crowell, 1937; Stuart and Currie, 2001). Temporary cases are constructed with materials attached rapidly to cover the entire larval body, while permanent cases are constructed slowly (Stuart and Currie, 2001). Cases are constructed by adding more materials to the anterior end of the cases until the construction is completed (Copeland and Crowell, 1937). Larvae continue to add more construction materials to their cases until they are enclosed in a protective case that covers the entire larva, and stopping only when the case is bigger than the larva itself (Prestidge, 1977). Most caddisfly larvae are known to reconstruct new cases when forced out of their old cases either by pushing them gently from the posterior end of their abdomen with soft-tipped forceps (Boyero, 2011; Correa-Araneda *et al.*, 2017; Gaino *et al.*, 2002), or when the old case is abandoned because of pesticide pollution or disturbance (Johnson *et al.*, 2008; Wiggins, 2004).

1.3.1: Costs Associated with Case Construction

Though structures constructed by animals are beneficial, it is important to investigate the tradeoff between the costs of constructing these structures and the benefits accrued from them (Okano and Kikuchi, 2009). The weight of caddisfly larval cases can affect larval foraging behaviour. Otto (1987) observed that larvae with lightweight cases were more successful in catching isopods than those with heavy cases. Also, the highest cost associated with the constructed cases, other than cost of foraging with the case, is the energy expended in searching for construction materials and the secretion of silk to bind them (Okano and Kikuchi, 2009).

According to Otto (1987), the energetic cost of case construction may be substantial. Otto (1975) observed that 12% of the larval energy content is expended in silk production in the final instar of *Potamophylax cingulatus* larvae. In another species, *Agrypnia pagetana*, larvae construct their cases using either small pieces of plant materials that require a higher amount of silk for binding them together, or from hollow stems that require less silk during case construction. This material selection behaviour indicates that inhabiting hollow stems is less costly because of the low amount of silk used in constructing them (Otto, 1987). Some of the cased larvae made new cases from rigid hollow stems, which could sustain more pressure than the abandoned cases (Otto, 1987). Although larvae with cases made of small pieces of materials already invested energy in constructing their cases, these larvae abandoned their cases when presented with hollow stem cases (Otto, 1987). The substitution of cases made of small pieces of materials with hollow stem cases indicate that hollow stem cases are more valuable, and the benefits of these hollow stems outweigh the cost of constructing the abandoned cases. The rigid hollow stem cases might enhance survival because of its protective benefits, hence the reason why the larvae opted for them. Further, when denuded larvae were provided with both case types, they preferred hollow stem cases than cases made from small pieces of plant materials irrespective of their original case type (Otto, 1987). Similarly, de Gispert *et al.* (2018) also observed that larvae survival was prioritized during case construction. Larvae constructed their cases with larger grains which is faster than using smaller grains, even though construction with larger grains is more costly than smaller grains because it requires more silk to bind them.

Animals must allocate an appropriate amount for energy for the construction of their structures to enhance fitness (Okano *et al.*, 2010). In caddisfly larvae, the construction process involves two main processes: the selection and gathering of appropriate case construction materials and the construction of cases using these materials (Okano *et al.*, 2010). Thus, it is essential for larvae to expend the right amount of energy in each process. The selection of unsuitable materials for case construction could result in a costly process of modifying the selected materials (Okano *et al.*, 2010). For instance, *Goera japonica* larvae secreted more silk to smoothen the inner walls of their cases when provided with rougher particles compared to those provided with smoother particles for case construction (Okano and Kikuchi, 2009). The smooth inner walls of larval cases help prevent friction between the abdomen and larval cases during the undulation of their abdomen, thus enhancing efficient respiration (Okano *et al.*, 2010). This silk lining behaviour of the inner walls of larval cases suggests that there is a tradeoff between the cost of searching for smooth particles and the cost of silk secretion during the case construction process (Okano and Kikuchi, 2009). Animal structures affect other aspects of the animal's lifestyle; therefore, these animals need to adapt by modifying the structures to suit their lifestyle (Hansell, 2005).

The cost of constructing and transporting mineral cases is higher than that of organic cases because they are heavier, and contain more particles, requiring more silk to bind them together (Otto and Svensson, 1980). However, mineral cases constructed by *Potamophylax cingulatus* are able to withstand pressure and are less susceptible to brown trout (*Salmo trutta*) predation than organic cases (Otto and Svensson, 1980). They suggested that portable case makers either invest a relatively large amount of energy to construct mineral cases, which are energetically costly, and the larvae become smaller emerging into smaller adults producing fewer eggs, or they expend more energy in growth while they construct organic cases which produce larger larvae and adults with high fecundity. The choice of case construction material selection is not well understood; however, Otto and Svensson (1980) suggested that predation pressure could influence case polymorphism in *P. cingulatus* larvae, with larvae using stronger cases as they grow. Since larval survival is known to be prioritized, larvae could prefer to pupate in mineral cases because they offer more protection, thus increasing their chances of emerging into adults.

Case construction also comes with a cost that can be measured as protein loss in both larvae and adults, and it also increases the larval consumption of oxygen (Mondy *et al.*, 2011). Increased

investment in case construction delayed adult emergence, and it also affected resource allocation in adults (Mondy *et al.*, 2011). The search for materials, construction of cases and production of silk are all at a cost whose return is to protect them from predators as well as increasing respiration efficiency (Wiggins, 2004).

1.3.2: Variation in Materials Used in Case Construction

Benthic organisms in the aquatic environment often inhabit the inorganic substratum underlying their habitat, and the composition of these substrata influences the organisms (Okano *et al.*, 2012). Caddisfly larvae inhabit the benthic habitat, and they construct their cases using the materials in their environment. The surface texture of case construction materials can influence larvae case construction behaviour (Okano and Kikuchi, 2009; Okano *et al.*, 2012). For instance, caddisfly larval preference for case construction can be influenced by the materials available in their environment; Okano *et al.* (2012) observed that the preference of *Perissoneura paradoxa* larvae for case construction materials varies based on the materials available in their environment and the surface texture of these materials. Field survey of the surrounding sediments revealed that the surface roughness of quartz, the smoothest particle available in their natural habitat, increases as the particle size increase, while the abundance of quartz decreases as quartz particles increase in size (Okano *et al.*, 2012). Also, the assessment of the anterior portion of larval cases constructed in their natural habitat showed that larvae use larger and rougher particles as they grow, although the particles used in constructing larval cases were smoother when compared with the surrounding sediments (Okano *et al.*, 2012). When *P. paradoxa* larvae were provided with equal proportion of smooth and rough particles to repair the anterior portion of their damaged cases, larvae decrease their preference for smooth particles as the larvae increase in size (Okano *et al.*, 2012). Another study conducted by Okano *et al.* (2011) compared some sand particles of *P. paradoxa* and *Psilotreta kisoensis* larval cases built in their natural environment with the sediment particles collected from the larval habitat, they found that the surface texture of the surrounding sediments which the larvae were gotten from was rougher than the sand particles used in constructing the cases. The result suggests that larvae prefer to construct their cases using materials with smooth surface texture. The study also demonstrated that larvae that inhabited areas with fewer smooth particles had low preference for smooth sand particles when provided with equal amount of smooth and rough particles during case repair.

Thus, they suggested that the use of smooth particles is advantageous for higher instar larvae, but the lack of smooth large particles in their natural habitats results in their preference for rough particles, indicating that the ontogenetic change in material selection is influenced by the surrounding sediments (Okano *et al.*, 2012).

Several studies have also demonstrated that larvae will construct their cases using non-native materials in the absence of their native case construction materials (Anderson, 1974; De Gispert, 2018; Gaino *et al.*, 2002; Okano *et al.*, 2012). Caddisfly larvae are also selective when choosing case construction materials; their preference for the materials is not only influenced by their surrounding sediments (Okano *et al.*, 2011), but also by predation risk (Boyero, 2011; Boyero *et al.*, 2006) and their instar level (Otto and Svensson, 1980). Larvae belonging to the same species and instar level mostly use the same type of material for case construction (Johansson, 1991; Otto and Svensson, 1980). Prestidge (1977) observed that higher instar larvae with longer cases used larger case construction materials than lower instars with smaller cases. Although larvae showed a preference for the size of case construction materials, there was no preference for colours (Prestidge, 1977). Otto and Svensson (1980) also observed that *Potamophylax cingulatus* larvae change their case construction materials from organic materials to mineral materials as they grow.

Gaino *et al.* (2002) suggested that caddisfly larvae expend the minimum amount of energy while trying to achieve maximum energetic gain by evaluating the quality of the construction materials during case construction. Consequently, this variation in larval preference for the particles in their environment based on their surface roughness can be used in understanding the cost-benefit relationship of case construction (Okano *et al.*, 2011). The variation in case construction material selection within species can be caused by innate ontogenetic differences or influenced by the environment (phenotypic plasticity), but the mechanisms controlling case polymorphism still remain unclear (Okano *et al.*, 2011). Also, the selection of case building materials by caddisfly larvae based on the surrounding sediments (Okano *et al.*, 2011), and the source and surface texture of the material, needs more attention (Okano *et al.*, 2010).

1.3.3: Other Environmental Parameters influencing Larval Case Construction Behaviour

1.3.3.1: Adult Female Oviposition Sites

Oviposition sites influence the survival of progeny; thus, it is important for the insects to select an appropriate oviposition site (Lancaster *et al.*, 2010). Studies have shown that only a few available oviposition sites are utilized by aquatic insects. Egg masses are often clumped, with many apparently appropriate sites with few or no eggs (Hoffmann and Resh, 2003; Lancaster *et al.*, 2010). This aggregation of egg mass behaviour suggests that females either experience high mortality in some oviposition sites while laying eggs, or that the survival of the eggs or newly hatched larvae vary significantly in different sites (Lancaster *et al.*, 2010).

Lancaster *et al.* (2010) observed that *Polycentropus flavomaculatus* and *Hydropsyche siltalai* mostly lay their eggs on submerged rocks at the edge of the pools. The female legs of *P. flavomaculatus* and *H. siltalai* were adapted for swimming, hence allowing the adults to swim under the water to lay their eggs on submerged rocks. In contrast, *Rhyacophila dorsalis* adult females have a preference for the underside of emergent rocks in riffles, and the female legs are not modified for swimming. The selection patterns of oviposition sites displayed by females are influenced by the behaviours and morphology of the female, however, the casual mechanisms influencing these patterns (e.g., how the oviposition sites are located) needs more attention (Lancaster *et al.*, 2010).

H. siltalai adult female, whose larvae are filter feeder feeders, prefer to lay their eggs in pools (slow-moving water) instead of riffles (fast-flowing water) where most filter-feeding larvae occur (Lancaster *et al.*, 2010). These newly hatched larvae must be able to move from areas with slow moving water where they hatched to fast flowing areas where older instar larvae occur. However, *P. flavomaculatus* and *H. siltalai* adult females laid their eggs in the usual habitat of their larvae (Lancaster *et al.*, 2010).

Hoffmann and Resh (2003) demonstrated that females of three limnephiloid caddisflies (*Hydatophylax* sp., *Neophylax rickeri* and *Onocosmoecus unicolor*) were selective while choosing their respective oviposition sites based on the features of the habitats. The egg masses of *Hydatophylax* sp. were mostly found in pool areas of the stream, with the eggs located at or above the surface of the water. *O. unicolor* displayed no preference for microhabitats (like riffles

or pools of the streams), and their eggs were mostly laid on damp surfaces of woody substrates emerging from the water. However, *N. rickeri* eggs were predominantly found in riffles (with high current velocity), where females deposited their eggs under water with the eggs attached to submerged parts of the stones (Hoffmann and Resh, 2003).

There are species-specific or habitat-specific variations in the female's preference for oviposition site i.e., laying eggs on either submerged or emergent rocks. Thus, Lancaster *et al.* (2010) suggested these differences could be influenced by the foraging behaviour of the potential predators of the eggs and newly hatched larvae, the adverse effect of siltation, the ability of the newly hatched to disperse, and the patterns of water movement around the substrate in which the eggs were attached. Unsuitable environmental conditions, e.g., drought and flood, are known to affect aquatic insect eggs, thus, to ensure the survival of their offspring, females should be under strong selection pressure to avoid exposing their eggs to such adverse conditions (Hoffmann and Resh, 2003).

These above-mentioned studies indicate that successful hatching of the eggs seems to be prioritized over the larval lifestyle, which is why females prefer suitable oviposition sites that will ensure hatching success and the survival of the newly hatched larvae over environment with adequate larval case construction materials. Caddisfly species displaced selective choices for their oviposition sites, which resulted in spatial distribution of eggs and newly hatched larvae. Hoffmann and Resh (2003) observed that these females of the three limnephiloid caddisflies (*Hydatophylax* sp., *Neophylax rickeri* and *Onocosmoecus unicolor*) made distinct choices while selecting their oviposition sites, and the selected microhabitats have balanced and quite predictable environmental conditions that are suitable for egg development and post-hatching survival. These studies suggest that the effect of the oviposition site selected on larval survival might vary between species. Some lay their eggs in a suitable larval habitat with appropriate case construction materials, while others lay their eggs far away from the larval typical habitat.

1.3.3.2: The Effect of Water Flow and Habitat Structure on Larval Case Construction Behaviour

Caddisfly larval case construction behaviour can be influenced by the habitats they inhabit and the rate of water flow in these habitats. A field survey conducted by Okano and Kikuchi (2012)

revealed that *Glossosoma* spp. larvae mostly inhabit areas with high velocity current like fast riffles. *Glossosoma* spp. larvae build dome-shaped cases with gaps to ensure passive respiration through water flow because they do not undulate their abdomen, and they do not have anterior and posterior openings for water passage. They suggested that the lack of this undulatory behaviour explains the high preference for areas with high current velocity because they need to obtain sufficient oxygen through fast-flowing water. The rate of larval mortality was high under lentic conditions, and this could be a result of insufficient oxygen. Also, larvae died more when the case perforations were closed in both lotic and lentic habitat. This result demonstrated that glossosomatid larval cases does not ensure respiration, rather their selected microhabitat (i.e., areas with fast-flowing water like riffles) supplies sufficient oxygen to ensure larval survival.

Studies have shown that some caddisfly larvae that inhabit lotic habitats with fast-flowing water build cases with lateral extensions to serve as ballast, hence ensuring their stability while moving in their natural habitat. Otto and Johansson (1995) observed that the *Silo pallipes* larval cases with ballast stones were more resistant to water currents than those without ballast stones. *Dicosmoecus gilvipes* larval cases with lateral extension regained their stability faster when dislodged than larval cases without the lateral extensions (Limm and Power, 2011). Larvae without lateral extensions serving as ballast rotated three times more than those with lateral extensions before regaining their stability when dislodged in high water currents. Otto (2000) also demonstrated that *Molanna angustata* larval cases with lateral extensions were more resistant to overturning than those without the lateral projections when the larvae were exposed to simulated wave actions. These results show that the lateral projections of larval cases help reduce the risk of being overturned or accidentally dislodged in lotic habitats.

These lateral projections also serve as a predator defence strategy in some species. Larval cases without ballast stones were attacked more by small bullhead (*Cottus gobio*) and brown trout than those with the lateral extension (Otto and Johansson, 1995), indicating that these extensions also serve as a defence strategy against predation. Similarly, the lateral extensions of *Molanna angustata* larval cases provided protective benefits against predation attack from perch (*Perca fluviatilis*). However, Limm and Power (2011) observed that larval cases of *Dicosmoecus gilvipes* provided protection against predatory attack from steelhead trout (*Oncorhynchus mykiss*) even when lateral extensions of the cases were absent. Steelhead trout did not eat any cased *D.*

gilvipes larva with or without lateral extension, but all caseless larvae were consumed. These results showed that larval projections in *D. gilvipes* larval cases could serve as ballast stones only, or as a predator defence strategy to other predators with a different foraging strategy than steelhead trout.

The extension of larval cases laterally using additional case construction materials is beneficial in maintaining balance and resisting currents in fast-flowing water by increasing their resistance to dislodgement. These above-mentioned studies have shown that water flow and habitat structure can influence larval case construction behaviour and distribution, with larvae building perforated cases that provide efficient respiration, or cases with lateral extensions that ensures larval resistance to dislodgement and prevents predation attacks in fast-flowing water.

1.3.3.3: The Selection of Microhabitats within Larval Habitat

Stream water flow is an essential tool responsible for the erosion and deposition of materials. Increase in the velocity of water flow results in the eroding and transportation of materials, which are later deposited as stream substrates. Fluctuations in water movement changes stream substrate over time, thus stream substrates vary greatly based on habitat patches (Skuja, 2011). Studies have described the relationship between many caddisfly larvae taxa and their environment, focusing on factors like available substrate (Okano *et al.*, 2012) and feeding behaviour (Wiggins, 2004). Caddisfly larvae are used in examining the distribution of benthic organisms based on stream substrates, because most larval species use these substrate materials for case construction (Cummins, 1964). Hence, a comprehensive analysis of larval case building behaviour can help understand the importance of the substrate materials used for case construction, because these substrates have been described to influence larval distribution in their habitat (Cummins, 1964).

Skuja (2011) observed three distinct groups of caddisfly larvae based on the microhabitats they inhabit, and these microhabitats differed in water current velocities and the sizes of the organic materials present in these environments, with the materials ranging from fine particulate organic matters (FPOM) to submerged macrophytes (plants). One of these three distinct larval groups inhabit microhabitats with FPOM, coarse particulate organic matters (CPOM) and akal (>2mm-2cm particle size), the other group of larvae inhabits microlithal, mesolithal and macrolithal

microhabitats (>2cm - >20cm particle size), while the last groups inhabit areas with macrophytes. Larvae prefer microhabitats containing great amount of their preferred food material (Skuja, 2011). This result is also in accordance with Urbanic *et al.* (2005) that observed that the microhabitat preferences of caddisfly larvae are influenced by their feeding behaviour.

Studies have shown that water current velocity, available food materials and substrate particles are essential components that determine the distribution of benthic invertebrates in microhabitats. For instance, *Glossosoma* spp. larvae have high preference for areas with high water currents like riffles because of their oxygen requirements; microhabitats with fast flowing water provides sufficient oxygen and ensures efficient respiration to this larval species (Okano and Kikuchi, 2012). Thus, it is important to consider all physical, chemical, and biological factors while predicting the distribution of benthic invertebrates (Cummins, 1964). When observing the behaviour and morphological traits of stream invertebrates, the effect of water flow (Okano and Kikuchi, 2012), and the resulting effect of these flow (e.g., the particles deposited and substrate materials) on the distribution of these organisms needs to be considered.

1.4: Caddisfly Larvae Case as a Predator Shelter

1.4.1: The Protective Function of Caddisfly Larvae Cases and Their Predator's Foraging Behaviour

Caddisfly larvae are exposed to various freshwater predators, including fish, aquatic birds such as ducks (e.g., blue duck, *Hymenolaimus malacorhynchos*) (Collier, 1991) and dippers (e.g., the Eurasian dipper, *Cinclus cinclus*) (Ormerod and Tyler, 1991), salamanders (Boyero *et al.*, 2006; Wissinger *et al.*, 2006), dragonfly naiads (Boyero *et al.*, 2006), beetles (Wissinger *et al.*, 2006), crayfish (Boyero, 2011) and other caddisfly larvae (Wissinger *et al.*, 2004a,b). Predators have developed multiple hunting strategies to deal with the protection cases of the caddisfly, for example, the sculpin (*Cottus gobio*) pull out the larvae from the cases and leave the cases intact while others such as brown trout (Johansson, 1991) and ducks (Wiggins, 2004) crush the larvae together with the case. Dragonfly nymphs either crush or puncture larval cases to consume the larvae, whereas salamanders separate the larvae from the case before feeding on them (Boyero *et al.*, 2006) or feed on both larvae and case when the cases are small and can be easily swallowed (Wissinger *et al.*, 2006). The Eurasian dippers also remove the larvae from their cases before

feeding on them, and this is done by repeatedly hitting the case against a rock to separate it from the larva (Tyler and Ormerod, 1994). Beetles (*Dytiscus dauricus*) attack caddisfly larvae by grasping the larval case and probing it with its mandibles to inject proteolytic enzymes that kill the larva (Wissinger *et al.*, 2006).

Caddisfly larval cases aid in avoiding predation by acting as a defensive mechanism (Boyero *et al.*, 2006; Ferry *et al.*, 2013; Johansson, 1991; Otto, 2000; Wiggins, 1977). These cases could also serve as a camouflage by matching their background (Otto and Svensson, 1980). For instance, *Potamophylax cingulatus* larvae with leaf cases experienced lower predation rate when on a leafy substrate than organic cases on a sandy substrate, indicating that larval cases serve as a camouflage (Otto and Svensson, 1980). However, *P. cingulatus* larvae with mineral cases on a sandy bottom and those with organic cases on a leafy bottom both experienced a similar predation rate from predatory brown trout (Otto and Svensson, 1980). Also, larvae with mineral cases were consumed less frequently by predatory brown trout than larvae with leaf cases when placed in an aquarium without underlying substrates; this indicates that mineral cases offer more protection than organic cases (Otto and Svensson, 1980) because mineral cases resist crushing better than organic cases (Boyero *et al.*, 2006; Johansson, 1991; Otto and Svensson, 1980). However, Ferry *et al.* (2013) found that larval cases offered protection against a predator, dragonfly nymph (*Anax junius*), irrespective of the material used in constructing their cases. Larval cases also reduce aggression between conspecifics and heterospecifics; it also prevents cannibalism and intraguild predation in species inhabiting temporary habitats with developmental time constraints such as ponds and wetlands (Wissinger *et al.*, 2004a) because larvae inhabiting temporary habitats need to supplement their plant diets with animal material (Wissinger *et al.*, 2004b). However, the ability of caddisfly larvae to escape predation does not depend only on their case construction and antipredator behaviour, but also on the adaptive foraging behaviour of the predator based on the larval case type (Johansson, 1991).

Larval cases greatly influence the behaviour of the larva, with rigid (Johansson, 1991), broad (Otto, 2000; Wissinger *et al.*, 2006), and longer cases protecting them against fish predators, even though longer cases makes them less mobile (Otto, 1987). Caddisfly larval cases are for protection against predators in addition to other functions like physical protection of larval soft bodies, and respiration; thus, the amount of energy used in case construction seems to be related

to their susceptibility to predation (Otto and Svensson, 1980). Hence, Okano *et al.* (2017) suggested that the assessment of caddisfly case construction behaviour may be easily used to estimate predation pressure on prey, making it a beneficial indicator to assess predator-prey relationships in organisms.

1.4.2: Caddisfly Larvae Response to Chemical Cues

Organisms interact with the environment using their sensory systems, and this interaction is essential for their survival and reproduction; sensory systems are used for foraging, communicating, detecting predators etc. (Stevens, 2013). Sensory systems are used to detect information from the environment through various sensory modalities (e.g., chemical, sound, light, electricity, magnetic, mechanical, and heat) (Stevens, 2013). These sensory systems are shaped by selection pressures through the information acquired from their environment, thus influencing the animal's response through the evolution of diverse behaviours (Stevens, 2013). The type of sensory system used by organisms is often associated with the organisms' habitat and their relationship with the environment (Stevens, 2013). For instance, aquatic organisms often specialize in detecting predation risk using chemical cues (Hettyey *et al.*, 2015).

Detecting risk often leads to changes in morphology and the initiation of behavioural responses (Dodson *et al.*, 1994). Caddisfly larvae can respond to chemical cues from predators (Boyero, 2011; Boyero *et al.*, 2006; Okano *et al.*, 2017) or chemicals from damaged conspecifics (Gall and Brodie Jr, 2009). These chemical cues affect larval case material selection (Boyero, 2011; Boyero *et al.*, 2006); For example, Boyero *et al.* (2006) demonstrated that caddisfly larvae (*Potamophylax latipennis*) exposed to chemical cues from different predators (dragonfly naiads, *Cordulegaster boltonii*, fire salamander larvae, *Salamandra salamandra*, and brown trout) were able to distinguish between these predators using predator odour, and these affected their case type selection. They observed that the foraging behaviour of the predator influenced the larval choice of case type. Chemical cues also affected larvae construction behaviour, with larvae constructing stronger and rigid cases when exposed to predator odour (Correa-Araneda *et al.*, 2017; Boyero, 2011; Okano *et al.*, 2017). For instance, *Perissoneura paradoxa* added more particles to their cases in the presence of white-spotted char (*Salvelinus leucomaenis*) odour than those exposed to river water (with no predator odour) only. Predator-related chemical cues can

also influence larval antipredator behaviour such as reduced activity and predator avoidance by retreating in their case (Gall and Brodie Jr, 2009; Malmqvist, 1992). Gall and Brodie Jr (2009) observed that *Hesperophylax occidentalis* larvae reduced their movement activity when exposed to predator odour and damage-released conspecific alarm cues. Similarly, *Agapetus ochripes* larvae reduced their movement activity when exposed to predatory stonefly (*Dinocras cephalotes*) and sculpin odour (Malmqvist, 1992). Thus, the type of information acquired from the environment using the sensory systems can be understood by studying the behaviour of the animals (Stevens, 2013).

1.5: Caddisfly Larval Case Construction Behaviour and Phylogeny

Caddisfly larval case construction methods and shapes are species-specific irrespective of the kind of material used (Copeland and Crowell, 1937; Otto and Svensson, 1980), and this similarity in larval case building behaviour can be used in identifying the larvae (Hickin, 1967; Wiggins, 1977, 2004). The similarities in case construction behaviour has made larval cases a means of identifying caddisflies at the family level (Otto, 2000) and usually also at the genera level (Wiggins, 2004). Although the case construction behaviour is similar, the methods of searching, handling, modifying, fitting, and attaching materials to the case varies among genera (Stuart and Currie, 2001). Therefore, a detailed understanding of this behavioural variation among caddisflies larvae genera can provide behavioural data that can be used for phylogenetic analyses of the case-making caddisflies (Stuart and Currie, 2001).

One of the ways in which behavioural information can be inferred from constructed structures is to examine these structures. According to Stuart and Currie (2002), the animal's movement during construction and the constructed structures (end-products) can be used to describe building behaviours; for instance, if similar structures are built using similar pattern, that could suggest behavioural homology. Stuart and Currie (2002) examined if the structures constructed by case-building caddisfly larvae are related to the larval construction behaviour (movements during construction), and if this information can be used to determine behavioural evolution. They observed that some larvae in the same family constructed different case structures using similar building behaviours (movements during construction); for example, *Oecetis* spp, larvae with tube-like mineral case, and *Triacnodes* spp, organic case builders, both in the family Leptoceridae, constructed different cases using similar behaviours. Also, some larvae

constructed similar case structures using different construction behaviours; for example, *Ceraclea* spp. (family Leptoceridae) constructed a case that resembles *Molanna* spp. (family Molannidae) case using a different building behaviour. Stuart and Currie (2002) concluded that the type of case constructed by caddisfly larvae and the case construction behaviour are sometimes not directly related because larvae of the same family with different case structures can exhibit similar case construction behaviour than those with similar case structures (Stuart and Currie, 2002).

Although case construction behaviour data are less useful in establishing the relationships among families, they can be used in classifying genera into families (Stuart and Currie, 2001). Case construction behaviour and material selection vary within species, and this variation can be influenced by innate ontogenetic differences (instar level) or the environment (predation risks and surrounding sediments). Thus, researchers should be cautious when using behavioural data, such as the case construction behaviour of animals and the constructed structures, to determine behavioural evolution (Stuart and Currie, 2002).

1.6: Research Objectives and Thesis Structure

My research focused on the case construction behaviour of caddisfly larvae and the role of risk (from damage-released conspecific alarm cues) and substrate material influencing this behaviour. Furthermore, I investigated the effectiveness of larval cases in protecting them against predatory attacks. Lastly, this study also examines if crayfish (predator) recognize empty caddisfly larval cases as a source of food.

The first experiment, presented in Chapter Two, examined the effect of damage-released conspecific alarm cues on *Limnephilus* spp. larval case construction behaviour. Larval case construction behaviour may be influenced by predator odour. However, the effect of damage-released conspecific alarm cues on larval case construction behaviour has not been documented. Thus, larval case construction behaviour and material selection in response to alarm cue exposure were examined. The second experiment, presented in Chapter Three, examined *Phryganea* spp. larval preference for case construction materials, the protective effect of the constructed cases, and the rate at which crayfish attack empty larval cases.

Chapter 2: The Role of Damage-Released Conspecific Alarm Cues on *Limnephilus* spp. (Trichoptera: Limnephilidae) Case Construction Behaviour

2.1: Abstract

The transportable case of a caddisfly larva is generally agreed to have evolved as a predator defence strategy. Caddisfly larvae use various materials present in their environment to construct mobile cases of different sizes and shapes using self-secreted silk to bind them together. Caddisfly larvae inhabit most freshwater habitats, and studies have shown that they can produce and perceive chemical cues that affect their case construction behaviour. Caddisfly larvae display both morphological changes and behavioural responses when exposed to predation risk. Prey can detect predators using chemical cues from either the predator, damaged conspecifics or known heterospecifics. These chemical cues affect case construction material selection and antipredator behaviour in caddisfly larvae. This study examined the case construction behaviour of caddisfly larvae, *Limnephilus* spp., in response to conspecific damage-released alarm cues. Exposure to alarm cues had no effect on the proportion of larvae that constructed cases when compared to those in the control group. Also, alarm cue had no effect on larval case length when compared to the cases constructed by the larvae in the control group. However, larvae in the alarm cue treatment group added more sticks to their cases after 24 hours of exposure than those in the control group. This study shows that *Limnephilus* spp. responds to alarm cue by adding more sticks to their cases to offer more protection, indicating that alarm cues affect case construction material selection in this species.

2.2: Introduction

Defence against predation is essential for the survival of an organism; thus, antipredator responses should reflect the intensity of the current predation risk (Lima and Dill, 1990). Although predation risk varies temporally, prey can detect and assess these variations, thus adjusting their decision-making process (Lima and Dill, 1990). These antipredator behaviours may occur in the form of morphological, behavioural (Boyero, 2011; Evans and Schmidt, 1990) and physiological defences (Hettyey *et al.*, 2015). Some antipredator behaviours combine morphological adaptations with the behavioural decision-making process (Lima and Dill, 1990).

Prey inhabiting terrestrial, marine and freshwater habitats can assess predation risks using predator-related chemical cues (Kats and Dill, 1998). Chemical cues that are released by aquatic predators or prey are used by prey to mitigate predation risk (Hettyey *et al.*, 2015; Wisenden, 2015). According to Hettyey *et al.* (2015), prey-borne cues can be classified based on the time of release as either pre-consumption prey-borne cues or post-consumption prey-borne cues. Pre-consumption prey-borne cues are released during stress (disturbance cues), and during attack or capture by a predator (damage-released alarm cues) while post-consumption prey-borne cues are released as constituents of digested prey (Hettyey *et al.*, 2015). Predator-borne cues can be classified into pre-consumption predator-borne cues, post-consumption predator-borne cues and continuously released predator-borne cues. Predator-borne cues are released in these forms: continually released (kairomones), after the consumption of prey (kairomones and digestion released cues), or without consuming a captured prey (e.g., saliva). Thus, when examining predator-prey interactions, it is important for researchers to know which cue is being detected by the prey when observing the anti-predator responses (Hettyey *et al.*, 2015).

The portable case of an actively foraging caddisfly larva is generally agreed to have evolved as a defence against predators (Wiggins, 2004). Studies have shown that predator-related chemical cues affect larval case material selection and construction behaviour. Larvae can distinguish between predators using chemical cues, and this ability to differentiate between predators can affect their choice of case type. For instance, caddisfly larvae (*Potamophylax latipennis*) are able to distinguish between predators (dragonfly naiads, fire salamander larvae, and brown trout) using chemical cues based on their experience with these predators, and this influences their choice of case type (Boyero *et al.*, 2006). Larvae exposed to dragonfly naiads and brown trout displayed a greater preference for mineral cases than organic cases when compared to those exposed to fire salamander larvae (Boyero *et al.*, 2006). Thus, they suggested that larval choice of case type could be explained by the foraging technique of these predators; dragonfly naiads and brown trout damage larval cases during consumption, hence the need for mineral cases which are more resistant to crushing. However, fire salamander larvae remove the larvae from the case before feeding on them, therefore both case types seem to offer similar protection against the predator. Larvae can also detect predators and adjust their case type accordingly (Boyero *et al.*, 2006). For example, *Sericostoma pyrenaicum* larvae constructed more defensive cases which

are longer, tougher and well-cemented when exposed to predator (brown trout) odour (Correa-Araneda *et al.*, 2017). Similarly, Boyero (2011) observed that larvae of *Calamoceras marsupus* responded to fish (*Squalius pyrenaicus*) and crayfish (*Procambarus clarkii*) odour by constructing more protective cases using sticks instead of leaves. In contrast, most larvae used leaves in the absence of predator odour. Okano *et al.* (2017) also found that larvae of *Perissoneura paradoxa* repaired their cases faster when exposed to white-spotted char odour, and they added more sand to enlarge their case compared to those in the control group.

Predator-related chemical cues do not only affect larval case construction behaviour, but they also influence larval antipredator behaviour. Boyero *et al.* (2006) observed that caddisfly larvae (*Potamophylax latipennis*) entered their cases faster when exposed to brown trout (a potential predator) odour, than those entering their case in the absence of predator odour. Similarly, caddisfly larvae (*Hesperophylax occidentalis*) increase the time to emerge from their case to move around when exposed to either alarm cues from conspecifics or predator odour (Gall and Brodie Jr, 2009). The above-mentioned studies have shown that caddisfly larvae are able to produce and perceive chemical cues such as alarm cues from conspecifics as well as predator odours, and these cues are used in detecting and avoiding predators. However, the effect of conspecific damage-released alarm cues on caddisfly larval case construction behaviour is still unknown.

This experiment examined the effect of damage-released alarm cues on caddisfly larvae case construction behaviour. Although the effect of alarm cues on larval case construction behaviour has not been documented, this stimulus has been reported to have elicited antipredator response (reduction in the latency to emerge and move) in caddisfly larvae (Gall and Brodie Jr, 2009). Predator-related chemical cues can also affect the rate at which larvae construct new cases. Boyero (2011) also observed that predator (fish, *Squalius pyrenaicus*, and crayfish, *Procambarus clarkii*) odour could affect the rate at which larvae construct new cases, and the nature of the predator can influence the protection level of these cases. Thus, to determine if damage-released conspecific alarm cues affect larvae case construction behaviour, the following questions were asked: a) Can exposure to alarm cue affect the proportion of larvae that construct new cases within the given time period? b) Will alarm cue affect larval case length? c) Will larvae exposed to alarm cue add more sticks to their cases than those in the control group?

2.3: Methods

2.3.1: Larvae Collection and Maintenance

The larvae used for this experiment were collected from the Waskesiu River, Saskatchewan (54°04'40.5"N, -105°56'22.2"W), in May 2019. The larvae were brought to the laboratory and maintained in a 73-L aquarium (62cm x 53.5cm x 22cm) with a mixture of their own river water and dechlorinated water (50:50) for them to acclimate. The tank water was fully changed to dechlorinated tap water after three days of acclimation. They were fed *ad libitum* with bloodworms before the experiment.

This larval species constructs cylindrical cases using sand grains, and they sometimes attach sticks of different diameters/widths and lengths to the case (Figure 2.1). They were identified as *Limnephilus* spp. using identification keys from Morse and Holzenthal (2008) and Wiggins and Currie (2008).

Smith (1984) collected 28 *Limnephilus* species in Saskatchewan, of which most species (16 species) were collected from the southern part of the northern boreal forest. Waskesiu river at Hwy. 2, which is the collection site of the species used in this experiment, was categorized as the southern part of the northern boreal forest. The sixteen species collected from the southern part of the northern boreal forest are *L. rhombicus*, *L. sansoni*, *L. sp. 1*, *L. extractus*, *L. infernalis*, *L. ornatus*, *L. externus*, *L. thorus*, *L. janus*, *L. secludens*, *L. hyalinus*, *L. parvulus*, *L. canadensis*, *L. nigriceps*, *L. argenteus* and *L. rossi*. Nine out of these sixteen *Limnephilus* species were collected from Waskesiu river at Hwy. 2, and they are *L. rhombicus*, *L. infernalis*, *L. ornatus*, *L. externus*, *L. thorus*, *L. janus*, *L. parvulus*, *L. canadensis* and *L. argenteus*.



Figure 2.1: *Limnephilus* sp. larva in its case (Photograph: Denis Meuthen)

2.3.2: Treatment Preparation

Alarm Cue Treatment: The caddisfly larvae used for the damaged-released alarm cues preparation were starved for three days prior to stimulus preparation to remove any food cues. The alarm cue was prepared by crushing 1g of caseless larvae (of varying instars) in 200mL of water. The solution was filtered to remove large solid particles while the filtrate was used as alarm cues. The filtrate was stored in plastic bags and frozen at -20 °C for subsequent use as the alarm cue stimulus. The frozen alarm cue was thawed at room temperature before use.

Control Treatment: Dechlorinated tap water, stored in plastic bags, were frozen at – 20 °C and thawed at room temperature before use.

The alarm cue and control treatments were stored in aliquots and were only thawed when needed.

2.3.3: Case Construction Behaviour

Larvae were removed from their cases by pushing them gently from the posterior end of their abdomen with soft-tipped forceps (Boyero, 2011; Correa-Araneda *et al.*, 2017). The larvae were weighed and placed individually into containers containing 150mL of dechlorinated water and case construction materials. These containers were placed in a water bath of 15⁰C. Larvae were well fed *ad libitum* for 24 hours before the experiment, and each container was aerated using air stones. Each larva was provided with half a tablespoon of sand grains and four sticks for case reconstruction. These sand grains and sticks were collected from their habitats, and the sand grains were sorted using a 4mm mesh size. Assessment of previous cases showed that they construct their cases with sand grains of 4mm or less, and they added an average of four sticks to their cases. Some of the sticks used for reconstruction were from their old cases. These sticks were washed to remove traces of silk (e.g. Boyero, 2011).

Each larva received 5mL of their respective treatment (dechlorinated water and alarm cue) at the beginning of the experiment after some acclimation (until the larvae begin to move around) and after 24 hours. The number of larvae that constructed new cases, their case length (in millimetres) and the number of sticks added to each case was recorded after 24 hours (before receiving the second treatment) and 48 hours (at the end of the experiment) of receiving their

respective treatment. In this experiment, the control group had a sample size of 18 larvae, while the experimental group had a sample size of 29 larvae.

2.3.4: Statistical Analysis

Larval mass: The data for larval mass was parametric for the control group (KS-test, $Z = 0.158$, $P = 0.200$), and non-parametric for the alarm cue treatment group (KS-test, $Z = 0.165$, $P = 0.042$). Thus, to determine if larvae of similar masses were used in the experiment, a Mann Whitney U test was performed to compare the mass of larvae in both treatment groups.

Proportion of case constructed: To determine whether the proportion of larvae that constructed new cases after 24 and 48 hours is dependent on the type of treatment (dechlorinated water or alarm cue) received, the number of constructed cases and the type of treatment they received were compared using a 2-way contingency table.

Case length: The data for larval case length was normally distributed (K-S test on each sample: $0.159 \leq Z \leq 0.177$, $0.164 \leq P \leq 0.200$), but it failed to meet the assumption for homoscedasticity (24 hours: $F_{1,15} = 8.95$, $P = 0.009$, 48 hours: $F_{1,15} = 17.707$, $P = 0.001$). A Welch's Approximate t' test ($\alpha = 0.05$, 2-tailed test) was used to determine if the type of treatment received affected larval case length after 24 and 48 hours of exposure to their respective treatment.

Number of sticks used: A Mann-Whitney U test was used to determine if the type of treatment received affected the number of sticks added to the cases after 24 and 48 hours of exposure to their respective treatment. A McNemar's test was also used to determine if larvae reduced the number of sticks added to their cases after the second exposure to their respective treatment.

Larvae that did not construct cases after 48 hours of exposure were excluded from the 'case length' and 'number of sticks' analyses. All analyses were performed using SPSS 23.0 (SPSS Inc. IBM).

2.4: Results

Larval mass: Larvae from the two treatment groups did not differ in mass ($Z = -0.547$, $P = 0.584$).

Proportion of cases constructed: Few larvae constructed new cases in both treatment groups. The results indicated that 27.7% (11 out of 47) and 36.2% (17 out of 47) of the larvae in both treatment groups constructed cases after 24 and 48 hours, respectively.

The type of treatment received by the larvae did not affect the number of larvae that constructed new cases after 24 hours ($\chi^2_1 = 0.469$, $P = 0.493$) (Figure 2.2) and 48 hours ($\chi^2_1 = 0.102$, $P = 0.750$) (Figure 2.2). This indicated that there was no difference between the number of larvae that constructed new cases in the alarm cue group and the control group after 24 and 48 hours of exposure to their respective treatments.

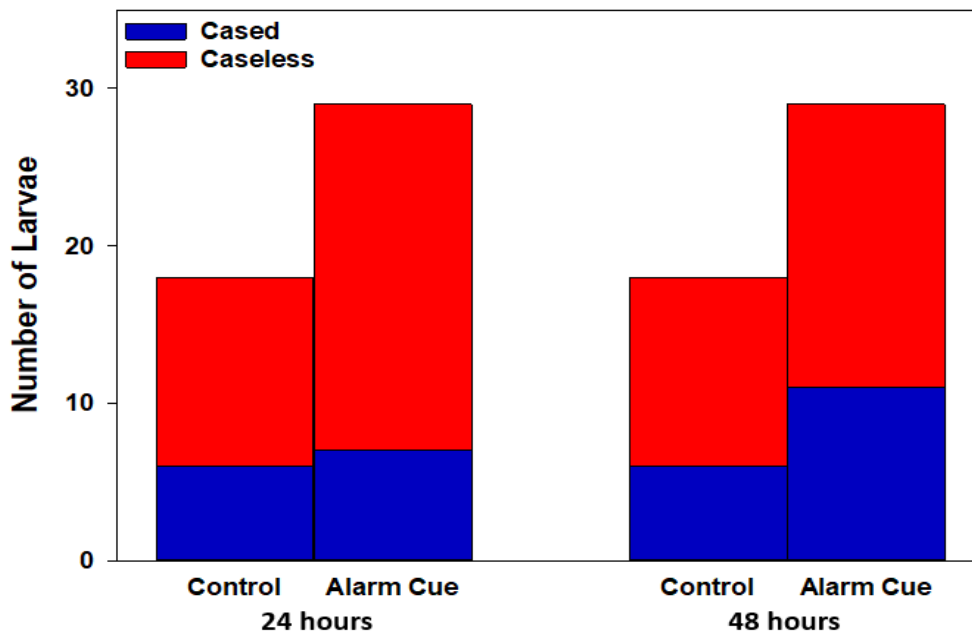


Figure 2.2: Number of cased and caseless larvae in both treatment groups after 24 hours and 48 hours of exposure to their respective treatment (dechlorinated tap water or alarm cue)

Case length: The treatment type did not have an effect on larval case length after 24 hours ($t'_{10.47} = -0.346$, $P = 0.736$; control: $n = 6$, alarm cue: $n = 7$) and 48 hours ($t'_{12.13} = -0.672$, $P = 0.514$; control: $n = 6$, alarm cue: $n = 11$) of exposure to their respective treatments. This indicates that the case length of larvae exposed to alarm cues did not differ from those in the control group.

Number of sticks used: The number of sticks added to the constructed cases after 24 hours of exposure differed in both treatment groups ($Z = -3.210$, $P < 0.001$; control: $n = 6$, alarm cue: $n = 7$) (Figure 2.3), indicating that larvae exposed to alarm cues added more sticks to their cases than those in the control group after 24 hours of exposure. However, the type of treatment received by the larvae did not affect the number of sticks added to their cases after 48 hours ($Z = -1.172$, $P = 0.241$; control: $n = 6$, alarm cue: $n = 11$)

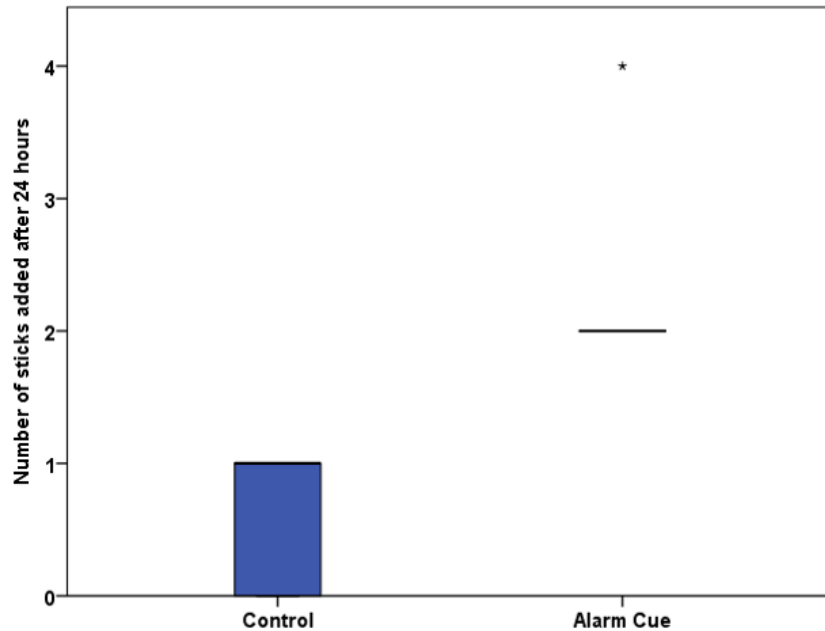


Figure 2.3: Box plot of the number of sticks added to larval cases constructed in the control and alarm cue treatment group after 24 hours of exposure to their respective treatment. The asterisk indicates a datapoint standing more than 3 IQR respectively (interquartile range) away from the median.

There was no difference between the number of sticks added to larval cases after 24 and 48 hours of exposure to their respective treatment (McNemar's test: $\chi^2_1 = 2.778$, $0.05 \leq P \leq 0.10$), indicating that larvae did not reduce the number of sticks added to their cases after the second exposure to their respective treatment.

2.5: Discussion

An exceptional characteristic of caddisfly larvae is the ability to elicit both morphological changes and behavioural responses within a short period of time when exposed to predation risk (Boyero, 2011). Unlike other caddisfly larvae species (e.g., *Phryganea* spp.) that construct cases faster (Chapter Three), the species used for this experiment did not construct new cases faster. Also, this species did not abandon their cases when disturbed when compared to other species, e.g., family Phryganeidae (Wiggins, 2004); this unwillingness to abandon their cases could be the reason why the larvae did not reconstruct new cases faster when forced out of their old cases.

The cost of case construction could also affect case construction behaviour. Mineral cases (cases constructed with stones, sand grains, small rocks, etc.) are more energetically costly to construct (Otto and Svensson, 1980). Also, cases constructed with sticks require more silk to bind particles together, thus increasing the energetic cost of case construction (Boyero, 2011). The species used in this experiment construct their cases using sand grains with sticks attached to them, indicating that case construction in this species is more costly because the materials (sand grains and sticks) used in the construction requires more silk to bind them together. This could also explain why they did not abandon their cases easily and why they did not construct new cases faster like the members of the family Phryganeidae (e.g., the species used for Chapter Three experiment) that construct their cases with leaves.

Subsequent experiments (Chapter Three) using another larval species (*Phryganea* spp.) shows that the quantity of materials available for case construction also influences this behaviour. Thus, case construction materials should be abundant enough to cover the entire bottom of the plastic container to influence case construction behaviour. Similarly, Copeland and Crowell (1937) observed that case construction is often difficult and unsuccessful in *Molanna* spp. larvae (a species that constructs its cases using sand grains) when there is an insufficient amount of sand.

Hanna (1960) also demonstrated that species that construct their cases with sand grains often burrow into the sand for case construction. It could also be possible that the case construction materials provided in this experiment are not enough to enhance case construction behaviour, hence the reason why fewer number of larvae constructed new cases. Also, each plastic container was aerated, and larvae were sometimes found attaching themselves to air stones instead of constructing new cases. Since caddisfly larval cases have also been proposed to aid respiration (Wiggins, 2004; Williams *et al.*, 1987), efficient oxygen from air stones could possibly reduce the number of larvae constructing new cases, or serve as a distraction.

Although the alarm cue had no effect on larval case length, it does affect the number of sticks attached to the constructed cases after 24 hours of exposure to the treatment. According to Boyero (2011), the predator's foraging mode determines the effectiveness of the larval case as a defence against predation, because a case that provides adequate protection against a vertebrate predator can be susceptible to a predation attack from an invertebrate predator with a different hunting behaviour (Johansson, 1991). Hence, the predator defence strategy should be appropriate for the foraging mode of the predator inhabiting the same habitat as the larva (Johansson, 1991). For example, *Calamoceras marsupus* larvae constructed more protective cases using sticks instead of leaves when exposed to crayfish than when exposed to fish. This outcome could be because crayfish are likely to be more efficient in feeding on leaf-litter invertebrates compared to fish, which inhabit water columns and feed on drifting invertebrates (Boyero, 2011). Broad (Otto, 2000; Otto and Johansson, 1995; Wissinger *et al.*, 2006) and rigid cases help caddisfly larvae escape predators (Johansson, 1991; Otto and Svensson, 1980). Otto and Johansson (1995) observed that *Silo pallipes* larvae with ballast stones on the lateral sides of their cases experienced lower predation attack from small bullhead and brown trout because these cases make the prey look too large for the predators to attack. Similarly, *Molanna angustata* larvae that constructed broad cases with lateral extensions suffered fewer predation attacks from perch (Otto, 2000). Perch prefer larvae with reduced cases because the lateral extensions of larvae cases make them look too large for the predator to handle (Otto, 2000). Johansson (1991) also observed that the survival rate of larvae attacked by sculpin and brown trout depends on the rigidity of the larval case. Since alarm cue is an indication of predation risk, this study showed that *Limnephilus* spp. larvae responded to predation risk by adding more sticks to their cases to

make it broad and rigid. Thus, the result of this experiment suggests that the addition of more sticks to larval cases is a predator defence strategy in this species.

There was no difference between the proportion of larvae that constructed cases in our treatment groups, suggesting that alarm cue does not influence the proportion of larvae that constructed new cases in *Limnephilus* spp. Boyero (2011) found that all the larvae provided with sticks and leaves constructed new cases, regardless of predator cues. However, the type of predator cues received affected the case construction material used by the larvae; larvae exposed to predator odour mostly constructed new cases using sticks, while those without predator stimuli used leaves most times. Case construction is energetically costly, especially the secretion of silk to attach materials together and foraging with the case. Although cases constructed with sticks require more energy and silk to bind them together, these cases are more efficient in preventing predatory attacks (Boyero, 2011). This factor explains why larvae in the control group added fewer sticks to their cases after 24 hours of case construction to reduce the cost of construction. Thus, alarm cue affects the case construction behaviour of this species by influencing case material selection.

Chapter 3: The Effectiveness of Larval Cases in Protecting *Phryganea* spp. (Trichoptera: Phryganeidae) Larvae Against Predatory Crayfish

3.1: Abstract

Defence against predation is an essential component for prey survival. In addition to physical protection, the portable case constructed by caddisfly larvae using various materials present in the environment is also agreed to have evolved as a means of avoiding predator attacks through camouflage. Studies have shown that larval preference for case construction materials can be influenced by predation risk, larval instar, or the surrounding substrate. In this experiment, *Phryganea* spp. larval preference for different case construction materials was examined based on their surrounding substrate materials. The experiment also investigated which material provided more protection when the larvae were exposed to predatory crayfish. As an addition to this study, larval recognition by predatory crayfish using empty larval cases was examined. Larvae were divided into three treatment groups, and each group was provided with either leaves, plastics, or the combination of both materials for case construction, after which they were exposed to crayfish to determine which case offers more protection against predation. The crayfish were later provided with empty larval cases to determine if the crayfish would recognize the cases as a source of food. The result of the experiment showed that *Phryganea* spp. larvae constructed new cases using the materials provided, and these materials did not affect the proportion of larvae that constructed new cases in each group. However, larvae provided with the mixture of leaves and plastics preferred to construct their cases using their native case construction material (leaves). Also, larval survival during predator encounters was not dependent on the case construction material; rather, it was dependent on whether the crayfish had previous experience with larvae. When crayfish were provided with empty *Phryganea* spp. larval cases, only those that had previously eaten caddisfly larvae attacked the empty cases. Although larval cases have been proposed to serve as a protection to caddisfly larvae, the protective effect of the cases constructed in this experiment could not be determined because most of the crayfish were naïve predators of case-building caddisfly larvae.

3.2: Introduction

The case construction behaviour of caddisfly larvae varies widely among different species (Hanna, 1960). Many caddisfly larvae species use different materials present in their environment to construct transportable cases using self-secreted silk to hold them together. Examples of the materials used in case construction are sand grains, larger rock fragments, twigs, leaves, the bark of trees, mollusc shells, and even cases of other caddisfly larvae. For instance, *Potamophylax* spp. (Limnephilidae) larvae build their cases from the empty cases of other caddisfly larvae (tube-like cases of Sericostomatidae) (Boyero and Barnard, 2004). Despite the variation in the case construction behaviour, the portable case of caddisfly larvae is generally agreed to have evolved as a protective device (Wiggins, 2004).

It has been hypothesized that the tubular case of a caddisfly larva has two functions, which are protection and respiration. Tubular cases of caddisfly larvae serve as camouflage by matching with their background and resisting crushing from predators (Williams *et al.*, 1987).

Trichopterans use their silk for different purposes (Williams *et al.*, 1987). Okano and Kikuchi (2009) suggested that the silk used for case construction by caddisfly larvae is used in two ways; (i) to join case construction materials and maintain an intact case, and (ii) to coat the inner walls of the constructed case. They observed that *Goera japonica* larvae secrete two kinds of silk; filamentous silk used in lining the inner surface of the case, and non-filamentous silk used in binding case construction materials and covering the gaps among the materials. Many case-building caddisfly larvae line the inner surface of their cases with self-secreted silk to prevent friction while undulating their abdomen (Okano and Kikuchi, 2009; Williams and Penak, 1980). However, larvae in the family Odontoceridae only use their silk in binding case construction materials rather than lining the interior surface of their cylindrical cases (Okano *et al.*, 2010; Wiggins, 2004). Similarly, *Glossosoma ussuricum* (family Glossosomatidae) larvae construct a dome-shaped case, but barely line the inner walls of their cases because they do not undulate their abdomen (Okano *et al.*, 2010). Williams *et al.* (1987) observed that the larval case aids respiration in some species, although it does not affect respiration in other case-building species, especially the Limnephilidae. Thus, larval cases could probably serve as camouflage or physical protection only in species where the cases do not aid respiration (Williams *et al.*, 1987). These studies suggest that larval case construction behaviour, and the structure and function of these

cases can be influenced by the information detected from their environment (e.g., surface texture of the surrounding sediment) using various sensory modalities.

Animals use various sensory modalities to detect information from their environment, and this information influences their decision-making process (Stevens, 2013). These sensory systems, which are mostly related to the ecology of the species, are used to locate food, detect predatory threats, communicate, navigate etc. (Stevens, 2013). The most suitable sensory modalities used by animals are determined by the ecology of these animals, their activities, and the environment they inhabit. Some examples of the sensory modalities used by animals are chemical, sound, light, electricity, magnetic, mechanical, and heat (Stevens, 2013). Some of these modalities are more useful in some environments than the other; for instance, electroreception is only used by aquatic organisms, because air is a poor conductor of electricity (Stevens, 2013). Non-visual signals released into the aquatic habitat in the form of chemicals or vibrations are essential for animals without image-forming eyes (e.g., some insect larvae, molluscs, and zooplankton) or those that inhabit dark or murky environments (Dodson *et al.*, 1994). Chemoreception is possibly the most used animal sense (Stevens, 2013) because chemicals persist longer in the environment than other information sources (Dodson *et al.*, 1994).

Predators use chemical cue to detect prey, and this influences the activity of predators by enhancing their success in locating and identifying prey (Dodson *et al.*, 1994). In cases where visual detection of food is impaired due to insufficient light, chemical cues can be useful in detecting prey while foraging (Rebach, 1996). Rock crabs, *Cancer irroratus*, can detect prey odours, and these odours can be used to locate and identify their prey. Rebach (1996) observed that visual cues are not enough to stimulate the predatory attack, rather the prey odour from mussels (prey) stimulates feeding activity in rock crabs.

Prey inhabiting various habitats also use predator-related chemical cues to detect and assess predation risk (Kats and Dill, 1998), and this ability to perceive these predation risks help prey to reduce their likelihood of consumption (Dodson *et al.*, 1994). Several studies have described how prey respond to predator-related cues, and this has been observed in caddisfly larvae case construction behaviour (Boyero, 2011; Boyero *et al.*, 2006; Okano *et al.*, 2017) and antipredator behaviour (Gall and Brodie Jr, 2009). Caddisfly larvae are selective when choosing case

construction materials, and their preference for the materials can be influenced by predation risk (Boyero, 2011; Boyero *et al.*, 2006).

Larva instar level also influences case construction behaviour and materials selection (Otto and Svensson, 1980; Prestidge, 1977). Some caddisfly larvae change the materials used in case construction as they grow older. For instance, *Potamophylax cingulatus* construct their cases with leaf materials during young instars, and later use pieces of bark, and then switch to mineral cases as they grow to reduce the risk of predation (Otto and Svensson, 1980). Cases constructed with mineral materials (e.g., stones, sand grains, small rocks, etc.) are costly and require more silk than those constructed with organic materials (e.g., leaves). They are also heavy and serve as a burden to the carrier, making it difficult for larvae to forage (Otto and Svensson, 1980). Thus, early instar larvae could reduce the cost of case construction by using materials that require less silk (e.g., leaves), thereby reserving more energy for growth (Otto and Svensson, 1980). Gaino *et al.* (2002) also observed that case material selection was determined by the instar level of the larva; early instars of *Sericostoma pademontanum* larvae were more selective while picking case construction materials than late instars (Gaino *et al.*, 2002). Early instar larvae are more active, and they build faster than inactive late instars. Also, Hanna (1960) demonstrated that larvae in their pre-pupal stage did not construct new cases when removed from their old case, thus suggesting that larvae tend to lose the urge to construct new cases as they grow older.

Since caddisfly larvae construct cases using materials available in their environment, their preference for the case construction materials is not only influenced by predation risk and instar level, but also by their surrounding substrate (Okano *et al.*, 2011). For example, *Pseudostenophylax edwardsi* larvae showed some level of flexibility when constructing their cases in the laboratory by making use of sand provided to construct a normal-shaped solid case, although they use small stones while in their natural environment (Anderson, 1974). However, in the absence of case construction materials (small stones), some *P. edwardsi* larvae fed with filamentous algae constructed cases from algal tubes (Anderson, 1974). *Sericostoma pademontanum* larvae largely prefer travertine grains because they naturally build their cases with travertine grains found in their natural habitat. However, they constructed their cases with quartzite (unfamiliar) grains when these were the only grains provided (Gaino *et al.*, 2002).

The surface texture of the case construction materials available in the environment also alters case-building behaviour (Okano and Kikuchi, 2009). *Goera japonica* larvae strongly prefer smooth particles to rough ones, and these help them reduce the quantity of silk to be secreted in lining the inner surface of their case. Furthermore, *Psilotreta kisoensis* and *Perissoneura paradoxa* larvae preferred smoother particles, even more than *G. japonica* (Okano *et al.*, 2010). As members of the Odontoceridae, both *P. kisoensis* and *P. paradoxa* do not line the inner walls of their cases with silk; hence their high preference for smooth particles ensures efficient respiration by preventing friction while undulating their abdomen (Okano *et al.*, 2010). These studies have shown that the surrounding materials alter larval preference for construction materials, and the urge to construct cases in caddisfly larvae thus seems to prevail over the nature of the construction materials available in their environment.

This experiment examined *Phryganea* spp. larval preferences for case construction materials and the effectiveness of these cases in protecting caddisfly larvae. Although larvae prefer their native case construction materials, it is unknown if cases constructed with unfamiliar materials will offer more protection through camouflage than those constructed with native case construction materials. Since larvae will construct cases with the materials available in their environment, to determine if case construction material type affects larval case construction behaviour and survival, the following questions were asked: a) Will case construction materials affect *Phryganea* spp. larval case construction behaviour (rate of case construction and material preferences)? b) Will non-native case construction material offer more protection against predatory attack? Also, crayfish were provided with an empty larval case to determine if they can recognize larval cases as a source of food.

3.3: Methods

3.3.1: Larvae Collection and Maintenance

The larvae used for this experiment were collected from Bittern Creek, Saskatchewan (53° 52' 34.5" N, 105° 55' 42.8" W), in October 2019. They were brought to the laboratory and maintained in a 73-L aquarium (62 x 53.5 x 22 cm) with a mixture of the creek water and dechlorinated water (50:50) for them to acclimate. The tank water was fully changed to dechlorinated tap water after

three days of acclimation. They were fed *ad libitum* with bloodworms before the experiment. The larval species used in this experiment construct cylindrical cases using plant materials (brown leaves) (Figure 3.1). They were identified as *Phryganea* spp. using identification keys from Morse and Holzenthal (2008) and Wiggins and Currie (2008).



Figure 3.1: A larval case constructed by *Phryganea* sp. in its natural environment.

3.3.2: Crayfish Collection and Maintenance

The crayfish (*Faxonius virilis*) used for this experiment were collected from the South Saskatchewan River near Clarkboro Ferry, Saskatchewan (52° 19' 14.0" N, 106° 27' 27.5" W) in May and October 2019. They were brought to the laboratory and maintained in a 73L aquarium (62 x 53.5 x 22cm) with a mixture of river water and dechlorinated water (50:50) for them to acclimate. The tank water was fully changed to dechlorinated tap water the next day, and the crayfish were fed *ad libitum* with bloodworms before the experiment. However, they were not fed for at least 24 hours before they were used for the survival experiment.

3.3.3: Experimental Design

Larvae were divided into three treatment groups: a 'leaf-only' control group, a 'leaf-plastic' group and a 'plastic-only' group. Larvae in the 'leaf-only' group were provided with approximately a handful of leaves (unidentified, native case construction material) (Figure 3.2a) collected from their natural habitat. Larvae in the 'leaf-plastic' group were provided with a similar amount of leaves as the control group (Figure 3.2a) and a half tablespoon of brown plastic cable ties (Figure 3.2b) for case construction. The 'plastic-only' group was provided with

a half tablespoon of small pieces of brown plastic cable ties (Figure 3.2b). Brown cable ties between 6 – 8mm long (Figure 3.2c) were used because larvae construct their cases using brown leaves. Cable ties are made from nylon material, so they sink in water. These cable ties were soaked in water for at least 24 hours before using them for the experiment.

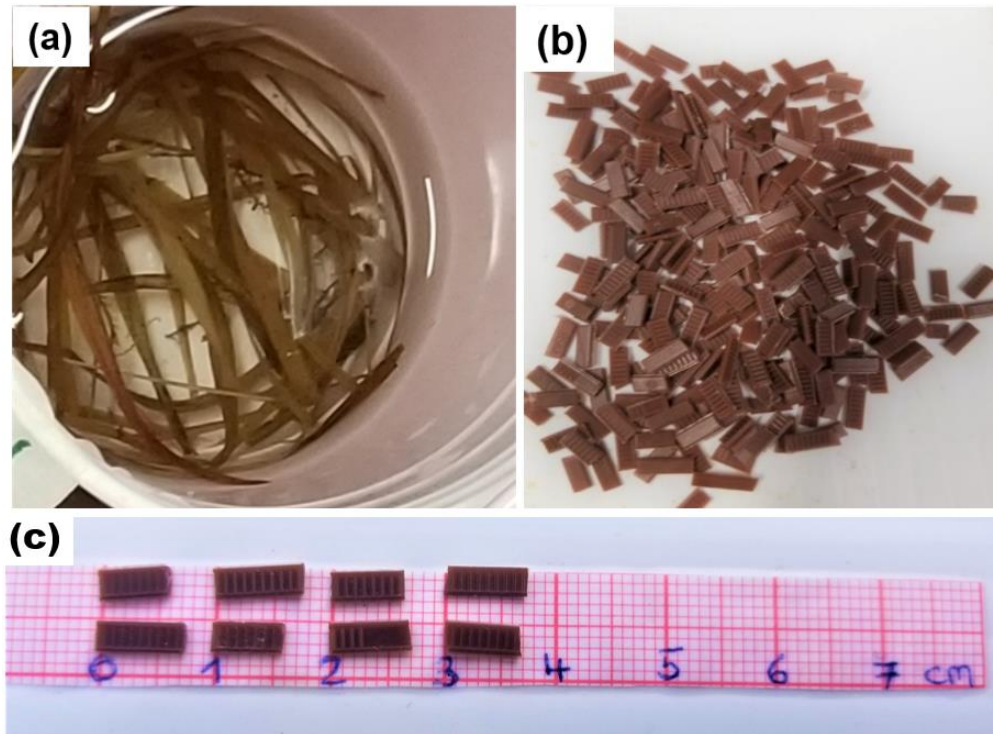


Figure 3.2: (a) Typical quantity of leaves provided for case reconstruction, depicted in a 0.5 L plastic cup, (b) Pieces of cable ties provided for case construction, and (c) Pieces of cable ties on a centimeter scale.

3.3.3.1: Experiment 1: Case Construction Behaviour

Larvae were removed from their cases by pushing them gently from the posterior end of their abdomen with soft-tipped forceps (Boyero, 2011; Correa-Araneda *et al.*, 2017, Gaino *et al.*, 2002). They were placed individually into plastic cups (473 mL; upper diameter: 97mm, lower diameter: 60mm, height: 118mm) containing case construction materials and 150 mL of dechlorinated tap water. These containers were placed in a water bath of 5°C. A preliminary experiment showed that larvae would only construct cases if the construction material is covering the entire bottom of the container. Thus, larvae in each experimental group were

provided with enough case construction materials to cover the entire bottom of their container. Larvae were allowed to construct their new cases for 72 hours before exposing them to crayfish. After 72 hours of case construction, the larvae were exposed to predators (crayfish) for one hour to determine which case offers more protection against predator's attacks. Twenty crayfish were used as predators for the survival experiment, and each crayfish was used three times, except if the larva assigned to the crayfish was dead. To block for experience effect, each crayfish only received one larva from each treatment group, which was randomly assigned (Table 1). These crayfish were identified with numbers from 1 – 20. Six out of these twenty crayfish were previously fed with another species of caddisfly larvae (Figure 2.1) six months before this experiment, although these caddisfly larvae construct their case using sand grains and sticks. However, the remaining fourteen crayfish were just collected from a habitat without case-building caddisfly larvae. Crayfish were placed into containers containing 320 mL of dechlorinated tap water, after which they received their respective larvae assigned to them.

Table 3.1: Table showing the randomization of larvae (*Phryganea* spp.) exposure to crayfish (*Faxonius virilis*). Replicate 1, 2, and 3 represent the first, the second and third time a crayfish was used, respectively. L – Leaves, P – Plastics, LP – a mixture of leaves and plastics, Rep. – replicate

Crayfish	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Rep. 1	P	LP	L	LP	L	P	L	P	LP	P	LP	L	LP	L	P	L	P	LP	P	LP
Rep. 2	LP	L	P	L	P	LP	P	LP	L	LP	L	P	L	P	LP	P	LP	L	LP	L
Rep. 3	L	P	LP	P	LP	L	LP	L	P	L	P	LP	P	LP	L	LP	L	P	L	P

3.3.3.2: Experiment 2: Do Crayfish Recognize the Casing as a Source of Food?

Crayfish were provided with empty larval cases built with leaves only (Figure 3.1) to determine if they will attack the cases or not. These empty cases were the old larval cases from Experiment 1 above. Larvae have been removed from these cases for at least three days before presenting these empty cases to the crayfish. The twenty crayfish used in the experiment above were reused for this experiment, and each predator was provided with an empty larval case for one hour. These crayfish had already been exposed to caddisfly larvae at least two times before this

experiment, and five out of these twenty crayfish already ate at least one larva during previous exposures.

The mass of each larva was measured before the experiment. Larval case lengths were also measured in millimetres after every 24 hours for three days. The number of plastics added to each case in the 'leaf-plastic' group was also counted after measuring case lengths every 24 hours. The status of the crayfish (experienced or naïve) was recorded. Larvae survival (i.e., survived or dead) was recorded after 1 hour of exposure to crayfish. The number of attacked empty larval cases was also recorded for the second experiment.

3.3.4: Statistical Analysis

3.3.4.1: Experiment 1: Case Construction Behaviour

Larval mass: The data for larval mass met assumptions for normality for the 'leaf-only' group (K-S test: $Z = 0.175$, $P = 0.150$), and the 'leaf-plastic' group (K-S test: $Z = 0.146$, $P = 0.200$). However, the data for larval mass in the 'plastic-only' group is non-parametric (K-S test: $Z = 0.232$, $P = 0.028$). Thus, a Kruskal-Wallis test ($\alpha = 0.05$, 2-tailed test) was performed to determine if larvae of similar sizes were used across the three treatment groups.

Rate of case construction: To determine if the type of material provided for case construction affected larval case construction behaviour (i.e., the rate of case construction), a 2-way contingency table was used to compare the number of larvae that constructed new cases in each group after 72 hours of construction and the type of case construction material provided.

Case length: The data for the length of larval cases was not normally distributed (KS test on each sample; $0.121 \leq Z \leq 0.264$, $0.002 \leq P \leq 0.200$). Thus, a Kruskal-Wallis test ($\alpha = 0.05$, 2-tailed test) was performed to compare larval case length across the groups after 24 hours, 48 hours and 72 hours of case construction. Due to the significant difference in larval case length among different groups after 48 and 72 hours of construction, post-hoc tests were performed using Mann-Whitney U tests to determine these differences. Post-hoc test was carried out by hand, the α level was corrected following a Bonferroni correction whereby $\alpha' = \alpha / 3$ (#/comparisons) = 0.0167. P^* denotes P-values that were compared to α' .

Percentage of plastic particles used: The percentage of larvae that added at least one plastic piece to their case after 72 hours of construction was recorded to determine larval preference for case construction material in the group provided with the mixture of leaves and plastic.

Survival: A 2-way contingency table was used to compare larval survival with their case construction material type to determine if the type of material provided affects larval survival when exposed to predators. Due to the varying experience of the predators, the crayfish status (experience) and larval survival were also compared using a 2-way contingency table to test whether the survival of the larvae depends on crayfish experience.

Larvae that did not construct new cases after 72 hours of case construction were excluded from the ‘case length’, ‘percentage of plastics used’ and ‘survival’ analysis.

3.3.4.2: Experiment 2: Do Crayfish Recognize the Casing as a Source of Food?

Frequency of attacks on cases: To determine if crayfish recognized larval cases as a source of food, a 2-way contingency table was used to compare crayfish experience (those that ate at least one larva in the Experiment 1) with the number of attacked cases.

All analyses were performed using SPSS 23.0 (SPSS Inc. IBM).

3.4: Results

3.4.1: Experiment 1: Case Construction Behaviour

Larval mass: Larvae of similar sizes were used for this experiment; there was no difference in larval mass between the treatment groups (Kruskal – Wallis test: $\chi^2_2 = 2.09$, $P = 0.352$).

Rate of case construction: The number of larvae that constructed cases in each group was not dependent on the type of case construction material provided ($\chi^2_2 = 1.394$; $P = 0.498$), indicating that case construction material did not affect the number of larvae that constructed and those that did not construct new cases in each treatment group after 72 hours (Figure 3.3).

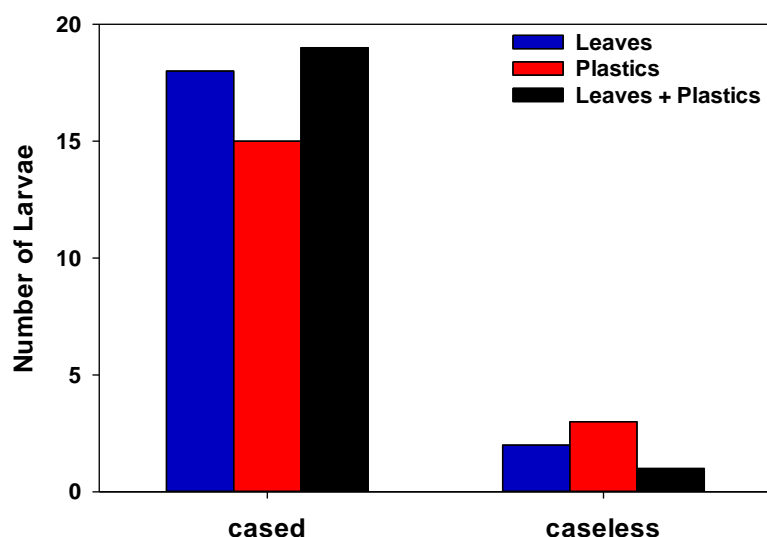


Figure 3.3: Count of larvae that constructed and those that did not construct new cases in each treatment group after 72 hours (n= 20/group except the ‘plastic-only’ group where n = 18)

Case length: There was no difference in larval case length constructed after 24 hours when the three groups were compared (Kruskal Wallis test: $\chi^2_2 = 1.947$, $P = 0.378$). However, the length of larval cases constructed after 48 hours (Kruskal Wallis test: $\chi^2_2 = 8.947$, $P = 0.011$) and 72 hours (Kruskal Wallis test: $\chi^2_2 = 15.074$, $P = 0.001$) differ among groups. Larvae provided with leaves alone constructed longer cases than those provided with plastics alone after 48 hours of case construction ($Z = -2.375$, $P^* = 0.016$). Similarly, larvae provided with a mixture of leaves and plastics also constructed longer cases than those provided with plastics alone after 48 hours of case construction ($Z = -2.730$, $P^* = 0.006$). However, there was no difference between the case length of larvae provided with leaves alone and those provided with a mixture of leaves and plastics after 48 hours of case construction ($Z = -0.807$, $P^* = 0.420$) (Figure 3.4).

Larvae in the ‘leaf-only’ group constructed longer cases than those in the ‘plastic-only’ after 72 hours of case construction ($Z = -3.048$, $P^* = 0.002$). Similarly, larvae in the ‘leaf-plastic’ group also constructed longer cases than those in the ‘plastic-only’ group after 72 hours of case construction ($Z = -3.527$, $P^* < 0.001$). However, there was no difference between the case length of larvae in the ‘leaf-only’ group and those in the ‘leaf-plastic’ group after 72 hours of case construction ($Z = -1.203$, $P^* = 0.229$) (Figure 3.4).

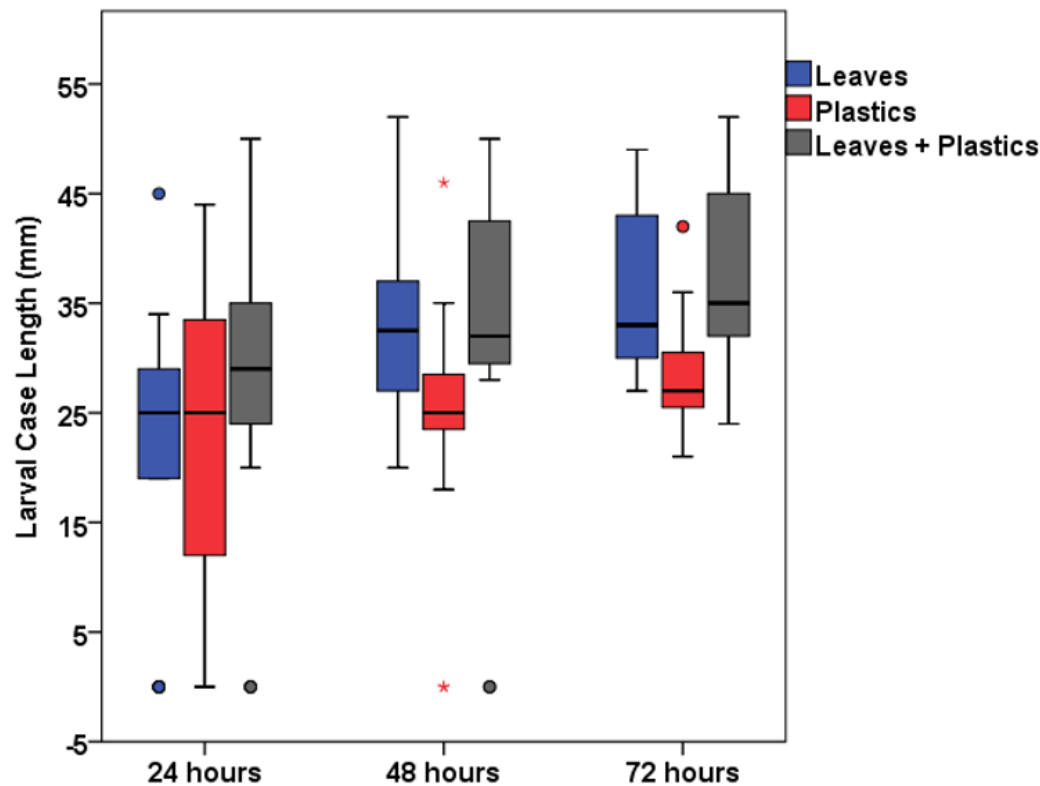


Figure 3.4: Box plot of the length of cases constructed with leaves alone, plastics alone, and a mixture of leaves and plastics after 24, 48 and 72 hours of case construction, showing the 1st quartile, median, 3rd quartile, minimum and maximum value. The circles and asterisks indicate datapoints standing more than 1.5 and 3 IQR, respectively (interquartile range) away from the median.

Percentage of plastic particles used: The result of the experiment also showed that only 16% of the larvae that constructed new cases after 72 hours of construction in the ‘leaf-plastic’ treatment group added at least one plastic material to their case (Figure 3.5), indicating that larvae prefer their native case construction material (leaves).

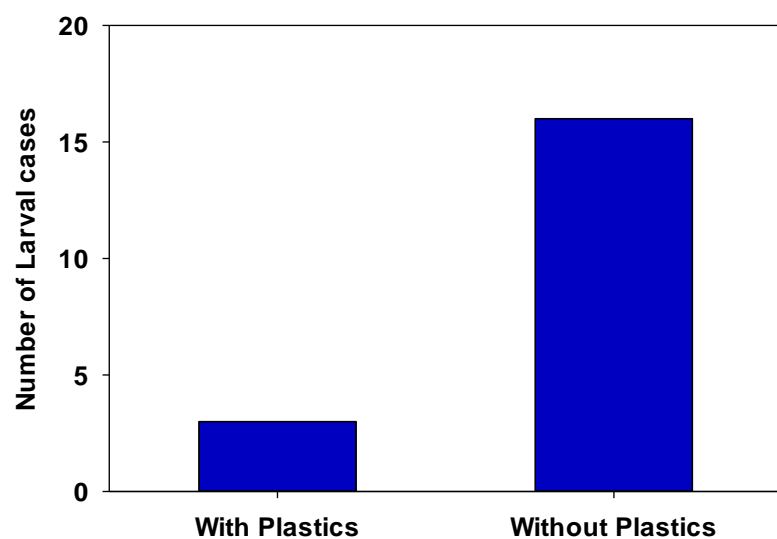


Figure 3.5: The number of larval cases in the 'leaf-plastic' group with and without plastic after 72 hours of case construction

Survival: Larval case construction materials did not affect larval survival when exposed to predators ($\chi^2_2 = 0.357$; $P = 0.837$) (Figure 3.6).

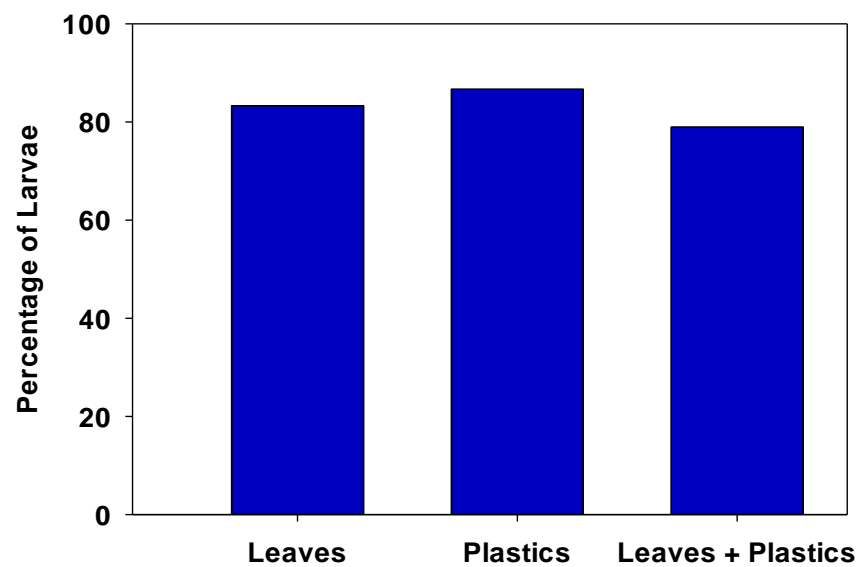


Figure 3.6: Bar chart showing the percentage of larvae that survived based on case type after one hour of exposure to predators.

Larval survival is dependent on the experience of the crayfish to which they are exposed to ($\chi^2_2 = 15.620$; $P < 0.001$) (Figure 3.7). Crayfish with previous experience with caddisfly larvae ate more larvae than naïve crayfish without experience with case-building caddisfly larvae.



Figure 3.7: The percentage of larvae that survived or died when exposed to experienced and naïve crayfish.

3.4.2: Experiment 2: Do Crayfish Recognize the Casing as a Source of Food?

All experienced crayfish attacked the empty cases they were provided with, and none of the naïve crayfish attacked an empty case ($\chi^2_1 = 20.00$, $P < 0.001$) (Figure 3.8), indicating that all experienced crayfish recognize larval cases as a source of food.

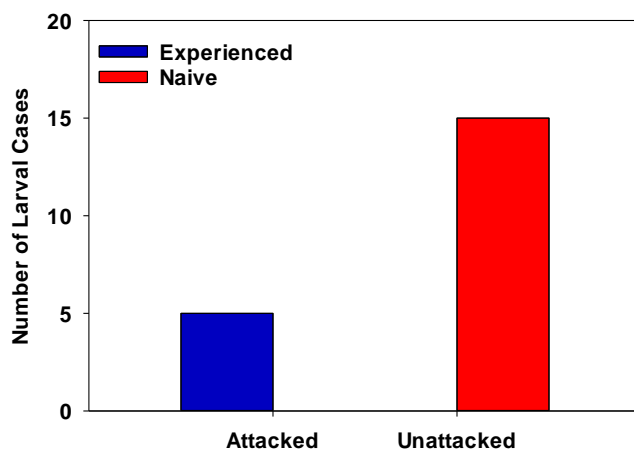


Figure 3.8: Bar chart comparing the number of attacked and unattacked larval cases based on crayfish experience.

3.5: Discussion

Case material selection is an essential process during the construction of a functional and effective caddisfly larval case (Okano and Kikuchi, 2009). This study shows that *Phryganea* spp. larvae will construct their cases with the materials provided, and the type of construction material provided does not affect larvae case construction behaviour (i.e., the rate at which larvae construct their cases). Although an important behaviour of many animals is the building of biological structures, it is usually assumed that this behaviour does not vary within species, and it is not influenced by the components of their habitats (Okano *et al.*, 2012). However, this result shows that the construction of biological structures (e.g. caddisfly larval cases) can vary within species, and it can be influenced by the surrounding materials. Similarly, Okano *et al.* (2011) tested the larval preference of two caddisfly species (*Psilotreta kisoensis* and *Perissoneura paradoxa*) for rough and smooth particles using two types of artificial inorganic materials with different surface texture; brick (rough) and glass (smooth). The level of preference for smooth particles was lower among larvae that live in areas with less abundant smooth particles, indicating that the surrounding sediment influences case construction material selection. In this experiment, *Phryganea* spp. larvae also display a lower preference for non-native case construction material (plastics) when provided with both leaves (native case construction material) and plastics for case construction. The type of case construction material used by caddisfly larvae and the shape of these cases varies significantly, but the adaptive mechanisms behind these variations are not clearly understood (Okano *et al.*, 2012).

Caddisfly larvae show some flexibility during case construction material selection. Several studies (e.g., Copeland and Crowell, 1937; Gaino *et al.*, 2002) have shown that larvae will construct cases with the available materials in the absence of their native case construction materials. This experiment also shows that *Phryganea* spp. larvae provided with non-native case construction materials (plastics) will construct their cases using the available material; however, larvae prefer their native case construction material. Hence, few larvae in the 'leaf-plastic' group added some plastics to their cases on the first, second and third day of case construction (Figure 3.5). Similarly, when Gaino *et al.* (2002) provided *Sericostoma pedemontanum* larvae with a mixture of calcareous and siliceous grains, larvae constructed their cases using both materials, although they largely prefer calcareous grains which is their native case construction material.

The result is also in accordance with De Gispert *et al.* (2018), who observed that larvae built their cases using unfamiliar grains when that was the only material provided.

Case construction is a continuous process in caddisfly larvae, with larvae increasing or modifying their cases as they grow. Anderson (1974) observed that larvae increase the size of their cases with time. This study also shows that larvae in the ‘leaf-only’ and the ‘leaf-plastic’ group increase their case length with time when compared to those in the ‘plastic-only’ group. Similarly, Copeland and Crowell (1937) observed that larvae that constructed their cases with non-native case construction materials do not add more materials to their cases on the second day. It could also be possible that the cost of secreting silk to construct cases using plastics is higher than those constructed with leaves. Inappropriate case construction materials could result in a costly process during construction by secreting excess silk to modify the selected materials (Okano *et al.*, 2010). This result is also in accordance with de Gispert *et al.* (2018), who found that larvae used more silk to construct their cases when provided with unfamiliar materials compared to those provided with their native case construction materials. Animals have a limited amount of energy in a given time, and this energy needs to be allocated appropriately for growth and other uses (Otto and Svensson, 1980). Thus, *Phryganea* spp. larvae provided with plastics only for case construction might not increase their case length by adding more case construction material because they need to allocate their energy for uses other than case construction. Also, studies have shown that longer and rigid cases offer more protection to larvae (Correa-Araneda *et al.*, 2017; Johansson, 1991; Otto, 1987). In this experiment, larval cases in the ‘plastic-only’ group could offer more rigidity than those in the ‘leaf-only’ and ‘leaf-plastic’ group; hence, a potential reason why larvae did not construct longer cases.

Larval case construction materials did not affect their survival when exposed to predators (Figure 3.6). Larvae survived at the same rate irrespective of the materials used in constructing their cases. This result is possibly because of the naivete of the prey and most of the predators used in the experiment. Naivete is an essential component that affects the relationship between novel organisms and their predators (Martin, 2014). Most of the prey (82.7%) survived after one hour of exposure to predators because there were fewer experienced predators used in this experiment when compared to the naïve predators. Also, almost all the crayfish that had previous experience

with caddisfly larvae of another species ate the larvae assigned to them in this experiment, suggesting that prey recognition needs to be learned to initiate a predatory attack.

Caddisfly larvae tubular cases serve as protection and camouflage (Otto and Svensson, 1980; Williams *et al.*, 1987). Larvae of *Potamophylax cingulatus* with leaf cases were more noticeable by brown trout when on a sandy substrate than when on a leafy substrate, indicating that larval cases serve as a camouflage to avoid predator detection (Otto and Svensson, 1980). However, the protective function of *Phryganea* spp. larval cases could not be determined in our study because of the naivete of the prey and some of the predators used in this experiment. Also, only the crayfish that had previously eaten caddisfly larvae attacked an empty case in Experiment 2, and some of these experienced predators attacked the cases until the end of the experiment. These experienced crayfish attacked the empty cases despite the absence of larvae in these cases; it could mean that these crayfish recognize larval cases as a source of food using visual cues instead of prey odour. It is also possible that crayfish recognized the cases as a source of food using larval signature left behind in these cases (e.g., silk used in binding the case construction materials). Larvae were removed from the cases at least three days before the empty larval cases were presented to the crayfish. Hazlett (1985) observed that disturbance cues from virile crayfish (*Faxonius virilis*) persisted for about one hour at room temperature. Thus, if these crayfish were to recognize the larval cases using any odour, it might be the odour of the silk or the leaves used in case construction because the larval odour would have degraded within those three days.

The relationship between the function of larval cases and the environmental factors influencing their construction behaviour has been investigated and discussed by some researchers. Okano *et al.* (2010) demonstrated that the relationship between the mineralogical components of the aquatic environment and the larvae inhabiting these environments depends on the surface texture of the substratum components. Okano *et al.* (2012) observed that caddisfly larvae, *Perissoneura paradoxa*, used larger and rougher particles as they grow because of the absence of enough smooth larger particles in their surrounding sediments. As the larvae grow in size, there is a tradeoff between the cost of searching for smooth particles and the respiratory benefits of smooth cases, thus making a decrease in their preference for smooth particles advantageous (Okano *et al.*, 2012). In contrast, the ability of *Goera japonica* larvae to line the inner walls of their case made them less selective than *P. paradoxa* larvae during case construction material selection

(Okano *et al.*, 2010). Structures constructed by animals enhance the animal's fitness, and the functions and benefits of these structures seem to be influenced by the quality of the construction materials present in the surrounding sediments (Okano *et al.*, 2011).

Several studies have investigated how organic materials, e.g. leaves and bark (Boyero, 2011), algae (Anderson, 1974), and mineral materials, e.g., stones (Okano *et al.*, 2012), sand grains (Anderson, 1974), influence case construction behaviour. However, there is no study on the effect of synthetic material (e.g., plastics) on case construction behaviour. Anthropogenic activities pollute the aquatic environment; according to data from volunteer beach cleanups, over 80% of the pollutants along the shoreline of Great Lakes are plastics (Driedger *et al.*, 2015). All around the world, plastic pollutants in the aquatic environment are present in different sizes and shapes (Driedger *et al.*, 2015; Imhof *et al.*, 2013). This study shows that anthropogenic activities can affect larval case construction behaviour via case construction material selection. Larval case construction behaviour, the type of material used in construction, and the shape of larval cases are sometimes used in identifying caddisfly larvae (Hickin, 1967; Wiggins, 1977, 2004). Thus, researchers (e.g. Weaver and Morse, 1986; Stuart and Currie, 2002) have suggested the use of case construction behaviour in constructing Trichoptera phylogenies. With the variation in the materials used for case construction, and the influence of anthropogenic activities such as plastic pollution, it might be difficult for researchers to rely on case construction behaviour for phylogeny construction. Also, as some predators ingest caddisfly larvae together with their cases, these predators might be at risk of ingesting plastics if plastics are used for constructing some part of the larval case. This route could be another means of ingesting plastics by fish and other predators in addition to generally mistaking plastic particles for food items.

Chapter 4: General Discussion

4.1: Summary of Findings

The experiments presented above indicate that caddisfly larval case construction behaviour can be influenced by predation risk and their surrounding materials. In Chapter Two, the exposure to alarm cues affected *Limnephilus* spp. larval case construction material selection, with larvae adding more sticks to their cases after the first exposure. However, the exposure to alarm cues did not affect *Limnephilus* spp. larval case length and the rate at which larvae construct new cases when compared to those exposed to dechlorinated tap water (control). In Chapter Three (experiment one), *Phryganea* spp. larval preference for case construction material was determined. The result indicates that they largely prefer their native case construction materials; however, they will construct their cases with the available material in the absence of their native case construction materials. Although the protective benefits of the constructed cases could not be examined, the results show that prey recognition also needs to be learned to initiate an attack. The second experiment in Chapter Three on the crayfish recognition of *Phryganea* spp. larval cases as a source of food showed that all experienced crayfish recognized and attacked larval cases despite the absence of larvae in these cases. Visual cue and the crayfish experience seem to play an important role in larval case recognition because some of the crayfish attacked the larvae till the end of the experiment despite the absence of larvae in these cases.

4.2: Future Research Directions

The concentration of alarm cues used in Chapter Two experiment influenced case material selection in *Limnephilus* spp. larvae, indicating that the concentration could be used for future studies to examine the effect of alarm cue on larval case construction behaviour. The experiment also showed that few larvae in both the control group and the alarm cue treatment group constructed new cases after 24 and 48 hours of exposure to their respective treatments; this shortfall in the number of larvae that constructed new cases could be because of insufficient case construction materials. Copeland and Crowell (1937) observed that case construction is often difficult in *Molanna* larvae when there is not enough quantity of sand for construction. Thus, larvae were provided with about a centimetre depth of sand for case construction (Copeland and Crowell, 1937) because species that construct their cases with sand often burrow into the sand (Hanna, 1960). Also, Boyero (2011) provided *Calamoceras marsupus* larvae with several sticks

of 15 – 20 units for case reconstruction; this species constructs its cases with either sticks or leaves. However, in this study, it is unknown if insufficient case construction materials was the reason why few *Limnephilus* spp. larvae constructed new cases in both treatment groups, or if the species do not construct new cases as fast as other species. e.g., *C. marsupus* (Boyero, 2011). Thus, future studies should investigate if the quantity of case construction materials available influences case construction behaviour.

The first experiment showed that the rate at which larvae construct new cases do not differ between treatment groups. This could be because the treatment was not persistent enough to influence the rate at which larvae construct new cases. Boyero (2011) examined the effect of predator odour on *Calamoceras marsupus* larvae case construction behaviour and material selection, and the larvae were exposed to 15mL of predator odour four times within 12 hours. In another study conducted by Okano *et al.* (2017), larvae were exposed to a mixture of 6 mL of predator odour and 4 mL of river water once (at the beginning of the experiment) within 22 hours for larvae to repair one-fourth of their case that was damaged. In this study (Chapter Two), larvae were only exposed to 5 mL of alarm cue introduced into 150 mL of water every 24 hours for two days. Thus, future studies should investigate if the concentration and time of exposures to alarm cues influence larvae case construction behaviour.

Previous studies have shown the effect of predator odour on case construction, case material selection and anti-predator behaviour in caddisfly larvae. However, these studies (Boyero *et al.*, 2006; Gall and Brodie Jr, 2009; Okano *et al.*, 2017) did not indicate the role of the predator's diet. Also, the effect of predator diet (digestion-released cues) on caddisfly larva antipredator and case construction behaviour is still unknown. Pre-consumption prey-borne cues like damage-released alarm cues and disturbance cues do not provide any information about the predator, unlike predator odour and digestion-released cues. Hettyey *et al.* (2015) observed that tadpoles elicited a stronger response to cues from predators fed with conspecifics than cues from homogenized tadpoles, starved predator, and the combination of both homogenized tadpoles and starved predators. This shows the role of digestion-released cues in anti-predator response. Since the intensity of prey's antipredator responses should reflect the current predation threat posed by the predator (Lima and Dill, 1990), it is unknown if larvae case construction behaviour will vary

based on the type of predation risk. Thus, future studies should investigate the influence of different predation risks on caddisfly larvae antipredator and case construction behaviour.

Prey can recognize and respond to predators using various predator-related chemical cues. For instance, Chivers *et al.* (1996) demonstrated that damselfly larvae (*Enallagma* spp.) learned to recognize pike (*Esox lucius*) as a predator using the diet-related cues in the predator's diet. Prey can also be conditioned to recognize a novel predator by pairing damage-released alarm cues with the predator odour. This has been demonstrated in some aquatic insects, e.g., damselfly larvae (*Enallagma boreale*) (Wisenden *et al.*, 1997) and larval mosquitoes (*Culex restuans*) (Ferrari *et al.*, 2008). However, the ability of caddisfly larvae to learn to recognize and respond to novel predators using either diet-related cues or the pairing of alarm cue with novel predator odour remains unknown.

The mechanisms influencing case construction behaviour and case polymorphism in caddisfly larvae have been described as unknown because of the various factors influencing case construction behaviour, e.g., predation risk (Boyero, 2011), the environment and ontogenetic differences (Okano *et al.*, 2011). Boyero (2011) demonstrated that *Calamoceras marsupus* larvae displayed case polymorphism, which is influenced by behavioural responses when exposed to varying predation risk from different predators (Fish, *Squalius pyrenaicus* and crayfish, *Procambarus clarkii*). Okano *et al.* (2012) investigated the effect of the surface texture of case construction materials on the different instar levels of *Perissoneura paradoxa* larvae. The effect of poor-quality diet, temperature difference, and predation risk on *Sericostoma pyrenaicum* larvae case construction behaviour have also been documented (Correa-Araneda *et al.*, 2017). Johnson *et al.* (2008) also observed that the case construction behaviour of *Brachycentrus americanus* larvae could be adversely affected by exposure to neurotoxic insecticides. Larvae exposed to insecticide abandoned their case and reduced the rate at which they constructed new cases, and those that constructed new cases built disorganized and non-intact cases that are less rigid and more vulnerable to predation attack from stonefly nymphs (*Hesperoperla pacifica*) (Johnson *et al.*, 2008). Most studies have focused on the effect of a single factor, e.g., predation risk (Boyero, 2011; Boyero *et al.*, 2006; Okano *et al.*, 2017), surface texture (Okano *et al.*, 2010), insecticides (Johnson *et al.*, 2008), on larval case construction behaviour and material selection. The underlying mechanisms influencing larval case construction behaviour and

material selection can be better understood by examining the effect of multiple factors on this behaviour.

In this study, the protective benefit of *Phryganea* spp. larvae cases constructed with non-native case construction materials (plastics) could not be determined because of the naivete of the prey and most of the predators. Thus, future studies should consider getting both the prey and the predators from the same environment. This recommendation would help understand if cases constructed with unfamiliar materials offer more protection through camouflage or because they are more rigid than the cases constructed with the native case construction materials.

In the second experiment in Chapter Three on the recognition of *Phryganea* spp. larval cases as a source of food by the crayfish, it is unclear if the crayfish recognized the empty larval cases using visual cues only or by the larval signature on the empty cases (e.g., silk used for case construction). Hence, future experiments should consider providing the crayfish with empty fake cases constructed without larval silk to determine if the attack on these cases was a result of visual cues only.

4.3: Conclusions

Case architecture of caddisfly larvae and their lifestyle are closely related, and this is consistent with the morphological features of all their life stages (Wiggins, 2004). The exceptional variations present in caddisfly larval construction behaviour and material selection indicates that the ecological complexities of this behaviour are yet to be fully known (Wiggins, 2004). Both studies in this thesis highlight the different factors (predation risk and surrounding materials) that can influence larval case construction behaviour, thus providing an insight into the ecological complexities of this behaviour.

Organisms can show morphological changes in response to perceived predation risk, and these changes make them less susceptible to predators (Chivers and Smith, 1998). According to Gall and Brodie Jr. (2009), caddisflies can reduce the predation risk they are exposed to by combining their immediate response to the perceived predatory threat with their behavioural responses. Thus, larvae that are able to modify their behaviour to match the intensity of the current predation risk should have a greater chance of survival (Gall and Brodie Jr, 2009). The assessment of larval case construction behaviour can provide an insight into the predator-prey

interaction in an aquatic environment (Okano *et al.*, 2017) and how human activities (e.g., the use of pesticides) can increase larval vulnerability to predation (Johnson *et al.*, 2008).

Stuart and Currie (2002) suggested that the assessment of larval case construction behaviour can be used to determine the behaviour and its evolution; however, constructed cases should be used to address the structure such as the design, adaptation, and evolution of the structure. The constructed cases can be used to explain the evolution of case construction behaviour only if there is a relationship between the case construction behaviour and the cases constructed (Stuart and Currie, 2002). With the increase in human activities (e.g., plastic pollution) and how they influence larval case construction behaviour, researchers should be careful while using case construction behaviour to determine the behaviour and the evolution of case building.

Caddisfly larvae constitute a significant component of the freshwater habitat, and they play important roles serving as food and bioindicator organisms as well as decomposing plant materials. Case construction in caddisfly larvae has been linked to their survival (De Gispert *et al.*, 2018), and these cases have been described as a tool that enhances their diversity within the freshwater habitat (Wiggins, 1998; 2004). Thus, activities (natural and anthropogenic) that influence this construction behaviour need to be examined and monitored to ensure that their role within the ecosystem is not altered. Ultimately, much more study is needed to determine the mechanisms influencing larvae case construction behaviour and how the variation in construction behaviour influences their survival in their natural habitat.

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