A novel test system for the measurement of Atlantic salmon (*Salmo salar*) attractiveness for *Lepeophtheirus salmonis* copepodids

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Lepeophtheirus salmonis is an ectoparasite responsible for severe economic losses in the Atlantic salmon industry (1–3). Regarding the life cycle, the most critical stage in this parasitosis is the copepodid. Copepodids are able to detect vibrations in the water and shadows created by the fish swimming (4). Upon detection of these signals, the copepodid propels to the fish and analyses the mucus to determine whether the fish is a suitable host (4). Some authors suggest that this attachment behavior is also triggered by the detection of semiochemicals from the fish (5). Such test could allow to screen any putative treatment (like semiochemicals) to prevent the infestation.

An in vivo test has been created to evaluate the attractiveness of Atlantic salmon for *L. salmonis* copepodids

The aim of this study is to evaluate 3 parameters likely to influence the results of this test

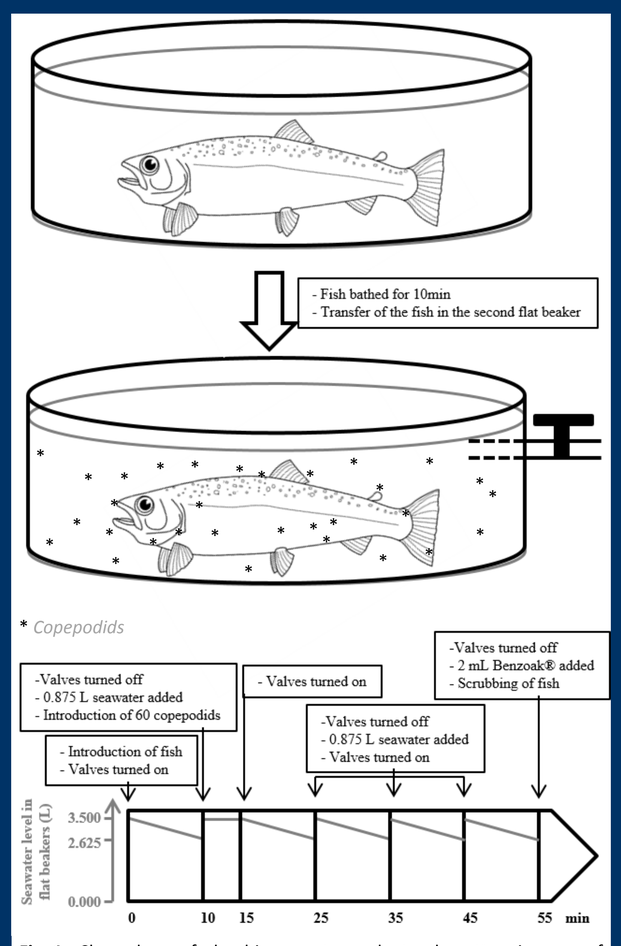

Fig 1. Chronology of the bioassay to evaluate the attractiveness of Atlantic salmon smolt to *L. salmonis* copepodids. The first flat beaker could allow to apply a putative treatment.

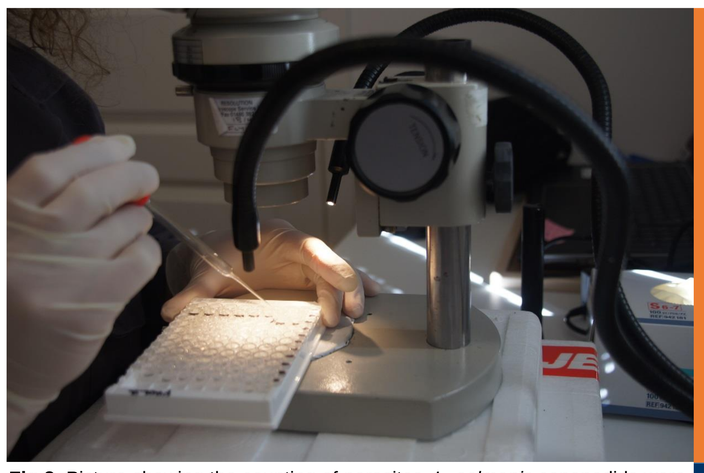

Fig 2. Picture showing the counting of parasites. *L. salmonis* copepodids were counted under stereoscopic microscope using a clean ELISA plate.

A smolt salmon (*n*=82) is bathed in a flat beaker of seawater for 10min (Fig. 1). This period could allow to apply the tested treatment in the water. Then, the fish is transferred in a second flat beaker equipped with a valve allowing to empty 0.875L of water in 10min. 10min after introduction of the smolt, 60 copepodids are added in the latter flat beaker (Fig. 2). The water is renewed every 10min to maintain appropriate oxygen level.

45min later, the fish is euthanized by lethal dose of anesthetic. The salmon's body is scrubbed above a plastic bag (Fig. 3). The content is filtered to count the number of parasite. This procedure was tested by different operators (A and B), for different body surfaces (S calculated from the body mass W by using the equation $S = 14.53W^{0.6044}$ (6)), and at different temperatures (6.1; 9.2; 10.3; 12.3; 13.8°C).

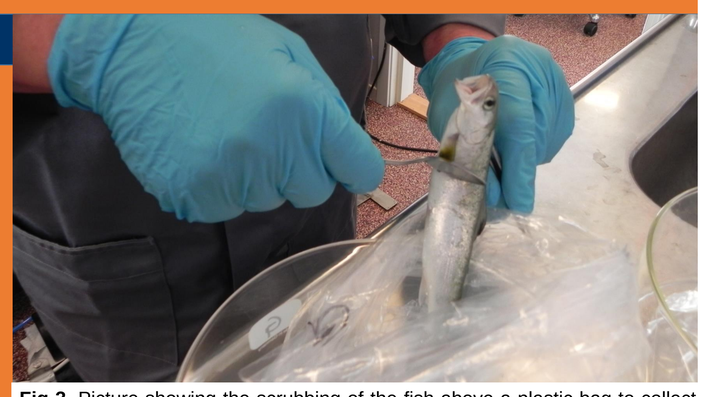

Fig 3. Picture showing the scrubbing of the fish above a plastic bag to collect attached copepodids.

Regarding the operator effect, no significant difference (Student t test, t-value = 0.17, DF = 80, p = 0.86) was found between the operators A (Mean ± SEM = 21.1 ± 0.6) and B (Mean ± SEM = 20.9 ± 0.9) (Fig. 4).

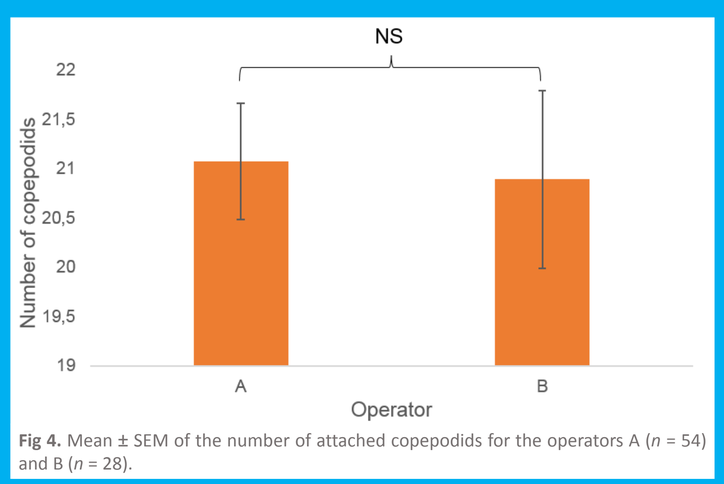

Fig 4. Mean ± SEM of the number of attached copepodids for the operators A (*n* = 54) and B (*n* = 28).

Scrubbing the fish body is certainly the most critical step affecting the final number of attached copepodids found on each fish. The description of this manipulation in the protocol was clear enough to be applied by any operator. The three-phase scrubbing (front area, back area, entire body) appears to be effective in removing all copepodids.

The correlation between body surface and the number of attached copepodids was not significant (Spearman coefficient correlation, ρ = 0.051, p = 0.65) (Fig. 5).

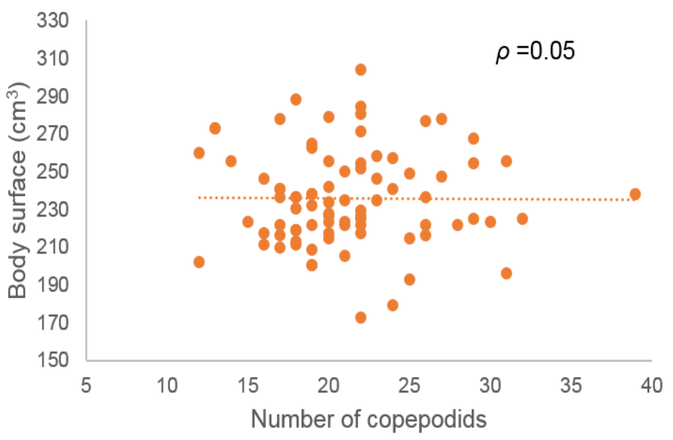

Fig 5. Scatter plot (n = 82) representing the number of attached copepodids according to the body surface area of Atlantic salmon smolts.

This result could be explained by copepodids' preference for certain areas of the body and especially the dorsal and pectoral fins (8). The surface of these areas remains nearly unchanged for the range of fish masses tested.

The bioassay highlighted significant differences in the number of attached copepodids according to water temperature (One-way ANOVA, F-value = 6.68, DF = 81, p < 0.001) (Fig. 6). Smolts presented significantly more attached copepodids at a higher water temperature (13.8 °C, mean ± SEM = 24.6 ± 1.3) compared to smolts at a lower temperature (6.1 °C, mean ± SEM = 18.6 ± 0.7) (Tukey test, p = 0.011).

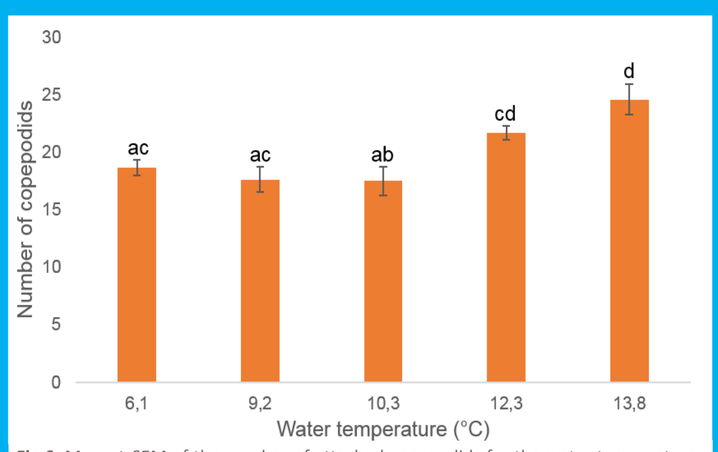

Fig 6. Mean ± SEM of the number of attached copepodids for the water temperatures 6.1 °C (*n* = 8), 9.2 °C (*n* = 8), 10.3 °C (*n* = 8), 12.3 °C (*n* = 12) and 13.8 °C (*n* = 46). Different lowercase letters indicate significant differences between water temperature groups.

The reasons for the increased attachment of copepodids at higher temperature are not well known, but water temperature has certainly a direct effect on the metabolic rate of copepodids and thus affects their activity (7).

This reliable and standardized bioassay can be used to successfully evaluate the attachment behaviour of *L. salmonis* copepodids on Atlantic salmon, making it an interesting tool to screen several putative semiochemicals contributing to the communication between the parasite and the host.

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