Research Article

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

Tor A. Bakke^{1*}, Rachel A. Paterson^{2,3} and Jo Cable²

¹ Natural History Museum, University of Oslo, Department of Research and Collections, Oslo, Norway;

² School of Biosciences, Cardiff University, Cardiff, United Kingdom

³ The Norwegian Institute for Nature Research, Trondheim, Norway

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 relatively 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, Misvaer, 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 charr (*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 charr 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 (*Lampetra 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

* Address for correspondence: Tor A. Bakke, Natural History Museum, Postbox 1172 Blindern, 0318 Oslo, Norway. Phone +047 22851678; +047 95466130 E-mail: t.a.bakke@nhm.uio.no

Gyrodactylids were previously recorded on 98% of alpine bullhead co-occurring with Atlantic salmon in Signaldalselva (Troms 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 epizootic infection.

Alpine bullhead (*Cottus poecilopus*) (ca. 50) young-of-the-year (0+) were collected by electrofishing on 14 December 2001

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

Fig. 2. Course of infection of *Gyrodactylus salaris* Malmberg, 1957 on individually isolated alpine bullhead, *Cottus poecilopus* Heckel, from the River Nitelva, southeastern Norway at low temperature ($6.5 \pm 0.5^\circ\text{C}$; $n = 18$). The individual trajectories demonstrate that all fish were initially resistant but after a week individual variations were apparent. Black lines indicate individual fish where parasite reproduction may have occurred. The parasite was eliminated on the alpine bullheads after 47–48 days.

(killed by a blow to its head) in plastic boxes ($37 \times 28 \text{ cm}$, 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 $5 \times 5 \text{ mm}$) placed in a grey, plastic tank ($100 \times 100 \text{ cm}$, 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 ($\text{GLM}_{\text{TEMP}}: F_{1,25} = 46.16, p < 0.001$), 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 ($\text{GLM}_{\text{TEMP}}: F_{1,25} = 12.39, p = 0.002$: 47–48 and 8–9 days for low and high temperature, respectively) and was also influenced by the initial infection ($\text{GLM}_{\text{DAY}_0}: F_{1,26} = 4.67, p = 0.044$; 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, Svenning 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.

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