Alpine bullhead (Cottus poecilopus Heckel): a potential refuge for Gyrodactylus salaris Malmberg, 1957 (Monogenea)

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Abstract: The notifiable freshwater pathogen Gyrodactylus salaris Malmberg, 1957 tends to be a generalist in contrast to other monogeneans. Whilst it causes most damage to its primary host, the Atlantic salmon (Salmo salar Linnaeus), transport and reservoir hosts likely play a key role in maintaining the parasite in the environment. Here, we tested the ability of G. salaris (strain River Lierelva, southern Norway) to infect and reproduce on a population of wild caught alpine bullhead (Cottus poecilopus Heckel). Exposure of alpine bullhead yearlings (0+) to G. salaris for 24 h at low (6.5 °C) or high temperature (11.5 °C) resulted in the establishment of 1 to 104 parasites per fish. Eight to nine days post-infection at high temperature, the infection of G. salaris was eliminated, indicative of innate host immunity. In contrast, at low temperature G. salaris infections persisted for 47–48 days. The relative lengthy infection of alpine bullhead with G. salaris compared to other non-salmonids tested may be due to low temperature and high initial infection load in combination with an epibiont infection. The present results suggest that this non-salmonid may function as a temperature-dependent transport or reservoir host for G. salaris.

Keywords: Gyrodactylidae, Siberian bullhead, Atlantic salmon, reservoir host, experimental infection, Norway

Successful establishment of invasive species in new regions is largely determined by the ability to find suitable resources to fulfill their habitat requirements (Torchin et al. 2003). For parasite species, invasion success is dependent on the presence of suitable hosts to maintain all life cycle stages, whether in the form of native species, or previously or co-introduced invasive hosts (i.e., parasite host switch; Bakke et al. 1992, 2002, Dunn 2009, Pettersen et al. 2016). In the case of parasites with direct life cycles such as monogeneans, invasion success is largely dependent on the presence of a suitable fish host (Bakke et al. 2007).

The invasive monogenean Gyrodactylus salaris Malmberg, 1957 was first detected in Norway in 1975 on a population of Atlantic salmon (Salmo salar Linnaeus) in Lakselva, Mivaer, Nordland County (Johnsen 1978). However, subsequent surveys revealed a widespread distribution of G. salaris (see Heggberget and Johnsen 1982, Bakke et al. 2007, Høgåsen et al. 2016). The invasive monogenean appeared highly pathogenic to the juvenile Atlantic salmon populations, with average losses of 86% (Johnsen et al. 1999).

Gyrodactylus salaris was also found to infect and reproduce on other salmonids, such as Arctic char (Salvelinus alpinus [Linnaeus]), brook trout (Salvelinus fontinalis [Mitchill]), brown trout (Salmo trutta Linnaeus), rainbow trout (Oncorhynchus mykiss [Walbaum]), grayling (Thymallus thymallus [Linnaeus]), and salmon-brown trout hybrids (Bakke et al. 1992, 1999, 2002, Paladini et al. 2014). Amongst these alternative hosts, Arctic char turned out to play a most significant role in the epidemics and dissemination of G. salaris (e.g., Bakke et al. 1996, Paladini et al. 2014).

Non-salmonid species may also have a role in the dissemination of G. salaris as transport or reservoir hosts (Bakke et al. 1992, 2002). However, in the eight non-salmonid species experimentally tested, namely three-spined stickleback (Gasterosteus aculeatus Linnaeus), nine-spined stickleback (Pungitius pungitius [Linnaeus]), perch (Perca fluviatilis Linnaeus), roach (Rutilus rutilus Linnaeus), minnow (Phoxinus phoxinus [Linnaeus]), eel (Anguilla anguilla [Linnaeus]), flounder (Platichthys flesus [Linnaeus]), brook lamprey (Lamproptera planeri [Bloch]), only a maximum infection for seven days (mean 4.1 days) was observed (reviewed by Bakke et al. 2007), similar to the maximum life span of G. salaris on dead salmonids (Olstad et al. 2006).

Temperature and climatic change may influence the dynamics between hosts and invasive monogeneans in freshwater ecosystems through various mediated mechanisms.

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including reproduction and survival (e.g., Jansen and Bakke 1991, Bakke et al. 2007), transmission (Soleng et al. 1999, Marcogliese 2008) and infection seasonality (Jansen and Bakke 1993a, b). All of them are largely mediated by host immune responses (Sakai 1992, Muiswinkel and Der Wal 2006, Brooks and Hoberg 2007).

The fact that low environmental temperature can be immunosuppressive (Bly and Clem 1992) is suggestive of a thermal refuge for gyrodactylids poorly adapted to their hosts, allowing them to persist for sufficient time to further disseminate or allow genetic adaptation (Bakke et al. 2007). Gyrodactylus salaris, which is genetically highly variable throughout its range (Hansen et al. 2007, Mieszkowska et al. 2018), could also have been subject to substantial selection pressure based on host species or where it extends across different climatic zones (Dobson and Carpenter 1992, Olstad et al. 2007).

Gyrodactylids were previously recorded on 98% of alpine bullhead co-occurring with Atlantic salmon in Signaldalselva (Trøms County, North Norway), at a mean intensity of 6.1 (Knudsen et al. 2004), although this probably represented a mixed infection of Gyrodactylus mariannae Winger, Hansen, Bachmann et Bakke, and G. salaris (see Winger et al. 2008). The lithophilous alpine bullhead, living in running water and also the littoral zone of lakes, has not previously been experimentally assessed for susceptibility to G. salaris (see Bakke et al. 2002). Hence, the current study assesses the ability of alpine bullhead to act as an alternative host for G. salaris under experimental conditions when subject to environmental stress based on temperature and a concurrent epizoic infection.

**MATERIAL AND METHODS**

**Host and parasite origin and maintenance**

Alpine bullhead (Cottus poecilopus) (ca. 50) young-of-the-year (0+) were collected by electrofishing on 14 December 2001 from the River Nitelva (60°13’47”N; 10°30’44”E), Oppland County, Norway and transported live to the Natural History Museum’s Aquarium Unit, University of Oslo. The status of infection with Gyrodactylus salaris was examined by screening all fish surfaces (fins, skin, mouth cavity, gills) from a random subsample of ten alpine bullhead euthanised by a blow to their head. No infection with Gyrodactylus spp. was detected. This is consistent with the alpine bullhead (n = 87) surveyed in 2002/2003 from the same river (Winger et al. 2008). Prior to the experiment, alpine bullhead were maintained under continuous dim illumination at 11 ± 0.5 °C in one grey, plastic tank (100 × 100 cm, 30 cm water depth) supplied with charcoal-filtered, dechlorinated tap water and fed with unmedicated pellet food (Ewos Cermaq ASA, Oslo, Norway) ad libitum.

The strain of G. salaris (haplotype F; Hansen et al. 2003) was obtained from infected Atlantic salmon parr caught by electrofishing in Glitra, a tributary of the River Lierelva, Buskerud County (59°50’3”N; 10°14’49”E). This strain of G. salaris was thereafter transferred to a laboratory stock of naïve River Lierelva salmon parr exposed to infected salmon fins to increase the average infection level of G. salaris before the start of the experiments.

**Experimental design**

Twenty-seven naïve alpine bullhead were randomly assigned to two temperature treatments (6.5 ± 0.5 °C and 11.5 ± 0.3 °C) and acclimatised to these conditions under continuous dim illumination seven days before the infections. Due to space restrictions, the experiments were conducted over two time periods (low temperature: 27 February – 16 April; high temperature: 24 April – 4 May); all laboratory conditions except for water temperature were kept constant. Each alpine bullhead was measured (average with range in parentheses: fork length, FL, mm; weight, W, g) at the start of the experiments low temperature (n = 18): FL 42.1 (34–47), W 0.68 (0.33–0.99); high temperature (n = 9): FL 44.4 (42–50), W 0.70 (0.60–0.95).

The infections with G. salaris were established by exposing alpine bullhead to fins of a heavily G. salaris infected salmon

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**Fig. 1.** Effect of temperature on infection with Gyrodactylus salaris Malmberg, 1957 on alpine bullhead, Cottus poecilopus Heckel, from the River Nitelva, southeastern Norway. **A** – mean infection intensity and duration; **B** – the relationship between initial infection load (Day 0) and infection duration. Open circles = low temperature (6.5 ± 0.5 °C; n = 18); closed circles = high temperature (11.5 ± 0.3 °C; n = 9); vertical bars and values = range (minimum, maximum) and prevalence (%).
(killed by a blow to its head) in plastic boxes (37 × 28 cm, 8 cm water depth) for 24 hours at the two temperatures. The alpine bullhead were then individually isolated into one of three compartments in floating plastic boxes (bottom wire mesh 5 × 5 mm) placed in a grey, plastic tank (100 × 100 cm, 30 cm water depth). Gyrodactylus transmission between the compartments has previously never been observed in numerous similar experiments over two decades (see Bakke et al., 2002).

Changes in infections with G. salaris on the skin, fins and mouth cavity over time were quantified by use of a dissecting microscope every second day post-exposure by anaesthetising each bullhead in a 0.04% chlorobutanol (trichloro-2-methyl-2-propanol) solution immediately prior to examination in accordance with previous experiments at different temperatures on non-salmonids (Jansen and Bakke, 1991, Bakke et al. 2002). The gill arches were not examined to avoid physical damage to live fish. Each alpine bullhead was kept in their respective compartments until no infection with G. salaris was detected following two examinations. No fish mortality occurred. At low temperature, a co-occurring infection with epibionts (peritrichid ciliates) became apparent when the experiments started but was not quantified.

Statistical analyses

All analyses were conducted in R version 3.4.2 (R Development Core Team 2017). The effect of temperature on the duration of infection with G. salaris on alpine bullhead was examined using a generalised linear model (GLM) fitted with a quasipoisson distribution to account for over-dispersed count data. Preliminary data exploration indicated that mean infection intensity at Day 0 differed between temperature treatments. Therefore, infection intensity at Day 0 and fork length were included as covariates in our model, with both continuous measures scaled and centered on the mean before analysis.

RESULTS

Initial infection differed between the temperature treatments (GLM$_{\text{TEMP}}$: $F_{1,25} = 46.16$, $p < 0.001$), with alpine bullhead acquiring on average 71.3 and 7.7 specimens of Gyrodactylus salaris at low and high temperatures, respectively (Fig. 1A). Fish in both temperature treatments demonstrated a general pattern of declining infection intensity and prevalence with time (Fig. 1B). A dramatic decline in parasite numbers occurred in the first two days post-infection in both treatment groups, but thereafter the decline levelled out. Maximum duration of infection differed between the temperature treatments (GLM$_{\text{TEMP}}$: $F_{1,25} = 12.39$, $p = 0.002$: 47–48 and 8–9 days for low and high temperature, respectively) and was also influenced by the initial infection (GLM$_{\text{DAY}}$: $F_{1,28} = 4.67$, $p = 0.044$; Fig. 1B). At low temperature, one fish still harboured 15 parasites at Day 21, but no parasites remained by Day 48 (Fig. 2). At high temperature only one fish remained infected at Day 7 and even this fish had lost all parasites by Day 8–9. The heaviest infected fish (39 G. salaris) at high temperature had eliminated the infection between Day 3 and 4 post-infection.

The overall parasite burden also declined on the low temperature fish, but there was evidence of an increased intensity of infection on four alpine bullheads (increasing from 4 to 9, 14 to 19, 17 to 19 and from 16 to 20) (Fig. 2).

DISCUSSION

The maximum duration of infection with Gyrodactylus salaris recorded on alpine bullhead in this study represents the longest continuous infection with this monogenean observed on a non-salmonid host. Previous studies suggested maximum persistence of one week on other non-salmonid species (Bakke et al. 2002). Jansen and Bakke (1991) experimentally observed a negative relationship between water temperature and the maximum lifespan of individual G. salaris on salmon parr, increasing from 8 days at 19°C to a peak of 58 days at 6.5°C, with the life span declining when approaching 0°C.

Whilst the maximum lifespan of 48 days on alpine bullhead at 6.5°C observed in the current study is less than the lifespan on salmon parr, it is significantly greater than that observed on any non-salmonid host. This prolonged infection on alpine bullhead may be due to the persistence of an individual parasite on the skin, migration from potentially infected gills, or even parasite reproduction at this temperature. If the latter, even though G. salaris is known to have a wider host range than most other gyrodactylids (Bakke et al. 1992, 2002), this study represents the first crossing of the parasite-host family border: salmonid to non-salmonid, providing an increased opportunity for speciation (Ziętara and Lumme 2002).

Alpine bullhead share the same microenvironment (shelter between gravel and stones, depth and river velocity) as...
salmon fry and parr, resulting in competition for both space and food. In fact, alpine bullhead may be the dominant of these two species, especially in high density populations (Gabler and Amundsen 1999, Svemning 2000) as also observed for Cottus gobio Linnaeus (see Gabler et al. 2001). The sympatry and niche overlap with salmon provides ideal conditions for G. salaris transmission and host switching (see Bakke et al. 2007).

In accordance with previous studies on other non-salmonid species experimentally infected with the haplotype F of G. salaris, once infected the population of G. salaris on alpine bullhead declined to elimination. The maximum average life span of individual G. salaris when feeding on its natural salmon host, or indeed when detached from a host, is negatively correlated with water temperature (Olstad et al. 2006). Environmental temperature not only impacts survival but also affects most other gyrodactylid functions (e.g., Gelnar 1991, Mohammed et al. 2016) and in the host both the alternative and classical complement pathway activities can be depressed during cold periods (Hayman et al. 1992, Collazos et al. 1994, Harris et al. 1998). In northern parts of Norway, river temperature is around 0°C during winter (see The Norwegian Water Resources and Energy: Directorate; https://www2.nve.no/h/hd/plotreal/WT/index.html). Such low temperatures may provide a refuge on non-salmonid hosts for G. salaris normally poorly adapted to these fish.

The two related sessile peritrich species, Vorticella sp. and Epistylis sp., which were observed in the current study attached by their stalks to the alpine bullhead skin at low temperature, are common ectocommensals on bottom dwelling fish and potential stressors. In general, fish ciliates tend to be pathogenic causing losses under aquaculture conditions (Lom and Dyková 1992) and red-sore disease epizootics caused by Epistylis sp. are considered to be most common during winter and spring months (Rogers 1971). Stress-induced immunosuppression of salmonids leading to increased susceptibility has previously been reported in salmonids (Buchmann 1997, Harris et al. 2000). The low temperature, the epibionts and lack of feeding during the experiment may all represent stressors, potentially interacting and negatively influenced alpine bullhead resistance to G. salaris.

In conclusion, we show that the alpine bullhead population (River Nitelva) may function as a transport host and even as a potential reservoir host for G. salaris (haplotype F) dependent on the ambient water temperature and environmental stressors.

Acknowledgements. This work was conducted at the Research Animal Department for Freshwater Fish (no. 166) at the Natural History Museum (NHM), University of Oslo. We thank Ann-Helén Rønning for technical assistance. This study was funded by the NRC Wild Salmon Program (project no. 145861/720), the National Centre for Biosystems (project no. 146515/420) and the European Union’s Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement (Project no. 663830).

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