In 1964, van Bergeijk asked, "...given that a fish can discriminate between sounds A and B when they are presented separately, can he still discriminate either one when both are presented simultaneously? Or do the two sounds blend to form a new entity (such as a chord)." This question was a very early query about sound source segregation by fishes. We have investigated the role that frequency separation plays in this phenomenon using classical respiratory conditioning with a stimulus generalization paradigm. Groups of animals were first conditioned to 2-tone mixtures comprised of 150 Hz (A) and another frequency (B) ranging from 300 Hz to 750 Hz. Generalization tests were then carried out using single tones between 50 and 900 Hz. Group mean generalization gradients showed that the two tones were segregated at the widest A-B spacings, and tended to be segregated as the A-B spacing was reduced. The limited resolution of the test procedure did not allow an answer at the narrowest spacings. Source segregation, even of abstract tones, may be a fundamental feature of the sense of hearing of all organisms, and occurs in the absence of auditory cortex and complex cognitive abilities.