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Products of Caddis-fly Larvae (*Trichoptera*) Silk Glands as a New Natural Textile Fibre

Abstract

Among the invertebrates, some insects are able to produce natural silk fibres which can serve as an alternative to the threads of the spider's web. The larvae of the caddis-fly make use of a multifunctional silky floss in producing hunting nets and residential tubular structures. Many species of caddis-fly build their capture webs in fast-flowing rivers. Therefore we suggest that such fibres must be strong. However, these webs have been studied and researched previously, taking into consideration the webs' shape and the geometry of the meshes. This paper presents preliminary results concerning some of the web material's morphological properties and tensile strength. We investigated the product of silk gland coming from *Hydropsyche pellucidula*, which is one of the most common caddisflies in Polish lowland rivers. Our researches proved that a cross-section of caddis-fly fibres is circular, with an average diameter of 10 μm . The mean tensile strength of the fibres investigated is 45 cN/tex ($5.7 \cdot 10^9 \text{ N/m}^2$), and is comparable to the strength of most spider thread.

Key words: natural silk fibers, silk gland, morphological properties, caddis-flies, tensile strength, *Hydropsyche pellucidula*.

Caddis-fly larvae are similar to spiders, butterflycaterpillars (e.g. silkworms) and blackflies (*Simuliidae*, *Diptera*) in their ability to produce a cocoon shell in the form of a multifunctional silky floss. Taking into account the variety of forms and versatility of silken products, it may be concluded the mastery of making cocoons is comparable in *Trichoptera* and *Araneinae*.

The fact that the caddis-fly's ability to spin silky floss was known in the past, not only to biologists, is confirmed by the text:

"O what tangled webs the caddis weave,
when first they practise to deceive!"

'Marmion, Sir Walter Scott, 1808.

Larvae of caddis-flies use a silky floss which is a product of their spinning glands (Figure 1) to build various accommodation structures. The 'weaving' is done by the elements of the mouthpart and not by the abdomen, as in the case of spiders, which makes it much more precise.

Some caddis-fly larvae build only simple structures: padded holes, corridors, hunting tunnels (Figure 2). The larvae can easily move inside them. Such holes may have continuations in the form of 'signaling threads', which inform the predatory caddis-flies about the prey that stumbles over the threads, or about intruders [5]. Others build fully-fledged webs. These have a shape of a tunnel-like sieve, inside which the caddis-fly awaits its prey. The inlet of such a structure is always directed against the water current, so not only live organisms but also fragments of plants

and detritus (unidentified, disintegrated organic matter) can fall into it [19].

Each species has its own web mesh shape and diameter, and the way the web is mounted depends on environmental conditions (Figure 3) [10].

Irrespective of hydrological conditions, webs are usually highly regular in their mesh size and pattern (Figure 4).

The structure and shape of a web may also depend on temperature [6]. The minimum temperature at which webs are built is also a characteristic feature

Introduction

Insects are the most widely distributed and frequently the most abundant group of invertebrates in the world. They include caddis-flies (*Trichoptera*), which are merolimnic insects, whose larvae and pupae, with few exceptions, prefer aquatic ecosystems, while the adults (imago) are pharate stages. The larvae of caddis-flies are found in all types of freshwater and marine environments.

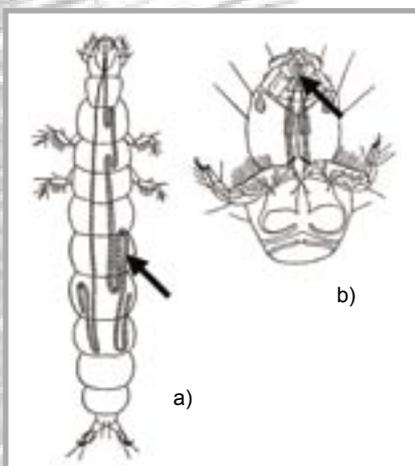


Figure 1. Location of spinning glands: a) in the body of the caddis-fly *Neureclipsis bimaculata*, b) the opening of silk glands at the haustellum [2].

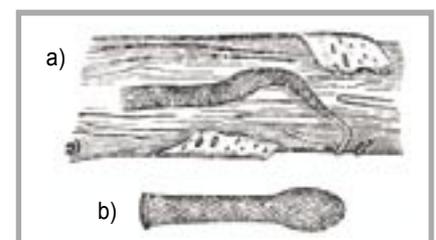


Figure 2. Simple structures in the form of holes and hunting tunnels of caddis-flies: a) *Lype reducta* [9], b) *Wormaldia occipitalis* [16].

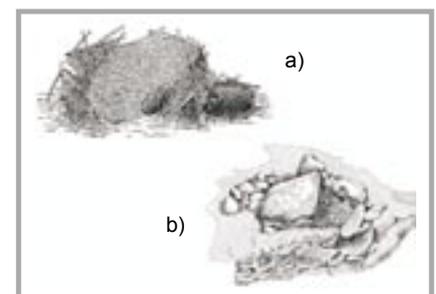


Figure 3. Web shape of *Hydropsyche angustipennis*, depending on the substratum: a) plant [13], b) stone [19].

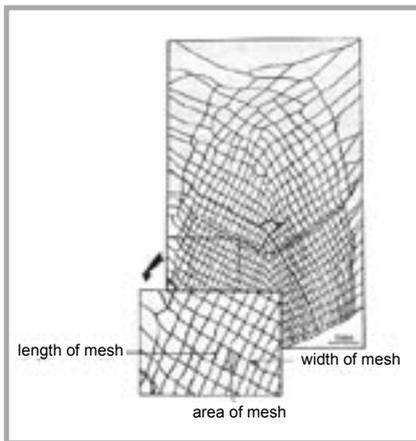


Figure 4. Web of caddis-fly larvae of the genus *Hydropsyche* [20].

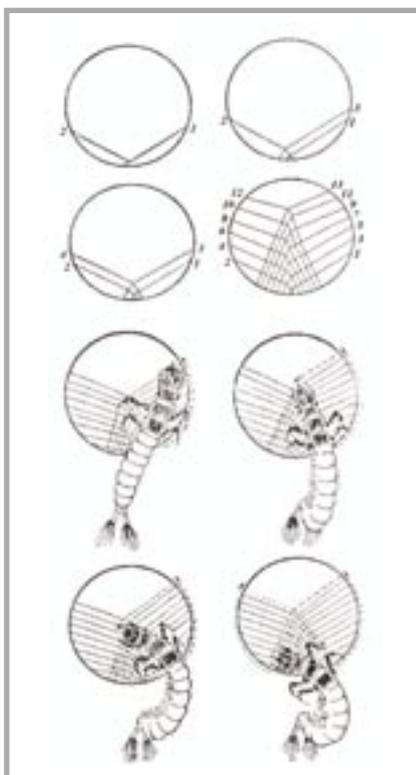


Figure 5. Scheme of web building by caddisfly *H. angustipennis* [19].

for a given species (in low temperatures some *Hydropsychidae* species, e.g. *H. angustipennis*, do not build webs) [18]. However, web building always consists of a set of similar actions, which are shown in Figure 5.

One of the most impressive webs, tubular in shape, is built by *N. bimaculata* (*Polycentropodidae*); it may be 20 cm long, whereas the larva itself is no longer than 2 cm. Despite being so big, its mesh is incredibly small, $0.45 \times 0.45 \mu\text{m}$; this is one of the denser webs built by caddisflies [17]. Larvae of the family *Polycentropodidae*, with a few exceptions, do not

build their webs in river currents. They are usually stretched between aquatic plants, mainly submerged macrophytes or floating leaves (Figure 6).

The most spectacular builder in this family is *Plectrocnemia conspersa*. We know [26] at least three types of webs built by *P. conspersa*, depending on water depth and velocity. In rivers of fast current, the web resembles a –‘swallow’s nest’ (Figure 7a), and a ‘hammock’ in shallow streams (up to 5 cm deep) (Figure 7b). In deeper rivers the web is placed on the bottom, with the funnel-like openings turned upwards, in this way catching victims moving along the bottom (invertebrates) or settling organic matter (Figure 7c).

Using the silky floss to build a web is not this fabric’s only application. Without the floss, it would be impossible to form another characteristic structure, the house. Usually, the larva begins building its house by spinning a silky tubular structure, into which it builds elements forming the house (Figure 8): fragments of plants, detritus, sand, small stones and so on [14, 21, 15].

A strong floss and an organic or inorganic substrate are also the basic components of the house built for the pupa (Figure 9 - see page 30). All types of caddis-flies, in the fifth larval stage, start building this, because without the cocoon it would be impossible for the specimen to metamorphose into the later stage.

Caddis-fly larvae usually build their webs in fast-flowing rivers, and so the cocoon shell they produce must be strong.

The product of *Trichoptera*’s spinning (silk) glands could be an alternative to a spider’s floss. Unlike spiders, caddis-flies fairly often produce cocoon shells in man-made conditions. It is much easier to breed them, because cannibalism among them is not observed, which does happen in spiders (*Arachnida*). Unfortunately, we do not find much significant data in literature on the properties of fibres made by caddis-flies. Therefore the aim of the studies that have been undertaken is to analyse the physical-mechanical properties, composition and chemical structure of the cocoon shell which these insects produce.

■ Study material

For microscopy observations, samples were selected for the present studies from different types of cocoon shell made by

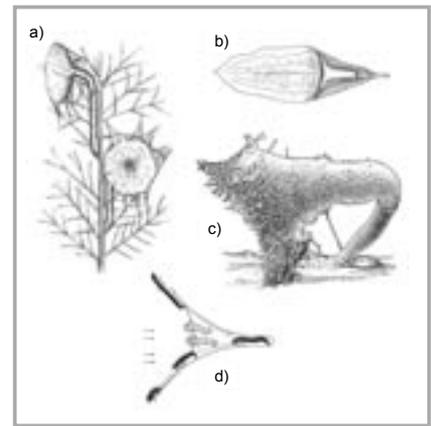


Figure 6. Different types of webs and web-houses of caddis-fly larvae from the family *Polycentropodidae*: a) *Holocentropus dubius*, b) *Cyrnus flavidus*, c) and d) *N. bimaculata*. a and b [23], c [13], d [2].

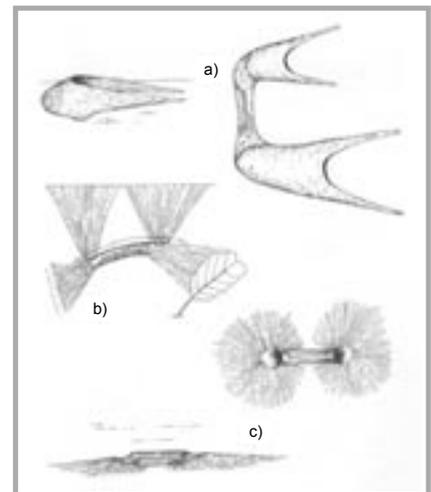


Figure 7. Different types of webs made by *P. conspersa* depending on environmental conditions in running waters: a. fast-moving river, b. shallow river, c. deep river [5].

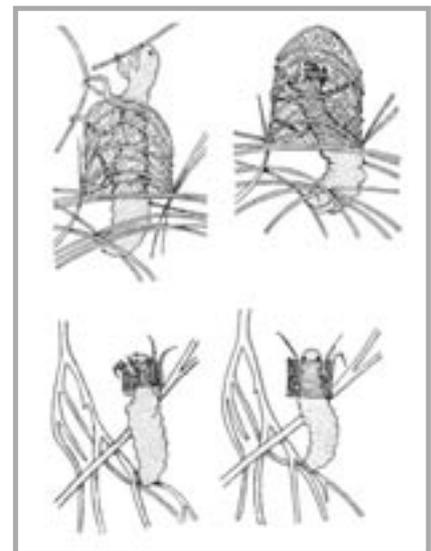


Figure 8. The course of the initial phase of house-building whose construction requires the product of spinning glands [16].

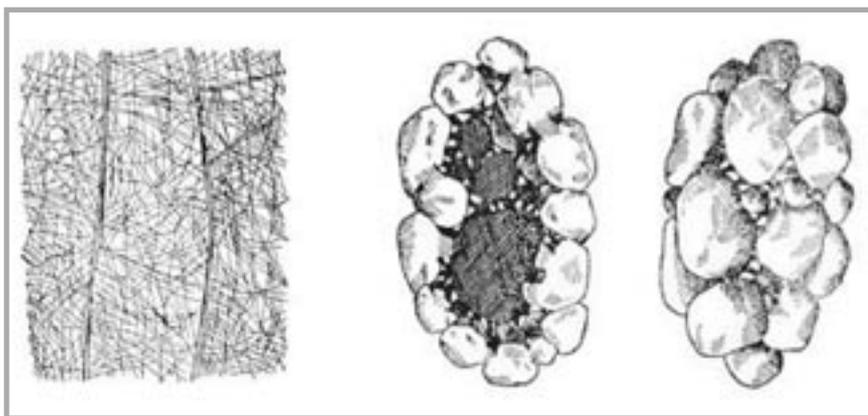


Figure 9. Pupal case and the structure of floss weave in the cocoon of larva *Rhyacophila subovata* [13].

Table 1. Tensile properties of Caddisfly fibres.

| No. of sample | F, cN | ϵ , % | σ , cN/tex | L, cN cm | d_{av} , μm | Tt, tex |
|---------------|-------|----------------|-------------------|----------|--------------------------|---------|
| 1 | 10.31 | 23.8 | 26.2 | 2.45 | 25 | 0.393 |
| 2 | 5.40 | 16.0 | 21.5 | 0.86 | 20 | 0.251 |
| 3 | 5.40 | 18.0 | 21.5 | 0.97 | 20 | 0.251 |
| 4 | 5.45 | 22.5 | 16.0 | 1.23 | 22 | 0.304 |
| 5 | 4.41 | 14.6 | 19.4 | 0.64 | 19 | 0.227 |
| 6 | 2.06 | 6.8 | 22.9 | 0.14 | 12 | 0.090 |
| 7 | 5.40 | 18.0 | 21.5 | 0.97 | 20 | 0.251 |
| 8 | 7.95 | 21.5 | 23.9 | 1.71 | 23 | 0.332 |
| 9 | 3.38 | 11.2 | 13.5 | 0.38 | 20 | 0.251 |
| 10 | 4.75 | 14.4 | 44.8 | 0.68 | 13 | 0.106 |
| 11 | 5.53 | 11.9 | 24.5 | 0.66 | 19 | 0.226 |
| 12 | 4.21 | 10.7 | 12.7 | 0.45 | 23 | 0.332 |
| 13 | 4.35 | 9.6 | 17.3 | 0.42 | 20 | 0.251 |

larvae of *Hydropsyche pellucidula* (*Trichoptera, Hydropsychidae*). These caddisflies inhabit the lowland River Drzewiczka (the biggest right-bank tributary of the River Pilica). The material studied included webs, structural floss forming a construction skeleton of shelters for caseless larvae, and floss from cocoon shell. Some samples were taken directly from the river and preserved in 70% ethanol. Other samples originated from laboratory-bred insects and were stored in distilled water. The tensile test were carried out on only one type of fibre material, on the structural flosses from cocoon shells.

Methodology of studies

Microscopy observations

Fibres selected from the studied samples were observed using computer image analysis, on a measuring stand composed of a microscope (Biolar), a CCD Elemis KK 35 video camera and a PC computer. Lucia software was used, enabling the automatic separation of objects from the background [12].

Samples for microscopic studies were prepared in the form of permanent microscopy slides (placed on micro-slides) in the environment in which they were stored; for studies of tensile strength, the samples were taken at random only from structural floss stored in ethyl alcohol.

Tensile strength tests

Tensile strength tests were carried out using an Instron tensile tester. The distance between clamps was 10 mm, and the elongation speed 10 mm/min. The length of the fibres did not enable them to be clamped directly in the tensile tester, so before measurements they were placed in paper frames and stored in alcohol. Measurements of tensile strength were carried out just after they were removed from the alcohol. In the laboratory, climate conditions were normal ($t = 20\text{ }^\circ\text{C} \pm 2\%$, $R_h = 65\% \pm 5\%$). For every sample we determined the values of breaking force, of absolute elongation and relative elongation at break. On the basis of breaking force measurements, the tenacity was calculated. To make calculations, it is neces-

sary to determine the linear density of the fibres studied. In order to do this, after determining the breaking force and elongation, the broken parts of the fibres were placed on a micro-slide in an alcoholic medium, and then under the microscope; the crosswise dimensions of fibres were determined. In this case a Lanametr projection microscope was used. The value of the crosswise dimension as determined by an optical method was considered on the fibre diameter. The mean diameter d_{av} was determined for each fibre on the basis of 20 measurements. Later, its linear density T_t was calculated.

Results

Microscopy observations

Images of fibres taken from the studied samples are presented in Figures 10a - 10g. They were registered on the stand for computer image analysis. The figures show the lengthwise views of the fibres. All the images were obtained at the same magnification. In order to make the visual analysis of the images easier, a micrometric grid with a scale, obtained at the same magnification at which the photographs presented in Figures 10a-10g were taken, is presented on each figure.

The images obtained are insufficiently accurate to enable clear determination of the shape of the fibre's cross-section. Additional investigations made with a scanning electron microscope (SEM) showed that the fibres' cross-sections were circular. An example of the results obtained is presented in Figure 11. In some cases two fibres were connected together, as we can see in the weave structures presented in Figures 10a and 10f, and as a double fibre cross-section in Figure 12. In such a case, the proportion of the largest to the smallest crosswise dimensions can be more than 2:1, and the fibres therefore appear to be flat (Figure 10f).

Tensile strength tests

The test results are given in Table 1. The table contains values of breaking force F for each sample studied, expressed in cN, values of relative elongation ϵ , in%, read at the peak of the curve of the drawing, the crosswise dimension of each fibre subjected to breaking d_{av} , expressed in μm . The values of thickness are mean values of 20 measurements for each fibre. d_{av} values are given in μm . The samples were numbered according to the order of measurements.

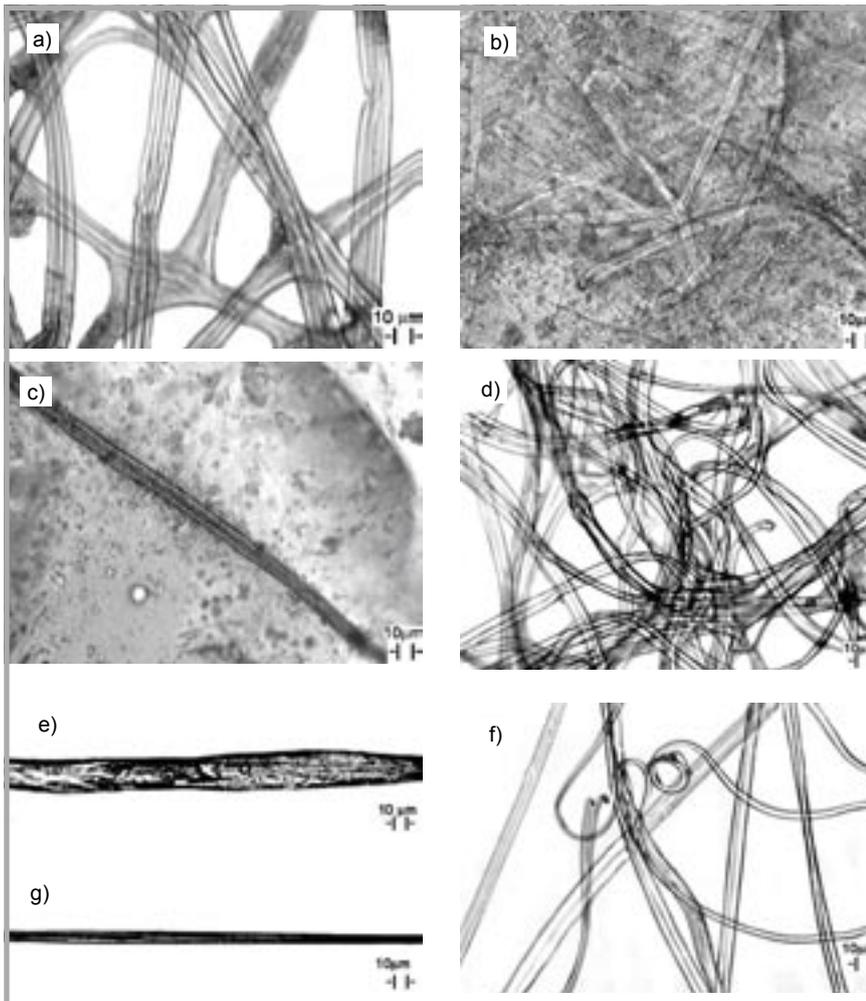


Figure 10. Microscopic views of weave structure and flosses; a) weave structure in the web with places marked where single fibres are knotted, b) weave structure in the cocoon shell, c) floss from a cocoon shell, d) structural floss forming a construction skeleton of the shelter, e) lengthwise projection of a floss taken from a cocoon shell, f) structural floss forming a construction skeleton of the shelter, g) lengthwise projection of structural (skeleton) floss.

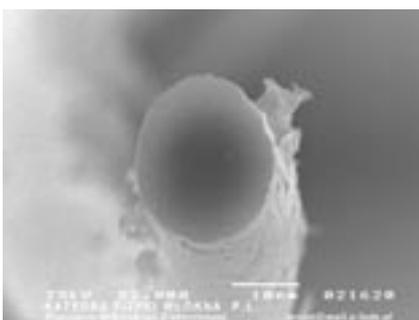


Figure 11. Fibre cross-section of a structural (skeleton) floss.

Assuming that d_{av} is the fibre diameter, the linear density Tt of each fibre studied was calculated. The density of fibres was assumed to be the value of density of ethyl alcohol, that is 800 kg/m^3 . This value was assumed on the base of observing the behaviour of the studied fibres in ethyl alcohol. The linear density values are given in tex.

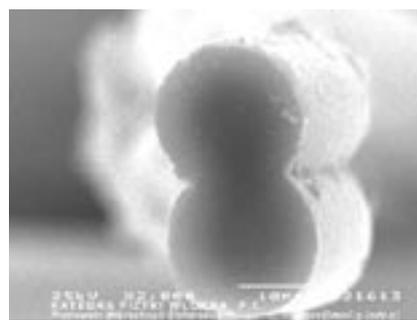


Figure 12. The cross-section of a double fibre of the structural (skeleton) floss.

On the basis of these results, the specific strength of each fibre and the mean value of specific strength were calculated. Values of tenacity range from 12.7 cN/tex to 44.8 cN/tex . The mean value of tenacity is 22.1 cN/tex , and the mean relative elongation at break is 15.3% . There is a great dispersion of measurement results. The variation coefficient of specific

strength is 37.26% , and of elongation at break 34.4% . Additionally, the work to break (in cN cm) was calculated for every sample and also presented in Table 1. It is clearly visible that high elongation at break is decisive for a great work needed to break the fibre.

Discussion

This work presents preliminary results obtained for the structural floss of only one type of cocoon shell.

The preliminary results show that the specific strength of fibres produced by caddis-flies is very high, reaching 45 cN/tex ($5.7 \times 10^9 \text{ N/m}^2$). The value of specific strength estimated on the basis of the results obtained for Trichoptera fibres is 1.5 times higher than the specific strength of steel [26], and is also as high as the strength of spider's floss, which is only two times higher than the strength of steel [27]. However, the specific strength of Trichoptera fibres is four times lower than the specific strength of aramid fibres [7] - ($180 - 190 \text{ cN/tex}$). According to [3], caddis-fly silk is one of the weakest natural silks so far reported, with a mean tensile strength of $(221 \pm 22) \times 10^6 \text{ N/m}^2$. Our results ($5.7 \times 10^9 \text{ N/m}^2$) show that the product of the trichoptera salivary gland reaches the breaking strength and elastic modulus of spider dragline silk (up to $2 \times 10^9 \text{ N/m}^2$ and $30 \times 10^9 \text{ N/m}^2$ [25-11] and highly drawn nylon ($0.7 \times 10^9 \text{ N/m}^2$ and $2.4 \times 10^9 \text{ N/m}^2$ [1]. The tensile strength of caddis fibres is comparable to the same parameter of the threads of a spider's web. However, if we compare spider dragline silk to Kevlar, we find that the silk has lower strength [8].

If further studies confirm these preliminary results, it will be possible to use caddis-fly fibres for different technical purposes, especially for use in aquatic conditions, because natural caddis floss is made and used in water. It is quite possible that they can also be used as surgical threads or bio-bandages, if they turn out to be biocompatible with human tissues, especially as nature created them to be used in an aquatic environment. Studies are currently under way on the chemical composition of caddis floss and on its biocompatibility with tissues of live organisms.

Studies conducted in [24] on the cocoon shells of *Rhyacophila fasciata* demonstrated the exceptional properties of this

material. This regular matter was formed from floss 5 µm in diameter and a mesh of 30×40 µm, and it has the properties of a semi-permeable membrane, selective only to some ions: CO₃²⁻, Cl⁻, NO₃⁻, SO₄²⁻, N⁺, K⁺, Mg²⁺ or Ca²⁺. Thus there are chances that this structure could be used as intelligent clothing. Each species has its own characteristic parameters of floss diameter and mesh density. Therefore they can be used for selections of ions. Additionally, it should be taken into account that caddis floss is sometimes as fine as spider floss (starting from 0.5 µm in the case of floss from the cocoon shell of *Limnephilus vittatus* [4]). This is of crucial importance in various measuring optical devices. Now we can be even more precise, thanks to the *Trichoptera*'s cocoon shell. Certainly, production costs will decide whether caddis floss can be successfully competitive with nanofibres, whose technology is being worked on.

Conclusions

1. The investigations presented were in the nature of a 'reconnaissance', and did not encompass all the possible physical and chemical properties of caddis threads. Therefore our data does not finish such research.
2. The tensile strength of *Trichoptera* salivary glands does not differ significantly from the strength of spider thread or anthropogenic fibres such as nylon or aramid fibres. This data is comparable.
3. The small diameter of thread and regular, dense meshes in pupal cocoons function as a semi-permeable membrane, which gives medicine hopes of devising an intelligent bandage on their basis.
4. So far, the special 'sister' structure (visible on the cross-section) of caddis fibres are unsubstantiated. It may yield exceptional properties. This aspect will be investigated in subsequent research.
5. Further investigation into the structure and properties of fibres produced by caddis-fly larvae should enlarge the current state of knowledge of this kind of fibres. However, the test material should be considerably larger and more differentiated.

Acknowledgment

We would like to express our gratitude to Maria Grzybkowska and Janusz Majecki for their comments and help in preparing biological material, as well as Henryk Wrzosek for taking microscope pictures.

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Received 05.04.2005 Reviewed 24.10.2005