El Colegio de la Frontera Sur

Efecto de los lixiviados de *Sargassum* spp. pelágico en el comportamiento natatorio de las larvas del coral *Acropora palmata*

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Las personas abajo firmantes, miembros del jurado examinador de: **Francisco Antonio Martínez**. Hacemos constar que hemos revisado y aprobado la tesis titulada: **"Efecto de los lixiviados de *Sargassum* spp. pelágico en el comportamiento natatorio de las larvas del coral *Acropora palmata*"**. Para obtener el grado de **Maestro en Ciencias en Recursos Naturales y Desarrollo Rural.**

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A mis papás, Rosalía Martínez y Francisco Antonio,

porque su amor y esfuerzo me han permitido

cumplir un sueño más.

En memoria del Dr. Julio Espinoza Avalos.

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RESUMEN

Una perturbación emergente para los arrecifes del Caribe es el arribo masivo de sargazo pelágico, el cual deteriora la calidad del agua debido a la producción de lixiviados. Los picos de la afluencia de sargazo en el Caribe mexicano pueden coincidir con el periodo de desove de corales liberadores de gametos. En este estudio, las larvas del coral *Acropora palmata* (Lamarck, 1816) fueron expuestas a cinco tratamientos (testigo, colorante café y tres concentraciones de lixiviados de sargazo al 25 %, 50 % y 100 %) durante 30 minutos (10 minutos de filmación + 20 minutos de observaciones) para determinar si los lixiviados modifican el comportamiento natatorio de las larvas. En las filmaciones se observó que las larvas con lixiviados redujeron la velocidad de nado, aumentaron el porcentaje de individuos nadando en espiral, fueron geotácticamente positivas y la mayoría de los pares de comportamiento cinético mostraron baja frecuencia comparado con las larvas sin lixiviados. Las respuestas de las larvas ocurrieron independientemente de las concentraciones de lixiviados. El comportamiento sintomático de nado en espiral fue mayor en presencia de lixiviados, sugiriendo que este comportamiento podría ser un efecto de la contaminación. Durante las observaciones posteriores a las filmaciones, la mayoría de las larvas con lixiviados permanecieron inmóviles comparado con las larvas sin lixiviados. Por primera vez, se reporta a los lixiviados de sargazo como un disturbio que modifica el comportamiento natatorio de las larvas, los cuales podrían reducir la dispersión de las larvas y el incremento de colonias provenientes de la reproducción sexual de *A. palmata*. Se sugiere evaluar los efectos de los lixiviados en larvas expuestas a menores concentraciones y con mayor tiempo de exposición. La resiliencia de los corales puede ser rebasada si el arribo masivo de sargazo se convierte en un evento frecuente.

Palabras clave: *Sargassum fluitans* · *Sargassum natans* · especie en peligro crítico · florecimientos algales · Caribe mexicano.

INTRODUCCIÓN

Los arrecifes de coral poseen una alta importancia ecológica, económica y de protección costera, ya que proporciona complejidad estructural, alta diversidad biológica, recursos pesqueros y atractivos turísticos, así como reducir el impacto de los huracanes en las zonas costeras (Kuffner y Paul 2004). Sin embargo, los arrecifes están siendo degradados en una tasa alarmante (Szmant 2002), especialmente los del Mar Caribe (Pandolfi et al. 2003). En algunos de estos arrecifes, la cobertura de corales ha disminuido hasta un 80 % (Dixson et al. 2014). En contraparte, las macroalgas están ocupando estos nuevos espacios disponibles, situación que se ha reportado en diversos arrecifes de coral, incluyendo los del Mar Caribe (Lirman 2001; Birrell et al. 2008; Arias-González et al. 2017). La presencia de macroalgas solía ser discreta en la mayoría de las comunidades de arrecifes coralinos antes de la década de los 80; sin embargo, su presencia ha aumentado en los últimos años (Birrell et al. 2008). A partir de los 80, la degradación de los arrecifes coralinos ha ocurrido gradualmente a escala global (Hughes et al. 2010), lo que ha propiciado que se incrementen las interacciones entre macroalgas y corales (Lirman 2001).

Un estresor emergente para los arrecifes del Caribe es la llegada masiva de dos especies de macroalgas pelágicas, *Sargassum fluitans* (Børgesen) Børgesen, 1914 y *S. natans* (Linnaeus) Gaillon, 1828 (van Tussenbroek et al. 2017). El sargazo pelágico se encuentra a la deriva en la superficie del agua de mar, lo que proporciona un hábitat importante para una gran diversidad de organismos (Wells et al. 2004; Witherington et al. 2012). El incremento excesivo de la biomasa de *Sargassum* spp. pelágico se detectó en 2011 en el Océano Atlántico central y el Mar Caribe (Wang et al. 2019). Sin embargo, la afluencia masiva de *Sargassum* pelágico en el Caribe mexicano ocurrió hasta el 2014, con los picos más altos reportados en septiembre de 2015 y mayo de 2018 (Rodríguez-Martínez et al. 2019). Desafortunadamente, se predice que la llegada masiva del sargazo pelágico será una perturbación frecuente (Wang et al. 2019).

La descomposición de la excesiva biomasa acumulada de *Sargassum* pelágico produce lixiviados, los cuales reducen el oxígeno disuelto (OD) y el pH, aumentan la turbidez y la temperatura, y deterioran la calidad del agua (van Tussenbroek et al. 2017; Rodríguez-Martínez et al. 2019). Estos lixiviados, que crean condiciones similares a la eutrofización, pueden contener metales pesados como mercurio (Hg), metilmercurio (MeHg) (Vieira et al. 2017), metano (Herrmann et al. 2015), ácido láctico (Milledge y Harvey 2016) y altas concentraciones de amonio y fósforo (Rodríguez-Martínez et al. 2019). En el Caribe mexicano, existen diversas especies de corales (constructora de arrecifes y oportunistas) que habitan en la laguna arrecifal (i.e., cercanas a la costa), donde se ha observado la presencia de lixiviados de *Sargassum*. Estos lixiviados se dispersan de la costa hacia mar adentro, en algunos casos hasta la cresta arrecifal. En este escenario, algunas especies de corales han presentado estrés y mortalidad (parcial o total), debido a la presencia de *Sargassum* y su descomposición (van Tussenbroek et al. 2017).

La especie de coral *Acropora palmata* es un elemento primario para la formación de arrecifes de coral en áreas poco profundas del Mar Caribe (Szmant 1986). Es una especie hermafrodita que libera sincrónicamente paquetes (con gametos masculinos y femeninos) en un desove anual que puede ocurrir entre julio y septiembre (van Woesik et al. 2006). El coral *A. palmata* se encuentra en la categoría "En peligro crítico" de acuerdo a la Unión Internacional para la Conservación de la Naturaleza (UICN) (Aronson et al. 2008) y “Sujeta a protección especial” decretado en la NOM-059-SEMARNAT-2010 en México (SEMARNAT 2010) debido a su vulnerabilidad a enfermedades emergentes (Aronson y Precht 2001; Baums et al. 2005; Williams et al. 2008), blanqueamiento (Muller et al. 2008) y huracanes (Aronson y Precht 2001; Alcolado et al. 2009), los cuales han diezmado sus poblaciones con tasas bajas de recuperación (Rodríguez-Martínez et al. 2014). La reproducción sexual de *A. palmata* es casi inexistente debido a la vulnerabilidad de sus etapas tempranas (e.g., larvas) (Albright et al. 2010; Denis et al. 2014; Humanes et al. 2016; Mora et al. 2016). En contraparte, la reproducción asexual (i.e., la clonación; Lirman 2000) es la fuente que contribuye al incremento de sus colonias.

El comportamiento de las larvas de corales se ha utilizado para evaluar la respuesta de las primeras etapas de vida de los corales bajo diferentes condiciones ambientales o estresores como petróleo (Hartmann et al. 2015), dispersantes de petróleo (Epstein et al. 2000), compuestos alelopáticos de algas (Denis et al. 2014; Dixson et al. 2014), enriquecimiento de nutrientes, aumento de temperatura (Humanes et al. 2016), y presencia de macroalgas (Vermeij et al. 2009) y cianobacterias (Kuffner et al. 2006). El comportamiento de las larvas de coral se ha clasificado por cambios en el patrón de natación (lineal, sinuoso, circular, de inmersión, en espiral, balanceo, hacia arriba, hacia abajo, con paradas, en reversa y al azar), velocidad de natación (mm/s), evaluaciones o pruebas (también denominado "testing") del sustrato (n s^{-1}), desplazamiento a lo largo del fondo y capacidad de asentamiento. Además, el análisis de trayectometría en animales (registro del desplazamiento de animales individuales que se mueven a través del espacio y el tiempo evaluados en dos dimensiones). La trayectometría también se ha utilizado para responder preguntas sobre alimentación, navegación, ecología de dispersión, migración e imitación del comportamiento animal (Codling et al. 2008).

En este estudio expusimos las larvas de *A. palmata* bajo cinco tratamientos (testigo, colorante (simulando el color de los lixiviados) y tres concentraciones de lixiviados (25 %, 50 % y 100 %) para observar (durante 30 minutos) si los lixiviados de *S. fluitans* y *S. natans* modifican el comportamiento de las larvas bajo condiciones de acuario. Se utilizaron filmaciones de 10 minutos para obtener índices de trayectometría globales y específicos (e.g., distancia, tiempo y velocidad del desplazamiento de las larvas) y la frecuencia del comportamiento cinético. Después de las filmaciones, se realizaron 4 observaciones de la actividad de las larvas durante 20 minutos (a los 5, 10, 15 y 20 minutos). En este trabajo, *S. fluitans* y *S. natans* fueron sumergidos en agua de mar filtrada durante cuatro días para obtener los lixiviados. Esta es la primera contribución que demuestra los efectos negativos de los lixiviados de *Sargassum* pelágico en el comportamiento de las larvas de coral. Sin embargo, el estudio de otras etapas tempranas de vida de los corales en concentraciones menores podría ayudar a obtener resultados más cercanos a los que ocurren en el campo, especialmente porque la llegada masiva de *S. fluitans* y

S. natans parece ser una perturbación que se puede seguir presentando en las costas del Mar Caribe. El presente trabajo demuestra que la afluencia excesiva de *Sargassum* pelágico en el Caribe mexicano es una amenaza adicional que puede afectar la reproducción sexual de los corales.

ARTÍCULO

Leachate effects of pelagic *Sargassum* spp. on larval swimming behavior of the coral *Acropora palmata*

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Leachate effects of pelagic *Sargassum* spp. on larval swimming behavior of the coral *Acropora palmata*

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An emerging disturbance for Caribbean reefs is the massive arrival of pelagic *Sargassum*, which deteriorates coastal water quality due to the production of leachates. Highest arrivals may occur during the annual broadcast spawning corals (July-October). We evaluated, through five treatments (control, stain (simulating 100% leachate color) and 25%, 50% and 100% *Sargassum* leachate concentrations) during 30 min (10 min of shooting + 20 min of post-observations), if *Sargassum* leachates modify swimming behavior of *Acropora palmata* larvae. During shooting, larvae with leachates reduced swimming speed, increased the percentage of individuals swimming in spiral, were positively geotactic and most kinetic behavior pairs presented lower frequencies than larvae without leachates. These behaviors occurred for all leachate concentrations. Moreover, symptomatic spiral behavior was higher in the presence of leachates, suggesting that this behavior may be an effect of pollution. During post-observations, most larvae with leachates were motionless. This is the first time that *Sargassum* leachates are reported as a disturbance that modifies larval swimming behavior, which may reduce larval dispersion and genetic diversity. We suggest an evaluation of the effects of leachates at lower concentrations and longer periods of exposure. The resilience of corals may be exceeded if *Sargassum* arrivals become a frequent event.

Introduction

Globally, coral reefs are being degraded by several stressors (e.g., global change, bleaching, overfishing and emergent diseases[1–5], especially those in the Caribbean[6]. An emerging stressor for Caribbean reefs is the massive arrival of two pelagic macroalgal species, *Sargassum fluitans* and *S. natans*. Pelagic *Sargassum* drifts on the sea surface, providing an

important habitat for a great diversity of organisms[7, 8]. The presence of high biomass of pelagic *Sargassum* spp. began in 2011 in the central Atlantic Ocean and the Caribbean Sea[9]. However, *Sargassum* massive influx in the Mexican Caribbean has occurred since 2014, with the highest peaks reported in September 2015 and May 2018[10]. Unfortunately, it is predicted that massive arrival of pelagic *Sargassum* will be a recurring disturbance[9].

Decomposition of the accumulated biomass of pelagic *Sargassum* produces leachates that reduced dissolved oxygen (DO) and pH, and increase turbidity and temperature, deteriorating water quality[10, 11]. These leachates create conditions similar to eutrophication and may contain heavy metals such as mercury (Hg) and methylmercury (MeHg)[12], methane[13], lactic acid[14] and high concentrations of ammonium and phosphorus[10]. In the Mexican Caribbean, there are various coral species that inhabit lagoon reefs (i.e., near to the coastline) where leachates of pelagic *Sargassum* are observed and dispersed from the coastline toward the reef crest. In this context, some coral species were stressed and presented partial or total mortality due to *Sargassum* presence and their decomposition[11] (Fig. 1).

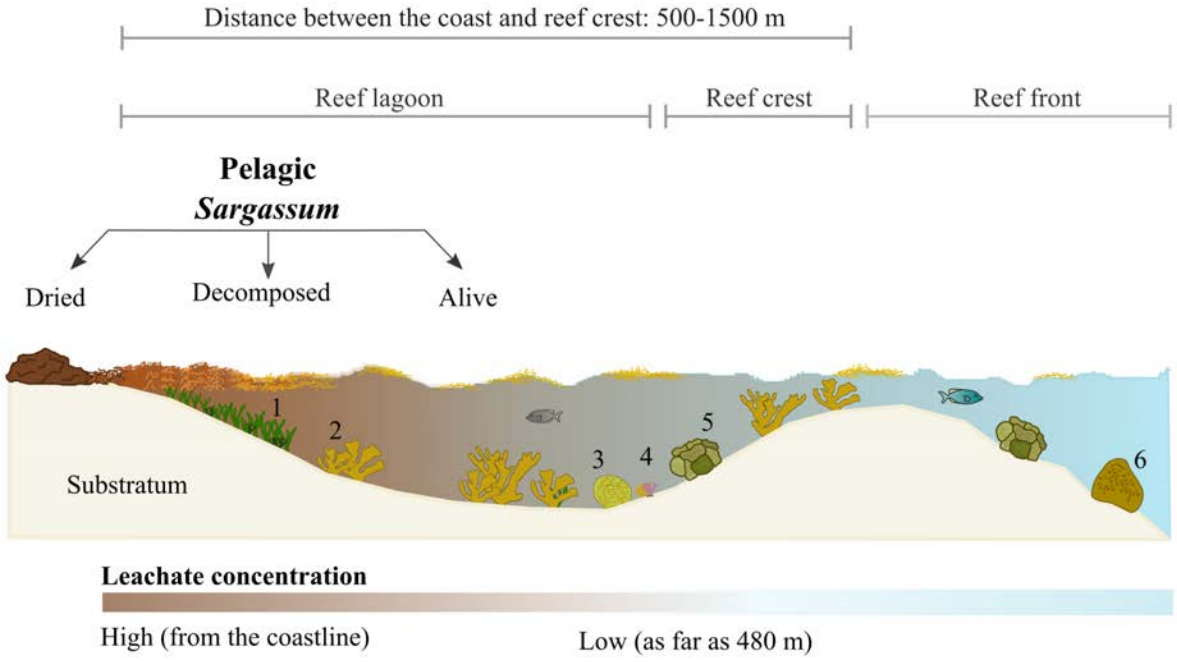

Figure 1. Schematic profile of location at Punta Venado, Quintana Roo, Mexico. Here, thick caps of pelagic *Sargassum* that accumulate at the coast, produce leachates when decomposing. Leachate concentration decreases with distance from the coast. 1= seagrass meadows mixed with seaweeds. Reef-building corals: 2= *Acropora palmata*; 3= *Pseudodiploria*; 5= *Orbicella annularis*; and 6= *O. faveolata*. Weedy corals: 4= *Agaricia* and *Porites*. Based on[10, 11, 52] and pers. obs. in the field. See References and text for more details.

Acropora palmata is a primary element for coral reef formation in shallow areas of the Caribbean Sea[15]. This hermaphrodite species synchronously release bundles with both gametes in an annual spawning that may occur from July to September[16]. This coral species is classified as “Critically endangered" by the International Union for the Conservation of Nature (IUCN)[17] and subject to special protection by NOM-059-SEMARNAT-2010 in Mexico[18] as emergent diseases[19–21], bleaching[2], and hurricanes[19, 22] have decimated its

populations with low rates of recovery[23]. Moreover, the increment of its population is predominantly a result of asexual[23] rather than sexual reproduction because early life stages (e.g., coral larvae) appear to be vulnerable[25–28].

Coral larvae behavior has been used to assess the response of early coral stages under different environmental conditions or stressors such as oil[29], oil dispersants[30], allelopathic algae compounds[26, 31], nutrient enrichment, temperature increase[27], macroalgal[32] and cyanobacteria presence[33]. Larval behavior has been classified by changes in swimming patterns (linear, sinuous, circular, immersion, spiral, swinging, up, down, with stops, in reverse and random), swimming speed (mm/s), evaluations or tests ('tests') of the substrate (n s^{-1}), displacement along the bottom and settlement capacity. In addition, trajectometry analysis in animals (registered tracks of individual animals that move through space and time evaluated in two dimensions) has also been used to answer questions related to foraging, navigation, dispersion ecology, migration, and mimicry of animal behavior[34].

In this study, we exposed competent larvae of *A. palmata* under five treatments (control, stain (caramel artificial food color simulating 100% leachate color), and 25%, 50% and 100% *Sargassum* leachate concentrations) to observe if leachates of *S. fluitans* and *S. natans* modify larval swimming behavior under aquarium conditions. Ten min shooting was used to obtained global and specific trajectometry indexes (e.g., distance, time, and speed of larvae) and frequency of kinetic behavior pairs; subsequently, larval activity was observed during 20 min (hereafter called “post-observations”). Larvae with leachates swam slower displaced over shorter distances and were positive geotactic. The majority of kinetic behavior pairs (i.e., partial displacements) displayed low frequency and most were motionless. Our results revealed that larval dispersal capacity may be reduced and even stopped in presence of *Sargassum* leachates. In addition, *A. palmata* larvae in

contact with *Sargassum* leachates displayed a spiral movement, which could be a symptomatic behavior of coral larvae exposed to pollution. This is the first contribution that demonstrates the negative effects of *Sargassum* leachates on swimming behavior of coral larvae; however, exploration of other life stages at lower concentrations will provide more accurate results that occur in environmental conditions given that massive arrivals of *Sargassum* appears to be a new frequent disturbance in the Caribbean Sea. Under this possible scenario, we showed that *Sargassum* arrivals in the Caribbean might be a major threat for sexual coral reproduction.

Results

Global and specific trajectometry indexes

In total, 23 trajectometry indexes were evaluated in *A. palmata* larvae: 11 global and 12 specific. Irrespective of concentration, *Sargassum* leachates had negative effects on 9 global and 9 specific trajectometry indexes as mentioned below. *Sargassum* leachates affected larval performance; larvae in contact with leachates became slower (hypoactive) and swam shorter distances, decreasing dispersion capacity. In addition, larvae in contact with leachates became positively geotactic, while larvae in control and stain treatments became negatively geotactic.

Global trajectometry indexes

Displacement-length of larvae in contact with leachates (25%: 75.3 ± 11.0 mm, 50%: 65.3 ± 7.1 mm, and 100%: 74.9 ± 8.3 mm) was 2.5 times lower than larvae in both control (190.4 ± 16.8 mm) and stain (188.6 ± 20.9 mm) treatments (KW: $H_{(4,244)}$=59.04, P<0.001; Fig. 2A). In contrast, displacement-time without stops of larvae in contact with leachates

(25%: 369.0 ± 23.6 s, 50%: 355.7 ± 22.8 s, and 100%: 355.1 ± 21.0 s) was greater compared to larvae without leachates (control: 235.1± 16.4 s, and stain: 246.5 ± 20.3 s; ANOVA: $F_{(4,244)}$=9.80, *P*<0.001; Fig. 2B).

Larvae with leachates were twice as slower with regard to their displacement-speed with stops (25%: 0.13 ± 0.02 mm/s, 50%: 0.11 ± 0.01 mm/s, and 100%: 0.13 ± 0.01 mm/s) compared with larvae not exposed to leachates (control: 0.32 ± 0.03 mm/s, and stain: 0.31 ± 0.03 mm/s; $H_{(4,244)}$=59.29, *P*<0.001; Fig. 2C). However, without stops, the displacement-speed of larvae with leachates (25%: 0.21 ± 0.02 mm/s, 50%: 0.19 ± 0.02 mm/s, and 100%: 0.21 ± 0.02 mm/s) was four times slower than larvae in absence of leachates (control: 0.80 ± 0.05 mm/s, and stain: 0.75 ± 0.06 mm/s; $H_{(4,244)}$=144.24, *P*<0.001; Fig. 2D).

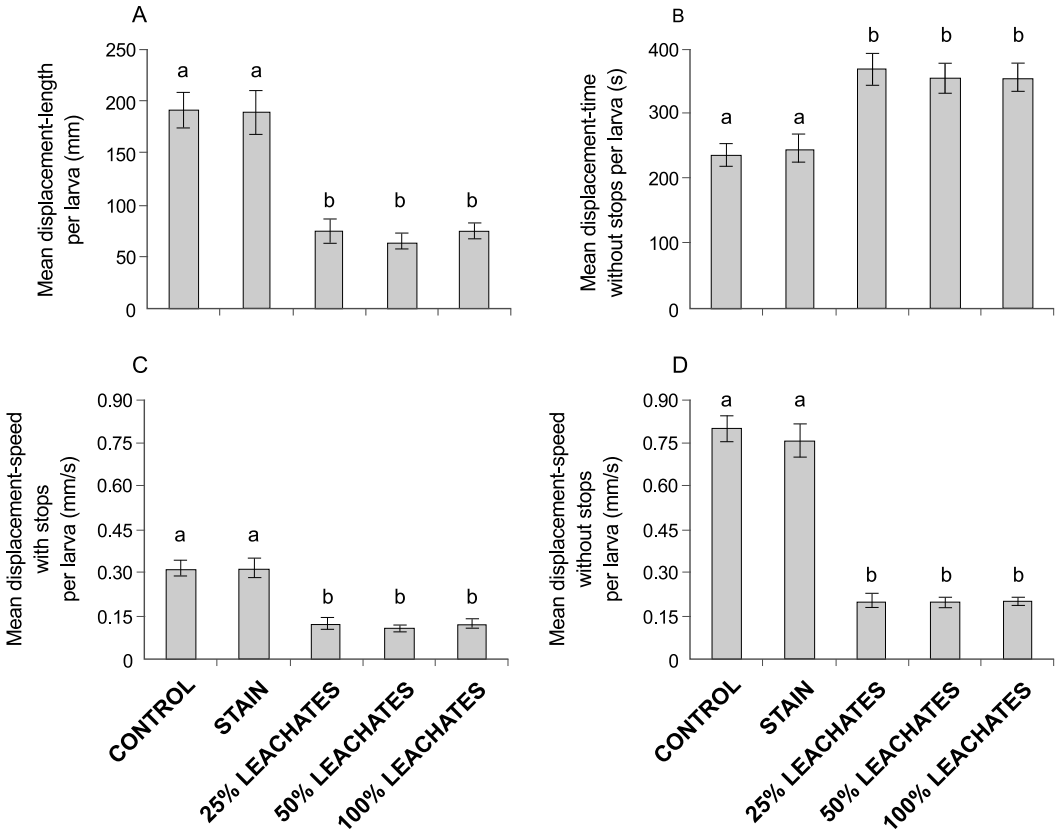

Figure 2. Global indexes evaluated through five treatments (control, stain, and 25%, 50% and 100% leachates) in *Acropora palmata* larvae: (**A**) Mean displacement-length, (**B**) mean displacement-time without stops, (**C**) mean displacement-speed with stops, and (**D**) mean displacement-speed without stops per larva. Letter above the bars indicate significant differences according to post hoc analyses. Error bars represent standard error. See text for more details.

Number of displacements (25%=5.6 ± 0.5, 50%=5.1 ± 0.5, and 100%=4.9 ± 0.5 vs. control=9.0 ± 0.6, and stain=8.5 ± 0.7; $H_{(4,244)}$=37.70, P<0.001; Fig. 3A) and number of stops (25%=1.7 ± 0.1, 50%=1.7 ± 0.1, and 100%=1.6 ± 0.1 vs. control=3.3 ± 0.2, and stain=3.1 ± 0.2; $H_{(4,244)}$=68.88, P<0.001; Fig. 3B) of larvae in contact with leachates were almost half compared with larvae in absence of leachates. In addition, time stops per larva in contact with leachates (25%: 236.8 ± 23.8 s, 50%: 246.2 ± 23.2 s, and 100%: 255.1 ± 20.5 s) was at least 100 s lower compared with larva in absence of leachates (control: 364.9 ± 16.4 s, and stain: 353.5 ± 20.3 s; $H_{(4,244)}$=32.34, P<0.001; Fig. 3C).

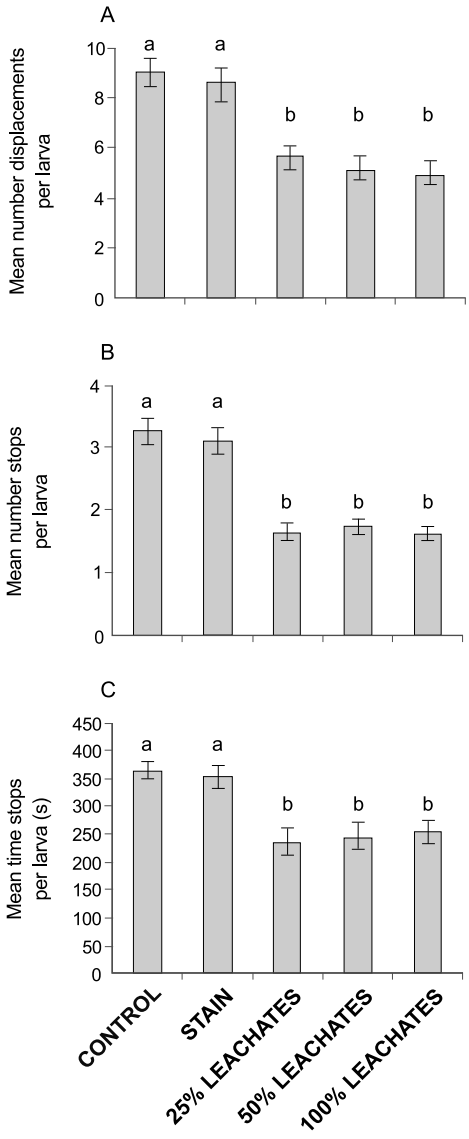

Figure 3. Global behavior indexes evaluated on coral larvae under five conditions (control, stain, and 25%, 50% and 100% leachates): (**A**) Mean number displacements, (**B**) mean number stops, and (**C**) mean time stops per larva. Letter above the bars indicate significant differences according to post hoc analyses. Error bars represent standard error.

Spiral movement is characterized by a rotation movement on its own axis either clockwise or counterclockwise; however, this movement was observed without

displacement or with slow displacement in the tank. Spiral movement showed differential responses in two of four trajectometry indexes evaluated among treatments as followed (Fig. 4). The percentage of larvae with spiral displacement in 25% (82 ± 6%) and 50% leachates (58 ± 7%) was almost double than in the control (12 ± 4%) and 100% leachates (30 ± 11%) treatments, while larvae in 25% leachates displayed almost three times lower percentage of spiral displacement than larvae in stain (30 ± 8%; $H_{(4,107)}$=25.76, P<0.001; Fig. 4A). The displacement-time in spiral per larva in leachates treatments (25%: 336.4 ± 22.7 s, 50%: 291.9 ± 27.8 s, and 100%: 296.0 ± 30.9 s) was three times greater than in the stain treatment (86.7 ± 14.9 s), while control (152.0 ± 80.4 s) was similar to stain and leachate treatments ($H_{(4,107)}$=32.06, P<0.001; Fig. 4C). In contrast, the displacement-length (8.4–34.3 ± 3.1–7.3 mm; $H_{(4,107)}$=12.29, P>0.05; Fig. 4B) and the displacement-speed (0.10–0.20 ± 0.02–0.05 mm/s; $H_{(4,107)}$=5.46, P>0.05; Fig. 4D) in spiral of larvae were not different among treatments.

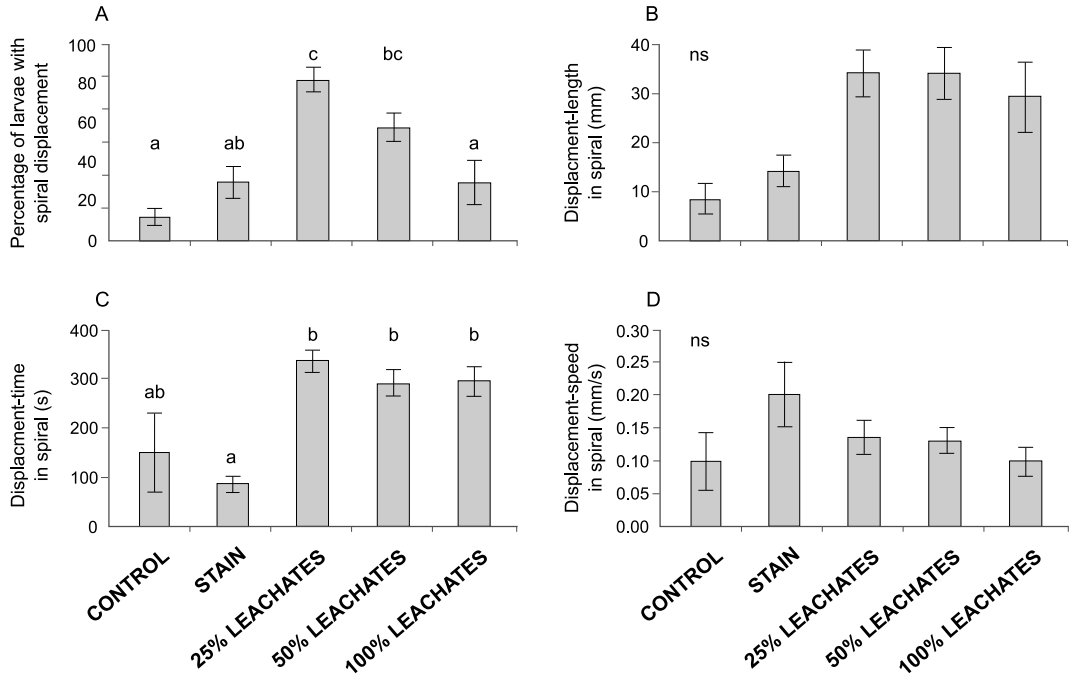

Figure 4. Four trajectometry indexes to evaluate spiral movement in *Acropora palmata* larvae under five treatments (control, stain, and 25%, 50% and 100% leachates). (**A**) Percentage of larvae with spiral displacement. (**B**) Displacement-length in spiral. (**C**) Displacement-time in spiral. (**D**) Displacement-speed in spiral. Letter above the bars indicate significant differences according to post hoc analyses; ns=not significant. Error bars represent standard error.

Specific trajectometry indexes

On the surface, displacement-length of larvae with leachates (25%: 36.5 ± 13.1 mm, 50%: 18.6 ± 5.1 mm, and 100%: 19.4 ± 4.9 mm) was at least three times shorter when compared with larvae without leachates (control: 111.1 ± 15.0 mm, and stain:127.7 ± 19.5 mm; $H_{(4,89)}$=36.77, P<0.001; Fig. 5A). No difference in displacement-time per larva among treatments was found (110.0–206.2 ± 13.7–48.1 s; $H_{(4,89)}$=5.07, P>0.05; Fig. 5B). Displacement-speed per larva with leachates (25%: 0.34 ± 0.08 mm/s, 50%: 0.10 ± 0.02 mm/s, and 100%: 0.17 ± 0.04 mm/s) was at least three times lower when compared with larvae in absence of leachates (control: 1.12 ± 0.10 mm/s, and stain: 1.29 ± 0.11 mm/s; $F_{(4,89)}$=57.51, P<0.001; Fig. 5C).

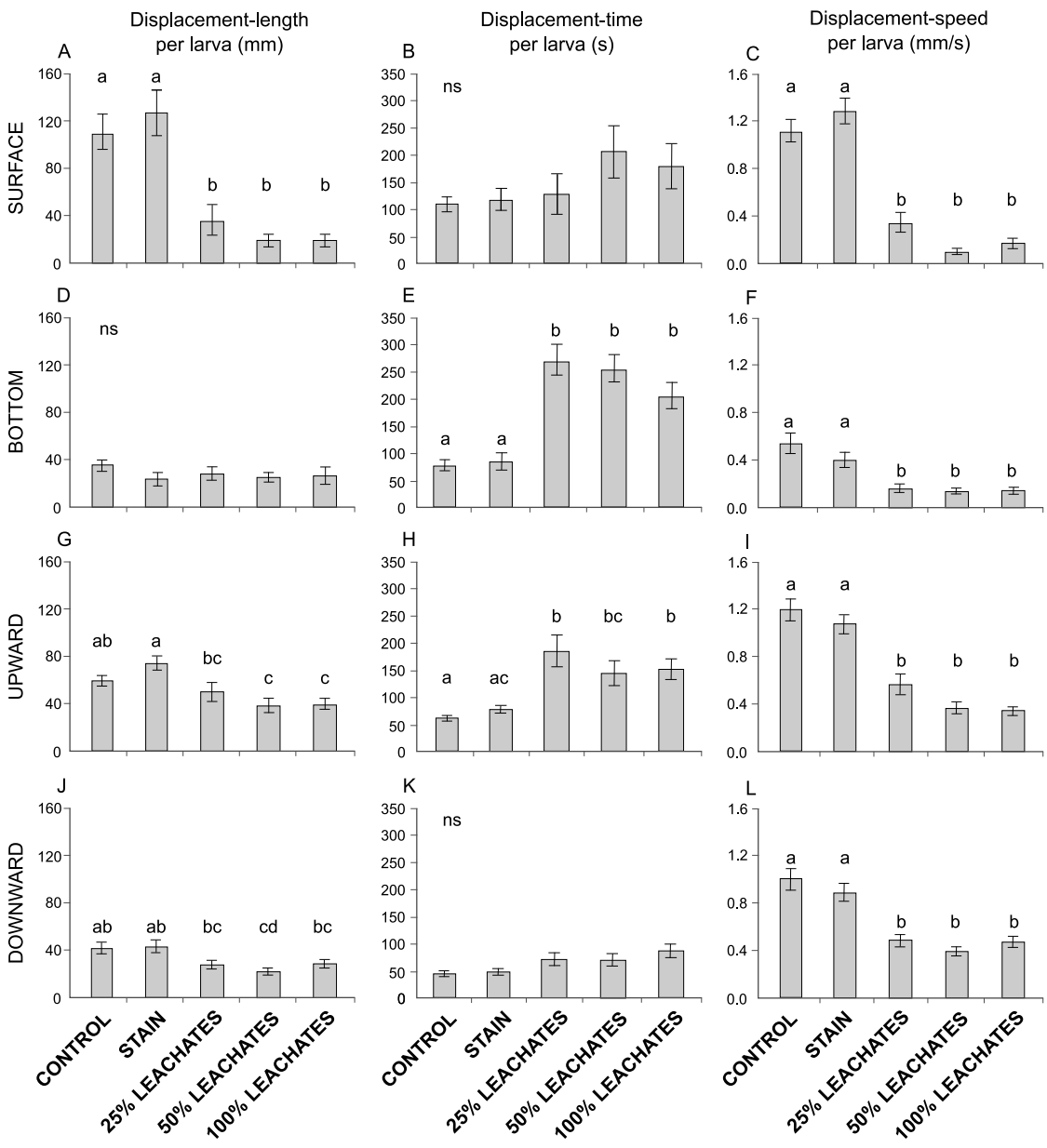

Figure 5. Specific trajectometry indexes evaluated per larva of *Acropora palmata* under five treatments (control, stain, and 25%, 50% and 100% leachates). (**A**,**D**,**G**,**J**) Displacement-length per larva. (**B**,**E**,**H**,**K**) Displacement-time per larva. (**C**,**F**,**I**,**L**) Displacement-speed per larva. (**A**,**B**,**C**) Trajectometry indexes per larva swimming on surface. (**D**,**E**,**F**) Trajectometry indexes per larva moving at the bottom. (**G**,**H**,**I**) Trajectometry indexes per larva swimming upward. (**J**,**K**,**L**) Trajectometry indexes per

larva swimming downward. Letter above the bars indicate significant differences according to post hoc analyses; ns=not significant. Error bars represent standard error.

At the bottom of the tank, no difference in displacement-length per larva among treatments was found (24.5–35.8 ± 5.0–7.3 mm; $H_{(4,168)}$=3.73, P>0.05; Fig. 5D). Displacement-time per larva in contact with leachates (25%: 276.5 ± 27.9 s, 50%: 260.8 ± 25.1 s, and 100%:208.4 ± 24.3 s) was three times higher when compared with larva in both control (78.9 ± 10.3 s) and stain (86.0 ± 15.4 s; $H_{(4,168)}$=57.69, P<0.001; Fig. 5E). In contrast, displacement-speed per larva with leachates (25%: 0.16 ± 0.03 mm/s, 50%: 0.14 ± 0.02 mm/s, and 100%: 0.14 ± 0.03mm/s) was at least twice lower when compared with both control (0.54 ± 0.09 mm/s) and stain (0.40 ± 0.06 mm/s; $F_{(4,168)}$=8.17, P<0.001; Fig. 5F).

In upward swimming, displacement-length per larva in 50% (38.6 ± 6.1 mm) and 100% leachates (100%: 39.7 ± 4.5 mm) was lower than in both control (59.8 ± 4.0 mm) and stain (74.3 ± 6.6 mm); however, there were no differences observed between displacement-length per larva in control and 25% leachates (50.6 ± 8.1 mm) ($H_{(4,155)}$=28.67, P<0.001; Fig. 5G). Displacement-time per larva in 25% (185.0 ± 28.9 s) and 100% (153.0 ± 18.7 s) leachates was twice as long when compared to control (60.7 ± 5.0 s) and stain (77.3 ± 6.8 s); however, 50% leachates (143.4 ± 23.9 s) presented similar results to those observed with stain, but not control ($H_{(4,155)}$=22.78, P<0.001; Fig. 5H). Displacement-speed per larva with leachates (25%: 0.57 ± 0.09 mm/s, 50%: 0.37 ± 0.05 mm/s, and 100%: 0.35 ± 0.04 mm/s) was 50% slower than larvae without leachates (control: 1.20 ± 0.09 mm/s, and stain: 1.09 ± 0.08 mm/s; $H_{(4,155)}$=73.67, P<0.001; Fig. 5I).

In downward swimming, displacement-length per larva in 50% leachates (22.3 ± 3.2 mm) was half of that found with both control (41.6 ± 4.9 mm) and stain (43.0 ± 5.4 mm; $H_{(4,232)}$=15.73, P<0.001; Fig. 5J). Displacement-time per larva was similar among treatments (45.6–88.0 ± 5.3– 12.4 s; $H_{(4,232)}$=4.06, P>0.05; Fig. 5K). In contrast, displacement-speed per larva with leachates (25%: 0.51 ± 0.05 mm/s, 50%: 0.41 ± 0.04 mm/s, and 100%: 0.50 ± 0.05 mm/s) was 50% slower than larvae without leachates (control: 1.05 ± 0.09 mm/s, and stain: 0.93 ± 0.08 mm/s; $H_{(4,232)}$=65.70, P<0.001; Fig. 5L).

Frequency of kinetic behavior pairs

Overall, 25 behavior pairs were recorded considering all treatments of *A. palmata* larvae, which were assigned to one of three ranks according to their frequencies: high (0.67–∞), medium (0.34–0.66) and low (0.00–0.33) (Fig. 6 and Suppl. Table 1). Larval behavior pairs recorded were 18 (5 high, 8 medium, and 5 low) in control (G=0.9, df=2, P>0.05), 22 (5 high, 5 medium, and 12 low) in stain (G=4.1, df=2, P>0.05), and 22 (2 high, 4 medium, and 16 low) in 25% (G=14.9, df=2, P<0.001), 24 (2 high, 2 medium, and 20 low) in 50% (G=25.5, df=2, P<0.001) and 23 (5 medium, and 18 low) in 100% leachates (G=7.7, df=1, P<0.001).These results show that, most frequencies of behavior pairs observed from larvae in contact with leachates belonged to the low rank, while with control and stain the frequencies of behavior pairs observed did not present any differences among ranks. Spiral movement of coral larvae showed low frequency in all treatments, but only larvae exposed to 25% leachates presented a behavior in spiral with medium frequency (Fig. 6 and Suppl. Table 1).

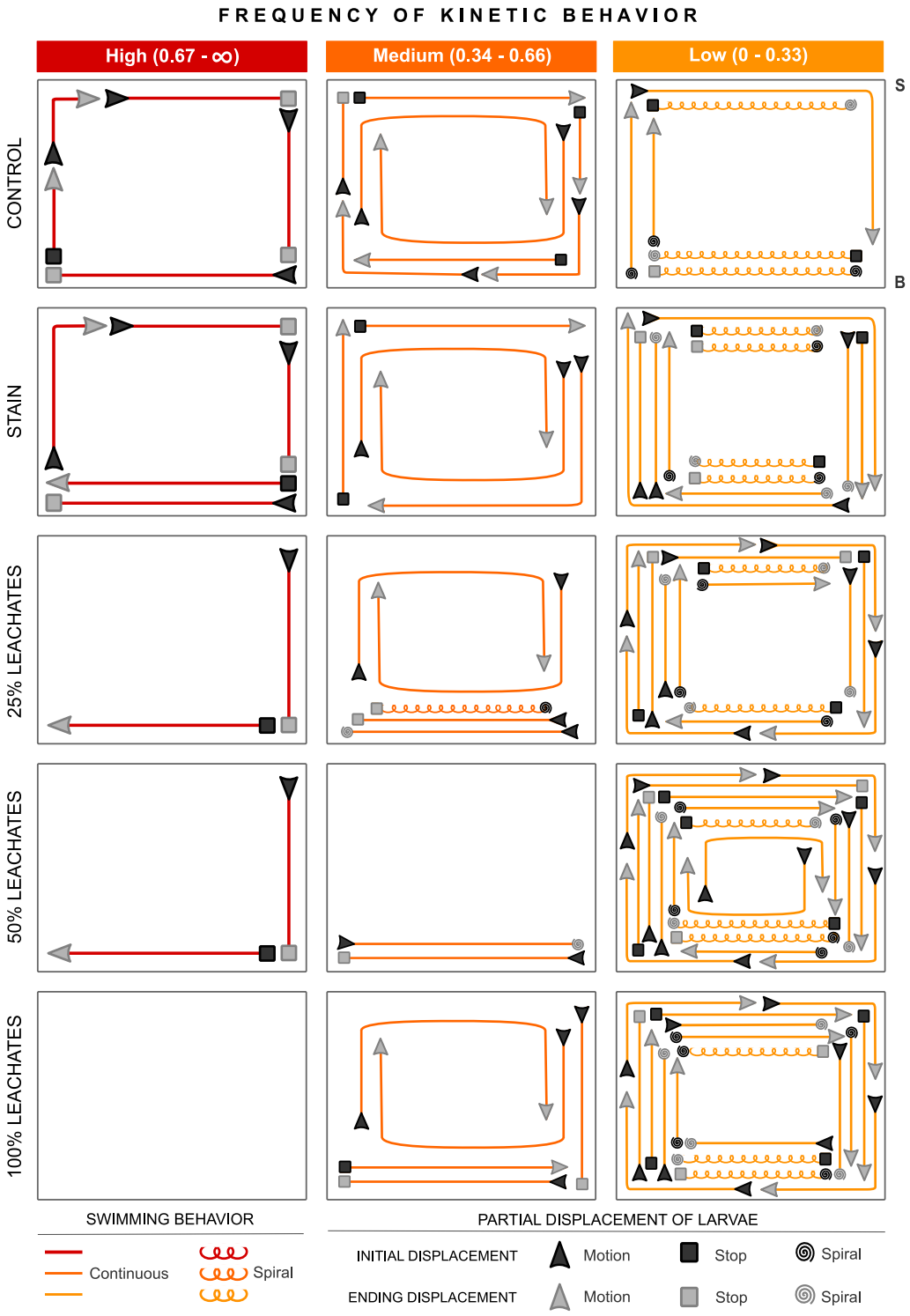

Figure 6. Schematic representation of the frequency of kinetic behavior of *Acropora palmata* larvae under five treatments (control, stain, and 25%, 50% and 100% leachates). Each movement frequency was assigned to one of three ranks: high (0.67–∞), medium

(0.34–0.66), and low (0.00–0.33) frequency. B=movements recorded at the bottom; S=movements recorded in surface.

Post-observations

During post-observations, all larvae in both control and stain were active swimmers, mainly exploring the surface at a high velocity. In contrast, larvae with leachates moved slowly and eventually became motionless, depending on leachate concentration. Most larvae in contact with leachates became motionless at the end of 20 min: 80% of larvae in 25%, and 100% of larvae in 50% and 100% leachates.

Control

During 20 min of observation, all larvae were always active (swimming quickly) compared to larvae in presence of leachates. Larvae mainly swam on the surface and bottom, but they also had the capacity to swim upward, downward and side to side.

Stain

Activity of larvae in contact with food colorant was similar to larvae in control. During 20 min of observation, all larvae displayed a fast motion; their movements were primarily on the surface and on the bottom; however, they also moved up, down, and side to side.

25% leachates

During the first 5 min, almost 80% of larvae were active, but were slower when compared to control and stain, either moving up and down or from side to side. Between 5 to 10 min, the larval activity decreased (i.e., hypoactive) or stopped (i.e., motionless), and larvae were located floating (on the surface or in the middle of the water column of the tanks) or sinking (lying down on the bottom). At 15 min, 80% of larvae were motionless, mostly found on the bottom (but also on the surface or in the middle of water column). In the last 5

min (i.e., 20 min), larvae were located again on the bottom, in the middle water column or on the surface; in addition, 80% of larvae were motionless and 20% of larvae displayed extremely slow mobility.

50% leachates

During the first 10 min, mobility was slow in 60% of larvae. They were located close to the bottom or in the middle of water column of the tanks. During the last 10 min, all larvae were motionless, lying down on the bottom or suspended in the middle of the water column, but not on surface.

100% leachates

During the first min, all larvae reduced their activity and at the end of 5 min, 70% of larvae were motionless and 30% hypoactive. Those larvae moved up and down or erratically. After 5 min, all larvae lost mobility (i.e., completely stopped), and were located on the surface or bottom.

Discussion

This is the first study that shows the effects of pelagic *Sargassum* leachates on coral larval behavior, affecting swimming performance of *A. palmata* larvae. Most of the global and specific trajectometry indexes showed differences among larvae with leachates (25%, 50% and 100%) vs. without leachates (control and stain). Of all trajectometry indexes evaluated, displacement-speed per larva was a pattern that reflected negative effects on larvae exposed to leachates, reducing larval speed up to four times. It has been suggested that stressful environments may influence offspring performance of corals[35], because larvae may respond to environmental changes[36]. In this study, hypoactive larvae in leachates became positively

geotactic (i.e., spent more time at the bottom instead of the surface), which also occurs in coral larvae under stressful conditions[36]. Larvae of *A. palmata* can be competent up to 3 weeks (Szmant unpubl. data, see[37]) but slower speed and lost activity of larvae in contact with leachates in our study suggest that larval dispersal capacity is highly reduced. Larvae with lower activity and positively geotactic behavior in contact with leachates may reduce dispersion that normally occurs on the top of the water column and therefore their recruitment in suitable environments may also be reduced[38, 39]. Stressful environmental conditions and benthic biota may harm early life stages of many organisms. The presence of oil, oil dispersants, nutrient enrichment, ocean acidification, ultraviolet rays and temperature, and the presence of allopathic algae compounds, macroalgae and cyanobacteria might reduce settlement and survivorship of coral larvae[25–27, 29, 32, 33, 40, 41]. Thus, *Sargassum* leachates are a new disturbance that affect coral larvae behavior of *A. palmata* larvae. Therefore, the replacement of *A. palmata* colonies from sexual reproduction might be further reduced.

Little is known about spiral movement as those observed in coral larvae, mostly in presence of *Sargassum* leachates. These particular movements have been reported in larvae of marine invertebrates such as sponges[42, 43], mussels[44] and corals[30, 45, 46]. Spiral movement is usually described as a clockwise rotation in one place or with a slow or erratic displacement[46]. Under increased pressure, coral larvae may swim upwards in spiral, as a compensatory response due to pressure changes[45]. However, spiral motion has also been referred to as a negative response under different concentrations of oil dispersants, affecting normal behavior of swimming and promoting disoriented spiral movement after several hours[30]. In our study, the percentage of larval with spiral movement was higher in contact with 25% and 50% leachates. However, similar responses among control, stain and 100%

leachates may have an alternate explanation. In control and stain, low percentage of spiral movement may be related to normal reproductive failure as occurs in juvenile marine invertebrates with high rates of mortality in natural conditions[48]. In contrast, low percentage of larvae that swim in a spiraling motion in contact with 100% leachates may be because most kinetic behavior pairs showed low frequencies, but movements of high frequency were absent. In another study of marine invertebrates, it has been reported that less active sponge larvae swim in a spiral or responded weakly when faced with a stimulus such as light; in contrast, active larvae displayed a negative swimming from light[42]. Thus, *Sargassum* leachates affected larvae, reducing swimming performance and possibly altering sensory perception as has been demonstrated in fish exposed to metals[48].

Larval activity in contact with leachates decreased as leachate concentration increased. Thirty minutes (10 min of shooting + 20 of post-observations) of leachate exposure were sufficient to observe that all coral larvae were motionless in 50% (until 10 min) and 100% leachates (until 10 and 5 min, respectively), while 80% of larvae were motionless in 25% leachates (until 15 min). Nevertheless, if larvae were exposed over a longer time period in 25% leachates, they would probably have become motionless. We cannot be certain that motionless larvae at the bottom settled or died, but slower speeds, lower dispersion and low frequency of kinetic behavior pairs exhibited might indicate that leachates eventually killed *A. palmata* larvae. Coral larvae exposed to dispersants may survive more than 96 h, but those larvae (with deformations, and atypical swimming and searching behavior of substratum) sooner or later died[30]. Therefore, larvae exposed to leachates may reduce dispersion, decreasing their capacity to find better conditions to settle and recruit.

In this study, values of DO (1.10 mg L^{-1}) and pH (7.4) in 25% leachate concentration were similar to those reported in two Mexican Caribbean reefs[10,11] at similar distances where *A. palmata* gametes were collected (from 60 m away of the coast; Fig. 1 and Suppl. Fig. 1) as mentioned below. On August 2015, a southern coral reef showed values ~1.00 mg L^{-1} of DO between 50 and 100 m away from the coastline, while pH of 7.4 was recorded at 50 m[11]. On May 2018, a northern coral reef (closer to our study site) showed values of 1.90 mg L^{-1} of DO at 130 m away from the coastline[10]. Because larval responses in contact with leachates were deleterious, whatever the concentration, the effects on *A. palmata* larvae exposed to leachates in natural conditions may also be negative. Higher *Sargassum* biomass was reported from May to July on 2018 in the Caribbean Sea[9]; however, fauna mortality was observed from May to September[11]. This information suggests the possibility of *Sargassum* leachates producing negative effects during spawning of *A. palmata*.

Leachates derived of *Sargassum* decomposition is an additional source of stress for coral larvae of *A. palmata*; however, leachates may affect other early life stages of corals, such as fecundity, embryogenesis, settlement and recruitment. Larvae affected by leachates in their natural environment is possible because the highest biomass period of *Sargassum* arrival[9] and high fauna mortality[10] coincide with the months of broadcast spawning corals in the Caribbean (July-October)[16]. In addition, *Sargassum* leachates may reduce the input of sexual recruits of *A. palmata* and other coral species that live in shallow environments. Another concern for the early life stages of corals is the possibility that drifting *Sargassum* could trap gametes and embryos because of buoyancy[38], and larvae due to negative geotactic behavior[36], at least during the first few hours[49], which may increase mortality. If massive arrival of *Sargassum* on the coast of the Mexican Caribbean promotes death in

adult corals[11], drifting *Sargassum* and leachates may have more deleterious effects on their early life stages due to higher mortality rates[47]. The future of coral reefs is uncertain because reef-building corals are decreasing, while small and opportunistic corals species appear to be increasing in abundance[50]. However, reef-building (this study) and opportunistic corals have been affected by *Sargassum* arrivals[11], which may reduce genetic diversity. Another concern is the implementation of barriers in the reef lagoons to trap *Sargassum* (placed in the north of the Mexican Caribbean[11]). This may increase leachate concentration if *Sargassum* collection does not occur before decomposition begins (between hours and days after getting trapped), depending on environmental conditions. Although 25% leachates affected larval swimming performance of *A. palmata*, we recommend an evaluation of lower leachate concentrations and more exposure time to obtain results that are closer to those that occur in the field. The resilience of corals (reef-building and opportunistic) may be exceeded if massive *Sargassum* arrivals become a frequent event in the Caribbean, which has been recurrent since 2011 in this region[9].

Materials and Methods

Study area

Gamete bundles of *Acropora palmata* were collected at Punta Venado (20º31'57" N, 87º10'26" W), the northern part of Quintana Roo, in the Mexican Caribbean (Suppl. Fig. 2). Punta Venado is part of the Mesoamerican Barrier Reef[51], which is considered as the second largest coral barrier after the GBR in Australia. *A. palmata*, an abundant reef-building coral species in the northern part of the Mexican Caribbean[23], may experience

contact with *Sargassum* leachates (Suppl. Fig. 3). In addition, *A. palmata* colonies are found 60 m away from the coastline at Punta Venado (Fig. 1).

Elaboration of coral larvae tanks and substrates

Small tanks (5 x 5 x 1.2 cm) for coral larvae were constructed with slides of 7.5 x 5 cm, which were cut and glued (with Norland Optical Adhesive 81) in the optical workshop at Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE). To prepare substrates, polystyrene rectangles (5 x 1.2 cm) were cut and sanded, while tiles of limestone were mashed and filtered in a strainer to obtain small grains. Later, limestone grains were glued on polystyrene with no toxic silicone (Sista F109). Substrates were submerged in a tank with presence of coralline crustose algae three weeks prior to coral spawning to favour recruitment (called "conditioned substrates").

Sampling collection

Coral egg-sperm bundles of ten colonies of *A. palmata* were collected at 22:06 h on August 3, 2018 (six days after full moon). Due to the buoyancy of gametes, egg-sperm bundles were collected with inverted conical nets (made with 100 μm filter mesh), adapted from a transparent flask to contain gametes. Flasks were capped and transported (upside down) to the boat without shaking in order to avoid breaking packages. Once all the containers were gathered, the content (gamete bundles + seawater) were gently released and mixed in a transparent container with 10 L of filtered seawater to begin fertilization. Gametes were transported at Xcaret Aquarium to stop fertilization (~2 h) through several washes, with seawater spiked with several meshes (from 100 to 5 μm) and UV filters, to eliminate excess sperm, avoiding polyspermia.

Coral embryos and larvae culture

After fertilization, 3 ml of embryos were transferred to three incubators (1 ml per incubator). An incubator, consisting of a 20 L plastic bucket divided in half to insert a micro nylon filter mesh (75 µm) between them that allows water interchange, but not the exit of embryos. The incubators were semi-submerged in a tank with closed flow; however, an in-line pump enabled seawater recirculation through sprayer nozzles inside incubators to create smooth irrigation and avoid embryos agglomeration.

Leachates preparation

Non-decomposed *Sargassum* spp. were collected and washed several times with filtered seawater to remove organisms and sediment. Subsequently, 1.5 kg of these brown algae (wet weight) were mixed with 10 L of sea water filtered with micro (5 µm) and UV filters. This mixture was left outdoors for 4 days before filtering leachates with a nylon mesh (75 µm) in order to prepare different concentrations (25%, 50% and 100%).

Experimental design

The experiment of larval swimming behavior was evaluated through five treatments on August 08 2018 during daylight hours (11:30–14:00 h). Treatment 1 (hereafter called "control") consisted in larvae exposed to filtered seawater. Treatment 2 (hereafter called "stain") consisted in larvae exposed to filtered seawater with caramel artificial food color to simulate the color of the highest concentration of leachate (100%) with the aim of evaluating if color affects coral larvae. In the last three treatments (T3-T5), larvae were exposed to different leachate concentrations to evaluate their impact on *A. palmata*: T3 (hereafter called "25% leachates") comprised larvae swimming on leachates at 25% concentration, T4 (hereafter called "50% leachates") contained larvae exposed to leachates at 50% concentration, and T5 (hereafter called "100% leachates") included larvae in

contact with leachates at 100% concentration. Five competent coral larvae, with similar size and shape (i.e., bowling pine or elongated) were deposited at the fifth day after fertilization per tank or replicate. Each small tank contained a conditioned substratum of limestone at the bottom and 25 ml of filtered seawater, with or without leachates, depending on treatment (Fig. 7). In total, 250 larvae were used (5 larvae x 5 treatment x 10 replicates). Previously, seawater of each treatment was placed in a plastic bottle; in total, five bottles (one per treatment) were semi-submerged in a tank with continuous seawater flow in order to ensure that the seawater within the bottles remained at a constant temperature. Before shooting, values of practical salinity unit (PSU), pH, dissolved oxygen (DO) and temperature (TT) were recorded using a PRO DSS probe 4-port DIGITAL. Mean data of physical parameters were obtained in the seawater used in each treatment as follows: 1) control (PSU=37.40, pH=8.13, DO=5.99 mg/L, and TT=29.2ºC); 2) stain (PSU=37.30, pH=8.12, DO =5.89 mg/L, and TT=29.2ºC); and 25% (PSU=40.64, pH=7.41, DO=1.10 mg/L, and TT=29.3ºC), 50% (PSU=41.54, pH=7.15, DO=0.10 mg/L, and TT=29.3ºC) and 100% (PSU=43.45, pH=6.97, DO=0.06 mg/L, and TT=29.3ºC) leachates. Subsequently, a 10 min movie was filmed per replicate using three CANON PowerShot cameras (G10, G11 and G12).

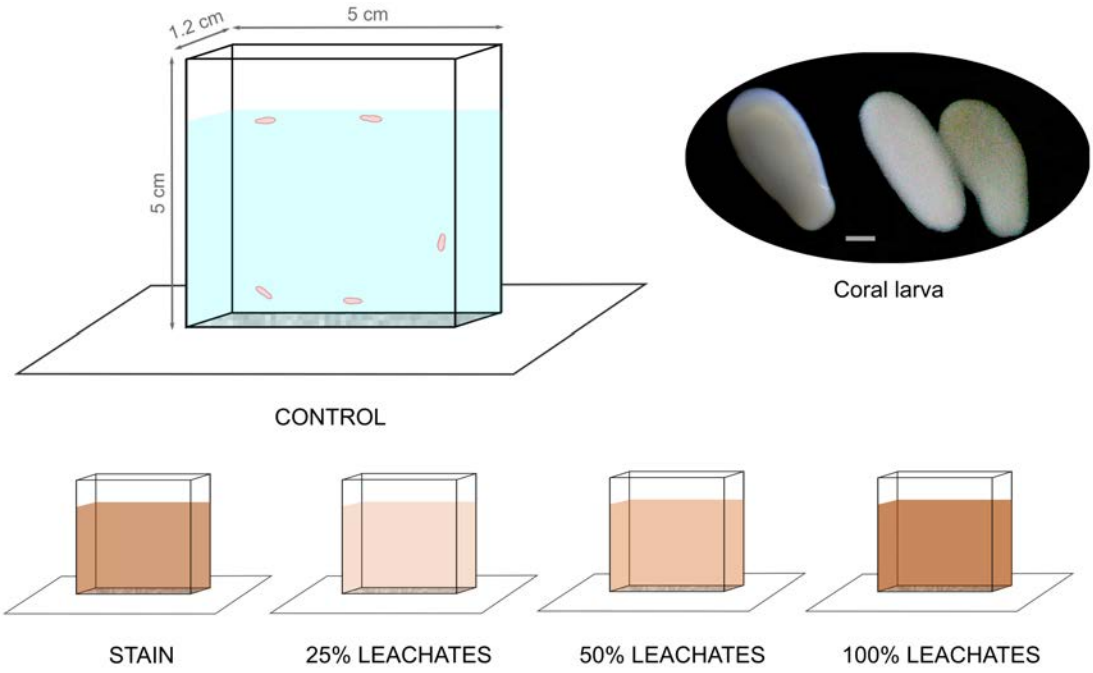

Figure 7. Experimental design to evaluate larval swimming behavior through five treatments (control, stain, and 25%, 50% and 100% leachate concentrations). Scale: 200 μm. See text for more details.

Global and specific trajectometry indexes

Shootings were recorded in .AVI format and projected on a 13-inch computer monitor. An acetate paper was placed on the monitor to trace (with permanent marker) all displacements of each larva (hereafter called "trajectometry indexes") (Table 1). Displacement-time and -length of each larva were recorded in order to obtain larval speed. Later, all trajectometry indexes were assigned in two categories: global (11) and specific (12). In specific category,

12 trajectometry indexes were evaluated in four directions in tanks: surface (3), bottom (3), upward (3), and downward (3) (Table 1).

Trajectometry indexes	**Unit**
Global	
1) Displacement-length per larva	mm
2) Displacement-time without stops per larva	s
3) Displacement- speed with stops per larva	mm/s
4) Displacement- speed without stops per larva	mm/s
5) Numbers displacements per larva	count
6) Number stops per larva	count
7) Time stops per larva	s
8) Percentage of larvae with spiral displacement	%
9) Displacement-length in spiral per larva	mm
10) Displacement-time in spiral per larva	s
11) Displacement-speed in spiral per larva	mm/s
Specific	
Surface	
12) Displacement-length per larva	mm
13) Displacement-time per larva	s
14) Displacement-speed per larva	mm/s
Bottom	
15) Displacement-length per larva	mm
16) Displacement-time per larva	s
17) Displacement-speed per larva	mm/s
Upward	
18) Displacement-length per larva	mm
19) Displacement-time per larva	s
20) Displacement-speed per larva	mm/s
Downward	
21) Displacement-length per larva	mm
22) Displacement-time per larva	s
23) Displacement-speed per larva	mm/s

Table 1. Trajectometry indexes and their units evaluated in all displacements of *Acropora palmata* larvae under five treatments (control, stain, and 25%, 50% and 100% leachates).

Frequency of kinetic behavior pairs

Frequency of kinetic behavior of coral larvae was obtained from the number of all movement pairs recorded in each treatment, which were divided by the number of larvae by each treatment, to assign each value obtained in one of three ranks (low, medium, and high frequency of movement pairs). Then data of all movement pairs and their frequency were placed in a table and represented in a diagram.

Post-observations

After shooting, four post-observations were recorded over 20 min (i.e., at minute 5, 10, 15 and 20) to register overall activity of larvae in each tank per treatment.

Statistical analyses

Shapiro-Wilk and Levene tests were used to check normality and homogeneity of variances of trajectometry indexes data. In normal and homogeneous data, one-way analyses of variance (ANOVA; factor: treatment) were performed followed by Tukey *post hoc* tests. In data with lack of normality and homogeneity of variances, Kruskal-Wallis (KW) tests were performed followed by *a posteriori* tests in IBM SPSS Statistics for Windows version 25 (IBM, Armonk, N.Y.). Due to the lack of normality and homogeneity of variances in the frequency of the kinetic behavior of each pair among treatments, KW tests were performed followed by *a posteriori* tests. In addition, G tests were used to compared the three ranks of all frequencies of kinetic behavior pairs within each treatment.

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Author contributions

J.E.-A., N.P.C.-N., and Y.H. conceived the study. F.A.-M., N.P.C.-N., A.V.-Z., A.I.C.-F., and R.R.-F. accomplished the fieldwork and laboratory work. F.A.-M., N.P.C.-N., and Y.H. analysed the data. F.A.-M., N.P.C.-N., Y.H., A.V.-Z., A.I.C.-F., and R.R.-F. wrote the manuscript.

Additional information

Competing financial interests: The authors declare no competing financial interests.

Supplementary Information

Leachate effects of pelagic *Sargassum* spp. on larval swimming behavior of the coral *Acropora palmata*

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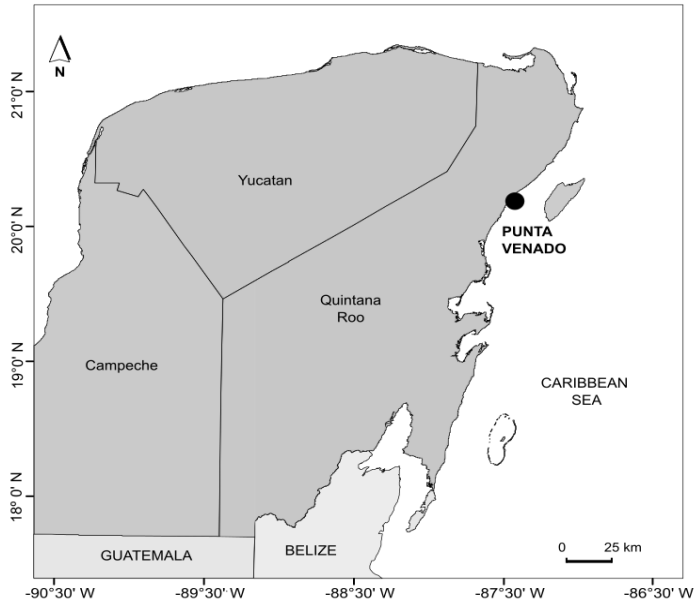
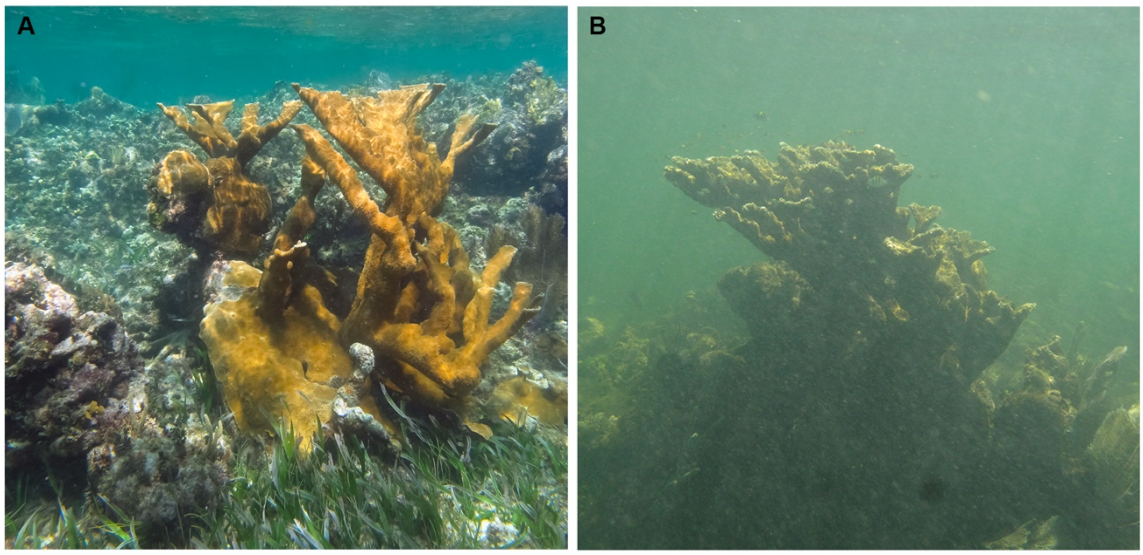
Supplementary Figure 1. Two coral reef located in the northern part of the Mexican Caribbean. (**A**) Study site located at Punta Venado with *Acropora palmata* colonies around 2 m wide (yellow box). (**B**) Puerto Morelos (a closer reef of our study site) with presence of leachates, but low presence of *Sargassum* spp. at the coastline. Scale bar: 5 m. Photos by V.M. Rodríguez-Cervantes.



Supplementary Figure 2. Map of study site located at Punta Venado (black dot), in the Mexican Caribbean.



Supplementary Figure 3. Colonies of *Acropora palmata* in the Mexican Caribbean. (**A**) Colonies of *A. palmata* before arrival of *Sargassum* spp. (**B**) Colony of *A. palmata* exposed to *Sargassum* leachates in May of 2019. Photo (A) by H. Bahena-Basave, and (B) by A. Vega-Zepeda.

Behavior pairs recorded	Control	Stain	Leachates			KW	
			25%	50%	100%	$H_{(4,244)}$	*P*
1) Surface–stop	1.12 ± 0.11	0.87 ± 0.09	0.04 ± 0.02	0.09 ±0.05	0.12 ± 0.05	118.37	0.001
2) Downward–stop	0.98 ± 0.10	0.98 ± 0.08	0.76 ± 0.06	0.70 ± 0.09	0.66 ± 0.07	10.55	>0.05
3) Upward–stop	0.96 ± 0.10	0.79 ± 0.08	0.12 ± 0.04	0.09 ± 0.04	0.24 ± 0.06	85.40	0.001
4) Bottom–stop	0.72 ± 0.10	0.72 ± 0.10	0.42 ± 0.10	0.55 ± 0.10	0.52 ± 0.10	12.69	0.001
5) Stop–upward	0.68 ± 0.09	0.51 ± 0.07	0.22 ± 0.05	0.13 ± 0.04	0.12 ± 0.04	45.06	0.001
6) Downward–upward	0.62 ± 0.07	0.64 ± 0.07	0.34 ± 0.06	0.21 ± 0.06	0.48 ± 0.07	24.67	0.001
7) Stop–downward	0.62 ± 0.06	0.28 ± 0.06	0.06 ± 0.03	0.09 ± 0.04	0.14 ± 0.04	57.87	0.001
8) Stop–bottom	0.60 ± 0.08	0.89 ± 0.05	0.78 ± 0.05	0.83 ± 0.07	0.52 ± 0.07	20.92	0.001
9) Upward–stop	0.52 ± 0.07	0.30 ± 0.06	0.10 ± 0.04	0.17 ± 0.05	0.20 ± 0.05	25.52	0.001
10) Stop–surface	0.46 ± 0.07	0.43 ± 0.07	NA	0.13 ± 0.04	0.16 ± 0.05	43.50	0.001
11) Upward–downward	0.40 ± 0.06	0.62 ± 0.08	0.44 ± 0.07	0.30 ± 0.06	0.42 ± 0.07	8.32	>0.05
12) Bottom–upward	0.38 ± 0.07	0.23 ± 0.06	0.14 ± 0.04	0.04 ± 0.02	0.06 ± 0.03	24.53	0.001
13) Downward–bottom	0.38 ± 0.06	0.36 ± 0.07	0.18 ± 0.05	0.11 ± 0.04	0.26 ± 0.06	12.95	0.001
14) Surface–downward	0.20 ± 0.05	0.28 ± 0.06	0.12 ± 0.04	0.11 ± 0.04	0.22 ± 0.05	6.41	>0.05
15) Stop–spiral (S)	0.14 ± 0.04	0.02 ± 0.02	0.08 ± 0.03	0.04 ± 0.02	NA	11.10	0.001
16) Spiral–stop (B)	0.08 ± 0.03	0.04 ± 0.29	0.34 ± 0.06	0.26 ± 0.06	0.08 ± 0.03	24.73	0.001
17) Spiral–upward	0.08 ± 0.03	0.17 ± 0.05	0.04 ± 0.02	0.11 ± 0.04	0.04 ± 0.02	7.16	0.001
18) Stop–spiral (B)	0.06 ± 0.03	0.02 ± 0.02	0.14 ± 0.04	0.19 ± 0.05	0.04 ± 0.02	12.15	0.001
19) Spiral– bottom	NA	0.26 ± 0.06	0.26 ± 0.06	0.13 ± 0.04	NA	29.37	0.001
20) Upward–spiral	NA	0.02 ± 0.02	0.18 ± 0.05	0.09 ± 0.04	0.04 ± 0.02	16.45	0.001
21) Downward–spiral	NA	0.02 ± 0.02	0.16 ± 0.05	0.17 ± 0.05	0.08 ± 0.03	14.88	0.001
22) Spiral–stop (S)	NA	0.02 ± 0.02	NA	NA	0.06 ± 0.03	8.38	>0.05
23) Bottom–spiral	NA	NA	0.60 ± 0.7	0.36 ± 0.7	0.12 ± 0.04	75.10	0.001
24) Spiral–surface	NA	NA	0.06 ± 0.03	0.11 ± 0.04	0.18 ± 0.05	17.63	0.001
25) Spiral–downward	NA	NA	NA	0.09 ± 0.04	0.10 ± 0.04	14.25	0.001

Supplementary Table 1. Mean values of the frequency of kinetic behaviors showed by *Acropora palmata* larvae under five treatments (control, stain, and 25%, 50% and 100% leachates). Frequency data were assigned to one of three ranks: high =0.67–∞ (red underline); medium =0.34–0.66 (orange underline); and low =0.00–0.33 (yellow underline). KW=Kruskal Wallis. (S)=Behavior pairs performed on surface. (B)=Behavior pairs performed at the bottom. NA=not applicable. See text and Fig. 6 for more details.

CONCLUSIONES

- Se demuestran los efectos negativos de los lixiviados de sargazo pelágico en el comportamiento larval de los corales, afectando el desempeño natatorio de las larvas de *A. palmata.*

- La mayoría de los índices de trayectometría (globales y específicos) mostraron diferencias entre las larvas con lixiviados de sargazo (25 %, 50 % y 100 %) *vs*. las larvas sin lixiviados (testigo y colorante café).

- De todos los índices de trayectometría evaluados, la velocidad de desplazamiento fue un patrón que reflejó los efectos negativos de las larvas expuestas a lixiviados, disminuyendo hasta cuatro veces su velocidad.

- Las larvas en contacto con los lixiviados de sargazo fueron hipoactivas (*i.e.*, disminuyeron su actividad) y positivamente geotácticas (*i.e.*, pasaron más tiempo en el fondo en lugar de la superficie), lo que puede disminuir la dispersión de las larvas que usualmente se encuentran en la superficie del agua.

- Los lixiviados de sargazo (*S. fluitans* y *S. natans*) son una perturbación para las larvas de *A. palmata*, por lo que el incremento de sus colonias a través de la reproducción sexual podría reducirse aún más.

- El porcentaje de larvas con movimiento en espiral fue mayor en contacto con lixiviados de 25 % y 50 % comparado con el testigo, el colorante café y la concentración de lixiviados al 100 %. El bajo porcentaje de larvas con movimiento en espiral en el testigo y el colorante café podría estar relacionado con fallas reproductivas naturales o esperadas, mientras que las larvas con lixiviados al 100 % podría deberse a que la mayoría de los pares de comportamientos cinéticos mostraron baja frecuencia, pero ningún movimiento de alta frecuencia.

- En las observaciones posteriores a las filmaciones se observó que la actividad de las larvas en contacto con los lixiviados disminuyó a medida que aumentó la concentración de lixiviados.

- Los lixiviados, derivados de la descomposición del sargazo pelágico, son una fuente adicional de estrés para las larvas de coral de *A. palmata;* sin embargo, éstos podrían afectar otras etapas tempranas de la vida de los corales (*e.g.*, la fecundidad, la embriogénesis, el asentamiento y el reclutamiento).

- Se plantea la posibilidad de que las larvas hayan sido afectadas por los lixiviados de sargazo pelágico en el medio natural, ya que la mayor afluencia de sargazo pelágico y la alta mortalidad de fauna asociada a estos arribos coinciden con los meses de desove anual de los corales liberadores de gametos en el Caribe mexicano (julio-octubre).

- Debido a que los lixiviados de *Sargassum* spp. se pueden extender a distancias mayores a 400 m alejados de la costa, éstos pueden afectar a *A. palmata* y a otras especies de corales que viven en ambientes someros.

- Aunque la concentración de los lixiviados de sargazo al 25 % afectó el desempeño natatorio de las larvas de *A. palmata*, sugerimos evaluarlas en menores concentraciones de lixiviados y con mayor tiempo de exposición para obtener resultados más cercanos a los que se pueden presentar en el medio natural.

- La resiliencia de las especies de corales (constructores de arrecifes y oportunistas) puede ser rebasada si el arribo masivo de sargazo pelágico se convierte en un evento frecuente en el Caribe mexicano.

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