Annual Report

The surveillance programme for Aphanomyces astaci in Norway 2017

Veterinærinstituttet
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The surveillance programme for Aphanomyces astaci in Norway 2017

Content

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Preface

Until 2015 surveillance of *Aphanomyces astaci* (crayfish plague), commissioned by the Norwegian Food Safety Authority (NFSA), was conducted by the Agency for outlying fields, Akershus & Østfold (AAØ) on the basis of cage experiments with live noble crayfish. In cases of mortality where crayfish plague could not be excluded, crayfish were sent to the Norwegian Veterinary Institute (NVI) for diagnostics.

Decapods including Noble crayfish are covered by the animal welfare act (LOV-2009-06-19-97). Thus, if an alternative method is available, the use of live animals for disease surveillance should be reduced.

In 2016, the NVI designed a new surveillance program on the basis of environmental DNA (eDNA) monitoring of the crayfish plague pathogen *Aphanomyces astaci,* combined with the traditional cage experiments. A collaborative pilot surveillance project (*NOK A. astaci 2016*) that was in part funded by the NFSA and in part funded by the research project TARGET (NRC- 243907) was offered.

In 2017, a similar collaborative project formed the basis for the *A. astaci* surveillance program (*NOK A. astaci 2017*), but this time the cage experiments were no longer included. To expand the synergies with on-going monitoring programs, we initiated collaboration with the National surveillance program for noble crayfish (*Astacus astacus*). This program, commissioned by the Norwegian Environmental Agency (NEA), was conducted by the Norwegian Institute for Nature Research (NINA) in 2017. The aim was to utilize the same water samples and downstream laboratory work for eDNA monitoring of *A. astaci* and noble crayfish in overlapping surveillance areas.

TARGET aims to develop cost-effective and environmentally friendly monitoring tools and control strategies for better safeguarding of noble crayfish in collaboration with the user group, on-going monitoring programs and project partners. The joint activity with *NOK A. astaci 2016 & 2017* therefore naturally fell within the scope of the TARGET project.

In the current project, the NVI (through the TARGET project) and the NFSA agreed on sharing the costs at ~40/60%, respectively.

Oslo March 20th 2018

Trude Vrålstad

Project coordinator (TARGET & NOK *A. astaci* 2017)

Summary

In this surveillance program, we used environmental DNA (eDNA) monitoring of the water as an alternative method to the traditional cage experiments with live noble crayfish. Here, DNA from spores of *Aphanomyces astaci* are detected directly from water filtrates. We also determine the presence/absence of eDNA from noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) to supplement the results and to gain the possibility to evaluate the habitat status in more detail. The main geographic focus of this surveillance program has been on the Halden watercourse and neighbouring risk areas. Other covered geographic areas include the Mosse watercourse, Glomma watercourse, and selected areas in Eidskog including the Buåa watercourse, the Vrangselva watercourse and River Finnsrudelva.

In total, 57, 32, 16 and 32 water samples were collected from selected sites in the Halden-, Mosse-, Glomma watercourse regions and in the Eidskog region, respectively. Locations for sampling water were strategically selected and focused on both control zones and in the risk areas adjacent to crayfish plague control zones. The presence/absence of the three target species was determined simultaneously through screening with species-specific qPCR assays.

In 2017, no spread of *A. astaci* was observed in any of the monitored areas.

- In the control zone of the Halden watercourse, *A. astaci* eDNA was only detected in the southern part of Lake Rødenessjøen. Here, the known presence of signal crayfish was confirmed by eDNA detection. No sign of crayfish plague was observed from the northern part of Lake Rødenessjøen (Kroksund) up to the control zone border at Fosserdam. This result was supported by positive detections of noble crayfish eDNA in all water samples from River Hølandselva and upstream. All water samples in this risk area were negative for *A. astaci* and signal crayfish, while positive for noble crayfish eDNA.
- In the Mosse watercourse, no eDNA of *A. astaci* and signal crayfish was detected, while noble crayfish eDNA was detected in Lake Våg.
- In the Glomma watercourse, no sign of crayfish plague was found, and the samples were negative for all screened targets.
- In Eidskog municipality, no samples were positive for signal crayfish or *A. astaci,* while several were positive for noble crayfish eDNA in the Vrangselva watercourse and River Finnsrudelva.

Introduction

The oomycete *Aphanomyces astaci,* the causative agent of crayfish plague, is a lethal pathogen on native European freshwater crayfish (1-3). It is carried and transmitted by North American freshwater crayfish, which act as healthy carriers of the pathogen. *Aphanomyces astaci* reproduces and spreads with swimming zoospores, the infective stage of the pathogen. It was accidentally introduced to Europe ~160 years ago, and resulted in mass-mortalities of freshwater crayfish all over Europe. It was later re-introduced through many independent introductions of alien North American carrier crayfish (3), in particular signal crayfish.

Crayfish plague is a list 3 disease in Norway, according to the "*Regulation on animal health requirements for aquaculture animals and products thereof, prevention and control of infectious diseases in aquatic animals*" FOR 2008-06-17-819.

Since 1971, seven water systems in Norway have been affected by crayfish plague outbreaks once or several times (4-5). These include the Vrangselva watercourse and River Veksa (1971), the Glomma watercourse (1997 and 2003), Lake Store Le (1989), the Halden watercourse (1989, 2005, 2014), River Lysakerelva (1998), Buåa watercourse (2010) and Mosse watercourse (2016). In 2016-2017, the border watercourse Vrangselva and River Billa (named River Finnsrudelva on the Norwegian side) were confirmed to be struck by crayfish plague on the Swedish side of the border, but the infection has not yet reached the Norwegian side. In addition, four further localities have been (or are still) under crayfish plague regulations due to illegally introduced and confirmed *A. astaci* positive signal crayfish (4).
Until 2015, surveillance of crayfish plague relied on cage experiments with live noble crayfish. In 2016, the *A. astaci* surveillance program combined the classical cage experiments with eDNA monitoring (6).

Here, the eDNA monitoring of *A. astaci* worked as intended, and in combination with the complementary eDNA targets noble- and signal crayfish, it was possible to produce a snapshot of the relevant habitat status. Within the cage experiments, the crayfish mortality was 24% despite that no crayfish plague was detected in any of the 10 cages. Furthermore, another 34% of the crayfish escaped, probably as a result of human interference (vandalism) (6). Based on an overall assessment taking crayfish welfare and cost-benefit into account, the cage experiments were excluded from the surveillance program in 2017.

The focus areas of the 2017 surveillance program for crayfish plague cover the

- Halden watercourse (under regulation FOR-2015-05-26-592)
- Mosse watercourse (under regulation FOR-2016-12-13-1523)
- Glomma watercourse (under regulation FOR-2005-06-20-652)
- Eidskog municipality, including Buåa watercourse, Vrangselva watercourse and River Finnsrudelva (under regulation FOR-2016-08-17-972)

The Halden watercourse was hit by crayfish plague in 1989, re-stocked with noble crayfish in the 1990s, and the population successfully recovered until the crayfish plague returned in 2005 (7). Quick closure of the Ørje water locks prevented upstream spread. In 2008, illegally introduced *A. astaci* positive signal crayfish were found in Lake Øymarksjøen (8), leading to the permanent closure of the water locks. This prevented further spread, until illegally introduced signal crayfish were found upstream of the water locks in 2014. The re-established noble crayfish population in Rødenessjøen was lost during the following plague outbreak. In this period, the TARGET project compared cage-based surveillance with eDNA-monitoring according to Strand et al (9). Here, we followed the infection front through analysis of water, and eDNA of *A. astaci* in the water was sometimes detected prior to crayfish mortalities in the cages. Noble crayfish and signal crayfish eDNA was also detected in the water where they are known to occur (10).

The Mosse watercourse was struck by crayfish plague in 2016. When the crayfish season started in August, the NFSA received concerning reports regarding the absence of noble crayfish from Lake Mjærvann and river Hobølelva. No dead crayfish could be found, but eDNA-analyses of water from the small stream Tangenelva upstream of lake Mjærvann (Enebakk) conducted at the NVI confirmed high levels of *A. astaci* eDNA, corresponding to an out-break situation (9). The NFSA established zone regulations, and initiated surveillance with cages in infected areas. In the cage upstream of the lower dam in the pond Steinkistedammen, the spread of crayfish plague was detected in December 2016 (11), while the cage placed in Lake Våg was not affected until the monitoring was terminated for the season.

The Glomma watercourse was struck by crayfish plague in July 1987, from Kirkenær in Solør and further downstream including Lake Vingersjøen and Lake Storsjøen/Oppstadåa (4). Environmental authorities and landowners cooperated to re-establish crayfish in the river system, but the plague struck again in 2003. Cage experiments combined with crayfish plague diagnostics confirmed active crayfish plague in the system from 2005 until 2015 (4-7). The last detection was in the tributary Opstadåa in 2015.

The Buåa system was struck by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river. A barrier built for preventing the spread of signal crayfish did not stop the infection from spreading, but hopefully stopped the signal crayfish (4). Cage experiments in the area have to date not revealed any active infection source (6).

Aims

This surveillance program aims to

- Monitor the infection pressure and spread of the crayfish plague pathogen *A. astaci* in zone regulated areas as a result of earlier detection of disease (referred to as control zones).
- Substantiate disease free waterbodies in neighboring areas of the control zones (= risk areas).
- Alert the authorities of any eventual spread of the disease from control zone to risk areas.

- Continue to evaluate eDNA as a monitoring tool for *A. astaci* - alone, and in combination with complementary eDNA targets including both the carrier- and susceptible crayfish host species

Materials and methods

Work plan

The surveillance program is based on eDNA monitoring of water, where DNA from spores of A. astaci are detected directly from water filtrates. To complement information on the habitat status, eDNA from the native and susceptible noble crayfish A. astacus and the alien carrier signal crayfish P. leniusculus is monitored within the same water samples. The logistics and analyses are conducted in collaboration with the NFR-research project TARGET (Figure 1).

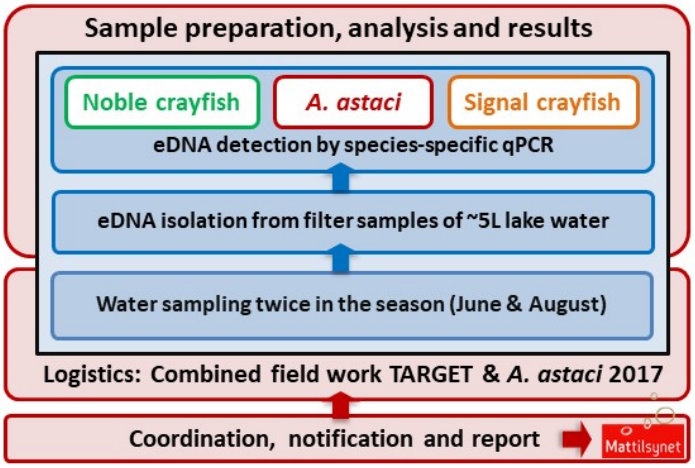

Figure 1. Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with TARGET (NFR-243907).

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse on both sides of the dam and control zone border Steinkistedammen, the Glomma watercourse, and Eidskog municipality including the Vrangselva watercourse, Buåa watercourse and River Finnsrudelva. Plotted locations for water sampling, in total 32 sites, as well as the crayfish plague zones, are given in Figure 2. Supplementary details are summarised in Appendix 1 (Table S2-S5).

Halden watercourse: The control zone was monitored in a total of 6 sites from Lake Fossersjøen to the outlet of Lake Rødenessjøen (Ørje water locks). Live noble crayfish were still expected to be present within the control zone in the upper parts of the system, awaiting the outbreak. Crayfish localities adjoining the control zone or in geographical close proximity are vulnerable to further spread, and referred to as "risk zone" (Table S2, Appendix 1). In total, 7 sites were monitored in the risk zone.

Mosse watercourse: The control zone was monitored from the dam Steinkistedammen and to River Hobølelva, in total 4 sites. The risk zone upstream the dam was monitored in Lake Våg and up to the outlet of Lake Langen, in total 3 sites (Table S3, Appendix 1).

Glomma watercourse: The control zone comprises the main passageway downstream Braskereidfoss in Våler. Only four sites within the control zone were possible to be monitored in 2017 because of resource allocation to new crayfish plague regulated areas in Norway (Table S4, Appendix 1).

Eidskog: the control zone (defined by the municipality boarder) was monitored in the Vrangselva (4 sites) and Buåa (2 sites) watercourses and River Finnsrudelva (2 sites) (Table S5, Appendix 1).

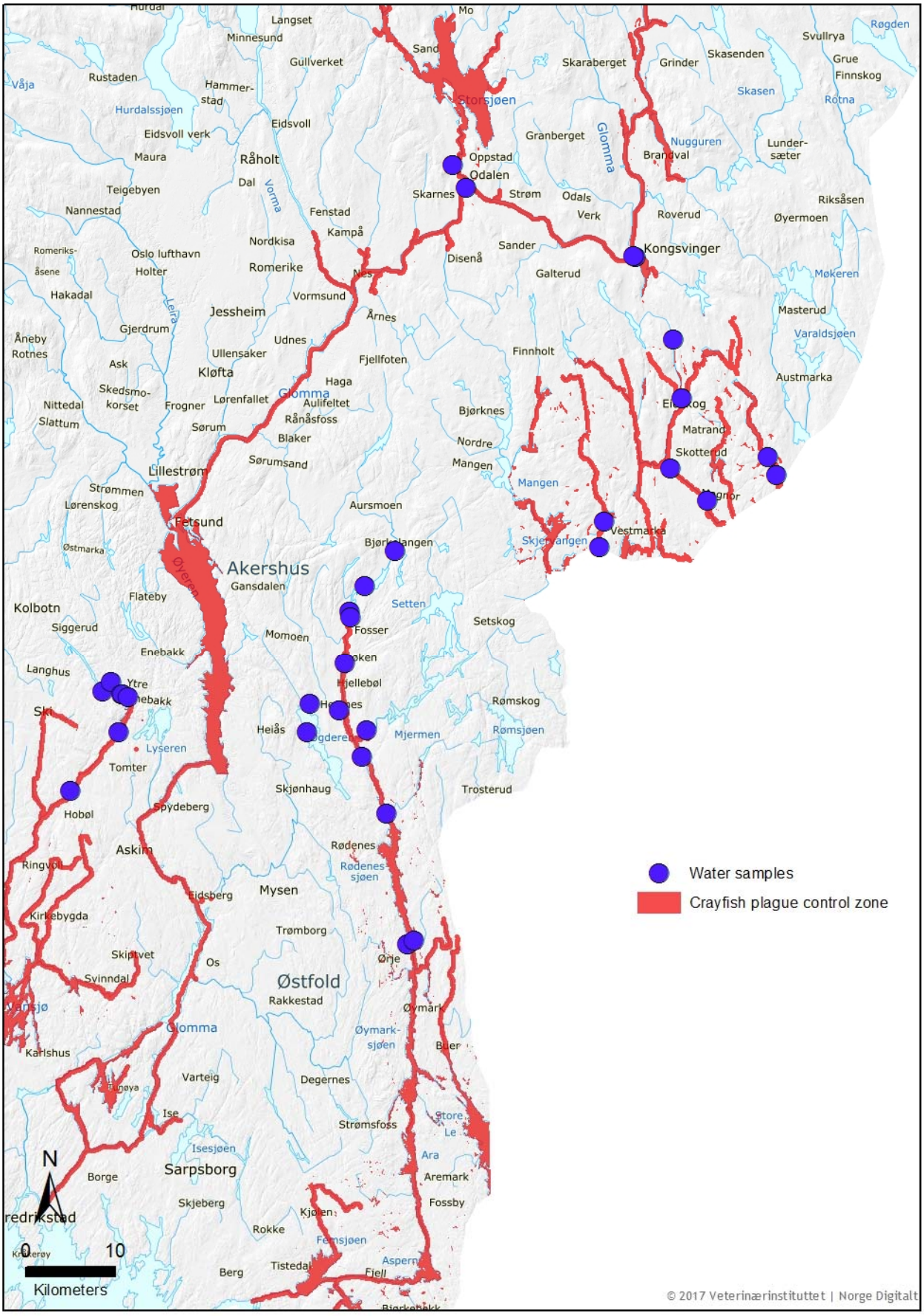

Figure 2. Surveilled sites in Eastern Norway 2017. Water samples (blue dots) were collected in June and August-September. Regulated areas (crayfish plague control zones) are marked in red. Note: For Glomma, the control zone is an approximation.

eDNA monitoring

In the Halden- and Mosse watercourse, water samples were collected in June and September 2017, while in the Glomma watercourse and Eidskog, water samples were collected in June and August 2017.

From each site, two samples of ~5 L water were filtered on-site onto sterile glass fibre filters (9). Ideally, 5 L water was filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower. In some of these cases, we therefore included extra samples to partly compensate for the reduced water volume. This explains the increased number of samples at some sites (Table S2-S5) compared to the agreed number of samples (Table S1).

The filters were transferred with a clean forceps to a sterile falcon tube immediately after filtration, kept on ice during transport back to the laboratory, frozen for a minimum of 24 hours and freeze dried before eDNA extraction (9).

The water samples were screened by qPCR for 3 DNA targets: the species specific qPCR assay for *A. astaci* (10, 12), and two crayfish species specific qPCR assays for noble crayfish and signal crayfish developed by Agersnap et al. (13). Figure 3 presents an overview of the eDNA monitoring procedure.

Figure 3. Water samples of ~5 L each were filtered on-site through glass fiber filters using a portable peristaltic pump (Masterflex E/S portable sampler). Each filter was carefully transferred to a sterile falcon tube, stored on ice before being frozen in the laboratory. DNA was isolated with a large volume extraction procedure, and presence/absence of eDNA from all target organisms was analysed with qPCR. Figure from Vrålstad et al (6).

Results and Discussion

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 57 water samples representing a total of ~230 L water were analysed. In the control zone, *A. astaci* eDNA was only detected in 2 water samples in June at low concentrations from the Southern part of lake Rødenessjøen, location Ysterud (Figure 4, Table S2). In areas with known presence of signal crayfish, this presence was confirmed by positive eDNA results in a total of 5 water samples (3 in June, 2 in September; Figure 4, Table S2).

No sign of crayfish plague was observed during the surveillance period in other part of the Halden watercourse control zone, from the outlet of Skulerudsjøen up to the border of the infection zone at Fosserdam (Figure 4). These results were supported by positive detections of noble crayfish eDNA in all water samples from Hølandselva and upstream, indicating the presence of live noble crayfish inhabiting the northern part of the Halden watercourse control zone. In total, 13 water samples were positive for noble crayfish eDNA on this stretch and in contrast to the 2016-result (6), none of these co-occurred with *A. astaci* eDNA. Thus, the previously reported "outbreak front" observed in the river Hølandselva in 2016

has seemingly not progressed upstream, but more likely has "burnt out". One reason for this may be a longer upstream river stretch with no, or very low densities of crayfish assisted by the continuous downstream water flow that reduces the risk of upstream infection spread.

In general, all water samples from the risk area surrounding the Halden watercourse were negative for *A. astaci* and signal crayfish eDNA, while positive for noble crayfish eDNA. In total, 21 water samples were positive for noble crayfish eDNA (Figure 4, Table S2). The combined absence of *A. astaci* eDNA and presence of noble crayfish eDNA suggest that there has been no further spread of the disease in the surveillance period, and that there are live noble crayfish in the monitored sites. This is supported by CPUE (catch per unit effort) data from the national surveillance program for noble crayfish 2017 (14), where live noble crayfish were documented in Lake Hemnessjøen of a density of 5.48 CPUE.

eDNA monitoring in the Mosse watercourse

In the Mosse watercourse, 32 water samples representing a total of ~113 L water were analysed. None of the analysed samples within and outside the control zone showed any sign of A. astaci or signal crayfish eDNA (Figure 5, Table S3). Seven samples from Lake Våg to River Tangenelva were positive for noble crayfish eDNA in June, but a worrying trend was that only one sample in the same stretch was positive in August. Combined with reports of empty crayfish traps in the national surveillance program for noble crayfish 2017 (14) the current status is unclear, and it cannot be excluded that the crayfish plague has spread unnoticed outside the period of eDNA monitoring.

eDNA monitoring in the Glomma watercourse

In the Glomma watercourse, 16 water samples representing a total of ~77 L water were analysed. No sign of A. astaci or signal crayfish was found through eDNA analysis (Figure 6, Table S4). In contrast to the 2016-results (6), no positive signal for noble crayfish eDNA was detected in the monitored area in 2017. Unfortunately, the number of monitored sites was considerably reduced compared to 2016. The results cannot verify any active A. astaci infection or infection source from the monitored sites in the Glomma.

eDNA monitoring in Eidskog municipality

In the Eidskog municipality, 32 water samples representing a total of ~144 L water were analysed. None of the analysed samples showed any sign of A. astaci or signal crayfish (Figure 6, Table S5). In the Vrangselva watercourse, 9 samples from Åbogen to Magnor were positive for noble crayfish eDNA (6 in June, 3 in August), suggesting that the river stretch is still inhabited by live noble crayfish. In River Finnsrudelva, 6 samples were positive for A. astacus eDNA (2 in June, 4 in August). Here, reports from the Swedish National Veterinary Institute (SVA) indicate that the crayfish plague was about to reach the Norwegian boarder in December 2017 (15).

In the Buåa watercourse, no positive samples were found for any of the screened target organisms. The Buåa watercourse has been monitored by cages for more than 5 years (6). Lack of crayfish plague detection could indicate disease free status. However, a new crayfish plague regulation from August 2016 affects the whole Eidskog municipality (FOR-2016-08-17-972), and replaces the old regulation for the Bua watercourse. Thus, as long as the Eidskog region is covered by one regulation, no conclusion can yet be drawn regarding disease freedom in the Buåa watercourse.

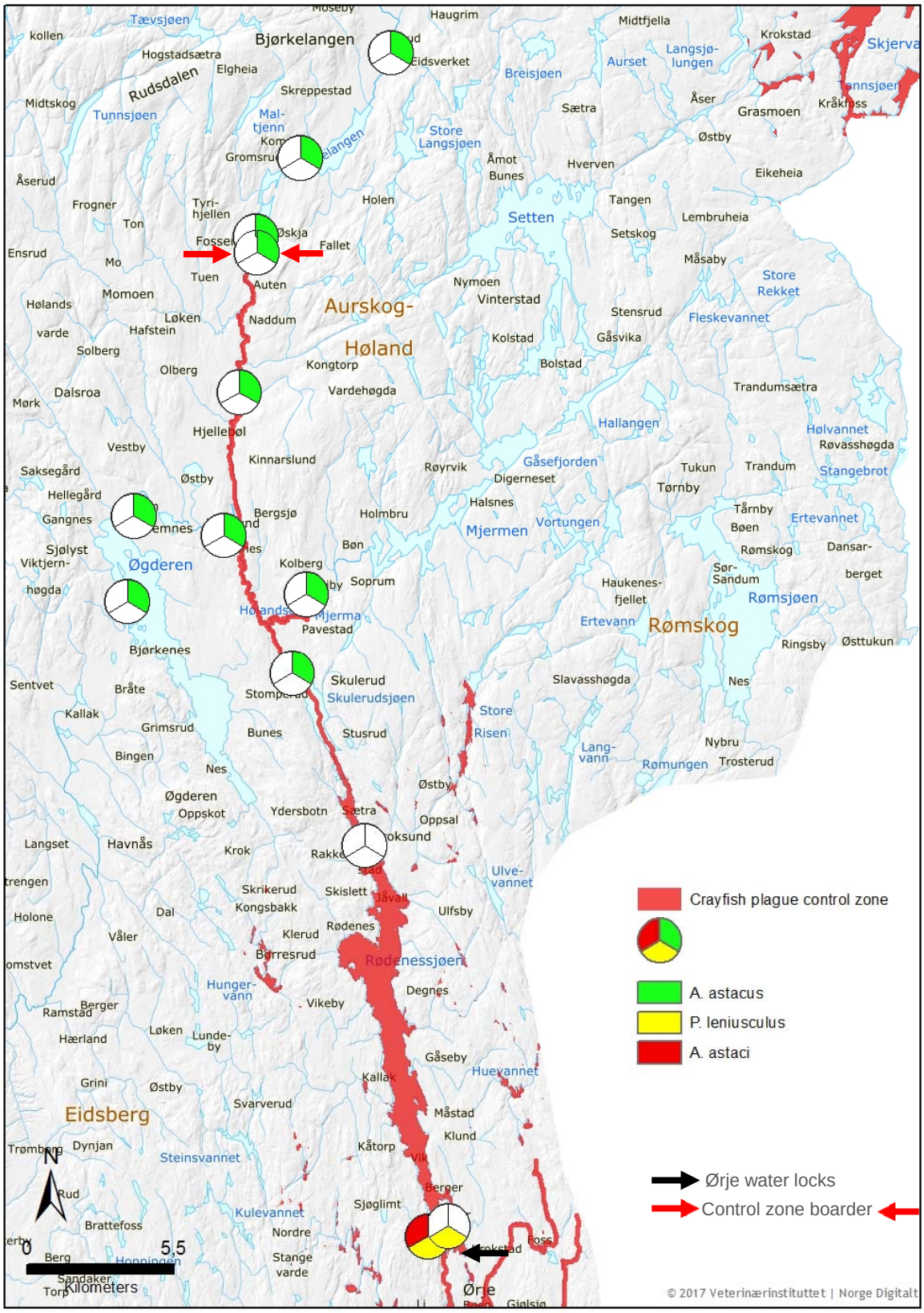

Figure 4. Overview map of the surveilled part of the Halden watercourse region in 2017, starting from the Ørje water locks (black arrow) in the south where signal crayfish is present. The control area is indicated by red colour on involved lakes and rivers, and ends at Fosserdam (red arrows), which is an artificial barrier for further spread. The pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. Positive *A. astaci* samples were only detected close to Ørje water locks together with *P. leniusculus* eDNA. Further north, only eDNA of noble crayfish was detected in the water from river Hølandselva and upstream. The same was observed in the risk area, suggesting no spread of *A. astaci* in the monitoring period.

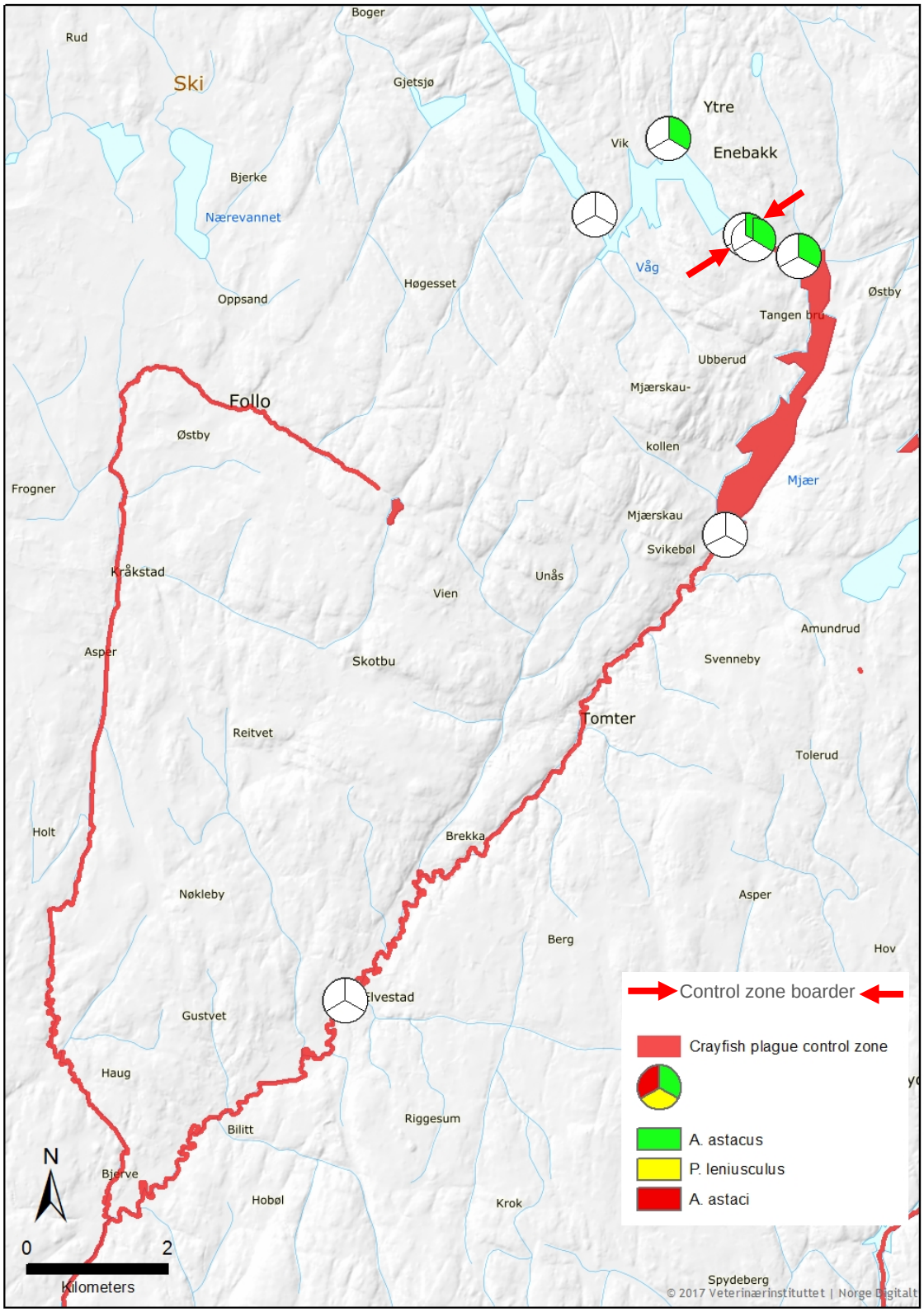

Figure 5. Overview map of the surveilled part of the Mosse watercourse. The control area is indicated by red colour on involved lakes and rivers and ends at Steinkistedammen in the river Tangenelva (red arrows), which is an artificial barrier for further spread. The pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. No eDNA of *A. astaci* and signal crayfish was detected, while eDNA of noble crayfish was detected in the river Tangenelva on both side of the control zone boarder and in Lake Våg, but not in by the outlet of Lake Langen.

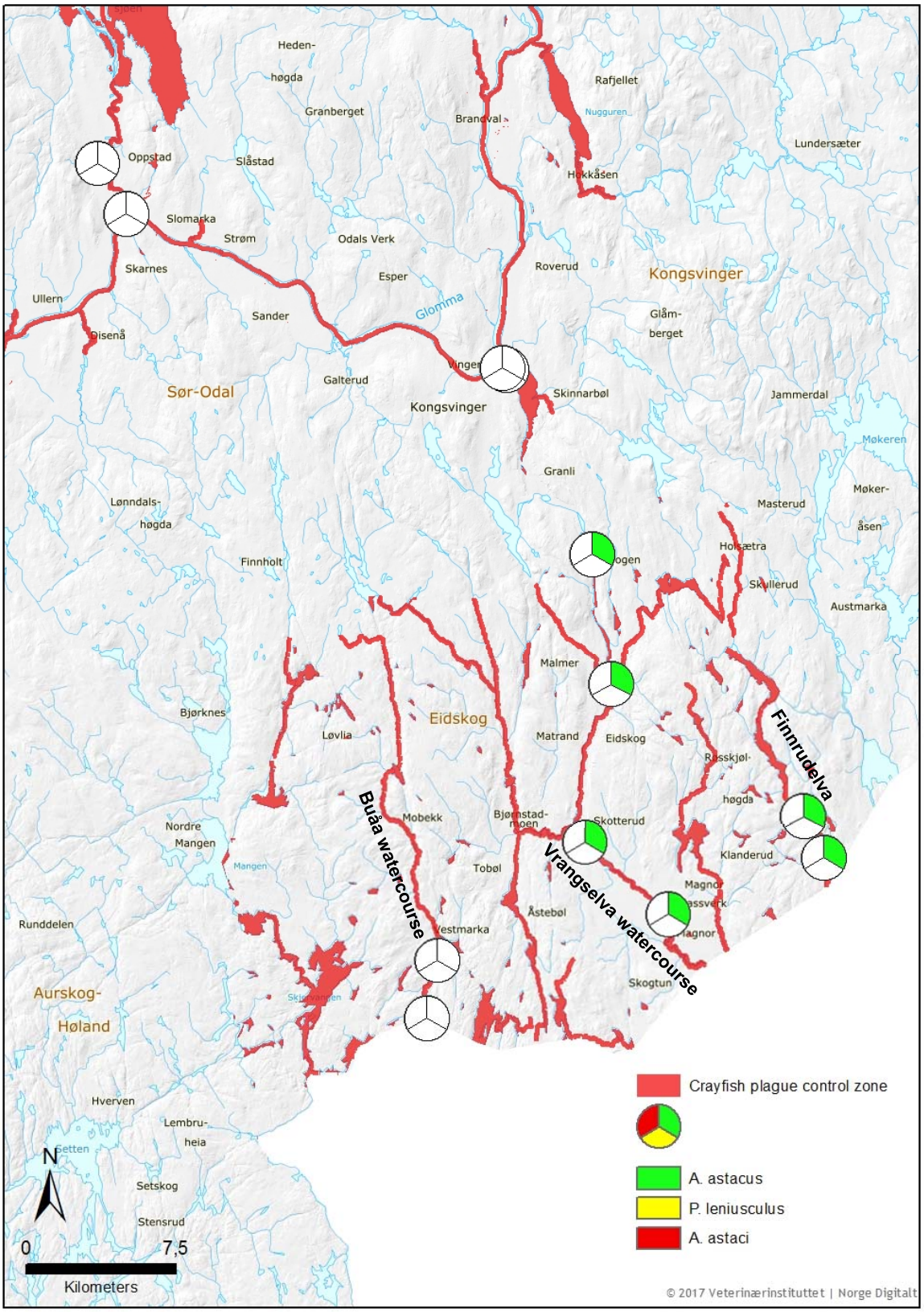

Figure 6. Overview map of the Glomma watercourse region and Eidskog municipality. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. None of the screened targets were detected in the Glomma watercourse. In the Vrangselva watercourse, no eDNA of *A. astaci* or signal crayfish was detected, while eDNA of noble crayfish was detected on the whole monitored stretch from Magnor to Åbogen. Also in the River Finnsrudelva, eDNA of noble crayfish was detected, but without signs of *A. astaci* and signal crayfish. None of the screened targets were detected in the Buåa watercourse.

Conclusion

In the Halden watercourse, combined eDNA monitoring of *A. astaci*, noble crayfish and signal crayfish largely confirmed that signal crayfish present in lake Rødenessjøen emit detectable, but low concentrations of *A. astaci* to the water. However, the observed infection front in the river Hølandselva in 2016 had seemingly not progressed upstream. This is supported by negative eDNA results for *A. astaci* in all samples apart from those for the Southern part of lake Rødenessjøen, and positive eDNA results for noble crayfish in the samples from the Northern part of the river Hølandselva and upstream. Similarly to 2016, there was no sign of *A. astaci* spreading to neighbouring risk areas.

No *A. astaci* or signal crayfish eDNA was detected in the Mosse watercourse. Positive eDNA results for noble crayfish in Lake Våg in June indicate that the infection front observed in 2016 had not spread upstream the dam, Steinkistedammen. However, reports of failure to catch crayfish during the trapping season (August) and only one eDNA sample positive for noble crayfish in September give reasons for concern. It is impossible to completely exclude that the crayfish plague has spread unnoticed outside the period of eDNA monitoring.

In the Glomma watercourse, no *A. astaci* or signal crayfish eDNA was detected. The status is still highly uncertain, given many years of recurrent crayfish plague detection in cage experiments. However, the results indicate at least that our sampling effort was not sufficient to reveal an eventual infection source in the watercourse. Unlike the 2016-results, we found no positive eDNA results for noble crayfish.

We found no sign of *A. astaci* in any of the monitored sites in Eidskog municipality. In the Buåa watercourse, none of the targets was detected, while in the Vrangselva watercourse and River Finnsrudelva, noble crayfish eDNA was detected at all monitored sites. This supports the view that the crayfish plague has not yet entered the Norwegian side of these river systems, and suggest the presence of live noble crayfish in both systems. We are aware - through notification from SVA - that this situation might have changed in December 2017 in River Finnsrudelva (15).

The eDNA monitoring of *A. astaci* worked as intended, and in combination with the complementary eDNA targets noble- and signal crayfish, it was possible to produce a snapshot of the relevant habitat status. The simultaneous monitoring of the three target species can facilitate more coordinated surveillance programs for crayfish plague, red-listed noble crayfish and black-listed signal crayfish, which will be tested in out in collaboration with the NEA and NINA in 2018.

Acknowledgements

We thank the Norwegian Food Safety Authority for accepting the idea of a second year of a joint collaborative project between TARGET and the surveillance programme for *Aphanomyces astaci* 2017. The TARGET project (NRC 243907; Targeted strategies for safeguarding the Noble crayfish against alien & emerging threats) is financially supported by the Norwegian Research Council through the “Environment 2015” (Miljø 2015).

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Appendix

Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2017" - Tables S1 – S5.

Table S1. Agreed areas and locations of the "*NOK A. astaci 2017*" program. We reserve the right to change and a reallocation of sample localities if new circumstances arise.

Location	Water course[1]/ municipality, county[2]	Location infection status	# water samples (site x samples x visits)
Halden watercourse			**Total samples 52**
Rødenessjøen	HW/Aurskog-Høland, A	Control zone	8 (2 x 2 x 2)
Hølandselva	HW/Aurskog-Høland, A	Control zone, outbreak expected	8 (2 x 2 x 2)
Fossersjøen	HW/Aurskog-Høland, A	Control zone, outbreak expected	4 (1 x 2 x 2)
Fosserdam	HW/Aurskog-Høland, A	Risk zone/control zone boarder	4 (1 x 2 x 2)
Bjørkelangen	HW/Aurskog-Høland, A	Risk zone	8 (2 x 2 x 2)
Lierelva	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Lundsfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Dalstorpfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)
Hemnessjøen	Lake, Aurskog-Høland, A	Risk zone	8 (2 x 2 x 2)
Mosse watercourse			**Total samples 28**
Hobølelva	MW/ Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Mjær	MW/ Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Tangenelva	MW/ Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Tangenelva	MW/ Enebakk, Ø	Risk zone	8 (2 x 2 x 2)
Våg-area	MW/ Enebakk, Ø	Risk zone	8 (2 x 2 x 2)
Glomma watercourse			**Total samples 16**
Vingersnoret	GW/ Sør-Odal, H	Control zone	4 (1 x 2 x 2)
Vingersjøen	GW/ Sør-Odal, H	Control zone	4 (1 x 2 x 2)
Oppstadåa	GW/Sør-Odal, H	Control zone	8 (2 x 2 x 2)
Eidskog			**Total samples 28**
Buåa	BW/Eidskog, H	Control zone	8 (2 x 2 x 2)
Finnsrudelva	RF/Eidskog, H	Control zone	4 (1 x 2 x 2)
Vrangselva	VW/Eidskog, H	Control zone	16 (4 x 2 x 2)
Total			**124**

[1] HW = Halden watercourse, GW = Glomma watercourse, MW = Mosse-watercourse, BW = Buåa watercourse, RF = River Finnsrudelva, VW = Vrangselva watercourse
[2] Ø = Østfold, A = Akershus, H = Hedmark.

Table S2. Location sites for water sampling in the Halden water course area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.

Location[1]	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Lierelva	HA1	R	59°53'8"N 11°34'29"E	5	15.9	0	3	0	0	1	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	4	20.0	0	1	0	0	1	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	20.0	0	2	0	0	0	0
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	18.3	0	1	0	0	2	0
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	20.0	0	2	0	0	2	0
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	4	19.5	0	1	0	0	2	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	4	15.8	0	2	0	0	0	0
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	5	10.8	*0*	2	0	0	2	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	6	16.0	0	3	0	0	3	0
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	5	13.7	0	1	0	0	3	0
Skulerudsjøen outlet	HA11	C	59°37'6"N 11°35'5"E	4	20.0	0	0	0	0	0	0
Rødenessjøen Ysterud	HA12	C	59°29'17"N 11°38'23"E	4	20.0	2	0	2	0	0	2
Rødenessjøen Ørje	HA13	C	59°29'31"N 11°39'10"E	4	20.0	0	0	1	0	0	0
Total				57	230.0	2	18	3	0	16	2

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (June & September summarized), L = total water volume summarized for all samples
[3] Number of samples in June and September with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

Table S3. Location sites for water sampling in Mosse-watercourse area with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Langen, bridge Skiveien	MO1	R	59°43'33.3"N 11°00'12.1"E	5	14.5	0	0	0	0	0	0
Våg, Badeplass	MO2	R	59°44'10.2"N 11°01'14.7"E	4	15.5	0	2	0	0	1	0
Våg, outlet	MO3	R	59°43'28.2"N 11°02'29.9"E	6	14.4	0	1	0	0	0	0
Tangenelva, downstream dam nt 1	MO4	C	59°43'26.3"N 11°02'36.9"E	5	15.8	0	3	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	4	18.0	0	1	0	0	0	0
Mjær, outlet	MO6	C	59°41'10.2"N 11°02'27.6"E	4	20.0	0	0	0	0	0	0
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	4	15.0	0	0	0	0	0	0
Total				32	113.2	0	7	0	0	1	0

[1] C = Crayfish plague control zone, R = risk area
[2] # = Total number of water samples (June & September summarized), L = total water volume summarized for all samples
[3] Number of samples in June and September with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

Table S4. Location sites for water sampling in the Glomma region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vingersnoret	GL1	C	60°11'36.3"N 12°01'54.5"E	4	20.0	0	0	0	0	0	0
North of Vingersnoret	GL2	C	60°11'39.7"N 12°01'41.2"E	4	20.0	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	20.0	0	0	0	0	0	0
Glomma, Skarnes	GL10	C	60°15'20.8"N 11°40'49.4"E	4	17.0	0	0	0	0	0	0
Total				16	77.0	0	0	0	0	0	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

Table S5. Location sites for water sampling in the Eidskog region with corresponding location and sample information. eDNA results are listed for crayfish plague, noble crayfish and signal crayfish.

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vrangselva, Åbogen	VR1	C	60°06'43.6"N 12°07'01.0"E	4	19.5	0	2	0	0	2	0
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	4	18.8	0	0	0	0	1	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	4	19.6	0	2	0	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	4	13.5	0	2	0	0	0	0
Finnsrudelva, Finnsrudvegen	FR1	C	59°59'50.7"N 12°19'05.4"E	4	20.0	0	0	0	0	2	0
Finnsrudelva, Billavegen	FR2	C	59°58'44.9"N 12°20'14.2"E	4	20.0	0	2	0	0	2	0
Buåa, Eidskog	BU1	C	59°55'31.1"N 11°59'37.0"E	4	19.5	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	4	13.0	0	0	0	0	0	0
Total				32	143.9	0	8	0	0	7	0

[1] C = Crayfish plague control zone
[2] # = Total number of water samples (June & August summarized), L = total water volume summarized for all samples
[3] Number of samples in June and August with positive detection of eDNA from crayfish plague (CP), noble crayfish (NC), and signal crayfish (SC).

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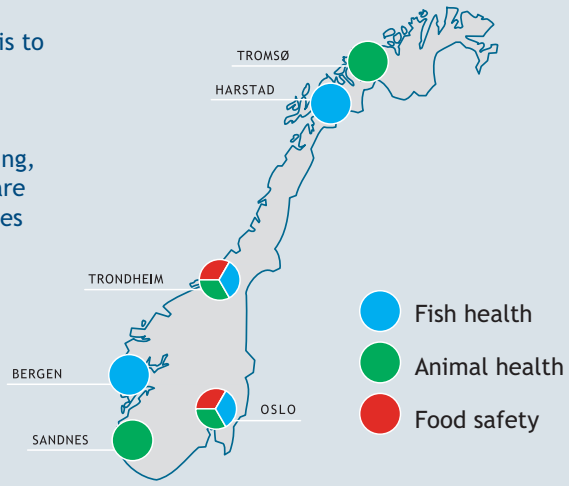

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