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Research article

Eradication of introduced signal crayfish *Pasifastacus leniusculus* using the pharmaceutical BETAMAX VET.®

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Abstract

Signal crayfish *Pasifastacus leniusculus* were first discovered in Norway in the Dammane area of Telemark County in October 2006. This introduced population was found to be infected with the oomycete *Aphanomyces astaci*, the causative agent of crayfish plague. The Dammane watershed consists of 5 small ponds, the largest with a surface area of approximately 2000 m^2. The Norwegian National Veterinary Institute conducted a feasibility study for the eradication of the Dammane signal crayfish population at the request of the Norwegian Food Safety Authority and Directorate for Nature Management. This study recommended the use of the pharmaceutical BETAMAX VET.®, followed by pond drainage as a feasible course of action. BETAMAX VET.® is a cypermethrin-based pharmaceutical developed for treatment of salmon louse (*Lepeophtherius salmonis*) infestations of farmed Atlantic salmon (*Salmo salar*). Cypermethrin is a synthetic pyrethroid and a common agent in many insecticides licensed throughout Europe. Following a comprehensive mapping of the Dammane watershed, the ponds were treated with BETAMAX VET.® on the 14 and 28 May, 2008. Subsequently, the ponds were drained by pumping out the water in two separate stages on 2-4 June, 2008 and 22-24 December 2008. During the first treatment with BETAMAX VET.®, signal crayfish were captured in the two upper ponds. During and following the second treatment and draining of the ponds, no signal crayfish were found. The ponds were gradually re-filled with water during the spring of 2009. It is too early to conclude whether the treatment has led to the complete eradication of the signal crayfish, but the results so far are promising. We believe that BETAMAX VET.® can be a useful tool in managing alien crayfish populations.

Key words: invasive species, signal crayfish, eradication, pyrethroids, crayfish plague

Introduction

The introduction of non-native species is regarded as one of the five most important drivers of global biodiversity change (Sala et al. 2000). Moreover, the introduction of non-native species via intentional and unintentional release of organisms (Lodge et al. 1998) is considered to be more ecologically damaging in freshwater ecosystems (especially lakes) than in terrestrial ecosystems (Sala et al. 2000).

There have been numerous intentional introductions of crayfish throughout the world (Hobbs et al. 1989), and at least ten non-native species of crayfish have been introduced to Europe (Souty-Grosset at al. 2006; D. Holdich pers. comm.). Alien crayfish have the potential to alter freshwater ecosystems through trophic cascade effects (Rodríguez et al. 2005; Gherardi and Acquistapace 2007; Matsuzaki et al. 2009). In addition, North-American crayfish species pose a significant threat to native European crayfish species through competitive exclusion (Westman et al. 2002) and the transmission of diseases (Alderman and Polglase 1988; Alderman et al. 1990). Since the 1960s, the North-American signal crayfish [*Pasifastacus leniusculus* (Dana, 1852)] has been introduced and spread on a large scale in Sweden, and is now the dominating crayfish species in Swedish lakes and rivers (Souty-Grosset et al. 2006). Signal crayfish are natural hosts for the oomycete *Aphanomyces astaci* Schikora (Unestam 1972), the causal agent of crayfish plague, and a disease lethal to European freshwater crayfish (Alderman and Polglase 1988; Alderman et al. 1990). Signal crayfish currently represent the main reason for the decline of indigenous crayfish in Europe (Dièguez-Uribeondo 2006). *A. astaci* has been shown to exist naturally in a balanced host-parasite relationship with crayfish in North America (Unestam 1972). In Sweden, approximately 95% of the native populations of noble crayfish [*Astacus astacus* (Linnaeus, 1758)] are lost, mainly due to the crayfish plague (Edsman 2004). Moreover, 65 % of all registered incidences of crayfish plague in Sweden in the period 1907–2004 occurred after the introduction of signal crayfish in 1969 (Bohman et al. 2006). Despite the massive introduction in Sweden, signal crayfish were not recorded in neighbouring Norway until 2006, when the first individuals were identified in a small isolated pond in the Dammane area of Telemark County (Johnsen et al. 2007).

A quantitative TaqMan® minor groove binder (MGB) real-time polymerase chain reaction (RT-PCR) based assay for detection of the oomycete *Aphanomyces astaci* (Vrålstad et al. 2009) confirmed that the introduced signal crayfish population was infected with crayfish plague (Johnsen et al. 2007). Crayfish plague is categorized as a most severe, or group-A disease by the Norwegian Food Safety Authority. Thus, this introduced population of signal crayfish represented a vector reservoir for the crayfish plague in Norway. The discovery of a Group A diseases is, if possible, routinely followed by the eradication of the affected animals.

The noble crayfish is native to Norway and is listed as endangered in the Norwegian red list (Kålås et al. 2006). This classification is mainly based on the threat from introductions of the crayfish plague by non-native crayfish. To minimize the risk of further propagation of the plague, the Norwegian authorities decided to eradicate the signal crayfish population in Dammane.

Throughout Europe there have been several attempts to eradicate different crayfish species. A review of possible methods for controlling nuisance populations of alien crayfish is given in Holdich et al. (1999). These methods include different legislative, mechanical, biological and physical measures, including the use of biocides and pheromones. Mechanical methods, such as trapping, seining, and electrofishing can control, but not eradicate crayfish populations (Holdich et al. 1999; Hiley 2003; Peay and Hiley 2006). Biological control, including the use of predatory fish such as the European eel [*Anguilla anguilla* (Linnaeus, 1758)] probably has the greatest potential for controlling crayfish populations (Furst 1977). However, there are no known examples for the successful eradication of crayfish populations by increased fish predation (Ribbens and Graham 2004). A problem associated with pond drainage is the burrowing behaviour of most crayfish (Holdich et al. 1999) and their ability to survive for months in moist environments or bottom areas that remain in contact with ground water (Holdich and Reeve 1991). There are no known examples of successful eradication of crayfish through draining alone (Peay and Hiley 2006; Kozak and Policar 2003).

As biological or physical methods have so far failed to exterminate alien crayfish populations, it seems that only chemical based treatments offers any hope for effective eradication of such invasive species (Peay 2001). Chemical methods of eradication include the use of biocides, surfactants and pheromones. Ribbens and Graham (2004) review the use of biocides for control of crayfish populations. Organophosphates and organochlorines are reported to be effective, but these chemicals are known to bioaccumulate through the food chain (Holdich et al. 1999). In contrast, both natural pyrethrum (Pyblast) and synthetic pyrethroids (derivates of natural pyrethrum) have been shown to be effective at very low doses, break down rapidly and do not bioaccumulate (Holdich et al. 1999; Hiley 2003; Peay and Hiley 2006). In one of the very few published field trials conducted to eradicate signal crayfish, Peay et al. (2006) used natural pyrethrum in small ponds in Scotland.

The results of this treatment were promising, as no signal crayfish were found in a post-treatment survey. However, natural pyrethrum is very expensive compared to its synthetic derivates. Eversole and Seller (1997) concluded in a comprehensive study based on 35 different chemical groups that synthetic pyrethroids were most poisonous to crayfish. Holdich et al. (1999) and Morolli et al. (2006) also state that pyrethroids have the greatest potential for the eradication of crayfish, and the latter author concluded that the pyrethroids cyfluthrin, deltamethrin and cypermethrin all share characteristics that make them effective for the extermination of crayfish. Based on experiments using red swamp crayfish [*Procambrus clarkii* (Girard, 1852)], Morolli et al. (2006) further showed that cypermethrin was the most effective chemical. For this reason, we considered the use of the pharmaceutical BETAMAX VET.® containing the active ingredient cypermethrin.

In this paper we report the first attempt of signal crayfish eradication with BETAMAX VET.®, thus contributing to the limited knowledge in the field of eradication of invasive crayfish species.

Materials and methods

Study site

The Dammane watercourse has a total length of 1000 metres and is situated on the Eidanger Peninsula between Frierfjord and Eidangerfjord in Porsgrunn municipality, Telemark County (see Johnsen et al. 2007). The watercourse consists of a creek with five small ponds, the largest measuring approximately 2000 m^2 surface area. The outlet runs into the Frierfjord near the town of Trosvik. The relatively warm coastal climate and the limestone bedrock have led to a rich flora and fauna. The watercourse includes five artificial ponds, originally constructed for the production of ice during the eighteenth century. The small reservoirs were used, until 1970 as a source of water for the city of Brevik. The uppermost dam is situated 70 meters above sea-level, whereas the lowermost pond is at an elevation of 40 meters. Prior to the chemical treatments, signal crayfish were only recorded in the uppermost pond (pond 5, see Figure 1).

BETAMAX VET.® characteristics and dosage

BETAMAX VET.® is a cypermethrin-based pharmaceutical originally developed for treatment of salmon louse [*Lepeophtherius salmonis* (Krøyer, 1837)] infestation of farmed Atlantic salmon [*Salmo salar* (Linnaeus, 1758)]. Cypermethrin is a synthetic pyrethroid and a common agent in many insecticides licensed throughout Europe. Pyrethroids and their synthetic equivalents are toxic to coldwater fish, aquatic insects and crustaceans, whereas other invertebrates, mammals, and birds are relatively tolerant towards this group of chemicals (Hiley 2003; Van Wijngaarden et al. 2006). All pyrethroids induce an irreversible alteration of nervous impulse transmission resulting in rapid death. Synthetic pyrethroids are based on the chemical structure and biological activity of natural pyrethrum, an extract of plants of the genus *Chrysanthemum*. Compared to natural pyrethrum the synthetic forms are more toxic, less degradable by light, more readily available and less expensive (Morolli et al. 2006).

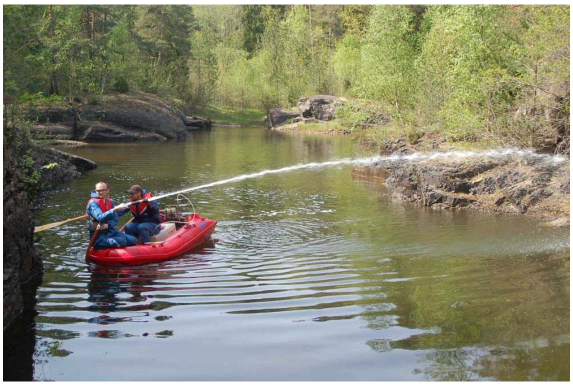

Figure 1. Treatment of pond 5 with BETAMAX VET.® using a pump to disperse the chemical. Photo by Roar Sandodden.

The environmental fate and degradation of pyrethroid insecticides were reviewed by Leahey (1979). The author concluded that pyrethroids do not persist in the environment for long periods, do not accumulate in the biosphere and do not biomagnify in the food chain. Ecosystem recovery is fairly rapid, with the toxic effect of pyrethroids lasting from days to months, and all major animal groups recovering within a year (Gydemo 1995).

Laboratory tests have shown that the synthetic pyrethroid Baythroid kills rusty crayfish [*Orconectes rusticus* (Girard, 1852)] at concentrations as low as 0.05 µl l^{-1} (Bills and Marking 1988) and noble crayfish at concentrations of 0.1 µl l^{-1} (Gydemo 1995). However, Bills and Marking (1988) found that concentrations as high as 25 µl l^{-1} were necessary to kill rusty crayfish in the field.

Preliminary work

Before the final planning of the treatment, the ponds of the Dammane watershed and adjacent tributaries were comprehensively mapped on 14 April, 2008. This date during the wet period was chosen to reduce the risk of missing any waterbodies. It was necessary to identify all water sources to the ponds, to make sure that every body of water within the watershed was treated with BETAMAX VET.® The final map included a description of the watersheds hydrology, and specified treatment details such as the amount of BETAMAX VET.® needed for each pond, creek and seep.

The surface area and water volume of each pond was calculated on 20 August, 2007, using a Garmin GPSmap 42 GPS mounted on a small radio controlled boat which moved around the ponds and recorded water depth. Depth contour maps were created in ArcView version 9.1 (ESRI). Pond 5 surface area and volume measured 1346 m^2 and 1996 m^3, respectively, whereas Pond 4 surface area and volume measured 6054 m^2 and 3154 m^3, respectively.

In situ toxicity test

To ensure that the correct concentrations of BETAMAX VET.® was applied for the eradication experiment an on-site toxicity-test using signal crayfish and pond water were performed the day prior to the first treatment. Thirty litres of water (15.5ºC) from pond 5 and 12 µl of BETAMAX VET.® (corresponding to a cypermethrin concentration of 20 µg l^{-1}) were mixed in a 60 l plastic tank. To which three male and two female crayfish (73-95 mm total length) were added. Crayfish behaviour was continuously observed. After 35 minutes, the five crayfish showed clear indications of irritation, including spontaneous tail flipping escape behaviour. After 45 minutes, none of the crayfish were able to regain correct orientation after being placed on their dorsal side (righting reflex). After 50 minutes, all crayfish were assumed to be dead. The test was stopped after 120 minutes of continuous observation. There seemed to be no obvious behaviour differences between male or female crayfish or between different size classes.

Equipment used and treatments performed

A first treatment of the five ponds with BETAMAX VET.® was conducted on 14 May, 2008. Team A treated ponds 1 and 2, starting in pond 1. Team B treated ponds 3 and 4, starting in pond 3. Both teams participated in treating pond 5 after finishing ponds 1–4. Pumps (250 litres min^{-1} capacity) that obtained water both from the pond and from a tank containing a diluted mix (1:100) of BETAMAX VET.® were placed in a boat or on the shore. The amount of chemical sucked from the tanks could be easily adjusted to the desired amount. The chemical was dispersed both on the water surface, along the pond bottom and on a 10 m onshore belt around each pond. To ensure that the chemical was well mixed in the ponds, we spent approximately one hour driving the pump around in each pond. One hour prior to BETAMAX VET.® dispersal in each pond, small drip stations were started in the tributaries. The drip stations were placed at the most upstream location of each creek or seep to ensure treatment of the whole drainage basin. This ensured a continuous, constant dosage of BETAMAX VET.® during the pumping treatment. The drip stations supplied a linear and constant dosage of 20 litres of diluted BETAMAX VET.® over four hours. In the smallest of seeps, enclosed water bodies and small upstream creeks watering cans were used to dispense the chemical.

To compensate for any possible uncertainty regarding our estimates of total water volume, we added 15% extra BETAMAX VET.® to our original estimate (20 µl l^{-1})and another 15% to compensate for the amount of chemical dispersed onshore. In the drip stations we used 0.05 litres BETAMAX VET.® A total of 10.6 litres of BETAMAX VET.® was used during the treatment of the 5 ponds; 5.37 litres during treatment one, and 5.22 litres during treatment 2.

The complete treatment of all five ponds was finished in approximately six hours. A similar water temperature of 14.0-16.5ºC was measured in all ponds and no stratification was observed. Prior to the first treatment, four traps each containing three signal crayfish were placed at different depths in pond 5. The second treatment was conducted on 28 May 2008 in an identical manner to that of treatment one. The water temperature was between 9.0 and 16.5ºC with no observed stratification. After each treatment, the shallow water of each pond was searched and all observed dead or dying crayfish were collected by hand or landing nets.

Draining of the ponds was conducted from 2-4 June, 2008 and again between 26 and 28 December, 2008. The second draining was performed to compensate for a partial refilling of the ponds, and to ensure empty ponds during the winter, and allow the bottom sediments to freeze.

Costs

Total cost for this project was 800 000 Norwegian kroner or approximately (95 000 Euro; October 2009 values). This does not include estimates of total water volume, and draining of the ponds.

Results

Prior to the first treatment of pond 5, no signal crayfish were observed from the shore. Thirty minutes after the treatment started, the first signal crayfish was detected in shallow water and displayed uncontrolled swimming/walking activity. The crayfish reacted by leaving their shelters exposing themselves in a manner not observed during normal conditions. Approximately 90 min after the start of the treatment, no surviving crayfish were observed. During this period, no crayfish were seen trying to leave the pond. The traps with signal crayfish were checked 18 hours after the start of the treatment and all individuals were found dead.

In pond 5, a total of 312 signal crayfish with lengths varying from 24 to 135 millimetres (total length) was collected within 3-4 hours after completion of the first treatment with BETAMAX VET.® (Figure 2). In pond 4, two more dead crayfish were collected. No signal crayfish were detected in the three other ponds. During the second treatment and the subsequent draining of the five ponds, no signal crayfish were found.

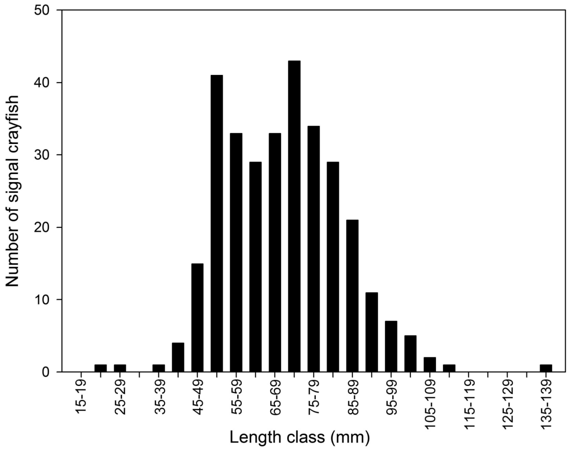

Figure 2. Length distribution (total length) of signal crayfish collected by hand and landing net during and after treatment with BETAMAX VET.® on the 14.05.2008 in pond 5 in Dammane.

Discussion

All indications are that the Dammane population was completely exterminated by chemical and physical treatment. In general, attempts to eliminate signal crayfish populations in European countries have proved difficult (e.g. Holdich et al. 1999; Kozac and Policar 2003). However, the present population of signal crayfish was restricted to small isolated ponds, and the chances of eradication, using a combination of chemical treatment and draining were believed to be good. However, eradication of non-native crayfish populations in very large watersheds is unlikely to succeed.

Mortality investigations and toxicity tests are usually performed in laboratories and under controlled conditions. In the field, chemical concentrations higher than those reported as 96h LC_{50} values are required to be effective in causing death of the target organism. Available information must be considered carefully when deciding on the concentration needed for use in eradication projects. In the eradication attempt performed by Peay et al. (2006), 100% mortality of caged crayfish was not reached until day 5 post-treatment. In our experiment, caged signal crayfish showed 100 % mortality within 18 hours, indicating that the chemical concentrations used were suitable for the eradication of the signal crayfish population.

In nature the chemical will not disperse perfectly. Dilution, dispersion, sedimentation, photolysis and degradation will start immediately after release into the recipient water body. In our opinion, a rapid response to a treatment is preferable when undertaking an eradication project. Once the decision to proceed with such a drastic measure is made, it should be performed in a manner that makes success probable at the first attempt.

No crayfish have been found after the first treatment. Pond 5 and 4 were controlled at night using light from 13-14 of June, 4-5 of July, 4-5 September and 17-18 September. Between 26 July and 18 September 2008 a total of 90 trap nights have been performed in ponds 4 and 5. Because of the ability of signal crayfish to survive for extended periods in moist sediments it is too early to conclude whether the treatment has been completely successful, but the results so far are promising. To report the ponds free of signal crayfish, surveys with traps or additional methods should be performed in upcoming years, at least throughout 2011. This is based on an expectation of very low numbers of survivors (if any), and that if the survivors consisted of small individuals they need some growth seasons to attain catchable size. Based on the results so far, we believe that BETAMAX VET.® can be a useful tool in managing alien crayfish populations.

During the 1960s, rudd [*Scardinius erythrophthalmus* (Linnaeus, 1758)] was introduced to Dammane, resulting in reduced diversity of aquatic insects and an apparent extermination of the Norwegian red list salamander species

Northern crested newt [*Triturus cristatus* (Laurenti, 1768)], listed as vulnerable, and the common newt [*Lissotriton vulgaris* (Linnaeus 1758)]), listed as near threatened (Saltveit et al. 2007). Thus, eradication of rudd was stated as a secondary aim of the project.

Stephenson (1982) reports a 96-h LC_{50} value of 0.4 µg cypermethrin l^{-1} at 15ºC for rudd. Hill (1989) further reports a general 96-h LC_{50} value of 0.4-2 µg l^{-1} for cold water adapted species and 2 µg l^{-1} for warm water adapted species. The cypermethrin concentrations used in this treatment were many times higher than the 96-h LC_{50} values for rudd, and three to four hours after the start of the treatment, no surviving rudd were observed. During the second treatment, no rudd was observed.

Health and safety issues

Health and safety advices concerning the handling of BETAMAX VET.® was given by the producer Novartis Pharmaceuticals. All participants in the project were instructed to follow these advices. When handling undiluted BETAMAX VET.®, face mask, safety glasses and gloves are necessary. When diluted no clear recommendation were given, but safety glasses and gloves were recommended. BETAMAX VET.® in boat tank was diluted approximately 1:50. This dilution was mixed with water from the recipient before leaving the pump (see "Materials and Methods" section. Equipment used and treatments performed).

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