

Laser-based killing of a macroparasite inside its live invertebrate host

Olivier Musset^{1,*}, Aude Balourdet², and Marie-Jeanne Perrot-Minnot²

¹Laboratoire Interdisciplinaire Carnot de Bourgogne, UMR 6303 CNRS, Université Bourgogne Franche-Comté, 9 Avenue Alain Savary, 21078 Dijon, France

²Biogéosciences, UMR 6282 CNRS, Université Bourgogne Franche-Comté, 6 Boulevard Gabriel, 21000 Dijon, France

Abstract. Whether host phenotypic alterations induced by parasites are reversible or not is a core issue to understand underlying mechanisms as well as the fitness costs of infection and recovery to the host. Clearing infection is an essential step to address this issue, which turns out to be challenging with endoparasites of large size relative to that of their host. Here, we took advantage of the lethality, contactless and versatility of high-energy laser beam to design such tool, using thorny-headed worms and their amphipod intermediate host as a model system. We show that laser-based de-parasitization can be achieved using blue laser targeting carotenoid pigments in *Polymorphus minutus* but not in the larger and less pigmented *Pomphorhynchus tereticollis*. Using DNA degradation to establish parasite death, we found that 80% *P. minutus* died from within-host laser exposure. Survival rate of infected gammarids to laser treatment was higher than uninfected ones. The failure to kill *P. tereticollis* was also observed with nanosecond-green laser, an alternative laser source targeting lipid. We discuss the possible causes of amphipod death following parasite treatment and highlight the perspectives that this technology offers.

1 Introduction

Host-parasite interactions are a driven force in the ecology and evolution of species and, as such, are extensively studied. Standard methodology to address the molecular, physiological, behavioral, and life-history responses of hosts to infection relies on the comparison of naturally or experimentally infected hosts to uninfected ones. In addition, curing host from infection allows addressing the reversibility of host responses, as well as assessing fitness costs associated with the parasite's strategy of host exploitation. This is generally achieved by pharmaceutical treatment in humans and in domesticated or captive animals. Alternatively, the technology of lasers could offer promising alternatives to clear parasites because it offers a contactless tool to deliver lethal high-energy beam and could be highly specific. Yet it could also be used to selectively damage macro-endoparasites within host body, as far as the parasite can be selectively targeted and concentrate most of the energy delivered to limit deleterious effects on the surrounding host tissues.

Our aim was to develop a method to kill a macro-endoparasite in vivo while preserving host viability, taking advantage of the great versatility of laser-based technology. Our host-parasite system involves the freshwater crustacea *Gammarus fossarum* as the intermediate host of two species of thorny-headed worms (Acanthocephala): *Pomphorhynchus tereticollis* and *Polymorphus minutus*. The last larval stage of the

parasite, called cystacanth, appears as an opaque ball yellow to red in color, oval-shaped, and ranging from 0.25 to 0.65 mm³ in volume depending on the species. The amphipod *G. fossarum* is approx. 0.5 to 0.8 cm length and hosts one to several cystacanths in its body cavity. It is visible through the translucent cuticle of the host. The Fig. 1. presents an example of the life cycle of the parasite and host couple considered.

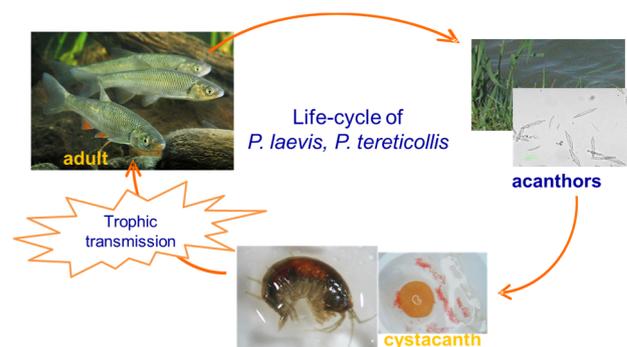


Fig. 1. Simplified diagram of macroparasite life cycle and reproduction with host *Gammarus fossarum*.

2 Experimental setup

We used a high-power multimode laser diode (BDL), capable of emitting 1.6W @ 450nm with an M² beam quality factor of about 4. As desired, BDL has its emission wavelength within the absorption maxima of carotenoids.

* Corresponding author: olivier.musset@u-bourgogne.fr

The laser beam at diode output was originally divergent and astigmatic, with strong divergences in the two main directions orthogonal to the direction of propagation. We added several optical components to generate a laser beam of diameter smaller than 100µm (focusing lens of 100m and a millimeter Rayleigh distance). The full setup was designed to configure and operate two laser sources (BDL and a Q-Switch green DPSSL) and to pilot sample holder from a single control box. The setup included the two pulsed and continuous laser sources with their optical shaping and focusing systems, a thermo-regulated sample holder mounted on a motorized XY displacement. The full setup is presented on Fig.2

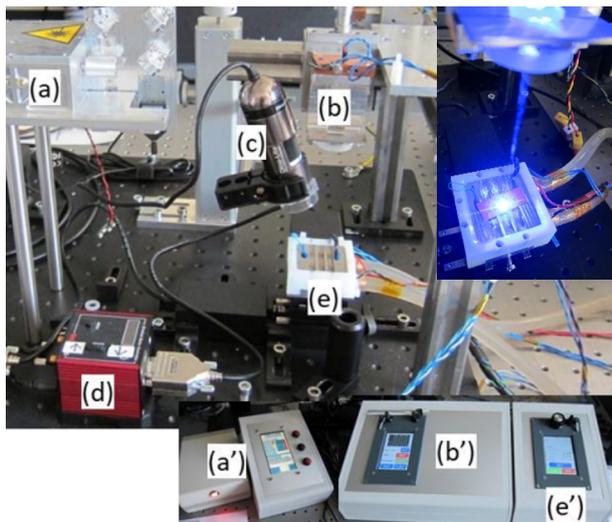


Fig. 2. Complete setup for irradiating acanthocephalan macroparasite inside live crustacean host, comprising two laser sources (532 nm) (a) and (450 nm) (b), a video camera (c), the 2D-displacement system of temperature-controlled sample holder (d, e), (a', b', e'): control units of lasers, and of refrigerated sample holder (thermostat), respectively.

3 Results

Several treatment protocols have been applied and tested to assess parasite mortality and host survival rates as a function of a large number of parameters. It was also necessary to develop a process of falling asleep by cooling and a chemical anesthesia protocol ([1]). Fig.3 presents as an example some photos of macroparasites and hosts after laser treatment.

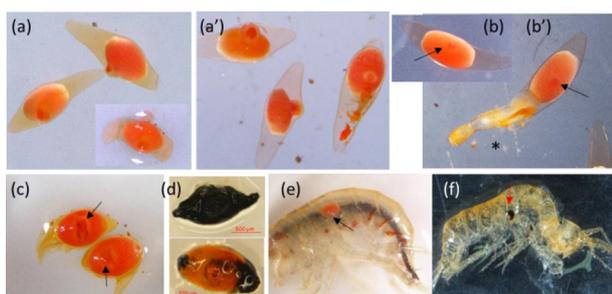


Fig. 3. Example of results obtained after laser treatment of hosts and parasites.

A delicate point in this study was to define what was a dead parasite compared to a living parasite. For this we had to develop DNA and RNA degradation tests, tests that are complex to implement given the very small volume of specimens processed. Fig. 4 gives an example of determining the live/dead state of these parasites.

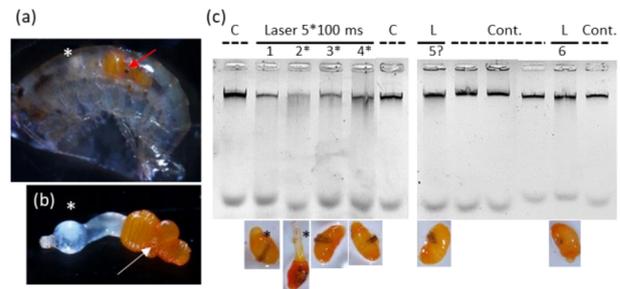


Fig. 4. Typical life/death test results performed on parasites exposed to laser

It is from these DNA/RNA analyzes that it was then possible to determine the survival rates of hosts with a dead parasite as a function of the laser exposure conditions. Fig.5 presents as an example a summary of survival rates proving the relevance of the method used based on laser irradiation.

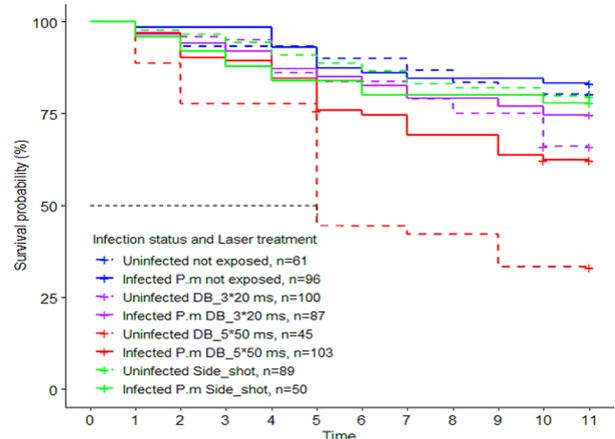


Fig. 5. Survival rate of *Gammarus fossarum* infected and treated with laser (with parasite validated as dead).

4 Conclusion

The macroparasite laser treatment method has been successfully validated. It should make it possible to study the link between the two organisms, which until now was very difficult to achieve with a chemical or mechanical technique (the host would die before the parasite). We will present examples of links highlighted thanks to this specimen preparation work (reproductive capacity, behavior in light, etc.).

References

1. M-J Perrot-Minnot, A. Balourdet, O. Musset. 2021 *Aquat. Toxicol.* **240**, 105981 (2021)