Wild aquatic birds are the natural reservoir of avian influenza virus (AIV), and the virus is transmitted among birds through a fecal-oral route. Infected birds excrete significant amounts of AIV into the environment, and thereby sustain the circulation of AIV in the bird populations. Improved knowledge on the influence of environmental factors on the persistence of AIV in natural habitats would be valuable for risk assessments. The presented work investigated the persistence of two low-pathogenic AIV subtypes in natural water samples. The study included two AIVs formerly isolated from wild ducks, which were suspended in filtered natural fresh, brackish or sea water with salinity of 0, 8000 and 20,000 parts per million (ppm), respectively. Also sterilized brackish and sea waters were included in order to examine the influence of microbial flora on virus persistence. All water samples were incubated at temperatures representative for seasonal variation of ambient temperatures in Northern Europe (4, 17 and 25°C). The results showed a clear correlation between persistence of viral infectivity and temperature, salinity and presence of microbial flora. While independent of virus subtype, the persistence of infectivity was negatively affected by increased temperature, salinity as well as presence of natural microbial flora. The study provides insight on impact of essential physical, chemical and biological parameters on persistence of AIV in aquatic environments. Studies determining the importance of additional environmental parameters and the detailed mechanisms of microbial inactivation of AIV should be encouraged.