Annual Report

The surveillance programme for *Aphanomyces astaci* in Norway 2016

Veterinærinstituttet
Norwegian Veterinary Institute

The surveillance programme for *Aphanomyces astaci* in Norway 2016

Content

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Preface

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

In Norway, Decapods including Noble crayfish are covered by the animal welfare act (LOV-2009-06-19-97). If an alternative method is developed, the use of live animals for disease surveillance should be reduced whenever possible. NVI has been working on the development of environmental DNA (eDNA) since 2009, monitoring the crayfish plague pathogen (*Aphanomyces astaci*) directly in the water. Proof of principle for the performance of this method in large freshwater systems was demonstrated by Strand et al (2014).

In 2015, the NFSA requested the NVI to design and suggest a new surveillance program for crayfish plague to be included in the NVI's NOK-AQUA portfolio from 2016 (hereafter referred to as "*NOK A. astaci 2016*"). The NVI offered a collaborative pilot project that was in part funded by the NFSA and in part funded by the research project TARGET (NRC- 243907).

The defined user group of the TARGET project is the National Working Group for freshwater Crayfish (NGC), where amongst others, NFSA and AAØ are represented. TARGET aims to develop cost-effective and environmentally friendly monitoring tools and control strategies for better safeguarding of noble crayfish in collaboration with the user group, on-going monitoring programs and project partners. The joint activity with "*NOK A. astaci 2016"* therefore naturally fell within the scope of the TARGET project.

TARGET is led by Trude Vrålstad, NVI. Research partners in this project relevant in the joint "*NOK A. astaci 2016"* project include the Norwegian Institute of Water Research (NIVA; represented by David Strand), the Norwegian Institute of Nature Research (NINA; represented by Stein Ivar Johnsen) and the University of Copenhagen, Denmark (UoC; represented by Peter Rask Møller).

New methods make it possible to detect virtually any organism that lives in water by analysing water samples for eDNA content, a concept that is about to revolutionize multi-species monitoring worldwide. The TARGET project will implement the methodology for simultaneous detection of eDNA from the crayfish plague pathogen *A. astaci*, red-listed *Astacus astacus* (noble crayfish), and black-listed *Pacifastacus leniusculus* (signal crayfish) which carry and transmit *A. astaci*. If successful, this will allow for collaborative monitoring programs between NFSA and the Environment Agency (EA).

In the current project, the NVI (through the TARGET project) and the NFSA agreed on sharing the costs at ~50%. The NFSA covers costs connected to six strategically placed cage experiments included for supplementary surveillance of risk areas and part of the costs covering logistics, field work and *A. astaci* screening analyses. The NVI uses the AAØ (represented by Øystein Toverud) as subcontractor to carry out these cage experiments. The remaining costs are covered by TARGET, including part of the costs covering logistics, field work and complementary screening analyses for eDNA of noble- and signal crayfish along with four cage experiments included for comparison of classic and new surveillance methods.

Oslo April 5th 2017

Trude Vrålstad

Project coordinator (TARGET & OK *A. astaci* 2016)

Summary

In this surveillance program, we used two methodological approaches. One is based on the traditional cage experiments, the other relies on eDNA monitoring of the water, where DNA from spores of *A. astaci* are detected directly from water filtrates. The main geographic focus of this surveillance program has been the Halden water course and neighbouring risk areas. Other geographic areas covered include the Glomma water course region and the Buåa water course region.

In total, 76 and 38 water samples were collected from selected sites in the Halden- and Glomma water course regions, respectively. For the cage experiments, 10 cages with live noble crayfish were distributed in strategically selected sites primarily in the risk areas outside crayfish plague control zones. The exceptions include cages placed in the Buåa, which is in the process of being declared disease-free area, and in the bordering areas of Glomma, where extra effort to pinpoint the long hunted infection source was prioritized. For comparison and evaluation, the TARGET-project provided eDNA monitoring data to this report of susceptible native noble crayfish *Astacus astacus*, and alien signal crayfish *Pacifastacus leniusculus* that carry and transmit *A. astaci*. The presence/absence of all three target species has been screened simultaneously with species specific qPCR assays.

No crayfish mortality was observed, that could be attributed to crayfish plague in any of the cages. In the control zone of the Halden water, *A. astaci* eDNA was detected in water samples in the stretch from Aremarksjøen (Skoteberg) to the Hølandselva outlet. In areas with known presence of *P. leniusculus*, this presence was confirmed by positive eDNA detection. No signs of crayfish plague were observed during the surveillance period from the northern part of Hølandselva up to the control zone border at Fosserdam. This result was supported by positive detections of *A. astacus* eDNA in all water samples from Hølandselva and upstream. All water samples in this risk area were negative for *A. astaci* and *P. leniusculus* eDNA, while positive for *A. astacus* eDNA. In Glomma, no sign of crayfish plague was found. The samples were negative for all screened targets apart from two weak *A. astacus* positives in Stortjennet and Opstadåa.

Introduction

The oomycete and crayfish plague agent *Aphanomyces astaci* is a lethal pathogen on native European freshwater crayfish (1-3). It is a specialized and relatively harmless parasite on North American freshwater crayfish, which consequently act as healthy carriers of the disease. *Aphanomyces astaci* reproduces and spreads with swimming zoospores, which is the infective stage of the pathogen. It was accidentally introduced to Europe nearly 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).

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

Since 1971, a total of 7 water systems in Norway have been affected by crayfish plague outbreaks once or several times (4-5). That includes Vrangselva/Veksa (1971), the Glomma watercourse (1997 and 2003), Lake Store Le (1989), the Halden watercourse (1989, 2005, 2014), the river Lysakerelva (1998), Buåa water course (2010) and Moss watercourse (2016). In addition, four other localities have been (or are still) under crayfish plague regulations due to illegally introduced and confirmed *A. astaci* positive signal crayfish (4).

The focus areas of the 2016 surveillance program for crayfish plague cover the

- Buåa water course (under regulation FOR-2011-08-05-831)
- Glomma water course (under regulation FOR-2005-06-20-652)
- Halden water course (under regulation FOR-2015-05-26-592)

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

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

The Halden water course was hit by crayfish plague in 1989, re-established with noble crayfish in the 1990s, and successfully recovered until the crayfish plague returned in 2005 (6). Quick closure of the Ørje water locks prevented upstream spread. In 2008, illegally introduced *A. astaci* positive signal crayfish was found in Lake Øymarksjøen (7), leading to permanent closure of Ørje water locks. This prevented further spread, until illegally introduced signal crayfish were found in upstream of the Ørje water locks in 2014. The re-established noble crayfish population in Rødnessjøen was lost during the following crayfish plague outbreak. In this period, the TARGET project compared surveillance with cages to environmental DNA (eDNA) monitoring according to Strand et al (8). Here, it was possible to follow the crayfish plague front through analysis of water, and eDNA of *A. astaci* in the water was sometimes detected prior to crayfish mortalities in the cages. Noble crayfish and signal crayfish eDNA was also detected in the water where it was known to occur (9).

Until 2015, surveillance of crayfish plague relied on cage experiments with live noble crayfish. In the current program, classical cage experiments are combined with eDNA monitoring.

Aims

This surveillance program aims to

- Monitor the infection pressure and spread of the crayfish plague pathogen *A. astaci* in zone regulated areas as a result of earlier detection of disease (referred to as control zones)
- Substantiate disease free waterbodies in neighboring areas of the control zones (= risk areas)
- Alert on any eventual spread of the disease from control zone to risk areas
- Evaluate eDNA as a monitoring tool for *A. astaci* monitoring - alone, and in combination with complementary eDNA targets including both the carrier- and susceptible crayfish host species

Materials and methods

Surveillance sites

The main areas for surveillance of crayfish plague in 2016 include the Halden water course and surrounding areas, the Glomma water course and surrounding areas, and two sites in the Buåa water course. The overall surveilled area with plotted locations for water sampling, cage experiments and crayfish plague zones are given in Figure 1. Supplementary details are summarized in Appendix 1.

The control zone of the Halden water course was monitored from Fossersjøen the northern part of Aremarksjøen. Live noble crayfish were still expected within the control zone in the upper parts of the system, awaiting the outbreak. Crayfish localities adjoining the control zone or in geographical close proximity are vulnerable to further spread, and referred to as "risk zone" (Table S1 in Appendix 1).

In the Glomma watercourse, the control zone comprises the main passageway downstream Braskereidfoss in Våler. Since the last detection of crayfish plague was in Oppstadåa at the end of Lake Storsjøen, this area was given main focus of the Glomma monitoring.

In Buåa, only cage experiments were conducted to follow up the long-term monitoring since 2010.

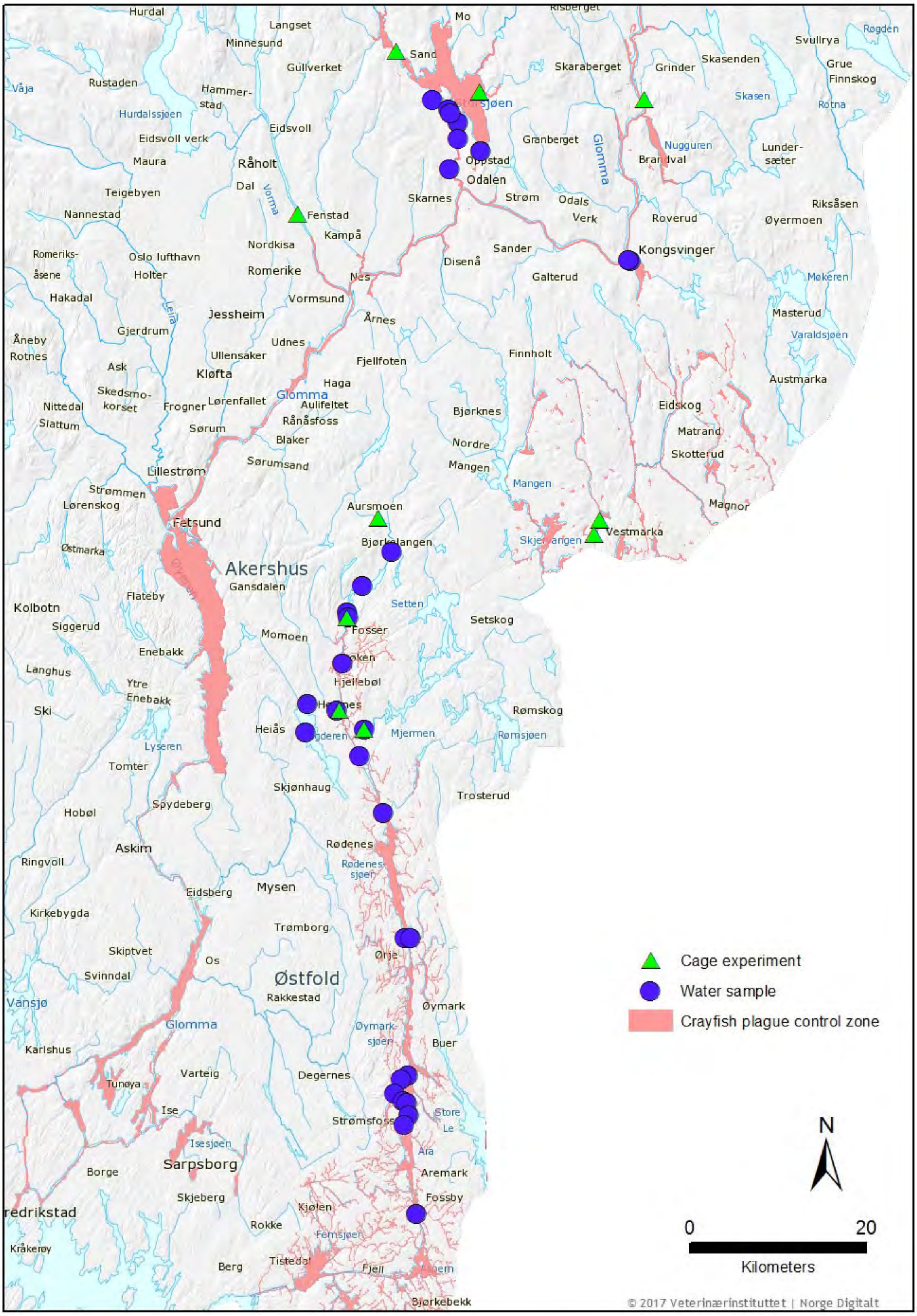

Figure 1. Surveilled sites in Eastern Norway 2016. Water samples (blue dots) were collected in June and August, and cage experiments (green triangles) were established in June and terminated (with some exceptions in October). Regulated areas (crayfish plague control zones) are marked in light red. Note: For Glomma, the zone is approximate.

Work plan

We follow two approaches for this surveillance program. One is based on the traditional cage experiments that have been used in surveillance of crayfish plague in Norway since the 1970s. The other approach relies 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. leniusculus* is monitored from the same water samples. The work plan for the surveillance program is illustrated in Figure 2.

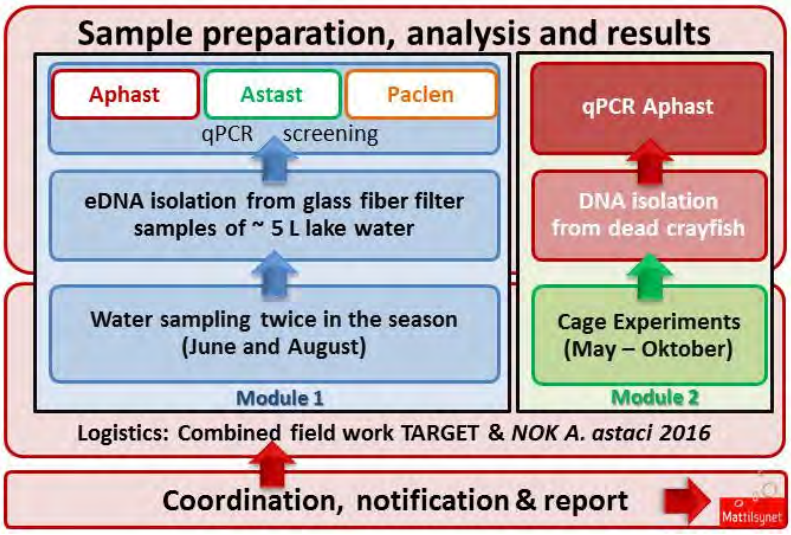

Figure 2. Work plan

The Norwegian Veterinary Institute (NVI) coordinates the project. Module 1 includes eDNA monitoring of lake water (new disease surveillance concept), and module 2 includes cage experiments (classical disease surveillance concept). The NVI organizes the eDNA water sampling and qPCR screenings in collaboration with the project TARGET. The Agency of outlaying fields (AAØ) is used as subcontractor of the cage experiments. The NVI conduct diagnostic services free of charge in cases of crayfish plague suspected mortalities outside control zones.

Cage experiments

The cage experiments were conducted by the Agency for outlying fields, Akershus & Østfold (AAØ). Disease free noble crayfish were bought from a noble crayfish farm at Hvaler, with permission from the Food Safety Authority. In total, 10 cages were set out with 10 noble crayfish in each cage. This involved 2 cages in the Buåa region, 4 cages in the Halden water course region, 3 cages in the Glomma region and 1 cage in Vorma (as part of the extended Glomma region) (Figure 1, Table 1).

The crayfish were cared for and monitored by local people with agreed contracts. The crayfish were fed weekly. If suspicious mortalities occurred, the instruction was to alert the NFSA and submit the dead animal(s) to the NVI. "Suspicious mortalities" does not involve crayfish cannibalism during moulting. In cases of crayfish loss in the cages which was not attributed to crayfish plague, new crayfish were retrieved as substitutes (Table 1).

Table 1. Location sites and obtained results for the cage experiments with live noble crayfish.

Location	Initial # crayfish in cage	Cage ID	Loss and substitutes of crayfish			
			Mortality *A. astaci*	Mortality other	Assumed escapes	Crayfish substituted
Lundsfoss	10	HA1	0	4	0	0
Daltorpsfoss	10	HA2	0	7	0	0
Lierelva	10	HA4	0	0	10	7
Fossersjøen	10	HA8	0	0	10	10
Upstream Svanefoss	10	V1	0	5	0	0
Hverbergåa outlet	10	GH15	0	2	8	0
Austvassåa, inlet Storsj.	10	Austvassåa	0	7	0	4
Outlet Råsen to Storsjøen	10	Sollauståa	0	5	0	2
Harstaddjøen outlet	10	BH1	0	0	4	0
Klanderudtjern outlet	10	BH2	0	0	10	0
Total	100		0	30	42	23

eDNA monitoring

A total of 21 and 9 sites were visited in the Halden- and Glomma water course area, respectively (Figure 1, Table S2-S3 in Appendix 1). The sites were visited in June and August 2016. From each site, two samples of ~5 L water were filtered on-site onto sterile glass fibre filters (8). Ideally, 5 L water was filtered per filter sample, but due to high turbidity or clay particles, the total filtered volume was sometimes lower. In some of these cases, we therefore included extra samples to partly compensate for the reduced water volume. The filters were transferred with a clean forceps to a sterile falcon tube immediately after filtration, kept on ice during transport back to the laboratory, frozen for a minimum of 24 hours and freeze dried before eDNA extraction (8). The water samples were screened by qPCR for 3 DNA targets: the species specific qPCR assay for A. astaci (8, 10), and two crayfish species specific qPCR assays for A. astacus and P. leniusculus developed by Agersnap et al. (11). Figure 3 presents an overview of the eDNA monitoring procedure.

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

Results and Discussion

Cage experiments

No crayfish plague was detected in any of the 10 cages included in the program, thus no sign of spread or active infection was detected in the monitored sites in the risk zone in the Halden water course, nor in the selected sites in the bordering zone of Buåa, Glomma and Vorma. A separate report from the AAØ is attached (Appendix 2). Of the initial 100 crayfish (10 per cage), several died or escaped early in the surveillance period, leading to the substitution of 23 extra crayfish. Of the total of 123 crayfish used, about ~ 24,4 % (30 crayfish) were found dead in the cage or had disappeared without trace, most likely as a result of cannibalism during moulting. These mortalities occurred without basis for crayfish plague suspicion, and were not analysed further. Another 42 crayfish (34%) probably escaped, mainly as a result of human interference (vandalism), and 50% of the cages had reports of vandalism/escapes. About 41% of crayfish were still alive and caged at the end of experiments. 20% of the cages were removed before October (July-August) due to the high probability of repeated vandalism if continued. Thus, it is challenging to manage the cage experiments according to plan.

eDNA monitoring in the Glomma watercourse

In the Glomma region, 38 water samples representing a total of ~144 L water were analysed. No sign of *A. astaci* nor of *P. leniusculus* from the eDNA analyses was found. In two cases (Oppstadåa and Stortjennet) a weak positive signal for *A. astacus* eDNA was detected in one water sample from each location (Figure 4, Table S2 in Appendix 1). The number of positive samples was too limited for conclusions, but indicate that some noble crayfish are about to re-establish themselves in the system. This corroborates observations from the area, with some smaller catches of noble crayfish (12). The results cannot verify any active *A. astaci* infection or infection source from the monitored sites in the Glomma region.

eDNA monitoring in the Halden watercourse

In the Halden watercourse region, 76 water samples representing a total of ~271 L water were analysed. In the control zone, *A. astaci* eDNA was detected in 20 water samples (9 in June, 11 in August) at low concentration from Aremarksjøen location Skoteberg to Hølandselva outlet (Figure 5-7, Table S3 in Appendix 1). In areas with known presence of *P. leniusculus*, this was confirmed by positive eDNA results in a total of 11 water samples (5 in June, 6 in August; Figure 5-7, Table S3 in Appendix 1).

No sign of crayfish plague was observed during the surveillance period from the northern part of the Halden watercourse control zone, from Hølandselva north until the border of the infection zone at Fosserdam (Figure 6). These results were supported by positive detections of *A. astacus* eDNA in all water samples from Hølandselva and upstream. In total 9 water samples were positive for noble crayfish eDNA on this stretch, of which 3 co-occurred with *A. astaci* eDNA at one site (HA10) (Figure 6, Table S3 in Appendix 1). This data is the closest we have got to the outbreak front at the time of sampling.

In general, all water samples from the risk area surrounding the Halden water course were negative for *A. astaci* and *P. leniusculus* eDNA, while positive for *A. astacus* eDNA. In total, 24 water samples were positive for *A. astacus* eDNA (Figure 5-6, Table S3 in Appendix 1). The combined absence of *A. astaci* eDNA and presence of noble crayfish eDNA suggest that there has been no further spread of the disease in the surveillance period, and that there are live noble crayfish in the monitored sites.

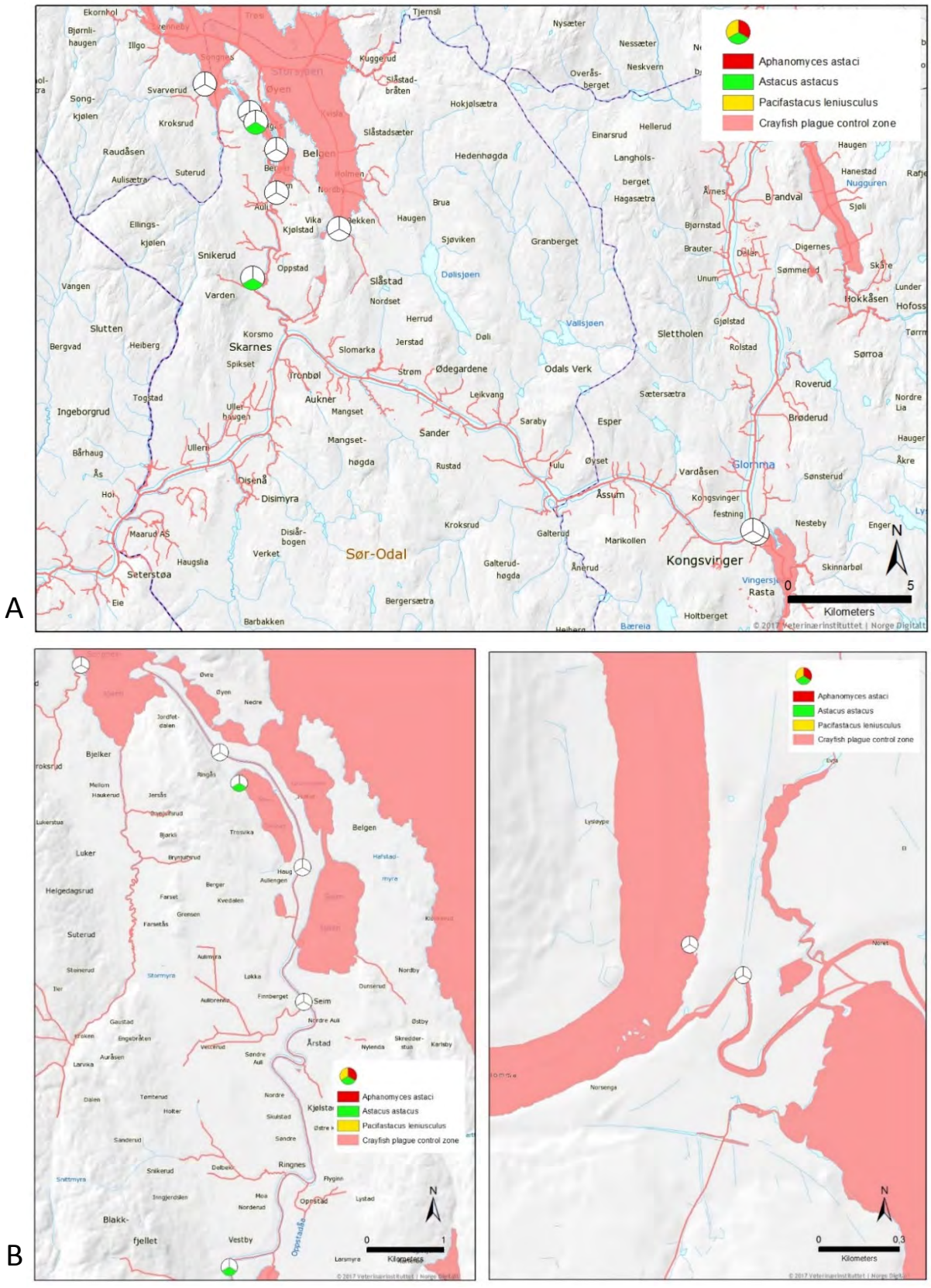

Figure 4. Overview map Glomma region (A) and close-ups for sample sites Oppstadåa in the west (B) and Vingersnoret in the east (C). The maps show eDNA results from surveilled sites in 2016. Regulated areas (crayfish plague control zones) are marked in light red. For each location site, the pie chart indicates presence (color) or absence (white) of *A. astaci* (red), *P. leniusculus* (yellow), and *A. astacus* (green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. Two *A. astacus* (noble crayfish) positive samples might indicate sporadic presence of this species in Stortjennet and Oppstadåa.

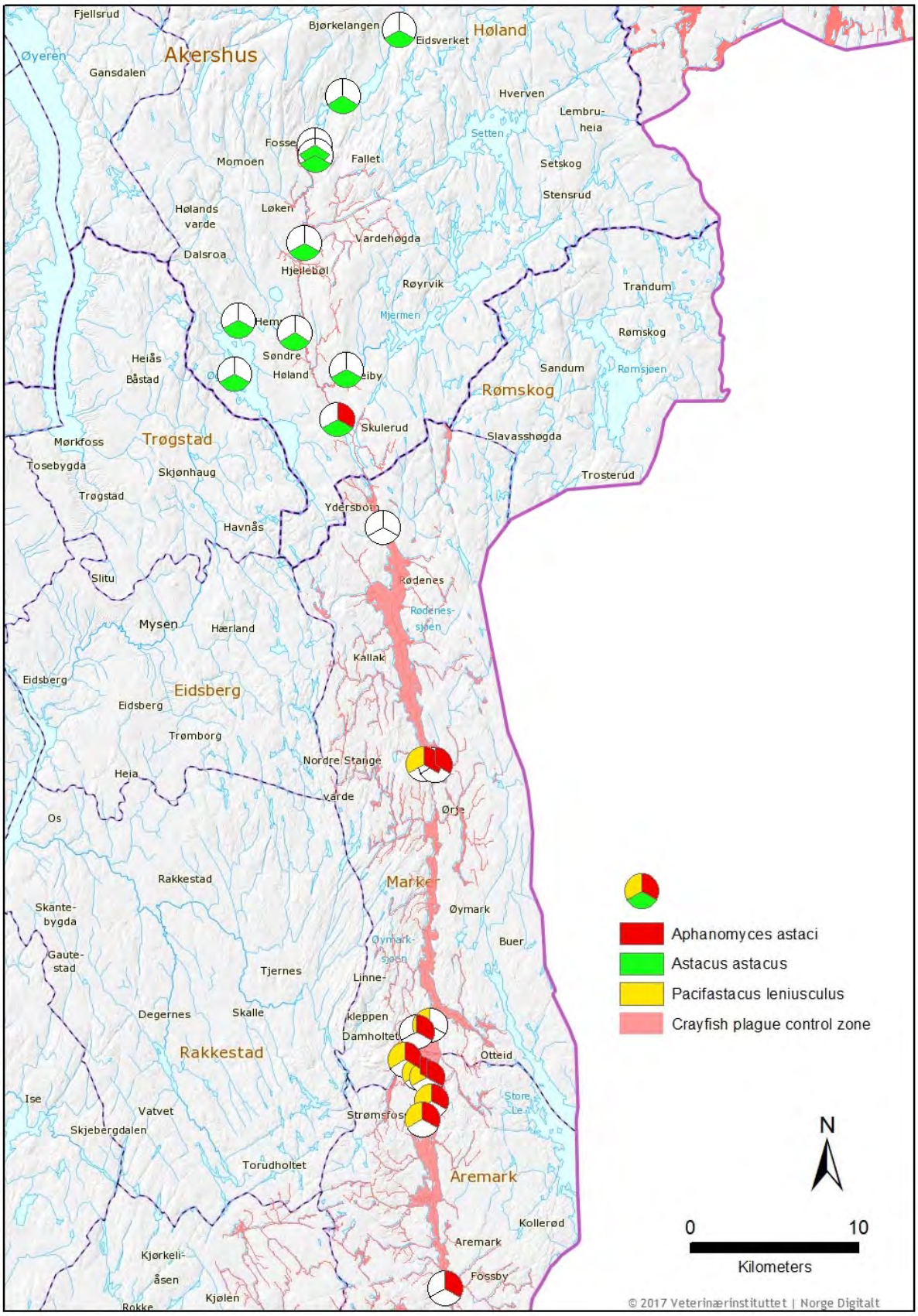

Figure 5. Overview map of the surveilled part of the Halden watercourse region in 2016. The control area is indicated by light red color on involved lakes and rivers, and ends at Fosserdam, which is an artificial barrier for further spread. The pie chart indicates presence (color) or absence (white) of *A. astaci* (red), *P. leniusculus* (yellow), and *A. astacus* (green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result.

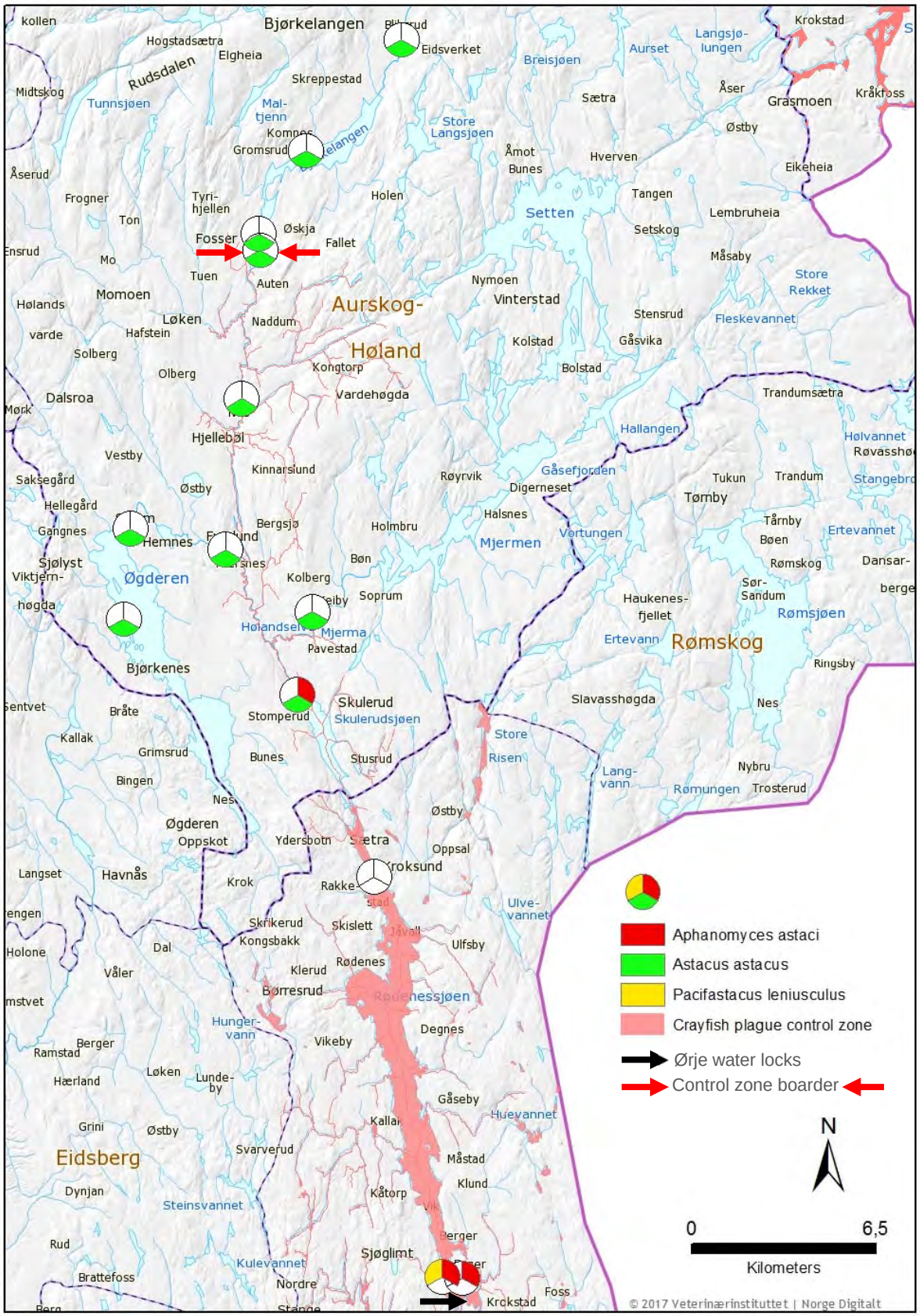

Figure 6. Close-up map for the Northern Halden watercourse region, starting from the Ørje water locks (black arrow) in the south where signal crayfish was revealed in 2014. The control area ends at Fosserdam (red arrows). The pie chart indicates presence (color) or absence (white) of *A. astaci* (red), *P. leniusculus* (yellow), and *A. astacus* (green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. At the last time point of surveillance (August), the infection front of *A. astaci* was detected in the Hølandselva outlet. Further north, only eDNA of noble crayfish is detected in the water. The same was observed in the risk area, suggesting no spread in the surveilled period.

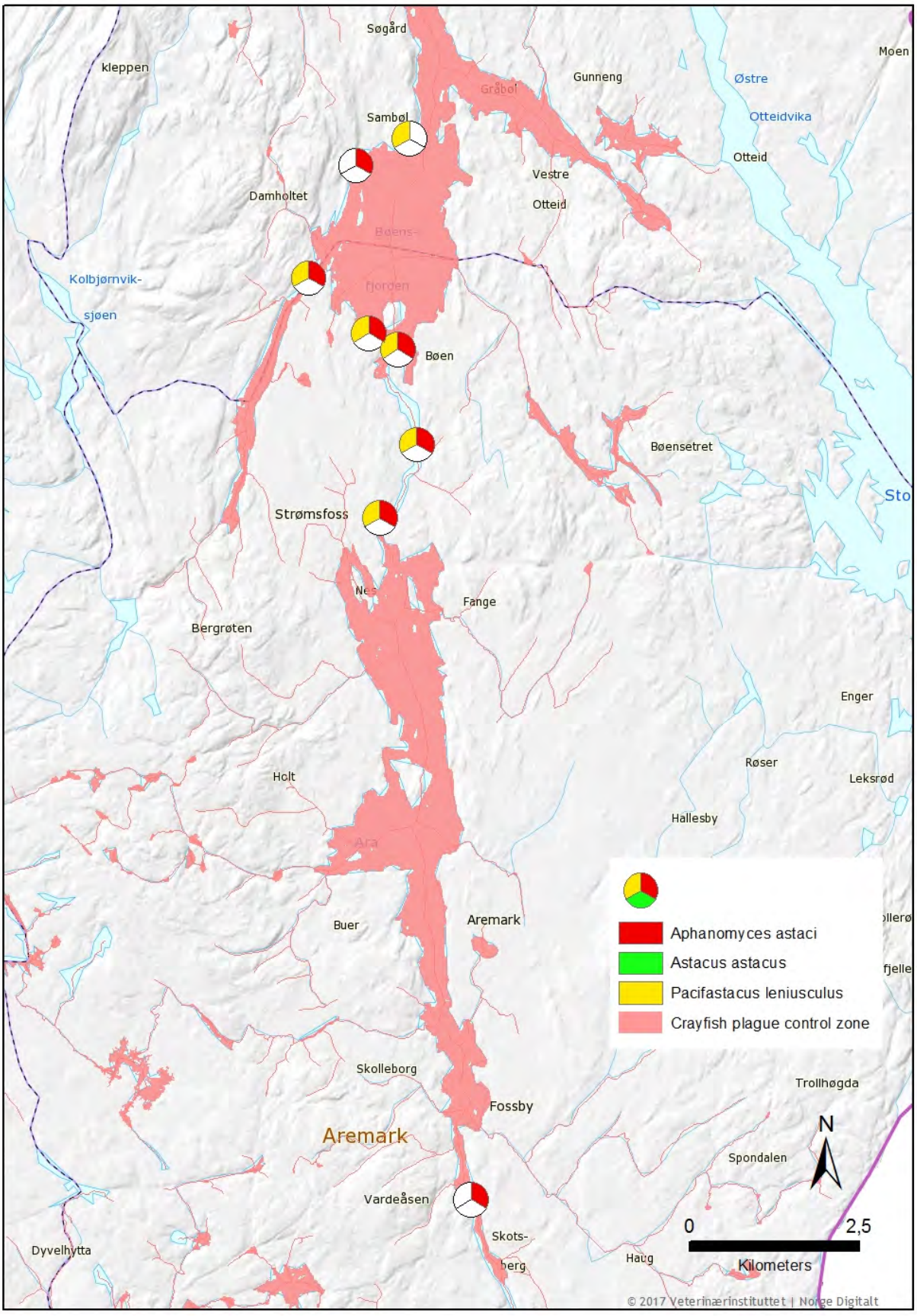

Figure 7. Close-up for maps for the southern part of Halden watercourse, focusing on Øymarksjøen where signal crayfish has been established for more than a decade. The pie chart indicates presence (color) or absence (white) of *A. astaci* (red), *P. leniusculus* (yellow), and *A. astacus* (green). Presence is listed if at least one of the tested water samples yielded a positive eDNA result. eDNA from signal crayfish and/or *A. astaci* was detected at all monitored sites, and co-detection of both targets appeared in five out of eight (62.5%) of the monitored sites.

Conclusion

In the Halden water course, combined eDNA monitoring of *A. astaci*, *A. astacus* and *P. leniusculus* largely confirmed the expected status: *P. leniusculus* present in Aremarksjøen, Øymarksjøen and Rødenessjøen emit detectable but low concentrations of *A. astaci* in the water. Further, it seems that the infection front has not yet reached the northern part of Hølandselva. The outbreak within the control zone was thus not yet completed at the last time-point of surveillance, since the upper part of the zone most likely hosted live noble crayfish in August 2016. It was no sign of *A. astaci* spread to the neighboring risk areas.

For Glomma, no *A. astaci* or *P. leniusculus* eDNA was detected. The status is 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. Two *A. astacus* positive samples could indicate sporadic presence of noble crayfish.

The Buåa water course has been monitored by cages for more than 5 years. Lack of crayfish plague detection could indicate disease free status. However, a new crayfish plague regulation from August 2016 affects the whole Eidskog municipality (FOR-2016-08-17-972). No conclusion can therefore yet be drawn.

The eDNA monitoring of *A. astaci* worked as intended, and in combination with complementary eDNA targets of noble- and signal crayfish, it was possible to receive a snapshot of the relevant habitat status. The simultaneous monitoring of the three target species could facilitate more coordinated surveillance programs for crayfish plague, red-listed noble crayfish and black-listed signal crayfish.

The cage experiments represent a valuable supplement and continuous monitoring. This is a powerful tool to detect crayfish plague in the whole season and/or monitor risk zones and particularly valuable localities. Drawbacks with the cage experiments include cannibalism in the cage, leading to moderate mortality rates also during the absence of disease, and escapes resulting from undesirable human interference. The latter also makes it demanding to manage the cage experiments.

Acknowledgements

We thank the Norwegian Food Safety Authority for accepting the idea of this joint pilot project in collaboration with the TARGET project in the first year of the new surveillance programme for *Aphanomyces astaci*. The TARGET project (NRC 243907; Targeted strategies for safeguarding the Noble crayfish against alien & emerging threats) is financially supported by the Norwegian Research Council through the “Environment 2015” (Miljø 2015).

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Appendixes

Appendix 1. Tables S1 – S3

Appendix 2. Report (in Norwegian) for the cage experiments from AAØ.

Appendix 1:
Supplementary information to the report "The surveillance programme for *Aphanomyces astaci* in Norway 2016"

Table S1. Agreed areas and locations of the "*NOK A. astaci 2016*" program

Location	Water course[1]/ municipality, county[2]	Location infection status	# water samples (site x samples x visits)	Cages
Aremarksjøen	HW/Marker, Ø	Control zone, signal crayfish	8 (2 x 2 x 2)	-
Øymarksjøen	HW /Marker, Ø	Control zone, signal crayfish	8 (4 x 1 x 2) (transect)	-
Rødenessjøen	HW /Marker, Ø	Control zone, signal crayfish	8 (2 x 2 x 2)	-
Skullerudsjøen	HW/Aurskog-Høland, A	Control zone, outbreak Aug. 2015	4 (1 x 2 x 2)	-
Hølandselva	HW/Aurskog-Høland, A	Control zone, outbreak expected	8 (1 x 2 x 2)	-
Fossersjøen	HW/Aurskog-Høland, A	Control zone, outbreak expected	4 (1 x 2 x 2)	1
Fosserdam	HW/Aurskog-Høland, A	Risk zone/control zone boarder	4 (1 x 2 x 2)	-
Bjørkelangen	HW/Aurskog-Høland, A	Risk zone	8 (2 x 2 x 2)	-
Lierelva	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)	1
Lundsfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)	1
Dalstorpfoss	HW/Aurskog-Høland, A	Risk zone	4 (1 x 2 x 2)	1
Hemnessjøen	Lake, Aurskog-Høland, A	Risk zone	8 (2 x 2 x 2)	-
Storsjøen inlet	GW/ Sør Odal, H	Control zone/boarder zone		2
Storsjøen	GW/ Sør Odal, H	Control zone	12 (3 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)	-
North of Vingersnoret	GW/ Sør-Odal, H	Control zone	4 (1 x 2 x 2)	-
Hvebergåa	GW/ Grue, H	Control zone	-	1
Upstream Svanfoss	V/ Eidsvoll, A	Risk zone	-	1
Harstaddjøen outlet	BW/ Eidskog, H	Control zone	-	1
Klanderudtjern outlet	BW/ Eidskog, H	Control zone	-	1
Total			**100**	**10**

[1] HW = Halden watercourse, GW = Glomma watercourse, V = Vorma, BW = Buåa watercourse.
[2] Ø = Østfold, A = Akershus, H = Hedmark.

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

Location	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Vingersnoret	GL1	C	60°11'36.3"N 12°01'54.5"E	6	18.6	0	0	0	0	0	0
North of Vingersnoret	GL2	C	60°11'39.7"N 12°01'41.2"E	4	19.5	0	0	0	0	0	0
Storsjøen South	GL3	C	60°17'51.1"N 11°42'49.2"E	4	20	0	0	0	0	0	0
Storsjøen Sagablom	GL4	C	60°20'52.4"N 11°36'34.0"E	4	17.5	0	0	0	0	0	0
Storsj. Ringsåsvn. pier	GL5	C	60°20'18.4"N 11°38'36.5"E	4	14.7	0	0	0	0	0	0
Engene farm	GL6	C	60°19'31.4"N 11°39'52.1"E	4	11	0	0	0	0	0	0
Stortjennet	GL7	C	60°20'05.7"N 11°38'54.3"E	4	19	0	0	0	0	1	0
Oppstadåa north	GL8	C	60°18'34.4"N 11°39'58.9"E	4	11	0	0	0	0	0	0
Oppstadåa south	GL9	C	60°16'40.3"N 11°39'06.9"E	4	13	0	1	0	0	0	0
Total				38	144.3	0	1	0	0	1	0

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

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

Location[1]	Location details			Water samples[2]		# eDNA positive samples[3]					
						June			August		
	ID	S[1]	GPS coordinates	#	L	*CP*	*NC*	*SC*	*CP*	*NC*	*SC*
Lierelva	HA1	R	59°53'8"N 11°34'29"E	4	6.5	0	0	0	0	1	0
Bjørkelangen	HA2	R	59°50'55"N 11°31'5"E	4	17.5	0	0	0	0	1	0
Fosserdam	HA3	R	59°49'17"N 11°29'27"E	4	14	0	2	0	0	2	0
Fossersjøen	HA4	C	59°48'58"N 11°29'32"E	6	12.4	0	0	0	0	2	0
Lundsfoss	HA5	R	59°42'7"N 11°32'14"E	4	20.0	0	2	0	0	2	0
Hemnessjøen pier	HA6	R	59°41'47"N 11°25'7"E	5	13.2	0	1	0	0	3	0
Hemnessjøen outlet	HA7	R	59°43'31"N 11°25'11"E	4	10.2	0	2	0	0	2	0
Daltorpsfoss	HA8	R	59°43'13"N 11°28'49"E	4	11.0	0	2	0	0	2	0
Hølandselva north	HA9	C	59°46'7"N 11°29'8"E	4	6.2	0	2	0	0	1	0
Hølandselva outlet	HA10	C	59°40'30"N 11°31'50"E	4	14	1	2	0	2	2	0
Skulerudsjøen outlet	HA11	C	59°37'6"N 11°35'5"E	4	14.5	0	0	0	0	0	0
Rødenessjøen Ysterud	HA12	C	59°29'31"N 11°38'25"E	4	20	2	0	0	1	0	1
Rødenessjøen Ørje	HA13	C	59°29'31"N 11°39'10"E	4	20	0	0	0	1	0	0
Øymarksj. Sambøl W	HA14	C	59°21'8"N 11°39'39"E	2	10	0	0	0	0	0	1
Øymarksj. Sambøl	HA15	C	59°20'54"N 11°38'50"E	2	9	1	0	0	1	0	0
Øymarksj. Sambøl S	HA16	C	59°19'59"N 11°38'11"E	2	10	1	0	1	0	0	0
Øymarksj.cabin village	HA18	C	59°19'34"N 11°39'10"E	2	9	1	0	1	1	0	1
Øymarksj. Blåsnuppen	HA21	C	59°19'27"N 11°39'38"E	3	11.5	1	0	1	2	0	1
Øymarksjøen Mokallen	HA22	C	59°18'42"N 11°40'0"E	2	10	1	0	1	1	0	1
Strømsfoss sluser Cafe	HA23	C	59°18'6"N 11°39'29"E	4	17.2	2	0	2	2	0	2
Aremarksj. Skoteberg	HA24	C	59°12'42"N 11°41'26"E	4	15	0	0	0	1	0	0
Total				76	271.2	10	13	6	12	18	7

[1] C = Crayfish plague control 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).

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Overvåkning av kreps med forsøksbur i Akershus og Hedmark 2016

Rapport nr:
14

Dato:
6.10.2016.

Forfatter :
Øystein Toverud

Oppdragsgiver:
Veterinærinstituttet ved Trude Vrålstad

Emneord:
Sykdomsovervåking, burforsøk, edelkreps

Ekstrakt:
I 2016 er det ikke påvist krepsepest i våre burforsøk.
Heller ikke noe tyder på krepsepest.

De fleste forsøkene ble startet 2.6. Det ble foretatt en etterfylling 17.6.

Alle forsøkene ble avsluttet 1.10.2016.

Arbeid 2016:

Forberedelser:

Et planleggingsmøte ble avhold 6.4.2016 i Oslo med tema «overvåkningsprogrammet» i regi av Trude Vrålstad, Veterinærinstituttet.
Rammer og krav til arbeidet om burforsøket ble deretter diskutert med Veterinærinstituttet og Trude Vrålstad.

Her er tabell over vedtatte burforsøk:

Lokalitet	Vassdrag[1]/Kommune	Status	Totalt antall vannprøver (prøvepunkt x prøver x besøk per år)	Burforsøk
HA8 Fossersjøen	HV/ Aurskog-Høland, A	Kontrollsone, utbrudd i vente?	4 (1 x 2 x 2)	1
HA4 Lierelva	HV/Aurskog-Høland, A	Risikosone	4 (1 x 2 x 2)	1
Overside Lundsfoss	Sjø, Aurskog-Høland, A	Risikosone	4 (1 x 2 x 2)	1
Utløpselva Hemnessjøen (Daltorpfoss)	HV/ Aurskog-Høland, A	Risikosone	4 (1 x 2 x 2)	1
Harstadsjøens utløp	Eidskog, H	Bekjempelsessone. Overvåkning med tanke på friskmelding eller innsirkling av smittekilde	Ikke satt av ressurser til vannprøver i 2016	1
Klanderudtjerns utløp	Eidskog, H	Kontrollområde. Overvåkning med tanke på eventuell friskmelding eller innsirkling av smittekilde	Ikke satt av ressurser til vannprøver i 2016	1
Innløp Storsjøen tilgrendende sideelver	GV/Sør-Odal, H	Bekjempelsessone/randsone		2
G15 Hvebergåa	Sideelv GV/Grue, H	Bekjempelsessone, forsøk på innsirkling av mulig smittekilde		1
VA 1 Vorma oppstrøms Svanfoss,(utløp Andelva)	Vorma/Eidsvoll, A	Risikosone		1

Tillatelse til å hente kreps hos Roger Strand, Hvaler:
Kontaktet Trude Vrålstad om dette var avklart med Roger Strand. Dette ble senere avklart med Jørn Våge, Mattilsynet, etter søknad fra Roger.

2016.05.24 ringte ØT til Strand om å få kreps i månedskifte mai-juni.

Arbeidet startet ved at vi fant fram til nye vertskap på de fleste steder fordi stasjonene var endret fra tidligere år. Disse fikk en instruks om hvordan arbeidet skulle utføres. Denne instruksen var på forhånd avklart med Trude Vrålstad.
Sammen med levert kreps fikk de utlevert bur. I hvert bur er det lagt ut 2 toms drensrør som er kappet ca 20 cm. De fungerer som skjul for krepsen.
Videre er det i hvert bur lagt ut et «varselsskilt» om at dette er et offentlig forsøk og at vi ber om at folk ikke skal røre buret.
I perioden mellom 2.6. og 1.10. har det vært jevnlig kontakt med vertskap.

Rapportering underveis:

Trude Vrålstad har fått en statusoversikt 11.7. som ble utvidet 13.7.

Videre ble det orientert 8.8. om status for G15 som var tømt.
17.8. ble det orientert om endringene i burforsøkene i Bua i Eidsskog.

Levert kreps:

2.6. 100 kreps ble hentet hos Roger Strand. Det var ekstremt varmt vær under transport. Selv om vi startet arbeidet kl.6.00 om morgenen, førte varmen til at en del kreps døde under transporten. Mye logistikk planlagt i flere uker var årsak til at vi ikke kunne endre dato.

17.6. Påfylling av kreps der kreps døde 2.6.
BH1 og BH2: Bua,(BH1 og BH2) 2 stasjoner, 20 kreps (de fikk ikke kreps 2.6.)
HA4: Lierelva: 7 kreps
HA8: Fossersjøen 10 kreps
Austvassåa, innløpselv Storsjøen, 4 kreps
Solauståa, innløpselv Storsjøen fra Råsen, 2 kreps
Totalt 43 kreps

Utvikling på de ulike forsøksstasjonene:
Øverst i Haldenvassdraget
HA1 Oppstrøms Lundsfoss i Mjerma før Hølandselva
2.6.: 10 stk
15.6.: melding og telefon, 2 kreps døde. Har vært svake lenge. Årsak: svekket ved utkjøring.
8.7.: 7 kreps
1.10.: 6 kreps

HA2: Utløpselv Hemnessjøen ovenfor Daltorpfoss før Hølandselva
2.6.: 10 kreps
30.6.: 10 kreps
12.7.: 4 kreps igjen, Kreps har forsvunnet og spist i skallskifte. Ingen rester.
19.9.:3 kreps
1.10.: 3 kreps

HA4: Lierelva, innløpselv i Bjørkelangensjøen
2.6.: 10 kreps
13.6.: Beskjed fra vertskap: Vi har allerede hatt en katastrofal burrømning. Teina lå på rygg og lukkemekanismen fungerte ikke 100 %. Status: Kun 3 kreps igjen.
17.6.: påfylling 7 kreps
8.7.: 10 kreps
22.8.: 7 kreps (rømt pga. åpning i lukkemekanisme og skakt bur).
1.10.: 7 kreps

HA8: Fossersjøen
2.6.: 10 kreps
6.6.: All kreps rømt fra teina pga folk har vært der. Satt på hengelås.
17.6.: Påfylling 10 nye kreps
8.7.: 10 kreps

15.7.: 10 kreps
1.10.: 10 kreps

V1.Oppstrøms Svanefoss i Vorma: (nytt sted)
2.6.: 10 kreps
8.7.: 10 kreps
15.7.: 9 kreps (spist, fryst det som er igjen)
23.8. : 6 kreps (ingen rester etter død kreps).
1.10.: 5 kreps

GH 15: Utløp Hverbergåa, sidevassdrag Glomma i Hedmark
2.6.: 10 kreps
8.7.: 8 kreps
28.7.: Alle krepsen er tatt. Luka på buret er åpen. Vanskelig med folk som går langs elva.

Storsjøen

Austvassåa, innløpselv på østsiden av Storsjøen:
2.6.: 10 kreps
7.6.: 4 døde kreps pga. at kreps døde etter varme under transport, 6 igjen.
17.6.: 4 kreps påfylling.
8.7.: 7 kreps
23.8.: 3 kreps (ingen rester av død kreps)
1.10.: 3 kreps

Sollauståa, utløpet av Råsen til Storsjøen:
2.6.: 10 kreps
6.6.: 2 døde kreps pga. varm transport.
17.6.: påfylling 2 kreps
19.6.: 8 kreps, 2 døde i fryseren.
22.6.: en død kreps
28.6.: en døde kreps
7.7.: 6 kreps igjen i teina.
23.8.: 6 kreps
1.10.: 5 kreps

Bua:

BH1: Utløp Klanderudtjern
17.6.: 10 kreps.
8.7.: 10 kreps
16.8.: 6 kreps, 4 kreps var blitt borte i løpet av en uke. Vertskapet mener at besøkende har tatt kreps fra buret. I forbindelse med krepsing har folk gått lags elva.
1.10.: 6 kreps

BH2: Utløp Harstadsjøen
17.6.: 10 kreps
8.7. 10 kreps

16.8.: Buret er tømt for kreps av mennesker. Ingen tegn til skall eller rester. Alt var i orden for noen dager siden. I forbindelse med krepsinga har folk gått langs strendene.

Resultat Generelt:

Det er ikke blitt oppdaget noe tegn til krepsepest i burforsøkene i 2016.

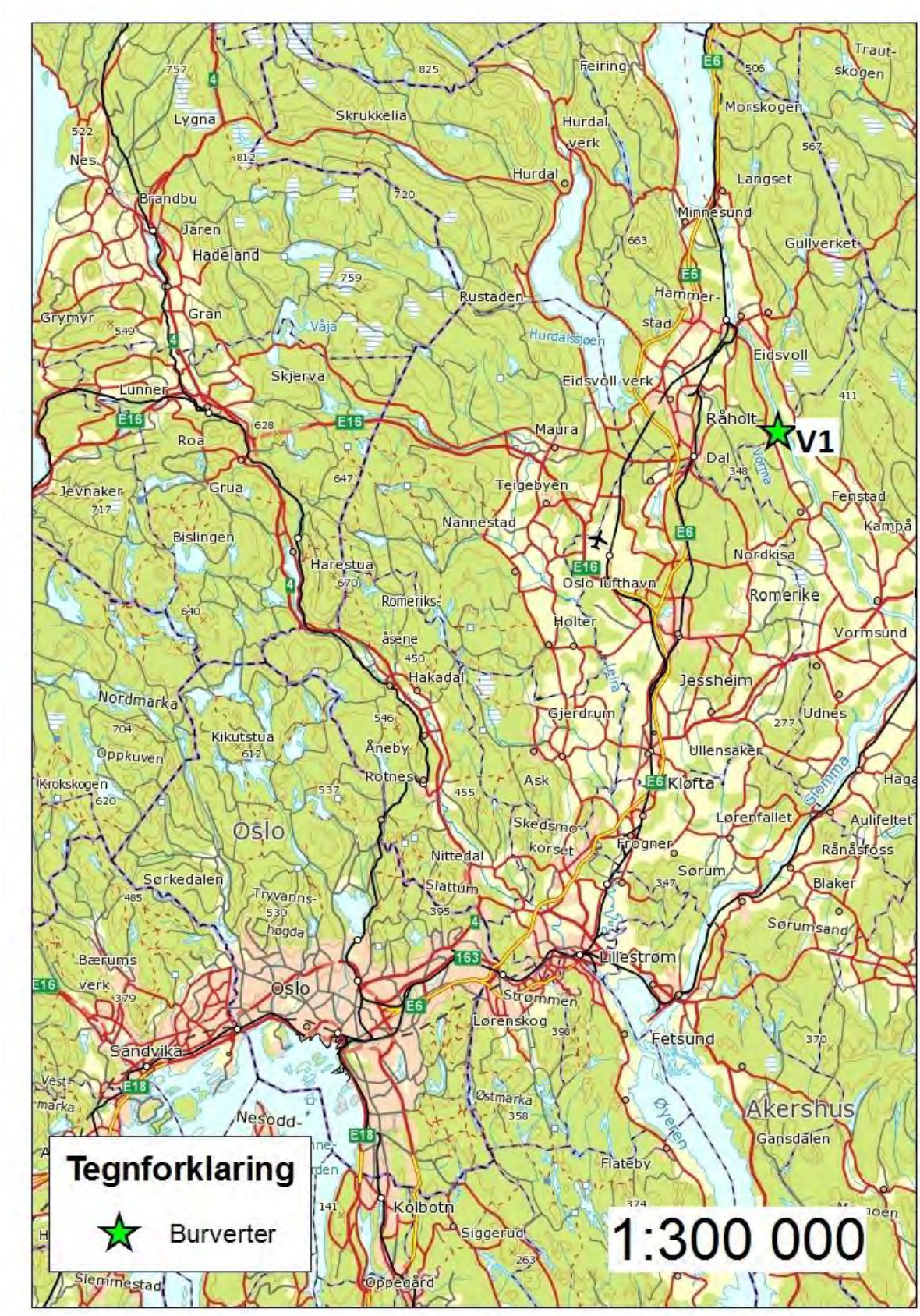
I flere teiner har vi hatt problemer med folk som enten ødelegger burene eller tar kreps. Dette selv med informasjon i burene om at de skal la burene ligge urørt. Vi har forsøkt med lås men det hjelper heller ikke hvis folk vil ødelegge.

Storsjøen, Hedmark:
Ingen ting tyder på at krepsepesten kommer fra sidevassdrag. UAØ har foretatt prøvekrepsing og vi fant heller ingen ting som tyder på signalkreps i Storsjøen. Det er etter mitt syn sannsynlig at årsaken til krepsepesten i 2015 ligger på Glommasiden av burforsøket fra 2015.

Bua, Hedmark
Ingen tegn til krepsepest i vassdraget.

2016.10.06 Øystein Toverud

Kart burforsøk Aurskog-Høland i Akershus



Kart burforsøk Vorma i Akershus

Kart burforsøk i Hedmark med spesielt fokus på Storsjøen og Bua

Scientifically ambitious, forward-looking and cooperatively oriented – for integrated health

The Norwegian Veterinary Institute is a national research institute that operates in the fields of animal and fish health, food safety and feed hygiene; its primary task is to provide the authorities with independently generated knowledge.

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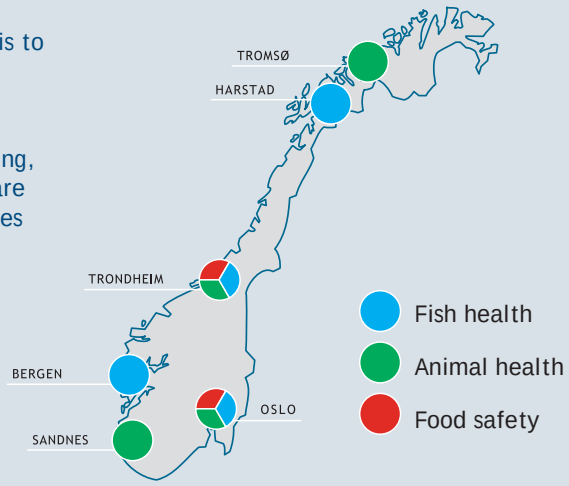
The Norwegian Veterinary Institute's central laboratory and administration lie in Oslo, and we operate regional laboratories in Sandnes, Bergen, Trondheim, Harstad and Tromsø.

The Norwegian Veterinary Institute collaborates with a large number of national and international institutions.



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