Marine Ecology Enhancement Fund (MEEF)
Declaration

To: The Secretariat of the MEEF

Reference No.: MEEF2018001

Project Title: Unravelling the strength behind the ecosystem resilience of Tung Chung mangrove: A high-resolution mapping of its food web

Name of Project Leader: Dr Benoit Thibodeau

I hereby irrevocably declare to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that all the dataset and information included in the completion report has been properly referenced, and necessary authorisation has been obtained in respect of information owned by third parties.

Signature: 

Project Leader, Dr Benoit Thibodeau

Date: 25/01/2020

THE UNIVERSITY OF HONG KONG

Final Report of Project MEEF2018001

Unravelling the strength behind the ecosystem resilience of Tung Chung mangrove: A high-resolution mapping of its food web

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I. Executive summary

The objective of this project was to provide a tool to quantitatively asses the resilience of the Tung Chung mangrove. This challenge was tackled by combining traditional and novel isotopic techniques to position a wide range of organisms into the food web and evaluate its redundancy. We were therefore able to evaluate the resilience of the food web to perturbation either natural or driven by anthropogenic activities.

First, our data show that the crab populations of Tung Chung can rely on both terrestrial and marine sources of both N and C in their diet. This means that this forest still benefits of a natural input of nutrients from both the Tung Chung river and the sea and shows how the natural and healthy hydrology of the whole ecosystem is still working. **Such an information is critical in terms of resilience of Tung Chung mangroves to anthropogenic and natural alterations** as well as in terms of possible rehabilitation programs, which proved to be successful in mangroves only when the natural hydrology is preserved.

Second, the food web we drawn is redundant, with a number of species of gastropods and crabs occupying very similar feeding niches. This is another **strong signal of good resilience** since a functionally redundant system could cope with local population extinctions, due to altered ecological conditions, better than a low redundant one.

Therefore, we showed that using these techniques it is possible to ascertain the level of resilience of a mangrove and better forecast its respond to potential disturbance. This is a key information regarding the conservation of this highly important environment in Hong Kong. Moreover, it is likely that our holistic view of Tung Chung mangrove food web could be used as a template to test the resilience of the other mangroves of Hong Kong. This food-web comparison could prove to be a powerful tool to ascertain healthy as well as endangered mangrove forests and help manage and preserve them.

II. Project title and brief description of the Project

Title:

Unravelling the strength behind the ecosystem resilience of Tung Chung mangrove: A high-resolution mapping of its food web

Description:

Despite the multiple environmental stressor, the Tung Chung mangrove appears to be fully functional and have strong resilience. In an attempt to better understand the success of this mangrove compared to other ones, we hypothesis that the presence of a strong food-web is key in this resilience. Thus, this project aims at providing the first, high-resolution mapping of the Tung Chung mangrove food web. The results of this project will also provide a holistic high-resolution conceptual model of the food web characterizing Hong Kong's mangrove ecosystems

The Tung Chung mangrove forest is one of the ten largest mangroves areas in Hong Kong SAR, the third largest on Lantau Island and the largest one by far on the northern coast of the island. Although deeply impacted by the construction of the new Hong Kong International Airport (Tam et al. 1997; Tam and Wong 2000), this forest showed strong resilience and rapid recovery. At present, Tung Chung mangroves look like a fully functional forest, as many important ecological indications testify, and it was recently put forward as an ideal MPA candidate (Kwan, Hsieh et al. 2016). Among the other signals of recovery it is worth to cite the increase in total surface area covered by mangroves, which grew from 2.21ha in 2001 (Tam et al. 1997) to 3.86 ha at present (ECF, 2017). Although the whole area is obviously impacted by a number of anthropogenic factors, it is still an important spawning/nursery area for two Asian horseshoe crab species, *Carcinoscorpius rotundicauda* and *Tachypleus tridentatus* (Kwan, Hsieh et al. 2016) and it harbours a diverse fauna of crabs and molluscs (ECF, 2017).

The determination of the vertical and horizontal levels of a food web will help unravel how ecosystem functioning might be affected by consumptive interactions across trophic levels and/or competition with levels (Duffy et al., 2007). However, the precise determination of the trophic niche of a species remains a challenge mostly due to the absence of robust empirical methods to assess the feeding behaviour of an organism, especially for omnivores (Chikaraichi et al., 2011; Steffan et al., 2013). This is probably why the determination of a reliable mangrove

food web proves to be very difficult. In fact, omnivorous crabs are the most abundant macrofaunal group in mangrove forests and, although their crucial role in the food chain has been demonstrated, it is still difficult to ascertain their tropic niche.

Analysis of amino acids nitrogen isotope (^{15}N) is a cutting-edge technique that provides an accurate and precise estimate of the trophic position of aquatic and terrestrial organisms (e.g., McClelland and Montoya, 2002; McCarthy and al., 2007, Chikaraishi et al., 2009). This method can be used to better resolve 1) intra-species variations as the degree of omnivory (vertical niche extent) or primary resource generalism (horizontal niche extent) and 2) inter-species variations to unravel the number of trophic levels in vertical dimensions and the number of species within each of these trophic levels. This method will be used to build a holistic high-resolution conceptual model of the food web characterizing Hong Kong's mangroves ecosystems Moreover, this method also allows for the determination of the base of the food web, namely the primary source of nitrogen for this specific food web. With this information, we will also be able to quantify the importance of different sources of nitrogen for this specific ecosystem, including seasonal variations (i.e., N_2-fixation, dissolved nitrate, etc.).

III. Completed activities against the proposed Work Schedule

Following the proposed work schedule, we have completed the recruitment of a research assistant (July 2018), the planning of the field trips (July 2018), the field sampling (August to October 2018) and the samples preparation and analysis (November 2018 to November 2019). The sample analysis was longer than planned due to the maintenance and upgrade of the instrument at HKU (gas-chromatography isotope ratio mass spectrometer). Moreover, the final redaction of the report was perturbed by the events occurring on campus during the first semester of the A.Y. 2019-2020.

Despite these events, all sample were analysed in the laboratory and we submit the final report on time.

IV. Results / descriptions on the completed activities, with the support of photos, videos, social media platform, etc. (if any)

1. Field sampling

All field sampling was conducted successfully, and all required specimens were collected. In total 47 different type of samples were collected (including mangrove leaves, microalgae, crabs, gastropods, etc), lower than what we estimated in the proposal (75), this was mostly due to the merge of many specimen that were actually from the same species. Species, type of samples, grouping and abbreviation can be found in the ANNEX 1. Details of sampling methods are described below.

Fig 1. Sampling crabs, snails, algae and mangroves in Tung Chung

Three replicate samples of leaves for the four most common tree species present in Tung Chung were collected. Brown leaves were chosen as a possible food source because of their higher presence on the forest floor making them more directly accessible than green leaves and because it has been shown that crabs prefer decayed to senescent leaves (Giddins et al., 1986; Camilleri, 1989; S. Y. Lee, 1989; Skov & Hartnoll, 2002). Therefore, two to three senescent leaves of each species were gathered from the sediment in close vicinity to the tree trunk. A distance of at least 3m was maintained between each tree. Microphytobenthos (MPB), later referred to as algae, was collected by scraping the top 1 cm of sediment using a spatula. Three replicate samples were randomly collected across a transect traversing the forest from sea to landward. Sediment samples were transported to the lab, refrigerated and processed the same or following day. MPB was isolated from the sediment through density gradient centrifugation using colloidal silica (Hamilton et al., 2005). Each surface sediment replicate was sieved to

remove large grains, detritus and larger nematodes using Milli-Q water. The filtrate was then run through a pre-combusted glass filter to obtain a higher concentration. The residue was removed from the filter and divided into aliquots of around 5 ml in individual centrifuge tubes. The aliquots were lightly shaken to prevent clumping when adding the colloidal silica. Colloidal silica was then added until a density of 1.27 g cm^{-3} was reached (Hamilton et al., 2005). The silica-surface sediment mixture was centrifuged at 10000 rpm for 10 min. The presence of MPB was confirmed by microscopic examination prior to pipetting off the distinct supernatant layer. If no MPB was observed the aliquot was divided up again to ensure the MPB was not obstructed to move vertically. Pre-combusted glass filters were loaded with the supernatant layer while adding Milli-Q water to remove as much of the silica as possible. Each filter was loaded with the suspension until it was clogged to a maximum amount. Filters were then in their entirety freeze dried for 24 hours after which the dried MPB was scraped off. Leaf samples were freeze-dried for a maximum period of 96 hours. To obtain sufficient material, small sized leaves from the same species were pooled together. Samples were homogenized with a pestle and mortar into a fine powder. Once collected and transported to the lab, the crabs and snails were rinsed with deionized water to remove all sediment and frozen at -20°C. Thawing of the individual was allowed no more than 5 min to prevent bacterial decomposition activities. After tissue extraction crab samples and MPB filters were freeze-dried for 24 hours. Samples were homogenized with a pestle and mortar into a fine powder. All samples were weighed to the nearest 0.001 mg.

Fig 2. Crabs in Tung Chun

2. Preparation and Analysis

The preparation included freeze-drying the sample, grounding it to powder and weighting the sample into a tin capsule. All samples were prepared for isotope analysis following Chikaraishi et al. (2014). Briefly, sample were cleaned with distilled water (except sediment) to remove surface contaminants and stored at -20°C. For marine invertebrates, a small sample of muscle tissue (~10 mg) was taken. For other sample types (ie., macroalgae, leaves) the whole sample was used for isotopic analysis (~30 mg). The dry and crushed samples were then be analysed using a GC-IRMS for the amino-acids and an EA-IRMS for the bulk isotopes (e.g., Chikaraishi et al., 2014).

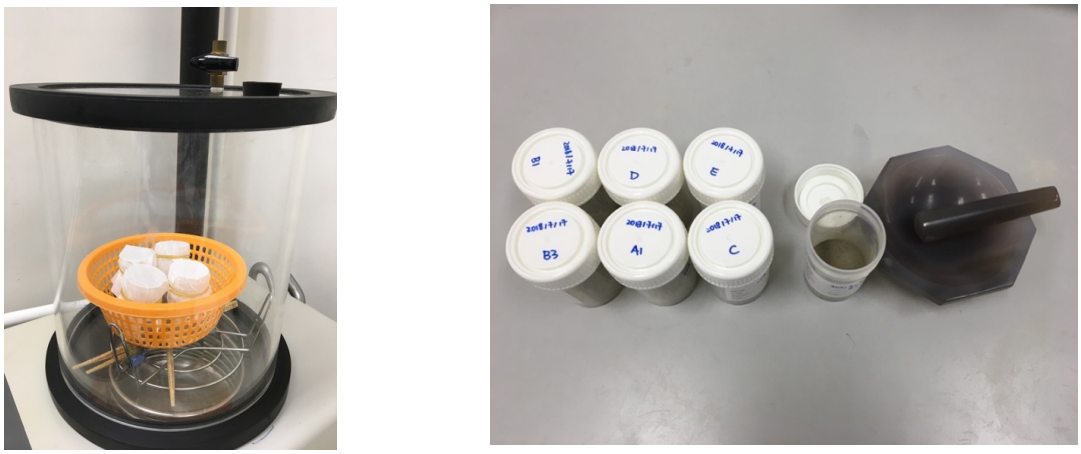

Fig 3. Sediment and organic material preparation: freeze drying (left) and grinding (right)

For bulk analysis, bulk material (sediment or dried, powdered organisms) was placed in tin capsule. The tin capsule was then placed in the EA-IRMS, in a combustion furnace at around 1000°C. This cause the tin capsule to flash combust rising the temperature at the vicinity of the sample to about 1700°C.

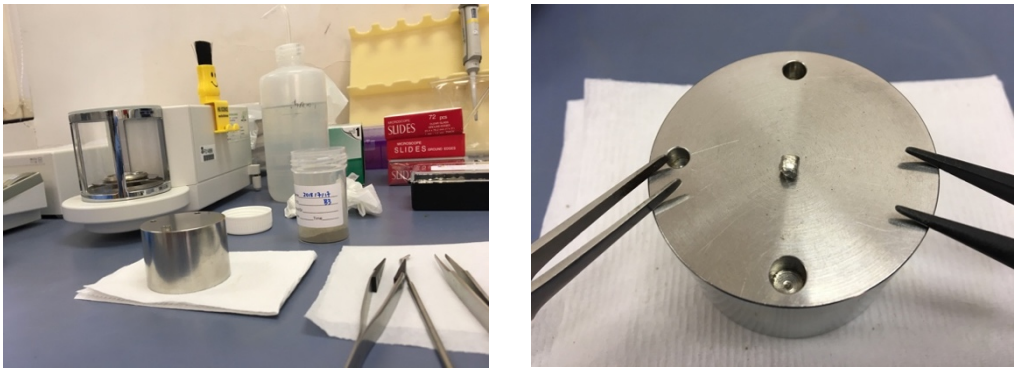

Fig 4. Samples preparating for EA analysis: weighting (left) and inserting in tin capculse (right)

The gases were then purified using Cr_2O_3 combustion catalysis, CuO wire and silver wool. The resulting purified gases (NO_x, H_2O, O_2, CO_2 and N_2) passesd by a reduction step to remove oxygen and transform NO_x to N_2 and then into a water trap. Then, CO_2 and N_2 are separated by a gas chromatographic column and enter the ion source of the IRMS sequentially. They were then ionized and accelerated and measured simultaneously by a faraday cup after being separated by a magnetic field. The ratio of heavy isotope (^{13}C, ^{15}N) on the light (^{12}C, ^{14}N) isotope of the sample is then compared to the same ratio of a known standard and reported in delta notation (only shown for carbon here):

$$\delta^{13}C_{sample} = \left[\left(\frac{{}^{13}C_{sample} / {}^{12}C_{sample}}{{}^{13}C_{STD} / {}^{12}C_{STD}} \right) - 1 \right] \times 1000$$

Eq 1. Delta notation for carbon 13 isotope

For amino-acid analysis we followed the protocol of UC-Davis stable isotope facility (https://stableisotopefacility.ucdavis.edu/gcaminosampleprep.html). Acid hydrolysis was performed to liberate individual amino acids from proteinaceous samples. Dry, homogenized sample materials were placed in new borosilicate vials with heat- and acid-resistant caps, 0.5mL (animal tissues) or 2mL (plant tissues) of 6M hydrochloric acid added, and vial threads wrapped with PTFE-tape.

Fig 5. Samples after acid hydrolyzation

Vials were then flushed with N_2, sealed, and placed in an oven at 150 °C for 70 minutes. After cooling, 200µL of heptane:chloroform (6:5, v:v) was added to the acid hydrolysates of sample materials, the vials briefly vortexed, and the organic layer discarded in order to remove any remaining lipophilic compounds prior to drying. Samples were then dried in a heating block at 60 °C under a gentle stream of N_2. For leaves the lipid removal step was not performed. For samples with a significant inorganic matrix, additional clean-up steps were required, including cation-exchange chromatography.

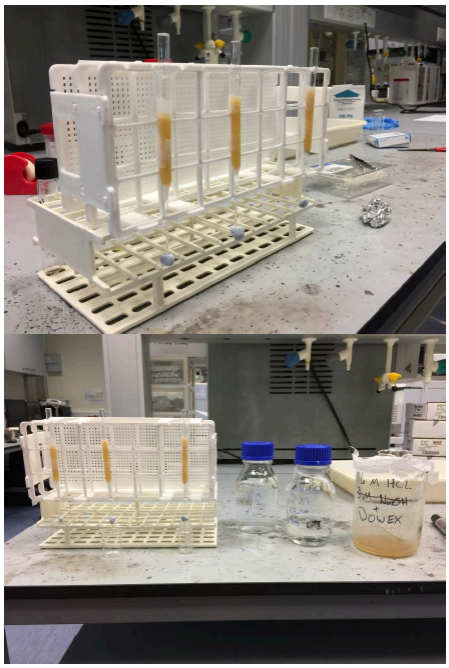

Fig 6. Cation-exchange chromatography

The sample acid hydrolysates (<10µmoles total) and 20µL of the internal reference solution were combined and then dried under a stream of nitrogen. 1mL of 1.85M acidified isopropanol was then added to each reaction vial and the solution heated at 100°C for 1h. The remaining isopropanol was evaporated under nitrogen at 40 °C. Chloroform is added (250µL) and evaporated under nitrogen in a cold block to remove remaining excess reagents. The partial derivatives were then acetylated with a mixture of acetic anhydride, trimethylamine, and acetone (1mL; 1:2:5, v/v/v; 10min., 60 °C) and the reagents evaporated under nitrogen gas in a cold block (0 °C). Once dry, ethyl acetate was added (2mL), along with a saturated NaCl solution (1mL), and the solution vortexed. Following phase separation, the aqueous phase was discarded, and the ethyl acetate removed under nitrogen gas in a cold block (0 °C). Trace water was removed with two additions of chloroform (1mL). Finally, ethyl acetate was added (100µL)

and the N-acetyl isopropyl esters transferred to a GC vial with insert. Prepared samples were generally analyzed within 24 hours of preparation.

Fig 7. Sample hydrolysates drying under nitrogen stream

The resulting N-acetyl amino acid isopropyl esters were then suitable for GC-IRMS analysis. The amino acids suitable to derivatization by this method include: Alanine (Ala), Aspartic acid (Asp), Glutamic acid (Glu), Glycine (Gly), Histidine (His), Hydroyproline (Hyp), Leucine (Leu), Lysine (Lys), Methionine (Met), Proline (Pro), Serine (Ser), Threonine (Thr), Tyrosine (Tyr), and Valine (Val). This method is not well suited for the analysis of Ile. Measurement error was variable between amino acids but was always better than ±1‰.

Fig 8. The GC-IRMS system at HKU

3. Results

a. Bulk analysis of carbon and nitrogen isotope

We analysed 111 specimens of 47 unique types of samples for bulk isotopes. To simplify the analysis, we grouped the crabs, snails, mangroves leaves and algae (micro and macro) together in four main groups. Bulk isotope is often used to calculate trophic position of organisms. Generally, for marine organisms, we consider that for every increase in trophic position the isotopic composition will increase between 0-1 ‰ and 2-3 ‰ for $\delta^{13}C$ and $\delta^{15}N$ respectively (e.g., Kristensen et al., 2017).

Nitrogen isotope

Taking the averaged $\delta^{15}N$ values of the different groups (Table 1) the picture seems fairly straightforward with the crabs (11.4 ‰) having an isotope values enriched by about 3 ‰ compared to the mangrove leaves, (8.6 ‰) suggesting that they are mostly using leaves as their primary source of food. Interestingly, the snails have lower $\delta^{15}N$ values of about 9 ‰, which is very close to the mangrove leave and thus suggest the leaves are not part of their diet and that the snail's source of nitrogen is probably completely different than the one from the crabs. In fact, the snails average isotopic $\delta^{15}N$ signature (9 ‰) is 2.4 ‰ higher than the algae (6.6 ‰) we managed to measure, suggesting that snails feed mostly on algae.

Descriptive statistics		A	B	C	D	E	F	G
		Leaves	Sails	Crabs	Sediment	Propagule	Grass	Algae
		Y	Y	Y	Y	Y	Y	Y
1	Number of values	39	16	45	2	2	3	2
2								
3	Minimum	1.500	1.900	8.100	7.500	5.500	6.400	6.600
4	Maximum	12.50	11.30	13.40	7.600	5.700	10.90	6.613
5	Range	11.00	9.400	5.300	0.1000	0.2000	4.500	0.01254
6								
7	Mean	8.621	9.013	11.38	7.550	5.600	7.967	6.606
8	Std. Deviation	2.536	2.582	1.505	0.07071	0.1414	2.542	0.008869
9	Std. Error of Mean	0.4060	0.6455	0.2243	0.05000	0.1000	1.468	0.006271
10								
11	Lower 95% CI of mean	7.799	7.637	10.93	6.915	4.329	1.651	6.527
12	Upper 95% CI of mean	9.443	10.39	11.83	8.185	6.871	14.28	6.686

Table 1. Statistical analysis of the $\delta^{15}N$ of all samples analysed

Carbon isotope

The carbon isotope value ($\delta^{13}C$) is used to identify the main source of carbon in a food web. Usually, little fractionation (~1 ‰) is observed between the primary source of carbon and each

trophic level. However, in this mangrove (Table 2) we observe a highly fractionated pattern between the primary sources as leaves (-28.7 ‰) and algae (-30.2 ‰) and the organism at upper trophic level like crabs (-16.9 ‰) and snails (-18.1 ‰). Interestingly, the offset between the higher organism and their suspected primary source of food (leaves → crabs and algae → snails) is extremely high (> 10 ‰). This is much higher than the observed offset between mangrove sesarmid crabs and their primary source of carbon in controlled experiment (5.45 ‰, Bui and Lee, 2014) and in different type of mangroves (1.5 to 6 ‰ Kristensen et al., 2017). This also might be due to the very large range of $\delta^{13}C$ value in each category, which is about 10 ‰ for leaves and between 12 and 13 ‰ for crabs and snails. This highlight a very large heterogeneity in the source of carbon and the potential for very strong biological fractionation in this environment. This might be linked to the highly variable amount of lipid between these groups; lipids are depleted in respect to ^{13}C and can significantly alter the trophic signal in $\delta^{13}C$ (Arostegui et al., 2019). Here, we suggest future work to use lipid-extraction method on crabs and snails to better understand the $\delta^{13}C$ signal.

Descriptive statistics		I	J	K	L	M	N	O
		Leaves	Sails	Crabs	Sediment	Propagule	Grass	Algae
		Y	Y	Y	Y	Y	Y	Y
1	Number of values	39	16	45	2	2	3	2
2								
3	Minimum	-31.30	-24.90	-25.10	-24.90	-32.30	-15.10	-31.10
4	Maximum	-21.40	-11.20	-12.30	-24.80	-27.60	-14.90	-29.29
5	Range	9.900	13.70	12.80	0.1000	4.700	0.2000	1.807
6								
7	Mean	-28.69	-18.09	-16.86	-24.85	-29.95	-15.00	-30.20
8	Std. Deviation	1.574	4.666	3.315	0.07071	3.323	0.1000	1.278
9	Std. Error of Mean	0.2521	1.166	0.4942	0.05000	2.350	0.05774	0.9034
10								
11	Lower 95% CI of mean	-29.20	-20.58	-17.86	-25.49	-59.81	-15.25	-41.67
12	Upper 95% CI of mean	-28.18	-15.61	-15.86	-24.21	-0.09042	-14.75	-18.72
13								

Table 2. Statistical analysis of the $\delta^{13}C$ of all samples analysed

Isotopic biplot Analysis

The extent of $\delta^{13}C$ value is extremely large and encompass typical value of both marine and terrestrial environments (Fig 9 and 10), which highlight the complexity of mangroves. This huge range of carbon value makes the food web analysis extremely difficult when taking the $\delta^{13}C$ values into account. Moreover, while the average value of $\delta^{15}N$ seems to indicate a direct link between crabs and mangroves leaves, the variability between species makes the analysis convoluted Fig X and X). Therefore, traditional isotopic techniques prove to lack precision when performing food web analysis of mangrove. This might be due to the highly variable

composition of the organisms in respect to lipids and amino acids. Thus, we expect the more refined technique of compound specific isotope to yield more usefull results.

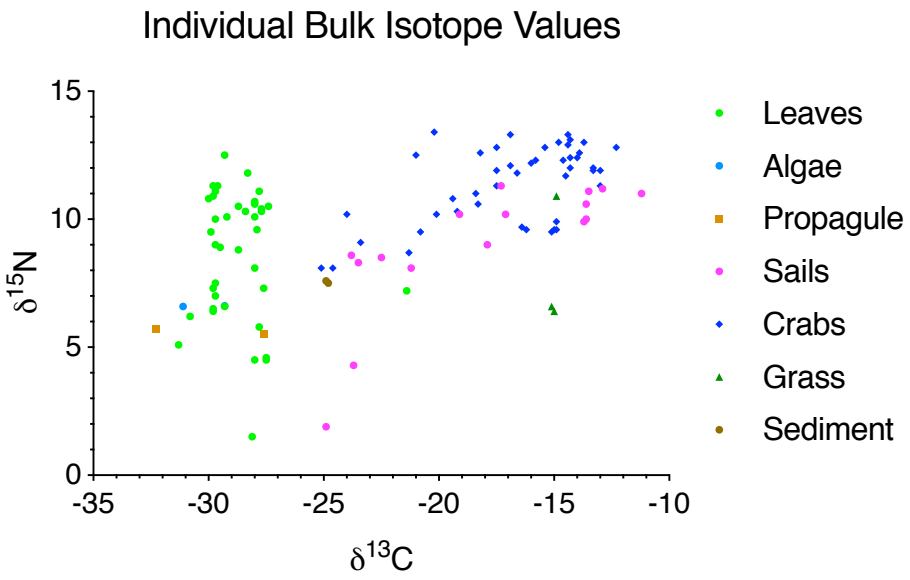

Fig 9. $\delta^{13}C$ and $\delta^{15}N$ of individual specimen

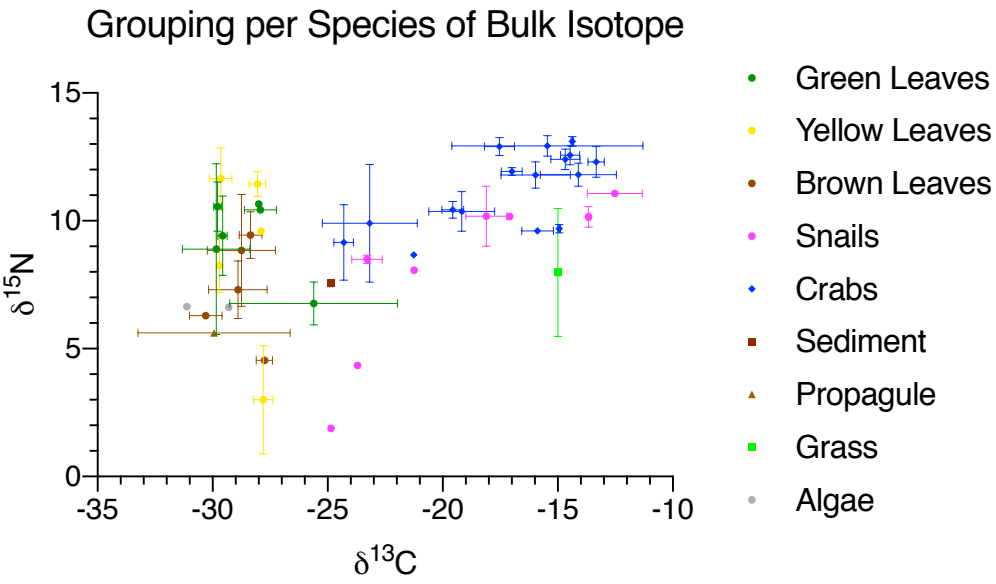

Fig 10. Averaged $\delta^{13}C$ and $\delta^{15}N$ of different species

b. Analysis of nitrogen isotope on amino acid

We analysed 32 unique species/types of samples for nitrogen isotope of 12 single amino acids with up to 6 different specimen per species/type. To simplify the analysis, we grouped the crabs, snails, mangroves leaves and algae (micro and macro) together in four main groups.

We focus the analysis on the two most representative amino acid: Glutamic acid (Glu) and Phenylalanine (Phe), which are respectively controlled by the trophic position of the organism and the primary source of nitrogen. This means that the higher the $\delta^{15}N$ of Glu is, the higher in the food web the organism is. This also means that organism using the same source of nitrogen should possess the same $\delta^{15}N$ of Phe.

We observe that both mangrove and algae have a similar, relatively low, value of Glu (11.7 and 11.9 ‰ respectively), indicating that they are both around the same trophic level (Fig 11). This is coherent with the fact that they are both primary producers. The snails are higher at 16.7 ‰ with the crabs being even slightly higher at 19.4 ‰. The value of Phe confirm that snails (7.6 ‰) feed exclusively on algae (7.7 ‰). However, the signal from crabs (10.8 ‰) seems to be composed of a mixture of algae (7.7 ‰) and mangroves (22.3 ‰).

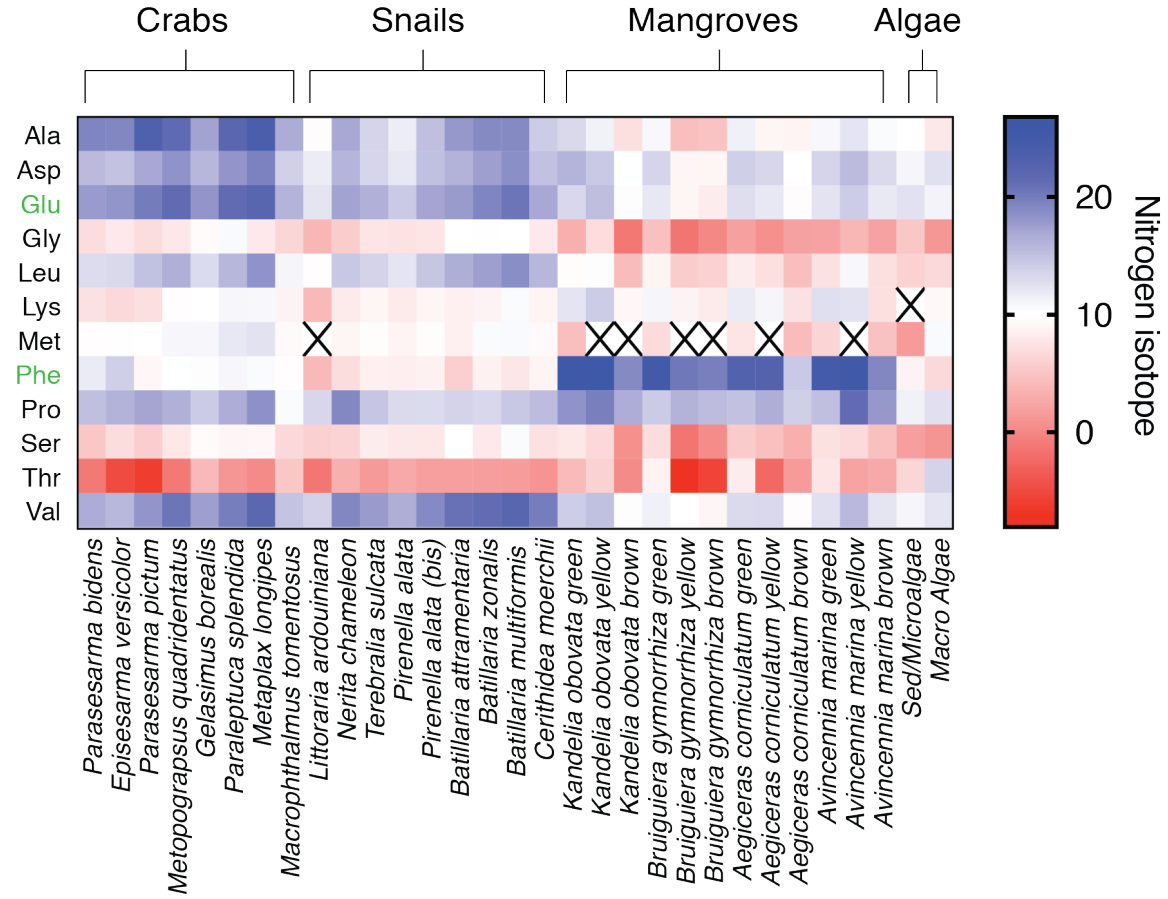

Fig 11. Heat map of nitrogen isotope of individual amino acid for each species

As there is still some debate related to the level of senescence of the leaves commonly eaten by crabs, although most studies showed that brown leaves are the most susceptible to be the start of the food-web since they have a better N/C ratio and are readily available (green leaves fall from the trees only occasionally). We thus sampled different leaves at different levels of senescence (i.e. from green leaves attached to the branches, through yellow leaves just fallen down to brown leaves decaying on the mud) from 4 different tree species: *Aegiceras corniculatum* (Ac), *Avicennia marina* (Am), *Bruguiera gymnorrhiza* (Bg) and *Kandelia obovata* (Ko). Interestingly, the $\delta^{15}N$ of Phe is lower in the brown leaves (18.1 ‰) compared to yellow (23.9 ‰) and green leaves (25.0 ‰). The fact that the $\delta^{15}N_{Phe}$ of yellow and green leaves is much higher than crabs suggests that they are probably not part of the crab's diet.

Trophic position calculation

The trophic position (TP) was calculated using the formula:

$$TP_{Glu/Phe} = [(^{15}N_{Glu} - {}^{15}N_{Phe} + \beta)/TDF] + 1$$

Where:

$\beta = {}^{15}N_{Glu} - {}^{15}N_{Phe}$ in primary producers (-3.4 ± 0.9 ‰ for aquatic cyanobacteria and algae, +8.4 ± 1.6 ‰ for terrestrial C_3 plants and -0.4 ± 1.7 ‰ for terrestrial C_4 plants) and

TDF = trophic discrimination factor for each shift of trophic level ($^{15}N_{Glu} - {}^{15}N_{Phe}$ = 7.6 ± 1.2 ‰).

It is difficult to choose β in such environment since we have mangrove trees that are C_3 plant but can switch to the C_4 pathway under certain condition and algae that can be found at the bottom of the food web. Moreover, while Phe values of snails suggest they feed exclusively on algae, Phe values of crabs suggest that both mangrove leaves and algae are contributing to a portion of the nitrogen that is found within the food web. Thus, it is probably unwise to simply use a single β value.

Here, we can calculate β in our specific environment. The average difference between $^{15}N_{Glu}$ and $^{15}N_{Phe}$ in our algae samples is -4.2 ‰, while it is of 10.6 ‰ in mangroves leaves. While both are within the expected range, it is noteworthy that brown leaves have a β of 8.1 ‰, much closer to the typical range, suggesting again that yellow and green leaves are probably not very

important to the food web. Thus, when using $\beta = 8.4 \pm 1.6$ ‰ for terrestrial C_3 plants, the brown leaves plots around the TP =1 (Fig 12), where they should be theoretically if they are the main source of food. This validate the quality of our data. The same is true with the algae, which plots also around TP =1 when using $\beta = -3.4 \pm 0.9$ ‰ for aquatic cyanobacteria and algae.

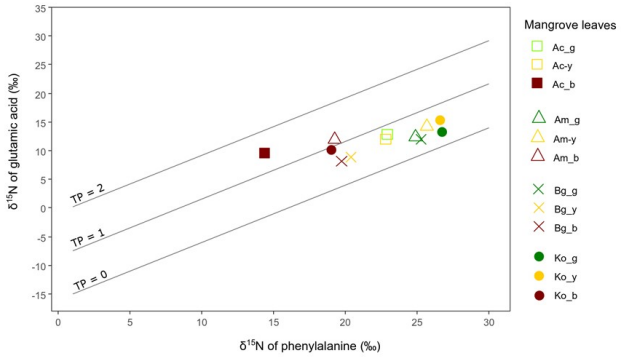

Fig 12. Plot of $\delta^{15}N$ of glutamic acid vs phenylalanine for different species of mangrove leaves (brow, yellow and green leaves) with theoretical trophic position (black lines)

Marine food web

Because snails possess the exact same $\delta^{15}N_{Phe}$ value as algae we used a specific β (-4.2 ± 0.9 ‰) that was calculated from our data and thus applies to the algae of the mangrove of Tung Chung. We can then calculate the trophic position of these snails under the hypothesis that they exclusively feed on algae (Fig 13). Most of the snails appear to be fairly low within the food web, with TP between 1.5 and 2.

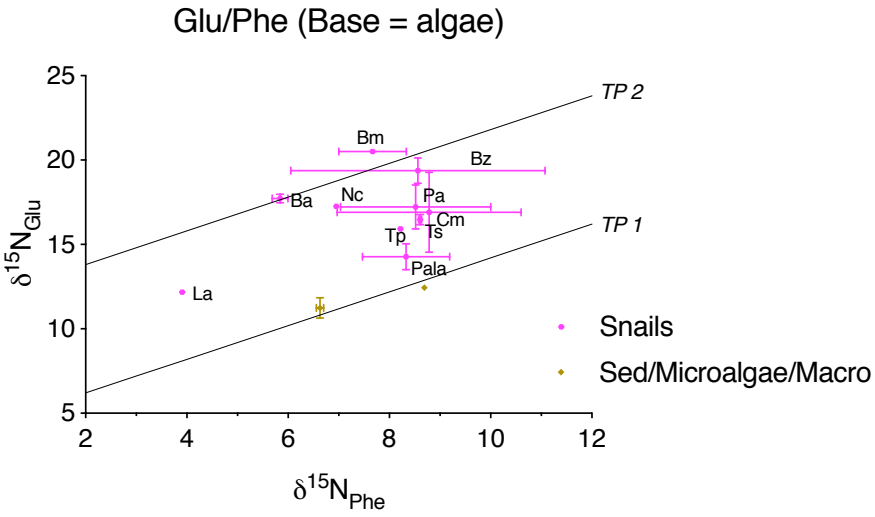

Fig 13. Plot of $\delta^{15}N$ of glutamic acid vs phenylalanine for snails (pink) and algae (brown) with theoretical trophic position based on algae as primary producer (black lines)

Terrestrial food web

Based on $\delta^{15}N_{Phe}$ and bulk $\delta^{15}N$ it appears that crabs are feeding on mangroves leaves, but probably also on another source that uses marine nitrogen as the primary source of nitrogen since their $\delta^{15}N_{Phe}$ is lower than the leaves (i.e., organisms feeding on algae). When using C3 plants as the base of the food web for the crabs we can calculate the trophic position for crabs to be generally above 3, which suggest that they are completing their diet with marine-based organisms (Fig 14).

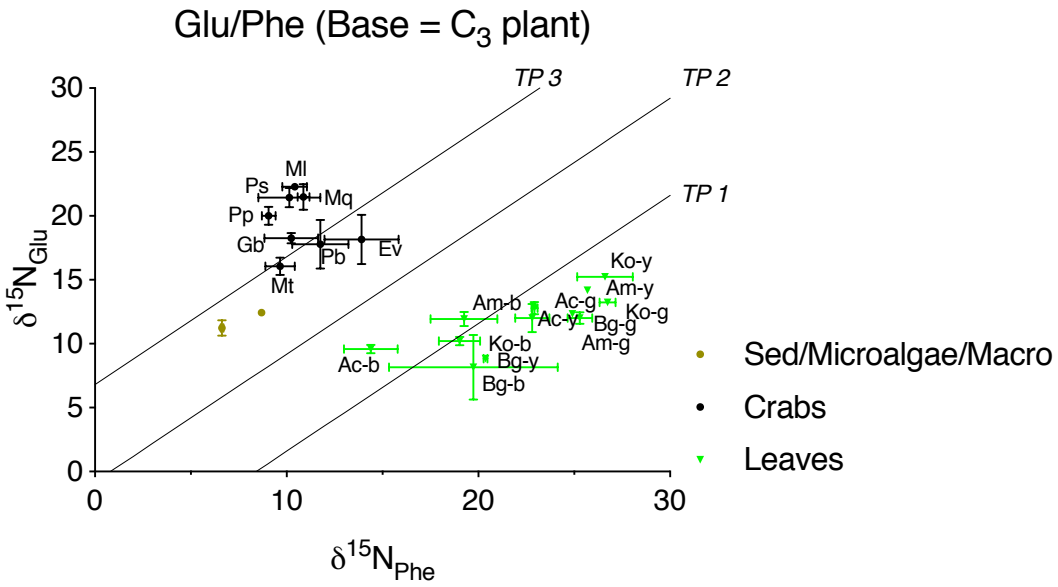

Fig 14. Plot of $\delta^{15}N$ of glutamic acid vs phenylalanine for crabs (black) and mangrove leaves (green) with theoretical trophic position based on mangrove leaves as primary producer (black lines)

Mixed food web

Elucidating the diet of sesarmid mangrove crabs has proven challenging (Bui & Lee 2014; Kristensen *et al.* 2017), which inhibited our ability to create robust models for tropical mangroves food web. While leaf litter is rich in carbon, it is fairly poor in nitrogen (e.g., Cannicci *et al.* 2008) and thus it has been debated if this nutrient poor diet can be sustainable for crabs (e.g., Kristensen *et al.* 2010). Attempts to solve this question using bulk isotope were diminished due to the large range of trophic disclination factors encountered in mangroves forest and thus the state-of-the-art knowledge is based on model scenarios using threshold values of trophic discrimination factor encountered on the field and in controlled experiments (e.g., Bui & Lee 2014; Kristensen *et al.* 2017). Our results are thus the first to provide a

quantitative partitioning of N sources in leaf-eating crabs. We used a simple two endmembers model using terrestrial (brown leaves) and marine (MPB) source of nitrogen. Brown leaves were chosen as a possible food source because of their higher presence on the forest floor making them more directly accessible than green leaves and because it has been shown that crabs prefer decayed to senescent leaves (Giddins *et al.* 1986; Camilleri 1989; Lee 1989; Skov & Hartnoll 2002). We calculated that 13 to 60 % of the crabs' diet is based on terrestrial N supplied by the mangrove leaves. In opposition to crabs, snails appear to feed exclusively on the marine part of the food web according to their $\delta^{15}N_{Phe}$ similar to MPB.

By combining the marine- and terrestrial-based food web we can craft a holistic view of the mangrove food web (Fig 15). We can calculate a trophic position for each species. We notice that once combined, the food web looks complete, with leaves and algae at the base, snails in the middle and crabs at the upper end, feeding on both the marine and terrestrial food web. Also, we can use the results calculated above to build an holistic food web based on the percentage of N originating from terrestrial and marine environment. In this case we use β = 2.1, which is the middle ground between leaves and algae (Fig 16). In this case, crabs' plots between TP 1.8 to 2.8.

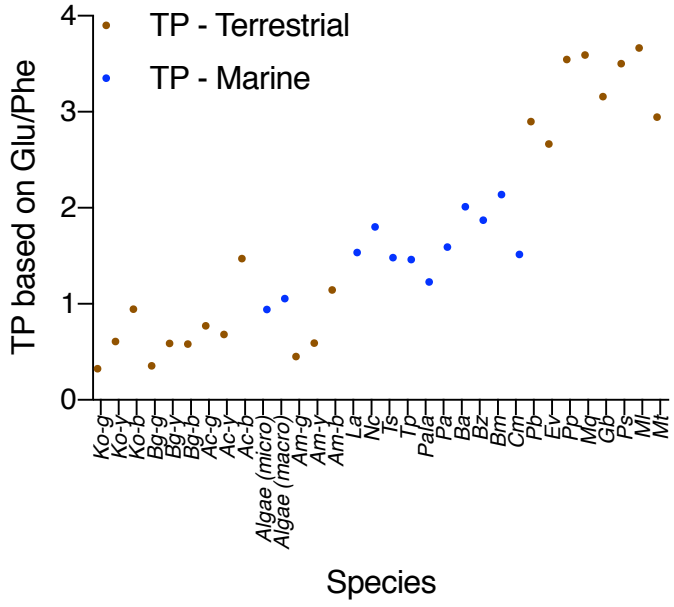

Fig 15. Trophic position (TP) of Tung Chung's species based on nitrogen isotope of amino acid. TP are calculated using a terrestrial food web for mangroves leaves and crabs (brown) and a marine food web for algae and snails (blue)

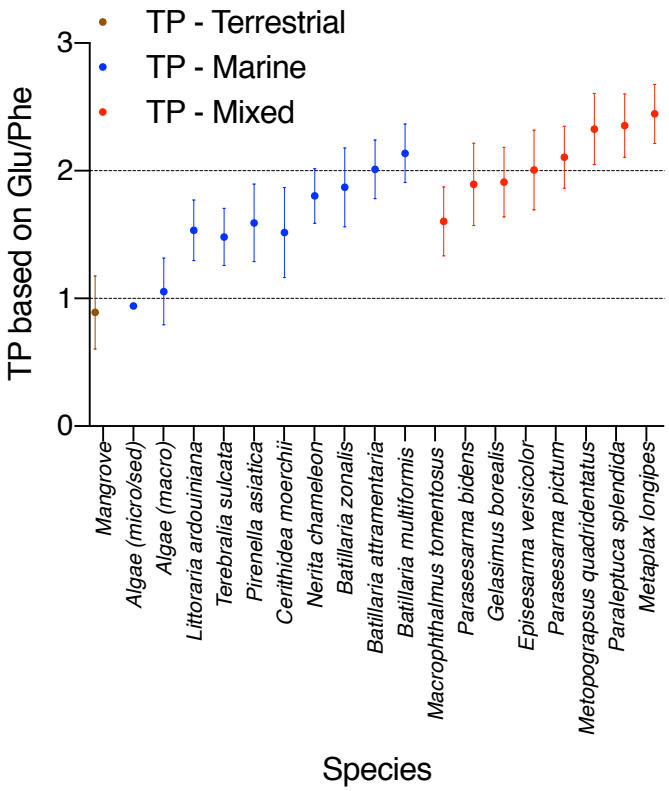

Fig 16. Trophic position (TP) of Tung Chung's species based on nitrogen isotope of amino acid. TP are calculated using a terrestrial food web for mangroves leaves (brown), a marine food web for algae and snails (blue) and a mixed food web for the crabs (red). Terrestrial-based TP for crabs is still show for comparison

V. Evaluation of the project effectiveness in achieving the proposed objectives as well as the impact (benefits) of the Project

Besides the one preserved within the Mai Po Nature Reserve, the residual mangroves forests of Hong Kong are small and patchy distributed (Tam et al., 1997: ECF, 2017). For this reason, most of them have been considered of low conservation value, since they could not provide the ecosystems services well-known for these coastal environments (Tam & Wong, 2002). The above view, however, was challenged, in general, by new perceptions about the value of small mangrove forests (Curnick et al., 2019) and, in particular for Tung Chung, by the results of a recent biodiversity survey funded by ECF (ECF, 2019). The latter survey found that Tung Chung is characterised by a high diversity of macrofaunal species and very high densities of sesarmid crabs and the commonest and exceptionally high abundance of the crabs *Parasesarma bidens*, *Metopograpsus quadridentatus* and *Macophthalmus tomentosus* and of the gastropods

Batillaria attramentaria, *Littoraria articulata*, *L. melanostoma*, *L. ardouiniana* and *Pirenella asiatica*. The results of the present project give us a crucial explanation of such diversity and densities. Our data show that the crab populations of Tung Chung can rely on both terrestrial and marine sources of both N and C in their diet. This means that this forest still benefits of a natural input of nutrients from both the Tung Chung river and the sea and shows how the natural and healthy hydrology of the whole ecosystem is still working. **Such an information is critical in terms of resilience of Tung Chung mangroves to anthropogenic and natural alterations as well as in terms of possible rehabilitation programs, which proved to be successful in mangroves only when the natural hydrology is preserved (Lee et al. 2019).**

Our data also showed that the food web in Tung Chung is complex and somehow redundant. Indeed, food-web complexity and functional redundancy are key features to assess the health and resilience of a natural system (Mouchet et al., 2010), and Tung Chung mangroves appeared to be ecologically well-structured and resilient. First, we could observe a nice and neat division of C and N sources between crabs and molluscs, which feed on mangrove leaves and other marine-borne material and microphytobenthos, respectively. This result is of outmost importance to understand and explain the exceptional diversity and abundance of both crabs and gastropods recently found in Hong Kong mangroves. Previous analyses, based on techniques such as stomach content and bulk isotopes analyses (Olive et al., 2001, Poon et al., 2010) were neither able to clearly identify the feeding habits of sympatric crabs and snails nor to evaluate niche overlap between these two groups. **For the first time, we could prove that these two groups are not competing for food in Hong Kong mangroves, and their abundant populations exploit different, and abundant, sources of nutrients.**

The food web we draw is also redundant, with a number of species of gastropods and crabs occupying very similar feeding niches. This another strong signal of good resilience since a functionally redundant system could cope with local population extinctions, due to altered ecological conditions, better than a low redundant one (Mouchet et al., 2010). **In our case, in synthesis, if one population of gastropods or crabs could disappear, due to anthropogenic pressure, there will be other specie performing the peculiar ecosystem functions of the extinct one, thus preserving the ecological balance of the whole mangrove forest.**

Our results are even more impactful if viewed in terms of global, Hong Kong, scale. The great majority of the species we analysed are widespread across the Hong Kong territory and they

are the most common and dominant tree, crab and gastropod species in our mangroves. Although local differences should be considered (i.e. between the West coast mangroves affected by the Pearl River and the more oceanic mangroves on the East coast), **it is highly likely that our holistic view of Tung Chung mangrove food web could be used as a template to test the resilience of the other mangroves of Hong Kong. This food-web comparison could prove to be a powerful tool to ascertain healthy as well as endangered mangrove forests and help manage and preserve them.**

VI. Summary and way forward

A natural ecosystem is a complex interplay of roles and relationships between and within its floral and faunal components. The precise representation of an ecosystem's food web is crucial to understand it status, its resilience to anthropogenic changes and, ultimately, to design local management and conservation strategies. However, the determination of a reliable food web proved to be very difficult for mangroves ecosystems, since the precise determination of the trophic niche of dominant omnivorous crabs remains a challenge, due to the lack of robust empirical methods to assess their feeding behaviour. Using the cutting-edge technology represented by the analysis of amino acids nitrogen isotope (^{15}N), the present project successfully accepted this challenge. We were able to quantify the importance of different sources of nitrogen for macrofaunal populations colonising Tung Chung mangroves and to show that the food web in Tung Chung is complex and redundant, indicating that this mangrove forest is still ecologically well-structured and resilient.

Our results will have a wide impact in the assessment of mangrove health and resilience at global, Hong Kong, scale, since our holistic high-resolution conceptual model of food web could be extended to most of Hong Kong's mangrove ecosystems. The way forward is definitely to apply our amino acids nitrogen isotope approach to other West and East coast mangrove forests of Hong Kong and to test their resilience, using the present data as a template. These comparisons could prove to be a powerful tool to ascertain healthy as well as endangered mangrove forests and help manage and preserve them.

VII. Financial position of the project with receipts for the expenses to be reimbursed for final payment

See attached

VIII. Complete statement of accounts

See attached

I hereby irrevocably declare, warrant and undertake to the MEEF Management Committee and the Steering Committee of the relevant Funds including the Top-up Fund, that I myself, and the Organisation:-

1. *do not deal with, and are not in any way associated with, any country or organisation or activity which is or may potentially be relevant to, or targeted by, sanctions administered by the United Nations Security Council, the European Union, Her Majesty's Treasury-United Kingdom, the United States Department of the Treasury's Office of Foreign Assets Control, or the Hong Kong Monetary Authority, or any sanctions law applicable;*

2. *have not used any money obtained from the Marine Ecology Enhancement Fund or the related Top-up Fund (and any derived surplus), in any unlawful manner, whether involving bribery, money-laundering, terrorism or infringement of any international or local law; and*

3. *have used the funds received (and any derived surplus) solely for the studies or projects which further the MEEF Objectives and have not distributed any portion of such funds (including any derived surplus) to members of the recipient organisation or the public.*

----------END

Prepared and approved by

Dr. Benoit Thibodeau Dr. Stefano Cannicci

ANNEX 1.

List of species and type of samples

Species/Samples	Group	Abbreviations
Parasesarma bidens	Crabs	Pb
Episesarma versicolor	Crabs	Ev
Parasesarma pictum	Crabs	Pp
Metopograpsus quadridentatus	Crabs	Mq
Gelasimus borealis	Crabs	Gb
Paraleptuca splendida	Crabs	Ps
Metaplax longipes	Crabs	Ml
Macrophthalmus tomentosus	Crabs	Mt
Littoraria ardouiniana	Snails	La
Nerita chameleon	Snails	Nc
Terebralia sulcata	Snails	Ts
Pirenella alata	Snails	Pa
Pirenella alata (bis)	Snails	Pa-1
Batillaria attramentaria	Snails	Ba
Batillaria zonalis	Snails	Bz
Batillaria multiformis	Snails	Bm
Cerithidea moerchii	Snails	Cm
Kandelia obovata green	Mangroves Leave	Ko-g
Kandelia obovata yellow	Mangroves Leave	Ko-y
Kandelia obovata brown	Mangroves Leave	Ko-b
Bruiguiera gymnorrhiza green	Mangroves Leave	Bg-g
Bruiguiera gymnorrhiza yellow	Mangroves Leave	Bg-y
Bruiguiera gymnorrhiza brown	Mangroves Leave	Bg-b
Aegiceras corniculatum green	Mangroves Leave	Ac-g
Aegiceras corniculatum yellow	Mangroves Leave	Ac-y
Aegiceras corniculatum brown	Mangroves Leave	Ac-b
Avincennia marina green	Mangroves Leave	Am-g
Avincennia marina yellow	Mangroves Leave	Am-y
Avincennia marina brown	Mangroves Leave	Am-b
Sed/Microalgae	Algae	
Macro Algae	Algae	