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The surveillance programme for *Aphanomyces astaci* in Norway 2023

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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. 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. These analyses are part of the collaboration and coordination with the national surveillance programme for noble crayfish. The geographic focus of the surveillance programme in 2023 is the Halden watercourse and neighbouring risk areas; the Mosse watercourse, Glomma watercourse, River Hæra and areas in the Eidskog municipality including the Buåa watercourse and the rivers Vrangselva and Finnsrudelva.

- In the Halden watercourse, eDNA of *A. astaci* was detected in the southern part of Lake Rødnessjøen within the restriction zone. eDNA of signal crayfish was also detected in the south of Lake Rødnessjøen, where there is a known signal crayfish population. All water samples in the risk area of the Halden watercourse region were negative for eDNA from *A. astaci* and signal crayfish, while most samples were positive for noble crayfish eDNA.
- In the Mosse watercourse, no eDNA from *A. astaci* or signal crayfish was detected, while noble crayfish eDNA was detected upstream of Lake Langen.
- In the Glomma watercourse, no eDNA from *A. astaci*, signal crayfish or noble crayfish was detected at any of stations in Glomma.
- In River Hæra, eDNA from both *A. astaci* and noble crayfish were detected at one location in June. Additional sampling confirmed an active crayfish plague outbreak in the risk area, upstream the restriction zone in the river. No eDNA from signal crayfish was detected.
- In Eidskog, all samples were negative for signal crayfish and *A. astaci*, while several samples were positive for noble crayfish eDNA in the rivers Buåa, Vrangselva and Finnsrudelva.
- Illegally introduced signal crayfish was discovered in Lake Østersjøen, which is connected to Glomma, after tip from the locals. The signal crayfish was confirmed to host *A. astaci*.

In summary, eDNA from *A. astaci* was detected within the restriction zone in Lake Rødnessjøen in the Halden watercourse, and in River Hæra within the risk area. The detection of *A. astaci* eDNA in Hæra initiated further sampling and an active crayfish plague outbreak was confirmed. By using eDNA we were able to follow the outbreak front moving upstream. Frequent detections of noble crayfish eDNA within the regulated *A. astaci* restriction zones of the Halden watercourse, Mosse watercourse, and the rivers Vrangselva and Finnsrudelva in Eidskog, suggest the presence of vital noble crayfish populations within *A. astaci* restriction zones.

Sammendrag

Overvåkningsprogrammet benytter miljø-DNA overvåking for artsspesifikk påvisning av *Aphanomyces astaci* sporer (krepsepest agens) direkte fra filtrerte vannprøver. Tilstedeværelse/fravær av miljø-DNA fra edelkreps (*Astacus astacus*) og signalkreps (*Pacifastacus leniusculus*) undersøkes også for å supplere resultatene, og for å evaluere habitat status. Påvisning av miljø-DNA for edelkreps, kombinert med fravær av miljø-DNA fra *A. astaci* og signalkreps, støtter tilstedeværelsen av ikke-infisert edelkreps som utgjør ønsket habitatstatus. Disse analysene er en del av samarbeidet og koordineringen med det nasjonale overvåkingsprogrammet for edelkreps. Geografisk fokus for overvåkingsprogrammet i 2023 er Haldenvassdraget og nærliggende risikoområder; Mossevassdraget, Glommavassdraget, Hæra og områder i Eidskog kommune inkludert Buåavassdraget og elvene Vrangselva og Finnsrudelva.

- I Haldenvassdraget ble det påvist miljø-DNA fra *A. astaci* i den sørlige delen av Rødnessjøen innenfor restriksjonssonen. Miljø-DNA fra signalkreps ble også påvist sør i Rødnessjøen, hvor det er en kjent signalkrepsbestand. Alle vannprøver i risikoområdet til Haldenvassdragsregionen var negative for miljø-DNA fra *A. astaci* og signalkreps, mens de fleste prøvene var positive for edelkreps miljø-DNA.
- I Mossevassdraget ble det ikke påvist miljø-DNA fra *A. astaci* eller signalkreps, mens det ble påvist edelkreps miljø-DNA oppstrøms Langen.
- I Glommavassdraget ble det ikke påvist miljø-DNA fra *A. astaci*, signalkreps eller edelkreps ved noen av stasjonene i Glomma.
- I Hæra ble det påvist miljø-DNA fra både *A. astaci* og edelkreps ved en stasjon i juni. Ytterligere prøvetaking bekreftet et aktivt krepsepestutbrudd i risikoområdet, oppstrøms restriksjonssonen i elva. Det ble ikke påvist miljø-DNA fra signalkreps.
- I Eidskog var alle prøver negative for miljø-DNA fra signalkreps og *A. astaci*, mens flere prøver var positive for edelkreps miljø-DNA i Vrangselva og Finnsrudelva.
- Ulovlig utsatt signalkreps ble funnet i Østersjøen, som er forbundet med Glomma, etter tips fra lokalbefolkningen. Det ble påvist *A. astaci* på signalkrepsen.

Kort oppsummert, så ble det påvist miljø-DNA fra *A. astaci* innenfor restriksjonssonen i Rødnessjøen i Haldenvassdraget, og i Hæra innenfor risikosonen. Påvisningen av *A. astaci* miljø-DNA i Hæra førte til ytterligere prøvetaking og et aktivt krepsepestutbrudd ble bekreftet. Ved hjelp av miljø-DNA var man i stand til å følge utbruddsfronten bevege seg oppstrøms. Flere påvisninger av edelkreps miljø-DNA innenfor de regulerte restriksjonssonene for bekjempelse av krepsepest i Haldenvassdraget, Mossevassdraget og elvene Vrangselva og Finnsrudelva i Eidskog, tyder på tilstedeværelse av vitale edelkrepsbestander innenfor restriksjonssonene.

Introduction

Aphanomyces astaci in Norway

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 category F disease in Norway, according to the "*The animal health regulations" Chapter II, § 6"* FOR-2022-04-06-631.

Since 1971, nine 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 Hæra (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 also called River Finnsrudelva on the Norwegian side), but the infection has not been detected on 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 (Telemark), Ostøya (Akershus), 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].

Focus areas 2023

The focus areas of the 2023 surveillance programme for crayfish plague cover the

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

Halden Watercouse

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 [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, and further upstream in 2021 and 2022 ([6, 10, 11]; **Figure 1**). Noble crayfish eDNA has been detected at Hølandselva and upstream from 2016-2022 ([7, 11-16]; **Figure 1**). While one sample was positive for signal crayfish eDNA in River Lierelva in 2021 ([16]; **Figure 1**), no signal crayfish were detected in Lierelva in 2022 after additional eDNA sampling and crayfish trapping [11].

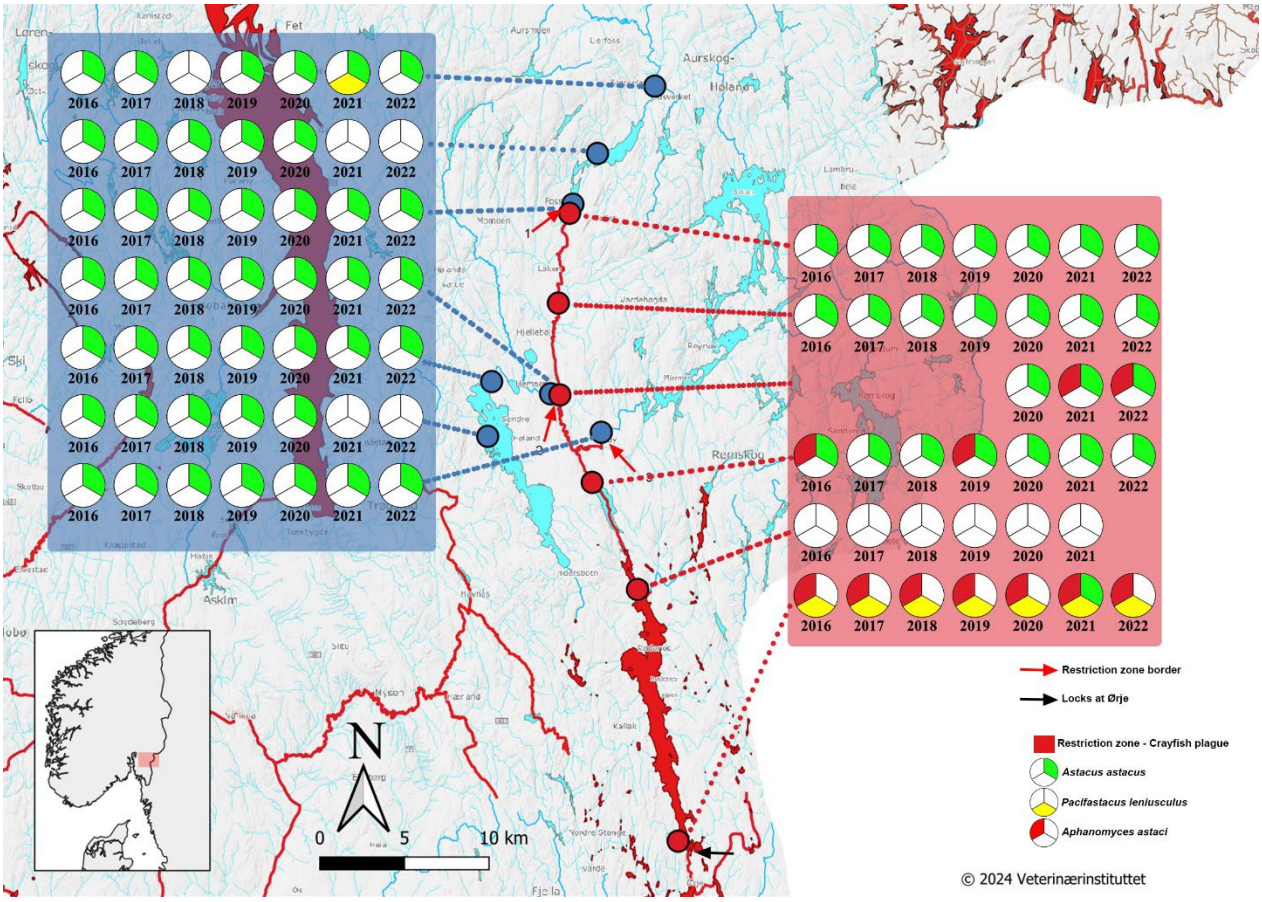

Figure 1. Recurring maps of the years 2016 – 2022, showing the stable detection of noble crayfish eDNA within the crayfish plague restriction zone from the middle part of River Hølandselva (stippled red line) up to the boarder of the restriction zone at Fosserdam (solid green line).

The Mosse watercourse

The Mosse watercourse was struck by crayfish plague in 2016 [17]. 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 [17]. 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 [13]. No *A. astaci* eDNA was detected in the Mosse watercource 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 2019-2022 [11, 14-16].

The Glomma watercourse

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 2016-2021 [7, 12-16], while noble crayfish eDNA was detected in River Oppstadåa in 2016 [7] and at Skarnes and Kongsvinger in 2019 [14]. Signal crayfish, and confirmed carriers of *A. astaci*, was discovered in Glomma at Fossum bridge, downstream Solbergfoss in 2020 [18, 19], and both signal crayfish and *A. astaci* eDNA was detected at this location in 2022 [11].

River Hæra

River Hæra, which drains into Glomma was struck by crayfish plague in 2021 [6, 16]. *A. astaci* was detected on dead crayfish found in the river. The outbreak was limited to the river downstream of Rustadfossen, where a constructed dam could act as a barrier. In august 2022 at the station upstream Rustadfossen in the risk area, two eDNA samples amplified for *A. astaci*, but below the detection limit [11]. Thus, indicating the need for follow up in 2023.

The rivers Vrangselva and Finnsrudselva

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 2016-2022 [7, 11-16].

The Buåa watercourse

The Buåa system was struck by crayfish plague in 2010 caused by the presence of signal crayfish on the Swedish side of the river [20]. 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 2017-2021 [12-16]. In 2021 and 2022 an extensive monitoring program, utilising eDNA,

trapping and cage experiments, was conducted in Buåa to evaluate the watercourse for disease freedom and no trace of *A. astaci* or signal crayfish were detected [11].

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 [12]. 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 areas regulated as a result of earlier detection of the pathogen (referred to as restriction zones[1]).
- Substantiate disease free waterbodies in neighbouring areas of the restriction zones (referred to as risk areas[2]).
- Alert the authorities of any eventual spread of the disease from restriction zones to risk areas.

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 and the alien carrier signal crayfish 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**).

[1] The «restriction zone» refers to the complete restriction zone covered by each of the regulations. For all practical purposes, a crayfish plague restriction zone does not differentiate between a protection zone and a surveillance zone.

[2] Risk area is not an official term according to the animal health regulations, but a term we have chosen to use for areas adjacent to or geographically close to the crayfish plague restriction zones covered by the regulations. These areas host healthy noble crayfish populations that face a high risk for spread of the infection from the restriction zones.

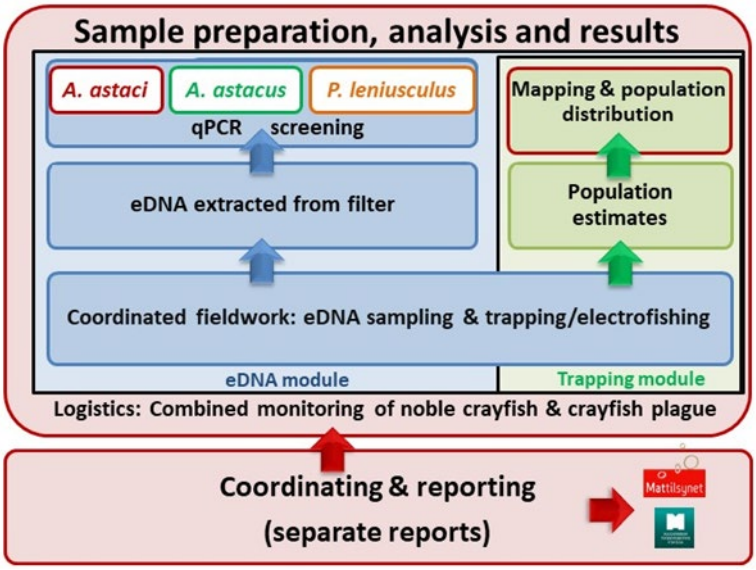

***Figure 2**. Work plan: The Norwegian Veterinary Institute (NVI) coordinates the project, and organises the eDNA water sampling and qPCR screenings in collaboration with the national surveillance of noble crayfish (Funded by the Norwegian Environment Agency (NEA).*

Surveillance sites

The main areas for surveillance include the Halden watercourse and surrounding areas, the Mosse watercourse, the Glomma watercourse, the River Hæra and Eidskog municipality including the Vrangselva watercourse, River Finnsrudelva and Buåa watercourse. Plotted locations for water sampling, in total 48 sites, as well as the crayfish plague zones, are displayed in **Figure 3**. Supplementary details are summarised in Appendix 1 (**Table S1-S6**).

Halden watercourse: The restriction zone was monitored at a total of 5 sites from Lake Fossersjøen to the south of Lake Rødenessjøen (Ysterud). Previous detection of noble crayfish eDNA within the crayfish plague restriction zone from the middle part of river Hølandselva (**Figure 1**) suggests that the upper parts of the system so far has escaped an outbreak. In total, 7 sites were monitored in the risk area (**Table S2**, Appendix 1).

Mosse watercourse: The restriction 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 restriction zone comprises the main passageway downstream Braskereidfoss in Våler. Six sites within the restriction zone were monitored. (**Table S4**, Appendix 1).

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

River Hæra: The restriction zone comprises the main passageway downstream Rustadfossen in Mysen. Two sites in the risk area and two sites within the restriction zone were monitored (**Table S6**, Appendix 1). Additional sites were monitored in the risk area after detection of *A. astaci* in eDNA samples at one site in the risk area, and the restriction zone expanded as a result if the confirmation of *A. astaci* caused mortality.

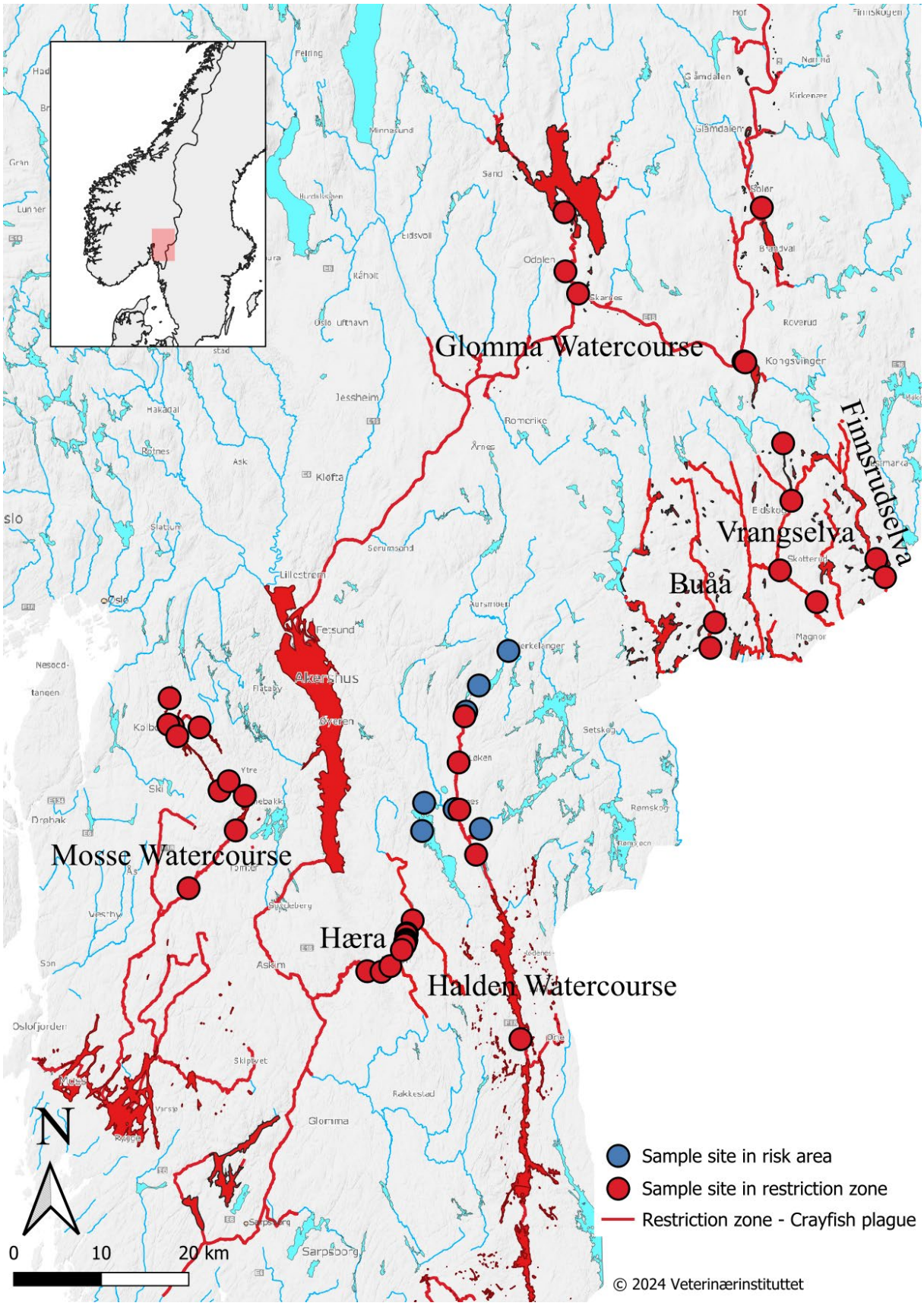

***Figure 3**. Surveillance sites in South-Eastern Norway 2023. Water samples (circles) were collected in June and August or September, and additional samples were collected in July and October in Hæra. Regulated areas (crayfish plague restriction zones) are marked in red. Note: For Glomma, the restriction zone is an approximation.*

eDNA sampling

The filter samples were collected in June and August/September. Additional samples were collected in Hæra in July and October. From each site, two samples of up to ~5 L water were filtered through sterile glass fibre filters on-site [10]. Ideally, 5 L was to be filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower.

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 [21, 22]. The extracted DNA samples were screened by qPCR for three DNA targets: the species-specific qPCR assay for *A. astaci* [10, 23] and two crayfish species specific qPCR assays for noble crayfish and signal crayfish developed [24]. **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. 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. 2016 [7].*

Result and Discussion

eDNA monitoring in the Halden watercourse

From the Halden watercourse region, 48 water samples were collected from 12 stations during the sampling in June and August 2023. In the restriction zone, *A. astaci* eDNA was detected in two water samples (two in June) at the Southern part of Lake Rødenessjøen where signal crayfish were confirmed to be present by positive eDNA results in a total of four water samples (two in May, two in September; **Figure 5**, **Table S2**). eDNA from *A. astaci* was not detected from samples collected in the River Hølandselva, as in 2019, 2021 and 2022. The positive detections of noble crayfish eDNA in samples from River Hølandselva and upstream (within the restriction zone), as observed in the previous years (**Figure 1**), support the presence of live noble crayfish inhabiting the northern part of the Halden watercourse restriction zone. In total, noble crayfish eDNA was detected in 13 water samples from River Hølandselva and upstream (within the restriction zone).

All water samples from the seven stations in the risk area surrounding Halden watercourse were negative for *A. astaci* eDNA, while most samples (20 samples) were positive for noble crayfish eDNA (**Figure 5**, **Table S2**), demonstrating the presence of noble crayfish within most of the monitored risk area. While signal crayfish eDNA was detected in one samples from River Lierelva in 2021, there were no detection of signal crayfish during the extended sampling in 2022 [11] or in the 2023 monitoring.

eDNA monitoring in the Mosse watercourse

From the Mosse watercourse, 40 water samples were analysed from 10 stations. 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 six samples (four in June and two in August) 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 detection in one dead crayfish found at Kilevika [13].

eDNA monitoring in the Glomma watercourse

From the Glomma watercourse, 24 water samples were analysed from six stations. No sign of *A. astaci* or signal crayfish was found through eDNA analysis (**Figure 7**,**Table S4**) at any of the monitored stations. Neither were from noble crayfish detected in any of the samples.

In cooperation with NINA, after receiving a tip from the locals, signal crayfish was discovered in Lake Østersjøen in Åmot municipality [25]. Analysis conducted at NVI confirmed that the signal crayfish was carriers of *A. astaci*. Lake Østersjøen is connected to the Glomma watercourse, via the outlet River Gjesa, River Julussa and River Rena into River Glomma.

eDNA monitoring in the rivers Buåa, Vrangselva and Finnsrudselva

In the Eidskog municipality, 32 water samples were analysed from eight stations. All samples were negative for eDNA from *A. astaci* or signal crayfish (**Figure 7**, **Table S5**). In the Vrangselva watercourse, four samples from Åbogen were positive for noble crayfish eDNA (2 in June, 2 in September), suggesting that the river stretch is still inhabited by noble crayfish. In River Finnsrudelva, eight samples were positive for noble crayfish eDNA (4 in June and 4 in September,

Figure 7, **Table S5**). In River Buåa, all samples were negative for the presence of eDNA from noble crayfish, signal crayfish and *A. astaci* (**Figure 7**, **Table S5**).

eDNA monitoring in River Hæra

From River Mysenelva, a total of 34 water samples were analysed from 12 stations. Two samples collected in June, from a station in the risk area upstream Rustadfossen, were positive for *A. astaci* and noble crayfish eDNA (**Figure 8**, **Table S6**). This led to additional eDNA sampling and trapping after noble crayfish upstream the positive station. Both new eDNA samples and dead crayfish found in the river in July, confirmed an active outbreak in the risk area. Using eDNA sampling, we could follow the spread of crayfish plague upstream, and by October 2023 the outbreak had reached just downstream of Krogstad Bruk in October 2023 (**Figure 8**, **Table S6**). The restriction zone in Hæra has been expanded as a result of the crayfish plague outbreak (see FOR-2005-06-20-652).

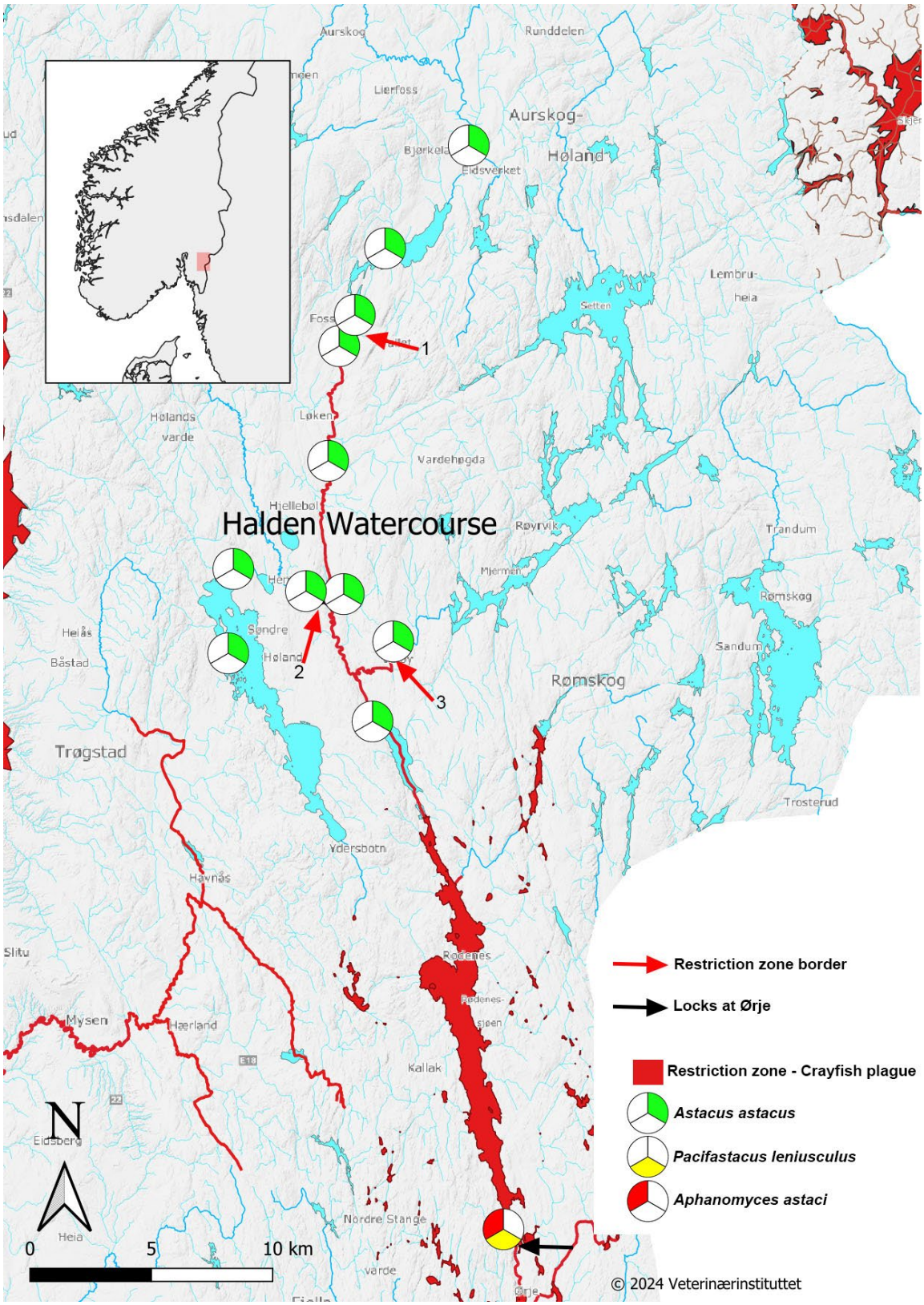

***Figure 5**. Overview map of the surveyed part of the Halden watercourse region in 2023, starting from the Ørje locks (black arrow) in the south where signal crayfish is present. The restriction zone is indicated by red colour on involved lakes and rivers, and ends at Fosserdam, Daltorpsfoss and Lundfoss (red arrows 1, 2 and 3 respectively), where dams act as 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.*

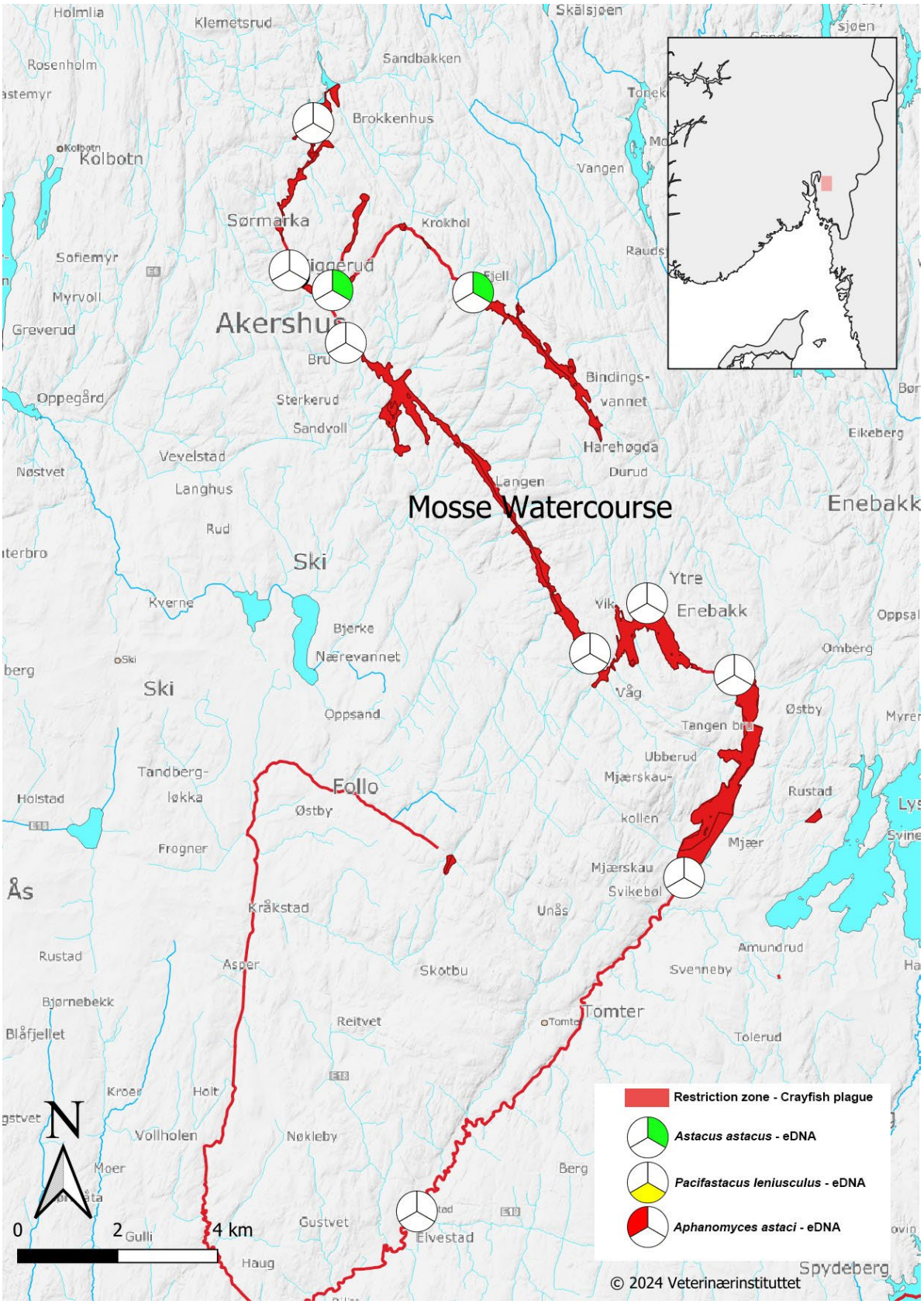

***Figure 6**. Overview map of the surveyed part of the Mosse watercourse in 2023. The restriction zone is represented by red colour. The pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*P. leniusculus*; yellow), and noble crayfish (*A. astacus*; green). 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.*

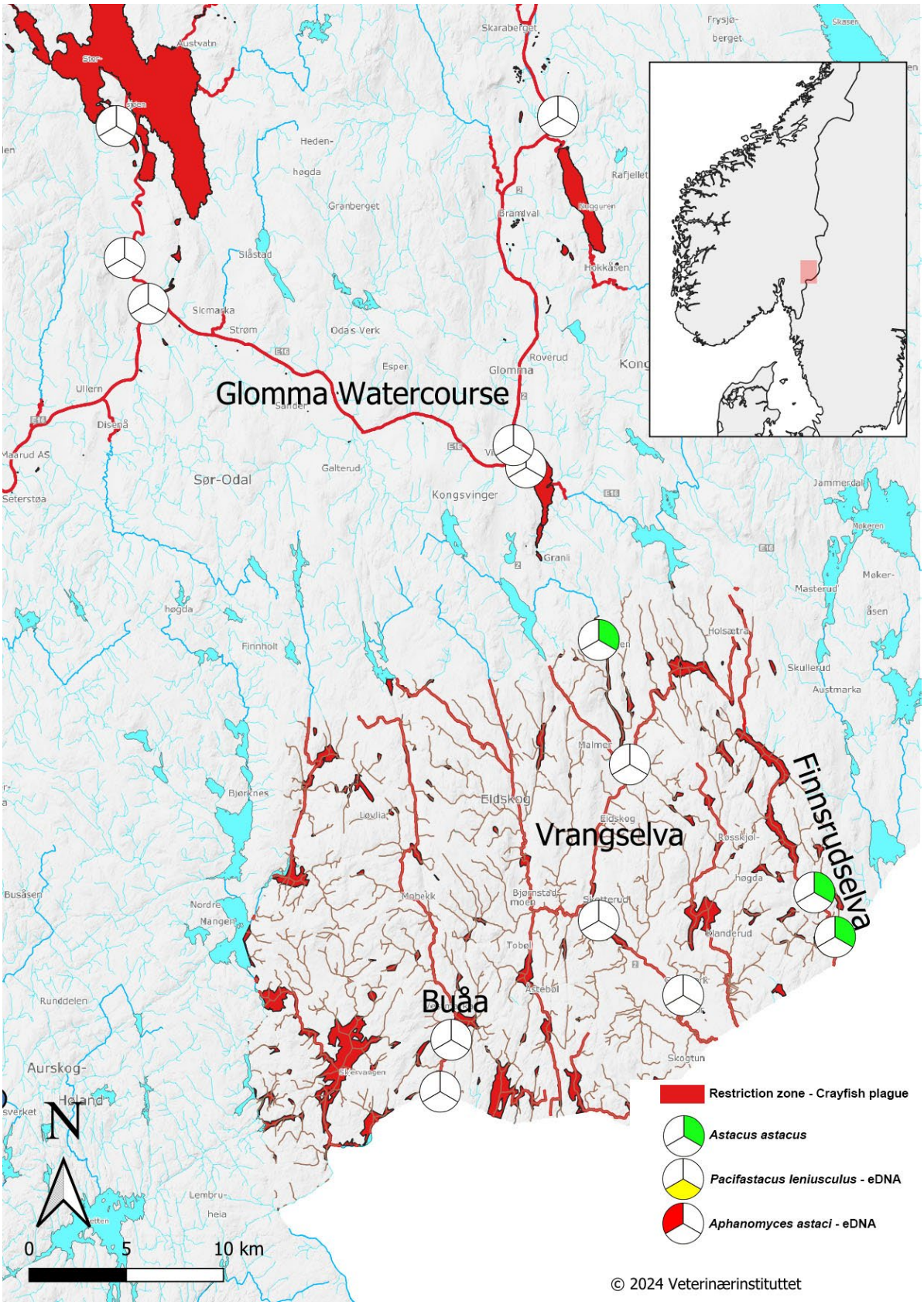

***Figure 7**. Overview map of the surveyed part of the Glomma watercourse region and Eidskog municipality in 2023. Regulated areas (crayfish plague restriction zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal 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.*

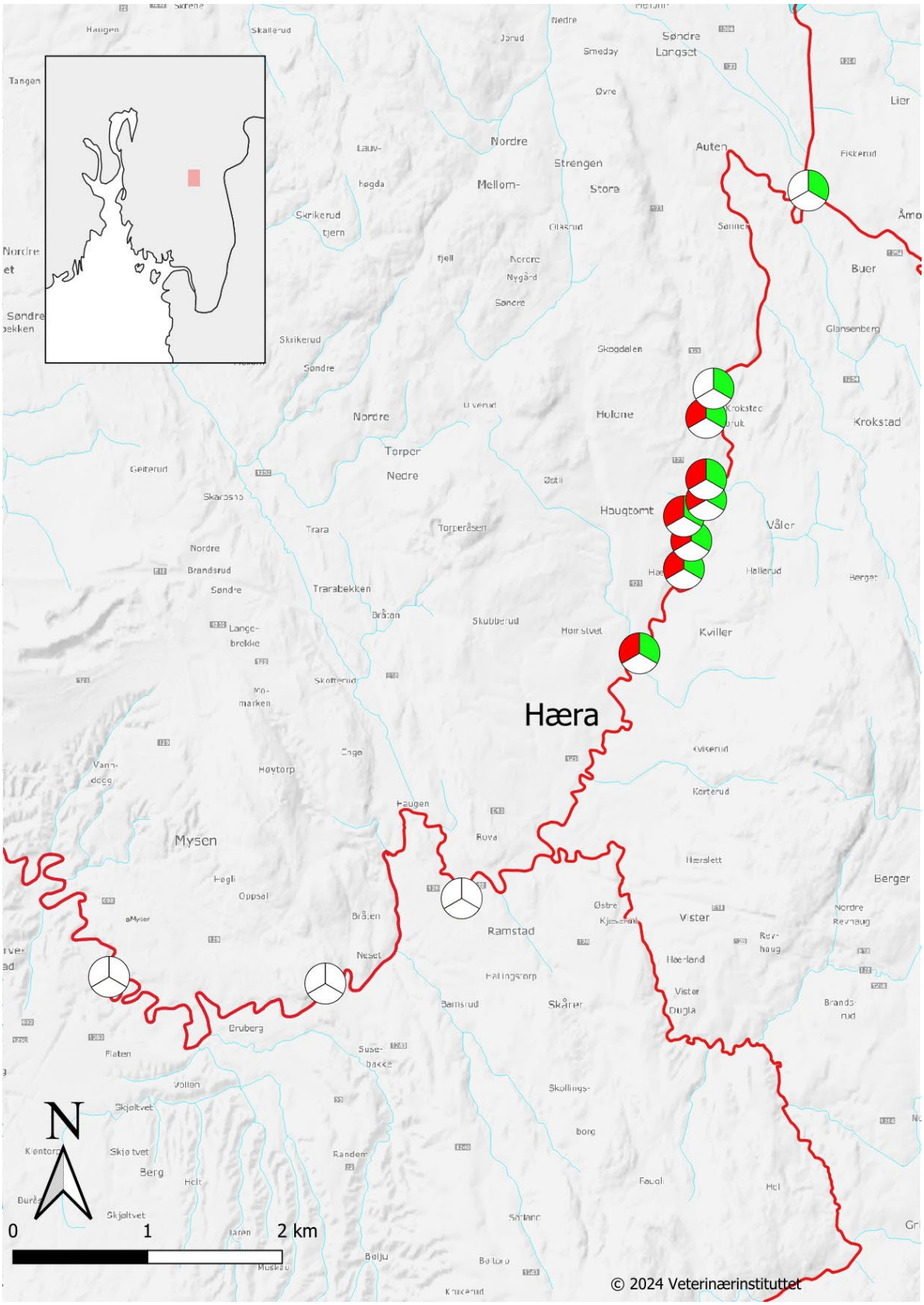

***Figure 8**. Overview map of the surveyed part of River Hæra in 2023, including additional sites in the river. Regulated areas (crayfish plague restriction zones) are marked in red. For each location site, the pie chart indicates presence (colour) or absence (white) of* A. astaci *(red), signal crayfish (*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, the combined eDNA monitoring of *A. astaci*, noble crayfish and signal crayfish demonstrates that signal crayfish present in Lake Rødenessjøen release detectable concentrations of *A. astaci* to the water. While *A. astaci* had been detected in River Hølandselva in 2019, 2021 and 2022, *A. astaci* was not detected at any of the stations of River Hølandselva in 2023. It is uncertain if *A. astaci* has disappeared from the river or is still present within the restriction zone, albeit at very low abundance. 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 detection of noble crayfish eDNA at most of the stations upstream. The single positive detection of signal crayfish eDNA in Lierelva in 2021 was not confirmed in 2022 in spite of increased sampling (three sites) in the river, nor in the 2023 sampling.

No eDNA samples were positive for *A. astaci* in the Mosse watercourse in 2023. While the crayfish plague reached Lake Langen in 2018, 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 upstream Solbergfoss. The status is still uncertain, given many years of recurrent crayfish plague detection in cage experiments up until 2015. However, the results indicate that our sampling has not been sufficient to reveal a suspected infection source in the watercourse upstream Solbergfoss. The discovery of *A. astaci* positive signal crayfish in Lake Østersjøen, which drains into Glomma via the outlet River Gjesa, River Julussa and River Rena, documents a permanent *A. astaci* reservoir with connection to Glomma watercourse. It is unknown when signal crayfish was illegally introduced into the lake, and if this populations can be related to the historical crayfish plague outbreaks in Glomma. None the less, a permanent *A. astaci* reservoir hinders reestablishment of noble crayfish in the watercourse.

Both noble crayfish and *A. astaci* eDNA was detected at one station in the risk area of River Hæra, indicating an outbreak of crayfish plague upstream of the restriction zone. Additional eDNA sampling and trapping was initiated in the river. The crayfish plague outbreak was subsequently confirmed with the detection of *A. astaci* in dead noble crayfish found in the river during trapping. Using eDNA we were able to identify and follow the outbreak front as it progressed upstream in the river.

We found no sign of *A. astaci* or signal crayfish in any of the monitored sites in Eidskog municipality. Similar to the results of 2017-2022, 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. While in Buåa, none of the samples were positive for *A. astaci*, signal crayfish or noble crayfish.

In summary, eDNA from *A. astaci* was detected in River Hæra, and an active crayfish plague outbreak was revealed in the river. Additionally, we detected eDNA from *A. astaci* within the restriction zone in the Halden watercourse where a known signal crayfish population is present. The frequent detections of noble crayfish eDNA within the regulated *A. astaci* restriction and risk areas 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). We also thank the County Governor of Oslo and Viken, the County Governor of Innlandet, Utmarksforvaltningen AS and local stakeholders for their contribution to the extended monitoring in Buåa.

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Appendix

Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2023" - Tables S1 – S6.

***Table S1**. Agreed areas and locations of the "NOK A. astaci 2023" program. However, due to ambiguous results from one station in River Hæra in autums 2022, the station "Glomma, Fossum" was replaced with one addition station in the risk area of River Hæra. After detection of A. astaci in River Hæra additional stations (see table S6) were monitored to follow the active crayfish plague outbreak in the river.*

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	Restriction zone	4 (1 x 2 x 2)
Hølandselva	HW/Aurskog-Høland, A	Restriction zone	12 (3 x 2 x 2)
Fossersjøen	HW/Aurskog-Høland, A	Restriction zone, outbreak expected	4 (1 x 2 x 2)
Fosserdam	HW/Aurskog-Høland, A	Risk area/restriction zone border	4 (1 x 2 x 2)
Bjørkelangen	HW/Aurskog-Høland, A	Risk area	4 (1 x 2 x 2)
Lierelva	HW/Aurskog-Høland, A	Risk area	4 (1 x 2 x 2)
Lundsfoss	HW/Aurskog-Høland, A	Risk area	4 (1 x 2 x 2)
Dalstorpsfoss	HW/Aurskog-Høland, A	Risk area	4 (1 x 2 x 2)
Hemnessjøen	Lake/Aurskog-Høland, A	Risk area	8 (2 x 2 x 2)
Glomma watercourse			**Total samples 28**
Oppstadåa	GW/Sør-Odal, I	Restriction zone	8 (2 x 2 x 2)
Skarsnes	GW/ Sør-Odal, I	Restriction zone	4 (1 x 2 x 2)
Vingersnoret	GW/ Sør-Odal, I	Restriction zone	4 (1 x 2 x 2)
Vingersjøen	GW/ Sør-Odal, I	Restriction zone	4 (1 x 2 x 2)
Glomma, Fossum	GV/Indre Østfold, Ø	Restriction zone	4 (1 x 2 x 2)
Glomma, Strategic	GV/Indre Østfold, I	Restriction zone	4 (1 x 2 x 2)
River Hæra			**Total samples 12**
Upstream Rustadf.	RM/Indre Østfold, Ø	Risk area	4 (1 x 2 x 2)
Downstr. Rustadf.	RM/Indre Østfold, Ø	Restriction zone	4 (1 x 2 x 2)
Downstr. Susebakk.	RM/Indre Østfold, Ø	Restriction zone	4 (1 x 2 x 2)
Eidskog			**Total samples 32**
Buåa	BW/Eidskog, I	Restriction zone	8 (2 x 2 x 2)
Finnsrudelva	RF/Eidskog, I	Restriction zone	8 (2 x 2 x 2)
Vrangselva	VW/Eidskog, I	Restriction zone	16 (4 x 2 x 2)
Mosse watercourse			**Total samples 40**
Hobølelva	MV/Enebakk, Ø	Restriction zone	4 (1 x 2 x 2)
Mjær	MV/Enebakk, Ø	Restriction zone	4 (1 x 2 x 2)
Tangenelva	MV/Enebakk, Ø	Restriction zone	4 (1 x 2 x 2)
Våg	MV/Enebakk, Ø	Restriction zone	4 (1 x 2 x 2)
Langen	MV/Enebakk, Ø	Restriction zone	8 (2 x 2 x 2)
Upstream Langen	MV/Enebakk, Ø	Restriction zone	16 (4 x 2 x 2)
Total			**160**

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

***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			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Lierelva, Bjørkelagen	HA1	R	59°53'8"N 11°34'29"E	4	13	0	0	0	0	0	2
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	4	20	0	0	2	0	0	2
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	15	0	0	2	0	0	2
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	4	14	0	0	2	0	0	2
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	20	0	0	2	0	0	2
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	4	10	0	0	0	0	0	2
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	4	10	0	0	1	0	0	1
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	4	18	0	0	2	0	0	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	4	15	0	0	2	0	0	2
Hølandselva middle	HA14	C	59°43'13"N 11°29'31"E	4	20	0	0	2	0	0	1
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	4	17	0	0	0	0	0	2
Rødenessjøen Ysterud	HA12	C	59°29'17"N 11°38'23"E	4	20	2	2	0	0	2	0
Total				48	191	2	2	15	0	2	18

[1] C = Crayfish plague restriction zone, R = risk area
[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 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			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Bindingsvann, outlet	MO11	C	59°47'22.1"N 10°57'17.6"E	4	16	0	0	2	0	0	0
Tangentjern, inlet, bridge on brusagav.	MO12	C	59°47'18.2"N 10°54'02.9"E	4	20	0	0	2	0	0	2
Sværsvann	MO8	C	59°49'03.2"N 10°53'25.3"E	4	19	0	0	0	0	0	0
Tangentjern, inlet, bridge on Hareveien	MO10	C	59°47'25.7"N 10°53'27.5"E	4	10	0	0	0	0	0	0
Langen, inlet, bridge on Bru-fjellv.	MO9	C	59°46'44.7"N 10°54'38.6"E	4	15	0	0	0	0	0	0
Langen, bridge on Skiveien	MO1	C	59°43'33.3"N 11°00'12.1"E	4	7	0	0	0	0	0	0
Våg	MO2	C	59°44'10.2"N 11°01'14.7"E	4	15	0	0	0	0	0	0
Tangenelva, bridge on Tomterveien	MO5	C	59°43'19.9"N 11°03'18.9"E	4	8	0	0	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
Hobølelva, Elvestad	MO7	C	59°37'26.5"N 10°57'09.2"E	4	17	0	0	0	0	0	0
Total				40	146	0	0	4	0	0	2

[1] C = Crayfish plague restriction zone, R = risk area
[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 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*	*SC*	*NC*	*CP*	*SC*	*NC*
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	15	0	0	0	0	0	0
Storsj. Ringåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	4	19	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	19	0	0	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
Glomma, Hvebergåa		C	60°21'11.5"N 12°03'06.0"E	4	17	0	0	0	0	0	0
Total				24	110	0	0	0	0	0	0

[1] C = Crayfish plague restriction 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.*

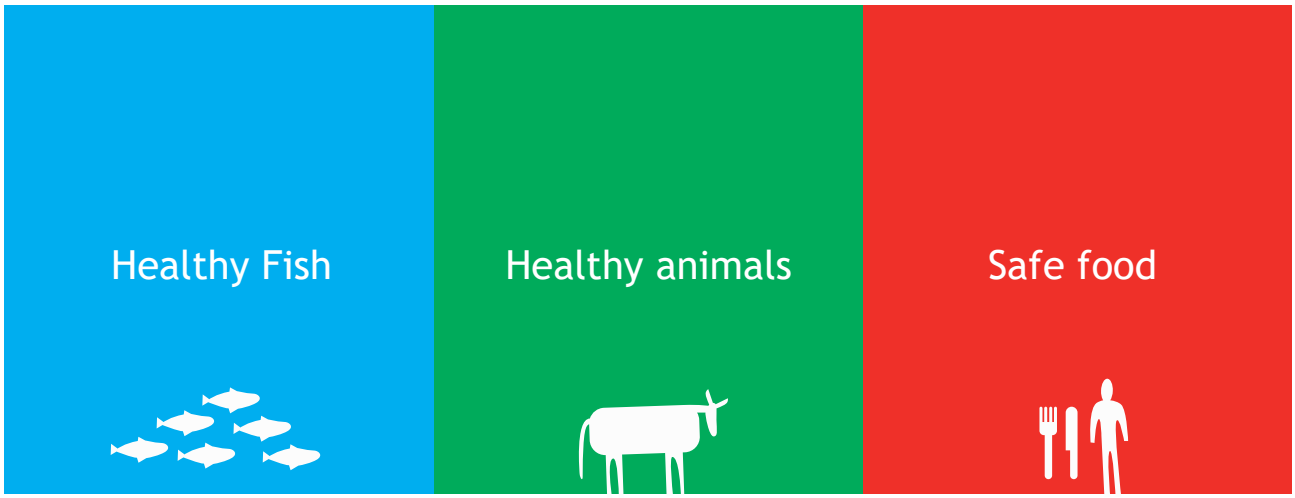
Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			September		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Vrangselva, Åbogen	VR1	C	60°06'43.6"N 12°07'01.0"E	4	20	0	0	2	0	0	2
Søndre Åklangen, Badeplass	VR2	C	60°03'12.8"N 12°08'20.8"E	4	20	0	0	0	0	0	0
Vrangselva, Skotterud	VR3	C	59°58'53.8"N 12°07'19.1"E	4	17	0	0	0	0	0	0
Vrangselva, Magnor bad	VR4	C	59°57'02.7"N 12°11'58.8"E	4	16	0	0	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
Finnsrudelva, Billavegen	FR2	C	59°58'44.9"N 12°20'14.2"E	4	20	0	0	2	0	0	2
Buåa, Eidskog	BU1	C	59°55'31.1"N 11°59'37.0"E	4	20	0	0	0	0	0	0
Buåa, Riksgrense	BU2	C	59°53'56.4"N 11°59'12.0"E	4	14	0	0	0	0	0	0
Total				32	146	0	0	6	0	0	6

[1] C = Crayfish plague restriction 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 S6**. Locations for water sampling in the River Hæra 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			July			August			October		
	ID	S[1]	GPS coordinates	#	L	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*	*CP*	*SC*	*NC*
Hæra, Dalselva		R	59°36'15.4"N 11°24'33.8"E	2	10	-	-	-	-	-	-	-	-	-	0	0	2
Hæra, Krogstad Bruk		R	59°35'26.7"N 11°23'56.1"E	4	30	-	-	-	-	-	-	0	0	2	0	0	2
Hæra, Krogstad Bruk Downstream		R	59°35'20.9"N 11°23'53.3"E	2	20	-	-	-	-	-	-	-	-	-	2	0	2
Hæra, Åmot		R	59°35'02.6"N 11°23'54.8"E	6	30	-	-	-	0	0	2	2	0	2	2	0	2
Hæra, Åmot Downstream #1		R	59°34'60.0"N 11°23'54.7"E	4	20	-	-	-	2	0	2	2	0	2	-	-	-
Hæra, Åmot Downstream #2		R	59°34'56.7"N 11°23'48.4"E	2	10	-	-	-	2	0	2	-	-	-	-	-	-
Hæra, Åmot Downstream #3		R	59°34'52.8"N 11°23'49.6"E	2	10	-	-	-	2	0	2	-	-	-	-	-	-
Hæra, Åmot Downstream #4		R	59°34'44.8"N 11°23'47.1"E	2	10	-	-	-	2	0	2	-	-	-	-	-	-
Hæra, Kvillerveien		R	59°34'24.1"N 11°23'27.1"E	6	29	2	0	2	2	0	2	2	0	2	-	-	-
Hæra, Ramstad	MY1	R	59°33'22.1"N 11°22'09.0"E	2	6	0	0	0	-	-	-	-	-	-	-	-	-
Hæra, Susebakkefossen	MY2	C	59°32'59.3"N 11°21'07.7"E	2	6	0	0	0	-	-	-	-	-	-	-	-	-
Hæra, Kapellveien	MY3	C	59°32'58.8"N 11°19'23.8"E	2	10	0	0	0	-	-	-	-	-	-	-	-	-
Total				34	180	2	0	2				6	0	8			

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



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