Supporting Information. A colorful killer: *Daphnia* infected with the bacterium *Spirobacillus cienkowskii* exhibit unexpected color variation. Nina Wale, McKenna L. Turrill, and Meghan A. Duffy. *Ecology*. 2018.

Appendix S1

Below we provide more details of the methods used to study *Spirobacillus cienkowskii* in the laboratory. Two additional figures are also included: the first shows how the colorful symptoms of *Spirobacillus* infection develop (Fig. S1), the second contrasts the appearance of *Spirobacillus*-infected and hemoglobin-producing *Daphnia* to help the reader distinguish between these host types (Fig. S2).

Materials & Methods

Spirobacillus cienkowskii has been maintained in *in vivo* culture in the Duffy Lab at the University of Michigan since February 2016, having originally been established by Alex Strauss (Indiana University). Cultures are set up in 1000ml beakers filled with 800ml filtered lake water (FLW) collected from North Lake (Washtenaw County, Michigan, USA) and initiated with 6 infected and 75 uninfected *D. dentifera* of the L6D9 clone, originally collected from Dogwood Lake (Greene-Sullivan State Forest, Indiana, USA). An alternative protocol, using 250ml beakers containing 200ml FLW, 20 uninfected animals and 5 infected animals is also used. New cultures are made every 10-14 days, using infected animals from previously established cultures and uninfected L6D9 individuals; the latter are collected from uninfected stock cultures that are maintained separately.

Infection assays

We present representative methods and data (Fig. S1) for individual-level infection experiments. For these individual exposure assays, 5 day old *D. dentifera* of the L6D9 clone were placed individually in 50ml beakers filled with 25ml FLW. Each beaker was fed 0.25ml of a 1,000,000 cells per ml solution of *Ankistrodesmus falcatus* daily, and maintained at 22°C in an incubator on a 16-8 hour light-dark cycle. Infected animals were collected from *in vivo* cultures into a 1.5ml tube and crushed using a motorized pestle; the contents were carefully mixed and evenly distributed among the beakers. Daily, individuals were checked for symptoms of infection by holding each beaker over white paper to better facilitate the detection of colored hosts. In this experiment, red animals were preserved before they died for further analysis. However, we have never seen a *Spirobacillus*-exposed animal recover after turning red. Indeed, in a recent experiment, red animals had an hourly mortality rate of 5%.

Assessment of infection status by PCR

DNA was extracted from animals using the DNeasy Blood & Tissue kit (Qiagen) according to manufacturer's instructions. The presence of *Spirobacillus* infection was assessed using a species-specific PCR assay. Each 50µl reaction contained GoGreenTaq Mastermix (Promega) and primers (0058F, 462R (Rodrigues et al. 2008, Thomas et al. 2011)) at a final concentration of 1x and 400nM**,** respectively, and 10µl of extracted DNA. Cycling conditions were the same as those used in Rodrigues et al. (2008), with the exception that 40 rather than 30 cycles of denaturation/annealing/extension were used. Gel electrophoresis was used to confirm that a fragment of the appropriate length had been amplified and hence that *Spirobacillus* DNA was present.

The impact of light on the color of infected Daphnia

12 250ml beakers were filled with 200ml FLW and 20 4-5 day old uninfected hosts of the L6D9 clone. 45 infected hosts were collected, crushed and distributed evenly among 9 of the beakers; 3 beakers were left unexposed in order to assess the impact of darkness on the color of uninfected hosts. 3 'exposed' cultures were placed in a clear plastic box with a lid; the remaining 6 'exposed' and further 3 'unexposed' in a similar box completely covered in light-blocking vinyl (BlackOut Cling Vinyl, Delta Photography Supplies, USA). Both totes were then placed in an incubator on a 16-8 hour light-dark cycle. Each beaker was fed 4ml of 1,000,000 cells per ml solution of *Ankistrodesmus falcatus* daily; animals in the 'dark' treatment were fed in a dark room devoid of light except for a red headlight worn by the experimenter (NW). 6 days after they were established**,** the cultures were inspected and the brightest colored animal from each of the replicate cultures selected and photographed (as shown in Fig. 2). Light had no apparent impact on the color of the unexposed hosts. This experiment does not preclude the possibility that *Spirobacillus* infection, and the characteristic red symptoms associated with it, takes longer to develop in the dark etc. and further investigations of the impact of light on carotenoid production are needed.

References

Rodrigues, J. L. M., M. A. Duffy, A. J. Tessier, D. Ebert, L. Mouton, and T. M. Schmidt. 2008. Phylogenetic characterization and prevalence of *Spirobacillus cienkowskii*, a red-pigmented, spiral-shaped bacterial pathogen of freshwater *Daphnia* species. Applied and Environmental Microbiology 74:1575–1582.

Thomas, S. H., C. Bertram, K. van Rensburg, C. E. Cáceres, and M. A. Duffy. 2011. Spatiotemporal dynamics of free-living stages of a bacterial parasite of zooplankton. Aquatic Microbial Ecology 63:265–272.

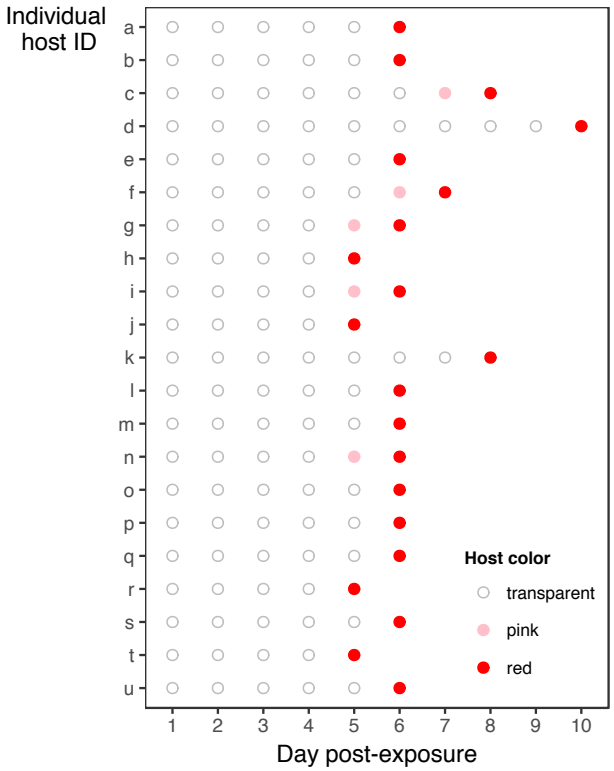

Fig. S1: The red color associated with *Spirobacillus* infection appears at the end of infection, 5-6 days after exposure. Each row represents an individual *Daphnia* exposed to *Spirobacillus* in an individual beaker (see supplementary text for details). The appearance of each host to the naked eye was recorded daily and is indicated by the circles' fill color. In this experiment, red animals were preserved before they died for further analysis but we have never observed a *Spirobacillus*-infected animal recover from infection after turning red.

Fig. S2: *Spirobacillus*-infected animals can be distinguished from hemoglobin-rich animals using dark field microscopy. The animal on the left of each photograph is infected with *Spirobacillus*, while the animal on the right is uninfected but producing hemoglobin in abundance. When viewed with bright field microscopy (left photograph), it can be challenging to distinguish *Spirobacillus* from hemoglobin. However, when viewed with dark field microscopy (right photograph), the *Spirobacillus*-infected *Daphnia* is more opaque than its hemoglobin-producing counterpart. In addition, when viewed live the hemolymph of *Spirobacillus*-infected hosts is characterized by a glittery appearance that hemoglobin-rich hemolymph lacks. Note that, due to the limited availability of hemoglobin-producing *D. dentifera* at the time that these photographs were taken, this figure contrasts a *Spirobacillus*-infected *D. dentifera* and a hemoglobin-producing *D. pulicaria*. The increased opacity of *Spirobacillus*-infected hosts is consistent across species.