Title- Death Trap or Dispersal Barrier: Can larval fish detect and avoid oil pollution, and what are the effects on larval dispersal and population connectivity?

Keywords- marine pollution, larval dispersal and connectivity, larval fish, orientation

Scientific abstract – Marine oil pollution is a major environmental stressor on the marine environment. It is widespread in all populated coastal areas, impacts most coastal marine species, and is particularly harmful to the early life stages of fish. Despite the high frequency of oil pollution incidents, their broad ecological impact is rarely accounted for in population connectivity studies. A fundamental knowledge gap is whether larval fish can detect and avoid contaminated areas or remain and suffer lethal or suethal consequences. These basic responses are expected to result in different ecological outcomes regarding larval dispersal and connectivity. A lack of representation of these fundamental pollution effects in models of large-scale ecological processes can lead to an inaccurate understanding of marine ecosystem dynamics, resulting in sub-optimal policy- and decision-making.

Here, we propose to bridge this critical gap by addressing three primary research aims, representing three methodological approaches: (1) Determining if larval fish can detect and avoid petroleum pollution using behavioral lab experiments, in situ sampling, and orientation experiments, (2) Translating small-scale (10-2 m and seconds) experimental results to large-scale (104 m and weeks) of dispersal and connectivity processes, and (3) Incorporating the formulation and parameterization developed in Aim 2 into a biophysical model of the Eastern Mediterranean to examine how experimentally obtained behavior affects spatiotemporally explicit modeled larval dispersal and population connectivity. As preliminary data, we include three resources: A set of preliminary experiments using the shuttle tank choice chamber, a recently developed biophysical larval dispersal model for the Eastern Mediterranean, and an oil spill simulation of a major oil/tar pollution event that occurred in the Eastern Mediterranean in Israel during Feb. 2021.

***The essence of the proposal is to empirically determine the behavioral response of larval fish to oil pollution and understand how this behavior affects the broad ecological aspects of larval dispersal and connectivity.***

The study area is the Israeli region of the Eastern Mediterranean, and the model animals are the widespread coastal species of *Mugil cephalus* (flathead grey mullet), *Dicentrarchus labrax* (sea bass), and *Sparus aurata* (gilt-head bream), whose larvae are artificially reared and readily obtained at various developmental stages.

This proposal is the first time larval orientation response to oil pollution will be quantified and implemented in biophysical models of larval dispersal to inform us about the effect of oil pollution on larval dispersal and connectivity dynamics. The anticipated outcome of this research includes elucidating how larval fish behaviorally respond to oil pollution and quantifying the effects on the broad process of larval dispersal and population connectivity. Our proposed research represents a novel combination of lab and in situ pollution-response experiments, movement modeling, marine pollution modeling, and dispersal and connectivity modeling. In addition, the project will form a general framework for translating small-scale behavioral experiments related to marine pollution to large-scale spatiotemporally explicit larval dispersal and pollution simulations. The information from this project will be useful for marine resource managers, supporting informed decision-making and marine spatial planning. Hence, this knowledge will ultimately serve the public, the main stakeholder of life-supporting marine ecosystems.

**Research Program**

1. **Scientific background**

Oil pollution is a major stressor on the marine environment that totals more than a million tons per year, primarily from terrestrial runoff and marine transportation 1–3, causing mortality and deterioration of marine organisms and habitats 3. Accordingly, areas of global marine oil pollutionspatially correlate with commercial shipping lanes and dense population centers 2. Remote sensing of oil pollution indicates an average of nearly two detected oil slicks per day in the Mediterranean alone 4. Other ecologically important marine systems, such as the Gulf of Mexico, the coral triangle, and the Yellow Sea, are also experiencing severe and chronic oil pollution 5.

The main toxic oil components are polycyclic aromatic hydrocarbons (PAHs) that are especially harmful to the early life stages of fish and invertebrates 6. A vast body of literature demonstrates the high toxicity of oil compounds to a wide diversity of marine organisms, including marine mammals, fish, invertebrates, corals, zooplankton, and phytoplankton 7,8. Concurrently, a global crisis of marine and terrestrial biodiversity has been declared that threatens fundamental life-supporting ecosystem services 9,10. Despite this severe crisis, the study of the effects of oil pollution on broad-scale marine ecological processes is minimal. By neglecting key stresses like marine pollution, a critical knowledge gap is created that limits our ability to quantify and understand natural marine processes, such as larval dispersal and connectivity 11.

Indeed, the life cycle of most marine animals involves a pelagic phase, in which eggs or larvae set out to the open ocean for up to several weeks. Only a tiny fraction of larvae survive the pelagic stage, use their sensory capacities to find suitable habitats and be recruited into the adult population 12. This pelagic stage is critically important because it is the main dispersive phase and governs population connectivity and biogeography 13. Whereas marine population dynamics are driven by larval growth, mortality, and dispersal 14, the impact of oil pollution on larval behavior and the role of pollution-related behavior on larval dispersal, recruitment success, and connectivity remain unknown.

Previous studies shed light on sensory capacities relevant to larval response to the presence of oil, indicating that fish larvae effectively use chemical cues in homing 15,16, settlement habitat 17,18, and conspecifics 19, for example. Moreover, a few oil-related studies indicate that juvenile *Dicentrarchus labrax* 20 and adult *Gadus morhua* 21 demonstrate avoidance behavior from oil-contaminated waters. In contrast, *Pleuronectes bilineatus*, *P. asper*, and *Hippoglossus stenolepis* do not exhibit avoidance behavior at environmental PAH concentrations in the sediment 22. Other studies indicate that the presence of low concentrations of oil reduces the in situ swimming speed of larval fish 23–25. These traits are important as they can impact these animals' fitness by affecting the larval fish's capacity to avoid threats while effectively locating and entering a proper settlement habitat. *While these studies provide information about the behavioral response of several species to the presence of oil pollution, the explicit effects of oil on the broad ecological processes of larval dispersal and connectivity are unknown.*

In the local context of the Eastern Mediterranean, Israel is a hotspot for marine shipping connecting the Mediterranean (and Atlantic Ocean) with the Red Sea (and Indian Ocean). Such traffic hotspots are characterized by frequent offshore oil spills from ships and tankers 2 that normally receive little attention but occasionally interact with the coastline as massive oil or tar pollution. Such an event occurred in Feb. 2021 when crude oil leaked from a tanker, resulting in a small-to-medium (7 - 700 ton) spill in the Israeli Mediterranean. The spill primarily contaminated the Israeli coastline and represents Israel's most severe coastal oil contamination to date 26.

**Importantly, existing models of larval dispersal and connectivity do not account for spatially explicit pollution. Hence, these models also fail to incorporate pollution-related mortality or orientation behaviors, specifically avoidance or changes in swimming speed. The resulting knowledge gap includes (1) A lack of knowledge about larval behavioral responses to oil pollution, (2) A lack of a spatiotemporally explicit modeling framework combining larval movement and marine pollution, and (3) A lack of understanding about the broader ecological effects of oil on larval behavior and the resulting impacts on larval dispersal and connectivity.** This knowledge is important because if larvae fail to recognize and respond to the threat of oil contamination, the waters become a death trap. Alternatively, if larvae successfully recognize and avoid the threat, the contaminated area may become a dispersal barrier hindering population connectivity, particularly if the pollution is persistent or chronic2. Either way, pollution-related outcome, whether orientation or mortality, is expected to substantially influence larval dispersal and connectivity. To bridge this critical knowledge gap, we propose to address two hypotheses. **We hypothesize that (1) larval fish will exhibit a significant behavioral response to oil pollution and (2) this response will significantly affect larval dispersal and connectivity patterns.**

1. **Research objectives and expected significance**

We propose to address these two overarching hypotheses through three interconnected Specific Aims, representing a comprehensive combination of experiments and modeling (**Fig. 1**).

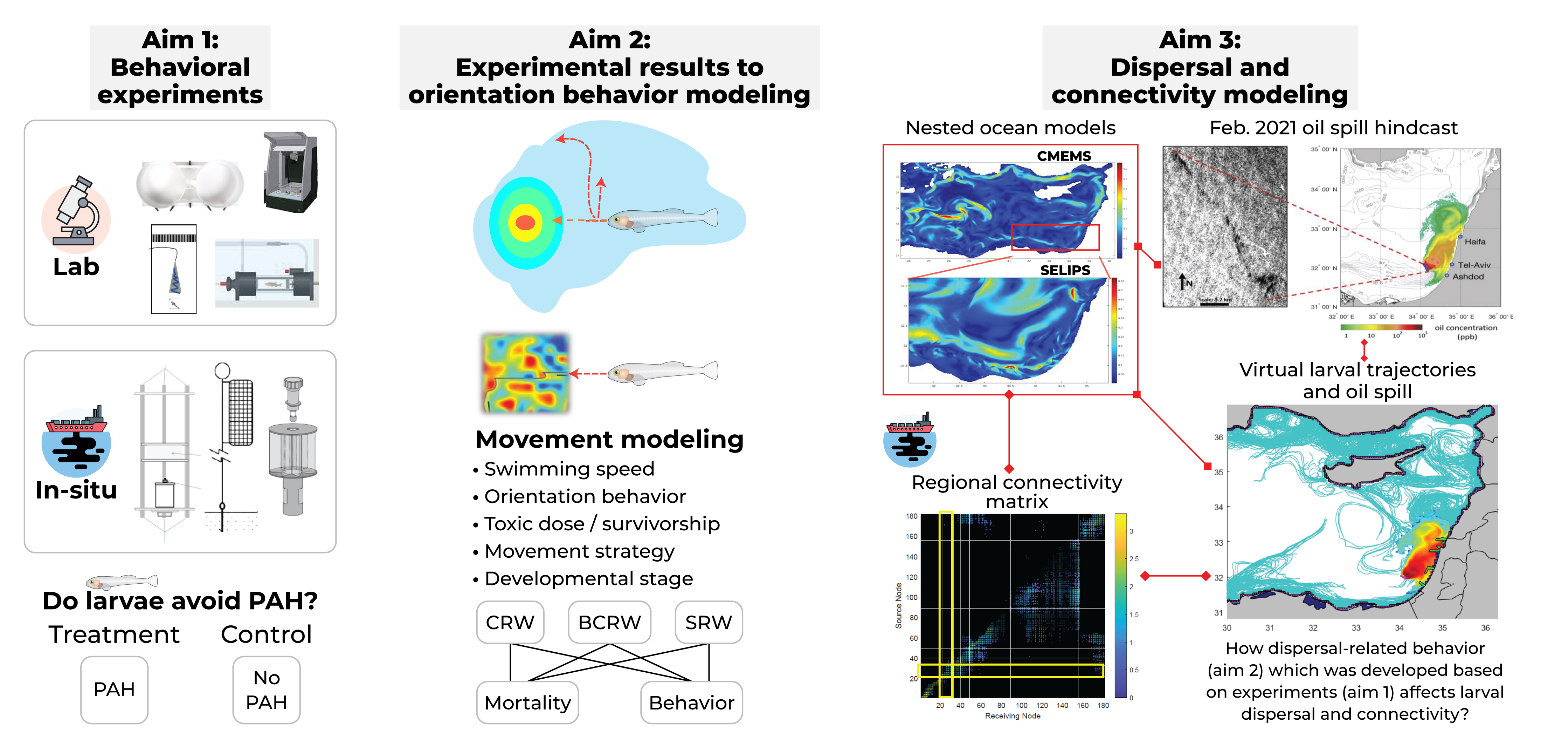
**Specific Aim 1 – Behavioral experiments*.* We will quantify the behavioral response of larval fish to PAHs, including avoidance/attraction, swimming speeds, escape response, mortality, and recruitment rate.** By examining larval behavioral traits in response to PAH, we will establish modeling parameters for orientation, swimming speed, and mortality in a virtual 3-D modeling arena (Specific Aim 2).

**Specific Aim 2 – Experimental results to orientation modeling. We will translate experimental results to modeling traits and parameters and bridge experimental small scales (seconds and centimeters) and dispersal-related scales (tens of kilometers and days).** This Aim will also include the implementation of classical movement strategies of correlated random walk (CRW), biased correlated random walk (BCR), and PAH-related orientation behavior (avoidance, attraction, indifference). The strategies will be employed while keeping track of the toxic-dose state of each virtual larva. In addition, this Specific Aim will provide the quantitative framework and mathematical formulation and parametrization to examine pollution and dispersal within a single framework. This framework has never been developed or applied before. The resulting formulation and parametrization of this Aim will be incorporated into biophysical models of larval dispersal (Specific Aim 3).

**Specific Aim 3 – Dispersal and connectivity modeling. We will incorporate experimentally obtained biophysical larval traits formulated in Specific Aim 2 into models of biophysical larval dispersal, including spatiotemporally explicit PAH concertation fields.** Using the parametrization and formulations obtained in Specific Aim 2, we will run a series of larval dispersal models for the Eastern Mediterranean, examining the effects of larval response to PAH pollution on larval dispersal and connectivity. Dispersal and connectivity-related variables, such as dispersal distance, recruitment success, and number of connections, will be quantified as part of this Aim.

1. **Detailed description of the proposed research**
   1. **Working hypotheses**

For Specific Aim 1, we hypothesize that larval fish will exhibit a significant response to the presence of PAH, which is to avoid PAH-contaminated waters. For Specific Aim 2, we expect that behavioral responses obtained in Specific Aim 1 will significantly affect movement pattern parameters in the virtual 3-D area, and movement parameter estimations will be affected by the simulated spatiotemporal resolution.

For Specific Aim 3, we hypothesize that implementing larval behavioral responses to PAH pollution (obtained in Specific Aim 1 and formulated in Specific Aim 2) in our larval dispersal model will generate significantly different dispersal outcomes compared to unpolluted conditions (no PAH). The differences will be expressed by variable connectivity matrices, dispersal kernels, and betweenness centrality estimates.

***Figure 1****. A schematic description of the proposed research. Abbreviations: CRW- Correlated Random Walk, BCRW- Biased Correlated Random Walk, SRW- Simple Random Walk.*

* 1. **Research design and methods**
     1. **Laboratory and in situ experiments (Specific Aim 1)**
        1. **Rationale:** We will conduct behavioral experiments in the laboratory and in situ at sea (**Table 1**). All experiments are based on the principle of quantifying biological/ecological responses in the presence versus absence of PAH pollution as part of the water-soluble fraction of oil. Quantification will allow us to understand if larvae can detect and avoid PAHs specifically. PAH concentrations applied in the lab experiments will include 1 and 10 µg L-1 total PAH (see section **3.2.1.11** **PAH concentrations and measurement)**. We will use commercially reared larvae of *Mugil* *cephalus,* *Dicentrarchus* *labrax,* and *Sparus* *aurata* provided by Dag On Inc. (Ma'agan Michael, Israel). For these experiments, we will focus on intermediate (14 Days Post Hatch; DPH) to late-stage (18 DPH) larvae. In Collect by Artificial Reef Eco-friendly (CARE) light trap (section **3.2.1.7**) and SMURF (section **3.2.1.8**) field recruitment experiments, we will capture wild larvae that will be tested individually for 20 min per trial using the Shuttle Box (section **3.2.1.2**), Danio-Vision (section **3.2.1.4**), and Drifting in situ chamber (DISC) (section **3.2.1.6**) methods described in the following sections. We will use longer durations for the mini-swim flume (~90 min; section **3.2.1.3**), CARE (12 h), and SMURF (30 d) experiments.
        2. **Shuttle Box (Loligo Systems, Fig. 1).** This system is a binary choice chamber used to assess larval preference for PAH. The Shuttle Box system consists of two 10 cm diameter circular chambers connected by a short narrow pathway allowing free passage of larvae 27. We will use paired t-tests to examine differences in the relative amount of time and swimming speed exhibited in the polluted chamber compared to the unpolluted chamber 18. For additional details about the Shuttle Box system, see the **Preliminary results** section.
        3. **Mini-swim flume (Loligo Systems).** This system is used to study the swimming performance and behavior of fish in a controlled laboratory setting 28. We will use the swim flume to test the effects of variable PAH concentrations on the swimming performance of larval fish. Specifically, critical swimming speeds will be measured by gradually increasing flow speed until larvae cannot maintain their position in the tunnel 29. We will examine the significance of differences in critical swimming speeds between the different PAH concentration groups using one-way ANOVA and Tukey post-hoc tests 30.
        4. **Danio-Vision (Noldus).** This system will be used for high throughput tracking of larval fish. We will use the Danio-Vision to test how variable PAH concentrations affect the movement patterns of larval fish, specifically their escape response pattern 31. We will use paired sample t-tests to examine differences in the escape response-related swimming speed in the polluted setting compared to the unpolluted setting. Escape response will be elicited using a startle/tapping disturbance as part of the Danio-Vision functionality 31.
        5. **Odor experimental flume.** This system is a custom experimental flume based on Gardiner and Atema (2007)32 in which the small-scale orientation behavior of larval fish is recorded in response to the spatial position of a chemical plume. Our analysis will identify larval responses, divided into four categories: turn right/left, up/down, swim towards/away from the plume, and increased/decreased swimming speed. We will determine the frequency differences between the categories using one-way ANOVA.
        6. **Drifting in situ chamber** (DISC; Paris *et al.*, 2008)**.** This deviceis a circular, transparent, symmetric behavioral chamber deployed from a boat that drifts in the water column. A larva is inserted into the DISC to record larval orientation behavior under natural and artificial conditions in situ 34,35. We will use quantitative analyses based on comparisons of mean swimming direction, resultant vector, and swimming speeds between “unpolluted” and “polluted” trials. We will induce PAH pollution with a piece of tar (8 gr; diameter ~2.5 cm) below the behavioral chamber. In the unpolluted trials, a black piece of cloth will be located at the same location to control for the visual appearance of the tar.
        7. **Light trap: Collect by artificial reef eco-friendly (CARE;** Lecaillon, 2004). This light trap is designed to attract and collect settlement-stage post-larval fish in the cod end during a single night.
        8. **Standard monitoring units for recruitment of temperate reef fishes (SMURF;** Ammann, 2004)**.** This moored cylinder mesh structure attracts settlement-stage post-larval fish. We will place light traps and SMURFS off the coast of Nahariya, Haifa, Sdot Yam, and Ashdod above a bottom depth of 10–20 m once per month during the recruitment season (May to September). For each site, we will place the installations in pairs with about 10 m between the treatment unit (with tar) and the control unit (without tar). This distance is sufficient because detectable PAH concentrations emitted from the tar do not exceed 1 m from the source based on our preliminary in situ measurements.
        9. **Molecular identification of wild-caught larvae**We will identify wild-caught larvae using previously applied sequencing methodology 38,39. Briefly, we will extract individual total genomic DNA and PCR amplify the cytochrome c oxidase subunit encoding gene (COI). Amplification will utilize previously developed primers. Sequences with reliable chromatograms will be identified based on NCBI (https://[www.ncbi.nlm.nih.gov/)](http://www.ncbi.nlm.nih.gov/)) and BOLD (https://[www.boldsystems.org/)](http://www.boldsystems.org/)) databases (Karahan et al., 2017; see Support Letter #1).
        10. **Quantitative analyses**

Statistical assumptions for parametric tests of paired t-test and ANOVA will be examined, and if these assumptions are not met, non-parametric tests will be applied, such as the Wilcoxon signed-rank and Kruskal-Wallis tests instead of paired t-test and one-way ANOVA, respectively 30. To infer higher-level differences between the species and between responses to the different PAH concentration levels, we will perform a meta-analysis of the effect sizes of the pairwise tests 40. A custom MATLAB-based fish algorithm will track larval fish in the Shuttle Box, mini-swim tunnel, Danio-vision, odor experimental flume, and DISC. Each treatment group per experimental method will include a default of N=20 larvae but may consist of up to N=30 according to a preliminary power analysis. For example, for the shuttle tank experiments, we will test 20 larvae per species for each of six PAH concentrations, resulting in 120 larvae. We will perform a power analysis of required sample sizes after preliminary trials, and sample sizes may be updated accordingly. The proposed sample size per experiment is given in **Table 1**.

* + - 1. **PAH concentrations and measurement**

Our laboratory experiments will establish PAH concentrations using seawater stiped with tar (8 gr; diameter ~2.5 cm) for one hour. Required concentrations will be achieved using a series of dilutions with unpolluted artificial seawater. We will measure PAH concentrations using an enviroFlu (TriOS Optical Sensors) PAH sensor, calibrated using PAH calibration solution (SV Mix, CLP method Calibration Std #5 PAH Mixture) and verified using gas chromatography-mass spectrometry (GC-MS) measurements. CG-MS will be performed at a certified lab (Bactochem, Israel) following the US Environmental Protection Agency (EPA) standard method EPZ 8270. The PAH concentration will be measured around each in situ experimental unit using the enviroFlu PAH meter to ensure that the values match the following expectations: ≥ 10 µg/L total PAH/L at <0.5 m radius from treatment and ~0 µg/L total PAH/L at <0.5 m radius from control. The PAH concentrations we apply in the laboratory experiments will represent sub-lethal to mildly lethal (1 and 10 µg/L total PAH/L, respectively) and lethal concentrations, respectively 6,41.

* + - 1. **Anticipated outcome**Specific Aim 1 will provide a comprehensive answer for how larvae behave in response to PAH contamination in water by focusing on avoidance, attraction, swimming speed, survivorship, and spatial movement patterns, both horizontally and vertically.

1. The results of this Aim will provide the basis for the model parametrization that will be developed in Specific Aim 2 and implemented in Specific Aim 3.
2. From a broader perspective, we will elucidate the fundamental question of whether larval fish can detect and avoid oil pollution or remain in the contaminated area and potentially suffer lethal or sub-lethal consequences.

***Table 1****. Proposed methods for Specific Aim 1 and the model parametrization associated with these experiments. The sample size represents the expected number of larvae from our focal species: Mugil cephalus, Dicentrarchus labrax, and Sparus aurata will be tested by the relevant experimental methods.*

|  |  |  |
| --- | --- | --- |
| Method | Location (sample size) | Specific question/insight | Explanation and modeled parameter. |
| Shuttle box  Lab. (120) | Do larval fish actively avoid PAH pollution? | Compute the probability of larval fish to elicit avoidance/attraction behavior. In other words, the number of virtual larvae that will elicit this behavior in the model is based on the relative number of larvae that demonstrate this behavior in the experimental results (Foretich *et al.*, 2017). |
| Mini-swim flume  Lab. (120) | How does PAH pollution affect larval swimming speeds? | Compute larval swimming speed in polluted and unpolluted waters. The relative change in swimming speed will be implemented in the modeled ontogenetic swimming speeds 28,42. |
| Danio-vision  Lab. (120) | How does PAH pollution affect larval movement patterns and escape/startle response? | Compute predation mortality. If larvae exhibit a reduced response to startle, we will assume an increase in predation mortality 43. |
| Odor preference Experimental flume  Lab (120) | What is the small-scale orientation strategy of larval fish with respect to PAH pollution? | Compute small-scale orientation strategy in response to PAH plume if individual larvae have a distinct directional preference (e.g., 90° right turns) 32. |
| DISC- Drifting In Situ Chamber  Sea (120) | How does PAH pollution affect larval orientation behavior in situ? | Compute small-scale orientation strategy in response to PAH pollution in situ, movement pattern associated with CRW, BCRW, etc. 33,34,44. |
| CARE-  light trap  Sea | How does PAH pollution affect larval fish recruitment probability? | Compute recruitment probability under PAH-polluted vs. unpolluted habitats for phototactic settlement-stage larvae attracted to light 45. |
| SMURF-  Sea | How does PAH pollution affect larval fish recruitment? | Compute recruitment probability under PAH-polluted vs. unpolluted habitats for larval fish attracted to artificial substrate 37. |

* + 1. **Experimental results to orientation modeling (Specific Aim 2)**
       1. **Rationale**: One of the main challenges of the proposed research is to translate small-scale (centimeters and seconds) experimental information to large-scale (tens of kilometers and days) biophysical modeling traits, representing a difference of five orders of magnitude. Specific Aim 2 is focused on addressing this challenge and consists of a required intermediate step for developing and implementing our larval behavioral model in response to pollution. We will use MATLAB-based virtual arenas where movement and pollution can be effectively represented and examined within a single framework at variable scales. The framework will allow us to implement scenarios with variable classic movement strategies (CRW, BCRW) and PAH-related orientation behavior (avoidance, attraction, indifference) while recording the toxic-dose state of each virtual larva. Our scenarios will be based on the characteristics and statistical results from Specific Aim 1. The scenarios will represent (1) a confirmation of the hypothesis that larvae avoid PAH pollution or a rejection of the hypothesis reflecting a lack of effect, (2) a size effect on the population proportion that responds to PAH pollution and the magnitude of the response, for example, and (3) the response variation, representing the natural variability of behavioral responses in the population. We will model these traits at the individual level using an individual-based model (IBM). Toxicity-related mortality will be applied based on the cumulative exposure of virtual larvae to PAHs, considering the PAH elimination rate in unpolluted water46. We will compare the virtual larval movement characteristics of each type of behavioral variant to other behavioral variants. For example, the mortality rate, displacement, and tortuosity of virtual larvae that avoid PAH will be compared with virtual larvae that ignore PAHs. We will also include classic movement patterns of CRW, Biased Random Walk (BRW), and BCRW in these scenarios. Our proposed set of scenarios represents a wide range of possibilities given the uncertainty and variability of larval orientation behavior in general and in response to PAH pollution in particular. The transition between scales will be based on statistical modeling of movement parameters using moving windows across sub-sampled trajectories, similar to the methodology applied in Berenshtein *et al.* (2018a).

A fundamental concept related to larval navigation in the absence of external directional cues is infotaxis, which refers to the movement of organisms in response to localized information gradients in their environment 48. Interestingly, reverse-infotaxis, where organisms move away from the source of information gradients, could also be similarly considered 49. This behavior is observed in various contexts, including chemical cues, suggesting that larvae might actively move away from unfavorable conditions or potential risks 50. By integrating traditional and reverse infotactic principles into micro-scale larval motion models, we will gain insights into how larvae optimize their movement patterns based on local environmental cues or lack thereof.

We will model PAH pollution based on a smoothed gradient in a 10x10 square matrix where each cell of the matrix contains a random variable pulled from an even distribution in the range [0,1] multiplied by a descending function of the distance of the cell. The matrix will then be smoothed to achieve a realistically gradual gradient.

***Figure 2****. A diagram of multi-scale movement simulation in response to pollution (Specific Aim 2). The basic movement strategy is BRW and an avoidance strategy of 20° right turns if the larva encounters PAH pollution greater than a threshold.*

* + - 1. **Anticipated outcome:** (1) Specific Aim 2 will increase our understanding regarding fundamental concepts of animal movement in contaminated regions and how their response to contamination affects movement trajectories expressed in displacement and tortuosity, for example.

(2) Specific Aim 2 will create the first “sandbox” virtual arena where virtual animal responses to marine pollution can be examined at variable scales. Our platform will be open access for the scientific community, enabling the simulation of animal movement in contaminated regions.

(3) Specific Aim 2 will create the quantitative framework and intermediate connecting link required to incorporate the experimental results of Specific Aim 1 into our larval dispersal biophysical models (Specific Aim 3). Specific Aim 2 will also allow a quantitative transition between scales using sub-sampling statistics.

* 1. **Dispersal and connectivity modeling** 
     1. **Rationale**: We will use dispersal and connectivity modeling to simulate spatiotemporally explicit virtual larval trajectories based on the connectivity model system (CMS) framework of Paris *et al.* (2013b) developed for the Eastern Mediterranean (**Fig. 3**). The spatial domain of this framework includes the Eastern Mediterranean east of 31.4°E, including coastal and continental shelf polygons, marine protected areas (MPAs), and habitat types for the Israeli region. The physical component of this framework includes nested ocean models of CIMEMS 51 as the outer domain and the South Eastern Levantine Israeli Prediction System (SELIPS) as the inner domain 52 (**Fig. 1**). Copernicus Marine Environment Monitoring Service (CMEMS) is a Copernicus-based hydrodynamic model implemented over the Mediterranean Basin with a horizontal grid resolution is 1/24˚ (~ 4 km) and 141 vertical levels. The hydrodynamics are supplied by the Nucleus for European Modeling of the Ocean (NEMO; (Clementi *et al.*, 2019). SELIPS is a sub-regional high-resolution circulation model operated by Israel Oceanographic and Limnological Research (IOLR) that generates daily forecasts of temperature, salinity, and sea currents in the southeastern region of the Levantine basin (**Fig. 1**). We will use the available four years (2017-2020) of ocean current data from the nested modeling framework to capture the inter-annual variability of the system.

We will enter the biological information and 4D current fields into the CMS dispersal model, which computes individual larval trajectories. The biological component in this model includes species-specific traits such as spawning season, spawning locations, and pelagic larval duration (PLD), as well as our behavioral traits experiments (Specific Aim 1). The data will be parametrized to fit our larval dispersal model (Specific Aim 2). Specifically, the dispersal outcome of control trajectories with no PAH pollution will be compared to “treatment” trajectories with PAH pollution. Currently, larval dispersal models neither implement spatially explicit pollution maps nor larval avoidance behavior. We propose two major tasks as part of Specific Aim 3. (1) We will combine spatiotemporally explicit PAH concentration maps (Specific Aim 2) with spatiotemporally explicit larval trajectories (Specific Aim 3). We will then apply concentration-dependent mortality rates based on existing LC50 responses of larval fish. (2) We will implement the responsive behavior from the experimental results into our individual-based dispersal model. If no avoidance behavior is detected, we will conclude that larvae cannot detect and avoid oil pollution. Hence, only mortality associated with PAH concentration will be utilized in our model.

We will compare simulation scenarios where we implement larval-responsive behavior and mortality at the regional (Eastern Mediterranean) and local (Israel) domains. We will examine differences in recruitment success, the degree of self-recruitment for continental shelf habitats, and MPA connectivity levels, such as the number of connections. Synthesis of the different variables will be applied by standardization of the variables, with a subsequent meta-analysis53. In addition, our approach will allow the quantification of the effect of the Israeli oil spill on larval survivorship, dispersal, and connectivity. This quantification is important because the Israeli oil spill was the greatest coastal/marine contamination event in the Israeli Mediterranean, which thus far had not been accounted for.

* + 1. **PAH pollution hindcast simulation**

We will make use of a hindcast of the Israeli oil spill (Feb. 2021) that successfully reconstructed the time and extent of shore contamination that was observed in the field (**Fig. 3**). The modeling is based on the oil-CMS model (Paris *et al.*, 2012). The oil-CMS model is an oil transport and fate model based on the open-source particle-tracking CMS 54,55. The oil-CMS accounts for key oil transport and fate processes in the marine environment, including biodegradation, dissolution, evaporation, wind-drift, and sedimentation 41,56–59.

The original oil spill output includes spatially explicit oil concentrations during the duration of the spill (22 days during Feb-Mar 2022). We will expand our examination of the impacts beyond the limited duration of the actual oil spill. The spill's spatially explicit PAH concentrations will be applied as three additional dates across different months (June, September, and December) using our four years of ocean currents data (2017-2020). Our results with the light trap and SMURF show that larval recruitment to contaminated units is indeed lower than the control units. Thus, in our simulations, contamination of the coast as defined by oil on the shoreline may deter, attract, or kill recruiting virtual larvae. We will quantify the effect of this behavior on larval dispersal and connectivity.

At the regional Eastern Mediterranean and local Israeli domains, we will compare control scenarios to those where we implement larval PAH-related behavior and mortality. The variables for comparison will be recruitment success for coastal/continental shelf polygons, recruitment success for MPAs polygons, the number of connections, the number of MPA connections, and the degree of self-recruitment. In addition, our approach will quantify the effect of the Israeli oil spill on larval survivorship, dispersal, and connectivity.

* + 1. **Anticipated outcomes**

(1) Specific Aim 3 will generate virtual trajectories under conditions with and without PAH pollution while considering a realistic pollution event that occurred during 2021.

(2) Specific Aim 3 will compute the effects of PAH-related larval behavior on larval dispersal and connectivity estimates such as connectivity matrices, dispersal kernels, and betweenness centrality.

(3) From a broader perspective, our proposal will push the envelope in increasing the biological realism of biophysical models, promoting suitable marine conservation and policy efforts.

* 1. **Preliminary results**

**Specific Aim 1** - We conducted 29 preliminaryshuttle tank trials, which indicated a clear preference at the individual level for either polluted (52%) or non-polluted (48%) chambers (**Fig. 3A**). The clear preference is expressed in the fact that at the individual level, larvae spent the great bulk of their time either in the polluted or in the unpolluted chambers and did not exhibit a balanced time expenditure between the chambers within each trial (**Fig. 3B**).

**Specific Aim 2** - We developed the “sandbox” MATLAB-based virtual arena and simulated virtual larval movement trajectories in response to the artificial gradient of PAH pollution (**Fig. 3**).

**Specific Aim 3** - We have fully developed the biophysical framework, and the modeled current field was successfully validated against in situ measurements (**Fig. 3**). We also completed the hindcast simulation for the oil spill that occurred in Feb. 2021 (**Fig. 3**).

***Figure 3.*** *Preliminary results and available resources. (Specific Aim 1). (****A****) The relative amount of time spent in the contaminated versus unpolluted chambers in preliminary experiments (n=29). (****B****) The histogram of the relative time spent in the polluted chamber (n=29). (****C and D****; Specific Aim 2) The “sandbox” intermediate modeling framework was developed for simulating movement and pollution at variable scales for BRW with (****C****) and without* ***(D****) a predetermined direction and a PAH avoidance strategy of 20° left turns upon encounter with above-threshold PAH concentrations. (****E****; Specific Aim 3) The Israeli oil spill hindcast simulation (Feb. 2021). Oil slick identified using Sentinel 1 satellite synthetic aperture radar SAR data. (****F****) Simulation of the Israeli oil spill using the oil-CMS model. Example results from the connectivity modeling framework for the Eastern Mediterranean were provided for larval Parupeneus forsskali and characterized by a PLD of 25-37 days and shallow coastal habitat (adult fish) at depths of 10-40 m. (****G****) The regional connectivity matrix for the same simulation, where the area between two adjacent white lines (vertical: bottom to top and horizontal: left to right) represents the coastal areas of Egypt, Israel, Lebanon, Syria, Turkey, Greece, and Cyprus, respectively.*

* 1. **Research resources**

Our resources include laboratory space, the shuttle tank, the enviroFlu PAH sensor (TriOS Optical Sensors, Germany), the CMS modeling framework, and SMURFS. In addition, the molecular identification of newly recruited post-larvae will be provided by the Laboratory of Dr. Nir Stern from IOLR (see **Support Letter #1**).

* 1. **Expected results and possible pitfalls**

We expect to bridge the critical knowledge gap of how larval fish respond to PAH pollution. We will provide a detailed description of the larval response and how it impacts regional and local larval dispersal and connectivity patterns. As in most behavioral experiments, we expect the experimental results to be highly variable among individuals and species and not necessarily provide significant results for all experiments. We will address this challenge systematically per experiment such that the effect and its variation will be implemented in the modeling frameworks of Specific Aims 2 and 3. In addition, the results might be contradictory between different methods. For example, we may observe increased recruitment to contaminated SMURF units and decreased recruitment in contaminated light traps. We will look for explanatory external variables that explain differences in such cases. If a variable is not found, we will simulate increased and decreased recruitment as part of a sensitivity analysis, adding this possible variability to the overall uncertainty of the system. Similarly, we know little about small-scale PAH distribution and patchiness during pollution events. To address this, we will simulate a range of patchiness patterns, using sensitivity analysis of the effect of patchiness on larval dispersal patterns. In addition, as larval fish recruitment is affected by a wide range of factors beyond the potential impact of PAHs, we expect variability in the SMURF and light trap results. To isolate the effect of PAH, we will use paired units spaced 10 m apart that will be randomly orientated to each other, with positions exchanged every consecutive deployment.

1. **Summary**In summary, we propose comprehensive research to address the fundamental question of how larval fish respond behaviorally to oil pollution and how the response affects broad ecological processes of larval dispersal and connectivity. We propose a robust combination of lab, in situ, modeling experiments, and simulations to provide a novel multi-scale solution. Oil pollution has proven to be a chronic global problem 60. Understanding the effect of pollution on larval dispersal and connectivity is at the forefront of contemporary marine ecology, which strives to realistically recreate the marine environment, including widespread anthropogenic stressors such as oil spills. Our results will serve marine resource managers and promote science-based marine decision-making and spatial planning. Spatial planning is a critical global trend that prioritizes protecting and conserving marine ecosystems61.

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