Influence of timing of space availability (slide exposure) on the development of natural biofilm communities was studied in a shallow area of a temperate lake in Orillia, Ontario, Canada. Four sets of glass slides were suspended in the sub-surface waters and the biofilm growth was monitored with intermittent sampling for a maximum period of 120 h. One set each of the glass slides was exposed in the lake at 6am, 12pm, 6pm and 12am on the starting day. The microalgal species composition and density, and biofilm thickness were monitored. The results showed two major trends: (1) the species composition and biofilm thickness on slides with different times of initiation varied significantly and (2) the initial species composition significantly influenced the subsequent growth of biofilm community. The results of this study provide better understanding of the initial community dynamics in natural biofilms in inland waters.