

Memo

To Dhr. J. Oosterbaan; Rijnland Water Board
From Rineke Keijzers & Jaap Postma, Ecofide
Date June 27th, 2017
Concerning Field research regarding possible effects of ultrasonic sound on zooplankton

Confidential Urgent Normal

Occasion

In cooperation with the municipality of Zoetermeer and the Rijnland Water Board, LG Sonic will conduct a two-year pilot regarding the possibilities to decrease the nuisance caused by blue-green algae in the Zoetermeerseplas with ultrasonic sound. The pilot has started in 2016 and 2017 will be used for further optimization of this control method.

One of the focus points is the question how much side effects this ultrasonic sound might have on the zooplankton. Previous research in lab conditions shows that a rise in death may occur (Lüring & Tolman, 2014). At the same time LG Sonic indicates that effects might not be that bad, based on different research (Herrewijn, 2012). One of the uncertainties is the question if the results from the laboratory can be translated directly to a field situation. Therefore, the Rijnland Water Board has asked Ecofide to perform research regarding the possible side effects, by conducting several field experiments with the water flea *Daphnia magna*.

Objective

The research should answer the question if applying ultrasonic sound in field conditions may cause negative effects on zooplankton.

Activities

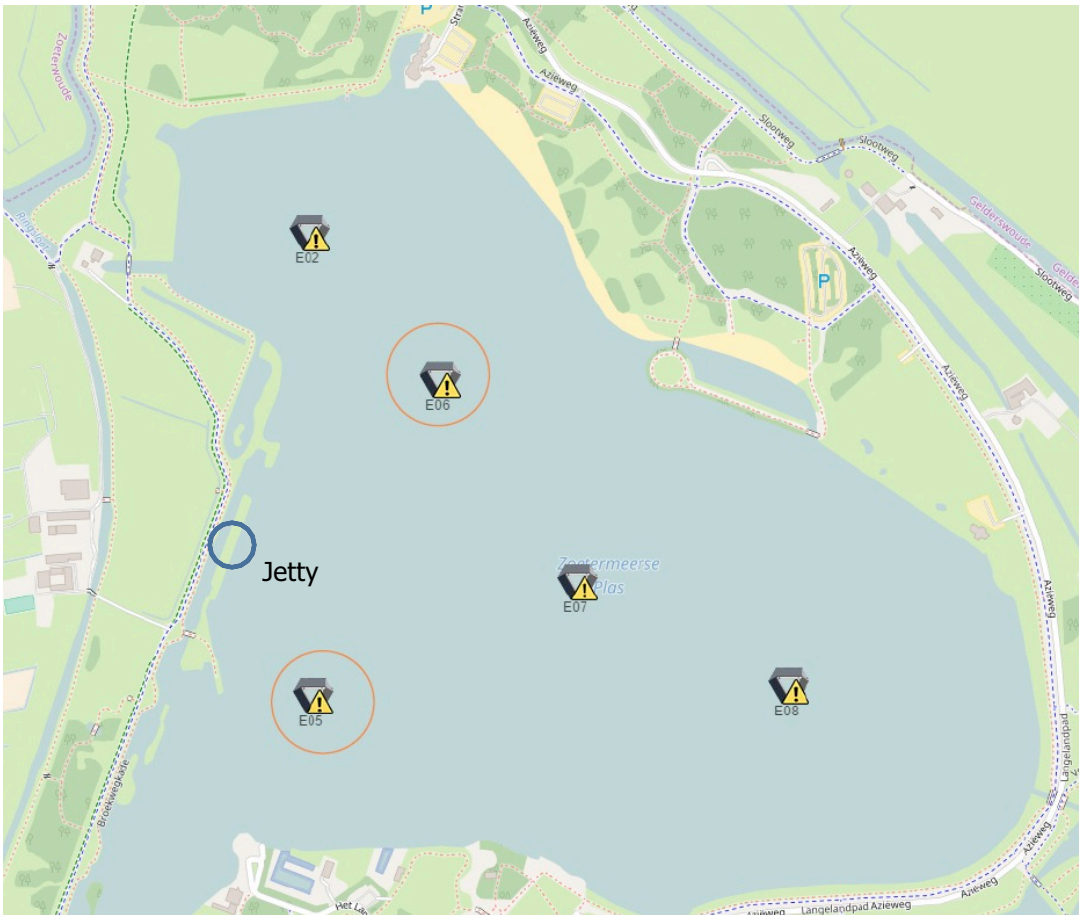
To answer the question as stated above, so called in situ experiments have been conducted. In these experiments, lab-bred water fleas (10 days old) are being released in cages for a period of 7 days (10 animals per cage). After 7 days, the number of surviving water fleas and the number of produced young are being counted. This testing method is and has been applied often to field research regarding the effects on zooplankton. For example, the Delfland Water Board has been using this method for years to study the effects of pesticides (for example see Ecofide overview report 2008), as well as in the current Waternet research (Simoni, part of Ecological Key Factor toxicity) these field experiments form an important part (van der Oost et al., 2017; Stowa, 2016).

Experimental setup

Logically the intensity of the applied ultrasonic sound is highest when there are actually blue-green algae present in the Zoetermeerseplas. This will most likely be the case in late summer, beginning of autumn. Seen from the experimental setup, this is a complicating factor, because blue-green algae themselves could have negative effects on the water flea. To exclude the negative effects of blue-green algae, research will be conducted earlier in the season, in a more experimental situation. After the buoys have been placed in the Zoetermeerseplas in spring, a control test had been conducted. This test considered the survival of the water fleas, without the buoys being on. This control test had been deployed on May 8th 2017. In the following week, the in situ experiment had been repeated, whereby two ultrasonic buoys were transmitting sound on an intensity and frequency, representative for the actual control situation of autumn 2016. A so called 'broadband program' had been used, in which multiple frequencies with a reach between 20 and 80 khz had been used at approximately 5 watts. This test, in which two ultrasonic buoys were sending out sound, has been deployed on May 15th 2017, and was pulled out a week later, on May 22nd.

The Zoetermeerseplas had five buoys, with two buoys (E05 and E06) having their transmitter on during the second week and three buoys (E02, E07 and E08) measuring water quality online (see chart below). The buoys are separated from each other by about 300 meters. This means a gradient with a distance of about 0, 300 and 600 meters from a transmitter. The transmitter's reach was estimated by LG Sonic to be approximately 500 meters. This means that E02 on 300 m is being influenced by 1 transmitter, E07 on 300 m by 2 and E08 at a 600 m distance by none. This setup also means that in the 2nd series of field experiments (in which the transmitter of the two buoys was switched on) there was a reference situation (buoy E08). Besides the filed cages connected to the buoys, cages have been hung to the jetty along the west side of the lake, about 150m away from buoy E05, to increase the distance range, during both series in situ experiments

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During both the 1st and the 2nd series in situ experiments, in principle three cages each containing 10 water fleas have been hung out at the five available buoys (triplo implementation). Three cages were also attached every time to the westside jetty.



Buoy with transmitter



Field cage attached to the jetty

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Results

The results of the two field experiment series are being presented in chart 1. Both the survival of the mature water fleas as well as the reproduction (number of young) after 7 days are being presented.

During the pulling out in the first experiment it turned out that the buoys on the lake were moving around considerably and much wear had been caused to the ropes, with which the cages were attached to the buoy. This has resulted in the loss of several cages in the first experiment (marked 'x' in the chart). Despite the availability of several reserve cages, not everything could be conducted threefold in the 2nd series (open cells in chart 1). The field cages had been extra secured in the second series, resulting in no loss.

Chart 1. Survival and reproduction of water flea *Daphnia magna* after 1 week hung out in the field

Location	Interference	Survival <i>Daphnia</i> (%)				Reproduction <i>Daphnia</i> (Number of juveniles)			
		Replica				Replica			
		1	2	3	gem	1	2	3	gem
Series 1 (control)									
Buoy E 05	none	100	90	x	95	99	94	x	97
Buoy E 06	none	100	x	x	100	114	x	x	114
Buoy E 02	none	100	100	x	100	97	101	x	99
Buoy E 07	none	100	100	100	100	92	83	119	98
Buoy E 08	none	100	x	x	100	103	x	x	103
Jetty	none	100	100	100	100	112	87	107	102
Series 2 (Exposure to ultrasonic sound)									
Buoy E 05	0m	100	90	100	97	58	41	22	40
Buoy E 06	0m	100	100		100	59	51		55
Buoy E 02	300m by 1	100	90		95	51	39		45
Buoy E 07	300m by 2	100	100	100	100	48	51	42	47
Buoy E 08	none (600m)	100	100		100	50	49		50
Jetty	150m by 1	100	100	0	67	41	63	29	44

Discussion

No negative effects caused by ultrasonic sound have been found regarding the survival of water fleas. Both in series 1 (without exposure) as well as in series 2 (with exposure) the average survival rate for water fleas is 95-100%. The only exception is 1 cage, which was attached to the jetty in series 2. All water fleas died after a week, but besides water fleas, the growth of thread algae could also be observed in this cage. The high mortality rate could not be explained as a result of ultrasonic sound and is most likely been caused by other factors.

The reproduction of adult water fleas from the 2nd series was generally speaking half of the water fleas from series one, but was the same across the several attachment points. The 50% lower reproduction seems graver than it actually is. The number of juveniles in the 2nd series was indeed 46 compared to 101 from the control week, but there were 10 animals in a cage. This means that the animals had each produced 5 fewer juveniles. It is good to know that 10-day old animals are starting to reproduce and that 5 juveniles more or less fall in the grey area. Whether the animals were 10,0 days or 10,9 days when put in, can mean that mothers just, or just not produce another extra hatch during the week hung out. Thereby, reproduction is more or less a variable parameter, also depending on factors like food supply. That makes this parameter suited to check if differences occurred within a series in relation to the distance compared to the buoys. Therefore it is determined whether the animals in the five cages with a distance from 0 meter (E05 and E06) had a significantly lower reproduction, when compared to the animals at a distance of 150, 300 or 600 meters. This was not the case.

Conclusions

The conducted research didn't provide any indications for a possible negative effect of ultrasonic sound on zooplankton.

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References

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