SUPPLEMENTARY METHODS

Aquaculture fish collection and care

Aquaculture fish were originally collected off southern California via lampara purse seine or lampara set and on display at MBA since May 30, 2012. They were housed in a fiberglass-reinforced plastic donut shaped tank (3 m W x 3 m H x 6.9 m L) with acrylic windows that contained 38,989 L water. 349 Pacific mackerel were on display with a total biomass of 195.4 kg. The average individual mass was 559.8 g ± 18.2 SE. MBA pumps seawater directly from Monterey Bay with a filtration system that uses a semi-closed, reservoir, sand filters, and de-aeration tower. In the month of collection, water quality measured 0.03 ppm Hach total NH_3, 0.02 ppm NO_2, 0 ppm un-ionized NH_3, 5.15 ppm MLML NO_3^-, 8.0 ppm DO, 99% saturation DO, 16.3 °C, and 7.63 pH. The mackerel were fed 4.5 kg squid, 1.5 kg white bait, and 4.86 kg Skretting classic trout pellets per week, with a feeding ration of 19.1 kcal kg^{-1} d^{-1}.

Deerinck Protocol for EM sample preparation

We followed the Deerinck Protocol modified to use sodium cacodylate, to prepare samples for EM. Samples were incubated in 0.2% tannic acid in water for 10 minutes, then washed 3x times for 10 min each with distilled water (DI). Samples were then incubated in 1% osmium tetroxide for 30 min at room temperature (RT), washed 3x for 10 min each with DI, and incubated in 1% thiocarbohydrazide for 20 min at RT and again washed 3x for 10 min in DI. The samples were again incubated in 1% osmium tetroxide for 30 min and washed 4x for 10 min with DI. The samples were then incubated in 0.5% uranyl acetate in 25% methanol at 4 °C overnight. The second day the samples were washed with DI 3x for 10 min RT, then incubated en bloc with 0.02 M lead nitrate in 0.03 M sodium aspartate pH 5.5 for 30 min at 60 °C and washed 2x for 5 min with DI. Afterwards, the samples were dehydrated using a graded series of ethanol: 2x for 7 min each and pure acetone 3x for 7 min, then dried on a molecular sieve. The ethanol series used was: 30%, 50%, 70%, 80%, 90%, and 96%. After the samples were dehydrated, they were infiltrated in series with a 3:1, 1:1, and 1:3 acetone:Araldite mixture for 1 h each at RT. The samples were then infiltrated overnight in a 3:1 acetone:Araldite mixture on a rotation plate. The third day the samples were put into pure Araldite under a vacuum 2x for 1 h. Then the samples were flat embedded in pure Epon for 6 h.

Notes on a single yellowfin tuna *Thunnus albacares*

In addition to Pacific mackerel, we imaged one aquaculture, symptomatic yellowfin tuna *(Thunnus albacares)* as an outgroup. The yellowfin tuna was captured at sea off San Diego, CA, USA, using conventional sportfishing techniques and arrived at MBA's Animal Research and Care Center on July 13, 2018 with 13 similarly sized fish in its cohort. It measured 47 cm curved fork length (CFL) at capture. It was housed in a fiberglass-reinforced plastic oval tank (60' x 40', 8' deep) that contained 502,092 L water. The 14 fish were joined by 4 other yellowfin tuna immediately and an additional 22 in September 2018. The fish were fed a squid, sardine, low-fat Mazuri Gel diet 5 days per week. Based on condition and behavior, they were fed 36–40 kcal kg^{-1}. Water chemistry (performed 2x per week) and nitrate and phosphate (performed monthly) measured 80–100% DO, 7.7–8.4 pH, 33–36 ppt salinity, temperature always within 1°F of

system set point, > 1.0 meq L^{-1} alkalinity TA, <0.1 ppm total ammonia (NH_3+NH_4^+), <0.1 ppm unionized NH_3, <50 ppm NO_3^-, <0.1 ppm NO_2, and 0 phosphates. The euthanized yellowfin tuna weighed 10.9 kg and measured 76 cm CFL at necropsy on June 27, 2019. The yellowfin tuna was sampled and processed following the protocol used for Pacific mackerel (see main text Methods).

VLPs were not observed in the aquaculture yellowfin tuna with moderate PSS symptoms. Sample analysis from a single yellowfin tuna specimen contained 51.5% malformed mitochondria.

We did not observe VLPs in the symptomatic aquaculture yellowfin tuna which could indicate that a different etiological agent causes PSS in yellowfin tuna than in Pacific mackerel, the symptoms observed in the yellowfin tuna may have appeared to be PSS but could have been due to mechanical injuries, or it is possible we did not have enough samples to capture images of the VLPs. Alternatively, the malformed mitochondria observed in the yellowfin tuna may indicate the damaged organelles are unrelated to the potential viral-infection in Pacific mackerel and are a response to aquaculture-induced stressors. The malformed mitochondria may be a response to both PSS and mechanical injuries.