GRAIN SIZE SELECTION IN CASE BUILDING BY THE MOUNTAIN CASED- CADDISFLY SPECIES POTAMOPHYLAX LATIPENNIS (CURTIS, 1834)

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Abstract

Among all aquatic macroinvertebrates, many Trichoptera species build cases to facilitate breathing, provide physical protection, reduce predation, avoid being drift, or prolong survival during drying conditions. However, building requires significant energetic costs related to grain searching and silk production, which may involve a trade-off with the size of grain used. Thus, building cases with large grain sizes would require less time (i.e. a trait related to survival) but higher silk production (i.e. a trait related to fecundity), whereas building with small grain sizes would show the contrary pattern. We evaluated grain size selection, time spent, and energetic costs linked to silk production in a population of the Limnephilidae Potamophylax latipennis from the Ritort river (Catalonia). Laboratory experiments were conducted to force individuals to rebuild using 7 different experimental conditions varying the grain size available. According to our results, P. latipennis presents a trade-off between time and energetic costs. P. latipennis prioritized building cases with grain sizes that provide a faster building despite spending higher amount of silk. In addition, when individuals were forced to rebuild twice using both a natural and an artificial substrate, the natural substrate was preferred and individuals with cases composed of artificial substrate repaired it with natural substrate even though it represented an extra cost for the individuals. Despite the high energetic costs of building cases in Trichoptera and their potential implications to reproduction traits in the adult stage, individual survival was prioritized.
1. INTRODUCTION

Many organisms build structures to protect themselves or to assist in feeding or reproduction, such as nets or cases (Dudgeon 1990; Bucheli et al. 2002; Statzner et al. 2005; Chaboo et al. 2008). These structures are built using surrounding, self-secreted or both types of material. In the freshwater world, the underwater architects are Trichoptera. Despite the fact that case building is not universal in this group of insects, most families of Trichoptera build cases of a wide variety of sizes, shapes, and materials, including self-produced silk, mineral grains, detritus, or alive organisms such as algae or molluscs (Wiggins 2004). The benefits of building cases in Trichoptera have been largely discussed in the literature and have mainly associated to increase survival. Cases assist in respiration by facilitating unidirectional flow when larvae move their abdomens, provide physical protection, serve as camouflage against predators reducing predation, give extra weight to the individual to avoid being drift, or prolong survival during drying conditions (Hansell 1974, Otto & Svensson 1980, Nislow & Molles 1993, Wiggins 1996, Zamora-Muñoz & Svensson 1996, Otto & Johansson 1995, Wissinger et al. 2004).

Case-building and repair has been analysed from various points of view, including animal behaviour, evolutionary biology, or basic life history characteristics. (e.g., Houghton & Stewart 1998, Gupta & Stewart 2000, Norwood & Stewart 2002, Mendez & Resh 2008). Different studies, have demonstrated that larvae can use a wide range of materials when the most favoured material for building cases is not accessible (Gorter 1931, Gaino et al. 2002). For example, Gaino et al (2002) showed that larvae that prefer travertine for case building may switch to quartzite if the former is unavailable. In addition, the type of material used can vary along the ontogeny of a particular species and depending on the presence of predators or other environmental conditions (Boyero et al. 2006), indicating that species can be flexible in the material used for building.

Not only the type of material, but also other aspects, such as the weight or the size of the grain, are also important during case building. For example, species building mineral cases can switch to grain sizes near the range limits of the most preferred grain size when this is not available (Hanna 1961, Tolkamp 1980). Thus, most Trichoptera species exhibit grain size selection depending on the past experience (i.e., allowing the insect to evaluate the quality of a particle in relation to a previous one) (Nepomnyaschikh, 1993). This suggests that caddisflies try to achieve the minimal energy expenditure during the grain searching.

However, besides the energy spent to collect the building material, there is also a direct cost of silk production by the larval labial glands. This occurs mainly because silk
used to build cases is energetically expensive (Stevens et al. 1999, Stevens et al. 2000). Especially in the case of the Limnephilidae, the costs associated with silk production are very high because large amounts of silk are produced not only to glue grains but also to cover the inner lining of the case to obtain a smooth surface. For example, Otto found that the cost of silk production for case construction during the last instar of Potamophylax cingulatus might represent 12% of the energy expenditure (Otto 1974). In addition, building also represents significant losses of larval protein (e.g., of about 35% in Limnephilus rhombicus; Mondy et al. 2012), and might have an impact in the fitness of the adults, despite the fact that costs for case building can be mitigated by the reallocation of resources during metamorphosis (Jannot et al. 2007). Costs for case building have also been linked to a reduction of the thoracic mass and the wing length, or to the incapacity of synthesising yolk and maturing eggs (Wheeler 1996, Stevens et al. 2000, McKie 2004). Therefore, silk production is ultimately associated with the fecundity traits of the adult phase. Because of these important energetic costs, repair behaviour may be more beneficial than rebuilding the entire case for larvae (Kwong et al. 2011).

2. HYPOTHESIS

The aim of this work was to investigate case building behaviour and repair in the Limnephilidae species *Potamophylax latipennis*, focusing on two aspects: grain size selection and energetic costs. Despite all integripalpian caddisfly families build portable tube-shaped cases, Stuart (2000) and Stuart & Currie (2001) observed that there is variation in the patterns of searching, handling, fitting, and attaching pieces to the case among different taxa. However, grain selection behaviour within a genus or species might respond to the availability of material and the associated energetic costs. Therefore, our first hypothesis is that grain size selection during the building process in *P. latipennis* should reach an optimal balance between time for building (i.e., protection which can have consequences on several survival traits, see above; e.g., Hansell 1974, Otto & Svensson 1980, Nislow & Molles 1993) and silk used (i.e., energetic costs which can have consequences on several fecundity traits, see above; Wheeler 1996, Stevens et al. 2000, McKie 2004, Jannot et al. 2007) (Figure 1). Building cases with a higher proportion of larger grain sizes than the original cases would require less time to build the case and provide a faster protection of larvae, but would imply a higher silk production to glue these large particles. In contrast, building cases with smaller proportion of grain sizes than the original cases would require more time despite gluing these small particles would require less silk production (Figure 1). On the other hand,
grain selection in Trichoptera has also been related to the smoothness of the grain or its chemical composition (Okano & Kikuchi 2009, Okano et al. 2012, Okano et al. 2010). Additionally, most Trichoptera species tend to partially or completely rebuild the case after eliminating damaged or less-suitable parts (Kwong et al. 2011). Therefore, our second main hypothesis is that building with mineral grains that differ from the original ones in these two aspects may involve an increase of the energetic costs due to an increase of the necessary silk to build the case and therefore, individuals of *P. latipennis* will try to rebuild when the preferred material is again available.

![Figure 1](image.png)

**Figure 1.** Scheme to illustrate the main hypotheses tested in this study.

3. **METHODOLOGY**

**Species description**

The species *P. latipennis* inhabits high mountain rivers with cold waters. It has a large altitudinal range and, in some cases, it coexists with *P. cingulatus*, which is usually found in much higher altitudes (Bonada et al. 2004). *P. latipennis* can also be found in margin shallow waters of mountain lakes. Larvae are shredders, feeding mainly on leaves and stems, and can be very abundant in well-oxygenated pools (Graf et al. 2008). Pupae aggregate under cobbles located in riffles to facilitate oxygen uptake. (Hynes, 1970; Newbury & Gaboury, 1993). The species has a univoltine cycle with a flying period from summer to autumn (Graf et al. 2008) and a Palearctic distribution. In the Iberian Peninsula, where this study was carried out, it has been...
found in mountain rivers in the Pyrenees, Granada, Madrid, or Teruel (González et al. 1992) (Figure 3).

**Figure 2.** Larva of the species *Potamophylax latipennis* used in the experiments. Photo credit: [http://www.biopix.com](http://www.biopix.com).

**Figure 3.** Distribution of *Potamophylax latipennis* in Spain according to the DAET database (Geo-referenced site scale data of European Trichoptera; [http://project.freshwaterbiodiversity.eu/index.php/geo-referenced-site-scale-data-of-european-trichoptera-daet](http://project.freshwaterbiodiversity.eu/index.php/geo-referenced-site-scale-data-of-european-trichoptera-daet))
Experimental design
The specimens were collected in a pool of the Ritort River in the locality of Espinavell (Girona, North-East of Spain) (Figure 4). This river is a tributary of the Ter River in its left side. It has a siliceous geology mainly composed of schist with limestone, dolomite, and marble. Most of the river has a very high ecological quality with a dense riparian forest of *Alnus glutinosa*.

![Figure 4. Pool in Ritort river where larvae were collected (Photo: Tony Herrera)](image)

About 170 individuals of the last instar were collected, in May 2013 (for Experiment 1, see below) and in June 2014 (for Experiment 2, see below) and brought alive to the laboratory where the experiments were conducted. To recreate the original substrate, a large sample of sand, gravels, pebbles, and little branches were collected in the same pool where individuals were sampled. Several water tanks were also collected from a nearby fountain to have water with similar chemical properties. Finally, dry leaves from *A. glutinosa* and *Corylus avellana* were collected from the riverbanks to feed the larvae.

Larvae were acclimatized during one week in an aquarium that recreated the original river conditions, providing food as required (Figure 5). The aquarium had a water recirculation system with an active carbon filter that cleaned and oxygenated the water continuously, and a refrigeration system that maintained the water temperature at 6.6°C, simulating river conditions.
Figure 5. Aquarium recreated in the laboratory with different cages were the experiments were performed.

Experiment 1: Grain size selection
A total of 35 larvae were randomly selected from the aquarium and were removed from their cases. The original cases were kept in dry conditions while larvae were put into cages made of a plastic net of about 1 mm of mesh size and filled with combinations of 3 different grain sizes (Figure 3): small (0.5-1 mm), medium (>1-1.5 mm), and large (>1.5-2 mm).

These 3 types of substrate were obtained by sieving gravel from the pool where we collected the larvae through different sieves. We set up 7 experimental conditions that included different proportions of the 3 grain sizes: 3 different cages with 100% of small, medium, and large grain sizes; 3 cages included only 2 grain sizes with 50% each; and 1 cage had the 3 grain sizes respectively with 33% each (Figure 6). Each experimental condition was replicated 5 times.
Figure 6. Scheme of the different experimental cages. Each blue circle represents a cage with the proportion of each grain size. L for large grain sizes (>1.5-2 mm), M for medium grain sizes (>1-1.5 mm), and S for small grain sizes (0.5-1 mm).

Each experimental condition had only one larva. We placed 2 overlapped pebbles to facilitate the rebuilding and covered the cages with a plastic net to avoid larvae escaping from the cage (Figure 7). The time for rebuilding was considered from the beginning of the reconstruction until we visualized the larvae with rebuilt cases moving completely free inside the cage. These larvae were preserved in alcohol and removed from the rebuilt cases. The original and the rebuilt cases were dried in a stove and weighed. Afterwards, cases were muffled at 400°C during 6 hours to burn the silk used and weighed again. The weight difference between the dry and muffled cases divided by the weight of the dry cases was used to calculate the silk expenditure in the original and the rebuilt cases. Finally, the grains used in the original and the rebuilt cases were sieved through different sieves to determine the proportion of large, medium, and small particles used.

Figure 7. Detail of the cages used in the experiments.
Experiment 2: Case rebuilding

A total of 110 larvae were randomly selected from the aquarium despite the fact that we were only able to use 92 individuals. Larvae were removed from their cases and forced to rebuild over 3 different experimental conditions: 30 larvae were placed in cages with the original river substrate (i.e., large, medium, and small grains obtained from the pool where larvae were collected and cleaned to remove any organic substrate); 30 larvae were placed in cages with an artificial substrate composed by a mixture of large, medium, and small grains of quartz (i.e., 100% natural, substrate designed for aquariums by Jardiland©; Figure 8); and 32 larvae were placed first in cages with the artificial substrate and, once they built the cases, were placed in the original river substrate to assess repairing behaviour during the next 48 hours. When analysed under a stereoscope, the artificial substrate had a visibly smoother surface but a higher waviness (larger scale undulation) than the natural substrate (Figure 8). All larvae were preserved in alcohol and removed from the rebuilt cases. The original and the rebuilt cases were dried in the stove and weighed before they were muffled at 400ºC for 6 hours to remove the silk used and weighed again. As in experiment 1, the weight difference between the dry and muffled cases divided by the weight of the dry cases was used to calculate the silk expenditure in the original and the rebuilt cases.

Figure 8. Natural (river) and artificial (quartz from Jardiland©) used in the second experiment. A general (left) and detailed (right) view is provided.
Data analysis

Data were analyzed using descriptive statistics and regression analyses. Linear models were applied when comparing pairs of continuous variables. The non-parametric Mann-Whitney-Wilcoxon tests were used for comparisons among grain sizes used and experimental conditions. This test was preferred over other non-parametric tests because it allowed pairwise comparisons that are corrected for multiple testing. When tests included more than 2 comparisons, the non-parametric Kruskal-Wallis test was used. All analyses were computed using R (R core development team, 1996; version 0.98.945 – © 2009-2013 RStudio, Inc.)

4. RESULTS

Experiment 1: Grain size selection

When comparing the total weight of the particles used in the original and the rebuilt cases, we found that almost every individual used more grains during the rebuilding in all experimental conditions (Figure 9). However, this difference decreased with increasing weight of the original cases (Slope = 0.78): as the original cases were heavier, the rebuilt ones were proportionally less heavy (Figure 9).

![Figure 9. Total particles used per each individual in the original and the rebuilt cases. The black line shows the x=y relationship whereas the red dashed line is a linear model fit showing that there is a significant and positive relationship between both variables (p< 0.005), (Adjusted $r^2 = 0.18$), (Slope = 0.78), (Intercept = 0.18).]
Original cases were composed by a mixture of L, M, and S grain sizes, although M was the preferred size followed by L and S (M=0.51%, L=0.29%, and S=0.20%; Figure 10). Pairwise Wilcoxon tests between each size were significant (pairwise Wilcoxon tests: L-M \( p=3.3\times10^{-10} \), L-S \( p=0.001 \), M-S \( p=4.3\times10^{-15} \)).

![Particle preference of original cases](image)

**Figure 10.** Boxplot showing the weight percentage of each grain size used in the original cases.

When rebuilding, individuals needed more time to rebuild the new case as the grain size decreased (Figure 11), being S the experimental condition which required more time. In those experimental conditions where S was present in combination with M or L, larvae also needed more time than where S was not present (Figure 11) (pairwise Wilcoxon tests: LM-S \( p=0.010 \), LM-MS \( p=0.022 \), LM-LS \( p=0.012 \)).

![Time spent to rebuild](image)

**Figure 11.** Time spent (hours) to rebuild the new cases in each experimental condition.

Individuals exposed to different experimental conditions negatively selected S grain size and preferred M and L sizes (Figure 10), with significant differences using a pairwise Wilcoxon tests in the LS experiment (\( p=0.007 \)), MS experiment (\( p=0.007 \)),...
and S size in LMS experiment (L-S p= 0.024, M-S p= 0.024). In contrast, in the LM experimental condition, both grain sizes were equally selected in combination (pairwise Wilcoxon test: p= 0.841) (Figure 10).

Figure 12. Boxplots showing the weight percentage of each grain size used each experimental condition.

When the proportion of particles in the original cases was compared with that of the rebuilt cases in the LMS experimental condition, we found no significant differences for L and M (pairwise Wilcoxon test: L-M p= 0.310) (Figure 13). In contrast, in the original cases there was a significantly higher proportion of M than L (pairwise Wilcoxon test: L-M p= 3.3e-10). For both, the original and the rebuilt cases in the LMS experimental condition, the proportion of S was significantly lower than the other grain sizes used (pairwise Wilcoxon tests: Original: L-S p= 0.001, M-S p = 4.3e-15; Rebuilt: L-S p= 0.024, M-S p= 0.024) (Figure 11).
Figure 13. Boxplots showing the weight percentage of each grain size used in the original and the LMS experimental condition.

In terms of the silk used per individual when comparing the original and the rebuilt cases, there was not a clear pattern (p > 0.05, Adjusted \( r^2 = -0.006 \), Slope = -0.011; Figure 14). Rebuilt cases used a similar amount of silk regardless of the silk used in the original case. Thus, cases with low silk values in the original cases used more silk when rebuilding and cases with large silk values in the original cases used less silk when rebuilding. However, the amount of silk used for building cases in the different experimental conditions differed (Kruskal-Wallis test: chi-squared = 18.0289, \( p = 0.00616 \)), being L, the size that requires the highest amount of silk and S the lowest (Figure 15).
Figure 14. Total amount of silk (g) used per each individual in the original and the rebuilt cases. The black line shows the x=y relationship. No significant relationship was found between both variables after applying a lineal model (p > 0.05), (Adjusted $r^2 = -0.006$), (Slope = -0.011), (Intercept = 0.006).

Silk spent in the rebuilt cases

Figure 15. The amount of silk spent to rebuild the new cases per each experimental situation.

Finally, when relating the time spent to rebuild with the amount of silk used, we found a significant and negative relationship (Figure 16): individuals that needed less time (i.e., those in the L experimental condition followed by LM) spent more silk when rebuilding, whereas individuals that needed more time to rebuild (i.e., those in the S experimental condition) spent less silk.

Silk spent in the rebuild cases

Figure 16. Relationship between the time (hours) and the amount of silk (g) used per individual to rebuild. The black line shows the x=y relationship whereas the red dashed line is a linear model fit showing that there is a significant and negative relationship between both variables (p< 0.011), (Adjusted $r^2 = 0.163$), (Slope = -0.0004), (Intercept = 0.009).
Experiment 2: Case rebuilding

In the first two experimental conditions, individuals were forced to rebuild a case with natural and non-natural substrates respectively. Larvae used a similar proportion of silk rebuilding with the original river substrate (grey) than with the artificial substrate (white), with no significant differences between them (Figure 17) (Wilcoxon test: $w = 458$, $p = 0.911$).

Figure 17. Amount of silk used to rebuild (g) with the two types of substrate (grey for the river substrate, white for the artificial substrate).

In the third experimental condition, when larvae were placed first in cages with the artificial substrate and later in the original river substrate to assess repairing behaviour, we found that the amount of silk used was dramatically reduced and independent of the amount of silk used in the original cases (Figure 18).

Figure 18. Relationship between the amount of silk used in the original natural cases and after the second rebuilding phase (artificial substrate). The black line shows the $x=y$ relationship. No significant relationship between both variables was found after applying a lineal model ($p > 0.005$), (Adjusted $r^2 = 0.006$), (Slope = -0.011), (Intercept = 0.006).
On the other hand, the cases of these larvae were partially repaired and percentage of the natural material used was higher in average (Figure 19) (Wilcoxon test: $w = 806, p = 8.11 \times 10^{-05}$)

Figure 19. Percentage of each substrate found in the rebuilt cases (grey for the river substrate, white for the artificial substrate).

5. DISCUSSION

Larvae of *P. latipennis* used more particles (in weight) when rebuilding but larger individuals used proportionally less. One possible explanation for this could be that, despite the potential higher costs of building (Otto 1974, Mondy et al. 2012), *P. latipennis* prioritize fast building instead of using the optimal quantity of grain (i.e., the one in the original case). When we analysed the silk expenditure, larvae used a similar amount of silk in the rebuilt cases regardless of the silk used in the original case. However, cases with low silk values in the original ones used more silk when rebuilding and cases with large silk values in the original cases used less silk when rebuilding. According to our first main hypothesis, time and silk expenditure were related to grain size and both variables were negatively associated, indicating that there is a trade-off between a variable related to survival (time, protection) and a variable related to fecundity (silk expenditure, energy costs). In natural conditions, *P. latipennis* larvae used an optimal combination of grain sizes that provide an optimal balance between protection and energy costs. However, once larvae are forced to rebuild in the laboratory, the higher amount of L grains used when all grain sizes were available, indicated that, even with higher costs of building, survival may be prioritized. There are several studies indicating that having a case increases the survival of caddisflies against cannibalism and predators (Wisinger et al. 2004; Wisinger et al. 2006; Ferry et al 2013) causing individuals to build as fast as they can when their case is removed.
Houghton & Stewart (1998) also discovered that an emergency-case is immediately produced when insects are experimentally deprived of their case.

Concerning Trichoptera, there are some examples of trade-offs in literature. Okano and Kikuchi (2009) described a possible trade-off in Goera japonica between the costs of material selection and silk secretion for case construction by caddisfly larvae; saving energy by secreting less silk on smoother particles allows more energy to be spent on selecting the preferred smoother particles or on other activities. A trade-off between larval and adult stages was also described by Stevens (2000). In this case, an increased larval expenditure of silk by fifth-instar larvae of Glyphotaelius pellucidus and Odontocerum albicorne was associated to a reduced size of some parts of the adult body.

In natural environments, we found that the M grain size seemed to be positively selected for, as it has been described in other species building cases preferably with a certain grain size range of mineral grain, but if the size is not available those species can switch to grain sizes near the range limits of the most preferred one (Hanna, 1961, Tolkamp, 1980). In our case, we found that larvae has no problem building cases outside the optimal grain size values despite it requiring more time or more resources. When all grain sizes were available, unprotected larvae expanded the preferable grain size range from 1-1.5 mm to 1.5-2 mm including M and L sizes, indicating again that getting a faster protection is much more important than energy expenditure. According to this, larvae did not prefer S sizes for building, as this grain size required more time to build a case. In contrast, the small grain size was selected and used in a small proportion in the original cases.

The selection of the natural material over the artificial substrate must have somehow increased the fitness of the builder. Our results on material preference showed that larvae of P. latipennis preferred the natural substrate more than the artificial one because most of the individuals rebuilt more than 50% of the case. Although Okano et al. (2009, 2010) reported that the larvae of caddisfly species Goera japonica selected smoother particles because less silk was used than when selecting rough particles, we observed that the preference of the larvae of P. latipennis for the natural material was not related to energetic reasons: the same amount of silk was used when constructing independently with the natural and the artificial substrates despite the artificial substrate was smoother than the natural one. Then, the preference of the natural substrate could be more related to a better adhesion of the silk in a rougher particle or
to phylogenetic issues. Although the high energetic costs and their potential implications to reproduction traits in the adult stage of Trichoptera building cases is always more beneficial for the individuals, indicating that survival is prioritized.

6. CONCLUSIONS

According to our initial hypothesis we found a trade-off between time and energetic costs. When larvae of *P. latipennis* were forced to rebuild, they prioritized fast building to be protected quickly, despite the high energetic costs that this represents. This was clearly observed in the LMS experimental situation where individuals used more L grains than in the original cases.

The different chemical composition and texture of the artificial material did not represent an impediment to *P. latipennis* to build a case. However, the individuals of *P. latipennis* preferred the natural substrate and cases built with the artificial substrate were rebuilt with the natural substrate.

Despite the high extra energetic cost that rebuilding represents for the individuals and its potential implications in their fecundity traits, our results suggest that individual survival was prioritized.

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8. REFERENCES


• Okano, J. I.; Kikuchi, E. & Sasaki, O. (2010). The role of particle surface texture


Agricultural Research Reports, Wageningen 907: 1–211.


