In recirculating aquaculture systems (RAS), the water quality changes continuously. Organic and inorganic compounds accumulate creating toxic conditions for the farmed organisms. Ozone improves water quality diminishing significantly both bacteria load and dissolved organic matter. However, in a non-meticulously designed system, residual ozone might reach the culture tanks causing significant harm to cultured species or excess costs. The aim of the study was to predict the suitable ozone dosage in pilot RAS, for water treatment purposes, based on laboratory studies. The ozone effect on water quality of freshwater RAS and system’s ozone demand was investigated. Bench-scale ozonation experiments revealed the ozone demand of the system to be 180 mg O3/h. Three different ozone dosages were applied to four replicated systems with fixed feed loading (1.56 kg feed/m³ make up water). Results suggested that the optimal ozone dosage was 15g O3/kg feed. Selected water quality parameters were measured, assessing biofilters performance as well as nitrogen and carbon–based compound concentration change during ozonation. Overall, this study contributed to a better understanding of the challenges of an ozonated RAS leading to the optimal design of such systems.