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Predicting *Cherax quadricarinatus* Habitat Distribution Patterns Through the Usage of GIS and eDNA Analysis in Terengganu, Malaysia

(Meramalkan Corak Taburan Habitat *Cherax quadricarinatus* melalui Penggunaan GIS dan Analisis eDNA di Terengganu, Malaysia)

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ABSTRACT

Cherax quadricarinatus (von Martens, 1868) is a highly invasive species that is resilient and highly adaptable to environmental conditions in new locations. Its presence brings along ecosystem imbalances and causes socioeconomic losses in invaded areas. Due to the destructive behaviour, it is important to conduct preliminary assessment methods and scientific studies especially on its behavioural and dispersal patterns. Thus, this study aims to determine the habitat distribution patterns of *C. quadricarinatus* based on the methods of GIS and environmental DNA (eDNA) analysis. The study was done at Felda Tenang, Terengganu, Malaysia. Based on the geographical features and climate conditions of the study area, we theorized that there are fewer and older *C. quadricarinatus* present at higher areas but more and younger *C. quadricarinatus* at lower areas. We found that 91.67% of the study area to be invaded, thus imploring the need for future mitigation plans to curb their dispersal into new areas. Future studies should also be done to determine the habitat distribution patterns of *C. quadricarinatus* in other areas.

Keywords: Asia; crayfish; distribution; invasive species; Parastacidae; redclaw

ABSTRAK

Cherax quadricarinatus ialah spesies yang sangat invasif yang mempunyai daya tahan dan mudah menyesuaikan diri dengan keadaan persekitaran di lokasi baharu. Kehadiran mereka membawa ketidakseimbangan ekosistem sekali gus merugikan sosioekonomi di kawasan yang diceroboh. Oleh kerana tingkah laku mereka yang membinasakan, adalah penting untuk kajian saintifik dan kaedah penilaian awal dapat dijalankan terutamanya untuk mengenal pasti corak tingkah laku dan penyebaran mereka. Justeru, kajian ini bertujuan untuk menentukan corak taburan habitat *C. quadricarinatus* berdasarkan kaedah GIS dan analisis DNA persekitaran (eDNA). Penyelidikan telah dijalankan di Felda Tenang, Terengganu, Malaysia. Berdasarkan ciri geografi dan pola iklim di kawasan kajian, kami berteori bahawa terdapatnya *C. quadricarinatus* yang lebih dewasa namun sedikit di kawasan yang lebih tinggi tetapi semakin banyak *C. quadricarinatus* yang lebih muda di kawasan yang lebih rendah. Kami mendapati bahawa 91.67% daripada kawasan kajian telah diceroboh, lantas membuktikan akan perlunya rancangan mitigasi dilaksanakan pada masa hadapan untuk mengekang penyebaran mereka ke kawasan baharu. Kajian lanjut juga perlu dilakukan untuk menentukan pola taburan habitat *C. quadricarinatus* di kawasan lain.

Kata kunci: Asia; Parastacidae; pola; sepit merah; spesies invasif; udang kara air tawar

Introduction

Australian redclaw crayfish (*Cherax quadricarinatus* (von Martens, 1868)) is a species of freshwater crayfish from family Parastacidae that first originated from Australia and New Guinea (Austin, 1996; Bláha et al. 2016). The species has spread to many countries worldwide (Haubrock et al. 2021) including Malaysia (Naguib et al. 2021; Naqiuddin et al. 2016; Norshida et al. 2021), Indonesia (Akmal et al. 2021; Patoka et al. 2018, 2016; Wicaksono, Mashar & Wardiatno 2021), China (Yau & Lau 2021), United States (Morningstar et al. 2020), Mexico (Tapia-Varela et al. 2020), Puerto Rico (Macias, Torres & Colon-Gaud 2021), Martinique Islands (Baudry et al. 2020), Africa (Maurice et al. 2019; Madzivanzira et al. 2020), and even Europe (Arias & Torralba-Burrial 2021; Weiperth et al. 2019). The species is therefore well-known to be a successful invasive species in the tropics and subtropics (Haubrock et al. 2021), and new established population has been recently discovered within Malaysia territory (Sallehuddin, Kamarudin & Ismail 2021).

Cherax quadricarinatus was first introduced commercially in the aquaculture and ornamental trade industries in Malaysia since the 1980s (Naqiuddin et al. 2016) and it has since gained interest due to its characteristically unique lobster-like blue appearance and tough survival conditions. *C. quadricarinatus* is indeed able to withstand a wide variety of temperatures together with low dissolved oxygen concentration levels (Naguib et al. 2021). Besides, they also have a simple reproductive cycle thus granting them to have rapid growth rates (Naguib et al. 2021; Nasir et al. 2020) and attract local aquarium enthusiasts for easy captive breeding. These impressive intrinsic characteristics as well as the similarities with its native environment allow *C. quadricarinatus* to establish itself effectively and quickly in new areas. Thus, in Malaysia, it finds an optimal environment for its development and wild populations have recently been discovered by Norshida et al. (2021), in the state of Terengganu, in the east coast of Peninsular Malaysia.

Since *Cherax quadricarinatus* is an invasive species, there are a few regulations in Malaysia that may be imposed on their presence. For example, the government through the Animals Act (1953) regulates the import and export of animals, avoiding the spread of diseases, besides preserving and enhancing the welfare of animals in general (FAO 2022a). Moreover, the Biosafety Act (2007) controls the discharge of living organisms and their importation in order to conserve the ecosystem and biological diversity (FAO 2022b). However, these regulations do not stop *Cherax quadricarinatus* from still being commercially bred by local farmers associated with the fishing, ornamental and aquaculture industries. This may be because of the lucrative market price for *Cherax quadricarinatus* in Malaysia, which can go to up 30USD/kg (Norshida et al. 2021). The major reason for redclaw crayfish entering the wild habitat is either by accidental escape or planned release from aquaculture facilities and hatcheries. Plus, most invasive crayfish introduced to the wild were due to releases from aquariums or escape from ponds (Patoka, Kalous & Kopecký 2014). Moreover, according to Yuliana et al. (2021) the species can be easily transported by humans in huge quantities. Besides, the success of its invasion can also be linked to the environmental similarities between Malaysia and their native range. The crayfish was first reported to be present in the east coast of Peninsular Malaysia by Norshida et al. (2021), in which the invasive crayfish has already successfully established itself in the local wild ecosystem of a rural village in the state of Terengganu.

Cherax quadricarinatus are also known to cause many damages both to the ecosystem and economy (Akmal et al. 2021). In Malaysia, the presence of *C. quadricarinatus* has piqued the interest of local fishermen, who have suffered losses as a result of damage to their fishing nets and catch (Naqiuddin et al. 2016), which in turn causes their fishing yield to decrease. Besides, the crayfish (and other crayfish species in general) also affects the natural balance of the local ecosystem and are recognised to pose major challenges to local biota and have far-reaching effects on the environment, including habitat dominance, competition (outcompeting local species), predation on native species, stunted growth, transmission of non-native pathogens, bioaccumulation, food web and habitat modifications (Akmal et al. 2021; Souty-Grosset et al. 2016; Strauss, White & Boots 2012). On the other hand, the red swamp crayfish (*Procambarus clarkii*) alters the benthic invertebrate community structure with a variety of functional effects, frequently mediated by trophic cascades, reducing macrophyte plants which in turn caused losses in macroinvertebrate genera, reduction in waterfowl, amphibian and duck species (Souty-Grosset et al. 2016).

Due to their destructive behaviour on the local ecosystem, some efforts, such as preliminary assessment methods, scientific studies on their dispersal patterns, are needed to prevent new introduction of *C. quadricarinatus* in the wild. Environmental DNA (eDNA) studies may provide a clearer and better view

of the survey on the specific locations pertaining to the crayfish's targeted and specific habitats. Environmental DNA is the DNA sample collected from environmental materials such as water, soil, and air without removing physical tissue from the actual target organism (Dougherty et al. 2016). The use of DNA as a monitoring tool in the management of invasive alien species can be considered as a relatively novel and developing technique that has been used in many studies (Nasir et al. 2020). According to Chucholl et al. (2021), eDNA is a great tool for preliminary large-scale survey that is ideal as an identifying method for both native and invasive crayfish species throughout the year. Even in natural settings with numerous or inaccessible shelters, where detection with conventional approach is challenging and labor-intensive, eDNA-based monitoring enables crayfish detection regardless of their activity pattern. Previously, Baudry et al. (2021) have examined and validated the viability of using eDNA in a large-scale study to actualize the distribution map of *C. quadricarinatus* to be effective. They concluded that the use of eDNA-based monitoring required less time and was less affected by inconsistent distribution patterns of *C. quadricarinatus*. An early detection of *C. quadricarinatrus* using eDNA as well as their abundance may be critical for species distribution monitoring, invasion control management, and limiting the threats and damage that they may pose to the local environment.

Based on Bajjali (2018), Geographic Information System (GIS) is a computer-based information technology system that stores all digital data in one location and enables for quick data retrieval and analysis in map format. These data, including the eDNA data, can be processed together to obtain multiple layers of related information such as elevation and climatic data using spatial and temporal data. The use of GIS data may facilitate by providing a clearer and better image of the survey on the precise locations relevant to the crayfish's targeted and specific habitats, which can also be applied to other IAS species. The study done by Sakai et al. (2019) has successfully shown how eDNA and GIS can be used concurrently to detect the potential habitats of the endangered species *Hynobius vandenburghi*. Their study proves that GIS is able to handle massive datasets and covering wide areas, which will allow researchers to evaluate spatial data to anticipate and locate habitats of targeted species.

This study aims to determine the preliminary habitat distribution patterns of *C. quadricarinatus* based on the methods of GIS and environmental DNA analysis in Terengganu, Malaysia. The results from this study can help us to detect the presence of *C. quadricarinatus* in potential new areas. Thus, we will be able to study its distribution behaviour related to the geographical conditions of the invaded area. This will eventually assist targeted efforts to prevent further spread of this invasive crayfish into different areas based on certain parameters.

Materials and Methods

Field sampling

The IAS studied in this paper is the Australian redclaw crayfish, *Cherax quadricarinatus*. Field sampling to detect *C. quadricarinatus* DNA samples was conducted from 22 to 24 March 2021 at a rural area (Felda Tenang: 5°33'42.6"N, 102°29'50.0"E) in the east coast, state of Terengganu, Peninsular Malaysia (Figure 1). Felda Tenang is a residential village enclosed by elevated areas such as hills that is surrounded by forests and palm plantations with connected water bodies. The sampling area, Felda Tenang, was chosen after taking into account the study done by Norshida et al. (2021) that confirms the first wild record of *C. quadricarinatus* in the East Coast of Peninsular Malaysia. Furthermore, the sampling sites were selected based on their local aquatic conditions, with a particular focus on connected streams, flood histories and geographical terrain.

Field sampling was conducted in the span of 3 days, with the second and third day carried out under heavy rainfall conditions. The sampling included taking water samples for eDNA analysis at 24 stations across the study area, which were divided into 4 zones namely the Red Zone, Blue Zone, Yellow Zone and Green Zone (Figure 2). Due to Felda Tenang being a flood prone area, the four zones were divided based on their average elevation range (Figure 3).

The study area was divided into 4 sampling zonation; namely the Red Zone, Yellow Zone, Blue Zone and the Green Zone (Figure 2). The zonation was divided based on their geographical terrain and elevation above sea level, for which the ranges are: Red (20-30 m), Blue (31-35 m), Yellow (36-39 m) and Green (40-50 m). The elevation was analysed digitally using ESRI ArcGIS 10.7 at ESERI, UniSZA. The Red Zone comprises of residential areas and community centers that are frequently hit with flooding, especially during the Northeast Monsoon from the month of October to March each year (World Bank Group 2021). The Red Zone is the most frequent area of which flooding occurs. The Red Zone comprises mostly of residences and community centers such as school, police station and

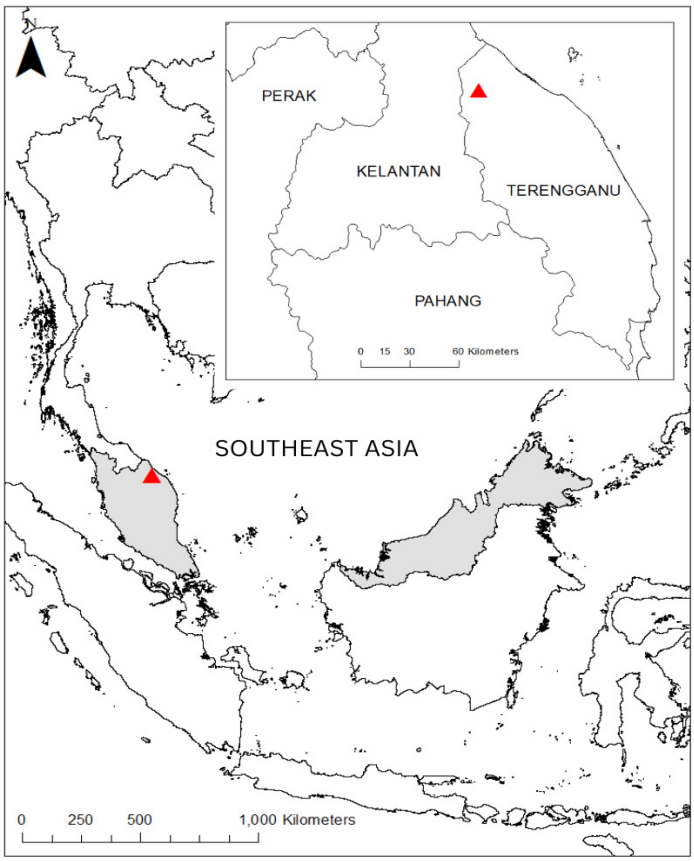

FIGURE 1. The pinned location of Felda Tenang, situated in the Terengganu state of Peninsular Malaysia

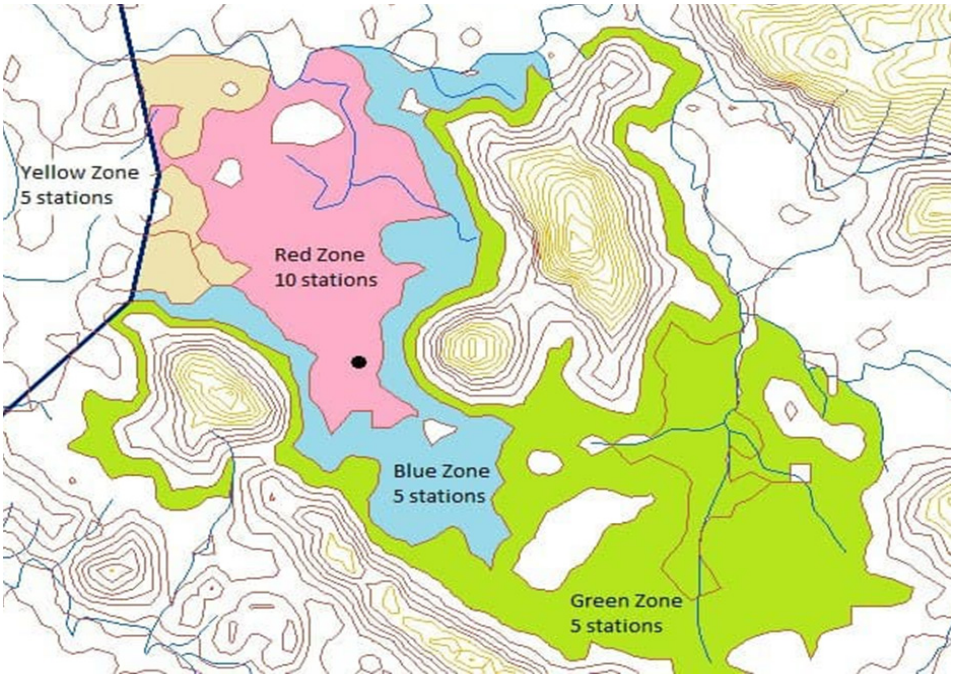

FIGURE 2. The sampling zones in Felda Tenang, Terengganu. The pinned location (black dot) is Station R1

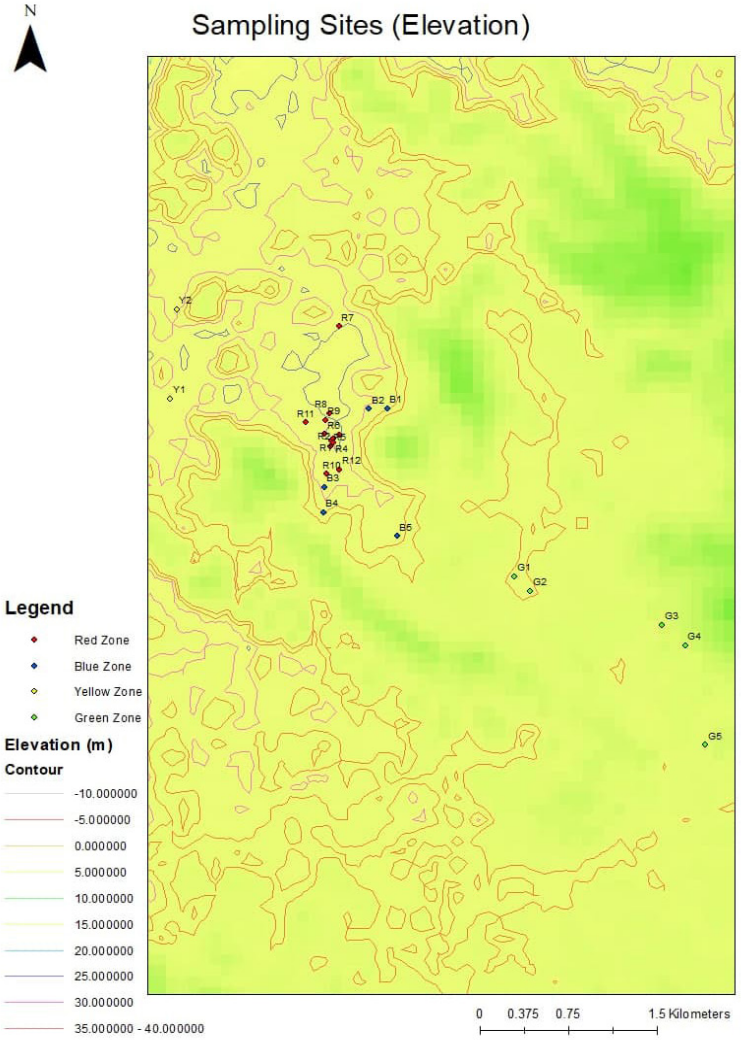

FIGURE 3. The contour map of the study area

mosque. The Green Zone is the highest elevated terrain and furthest from the flooding zone and residential area, in which all of the sites in the Green Zone were located in palm plantations. One litre of water samples were taken at each of the designated 24 sampling sites. The water samples were collected using modified disposable plastics syringe, which was a tube attached to it to the syringe nozzle to collect water samples at tight location and the water samples were kept in two new 500 mL bottles, for each of the sampling sites. To avoid cross contamination, the syringe was rinsed twice, thoroughly using autoclaved distilled water between sampling sites. To preserve the eDNA concentration in the water samples, the water bottles were kept in an ice box right after the acquisition before being transported back to the Molecular Biology Lab, UniSZA to be stored in the chiller.

LABORATORY EXPERIMENT

Water filtration and DNA extraction

Each of the water samples from the sampling sites was filtered using 0.22 µM PES 500 mL Upper Cup Filter (JetBiofil, China), attached to an electric motor pump. All the water samples were completely filtered and filter paper was then taken to be used for DNA extraction process. DNA analysis was done according to the FavorPrepTM Tissue Genomic DNA Extraction Kit kit's protocol (Favorgen Biotech Corp Taiwan, 2017) with minor modification. The modification made was that the filter

paper was used instead of living tissue. The filter paper was first cut into half and put into two 1.5 mL micro centrifuge tubes respectively before grinded into smaller pieces and labeled as 'a' and 'b'. These two replicates were combined again during the final step, which was the elution step where each of the two replicates were eluted at volume of 45 µL into one single 1.5 mL tube, making the total eluted DNA was 70 µL. The process was repeated for the rest of the samples.

PCR amplification and gel electrophoresis

For each extracted sample, PCR amplification of the Cytochrome Oxidase I (COI) mitochondrial (mtDNA) region was carried out according to Nasir et al. (2020), with a pair of *Cherax quadricarinatus* species-specific primer. The primer sequences for COI region of invertebrates used were the forward CQ_COI_F2 (5'– AGC CCC TGA TAT AGC CTT CCC TCG AAT AAA-3') and reverse, CQ_COI_R3 (5'– GCC TAG GTC GAC TGA TGC TCC TGC A-3). The total volume of the PCR reaction used, which included 2.0 µL of double distilled water (ddH_2O), 13.0 µL of MyTaq™ DNA Polymerase mixture/master mix (Bioline, Meridian Bioscience International Limited, United Kingdom), 0.5 µL (10uM) for each forward (CQ_COI_F2) and reverse (CQ_COI_R3) primer and 2.0 µL of DNA template (100 ng/µL). The PCR amplification cycle began with a 5-minute initial denaturation phase at 95 °C; followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 66 °C for 45 s, and elongation at 72 °C for 60 s; before concluding with a 10-minute final elongation phase at 72 °C. Gel electrophoresis was used to check for the presence of DNA band in the amplified PCR product. In a conical flask, 0.40 g of agarose powder was mixed with 20 mL of 1x TBE buffer before being microwaved for 40 s until the agarose powder was completely melted. After that, the gel solution was allowed to cool for about 5 min before 1.0 µL of FloroSafe DNA Stain (Axil Scientific Pte. Ltd., Singapore) was inserted. The solution was then thoroughly mixed by gentle swirling. A gel cast including gel comb was also used to pour the solution into. The gel was then allowed to solidify. The comb was then carefully removed from the agarose gel after it had set before being placed in the gel electrophoresis tank. In the electrophoresis tank, 1x TBE buffer was poured until the gel was completely submerged. Each sample received 3.5 µL of PCR products and 2.5 µL of 100 bp ladder pipetted into separate wells. For the electrophoresis procedure, the tank was then linked to a power supply. The power supply was set to 100 V for 40 min at 500 mA. The gel was seen using a transilluminator and FluorChem E (Protein Simple, California, USA) once the electrophoresis process was completed. Since the amplicon for the species-specific primer utilised in this investigation was around 175 bp, any samples with a band between 100 bp and 200 bp were considered positive.

qPCR amplification

Quantitative PCR (qPCR) analysis was conducted according to Nasir et al. (2020), utilizing the designed *Cherax quadricarinatus* species specific primer and probe. All of the PCR products from each sampling sites were sent to Lab-Ind Resource Sdn. Bhd. Amplification through qPCR was done to obtain a more accurate result. The analysis was conducted on DTprime Real-time Detection Thermal Cycler. The thermal profile conducted was as follows: a cycle of Uracil-dehydrogenase treatment phase at 50 °C for 2 min; initial denaturation phase at 95 °C for 10 min; 40 cycles of denaturation phase at 95 °C for 30 s; and annealing phase at 62 °C for 30 s. The qPCR reagents used were 0.5 µL for each of the forward and reverse primers (10µM), 10 µL WizPureTM qPCR 2X Master-UDG (PROBE), 0.4 µL probe (10µM): 5' - /56-FAM/ CTA GCA GCA/ ZEN/ TCA ATC GCC CAT GCA/ 3IABkFQ/ -3' (10 µM), 2 µL of extracted DNA and 6.6 µL nuclease free water. The qPCR results were analysed using the RealTime_PCR v7.9 (DNA Technology, Russia). The analysis was evaluated by comparing the standard curve to the qPCR graph generated by qPCR amplification. A 10-fold serial dilution from cloned positive plasmid from 10^6 to 10^1 copies/µL was used to generate the standard curve. The standard curve is further used to determine the number of copies of test samples from the Ct value obtained.

GIS TREATMENT

The data processing software used was ESRI ArcGIS 10.7. To draft the database design of the study area, there are several particular feature included, which comprise of evaluating and analyzing available data; describing the study area and objects within such as vegetation, residential area and water bodies; linking the available spatial data with the database; physical design; and lastly, implementation. The geographical features, terrain/ elevation and climate data were obtained from existing databank available on the Internet (www.diva-gis.org). The Digital Elevation Model (DEM) that shows the 3D terrain of the study area was done using the ArcScene function in ArcGIS 10.7. The historical record of *C. quadricarinatus* presence; the physical survey and observation of the crayfish; and the climate history and

pattern in the study area were then compared with the generated database design to determine the possible relationship for *C. quadricarinatus* dispersal.

Results and Discussion

According to Haubrock et al. (2021) and Madzivanzira, Weyl and South (2022), freshwater crayfishes are one of the most notorious and harmful IAS worldwide due to their large size when comparing to other invertebrates, their omnivorous diet behaviour and their interest as a food item for human consumption. Freshwater crayfish such as *Cherax quadricarinatus* has resilient characteristics that make them easily adaptable to establish and expand their habitat in tropical and subtropical regions that are mostly similar or identical to their native habitats in northern Australia and southeastern New Guinea (Naqiuddin et al. 2016). *C. quadricarinatus* is highly reproductive, has a rapid growth rate and high survival rate. Apart from being tolerant to a wide dietary content, they are also tolerant to wide ranges of water quality and environmental parameters such as being able to survive at circa 10-31 °C temperature, highly adaptable to various levels of pH, alkalinity, water hardness, oxygen and ammonia (Akmal et al. 2021; Haubrock et al. 2021; Sallehuddin, Kamarudin & Ismail 2021). They can live at both lotic and lentic ecosystems, thus allowing them to establish their habitat in various types of water bodies such as drainages (Norshida et al. 2021), streams, canals (Naguib et al. 2021), rivers, and lakes (Naqiuddin et al. 2016). *C. quadricarinatus* might also carry foreign diseases or pathogens. Besides, redclaw crayfish can destroy fishermen nets and traps (Naqiuddin et al. 2016). Their dangerous and disruptive behaviour will eventually disrupt the local aquaculture industry in invaded areas, thus not only affecting the local ecosystem but also the aquaculture yield and income of local fishermen.

All of the sampling sites in the study area are water bodies that are connected to each other. There are comprised of drainages systems, streams and rivers, making it easier for *C. quadricarinatus* to spread to other locations. In the study area, The Red Zone is also encompassed by elevated areas such as hills to the northeast, southwest and southeast. The enclosed nature of the Red Zone has made them susceptible to flooding. On the other hand, Moorhouse and Macdonald (2014) and Norshida et al. (2021) described that confined geographical nature can affect the invasiveness of a species to be more probable. The Blue and Green Zones are located within the proximities of elevated areas. The Blue Zone consists of residential areas and forests meanwhile the Green Zone is purely within palm plantations and the furthest from the flood-prone Red Zone. Meanwhile, the Yellow Zone is located near the main access road to the entrance of Felda Tenang. Comparing to the Red and Blue Zones, of which the locals were already aware of redclaw crayfish presence, the detection of *C. quadricarinatus* at both the Yellow and Green Zones should be noted since there were no records of their physical presence at both zones yet. Since the Yellow and Green Zones are located far from residential areas, the establishment of *C. quadricarinatus* can persist without anthropogenic disturbance.

Based on the results of conventional PCR, we detected DNA traces of *C. quadricarinatus* from the waters at Stations R1, R2, R3, R4, and R5. During eDNA water sampling (only in Day 1), we even detected juvenile *C. quadricarinatus* at Stations R1, R3 and R4 by accident, which were caught using small fish nets. However, we were unable to acquire juveniles during the rest of the sampling days due to increased water flow and accumulation under the raining conditions. This outcome does not affect the eDNA results in this study since we were only focused on acquiring water samples. Meanwhile, there was no presence of *C. quadricarinatus* detected at all of the remaining stations. However, the qPCR result indicates their DNA presence at all of the sampling stations with the exception of Stations B2 and G4. We conducted qPCR analysis to ensure a more detailed accuracy of *C. quadricarinatus* detection in the eDNA samples. Since the sampling on the second and third day (comprising of Yellow and Green Zones) was done under raining condition, the water samples collected were mixed with rainwater thus diluting the overall biomass but nevertheless, traces of *C. quadricarinatus* DNA can still be detected in both zones albeit with increased threshold (Ct) value (Table 1). The planned field sampling period proceeded even during the rain since it can only be done during the relaxed period of Movement Control Order (MCO), implemented by the Malaysian government to curb the spread of COVID-19. The most influential factors during eDNA sampling includes the number of days of previous rainfall, water temperature and maximum flow accumulation, apart from the flow rate and turbidity change. Due to the changes in these factors, the Ct value in Felda Tenang was increased thus lowering the detection probabilities. Unlike traditional PCR, which relies on the visual inspection of PCR band, qPCR is more sensitive and specific due to application of TaqMan-probe hence its usage to overcome this sampling challenges to

acquire indicative results as shown in Table 1. Table 1 shows the average Ct value of the study area, in which the Red Zone has the lowest Ct value. This emphasizes that the DNA traces of *C. quadricarinatus* is higher in the flood-prone Red Zone compared to the other zones. Hence, we evaluated that there are fewer but older *C. quadricarinatus* adults in streams and drainages at higher elevated residential area but more younger *C. quadricarinatus* at the lower elevated residential areas. During heavy rainfall, the increased water flow and accumulation in the waterways will disperse the juveniles into the lower areas, thus introducing them into new areas. Nunes et al. (2017) found that different elevations, barriers and changes in temperature contribute to the dispersal pattern of *C. quadricarinatus*. The dispersal pattern of *C. quadricarinatus* is higher for upstream movements, making it easier for potential new areas to be invaded especially via irrigation channels.

The heavy amount of precipitation in the Blue Zone (Figure 4) acts as a catalyst for *C. quadricarinatus* to be dispersed into the lower areas. Thus, we hypothesized that the Blue Zone is the first occurrence of *C. quadricarinatus* introduction in Felda Tenang. Moreover, during eDNA sampling, we detected adult crayfishes at Station B1. Upon closer survey, the locals confirmed the sizes of the crayfishes are among the biggest they have ever seen in Felda Tenang, adding to the speculation that this area might be the initial point of introduction of the species in Felda Tenang. Nunes et al. (2017) indicated that crayfishes were generally heavier and bigger in areas close to the point of introduction with the sizes decreasing over distance travelled into new areas. The adult crayfish found in the Blue Zone might have lived longer than the crayfishes that were usually found in the Red Zone due to less human disturbance in higher elevated areas that might affect their diet. Plus, the rich and undisturbed environment may provide more variety of diet and shelter for *C. quadricarinatus*. However, further inspection was made in the Blue Zone for the occurrence of younger *C. quadricarinatus* to no avail.

TABLE 1. The presence of *Cherax quadricarinatus* at each sampling stations based on conventional and quantitative PCR analysis

Zone	Station	Presence		Ct value	Ave. Ct value	Zone	Station	Presence		Ct value	Ave. Ct value
		PCR	qPCR					PCR	qPCR		
Red	R1	+	+	32.3	29.81	Blue	B1	-	+	30.3	32.97
	R2	+	+	32.4			B2	-	-	-	
	R3	+	+	32.2			B3	-	+	32.2	
	R4	+	+	30.7			B4	-	+	35.3	
	R5	+	+	27.2			B5	-	+	34.1	
	R6	-	+	31.1		Yellow	Y1	-	+	32.8	32.95
	R7	-	+	29.4			Y2	-	+	33.1	
	R8	-	+	30.5		Green	G1	-	+	34.0	34.00
	R9	-	+	30.4			G2	-	+	34.1	
	R10	-	+	24.8			G3	-	+	34.0	
	R11	-	+	28.9			G4	-	-	-	
	R12	-	+	27.8			G5	-	+	33.9	

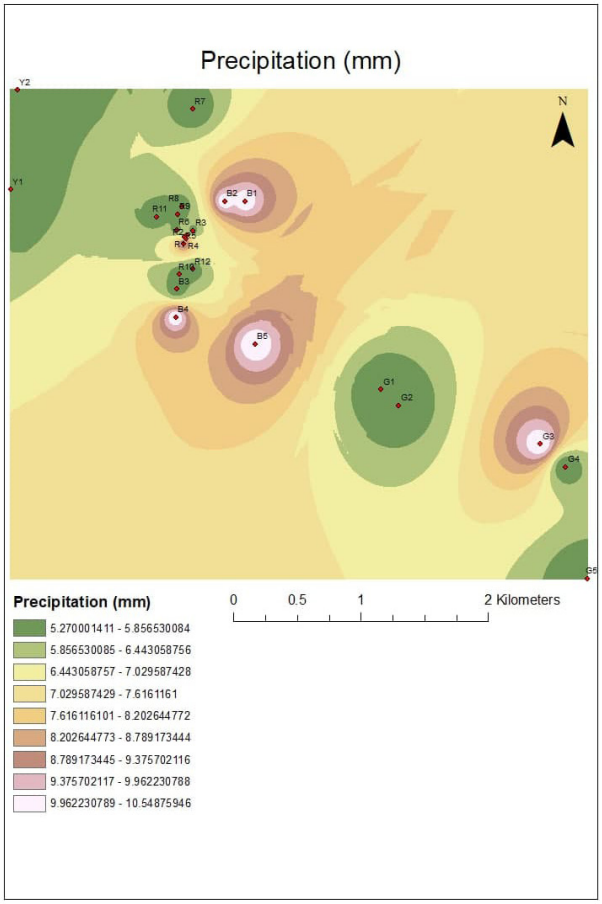

FIGURE 4. Average precipitation at Felda Tenang

The distribution of *C. quadricarinatus* is made worse during the Northeast Monsoon. The heavy rainfall during monsoon produced a great amount of water flowing into the streams and drainages. Since they cannot handle the increased volume of rainwater, the excess water has overflowed and cause flooding to happen, especially in the Red Zone as the lowest elevated area that has become the retention area for excess rainwater. Flood that happens causes *C. quadricarinatus* to cross physical boundaries into new locations. This is confirmed when the locals mentioned of crayfishes found crawling on the roads and into their houses during flooding. Moreover, the water flow has dispersed the juveniles of *C. quadricarinatus* into another area, causing the invasion to happen. Figure 5 shows the DEM of the study area, from which the blue-shaded region shows the flood-prone area of Felda Tenang. The blue region might also indicate possible habitat distribution of *C. quadricarinatus* outside of Felda Tenang. Interestingly, there are *C. quadricarinatus* detected at the blue-shaded Green Zone even if they are separated from the remaining zones by elevated area. This implies that their dispersion might originate from a single point of occurrence (Blue Zone). Further eDNA studies covering a larger area within the blue-shaded region should be done in the future to detect the possible invasion of *C. quadricarinatus*.

By determining *C. quadricarinatus* habitat distribution, we can evaluate its consequences from inflicting further damages to the local ecosystem and socioeconomy as implied by Madzivanzira, Weyl and South (2022). For example, barriers can be made at the potential spreading areas (Manfrin et al. 2019). These barriers might negatively affect the native species but currently there are no known native species documented in Felda Tenang waterways. Plus, mitigation measures to tackle flooding can also be enforced (especially in Felda Tenang, since flooding is the main reason of *C. quadricarinatus* dispersal). Since *C. quadricarinatus* is

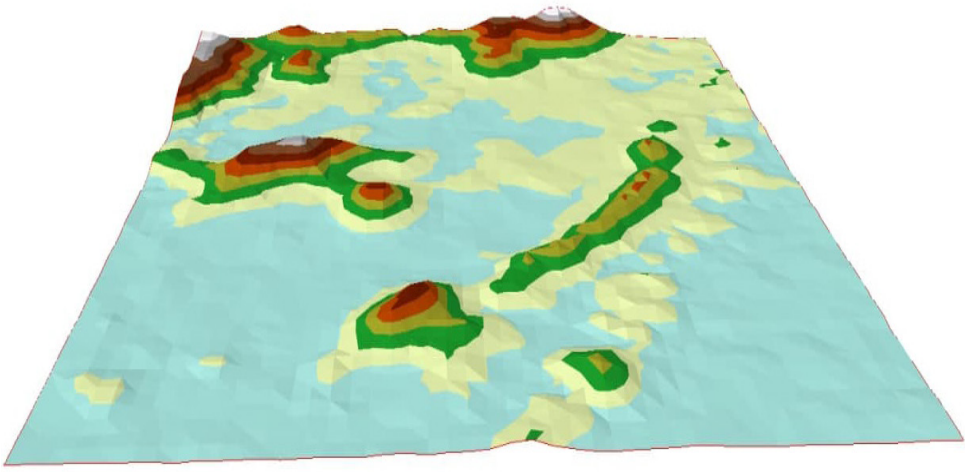

FIGURE 5. The DEM of Felda Tenang. The blue-shaded area is the flood-prone area

highly invasive and resilient, the species can outcompete native species by direct competition and predation. Thus, there will be food web imbalances and might cause native species disappearance. However, natural predator such as eels might be used as a biological control (Manfrin et al. 2019) to manage the population of crayfishes, as Aquiloni et al. (2010) experimented as biological control to red swamp crayfish (*Procambarus clarkii*). However, the fight against invasive crayfish is complicated. This is pointed out by Manfrin et al. (2019) that although the invasive crayfish species is diverse, widespread, and gifted with a high reproductive rate and a wide range of physiological tolerances in open systems like rivers and streams, no physical control strategies have demonstrated effectiveness in these environments. Thus, Manfrin et al. (2019) suggested that apart from mass trapping, baited trapping and fish predation, collective monitoring by the local citizens and stakeholders alike might also be advantageous especially when covering a large area that needs a lot of manpower for continuous observation and eradication efforts. In this case, it is important that necessary actions are taken collectively to ensure the successful restoration and preservation of natural biodiversity in Felda Tenang.

Conclusions

The usage of eDNA analysis correlated with GIS predictions has made the detection of *C. quadricarinatus* easier thus enabling us to determine their dispersal patterns and potential distribution area at Felda Tenang. The distribution of *C. quadricarinatus* at Felda Tenang is worrying as they have been detected in 91.67% of the stations in the study area. The ability of *C. quadricarinatus* to easily adapt in the wild has made them a worrying presence in the local ecosystem of Felda Tenang. Combined with the geographical terrain and frequent flood occurrence, it has undoubtedly made them easier to invade new areas. To prevent the future spread of *C. quadricarinatus*, we encourage the authorities, educational institutes, and non-governmental organizations to conduct proper community engagement programs to spread awareness and to guide the local residents to be citizen scientists. Besides, policies and regulations can be done to ensure that there will be less economic advantage for the aquaculture and ornamental trade of *C. quadricarinatus*. Finally, we also recommend for a thorough flooding mitigation plan to anticipate the annual supplemental rainfall in Felda Tenang during the Northeast Monsoon. Future studies can also be done to determine the habitat distribution patterns of *C. quadricarinatus* in other areas.

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