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Occurrence, toxicity and ecological risk of larvicidal oil in the coastal marine ecosystem of Hong Kong

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ABSTRACT

Application of larvicidal oil (LO) is the most common practice in Hong Kong to control mosquitos, and hence prevent mosquito-borne diseases and protect human health. Globally, this study represented the first comprehensive assessment of toxicity and risk posed by LO to marine organisms. We found concentrations of LO ranged from 0.08 to 0.66 mg/L in coastal seawaters of Hong Kong. Waterborne exposure to water-accommodated fractions of LO resulted in growth inhibition to two microalgal species (72-h EC_{50}: 1.92–2.90 mg/L) and acute mortality to three marine animals (96-h LC_{50}: 3.41–8.10 mg/L). From these toxicity results, a concentration that considered to be hazardous to 5% of species (HC_5) was predicted at 1.45 mg/L, while the predicted no-effect concentration was determined to be 0.29 mg/L. The hazard quotient of LO exceeded 1 at 9 out of 15 sites, indicating moderate-to-high ecological risk to exposure of LO in the marine environment of Hong Kong.

1. Introduction

Mosquitoes, due to their ability to transmit diseases to humans, are among the most influential insect in the world causing millions of deaths every year (WHO, 2016). These mosquito-borne diseases are usually caused by viruses or parasites, which are transferred from affected animals to mosquitoes through blood, after which the virus or parasite reproduces inside the mosquito's body and is then transferred to humans when the mosquito bites them (AMCA, 2014; NPIC, 2015).

In Hong Kong, dengue fever, Japanese encephalitis, malaria, West Nile virus infection, yellow fever and Zika virus infection are listed as notifiable infectious diseases by mosquitoes (CHP, 2020). Among these six diseases, dengue fever, Japanese encephalitis, malaria and Zika virus are regularly reported with a total of 1101 confirmed cases between 2008 and 2017 (CHP, 2018) (Fig. S1). Dengue fever is the most frequently reported mosquito-borne disease in Hong Kong; Malaria comes second while only a few cases of Japanese encephalitis have been recorded (Fig. S2). Given a vast amount of cases especially in summer, it is important to monitor and control the population of mosquitoes in Hong Kong to mitigate the mosquito-borne disease.

Application of pesticides, which are generally divided into larvicides and adulticides, is the most common way to control mosquitos in Hong Kong. Larvicides, which target larvae in surface waters, are more commonly used because it is easier to reach and kill the aquatic, immature mosquitoes than flying, mature individuals (AFCD, 2010). There are six registered larvicides in Hong Kong, including the bacteria *Bacillus thuringiensis* (B.t.i.) and *Bacillus sphaericus* (Bs), the insecticide chemical S-Methoprene, temephos, larvicidal oil and monomolecular film (MMF) (AFCD, 2010). Among these registered larvicides, larvicidal oil is commonly used on surfaces of drainage systems by the Government of the Hong Kong Special Administrative Region (HKSAR) due to its efficacy, ease of application and low cost (FEHD, 2015). In 2016, a total of 131,500 L was applied by the HKSAR Government (FEHD, 2017). Applied larvicidal oil is eventually discharged to the marine environment due to rainfall and surface runoff. Some local fish farmers operating in Tolo Harbour, northeastern Hong Kong suspected that occurrences of severe red tides and massive fish kills in fish culture zones might be associated with the discharge of larvicidal oil (AFCD, 2010; HKSAR, 2016). However, information regarding the composition of the larvicidal oil used in Hong Kong, its occurrence, potential toxic effects and ecological risks to marine organisms and the coastal marine ecosystem is lacking. This study, therefore, aimed to: i) characterize composition of the larvicidal oil used by the HKSAR government; ii) acquire information on the amount and distribution of the larvicidal oil used in various districts of Hong Kong; iii) conduct bioassays to determine thresholds for effects of the larvicidal oil to five marine species

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of different trophic levels including algae, crustacean and fish; and iv) evaluate ecological risks of the larvicidal oil to marine ecosystems by developing a species sensitivity distribution (SSD), determining a predicted no-effect concentration, and computing hazard quotients (HQs). In this study, we hypothesized that the larvicidal oil is toxic to non-target marine organisms and thus pose ecological risks to the coastal marine environment of Hong Kong.

2. Methods and materials

2.1. Characterization of larvicidal oil

The larvicidal oil was purchased from Pro-Link Asia Co. Ltd. (Hong Kong), which is the sole supplier for the HKSAR Government. It was characterized using a gas chromatograph (GC; Agilent 6890, Santa Clara, CA, USA) equipped with a mass spectrometer (MS; Agilent 5973, Santa Clara, CA, USA). The GC column used was 30 m DB-5MS fused silica capillary with 0.250 mm internal diameter and 0.25 μm film. The oven temperatures were programmed with initial 35 °C, maintained for 2 °C, and increase to 250 °C via ramping of 10 °C/min, then further increase to 320 °C via ramping of 20 °C/min and maintained at 320 °C for 10 min. Sample injection of 1 μL was in splitless mode with helium flow rate of 2 mL/min. Mass spectra were collected qualitatively in full-scan mode with m/z 50 to 500, while selected ion monitoring (SIM) mode was used for quantitation. Assignments of each compound in the larvicidal oil were done by matching the retention times in gas-chromatograms and ratios of fragment ions to corresponding parent ions of the compound obtained from the mass spectrum in the NIST 14 library (Bennett et al., 1990; NIST, 2016).

2.2. Surveillance of mosquitoes in Hong Kong

Since 2000, oviposition traps (ovitraps) have been used in designated areas in Hong Kong to monitor and investigate the occurrence of adult mosquitos of the genus *Aedes* by use of Area Ovitrap Index (AOI) and Monthly Ovitrap Index (MOI). AOI shows the extent of distribution of *Aedes* mosquitos in a surveyed area while MOI shows the average of all AOI values of the area within the same month, which indicate the territory-wide situation for occurrences of *Aedes* mosquitos (FEHD, 2016). The ovitrap index for *Aedes* is calculated (FEHD, 2016) as

Ovitrap Index
= Number of Aedes positive ovitraps/Number of ovitraps collected from the specific area × 100%

Values of MOI, during 2008–2017, were obtained from the Food and Environmental Hygiene Department (FEHD) of the HKSAR Government. Annual consumption of the larvicidal oil and number of infectious diseases cases, reported during 2008–2017, were obtained from FEHD and the Centre for Health Protection, respectively.

2.3. Quantification of the larvicidal oil in marine environments

2.3.1. Water sampling

Three replicate samples of one-litre surface water were collected from 0.5 m depth at locations A1–A4 along the coast of Hong Kong in both 2016 wet season (August–September) and 2017 dry season (January–March) whereas samples were collected at locations A1–A15 in 2017 wet season (June–August) (Fig. 1). All water samples were preserved with 5 mL 1:1 (v:v) hydrochloric acid (pH < 2) and stored in dark at 4 °C before extraction (ASTM, 2011; BS, 2008; USEPA, 1999). Dissolved oxygen (DO), salinity, temperature and pH were immediately measured after collection of water with DO meter (YSI Professional Plus, USA).

2.3.2. Extraction of larvicidal oil

The larvicidal oil in water samples was extracted thrice with *n*-hexane with a volume ratio of 20:1 with vigorous shaking (Ahmed et al., 1998; Bennett et al., 1990). The organic layer (upper layer) was collected and combined, followed by passing through 10 g anhydrous sodium sulphate (Sigma-Aldrich, St. Louis, MO, USA) and 1 g clean-up column, silica gel (Sigma-Aldrich, St. Louis, MO, USA) (BS, 2008; USEPA, 1999). The extract was then concentrated to 1 mL under a gentle stream of nitrogen.

2.3.3. Quantification of larvicidal oil

The resulting samples were quantified by use of GC–MS with the same conditions as were used to characterize larvicidal oil. A calibration standard curve was prepared using five calibration mixtures by diluting C_7 to C_{40} saturated alkanes standard (Sigma-Aldrich, St. Louis, MO, USA) with *n*-hexane at concentrations 0 (blank), 1, 5, 10 and 20 mg/L. Concentrations of individual petroleum hydrocarbon components were determined by integrating the area under the curve of the chromatogram and compared to the external standard curve. Concentrations of total petroleum hydrocarbons (TPH) (expressed in mg/L) were calculated by adding up individual petroleum hydrocarbon in samples (Reddy and Quinn, 1999).

2.4. Toxicity testing

2.4.1. Test species and conditions

All five species used in this study, including two marine microalgae *Isochrysis galbana* and *Chaetoceros gracilis*, the copepod *Tigriopus japonicus*, the brine shrimp cysts *Artemia franciscana* and the marine medaka fish embryos *Oryzias melastigma*, were cultured in the laboratory of School of Biological Sciences of the University of Hong Kong. *Isochrysis galbana*, *C. gracilis* and *T. japonicus* were acclimated to test conditions (20 ± 1 °C, 14 h, 10 h light: dark photoperiod) for one week prior to the experiments. *Artemia franciscana* of instar 2–3 stages and *O. melastigma* of 4 h post-fertilization (hpf) were used for experiments.

2.4.2. Preparations of chemicals

Test solutions were prepared by diluting 100% water-accommodated fractions (WAFs) to desired test concentrations with f/2 medium for algal tests or artificial filtered seawater for tests other than algae (Barron et al., 1999). WAFs were prepared by mixing one part larvicidal oil with nine parts of either f/2 medium for algal tests or artificial filtered seawater for copepod, brine shrimp and fish embryo tests (Barron et al., 1999; Girling et al., 1994; Martínez-Jerónimo et al., 2005; Neff et al., 2000; Swigert et al., 2014). WAFs were made by stirring water and oil for 24 h by use of a magnetic stirrer to ensure the mixture was at equilibrium. Mixtures were prepared in glass bottles that had been rinsed with acid and acetone. The mixture was then allowed to separate for at least 1 h at room temperature in the dark prior to use in tests (Girling et al., 1994; Neff et al., 2000; Swigert et al., 2014; Zhang and Yan, 2014). The aqueous phase, which was the test stock solution (i.e., 100% WAFs), was collected using a siphon tube inserted in bottles and collected WAFs from the below layer of oil (Barron et al., 1999).

2.4.3. Acute toxicity tests

72-h tests to determine inhibition of growth of algae and 96-h acute tests with lethality as the endpoint were performed according to OECD guidelines No. 201, 202 and 236 (OECD, 2004, 2011, 2013). There were seven treatments (including control) of the test chemical. Test species were not fed during the experiment (Kwok and Leung, 2005; Lee et al., 2007; Lee et al., 2013).

Isochrysis galbana and *C. gracilis* were exposed to 0, 0.1, 0.2, 0.4, 0.6, 0.8 or 1% WAFs and 0, 0.02, 0.05, 0.1, 0.2, 0.5 or 1% WAFs, respectively, with four replicates for each treatment. Experiments were conducted in 10 mL test vials with 8 mL test solution. Algal cell density was

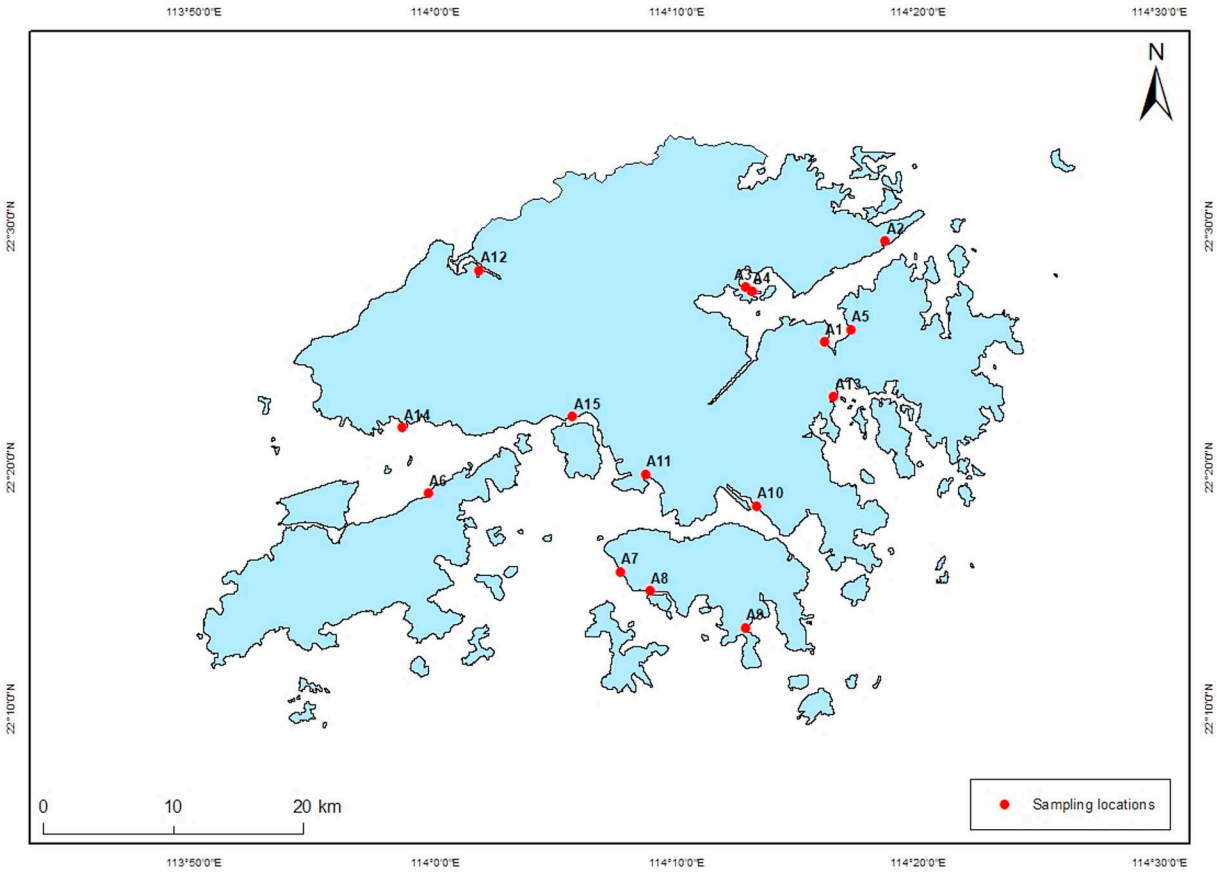

Fig. 1. Map of Hong Kong indicating the locations (A1–A15) of collecting seawater samples in this study: A1: Sai Keng; A2: Fung Wong Wat; A3: Sam Mun Tsai; A4: Yim Tin Tsai; A5: Yung Shue O; A6: Sham Shui Kok; A7: Cyberport; A8: Aberdeen; A9: Stanley; A10: Kwun Tong; A11: Lai Chi Kok; A12: Yuen Long; A13: Sai Kung; A14: Tuen Mun, and A15: Tsuen Wan.

determined daily during the test period by sampling 1 mL of algal culture from each replicate and preserved with 0.01 mL Lugol's solution prior to counting the cell number using a cell counter (Multisizer II, Coulter, Fulleron).

Tigriopus japonicus was exposed to 0, 0.5, 1, 2, 5, 8 or 10% WAFs with three replicates for each treatment. Six-well plates were used with 5 mL test solutions per well with one test organism in each well. One well plate was considered as independent replicate. *Artemia franciscana* was exposed to 0, 0.1, 0.2, 1, 2, 5 or 8% WAFs with three replicates for each treatment. Six-well plates were used with 5 mL test solutions per well with ten test organisms in each well. *Oryzias melastigma* was exposed to 0, 0.1, 0.2, 0.5, 2, 5 and 8% WAFs with six replicates for each treatment. Twenty-four-well plates were used with 2 mL test solutions per well with five embryos in each well. Individual wells were considered as an independent replicate for *A. franciscana* and *O. melastigma*. Immobilization and mortality were observed daily and dead animals were removed immediately.

2.4.4. Determination of end-point of experiment

Specific growth rate of microalgae *I. galbana* and *C. gracilis* were calculated as $(\ln X_j - \ln X_i)/(t_j - t_i)$ where X_i was the initial cell density at time i, X_j was the final cell density at time j. Percentage inhibition of growth rate ($\%I_r$) was calculated as $(\mu_C - \mu_T)/\mu_C \times 100\%$, where μ_C was the mean value for average specific growth rate (μ) in the control group, μ_T was the average specific growth rate for the treatment replicate (OECD, 2011). Lethality of the copepod *T. japonicus*, brine shrimp *A. franciscana* and embryos of the fish marine medaka (*O. melastigma*) were recorded for every 24 h. Percent inhibition and mortality were plotted as a function of logarithm of the test substance concentration plotted by use of the sigmoidal log (agonist)-response regression model (GraphPad Prism, Version 6.01, San Diego, USA). Median effect concentrations (EC_{50}) of larvicidal oil (in terms of % WAFs) were reported for microalgae *I. galbana* and *C. gracilis* while median lethal concentrations (LC_{50}) of larvicidal oil (in terms of % WAFs) were reported for copepod *T. japonicus*, brine shrimp *A. franciscana* and fish embryos of marine medaka *O. melastigma* (Lee et al., 2007). The EC_{50}s and LC_{50}s of test species were converted from % WAFs to mg/L based on the measurement composition of 100% WAFs prepared for toxicity testing described in Section 2.4.5.

2.4.5. Chemical analyses

Compositions of 100% WAFs prepared for toxicity testing were measured by use of GC–MS with the same conditions as the characterization of the larvicidal oil. Quantification method of 100% WAFs was the same as the one used to quantify the larvicidal oil in water samples.

2.5. Ecological risk assessment of larvicidal oil

A species sensitivity distribution (SSD) was constructed by use of a cumulative distribution plotted against rank-assigned centile of $E(L)C_{50}$ of larvicidal oil, i.e., WAFs, on various species obtained from current results based on log-normal distribution model (Jin et al., 2012, 2013; Wang et al., 2008; Wheeler et al., 2002; Zhou et al., 2019). A hazardous concentration for 5% of species (HC_5) was derived from SSD. The predicted no-effect concentration (PNEC) was derived by dividing HC_5 with an assessment factor of 5 (European Commission, 2003). To evaluate the ecological risks of larvicidal oil to marine ecosystem, hazard quotients (HQs) were calculated as by dividing measured environmental concentration (MEC) with the PNEC. If computed HQs

were greater or equal to one, the marine species were at risk due to the exposure of the larvicidal oil, and vice versa.

2.6. Statistical analysis

The data of MOI, mean larvicidal oil consumption in summer were tested for normality using Kolmogorov-Smirnov one-sample test and for homogeneity of variance using Levene's test, followed by one-way Analysis of Variance (ANOVA) where 'district' was fixed factor. Post hoc Tukey's multiple comparison was performed following one-way ANOVA if differences among mean values were confirmed. If the data did not exhibit homogeneity of variance, they were logarithmic-transformed (base 10), then proceeded to ANOVA, or performed non-parametric Kruskal-Wallis test followed by Dunn's test. Pearson's Correlation Analysis were performed among MOI, the mean larvicidal oil consumption and the number of reported infectious diseases cases in summer.

The mean concentrations of larvicidal oil in water samples at locations A1–A4 were temporally compared among 2016 wet season, 2017 dry season and 2017 wet season using one-way ANOVA followed by Tukey's test whereas the mean concentrations of larvicidal oil in water samples were spatially compared among locations A1–A15 using non-parametric Kruskal Wallis test followed by Dunn's test because the data failed the assumption of homogeneity of variances after they were logarithmic-transformed (base-10). All statistical tests were conducted by IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA, 2011). Significance levels were set at 0.05. Statistical power was checked using G* Power 3.1 (Heinrich-Heine-Universität Düsseldorf, Germany).

3. Results

3.1. Composition of the larvicidal oil

Both gas-chromatographs and mass-spectra showed that the larvicidal oil was a mixture of aliphatic hydrocarbons consisting mainly of *n*-octane (C_8) to *n*-pentacosane (C_{25}) (Fig. 2). Shorter-chain petroleum hydrocarbons (PH) eluted earlier than longer-chain PH. C_{12} and C_{14} were the most abundant compounds present in larvicidal oil (Fig. S3), based on the largest area under the peak among all compounds.

3.2. Monthly ovitrap index and consumption of the larvicidal oil

Both MOI and consumption of the larvicidal oil were significantly greater in summer than in winter over the 18 districts of Hong Kong, and both showed significant difference among districts. MOI was the least in the Tsuen Wan district and the greatest in Shatin district (one-way ANOVA; $F_{17,\ 144} = 3.164$, $p < 0.05$, Fig. S4). Also, there was no significant correlation between MOI and the number of cases of dengue fever reported between January 2008 and October 2017 (Pearson's product-moment correlation $r_{0.05(2),116} = 0.114$, $p > 0.05$, Fig. S5). For consumption of the larvicidal oil, it was the least on Islands and the greatest in Yuen Long district (Kruskal-Wallis test; $\chi^2_{0.05,17} = 116.683$, $p < 0.05$, Fig. S6). However, there was also no significant correlation between MOI and consumption of the larvicidal oil during either winter ($r_{0.05(2),160} = 0.103$ $p > 0.05$, Fig. S7a) or summer ($r_{0.05(2),160} = 0.105$, $p > 0.05$, Fig. S7b).

3.3. Concentrations of the larvicidal oil in marine environments of Hong Kong

The mean concentration of larvicidal oil collected at locations A1–A4 during 2017 wet season was 0.60 mg/L, which was significantly greater than that collected during 2016 wet season at 0.28 mg/L and 2017 dry season at 0.16 mg/L (one-way ANOVA; $F_{0.05(2),2,33} = 22.271$, $p < 0.05$) (Fig. 3). The mean concentration of the larvicidal oil was significantly different among sampling locations A1–A15 collected during 2017 wet season, with the least concentrations observed in A11 and A15 at 0.08 mg/L while the greatest concentration was observed in A2 at 0.66 mg/L (Kruskal-Wallis test; $\chi^2_{0.05,14} = 36.209$, $p < 0.05$) (Fig. 4). Environmental parameters of water samples are presented in Table S1.

3.4. Toxic effects and ecological risks of larvicidal oil

The 72-h EC_{50}s of the larvicidal oil to marine microalgae *I. galbana* and *C. gracilis* were 1.92 mg/L (0.43% WAFs) and 2.90 mg/L (0.65% WAFs), respectively, with the measured concentration of 100% WAFs at 445.70 mg/L. The 96-h LC_{50}s of the larvicidal oil to the copepod *T. japonicas*, brine shrimp *A. franciscana* and marine medaka fish embryos *O. melastigma* were 8.10 mg/L (7.15% WAFs), 3.41 mg/L (2.82% WAFs) and 4.39 mg/L (3.88% WAFs), respectively, with the measured

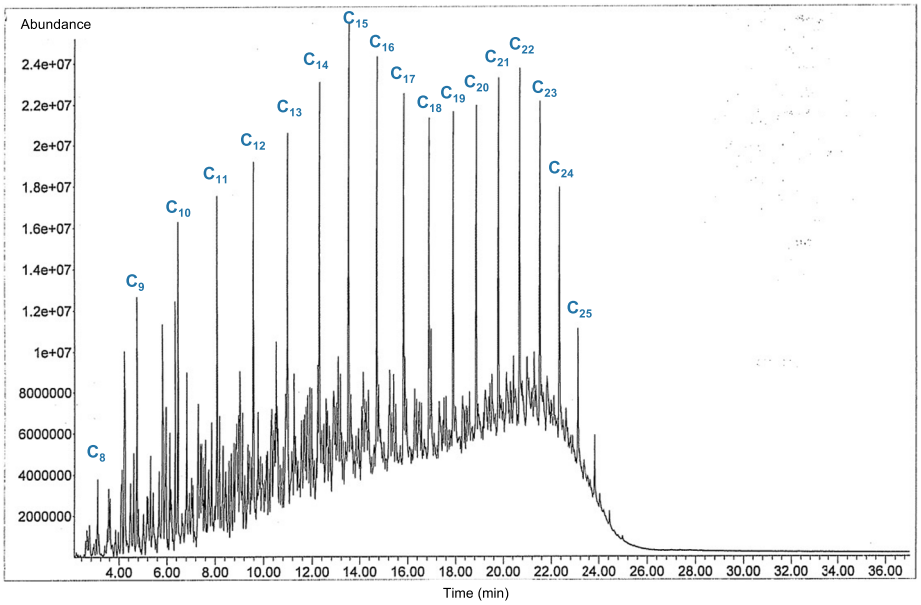

Fig. 2. Total ions chromatogram (TIC) of the larvicidal oil obtained from gas chromatography, with *n*-alkanes labeled from C_8 (*n*-octane) to C_{25} (*n*-pentacosane).

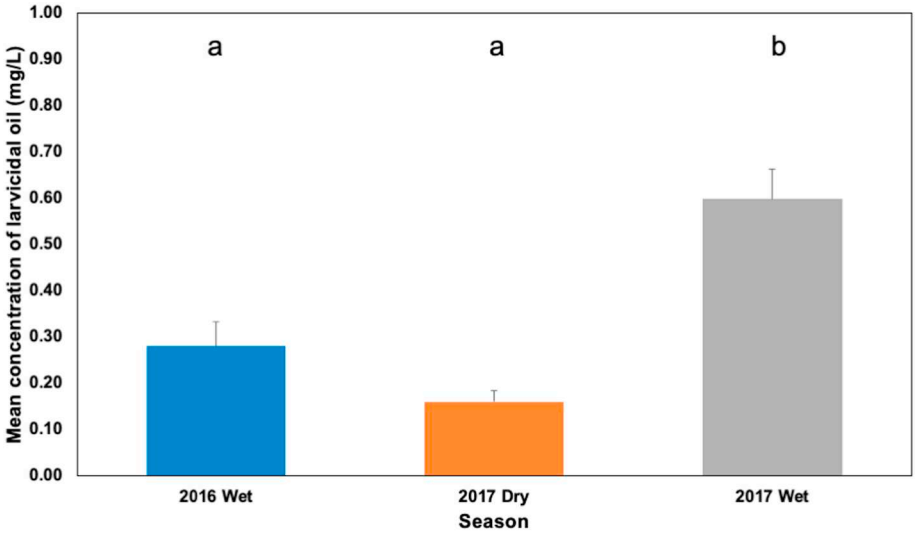

Fig. 3. Concentration of the larvicidal oil in seawater samples collected along the coastal sites of A1–A4 in Hong Kong in 2016 wet, 2017 dry and 2017 wet seasons (mean + SE). Different letters on top of the bars denote a significant difference for the concentration of larvicidal oil in seawater samples among seasons at $p < 0.05$.

concentration of 100% WAFs at 113.27 mg/L. *Isochrysis galbana* was the most sensitive while *T. japonicas* was the most tolerant taxon studied (Fig. S8; Table 1). The calculated HC_5 was determined to be 1.45 (0.97–2.16) mg/L (95% confidence interval) from the SSD (Fig. 5) whereas the PNEC was determined to be 0.29 mg/L. The HQs computed for 2016 wet season and 2017 dry season at A1–A4 were ranged 0.60–1.81, 0.27–0.77, respectively whereas HQs computed for 2017 wet season at A1–A15 were ranged 0.26–2.29, meaning that some sampling locations in the study were at risk (Fig. 6). Among the 15 sites, 9 sites had HQ > 1 indicating moderate-to-high risk of the larvicidal oil in the coastal marine environmental of Hong Kong.

4. Discussion

4.1. Identification of the larvicidal oil composition

Oil spills commonly happen worldwide and thus mixtures of petroleum hydrocarbons are one of the important sources of pollution in marine ecosystems. It is important to develop a method to identify the types of oil present in the marine environment for corresponding management. Among various techniques available for quantification of hydrocarbons in oil, gas chromatography (GC) is the most widely used method because it can identify marker compounds (Wang and Fingas, 2003) that can be used to characterize sources of petroleum hydrocarbons. While flame-ionization detectors can be used, mass spectrometry (MS) is usually coupled with GC because it is a sensitive detector that has low background signals and can provide unique mass spectra for each unknown compound. In this study, using GC–MS, we revealed that the larvicidal oil used in Hong Kong is a mixture of aliphatic hydrocarbons, ranging from *n*-octane to *n*-pentacosane. This pattern is consistent with Diesel No. 2, since it matches the carbon range with maximum concentrations for C_{12} to C_{14} and a central "hump" (Fig. 2) (Wang and Fingas, 2003).

After being released into the environment, mixtures of petroleum hydrocarbons would undergo weathering. Due to differential vapour pressures, solubility and sorption coefficients among individual petroleum hydrocarbons because of differential rates of degradation and partitioning, the relative proportions of individual constituents in mixtures would change (Neff et al., 2000). Therefore, it might not be possible to identify and distinguish original compositions of the larvicidal oil in the environment.

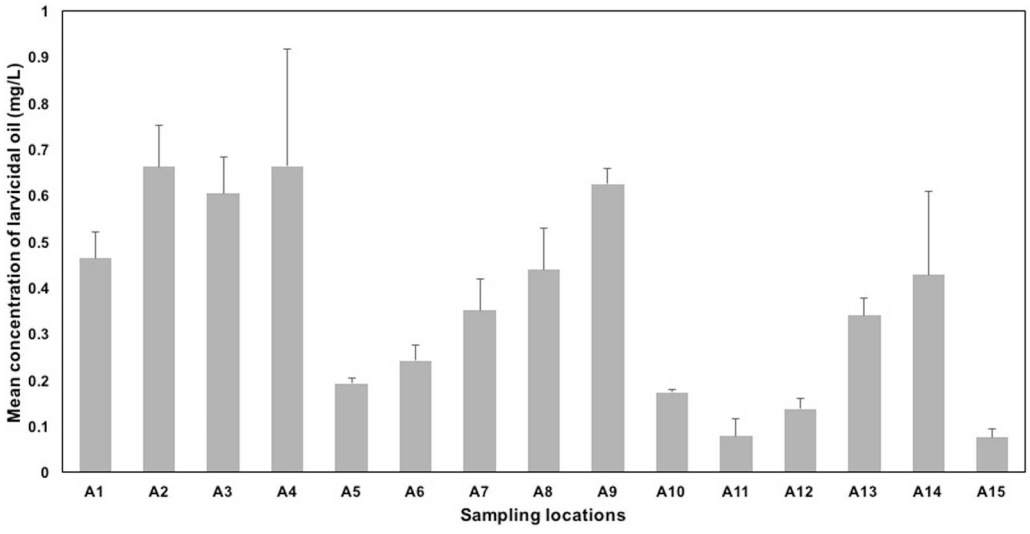

Fig. 4. Concentration of the larvicidal oil in seawater samples collected in 15 coastal sites in Hong Kong during July and August 2017 (mean + SE). The Kruskal-Wallis test detected significant differences for the concentration of larvicidal oil in seawater samples among the sampling sites ($\chi^2_{0.05,14} = 36.209$, $p < 0.05$; $\alpha = 0.05$) while the post hoc Dunn's test failed to detect significant differences between pairs of sampling sites due to large variation within site ($\alpha = 0.05$). The least p values were found between pairs A2 and A11; and A2 and A15, that lied between $0.1 < p < 0.2$.

Table 1
Median effect/lethal concentrations (EC_{50}/LC_{50}) from acute toxicity tests of the larvicidal oil on selected marine species (mean ± SD, n = 4 for microalgae, n = 3 for copepod and brine shrimp, and n = 6 for fish embryo).

Species	Endpoint	Duration (h)	n	Concentration-response equation	r^2	E/LC_{50} (%WAFs)	E/LC_{50} (mean ± SD; mg/L)
Isochrysis galbana	Growth inhibition	72	4	y = 100/(1 + 10^((0.43-x) * 8.903))	0.908	0.43	1.92 ± 0.22
Chaetoceros gracilis	Growth inhibition	72	4	y = 100/(1 + 10^((0.65-x) * 2.145))	0.877	0.65	2.90 ± 0.58
Tigriopus japonicus	Mortality	96	3	y = 100/(1 + 10^((7.15-x) * 0.130))	0.793	7.15	8.10 ± 1.30
Artemia franciscana	Mortality	96	3	y = 100/(1 + 10^((2.82-x) * 0.169))	0.796	2.82	3.19 ± 0.87
Oryzias melastigma	Mortality	96	6	y = 100/(1 + 10^((3.88-x) * 0.189))	0.704	3.88	4.39 ± 0.96

y, % of affected test population.
x, log-transformed larvicidal oil (% WAFs).
WAFs, water-accommodated fractions.

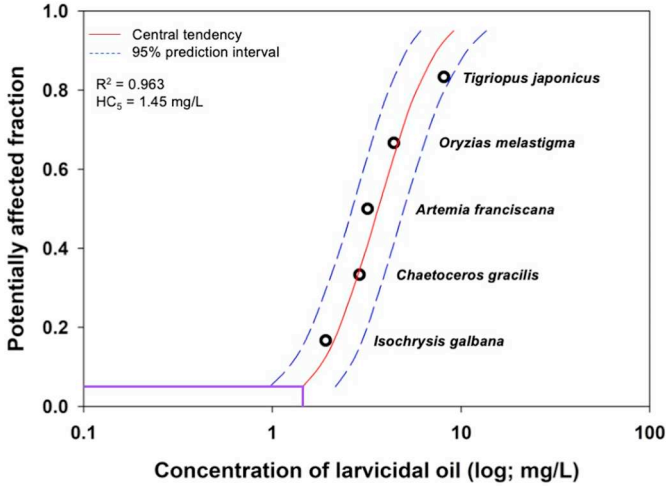

Fig. 5. Species sensitivity distribution of the larvicidal oil constructed based on toxicity results from this study.

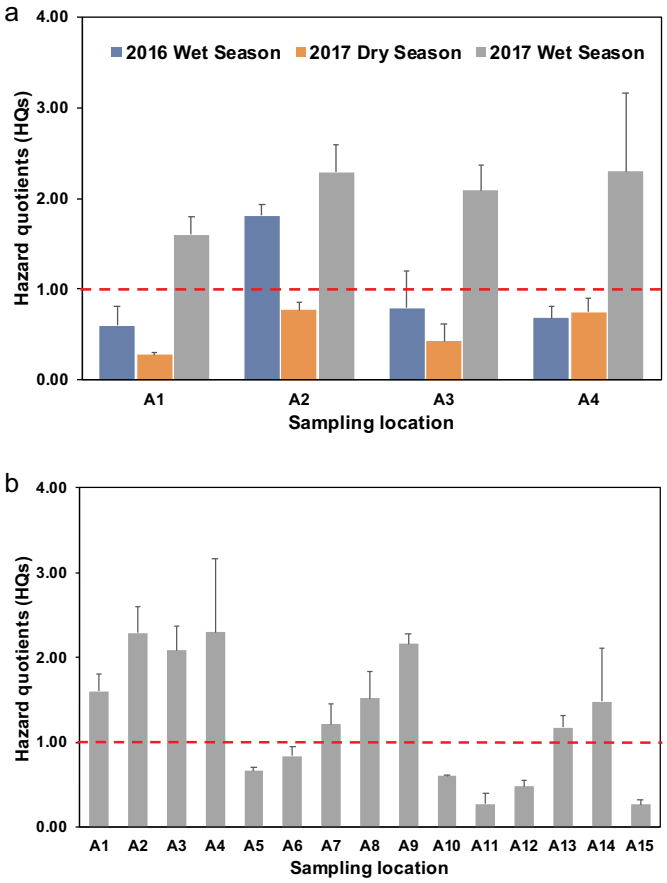

Fig. 6. Hazard quotients (HQs; mean + SE) were computed for a) four water sampling locations collected in 2016 wet, 2017 dry and 2017 wet seasons; and b) 15 sampling locations in 2017 wet season, based on the MECs of the larvicidal oil and its PNEC value derived from the SSD.

4.2. Monthly ovitrap index and consumption of the larvicidal oil

The fact that MOI is consistently greater in summer than that in winter can be explained by the weather in Hong Kong. Hong Kong is situated in the sub-tropical region where summer is very warm (up to 35 °C air temperature) and humid (up to 100% humidity) with heavy rainfall (up to 600 mm/month) that certainly favours growth of mosquitoes (HKO, 2017). However, MOI was not significantly correlated with number of dengue fever cases reported, suggesting that the number of reported dengue fever cases might not be due to local infection but might have originated from overseas. Previous data supported this explanation since >95% of reported cases were imported (CHP, 2011; HKSAR, 2016, 2017).

Alternatively, the fact that the larvicidal oil applied to aquatic environments was significantly greater in summer than in winter can be explained by a greater MOI observed in summer since the warm, humid weather favours the increase of mosquito populations (Reiter, 2001). More pesticides were thus applied in summer to control populations of mosquito. In Hong Kong, application of the larvicidal oil was the least on Islands whereas the greatest amount was applied in Yuen Long. Applications might be, in part, due to the distribution of populations in various districts where the Islands district had a small population size (2.1%) while Yuen Long district contributed the third highest percentage (8.4%) of population of Hong Kong (CSD, 2016).

Interestingly, there was no significant correlation between MOI and amounts of the larvicidal oil consumed. This might be because use of the larvicidal oil was not based on appearance of mosquitoes but was applied periodically and regularly as part of a general program to suppress the presence of mosquitoes. Another possible reason was that MOI referred to the trapping of *Aedes* mosquitoes only, but not other species (Chadee and Ritchie, 2010; FEHD, 2016), which resulted in under-estimation of the overall population of mosquitoes. Furthermore, the function of the larvicidal oil was applicable to all mosquito species, resulting to the lack of correlation between MOI and the amount of the larvicidal oil applied. As mosquitoes can breed in small areas where application of the larvicidal oil is not accessible (FEHD, 2005), this also leads to the lack of correlation of MOI and consumption of the larvicidal oil.

4.3. Toxic potency and ecological risk posed by the larvicidal oil

The octanol/water partition coefficient (log K_{ow}) is commonly used to characterize the lipophilicity of a compound including petroleum hydrocarbons (PH). The shorter the PH chain, the less its log K_{ow} value, implying a higher solubility of the substance in water, and vice versa. Therefore, shorter-chain PH may have greater toxic potential than longer-chain PH which have greater log K_{ow} (Di Toro et al., 2007; Tang

et al., 2011). In this study, the sum of concentrations of C_8 to C_{25} was used in determining the concentration of 100% WAFs, E(L)C_{50}s and the generation of SSD because these hydrocarbons are the main composition of the larvicidal oil. Although 100% WAFs showed that C_{12} to C_{14} (Fig. 2) have the maximum concentration among all PH, all compounds in larvicidal oil contributed to toxic potencies of the larvicidal oil.

The microalga *I. galbana* was the most sensitive species to the larvicidal oil, which might be due to the penetration of chemicals through the cell and thus altering photosynthesis and inhibiting growth (Pérez et al., 2010). The result deviated from the expectation that embryos of the small fish, marine medaka (*O. melastigma*) were expected to be the most resistant species to the larvicidal oil because they were at the highest trophic levels. Instead, the copepod *T. japonicus* was the species most tolerant to effects of larvicidal oil. The result was consistent with that from other organisms in freshwater environments that microalgae were more sensitive to petroleum distillates while the small planktonic crustacean (*Daphnia magna*) was less sensitive than rainbow trout (*Oncorhynchus mykiss*) (Swigert et al., 2014).

In this study, the derivation of SSD used data of five species only because there was no existing data from ECOTOX database showing the toxic potency of the larvicidal oil such that we cannot obtain previous data in addition to generate a higher resolution of SSD. Derivation of PNEC is essential for ecological risk assessment. Assessment factor of 1 to 5 were usually applied when using the SSD method (European Commission, 2003). Although there are always critics on the reliability of small sample size for SSD, recent studies showed that sample size of 5 (i.e., the number of test species in this study) still performed satisfactorily with using the SSD method (Sorgog and Kamo, 2019; Yeung et al., 2020).

Although the toxicity of petroleum had long been studied due to its persistence and bioaccumulative potential along the food chain (Garr et al., 2014), concentrations of aromatic hydrocarbons have been more reported than aliphatic hydrocarbons for water samples. It is because aromatic hydrocarbons are more soluble in water and they are more bioavailable and thus more toxic to aquatic organisms (Barron et al., 1999; Swigert et al., 2014). There have been few studies of toxicity of aliphatic hydrocarbons because of its hydrophobicity and assumed that only soluble petroleum hydrocarbons in crude oil were toxic to aquatic life. Therefore, the present study presented as the first study to determine toxic potencies of the larvicidal oil, mainly consisting aliphatic hydrocarbons.

Even though concentrations of TPH were measured at several locations in Hong Kong, the HQs exceeded 1.0 at 9 out of 15 sampled locations indicating that some adverse effects might be expected to aquatic environments at these particular locations. However, it was impossible to conduct an assessment of hazards or risks posed solely by use of the larvicidal oil to the coastal marine environment due to the fact that it was impossible to distinguish between PHs applied as the larvicidal oil and those released from other sources. In addition, even if there were markers specific to larvicidal oil or other diesel fuels, differential weathering in the environment made it impossible to partition the PHs from various sources. It is also impossible to know the proportion of larvicidal oil contributed to measure PHs in the marine environment as well as the proportion of the larvicidal oil contributing the hazard determined based on concentrations of PH in coastal marine waters of Hong Kong. Therefore, apart from the larvicidal oil, it is very important to explore a more sustainable and environmentally friendly mosquito control pesticide to minimize impacts to the marine environment.

5. Conclusions

This study comprehensively investigated, for the first time, the toxic potency and ecological risk of the larvicidal oil commonly used as a mosquito-control agent in Hong Kong. Aliphatic petroleum hydrocarbons were the main composition of the larvicidal oil which was largely applied by the HKSAR Government to surface drainage systems in Hong Kong every year especially during summer to eliminate mosquitoes, resulting in relatively elevated concentrations in the marine environment. Standard acute toxicity tests were conducted on five selected species, and the microalga *Isochrysis galbana* (a primary producer) showed to be the most sensitive. A hazardous concentration for 5% of species (HC_5) of 1.45 mg/L was derived from the SSD while hazard quotients showed that the coastal marine ecosystem of Hong Kong was at moderate-to-high risk of exposure to the larvicidal oil. More sustainable and environmentally friendly formulas of mosquito control pesticides are required to minimize its impact to the marine environment.

Credit authorship contribution statement

Katie W.Y. Yeung: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **John P. Giesy:** Writing - original draft, Writing - review & editing. **Guang-Jie Zhou:** Writing - review & editing. **Kenneth M.Y. Leung:** Conceptualization, Methodology, Formal analysis, Supervision, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111178.

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Occurrence, toxicity and ecological risk of larvicidal oil in the coastal marine ecosystem of Hong Kong

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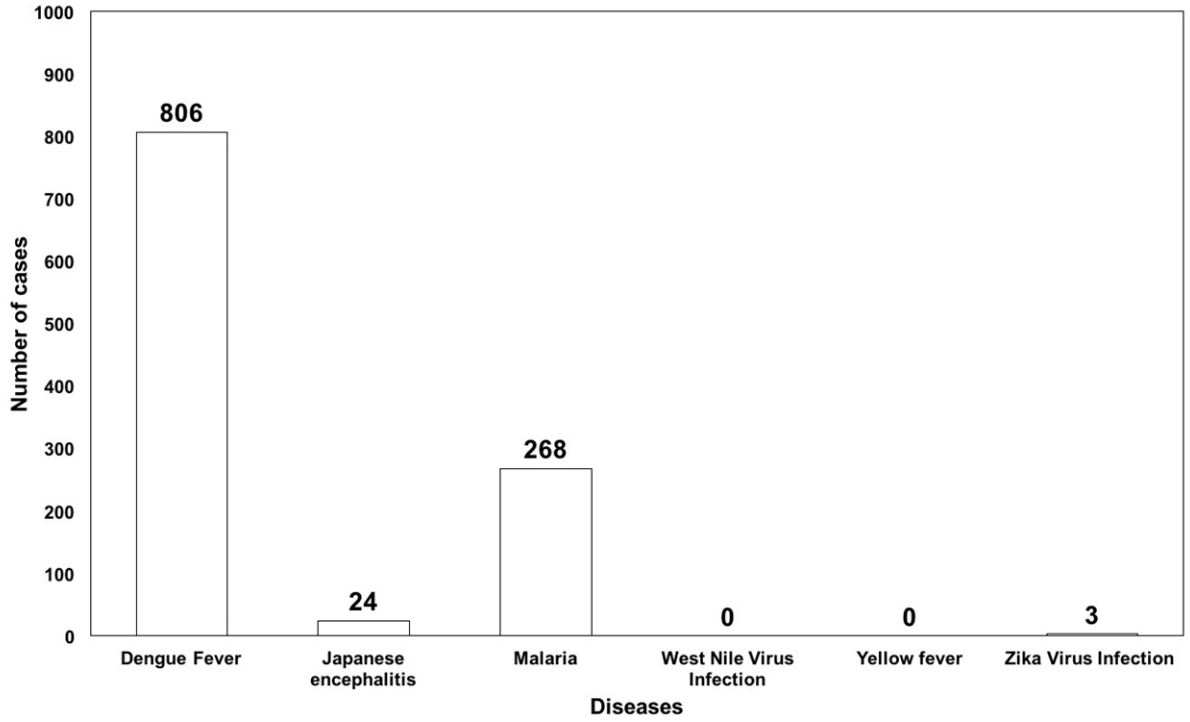


Figure S1 Number of cases of mosquito-borne diseases reported to the Department of Health of Hong Kong Special Administrative Region during 2008–2017 (CHP 2018).

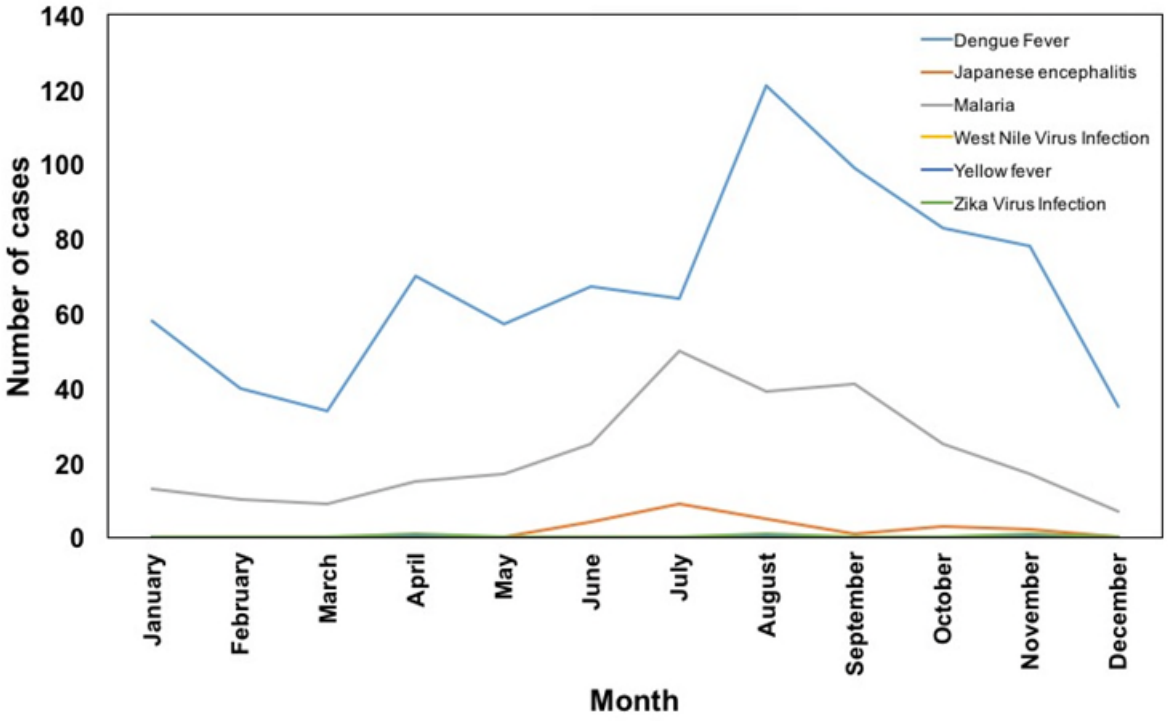


Figure S2 Monthly trend of number of cases of mosquito-borne diseases reported to the Department of Health of Hong Kong Special Administrative Region during 2008–2017 (CHP 2018). Note: no cases of West Nile Virus Infection and Yellow Fever were recorded during 2008–2017.

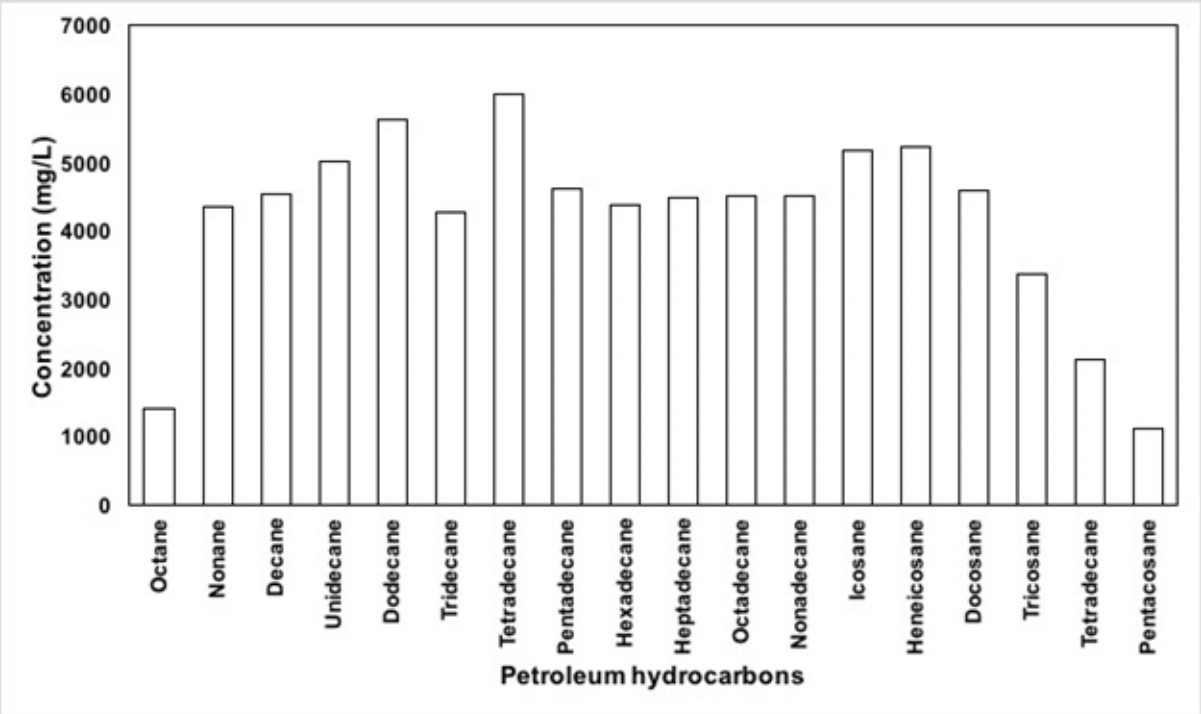


Figure S3 Composition and concentration of hydrocarbons (C_8 to C_{25}) in larvicidal oil.

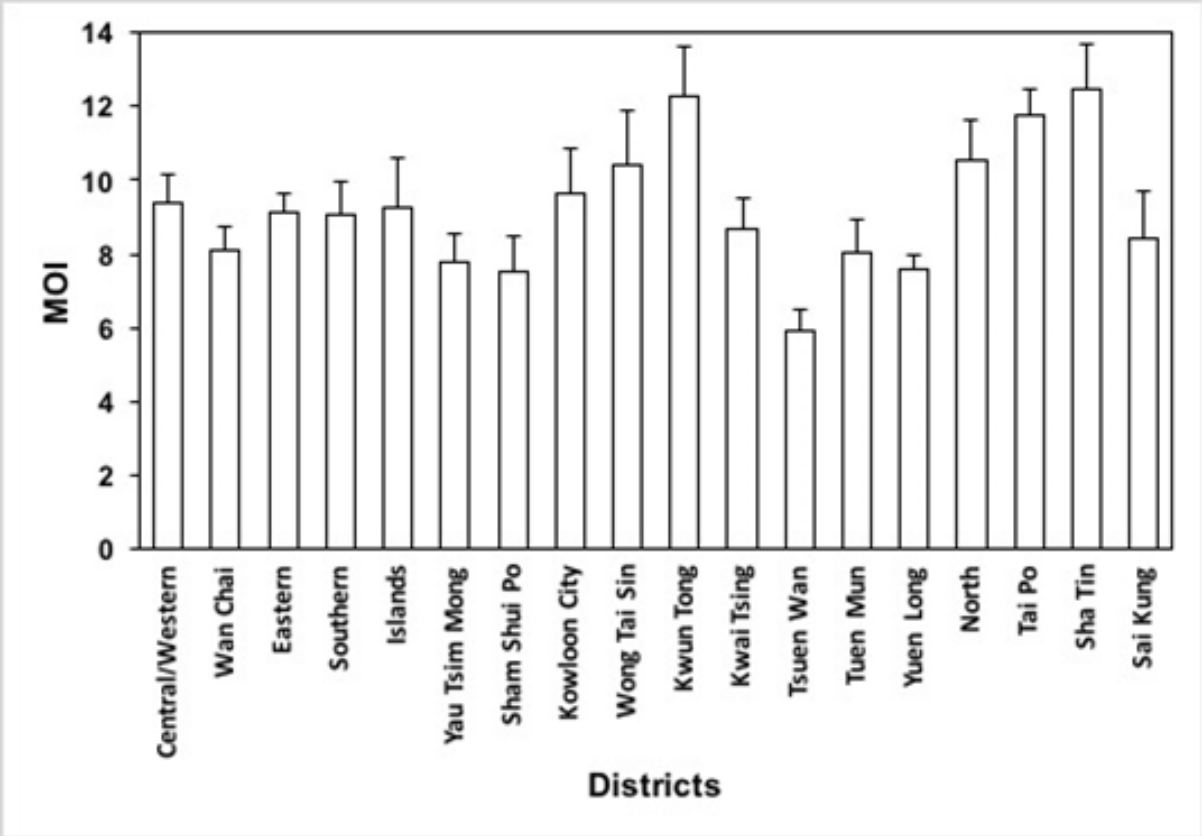


Figure S4 Monthly Ovitrap Index (MOI) in summer (June-September) during 2008–2017 in all 18 districts in Hong Kong (mean + SE).

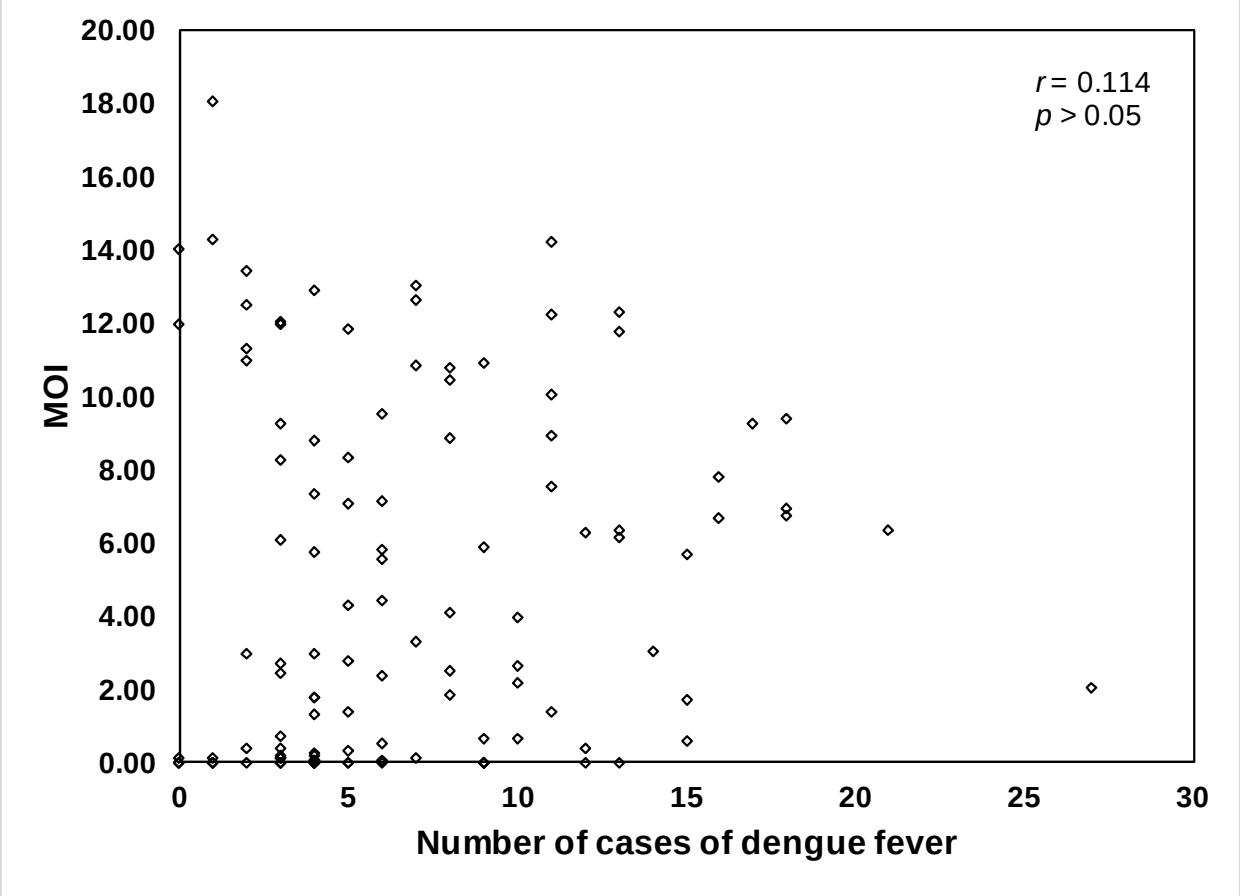


Figure S5 Relationship between Monthly Ovitrap Index (MOI) and number of cases of dengue fever reported every month between January 2008 and October 2017. No significant correlation was recorded ($p > 0.05$).

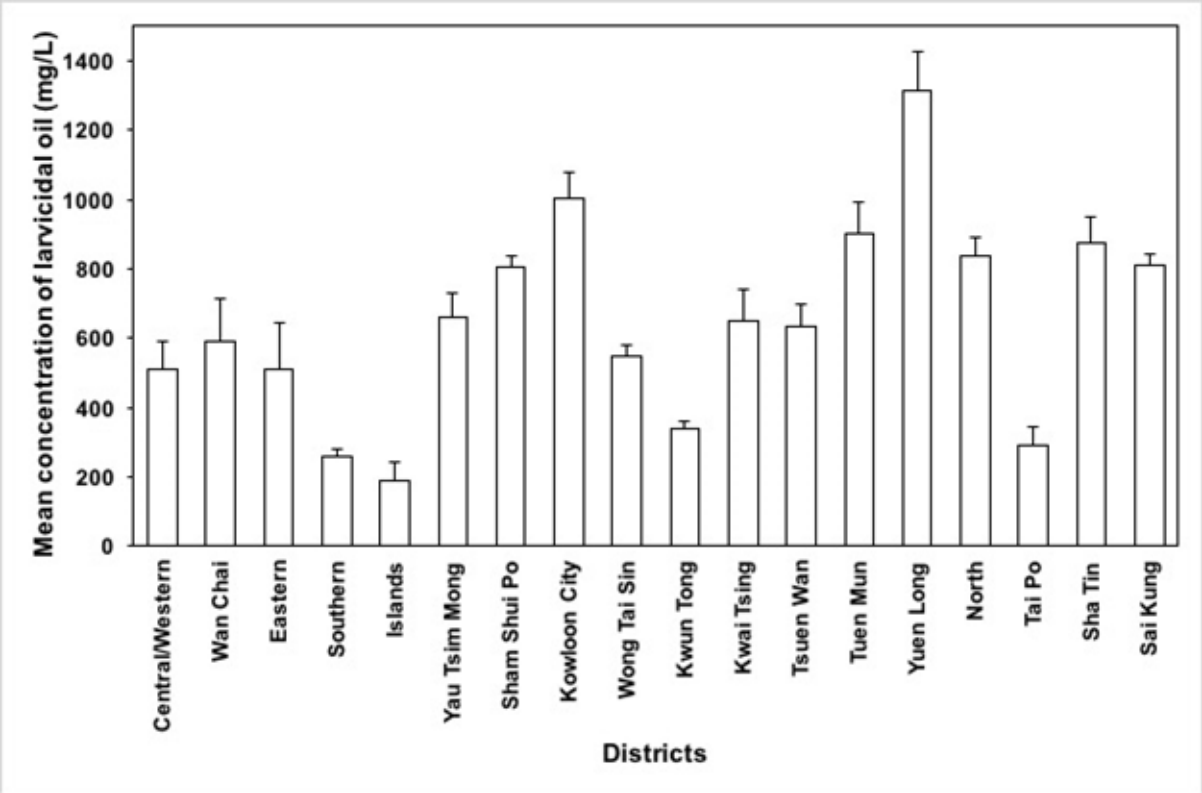
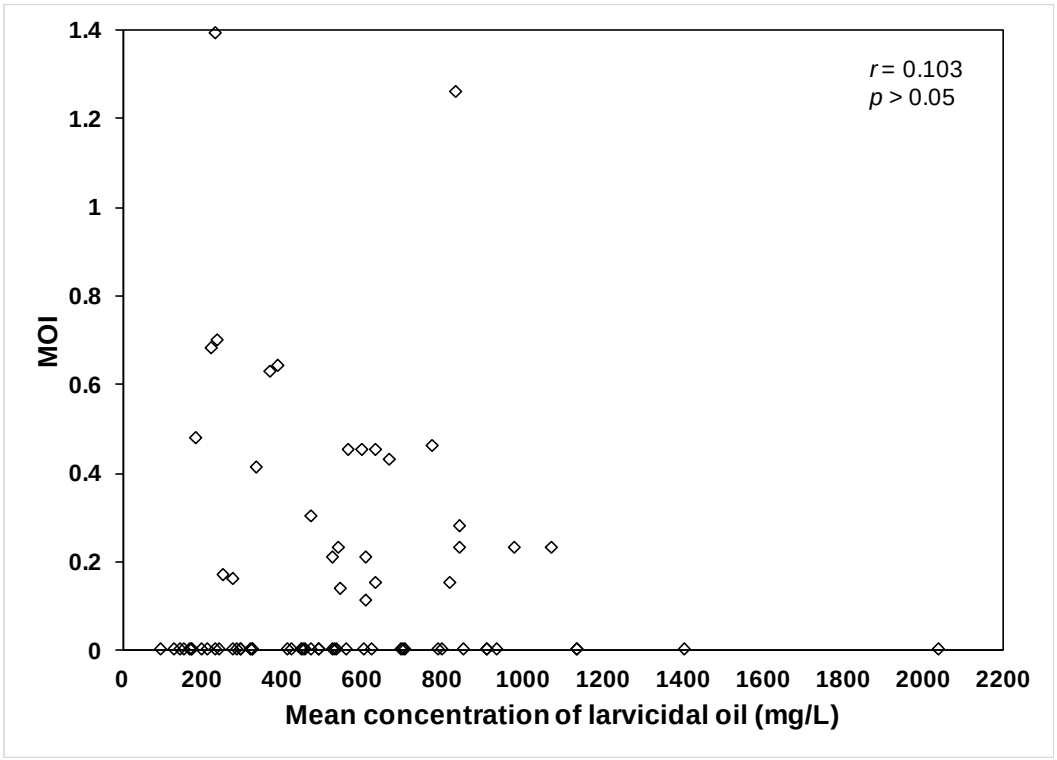


Figure S6 Mean larvicidal oil consumption (mg/L) in all 18 districts in Hong Kong during 2008–2017 (mean + SE).

a



b

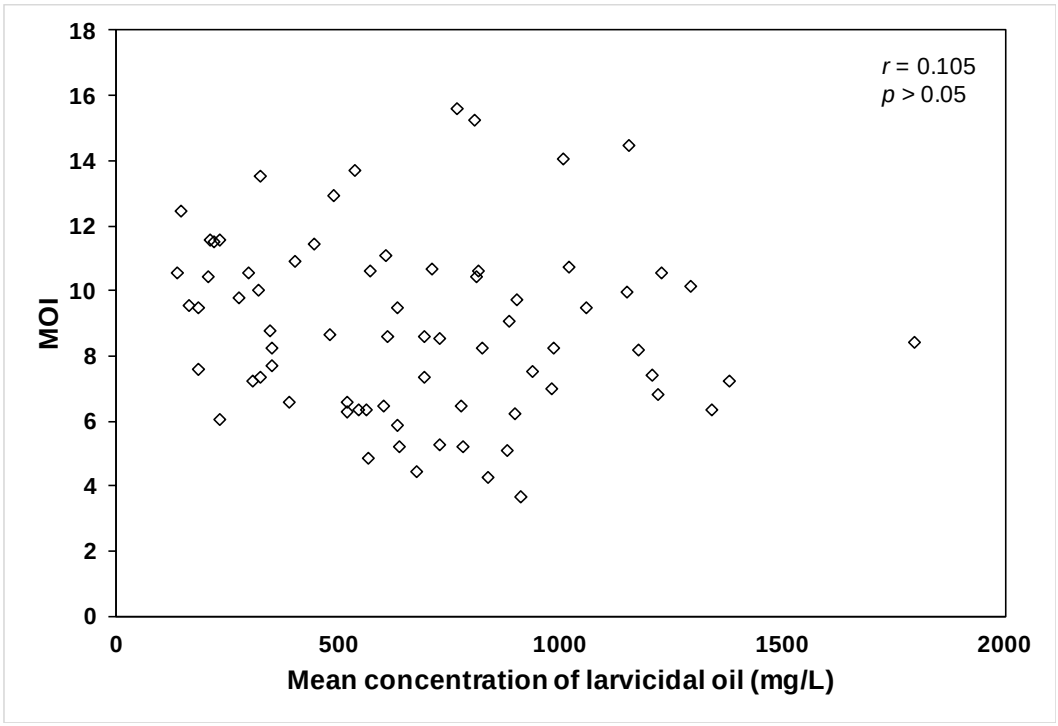


Figure S7 Relationship between Monthly Ovitrap Index (MOI) and mean monthly consumption of oil consumption in a) winter, and b) summer. No significant correlations were recorded ($p > 0.05$)

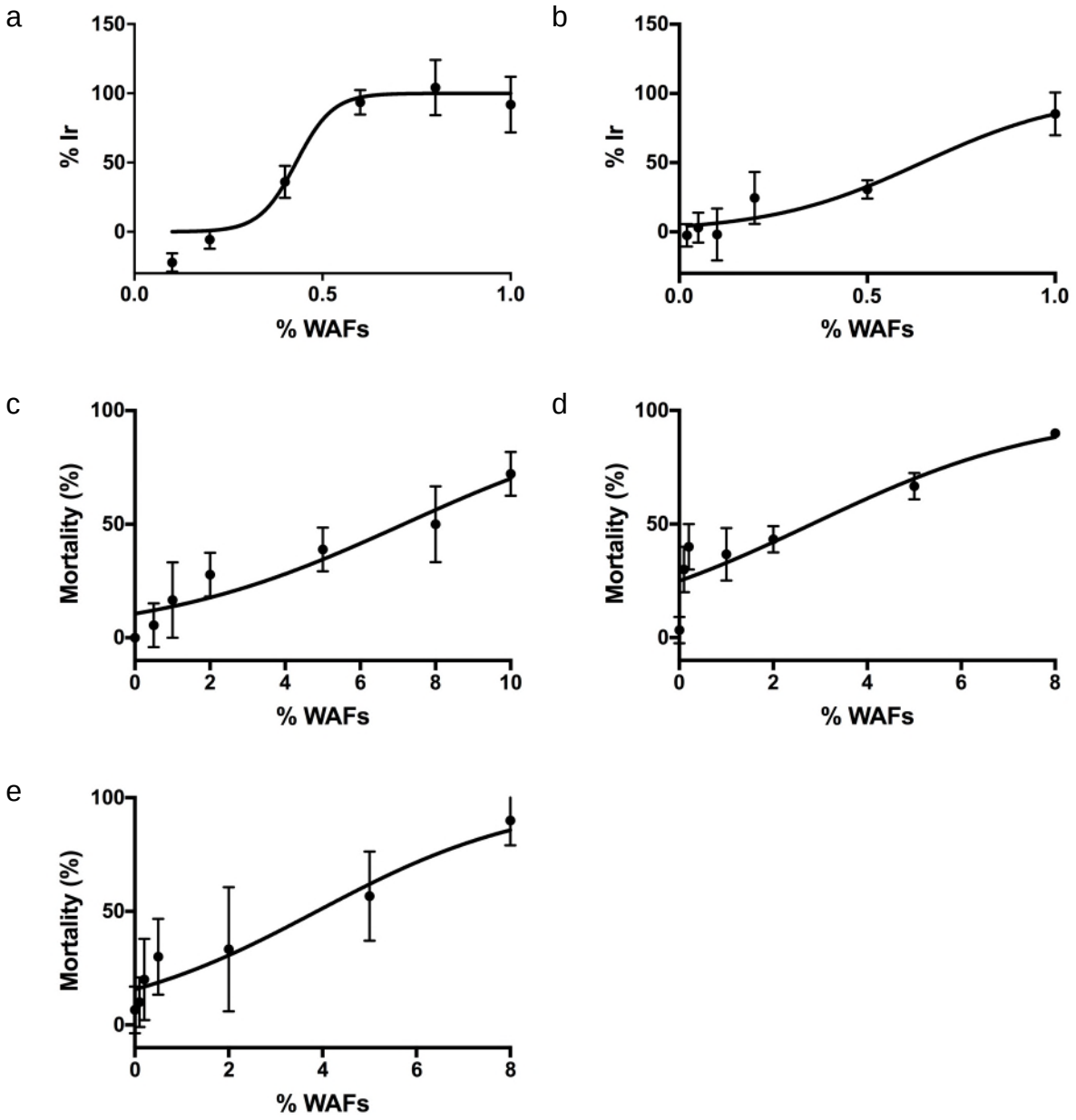


Figure S8 Concentration (% water-accommodated fractions) (% WAFs)-response (growth inhibition effects) of larvicidal oil on the percentage inhibition of growth rate (%Ir) of: a) the microalga *Isochrysis galbana* for 72 h (*n* = 4); and b) microalga *Chaetoceros gracilis* for 72 h (*n* = 4). Concentration (% water-accommodated fractions) (% WAFs)-response (mortality) of larvicidal oil on the mortality of: c) copepod *Tigriopus japonicus* for 96 h (*n* = 3); d) brine shrimp *Artemia franciscana* for 96 h (*n* = 3); and e) marine medaka fish embryo *Oryzias melastigma* for 96 h (*n* = 6) (mean ± standard deviation).

Table S1 Environmental parameters of water samples upon collection at A1 – A4 during 2016 wet, 2017 dry and 2017 wet seasons; and environmental parameters of water samples upon collection at A5 – A15 during 2017 wet season. (*n* = 3; mean ± SE).

Sampling locations		**pH**	**Temperature (ºC)**	**Salinity (PSU)**	**DO (mg/L)**
A1	Sai Keng	8.05 ± 0.02 (2016 wet) 8.01 ± 0.03 (2017 dry) 8.11 ± 0.02 (2017 wet)	29.45 ± 0.06 (2016 wet) 21.43 ± 0.03 (2017 dry) 28.43 ± 0.23 (2017 wet)	23.50 ± 0.71 (2016 wet) 26.43 ± 0.03 (2017 dry) 25.28 ± 0.15 (2017 wet)	4.73 ± 0.11 (2016 wet) 5.68 ± 0.05 (2017 dry) 7.30 ± 0.17 (2017 wet)
A2	Fung Wong Wat	8.51 ± 0.02 (2016 wet) 8.17 ± 0.07 (2017 dry) 7.99 ± 0.01 (2017 wet)	30.63 ± 0.06 (2016 wet) 18.00 (2016 dry) 29.73 ± 0.03 (2017 wet)	29.77 ± 0.05 (2016 wet) 27.93 ± 0.03 (2017 dry) 31.63 ± 0.12 (2017 wet)	8.25 ± 0.10 (2016 wet) 7.31 ± 0.03 (2017 dry) 6.63 ± 0.03 (2017 wet)
A3	Sam Mun Tsai	8.52 ± 0.01 (2016 wet) 7.81 ± 0.10 (2017 dry) 8.40 ± 0.01 (2017 wet)	31.60 ± 0.12 (2016 wet) 20.07 ± 0.17 (2017 dry) 32.20 ± 0.03 (2017 wet)	29.10 ± 0.04 (2016 wet) 30.00 (2017 dry) 28.40 ± 0.25 (2017 wet)	6.93 ± 0.06 (2016 wet) 5.62 ± 0.04 (2017 dry) 5.28 ± 0.03 (2017 wet)
A4	Yim Tin Tsai	8.62 (2016 wet) 8.01 ± 0.03 (2017 dry) 8.05 ± 0.02 (2017 wet)	30.90 (2016 wet) 19.20 (2017 dry) 27.77 ± 0.03 (2017 wet)	31.22 (2016 wet) 27.60 (2017 dry) 19.01 ± 0.03 (2017 wet)	10.70 (2016 wet) 5.96 ± 0.11 (2017 dry) 6.83 ± 0.06 (2017 wet)
A5	Yung Shue O	7.59 ± 0.04	30.03 ± 0.04	14.39 ± 0.78	6.47 ± 0.20
A6	Sham Shui Kok	8.53	29.80	19.35	7.77 ± 0.04
A7	Cyberport	8.36	28.37	20.20	5.82 ± 0.07
A8	Aberdeen	8.15 ± 0.01	28.40	20.50	6.32 ± 0.01
A9	Stanley	8.70	30.60	14.90	6.27 ± 0.03
A10	Kwun Tong	8.37	28.20	23.40	6.33 ± 0.08
A11	Lai Chi Kok	7.79 ± 0.06	28.40	21.90 ± 0.03	4.09 ± 0.03
A12	Yuen Long	7.71 ± 0.06	32.80	0.70 ± 0.03	4.66 ± 0.03
A13	Sai Kung	7.79 ± 0.01	30.07 ± 0.01	28.90	4.54 ± 0.13
A14	Tuen Mun	7.94 ± 0.04	31.00 ± 0.04	16.80	4.62 ± 0.03
A15	Tsuen Wan	7.84 ± 0.01	29.80	18.60	5.41 ± 0.01

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