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Test of the host fish species of a unionoid mussel: A comparison between natural and artificial encystment☆

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ABSTRACT

Many of the unionoid mussel species are threatened, and to be able to develop strategies for effective conservation, one of the needs is to distinguish host fish species from non-host fish species using reliable methods. *Margaritifera margaritifera* lives as a parasite on brown trout (*Salmo trutta*) and/or Atlantic salmon (*Salmo salar*). The aim was to compare the reliability of two methods measuring the host specificity of *M. margaritifera* in two rivers that flow out into Skagerrak in the Atlantic Ocean. A second aim was to compare the time- and cost-efficiency of the two methods. The methods were (1) natural encystment abundances on fish in their native streams using electrofishing, and (2) encystment abundances from controlled artificial infestation in aquaria, on fish that were sacrificed. In both rivers, young-of-the-year (YOY), but not older brown trout, were naturally infested with relatively low loads of glochidia larvae, while the Atlantic salmon was not infested at all. When using artificial infestation, both YOY and older brown had encysted glochidia larvae on their gills, while glochidia larvae were not able to develop in Atlantic salmon at all. Here, the encystment was higher on the brown trout from the Lärje River, and older brown trout from the Lärje River did not seem to have as strong immunity response compared to older brown trout from the Brattefors River. In summary, brown trout is the only host fish for *M. margaritifera* in these rivers. Both methods can be used to discriminate between host fish species, but the method measuring natural encystment seems most time- and cost-efficient. In addition, natural encystment can be measured using a non-destructive photo-method, and is therefore suggested to be used when discriminating between host fish species for *M. margaritifera*.

Introduction

The unionoid mussels have an obligate parasitic stage on one or more host fish species (Strayer et al., 2004). A unionoid mussel species may be adapted to different fish species in different streams (Hastie and Young, 2001). As many of these mussel species are threatened, the parasitic stage may be the life stage where the mussel reproduction fails (Strayer et al., 2004). To restore threatened unionoid mussel populations, restoration measures can be directed towards the host fish species (Palm et al., 2007, 2010). Reliable, non-destructive methods to distinguish host fish species from non-host fish species are therefore needed.

One method to investigate the suitability of potential host fish is to infest the fish artificially with mussel larvae (Österling and Larsen, 2013). When using this method, the ripe mussel larvae are mixed with fish, and the number of juvenile mussels that hatches from each potential host fish species is counted. Alternatively, the fish is checked for encysted mussel larvae at a point in time when it is known that the fish will be a functional host for the mussel (Bauer and Vogel, 1987; Bauer, 1987a,b; Taeubert et al., 2010; Österling and Larsen, 2013). Another way to discriminate among potential host fish species is to investigate the encystment rates from fish that were naturally infested in the field (Österling et al., 2008)

The freshwater pearl mussel (*Margaritifera margaritifera*) is a threatened unionoid mussel (Endangered, EN, in the IUCN Red List), and lives as a parasite on brown trout (*Salmo trutta*) and/or Atlantic salmon (*Salmo salar*) for 10–12 months (Young and Williams, 1983, 1984; Bauer, 1987b,c; Hastie and Young, 2001, 2003). To be able to address strategies for effective conservation, there is a need to know if brown trout and/or Atlantic salmon are a functional host fish. This may be especially important as many of these fish populations are threatened themselves (Klemetsen et al., 2003; Griffiths et al., 2010; Österling et al., 2010; Arvidsson et al., 2012; Österling and Högberg, 2014) Therefore, it is also important to use non-destructive methods, such as photo-methods, when investigating potential host fish

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species (Österling, 2011). Time- and cost-efficient methods are also welcomed, since the cost often regulates how many fish individuals/populations that can be investigated.

Investigations of encystment load using artificial infestation on brown trout have been shown to be a reliable method (Young et al., 1987; Taeubert et al., 2010; Österling and Larsen, 2013). The method may however be destructive, as the fishes often have to be sacrificed, since it for example may not allowed to move fish from the lab back into the river. In contrast, electrofishing of naturally infested host fish is a method worth investigating, since some host fish individuals always seem to have encysted mussel larvae in *M. margaritifera* streams (Österling et al., 2008). This method also appears to be cost-efficient, since fish need to be monitored for a shorter time (days) than infestation in the lab (months). If the electrofishing method is shown to be a reliable measurement of encystment rate, it can be combined with the non-destructive photo-method, which estimates encystment without killing the fish (Österling, 2011). Then it should be possible to discriminate between host fish species without using destructive methods at all.

The aim was to compare the reliability of two methods of measuring the host specificity of *M. margaritifera* (i.e. to investigate the encystment of mussel larvae on *S. trutta* and *S. salar*) in two streams that flows out into Skagerrak in the Atlantic Ocean. A second aim was to compare the time- and cost-efficiency of the two methods. The methods were (1) encystment abundances from artificial infestation, and (2) natural encystment abundances on fish in their native streams using electrofishing.

Materials and methods

Electrofishing

Electrofishing was performed on 10 October and 14 October 2010 in the Brattefors River and in the Lärje River, respectively, at one site in each river, to investigate the natural encystment rates in the field (see Tables 1 and 2). Captured fish were anaesthetized with tricain methane-sulphonate (MS222), whereupon length and weight were measured. The brown trout and Atlantic salmon were preserved in 4% formaldehyde for later investigation of encysted glochidia on the gills. In the laboratory, the gills were removed from the fish using a razor blade, and the number of glochidia was counted under a microscope.

Artificial infestation

Ten gravid mussels were collected at sites that were electrofished (see below) from the Brattefors River and from the Lärje River, at 12 August 2010. Gravid mussels were investigated by careful opening using tongs (Bauer, 1987a; Hastie and Young, 2003; Österling et al., 2008). The mussels were then transported to Karlstad University, and were acclimatized at 16 °C. Brown trout and Atlantic salmon (young-of-the-year, YOY and one year old fish, OLD) were collected by electrofishing (petrol-driven electroshocker, LUGAB) in these rivers at the 12 and 14 August 2010 (Tables 1 and 2). The fish were transported to Karlstad University in aerated coolers, and were acclimatized at 16 °C in the aquaria facility at Karlstad University. The fish were visually examined for mussel encystment, but no larvae were found.

Inspection of released glochidia larvae was performed each day using microscope. At 20 August and 23 August, high densities of snapping glochidia larvae from the Brattefors River and from mussels from the Lärje River, respectively, were found suspended in the water. The experiment was initiated by placing each of the groups YOY and OLD brown trout and YOY and OLD Atlantic Salmon from the Brattefors River and the Lärje River respectively, in three separate containers (two containers for the four individuals for the OLD Atlantic Salmon), with water containing a concentration of 30 000 larvae l^{-1} for 30 min. The infestation was terminated by the movement of each year class group of brown trout and Atlantic salmon to three 100 l aquaria each (two to six individuals each; eight groups, see Tables 1 and 2). For the treatment "OLD Atlantic Salmon" the number of fish individuals was four, and two aquaria were therefore used. The water was changed once a week and filters (EHEIM 2217) were cleaning the water. The water temperature was 18.4 °C at the start of the experiment, and 13.1 °C when the experiment was terminated at 14 October. The light regime was 11 h light–11 h darkness, and 1 h dusk and 1 h dawn each day. The fish were fed with chironomid larvae 3 times each week.

The experiment was terminated at 12 October (the Brattefors River) and 14 October (the Lärje River), when four to eighteen brown trout and Atlantic salmon individuals the YOY and OLD brown trout were collected (see Tables 1 and 2). The length and weight of the fish were measured, and the fish were stored in formaldehyde for 4 weeks. The fish were then put in flow through tap water. The first (outer) gill arch on the left and the right sides were removed with a razor blade, whereupon the numbers of encapsulated larvae were counted under a microscope.

The results are reported as mean ± 1 SD. The data were non-normally distributed (Shapiro–Wilk test). The data were log-transformed, and were analyzed with Mann Whitney *U*-tests.

Results

The Brattefors River

Twenty-three brown trout and seventeen Atlantic salmon individuals were captured in the Brattefors River and investigated for natural glochidial encystment. Only YOY brown trout had larvae encysted on their gills, while OLD brown trout and Atlantic salmon did not. The encysted larvae among YOY brown trout ranged from zero to over 500 larvae per brown trout individual, and five of nineteen YOY individuals had larvae encysted on their gills (Table 1).

The aquaria experiment showed similar results, with the exception that OLD brown trout also had larvae encysted on their gills. A higher proportion of YOY than of OLD brown trout had larvae encysted on their gills. The encystment abundance (Mann Whitney *U*-test, $p = 0.02$) and the mass normalized encystment abundance (Mann Whitney *U*-test, $p = 0.003$) were higher for YOY than for OLD brown trout. The encystment loads were generally higher on the brown trout from the aquaria experiment than on the naturally infested brown trout. In the aquaria experiment, no individual of the Atlantic salmon had larvae encysted on their gills (Table 1).

The Lärje River

The results were similar for the naturally infested fish from the Lärje River, as the brown trout had glochidia larvae encysted on their gills. Likewise, no Atlantic salmon individual had larvae encysted on their gills. The percentage of brown trout from the field that had larvae encysted on their gills was 43%, which was higher than brown trout from the Brattefors River. However, the mean encystment abundance was higher in the Brattefors River than in the Lärje River (Table 2).

The aquaria experiment showed similar results as the naturally infested fish in the Lärje River with the exception that OLD brown trout also had larvae encysted on their gills, while the Atlantic salmon had no encystment at all. There was a relatively large variation in encystment, ranging from zero to nearly three-thousand larvae on one OLD brown trout. The encystment