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REGULAR ARTICLE

MASS MORTALITY EVENTS IN FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) POPULATIONS IN SWEDEN: AN OVERVIEW AND INDICATION OF POSSIBLE CAUSES

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ABSTRACT

The freshwater pearl mussel *Margaritifera margaritifera* is an endangered species in Sweden with more than 600 known populations distributed in 16 out of 21 counties. Only approximately one-third of these populations are considered viable and healthy with signs of recent juvenile recruitment. From 2011 to 2017, research documented an increased mortality in this species, of up to 100% in some populations, but no etiological cause of these mortalities has been identified. With this paper, we provide current knowledge of locations where mass mortality of freshwater pearl mussel has been found in Sweden and discuss possible causes. Postmortem sampling and histopathological findings from two counties in 2016–17 detected lesions in digestive glands indicating a reduced capacity for nutrient uptake. Results from these macroscopic and microscopic investigations also indicate a reduction in, or a lack of, reproductive output compared with reference populations.

KEY WORDS: *Margaritifera margaritifera*, die-offs, mortality, pathology, emaciated

INTRODUCTION

The Swedish fauna of freshwater mussels consists of seven native species (*Margaritifera margaritifera, Unio crassus, Unio pictorum, Unio tumidus, Anodonta anatina, Anodonta cygnea*, and *Pseudanodonta complanata)* and four nonnative species (*Dreissena polymorpha*, *Mytilopsis leucophaeatea, Rangia cuneata*, and *Sinanodonta woodiana;* von Proschwitz et al. 2017). All native species can be found in streams and lakes except for *M. margaritifera* and *U. crassus*, which only occur in streams. According to the Swedish Red List of Threatened Species, the conservation status of *A. anatina, A. cygnea*, and *U. tumidus* is listed as least concerned, *U. pictorum* and *P. complanata* as near threatened, and *U. crassus* and *M. margaritifera* as endangered (EN; Artdatabanken 2018a).

Margaritifera margaritifera has a Holarctic distribution and can be found in North America (common name: eastern pearlshell) and Europe (common name: freshwater pearl mussel [FPM]; Graf and Cummings 2007). Sweden has more than 600 populations of *M. margaritifera* distributed across the country in 16 of 21 counties (Söderberg et al. 2008; Fig. 1). The species is listed as EN because of limited juvenile (individuals <50 mm in length) recruitment and habitat loss (Artdatabanken 2018a). Juvenile recruitment occurs in 50% of

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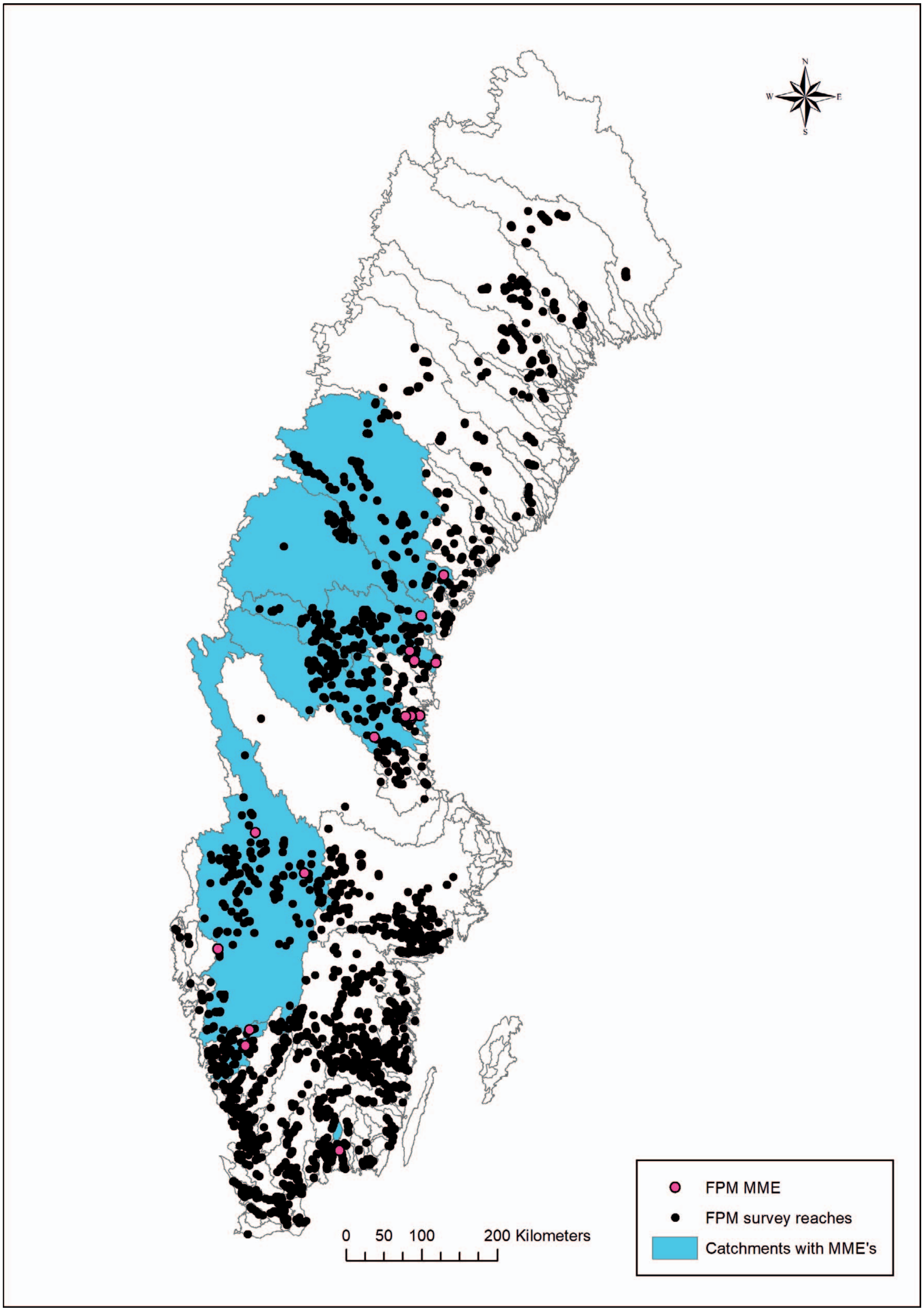

Figure 1. Locations of all freshwater pearl mussel (FPM) survey reaches in Sweden (black dots). Catchments with mass mortality events (MMEs) are indicated in blue and reaches with MMEs are indicated with red dots.

all populations, but only one-third of these are considered viable in terms of recent recruitment and proportion of juveniles (>20% of the population <50 mm in length; Söderberg et al. 2008). Recruitment failure is associated with turbidity and sedimentation of fine substrates and habitat alteration caused by historical use of streams for logging, mills, and hydropower plants (Österling et al. 2010; Degerman et al. 2013). Acidification of streams and lakes became one of Sweden's biggest environmental problems in the late 1960s, exacerbating habitat loss as it not only impacted FPM recruitment but also negatively affected brown trout (*Salmo trutta*), the host fish of FPM (Hesthagen et al. 1999; Taskinen et al. 2011). In recent years, the discovery of mass mortality events (MMEs) in *M. margaritifera* populations due to unknown causes has become a new threat (Fig. 1). Our definition of an MME is a >20% decrease in the number of individuals in a reach (minimum length of 3 m) between two or more surveys. Such mass mortality events in FPM populations have been reported from different countries and often the causes of these die-offs are unclear (Blodgett and Sparks 1987; Strayer et al. 2004; Downing et al. 2010; Southwick and Loftus 2017; Sousa et al. 2018). An MME affects all life stages in a population and can remove a substantial part of a population in a short time (Fey et al. 2015).

In this paper we report the occurrence of MMEs in Swedish populations of *Margaritifera margaritifera* and address possible causes by comparing histological examinations of mussels from four streams with die-offs to mussels from streams without MMEs.

METHODS

Since the 1980s, *Margaritifera margaritifera* populations have been sampled with both quantitative and qualitative methods in regional monitoring programs performed by county administrative boards (CABs). In preliminary surveys, every 20 to 50 m of a stream are searched with an aqua scope for 5 to 10 min. These preliminary surveys are used to determine the presence of mussel beds (densities >1 mussel/m^2). For the quantitative method, 15–18 randomly picked reaches (≤20 m in length) are searched within the known distribution of a population within a stream, and all mussels seen on the bottom are counted. In order to assess the demographic distribution of the population, the lengths of 15 mussels from each reach are measured to the nearest millimeter. For the qualitative surveys, the reach in a stream with the highest density (>1 mussel/m^2) and highest numbers of juveniles is sampled. In streams with low densities and no juvenile recruitment, the reach with the highest number of adults is sampled. A maximum of 100 individuals are collected and measured for length. All collected data are reported to the national database for freshwater mussels (Artdatabanken 2018b). Results of these surveys are compared with data from earlier surveys from the same streams and reaches using data from the national database. In total, 17 streams are included in this study (Table 1).

During recent field surveys (2016–17), *M. margaritifera* ($n = 21$) from four streams were transported alive to the National Veterinary Institute (NVI; Uppsala, Sweden) in boxes with water from the sampling sites; they were kept cold using cooling blocks. We collected mussels from two healthy reference populations (Älgån, Ålakarsbäcken) where no signs of MMEs have been reported and from two populations (Stampebäcken, Lillån) where MMEs have been reported. At the NVI, each animal was measured for length (mm) and wet weight (g) including shell (after emptying the body cavity of stored water). Each mussel was opened by cutting the anterior and posterior adductor muscles with a scalpel and letting out all water left from the mantle cavity. After removing the animal from the shell (by detaching the adductor muscles and mantle) we measured its soft body weight (g). We photographed each mussel using a mobile phone camera (Microsoft Lumia 640 LTE, file type: JPG-format; Redmond, WA USA) and inspected the mantle, gills, foot, and adductor muscles. Ocular characteristics, such as coloration and transparency of mantle, body thickness, condition of foot, coloration of digestive gland, and filling of gonads, were noted.

We examined cross sections of the bodies of these animals for signs of pathological alterations of the gonads or digestive glands. Two cross sections of each animal, including gonad, stomach, digestive gland, mantle, and gill, were collected and fixed in Davidson's freshwater fixative. Sampled organs were processed according to the standard routines for histological sectioning of samples embedded in paraffin blocks (Howard and Smith 1983). Sections of 5 μm were cut and stained with H&E. Stained sections of each sampled specimen were inspected by microscopy using both low power (Olympus SZ binocular stereo zoom microscope; Tokyo, Japan) and high-power magnification (Nikon labophot; Tokyo, Japan). Photos were taken with a Canon EOS 500D attached to a microscope by a lens-adaptor (Martin Microscope Company MM-SLR adaptor S/N:0468; Easley, SC USA).

RESULTS

Between 2006 and 2018, MMEs occurred in 17 streams belonging to nine different catchments from southern and central Sweden (Fig. 1). In five catchments, more than one stream had an MME and in catchment 46/47, all known populations suffered MMEs (Table 1). The mortality between two surveys ranged from 22% to 100%, and in 10 of the streams, the mortality was more than 90% (Table 1). In streams with more than one surveyed reach, MMEs were detected at all reaches in all cases except Örasjöbäcken, where an MME was detected at only one of 21 reaches (Tables 1 and 2). Length measurements indicate that 47% of the streams had recent juvenile recruitment (Table 2).

Table 3 lists ocular characteristics, such as coloration and transparency of mantle, body thickness, activity of foot, coloration of digestive gland, and filling of gonads. We found

Table 1. Streams with mass mortality events (MMEs) in Sweden and their association with catchments, counties, and streams. Mortality is based on the difference in numbers of live Freshwater Pearl Mussel (FPM) individuals between the survey years. The number of reaches per stream is associated with the different monitoring methods used in Sweden.

Catchment ID	No. of FPM Populations in the Catchment	County Code	Stream Name	Group	Year of MME Detection	Survey Years	% Mortality	No. of Reaches
38	43	Y	Tannån	MME	2016	2006, 2016	95	1
40	6	Y	Örasjöbäcken	MME	2013	2015–17	70	1
40	6	Y	Örasjöbäcken	Reference	—	2007, 2015	3	21
42	46	Y	Rännöån	MME	2004	2006, 2016	100	1
42	46	Y	Tälgslättån	MME	2017	2005, 2017	96	1
42/43	2	Y	Galtströmmen	MME	2017	2005, 2017	—*	1
46/47	3	X	Enångersån	MME	2011	2005, 2017	99	15
46/47	3	X	Grottsjöbäcken	MME	2012	2012–17	95	1
46/47	3	X	Grängsjöbäcken/ Tolockbäcken	MME	2012	2012–17	97	1
46/47	3	X	Lövåsbäcken	MME	2012	2012–17	100	1
46/47	3	X	Mjusbäcken	MME	2012	2012–17	100	1
48	57	X	Lillån	MME	2017	2011, 2016	69	15
84	2	K	Husörenbäcken	MME	2017	2005, 2017	97	5
105	15	O	Kovraån/Lillån	MME	2016	2005, 2017	84	1
105	15	O	Viskan	MME	2016	2015–17	93	1
108	52	O	Teåkersälven	MME	2017	2016, 2017	54	18
108	52	S	Stampbäcken	MME	2015	2009, 2015	81	15
108	52	S	Väråns	MME	2013	2014, 2017	22	19

FPM = freshwater pearl mussel; MME = mass mortality event; County Codes: K = Blekinge, O = Västra Götaland, S = Värmland, X = Gävelborg, Y = Västernorrland; * = data deficiency.

anatomical differences between mussels from sites with MMEs (Stampebäcken, Lillån) and those without (Älgån, Ålakarsbäcken). The mussels from healthy populations ($n = 6$) typically had firm, thick bodies, compact feet, and well-developed gonads filled with protruding gametocytes (Fig. 2a, b, e, f, j). In contrast, mussels from populations with MMEs ($n = 15$) were thinner, with more relaxed bodies, and with the foot halfway or fully released. Further, several individuals showed low or no presence of gonads (Fig. 2c, d, h, i, j). In many cases, the digestive glands in healthy mussels were filled with dark green, protruding food (Fig. 2b, g) and the inside of the shells were smooth and iridescent. In comparison, mussels from sites experiencing MMEs had digestive glands that were nearly empty and so they appeared pale (light green to pale brown) and more transparent (Fig. 2d, j). Individuals from one stream with MMEs (Stampbäcken) exhibited multiple small cavities in the mother of pearl layer on the inside of the shell, and one specimen also had small nodules on the otherwise smooth inside shell surface.

The microscopic investigation supported the observed external differences. We found different lesions in digestive glands and other organs in mussels from MMEs. When gonads were sectioned in specimens from a population with MMEs, the gametocytes/oocytes were often absent or low in numbers (Fig. 3g, j). These findings clearly differ when compared with a reference population (Fig. 3a, b, d), where one can clearly see follicles filled with oocytes. Declining populations in areas with MMEs generally showed empty follicles without gametocytes or follicles filled with other cell types. In some cases, hypertrophy, hyperplasia, or enlarged vacuolated epithelial cells could be detected in digestive glands in individuals from the MME population (Fig. 3g, h, k, l). Many digestive gland cells showed no presence of granular or vesicular content in the tubules. In some specimens, cellular infiltration was seen in the connective tissue areas surrounding the tubular digestive glands, expanding the distance between each tubular gland (Fig. 3k, l). In comparison with a normal tubular gland from the reference population (Fig. 3e), a fine band of connective cells separated the tubular glands and there was no cellular infiltration. In the external parts of the foot and body, where connective and muscle fibers dominate, we observed signs of hypertrophied muscular fibers or cellular swelling, as well as increased cellular infiltration, in some of the mussels from MMEs (Fig. 3i) compared with reference mussels (Fig. 3c, f).

DISCUSSION

Mass mortality events of freshwater mussel populations in Sweden have been reported only in *M. margaritifera* populations, and these have occurred only in southern and

Table 2. Streams with mass mortality events and measurements of percentage of juveniles; average, minimum, and maximum lengths; and number of dead and live mussels for each survey year.

Stream Name	Survey Year	% Juveniles	Average Length (mm)	Minimum Length (mm)	Maximum Length (mm)	No. of Dead Mussels	No. of Live Mussels
Tannån	2006	0	97	74	114	4	42
Tannån	2016	0	106	98	114	31	2
Örasjöbäcken*	2015	0	99	71	123	93	253
Örasjöbäcken*	2016	0	100	68	118	111	142
Örasjöbäcken*	2017	0	100	16	114	35	107
Örasjöbäcken*	2018	0	—†	—	—	60	58
Örasjöbäcken – reference	2007	4	81	30	111	11	377
Örasjöbäcken – reference	2015	34	67	18	110	16	417
Rännöån	2006	1	99	49	120	6	129
Rännöån	2016	0	—	—	—	6	0
Tälgslättån	2005	0	104	69	142	1	89
Tälgslättån	2017	0	96	92	112	29	4
Galtströmmen	2005	12	91	15	154	0	177
Galtströmmen	2017	3	96	33	144	10	431
Enångersån	2005	8	87	23	126	—	628
Enångersån	2017	—	—	—	—	—	3
Grottsjöbäcken	2012	20	—	34	—	69	20
Grottsjöbäcken	2017	0	—	—	—	0	2
Grängsjöbäcken	2012	—	—	—	—	26	33
Grängsjöbäcken	2013	—	—	—	—	34	3
Grängsjöbäcken	2014	—	—	—	—	18	2
Grängsjöbäcken	2015	—	—	—	—	10	0
Grängsjöbäcken	2016	—	—	—	—	0	0
Grängsjöbäcken	2017	—	—	—	—	0	1
Lövåsbäcken	2012	—	—	—	—	169	54
Lövåsbäcken	2014	—	—	—	—	36	0
Lövåsbäcken	2017	—	—	—	—	0	0
Mjusbäcken	2012	—	—	—	—	2	217
Mjusbäcken	2013	—	—	—	—	91	100
Mjusbäcken	2014	—	—	—	—	186	6
Mjusbäcken	2015	—	—	—	—	150	0
Mjusbäcken	2017	—	—	—	—	0	0
Lillån	2011	2	89	30	120	93	5,987
Lillån	2016	—	—	—	—	0	1,856
Husörenbäcken	2005	87	45	38	51	21	20
Husörenbäcken	2017	—	—	—	—	0	1
Kovraån/Lillån	2005	—	—	—	—	17	365
Kovraån/Lillån	2016	—	—	—	—	106	59
Viskan	2015	—	—	—	—	70	350
Viskan	2016	—	—	—	—	300	29
Viskan	2017	—	—	—	—	300	5
Teåkersälven	2016	0	—	—	—	—	553
Teåkersälven	2017	0	—	—	—	—	407
Stampbäcken	2009	0	68	54	80	—	5,314
Stampbäcken	2015	3	63	42	70	—	1,012
Väråån	2014	0	94	56	117	27	1,642
Väråån	2017	0	96	70	120	—	1,279

*Measurements taken from dead mussels.

†Dash indicates data deficiency.

Table 3. Biological parameters and macroscopic findings from examination of freshwater pearl mussels (*Margaritifera margaritifera*) from two unaffected streams (ref.) and two streams with mass mortality events.

Stream Name	Survey Year	Date	ID	Length (cm)	Weight (g) Whole Specmen	Weight (g) Soft Body	Gonads	Foot	Digestive Gland	Shell Inside
County of Värmland										
Älgån (ref.)	2017	November 1	5436	10.2	60.3	17.6	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Ålgån (ref.)	2017	November 1	5437	9.2	50.5	14.0	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Ålgån (ref.)	2017	November 1	5438	8.3	33.7	11.6	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Ålgån (ref.)	2017	November 1	5439	8.4	34.4	10.6	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Stampe-bäcken	2017	November 1	5440	6.3	12.6	3.4	Not detected	Relaxed,* fully released	Pale brown, no content	Small cavities, iridescent
Stampe-bäcken	2017	November 1	5441	7.0	24.1	4.5	Not detected	Compact, halfway released	Pale green, semitransparent, no content	Small cavities, iridescent
Stampe-bäcken	2017	November 1	5442	7.2	26.6	7.8	Small, semi-protruding	Compact, halfway released	Dark green, protruding content	Smooth, iridescent
Stampe-bäcken	2017	November 1	5443	6.9	20.4	5.3	Large, protruding	Compact, halfway released	Medium green, no content	Rough with nodules and small cavities
Stampe-bäcken	2017	November 1	5444	7.0	23.5	7.3	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Stampe-bäcken	2017	November 1	5445	7.1	26.5	6.4	Not detected	Relaxed, fully released	Pale green, no content	Small no. of cavities, iridescent
Stampe-bäcken	2017	November 1	5446	7.2	24.6	6.2	Not detected	Relaxed, fully released	Pale green, no content	Small no. of cavities, iridescent
County of Gävleborg										
Alakars-bäcken (ref.)	2016	June 15	1826	—†	132.4	45.2	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Alakars-bäcken (ref.)	2016	June 15	1827	—	153.3	41.7	Large, protruding	Compact, withdrawn	Dark green, protruding content	Smooth, iridescent
Lillån	2016	June 15	1818	11.1	109.1	30.7	Not detected	Relaxed, fully released	Pale brown, no content	Smooth, iridescent
Lillån	2016	June 15	1819	8.6	40.6	9.7	Not detected	Relaxed, fully released	Pale green, no content	Smooth, iridescent
Lillån	2016	June 15	1820	10.0	74.9	20.2	Not detected	Relaxed, fully released	Pale green, no content	Smooth, iridescent
Lillån	2016	June 15	1821	10.5	70.8	22.2	Not detected	Relaxed, fully released	Pale brown, no content	Smooth, iridescent
Lillån	2016	June 15	1822	10.8	81.7	23.7	Not detected	Relaxed, fully released	Pale brown, no content	Smooth, iridescent
Lillån	2016	June 15	1823	9.4	66.1	15.8	Not detected	Relaxed, fully released	Pale green, no content	Smooth, iridescent
Lillån	2016	June 15	1824	8.8	47.2	13.5	Not detected	Relaxed, fully released	Pale green, protruding content	Smooth, iridescent
Lillån	2016	June 15	1825	11.1	132.4	45.2	Not detected	Relaxed, fully released	Pale brown, no content	Smooth, iridescent

*This specimen died shortly before examination.
†Dash indicates data deficiency.

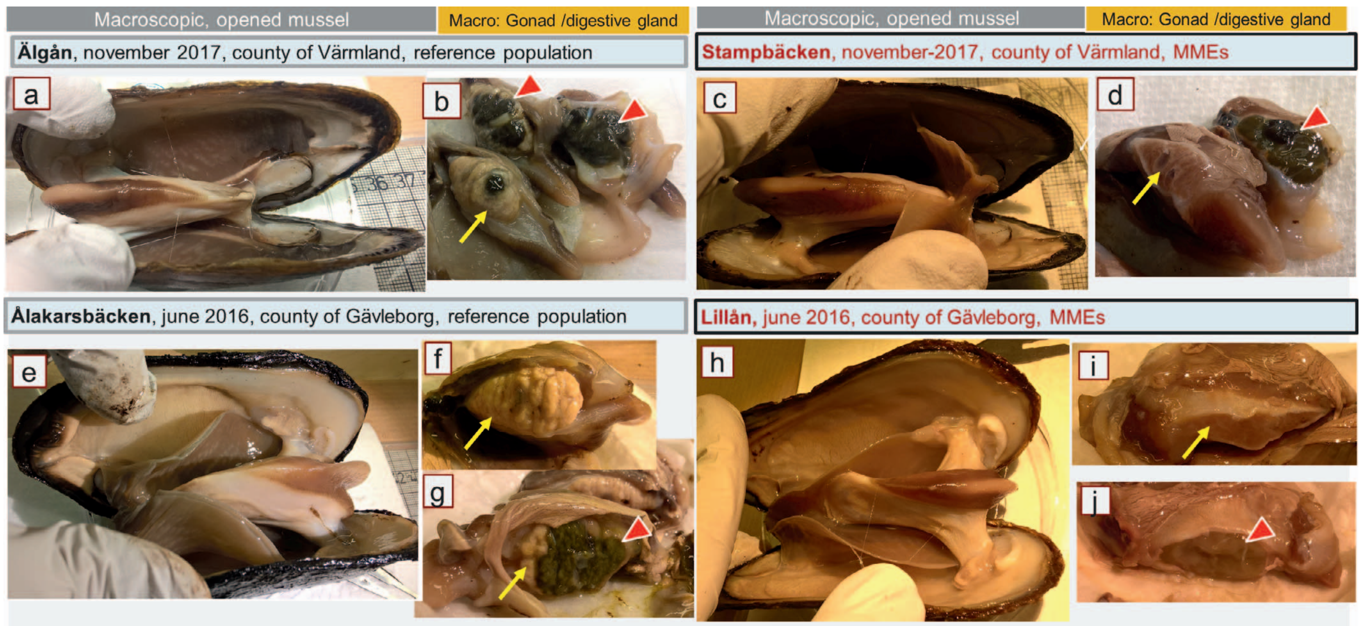

Figure 2. Macroscopic observation of *Margaritifera margaritifera* from reference populations (Ålgån, Ålakarsbäcken; left) and those from populations experiencing mass mortality events (Stampebäcken, Lillån; right). Differences between gonads are highlighted with yellow arrows, while differences between digestive glands are highlighted with red arrow heads.

central Sweden. As in the USA, the causes of MMEs in Sweden are uncertain (Downing et al. 2010). The geographic pattern in Sweden is unclear and warrants further investigation. Mass mortality events have not been detected in Västerbotten and Norrbotten, the two most northern counties in Sweden, but it is possible they have been missed, as reaches are investigated only every 6 yr. That was the case with Örasjöbäcken, where investigators found an isolated MME in 2013 while walking between two monitoring reaches. Between 2007 and 2015, the number of live animals increased by 10% at the 21 survey reaches in Örasjöbäcken and the proportion of juveniles increased from 4% to 34% (Table 2). In the isolated reach, the mortality was 70% between 2013 and 2017. This isolated MME needs further investigation.

In this study, we found some novel results regarding differences in histopathological structure in mussels from sites with and without MMEs. Individuals from MME sites seemed to be emaciated and may have lost the capacity to use and digest energy resources. This may be explained by alteration or damage to the epithelial cells of the digestive glands. The observed lesions in digestive glands and other organs are likely to adversely impact digestion and assimilation of food, thus indirectly affecting reproduction by decreasing energy resources and storage of ions and metabolites necessary for production of offspring. We also observed the absence, or decreased volumes, of gonads, findings that indicate a negative impact on the reproductive capacity of these mussels. The causes of our findings are still unresolved. Infections transmitted from vectors or conspecifics have been documented to affect reproductive capacity in other mussel species via direct or indirect castration (Rice et al. 2006; Lafferty and Kuris 2009), destruction of the gonads by intracellular infections of oocytes (Ngo et al. 2003), or targeting of epithelial cells that are important for food absorption and uptake of nutrients (Villalba et al. 1993). In the latter study, *Marteilia refringens* infections reported in the Mediterranean mussel (*Mytilus galloprovincialis*) were significantly linked to gonad development. Adipogranular (ADG) cells, which store energy and promote the initiation of gonad development, were investigated in mussels from farming areas. A negative correlation was found between the degree of parasitic infection and the abundance of ADG cells in the mantles of these mussels. The conclusion was that *Marteilia refringens* infection was clearly associated with the inhibition of ADG cells and also the development of the gonads in both males and females (Villalba et al. 1993). Whether or not this could account for the observed pattern in our study is unclear as no obvious infectious diseases or parasites were detected.

The qualitative survey method examines only one reach in a stream so it is impossible to state whether the whole stream is affected or just a part of the stream. In some of the streams, it is also difficult to say when the start of the MME occurred because of the 6-yr monitoring interval and the fact that monitoring started in only the mid-1990s. In all streams with more than one surveyed reach, all reaches were affected except for Örasjöbäcken. This finding suggests that MMEs tend to occur throughout a given stream rather than being limited to single reaches. Thus, causes are likely to be characteristic of a stream. The five most common causes of MMEs identified in

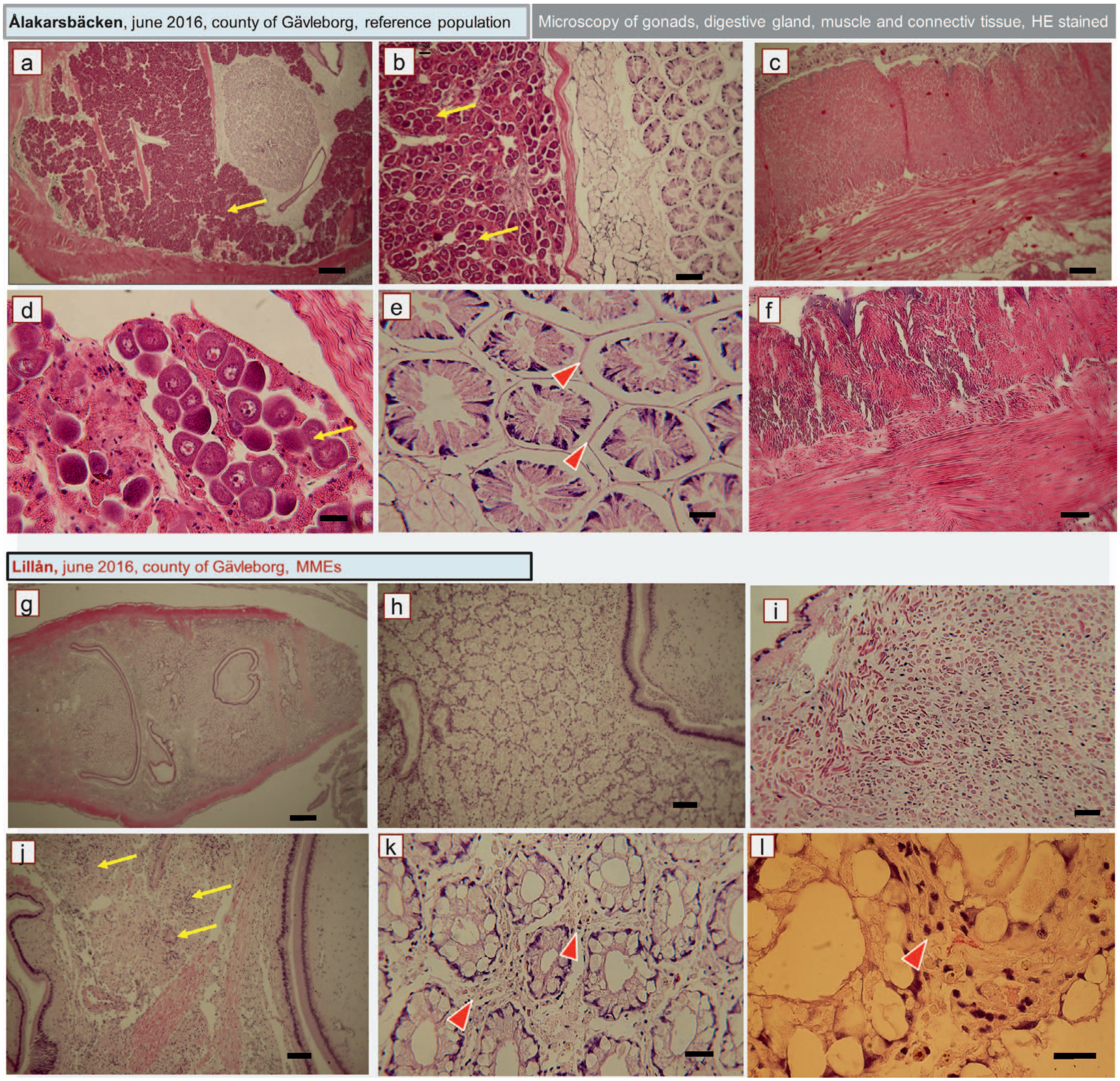

Figure 3. Histopathology of fixed cross sections of *Margaritifera margaritifera*. Differences in morphology and the presence of lesions were detected when comparing reference individuals (a–f) with those collected from sites experiencing mass mortality events (MMEs; g–l). Tissue sections were stained with H&E. Scale bars are as follows: a,g = 1,000 μm, b,c,h,i,j = 100 μm, d,e,f,k = 40 μμm, l = 20 μm. Cellular infiltration between the digestive glands is highlighted by red arrowheads (k,l). This infiltration is absent in specimens from the reference population (e). Normal gonads are highlighted with yellow arrows (a,b,d), but were not detected in many mussels from sites experiencing MMEs (j). The external layer of the foot had thick bundles of muscle fibers that were tightly packed in the reference mussels (c,f) but hypertrophied in many of the MME individuals (i).

the USA are pollution, habitat destruction, hydrologic change, presences of dams, and lack of host fishes (Downing et al. 2010). Populations dominated by single, old cohorts of mussels might suffer MMEs if these cohorts die of natural causes. Old and senescing FPM populations are common in Sweden. However, our length data suggest that multiple cohorts are affected, and the histological analyses indicate other reasons besides age-related death. At this point, we are unable to determine what factors account for MMEs in Swedish streams.

During the last decade, mortality of up to 100% in Swedish populations has been reported but the possible causes of MMEs in *M. margaritifera* populations are still unresolved. However, we detected lesions in digestive glands indicating a reduced capacity for uptake of nutrients, and both macroscopic and microscopic investigations indicated reduced reproductive capacity compared with reference populations. Clearly, more investigations are needed to determine the causes of declines in Sweden's mussel populations.

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