Vol. 112: 219–228, 2015
doi: 10.3354/dao02811

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published January 15

Eroded swimmeret syndrome in female crayfish *Pacifastacus leniusculus* associated with *Aphanomyces astaci* and *Fusarium* spp. infections

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ABSTRACT: We describe a novel syndrome in crayfish, eroded swimmeret syndrome (ESS), affecting wild female signal crayfish *Pacifastacus leniusculus*. ESS causes partial or total swimmeret erosion. We observed ESS only in female signal crayfish larger than 40 mm carapace length, i.e. sexually mature and probably having carried eggs at least once. The eroded swimmerets were melanised, indicating a crayfish immune system response. We isolated *Fusarium tricinctum* species complex (SC), *F. sambucinum* SC, *Saprolegnia parasitica* and *S. australis* from the melanised tissue of the eroded swimmerets. ESS includes chronic *Aphanomyces astaci* infection and a secondary infection by *Fusarium* sp. In Sweden, we found female signal crayfish with ESS in 6 out of 11 populations with a prevalence below 1% in lakes with commercially productive signal crayfish populations and higher than 29% in lakes with documented signal crayfish population crashes. In Finland, the ESS prevalence was from 3.4 to 6.2% in a commercially productive population. None of the sampled male signal crayfish showed signs of ESS. A caging experiment indicated that females with at least 1 lost swimmeret carried on average 25% fewer fertilized eggs compared to females with intact swimmerets. ESS could significantly reduce individual female fecundity and thus could also affect fecundity at the population level. The decline in reproductive success due to ESS could be among the factors contributing to fluctuations in wild signal crayfish populations.

KEY WORDS: Signal crayfish · Reproduction · ESS · Crayfish plague · Opportunistic infection

INTRODUCTION

Populations of signal crayfish *Pacifastacus leniusculus* have been shown to suffer from increased mortality due to severe crayfish plague *Aphanomyces astaci* infection (Jussila et al. 2013, Aydin et al. 2014, Sandström et al. 2014), contrary to what was assumed when this alien crayfish was introduced to Europe more than 50 yr ago (Unestam & Weiss 1970, Westman 2000, Holdich et al. 2009). The *A. astaci* infection seems to be more severe if the signal crayfish population is exposed to other stressors such as contaminants or parasites (Persson & Söderhäll 1983, Svärdson et al. 1991, Thörnqvist & Söderhäll 1993, Ward et al. 2006, Aydin et al. 2014). These long discussed and recently verified findings indicate that the role and potential of the signal crayfish fisheries in Europe should be seriously revised.

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The fungal genus *Fusarium* causes melanisation and cuticle erosion in several crustacean species (Hose et al. 1984, Alderman & Polglase 1985, Chinain & Vey 1988, Khoa et al. 2004, Makkonen et al. 2013). Other possible chitinoclastic pathogens have also been named as disease agent candidates (Edgerton et al. 2002). Secondary opportunistic parasites can cause severe gross signs in signal crayfish that have been chronically infected with *A. astaci* (e.g. Jussila et al. 2013), while crayfish in North America show only minor visible signs (Souty-Grosset et al. 2006), often only tiny melanised spots in their carapace (J. Makkonen pers. obs.). The observed melanisation among Nordic signal crayfish stocks is so severe that one would suspect difficulties and failures during moulting, which in turn could result in decreased production in wild stocks and even population collapses in the most serious cases.

Lake populations of commercially harvestable signal crayfish fluctuate by several orders of magnitude from year to year in Swedish and Finnish lakes (Westman et al. 1999, Olsson et al. 2010, Sandström et al. 2014). At least 10% of the Finnish signal crayfish stocks have collapsed for unknown reasons (authors' unpubl. data), and there is concern arising from wild population collapses (Edgerton et al. 2004, Pakkasmaa 2006). One of the suspected factors behind the collapses is *A. astaci* infection (Jussila et al. 2014a, Sandström et al. 2014), but only a limited number of publications have discussed the possibility of signal crayfish being susceptible to *A. astaci* infection (Thörnqvist & Söderhäll 1993, Jussila et al. 2013, Aydin et al. 2014). Indirect evidence from wild populations strongly suggests that signal crayfish under European, and especially Nordic, conditions show increased mortality if affected by chronic *A. astaci* infection (Sandström et al. 2014, Jussila et al. 2014a,b, 2015).

During field studies, signal crayfish females with lost swimmerets have been observed in natural populations in both Sweden (Sandström et al. 2014) and Finland. Since females attach their fertilized eggs to the swimmerets, females with lost swimmerets would carry fewer eggs than females with intact swimmerets, which could then be among the factors behind density declines or even population crashes.

In this study, we further explored this phenomenon with several aims: (1) describe the eroded swimmeret syndrome, i.e. the loss of female signal crayfish swimmerets; (2) provide suggestions regarding possible candidates for the cause of the swimmeret erosion based on the isolation of micro-organisms from the melanised lesions at the sites of eroded swimmerets; (3) determine the frequency of occurrence of the eroded swimmeret syndrome in Swedish and Finnish signal crayfish populations; and (4) test whether the number of eggs attached to swimmerets differs between females with or without lost swimmerets. Based on our results, we discuss the consequences of swimmeret loss to the dynamics of wild signal crayfish populations.

MATERIALS AND METHODS

Sampling and data collection from Lake Saimaa (Finland)

The Lake Saimaa signal crayfish population has been routinely monitored since 2009 (Jussila et al. 2013) as part of fisheries monitoring projects. The stock is a reservoir of crayfish plague *Aphanomyces astaci* (Strand et al. 2011), with a high level of crayfish showing gross signs of infection (e.g. melanised spots, eroded shell and lost appendages; Fig. 1). The population has individuals with melanisation at a frequency of 40 to 80% in the commercial catch, with considerable variation within test sites both during the crayfish season and between years. In addition, the stock is parasitized by *Psorospermium haeckeli* (Jussila et al. 2013).

Since 2011, swimmeret losses have been detected and the classification of eroded swimmeret syndrome was added to the datasheet notes during the 2013

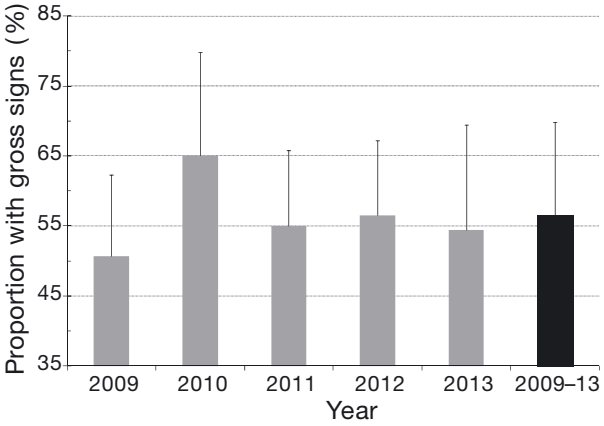

Fig. 1. Proportion of Lake Saimaa signal crayfish *Pacifastacus leniusculus* showing gross signs of *Aphanomyces astaci* infection in the test catches during 2009 to 2013. Signs include melanised spots, eroded shell and lost appendages indicative of *A. astaci* infection. Data were pooled from 5 different test sites, 4 measurements each, annually. Mean + SD annual values from these 5 sites are shown (grey bars) as well as the mean from the entire period (black bar)

catch monitoring. In total, 5 test sites from 3 commercial trappers were measured 4 times, i.e. every other week during the Finnish crayfish season from late July to early September, giving a total of 15 individual test sites and a total of 1235 signal crayfish with 553 females (44.8% of total) being examined. The level of the swimmeret losses were ranked as stage 0 (no erosion), stage 1 (melanisation with possibly partial erosion of swimmerets), stage 2 (melanisation and total erosion of 1 to 3 swimmerets) and stage 3 (total erosion of more than 3 swimmerets). The test site locations in Lake Saimaa were Saimaa1 (61° 9′ 59.4″ N, 28° 29′ 59.2″ E), Saimaa2 (61° 11′ 28. 7″ N, 28° 25′ 29.9″ E), Saimaa3 (61° 13′ 51.3″ N, 28° 27′ 7.4″ E), Saimaa4 (61° 12′ 57.0″ N, 29° 19′ 42.5″ E) and Saimaa5 (61° 15′ 51.0″ N, 28° 17′ 12.5″ E).

Female signal crayfish with swimmeret losses and apparently healthy female signal crayfish with intact swimmerets were collected from trapping sites and transferred to the University of Eastern Finland, Kuopio campus, for further identification of possible disease agent candidates in the swimmeret tissue. Crayfish from each site and category were kept separately during the transport.

Sampling and data collection from Sweden

The first observations of swimmeret losses in Swedish populations of signal crayfish were made during 2010 in 11 water bodies (see Table 4). Of these monitored populations, Lake Immeln and Lake Trehörningen signal crayfish populations had experienced recent collapses (Sandström et al. 2014) while the other 9 lakes had signal crayfish populations that were harvestable with catch per unit effort (CPUE) > 2 crayfish per trap per night. The Lake Immeln signal crayfish population showed a 94% drop in total catch from the 2009 season compared to the 2010 season (Sandström et al. 2014), and there was still no sign of population recovery in 2013.

During the test trapping, we used baited commercial traps that capture crayfish larger than 6.5 cm total length (TL) (Edsman & Söderbäck 1999). All captured crayfish were sexed, and the number of crayfish with lost swimmerets was recorded according to the erosion ranking criteria described above.

Lake Immeln (Sweden) cage experiment

In order to evaluate the possible effect of eroded swimmerets on the number of eggs attached to the females during hatching, we conducted a non-replicated *in situ* cage experiment in Lake Immeln in 2010, a year after the population collapse in the lake was documented. On 30 September, we examined and measured 27 sexually mature females and 7 males which had been previously caught in baited traps by local fishermen. Of these females, 8 had lost from 1 to 5 swimmerets (median value of 1.5 lost swimmerets), but all males had intact swimmerets. A total of 8 females with lost swimmerets were placed together with 3 males in a cage (1 × 1.5 m) with a coarse mesh (mesh size 1 cm), and the cage was placed in shallow shoreline water on the lake bottom (1 m depth) for spawning to occur. Next to this cage, a similar cage was placed with 8 females with intact swimmerets together with 3 similarly healthy males. The females with lost swimmerets were of similar size (mean ± SD: 11.2 ± 1.0 cm TL) as the females with intact swimmerets (10.8 ± 0.6 cm TL; *t*-test, $p > 0.39$). The cages were revisited after spawning on 1 November (water temperature 8.7°C), and all crayfish in the cages were frozen for later analyses (within 2 mo). After thawing, we carefully counted the number of eggs attached to each swimmeret as well as eggs attached to the abdomen by means other than attachment to swimmerets. In addition, we also checked for eggs still in the ovaries. This protocol enabled us to assess the consequences of lost swimmerets in terms of number of carried eggs.

Sample material for disease agent isolation and identification from Lake Saimaa (Finland)

Eroded and melanised swimmeret stumps were dissected from Saimaa female signal crayfish with swimmeret losses. Samples from 2 individuals were cultured. Eroded and melanised swimmerets were dissected, surface sterilized with 70% ethanol for 1 min and washed in sterile MQ-water. Tissue parts were mounted into 2 different agars, viz. mycological agar and potato dextrose agar (PDA) (both Difco), containing 1 g l^{-1} of streptomycin and ampicillin. Plates were incubated in a 20°C incubator for 2 wk, and new fungal growth observed during that period was transferred into new plates daily. Before the DNA extractions were started, single spore cultures were made following the methods of Makkonen et al. (2013).

For the DNA extractions, 50 mg of the aerial hyphae were cut from the PDA. DNA extractions from the pure cultures were made with an E.Z.N.A Insect

DNA isolation kit (Omega Bio-tek). For the DNA extraction, the tissue samples were disrupted for 2 × 30 s at full speed in TissueLyser II (Qiagen) with 350 µl lysis buffer of the DNA extraction kit, stainless steel beads (Qiagen) and sea sand (Merck). Then, 25 µl of Proteinase K (Omega Bio-Tek) were added and samples were lysed for 2 h at 60°C. The DNA was extracted using the E.Z.N.A Insect DNA isolation kit according to the manufacturer's instructions. The concentration and purity of the DNA was measured with NanoDrop (Thermo Scientific).

PCR of internal transcribed spacer regions and elongation factor 1 alpha gene

Internal transcribed spacer (ITS) regions surrounding the 5.8S rRNA gene were amplified with primers ITS1 and ITS4 (White et al. 1990). For the PCR reaction, DreamTaq Green 2× Master Mix (Thermo Scientific) was used with 10 µM of each primer and 50 to 100 ng of template DNA. The 50 µl reaction volumes were completed with PCR-grade water. The following program was used during the PCR procedure (PTC-200, MJ Research): initial denaturation at 95°C for 3 min, 35 amplification cycles (95°C for 30 s, 52°C for 30 s and 72°C for 45 s) and final elongation at 72°C for 10 min. An additional PCR-reaction was conducted using primer pair EF1αF and EF1αR to amplify the translation elongation factor 1 alpha (EF1α) gene, according to Geiser et al. (2004). Reaction conditions were as described above, except for an annealing temperature of 53°C.

DNA extraction from damaged tissue and PCR amplification of disease agents

The sample from site Saimaa1 contained female signal crayfish with (ESS stages 2 and 3, n = 6) and without clinical signs (ESS stage 0, n = 5). The sample from site Saimaa4 contained females at ESS stages 2 and 3 (n = 7). Swedish samples from Lake Hjälmaren (n = 10) contained ESS stages 0 (n = 2), 1 (n = 3), 2 (n = 4) and 3 (n = 1). The crayfish from Båven (n = 1) was at ESS stage 0. If an individual showing signs had several melanised swimmeret stumps, they were pooled as 1 sample for the DNA extraction. For the individuals showing no signs, 3 healthy swimmerets from each individual were dissected for the DNA extraction, which was made as described earlier.

qPCR amplification of the crayfish plague agent *Aphanomyces astaci* from eroded swimmerets

To confirm the presence of the crayfish plague agent *A. astaci* in the eroded swimmeret tissue samples, a qPCR was conducted according to Vrålstad et al. (2009) and samples of individual crayfish were analysed separately. The reactions were carried out in a LightCycler II 480 qPCR machine (Roche) in a 10 µl reaction volume from 1× and 10× diluted DNA samples. Agent level scoring was done against a calibrated standard curve according to Vrålstad et al. (2009). A0 and A1 were negative samples. Agent levels A2 (5–50 PCR forming units, PFUs) and A3 (51–1000 PFU) corresponded to very low and low agent levels of *A. astaci*, respectively. Agent level A4 (1001–10 000 PFU) corresponded to moderate agent levels, agent level A5 (10 001–100 000 PFU) to high agent levels and A6 (100 001–1 000 000 PFU) to very high agent levels.

Identification of the possible eroded swimmeret syndrome disease agent

Amplification of correct PCR-amplicons was checked on 1.5% agarose gel containing 0.5 µg ml^{-1} of EtBr. PCR products were purified with a QiaQuick Spin PCR-purification Kit (Qiagen) and sent to LGC Genomics (Germany) for sequencing. Resulting chromatograms were analysed with Geneious 5.4 (Drummond et al. 2011), and the species were identified with Blast-searches against the NCBI GenBank nr-database or FUSARIUM-ID (Geiser et al. 2004). Sequences were submitted to NCBI GenBank under accession numbers KJ920731 to KJ920740.

RESULTS

Description of eroded swimmeret syndrome

Eroded swimmeret syndrome (ESS) first appears as melanisation of the swimmerets. More severe ESS shows as either partially eroded swimmerets which also show melanisation or both fully eroded and partially eroded swimmerets with melanisation. In the final stages of ESS, the whole swimmeret has disappeared and there is a small, round melanised stump left where the swimmeret was joined to the abdomen (Fig. 2). We also observed scar-like tissue formation where the swimmeret had been in newly moulted crayfish during mid-

Fig. 2. Eroded swimmeret syndrome (ESS) signs (arrows) as observed in Lake Saimaa female signal crayfish *Pacifastacus leniusculus*. (A) swimmerets intact but show melanised spots (stage 1); (B) swimmerets partially or totally eroded with few intact swimmerets remaining (stage 2); (C) swimmerets completely eroded with melanised spots remaining (stage 3). ESS stage 0 shows intact swimmerets with no visible signs

August 2013 sampling in Lake Saimaa (Fig. 3). During the field surveys in the summer of 2013, there were no observations of a swimmeret regeneration process, normally observed in crayfish that have lost a limb. During test trappings in the summer of 2014, we made observations of partially regenerated swimmerets.

We isolated and identified several microbes from the ESS melanised stumps dissected from the Saimaa signal crayfish ESS trauma sites. The microbes include those routinely detected in water samples and aquatic animal samples, such as *Saprolegnia* (Table 1). We also obtained isolates belonging to the *Fusarium tricinctum* species complex (SC) (Table 1).

We observed a lower qPCR agent level rating in the individual crayfish swimmeret samples from those crayfish showing no signs of ESS compared to crayfish showing ESS signs in both Swedish and Finnish data (Table 2). The difference in the Swedish

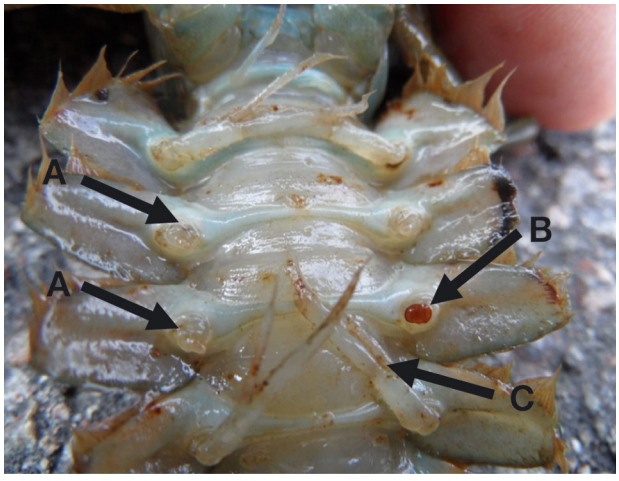

Fig. 3. Example of a moulted female signal crayfish *Pacifastacus leniusculus* with eroded swimmeret syndrome (ESS) and eroded swimmeret remains showing scar tissue (A) with the base of one swimmeret still melanised (B). Melanised spots are also visible in the intact swimmerets (C)

Table 1. Results of the isolation and identification of possible disease agents from the melanised and eroded swimmerets of Lake Saimaa female signal crayfish *Pacifastacus leniusculus*. The species identification is based on internal transcribed spacer (ITS) sequence and elongation factor 1 alpha (EF1α) gene blasts in NCBI GenBank (*Saprolegnia* species) and in FUSARIUM-ID (*Fusarium* species) with the GenBank accession numbers provided; na: not analysed

Isolate	Closest species	Match similarity (%)	Accession no.	Species complex	GenBank accession no. EF1α	GenBank accession no. ITS regions
Saimaa1 sampling site						
UEF-LIM5a	*Saprolegnia parasitica*	99.8	AM947031	–	na	KJ920734
UEF-LIM6	*S. australis*	99.9	JN662488	–	na	KJ920736
UEF-LIM5b	*Fusarium negundis*	97.05	NRRL 20682	*Tricinctum*	KJ920740	KJ920735
Saimaa5 sampling site						
UEF-ES6a	*F. tricinctum*	97.30	NRRL 36147	*Tricinctum*	KJ920737	KJ920731
UEF-ES6b	*F. graminearum*	99.84	NRRL 28336	*Sambucinum*	KJ920738	KJ920732
UEF-ES7a	*F. negundis*	97.64	NRRL 20682	*Tricinctum*	KJ920739	KJ920733

Table 2. *Aphanomyces astaci* agent levels obtained in qPCR from eroded and melanised swimmerets of signal crayfish *Pacifastacus leniusculus*. The results for each individual crayfish are pooled (number of individuals in each category in brackets). ESS: eroded swimmeret syndrome

ESS stage	n	*Aphanomyces* agent level prevalence (%)						
		A0	A1	A2	A3	A4	A5	A6
Finnish samples	**18**							
Stages 2 and 3	13			8 (1)	54 (7)	38 (5)		
Stage 0	5	20 (1)		40 (2)	20 (1)	20 (1)		
Swedish samples	**11**							
Stages 2 and 3	8				13 (1)		75 (6)	13 (1)
Stage 0	3	33 (1)	33 (1)	33 (1)				

Table 3. Percentage (%) of female signal crayfish *Pacifastacus leniusculus* showing eroded swimmeret syndrome (ESS) stage 1 in a commercial catch during the 2013 crayfish season (survey period 21 July to 10 September) in Lake Saimaa, Finland

Site	Location	Range (%)
Saimaa1	61° 09′ 59.4″ N, 28° 29′ 59.2″ E	24–41
Saimaa2	61° 11′ 28.7″ N, 28° 25′ 29.9″ E	11–57
Saimaa3	61° 13′ 51.3″ N, 28° 27′ 07.4″ E	6–32
Saimaa4	61° 12′ 57.0″ N, 29° 19′ 42.5″ E	5–37
Saimaa5	61° 15′ 51.0″ N, 28° 17′ 12.5″ E	3–28

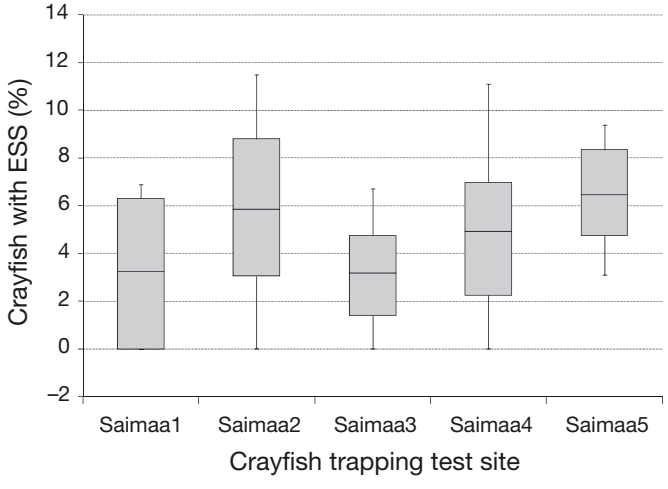

Fig. 4. Proportion of female signal crayfish *Pacifastacus leniusculus* with eroded swimmeret syndrome (ESS) stages 2 and 3 in the test catches in Lake Saimaa during the 2013 season, sorted by test site. Results are combined from 4 measurements made during the crayfish trapping season. Data are median (horizontal line inside the box), interquartile ranges (box) and ranges (min.–max.; whiskers)

data was statistically significant (χ^2 = 11.0, df = 5, p = 0.05), while the Finnish data showed only a tendency (χ^2 = 6.2, df = 3, p = 0.14).

ESS frequency in wild populations

Lake Saimaa wild population

In 2013, the proportion of crayfish with ESS stage 1 varied from 3 to 57% among the 5 surveyed Lake Saimaa signal crayfish test trapping sites (Table 3). The female signal crayfish showing ESS early stage syndromes (stage 1) were >42 mm carapace length (CL), while those showing ESS (stages 2 or 3) were >40 mm CL in Lake Saimaa samplings in 2013. All individuals showing ESS also had at least 1 gross sign of *Aphanomyces astaci* infection, i.e. melanised spots, carapace erosion or lost appendages.

Among the 5 Lake Saimaa sites, on average between 3.2 and 6.2% of the female signal crayfish showed ESS in stages 2 and 3 during the test catches in 2013 (Fig. 4), and this pattern was independent of test site (χ^2 = 12.6, df = 8, p > 0.12). In the majority of the test sites (4 out of 5), there were no female signal crayfish with ESS in the catches at least once during the season 2013. Thus, the variation in the proportion of females having ESS within each test site as the season progressed was considerable.

Swedish wild populations

We recorded ESS in 6 of the 11 surveyed Swedish lakes, and 34 out of more than 5200 examined crayfish had at least 1 lost swimmeret (Table 4). The per-

Table 4. Observations on female signal crayfish *Pacifastacus leniusculus* with eroded swimmeret syndrome (ESS; stages 2 and 3) during test fishing in Swedish lakes between 2010 and 2013. Prev.: prevalence

Lake	Samples (n)	ESS prev. (%)
Immeln[a]	42	38.0
Trehörningen[a]	7	29.0
Trummen	101	5.9
Läen	50	4.0
Bunn[b]	118	1.7
Hjälmaren	1561	0.3
Erken[b]	245	0.0
Hövern[b]	203	0.0
Båven[b]	191	0.0
Vättern	996	0.0
Sjögarpesjön	56	0.0

[a]Lake with significant population collapses
[b]No collapse (Sandström et al. 2014)

centage of female signal crayfish with ESS in these lakes varied from 0 to 38%. Two of the lakes with a previously documented population collapse stood out, and 18 of the female signal crayfish with ESS were captured in these 2 lakes. Here, the percentage of females with ESS was 29% and 38% in Lake Trehöringen and Lake Immeln, respectively. In the other lakes, the mean value for ESS prevalence was just below 1% and never exceeded 6% of the female signal crayfish (Table 4).

Lake Immeln (Sweden) cage experiment

In the caging experiment, all males survived, while 1 female from the control group (those with intact swimmerets) was found dead in the cage, and 2 females in the experimental group (those with at least 1 lost swimmeret) were found dead. All females had mated (i.e. they carried male spermatophores) and had laid eggs that appeared to be viable without any visible fungal infections.

Later inspection of female signal crayfish ovaries and egg counts showed that none of the females had eggs remaining in their ovaries. However, some eggs (range from 14 to 20 eggs) were found on smaller threads attached to the abdomen and not to the swimmerets. This number was less than 10% of the total number of eggs attached to the swimmerets. Those females with intact swimmerets (n = 7) had 322 ± 55 eggs (mean ± SD), whereas those females with at least 1 lost swimmeret (n = 6) had 240 ± 57 eggs, which was significantly lower than that of the females with intact swimmerets (*t*-test, p = 0.032). The mean reduction due to ESS in the number of eggs carried was 25.5%.

DISCUSSION

We have described a novel traumatic syndrome, ESS, affecting female signal crayfish *Pacifastacus leniusculus* in wild stocks both in Sweden and Finland. The syndrome has only been observed for the past 5 yr, and field notes have been made routinely since 2010 in Sweden and 2012 in Finland. The only previous reference to ESS-like syndromes is based on the same data (Sandström et al. 2014). To date, we have observed ESS to affect only female signal crayfish that are sexually mature and, most likely, have already produced at least 1 clutch. No similar signs have been recorded from wild native noble crayfish *Astacus astacus* populations in the Nordic countries. This indicates that ESS may be found only on signal crayfish which, as a precondition, have been chronically infected with *Aphanomyces astaci*. ESS would thus be caused by multiple infections, with the precondition of the *A. astaci* infection and then *Fusarium* sp. most probably causing swimmeret erosion as an opportunistic pathogen. If the proportion of individuals with ESS is high, our observations suggest that populations could be negatively affected through reduced fecundity resulting in decreased juvenile recruitment. Crayfish population dynamics can at least partly be explained by variations in recruitment (Hein et al. 2006, Sadykova et al. 2009, Skurdal et al. 2011).

In Lake Immeln, Sweden, about 60% of the PCR-examined signal crayfish were detected to be infected with *A. astaci* (Sandström et al. 2014). Furthermore, the prevalence of gross signs indicating *A. astaci* infection in the population of Lake Saimaa, Finland, was over 55% in this study, with an even higher PCR-based detection reported earlier (Strand et al. 2011). In our study, all of the crayfish in Lake Saimaa affected by ESS showed classic gross signs of *A. astaci* infection in the field surveys. The female signal crayfish exhibiting ESS collected from Lake Saimaa and Lake Immeln that were later dissected in the laboratory and analysed using qPCR were *A. astaci* positive. Thus, we conclude that the *A. astaci* infection is a precondition for ESS. In Lake Saimaa female crayfish, we identified several disease agent candidates from the melanised remains of the swimmerets, including *A. astaci*, *Saprolegnia parasitica*, *S. australis, Fusarium tricinctum* SC and *F. sambucinum* SC. Of these organisms, we conclude that *Fusarium* sp. is the most likely disease agent candidate in our data, as there is evidence in the literature that *Fusarium* spp. cause soft and chitinised tissue erosion of gills and exoskeleton in crustaceans (McAleer & Baxter 1983, Makkonen et al. 2010, 2013).

We conclude that the *Fusarium* SC likely includes the probable candidates for the ESS disease agent and that *A. astaci* infection is the precondition, based on how these organisms are reported to interact with crustaceans and crayfish. *A. astaci* invades the soft tissues of the crayfish and for that purpose has to penetrate the surface tissues, either exoskeleton or soft tissue membrane (Söderhäll et al. 1988, Souty-Grosset et al. 2006). In this instance, it pushes the hyphae through the exoskeleton and activates the crayfish immune system. The oomycete then penetrates the surface tissue or is blocked by the crayfish immune system, normally by the melanisation process. This creates the visible darkening of the tissue.

The penetration site is tiny, normally less than 1 mm in diameter. For *A. astaci*, it would not be evolutionarily rational to waste energy in a massive tissue breakdown. *Fusarium* SC, on the other hand, targets the chitinised tissues, either hard or soft, and causes massive tissue erosion (e.g. Makkonen et al. 2013).

The synergistic interaction of opportunistic pathogens, especially together with *A. astaci*, may cause severe multiple infections by overloading the crayfish defence system (e.g. Thörnqvist & Söderhäll 1993). This would allow new avenues for the pathogens, and even parasites, to infect crayfish and could cause traumatic conditions such as ESS. For *A. astaci*, multiple infections might be beneficial since they could create conditions where sporulation from the weakened North American crayfish host would be improved. This has been indicated by a slight elevation in the *A. astaci* spore release from signal crayfish during moulting (Strand et al. 2011).

Fusarium spp., including *F. avenaceum*, cause exoskeleton erosion, including burn spot disease (McAleer & Baxter 1983, Makkonen et al. 2013). Thus, *Fusarium* spp. benefit from already damaged, even infected, tissue being available. The role of opportunistic pathogens and multiple infections in signal crayfish needs to be studied further, and could be an interesting aspect highlighting the unexpected population dynamic patterns in wild signal crayfish.

Our data suggest a link between sexual maturity and initiation of ESS, since only those female signal crayfish that have carried eggs at least once seemed to show ESS signs. The triggering factor for ESS might be the hatching of the eggs, which provide suitable and preferred growth surface for the opportunistic pathogens. The egg mass can be easily infected with aquatic pathogens and parasites, including *Saprolegnia* sp. and *Fusarium* sp. The latter was isolated from the melanised remains of the swimmerets and, based also on other studies (Alderman & Polglase 1985, Chinain & Vey 1988, Vey 1988, Edgerton et al. 2002, Quaglio et al. 2006, Makkonen et al. 2010, 2013, Dörr et al. 2012), would be the main candidate for consideration as the ESS disease agent.

We observed indications of a severe *A. astaci* infection triggering ESS, as the Swedish data showed that signal crayfish females with ESS had higher qPCR *A. astaci* ratings compared to those showing no ESS signs. The Finnish data, on the other hand, did not show such clear differences between affected and non-affected individuals, although they followed a similar trend. Sandström et al. (2014) suggested that the loss of pleopods in signal crayfish females could be accompanied by *A. astaci* infection. This finding is based on the same data discussed here. Furthermore, there are several observations indicating that female signal crayfish could be more intensely infected with *A. astaci* compared to males (Vrålstad et al. 2011, Sandström et al. 2014), thus exposing them to other parasites and pathogens. Our sample sizes were low, and further studies are needed to fully understand the role of *A. astaci* infection in the development of ESS.

Our observations show that lake-dwelling signal crayfish females may suffer from higher degrees of *A. astaci* infection than males. Environmental stressors may suppress the immune responses in signal crayfish (Ward et al. 2006), although the stressors in the case of Lake Immeln, where the caging experiment took place and ESS was high among female signal crayfish, are unknown. We hypothesize that reproductive impairment caused by ESS may be among the factors explaining the population collapses in Lake Immeln, and potentially in other lakes as well.

In the case of Lake Saimaa, the signal crayfish had an acute *A. astaci* infection in 2007 (Jussila et al. 2014a) and the catch declined. Since then, the CPUE has remained low, around 1 (Jussila et al. 2013), and the crayfish show severe gross signs of *A. astaci* infection. Recruitment is suggested to have substantial effects on population growth in crayfish (Jones & Coulson 2006, Zimmerman & Palo 2012). As ESS is rather common in the Lake Saimaa stock, we hypothesize that ESS could also in this case be one of the key factors hindering a full recovery of the signal crayfish stock in this lake.

In Lake Immeln, which suffered a severe population collapse of signal crayfish (Sandström et al. 2014), we observed that 38% of the sexually mature females had lost at least 1 swimmeret. In our study, the loss of on average 1.5 swimmerets decreased the number of eggs laid by roughly 25%, which should reflect on juvenile recruitment. Thus, the effect of ESS could also be observed at the population level and, apart from direct effects on the number of eggs laid by females, it may also increase mortality of the infected and stressed signal crayfish females (e.g. Thörnqvist & Söderhäll 1993, Aydin et al. 2014), as was indicated in our cage experiment. There are also other candidates for the list of factors causing freshwater crayfish population collapses, including heavy exploitation (Hein et al. 2006), predatory fish (Olsson et al. 2006), low pH (France 1987) and a combination of diseases (Thörnqvist & Söderhäll 1993, Aydin et al. 2014, Jussila et al. 2014b).

In the case of Lake Saimaa, the whole catch from the test sites was removed as a result of the survey

measurements, which were repeated every other week during the crayfish season. Commercial trappers were also removing crayfish from those sites during the season. This includes females with ESS. In spite of that, there seemed to be a constant recruitment of females with ESS to the stock, with rather a high proportion of ESS stage 1 females in the test catch, indicating that ESS could be a persisting phenomenon among Lake Saimaa signal crayfish females. ESS-affected females have also been observed in other Finnish signal crayfish populations, i.e. in Lakes Unnukka (Savo), Jääsjärvi (Hartola) (J. Jussila pers. obs.), Päijänne (T. Ruokonen pers. comm.), Pyhäjärvi (Säkylä) (P. Mäkinen pers. comm.) and Pyhäjärvi (Tampere) (E. Erkamo pers. comm.). Stocks suffering ESS-like signs were detected in Lakes Päijänne, Näsijärvi (Tampere) and Pyhäjärvi (Säkylä) by the Finnish Game and Fisheries Research Institute during summer 2014 test trappings (M. Pursiainen pers. comm.).

We have shown that female signal crayfish in wild Nordic populations are suffering from a novel condition (ESS) which causes swimmeret erosion and loss. ESS has been shown to decrease clutch size, and would thus decrease juvenile recruitment. ESS could be among the factors adding to the signal crayfish population struggles observed in Nordic countries.

Acknowledgements. We thank all the landowners, fishermen and organizations who provided data on crayfish and for allowing us to collect additional data. L.E., P.N., A.S. and M.S. were partly financed by the European Union's Fisheries Fund. H.K., V.T., J.M. and J.J. were funded by Southeast Finland Centre for Economic Development, Transport and the Environment using fisheries funds. We are grateful to Saimaa crayfishermen Esko Toikka and Timo Myllynen for their collaboration. The language was corrected by Daniel Blande MSc (University of Eastern Finland).

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Editorial responsibility: Grant Stentiford, Weymouth, UK

Submitted: July 2, 2014; Accepted: September 30, 2014
Proofs received from author(s): December 11, 2014