Annual Report

The surveillance programme for Aphanomyces astaci in Norway 2018

Veterinærinstituttet
Norwegian Veterinary Institute

Mattilsynet

The surveillance programme for *Aphanomyces astaci* in Norway 2018

Content

Authors
David Strand[1], Johannes Rusch[1], Stein Ivar Johnsen[2], Attila Tarpai[1], Trude Vrålstad[1]
[1] Norwegian Veterinary Institute
[2] Norwegian Institute for Nature Research

Commissioned by
Norwegian Food Safety Authority

ISSN 1894-5678

Design Cover: Reine Linjer
Photos front page: Johannes Rusch, David Strand & Trude Vrålstad

Summary

In this surveillance program, environmental DNA (eDNA) monitoring of the water was used 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. The presence/absence of eDNA from noble crayfish (*Astacus astacus*) and signal crayfish (*Pacifastacus leniusculus*) was also determined to supplement the results and to gain the possibility to evaluate the habitat status in more detail, and as a part of the collaboration and coordination with the national surveillance program for noble crayfish. The main geographic focus of this surveillance program has been the Halden watercourse and neighbouring risk areas. Other covered geographic areas include the Mosse watercourse, Glomma watercourse, and selected areas in the Eidskog municipality including the Buåa watercourse, the Vrangselva watercourse and River Finnsrudelva.

In total, 56, 35, 21 and 34 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 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 2018, *A. astaci* spread upstream in the Mosse watercourse while no spread was observed in any of the other 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 in 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 most samples were positive for noble crayfish eDNA.
- In the Mosse watercourse, no eDNA of *A. astaci* or signal crayfish was detected. However, one dead crayfish found in Lake Langen was positive for *A. astaci*, demonstrating that crayfish plague has spread into Lake Våg and Lake Langen. Noble crayfish eDNA was detected at the inlet of Lake Langen.
- 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 either signal crayfish or *A. astaci,* while several were positive for noble crayfish eDNA in River Vrangselva 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 one 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, crayfish plague was confirmed in noble crayfish inhabiting the border watercourse Vrangselva and River Billa (named River Finnsrudelva on the Norwegian side), 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). These include Dammane (Telemark), Ostøya (Akershus), Fjelna-vassdraget (Sør-Trøndelag) and Kvesjøen (Nord-Trøndelag), where signal crayfish were discovered in 2006, 2009, 2011 and 2013, respectively. For two of these locations (Dammane and Ostøya), signal crayfish has been eradicated and the areas were recently declared disease free after several years of surveillance (4).

The focus areas of the 2018 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 first 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. Illegally introduced *A. astaci* positive signal crayfish were found in Lake Øymarksjøen in 2008 (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 Lake 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, the infection front was followed through analysis of water and eDNA of *A. astaci* was sometimes detected in the water samples prior to crayfish mortalities in the cages. Noble crayfish and signal crayfish eDNA was also detected in the locations where the crayfish are known to occur (10).

The Mosse watercourse was hit by crayfish plague in 2016. When the crayfish season started in August, the NFSA received 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 River 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 hit by crayfish plague in July 1987, from Kirkenær in Solør and further downstream including Lake Vingersjøen and Lake Storsjøen/Oppstadåa (4). Environment 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 hit by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river. A barrier built to prevent 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).

The rivers Vrangselva and Finnsrudelva in Eidskog municipality, that flow across the border into Sweden were hit by crayfish plague on the Swedish side of the border in 2016. No sign of crayfish plague has been detected on the Norwegian side of the border in either of these two watercourses.

The surveillance program for *A. astaci* is commissioned by the Norwegian Food Safety Authority (NFSA) and conducted by the Norwegian Veterinary Institute (NVI). 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 environmental DNA (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. Decapods including Noble crayfish are covered by the Norwegian animal welfare act (LOV-2009-06-19-97). When an alternative method is developed, the use of live animals for disease surveillance should be reduced. Within the cage experiments, the crayfish mortality was 24% despite that no crayfish plague was detected in any of the ten 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 program was co-funded by the research project TARGET (NRC- 243907) in the period 2016-2017. From 2018, a formal collaboration was initiated with the National surveillance program for noble crayfish (*Astacus astacus*), commissioned by the Norwegian Environment Agency (NEA) and coordinated by the Norwegian Institute of Nature Research (NINA). This involves joint field work and joint exploitation of water samples and molecular results in overlapping surveillance areas. These synergies enable analyses of a slightly larger sample size than the NFSA-programme alone would allow.

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 neighbouring 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 national surveillance of noble crayfish, funded by the Norwegian Environment Agency (NEA), and coordinated by the Norwegian institute for Nature research (Figure 1).

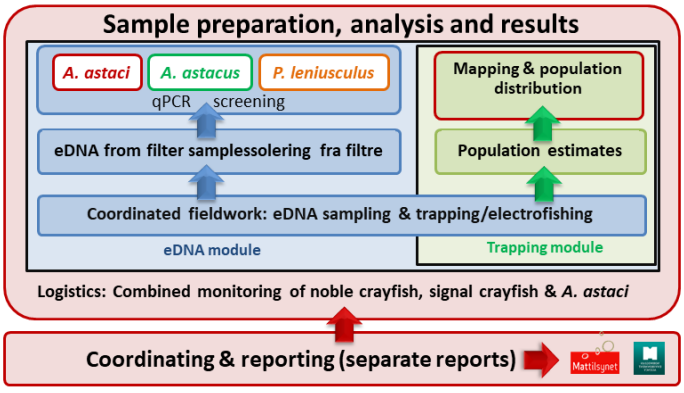

Figure 1. Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with the national surveillance of noble crayfish (NEA funded).

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse on both sides of the control zone border at Steinkistedammen, the Glomma watercourse, and Eidskog municipality including the Vrangselva watercourse, Buåa watercourse and River Finnsrudelva.

Plotted locations for water sampling, in total 36 sites, as well as the crayfish plague zones, are displayed in Figure 2. Supplementary details are summarised in Appendix 1 (Table S2-S5).

Halden watercourse: The control zone was monitored at 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 close geographical proximity are vulnerable to further spread, and referred to as "risk zones" (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, Lake Langen and Lake Sværsvann, in total 6 sites (Table S3, Appendix 1). The control zone was expanded to cover these sites after crayfish plague was confirmed from one dead crayfish found in Lake Langen in July.

Glomma watercourse: The control zone comprises the main passageway downstream Braskereidfoss in Våler. Only 5 sites within the control zone could to be monitored in 2018 due to 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 watercourse (4 sites), Buåa watercourse(2 sites) and River Finnsrudelva (2 sites) (Table S5, Appendix 1).

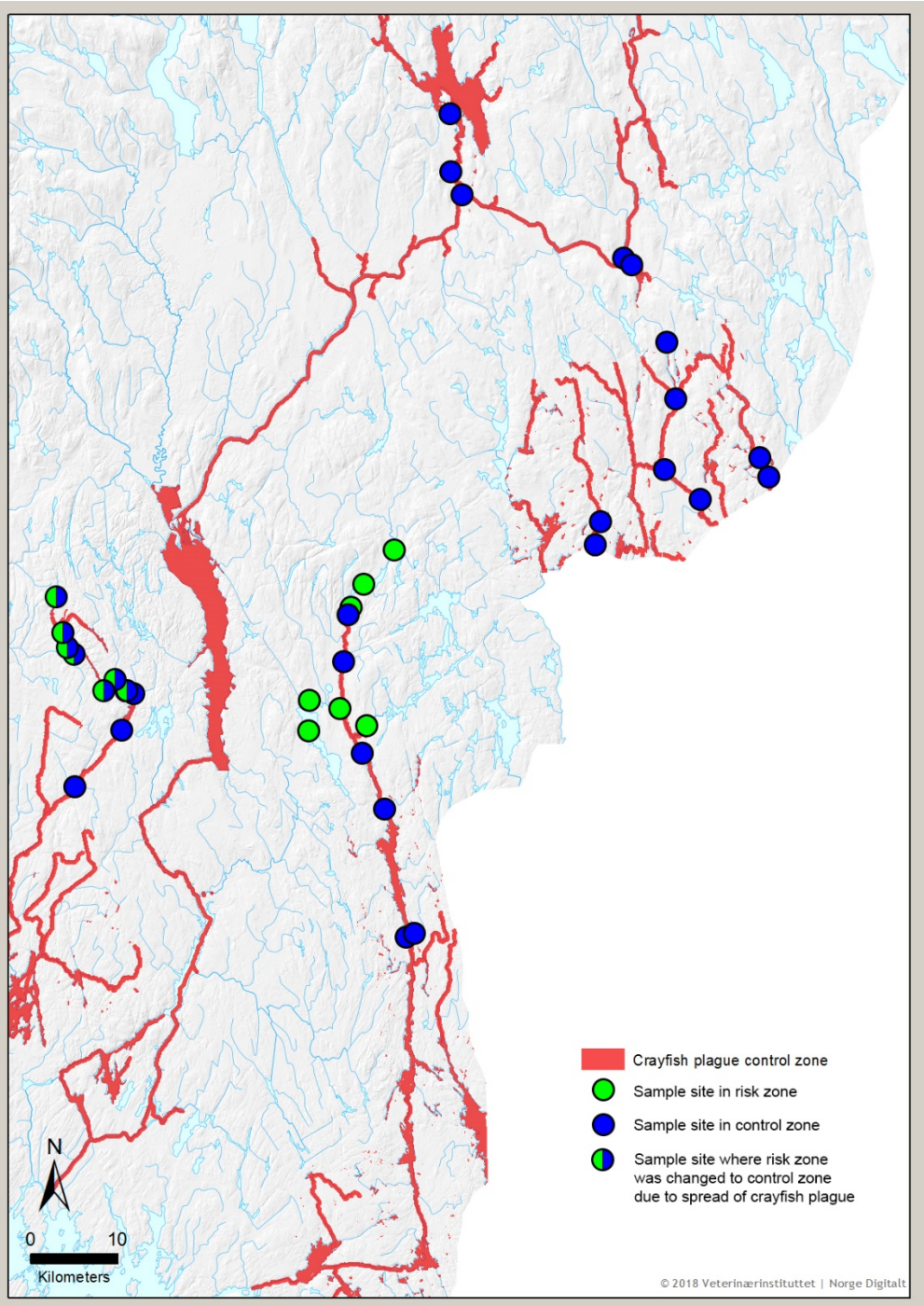

Figure 2. Surveillance sites in Eastern Norway 2018. Water samples (circles) were collected in June and September. Split circles (green/blue) represent the sample sites that were initially located in the risk zone, which was changed to a control zone after August 1st as a consequence of the spread of crayfish plague in the Mosse watercourse. Regulated areas (crayfish plague control zones) are marked in red. Note: For Glomma, the control zone is an approximation.

eDNA monitoring

The water samples were collected in June and September 2018. 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 extra samples were included 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 ziplock bag with silica-gel beads, which dry the filter to preserve biological material and DNA.

The water samples were screened by qPCR for three 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 fibre filters using a portable peristaltic pump (Masterflex E/S portable sampler). Each filter was carefully transferred to ziplock bag containing silica-gel beads for preservation until transportation to the laboratory. DNA was isolated with a large volume extraction procedure and presence/absence of eDNA from all target organisms was analysed using qPCR. Figure modified from Vrålstad et al (6).

Results and Discussion

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 56 water samples representing a total of ~228 L water were analysed. In the control zone, *A. astaci* eDNA was detected in six water samples (four in June and two in September) at the Southern part of lake Rødenessjøen (Figure 4, Table S2). Signal crayfish are known to be present at these two locations and this was confirmed by positive eDNA results in a total of eight water samples (four in June, four in September; Figure 4, Table S2).

No sign of crayfish plague was observed during the surveillance period in any other part of the Halden watercourse control zone, from the outlet of Lake 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 River Hølandselva and upstream (in the control zone), indicating the presence of live noble crayfish inhabiting the northern part of the Halden watercourse control zone. In total, eDNA of noble crayfish was detected in 9 water samples from River Hølandselva and upstream (in the control zone).

While *A. astaci* eDNA was detected at the outlet of River Hølandselva in 2016, no *A. astaci* eDNA was detected there in 2017 or 2018. Thus, the previously reported "outbreak front" observed in the river Hølandselva in 2016 has seemingly not progressed upstream, but has more likely "burnt out". One reason for this may be a longer upstream river stretch with no, or very low densities of crayfish, combined 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 most samples were positive for noble crayfish eDNA. In total, 25 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 suggests 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 was 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 at a density of 5.48 CPUE.

eDNA monitoring in the Mosse watercourse

In the Mosse watercourse, 35 water samples representing a total of ~148 L water were analysed. None of the analysed samples within or outside the control zone showed any sign of *A. astaci* or signal crayfish eDNA (Figure 5, Table S3). In 2017, noble crayfish eDNA was detected at two locations in Våg. In 2018, noble crayfish eDNA was only detected at the inlet to Lake Langen in June, but not in September, with no signs of noble crayfish eDNA in Våg. In July one dead crayfish was found at Kilevika in Lake Langen by locals and crayfish plague was confirmed through diagnostics carried out at the NVI (Figure 5). This confirms that the crayfish plague has spread upstream from Steinkistedammen into Lake Våg and Lake Langen. As a consequence, the control zone has been expanded by NFSA to include Lake Våg, Lake Langen and upstream locations from August 1st 2018 (FOR-2018-08-01-1214). The situation highlights that constant awareness from the public is very important to discover the spread of crayfish plague, and that eDNA monitoring alone may fail to detect the spread of crayfish plague that occurs outside of the collection sites and time-windows for sampling.

eDNA monitoring in the Glomma watercourse

In the Glomma watercourse, 21 water samples representing a total of ~89 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 results of 2016 (6), no positive signal for noble crayfish eDNA was detected in the monitored area in 2017 or 2018. 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, 34 water samples representing a total of ~143 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, five samples from Åbogen to Magnor were positive for noble crayfish eDNA (three in June, two in August), suggesting that the river stretch is still inhabited by live noble crayfish. In River Finnsrudelva, eight samples were positive for noble crayfish eDNA (four in June, four in August). Here, field sampling in cooperation with the Swedish National Veterinary Institute (SVA) and Swedish University of Agricultural Sciences (SLU) confirmed that crayfish plague was still active at the Swedish-Norwegian border in June 2018 (15, 16).

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 five years (6), and eDNA alone for another two years. Lack of crayfish plague detection could indicate disease free status. However, a new crayfish plague regulation from August 2016 covers the whole Eidskog municipality (FOR-2016-08-17-972), and replaces the old regulation for the Buåa 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.

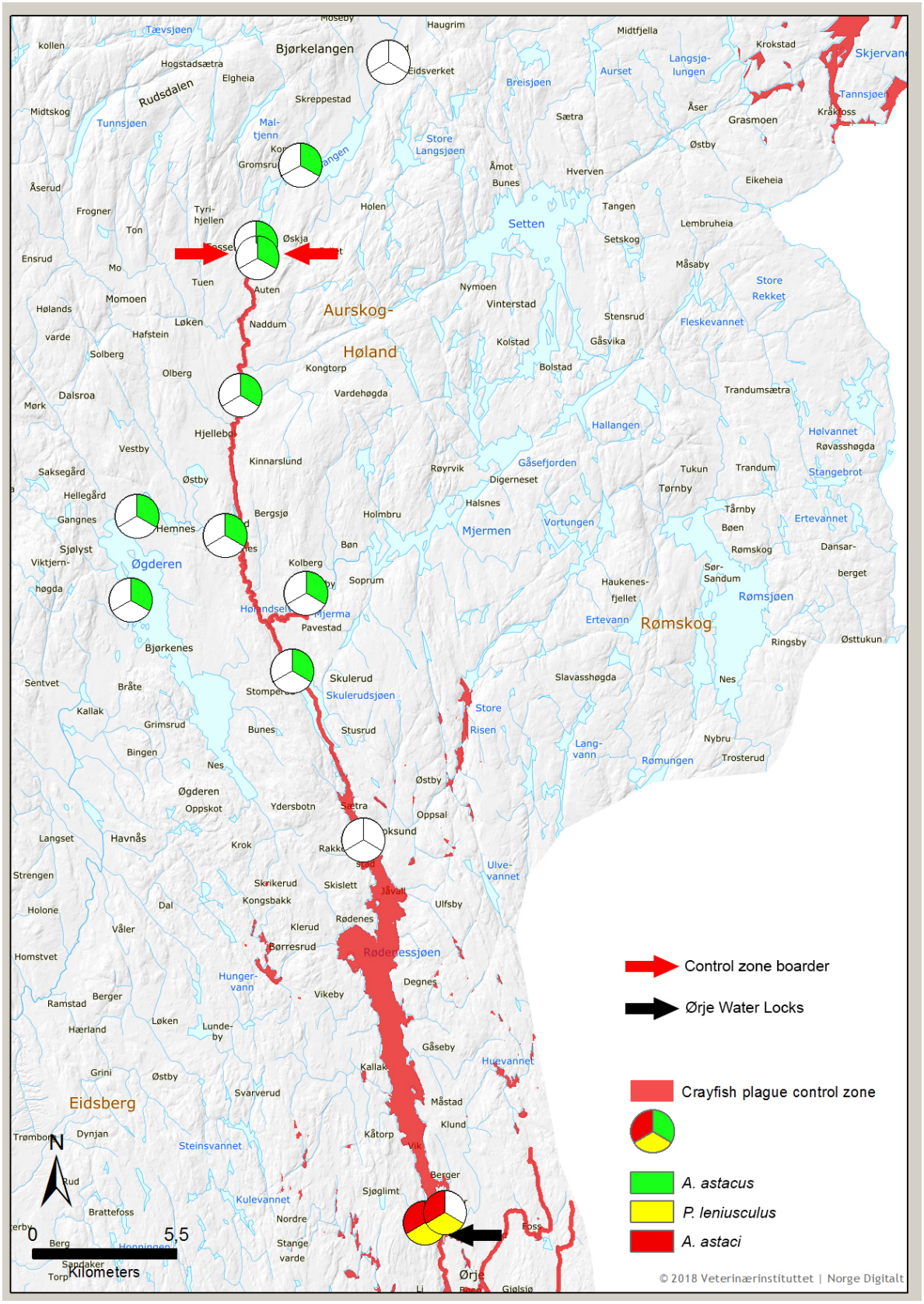

Figure 4. Overview map of the surveilled part of the Halden watercourse region in 2018, starting from the Ørje water locks (black arrow) in the south where signal crayfish is present. The control area is indicated by red colour on involved lakes and rivers, and ends at Fosserdam (red arrows), which is 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.

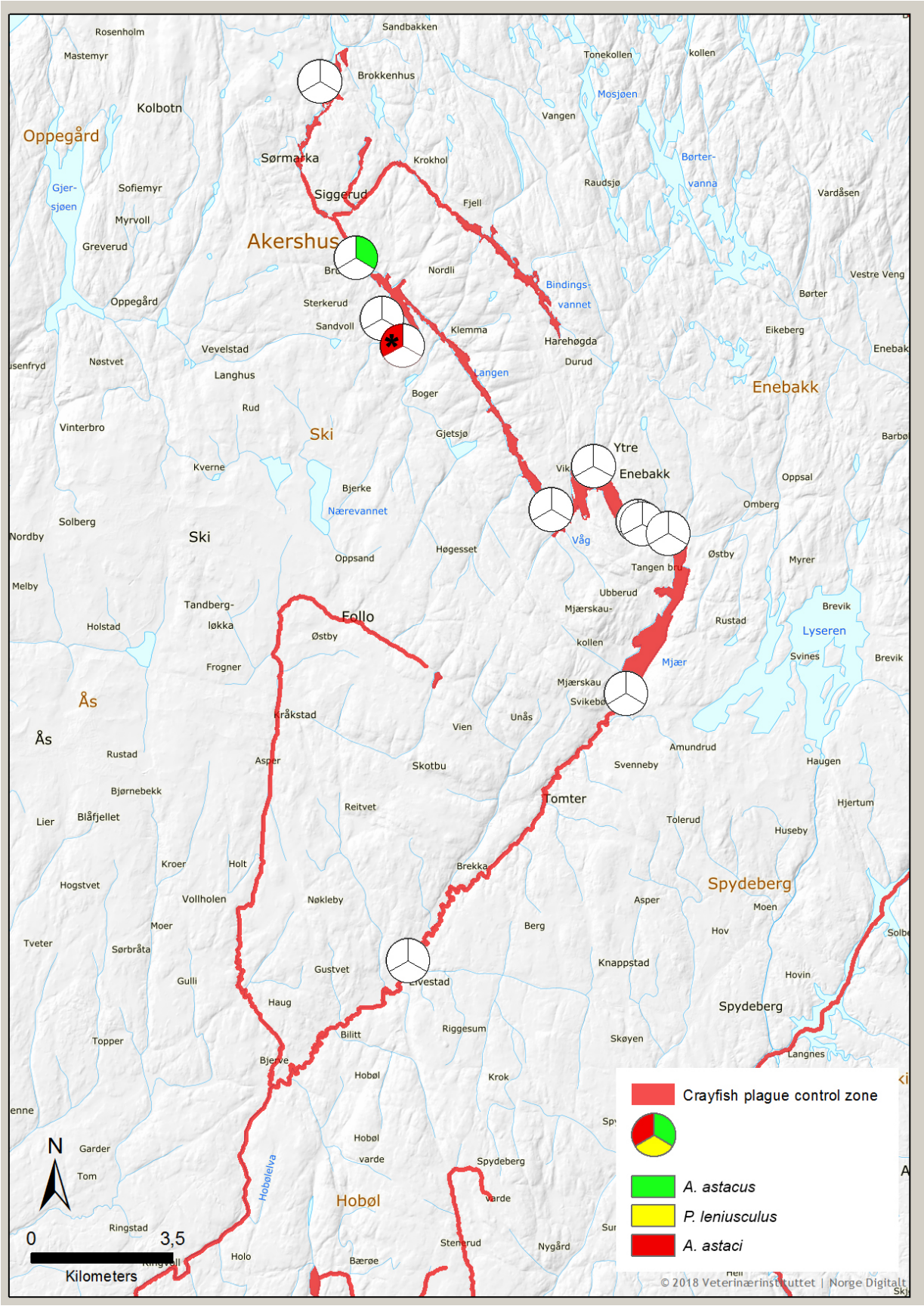

Figure 5. Overview map of the surveilled part of the Mosse watercourse. The control area, which was extended in 2018 due to crayfish plague in Lake Langen, is represented by red colour. The pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; 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. However one dead noble crayfish with confirmed *A. astaci* infection was found in July (marked with *). eDNA of noble crayfish was only detected in the river inlet to of Lake Langen.

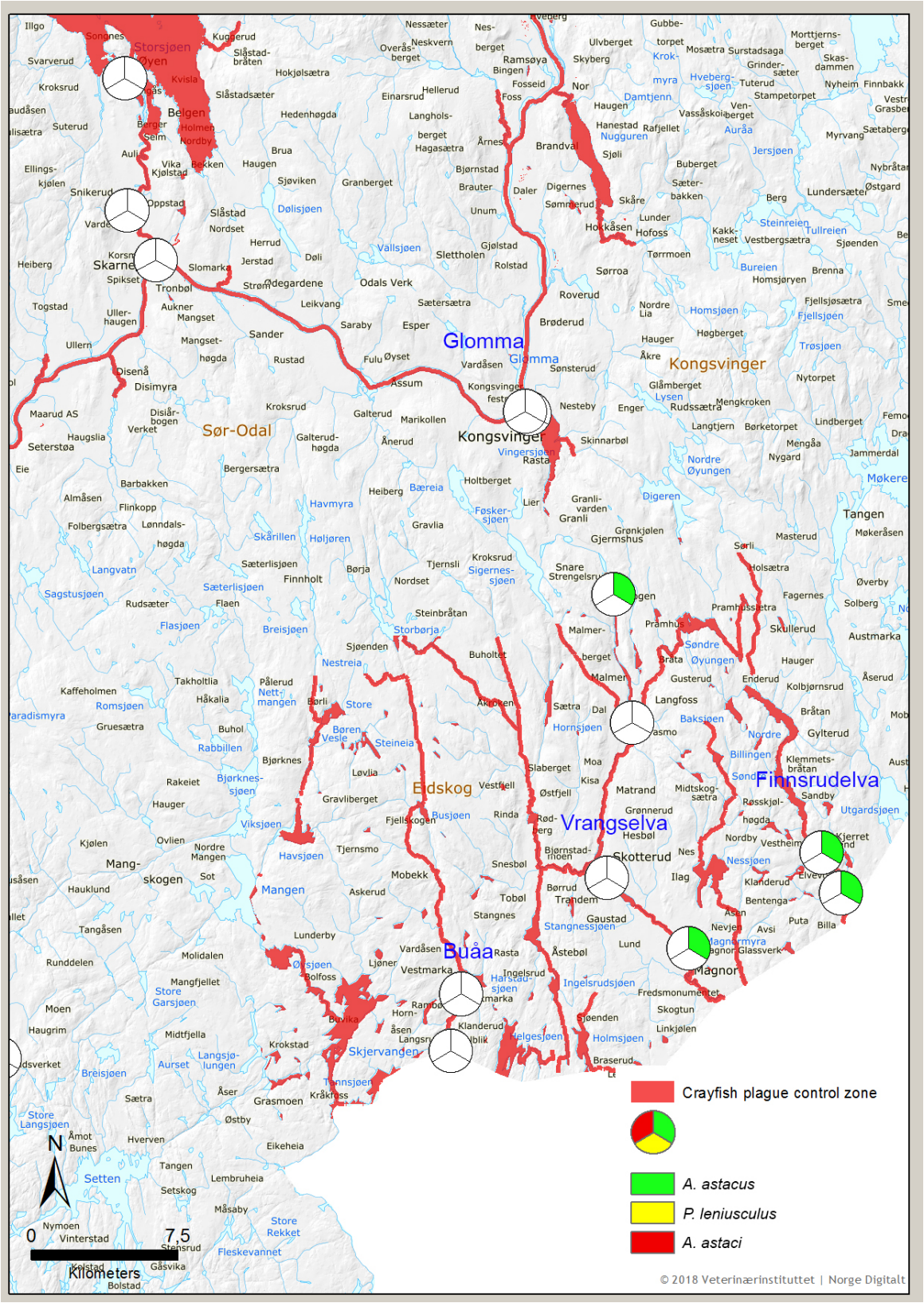

Figure 6. Overview map of the Glomma watercourse region and Eidskog municipality. Regulated areas (crayfish plague control zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of *A. astaci* (red), signal crayfish (*P. leniusculus*; yellow), and 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 at Åbogen and Magnor. Also, in the River Finnsrudelva, eDNA of noble crayfish was detected but without signs of *A. astaci* or signal crayfish. However, in another survey with Swedish collaborators from SVA and LSU, *A. astaci* eDNA was detected in the water on the Swedish-Norwegian border (not shown in this figure). 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. The observed infection front in River Hølandselva in 2016 has seemingly vanished in 2017, and the situation is the same in 2018. 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 River Hølandselva and upstream. Similarly to 2016 and 2017, there was no sign of *A. astaci* spreading to neighbouring risk areas.

No *A. astaci* positive eDNA samples were observed in the Mosse watercourse in this monitoring program in 2018 on the surveyed sites in June and September. However, a dead noble crayfish, found by locals was reported in Lake Langen in July and confirmed *A. astaci* positive as part of regular crayfish plague diagnostics at NVI. This confirmed that crayfish plague has spread upstream of the dam at Steinkistedammen, into Lake Våg and Lake Langen, and resulted in an expansion of the zone in August 2018. Thus, constant awareness from the public is also very important to discover crayfish plague spread since eDNA monitoring provides only a snap-shot of the situation at a specific date and sample location.

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. Positive eDNA results for noble crayfish was found in 2016, but not in 2017 and 2018.

We found no sign of *A. astaci* in any of the monitored sites in Eidskog municipality. In the Buåa watercourse, none of the targets were detected. Similar to the 2017 results, noble crayfish eDNA was detected at several of the monitored sites in the Vrangselva watercourse and River Finnsrudelva. This supports the view that the crayfish plague has still not yet entered the Norwegian side of these river systems, and suggests the presence of live noble crayfish in both systems. However, the crayfish plague was active in River Finnsrudelva at the border in June 2018 (16).

Acknowledgements

Both the surveillance program for *Aphanomyces astaci* in Norway and the national surveillance program of noble crayfish benefit from the coordinated and simultaneous monitoring of the three target species (crayfish plague, red-listed noble crayfish and black-listed signal crayfish). We thank the Norwegian Food Safety Authority (NFSA) for accepting the idea of a joint collaborative project between these surveillance programmes in 2018. The surveillance programme for *A. astaci* is funded by NFSA, and the national surveillance program for noble crayfish is funded by the Norwegian Environment Agency (NEA).

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Appendix

Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2018" - Tables S1 – S5.

Table S1. Agreed areas and locations of the "*NOK A. astaci 2018*" program. We reserve the right to change and a reallocation of sample localities if new circumstances arise. A few more sites were sampled in the Mosse watercourse due to the changed status there (spread of crayfish plague).

Location	Watercourse[1]/ municipality, county[2]	Location infection status	# water samples (site X samples X visits)
Halden watercourse			**Total samples 48**
Rødenessjøen	HW/Marker, Ø	Control zone	4 (1 x 2 x 2)
Hølandselva	HW/Aurskog-Høland, A	Control zone	8 (2 x 2 x 2)
Fossersjøen	HW/Aurskog-Høland, A	Control zone, outbreak expected	4 (1 x 2 x 2)
Fosserdam overside	HW/Aurskog-Høland, A	Risk zone/control zone border	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)
Dalstorpsfoss	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)
Glomma watercourse			**Total samples 20**
Storsjøen	GW/Nord & Sør Odal, H	Control zone	4 (1 x 2 x 2)
Oppstadåa	GW/Sør-Odal, H	Control zone	8 (2 x 2 x 2)
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)
Eidskog			**Total samples 32**
Buåa	BW/Eidskog, H	Control zone	8 (2 x 2 x 2)
Finnsrudelva	RF/Eidskog, H	Control zone	8 (2 x 2 x 2)
Vrangselva	VW/Eidskog, H	Control zone	16 (4 x 2 x 2)
Mosse watercourse			**Total samples 32**
Hobølelva	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Mjær	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Tangenelva	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Våg oppst. demning	MV/Enebakk, Ø	Risk zone / Control zone*	4 (1 x 2 x 2)
Våg badeplassen	MV/Enebakk, Ø	Risk zone / Control zone*	4 (1 x 2 x 2)
Langen	MV/Enebakk, Ø	Risk zone / Control zone*	12 (3 x 2 x 2)
Total			**132**

[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.
* Risk zone was changed to a control zone in August as a consequence of the spread of crayfish plague in the Mosse watercourse

Table S2. Location sites for water sampling in the Halden watercourse 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	14.7	0	0	0	0	0	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	5	18.1	0	2	0	0	3	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	14.2	0	2	0	0	2	0
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	13.8	0	2	0	0	1	0
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	19.5	0	2	0	0	2	0
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	5	17.6	0	2	0	0	1	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	5	15.1	0	2	0	0	3	0
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	4	18.0	*0*	2	0	0	2	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	4	17.0	0	2	0	0	1	0
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	4	20.0	0	2	0	0	2	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	2	0	2
Rødenessjøen Ørje	HA13	C	59°29'31"N 11°39'10"E	4	20.0	2	0	2	0	0	2
Total				56	228.0	4	18	4	2	17	4

[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			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Sværsvann	MO8	C	59°49'03.2"N 10°53'25.3"E	2	10.0	0	0	0	0	0	0
Langen, inlet Bru-fjellv.	MO9	C	59°46'44.7"N 10°54'38.6"E	5	12.0	0	2	0	0	0	0
Langen, Siggerudveien	MO10	C	59°45'57.5"N 10°55'25.7"E	2	10.0	0	0	0	0	0	0
Langen, Kilevika	MO11	C	59°45'37.0"N 10°56'01.0"E	2	10.0	0	0	0	0	0	0
Langen, bridge Skiveien	MO1	C	59°43'33.3"N 11°00'12.1"E	4	10.0	0	0	0	0	0	0
Våg, badeplass	MO2	C	59°44'10.2"N 11°01'14.7"E	4	16.0	0	0	0	0	0	0
Våg, outlet	MO3	C	59°43'28.2"N 11°02'29.9"E	4	20.0	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	4	20.0	0	0	0	0	0	0
Mjær, outlet	MO6	C	59°41'10.2"N 11°02'27.6"E	4	20.2	0	0	0	0	0	0
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	4	19.3	0	0	0	0	0	0
Total				35	147.5	0	2	0	0	0	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			September		
	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	16.0	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
Storsj. Ringåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	5	16.1	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	17.5	0	0	0	0	0	0
Glomma, Skarnes	GL10	C	60°15'20.8"N 11°40'49.4"E	4	19.5	0	0	0	0	0	0
Total				21	89.1	0	0	0	0	0	0

[1] C = Crayfish plague control zone
[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 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			September		
	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	20.0	0	2	0	0	2	0
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	4	20.0	0	0	0	0	0	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	5	16.0	0	0	0	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	5	17.0	0	1	0	0	0	0
Finnsrudelva, Finnsrudvegen	FR1	C	59°59'50.7"N 12°19'05.4"E	4	20.0	0	2	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	20.0	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	4	9.5	0	0	0	0	0	0
Total				34	142.5	0	7	0	0	6	0

[1] C = Crayfish plague control zone
[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).

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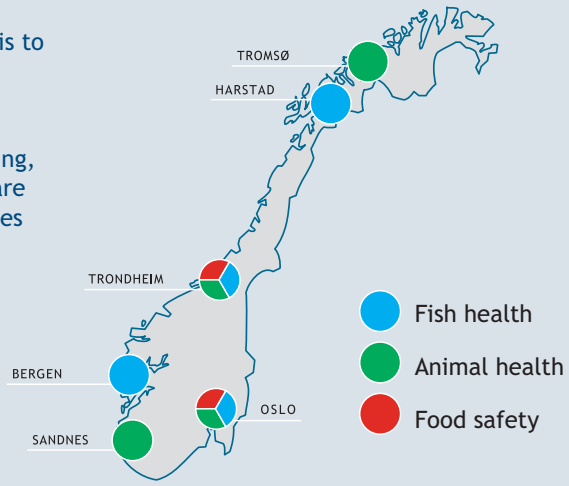

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