

limiting (~2 days), as indicated by the cessation of biomass accumulation (Fig. 3A) as well as the η_{elec} measured every 24 hours (Fig. 3B and fig. S13). With a titer of ~700 mg/liter, the 6-day average for PHB synthesis was $\eta_{\text{elec}} = 36 \pm 3\%$ (Fig. 2A, entry 9) with a 24-hour maximum of $\eta_{\text{elec}} = 42 \pm 2\%$ ($n = 3$) (Fig. 3B). In engineered strains (32, 33), this PHB pathway could be modified to excrete the fusel alcohols isopropanol (C_3), isobutanol (C_4), and 3-methyl-1-butanol (C_5), which possess energy densities of 24, 28, and 31 MJ/liter, respectively. The culture supernatant was then analyzed to quantify the secreted alcohols (23). The accumulation of these liquid fuels followed trends similar to those observed for PHB synthesis. As shown in Fig. 3, C and E, biomass production reached a plateau while isopropanol titers grew to ~600 mg/liter and $C_4 + C_5$ alcohol titers grew to ~220 mg/liter. An engineered *R. eutropha* strain produced isopropanol with a 6-day average $\eta_{\text{elec}} = 31 \pm 4\%$ (Fig. 2A, entry 10) and a 24-hour maximum of $\eta_{\text{elec}} = 39 \pm 2\%$ ($n = 4$) (Fig. 3D); a strain engineered to produce $C_4 + C_5$ alcohols averaged a 6-day $\eta_{\text{elec}} = 16 \pm 2\%$ (Fig. 2A, entry 11) with a 24-hour maximum of $\eta_{\text{elec}} = 27 \pm 4\%$ ($n = 3$) (Fig. 3F). The achieved titers are higher than previous reported values, and η_{elec} values have increased by a factor of at least 20 to 50 (10, 18). *R. eutropha* has demonstrated tolerance toward isopropanol (fig. S14), allowing for enriched product concentrations under extended operation.

Our combined catalyst design mitigates biotoxicity at a systems level, allowing water-splitting catalysis to be interfaced with engineered organisms to realize high CO_2 reduction efficiencies that exceed natural photosynthetic systems. Because E_{appl} required for water splitting is low (1.8 to 2.0 V), high η_{elec} values are achieved that translate directly to high solar-to-chemical efficiencies (η_{SCE}) when coupled to a typical solar-to-electricity device ($\eta_{\text{SCE}} = \eta_{\text{solar}} \times \eta_{\text{elec}}$). For a photovoltaic device of $\eta_{\text{solar}} = 18\%$, the Co-P[CoP]*R. eutropha* hybrid system can achieve $\eta_{\text{SCE}} = 9.7\%$ for biomass, 7.6% for bioplastic, and 7.1% for fusel alcohols. This approach allows for the development of artificial photosynthesis with efficiencies well beyond that of natural photosynthesis, thus providing a platform for the distributed solar production of chemicals.

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SUPPLEMENTARY MATERIALS

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ECOTOXICOLOGY

Environmentally relevant concentrations of microplastic particles influence larval fish ecology

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The widespread occurrence and accumulation of plastic waste in the environment have become a growing global concern over the past decade. Although some marine organisms have been shown to ingest plastic, few studies have investigated the ecological effects of plastic waste on animals. Here we show that exposure to environmentally relevant concentrations of microplastic polystyrene particles (90 micrometers) inhibits hatching, decreases growth rates, and alters feeding preferences and innate behaviors of European perch (*Perca fluviatilis*) larvae. Furthermore, individuals exposed to microplastics do not respond to olfactory threat cues, which greatly increases predator-induced mortality rates. Our results demonstrate that microplastic particles operate both chemically and physically on larval fish performance and development.

Global plastic production is estimated to be about 300 million metric tons (MMT) annually and is increasing by 20 MMT per year (1). As a direct consequence of the massive use of plastics in modern society, plastic waste is accumulating, especially in and around urbanized areas, where it often ends up in waterways and is ultimately transported into the ocean (2, 3). Because plastic polymers show minimal biological degradation, they remain in the environment for hundreds to thousands of

years, where they break down into smaller pieces owing to ultraviolet radiation, physical forces, and hydrolysis (4). Hence, plastic particles continue to accumulate as small fragments (hereafter termed microplastics, and defined as <5 mm in size) throughout the world's oceans (4, 5). Plastic debris can affect marine biota both physically (e.g., by blocking the alimentary tract when ingested) (6) and chemically (e.g., by leaching toxic pollutants that are part of the plastics or that have been absorbed by the plastic) (7).

To date, passive ingestion of plastic microdebris by filter feeders is known to occur, but the ecological significance of ingestion is poorly understood (3, 4, 8). There is increasing concern that

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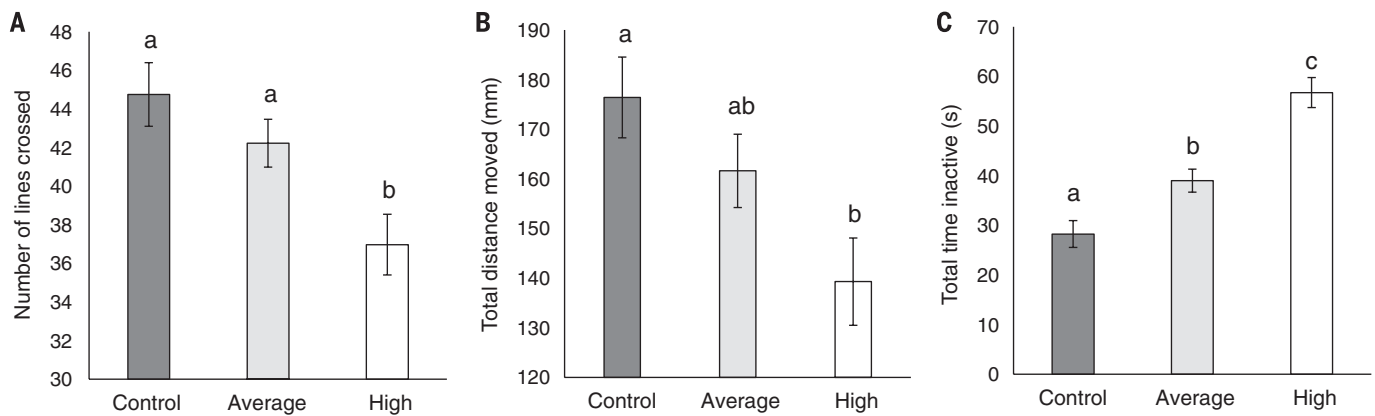


Fig. 1. Fish behavior when exposed to polystyrene microplastic particles. Mean (\pm SE) number of (A) lines crossed (a measure of activity), (B) total distance moved (mm), and (C) total time spent inactive (s) for 10-day-old *P. fluviatilis* were affected by microplastic concentration (control, average, or high).

the accumulation of microplastic waste could affect the functioning of marine ecosystems; however, the mechanisms by which effects will manifest have not been identified. This is especially true for eggs, embryos, and larvae of aquatic organisms, which are particularly vulnerable to water-borne pollutants owing to their limited ability to regulate their internal environment (9). In particular, early life stages of fishes are under strong selection, driven by high rates of predator-induced mortality (10, 11). Hence, selection is often mediated by antipredator behaviors and proximate factors (e.g., feeding history and growth) (12). To better understand potential effects of microplastic waste on the vulnerable younger life stages of fish, we examined how natural levels of microplastic particles affected the development, behavior, and survival of Eurasian perch (*Perca fluviatilis*).

The abundance of microplastic particles on the Swedish coast is in the range of 150 to 2400 particles/m³ to 68,000 to 102,000 particles/m³, with average values being 7000 to 10,000 plastic particles/m³, based on zooplankton sampling (net mesh size 10 to 300 μ m) (13, 14). Many juvenile fish are likely to encounter high concentrations of microplastic debris in their nursery habitats, as microplastic pollutants often accumulate in shallow coastal habitats (13–15). Polystyrene is one of the five major types of microplastic debris found in the marine environment (3, 16), and ingestion of polystyrene particles has been found to alter behaviors (17) and disturb the fat metabolism in freshwater fishes (18). Hence, in the current study, we used three different concentrations of polystyrene micro-

plastic particles (90 μ m): (i) no microplastics (0 particles/m³), (ii) average microplastic concentration (10,000 particles/m³), and (iii) high microplastic concentration (80,000 particles/

m³). Fish across all treatments were fed the same concentrations of newly hatched *Artemia* sp. nauplii twice daily (ad libitum, 75,000 nauplii/m³).

To assess direct chemical effects of polystyrene microplastics on fish, we collected fertilized egg strands of *P. fluviatilis* from natural populations in the Baltic Sea and placed them in 1000-ml glass aquaria that contained one of the three microplastic concentrations and filtered estuarine water (19). We then monitored the number of successful hatching events over a 3-week period ($N = 5$ with 58 to 60 eggs per replicate aquaria). Overall, successful hatching rates of fish were significantly related to microplastic concentration [analysis of variance (ANOVA): $F_{2,12} = 19.4$, $P = 0.0002$]. Fish that were not exposed to microplastics during egg development had high hatching rates typical of most teleosts [e.g., (20)], with 96% successfully hatching compared to eggs that were exposed to polystyrene particles. Fish in the high-concentration treatment had the lowest hatching rates, at 81%, whereas fish exposed to average microplastic concentrations displayed hatching rates of 89%. This suggests that polystyrene particles may be chemically affecting larvae in both average and high concentrations, as exposure potentially reduces hatching rates of fertilized *P. fluviatilis* eggs.

Behavior is a crucial determinant for essential fitness correlates (e.g., overall health), such as growth, reproduction, and survival (21). To investigate if exposure to microplastic particles during the first weeks of development altered fish behavior, we measured activity rates [defined as the number of lines crossed on a grid (5 mm by 5 mm) present on the

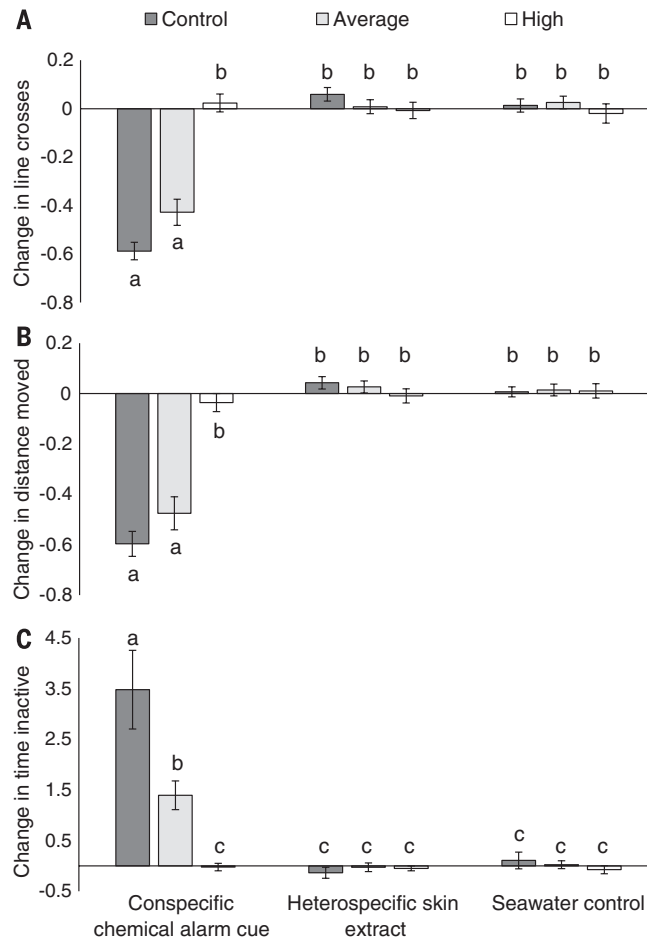


Fig. 2. Innate responses to olfactory threat cues are affected by exposure to microplastic particles. Fish exposed to high concentrations of microplastic particles did not significantly alter their proportional change in (A) activity, (B) area use (mm), or (C) freezing behavior after being exposed to a conspecific chemical alarm cue compared to the two controls [alarm cue from a heterospecific fish (flounder, *Platichthys flesus*) or water controls.

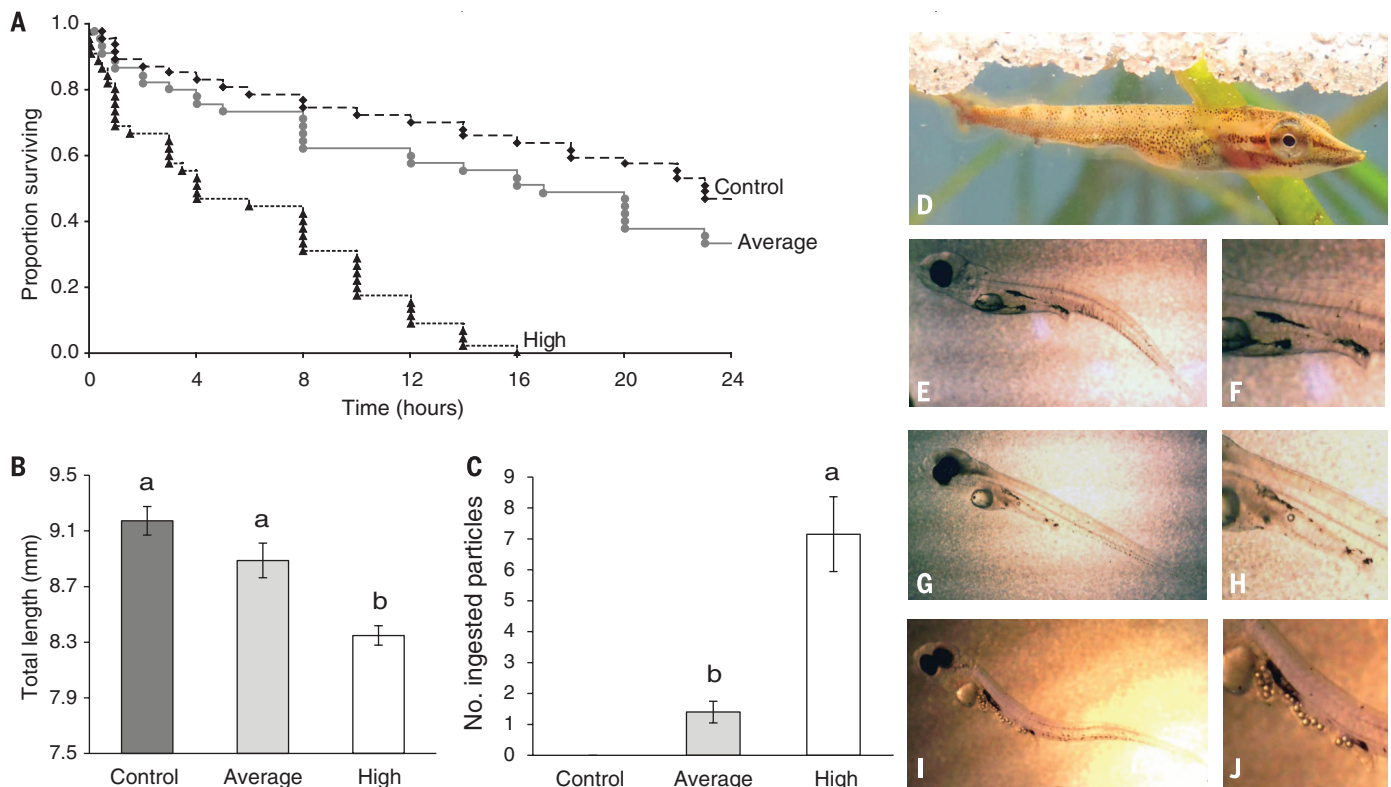


Fig. 3. Exposure to microplastic particles affects survival, growth, and mean number (\pm SE) of ingested microplastic particles. (A) Survival curves (Kaplan-Meier plot) of 10-day-old *P. fluviatilis* larvae from the three different microplastic treatments. (B) Standard length and (C) number of microplastic particles found in stomach contents were also affected by treatment. Juvenile pike [*Esox lucius* (D)] are a common and natural piscivore that preys on larval perch. Larvae exposed to the different treatments had consumed varying amounts of microplastic particles: no microplastics (E and F); average amounts of microplastics (G and H); or high amounts of microplastics (I and J).

bottom of the aquarium], total distance moved (total distance fish swam over the 3-min observation period), and the amount of time fish were immobile (s) using standardized protocols ($N = 36$) (12, 22). We found clear effects of exposure to polystyrene microplastics (average and high concentrations) on behavior of 10-day-old fish larvae [2-factor multivariate analysis of variance (MANOVA): $F_{6,180} = 8.47$, $P < 0.00001$; Fig. 1, A to C]. There was a nonsignificant effect of exposure tank on behaviors of individual fish (2-factor MANOVA: $F_{39,267} = 0.99$, $P = 0.49$). Hatched larvae that were reared under control conditions had higher activity rates (2-factor ANOVA: $F_{2,92} = 7.24$, $P = 0.0012$; Fig. 1A), swam greater distances (2-factor ANOVA: $F_{2,92} = 5.14$, $P = 0.0076$; Fig. 1B), and spent less time motionless (2-factor ANOVA: $F_{2,92} = 28.98$, $P < 0.00001$; Fig. 1C) compared to fish that were reared under microplastic treatment conditions.

Early life-history stages of many aquatic organisms are inherently vulnerable to predators, and an innate ability to detect predators is critical for survival (10–12). One way naïve prey avoid predators is through an innate response to damage-released chemical alarm cues, and although the olfactory sense in larval fish is sensitive to changes in habitat composition (22) and ocean chemistry (23), it is unknown if olfactory threat responses are affected by exposure to

microplastic pollutants. By recording behaviors of fish from the three different microplastic treatments before and after the injection of an alarm cue, we could determine innate fear responses of naïve 10-day-old *P. fluviatilis* ($N = 12$). We found a strong influence of microplastic exposure and concentration on the response of fish to olfactory threat cues (2-factor MANOVA: $F_{12,291} = 6.59$, $P < 0.00001$; Fig. 2, A to C). Fish reared under control conditions displayed lowered activity rates (2-factor ANOVA: $F_{4,97} = 29.72$, $P < 0.0001$; Fig. 2A), decreases in distance moved (2-factor ANOVA: $F_{4,97} = 23.44$, $P < 0.0001$; Fig. 2B), and a greater incidence of freezing behavior (e.g., time immobile: 2-factor ANOVA: $F_{4,97} = 12.94$, $P < 0.0001$; Fig. 2C) in response to conspecific alarm cues. Although there was a tendency of fish reared in the average microplastic concentrations to display weaker threat responses compared to control fish, they still displayed significantly stronger threat responses to chemical alarm cues compared to the two control cue treatments (heterospecific skin extract and water controls; Tukey's HSD (honest significant difference) test $P < 0.02$; Fig. 2, A to C). In contrast, *P. fluviatilis* larvae reared in high microplastic concentrations did not exhibit an antipredator response when exposed to threat cues compared to controls (Fig. 2, A to C).

To assess more direct ecological effects of microplastic exposure on fish, we measured indi-

vidual survival rates of 2-week-old larvae from the different treatments when exposed to a natural and common predator on larval perch, juvenile pike (*Esox lucius*, 31 ± 1.5 mm total length). Survival of fish was monitored every 2 to 6 hours over a 24-hour period in mesocosms simulating natural conditions ($N = 45$ to 47) (19). We found that microplastic exposure during development influenced survival rates of *P. fluviatilis* ($\chi^2_{2,0.05} = 34.02$, $P < 0.0001$). Survival of fish larvae was highest and most similar to natural survival rates at this life stage [e.g., (20)] when reared under control conditions, with 46% still alive after 24 hours (Fig. 3A). Fish reared in average microplastic concentrations had a lower survival rate, with 66% consumed after 24 hours. Larvae reared in high microplastic concentrations had the lowest survival rates, with 100% consumed by pike within 24 hours. Observed survival patterns in the current study emphasize the importance of behavioral responses to threat cues, as larval fish failing to respond to conspecific alarm cues had threefold (high microplastics = 37 out of 45) higher mortality rates compared to control larvae (no microplastics = 12 out of 47) in the first 10 hours after exposure to a predator (Fig. 3A) ($P < 0.001$).

Two weeks after hatching, total length (mm) differed significantly between fish exposed to the different microplastic concentrations (2-factor ANOVA: $F_{2,45} = 17.16$, $P < 0.0001$; Fig. 3B; $N = 20$).

Fish reared in the highest microplastic concentrations were significantly smaller (8.35 ± 0.07 mm) than fish reared in average concentrations (8.89 ± 0.12 mm) or than those without exposure to microplastics (9.17 ± 0.1 mm). There was also a significant difference in the number of ingested microplastic particles between the three treatments (2-factor ANOVA: $F_{2,45} = 79.24$, $P < 0.0001$; Fig. 3C; $N = 20$). Larvae from the high microplastics treatment had consumed an average of 7.15 ± 1.2 polystyrene particles, with stomachs containing solely plastic particles. In contrast, fish from the average microplastics treatment consumed 1.4 ± 0.35 plastic particles but also consumed the food source (*Artemia* sp. nauplii) that was available at similar concentrations across all three treatments. Fish that were reared in water that contained no microplastics only had *Artemia* sp. nauplii in their stomachs (Fig. 3, E to J). These results suggest that newly hatched larvae favor microplastic particles over the more natural food source of free-swimming zooplankton. Other aquatic organisms have been found to both passively and actively ingest plastic waste (8, 24). Here it appears that larvae preferentially feed on plastic particles.

Our results suggest that environmentally relevant concentrations of microplastic particles operate both chemically and physically on the early life stages of perch. Not only are crucial behaviors such as activity and feeding affected, but innate responses to olfactory threat cues are impaired. Such loss of predator-avoidance behaviors greatly increased predator-induced mortality rates of larvae.

Increases in microplastic pollution in the Baltic Sea and marked recruitment declines of the coastal keystone species (e.g., perch and pike) have recently been observed (25). It has been suggested that population decline is related to feeding in the juvenile stage, where resource deficits may have led to increased mortality (26). Our study suggests a potential driver for the observed decreased recruitment rate and increased mortality. If early life-history stages of other species are similarly affected by microplastics, and this translates to increased mortality rates, the effects on aquatic ecosystems could be profound. Our findings highlight ecologically important and previously underappreciated effects of microplastic particles that enter marine ecosystems and emphasize the need for new management strategies to control the release of microplastic waste products.

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SUPPLEMENTARY MATERIALS

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CELL REPROGRAMMING

Conversion of human fibroblasts into functional cardiomyocytes by small molecules

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Reprogramming somatic fibroblasts into alternative lineages would provide a promising source of cells for regenerative therapy. However, transdifferentiating human cells into specific homogeneous, functional cell types is challenging. Here we show that cardiomyocyte-like cells can be generated by treating human fibroblasts with a combination of nine compounds that we term 9C. The chemically induced cardiomyocyte-like cells uniformly contracted and resembled human cardiomyocytes in their transcriptome, epigenetic, and electrophysiological properties. 9C treatment of human fibroblasts resulted in a more open-chromatin conformation at key heart developmental genes, enabling their promoters and enhancers to bind effectors of major cardiogenic signals. When transplanted into infarcted mouse hearts, 9C-treated fibroblasts were efficiently converted to chemically induced cardiomyocyte-like cells. This pharmacological approach to lineage-specific reprogramming may have many important therapeutic implications after further optimization to generate mature cardiac cells.

Advances in reprogramming enable the fate of a cell to be changed, with potential applications for regenerative therapy. Cardiomyocyte (CM)-like cells can be reprogrammed from somatic fibroblasts by overexpression of cardiac genes in vitro (1–6) and in vivo (5, 7–10). However, efficiently transdifferentiating human noncardiac cells into highly functional CMs has remained a major challenge (1, 4, 6). In contrast to conventional reprogramming by genetic methods, a chemical reprogramming approach introduces small molecules that interact with and modulate endogenous factors in the starting cell type (e.g., fibroblast) in the absence of target cell type-specific proteins. Small molecules have certain advantages

over genetic methods: They are convenient to use, can be efficiently delivered into cells, provide

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Environmentally relevant concentrations of microplastic particles influence larval fish ecology

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Editor's Summary

Microplastic's triple threat

The billions of tons of plastics that we release into the environment for the most part do not biodegrade. But they do degrade, breaking into ever smaller particles that end up in the oceans. Lönnstedt *et al.* show that the impacts of these microplastics are multifold (see the Perspective by Rochman). Eurasian perch larvae exposed to microplastics were less active, less responsive to predator cues, more likely to be eaten, and less likely to thrive—preferring to eat plastic rather than their natural prey.

Science, this issue p. 1213; see also p. 1172

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