

## Aquatic macroinvertebrate richness, abundance and distribution in the Nee Soon freshwater swamp forest, Singapore

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**ABSTRACT.** The Nee Soon freshwater swamp forest is a vital area for biodiversity conservation in Singapore. A survey of the aquatic macroinvertebrates in the streams of the Nee Soon drainage was carried out to capture a representative sample of the communities present. Here, we present the different groups of macroinvertebrates sampled as well as their abundance and distribution within the freshwater swamp forest. An annotated checklist of the orders of the macroinvertebrates found in the freshwater swamp forest follows, together with information on their distribution and abundance within the Nee Soon catchment.

**Keywords.** Aquatic biodiversity, freshwater ecology, invertebrate surveys

### Introduction

The Nee Soon freshwater swamp forest is the last substantial area of freshwater swamp forest found in the island nation of Singapore and serves as a vital conservation area due to its rich biological diversity (Ng & Lim, 1992; Lim et al., 2011; O'Dempsey & Chew, 2013; O'Dempsey, 2014). It is also one of the most biologically diverse areas in Singapore (Lim et al., 2011), especially for freshwater fish and decapod crustaceans (Ng & Lim, 1992; Ng, 1997; Ng & Lim, 1997; Li et al., 2016). Additionally, the Nee Soon freshwater swamp forest is also an important conservation area for other groups of water-dependent (aquatic) macroinvertebrates, also known as the zoobenthos (Dudgeon, 1999), as these animals are usually found in stream beds. Among these lesser-known groups are the aquatic larvae of insects such as dragonflies and damselflies (Odonata), stoneflies (Plecoptera), mayflies (Ephemeroptera), and caddisflies (Trichoptera). Other groups which can be found among the zoobenthos of the freshwater swamp forest include leeches and other worms (Annelida) as well as crustaceans such as the seed shrimps (Ostracoda). Several species from these groups are found nowhere else in Singapore, such as the potbellied elf dragonfly (*Risiphlebia*

*dohrni*), and would be extirpated from Singapore should anything happen to the Nee Soon freshwater swamp forest (Tang et al., 2010).

Currently, information on aquatic macroinvertebrate groups in Singapore is scarce in the scientific literature, with information existing only for certain groups (Balke et al., 1997; Murphy, 1997; Yang et al., 1997; Hendrich et al., 2004). Additionally, even for relatively charismatic and well-studied groups such as the odonates, available information deals mostly with the adult stage of these insects (Murphy, 1997; Norma-Rashid et al., 2008; Tang et al., 2010), with not much being known about their aquatic larval stages. More obscure groups such as the ostracods and aquatic mites lack even basic information about their presence, distribution and diversity in Singapore. While several studies on macroinvertebrates in Singapore's urbanised water bodies exist (Loke et al., 2010; Blakely et al., 2014; Clews et al., 2014), the macroinvertebrates in forested areas are less well-known, with scattered reports on selected insect groups (e.g. Coleoptera, Hemiptera and Odonata) in the entire Central Catchment Nature Reserve (Balke et al., 1997; Murphy, 1997; Yang et al., 1997), as well as a single report on selected insect groups (Hemiptera, Coleoptera, and Odonata) of the Nee Soon freshwater swamp forest (Gani, 2013). In recent years, books focusing on specific insect groups, such as those by Tang et al. (2010) and Tran et al. (2015), also provide a general overview of their target groups in Singapore, including information on the populations inside the forests of Singapore.

The lack of information on the aquatic macroinvertebrates of Singapore, particularly baseline ecological information, is meant to be partially addressed by the current study, which aimed to undertake representative sampling of macroinvertebrate communities across the Nee Soon catchment. This enabled the identification of different groups of macroinvertebrates found in the streams, their abundance and distribution in the Nee Soon drainage. This is the first dedicated and relatively comprehensive quantitative survey of the presence of aquatic macroinvertebrates in the Nee Soon drainage, allowing for the establishment of baseline data regarding the aquatic macroinvertebrates found in the freshwater swamp forest. This data will allow for more informed management decisions to be made regarding the Nee Soon freshwater swamp forest, especially with regards to its conservation.

## Methods

### *Study sites*

The Nee Soon freshwater swamp forest is located in the Central Catchment Nature Reserve, roughly bounded by the Upper Seletar Reservoir, the Upper Peirce Reservoir and the Seletar Expressway. It is the last remaining freshwater swamp forest found in Singapore, and is accorded "protected" status as a nature reserve on account of its inclusion in the Central Catchment Nature Reserve, as well as its use for military training (Ng & Lim, 1992) with the presence of a military firing range within the Nee Soon catchment (Fig. 1). The Nee Soon freshwater swamp forest is drained by a single stream network, originating in central Singapore and draining roughly northeast

towards Lower Seletar Reservoir. The streams are typically shallow (8–70 cm in depth) and slow-flowing (mean velocity 1–18 cm/s). Tannins and other chemicals leaching from leaf litter stain the water a dark tea-colour, as well as reducing the pH to acidic levels (pH 4–6). Only a small amount of dissolved minerals can be found in the water. In periods of heavy rainfall, the streams tend to overflow and flood the surrounding area, creating small pools and flooded areas which may persist for indeterminate periods (Ng & Lim, 1992; Lim et al., 2011). In addition to ground and rainwater input, the Nee Soon drainage also receives periodic input from the Upper Seletar Reservoir near the northeast edge of the lower catchment. The environment in this area of the Nee Soon drainage is quite different from the rest of the swamp forest. Here, pH levels tend to be higher (> 5), reflective of the less acidic waters received as well as the more open forest canopy.

Additional information on the hydrology and geomorphology of the Nee Soon catchment relevant to the aquatic macroinvertebrates is given by Nguyen et al. (2018) and Sun et al. (2018).

### *Sample collection*

The diversity of aquatic freshwater macroinvertebrates in the Nee Soon freshwater swamp forest was represented by sampling a total of 40 sites throughout the freshwater swamp forest each on a single occasion between October 2013 and September 2014 (Fig. 1). Additionally, sites 18, 33 and 38 (Fig. 1) were surveyed every two weeks between December 2013 and January 2015. Macroinvertebrates were sampled with a qualitative kick sampling method described in Blakely et al. (2010). The sampling procedure involves kicking the stream substrata to disturb and release macroinvertebrates into a kick-net (36 × 30 cm, 250 µm mesh size) held downstream. Kick samples were collected from a wide range of microhabitats (e.g. leaf packs, cobbles, pools, log jams and stream margin) over a 2-minute period within a 10 m reach delimiting each site. This procedure facilitated the collection of comparable representation of macroinvertebrate communities, while maximising the likelihood of collecting all species present including rare and habitat-specific species. All macroinvertebrate samples obtained were then immediately preserved in the field with molecular grade isopropanol.

In the laboratory, samples were rinsed with water on a 250 µm mesh Endecott sieve, and all aquatic macroinvertebrates were removed and identified under ×100 magnification. Individuals were identified to family-level, except for class Ostracoda, subclass Acari, Collembola and Oligochaeta, order Araneae, and infraorder Brachyura. The main references used for taxonomic identification were Merritt & Cummins (1996), Yule & Yong (2004), and Blakely et al. (2010).

In this paper, the distribution of aquatic biodiversity in the Nee Soon drainage is represented by a series of maps that show the number of individuals (abundance) of each taxonomic group from each site during the spatially extensive survey (one sample per site). Taxonomic abundance is represented at the order level for all groups, except the Ostracoda that are identified to class-level and the Acari, Collembola and Oligochaeta that are identified to subclass-level. Biological notes for each taxonomic

group include morphological characteristics that describe each taxonomic level (up to order) and feeding habits. Biological notes are made with reference to Yule & Yong (2004), Tang et al. (2010), Tan et al. (2010), and Tran et al. (2015).

For each taxon, the distribution of the group is supplemented with family-level information. A checklist indicating the presence of families (or lowest practicable level of identification) at each site is also presented. This checklist is derived from all samples collected during the period between October 2013 and January 2015 (encompassing both the spatially extensive survey and higher frequency surveys of three sites). Organisms from this study of the aquatic fauna were provided to a parallel team working on genomics and imaging, for a depository of photographic images and of barcodes (Kutty et al., 2018).

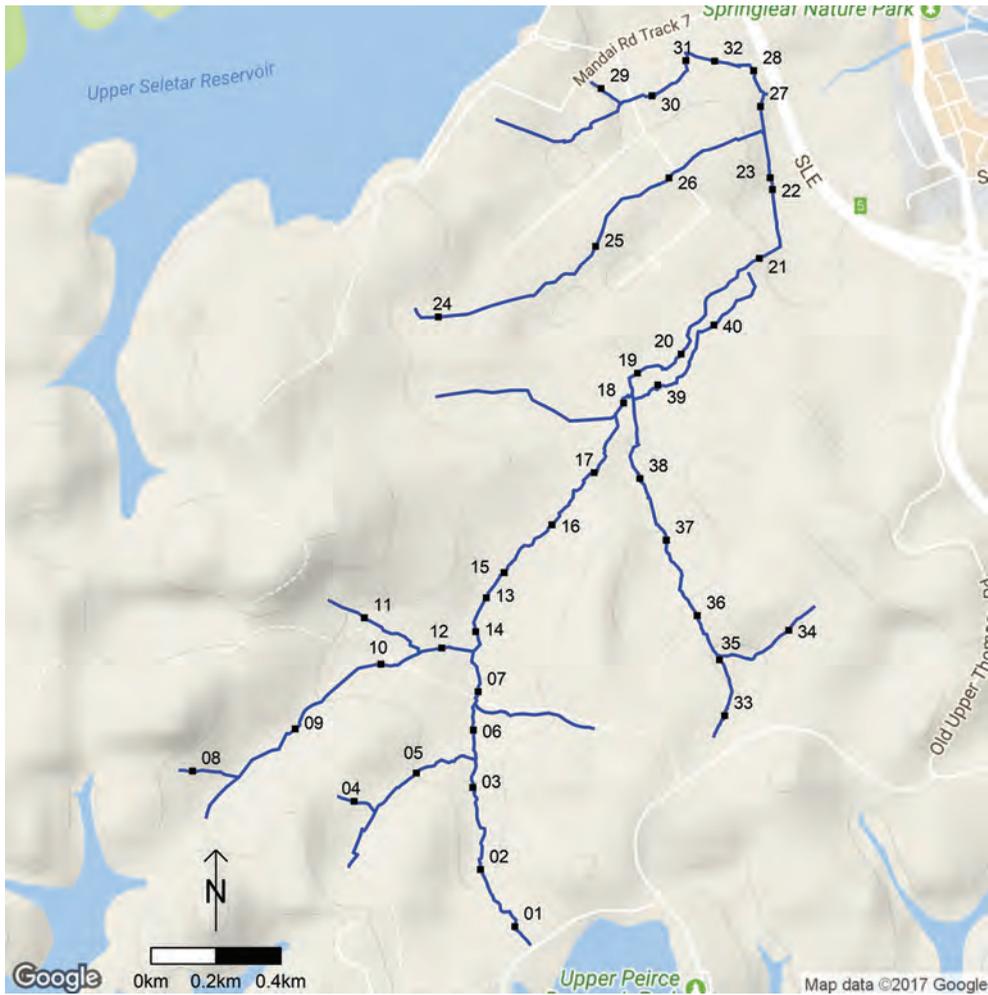
## **Results**

In total, 82 taxonomic groups (76 families and six higher taxa) of aquatic macroinvertebrates were found during the study throughout the Nee Soon freshwater swamp forest, with the majority of these families from the phylum Arthropoda (Table 1). The most diverse class of organisms recorded within Arthropoda was Insecta (66 families in total). Other arthropod classes recorded were Malacostraca (two families and one infraorder), Arachnida (two families) and Entognatha (one family). The phylum Mollusca was represented by two classes, namely Gastropoda (four families) and Bivalvia (two families). Finally, the phylum Annelida was represented by two classes, which were Clitellata (two families) and Oligochaeta (one family).

The macroinvertebrate classes in the freshwater swamp forest appear to have differing distributions, with both classes of Mollusca only being found in the northeastern section of the Nee Soon drainage, as well as the class Ostracoda (Table 2). The class Clitellata is found only at one site, with leeches from the family Erpobdellidae (order Arhynchobdellida) being recorded at site 11 and leeches from the family Glossiphoniidae (order Rhynchobdellida) being recorded at site 32. Other aquatic macroinvertebrate groups are more evenly distributed throughout the Nee Soon freshwater swamp forest, with Insecta, Malacostraca, and Arachnida being the most common. Entognatha and Oligochaeta are somewhat less common, but are also found throughout the Nee Soon drainage.

### **Class INSECTA**

The insects are the most diverse macroinvertebrate group found throughout the Nee Soon drainage, with a total of nine orders and 66 families recorded. Most insects found in the streams of the Nee Soon drainage are in their larval stage, with the adult stage being terrestrial rather than aquatic. Many of the classes recorded in the Nee Soon drainage such as the stoneflies (Plecoptera) and the mayflies (Ephemeroptera)



**Fig. 1.** The main drainage of the Nee Soon freshwater swamp forest, with 40 sites sampled from October 2013 to September 2014 for aquatic macroinvertebrates.

are indicators of good water quality (Blakely et al., 2014). Previous studies of the insect fauna of the Central Catchment Nature Reserve (which includes the Nee Soon drainage) covered several different insect orders (Balke et al., 1997; Murphy, 1997; Yang et al., 1997), which makes the insects among the better known groups of aquatic macroinvertebrates in the Nee Soon freshwater swamp forest.

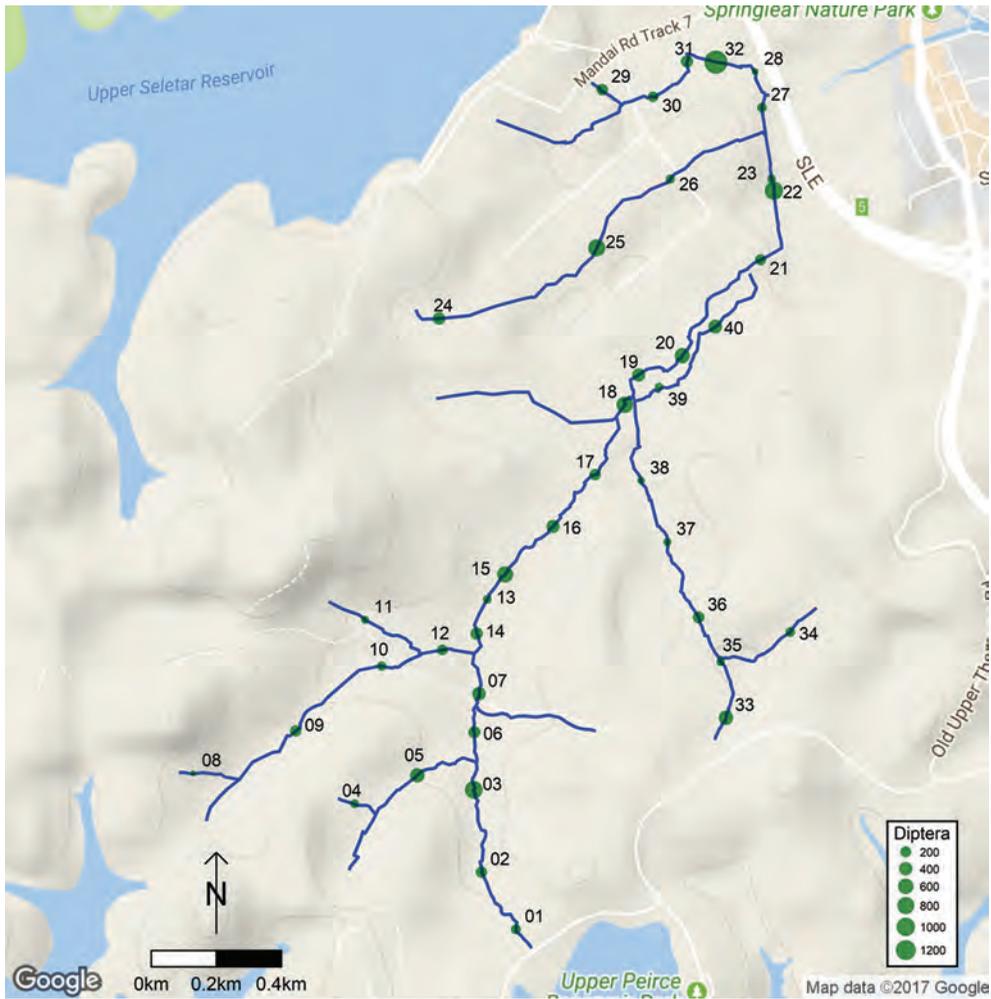
**Order Diptera**

The order Diptera, or the true flies, is an enormously successful and diverse group of insects. There are currently 17 families of Diptera recorded from the Nee Soon drainage. Overall, the Diptera are common throughout the entire Nee Soon drainage, being found in nearly all 40 sites (Table 2). The family Chironomidae, which occur naturally throughout the Nee Soon drainage, are the most common family. Other

**Table 1.** Summary of aquatic macroinvertebrates recorded across 40 sites in the Nee Soon Swamp Forest stream network. Macroinvertebrates identified are presented in respective taxonomic structure, with the number of invertebrate taxa (identified at Family level or higher) for each order denoted in No. of taxa. Total number of sites (No. of sites recorded) and the range of individuals (Individuals recorded/site) where each order was surveyed were also presented, along with the site number where the highest number of individual was recorded (Site with highest abundance). \*In this study, Ostracoda are identified at class-level; Acari, Collembola and Oligochaeta are identified at subclass-level; and Araneae are identified at order-level. #Within the Decapoda, three taxa were recognised, two families of shrimp (Atyidae and Palaemonidae) and one infraorder for crabs (Brachyura).

| Phylum     | Class        | Order          | No. of taxa<br>(Family level<br>and above) | No. of<br>sites<br>recorded | Individuals<br>recorded/<br>site | Site with<br>highest<br>abundance |
|------------|--------------|----------------|--|-----------------------------|----------------------------------|-----------------------------------|
| Arthropoda | Insecta      | Coleoptera     | 12   | 26                          | 1 - 15                           | 24                                |
|            |              | Diptera        | 17   | 40                          | 8 - 1316                         | 32                                |
|            |              | Ephemeroptera  | 5  | 38                          | 1 - 87                           | 32                                |
|            |              | Hemiptera      | 6  | 24                          | 1 - 123                          | 32                                |
|            |              | Lepidoptera    | 1  | 5                           | 1 - 4                            | 31                                |
|            |              | Megaloptera    | 2  | 6                           | 1 - 2                            | 9, 33                             |
|            |              | Odonata        | 10   | 32                          | 1 - 119                          | 32                                |
|            |              | Plecoptera     | 2  | 8                           | 1 - 3                            | 15                                |
|            |              | Trichoptera    | 11   | 31                          | 1 - 34                           | 32                                |
|            | Entognatha   | Collembola*    | 1  | 10                          | 1 - 4                            | 24                                |
|            | Arachnida    | Acari*         | 1  | 4                           | 1 - 4                            | 31                                |
|            |              | Araneae*       | 1  | 22                          | 1 - 6                            | 22                                |
|            | Malacostraca | Decapoda #     | 3  | 34                          | 1 - 73                           | 32                                |
|            | Ostracoda*   | -              | 1  | 2                           | 8 - 225                          | 32                                |
| Mollusca   | Gastropoda   | Mesogastropoda | 2  | 3                           | 1 - 87                           | 32                                |
|            |              | Basommatophora | 2  | 2                           | 8 - 29                           | 32                                |
|            | Bivalvia     | Veneroida      | 2  | 1                           | 127 - 127                        | 32                                |
| Annelida   | Clitellata   | Arhynobdellida | 1  | 1                           | 1                                | 11                                |
|            |              | Rhynobdellida  | 1  | 1                           | 1                                | 32                                |
|            |              | Oligochaeta*   | 1  | 24                          | 1 - 50                           | 29                                |

common dipteran families included Ceratopogonidae and Simuliidae, which were recorded at sites 33 and 15, respectively (Table 2). Diptera were found in relatively high numbers throughout the entire drainage (Table 1), but the abundance is not as high as other sites in Singapore (e.g. Clews et al., 2014). Diptera were most abundant



**Fig. 2.** Distribution and abundance of order Diptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

in the northeastern part of the Nee Soon drainage, with more than 1,300 specimens recorded in a single site (site 32) (Fig. 2).

**Order Coleoptera**

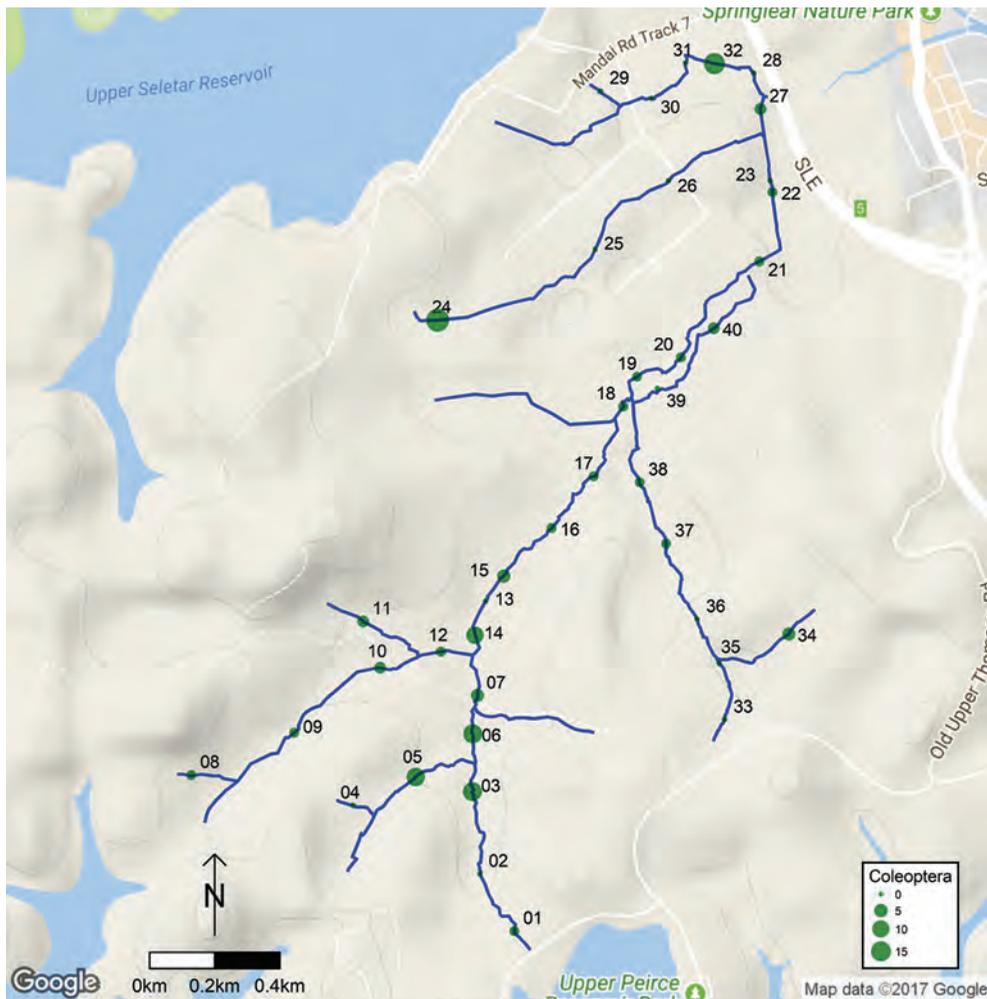
The order Coleoptera, also known as the beetles, is probably the most speciose order of eukaryotic organisms in the world. Almost all beetles possess elytra or wing-cases, a hardened first set of wings designed to protect the more delicate second set of wings from damage (Romoser & Stoffolano, 1994). In total, 12 families of beetles were found in the Nee Soon freshwater swamp forest throughout this study. In many aquatic species of beetle, both the larval and the adult stages are aquatic, unlike many other insect orders which only have aquatic larval stages (Yule & Yong, 2004). In the Nee





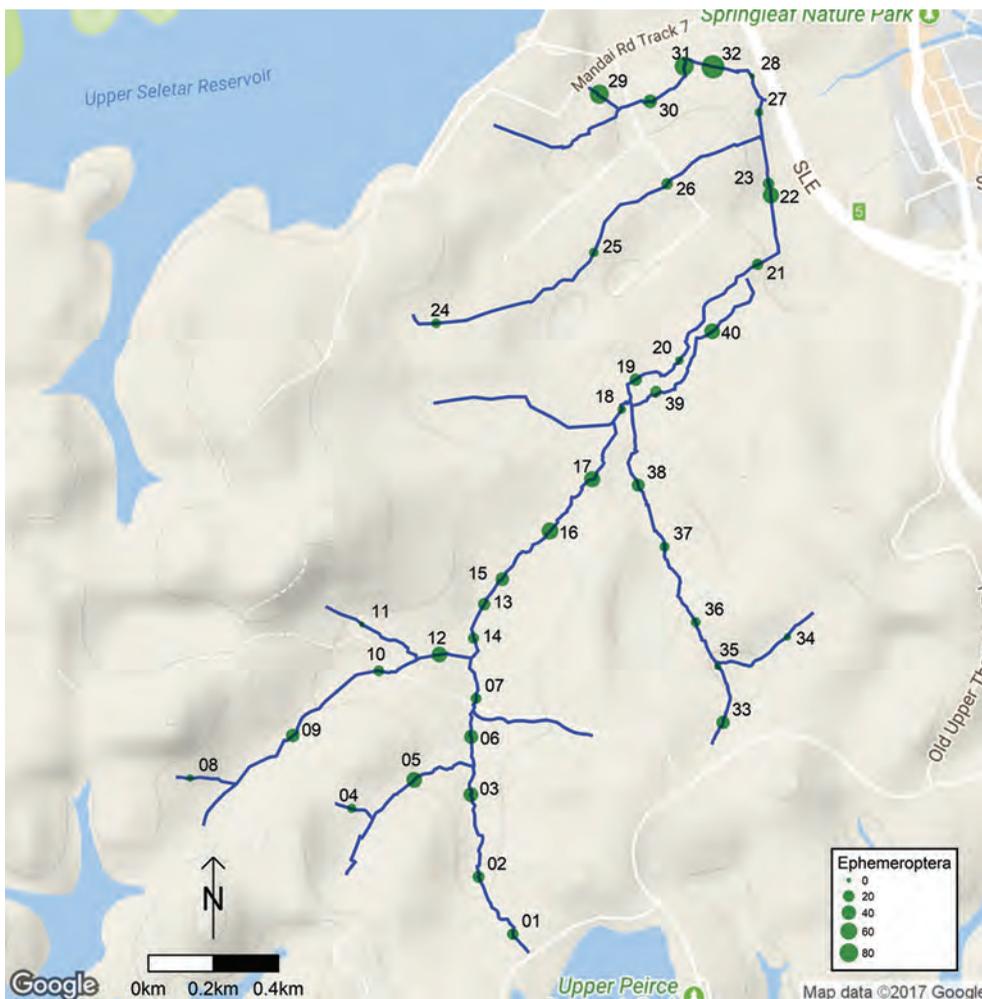






**Fig. 3.** Distribution and abundance of order Coleoptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

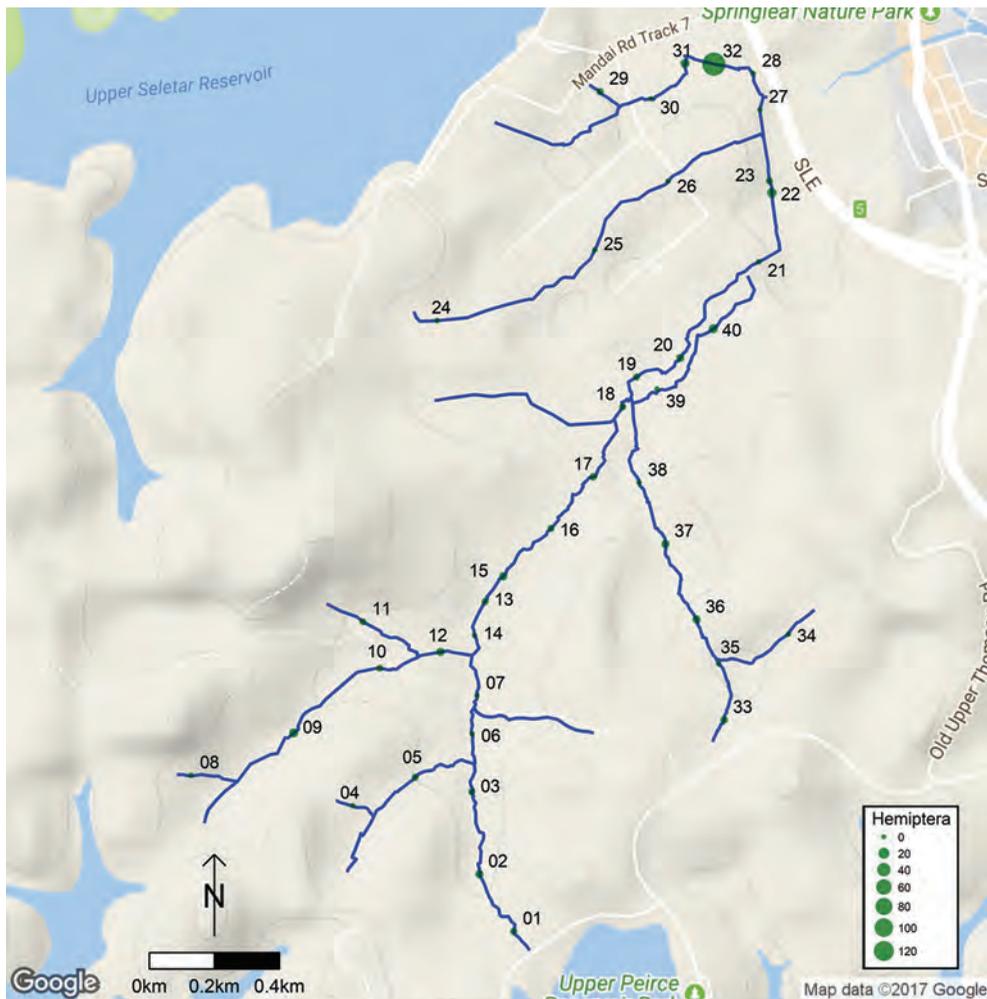
Soon freshwater swamp forest, the Coleoptera are not very evenly distributed, being found in several clusters throughout the swamp forest, including in the more upstream portions as well as the outskirts of the Nee Soon drainage (Fig. 3). They are relatively uncommon, being found in 26 sites. Of the 12 families recorded, the most common is the family Elmidae, which were recorded from 16 sites across the Nee Soon freshwater swamp forest (Table 2). The other 11 families were largely rare, occurring at one to four sites across the Nee Soon freshwater swamp forest. In general, their numbers are relatively low compared to other groups (Table 1), with the maximum abundance in a single site (site 24) being 14 specimens.



**Fig. 4.** Distribution and abundance of order Ephemeroptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Order Ephemeroptera**

Insects of the order Ephemeroptera are also known as the mayflies. The presence of this group of insects is generally an indication of good water quality (Blakely et al., 2014), as they are sensitive to pollution. Mayflies spend most of their life in the larval stage. When they reach adulthood, they usually live for a very short period of time (Romoser & Stoffolano, 1994). There are 5 families from this order found in the Nee Soon drainage. Mayflies are distributed throughout the Nee Soon drainage in large numbers, but are most abundant in the northeast of the Nee Soon drainage. They were found in 38 out of 40 sites (not found at sites 11 and 28), with the most abundant site having around 80 or so individuals (Fig. 4). Common Ephemeroptera families included Baetidae and Caenidae, which were recorded at 34 and 29 sites across the Nee Soon

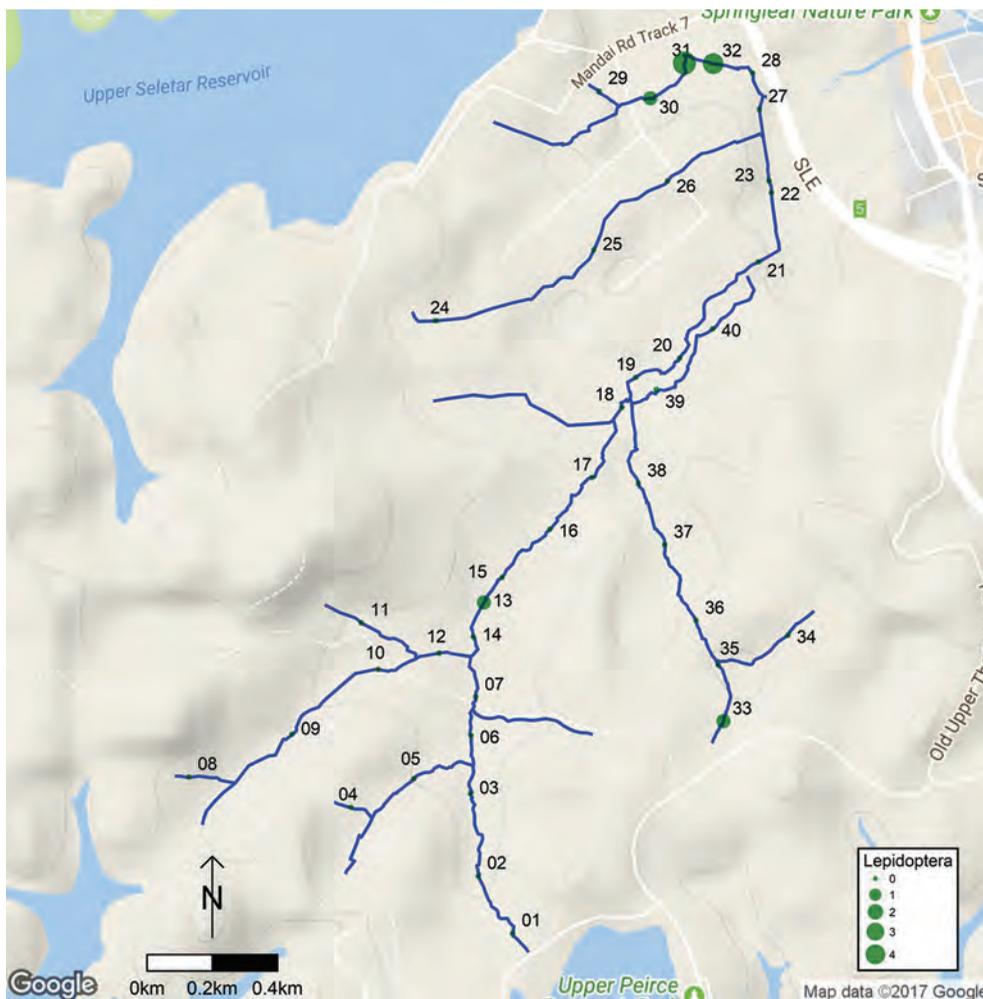


**Fig. 5.** Distribution and abundance of order Hemiptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

drainage, respectively (Table 2). Heptageniidae, Leptophlebiidae and Siphonuridae were, however, less common, and were recorded at 12 and five sites across the Nee Soon drainage, respectively.

### Order Hemiptera

Six families of Hemiptera were found in the Nee Soon freshwater swamp forest. Commonly known as the “true bugs”, hemipterans all possess a rostrum, a hollow feeding tube which is used to pierce and suck food, whether plant or animal in origin (Tran et al., 2015). Like the beetles, both the adults and the juveniles of this group are aquatic, with some living on the surface of the water and others swimming in the water column (Tran et al., 2015). Hemiptera are relatively uncommon in the Nee Soon

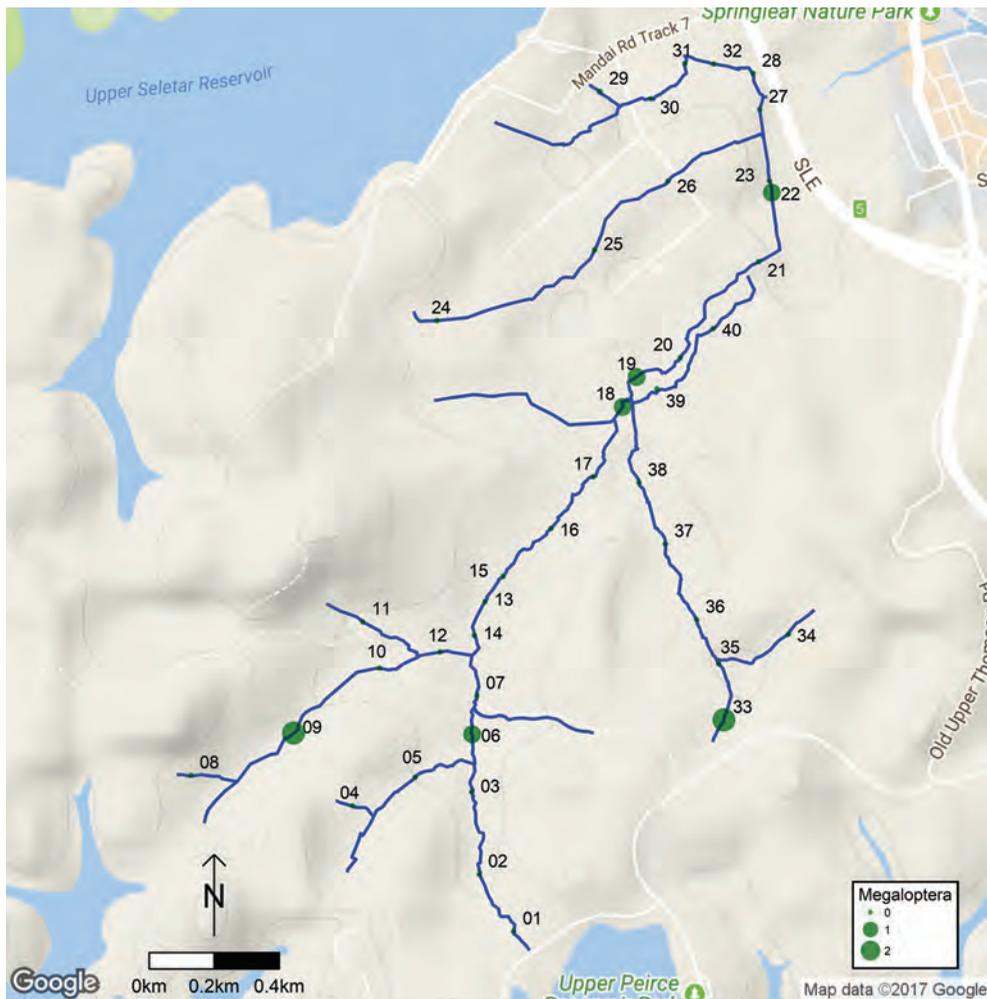


**Fig. 6.** Distribution and abundance of order Lepidoptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

drainage (recorded in 24 sites), and are typically found in low numbers (one to five specimens recorded for each family per site). Large numbers of Corixidae (122) were recorded at site 32 (Fig. 5). Out of the six families of Hemiptera recorded, Gerridae was the most common (recorded at 16 sites), while Corixidae (seven sites), Haliplidae (one site), Hebridae (one site), Mesoveliidae (two sites) and Veliidae (six sites) were uncommon (Table 2).

**Order Lepidoptera**

The order Lepidoptera includes the butterflies and the moths. While these insects are an extremely diverse and abundant group, only one family (Crambidae) has been recorded within the streams of the Nee Soon freshwater swamp forest, as most lepidopterans



**Fig. 7.** Distribution and abundance of order Megaloptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

have a terrestrial lifestyle (Romoser & Stoffolano, 1994). Lepidopterans were mostly found in the northeast of the Nee Soon drainage, occasionally being found deeper within the swamp forest. They are relatively uncommon (recorded at six sites) and were found only in small numbers, with the most abundant site (site 31) having only four individuals (Fig. 6).

### Order Megaloptera

Two families of Megaloptera were recorded from the Nee Soon freshwater swamp forest. The Megaloptera are also known as the dobsonflies, and are famous for the enormous mandibles which adult males from some genera possess (Cover & Resh, 2008). However, in most species, the adult form does not feed at all, and it is theorised

that the mandibles are a secondary sexual characteristic, meant to attract mates or drive off rivals (Cover & Resh, 2008). In contrast, the larvae are voracious predators, and feed on almost anything they can catch (Romoser & Stoffolano, 1994; Cover & Resh, 2008). Megaloptera are rare in the Nee Soon freshwater swamp forest (Fig. 7). Only one Corydalidae specimen was collected from site 22 and low numbers of Sialidae (1 to 2 individuals) were recorded at five sites (sites 6, 9, 18, 19 and 33) (Table 2).

### **Order Odonata**

The order Odonata, better known as the damselflies (Zygoptera) and dragonflies (Anisoptera), are among the best known groups of insects, due to the visibility and bright colours of the adults. Most of the information currently available on the Odonata of Singapore focuses on their adult stage (Murphy, 1997; Norma-Rashid et al., 2008; Tang et al., 2010; Ngiam & Cheong, 2016). However, knowledge of the aquatic larval stages is growing, with a recent study by Yeo et al. (in press) enabling the matching of adult and larval stages of 59 odonate species found in Singapore. Currently, 10 families of Odonata were documented from the Nee Soon freshwater swamp forest. The most commonly recorded dragonfly families were Gomphidae (recorded at 26 sites) and Corduliidae (recorded at 13 sites), while the most common damselfly family was Protoneuridae (recorded at 12 sites) (Table 2). The Odonata appear to be distributed throughout the entire Nee Soon drainage, as they were recorded from 32 out of 40 sites. However, they are most abundant in the northeast of the Nee Soon drainage (Fig. 8). For instance, at Site 32, 119 specimens were documented. These specimens were largely made up of damselfly larvae (94 Protoneuridae individuals, 1 Platystictidae individual and 1 Coenagrionidae individual), along with some dragonfly larvae (12 Corduliidae individuals, 6 Libellulidae individuals and 5 Gomphidae individuals).

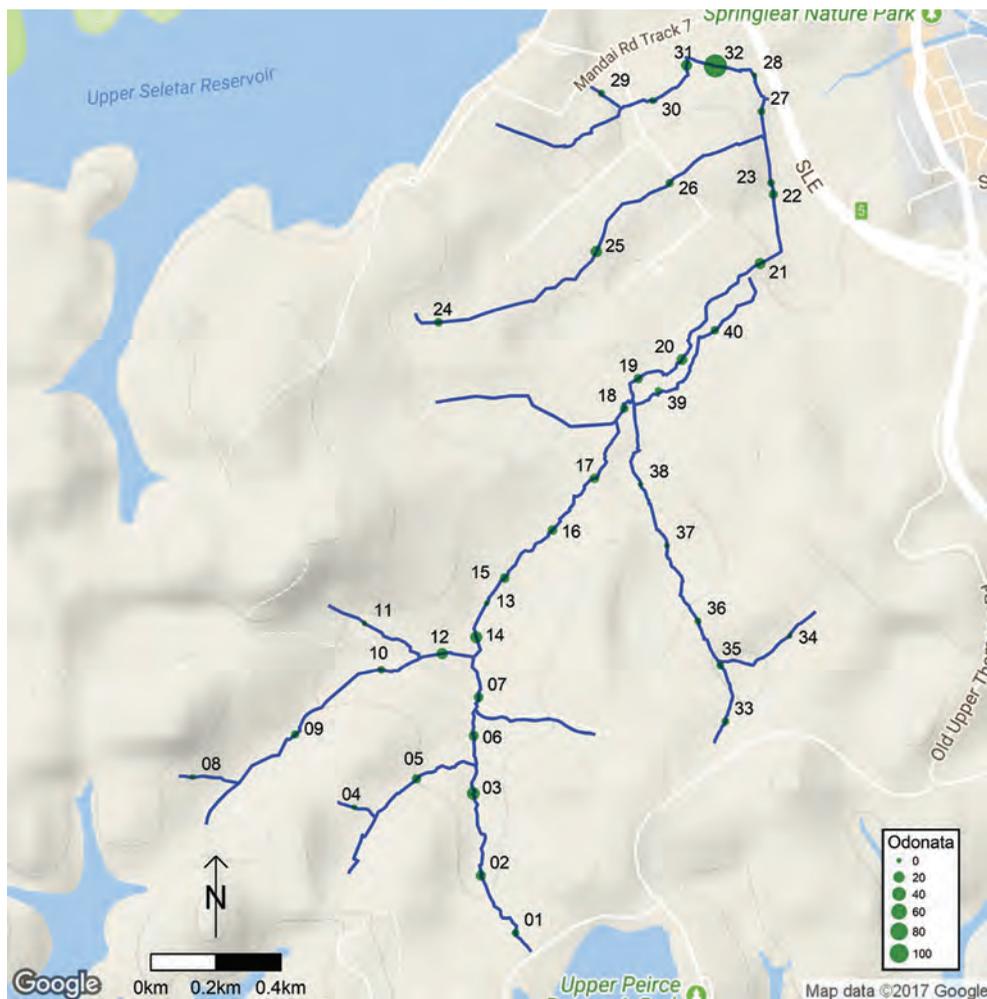
Further information on the Odonata recorded in the Nee Soon freshwater swamp forest is given by Cai et al. (2018).

### **Order Plecoptera**

Two families of Plecoptera have been recorded from the Nee Soon freshwater swamp forest. This group is commonly known as the stoneflies, and is commonly used as an indicator group, since the aquatic nymphs are generally intolerant of pollution (Blakely et al., 2014). Some species are herbivorous, while others are predatory (Romoser & Stoffolano, 1994). In the Nee Soon drainage, they are relatively rare, with one Leutridae individual recorded at sites 4 and 11, and low numbers of Perlidae (one to three individuals) recorded at seven sites (sites 1, 4, 5, 15, 24, 34, 40) (Table 2). Unlike other aquatic invertebrates, Plecoptera were absent in the northeast of the Nee Soon drainage (Fig. 9).

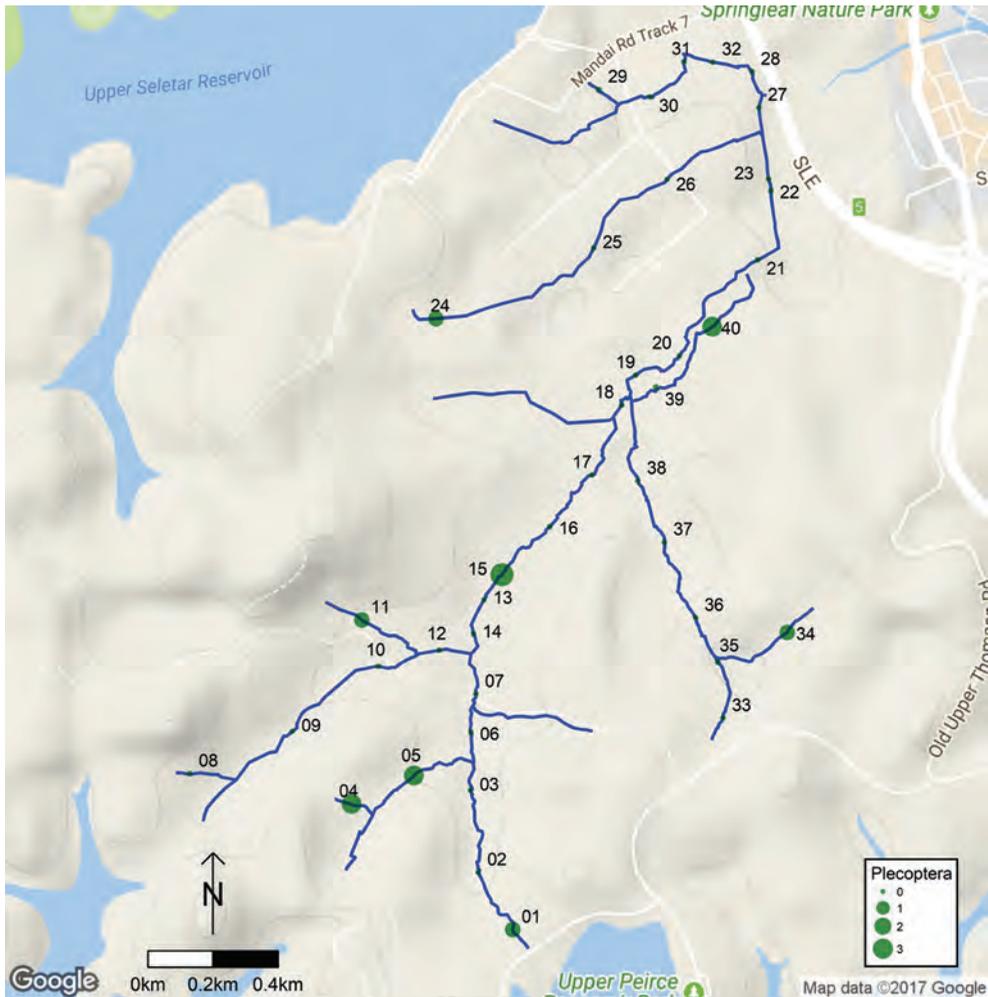
### **Order Trichoptera**

The order Trichoptera is commonly known as the caddisflies. These insects are well-known for the cases which their aquatic larval stages construct, using detritus and silk. This group is also commonly used as a bioindicator of aquatic pollution (Yule & Yong, 2004; Blakely et al., 2014). Eleven families of Trichoptera were found in the Nee Soon



**Fig. 8.** Distribution and abundance of order Odonata in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

drainage. Ecnomidae was the most common Trichoptera family recorded (found at 22 sites), while five other families – Calamoceratidae, Hydroptilidae, Leptoceridae, Odontoceridae and Polycentropodidae, were recorded at more than 10 sites across the Nee Soon drainage (Table 2) In total, trichopterans were found in 31 out of the 40 sampling sites, with their greatest abundance (30 individuals) recorded at site 32 (Fig. 10). Trichoptera individuals recorded at site 32 consisted of individuals from Hydroptilidae (fourteen individuals), Leptoceridae (eleven individuals), Ecnomidae (six individuals), Hydropsychidae (one individual), Calamoceratidae (one individual) and Polycentropodidae (one individual).



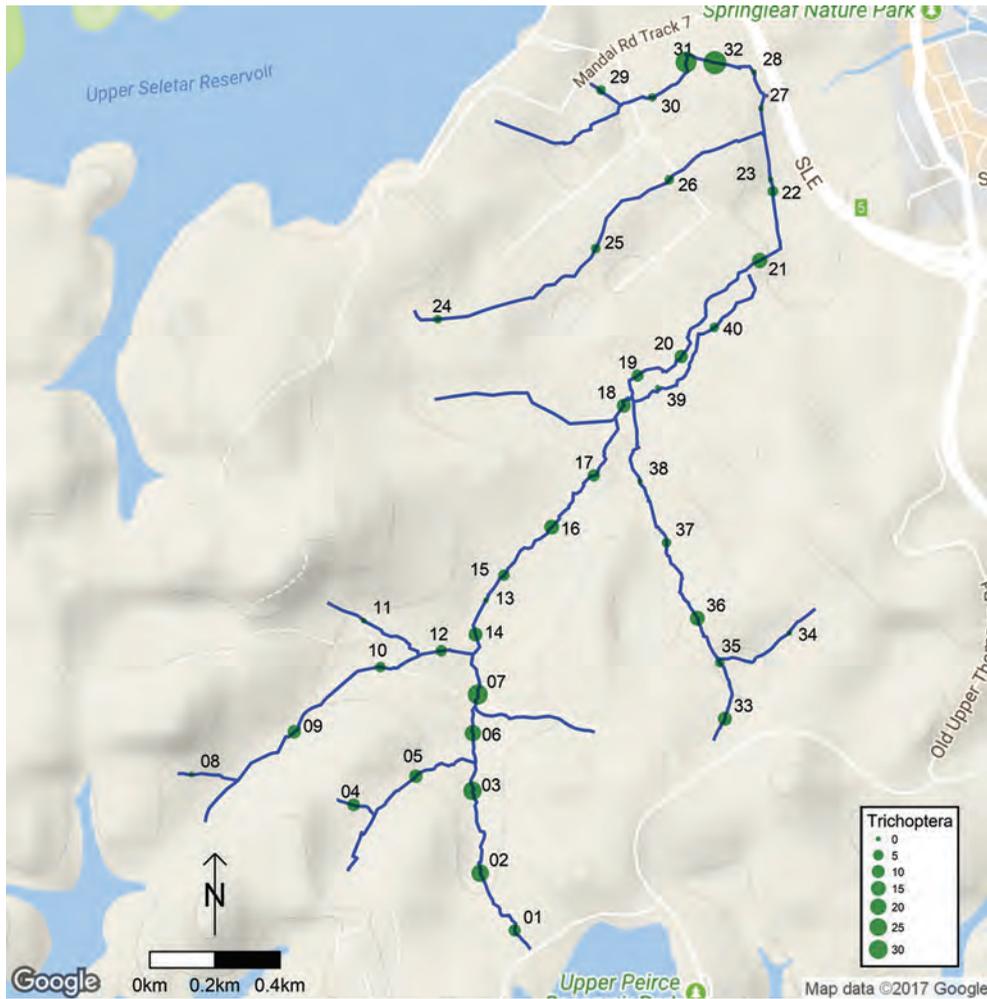
**Fig. 9.** Distribution and abundance of order Plecoptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Class ARACHNIDA

The arachnids include not only familiar organisms such as the spiders and the scorpions, but also several other groups such as mites and harvestmen. Arachnids are easily identified, as they typically possess four pairs of legs as well as a body divided into two parts, the cephalothorax and the abdomen. Two taxonomic groups have been recorded from the Nee Soon drainage in the course of this study.

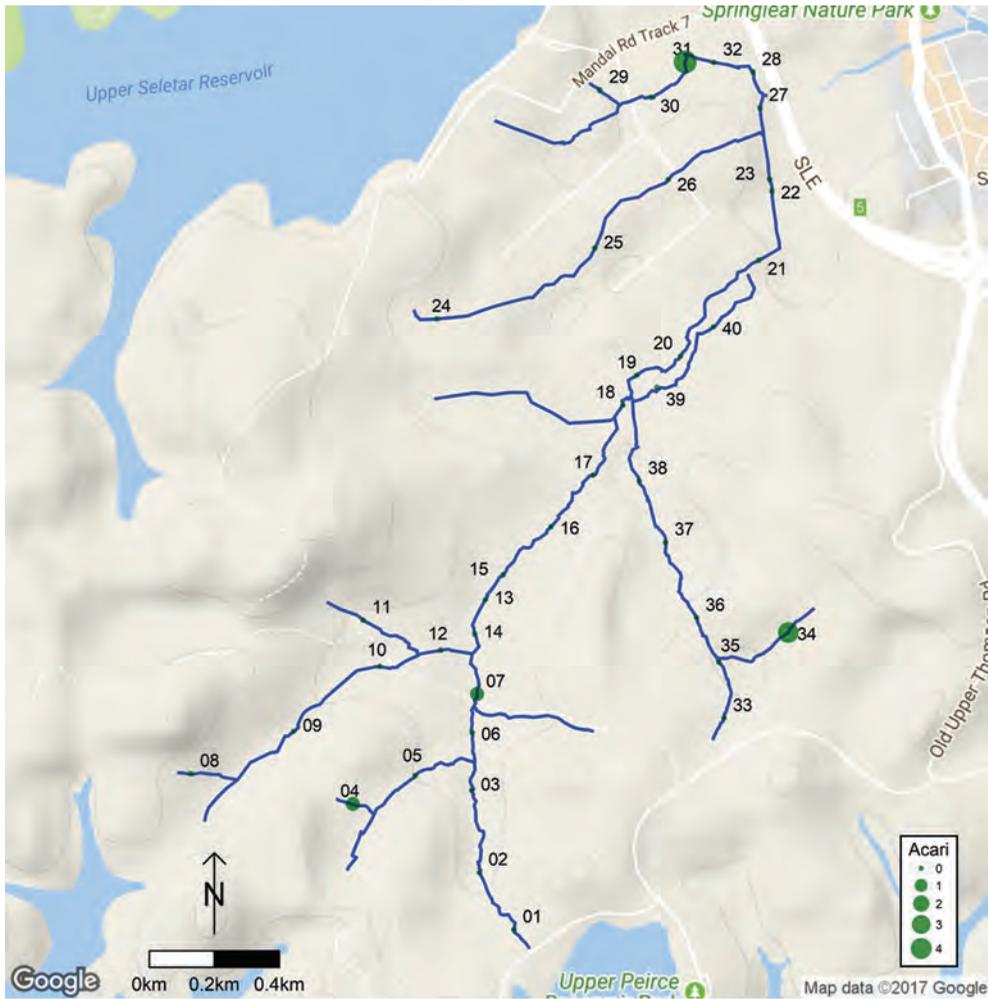
#### Subclass Acari

The subclass Acari was recorded from the Nee Soon freshwater swamp forest during this study. This subclass is more commonly known as the mites, and is extraordinarily



**Fig. 10.** Distribution and abundance of order Trichoptera in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

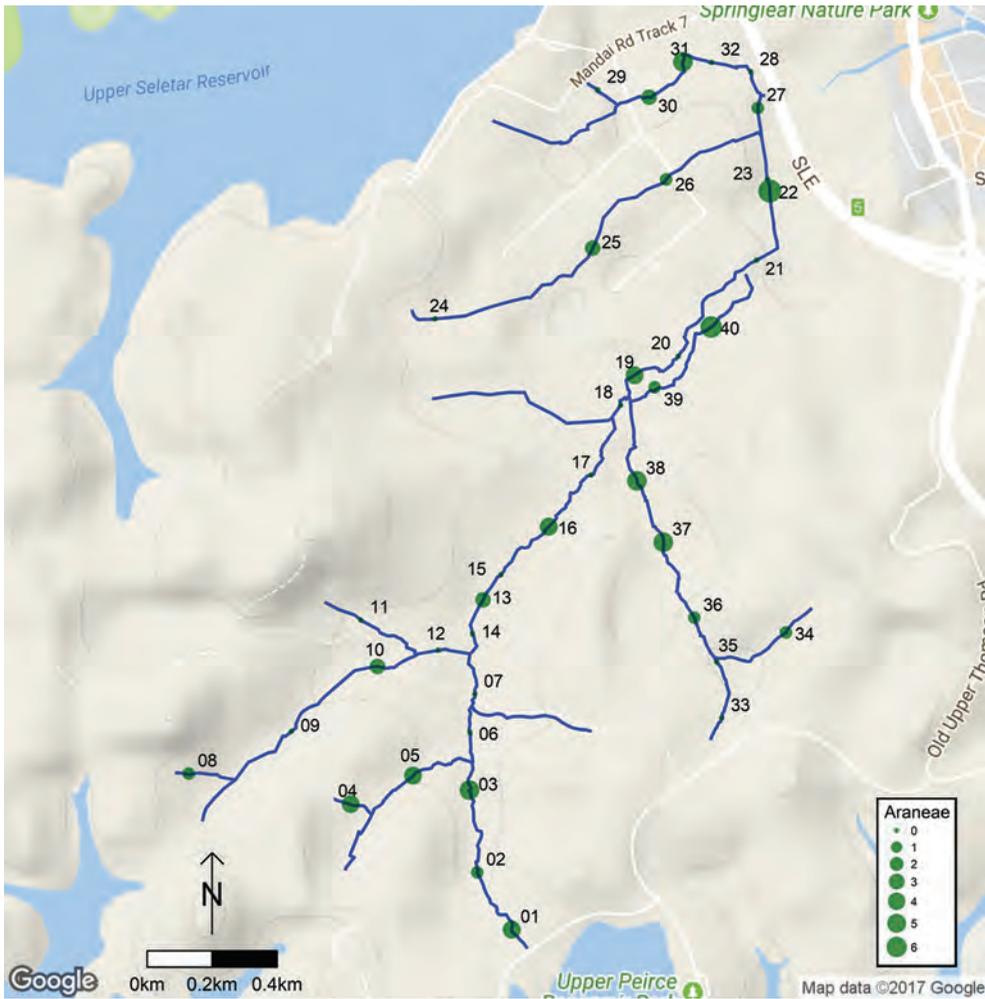
diverse and widespread worldwide, having adapted to many different environments and modes of life (Yule & Yong, 2004). However, in the Nee Soon catchment they appear to be quite rare, being only found in a few scattered locations in low numbers (Fig. 11). Members of this subclass were only found in four sites (sites 4, 7, 31 and 34), with the most abundant site (site 31) having a total of four individuals. Freshwater mites in both adult and larval stages are usually predators or parasites, feeding off other organisms such as insect larvae.



**Fig. 11.** Distribution and abundance of subclass Acari in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Order Araneae

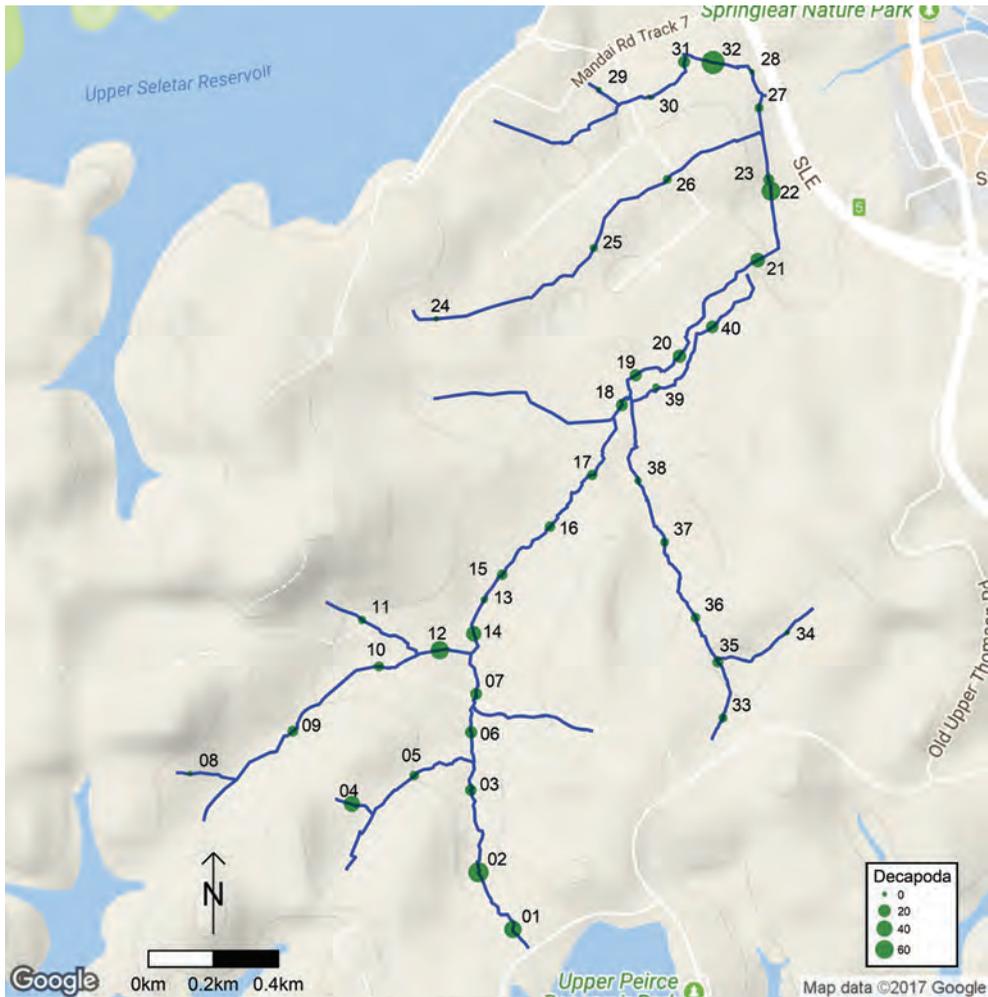
The Araneae are the arachnids most familiar to most people, as they comprise the familiar spiders. The aquatic spiders present in the Nee Soon freshwater swamp forest tend to remain on the water surface, attacking fish, aquatic insects and small crustaceans that venture too close to them (Ng et al., 2011). Aquatic spiders in the Nee Soon drainage are uncommon, and can be found distributed across 22 sites throughout the entire drainage of the swamp (Fig. 12). The highest number of individuals found in a single site (site 22) was six.



**Fig. 12.** Distribution and abundance of order Araneae in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Class MALACOSTRACA

The malacostracans are a class of crustaceans which include the familiar crabs, shrimp and lobsters, as well as perhaps less familiar amphipods and woodlice (isopods). They are extremely widespread, being found in marine, freshwater and even terrestrial environments worldwide. They can be identified by their body plan with 20–21 segments, and possess a head, thorax, and abdomen. Many species of malacostracans are commercially important.



**Fig. 13.** Distribution and abundance of order Decapoda in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Order Decapoda**

The decapods are so named due to the ten walking legs all members of this group possess. The crustaceans which most people are familiar with, such as crayfish, crabs, and shrimp, belong to this group. In the Nee Soon drainage, three groups of decapods – Atyidae, Palaemonidae (families of shrimp) and Brachyura (infraorder for crabs, in two families, Gecarcinucidae and Sesarmidae) can be found. The decapods are distributed throughout the entire drainage and are relatively common and abundant, being found in greatest numbers in the northeast and southwest areas of the Nee Soon

drainage (Fig. 13). Of the three groups recorded, Palaemonidae was the most common throughout the Nee Soon drainage (recorded at 32 sites), while the Brachyura (recorded at 18 sites), as well as the Atyidae (recorded at 13 sites), were mostly recorded at the southwest of the drainage (co-recorded at sites 1 to 13) (Table 2). In total, decapods were found in 34 sites in the Nee Soon drainage, with the most abundant site (site 32) having 73 Palaemonidae individuals.

### **Class OSTRACODA**

The tiny Ostracoda, or seed shrimps, are small crustaceans which are found in freshwater and marine environments worldwide. They can be either free-swimming or benthos-dwellers. Ostracods possess a carapace made out of a hinged valve or shell, somewhat resembling that of a bivalve, which protects them from predation. In some species, the carapace is ornamented with spikes or protrusions, while others have a smooth, featureless carapace (Yule & Yong, 2004). They have a wide dietary range, with some being predators, while others are scavengers or filter feeders (Ng et al., 2011).

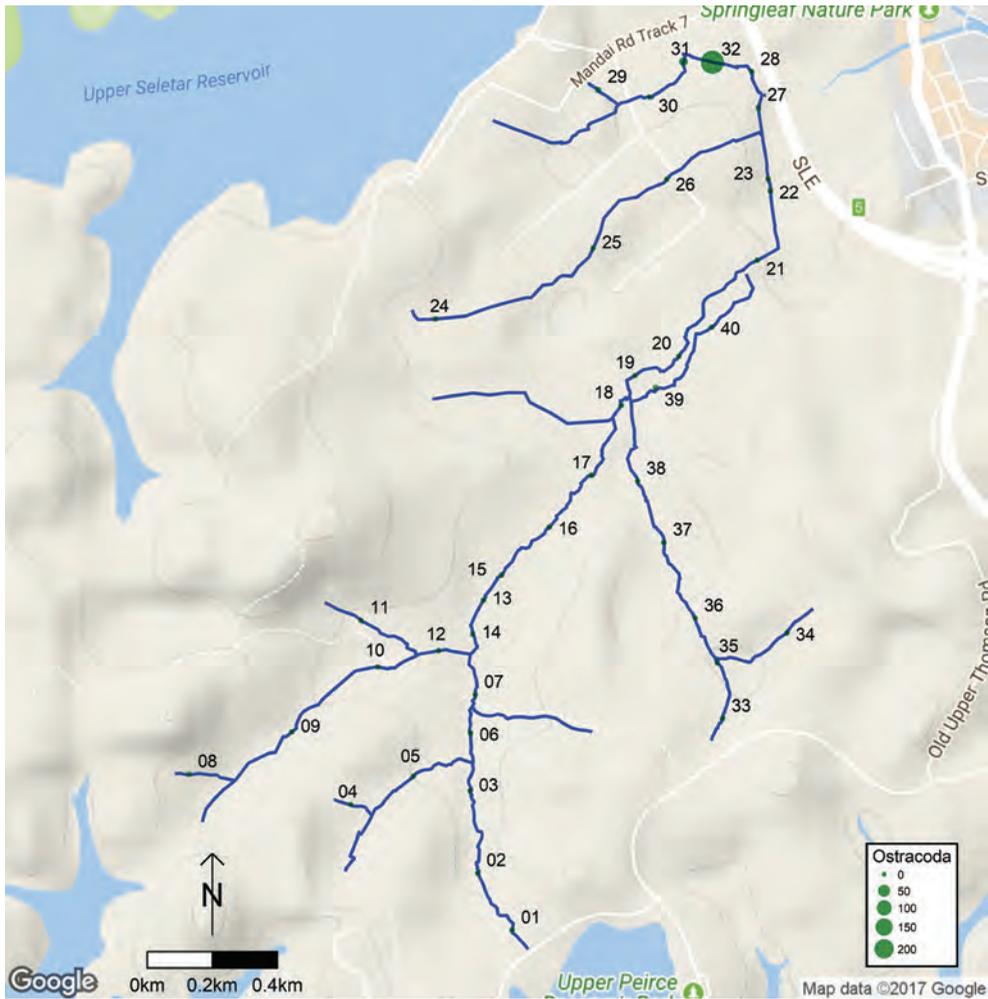
In the Nee Soon drainage, ostracods were found at three sites in the northeast areas in large numbers (the highest number of individuals recorded was 225 at site 32) (Fig. 14). They were almost completely absent from the rest of the swamp forest.

### **Class ENTOGNATHA**

The Entognatha were previously classified as insects, but have recently been separated and placed into their own class. They are wingless, six-legged arthropods which possess mouthparts recessed into the head, with only the tips of the mandibles and maxilla exposed. The subclass Collembola are perhaps the best known of the Entognatha, and are commonly called the springtails, due to their possession of a two-pronged appendage known as the furcula (Yule & Yong, 2004). This organ is usually found under the abdomen and is held under tension most of the time. When the springtail is alarmed, it releases the furcular, which then impacts against the substrate and propels the springtail into the air, away from the source of the alarm.

#### **Subclass Collembola**

In the Nee Soon freshwater swamp forest, Collembola are not very common, and were found in several scattered locations throughout the entire drainage. They were only found in 10 sites, with the most abundant site (site 24), only recording a total of four individuals (Fig. 15).



**Fig. 14.** Distribution and abundance of class Ostracoda in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Class GASTROPODA

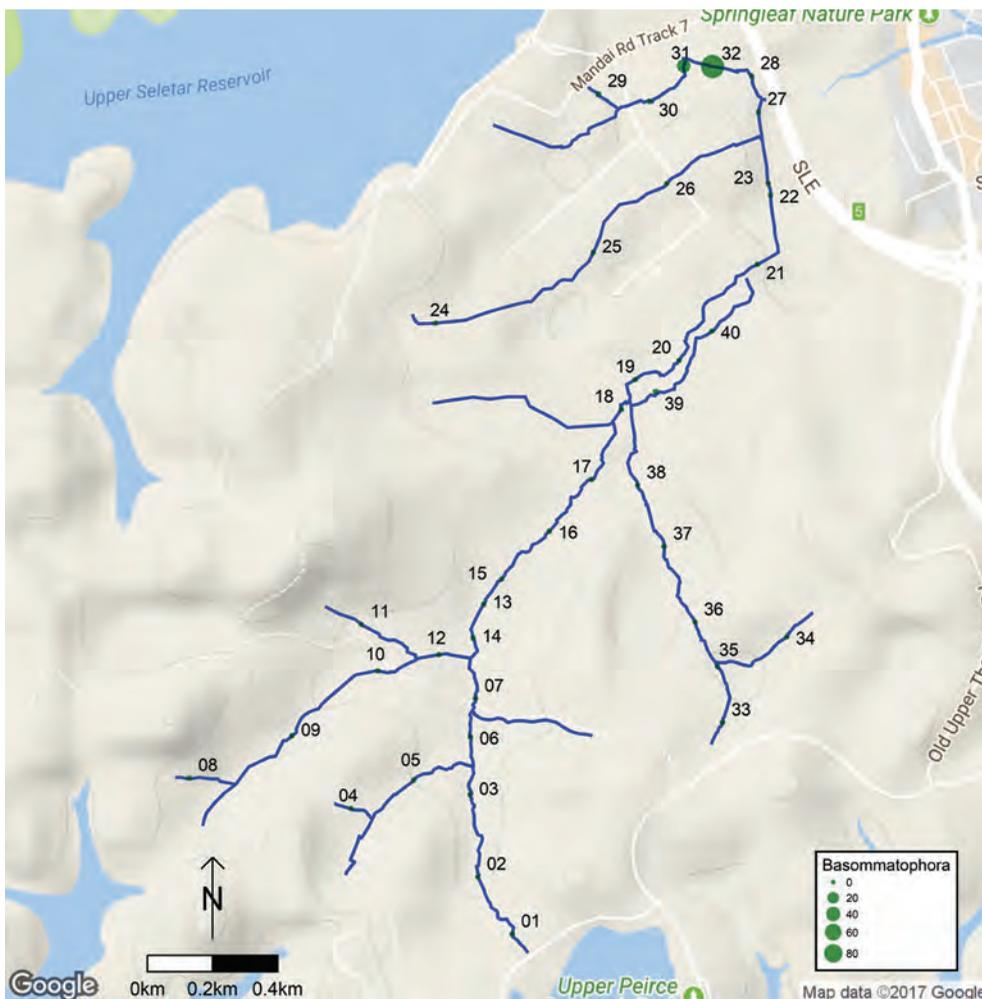
The gastropods are a group of molluscs possessing a large, fleshy organ known as a foot, which is used to move around. They are commonly referred to as snails or slugs, depending on whether they possess a shell or not. The gastropods are found in terrestrial, freshwater and marine environments worldwide. Many gastropods are hermaphrodites. While most feed on plant matter or are scavengers, some gastropods are predators instead (Ng et al., 2011). Terrestrial snails and slugs from the Nee Soon freshwater swamp forest have been surveyed by Lim et al. (2018).



**Fig. 15.** Distribution and abundance of subclass Collembola in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Order Basommatophora**

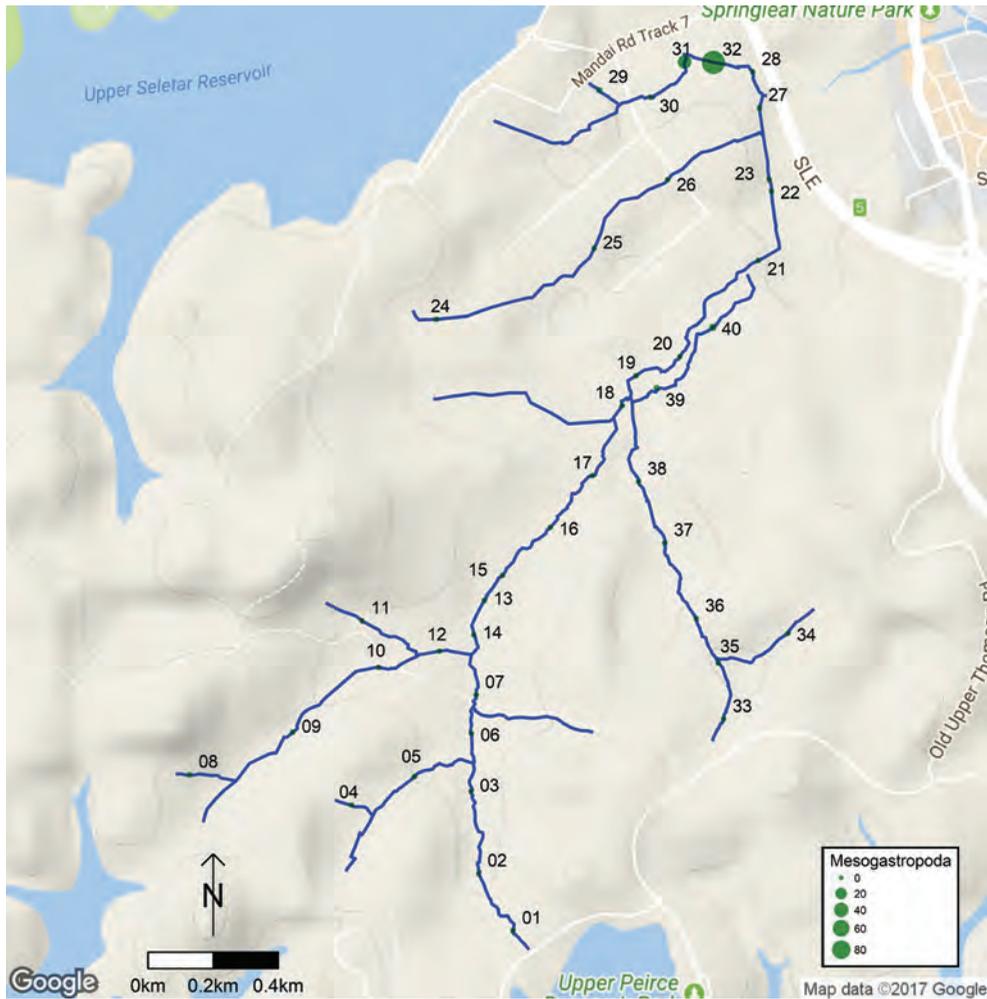
The order Basommatophora is part of a larger group called the Pulmonata, also known as the air breathing snails and slugs. Together with a modified mantle cavity, which allows them to breathe atmospheric air, the members of this order have their eyes on the base of their tentacles (Ruppert & Barnes, 1994). Two families from this order – Physidae and Planorbidae, were found in the Nee Soon drainage in large numbers, but only in the northeast of the Nee Soon drainage. Relatively larger numbers of Planorbidae (8 and 27 individuals) were recorded at sites 31 and 32, while only two Physidae individuals were recorded at site 32 (Fig. 16). Their absence from the rest of the swamp forest could possibly be due to the lower pH levels within the rest of the Nee Soon drainage, as snails are intolerant of acidic conditions (Zischke et al., 1983).



**Fig. 16.** Distribution and abundance of order Basommatophora in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Order Mesogastropoda**

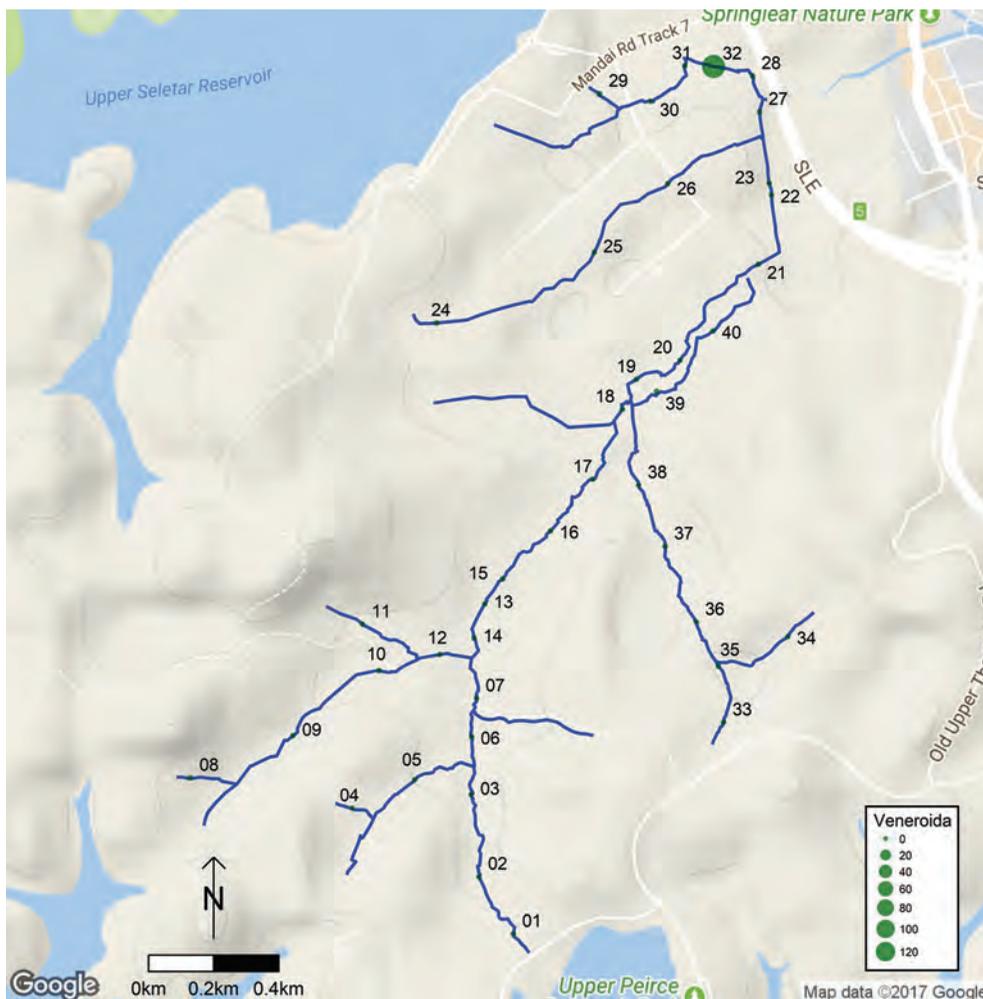
The gastropods of the order Mesogastropoda possess gills which are directly attached to the mantle wall, as well as an operculum, features which the Basommatophora lack (Ruppert & Barnes, 1994). Representatives from two families from this order – Assimineidae and Thiaridae were found in the Nee Soon drainage, but again only in the northeast areas and nowhere else within the Nee Soon freshwater swamp forest. Snails from the family Thiaridae were the most commonly recorded, with 19 and 87 individuals recorded at sites 31 and 32 respectively, while only one Assimineidae individual was recorded at site 40 (Fig. 17).



**Fig. 17.** Distribution and abundance of order Mesogastropoda in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Class BIVALVIA

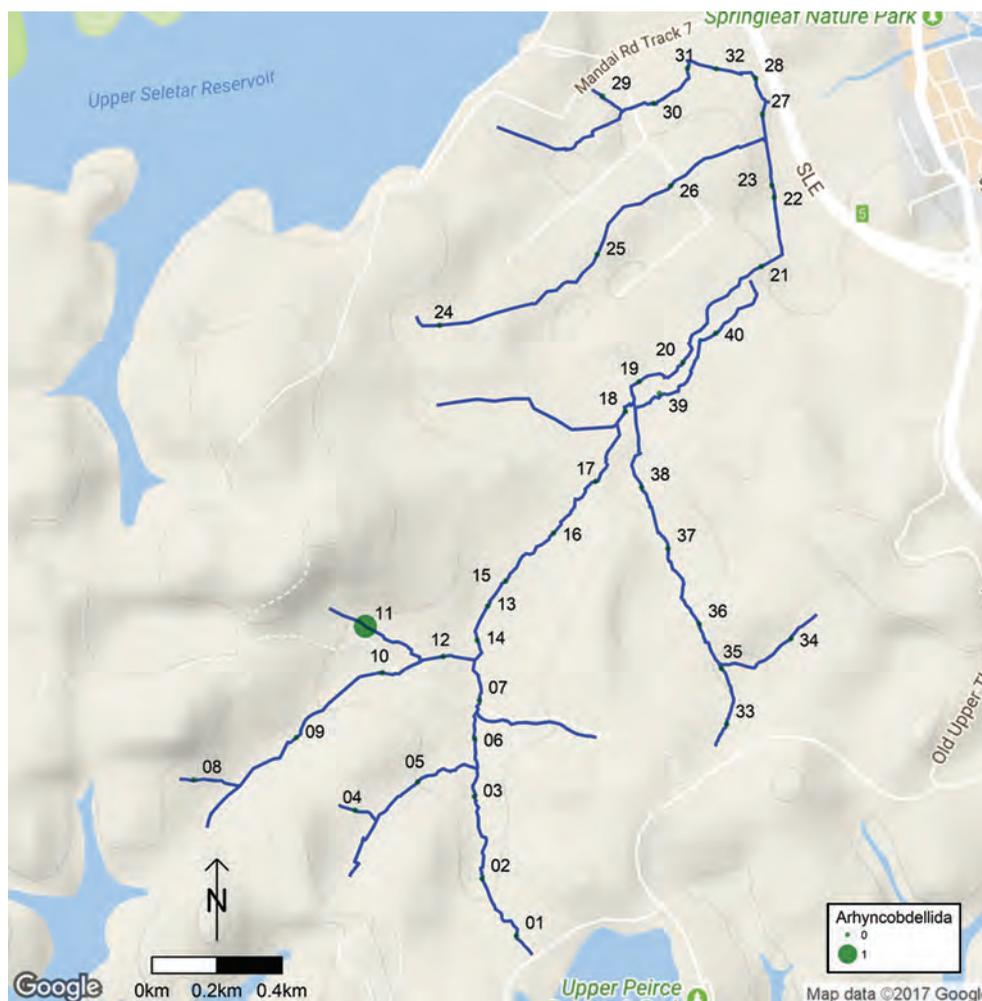
The bivalves are another group of molluscs, named after the two hinged shells all members of the class possess. Most bivalves are filter feeders (though some are deposit feeders), extracting food particles from the water they are submerged in using modified gills (Ruppert & Barnes, 1994). When startled, bivalves close their shells tightly and can hold them closed with surprising force. Bivalves are most abundant in the marine environment, but many families have adapted to a freshwater environment. In fact, some freshwater bivalves are among the most invasive species in the world, such as the Ponto-Caspian zebra mussel (*Dreissena polymorpha*), introduced into North America (Lowe et al., 2004). Ironically, native bivalves in North America are also among the most imperilled freshwater fauna (Ricciardi et al., 1998).



**Fig. 18.** Distribution and abundance of order Veneroida in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Order Veneroida**

The order Veneroida is generally made up of marine bivalves, but with some freshwater representatives. In the Nee Soon drainage, two families (Corbiculidae and Sphaeriidae) from the order can be found, and both were recorded at site 32 in relatively high numbers (Fig. 18). At site 32, 125 Corbiculidae individuals and three Sphaeriidae individuals were recorded. Like the gastropods, bivalves are intolerant of acidic conditions, which could affect their survival, growth and shell integrity (Bressan et al., 2014). They are completely absent from the rest of the Nee Soon drainage, possibly due to the effects of low pH on their shells.



**Fig. 19.** Distribution and abundance of order Arhynchobdellida in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

### Class CLITELLATA

The class Clitellata are annelids which possess a “glandular girdle”, also known as the clitellum, giving them their name. This organ produces a substance which forms a cocoon in which the juvenile worms develop (Ruppert & Barnes, 1994). They are found in the terrestrial, freshwater and in marine ecosystems. Among the more familiar members of this class are the earthworms and leeches.

#### Order Arhynchobdellida

The leeches found in this order do not possess a proboscis. Some members have jaws, while others are jawless. They also possess multiple feeding methods, with some

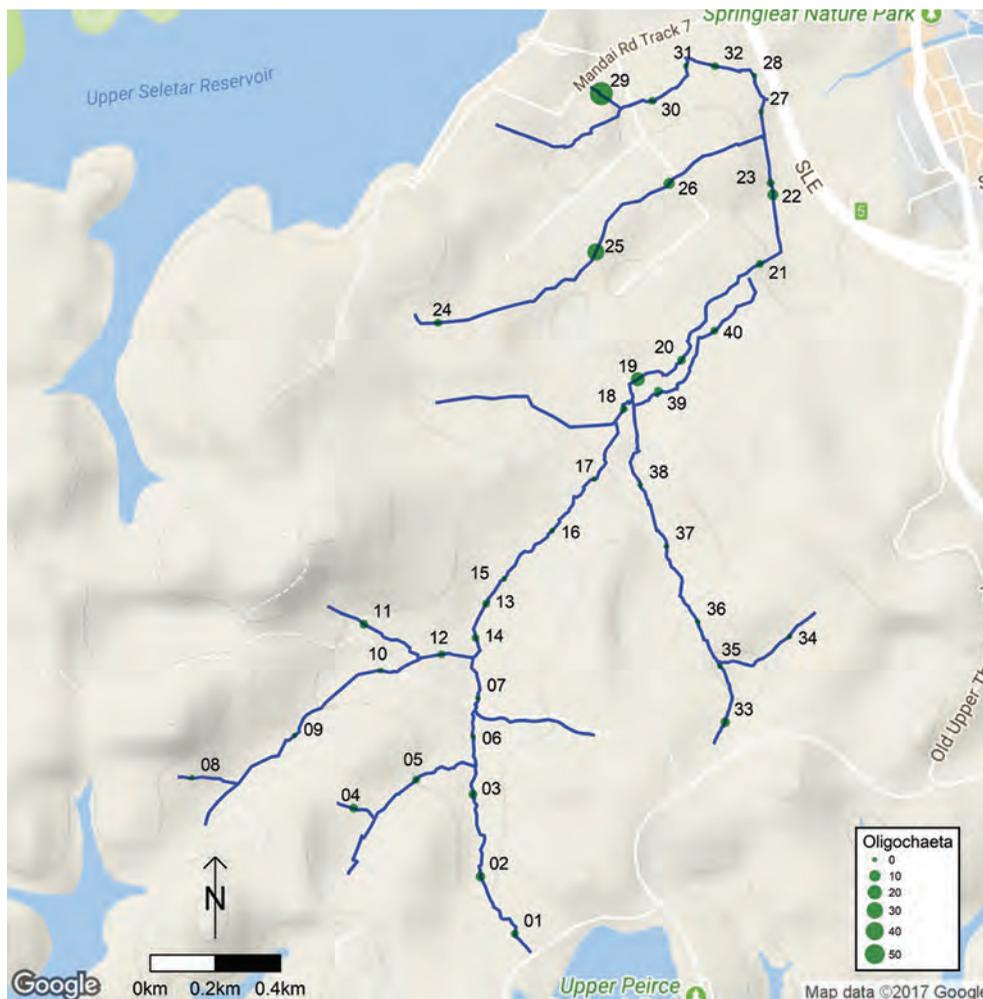


**Fig. 20.** Distribution and abundance of order Rhynchobdellida in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

being ectoparasites which feed on blood, and others being predators which hunt down and swallow smaller invertebrates whole (Ruppert & Barnes, 1994). In the Nee Soon drainage, one family (Erpobdellidae) from this order has been found in the course of this study, and only a single specimen was documented (Site 11) (Fig. 19).

**Order Rhynchobdellida**

The Rhynchobdellida are also known as the jawless leeches, but this common name is misleading due to the fact that many leech species from the order Arhynchobdellida are also jawless. The true mark of a member of the Rhynchobdellida is the possession of a proboscis, which is used to feed. In the Nee Soon drainage, one individual from the family Glossiphoniidae was documented (Site 32) (Fig. 20).



**Fig. 21.** Distribution and abundance of subclass Oligochaeta in the Nee Soon drainage. Solid circles indicate sites where representatives were collected and circle sizes are proportionate to abundance/numbers of individuals captured as reflected in the accompanying legend.

**Subclass Oligochaeta**

Oligochaeta contains the familiar terrestrial earthworms as well as more obscure annelids which live in freshwater habitats. They are distributed throughout the entire Nee Soon drainage (being found in 24 sites in total), and are typically found in relatively small numbers (1–12 individuals at 22 sites) (Fig. 21). However, large numbers of Oligochaeta, 22 and 50 individuals, were recorded at sites 25 and 29 respectively.

## Discussion

The Nee Soon freshwater swamp forest is a vital refuge for the biodiversity of Singapore, due to the large number of species which can be found nowhere else in Singapore (Clews et al., 2018; Davison et al., 2018). Furthermore, some species are endemic to the Nee Soon freshwater swamp forest and found nowhere else in the world (Ng & Lim, 1992; Cumberlidge et al., 2009; Lim et al., 2011). These species would likely be extirpated from Singapore or rendered globally extinct, should their habitat be damaged or severely altered. In order to conserve Singapore's native biodiversity, there is a need to understand the diversity and distribution of the aquatic macroinvertebrates in the Nee Soon freshwater swamp forest and develop a baseline dataset. Additionally, further studies can be built on this baseline dataset to inform policy makers and aid in the conservation efforts of the Nee Soon freshwater swamp forest.

The results of this study suggest that the conservation of the Nee Soon freshwater swamp forest is pivotal in protecting the rich aquatic macroinvertebrate biodiversity found there. Surveys during this study yielded a higher number of aquatic macroinvertebrate taxonomic groups (76 families and six higher taxa) as compared to the number of macroinvertebrate taxonomic groups (68 families and 6 higher taxa) collected across 47 urban concrete canals and natural forest streams within the Central Catchment Nature Reserve (Blakely et al., 2014). Moreover, only 66% of the taxa (54 out of 82 taxonomic groups) in this study are reported in the study by Blakely et al (2014). This means that the aquatic biodiversity in Nee Soon freshwater swamp forest consists of a large number of rare taxa (e.g. Psephenidae or water-penny beetle and the Xiphocentronidae caddisflies) that are not found in other habitats within Singapore. This further highlights the need to prioritise the conservation and the protection of the natural resources within the Nee Soon drainage.

The results of this study demonstrate variations in the distribution of macroinvertebrate groups within the forest streams with different communities represented in different parts of the forest. These communities reflect heterogeneous environmental conditions across the catchment. Some taxa, including both mollusc classes and the crustacean class Ostracoda, are confined to only the northeast area of the Nee Soon drainage. Common taxa, such as the Diptera and the Ephemeroptera, are also found in extremely large numbers in this area and in reduced numbers deeper inside (further upstream) the Nee Soon drainage. The northeast area of the catchment receives additional input of less acidic water from an adjacent reservoir. The decreased acidity here is more suitable for some of the mollusc taxa, since molluscs are sensitive to low pH levels due to the corrosive effects on their shells (Clements et al., 2006; Gazeau et al., 2013; Parker et al., 2013).

In addition to the less acidic conditions in the northeast area of the Nee Soon drainage, the streams are more open and lacking canopy cover, unlike the densely forested streams deeper within the freshwater swamp forest. Macroinvertebrate groups such as Plecoptera, Megaloptera and some of the Ephemeroptera (Family Heptageniidae and Leptophlebiidae) were absent in these less-forested areas.

Macroinvertebrate communities in forested streams are strongly influenced by debris input from riparian vegetation. The lack of canopy cover could result in a community that favours taxa with diverse feeding habits that can deal with the allochthonous debris (e.g. plecopteran shredders that feed by cutting and tearing large pieces of debris/organic matter) (Schmidt-Kloiber et al., 2006).

Habitats at the outskirts of the Nee Soon catchment are more exposed to potential encroachment of non-native taxa from other catchments. For example, a South American cichlid, *Acarichthys heckelii*, was recently reported for the first time within the outskirts of the Nee Soon freshwater swamp forest (Tan & Lim, 2008; Ng & Tan, 2010). Whilst existing environmental conditions may hinder the establishment of non-native species, the constant propagule pressure from the reservoir (Yeo & Chia, 2010) may pose a future threat (Holle & Simberloff, 2005; Lockwood et al., 2005). The Nee Soon streams also drain into the Lower Seletar Reservoir via Sungai Seletar, which may be another potential source of non-native taxa, since Lower Seletar Reservoir also contains many established populations of non-native species (Ng & Tan, 2010).

While the present study captured a representation of forest stream fauna across the catchment, there is always the possibility that certain groups were not obtained in the course of sampling due to limitations in the sampling methods. In the future, additional methods such as deployment of colonisers could be utilised in addition to traditional kick sampling methods, in order to ensure that a wider range of diversity is captured. As the life cycle of numerous insects are associated with different types of habitat (e.g. all aquatic dipteran larvae emerge as terrestrial adults), sampling methods that capture adult insects can also be useful to supplement the determination of aquatic macroinvertebrate diversity. Malaise traps (Gressitt & Gressitt, 1962) are an effective means of collecting many types of adult insects, and have been deployed in various parts of Singapore (including the Nee Soon freshwater swamp forest) for taxonomic work with adult insects (e.g. Grootaert, 2006; Ngiam & Cheong, 2016). Emergence traps have also been adapted for use in tropical streams (e.g. in the Philippines) (Freitag, 2004a, 2004b) and are useful for capturing emerging adults at specific riverine locations. Alternative sampling methods for larvae collection include electroshocking techniques (Taylor et al., 2001), which use electrofishing equipment to collect and quantitatively sample stream invertebrates. Processing of samples collected by electroshocking techniques has been reported to be 40% faster than traditional sampling methods, due to a reduction of debris collected in the samples (Taylor et al., 2001).

Unfortunately, knowledge of aquatic macroinvertebrate taxonomy is limited in the tropical Asian region. Many of the molluscs and decapods can be identified to the species level (Ng, 1988; Ng, 1990; Tan et al., 2012), and some taxonomic groups such as the Odonata or the Hemiptera can be identified to the genus level (Yeo, 2012; Tran et al., 2015). However, many other groups including the Trichoptera and Diptera cannot be identified beyond the family level without the assistance of taxonomic experts. This results in a loss of resolution and information about species distribution, especially with regards to species of conservation value. Identifying specimens down to the genus and the species levels could be achieved in future studies. For instance, in South Korea, dedicated studies of aquatic macroinvertebrate species facilitated

the identification and the designation of endemic Plecoptera (e.g. *Scopura gaya* and *Scopura jiri*) and Trichoptera species (e.g. *Agrypnia pagetana*) as threatened species (Kim et al., 2014). Incorporation of molecular techniques (e.g. DNA barcoding) into identification workflow in future studies would vastly improve this process, as molecular barcoding allows for the linkage of adult and juvenile forms (Zhou et al., 2007; Kutty et al., 2018), as well as allowing for the identification of cryptic species down to the species level. Indeed, previous work by Ball et al. (2005) and Pfenninger et al. (2007) has shown that high levels of accuracy from molecular techniques can be achieved in the identification of Ephemeroptera and Chironomidae, respectively.

**ACKNOWLEDGEMENTS.** We would like to thank Y. Cai, T. Li and W.H. Lim for their assistance in the field. This study was approved by the National Parks Board of Singapore (Permit no. NP/RP13-068-1). Funding for this study was received from the National Parks Board of Singapore (National University of Singapore grant number R-347-000-198-490).

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