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The surveillance programme for *Aphanomyces astaci* in Norway 2021

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Content

Summary

This surveillance programme uses environmental DNA (eDNA) monitoring for species specific detection of *Aphanomyces astaci* spores directly from water filtrates. The presence/absence of eDNA from noble crayfish (*Astacus astacus)* and signal crayfish (*Pacifastacus leniusculus*) is also determined to supplement the results, and to evaluate the habitat status. These analyses are part of the collaboration and coordination with the national surveillance programme for noble crayfish. The geographic focus of the surveillance programme is the Halden watercourse and neighbouring risk areas, the Mosse watercourse, Glomma watercourse, and areas in the Eidskog municipality including the Buåa watercourse and the rivers Vrangselva and Finnsrudelva. Detection of noble crayfish eDNA, combined with the absence of eDNA from *A. astaci* and signal crayfish, substantiate the presence of non-infected noble crayfish which constitutes the desired habitat status.

In 2021, a total of 58, 39, 20 and 36 water samples were collected from selected sites in the Halden-, Mosse-, Glomma watercourse regions and in the Eidskog region, respectively. Sampling locations 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 the Halden watercourse, eDNA of *A. astaci* was detected in the south of Lake Rødnessjøen and in River Hølandselva, further upstream than previously detected. No spread of the pathogen was observed in any of the other areas monitored with the eDNA methodology. However, one sample from River Lierelva (risk zone) was positive for signal crayfish eDNA in June. This coincided with the cessation of positive noble crayfish eDNA detections at this site in September. Also two other locations in the risk zone yielded no noble crayfish eDNA, which is a decline in positive noble crayfish detections from previous years.
- In the Mosse watercourse, no eDNA of *A. astaci* or signal crayfish was detected, while noble crayfish eDNA was detected upstream of Lake Langen.
- In the Glomma watercourse, eDNA from noble crayfish was detected at one station - Oppstadåa south. No eDNA was detected from signal crayfish or *A. astaci*.
- In Eidskog, all samples were negative for signal crayfish and *A. astaci*, while several samples were positive for noble crayfish eDNA in the rivers Vrangselva and Finnsrudelva.

In August 2021, the river Mysenelva that is part of the Hæra watercourse was struck by crayfish plague. The Norwegian Veterinary Institute confirmed the detection of *A. astaci* from dead noble crayfish. This further increases the number of *A. astaci* infected areas in Norway. The Norwegian Food Safety Authority extended the crayfish plague zone for Glomma to incorporate Mysenelva downstream of Rustadfossen east of Mysen.

In summary, the single positive detection of signal crayfish eDNA in Lierelva is a non-conclusive result and requires close follow-up in 2022 regarding the possible spread of crayfish plague. The observed crayfish plague outbreak in Mysenelva increases the number of infected water systems in Norway, and will be integrated in the crayfish plague surveillance in 2022. Also in 2021, frequent detections of noble crayfish eDNA within the regulated *A. astaci* control zones of the

Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog suggest the presence of vital noble crayfish populations within *A. astaci* regulated and restricted zones.

Introduction

The oomycete *Aphanomyces astaci*, the crayfish plague pathogen, is lethal to native European freshwater crayfish (1-3). It is carried and transmitted by North American freshwater crayfish, which act as healthy carriers of the pathogen. *A. astaci* reproduces and spreads with swimming zoospores, the infective stage of the pathogen. It was accidentally introduced to Europe in the 1860s, and resulted in mass-mortalities of freshwater crayfish all over Europe. It was later re-introduced to Europe 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, eight water systems in Norway have been affected by crayfish plague outbreaks one or several times (4-6). These include the Vrangselva watercourse and River Veksa (1971), the Glomma watercourse (1987 and 2003), Lake Store Le (1989), the Halden watercourse (1989, 2005 and 2014), River Lysakerelva (1998), Buåa watercourse (2010), Mosse watercourse (2016), and recently River Mysenelva (2021) (6). In 2016, crayfish plague was confirmed in noble crayfish inhabiting the bordering watercourse Vrangselva and River Billa between Norway and Sweden (which is called River Finnsrudelva on the Norwegian side), but the infection has not yet reached the Norwegian side. In addition, four more localities have been (or still are) subject to crayfish plague regulations due to illegally introduced and confirmed *A. astaci* positive signal crayfish (4). These include Dammane (Vestfold and Telemark), Ostøya (Viken), The Fjelna watercourse (Trøndelag) and Lake Kvesjøen (Trøndelag) where signal crayfish were discovered in 2006, 2009, 2011 and 2013, respectively (4-7). At two of these locations (Dammane and Ostøya), signal crayfish have been successfully eradicated and the areas were declared disease free (4).

The focus areas of the 2021 surveillance programme 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 struck 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 (8). Immediate closure of the Ørje locks prevented upstream spread to Lake Rødenessjøen. Illegally introduced *A. astaci* positive signal crayfish were found in Lake Øymarksjøen in 2008 (9), leading to the permanent closure of the locks. This prevented further spread, until illegally introduced signal crayfish were found upstream of the locks in 2014. The re-established noble crayfish population in Lake Rødenessjøen was lost during the following plague outbreak (10). In this period, the TARGET project (NRC- 243907) compared cage-based

surveillance with environmental DNA (eDNA) monitoring as described in Strand et al. (10). 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). After the main outbreak in Rødnessjøen and the spread of crayfish plague to the River Hølandselva in 2015, *A. astaci* was detected at the outlet of the river in 2016 and in 2019, but not further upstream in the water course (6, 10). Noble crayfish eDNA has been detected at Hølandselva and upstream from 2016-2020 (7, 11-14; Figure 1).

The Mosse watercourse was struck by crayfish plague in 2016 (15). When the crayfish season started in August 2016, the Norwegian Food Safety Authority (NFSA) received reports regarding possible 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 Norwegian Veterinary Institute (NVI) confirmed high levels of *A. astaci* eDNA, corresponding to an outbreak situation (15). 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, while the cage placed in Lake Våg was not affected in 2016 (12). No *A. astaci* eDNA was detected in the Mosse watercourse in 2017, but there was a significant drop in eDNA detection of noble crayfish from June to August in Lake Våg (12). A dead crayfish found in Lake Langen in 2018 was diagnosed with crayfish plague, confirming the upstream spread of crayfish plague in the watercourse (13). No *A. astaci* was detected in the watercourse in 2019 or 2020 (12-13).

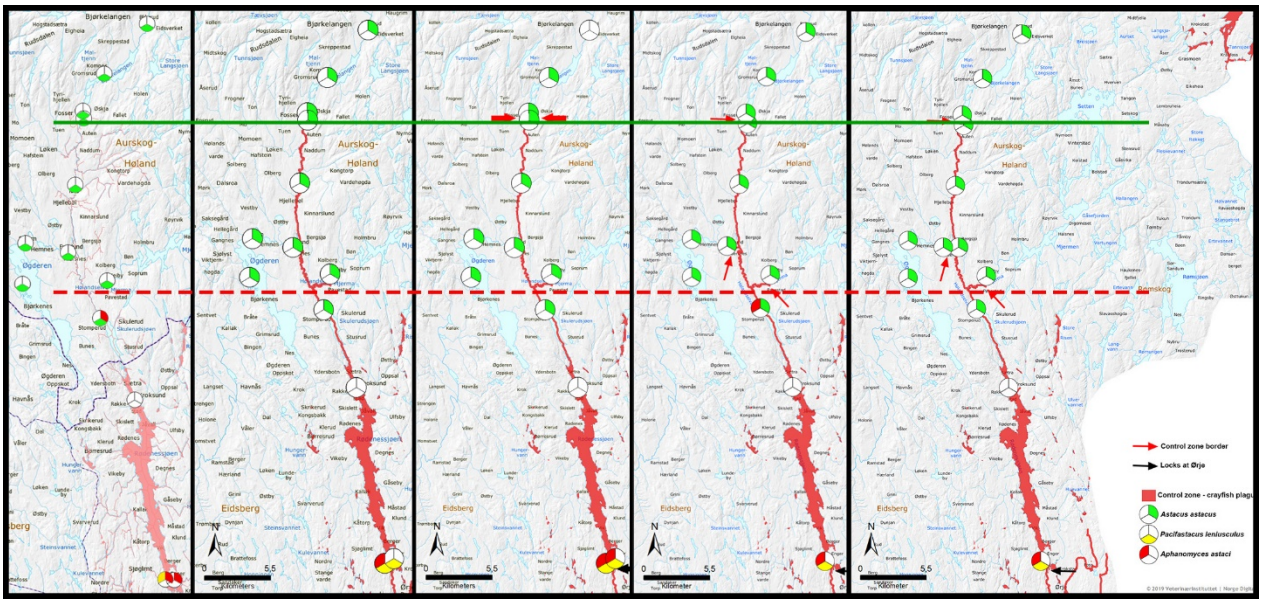

***Figure 1**. Recurring maps of the years 2016 – 2020, showing the stable detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of River Hølandselva (stippled red line) up to the boarder of the control zone at Fosser dam (solid green line).*

The Glomma watercourse was struck by crayfish plague in July 1987, from Kirkenær in Solør and further downstream including Lake Vingersjøen and Lake Storsjøen/River 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, 5, 7). The last detection was in the tributary Opstadåa in 2015. No *A. astaci* eDNA has been detected in the Glomma watercourse, the outlet of Lake Vingersjøen or Oppstadåa in the period 2016-2020 (7, 11-14), while noble crayfish eDNA was detected in River Oppstadåa in 2016 (7) and at Skarnes and Kongsvinger in 2019 (13).

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 to prevent the spread of signal crayfish did not stop the infection from spreading, but hopefully stopped the signal crayfish (4). Cage experiments were conducted in the area until 2016 without revealing any active infection source (7). eDNA analysis of samples for Buåa tested negative for *A. astaci* and signal crayfish in the period 2017-2020 (11-14).

The rivers Vrangselva and Finnsrudelva/Billa in Eidskog municipality that flow across the border into Sweden were struck by crayfish plague on the Swedish side of the border in 2016. The crayfish plague has been active and slowly spreading upstream in River Finnsrudelva/Billa on the Swedish side of the border in 2017 and 2018. However, no sign of crayfish plague has been detected on the Norwegian side of the border in either of these two watercourses in the period 2016-2020 (7, 11-14).

The surveillance programme for *A. astaci* is commissioned by NFSA and conducted by NVI. Until 2015, surveillance of crayfish plague relied on cage experiments with live noble crayfish. In 2016, classical cage experiments were combined with eDNA monitoring (7). Based on an overall assessment taking crayfish welfare and cost-benefit into account, the cage experiments were excluded from the surveillance programme in 2017 (11). From 2018, the program has collaborated with the National surveillance programme for noble crayfish, 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 programme aims to

- Monitor the presence and spread of the crayfish plague pathogen *A. astaci* in 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 programme is based on eDNA monitoring of water, where DNA from spores of *A. astaci* is 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. leniusc*ulus is monitored within the same water samples. The logistics and analyses are conducted in collaboration with the national surveillance of noble crayfish, funded by NEA, and coordinated by NINA (Figure 2).

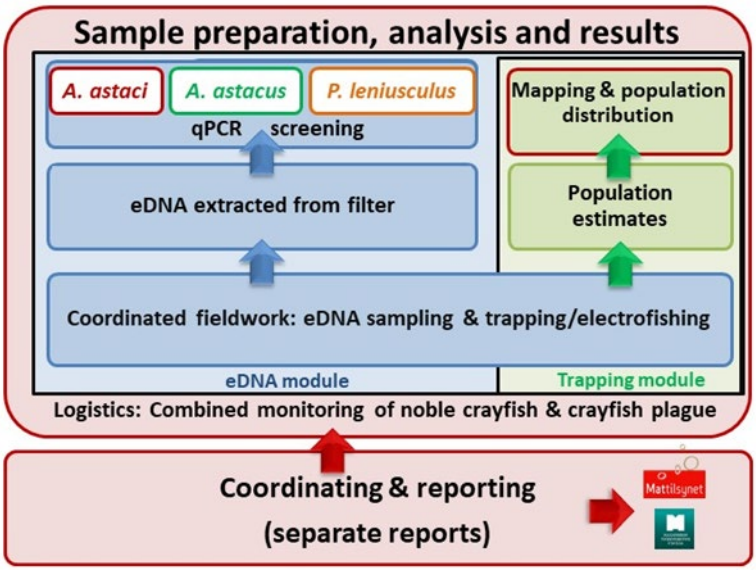

Figure 2. *Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with the national surveillance of noble crayfish (Funded by the Norwegian Environment Agency (NEA).*

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse, 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 3. 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 south of Lake Rødenessjøen (Ysterud). From monitoring in previous years the detection of noble crayfish eDNA within the crayfish plague control zone from the middle part of river Hølandselva (Figure 1) suggests that the upper parts of the system so far has escaped an outbreak. Crayfish localities adjoining the control zone or in close geographical proximity are nevertheless 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 Lake Sværsvann and Lake Bindingsvann and downstream to River Hobølelva, in total 10 sites (Table S3, Appendix 1).

Glomma watercourse: The control zone comprises the main passageway downstream Braskereidfoss in Våler. Five sites within the control zone were monitored. (Table S4, Appendix 1).

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

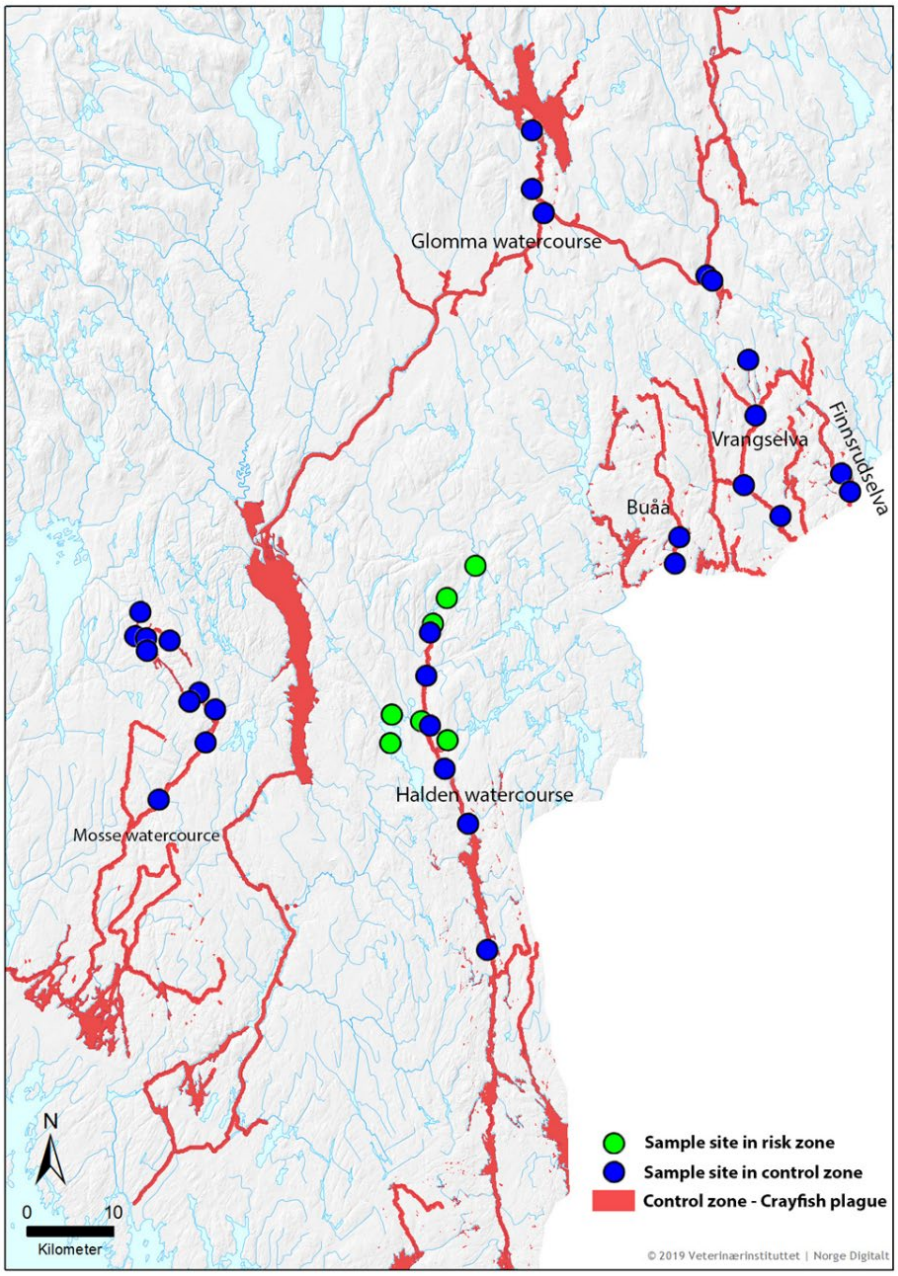

***Figure 3**. Surveillance sites in South-Eastern Norway 2021. Water samples (circles) were collected in June and September. Regulated areas (crayfish plague control zones) are marked in red. Note: For Glomma, the control zone is an approximation*

eDNA monitoring

The water samples were collected in June and September 2021. From each site, two samples of ~5 L were filtered through sterile glass fibre filters on-site (10). Ideally, 5 L was filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower. If less than 3 L were filtered, 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 15 ml falcon tube with ATL-buffer. DNA was extracted using a NucleoSpin Plant II Midi kit (Marcherey-Nagel) protocol (16). The extracted DNA samples were screened by qPCR for three DNA targets: the species-specific qPCR assay for *A. astaci* (10, 17) and two crayfish species specific qPCR assays for noble crayfish and signal crayfish developed by Rusch et al. 2020 (18). Figure 4 presents an overview of the eDNA monitoring procedure.

***Figure 4**. 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 a 15ml tube with buffer and stored there, until further processing in 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 (7).*

Result and Discussion

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 58 water samples representing a total of ~218 L water were analysed. In the control zone, *A. astaci* eDNA was detected in three water samples (two in June and one in September) at the Southern part of Lake Rødenessjøen (Figure 5, Table S2) where signal crayfish were confirmed to be present by positive eDNA results in a total of four water samples (two in June, two in September; Figure 5, Table S2). eDNA from *A. astaci* was also detected at low intensity in one sample in September at the middle station in the River Hølandselva (Figure 5, Table S2). This detection of *A. astaci* is further upstream in the control zone than previously observed, where *A. astaci* has been detected at the outlet of the Riverhølandselva in 2015, 2016 and 2019 (Figure 1). This indicates that *A. astaci* is still present in the river system and is slowly spreading upstream. The positive detections of noble crayfish eDNA in samples from River Hølandselva and upstream (within the control zone), as observed in the previous years (Figure 1), support the presence of live noble crayfish inhabiting the northern part of the Halden watercourse control zone. In total, noble crayfish eDNA was detected in 19 water samples from River Hølandselva and upstream (within the control zone).

All water samples from the risk area surrounding the Halden watercourse were negative for *A. astaci* eDNA. However, one sample was positive for signal crayfish eDNA at the station Lierelva in June. In addition, this coincided with the cessation of positive noble crayfish eDNA detections at this site in September. Another two locations (Lake Bjørkelangen and Hemnessjøen pier) in the risk zone had no positive noble crayfish eDNA samples, which is a decline in noble crayfish detections from previous years. Noble crayfish eDNA was detected at stations downstream from these two locations and in a total of 24 samples in the risk zone (Figure 5, Table S2), demonstrating the presence of noble crayfish within most of the monitored risk zone. The single detection of signal crayfish eDNA at River Lierelva is an uncertain result that will be followed up in 2022.

eDNA monitoring in the Mosse watercourse

In the Mosse watercourse, 39 water samples representing a total of ~161 L water were analysed. None of the analysed samples showed any sign of *A. astaci* or signal crayfish eDNA (Figure 6, Table S3). eDNA from noble cayfish was detected in nine samples (five in June and four in September) at two stations upstream of Lake Langen. This suggests that crayfish plague has not spread upstream from Lake Langen where crayfish plague was confirmed in 2018, after positive detection in one dead crayfish found at Kilevika (12).

eDNA monitoring in the Glomma watercourse

In the Glomma watercourse, 20 water samples representing a total of ~91 L water were analysed. No sign of *A. astaci* or signal crayfish was found through eDNA analysis (Figure 7, Table S4). One sample was positive for noble crayfish eDNA at the station Oppstadåa South, where eDNA of noble crayfish has been detected previously in 2016 (7). The results cannot verify any active *A. astaci* infection or infection source from the monitored sites in the Glomma. In September 2020 signal crayfish was discovered in Glomma during a crayfish survey (20) and

these were confirmed carriers of *A. astaci* (21). These signal crayfish were captured in traps downstream of Solbergfoss, which is in the lower part of Glomma and over 80 km downstream from the eDNA stations in Glomma. In August 2021 the NVI received dead noble crayfish found in River Mysenelva, which is part of River Hæra that drains into Glomma, and high levels of *A. astaci* were detected in the dead crayfish (6). Thus, the monitored area of Glomma should be extended to include River Mysenelva.

eDNA monitoring in Eidskog municipality

In the Eidskog municipality, 36 water samples representing a total of ~138 L water were analysed. None of the analysed samples showed any sign of *A. astaci* or signal crayfish (Figure 7, Table S5). In the Vrangselva watercourse, seven samples from Åbogen to Skotterud were positive for noble crayfish eDNA (four in June, three in September), suggesting that the river stretch is still inhabited by noble crayfish. At the Åbogen sampling site, noble crayfish was also visually confirmed in September. In River Finnsrudelva, eight samples were positive for noble crayfish eDNA (four in June, four in September).

In the Buåa watercourse, no samples were positive for noble crayfish eDNA and no sign of *A. astaci* or signal crayfish was found through eDNA analysis (Figure 7, Table S5). The Buåa watercourse has been monitored by cages for more than six years (7) and by eDNA alone for another five 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.

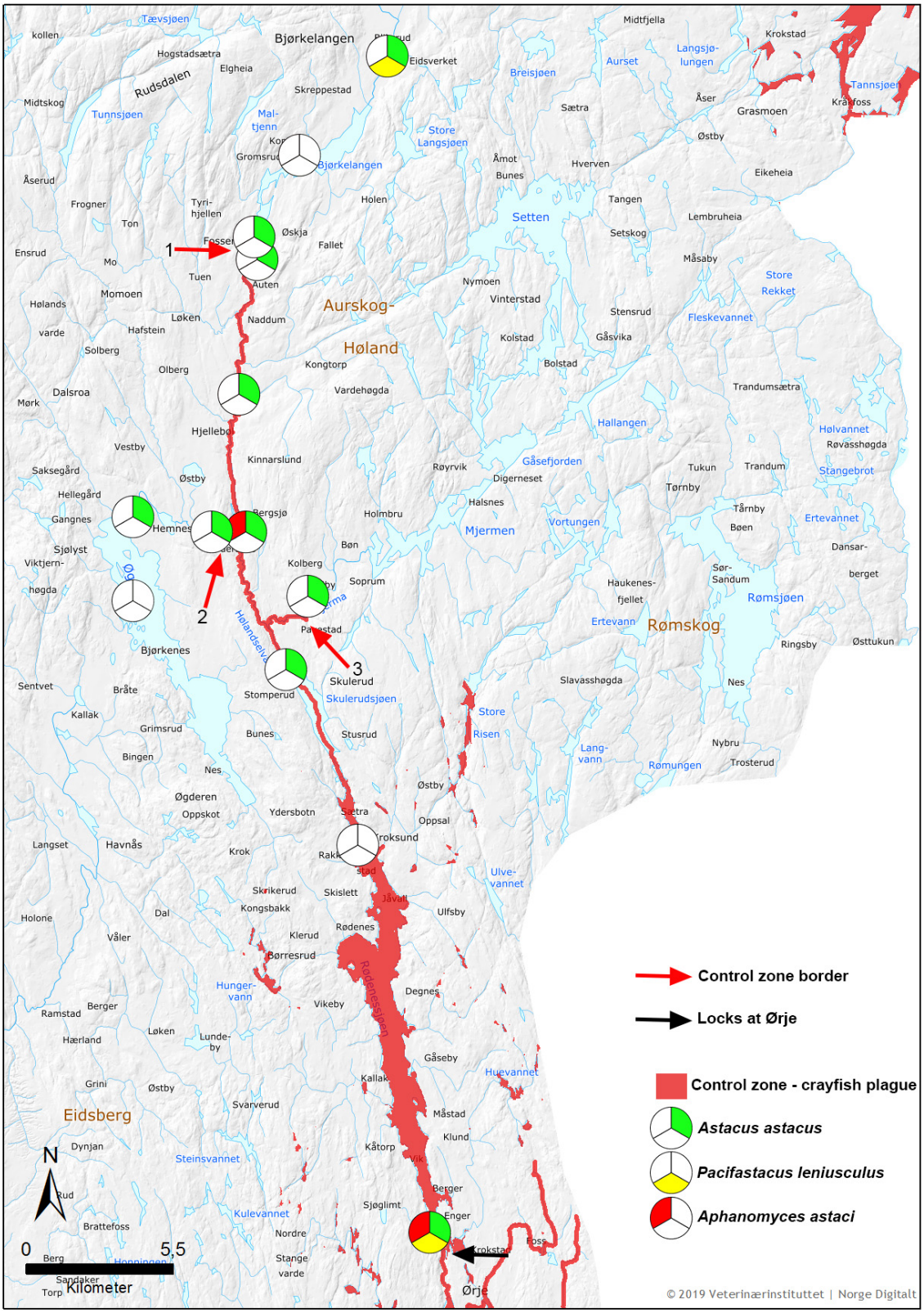

***Figure 5**. Overview map of the surveyed part of the Halden watercourse region in 2021, starting from the Ørje locks (black arrow) in the south where signal crayfish is present. The control area is indicated by red colour on involved lakes and rivers, and ends at Fosserdam, Daltorpsfoss and Lundfoss (red arrows 1, 2 and 3 respectively), where dams acts is artificial barriers 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.*

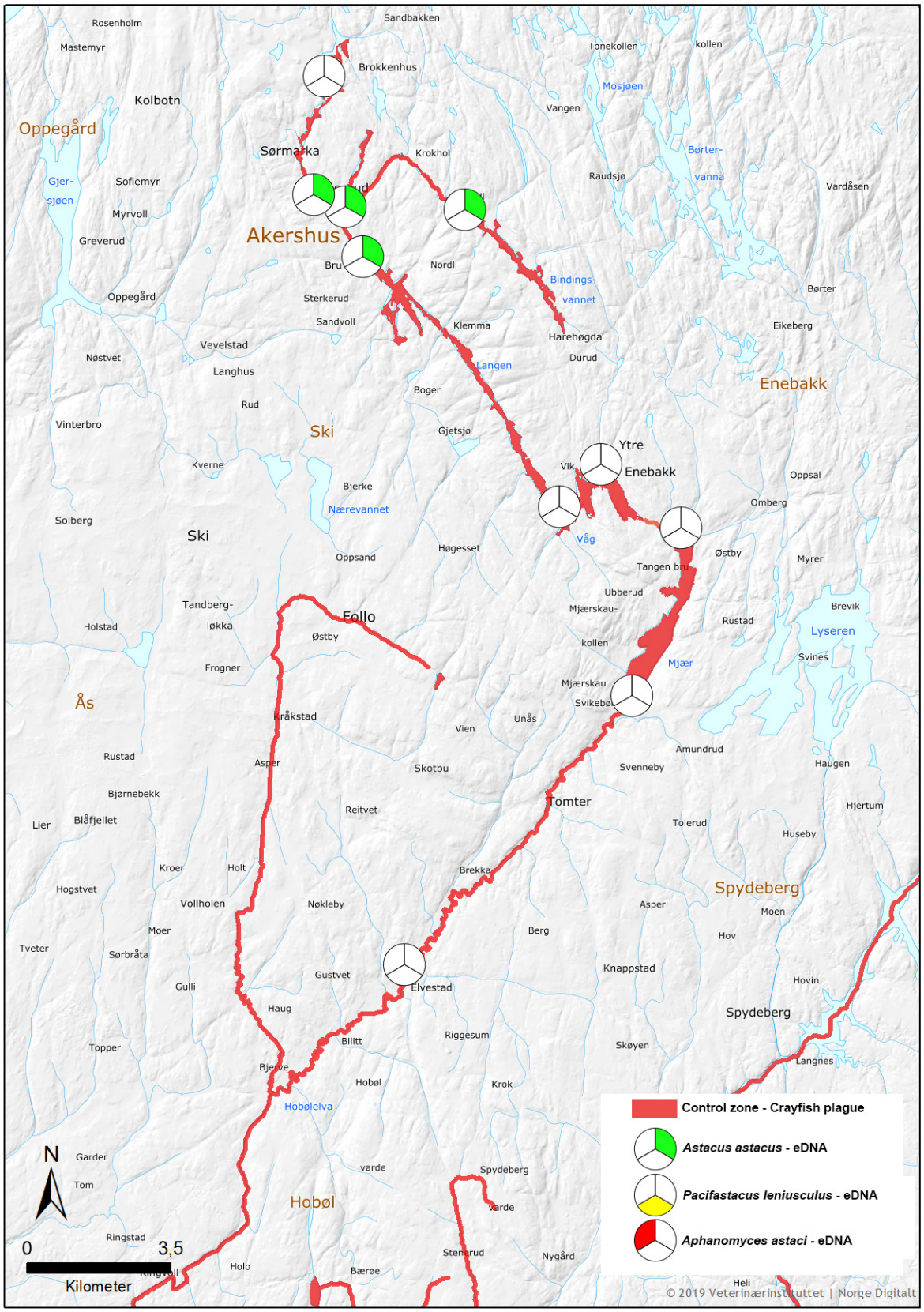

***Figure 6**. Overview map of the surveyed part of the Mosse watercourse. The control area is represented by red colour. The pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). Presence is 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.*

Figure 7. 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.*

Conclusion

In the Halden watercourse, combined eDNA monitoring of *A. astaci*, noble crayfish and signal crayfish confirmed that signal crayfish present in Lake Rødenessjøen release detectable, but low concentrations of *A. astaci* to the water. The detection of *A. astaci* in the middle part of River Hølandselva indicates that the pathogen is still present there in low abundance, and that it is slowly spreading upstream. There was no detection of *A. astaci* in the northern part of River Hølandselva or in any of the stations in the neighbouring risk areas indicating that the outbreak is limited to the lower part of River Hølandselva. This is also supported by positive samples for noble crayfish eDNA at most stations upstream. However, the single positive detection of signal crayfish eDNA in Lierelva combined with reduced number of positive noble crayfish samples compared to previous years is worrying, and must be followed up in 2022.

No eDNA samples were positive for *A. astaci* in the Mosse watercourse in 2021 at the surveyed sites in June and September. While the crayfish plague reached Lake Langen in 2018 (12), detection of noble crayfish eDNA upstream of the lake indicates no further spread.

In the Glomma watercourse, no *A. astaci* or signal crayfish eDNA was detected. The status is still uncertain, given many years of recurrent crayfish plague detection in cage experiments. However, the results indicate that our sampling effort was not sufficient to reveal an eventual infection source in the watercourse. The discovered signal crayfish population downstream of Solbergsfoss in Glomma watercourse does not explain the previous outbreaks further upstream, as Solbergsfoss works as a barrier for upstream spread. Positive eDNA results for noble crayfish were obtained in 2016 and in 2021 (at Opstadåa) and 2019 (at Skarsnes and Vingersnoret), but not in 2017, 2018 or 2020.

We found no sign of *A. astaci* in any of the monitored sites in Eidskog municipality. Similar to the results of 2017-2020, 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.

A new crayfish plague outbreak was discovered in River Mysenelva in 2021 (6), after a dead noble crayfish was found and molecular diagnostics confirmed an *A. astaci* infection.

In summary, eDNA from *A. astaci* was not detected anywhere else than in the control zone within the Halden watercourse, but the positive detection of signal crayfish eDNA in Lierelva is worrying and must be followed up in 2022. The observed crayfish plague outbreak in Mysenelva increases the infected water systems in Norway and the river will be integrated in the crayfish plague surveillance in 2022. Also in 2021, frequent detections of noble crayfish eDNA within the regulated *A. astaci* control and risk zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggest the presence of vital noble crayfish populations within several of the *A. astaci* regulated and restricted zones.

Acknowledgements

Both the surveillance programme for *Aphanomyces astaci* in Norway and the national surveillance programme of noble crayfish benefit from the coordinated and simultaneous monitoring of the three target species (crayfish plague, red-listed noble crayfish and alien invasive signal crayfish). We thank the Norwegian Food Safety Authority (NFSA) for accepting the idea of a joint collaborative project between these surveillance programmes. The surveillance programme for *A. astaci* is funded by NFSA, and the national surveillance programme 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 2021" - Tables S1 – S5.

***Table S1**. Agreed areas and locations of the "NOK A. astaci 2021" program.*

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	8 (2 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	4 (1 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 badeplassen	MV/Enebakk, Ø	Control zone	4 (1 x 2 x 2)
Langen	MV/Enebakk, Ø	Control zone	8 (2 x 2 x 2)
Upstream Langen	MV/Enebakk, Ø	Control zone	16 (4 x 2 x 2)
Total			**140**

[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**. Locations 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	18.5	0	2	1	0	0	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	3	15	0	0	0	0	0	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	20	0	2	0	0	2	0
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	16.7	0	2	0	0	2	0
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	17.5	0	2	0	0	2	0
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	4	16	0	0	0	0	0	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	5	13.8	0	3	0	0	1	0
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	6	11.9	0	3	0	0	3	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	5	17.5	0	3	0	0	2	0
Hølandselva middle	HA14	C	59°43'13"N 11°29'31"E	6	14.4	0	3	0	1	3	0
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	4	16.8	0	2	0	0	2	0
Rødnessjøen Kroksund	HA11	C	59°37'6"N 11°35'5"E	4	20	0	0	0	0	0	0
Rødenessjøen Ysterud	HA12	C	59°29'17"N 11°38'23"E	4	20	2	1	2	1	0	2
Total				58	218.1	2	23	3	2	17	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).
NA = Not available.

***Table S3**. Locations 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*
Bindingsvann, outlet	MO11	C	59°47'22.1"N 10°57'17.6"E	3	9.7	0	1	0	0	1	0
Tangentjern, inlet, bridge on brusagav.	MO12	C	59°47'18.2"N 10°54'02.9"E	5	17.9	0	2	0	0	3	0
Sværsvann	MO8	C	59°49'03.2"N 10°53'25.3"E	5	17.2	0	0	0	0	0	0
Tangentjern, inlet, bridge on Hareveien	MO10	C	59°47'25.7"N 10°53'27.5"E	4	20	0	1	0	0	0	0
Langen, inlet, bridge on Bru-fjellv.	MO9	C	59°46'44.7"N 10°54'38.6"E	4	16	0	1	0	0	0	0
Langen, bridge on Skiveien	MO1	C	59°43'33.3"N 11°00'12.1"E	3	11.6	0	0	0	0	0	0
Våg	MO2	C	59°44'10.2"N 11°01'14.7"E	4	16.8	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	3	15	0	0	0	0	0	0
Mjær, outlet	MO6	C	59°41'10.2"N 11°02'27.6"E	4	17.2	0	0	0	0	0	0
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	4	20	0	0	0	0	0	0
Total				39	161.4	0	5	0	0	4	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**. Locations 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.*

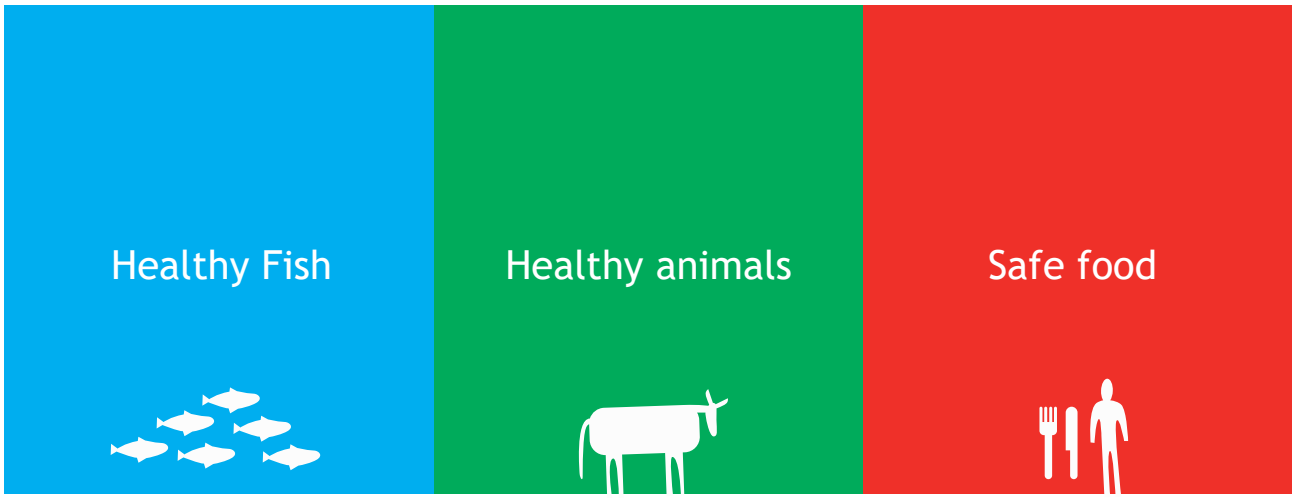
Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vingersnoret	GL1	C	60°11'36.3"N 12°01'54.5"E	4	20	0	0	0	0	0	0
North of Vingersnoret	GL2	C	60°11'39.7"N 12°01'41.2"E	4	17.6	0	0	0	0	0	0
Storsj. Ringåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	4	16	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	17.5	0	1	0	0	0	0
Glomma, Skarnes	GL10	C	60°15'20.8"N 11°40'49.4"E	4	20	0	0	0	0	0	0
Total				20	91.1	0	1	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**. Locations 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	2	0	0	2	0
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	6	18.7	0	0	0	0	1	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	5	14.9	0	2	0	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	4	15.5	0	0	0	0	0	0
Finnsrudelva, Finnsrudvegen	FR1	C	59°59'50.7"N 12°19'05.4"E	4	20	0	2	0	0	2	0
Finnsrudelva, Billavegen	FR2	C	59°58'44.9"N 12°20'14.2"E	4	20	0	2	0	0	2	0
Buåa, Eidskog	BU1	C	59°55'31.1"N 11°59'37.0"E	4	18	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	5	10.6	0	0	0	0	0	0
Total				36	137.7	0	8	0	0	7	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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