

# Supporting Information

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## SI Materials and Methods

**Bacterial Cultures.** Wild-type *Photobacterium leiognathi* and the dark mutant were cultured using liquid and solid seawater-based LB media consisting of 0.5% yeast extract and 1% (wt/vol) peptone in filtered sea water (FSW; filtered through 0.7- $\mu$ m Whatman glass microfibre filters) diluted with 25% (vol/vol) double-distilled water; solid medium contained 1.5% (wt/vol) agar. Bioluminescence of bacterial cultures was verified by visual inspection in a dark room and by measuring the relative luminance of 1 mL of culture using a custom-made luminometer (Model 597D; Technion). This luminometer is an analog photomultiplier calibrated using a 532-nm green laser (Lambdapro Technologies Ltd.) and a laser meter with a photodiode head (Ophir Optotronics).

**Production of Glowing Zooplankton Using Colony Particles.** A Petri dish with solid [1.5% (wt/vol) agar] seawater-based LB medium covered by ~24-h-old colonies of *P. leiognathi* was placed on the bottom of a 1-L plastic beaker with a small metal weight on top serving as an anchor. The beaker was filled gently with FSW through a capillary tube. Following the slow water motion, minute pieces of bacterial colonies detached from the agar and were suspended in the water. *Artemia salina* (hereafter “*Artemia*”) specimens were introduced into the suspension and allowed to swim/graze for 2.5 h. Afterward they were allowed to swim in FSW for 10 min and then were photographed in a drop of FSW, both in the light and in darkness (the latter without artificial illumination) using a Nikon D3 camera, exposure time 30 s, aperture  $f/5.6$ , ISO 25600, focal length 150 mm (Fig. 2 in main text).

**Fish-Feeding Experiment.** Feeding experiments with glowing and nonglowing control *Artemia* were carried out in a recirculating flume as described in ref. 1. The sides of the working section were made of transparent glass. Water flow was generated by a propeller with an electric frequency controller. Two plastic-coated screens (mesh size, 1  $\times$  1 cm) were placed in the downstream end of the working section, delimiting a 30-cm experimental arena in which an individual fish was held. The flow speed was 6  $\text{cm}\cdot\text{s}^{-1}$ . Runs were made with a single fish of the species *Apogon annularis* in the flume. The fish motions were recorded in 3D during the trials using an IR-sensitive video

camera and IR illumination (Watec 902H with a Tamron zoom lens, 8–80 mm,  $f/1.8$ ). Four molded packages, each containing 30 light-emitting diodes, were used as a submersible source for IR illumination (880 nm) as described in ref. 2. The IR illumination has no apparent effect on the fish feeding (2, 3). A mirror attached above the experimental arena at a 45° angle allowed the tracking of fish movements in 3D (1).

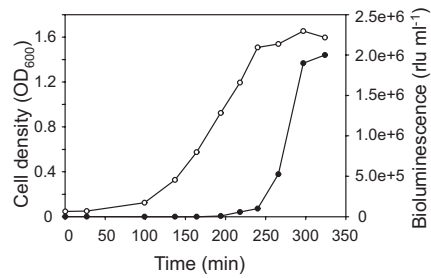
Each fish was allowed to acclimate in the flume for at least 4 d before the experiment started. A day/night cycle (10 h darkness/14 h light) was simulated using 12 fluorescent lamps (36 W, 115 cm long) ~70 cm above the flume. A small plastic pipe (20  $\times$  7  $\times$  7 cm) provided a shelter in which the fish hid during the 14 h of light. The fish was fed with 20 *Artemia* every evening during the acclimation period but was starved for 24 h before the experiment.

The *Artemia* were introduced into the flume by gradually releasing them above the flume’s propeller. The fish was allowed to feed for 2.5 min, during which time the water completed two to three revolutions of the flume. The run was terminated by inserting a plankton net (mesh size, 100  $\mu$ m) into the flume upstream of the working section. The net, mounted on a square aluminum frame and tightly fitting the flume’s inner cross-section, was used to collect the *Artemia* that survived predation by the fish. After the net was inserted fully, the flow speed was increased to 10  $\text{cm}\cdot\text{s}^{-1}$ , and the water was filtered through the net for 5 min. The number of *Artemia* in the net was subtracted from the number initially offered (10), yielding the number eaten by the fish during the trial. Control runs without a fish in the flume indicated a recapture success of 98%.

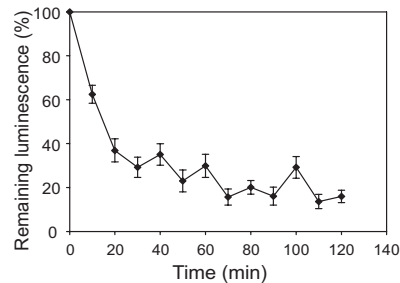
**Luminous Bacteria in Feces of Zooplankton.** The different pellets (first-step, second-step, and third-step as described in *Materials and Methods* in the main text) of the mysids *Anisomysis marisrubri* were processed identically as follows: Three to five fecal pellets of the respective type were washed three times in sterile FSW, torn apart with a sterilized needle, and suspended in 1 mL sterile FSW. Appropriate dilutions were made and plated on agar plates in duplicates followed by overnight incubation at 30 °C. Luminous colonies were counted, and the number of luminous cfu per fecal pellet was determined. The result of this experiment ( $n = 5$ ) is regarded as qualitative, because no estimation could be made of the number of bacteria eaten by the mysids.

1. Kiflawi M, Genin A (1997) Prey flux manipulation and the feeding rates of reef-dwelling planktivorous fish. *Ecology* 78:1062–1077.
2. Holzman R, Genin A (2003) Zooplanktivory by a nocturnal coral-reef fish: Effects of light, flow, and prey density. *Limnol Oceanogr* 48:1367–1375.

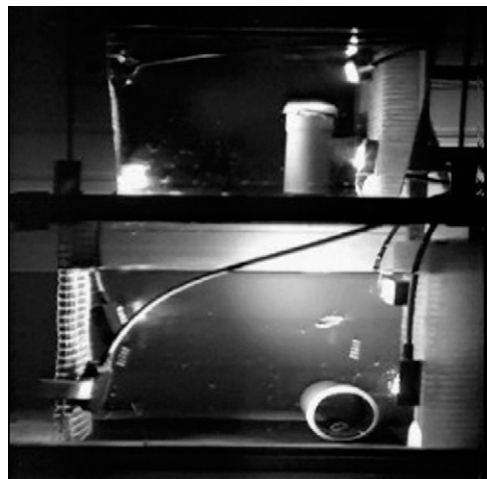
3. Ryer CH, Olla BL (1999) Light-induced changes in the prey consumption and behavior of two juvenile planktivorous fish. *Mar Ecol Prog Ser* 181:41–51.



**Fig. S1.** Quorum sensing in *P. leiognathi*, shown as the increase in bioluminescence intensity per milliliter (right axis; black circles; relative light units) after the cell density (OD<sub>600</sub>) (left axis; open circles) exceeded the threshold level ~200 min after the start of the experiment. This threshold is reached at an optical density of ~0.9, which corresponds to ~10<sup>8</sup> cells·mL<sup>-1</sup>.

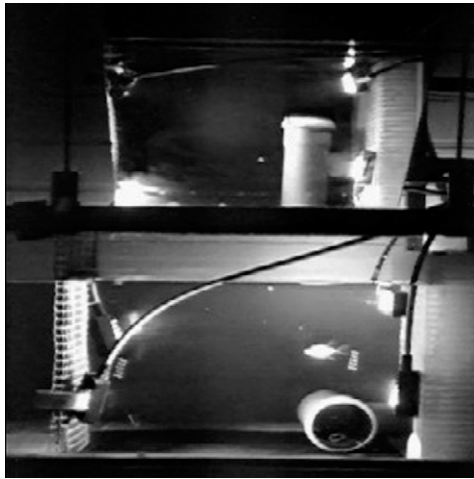


**Fig. S2.** The decrease in the intensity of bacterium-promoted glow in *Artemia salina* with time. A batch of 10 glowing *Artemia* was measured in the luminometer every 10–15 min. Although each animal was measured separately, the decrease in glow was calculated using the average value of each batch at each time interval ( $n = 5$  batches). The animals in a single batch were kept in fresh FSW between different measurements. Error bars indicate SE.



**Movie S1.** *Apogon annularis* detects and efficiently consumes glowing *Artemia* in the dark. These *Artemia* became glowing by contacting and ingesting bioluminescent bacteria. Infrared light was used to illuminate the flume for video recording. The lower part of the image (below the dark bar) shows the flume's working section as viewed from the side. The upper part shows the working section as viewed from above, through a 45° inclined mirror. For experimental details see *Materials and Methods* and *SI Materials and Methods*.

[Movie S1](#)



**Movie S2.** *Apogon annularis* does not detect nonglowing *Artemia* in the dark. These *Artemia* contacted and ingested non-bioluminescent mutant bacteria. Infrared light was used to illuminate the flume for video recording. The lower part of the image (below the dark bar) shows the flume's working section as viewed from the side. The upper part shows the working section as viewed from above, through a 45° inclined mirror. For experimental details see *Materials and Methods* and *SI Materials and Methods*.

[Movie S2](#)