Proc. Zool. Inst. Russ. Acad. Sci., 310, 2006: 107-112

Heterogeneity of the byssal threads in *Mytilus edulis* L. on different stages of attachment

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Mytilids, blue mussel (*Mytilus edulis* L.) in particular, spend most part of their life attaching to substrata. Blue mussels utilize byssal hold-fasts to secure attachment to hard substrata (Price, 1983; Berger *et al.*, 1985). For attachment mussels form from one to several tens of byssal threads. These threads keep the animals on an attachment site. Number and thickness of the byssal threads are determined by many factors, such as temperature, salinity, wave action, predators (Dolmer & Svane, 1984; Cote, 1995; Voronkov, 1995; Kulakowski *et al.*, 2000; Lin, 1991; Selin & Vekhova, 2004b).

Variations in byssal thread production and mussel mobility with changes on the ontogenic stage and environmental conditions have been reported for some species of mytilids (Berger *et al.*, 1985; Selin & Vekhova, 2004a). Some authors (Eckroat & Steel, 1993; Clarke & McMahon, 1996) stated that the structure and morphology of the byssal threads are invariable in the majority of cases.

However, in laboratory studies authors found that mussels formed different byssal threads during a short period of attachment. Significant differences in the diameter, number and structure of threads were detected. The present study dealt with byssal thread production and movement of *Mytilus edulis* L. at different stages of attachment.

Materials and methods

Laboratory experiments were carried out at the White Sea Biological Station of the Zoological Institute of the Russian Academy of Sciences (Kandalaksha Bay, White Sea). Juvenile mussels *M. edulis* with shell length of 15-20 mm were collected from artificial substrata used as collectors for aquaculture in the Kruglaya Bay. Collected mussels were acclimated to the constant temperature of 10 °C and salinity of 24‰ for a period of three days.

Mussels were placed into laboratory dishes with coordinate grid on the bottom (each specimen into a separate dish). Mollusks were kept during 24 hours. In most cases this interval was enough for attachment (Kulakowski *et al.,* 2000; Khalaman & Lesin, 2004). Mussels movement and attachment behavior on different stages of attachment was recorded using digital video camera. On completion of the exposition, video records were analyzed with the computer. For fixing of all the attachment places a coordinate grid was used. The time and continuance of the attachment were fixed by timeline of the video recording.

All byssus threads formed were collected from the dishes. When a mussel by completion of the experiment was attached to substratum by a thick byssal tuft, these threads were carefully cut from the animal and substratum. Collected byssal threads were examined under the microscope. Diameter and morphological features were registered.

Results and discussion

Mussels started to form byssal threads during the first hour of the experiment. We observed multiple attaching and reattaching during the experiment in most cases. Three basic stages (phases) of attachment were differentiated on the basis of video recording.

On the first stage of attachment the mussels actively moved and looked for suitable attachment site, forming sometimes single threads. This phase was named *quest stage*

On the second stage animals stopped and attached to substrate by 2-4 threads for a short time. This phase was named *preliminary attachment stage.* In some cases mussels broke preliminary attachment threads and returned to quest stage. On the most suitable attachment site mollusks formed the permanent byssal tuft. This phase was named *permanent at tachment stage.*

On the different stages of attachment mussels formed byssal threads different in size and morphological features.

Quest stage. Animals continued looking for an attachment site, generally, from 0.5 to 3 hours. During the movement mussels produced single thin threads. The size of these threads was approximately 42.4 ± 2.7 μm (see Table). The surface of the threads, formed on this stage, is smooth, transparent and non-structured (Fig. 1, *A).* However, in some cases mussels produced colored flat threads (Fig. 1, *B*). Some of these threads are stranded along longitudinal axis (Fig. 1, *C*).

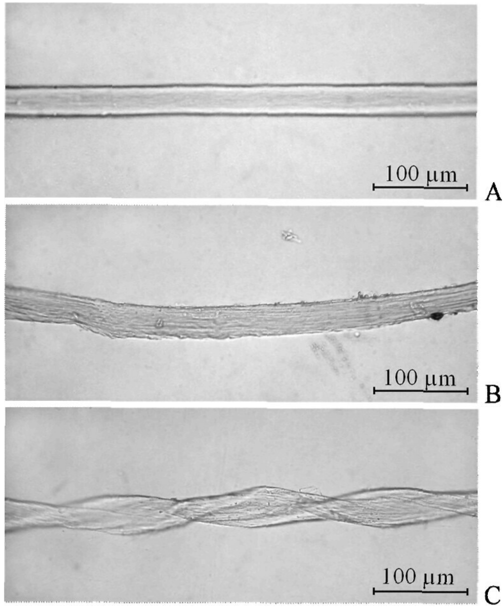

Fig. 1. Single byssal threads formed by mussels on the quest stage

Table. Characteristics of byssal threads of *M. edulis* on the different stages of attachment

	Quest stage	Preliminary attachment	Permanent attachment
Average diameter of threads, μm (mean ± 95% confidence interval)	42.4 ± 2.7	73.1 ± 6.9	63.1 ± 4.3
Minimum diameter of threads, μm	32.8	25.6	38.4
Maximum diameter of threads, μm	58.4	138.2	87.4
Number of threads	1	2-3	5-32
Continuance of the stage, *h*	0.5-3	1-3	–

Preliminary attachment stage. The byssal threads produced by mussels on this stage were different in their size and structural features. The diameter of the threads from the same tuft varied within the extremely wide range from 25.6 to 138.2 100 μm. (see Table, Fig. 2, *A)*. Average diameter of the threads, formed during the preliminary attachment stage was 73.1 $\pm$ 6.9 μm.

We observed a considerable diversity of the threads morphology in the provisional tufts, formed by mussels on this stage. Some threads were thin and either transparent or colored, just as threads produced on the quest stage (Fig. 2, *A)*. Most of the preliminary attachment byssal threads were thick and flat with smooth or folded edge (Figs 2, *B, C)*.

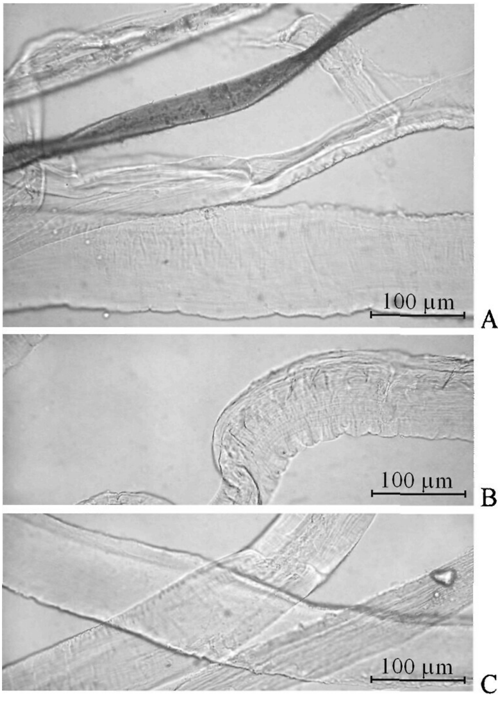

Fig. 2. Byssal threads formed by mussels on the stage of preliminary attachment

Permanent attachment stage. Mussels formed branchy byssal tuft on the permanent attachment stage. The number of threads in this tuft was within the limits of 5 to 32 (see Table). Average diameter of the threads, formed during the permanent attachment stage was 63.1 $\pm$ 4.3 μm. No significant difference in the diameter of threads produced on this stage was detected. All threads from the permanent byssal tuft were transparent with smooth or, occasionally, folded edge.

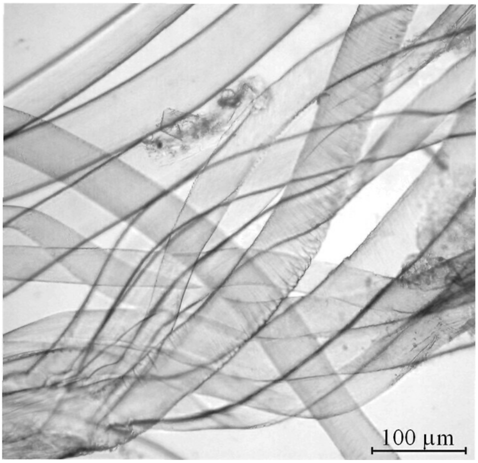

Fig. 3. Byssal threads from the permanent byssus tuft

Selin and Vekhova (2004a), pointed out that forming of the byssus tuft by some mytilids is a complex and prolonged process, the number of threads in the tuft depending on an attachment time. According to these authors, attachment includes several stages, one following the other. Continuation of these stages is approximately one month. Numbers of threads in the tufts are different. These data closely agree with our observations. However, forming of the byssus attachment is a more complicated and varied process. Different stages in the formation of attachment have been observed during the first hours of the experiment. The newly formed byssal threads on these stages differed in size and structure. The mussels changed their behavior from stage to stage as well as byssal threads morphology.

Capability for forming different in thickness and structure byssal threads was observed only for early post larval stages of *M. edulis* (Lane *et al.*, 1985). The above authors stated that young mussels formed special thin threads for a drifting. These drifting threads differed from the threads attaching to substrate in size characteristics and structure.

Similar behavior during the attachment was observed in our experiments in adult animals. We suppose that forming of single threads in adult mussels, possibly, resemble the analogous process in young post-larval *M. edulis.* Absence of the ultrastructure data does not permit us to compare threads for drifting with threads produced on the quest stage.

Up to now the authors cannot offer any plausible explanation for the functional role of different byssal threads and for their role in *M. edulis* attachment. We intend to conduct further studies for elucidation of this issue.

This study was supported by the Russian Foundation for Basic Research (grants nos. 03-04-49701 and 06-04-48789).

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