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# ASSESSMENT OF LANDSCAPE QUALITY BASED ON THE PERCEPTION OF PEOPLE: STUDY OF TWO PARKS IN NEWCASTLE UPON TYNE

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## ABSTRACT

People experience different types of landscapes every day. The quality of these landscapes has a major effect on their psychological well-being and general satisfaction of a place. Determining the quality of landscapes is an important issue for policy makers and planners, especially when making decisions about the conservation or transformation of urban landscapes. Defining quality based on people's perception has received significant attention from planners and decision makers as it can lead to greater public involvement and improve the reliability of the decisions. This research aimed to determine the criteria important for landscape quality assessment by using walk-and-talk interviews in two green landscapes in Newcastle upon Tyne (UK). The attributes affecting people's choices include naturalness, locomotion, smoothness, surprise, variety, sense of place, tranquillity, rarity, legibility, safety, complexity, mystery, openness, accessibility, maintenance and management. Not all of these attributes have the same weight, as some are more influential in determining people's preferences, however, categorizing them into primary and secondary attributes, provided a means of evaluating landscape that is less costly and time-consuming.

**Keywords:** landscape evaluation; landscape perception; landscape quality attributes; people's preferences; urban parks

## Introduction

The word landscape comes from the German word "Landschaft" meaning a bounded area and the Dutch word "landchap" meaning a visual or artistic perceived area (Selman 2012). Nan Fairbrother (1970) states that landscape is a separate concept from nature and describes the interaction between society and its habitat, and Cosgrove and Daniels (1988) define it as a cultural image of the surrounding area. In most contemporary definitions and more specifically the European Landscape Convention, landscape is seen and understood in the eyes, mind, and heart of beholders with an emphasis on cultural values (Fairclough 2002). Thus, currently landscape is regarded as "an area, as perceived by people, whose character is the result of the action and interaction of natural and/or human factors" (Ahern et al. 2009).

Landscapes are experienced by a range of people on a daily basis and affect their psychological well-being and general satisfaction of places (Ruelle et al. 2013). The quality of landscapes has a direct effect on the quality of life. It affects people's satisfaction with cities, their perspective of cities and personal situations. It can be regarded as one of the factors determining the quality of life for communities (Gavrilidis et al. 2016). Visual attributes of surrounding landscapes can affect a citizen's satisfaction and be a factor in determining whether they leave a city (Barreira et al. 2019). People's satisfaction with landscapes can provide an opportunity to make the local community liveable in, increase the sense of place and reduce un-civic behaviour (Ruelle et al. 2013). In contrast, several studies show that renewal programs and urban interventions that do not integrate people's perception and satisfaction are likely to result in boycotting the interventions or even vandalism (Sharp et al. 2005;

Orueta 2007; Ruelle et al. 2013). Therefore, considering people's preferences for landscapes is highly important for planners and policymakers.

This study seeks to determine people's perception of quality. What people perceive and the rationale of their preferences are important for determining how landscapes are evaluated. In order to address the aim of this research, the main characteristics influencing their judgments need to be identified. This will be done by comparing people's view of two urban landscapes (parks) one of which is reclaimed industrial wasteland. The attributes of these two parks will be compared in order to identify the attribute(s) important in determining their quality as landscapes and the rationale behind people's perception of the quality of landscapes.

To achieve this aim, three questions were addressed:

1. What are the important attributes?
2. How do these attributes determine quality?
3. Are all the attributes equally important?

## Methodology

A case study approach was used to provide detailed information on landscape quality in a real-life context (Crowe et al. 2011) and provide conceptual validity in terms of the identification and measurement of indicators of quality (Starman 2013). The data was obtained from walk and talk interviews recorded in two green spaces (parks) in Newcastle upon Tyne. Riverside Park, which is recently reclaimed industrial wasteland where the city landscaped the naturally grown vegetation. Exhibition Park is a well-designed urban park. These two parks differ in that one mainly consists of naturally developing vegetation and the other is well-designed with well-maintained

areas of grass. Comparing the reaction of people to these two parks will clarify the reaction of people to semi-natural landscapes and endemic flora and fauna. In addition, these two parks have different social characteristics. The nature of the questions asked in the interviews was determined by the attributes cited in the literature.

This study used semi-structured interviews with specific groups of people to determine why they have a particular attitude towards a place or phenomenon. Although most landscape preference studies use surveys and questionnaires along with photographs or computer visualizations (Ruelle et al. 2013), the responses are often limited by the quality of photographs or range of questions in the questionnaires.

Interviews provide scope for determining the rationale of each response. Interviews can be conducted with either groups or single individuals (Dicicco and Crabtree 2006). In one-to-one interviews, the interviewees are not challenged and may not provide considered answers, whereas group interviews often result in a discussion that results in a more considered response (Bryman 2001). It is suggested that the number of participants in such groups should be between 6 to 10 people (Bryman 2001) as when the number of interviewees exceeds 6 or 7, the answers tend to be the same (Coeterier 1996). If the interviewees are familiar with one another, the discussion is likely to be more natural (Bryman 2001). Based on this information, seven female master students of Newcastle University from five different countries participated in

this study. The interviewees were all in their 20s but with different educational backgrounds in that they were either studying Landscape Architecture, Urban Planning, Communication, Linguistics or Software Engineering. They were invited through social media.

One of the important characteristics of the interviews is that they were carried out while walking in a particular landscape. Mobile interviews result in the discovery of new spaces and subjects for discussion (Macpherson 2016). In addition, this is an ideal technique for obtaining more detailed data due to the connection between the interviewees and surrounding environment (Evans and Jones 2011). Mobility while talking is more likely to reveal issues (Jones et al. 2008) and facilitate discussion (Macpherson 2016). This approach is thought to more likely reveal, how different people value landscapes (Macpherson 2016), which is important for understanding landscape quality. Thus, the data used in this study were obtained from interviews, which was compared with that in the literature. The overall structure of this research is presented below (Fig. 1).

## Literature Review

### Paradigms of landscape quality assessment

Landscape quality evaluations can be categorized into monetary and non-monetary (Tempesta 2014). There are two categories of non-monetary: objectivist (expert-de-

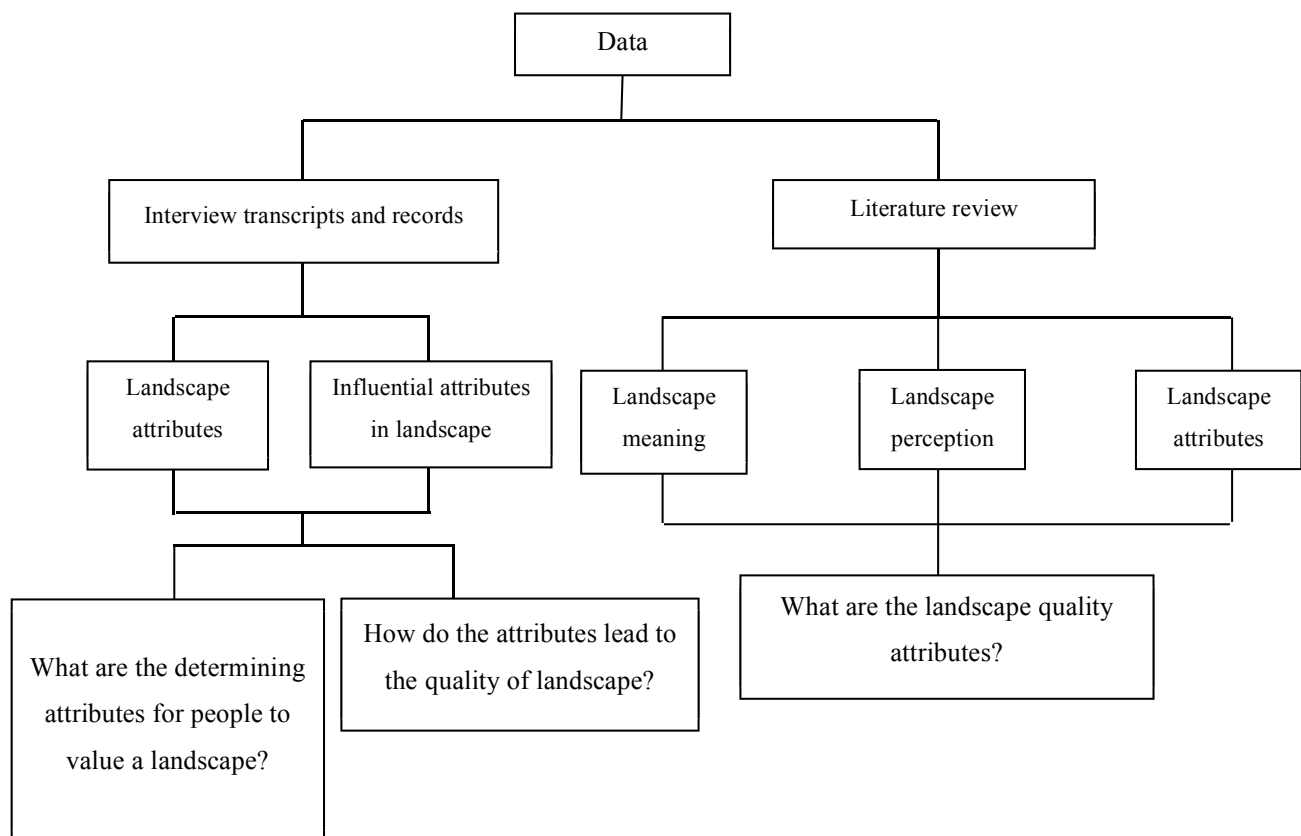


Fig. 1 Structure of the study.

sign approach) and subjectivist (community perception-based approach), which differ in terms of their specific views on landscapes (Pouya and Behbahani 2017). In the former, the visual quality is considered to be inherent to landscapes and in the latter the quality is in the eye of the beholder (Lothian 1999; De Val et al. 2006). The monetary approach has various categories: supply based, demand-based, revealed preferences and stated preferences, which can be useful for analysing the cost-benefit of large infrastructure projects, the effectiveness of subsidies given to farmers for improving the landscape, or payments for damage caused by landscape transformation (Tempesta 2014). The monetary approach is not used in this study, which is based entirely subjective.

An expert-approach evaluates and inspects landscapes based on the view of a trained expert (Daniel 2001). In this approach, the biophysical features of landscapes (mountains, lakes, etc.) are transformed into parameters (De Val et al. 2006) such as form, texture, colour and line and are ranked in terms of quality from low to high. This approach is objective and based on the assumption that landscape quality is determined by its physical features. The human aspect is acknowledged in terms of viewpoints, location, number of viewers and sensitivity (Daniel 2001). In contrast, the perception-based approach emphasizes the human view of landscapes (Wu et al. 2006) and regards landscape quality as being in the eye of the beholder (Pouya and Behbahani 2017). Various landscape studies using this approach introduce derived perceptual factors of landscape (Daniel 2001) and the emotional responses of people to different landscapes. More specifically it reveals the role of landscape in mediating emotional responses (Zube et al. 1974; Parsons et al. 1998) and/or its effect on health (Seymour 2016; Frumkin et al. 2017). That is this approach is based on the sensory, emotional and cognitive factors of the relationships of people with landscapes (Daniel 2001).

During the last decade, there were several subjective studies on landscape quality. In such a study of the visual quality of urban landscapes Keshtkaran et al (2017) use elements of visual design to assess their physical quality using pictures (Bell 2004). Pouya and Behbahani (2017), in a similar study, use pictures to assess the quality of the landscapes in two memorial gardens. In addition, to the physical elements of landscape other concepts such as stewardship, coherence, disturbance, historicity, visual scale, image ability, complexity and naturalness are identified in the review of the key concepts of landscape quality by Tveit and Fry (2006). The above highlight specific indicators of landscape quality and below the literature on the assessment of landscape quality will be reviewed.

### Attributes used to assess landscape quality

Most of the preference studies on landscape quality were done after 1960 by Appleton (1975), Anderson (1978), Kaplan (1979), Zube (1987), Nassauer (1995), Coeterier (1996), Swanwick (2002) and Burton and Rym-

sa-Fitschen (2008) were reviewed and analysed in this study in order to determine the attributes for assessing landscape quality.

Kaplan (1979) assumes preference is a complex process, which includes perception and reaction in terms of usefulness and supportiveness. He believes that it is important to understand people's preferences and show they are not idiosyncratic. There might be patterns in the preferences (Kaplan 1979) and differences between groups' people in their preferences for a landscape (Rogge et al. 2007). Kaplan (1979) propose two underlying purposes: 'making sense' and 'involvement', for identifying the preference of people for landscapes. Making sense is about understanding what is currently stimulated by it, with those landscapes inducing these responses being the preferred (Coeterier 1996).

Coeterier (1996) suggests complexity is an important attribute determining preference for a landscape. "Complexity" in terms of "diversity" or "richness" is seen as an important characteristic of landscapes. Kaplan et al. (1998) relate complexity to coherence and argue that too much complexity has a negative effect on coherence as it makes a landscape difficult to comprehend. Coherence is achieved by having identifiable components, such as "legibility", which is the ability to be able to interpret and understand the structure and perceive a landscape (Zube 1987). "Openness", which is 'the amount of space perceivable to a viewer', "smoothness" – the 'uniformity of ground texture', and "locomotion" – the 'ease of traversing without undue effort' are important features of a landscape that can affect people's preference (Kaplan et al. 1989). They investigated these attributes along with "spaciousness", which is the amount of room for wandering. Previous studies on spaciousness (Gallagher 1977; Anderson 1978) see it as a negative attribute. Some authors discuss different components of spaciousness, particularly openness and state that whether it is a positive or negative feature depends on the location (Rogge et al. 2007) and in some cases can even lead to the feeling of calmness (report of the Research Box (2009) for Natural England).

"Mystery" is another aspect, which may increase willingness to explore a landscape (Kaplan 1979), but might be mistaken for "novelty" or "surprise". Novelty implies exploring something new and surprise finding something unexpected (Zube 1987). Mystery can be provided by features like curved paths. Although some people think that dense vegetation increases the mystery of a place Kaplan et al. (1998) state that landscapes in which the view is limited lack mystery.

"Management" is considered to be important in terms of increasing the quality of a landscape (Rogge et al. 2007), especially if it includes maintenance, rules for safety issues and provision of facilities. The criteria of this attribute mean that its effects may depend on the landscape. Too much maintenance is viewed negatively, as it can result in an artificial and restrictive landscape, and too little, in an

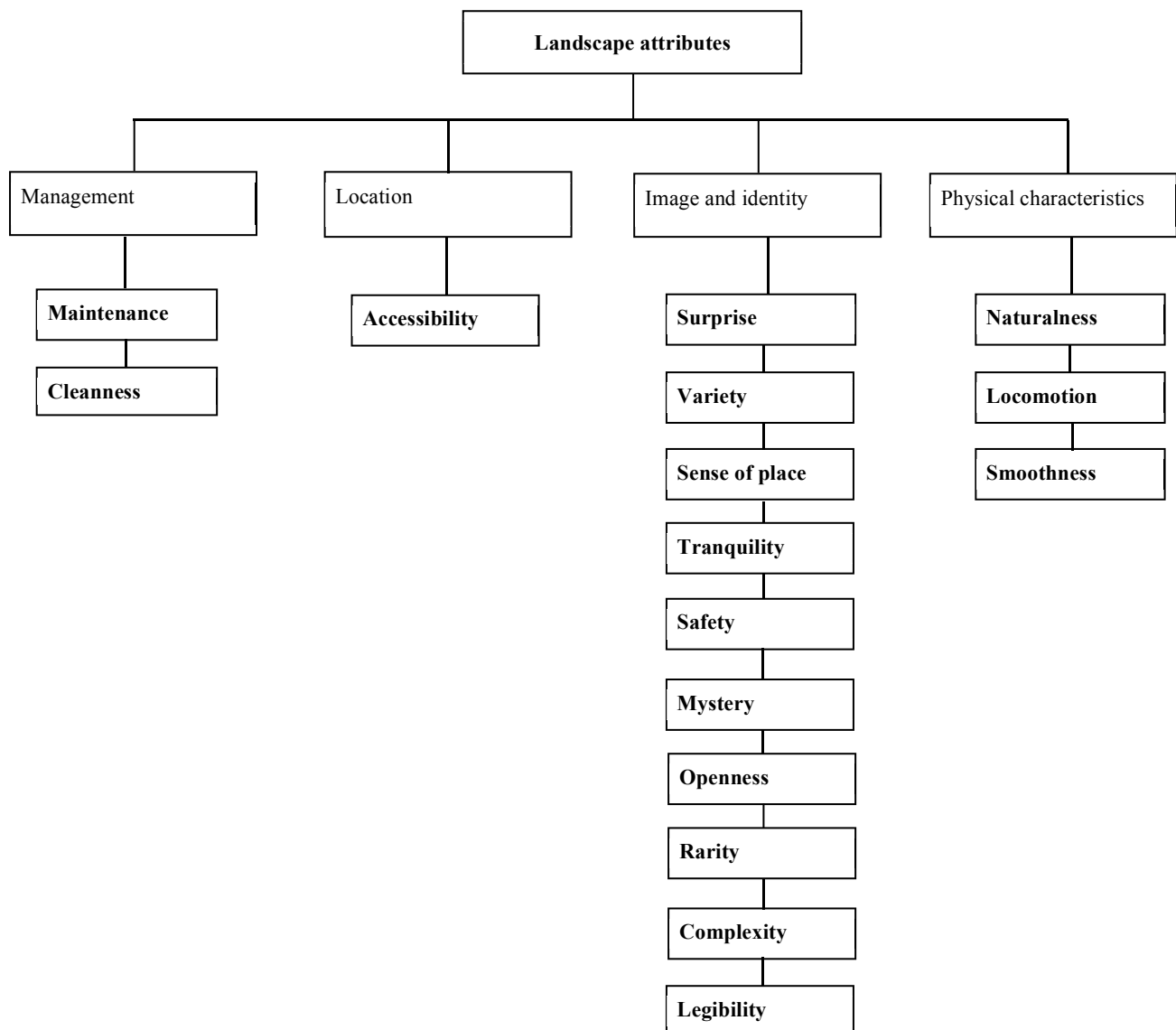
appearance of negligence or vandalism (Coeterier 1996). “Variety” is also of interest, as places with high variety are valued (Rogge et al. 2007) for providing people with chances for learning and discovery, however, simplicity is also appreciated as it can result in unity, especially in small-scale landscapes. In the report of Research Box (2009), simplicity is considered as a feature that can provide amenity benefits such paths for cycling or a playing field and a sense of tranquillity and calmness.

“Unity” is one of the attributes proposed by Coeterier (1996) and is defined as the situation when all the parts fit together and function as a whole giving the landscape an air of completeness.

“Naturalness”, which is the impression that the environment is natural in terms of the flora and fauna (Coeterier 1996), is always mistaken for ecological quality (Nassauer 1995). Appreciating naturalness and the extent to which this attribute is considered positive, depends on our view of culture (Appleton 1975). Even the most fundamental elements of nature and vegetation are perceived through

cultural lenses. Generally, it is believed that people prefer natural landscapes, however, what they are more likely to prefer is not viewed in terms of ecological quality because it tends to be messy (Nassauer 1995). They appreciate nature, but only when it is present in landscapes that are designed (Appleton 1975). People prefer natural landscapes, as they are green and predominantly rural, but clearly man-made features make them more desirable. The Research Box (2009) study revealed that people prefer rugged and isolated landscapes when seeking adventure and discovery, but otherwise designed green spaces and areas with few natural elements and more man-made features. Kaplan et al. (1998) considers natural landscapes to be unsuitable for most people, as they lack a clear focus and might provoke concerns of becoming lost.

Sensory impressions of people of colours, smell, taste, sound, humidity, temperature, light and shadow affect their perception and preference, and can give a “sense of identity” to a place (Coeterier 1996). “Safety” is another important attribute that can greatly affect people’s pref-



**Fig. 2** Attributes of Landscape Quality.





**Fig. 3** Route in Riverside Park along which people were interviewed (Source: Google maps).

erences. It is viewed as a sense of fear by Kaplan et al. (1998) that many people experience when entering an unfamiliar landscape, which engenders a sense of fear. In this context, visual access is important, as landscapes with dense vegetation that restricts their view is likely to increase their sense of fear. The sign of human involvement in a landscape, such as fences and walls, can reduce the fear factor (Kaplan et al. 1998).

“Location” of the site and “accessibility” are additional features that can affect people’s preferences (Burton and Rymsa-Fitschen 2008). Swanwick (2002) introduces features such as “rarity”, if there is a rare feature in a land-

scape and “tranquillity”, if the noise level and light from adjacent built up areas is low.

Based on the above people’s preferences for a landscape are affected by the following attributes: management, location, image and identity, physical characteristics, each of which are determined by one or more features (Fig. 2).

## Case Study and Findings

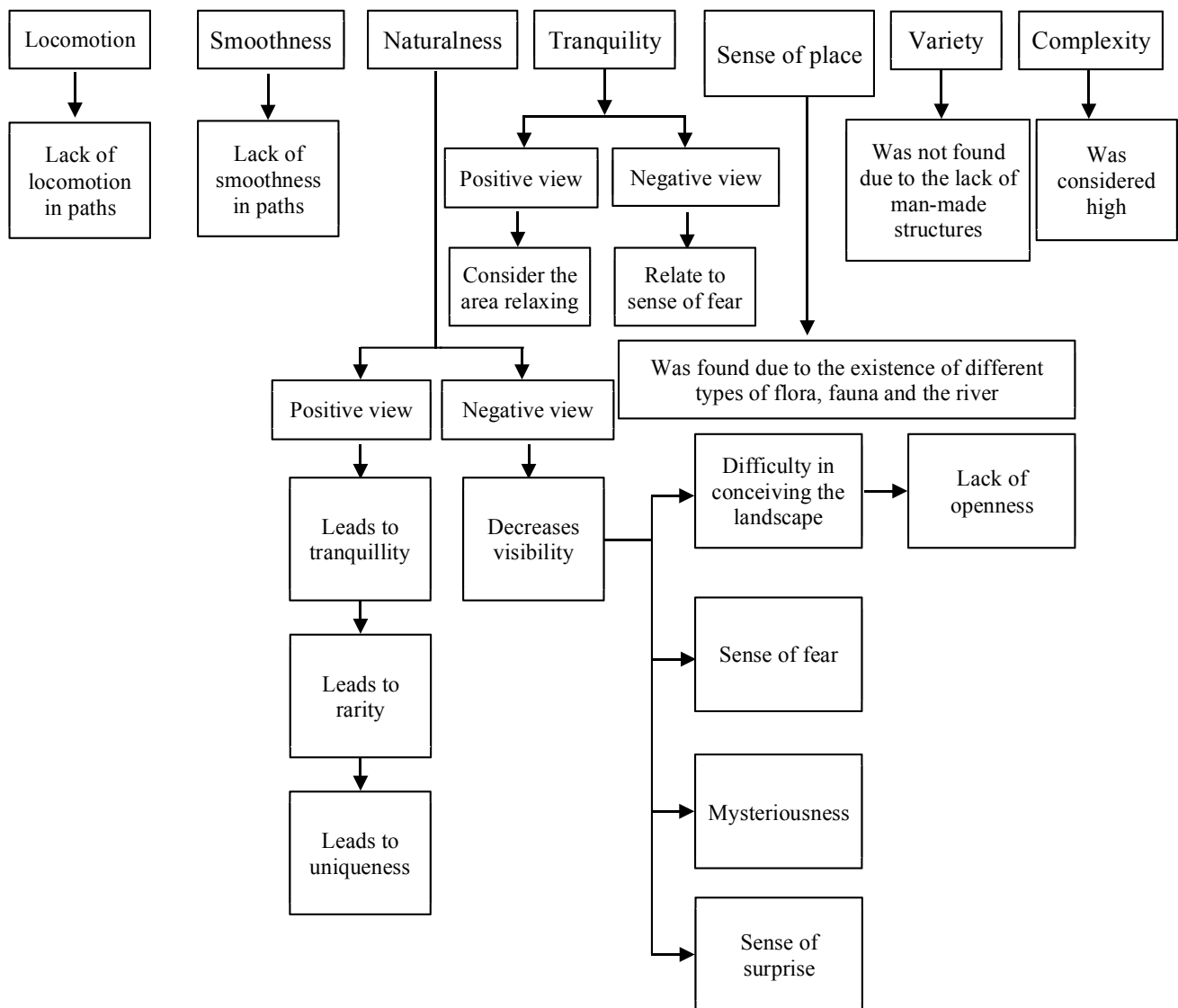
### Riverside Walker Park (St. Anthony’s), Newcastle upon Tyne

Riverside Park is located in the St Anthony’s area in south-eastern Newcastle upon Tyne. It was occupied by heavy industry in the past and after 1980 the land was reclaimed and now consists of large areas of grassland and woodland with a spectacular view of the River Tyne (Emms 2006). Like many other wastelands, this area became available as a result of industrial decline (Mah 2010), but subsequently became a site of high ecological value, with a great range of habitats and dense woodland and scrub inhabited by many species of animals, such as birds, foxes and rabbits (Emms 2006). Nevertheless, the City Council was worried by the level vandalism and decided to develop the area with the view of making it more attractive for the public. The interview route of Riverside Park is shown in the following map (Fig. 3).

It is a large area that has been recolonized by nature where one can walk, cycle or go fishing, as there are several cycle ways and footpaths through the site and along



**Fig. 4** Vegetation in Riverside Park (Source: author).



**Fig. 5** Summary of the results for Riverside Park.

the river. It is located near several bus stations with direct and quick (< 20 mins) access to the city centre. City council improved the foot and cycle paths, provided linkages for green corridors, cleared some of the vegetation and improved the public areas by improving security and providing facilities for a range of activities (Emms 2006).

The people interviewed had not previously visited the site and were brought there by bus. The discussions took place while walking along a route designed by the researcher so that it would not exhaust the interviewees.

As this landscape was a former industrial site, it was not viewed initially by the interviewees as attractive. More specifically, they found the paths inappropriate for easy walking and in terms of naturalness, some found it very natural, but its negative effect on visibility increased their feeling of fear. The poor visibility increased the element of surprise, but made it difficult to conceive it as a landscape. This attribute was also connected with the mysteriousness of this landscape. They believed that the lack of management resulted in the vast amount of vegetation

in this park. Others had a more positive view in that the naturalness increased their feeling of tranquillity. Almost half found it had a positive and relaxing effect while the other half found it increased their sense of fear and insecurity (Fig. 4).

Although this area is located near bus stations, the interviewees did not find it easily accessible, perhaps due to its emptiness. Smoothness was not an attribute of this area, which hindered its exploration. High ecological value associated with the river created a sense of identity and the sound of birds, the river and leaves resulted in a unique and enjoyable experience. Complexity was noted by some interviewees, which they attributed to the dense vegetation. Some of them related the poor visibility to a lack of variety but others did not know what constituted the landscape, as there was a lack of natural features, like the river. However, most of them viewed the landscape as boring, because of a lack of variety. Safety issues affected many other attributes or were associated with them. Lack of safety was viewed as the most important impediment to regularly visiting



**Fig. 6** Route in Exhibition Park along which people were interviewed (Source: Google maps).

and exploring this landscape, especially if alone. Thus, although some attributes of this landscape were viewed positively, safety is a major issue for people visiting this area (Fig. 5).

#### **Exhibition Park, Newcastle upon Tyne**

Exhibition Park is located in the centre of Newcastle and has existed since 1870 and was known previously as Bull Park. After 1885 North of England, Mining and Mechanical Engineering exhibitions were held in this space, which resulted in it being called Exhibition Park (Ainsley and Archbold 2013). It is one of the biggest parks in Newcastle upon Tyne with a variety of facilities including a coffee shop, basketball court, tennis court, skate-park, and a lake. This park, unlike Riverside Park is not covered with dense vegetation, but with cut grass and well laid out green spaces. It is now undergoing redevelopment funded by the Heritage Lottery and some parts are closed to the public. Fig. 6 shows the route of interview at Exhibition Park.

The attributes listed in the previous section were discussed while walking in this landscape. This site was visited after returning from Riverside Park. Of the 7 interviewees, 3 had previously visited and were familiar with this park. The attributes were physical characteristics, image, identity, location and management.

Unlike their responses to Riverside Park, almost all of the interviewees responded in the same way to the attributes of this landscape. They regarded the high quality paths as suitable for all types of activities and abundant enough to provide quick access to different parts of this park. Hence, “smoothness” and “locomotion” were valued as important attributes of this park. In terms of “naturalness”, they viewed wasteland as having a higher ecological value, but that naturalness and amount of vegetation in this park are sufficient. The large areas of grass were appreciated by some interviewees in that they provided suitable space for resting and chatting. Lack of dense vegetation was admired by the interviewees as it increased the “legibility” and “openness” of this site. They also related naturalness and the amount of vegetation to the “mysteriousness” of this site in terms of a lack of “surprise”. “Complexity” of this space was considered to be low and “variety” was based on man-made and natural elements and the range of activities it offers. They believed this Park was sufficiently “tranquil”, whereas Riverside Park was too complex and boring (Fig. 7).

“Rarity” and “sense of place” were the attributes most valued at this site and are associated with the existence of birds and swans near the lake. In terms of “location and accessibility”, this site was considered to be very accessible and well located. They even compared it with the previous site and argued that psychologically, they consider this area to be more accessible even though both are located at similar distances from the centre and in similar situations. “Management” and “safety” of the park were ranked very highly and even related to each other as good management results in good safety. Summary of results are represented in following chart (Fig. 8).



**Fig. 7** Panoramic views of Exhibition park (Source: author).

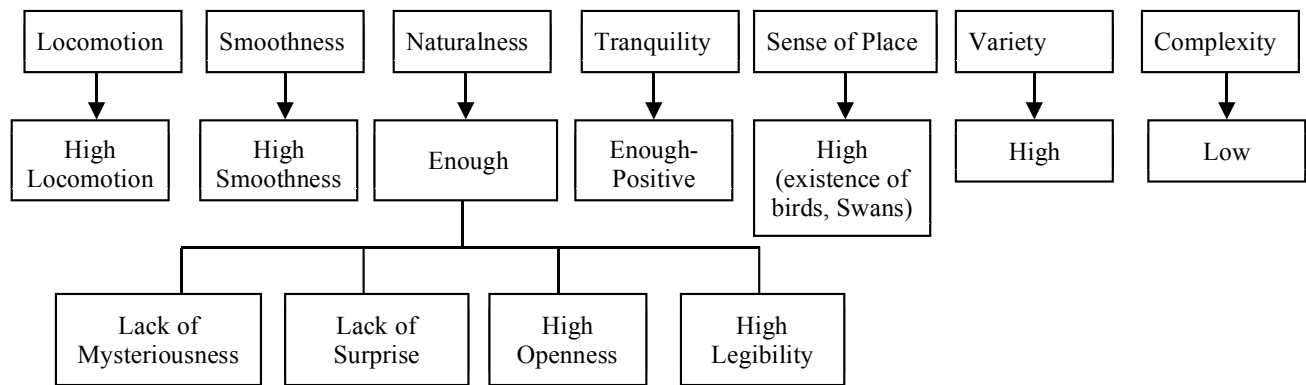


Fig. 8 Summary of the results for Exhibition Park.

## Discussion

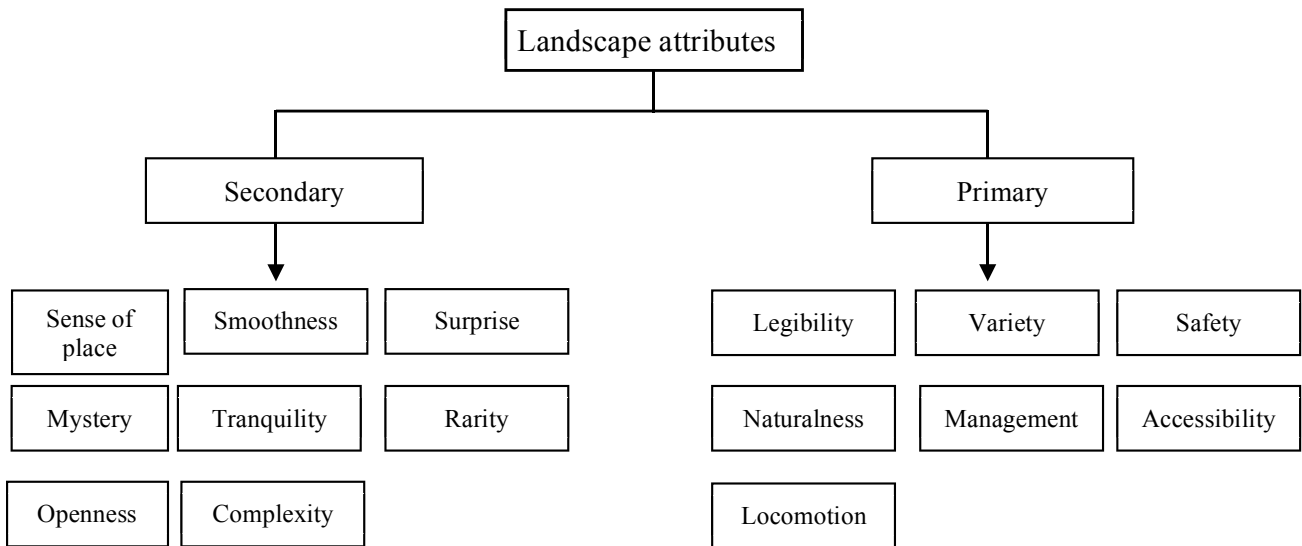
The subjective assessment of landscape quality is dependent upon people's status (Rogge et al. 2007), which influences their preferences. Attributes of landscape quality are those features of a landscape that are important in determining people's preferences. Attributes regarded as important were obtained from the literature published since 1970 by Kaplan (1979), Zube (1987) and Coeterier (1996), and categorized into four groups: physical characteristics that include naturalness, locomotion and smoothness, image and identity that includes surprise, variety, sense of place, tranquillity, rarity, legibility, mystery and openness, and location that includes accessibility and management especially maintenance and cleanness. Below is a table, in which the quality of the important attributes of the two landscapes studied are compared.

Table 1 Comparison of the quality of the attributes of Exhibition Park and Riverside Park.

Landscape attributes	Riverside Park	Exhibition Park
Locomotion	Low	High
Smoothness	Low	High
Naturalness	High	Medium
Tranquillity	High	Medium
Sense of Place	High	High
Variety	Low	High
Complexity	High	Low
Mysteriousness	High	Low
Surprise	High	Low
Openness	Low	High
Legibility	Low	High
Management	Low	High
Rarity	High	Low
Safety	Low	High
Accessibility	Low	High

The interviewees' responses to these attributes were similar to that reported in the literature reviewed. They valued some attributes similarly to that reported in the

literature as positive, such as sense of place, management, openness, variety, smoothness, locomotion, surprise and safety. Complexity, which is valued negatively in the literature, was also considered to have a negative effect in terms of the coherence and legibility of an area. Other attributes were challenged and some were valued differently. Naturalness is an attribute that despite what some studies report, is not viewed positively. Nevertheless, naturalness is viewed in the same way as by Appleton (1975) in that it largely depends on the cultural background of the people, with some preferring to be in natural environments while others do not. However, all of the interviewees in this study preferred Exhibition Park, which is in a less natural setting. Very tranquil sites are viewed negatively. Accessibility is a controversial attribute as it is viewed differently in this research as the interviewees considered more the situation rather than the accessibility of the site. They believed that empty landscapes, although highly accessible, are not as accessible as crowded space. Mysteriousness is another attribute challenged by this study and unlike in the literature it was not viewed positively in terms of attracting more people to explore a site (Kaplan 1979). The interviewees viewed Riverside Park as mysterious, however, were not eager to visit this site again. This is also true for the feeling of surprise as although Riverside Park was viewed as surprising they were nevertheless not eager to explore it. The quality of these attributes is affected by level of safety and variety at the sites. This study revealed that the attributes are not equally important for qualifying landscapes as the interviewees considered some features as more influential than others in determining their preferences. Riverside Park was valued for its sense of identity, rarity, mystery, tranquillity and complexity; however, almost all of the interviewees were not willing to visit the site again. The major problem was the lack of safety as those who appreciated the tranquillity and mysteriousness of the area, were not eager to walk there alone. The sense of fear is a major attribute affecting the view of the overall quality of a particular site. After safety, lack of variety, management, legibility and locomotion are considered the most important. In Exhibition Park the interviewees talked positively about their feelings for the area mainly



**Fig. 9** Categories in which the various attributes of landscapes were classified.

in terms of variety, legibility, accessibility, management, locomotion and safety.

Although this site lacks surprise, mysteriousness, complexity and rarity the interviewees were interested in visiting the site again. People tend to choose and value landscapes that provide them with the possibility of experiencing a wide range of activities. Accessibility, management and legibility are also significant attributes determining the quality of a landscape. Overall, although Riverside Park was valued for its tranquillity, sense of identity, sense of place, rarity and mystery, it was not viewed as a high-quality landscape as poor safety was a major issue and locomotion, management and lack of variety were also influential. Although Exhibition Park lacked rarity, mystery, and to some extent a sense of place, it was valued very highly by all the interviewees, with some attributes being more influential in determining the quality of this site.

This research considers the difference between the attributes of landscapes and categories them as either primary or secondary. Based on the views of the interviewees the primary attributes are locomotion, safety, variety, legibility, management, accessibility and naturalness, and secondary attributes smoothness, surprise, sense of place, complexity, tranquillity, rarity and mystery.

## Conclusions

Landscapes provoke unique feelings, meanings and concepts that can effect senses of identity (Pouya and Behbahani 2017), physical and psychological health (Abraham et al. 2010) and satisfaction of the environment (Kearney 2006). Understanding the quality of these landscapes in terms of people's perception can help planners to define the values of environments and result in better decisions. In order to achieve this one needs to be more rigorous in defining landscape quality and the ways, in

which perceptual quality is assessed. Using the methods of assessing landscape quality cited in the literature published over the last five years, a framework for assessing the quality of landscapes was developed. Although several recent studies on landscape quality were carried out using photographs (Keshtkaran et al. 2017; Gungor and Polat 2018; Roth et al. 2018), in this study people were brought to the landscapes and questioned about their feelings an analysis of which revealed that certain attributes, like safety, can have a marked effect on their assessment of the quality of the landscape. Visiting a landscape is more likely to give them an accurate picture of safety than looking at photographs. Some attributes, such as safety and accessibility, are more important for people when assessing the quality of a landscape and visiting the landscape gives them a better idea of the quality of these attributes. This systematic study of the assessment of landscape quality is based on people's views when actually visiting urban green landscapes. The results of this study will help with the planning and management of landscapes. Highlighting those attributes that are of primary importance (legibility, variety, safety, naturalness, management, accessibility and locomotion) should help policymakers and planners to quickly evaluate landscapes as it greatly reduces the number of attributes that need to be evaluated. This is in addition to existing park evaluator tools, such as The Trust for Public Land Park Score. It will place more emphasis on the quality of urban green spaces, rather than the existence of a park or green space.

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# POLYHYDROXYALKANOATES: ADVANCES IN THE SYNTHESIS OF SUSTAINABLE BIO-PLASTICS

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## ABSTRACT

The extensive use of petro-based plastics, lack of suitable waste management and casual behaviour of communities to their proper disposal has posed a significant threat to the environment. Currently, single-use petro-based plastics are being banned all over the world due to their inherent non-degradability and problems with disposal. Worldwide, these bans have proved an impetus for increasing research on bio-based polymers as a substitute for conventional plastics. Use of greener or biodegradable polymeric materials would have immense socio-economic and ecological significance.

Polyhydroxyalkanoates (PHAs) are eco-friendly alternatives to petrochemical plastics and are quickly biodegraded in aerobic or anaerobic environments such as a landfill, waste treatment facilities or oceans. PHAs have material and mechanical properties ranging from stiff and brittle crystalline to elastomeric and moulding, similar to petrochemical thermoplastics. In addition, they are hydrophobic, isotactic, biocompatible and have piezoelectric properties. PHAs in being environmentally friendly are seen as ideal candidates for replacing conventional petro-based plastics in numerous fields.

This review focuses on the biochemistry along with in-vitro reaction mechanism of PHA synthases. In addition to the applications and modes of production of PHAs already mentioned a few factors governing PHA synthesis will be discussed. Scope for improving PHA synthesis is also mentioned and some of the tools for enhancing the properties of PHAs are discussed.

**Keywords:** bioplastics; blending; co-culture; PHA; PHA synthase; polyhydroxyalkanoates; recombinant bacteria

## Introduction

Traditional petro-chemical based plastics have been a cause of concern for several decades, not only because of their resistance to biodegradation but also because of their toxic effect on terrestrial and marine environments. Currently, the main source of carbon backbone plastic production is fossil material, which is a non-renewable natural resource and, therefore, of not only environmental concern (Koller 2020). The search for a suitable substitute involved finding new polymers with similar physico-chemical properties and addressing the issue of biodegradation. The first milestone was the discovery by the French microbiologist Maurice Lemoigne in 1920 of a biopolymer, poly (3-hydroxybutyrate), PHB, involved in intracellular granule formation in the gram-positive bacterium, *Bacillus megaterium* (LeMoigne et al. 1926). This PHB is the most commonly accumulated member of polyhydroxyalkanoates (PHAs) family. PHAs act as microbial storage compounds with versatile plastic like material properties and a possible solution to the above problem.

Although conventional petro-based plastics may not be completely phased out, the increase in socio-consciousness is forcing various industries to change to using polyhydroxyalkanoates (PHAs) polymers for some products because of their eco-friendly properties coupled with thermal, mechanical and physico-chemical properties similar to those of conventional plastics.

They are produced by a wide range of bacteria, which accumulate PHAs intracellularly in granules that act as energy reserves (substitute for fatty acid for maintaining

metabolism when under stress) for the microbe. The trigger for this to occur is when a nutrient in a culture medium is limiting but there is an excess of carbon. Under natural conditions, these polymers can constitute nearly 90% of the dry weight of a cell (Madison and Huisman 1999).

Non-storage PHAs of low molecular weight occur in the cytoplasmic membrane and cytoplasm of *Escherichia coli*, however.

Chemically, PHAs are polyesters of various hydroxyl alkanates. More than 150 monomer subunits have been identified in PHAs synthesized by various plants and microbes. The different combinations of these individual subunits offer infinite scope for synthesis of various types of PHAs with different properties. The molecular weight of PHAs differ in different microbes and ranges between 50–1000 KDa. Structurally the individual subunits have the (D) configuration of stereo-specific catalytic enzymes (Senior et al. 1972; Dawes and Senior 1973; Oeding and Schlegel 1973; Wang and Bakken 1998).

An example of a low molecular weight PHA is PHB, which apart from being a green polymer has many of the chemical properties of a conventional plastic like polypropylene (Madison and Huisman 1999).

Microbes are versatile in their biosynthesis of PHAs and use various renewable substances like cellulose, triacyl glycerol, starch and sucrose as precursors. In addition, non-renewable substances such as coal, methane, lignite and mineral oil are also reported as precursors and some microbes also use by-products such as molasses, glycerol and whey, and gases like carbon dioxide as substrates for the synthesis of PHAs.



Depending on their chemical properties and structure, PHAs may be brittle or rubber-like. These biodegradable polymers are considered to be better than starch-based polymers (starch-polyethylene) and chemically synthesized ones like polylactic acid (PLA) and polyglycolic acid (PGA) due to their structural and tensile properties (Steinbüchel and Fuchtenbusch 1998).

PHAs are biogenic and can be produced naturally in microbial cultures (Steinbüchel and Lutke-Eversloh 2003). These microbial cultures are often referred to as bio-refineries because they are high yielding and tolerant of a wide range of environments. Some algae have also been found to biosynthesize PHAs (Bugnicourt et al. 2014).

The production of biopolymers increased recently because of their sustainability and a desire to increase the use of waste products. This is not only of environmental value, but is also economically beneficial.

## Classification of PHAs

Based on the number of carbon atoms in the monomers and the source of the enzyme, PHAs are classified into two categories: short chain length PHAs (scl-PHAs) and medium chain length PHAs (mcl-PHAs). There are 3 to 5 carbon atoms in the former and 6 to 14 in the latter. Both scl-PHAs and mcl-PHAs are synthesized by different species of bacteria. Examples of bacteria synthesizing scl-PHAs are *Cupriavidus necator* and *Alcaligenes latus*, and mcl-PHAs, *Pseudomonas putida* (Verlinden et al. 2007).

Their properties depend on the number of carbon atoms and their chemical structure. The most studied scl-PHA is PHB, which is stiff and brittle, and its crystalline structure makes it difficult to process. Therefore, it is customary to co-polymerize it with poly-3-hydroxyvalerate in order to produce a flexible, strong and thermally stable bio-plastic (Marchessault 1996). The resultant copolymer is 3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV), which is now widely used in the manufacture of items used for the storing and packaging of food. The mcl-PHAs are used in the production of biomedical products. In contrast to scl-PHAs, mcl-PHAs are more elastic and less crystalline (Gross et al. 1989; Preusting et al. 1990). In most cases, the structural monomeric component(s) of scl-PHAs is 3-hydroxybutyrate (3HB) and of mcl-PHAs 3-hydroxyoctanoate (3HO) and 3-hydroxydecanoate (3HD) (Anderson and Dawes 1990; Steinbüchel 1991; Steinbüchel and Schlegel 1991; Lee 1996).

## Biochemistry of PHA Synthases

Bacteria use enzymes to biosynthesize PHAs, which remain in the bacteria and act as energy reserves. The rate of biosynthesis and the quantity produced depends

on the enzymes involved and physiological status of the bacteria. To understand the complexity of the biochemical processes it is important to know, what enzymes are involved in these pathways.

Four different types of PHA synthases are reported, which differ in their size, number of subunits and substrate specificities. Types I and II specifically synthesize scl-PHAs and mcl-PHAs, respectively, and are composed of a single subunit (PhaC) (Bernd et al. 2003). Types III and IV are dimers composed of the subunits PhaC+PhaE and PhaC+PhaR, respectively. They are implicated in the synthesis of scl-PHAs. The subunits PhaE and PhaR have only an 18% sequence similarity, but despite this they function similarly in establishing contact between the enzyme and the hydrophobic polymer (Satoh et al. 2002).

Witholt and Kessler (1999) report the presence of an operon in *Cupriavidus necator*, a Gram negative soil bacterium with three genes, namely PhaA, PhaB and PhaC, which code for the enzymes  $\beta$ -ketothiolase, (R)-specific acetoacetyl-CoA reductase and PHA synthase, respectively. These enzymes are involved in the biosynthesis of PHA from acetyl CoA. The first step is the conversion of two molecules of acetyl CoA into acetoacetyl CoA by the enzyme  $\beta$ -ketothiolase. This reaction requires energy provided by the splitting of ATP into ADP and Pi. In the next step, the reduction of acetoacetyl CoA into 3-hydroxybutyryl CoA with the oxidation of NADPH occurs in the presence of the enzyme (R)-specific acetoacetyl-CoA reductase. Thereafter, 3-hydroxybutyryl CoA is polymerized into polyhydroxybutyrate by PHA synthase.

The mcl-PHAs are generally heteromeric molecules with more than one type of monomeric unit in their structure, whereas mcl-PHAs are usually heteromers of multiple subunits in which the dominant subunit is in direct contact with the carbon chain (Witholt and Kessler 1999). *Pseudomonas spp.* is the most commonly used for studying mcl-PHA production. The substrates for mcl-PHA are derived from the intermediates produced during biosynthesis and degradation of fatty acids, such as, (R)-3-hydroxyacyl-CoA, which are further polymerized by PhaC. PHA accumulators polymerize hydrophilic monomers into a hydrophobic polymer in the presence of soluble or membrane-bound PhaC (Nobes et al. 2000; Grage et al. 2009). The result is the production of intracellular granules with an amorphous hydrophobic core enclosed in a monolayer of phospholipids embedded in proteins like the PHA synthases, phasins (a regulatory protein) and intracellular depolymerases (Jurasek et al. 2004). The intracellular depolymerases belong to the PhaZ family and are implicated in the remobilization of a carbon supply, whereas phasins are small proteins that regulate the size and morphology of the granules (York et al. 2002).

Schuber et al. (1988) report the expression of recombinant PHA synthase in *Escherichia coli* and a similar granular morphology to that produced by *Pseudomonas*. Their genetically engineered *Escherichia coli* does not have active

PHA depolymerase and phasins so Gerngross et al. (1994) suggest that there is an indigenous component functionally similar to phasins present in *Escherichia coli*. When PHA is synthesized in the laboratory (*in vitro* conditions), the granules of PHA are five times larger than those synthesized by genetically engineered *Escherichia coli*. It is likely that this because the area available for the naked polymer to coalesce is greater in the absence of a regulatory protein (Gerngross et al. 1994; Nobes et al. 2000).

### The *in-vitro* Reaction Mechanism

Reaction mechanism, kinetics, substrate specificity and intermolecular interactions are critical parameters in vitro synthesis. Most of the studies on PHA production are on PHA synthases. Type I PHA synthase is the most studied because of its simple structure, though it is possible to purify type II and III enzymes (Jossek et al. 1998; De Roo et al. 2000; Yuan et al. 2001; Takase et al. 2004). The only other highly purified synthase is that of *Thermus thermophilus* and another from *Bacillus cereus* SPV, which is in a novel synthase class and class IV synthase, respectively (Lawrence et al. 2005). Purification from the host is a challenge as in this case the synthase is in the form of an aggregate, however, this can be overcome by using recombinant proteins and minute quantities of the detergent Hecameg (Stubbe and Tian 2003).

In-vitro kinetics of type I synthases deviate from the Michaelis-Menten model. The initiation of polymerization is marked by a lag phase, which is followed by an exponential phase. Chromatographic analyses indicate that the synthase remains in an equilibrium between an inactive monomer and active dimer. The in-vivo state still needs to be deciphered (Gerngross et al. 1994).

The lag phase is attributed to the time needed for priming the synthase and polymerization (Stubbe and Tian 2003). Homology studies on the four classes of enzymes indicate a strong similarity with the  $\alpha/\beta$ -hydrolase superfamily (Bernd 2003). These enzymes have a specific catalytic triad motif with histidine as the nucleophile, which is a suitable catalytic site for interaction between two monomers each contributing cysteine for dimer formation. This may apply to the rest of the three synthase classes and also fits with a polymer being associated with each dimer.

Another model is based on a post-translational modification for fulfilling the need of the second thiol, though this is unlikely for recombinant organisms (Stubbe et al. 2005). The yield of polymers using various classes of synthases indicate that class I and II synthases produce a single polymer chain per molecule of enzyme while class III synthases result in chain transfer reaction, which yield several polymer chains per enzyme molecule (Jossek et al. 1998). Nonetheless, in vivo studies class I synthases can yield 60 polymer chains (Tian et al. 2005).

It is suggested that the termination of polymerization is brought about by the attachment of a nucleophilic chain

transfer molecule at or near the active site (Lawrence et al. 2005). It is predicted that CoA (Kurja et al. 1995; Jossek et al. 1998), hydroxybutyrate (Madden et al. 1999), water (Kawaguchi and Doi 1992; Kusaka et al. 1997) or alcohol (Shi et al. 1996; Madden et al. 1999) may act as nucleophiles. The difference in the yield in-vivo and in-vitro conditions may be attributed to either the lack of phasin-like regulatory proteins in-vitro or high concentration of nucleophilic agent in-vivo. This would account for the high molecular mass of in-vitro synthesized PHAs as the scope there for chain transfer is greatly reduced (Daae et al. 1999; Lawrence et al. 2005).

The study of the biosynthesis and production of PHA is mainly dependent on *R. eutropha*. Two acetyl-CoA moieties are condensed to form acetoacetyl-CoA by  $\beta$ -ketothiolase (PhaA) in *R. eutropha*, which then undergoes reduction by NADPH-dependent reductase (PhaB) resulting in the formation of the (R)-isomer of 3-hydroxybutyryl-CoA.

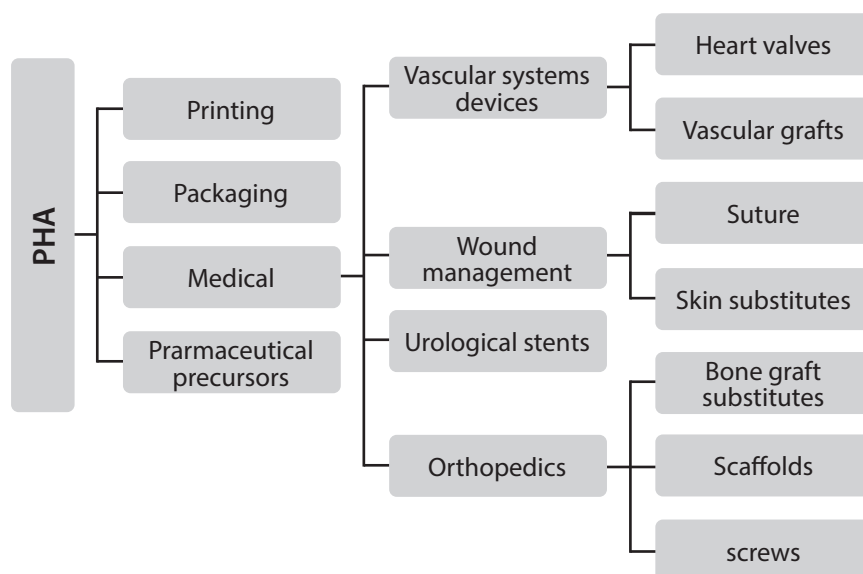
Similar PHA biosynthesis is also reported in *R. rubrum* in which the reductase is an NADH-dependent isoenzyme that produces (S)-isomers of 3-hydroxybutyryl-CoA. Here two groups of enoyl-CoA hydratases facilitate the conversion of (S)-type to (R)-type isomer, which is the only form of stereoisomer that is accepted by the polymerizing enzyme, PHA synthase. Studies on the biosynthesis of PHA in *R. eutropha* and *R. rubrum* have revealed a cascade of events that result in the efficient production of PHA in-vivo (Sudesh et al. 2000).

The biodegradability of the PHAs is another aspect that needs to be considered. PHAs act as energy resource for microbes, which enzymatically hydrolyse them into HB and HV units. The degraded products further facilitate biomass growth in culture. The rate of degradation is dependent on parameters like exposed surface area, pH, temperature, humidity and other ingredients in the system. P(HB-HV) remains stable in air for an indefinite period and is therefore resistant to degradation under ambient conditions. Under aerobic conditions, PHA degrades into water and carbon dioxide. The rate of degradation is fastest in oxygen-depleted sewage and slowest in marine environments (Doi et al. 1992; Lee et al. 1996).

### Applications

The PHA family of products have numerous uses, especially for making containers for food since these polymers are inert and non-toxic. In addition, because of their anti-microbial properties, they are used for manufacturing adhesive films and hygiene-related baby items, including nappies. They can also be used in printing as toners and adhesives for coating films (Madison and Huisman 1999).

Polyhydroxyalkanoates (PHAs) that accumulate as natural biogenic polyesters in microbial cultures, when formulated and processed, form highly flexible materi-



**Fig. 1** A flow chart indicating the various uses of PHAs.

als that are sustainable substitutes for traditional plastics. As bio-plastics, they are used in the manufacture of electronic products like mobile phones and computer tablets etc. Their amphiphilic property makes them suitable for encapsulating seeds and fertilizers (causing slow release), biodegradable films and containers for covering and storage of crops. In addition, the turnover in agriculture is likely to increase due to the use of their waste materials for the production of PHAs.

PHAs also have many medical uses because their biodegradable and non-toxic properties make them suitable for manufacturing biomaterial for insertion in the human body. They are used as surgical sutures, adhesive bandages, orthopaedic straps and pins, repair stents, in targeted drug delivery, patches and matrix for bone marrow. When combined with hydroxyapatite it becomes a bio-active composite suitable for bone tissue regeneration and replacement (Chen and Wu 2005). There are many possible commercial uses of PHAs derived bio-plastics. A number of the applications in which bioplastics are being used are depicted in Fig. 1.

## Production of PHAs

Biosynthetic polymers can be produced by plants or microbes. The mass production of bio-plastics with different properties using microbes is costly, requires skill, technical expertise and the yield is low compared to petro-chemical based plastics, which has inhibited the industrial use of PHAs (Ojumu et al. 2004).

## Natural Biosynthesis of PHAs

Various factors determine the subunit composition of a polymer, such as, type of microbe, composition of me-

dia, fermentation and mode of recovery. There are more than 300 species of Gram positive and Gram-negative microbes capable of PHA biosynthesis (Steinbüchel and Schlegel 1991; Suriyamongkol et al. 2007). These microbes have the necessary enzymes for the biosynthesis of PHAs and store them as granules in their cytoplasm as an energy reserve (Reddy et al. 2003). The stimulus for their biosynthesis is usually some kind of stress like lack of a nutrient (N or P) in the presence of excess carbon (Dawes and Senior 1973; Amass et al. 1998). However, a few species like *Azotobacter vinelandii* (mutant and wild type), *A. eutrophus* and *A. latus* also synthesize PHAs under non-stress conditions (Ojumu et al. 2004). Studies indicate that the most common limitation in almost all types of bacteria to the accumulation of PHA, is the availability of nitrogen. However, in some species of bacteria, such as *Azotobacter*, it is the availability of oxygen (Johnston et al. 2017).

## PHAs Production by Recombinant Bacteria

As it has been mentioned above, there are number of microbes, which synthesize PHAs as an energy reserve, when either stressed or routinely. These organisms, however, grow slowly and have a long generation time. The extraction of PHAs from these microbes is done using lysis, which results in a very low yield. It also depends on the physiological conditions of the cells since they also have enzymes, which degrade these PHAs, when they require the energy.

The wild type *E. coli* lack PHAs otherwise its ability to grow rapidly make it a good potential candidate for the production of PHAs. If *E. coli* can be genetically engineered to produce enzymes for synthesizing PHAs, then the production and isolation of PHAs is cost-effective (Steinbeuchel and Schlegel 1991; Madison and Huisman 1999; Kadouri et al. 2005).

There have been attempts to characterize the metabolic pathways, so that it is possible to insert various substances at different stages. Recombinant strains of *E. coli* that have genes for the biosynthesis of PHA from *Alcaligenes eutrophus* have been produced by metabolic engineering, which yield high levels of PHA (Zhang et al. 1994; Lee et al. 1996). *E. coli* is highly versatile in terms of the range of substances it can utilize from lactose, glucose and sucrose to xylose and more complex sugars, which further increases the cost-effectiveness of the process. Cheaper substrates like whey and hemicellulose hydrolyzates can also be used (Lee et al. 1996).

## Production of PHA by Genetically Engineered Plants

Not only microbes but plants also produce PHA, with crop plants high on the list, which are even more cost-effective than bacteria or yeast in terms of yield and effort. The production of PHA using crops requires the genetic engineering of transgenic varieties capable of high PHA production and remaining genetically stable for many generations (Snell and Peoples 2002). This would enable the production of these green polymers in greater quantities than other ways of producing PHA. Unlike bacterial cells, plant cells are compartmentalized and transformed phb genes allocated to locations with high concentrations of acetyl-CoA. *Arabidopsis thaliana* is the favoured choice for such transgenic transformation, as like *E. coli*, it is the equivalent in terms of plants (Madison and Huisman 1999). The innate presence of 3-ketoacyl-CoA thiolase in *A. thaliana* required the transfection with phbB and phbC genes from *R. eutropha* for it to accumulate PHB in the nucleus, cytoplasm and vacuoles of its cells; however the plant has several growth defects. This was resolved by targeting the phbCAB genes in the plastid of *A. thaliana*, which receive a high flux of carbon via acetyl CoA, which enhance the yield of PHA without adversely affecting the growth of the plant. The maximum yield in terms of dry weight percentage is 14% (Nawrath et al. 1994). Oilseed plants are also potential candidates for the production of seed specific PHAs. The common source of PHB and oil is acetyl-CoA, which is easily inserted into the biosynthesis pathway of PHA with a high innate carbon flux courtesy of acetyl CoA. Other plants being used in PHA production are *Gossypium hirsutum* and *Zea mays*.

## Co-culturing and the Biosynthesis of PHA

Co-culturing using two or more microorganisms to produce PHA from complex carbon feed (Choi and Lee 1999; Ganduri et al. 2005; Nikodinovic-Runic et al. 2009) is easy as several microbes are available. The synergistic production of PHA in such cultures is attributed to the specific utilization of the same substrate by different bac-

terial strains. The most well-known example is the utilization of toxic compounds like benzene, toluene, ethyl acetate, xylene (collectively called BTEX) and styrene in the synthetic plastic pyrolysis of oil for conversion to biodegradable PHA by three strains of *Pseudomonas putida*, namely F1, mt-2 and CA-3.

## Factors Governing PHA Synthesis

### Species of bacteria

A major factor determining the efficiency of PHA synthesis is a species of microbe that is high yielding when provided with a cheap substrate. Industrial application requires mass scale production for which rapidly growing species and strains are required, such as recombinant organisms with short generation times and are easy to lyse. A good example is recombinant *E. coli*, which is used to produce PHB along with other co-polymers (Schubert et al. 1988; Slater et al. 1988; Zhang et al. 1994; Reddy et al. 2003). Several strains of *Pseudomonas*, like *P. oleovorans*, have been engineered to produce various blends of the polymers scl- and mcl-PHA (Preusting et al. 1993).

### Culture medium

A culture medium, apart from providing conditions for optimal growth of the microorganisms, also determines the yield and cost of the product, as it is the major cost of producing PHA on an industrial scale. Therefore, there is a need to reduce the cost of making the medium without compromising on essential ingredients needed for high productivity (Lee 1996; Ojumu et al. 2004), which are corn steep liquor (Nikel et al. 2006), molasses (Solaiman et al. 2006), rice bran and wheat (Huang et al. 2006; Van-Thuoc et al. 2008), starch-rich waste (Kim and Chang 1998), activated sludge (Yuan et al. 2006; Jiang et al. 2009), effluents from olive and palm oil mills (Ribera et al. 2001; Pozo et al. 2002; Bhubalan et al. 2008) and whey (Marangoni et al. 2002; Koller et al. 2008). The medium selected depends on the culture organism. In addition, by varying the medium used to produce homopolymers or heteropolymers it is possible to produce more than 100 different monomers, which can be combined to produce homopolymers or copolymers with molecular weights ranging between 50 to 100 KDa (Taguchi and Doi 2004; Valappil et al. 2007).

### Fermentation conditions

This process involves first achieving a high density of microbes and then creating a nutrient-limited condition that enhances the synthesis of PHA (Asenjo et al. 1995; Madison and Huisman 1999; Tsuge 2002). The temperature and pH of the medium are determined by the species of microorganism used. The temperature is kept at 30–37 °C and slow stirring determines its oxygen concentration, while the pH is varied depending on substrate (Kim et al. 1994; Chung et al. 1997). Various

things can be done to produce fermentation conditions suitable for maximum yield, such as varying the source of carbon during the process (Kim et al. 1992; Koyama and Doi 1995; Henderson and Jones 1997; Durner et al. 2001; Zinn et al. 2003), diluting the medium (Sun et al. 2007), fed and unfed culture batches (Kim et al. 1992; Rhee et al. 1993; Kim and Chang 1995; Kim et al. 1997) and limiting the supply of nitrogen, phosphorus or oxygen (Sun et al. 2007).

### Recovery

The final step in the biosynthesis is recovery the cost of which is dependent on the method of purification. The most primitive of which is extraction using an organic solvent (Lee 1996), which is better in terms of the percentage recovery of PHA than cell lysis followed by aqueous extraction, since cell lysis alters the molecular mass of the polymer (Ojumu et al. 2004). Other methods include enzymatic digestion of non-PHA components, floatation-mediated extraction using supercritical carbon dioxide and spontaneous liberation of polymers. Because of their limitations, however, neither are suitable for industrial use.

## Methods for Improving the Properties of PHAs

### Blending

Blending of PHAs results in an appreciable improvement in the properties of the biopolymers. The raw materials determine the properties of the final product in terms of its physical and mechanical durability, whereas by varying their concentrations and optimizing the temperature and pH alter the biodegradability and toxicity of the bio-plastics. Currently, this is very important due to increase in societal and government concerns over non-sustainable energy resources. The blending has also proved to be a boon for the medical industry, which is using this method in the manufacture of implants and absorbable non-toxic surgical sutures.

## Components Used in Blending

### Natural raw material

Starch is the most widely used as it is highly biodegradable, readily available and convenient (Pilkington et al. 2015). The linear amylose and branched amylopectin structure of starch determines the mechanical properties of the final product. It is reported that it results in a significant improvement in the properties of PHB and a reduction in the cost of production (Godbole et al. 2003). PHB and all percentages of it in blends of starch have the same transition temperature ( $T_g$ ) and the final product in all cases is crystalline. The tensile strength of the product is best when the percentage of starch is 30% (Pilkington et al. 2015), which is what the packaging industry

requires for paper and cardboards packaging etc. The amends were also possible with the thermal and phase separation properties by starch blending. The final blend is immiscible as there is a direct relationship between  $T_m$  and percentage of starch (Zhang et al. 1997a). The enthalpy of melting did not change significantly while  $T_g$  of  $9^\circ\text{C}$  is required for all percentages of starch.

However, in spite of reducing manufacturing costs and improvements in mechanical properties, the final products are brittle and unsuitable for producing strong bio-films, but this can be overcome by using copolymer grafts of starch and glycidyl methacrylate (GM), which increase the compatibility between the bio-films and PHA matrix (Willett et al. 1998). Treatment of starch with GM results in the polymer being more durable and tensile and there is a direct correlation between this property and percentage of treated starch. Scanning electron microscopy revealed strong adhesion between the film and matrix. By using corn starch treated with polyvinyl acetate (PVAc) also increases the mechanical strength of the film (Lai et al. 2015). When the percentage is 27% the  $T_g$  increases to  $44^\circ\text{C}$  and the compatibility with PHB is maintained. This blend is also compatible with the recovery method as it does not affect the  $\alpha$ -amylase degradation of corn starch (Lai et al. 2015).

### Cellulose derivatives

Cellulose derivatives like cellulose acetate, cellulose butyrate, cellulose propionate or ethyl cellulose are potential candidates for PHB blends, especially for materials used in the biomedical industry (Zhang et al. 1997b). They are compatible with PHAs and are easily recoverable (Wang et al. 2015). Studies on crystallization, melting properties and miscibility of these blends indicate a concentration dependent increase in  $T_g$  with decrease in the percentage of PHB. These blends lack crystalline properties in non-isothermal crystallization tests, however, spherulite formation continues but is delayed (El-Shafee et al. 2001). The single  $T_g$  indicates the blends are miscible and the phase morphology analysis that the polymer is homogeneously amorphous. The presence of cellulose acetate butyrate in the inter-lamellar region of PHB enhances the mechanical properties of the final blend.

### Lignin

The functional groups of lignin (phenyl propane repeats and carboxylic acid groups) determine its amorphous nature, which in turn make it a suitable candidate for producing PHA blends. The phase morphology of lignin prevents crystal nucleation and spherulite formation, and decreases the brittleness reported when using cellulose derivatives. Lignin and its derivatives are highly miscible with PHB and prevent crystal formation and reduce the number and size of spherulites (Weihua et al. 2004; Mousavioun et al. 2013). Soda lignin is another modified derivative that has a significant effect on the thermal stability of PHB (Mousavioun et al. 2010). Ther-

mo-gravimetry and electron microscopy indicate that the optimal percentage of soda is 40%. The intermolecular interactions between the blending components are due to hydrogen bond formation between carbonyl groups of PHB and carboxyl groups of lignin. This is the major reason for the enhanced fractural stability of this blend and also prevents degradation (Mousavioun et al. 2012). During degradation, there is an increase in the unevenness of the surface of the bio-film, which indicates efficient biodegradation.

## Co-polymerization

### Different monomeric subunits of PHA

Production of copolymers using different combinations of monomers is a common commercial practice. The different monomers are important in determining the strength and durability of the biopolymer. PHB is brittle and tends to form spherulites. Blending PHB with PHA-mcl monomers imparts thermal and mechanical properties similar to polyolefin (Saad 2002). These combinations are also an effective way of producing polyester-like blends and products for biological applications. Different blends of poly-3-hydroxybutyrate (P3HB) and poly-4-hydroxybutyrate (P4HB) vary from crystalline to elastic and the use of different blends of PHBHHx with PHB have resulted in the production a wide range of biomedical products (Yoshie et al. 2004) and the modification of the properties of biological materials. PHB rapidly crystallizes and forms an inundated bio-film that is not a suitable matrix for mammalian cells, but by incorporating copolymers into blends alters the degree of crystallisation and results in an even surface for attachment and growth of cells (Kai et al. 2003).

## Synthetic Biodegradable Polymers

### Poly-lactic acid (PLA)

Poly-lactic acid is produced from lactic acid or by fermenting simple carbohydrates. It is biodegradable and therefore is widely used to produce biodegradable packaging and biocompatible devices. Despite being an environmentally friendly option, its poor durability and thermal stability hinders its use in the industrial production of polymers. Blending mineral fillers with PHA/PLA results in immiscible polymers with a nodular morphology (Koyama and Doi 1997; Ohkoshi et al. 2000; Gerard and Budtova 2012) and those with PHBV enhancing the thermal stability of PLA. Despite being brittle, these bio-films are very ductile and plastic (Gerard and Budtova 2012). The ductile strength can be increased by including more PHA-mcl than PHA-scl co-polymers in the blend., which resulted in Noda et al. (2004) hypothesizing that PLA blends with PHA can compensate for the limitations of the individual ingredients in a combination (Noda et al.

2004). An addition of 10% PHA to the blend transforms the phase morphology from crystalline to amorphous and PHA mcls and epoxy-PHA mcls results in amorphous elastomers. This is attributed to the interaction of the epoxy group with the hydroxyl in the PLA (Takagi et al. 2004). The method of blending also affects the miscibility and compatibility imparted to the final product. PHA/PLA blends prepared using solvent casting and melt blending methods vary in their miscibility (Zhang et al. 1996), with the former immiscible for a range of percentages and the latter appreciably miscible, which affect the crystalline properties of the product (Furukawa et al. 2007). Those PLA blends with low crystallization temperatures are miscible but those with high crystallization temperatures are not.

### Polycaprolactone (PCL)

Because of its mechanical and ductile strength, it is extensively used in medical applications, which have undergone several modifications. It is inherently semi crystalline and blending with PHBHHx yields a soft and flexible polymer suitable for musculoskeletal tissue engineering (Lim et al. 2013). The biodegradable and non-toxic nature of this blend makes it even more suitable for tissue engineering, adhesion and proliferation surfaces for human foetal mesenchymal tissue and human bone (Chen and Wu 2005). The ductility of PCL/PHBV blends is directly proportional to the percentage of PCL in the blend (Chiono et al. 2008). Even vascular grafts are prepared using these blends, which are biocompatible with the rat cerebral endothelial cells in terms of adhesion, proliferation, viability and migration over a period of two weeks (Del Gaudio et al. 2012).

## Chemical Modification of PHAs

This is another tool for enhancing the properties of PHAs, which is more regulated than blending as it modulates the structure of the polymer and the resulting changes are mostly predictable. This can be taken to the next level by controlled polymerization, which impart a new dimension to the architecture of the PHA. PHA graft graphene nano-composites degrade at higher temperatures and have a higher electrical conductivity than neat PHA (Yao et al. 2019).

### Grafting on natural polymers

Grafting PHA onto natural polymers have proved highly suitable for medical applications including tissue engineering, production of antimicrobial material and drug delivery. The interaction of the  $-NH_2$  group of chitosan with  $-COOH$  group of PHB and the ester formation between  $-COOH$  of PHB and  $-OH$  of cellulose produces two variants of PHB graft copolymers (Yalpani et al. 1991). Hydrophilic nature of chitosan imparts an amphiphilic tendency to the graft copolymer and there is

a direct correlation between the solubility of the final biopolymer and the degree of the graft (Arslan et al. 2007).

### PHA graft copolymers with vinyl or (meth)acrylate groups

Copolymerization of PHA grafts with oligomers containing vinyl or (meth)acrylate groups like 2-hydroxyethylmethacrylate, polyethylene glycol or poly(methylmethacrylate) by irradiation is highly effective in altering the property of PHA grafts (Hazer and Steinbüchel 2007). The presence of vinyl chains enhances the hydrophilicity of the polymer, which reduces the inter-facial tension and platelet adhering property of the product, hence making it suitable for the manufacture of blood containing bags and devices (Chung et al. 2003).

### Scope for Improving PHA Production

With increased demand for environmentally friendly PHA there is a need to consider the sustainability and cost-effectiveness of its synthesis.

### Screening of New Species of Microbes

There is a need to explore a wide range of habitats for more microorganisms for PHA production, especially those in estuaries, rhizospheres and wastewater effluents, and develop measures for distinguishing between efficient and non-efficient PHA producers. Genetic interventions like using recombinant organisms with suitable enzyme requirements and the identification of PHA producing species using FISH or PCR has facilitated this process. Mixed microbial cultures (MMC) are being widely adopted in preference to recombination as MMC creates an ecosystem with various species contributing to the biosynthesis of PHA. The medium for the microbes and synthesis conditions are provided by bioreactors (Duque et al. 2014). Use of agro-industrial waste as the raw material in these bioreactors for producing PHA has proved very successful. Similarly, the fermentation of agro-waste like sugar cane molasses, olive oil effluents and even food waste in MMC systems (Koller et al. 2017). The production of PHA in photo bioreactors with mixotrophic microbe culture containing cyanobacteria (recombinant or wild type) is sustainable and economic (Stal 1992; Narodslawsky et al. 2015), with the process optimal in terms of carbon dioxide emission and soluble organic compounds in cultures with *Nostoc muscorum* Agardh acting on poultry waste (Bhati and Mallick 2016).

### Genetic interventions for improvement of PHA producers

Molecular biology has provided tools that can be used to convert non-PHA synthesisers to producers or for improving yield and properties of PHA. Recombinant *E. coli* cloned with PHAc synthases on a lactose substrate produces PHB (Ahn et al. 2001). In another study, *Cupriavi-*

*duc necator* was engineered to exploit more substrates in a medium and produce more PHB. The lac system genes (lacO, lacZ and lacI) can be integrated into the bacterial genome for allowing the conversion of lactose to polyesters (Povolo et al. 2010). In addition, genetic tools have facilitated improvements in the recovery process, for example, PHAmcl can be produced in vivo if staphylococcus nuclease is integrated into the genome of *Pseudomonas putida*. Currently this is the most effective way for producing medium chain length PHA (Boynton et al. 1999). *P. putida* with knock-outs of fatty acid degradation enzymes and the substrate carrier protein, 3-hydroxyacyl-CoA-acyl, is able to produce homomers instead copolymers due to reduction of  $\beta$ -oxidation by these mutants (Liu et al. 2011).

### Utilization of inexpensive agro-waste as raw material

Fifty percent of the cost of producing PHA is the cost of the substrate and subsequent processing. The most common raw materials are simple sugars, fatty acids and oils, however, there are alternative carbon-rich resources. The waste from agriculture is a rich source of carbon that can be used as a substrate for the synthesis of PHA. Agro-waste is a by-product of a variety of crops, such as, the bagasse from fruit and sugarcane, peel from potato, orange and banana, and bran of wheat and rice (Stavroula et al. 2020). The geographical availability is restricted to tropical countries, like India. It is estimated that for every 10 tonnes of sugarcane, 3 tonnes of bagasse is produced (Kulkarni et al. 2014). Further, as India is the world's largest producer of bananas there is a great potential for it to use the peel to produce PHA. Sugarcane and corn cob are the richest sources of carbon used in media for culturing *C. necator*. When considering a substrate for synthesis of PHA it is important to determine whether it is readily available all year round, cheap and easy to store in good condition for long periods. The agricultural substrates are first converted to stable sugars like lactose via anaerobic fermentation and then aerobically used to produce PHA. In addition, green gas effluents from anaerobically produced silage can be added to *C. necator* cultures producing PHA (Koller et al. 2005). The major challenge in using agro- or industrial waste as substrates is the poor yield of PHAs. Purified sugars deliver higher yields as they provide higher concentrations of the substrate for synthases to act on, whereas agro- or industrial waste, not only is the concentration of the carbon source less but there are other ingredients inhibiting or slowing down the synthesis. The dilution of the substrate increases the production time, which increases the operation costs, especially, in mass scale production. There is, therefore, a need to look for ingredients rich in carbon that optimise the process and reduce the cost. Lactose-rich whey protein consists of about 4–5% carbon by weight, but after ultrafiltration, one is left with a lactose-rich feedstock (Ahn et al. 2001). Whey protein can also be enzymatically broken down into glucose and galactose and after dehydration the mix consists of 50% sugars by weight. Charcoal stripping wood is good

for removing inhibitors in the reaction mix (Koller et al. 2005). Liquefied wood is produced from spruce wood dust via a microwaved toluene sulfonic acid mediated hydrolysis. Although it is a good substrate for PHA production by *C. necator*, it also contains an abundance of inhibitors, which are removed cheaply by means of charcoal media filtration in order to restore the growth kinetics of the reaction. The use of liquefaction is widely criticised because it introduces toxins and inhibitory substances into the process. The extreme temperatures, pressures and pH leads to the production of inhibitors like furfural and 5-hydroxymethyl furfural. This can be avoided by using enzymatic hydrolysis under normal conditions, however, the many hydrolytic enzymes required make it very expensive (Koller et al. 2017). In the circumstances charcoal stripping is an effective way of producing a substrate (bagasse hydrolysate) suitable for *Burkholderia cepacia* and *B. sacchari* (Silva et al. 2004). As these two *Burkholderia* strains are able to utilise both pentose and hexose sugars in the bagasse hydrolysate, they give higher yields than those obtained using a purified sugar substrate (xylose). Charcoal stripping is also used for removing inhibitors from chicory root hydrolysate for fermentation by *C. necator* (Haas et al. 2015). An economic alternative to charcoal bed filtration for removing inhibitory components like free fatty acids, ash, residual biodiesel and methanol is demethanolization, which involves thermal or vacuum – assisted evaporation (Hájek et al. 2012). Phase separation techniques or even distillation and vacuum dehydration are useful for concentrating glycerol. Consequently, a multi-culture system can use both glycerol and methanol as substrates. *Methylomonas extorquens* has been used in such studies, in which a crude glycerol medium resulted the diauxic growth of the microbe and conversion of methanol before the utilisation of glycerol (Braunegg et al. 1999). Using crude glycerol as the sole carbon source results in a high yield of PHB in sequencing bioreactors (Moita et al. 2014). PHA production is also associated with the generation of biofuel. Integration of these two industries that have similar feed stocks would be a step closer to sustainability.

## Conclusion

PHAs are extremely important because they enable society to decouple economic growth from resource exhaustion and environmental ruin. With increase in the diversity of PHAs there will be a considerable increase in their application. However, the cost of production of PHAs constrains their large-scale industrialization and commercialization. A lower-cost can be achieved by integrating fields like synthetic biology, systems biology, morphology engineering, next generation industry biotechnology and high-cell-density cultivation, which will facilitate the synthesis of all kinds of PHA. Moreover, bacteria can be designed that have multiple metabolic

pathways and can synthesize a particular PHA on demand. Eventually, an economically, ethically and environmentally sustainable production of PHA is feasible.

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# ESTIMATES OF THE CONTRIBUTION OF FOG WATER TO WET ATMOSPHERIC DEPOSITION IN CZECH MOUNTAIN FORESTS BASED ON ITS STABLE HYDROGEN AND OXYGEN ISOTOPE COMPOSITION: PRELIMINARY RESULTS

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## ABSTRACT

Stable isotopes are increasingly being used in many scientific fields, including environmental sciences. In this study we measured the variation in the stable hydrogen and oxygen isotope composition of fog water, rain water (in the form of bulk falling precipitation) and throughfall water in the Šumava (the Bohemian Forest), Krkonoše (the Giant Mts.) and Jizerské hory (the Jizera Mts.) Mts. in October–November 2017. In total, 46 cumulative two-week samples were collected and analysed. Our results indicate that the overall stable hydrogen and oxygen isotope composition of fog and rain samples differed significantly, fog being isotopically enriched in the heavier isotopes <sup>2</sup>H and <sup>18</sup>O relative to rain. In contrast to our assumption, throughfall water was generally depleted in the heavier isotopes <sup>2</sup>H and <sup>18</sup>O relative to rainwater. Hence, the simple mixing model for most samples yielded an unrealistic percentage outside the reasonable range of 0–100%. For few samples, however, the estimated contribution of fog to throughfall ranged between 3 and 8% based on  $\delta^2\text{H}$  and 4 and 7% based on  $\delta^{18}\text{O}$ , which is lower than that estimated for the same mountain regions by other authors using different methods and significantly lower than that reported for mountain ranges in neighbouring countries. Although using stable isotopes is a promising tool for determining the contribution of fog to the hydrological budget when assessing atmospheric deposition, the critical limitations are in the collection, manipulation and storing of the samples.

**Keywords:** atmospheric deposition; fog; rain; stable hydrogen and oxygen isotopes; throughfall

## Introduction

Atmospheric deposition is important in contributing to the self-cleaning of the atmosphere while being a significant source of pollution for the Earth surface (Amodio et al. 2014). It is comprised of wet, dry and occult deposition (Seinfeld and Pandis 2006). While the wet deposition in the form of falling precipitation (episodically in occurrence, related to rain and snow) is relatively easy to measure, dry deposition (occurring continuously via gases or aerosols) is much more difficult to quantify and occult deposition (i.e. fog and rime) is mostly not accounted for at all. It is widely accepted, however, that an accurate quantification of the real atmospheric deposition flux is an essential prerequisite for linking it to its possible environmental effects. While the volume of rain or snow is fairly easy to determine, the water input of occult precipitation (deposition of fog and cloud droplets) and its contribution to meteoric water budget is mostly uncertain. It is well known that occult precipitation may play an important role in both water and ion inputs, particularly in mountain and coastal regions (e.g. Rogora et al. 2006; Schmid et al. 2011; Blaš et al. 2012). Nevertheless, the occult deposition is very difficult to measure and rarely recorded, and not included in regular monitoring networks (Schemenauer and Cereceda 1994).

Stable hydrogen and oxygen isotope composition of meteoric water is an elegant tool for studying different environmental processes (Sharp 2007; Šantrůček et al. 2018). In particular, it is widely used in hydrology for studying the hydrological cycle and water budget (e.g. Soulsby et al. 2000; Votrubová et al. 2017; Zhan et al. 2017). In addition, it has proved useful in climatology, as there are close relationships between climatically relevant meteorological variables, such as surface air temperature or moisture and stable hydrogen and oxygen isotope composition of precipitation (Rozanski et al. 1992; Ichimyanagi 2007). The isotopic approach is used in studies on fog, mostly for identifying its origin (Kaseke et al. 2018). In addition, it was successfully used to estimate the hydrological input of occult precipitation in atmospheric deposition (Dawson 1998; Scholl et al. 2007), as the stable isotope composition of local fog water differs significantly from local rainwater (Fischer and Still 2007).

Fog plays a significant role generally in the maintenance of ecosystems (Weathers et al. 2020) and specifically in the CR, based both on direct measurements (Tesař et al. 2000; Bridges et al. 2002; Bridgman et al. 2002; Fišák et al. 2002; Křeček et al. 2017; Palán and Křeček 2018) and data-driven models (Hůnová et al. 2011, 2016), in which it substantially enhances total atmospheric deposition of environmentally relevant substances, particularly in mountain forested areas (Igawa et al. 2002; Lange

et al. 2003). Stable hydrogen and oxygen isotope composition of rain has been used in the CR to study the role of rain in the hydrological balance in small water catchments (Šanda et al. 2011, 2019), whereas that of fog has not been measured.

The aim of this study, carried out at the Institute of Environmental Sciences (Hanusková 2018) was to measure the stable hydrogen and oxygen isotope composition of fog, rain and throughfall. We hypothesised that their isotopic compositions would differ significantly and that by using the simple isotopic mixing model, it would be possible to estimate the contribution of fog to wet atmospheric deposition in mountain forests in the Czech Republic.

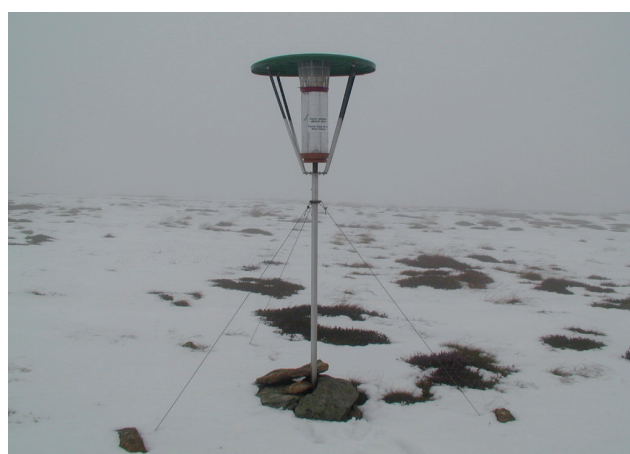
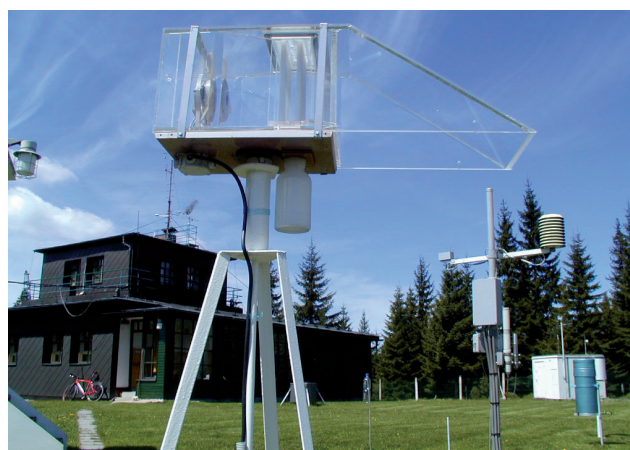
## Methods

### Sampling of fog, rain and throughfall

In order to collect samples of cloudwater, sampling devices were constructed. Two methods of sampling were used. In the first: the active cloud and fog water collector (see photograph in Fig. 1a), in which fog is collected by using a fan to draw air across 10 rows of vertical Teflon strands of 0.5–0.87 mm diameter, which are fixed to a support inside a Plexiglas box at one end of which is the fan. Air enters the box through an opening in the bottom of the box. These so-called active CWP collectors are described in literature by Daube et al. (1987) and were used extensively in research on fog in the U.S.A. (Weathers et al. 1988) and in Europe in the Bohemian Forest and Ore Mts in the Czech Republic (Tesař et al. 1995) and Taunus Mts. in Germany (Eliáš et al. 1995). In the second, so-called passive cloud and fog water collector, fog is collected passively (see photographs in Fig. 1b and 1c), i.e. fog droplets were captured on Teflon strings 0.5–0.87 mm in diameter mounted alongside one another to form a curtain in the form of a truncated cone. This “Grunow” type of fog gauge (Grunow 1952) is shielded from rain by a 0.5 m<sup>2</sup> roof. This passive cloud and fog water collector was first advertised at the 2nd International Conference on Fog and Fog Collection in Canada in 2001 (Tesař et al. 2001).

Samples collected were stored in 500 or 1000 ml polyethylene bottles at 4 °C in the dark. Prior to use, storage bottles were washed with 6N HCl, followed by several rinses with distilled water.

For this study, we sampled fog, rain and throughfall. In meteorology, fog is defined as an obscuration in the surface layer of atmosphere caused by a suspension of water droplets reducing horizontal visibility at least in one direction to less than 1000 m (ČMES 2020). Thus, apart from genuine fog, a cloud touching the Earth surface is also perceived as fog. The most accurate way to distinguish fog from rain is by droplet size, with fog consisting of tiny water droplets, typically 10–20 µm in diameter and rain of drops greater than 0.5 mm in diameter



**Fig. 1** Fog samplers: (a) active fog sampler, Sum\_CH, (b) passive fog sampler, KrK\_SH and (c) passive fog sampler in the background with rain sampler in the foreground, Jiz\_CN, photographer: M. Tesař.

(Scholl et al. 2011), i.e. large enough to fall into a conventional rain gauge. Throughfall is precipitation collected underneath vegetation, which consists of both precipitations intercepted by the canopy that then drips on to the ground and precipitation falling directly through gaps in the canopy. Throughfall is often used in environmental studies to quantify the input part of the geochemical balance (Bellot et al. 1999). Photographs of the fog and rain samplers used at some of our sites are presented in Fig. 1.

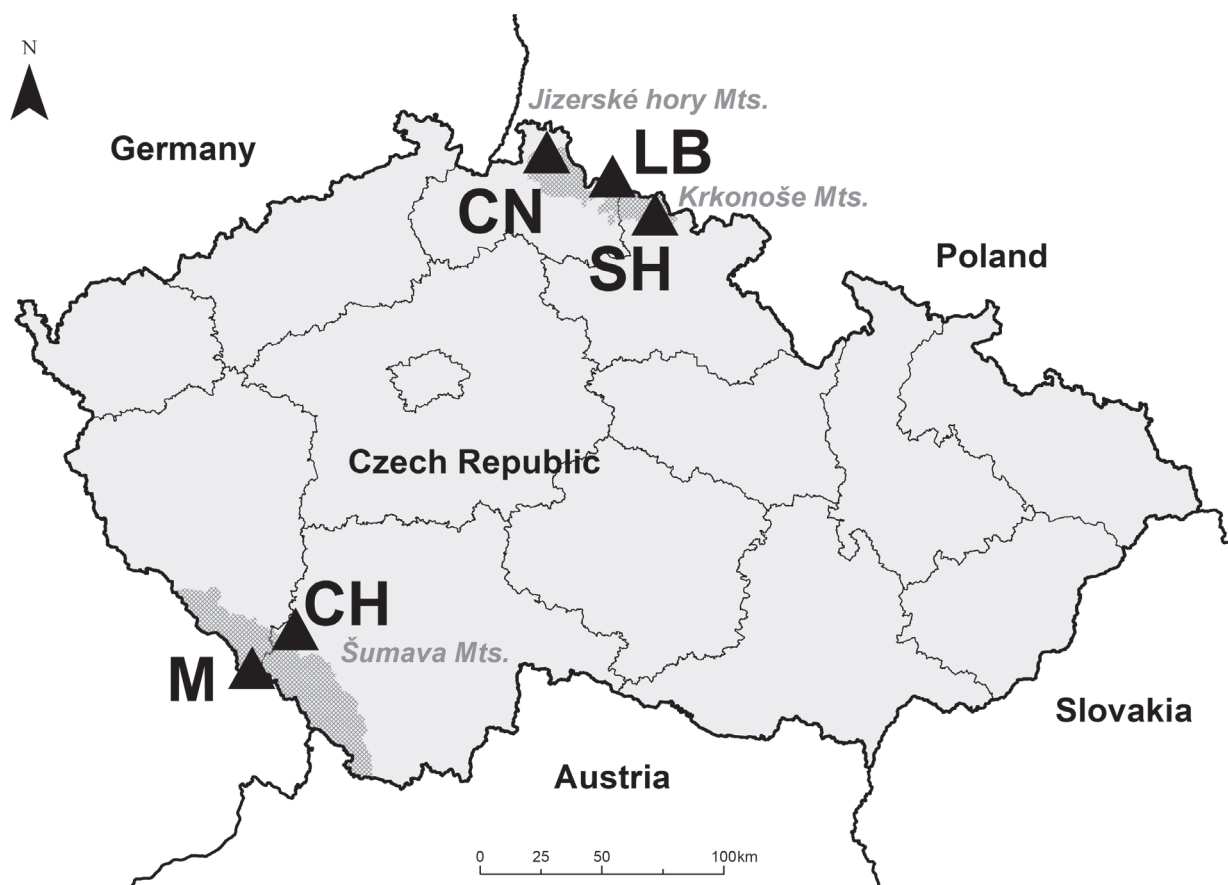


Fig. 2 Map showing the locations of the sites sampled.

The throughfall samples were assumed to be a mixture of fog and rainwater.

In this study, a bulk precipitation sampler for rainwater was located close to the fog sampler, whereas one identical precipitation sampler was placed under a tree for collecting throughfall (spruce trees in the Šumava Mts., dwarf pines in the Krkonoše Mts.). The throughfall sampler was placed under branches some distance from the tree trunk in order to ensure the representativeness of the samples as much as possible.

#### Sites sampled

For this study, we used five established sites situated in three Czech mountain regions: Krkonoše (the Giant Mts.), Jizerské hory (the Jizera Mts.) and Šumava Mts. (the Bohemian Forest) (Fig. 2, Table 1). The Krkonoše and Jizerské hory Mts., located in the northern part of the CR bordering on Poland, belonged in the past to the so called Black Triangle region, infamous for heavy ambient air pollution particularly in the 1970s and 1980s (Hůnová 2020). In contrast, the Šumava Mts., are in the southern part of the CR bordering on Austria and is the cleanest part of the CR (CHMI 2019).

In the Krkonoše Mts., Labská bouda (1355 m a.s.l.) and Studniční hora (1550 m a.s.l.) are the sites, where fog (passive samplers), rain and throughfall were sampled. The vegetation at these sites consists of artic-alpine tundra with dwarf pine, *Pinus mugo* (Šír and Tesař

2013). In the Šumava Mts., Churáňov (1123 m a.s.l.) and Malá Mokrůvka (1300 m a.s.l.) are the sites where fog (Churáňov active sampler, Malá Mokrůvka passive sampler), rain and throughfall (in spruce, *Picea abies*, stands) were sampled. The hilltop station at Churáňov is part of the network of professional meteorological stations run by the Czech Hydrometeorological Institute. In addition, it is the site with the longest record of horizontal (occult) deposition for the hydrological and geochemical balance of a small mountain water catchment (Tesař et al. 2000). Only one site in the Jizerské hory Mts., Prameny Černé Nisy, was sampled for fog and rainwater (for technical reasons the throughfall was not sampled). This site is situated within the Uhlířská water catchment and is part of the international monitoring of stable isotopes within the GNIP (Global Network of Isotopes in Precipitation) and GNIR (Global Network of Isotopes in Rivers) networks (Šanda et al. 2011).

#### Period sampled

Samples of fog, rain and throughfall were collected from October to November in 2017. The selection of October and November was because the highest occurrence of fog in the CR is in the late autumn/winter periods (Tolasz et al. 2007; Hůnová et al. 2020). For practical reasons, based on previous experience, it was most convenient to sample fog before December, as in January and February sampling (and generally access to

**Table 1** Characteristics of the sites sampled.

Mountain region	Site sampled	Code	Coordinates	Altitude [m a.s.l.]	Fog sampler	Samples
Krkonosé	Studniční hora	KrK_SH	50.7265272N, 15.7051425E	1550	passive	F, R, T
	Labská bouda	KrK_LB	50.7703075N, 15.5412983E	1355	passive	F, R, T
Šumava	Malá Mokrůvka	Sum_M	48.9702228N, 13.5123172E	1300	passive	F, R, T
	Churáňov	Sum_CH	49.0682161N, 13.6152428E	1123	active, passive	F, R, T
Jizerské hory	Prameny Černé Nisy	Jiz_CN	50.8400925N, 15.1518536E	820	passive	F, R

Note: F – fog, R – rain, T – throughfall

the sampling site) can be difficult due snow cover. Two-week cumulative samples were collected in both months. For the Šumava and Jizerské hory Mts., the samples were available for both October and November, whereas for the Krkonosé Mts. only for October. In total 46 samples were collected.

### Stable hydrogen and oxygen isotope analyses

Water samples were pipetted into 1.5-ml glass vials, crimped and stored upside down (to prevent water vapour exchange with ambient atmosphere) in a refrigerator prior to analyses. Stable isotope composition of water was determined using an isotope ratio mass spectrometer (IRMS) (DeltaPlus XL, ThermoFinnigan, Bremen, Germany) coupled to a high-temperature conversion elemental analyser (TC/EA ThermoFinnigan, Bremen, Germany) at the University of South Bohemia in České Budějovice. A volume of 1 µl was injected into the reactor heated to 1400 °C using a COMBI PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). Then, the gases produced were separated using a molecular sieve heated to 85 °C before finally entering the IRMS. There were three replicate injections of each sample, the first of which was not included in the calculations due to a memory effect. The hydrogen and oxygen isotope ratios are expressed as follows:  $\delta X [‰] = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$ , where X is  $^2\text{H}$  or  $^{18}\text{O}$  and R is the relative abundance of hydrogen or oxygen isotopes ( $R = ^2\text{H}/^1\text{H}$  or  $^{18}\text{O}/^{16}\text{O}$ ). The isotope ratios were normalised based on the international standards Vienna Standard Mean Ocean Water 2 (V-SMOW 2) and Greenland Ice Sheet Precipitation (GISP) and reported on the V-SMOW scale according to Nelson (2000). The standard deviations of repeatedly measured international standards indicating the precision of the measurements are shown in Table 2.

**Table 2** Precision of our measurements.

	Standard deviation, n = 10	
	$\delta^2\text{H} [‰]$	$\delta^{18}\text{O} [‰]$
V-SMOW 2	1.5	0.24
GISP	1.8	0.37

### Calculation of the hydrological contribution to atmospheric deposition

At localities, where fog and rain have distinct isotopic compositions, a simple linear mixing model may be used to determine the contributions of these two sources to throughfall (Scholl et al. 2011). The fraction of throughfall that comes from fog  $f_F$  is estimated using a simple mixing model:

$$f_F = (\delta_T - \delta_R) / (\delta_F - \delta_R)$$

Where:

$\delta$  denotes the isotopic values ( $\delta^2\text{H}$  or  $\delta^{18}\text{O}$ ),

$F$  denotes the sampled fog,

$T$  denotes the sampled throughfall,

$R$  denotes the sampled rain.

### Results

Our results for individual samples from particular sites are summarised in Table 3 and visualized in Fig. 3. The  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for individual samples regardless of their form range between  $-12.6‰$  and  $-6.1‰$ , and  $-84.6‰$  and  $-39.0‰$ , respectively. In terms of the form of the precipitation: the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for (1) fog range between  $-11.1‰$  and  $-6.1‰$ , and  $-75.4‰$  and  $-39.0‰$  respectively; for (2) rain between  $-12.6‰$  and  $-8.6‰$ , and  $-83.6‰$  and  $-56.8‰$ , respectively; and for (3) throughfall between  $-12.4‰$  and  $-8.0‰$ , and  $-84.6‰$  and  $-55.4‰$ , respectively. The measured  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of the rain sampled were placed along the local meteoric water line (LMWL) expressed by the equation  $\delta^2\text{H} = 7.028 \times \delta^{18}\text{O} + 4.78‰$ , which departs slightly from the global meteoric water line (GMWL), indicating the influence of evaporation and the fact that local precipitation originates from locally evaporated water.

Tables 4 and 5 presents average isotopic values for particular sites and regions, respectively. We have found that the fog samples were isotopically enriched in the heavier isotopes relative to rain in most cases with two



**Table 3**  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values for fog, rain and throughfall sampled in the Krkonoše, Šumava and Jizerské hory Mts.

Site	Date sampled	$\delta^2\text{H}$ [‰]			$\delta^{18}\text{O}$ [‰]		
		F	R	T	F	R	T
Krk_SH	2.10.	-54.8	-77.3	-84.6	-8.7	-11.3	-12.3
Krk_SH	16.10.	-49.1	-58.1	-62.2	-7.7	-8.7	-9.4
Krk_LB	2.10.	-64.9	-83.6	-84.5	-9.9	-12.6	-12.5
Krk_LB	16.10.	-55.3	-60.7	-61.9	-8.7	-9.3	-9.3
Krk_LB	27.10.	-41.4	-64.4	66.7	-7.3	-9.9	-10.0
Sum_CH	2.10.	-57.1	-59.9	65.1	-8.7	-9.5	-9.7
Sum_CH	24.10.	-60.1	-57.0	-55.7	-9.7	-8.6	-8.0
Sum_CH	7.11.	-54.9	-65.5	-65.2	-9.1	-10.0	-9.9
Sum_CH	9.11.	-61.0	-83.3	-82.6	-9.9	-12.6	-12.4
Sum_M	25.10.	-39.0	-56.8	-55.4	-6.1	-8.6	-9.0
Sum_M	14.11.	-75.4	-65.5	-63.7	-11.1	-10.3	-10.0
Jiz_CN	6.10.	-55.3	-60.0	n. s.	-9.1	-9.3	n. s.
Jiz_CN	6.11.	-47.1	-59.1	n. s.	-7.9	-9.5	n. s.

Note: F – fog, R – rain, T – throughfall, Krk\_SH – Krkonoše-Studniční hora, Krk\_LB – Krkonoše-Labská bouda, Sum\_CH – Šumava-Churáňov, Sum\_M – Šumava – Mokřůvka, Jiz – Jizerské hory Mts., Values when fog is not isotopically enriched in the heavier isotopes relative to rain are indicated in italics, n.s. – not sampled

**Table 4**  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of fog, rain and throughfall samples averaged for each of the sites sampled.

Site	Dates sampled	$\delta^2\text{H}$ [‰]			$\delta^{18}\text{O}$ [‰]		
		F	R	T	F	R	T
Krk_SH	2.10/16.10.	-54.8	-67.7	-73.4	-8.2	-10.0	-10.8
Krk_LB	2/16/27.10.	-53.9	-69.6	-71.1	-8.6	-10.6	-10.6
Sum_CH	2,24,10/7,9,11.	-58.3	-66.4	-67.1	-9.4	-10.2	-10.0
Sum_M	25.10/14.11.	-62.8	-53.8	-59.7	-8.2	-9.9	-9.5
Jiz_CN	6.10/6.11.	-51.1	-59.5	n. s.	-8.5	-9.4	n. s.

Note: F – fog, R – rain, T – throughfall, Krk\_SH – Krkonoše-Studniční hora, Krk\_LB – Krkonoše-Labská bouda, Sum\_CH – Šumava-Churáňov, Sum\_M – Šumava – Mokřůvka, Jiz – Jizerské hory Mts., n.s. – not sampled

**Table 5**  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of fog, rain and throughfall samples averaged for each of the regions.

Site	Dates sampled	$\delta^2\text{H}$ [‰]			$\delta^{18}\text{O}$ [‰]		
		F	R	T	F	R	T
Krkonoše	2/16/27.10.	-53.1	-68.8	-72.0	-8.5	-10.3	-10.7
Šumava	2,24,25.10/7,9,14.11.	-57.9	-64.7	-64.6	-9.0	-10.1	-9.8
Jizerské hory	6.10/6.11.	-51.2	-59.5	n. s.	-8.5	-9.4	n. s.

Note: F – fog, R – rain, T – throughfall, Krk\_SH – Krkonoše-Studniční hora, Krk\_LB – Krkonoše-Labská bouda, Sum\_CH – Šumava-Churáňov, Sum\_M – Šumava – Mokřůvka, Jiz – Jizerské hory Mts., n.s. – not sampled

exceptions (indicated in italics in Table 3), which was expected. Throughfall was generally depleted compared to rain, however, which is the opposite of what was assumed. Furthermore, the  $\delta^2\text{H}$  and  $\delta^{18}\text{O}$  values of rain and throughfall were quite similar in many cases.

Table 6 presents the percentage of fog in throughfall in individual samples. It is obvious that the percentage for most samples is unrealistic as they are outside the reasonable range of 0–100%. Only a few samples yielded realistic percentages ranging between 3 and 8% based on  $\delta^2\text{H}$  and 4 and 7% based on  $\delta^{18}\text{O}$ .

## Discussion

### Comparison of our results on the hydrogen and oxygen stable isotope compositions of fog, rain and throughfall with those reported in similar studies

The hydrogen and oxygen stable isotope composition of rain can be compared with that reported for other parts of the CR, as there is an ongoing long-term study of the experimental catchment Uhlířská in the Jizerské hory Mts., which is part of the international GNIP and GNIR networks (Vitvar et al. 2007; IAEA/WMO 2020). The

**Table 6** Percentage of fog in throughfall.

Site	Date	Percentage of fog [%]	
		based on $\delta^2\text{H}$	based on $\delta^{18}\text{O}$
Krk_SH	2.10.	-32.3	-38.4
Krk_SH	16.10.	-45.2	-70.9
Krk_LB	2.10.	-4.9	<b>3.5</b>
Krk_LB	16.10.	-21.5	-8,8
Krk_LB	27.10.	-10.4	-7.0
Sum_CH	2.10.	-181.1	-26.4
Sum_CH	24.10.	-44.7	-52.6
Sum_CH	7.11.	<b>3.2</b>	<b>7.4</b>
Sum_CH	9.11.	<b>3.2</b>	<b>5.4</b>
Sum_M	25.10.	<b>7.8</b>	112.8
Sum_M	14.11.	-18.3	142.5

**Note:** Krk\_SH – Krkonoše-Studniční hora, Krk\_LB – Krkonoše-Labská bouda, Sum\_CH – Šumava-Churáňov, Sum\_M – Šumava-Mokrávka, Jiz – Jizerské hory Mts., realistic fraction of fog in bold

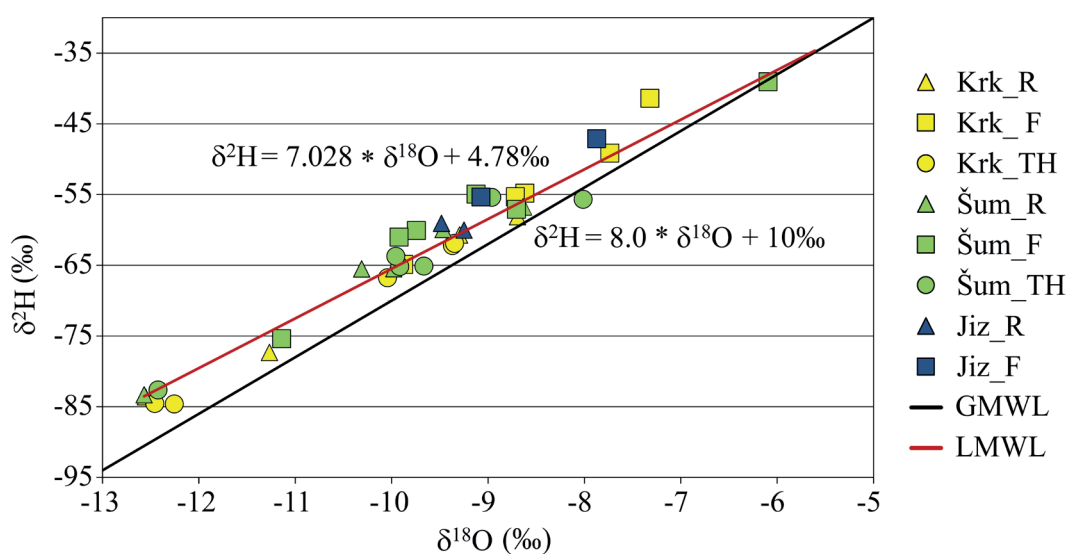
measurements for October–November 2007–2016 reported by Šanda et al. (2019) indicate that  $\delta^{18}\text{O}$  is around  $-10\text{‰}$ , which is similar to our results. For Churáňov in the Šumava Mts., Vystavna et al. (2018) report  $\delta^{18}\text{O}$  values in monthly samples of between  $-14.5\text{‰}$  and  $-7.1\text{‰}$  and  $\delta^2\text{H}$  values of between  $-100.3\text{‰}$  and  $-45\text{‰}$ , with volume-weighted averages for  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in precipitation over the entire period studied (November 2016 – October 2017) of  $-10.4\text{‰}$  and  $-71\text{‰}$ , respectively. For Vysoké Tatry in neighbouring Slovakia, Holko et al. (2018) report values ranging from  $-70\text{‰}$  to  $-45\text{‰}$  for  $\delta^2\text{H}$  and  $-10\text{‰}$  to  $-5\text{‰}$  for  $\delta^{18}\text{O}$ , and  $-50\text{‰}$  to  $-35\text{‰}$  for  $\delta^2\text{H}$  and  $-8\text{‰}$  to  $-6\text{‰}$  for  $\delta^{18}\text{O}$ , for Škaredý potok (altitude of 800–1800 m a.s.l.) and Jalovecký potok (altitude of 800–1500 m a.s.l.), respectively. The values for Slovakia are thus somewhat higher.

Throughfall is expected to be richer in heavier isotopes compared to rain due to increased evaporation and sublimation. The enrichment values in scientific literature are not substantial, however. For spruce trees in a densely forested catchment in Germany it is enriched by only up to 1‰ for  $\delta^{18}\text{O}$  (Brodersen et al. 2000) and the maximum throughfall  $\delta^{18}\text{O}$  enrichment of 1.61‰ is reported for a spruce forest located in central Pennsylvania, U.S., in spring (Dewalle and Swistock 1994). For pine forest the average  $\delta^{18}\text{O}$  enrichment is even lower,  $-0.3\text{‰}$  (Saxena 1986). In contrast to the above reports and our assumption, our throughfall samples were depleted in heavier isotopes relative to rain in most cases.

Our results for fog cannot be compared with other studies from Central Europe or even the CR, as such information is not available. They compare well with the values for different regions of the world cited by Scholl et al. (2011), which range, however, widely between  $-71$  and  $+13\text{‰}$  for  $\delta^2\text{H}$  and  $-10.4$  and  $+2\text{‰}$  for  $\delta^{18}\text{O}$ .

### The contribution of fog to throughfall

The contribution of fog to atmospheric deposition might be substantial, as it is usually much more concentrated than rain in particular regions. This is not surprising, as fog generally forms much lower in the atmosphere and close to the ground, in areas with higher particulate and gaseous concentrations than rain (Seinfeld and Pandis 2006; Hůnová et al. 2018). That fogs are usually substantially enriched in ions, in particular those of environmentally important substances, is documented for many regions of the world (Bridges et al. 2002; Blaš et al. 2012). However, it is difficult to estimate the contribution of fog water contribution and reliably quantify the deposition fluxes for respective ions. As fog and rain often co-exist in a mixed form, it is difficult to distinguish between them and trace those separately



**Fig. 3** The relationship between the  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  values for fog, rain and throughfall in the regions studied (R – rain, F – fog, TH – throughfall, Krk – Krkonoše Mts., Šum – Šumava Mts., Jiz – Jizerské hory Mts., GMWL – Global Meteoric Water Line, LMWL – Local Meteoric Water Line).

using traditional methods. Nevertheless, due to different condensation conditions and processes, fog water and rainwater have typical fingerprints and can be distinguished by differences in their isotopic composition (Scholl et al. 2002, 2011).

There are many studies on the stable isotope compositions of fog, rain and throughfall mainly in cloud montane forests (e.g. Chang et al. 2002; Eugster et al. 2006; Eugster 2007), some of which use simple mixing model to estimate the contribution of fog to throughfall. Simple isotope mixing model is a good tool for assessing the fog content of throughfall for many precipitation events. Nevertheless, some authors report unrealistic results. For example, Schmid et al. (2011) indicate that almost half of the events during a three-months long study conducted in cloud forest in Costa Rica in 2003 resulted in percentages outside the valid range of 0–100%, and conclude that this approach needs further refinements. In addition, the percentages obtained using this technique were lower than those obtained using the eddy covariance method (Eugster 2007).

In our study, the percentage of fog water fraction in throughfall was about 5%, which is lower than originally expected. Tesař et al. (2000), using a different approach, however, report that fog makes up 10% and 15–20% of precipitation in the Šumava and the Krkonoše Mts., respectively. As fog in mountain regions in the Czech Republic occur most frequently during autumn and winter (Tolasz et al. 2007; Hůnová et al. 2020), which is when we collected our samples, the expectation was that the percentage contribution of fog over an entire year would be considerably lower than that reported for October and November. The above is very different from Tesař et al. (2004) estimates despite the fact that they are based on the percentage of fog in precipitation collected from a sampler located outside the forest, whereas we measured the percentage of fog in throughfall collected under the canopy of trees. Generally, throughfall is some 20% lower than the amount in precipitation collected outside a forest (CHMI 2019). Interestingly, some studies in the Central European region, using different approaches, however, estimate much higher contributions of fog. For example, Lange et al. (2003) for a site in the Erzgebirge Mts. In Germany estimate that fog makes about 25% of the annual precipitation, whereas Blaš et al. (2010) report that the percentage for the Sudeten Mts. in neighbouring Poland might be 60%.

### The limitations of this study

Although the IRMS analysis is very reliable and accurate, the sampling procedure might have introduced errors. In contrast to sampling rain, which might be considered relatively easy and straightforward, the sampling of both fog and throughfall is challenging (Scholl et al. 2011). In areas with mixed fog and rain precipitation, passive fog samplers (which were used at all sites in this study except Churáňov), generally collect some wind-driven rain as

their shields are not capable of completely excluding rain (Fischer and Still 2007). This might be an issue especially in the Krkonoše Mts., where both sampling sites, due to their location in open un forested areas at high altitudes, were exposed to strong winds (Tolasz et al. 2007). For collecting throughfall, the positioning of the sampler underneath the canopy is reported to be important (Brodersen et al. 2000). A major difficulty in sampling throughfall is high spatial and temporal variability and consequent difficulties in ensuring sample representativeness (Beier et al. 1993; Keim et al. 2005; Hansen et al. 2013). In addition, whereas the positioning of the throughfall samplers in spruce forests in the Šumava and Jizerské hory Mts. was easy due to the canopy structure and architecture of the trees, it was much more challenging underneath dwarf pines in the Krkonoše Mts.

In addition, the storage and manipulation of samples could cause problems because the stable isotope composition can be substantially changed by evaporation and/or exchange with ambient water vapour (Ingraham and Criss 1993). The essential prerequisite is therefore to avoid changes in isotopic composition between collection and the IRMS analysis. For this reason, some authors recommend the sample to be covered with a *ca* 1cm-thick layer of mineral oil (Scholl et al. 2011). We did not do this, however, as a portion of each sample was used for the ion analysis and could not be used if it was contaminated with mineral oil.

In addition, there is an unassessed uncertainty because the dissolved organic matter in samples of throughfall contains O and H that might interfere with O and H incorporated in water molecules (Van Stan and Stubbins 2018).

### Conclusions

Our results indicate that fog water is enriched in heavier isotopes relative to rainwater in all three regions studied. The differences in  $\delta^{18}\text{O}$  and  $\delta^2\text{H}$  in rainwater and throughfall water were subtle, however, contrary to our assumptions, rainwater was often enriched in heavier isotopes relative to throughfall water. Hence the simple mixing model worked only in a few cases, indicating that fog contribution to throughfall is 5%, which is lower than that expected based on the results of other studies carried out in the same regions, and substantially lower than in adjacent mountain regions in Germany and Poland. Though the IRMS analysis is reliable, the sampling of precipitation, particularly in mountain regions with extreme meteorological conditions including strong winds make it very difficult to separate the different types of precipitation (i.e. fog, rain and throughfall). The stable isotopic approach is suitable for this kind of study providing careful attention is paid to sampling precipitation and avoiding changes in isotopic composition during storage.

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# PREDICTING THE DISTRIBUTION OF THE INVASIVE SPECIES *HYPTIS SUAVEOLENS* IN NIGERIA

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## ABSTRACT

*Hyptis suaveolens* is an exotic plant that has become established in Nigeria because it grows vigorously and produces allelochemicals that adversely affect the growth of adjacent plants. In this study, we predict the distribution of *Hyptis suaveolens* in Nigeria using a Maximum Entropy modelling approach. Pearson correlations were used to select five bioclimatic variables to which type of soil and altitude were added to give a total of seven environmental variables that were then included in the MaxEnt model. This model predicts that *Hyptis suaveolens* can colonize 25%, 45% and 25% of the total land area in FCT, Nasarawa and the River States in Nigeria. In addition, *Hyptis suaveolens* in Nigeria grows mainly in sandy clay and sandy loam soils. Bioclimatic conditions most suitable for this species include temperatures between 26–30 °C, 9–10 hour photoperiod, low altitudes of around 200–800 m and rainfall within the range of 200–800mm. The MaxEnt model indicates that *Hyptis suaveolens* is currently a threat to the survival of native species of plants in the North Central part of Nigeria. The MaxEnt model further reveals that because of its high adaptability and tolerance of changes in abiotic factors, *Hyptis suaveolens* is likely to invade a new environment, the humid forest zone in Nigeria. As this species can become very abundant, we recommend that its spread is monitored and controlled, particularly in areas of high suitability.

**Keywords:** climatic factors; *Hyptis suaveolens*; MaxEnt model; Nigeria; invasion

## Introduction

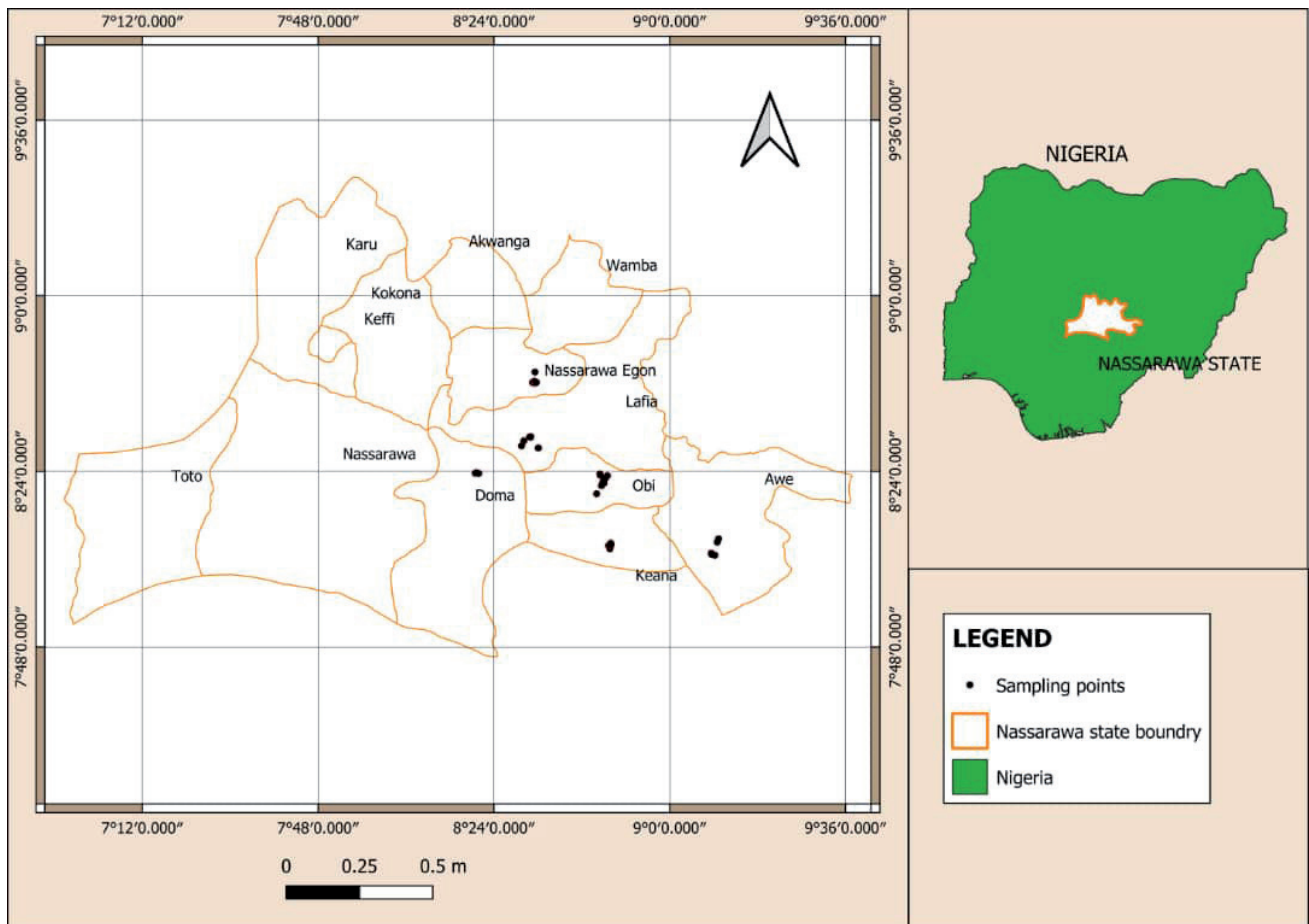
Biological invasions are seen as threats to plant biodiversity, as they can alter the vegetation and change habitats (Mack and D'Antonio 1998; Mack et al. 2000) and thus reduce diversity (Meiners et al. 2001). In addition, invasion can reduce species richness through interspecific competition (D'Antonio et al. 1998; Christian and Wilson 1999; Parker et al. 1999; Meiners et al. 2001). Invasive species usually compete with native plants, which eventually affects species evenness in communities (Sharma et al. 2009). Invasive alien species (IAS) affect native plants by competing for space and nutrients (Borokini 2011). Global invasive species database documents 6 species of grass, 6 shrubs, 8 trees, 8 herbaceous plants and 2 sedges occurring in Nigeria whereas, Borokini (2011) identifies 25 IASs in Nigeria, but does not include *Hyptis suaveolens*. In total, 487 protected sites in the world are under threat and protected areas in 106 countries are seriously under siege from IASs (De Poorter et al. 2007).

*Hyptis suaveolens* (L.) is among the most damaging of the invasive species invading a wide range of ecosystems in tropical and sub-tropical regions in the world (Sarmiento 1984; Afolayan 1993; Padalia et al. 2014). *Hyptis suaveolens* is an annual herbaceous plant belonging to the family Lamiaceae. It thrives in high rainfall warm tropical and sub-tropical regions. Also, it can survive in semi-arid areas, where it propagates by means of seed. Optimal growth of *H. suaveolens* is recorded be-

tween 25 to 30 °C and it can germinate at temperatures between 10 to 40 °C (Felippe et al. 1983). In savannah, dominance of *H. suaveolens* is mainly associated with land from which humans have removed the vegetation by burning or over-grazing (Wulff 1987). It grows rapidly in cultivated fields and disturbed sites, especially where mechanized farming and intense cattle grazing are practised (Holmes 1969). This plant also grows along roadsides and watercourses. It has a strong tendency to grow abundantly in open woodlands and rangelands. It produces a dense growth and releases allelochemicals, which inhibit the germination of the seeds and growth of indigenous species.

Moreover, prolific seed production (Raizada 2006), easy dispersal (Parsons and Cuthbertson 2000) and phenotypic plasticity means this species can colonize many habitats (Sharma and Raghubanshi 2009). The release of unpleasant scent makes it unpalatable for livestock and partly accounts for its abundance over a broad ecological range. The proliferation of *Hyptis* is characterized by some morphological features that enable it to grow in poor quality soils, such as the small size of its seed, seed dimorphism, auto gamic and allogamic reproduction and vigorous growth from perennating rootstocks (Raizada 2006).

Climate is an important factor affecting plant distribution (Catford et al. 2009; González-Moreno et al. 2013). Global climate change has focused attention on the prediction of plant distribution and likely future trends



**Fig. 1** The sites where *Hyptis suaveolens* occurred in Nasarawa State, Nigeria.

(González-Moreno et al. 2013). The MaxEnt model helps us understand the highly complex relationships between the occurrence of species and their environments (Busby 1991; Phillips et al. 2006). It compares their coordinated geographical data in terms of climate factors in order to predict their probable distributions (Guillera-Arroita et al. 2014; Shabani et al. 2018). Predicting the potential distribution of *H. suaveolens* will facilitate the development of the methods needed to curtail its adverse effect on native species. Currently, the potential distribution of *Hyptis suaveolens* in Nigeria is unknown. This study, therefore, aims to predict the potential distribution of *Hyptis suaveolens* in Nigeria using a Maximum Entropy modelling approach.

## Material and Methods

### Study area

This study was carried out in Nasarawa state, which is situated between latitude 8°25'40"N and longitude 8°39'19"E. The sampling was carried out in 6 local government areas (LGAs) including Awe, Keana, Obi, Eggon and Lafia. The spatial data for this plant at each site was collected using a quadrant of size 20 × 20 m. *Hyptis suaveolens* was sampled at 76 different locations for which

the latitude, longitude and altitude were determined using a GPS device (Fig. 1).

### Environmental data

Environmental variables used in the model were downloaded from WorldClim (worldclim.org). The bioclimatic data were obtained from monthly temperature and rainfall values and consist of 19 variables, which are listed in Table 1. The spatial resolution of these variables is 2.5 arc minutes. Digital Altitude Model (DEM) data were downloaded from USGS Earth Explorer. The soil type is a categorical variable and was obtained from data.mendeley.com (Nkwunonwo et al. 2020). ArcGIS 10.2.1 software was used for all the conversions and analyses used for the MaxEnt modelling.

It is likely that the bioclimatic variables are highly correlated with one another, which can affect the performance of the model. Therefore, a multicollinearity test was conducted, according to Pradhan (2016), using ENM Tools and based on Pearson correlation coefficients ( $r$ ). The VIFs (Variance Inflation Factor), which were obtained using the formula  $1/(1 - r^2)$ , were analysed using Excel 2015 software. Errors due to multicollinearity were screened out using the standardized values of  $r > 0.8$ ,  $r^2 > 0.8$  and VIF value  $> 10$ , but values of  $r > 0.8$  and VIF  $> 10$  were also considered following Pradhan (2016). The

Multicollinearity test resulted in five bioclimatic variables being selected (Table 2).

**Table 1** Description of bioclimatic variable prior to the Multicollinearity test.

Code	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal variation in temperature (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) ( $\times 100$ )
BIO4	Seasonal range in temperature (standard deviation $\times 100$ )
BIO5	Max Temperature in Warmest Month
BIO6	Min Temperature in Coldest Month
BIO7	Annual range in temperature (BIO5-BIO6)
BIO8	Mean Temperature in Wettest Quarter
BIO9	Mean Temperature in Driest Quarter
BIO10	Mean Temperature in Warmest Quarter
BIO11	Mean Temperature in Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation in Wettest Month
BIO14	Precipitation in Driest Month
BIO15	Seasonal variation in Precipitation (Coefficient of Variation)
BIO16	Precipitation in Wettest Quarter
BIO17	Precipitation in Driest Quarter
BIO18	Precipitation in Warmest Quarter
BIO19	Precipitation in Coldest Quarter

**Table 2** Bioclimatic variables remaining after the Multicollinearity test.

Code	Description
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal variation in temperature (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) ( $\times 100$ )
BIO8	Mean Temperature in Wettest Quarter
BIO19	Precipitation in Coldest Quarter

The Soil type and DEM were included in a raster and then converted to an ASCII file with the same extent and cell size using ArcGIS 10.2.1. The soil types were categorized based on their texture. The ArcGIS 10.2.1 was used to produce a map of the locations of the sites sampled, along with the density, soil type and kriging interpolation.

### Modelling

MaxEnt (Maximum entropy) version 3.4.1 was used to determine the potential distribution of *H. suaveolens* in Nigeria. Of the various models, the predictions of the MaxEnt model are often the most precise, possibly because they are based on applications, such as domain, and bioclimatic and logistic regressions (Hijmans and Graham 2006; Phillips and Dudik 2008). MaxEnt evaluates the habitat suitability of a targeted species by analysing the maximum entropy of the environmental variables of

species occurrence data. (Phillips et al. 2006). Even when there are data for only a few occurrences, this model still gives an accurate prediction. The MaxEnt generated continuous outputs with ten replicates using subsample replication and the averages of the replicates are presented. A maximum of 1000 iterations was allowed.

### Model evaluation

The curves of the species responses to predictors predicted by the model were analysed to determine the patterns. Jackknife test was used to assess the contributions of individual variables to the MaxEnt predictions. In the Jackknife test, the training loss/gains for all the variables, without the specific variable, and with each variable in isolation, were used to evaluate the model. This was done for all the predictor environmental variables used in the model (Padalia et al. 2014) Receiver operating characteristic (ROC) area under curve (AUC) method was used to determine the performances of the MaxEnt model (Shabani et al. 2018). The AUC provides a single measurement of model performance, independently of any particular choice of threshold. The value of the AUC ranges from 0 to 1, indicating the model performance. This method is widely used for comparing the performances of species distribution models (Padalia et al. 2014).

### Spatial distribution

The occurrence data for *H. suaveolens* was converted to Shp (Shapefile) and used to map the density of occurrence on-site and kriging interpolation generated for density.

### Results

The soils at locations where *Hyptis suaveolens* was recorded in Nasarawa state, Nigeria, varied from sandy clay-to-clay loam (Fig. 2). Considering the density of occurrence of *H. suaveolens*, the results show that the density of this plant ranged from 9–11 plants/m<sup>2</sup> at the six areas studied (Fig. 3). However, the highest density occurred in Awe LGA and lowest in some parts of Lafia, Doma, Obi and Keana (Fig. 4). The AUC test ranged from 0.972–0.991 and Training AUC ranged from 0.985–0.992 (Fig. 5). The average AUC for the replicate runs is 0.982 and the standard deviation is 0.007, which indicate that MaxEnt prediction of the distribution of *Hyptis suaveolens* in Nigeria is accurate (Fig. 5). The Maxent model revealed that the omission rate of the training and test portions of the data is zero. This indicates that this model correctly predicts the presence *H. suaveolens* in the areas that are 100% suitable for its invasion. The average AUC of the ROC for the training and test data of the MaxEnt model is 0.988 and 0.982, respectively, whereas that of random prediction is 0.5. This reveals that this model accurately discriminates between the suitable and unsuitable habitats for *H. suaveolens*.



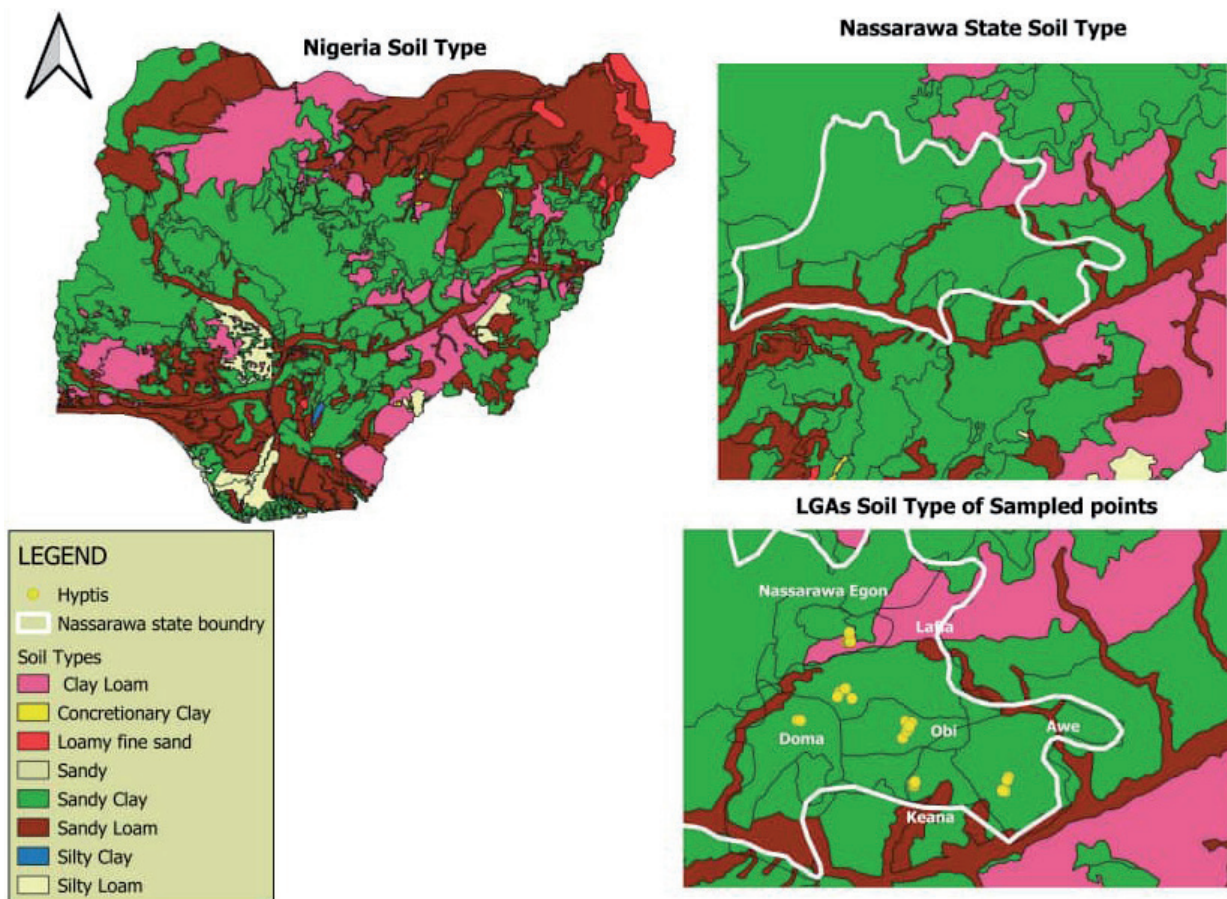


Fig. 2 The distribution of the different types of soil at localities where *H. suaveolens* occurred in the area studied.

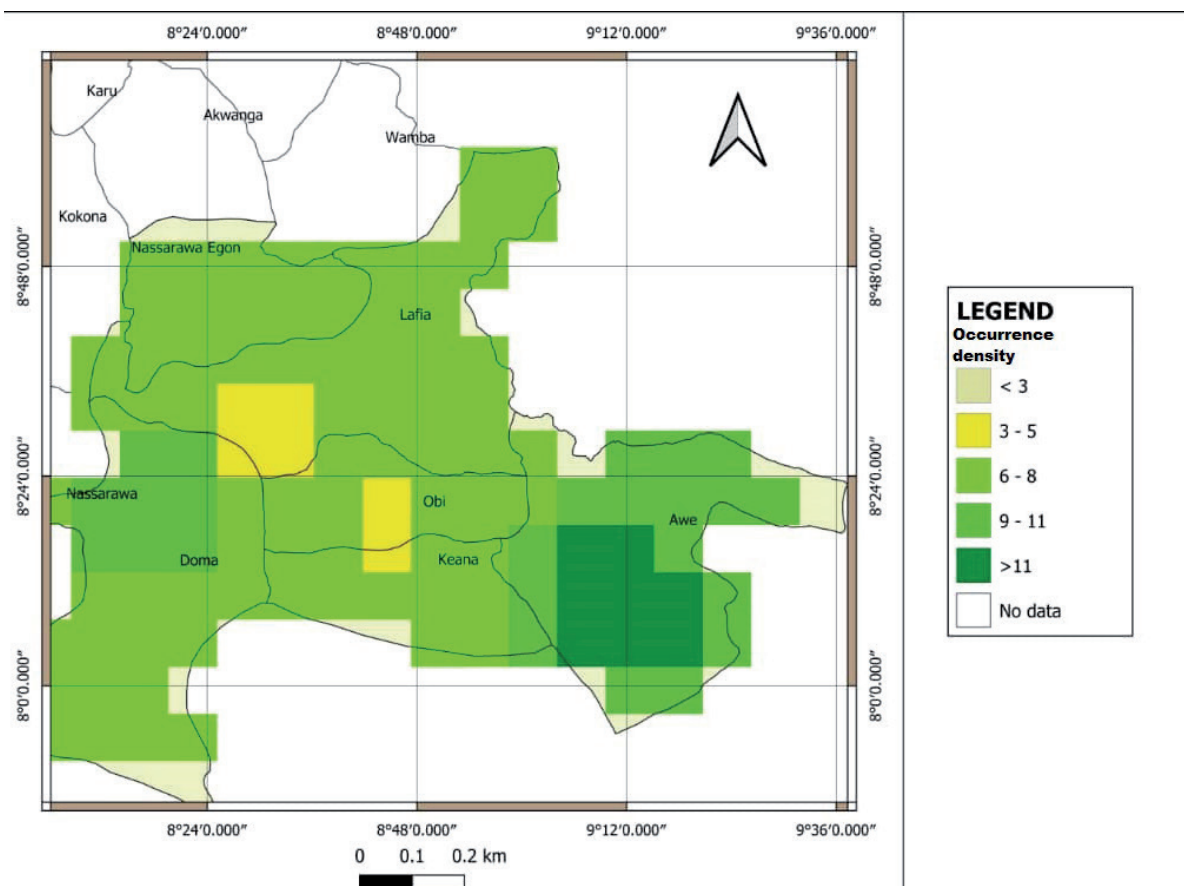


Fig. 3 The density of occurrence of *H. suaveolens* in particular areas in Nasarawa State, Nigeria.

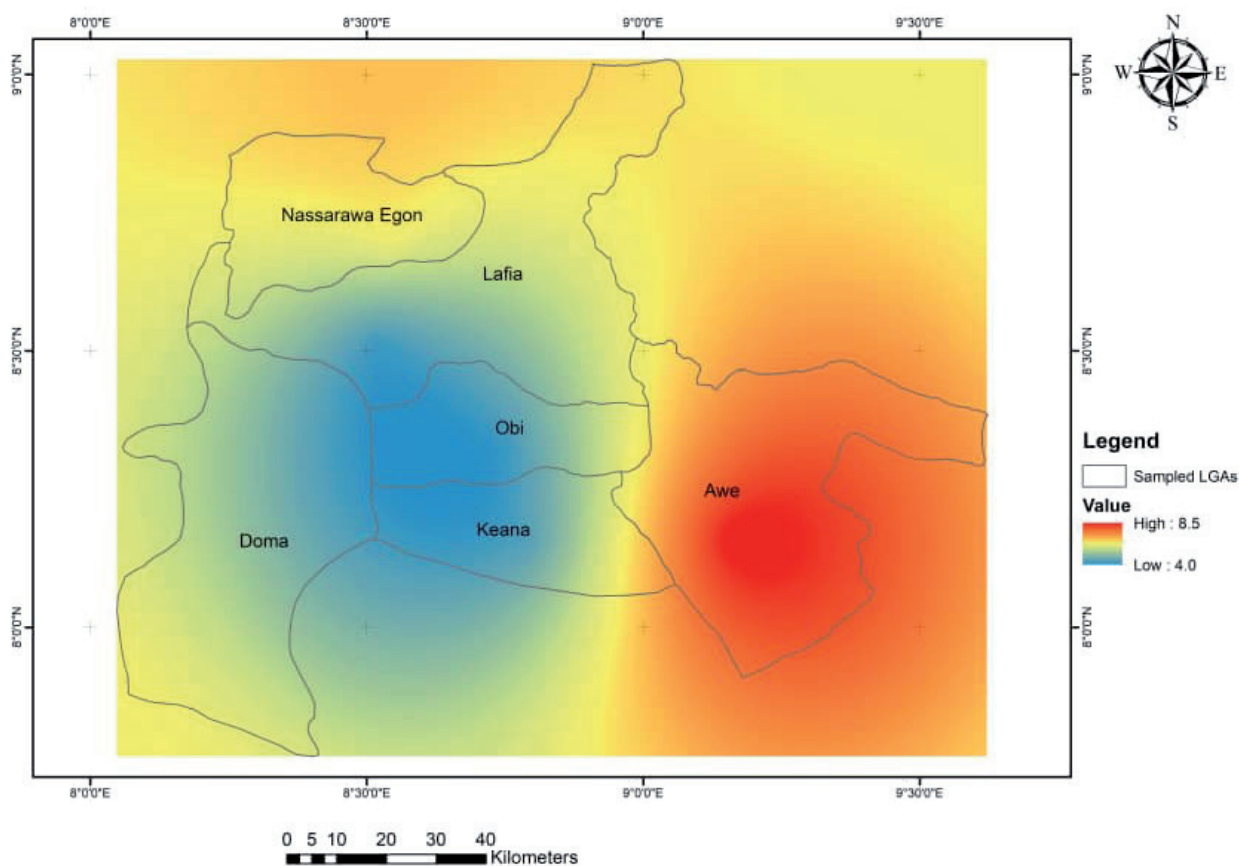


Fig. 4 Kriging Interpolation of the density of occurrence.

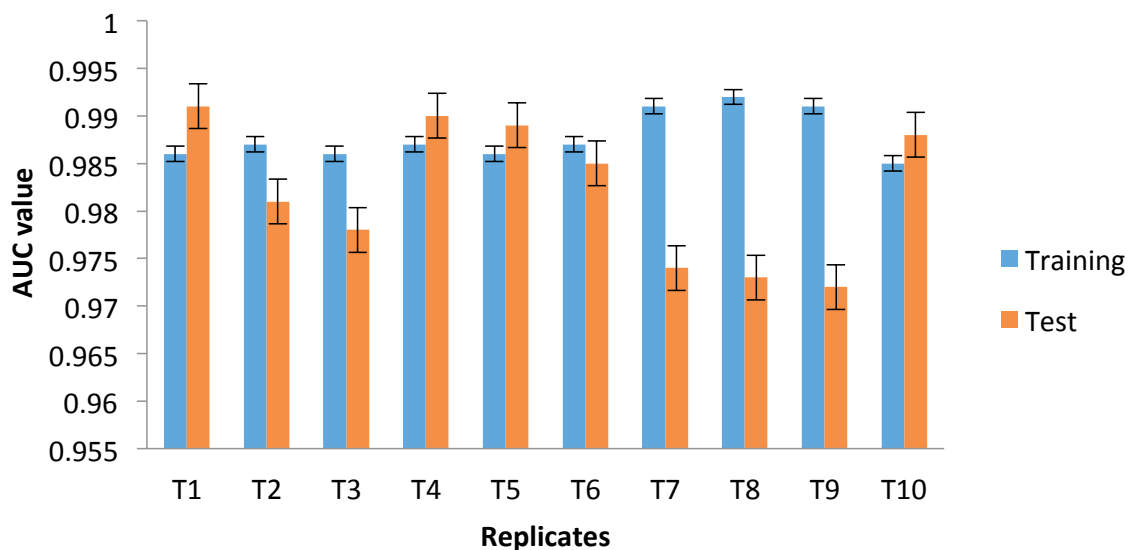


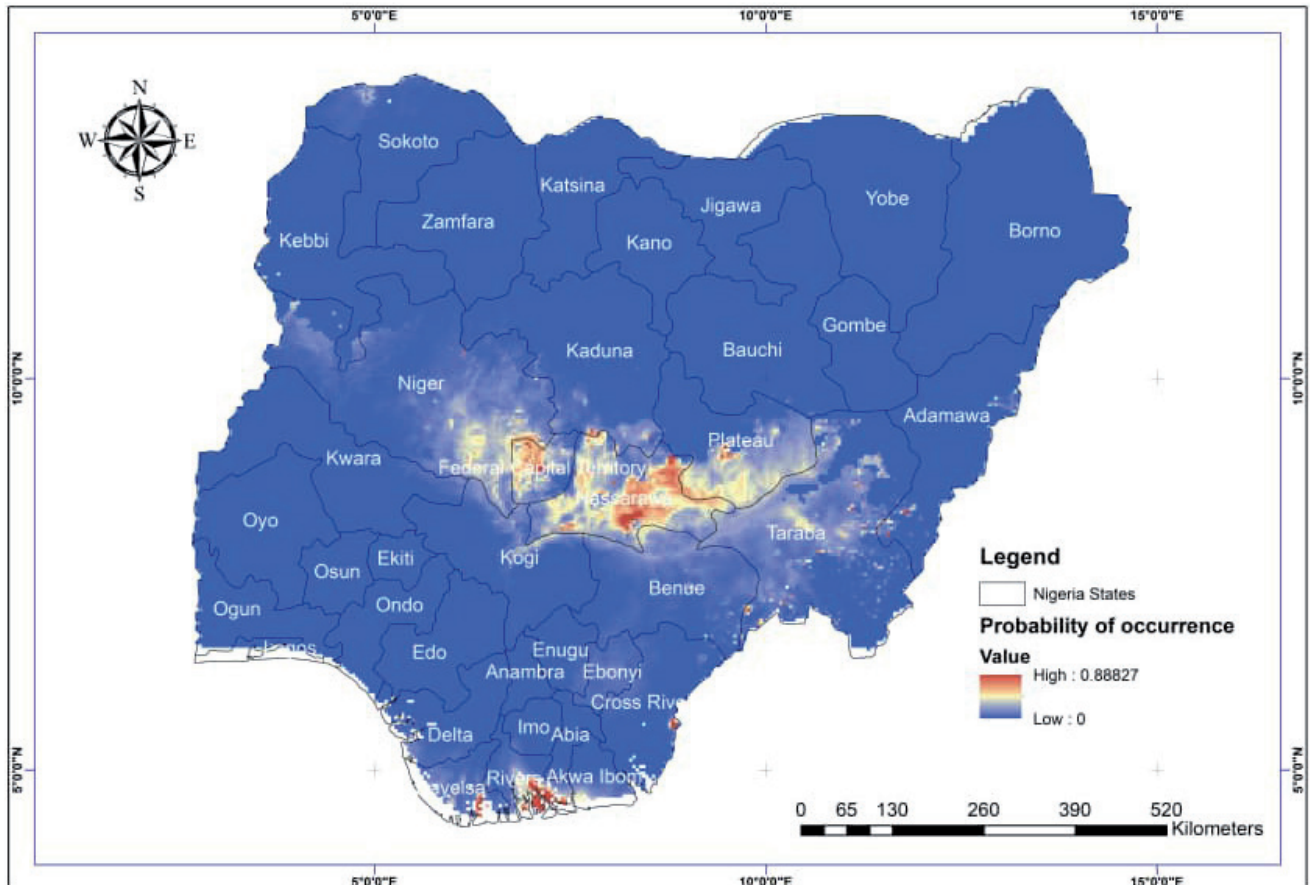
Fig. 5 Evaluation of the Test and training AUC values of the ten-cross-model validations.

The model can be used to predict with a probability of between 0.62–1.00 those areas in Nigeria that are suitable for *H. suaveolens*. The nine areas predicted to be most suitable for *H. suaveolens* in Nigeria include Abuja (FCT), Nasarawa, Plateau, Taraba, Adamawa, Cross River, Bayelsa Rivers and Abia (Fig. 6). Of the nine states, three are in the North Central, 2 in the North East, 3 in the South-South and 1 in the southeast zone (Table 3). All the other states in Nigeria are predicted to be less suitable

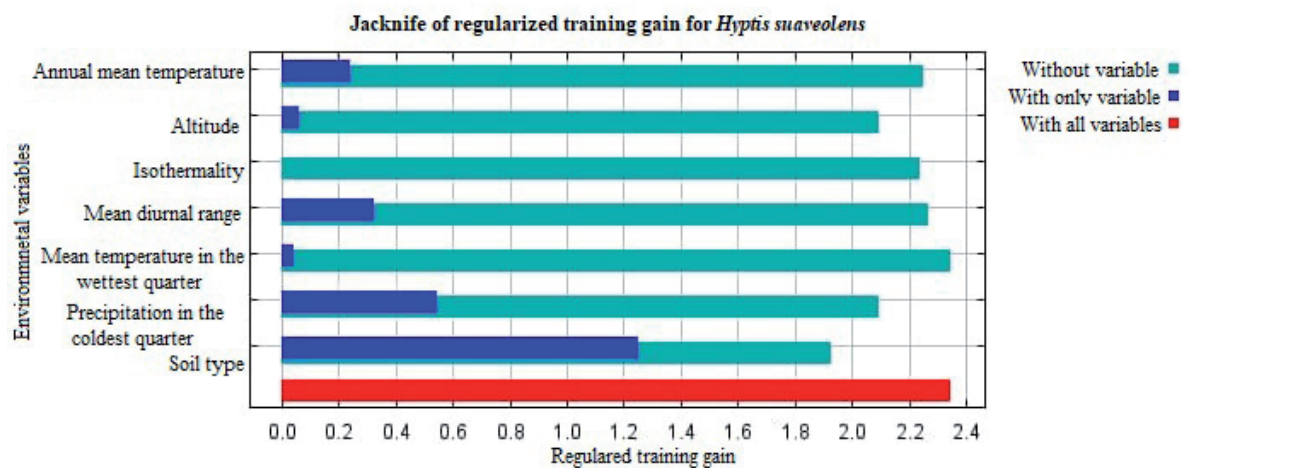
for this plant. It is predicted that the highest percentage of the land (47.5%) likely to be invaded by *H. suaveolens* in Nigeria is in Nasarawa State, followed by Rivers and Abuja, with 25% (Table 3). The least affected State is Adamawa for which the prediction is 1.49%. The Jackknife analysis used to identify the contributions of each of the predictor abiotic variables revealed that isothermality on its own was not important (Fig. 7). Soil type was the only variable that was important on its own. The effects of all

**Table 3** Areas of land predicted to be affected by *H. suaveolens* in Nigeria.

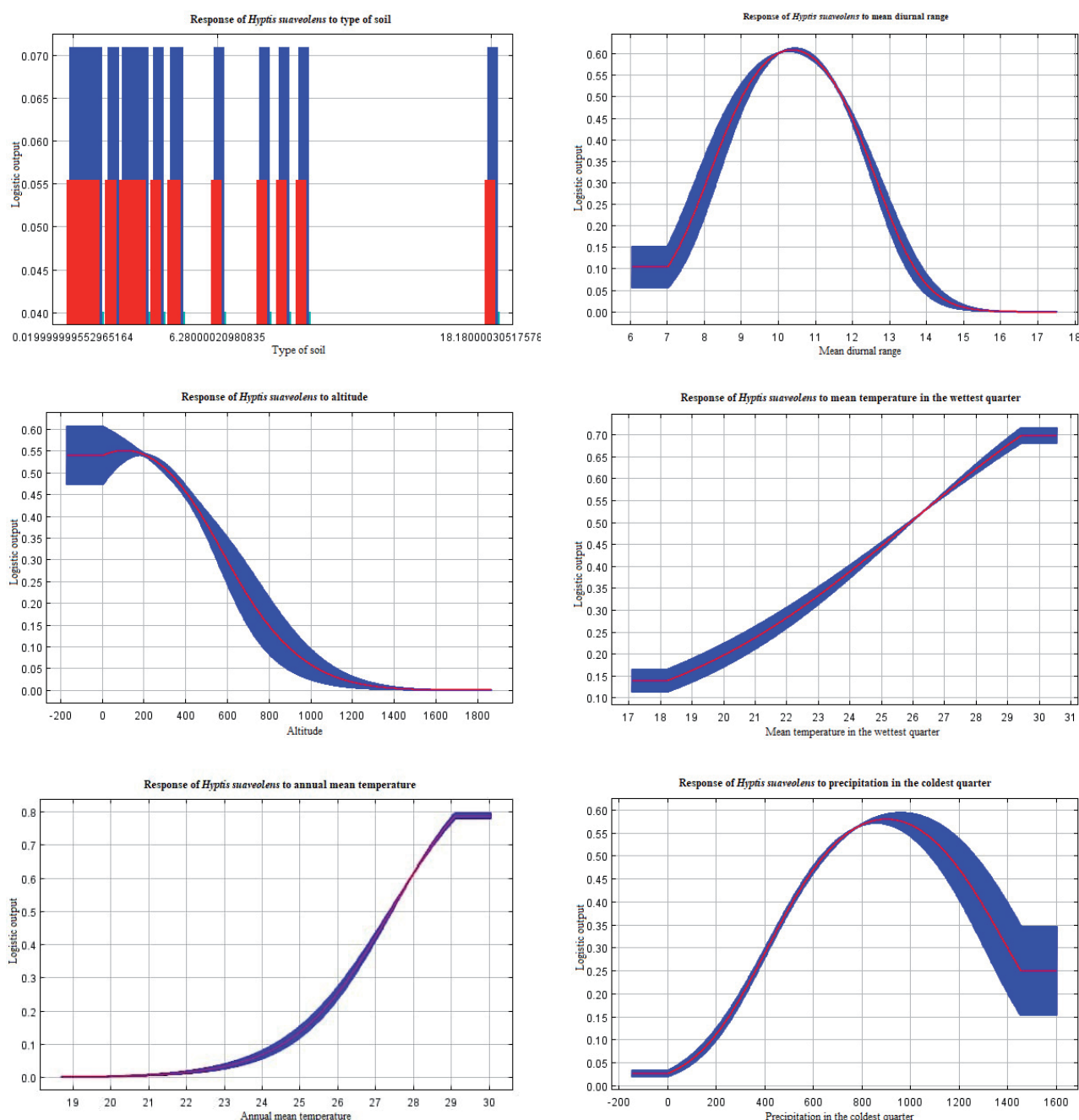
S/N	Name of State	Geopolitical Zone	Total Area (km <sup>2</sup> )	Area predicted to be affected (km <sup>2</sup> )	Percentage of the area affected (%)
1	Abia	South East	4723.46	429.41	9.09
2	Abuja (FCT)	North Central	7353.15	1838.29	25
3	Adamawa	North East	34323.63	512.29	1.49
4	Bayelsa	South-South	10173.35	678.22	6.67
5	Cross River	South-South	21153.35	515.94	2.44
6	Nasarawa	North Central	26313.58	12500	47.5
7	Plateau	North Central	27562.53	1968.75	7.14
8	Rivers	South-South	9100.54	2275.14	25
9	Taraba	North East	60115.8	1134.3	1.89



**Fig. 6** Map showing the predicted probability of occurrence of *H. suaveolens* in Nigeria.



**Fig. 7** Jackknife plots of the training data.



**Fig. 8** The responses of *H. suaveolens* to (A) type of soil, (B) mean diurnal variation in temperature, (C) altitude, (D) mean temperature in the wettest quarter, (E) annual mean temperature and (F) precipitation in the coldest quarter. Red curves = mean response and blue margins =  $\pm 1$  SD based on 10 replicates.

the variables based on their respective contributions are depicted in Figure 7. These are regarded as the most important predictors of the areas likely to be invaded by *H. suaveolens* in Nigeria. Their individual percentage contributions to the prediction are: soil type (52.08%), mean diurnal variation in temperature (12.5%), precipitation in the coldest quarter (22.08%), annual mean temperature (9.5%), mean temperature in the wettest quarter (2.08%) and altitude (3.7%). Probability of *H. suaveolens* occurring at high altitudes (above 1000 m) was  $< 0.05$  and for those occurring between 200–800 m ranged between 0.15–0.5. The probability of its occurrence ( $> 0.5$ ) increased with increase in the mean temperature in the

wettest quarter (26 °C to 30 °C). Favourable precipitation in the coldest quarter ranged from 200–800 m. The probability of an area being invaded by *H. suaveolens* ( $> 0.5$ ) increases when the mean diurnal variation in temperature is  $> 0.5$ , photoperiod less than 10 hours and the isothermal is 50–60 J K<sup>-1</sup> (Fig. 8).

## Discussion

*Hyptis suaveolens* in areas already invaded is abundant and spreading rapidly, resulting in the homogeneity of floras, reduced biodiversity and a reduction in the ecolog-

ical integrity of the native habitats and ecosystems functioning (Hulme et al. 2003; Borokini and Babalola 2012). It has caused a 46–52% decrease in species evenness in the periurban ecosystems in Chandigarh, north-western India (Sharma et al. 2017). There are invasive potential hotspots of *Hyptis suaveolens* in Abia, Adamawa, Bayelsa, Cross River, Nasarawa, Plateau, Rivers, Taraba and Rivers States, especially between latitude 8°52'N and 12°07'E in areas with derived savannah, which have suitable climatic conditions. Derived savannah is disturbed forest, which was cleared mechanically, or by burning or manual hand-weeding for agricultural purposes, which – when abandoned – are colonized by grasses. Recurrent fires and overgrazing are also frequent in this ecological zone. *Hyptis suaveolens* is described as an invader of grassland. This weed grows vigorously in agricultural fields, rangelands, pasture, floodplains, open woodlands, roadsides and disturbed areas (Raizada 2006; Padalia et al. 2014). It is predicted that the Federal Capital Territory (FCT) and Rivers State has suitable eco-climatic conditions and is very likely to be invaded by this plant, which could seriously reduce the biodiversity in this humid forest zone. This is because *H. suaveolens* thrives in a wide range of climatic conditions. The ecological amplitude and seed dimorphism of this species enables it to germinate over a wide range of temperatures, which results in year-round seed germination (Raizada 2006). The seeds of *H. suaveolens* are capable of germinating at temperatures between 10 °C and 40 °C, but 25–30 °C seems to be the optimum for growth (Felippe et al. 1983).

Importantly, this plant prefers sandy clay and sandy loam soils, which further broadens the range conditions in which it can survive. This may enable it to thrive in new environments such as Benue, Kogi, Niger, Kaduna and Bauchi States, in the future. Similarly, *H. suaveolens* thrives in sandy soil with a high pH, low fertility and is well-drained (Aboh et al. 2017), which with climate change could result in it invading new habitats (Padalia et al. 2015).

The results also indicate that the mean temperature in the wettest quarter, annual mean temperature, altitude, diurnal variation in temperature and precipitation in the coldest quarter were the most important bioclimatic factors determining the survival and growth of *H. suaveolens* in Nigeria. Low temperature and low moisture environments limit its invasive potential worldwide (Padalia et al. 2014). It is well known that other climatic factors, such as moisture balance and extreme temperatures, determine plant distribution (Stephenson 1990; Widrlechner et al. 2012; Zhang et al. 2020). Several studies report the effect of moisture and extreme temperatures on the physiological characteristics of plants, such as growth, yield, flowering and tolerance (Tandazo-Yung et al. 2017; Song et al. 2018).

Type of soil, diurnal variation in temperature, altitude, precipitation and temperature are significant factors determining the distribution of the targeted plant. In addition, areas with lower mean diurnal variation in

temperature at low altitudes are more prone to being invaded. High abundances of *H. suaveolens* in Nigeria occur mainly at altitudes between 200–800 m, where the temperature is between 26 °C and 30 °C, rainfall between 200–800 mm with a photoperiod of 9–10 hours. Probability of occurrence is very low at altitudes above 1500 m, where conditions are too cold for this species (Padalia et al. 2014). It occurs at an altitude ~1560 m in its native range in Venezuela, (GBIF 2013) and has invaded the central highlands at an altitude ~850 m in India (Padalia et al. 2014). It prefers wet and warmer environments, as it is an annual herbaceous plant originating from the tropics (Padalia et al. 2015). It is a short-day plant with optimum growth at temperatures ranging between 25 and 30 °C (Raizada 2006; Barbosa et al. 2013).

In this study, the MaxEnt model was used to predict the distribution of *H. suaveolens* in Nigeria. This revealed that abiotic factors such as type of soil, mean temperature in the warmest quarter, precipitation in the coldest quarter, altitude and diurnal variation in temperature are the most important factors determining its occurrence and distribution in Nigeria. Among these factors, type of soil was the most important. The model predicted a high occurrence of *H. suaveolens* in derived savannah in Nigeria (FCT, Nasarawa, and Plateau States) and because of its broad ecological tolerance a future occurrence in humid forest areas (Rivers and Cross Rivers States) in Nigeria, which would be a major threat to the native vegetation in humid forest. This invasion seriously threatens the survival of native plants in the most suitable areas, therefore, there is an urgent need to stop the spread and control the abundance of *H. suaveolens* in Nigeria.

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# HIGH BIODIVERSITY IN A CITY CENTRE: ODONATOFAUNA IN AN ABANDONED LIMESTONE QUARRY

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## ABSTRACT

Limestone quarries are known to be places where the diversity of xerothermophilic organisms is promoting diversity and in some, there are water bodies that potentially support the presence of hydrobionts. These include dragonflies (Insecta: Odonata), which, as amphibiotic insects, use both aquatic and terrestrial habitats. The purpose of this paper was to determine whether there was a high diversity of odonatofauna in an old limestone quarry with well-developed aquatic habitats, located in an urban environment in the Central-European city of Kraków (S Poland). For this purpose, dragonflies in the quarry were monitored regularly, focusing on the reproductive status and relative abundance of each species. In 2017–2018, 37 species belonging to seven families of Odonata were recorded in the quarry, which is 50% of the Poland's odonatofauna. Of these, 33 species were considered indigenous to the quarry. Among them, 30% were moderately urbanophobic or urbanophobic taxa. Habitat specialists made up 39% of the species. Some rare and declining species, i.e. *Leucorrhinia pectoralis* and *L. rubicunda*, were abundant at this site. The study shows that a well-preserved secondary habitat, located in the centre of a city and not subject to urban management, can support a high diversity of odonates. Such limestone quarries in highly transformed urban environments can be valuable sites for this indicator group of organisms and should be identified, evaluated and conserved.

**Keywords:** anthropogenic habitat; damselflies; dragonflies; novel ecosystem; Odonata assemblage; secondary habitat; urban environment

## Introduction

Progressive urbanization is considered to be one of the most important threats to biodiversity in the world (Grimm et al. 2008). In an increasingly degraded world, replacement (secondary) habitats are becoming increasingly important for maintaining local biodiversity (Tropek et al. 2010; Chester and Robson 2013; Buczyński 2015; Aronson et al. 2017). Their role is particularly important in strongly transformed urban environments, in which, among others, the size and character of patches of well-preserved habitats, their connectivity and diversity of management and history determine the level of biodiversity they support (Beninde et al. 2015; Aronson et al. 2017).

Examples of valuable replacement habitats are mineral extraction sites, such as quarries, mines, gravel- and sandpits (Chester and Robson 2013). During exploitation, such places undergo significant transformation in terms of the surface, soil, vegetation, water relations and pollution resulting in the destruction of the local ecosystem. However, when abandoned, they undergo spontaneous transition and develop valuable secondary habitats, which are colonized by numerous, often specialized species, typical of the early stages of ecological succession (Bétard 2013). Particularly high levels of biodiversity are present in abandoned limestone quarries, which despite their anthropogenic and replacement character, are often colonized by rich and diverse flora and fauna, including many of high conservation status or even by endangered species (Tropek et al. 2010). This is particularly well documented in the case of petrophilic and xerothermic organisms (Jefferson 1984; Beneš et al. 2003; Tropek and Konvicka 2008; Krauss et al. 2009; Tropek et al. 2010), which are favoured by rocky, calcium-rich soils and

strong insolation. However, organisms associated with the small water bodies that form in abandoned quarries are less well studied. These include dragonflies, which have high dispersal abilities and quickly colonize newly created water bodies (Conrad et al. 1999). What is more, dragonflies are bioindicators of the quality of both stagnant and flowing water (Bulánková 1997; Osborn 2005; Kutcher and Bried 2014; Kietzka et al. 2018). This also applies to urban landscapes and dragonflies are a good model group for determining the effect of urbanization on biodiversity (Villalobos-Jiménez et al. 2016).

Although there are quite a few papers on dragonflies in abandoned limestone quarries (e.g. Rudolph 1976; Koepfel et al. 1995; Kuňka et al. 2008; INULA 2011; Fröhlich et al. 2012; Czerniawska-Kusza and Brożonowicz 2014; Gwardjan et al. 2015) there is still a paucity of detailed studies on odonate biodiversity in such habitats. It is postulated that the main limitation on considering bodies of freshwater of anthropogenic origin as refugia for biodiversity is the lack of recognition of their biocenotic value (Chester and Robson 2013). The significance of artificial waterbodies is underestimated in many biodiversity management programs and are often destroyed in the course of standard water management practices and urban development, which do not take into account their ecological importance (Chester and Robson 2013). The biocenotic role of water bodies located in city centres is particularly interesting. On one hand, they constitute a distinctive element enhancing diversity in homogenous, urbanized landscapes, and on the other, compared to natural biotopes, their colonization by organisms may be more difficult, even for dragonflies. This is related to the fact that urban surface waters are often characterized by high levels of pollution, high levels of

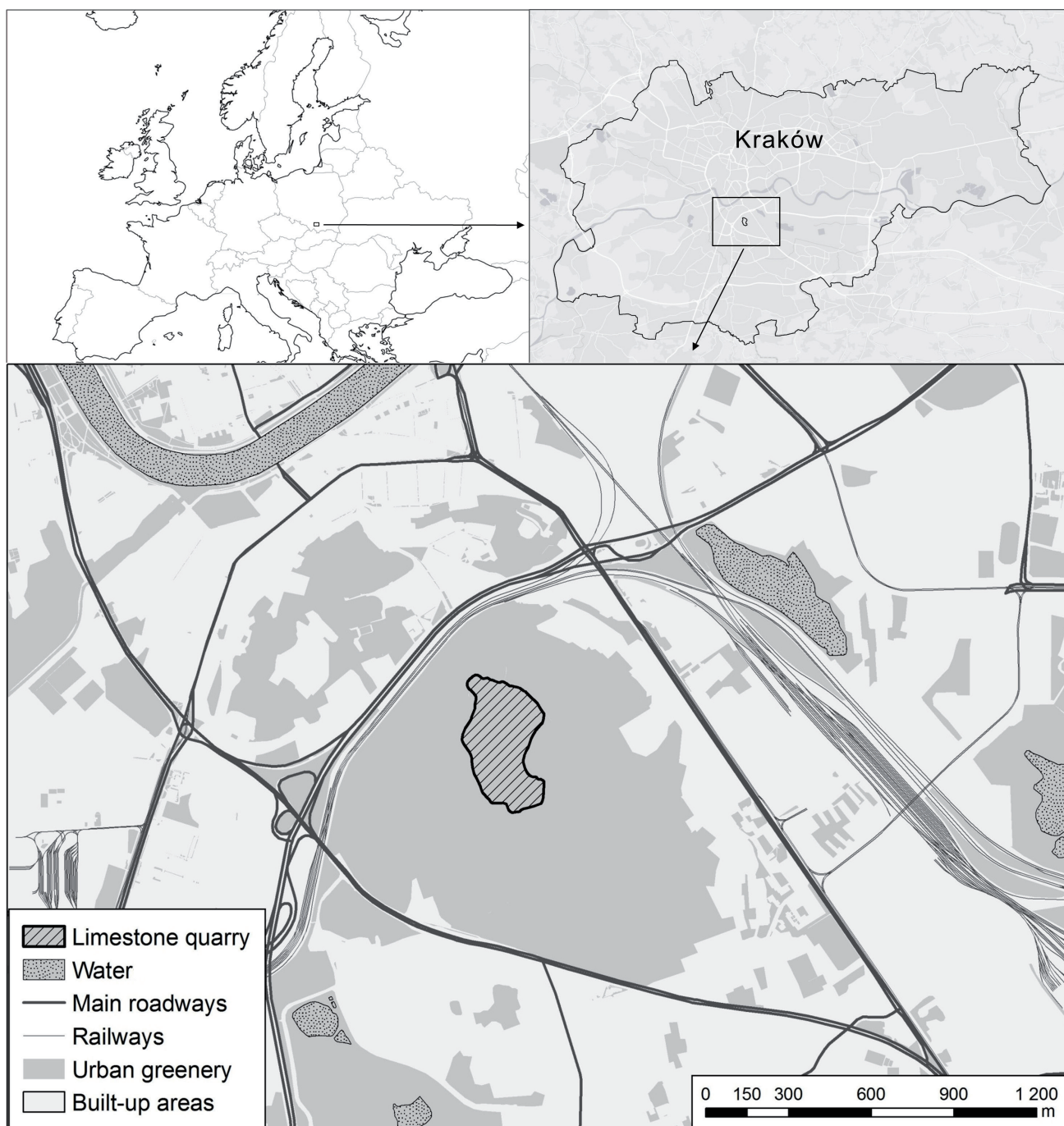
nutrients, poor and structurally undifferentiated vegetation or concrete sides (Vermondén et al. 2009; Buczyński and Lewandowski 2011). What is more, these secondary habitats are sometimes considered to be ecological traps, in which reproduction is ineffective, as the incidents of colonizations and extinctions are frequent in such habitats (Harabiš and Dolný 2012). It is not surprising, therefore, that in cities, which are usually poor in water habitats, increasing urbanization results in loss in species diversity of dragonflies (Willigalla and Fartmann 2012). Studies on urbanization (rural-urban) gradients clearly show that the diversity of dragonfly assemblages decrease towards inner city areas (review in: Villalobos-Jiménez et al. 2016). The aim of this paper was to check whether

a limestone quarry located in an urban environment in the core zone of a large city, can maintain a high species richness of odonatofauna. In addition, an attempt is made to assess whether this type of habitat could act as a refugium for water insects at local and supralocal scales.

## Material and Methods

### Study site

The research area is located in southern Poland, in Kraków, a city of around 800,000 inhabitants, covering an area of over 325 km<sup>2</sup> (Fig. 1). The research was carried out in an abandoned “Liban” limestone quarry, located in



**Fig. 1** Maps showing the location of the limestone quarry studied.



the historical district in the central part of the city, only 3 km from the main square of the old town and 1 km from the Vistula river. The quarry covers an area of 10 ha and is bounded by almost vertical, rocky slopes. Limestone was excavated from here from the 14th century until 1986. At present, this site is not economically used or managed for any urban purpose and is undergoing slow and gradual ecological succession (Górecki and Seremet 2010). Significant parts of the quarry are subject to tourist pressure as they are popular places for Kraków's residents and tourists to walk. The quarry and its surroundings (the Krakus Mound, cemetery and allotments, geological nature reserve, historical site etc.) constitute an "island of greenery" in a dense urban development with many routes carrying heavy traffic (Fig. 1).

The quarry is characterized by high habitat diversity, with water, marsh and dry biotopes, constituting a complex mosaic of habitats. The character of this mosaic is shaped by significant fluctuations in the water level throughout the year and between years, resulting in a large inundation zone. In wet periods, many water-filled cavities and depressions in the bottom of the quarry combine and form a flooded area of about 2 ha. The water reservoirs are shallow and have rocky bottoms, covered by a thin layer of silt. They are astatic, which means most of them dry out completely in dry periods. However, two pools are deeper and more permanent, retaining water even during droughts. Most of the water biotopes are strongly insolated and heat up quickly over the course of a season, although there are also parts shaded by vegetation. The water is clear, which indicates a low nutrient content. There is a lack of naturally occurring fish there, but there are introduced goldfish, *Carassius auratus*.

All of the water bodies except one are characterized by well-developed and diverse submerged and emergent vegetation. The elodeids are mainly *Myriophyllum* spp., *Ceratophyllum* spp., *Potamogeton lucens*, *Batrachium* spp. and water mosses. Nimfeids, occur in the deeper parts and are mainly *Potamogeton natans* and *Polygonum amphibium*. Extensive areas are occupied by helophytes, mostly *Juncus* spp., *Schoenoplectus* spp., *Eleocharis* spp., *Carex* spp., *Typha latifolia*, *T. angustifolia* and *Phragmites australis*, which are accompanied by species such as *Alisma plantago-aquatica*, *Solanum dulcamara* and *Lythrum salicaria*. The area surrounding the water bodies is overgrown with meadow, grassland and rock communities (wet as well as xerothermic), and large areas are also occupied by willow bushes, *Salix* spp. Willows definitely dominate the tree layer, which also includes *Betula pendula* and *Populus tremula* mainly in the higher and non-flooded areas. The habitat mosaic also includes the surfaces of bare rock and steep limestone walls along with the rocky heaps and rubble at the bases of these walls.

### Sampling and data analysis

Fieldwork in the quarry was carried out in 2017 and 2018. In 2017, between April 20 and August 18, there

were 7 visits (1–2 in a month) to the quarry and in 2018, 19 visits (on average every 10–14 days) between April 15 and October 14. Each visit took from 1 h 15 min to 5 h 10 min (average 3 h 44 min). Altogether, the 26 visits lasted a total of 97 h 25 min. Observations were made mainly during the period when the adult odonates were most active, which was between 11:30 and 15:30 h CEST (UTC + 02:00), usually on bright and sunny days, when it was not raining, there was low cloud cover and light wind.

The quarry was sampled each time along a similar route, when adult odonates were observed (with the naked eye and using binoculars) and, when necessary, the identification of the species was confirmed by catching individuals using a sweep net. Many observations were also photographically documented. In addition, exuviae were also collected. On each visit, information was collected on the reproductive behaviour and the relative numbers of each species. Each type of behaviour recorded was classified in terms of the probability of it indicating a species had reproduced at the quarry, as unconfirmed, probable or confirmed (Table 1). On the basis of all the field visits, the highest category of behaviour was determined for each species. Indigenous species were considered to be those, for which the reproduction category was probable or confirmed. A relative abundance index was used to determine the quantitative relationships between particular species. This index indicates the minimum number of dragonflies observed during a visit, for which there was no doubt that they were different individuals. The highest category of the relative number of individuals recorded in a visit (Table 1) was used as a general index of abundance for each species, regardless of the numbers recorded on other visits.

### Results

In 2017–2018, 37 species of odonates were recorded in "Liban" quarry, including 16 belonging to the suborder Zygoptera and 21 to the suborder Anisoptera (Table 1) belonging to seven families: Calopterygidae (2 species), Platycnemididae (1), Lestidae (5), Coenagrionidae (8), Aeshnidae (8), Corduliidae (1) and Libellulidae (12). Of these species 28 were confirmed as reproducing and five as probably reproducing there. That is, 33 species (12 Zygoptera and 21 Anisoptera) were considered to be indigenous to the quarry, although these species differed in their reproductive behaviour (Table 1). For the four remaining species (*Calopteryx splendens*, *C. virgo*, *Platycnemis pennipes*, *Pyrrhosoma nymphula*) there was no evidence that they reproduced in this quarry (Table 1), and were considered to be non-indigenous, vagrant species. Thus the odonates that reproduced in the quarry belonged to five families.

The seven most abundant odonates recorded are generally very numerous and formed the core of the local odonatofauna. These included the Zygoptera: *Coenagri-*

**Table 1** List of Odonata species recorded in the “Liban” quarry in Kraków in the years 2017–2018. The status of reproduction, behaviour and relative abundance are provided for each species. Reproduction was considered unconfirmed (REC – adult recorded), probable (TER – territorial behaviour, JUV – juvenile, TAN – tandem, COP – copulation ring) or confirmed (OVI – oviposition, EXU – exuvia, TEN – teneral imago). The code ‘REC’ is omitted for species for which any higher code of behaviour was recorded. Five categories of abundance were used: (i) 1 (single), (ii) 2–10 (scarce), (iii) 11–20 (fairly numerous), (iv) 21–50 (numerous), (v) >50 (very numerous). These terms are used throughout the text. The last column contains the classification (according to Willigalla and Fartmann 2010) of species based on the strength of their connection with urban environments.

No.	Species	Codes of behaviour	Reproduction	Abundance	Urban environment connection
1.	<i>Calopteryx splendens</i> (Harris, 1782)	REC	unconfirmed	scarce	urbanoneutral
2.	<i>Calopteryx virgo</i> (Linnaeus, 1758)	REC	unconfirmed	single	urbanoneutral
3.	<i>Lestes dryas</i> (Kirby, 1890)	TEN, OVI, COP, TAN, JUV	confirmed	very numerous	urbanoneutral
4.	<i>Lestes sponsa</i> (Hansemann, 1823)	TEN, OVI, COP, TAN, JUV, TER	confirmed	very numerous	urbanoneutral
5.	<i>Lestes virens</i> (Charpentier, 1825)	TEN, OVI, COP, TAN, JUV	confirmed	very numerous	moderately urbanophobic
6.	<i>Chalcolestes viridis</i> (Vander Linden, 1825)	OVI, COP, TAN	confirmed	scarce	moderately urbanophilous
7.	<i>Sympetma fusca</i> (Vander Linden, 1820)	TEN, OVI, COP, TAN, JUV	confirmed	numerous	urbanoneutral
8.	<i>Platycnemis pennipes</i> (Pallas, 1771)	REC	unconfirmed	scarce	urbanoneutral
9.	<i>Ischnura elegans</i> (Vander Linden, 1820)	TEN, COP, JUV	confirmed	very numerous	moderately urbanophilous
10.	<i>Ischnura pumilio</i> (Charpentier, 1825)	TEN, COP	confirmed	scarce	urbanoneutral
11.	<i>Enallagma cyathigerum</i> (Charpentier, 1840)	OVI, COP, TAN, JUV	confirmed	numerous	urbanoneutral
12.	<i>Coenagrion puella</i> (Linnaeus, 1758)	TEN, OVI, COP, TAN, TER	confirmed	very numerous	moderately urbanophilous
13.	<i>Coenagrion pulchellum</i> (Vander Linden, 1825)	OVI	confirmed	scarce	moderately urbanophobic
14.	<i>Erythromma najas</i> (Hansemann, 1823)	TEN, OVI, COP, TAN, TER	confirmed	numerous	urbanoneutral
15.	<i>Erythromma viridulum</i> (Charpentier, 1840)	OVI, TAN, TER	confirmed	fairly numerous	urbanoneutral
16.	<i>Pyrrhosoma nymphula</i> (Sulzer, 1776)	REC	unconfirmed	single	urbanoneutral
17.	<i>Brachytron pratense</i> (O. F. Müller, 1764)	TER	probable	scarce	moderately urbanophobic
18.	<i>Aeshna affinis</i> (Vander Linden, 1820)	COP, TER	probable	fairly numerous	moderately urbanophobic
19.	<i>Aeshna cyanea</i> (O. F. Müller, 1764)	EXU, OVI, TER	confirmed	scarce	moderately urbanophilous
20.	<i>Aeshna grandis</i> (Linnaeus, 1758)	OVI, TER	confirmed	scarce	urbanoneutral
21.	<i>Aeshna isocetes</i> (O. F. Müller, 1767)	OVI, TER	confirmed	scarce	moderately urbanophobic
22.	<i>Aeshna mixta</i> (Latreille, 1805)	EXU, TER	confirmed	scarce	moderately urbanophilous
23.	<i>Anax imperator</i> (Leach, 1815)	EXU, OVI, TER	confirmed	fairly numerous	moderately urbanophilous
24.	<i>Anax parthenope</i> (Sélys, 1839)	OVI, TAN, TER	confirmed	scarce	moderately urbanophobic
25.	<i>Cordulia aenea</i> (Linnaeus, 1758)	OVI, TER	confirmed	scarce	urbanoneutral
26.	<i>Libellula depressa</i> (Linnaeus, 1758)	TER	probable	scarce	urbanoneutral
27.	<i>Libellula quadrimaculata</i> (Linnaeus, 1758)	OVI, COP, TAN, TER	confirmed	very numerous	urbanoneutral
28.	<i>Orthetrum cancellatum</i> (Linnaeus, 1758)	OVI, JUV	confirmed	scarce	moderately urbanophilous
29.	<i>Crocothemis erythraea</i> (Brullé, 1832)	OVI, COP, JUV, TER	confirmed	fairly numerous	moderately urbanophobic
30.	<i>Sympetrum danae</i> (Sulzer, 1776)	OVI, COP, TER	confirmed	numerous	urbanoneutral
31.	<i>Sympetrum flaveolum</i> (Linnaeus, 1758)	TER	probable	single	urbanoneutral
32.	<i>Sympetrum meridionale</i> (Sélys, 1841)	TEN, JUV, TER	confirmed	scarce	urbanophobic
33.	<i>Sympetrum sanguineum</i> (O. F. Müller, 1764)	TEN, OVI, COP, TAN, JUV, TER	confirmed	numerous	moderately urbanophilous
34.	<i>Sympetrum striolatum</i> (Charpentier, 1840)	TEN, OVI, COP, TAN, JUV, TER	confirmed	very numerous	urbanoneutral
35.	<i>Sympetrum vulgatum</i> (Linnaeus, 1758)	TEN, JUV, TER	confirmed	single	urbanoneutral
36.	<i>Leucorrhinia pectoralis</i> (Charpentier, 1825)	OVI, COP, TAN, JUV, TER	confirmed	numerous	moderately urbanophobic
37.	<i>Leucorrhinia rubicunda</i> (Linnaeus, 1758)	TER	probable	fairly numerous	moderately urbanophobic

on puella, *Ischnura elegans*, *Lestes sponsa*, *L. dryas* and *L. virens* and Anisoptera: *Libellula quadrimaculata* and *Sympetrum striolatum* (Table 1). Fewer species were categorized as numerous or fairly-numerous (6 and 5 species, respectively). Fifteen species were categorised as scarce and single individuals of four species were recorded on only one visit. There were low numbers of four non-indigenous species, two of which were categorised as scarce and the other two were only recorded as single individuals (Table 1).

## Discussion

### Odonata assemblage in the limestone quarry

In Poland, there are currently 74 species of odonates recorded, 71 of which are indigenous species and breed here (Bernard et al. 2009; Buczyński et al. 2019). Therefore, the 37 species recorded in 2017–2018 in “Liban” quarry, make up 50% of the national odonatofauna. The share of indigenous species is similar with the 33 species breeding or probably breeding at this site making up 46.5% of such species recorded so far in Poland. All the species classified as non-indigenous in the quarry are ecologically associated mainly or exclusively with lotic habitats (Bernard et al. 2009), which are not present in the quarry. The results of irregular observations in the “Liban” quarry carried out previously (Miłaczewska 2019) indicate that there may be several other species of odonates that were not recorded in this study. Although these observations provide little evidence of these species breeding there, an irregular, indigenous occurrence seems possible at least in the case of the thermophilic *Orthetrum albistylum*, *O. brunneum* and *Sympetrum fonscolombii*.

Such a large richness of Odonata species in a single limestone quarry, as recorded at the site studied, is exceptional (Rudolph 1976; Koeppl et al. 1995; Kuńka et al. 2008; INULA 2011; Fröhlich et al. 2012; Czerniawska-Kusza and Brożonowicz 2014; Gwardjan et al. 2015). It is also different from that recorded typically at other types of small water habitats. For example, from 327 small anthropogenic water bodies of different kinds (e.g. fishponds, sandpits, clay pits, excavations in peat bogs, drainage ditches) examined by Buczyński (2015) in central-eastern Poland, only a few (fish ponds and excavations in peat bogs) supported similar or slightly higher (up to 38 species) numbers of indigenous species. It is exceptional to record as many as 40 odonate species at a single site (Stark 1977). The high species richness recorded in this study is particularly surprising, since “Liban” limestone quarry is located in the centre of a large city and surrounded by highly urbanized areas. Moreover, it was revealed during a short two-year study.

Central European dragonflies can be categorised by their degree of association with urban environments as: moderately urbanophilous, urbanoneutral, moderately

urbanophobic or urbanophobic species (Willigalla and Fartmann 2010). The species recorded in the “Liban” quarry categorized in this way is presented in Table 1. Of the 33 indigenous species, 8 are classified as moderately urbanophilous, and 15 as urbanoneutral. Therefore, 70% of all the species of indigenous dragonflies recorded in the quarry are considered to be adapted to urban environments, and for which urbanization is not a significant obstacle to their occurrence. On the other hand, the remaining 10 species (30%) are moderately urbanophobic (9) and urbanophobic (1) odonates. For such species, the colonization of this city environment is hindered by the high degree of anthropogenic transformation of the area. There are similar quantitative relations in terms of the degree of habitat specialization. The majority of the species (20), are generalists and the remaining 13 are habitat specialists (Willigalla and Fartmann 2012). Among the latter, is a species of conservation concern, *Leucorhinia pectoralis*, which is legally protected in Poland and also listed in Annexes II and IV of the EU Habitats Directive, for which Natura 2000 sites are being established. This species has already been recorded in Kraków in a small pond located in the southern part of the city (Piksa et al. 2006), so it is possible that “Liban” quarry is a part of a wider (meta)population. *L. pectoralis* and *L. rubicunda* are the only species in the quarry, the populations of which are declining in Europe (Kalkman et al. 2010). In addition, the number of sites with *L. rubicunda* in southern Poland is currently decreasing (Bernard et al. 2009). It is worth noting that these species of dragonflies in the quarry studied were categorized as numerous or fairly numerous. The occurrence of species of dragonflies that are habitat specialists or urbanophobic indicates that they are able to colonize areas even in highly urbanized centres of large cities. This also indicates that the local habitat conditions are suitable for many species of Odonata, which usually do not breed in transformed environments in central European cities (Willigalla and Fartmann 2010). On the other hand, the predominance of eurybionts in this quarry is typical of dragonfly assemblages in small water bodies (Steytler and Samways 1995). Their presence indicates an instability in environmental conditions (Buczyński 1999), which could be the marked inter- and intra-annual fluctuations in the water level, a common feature of the area studied. This result is in accord with the opinion that in cities, due to, among other things, the pollution and eutrophication of habitats, the proportion of resistant eurybiontic species is larger than in less urbanized environments (Buczyński and Lewandowski 2011; Villalobos-Jiménez et al. 2016).

### Causes of the local species richness

Recording in an area of only 10 ha as much as 50% of the odonate species occurring in the country, including numerous habitat-specialists and species that are declining in the region and throughout the continent, and a high proportion of urbanophobic species indicates

this area is a centre of biodiversity at the scale of a city or a “secondary biodiversity hotspot” (Harabiš and Dolný 2012). It is worth noting that the importance of the quarry is not only based on the number of species recorded there, but also that almost all of them breed and probably complete their life cycle in this quarry. The results for the “Liban” limestone quarry indicate that not only woodlands (Croci et al. 2008) and ponds (Gledhill et al. 2008), but also even small limestone quarries can play an important role in increasing biodiversity in cities. Thus, in cooperation with naturalists, urban planners and city managers, they should be identified, evaluated and conserved.

High biodiversity of plants and invertebrates in quarries is favoured by the geomorphological heterogeneity and structural diversity of such places. This provides many microhabitats (bare rocks, grassland, wetlands, water bodies, etc.), which can be colonized by various groups of organisms. Also the dynamic nature of these habitats that are mainly in early successional stages is not without significance (Tropek et al. 2010; Bétard 2013). The above factors may also positively contribute to the species richness of Odonata, e.g. the presence of numerous hollows in impermeable ground enable water to accumulate and form small reservoirs, which are valuable breeding habitats for numerous species of dragonflies. In cities, the key factors determining the high diversity of aquatic macroinvertebrates (including dragonflies) include the presence of reservoirs with low nutrient levels, clear water and a rich and diverse immersed and floating vegetation at an intermediate stage of succession (Gledhill et al. 2008; Vermonden et al. 2009; Buczyński and Lewandowski 2011; Goertzen and Suhling 2013; Villalobos-Jiménez et al. 2016). All these features are present in the limestone quarry studied. In addition, the astatic nature of the reservoirs and the high variability in the water level and the area flooded also play an important role. As this quarry has a rocky substrate, this hinders succession and stabilizes the favourable mosaic pattern of biotopes, which undoubtedly promotes the high diversity of dragonflies (Goertzen and Suhling 2013). A key factor in maintaining the alpha diversity of dragonflies is the structure of the vegetation (Steytler and Samways 1995; Goertzen and Suhling 2013; Buczyński 2015), which in the quarry is very strongly differentiated. This is the case for both aquatic, rush, riparian and terrestrial vegetation (meadows and grasslands, shrubby thickets, small stands of trees). These provide the dragonflies, as amphibiotic organisms, with access to suitable habitats at all stages of their life cycle (Willigalla and Fartmann 2012; Kietzka et al. 2018) and enables many species to survive and complete their development within a small area. In spite of this quarry’s downtown location and isolation, its proximity to the most important ecological corridor in the city, a large river, has advantages. This corridor provides connectivity and potential population exchange with areas located outside the city, which is of great importance

for biodiversity in such urban “green islands” (Snep et al. 2006; Bräuniger et al. 2010).

### Conservation of secondary habitats in cities

The basic threat to the richness of fauna and flora at post-mining sites is their planned transformation or restoration (technical reclamation), which is often synonymous with afforestation or transformation into a recreational area (Tropek and Konvicka 2008; Harabiš and Dolný 2012). This occurs despite the fact that leaving the area to natural, spontaneous succession is a less costly and more environmentally friendly (Beneš et al. 2003; Prach and Hobbs 2008; other references in: Tropek and Konvicka 2008). Reclamation was not necessary in the case of “Liban” quarry, because, as revealed, it is already a very suitable area for odonates. This supports the opinion that such anthropogenic habitats are suitable habitats for a wide range of dragonflies associated with natural water bodies (Buczyński 2015). Despite the fact that odonate communities in the centres of cities are generally poorer than those at the peripheries (Willigalla and Fartmann 2010, 2012; Buczyński and Lewandowski 2011; Villalobos-Jiménez et al. 2016) the quarry studied indicates that even highly modified environments may deserve the attention of conservationists. It is widely recognized that there should be areas with high levels of naturalness in cities, due to their role in increasing biodiversity and the functioning of urban ecosystems (Beninde et al. 2015; Aronson et al. 2017). Also, their psychological, recreational and educational significance for the urban community is very important (Fuller et al. 2007; Lemelin 2007) and the ecosystem services provided by such areas is extremely valuable (Bolund and Hunhammar 1999). Undoubtedly, the place studied is of key importance in determining the biodiversity of the entire city. This information should be used to set priorities for nature conservation in the city. Urban planners should use data on local biodiversity to improve the capacity of the city to host a variety of species. They should recognize that this is vital for both the conservation of natural resources and the needs of society.

One of the main threats to odonate biodiversity in cities is habitat fragmentation, barriers to dispersal and reduction in landscape connectivity (Sato et al. 2008), which may lead to the extinction of local populations (Harabiš and Dolný 2012). In order to maintain a high level of biodiversity (including endangered and urbanophobic species) a threshold value of at least 50 ha of a local habitat patch should be left for conservation in cities (Beninde et al. 2015). In the case of “Liban” quarry and its surroundings, the “green” habitat with diversified, semi-natural vegetation covers over 120 ha. Therefore, it should be treated as an important local centre of urban biodiversity, ensuring the existence of a high-quality habitat for water and terrestrial organisms (besides odonates, also plants, amphibians, reptiles, orthopterans etc.). The main conservation measures for the site can be

summarized as follows. The most important is to leave the area as it is and protect it against major changes in use, especially urbanization. Any development for tourism or recreation purposes (conversion into a city park with standard infrastructure and “arranged” greenery) poses a serious threat, as such a transformation would inevitably involve modification of the hydrological regime, drainage of flooded areas and changes in the structure of water and riparian vegetation, which would make the area less suitable for dragonflies (Goertzen and Suhling 2013). The local odonate assemblage would certainly benefit from the removal of artificially introduced, ornamental fish, as it is documented that their predation may negatively affect the diversity of dragonflies, including sites located in cities (Wittwer et al. 2010; Goertzen and Suhling 2013). It is also important to control the growth of riparian bushes and trees in order to avoid excessive shading of ponds, which is generally disadvantageous for dragonflies (Remsburg et al. 2008). Keeping this quarry free from urban infrastructure is an indispensable prerequisite for maintaining the species richness of the local odonatofauna. It is also a simple and almost costless way to protect anthropogenic habitats that are favourable breeding sites for dragonflies (Buczyński 2015), which decision makers are currently extremely resistant to do. The protection of this and similar patches of wetland would certainly benefit not only dragonflies, but also many organisms associated with water, as odonates are a good umbrella group (Bernard et al. 2002; Oertli 2008). Moreover, due to their attractiveness (Lemelin 2007), dragonflies can attract visitors to such urban oases of biodiversity, thus fulfilling the role of flagship species for wetlands located in cities (Goertzen and Suhling 2013; Villalobos-Jiménez et al. 2016). The assignment of this area for conserving biodiversity seems to be the best, the simplest and certainly the cheapest way to manage this type of post-exploitation site in cities. This will benefit not only nature, but also society, by providing citizens with easy access to biodiverse sites that are rich in plants and animals, which is becoming increasingly important in the modern world.

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# DIVERSITY OF MARINE FISH AND THEIR CONSERVATION STATUS IN PUSONG BAY, LHKSEUMAWE CITY, ACEH PROVINCE, INDONESIA

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## ABSTRACT

Marine fish have been decreasing in abundance over the last few decades due to environmental destruction and human exploitation. The aim of this study was to produce an inventory of the fish in Pusong Bay, Lhokseumawe City, Aceh Province, Indonesia by means of an exploratory survey of the area. The data was analysed descriptively using tables and graphs. A total of 71 species of fish were identified belonging to 54 genera, 37 families and 15 orders. Fish belonging to the Perciformes dominated the area with 33 species or 46.48% of the total number of fish identified. Based on the IUCN red list status, 62% of the species recorded in this study are categorized as of Least Concern, 35% Not Evaluated and 3% Data Deficient. Based on CITES, 71 species of the fish in Pusong Bay are categorized as Not Evaluated. Current data on these fish is important for future evaluations of their status.

**Keywords:** bay; order; Pusong; species

## Introduction

Bay waters are zones subject to high levels of human activity, such as industry, port facilities, tourism and fishing (Bunce et al. 1999; Walker 2001; Poulard and Léauté 2002; Wesley et al. 2016; Sahetapy et al. 2018), which result in sedimentation, pollution and erosion (Auernheimer and Chinchon 1997; Ruilian et al. 2008; Wei et al. 2008; Gao and Chen 2012). In addition, bays are habitats for marine organism and one of the main sources of livelihood for local communities (Deng and Jin 2000; Jin and Deng 2000; van-der-Meij et al. 2009; Zakaria and Rajpar 2015), which in Aceh is mainly fishing.

The coastline of Aceh province is 1865 km long and encompass 591,089 km<sup>2</sup> of ocean, which is utilized by industries, port facilities and fisheries. Aceh also includes 663 islands in the Straits of Malacca and Indian Ocean (BPS Aceh 2019). One of the bays in this province is Pusong Bay, which is governed by the Lhokseumawe City Government.

Pusong Bay is in the Fisheries Management Area (FMA) 571 of the Republic of Indonesia, which includes the Straits of Malacca and Andaman Sea (KKP 2014). Around 3500 fishermen fish in this area and harvest on average 8944 tones/year (DKP Kota Lhokseumawe 2018). Based on this data, Pusong Bay is one of the best fishing grounds on the north coast of Aceh. Therefore, it is essential for the future management of the fisheries to have an inventory of the species of fish in this area.

There are inventories of the species of fish for several areas in Aceh, such as Aceh Besar Regency (Rizwan et al. 2017; Dekar et al. 2018; Dewiyanti et al. 2019; Nur et al. 2019a), Banda Aceh (Muchlisin et al. 2017a; Fadli et al. 2018; Perdana et al. 2018), Sabang (Rudi et al. 2009; Rudi et al. 2012), Pidie (Nasir et al. 2018), Central Aceh (Muchlisin et al. 2013) and South-West Aceh (Muchlisin et al. 2015; Batubara et al. 2017; Muchlisin et al. 2017b; Batubara et al. 2018; Irhami et al. 2018; Timorya et al. 2018; Nur et al. 2019b), but not for Pusong Bay, Lhokseumawe. Therefore, the aim of this study is to collect data that will be important for the management of fisheries and conservation of endangered species of fish in the future. In addition, the diversity of fish is a good indicator of the effect of climate change, environmental degradation and pollution in an area (Fausch et al. 1990; Schiemer 2000; Daufresne and Boet 2007; Brodeur et al. 2008; Villéger et al. 2010).

## Material and Methods

This study was carried out from April to May 2019 at Teluk Pusong, Lhokseumawe City, Aceh Province, Indonesia (Fig. 1). Fish were sampled using a local fishing boat to sweep an area of the bay. A mini fish trawl with mesh size of 2 inches in the wing net, 1.5 inches in the body net and 0.5 inches in the cod end, was used. The Sparre and Venema (1992) equation was used to calculate fish stock abundance:

$$a = V \times t \times hr \times X2 \times 1.852 \times 0.001$$

$$D = (1/a) \times (c/f)$$

Where:

$a$  – swept area (km<sup>2</sup>),

$V$  – net pull velocity (knot),

$T$  – time for which the net was towed (hour),

$hr$  – head rope length (m),

$X2$  – head rope length fraction of 0.5 according to Pauly (1980) as the width swept by trawl,

1.852 – conversion from miles to kilometres,

0.001 – conversion from meter to kilometres,

$D$  – fish stock abundance (ton km<sup>-2</sup>),

$c$  – catch rate (kg hr<sup>-1</sup>),

$f$  – escapement factor of 0.5 as an estimate of the proportion of fish in the swept area that were caught by the net (Saeger et al. 1976).

The data collected was analysed in the Laboratory of Ichthyology, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia. This study used an explorative survey (Muchlisin and Siti-Azizah 2009) after identifying locations with a high abundances of fish using information provided by local fisherman. Fish caught were identified using Allen (2000), Schultz (2004), Vida and Kotai (2006), Ambak et al. (2010) and Kottelat (2013).

Samples were collected from several areas in Pusong Bay, the location of which was based on information from local fishermen. Sampling was carried out between 07:00 PM and 08:00 AM. Fish were caught using a mini trawl (net and pocket length of 6 meters). The gear was pulled randomly for 2 hours in a particular area of Pusong Bay at a depth between 8–40 meters. Hauling took 15 minutes during which the fish were grouped based on

species and the mini trawl was set for fishing. The trawl was operated approximately 5 to 6 times per night.

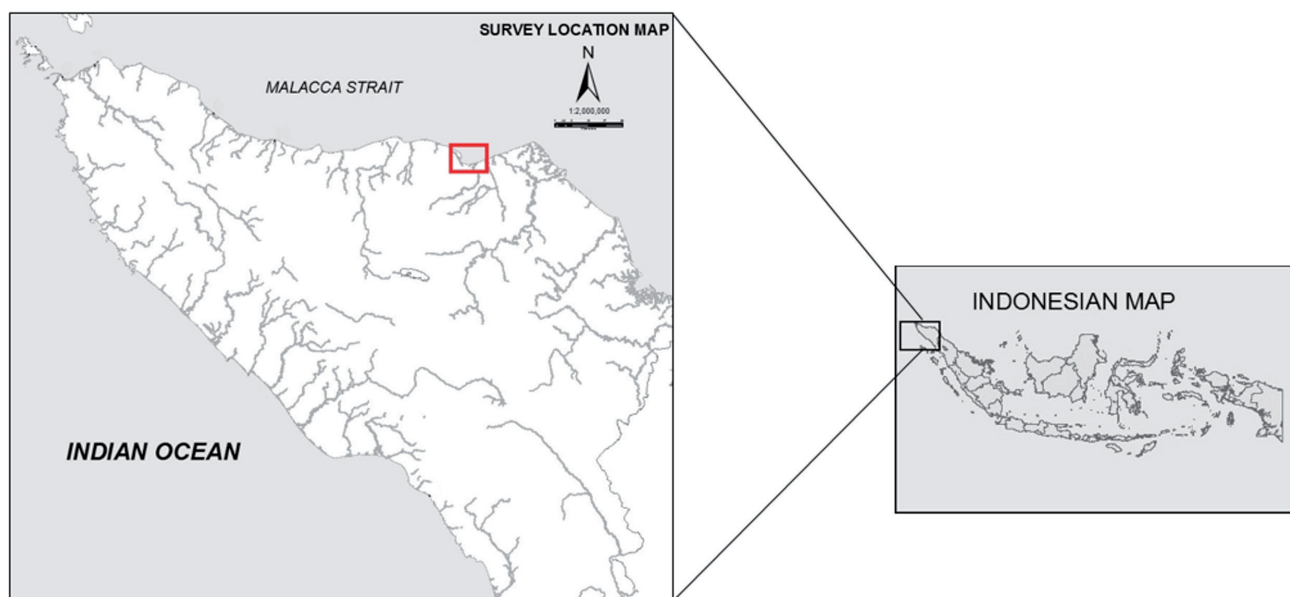
Fish caught were processed in terms of total and standard length measurement using a digital caliper (Mitutoyo, CD-6CS. Error = 0.01 mm) and weight using a digital scale (Toledo, AB-204. Error = 0.01 g). Fish were documented using a digital camera (Nikon D5300). Fish samples were then preserved in 10% formaldehyde solution.

The threat status of the identified species of fish was determined by reference to the IUCN red list of threatened species website (<https://www.iucnredlist.org/>) and its trade status using the CITES website (<https://checklist.cites.org/#/en>). Data was analysed descriptively by means of tables and graphs based on (Batubara et al. 2017) and compared with the results of similar studies.

Environmental data used in this study was obtained from Aqua-MODIS L3 8Day composite satellite images with a 4 km spatial resolution, which was downloaded from <http://oceancolor.gsfc.nasa.gov>. Chlorophyll-a and sea surface temperature (SST) data were analysed using the SeaDAS 7.5.3 program and visualized using QGIS 3.4.14.

## Results

In this study, 71 species of fish were recorded in Pusong Bay, Lhokseumawe City (Table 1), belonging to 15 orders, 37 families and 54 genera (Table 2). The dominant group were those belonging to the order Perciformes, based on the number of families (14 families), genera (24 genera) and species (33 species) (Table 2). Based on the number of species, the family Carangidae in the order Perciformes dominated the bay with 8 species (Table 1).



**Fig. 1** Maps showing the location (red square) of Pusong bay, the site of this study.



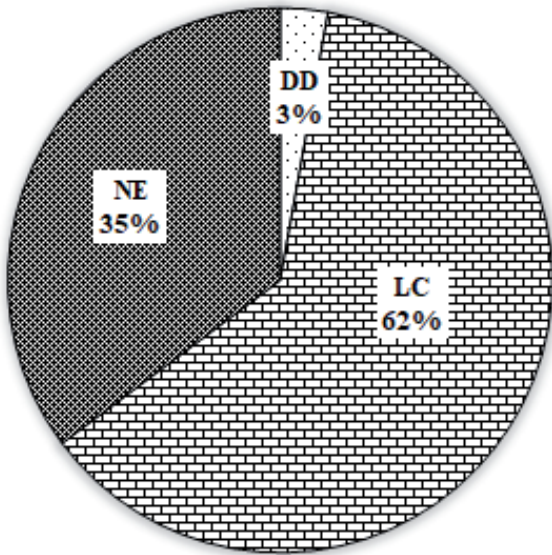
**Table 1** Species of fish recorded in Pusong Bay, Lhokseumawe.

No	Ordo	Family	Genus	Species	Common Name	IUCN Red List	CITES		
1.	Aulopiformes	Synodontidae	<i>Saurida</i>	<i>Saurida gracilis</i>	Gracile Lizardfish	LC	NE		
2.				<i>Saurida undosquamis</i>	Brush tooth Lizardfish	LC	NE		
3.	Beloniformes	Hemiramphidae	<i>Hemiramphus</i>	<i>Hemiramphus far</i>	Black-Barred Halfbeak	NE	NE		
4.	Clupeiformes	Clupeidae	<i>Sardinella</i>	<i>Sardinella gibbosa</i>	Gold stripe Sardinella	LC	NE		
5.		Engraulidae	<i>Stolephorus</i>	<i>Stolephorus indicus</i>	Indian Anchovy	LC	NE		
6.				<i>Thryssa</i>	<i>Thryssa hamiltonii</i>	Hamilton's Thryssa	LC	NE	
7.					<i>Thryssa mystax</i>	Moustached Thryssa	LC	NE	
8.	Elopiformes	Megalopidae	<i>Megalops</i>	<i>Megalops cyprinoides</i>	Indo-pacific Tarpon	DD	NE		
9.	Kurtiformes	Apogonidae	<i>Apogon</i>	<i>Apogon ellioti</i>	Flag-in Cardinalfish	NE	NE		
10.				<i>Apogon quadrifasciatus</i>	Broad banded Cardinalfish	NE	NE		
11.				<i>Apogon</i> sp.	Cardinalfish	NE	NE		
12.				<i>Apogon timorensis</i>	Timor Cardinalfish	NE	NE		
13.	Lophiiformes	Antennariidae	<i>Antennarius</i>	<i>Antennarius striatus</i>	Striated Frogfish	LC	NE		
14.	Myliobatiformes	Dasyatidae	<i>Himantura</i>	<i>Himantura toshi</i>	Black-Spotted Whip ray	LC	NE		
15.	Perciformes	Ambassidae	<i>Ambassis</i>	<i>Ambassis nalua</i>	Scalloped Perch let	LC	NE		
16.		Carangidae	<i>Alectis</i>	<i>Alectis indica</i>	Indian Thread fish	LC	NE		
17.				<i>Carangoides</i>	<i>Carangoides ferdau</i>	Blue Trevally	LC	NE	
18.			<i>Carangoides uii</i>		Coastal Trevally	LC	NE		
19.			<i>Caranx</i>	<i>Caranx sexfasciatus</i>	Bigeye Trevally	LC	NE		
20.			<i>Scomberoides</i>	<i>Scomberoides lysan</i>	Double spotted Queen fish	LC	NE		
21.			<i>Selaroides</i>	<i>Selaroides leptolepis</i>		Yellow stripped scad	LC	NE	
22.					<i>Ulua</i>	<i>Ulua aurochs</i>	Silver mouth Trevally	LC	NE
23.						<i>Ulua mentalis</i>	Longrakered Trevally	LC	NE
24.		Gerreidae	<i>Gerres</i>	<i>Gerres longirostris</i>	Strong spine Silver-Biddy	LC	NE		
25.		Gobiidae	<i>Istigobius</i>	<i>Istigobius spence</i>	Pearl Goby	LC	NE		
26.		Haemulidae	<i>Diagramma</i>	<i>Diagramma labiosum</i>		Painted Sweetlips	LC	NE	
27.					<i>Pomadasys</i>	<i>Pomadasys kaakan</i>	Javelin Grunter	NE	NE
28.		Leiognathidae	<i>Gazza</i>	<i>Gazza minuta</i>		Toothed Pony fish	LC	NE	
29.					<i>Leiognathus</i>	<i>Leiognathus equulus</i>	Common Pony fish	LC	NE
30.						<i>Leiognathus leuciscus</i>	Whip fin Pony fish	LC	NE
31.			<i>Leiognathus splendens</i>	Splendid Pony fish		LC	NE		
32.			<i>Secutor</i>	<i>Secutor</i> sp.		Pony fish	NE	NE	
33.				<i>Secutor indicus</i>	Pony fish	NE	NE		
34.				<i>Secutor interruptus</i>	Pig-Nosed Pony fish	NE	NE		
35.		Lutjanidae	<i>Etelis</i>	<i>Etelis carbunculus</i>		Ruby Snapper	LC	NE	
36.					<i>Lutjanus</i>	<i>Lutjanus lutjanus</i>	Bigeye Snapper	LC	NE
37.						<i>Lutjanus apodus</i>	Schoolmaster Snapper	LC	NE
38.	<i>Lutjanus fulviflamma</i>					Dory Snapper	LC	NE	
39.	<i>Pristipomoides</i>					<i>Pristipomoides multidentis</i>	Gold banded Job fish	LC	NE
40.	Mullidae	<i>Upeneus</i>	<i>Upeneus sulphureus</i>	Sulphur Goatfish	LC	NE			
41.	Nemipteridae	<i>Nemipterus</i>	<i>Nemipterus isacanthus</i>		Teardrop Threadfin Bream	NE	NE		
42.				<i>Nemipterus nematophorus</i>	Double whip Threadfin Bream	LC	NE		
43.	Polynemidae	<i>Polydactylus</i>	<i>Polydactylus nigripinnis</i>	Black fin Threadfin	NE	NE			
44.	Priacanthidae	<i>Priacanthus</i>	<i>Priacanthus hamrur</i>	Moon tail Bullseye	LC	NE			
45.	Sciaenidae	<i>Johnius</i>	<i>Johnius amblycephalus</i>	Bearded Croaker	NE	NE			
46.	Serranidae	<i>Epinephelus</i>	<i>Epinephelus lanceolatus</i>	Giant Grouper	DD	NE			
47.	Siganidae	<i>Siganus</i>	<i>Siganus canaliculatus</i>	White-Spotted Spine foot	LC	NE			

No	Ordo	Family	Genus	Species	Common Name	IUCN Red List	CITES	
48.	Pleuronectiformes	Bothidae	<i>Grammatobothus</i>	<i>Grammatobothus polyophthalmus</i>	Three spot Flounder	LC	NE	
49.		Cynoglossidae	<i>Paraplagusia</i>	<i>Paraplagusia bilineata</i>	Double lined Tongue sole	NE	NE	
50.		Paralichthyidae	<i>Pseudorhombus</i>	<i>Pseudorhombus arsius</i>	Large tooth Flounder	NE	NE	
51.				<i>Pseudorhombus</i> sp.	Flounder	NE	NE	
52.		Psettodidae	<i>Psettodes</i>	<i>Psettodes erumei</i>	Indian Halibut	NE	NE	
53.				<i>Psettodes</i> sp.	Halibut	NE	NE	
54.	Scombriformes	Sphyraenidae	<i>Sphyraena</i>	<i>Sphyraena novaehollandiae</i>	Australian Barracuda	NE	NE	
55.	Scorpaeniformes	Platycephalidae	<i>Inegocia</i>	<i>Inegocia harrisii</i>	Harris's Flathead	NE	NE	
56.		Scorpaenidae	<i>Brachypterois</i>	<i>Brachypterois serrulatus</i>	Saw cheek Scorpionfish	NE	NE	
57.				<i>Parascorpaena</i>	<i>Parascorpaena mcadamsi</i>	McAdam's Scorpionfish	LC	NE
58.				<i>Pterois</i>	<i>Pterois russelii</i>	Plain tail Turkey fish	LC	NE
59.				<i>Scorpaenodes</i>	<i>Scorpaenodes hirsutus</i>	Hairy Scorpionfish	LC	NE
60.				<i>Scorpaenopsis</i>	<i>Scorpaenopsis venosa</i>	Raggy Scorpionfish	LC	NE
61.		Synanceiidae	<i>Erosa</i>	<i>Erosa erosa</i>	Pitted Stonefish	LC	NE	
62.	Siluriformes	Plotosidae	<i>Plotosus</i>	<i>Plotosus lineatus</i>	Striped Eel Catfish	NE	NE	
63.	Syngnathiformes	Fistulariidae	<i>Fistularia</i>	<i>Fistularia petimba</i>	Red Cornet fish	LC	NE	
64.	Tetraodontiformes	Balistidae	<i>Balistoides</i>	<i>Balistoides viridescens</i>	Titan Triggerfish	NE	NE	
65.		Tetraodontidae	<i>Arothron</i>	<i>Arothron reticularis</i>	Reticulated Pufferfish	LC	NE	
66.				<i>Carinotetraodon</i>	<i>Carinotetraodon lorteti</i>	Redeye Puffer	LC	NE
67.				<i>Lagocephalus</i>	<i>Lagocephalus lunaris</i>	Lunar tail Puffer	LC	NE
68.		Triacanthidae	<i>Triacanthus</i>	<i>Triacanthus biaculeatus</i>	Short-Nosed Tripod fish	NE	NE	
69.	Trachiniformes	Uranoscopidae	<i>Uranoscopus</i>	<i>Uranoscopus cognatus</i>	Yellowtail Stargazer	NE	NE	
70.				<i>Uranoscopus kaianus</i>	Kai Stargazer	NE	NE	
71.		Labridae	<i>Xiphocheilus</i>	<i>Xiphocheilus typus</i>	Blue-Banded Wrasse	LC	NE	

**Table 2** Classification of the 71 species of fish recorded in terms of the order, family, genus and species to which they belong.

No.	Order	Family		Genus		Species	
		Number	%	Number	%	Number	%
1.	Aulopiformes	1	2.70	1	1.85	2	2.82
2.	Beloniformes	1	2.70	1	1.85	1	1.41
3.	Clupeiformes	2	5.41	3	5.56	4	5.63
4.	Elopiformes	1	2.70	1	1.85	1	1.41
5.	Kurtiformes	1	2.70	1	1.85	4	5.63
6.	Lophiiformes	1	2.70	1	1.85	1	1.41
7.	Myliobatiformes	1	2.70	1	1.85	1	1.41
8.	Perciformes	14	37.84	24	44.44	33	46.48
9.	Pleuronectiformes	4	10.81	4	7.41	6	8.45
10.	Scombriformes	1	2.70	1	1.85	1	1.41
11.	Scorpaeniformes	3	8.11	7	12.96	7	9.86
12.	Siluriformes	1	2.70	1	1.85	1	1.41
13.	Syngnathiformes	1	2.70	1	1.85	1	1.41
14.	Tetraodontiformes	3	8.11	5	9.26	5	7.04
15.	Trachiniformes	2	5.41	2	3.70	3	4.23
Total		37	100	54	100	71	100



**Fig. 2** Pie diagram of the IUCN red list status of the 71 species of fish species caught at Teluk Pusong, where DD is data deficient, LC least concern and NE not evaluated.

Threat status of the 71 species of fish in Pusong Bay based on The IUCN Red List indicates that 62% are categorized as of Least Concern (LC), 35% Not Evaluated (NE) and 3% as Data Deficient (DD) (Fig. 2). Moreover, based on the Convention on International Trade in Endangered Species (CITES), all of the species are categorized as Not Evaluated (Table 1).

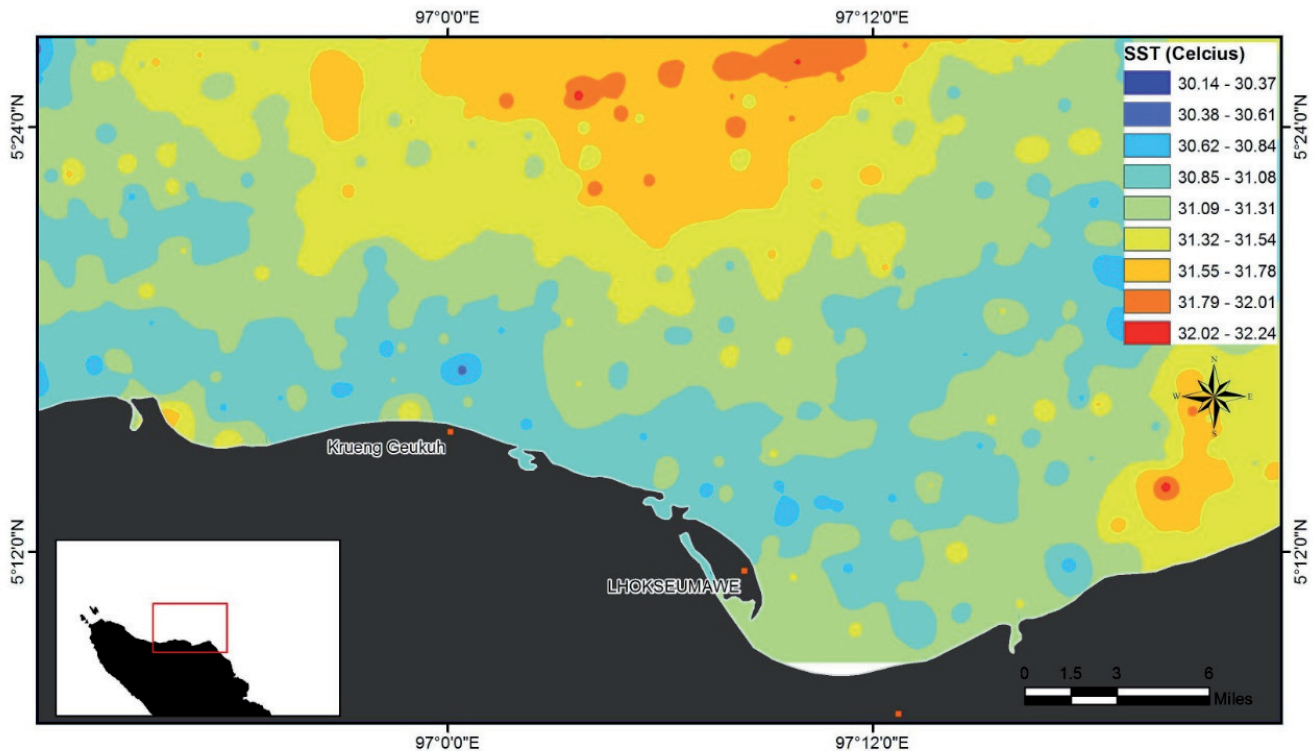
The mini fish trawl used in this study was used to sweep an area of 34.42 km<sup>2</sup> and caught 70.6 kg of fish. This indicates that the fish stock in Pusong Bay is

0.065 tonnes km<sup>-2</sup>. SST and chlorophyll concentrations also influence the abundance of fish in these waters. SST within Pusong Bay ranged between 30.9–31.1 °C (Fig. 3) and the chlorophyll concentration between 2–4 mg m<sup>-3</sup> (Fig. 4).

## Discussion

The 71 species of fish collected in Pusong Bay, Lhokseumawe City (Table 2) belonged to 15 orders, which is higher than that recorded in other areas in Aceh Province, such as Simeulue Island (12 orders) (Batubara et al. 2017; Batubara et al. 2018), Meurebo River (7 orders) and Mifa Bersaudara Inc. Area (7 orders) in the West Aceh Regency (Irhami et al. 2018; Nur et al. 2019b), Aceh River (12 orders) in the Aceh Province (Dekar et al. 2018), Sabee River in the Aceh Jaya Regency (Timorya et al. 2018) and Lhoknga and Lhok Mata Ie Coast (8 orders) in the Aceh Besar Regency (Nur et al. 2019a). Thus based on the number of orders of fish, there is a higher diversity fish in the bay than in the river and coastal waters in Aceh Province.

A higher number of families of fish (37) were recorded in Pusong Bay than at other locations in Aceh such as Simeulue Island (26 families) (Batubara et al. 2017) and Lhoknga and Lhok Mata Ie Coast in the Aceh Besar Regency (11 families) (Nur et al. 2019a). However, the number of families of marine fish recorded in Pusong Bay is lower than that of families of freshwater fish in Aceh Province, which is 41 families (Muchlisin and Si-



**Fig. 3** Map showing sea surface temperatures (SST) in the Pusong Bay when the fish were sampled.

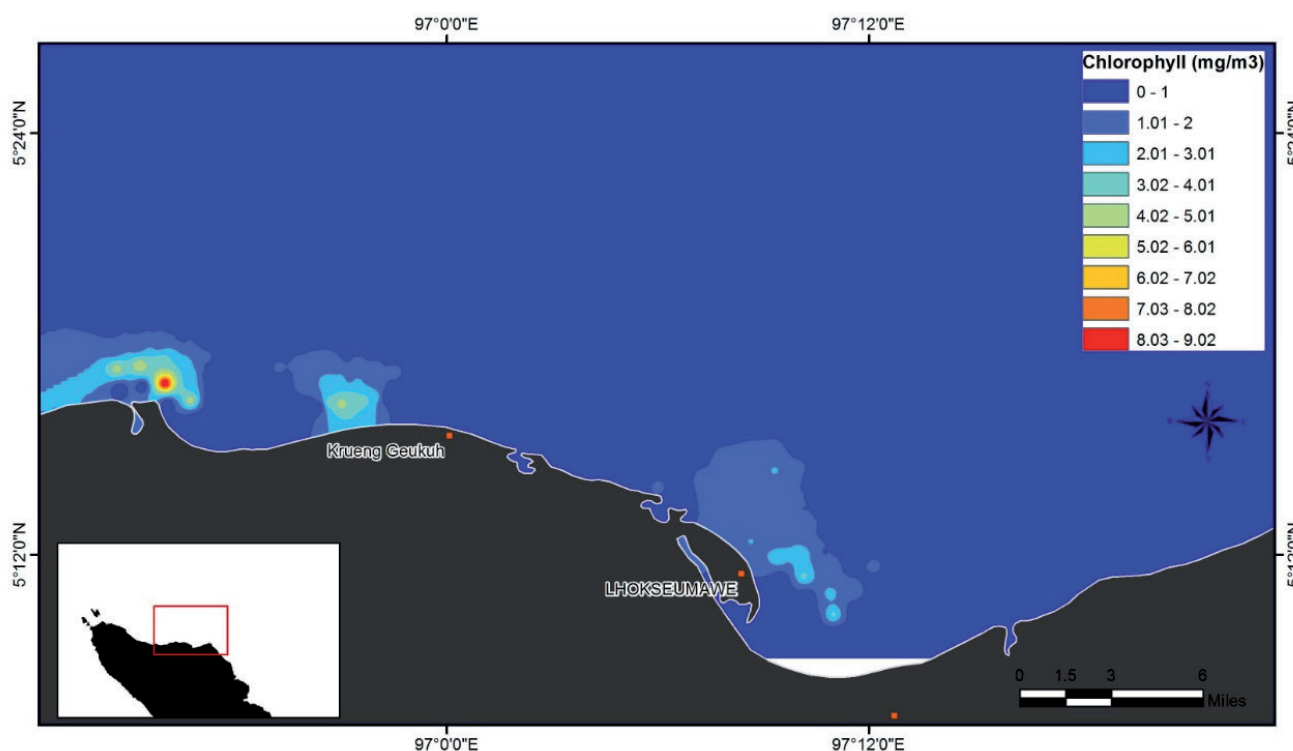


Fig. 4 Map showing the concentrations of chlorophyll recorded in Pusong Bay when the fish were sampled.

ti-Azizah 2009). The number of species of fish recorded in Pusong Bay (71) is also lower than the number recorded in several other areas in Aceh Province, such as Aceh River (44) (Dekar et al. 2018), Meureubo River (32) (Irham et al. 2018), Lhoknga and Lhok Mata Ie Coast (25) (Nur et al. 2019a) and Sabee River (12) (Timorya et al. 2018).

The order Perciformes dominated Pusong Bay in terms of the number of species (33) and percentage composition of the total catch (46.48%). The same order of fish dominates in other areas such as Simeulue Island (67 species) (Batubara et al. 2017) and Aceh River (20 species) (Dekar et al. 2018). However, the order Cypriniformes dominates in area such as Rawa Teripa waters in Nagan Raya Regency (19% of all species caught) (Muchlisin et al. 2015), Mifa Bersaudara Inc. Area (39%) (Nur et al. 2019b) and Meureubo River in West Aceh Regency (12.5%) (Irhami et al. 2018).

Based on the IUCN red list status, 62% of the fish in Pusong Bay are categorized as of Least Concern (LC) and 38% as Data Deficient (DD) or Not Evaluated (NE). LC is attributed to species not listed in a higher threat category, which are usually widely distributed and abundant (Rodrigues et al. 2006). DD is attributed to species for which there is little or no data on their distribution or abundance (Gärdenfors et al. 2001). While NE is attributed to species for which the threat risk has not been evaluated (Hoffmann et al. 2008). Based on CITES, the 71 species of fish recorded in Pusong Bay have not been evaluated (CITES 2020).

A precautionary approach is recommended for managing the resources of fisheries that have a NE status.

This approach aims to protect fish from both growth and recruitment overfishing (Lessa and Duarte-Neto 2004; Damora and Wagiyu 2012; Damora and Baihaqi 2013; Damora et al. 2018a, b).

The abundance of the fish stock recorded in this study is relatively low, compared with other studies in the Java Sea (Badrudin et al. 2011) that report fish stocks of 2.7–3.1 tonnes km<sup>2</sup>. East and west monsoons influence the abundance of fish in the Pusong Bay, as during the east monsoon demersal fish tend to gather in shore due to the murky waters associated with the strong southeast wind, which create a whirlpool of schools of fish as a result of an interaction between the water currents and fish (Badrudin et al. 2011). Meanwhile, this study was conducted in the transitional season between the west and east monsoon, which created strong currents and waves within Pusong Bay.

The average gradient in SST in tropical waters is about 0.3–1 °C (Choudhury et al. 2007). SST gradient affects change in the water resulting in upwelling and accumulations of plankton usually affects fish behaviour, as they respond by modifying their metabolism, spawning, migrating, increasing food consumption and the salt concentration in their bodies (Laurs et al. 1984; Arnone 1987).

The concentration of chlorophyll in Pusong Bay is lower than in other areas in the Andaman Sea, such as the coastal waters of Bangladesh, Myanmar, Thailand and Malaysia. The Andaman Sea and Pusong Bay are located in the same bio-ecoregion and the concentration of chlorophyll in these areas is between 5–15 mg m<sup>-3</sup>. In addition, Suwannathatsa et al. (2012)

report a concentration of 15–30 mg m<sup>-3</sup> in the Malacca Straits.

February and May are the transition period between the west to east monsoon season in which the divergence of Ekman transport, equatorial Kelvin waves and Rossby waves create a strong current that spreads through the Nicobar Islands within the Andaman Sea. This strong current is neutralized during the transition period between the northeast and southwest monsoons, which results in murky conditions and an increase in the average SST (Suwannathatsa et al. 2012). During this transition, there is no runoff of rainwater into the bay, which along with the turbulence result in a decrease in the concentration of chlorophyll to its lowest level (Suwannathatsa and Wongwises 2013). Fish generally are only abundant where there are high concentrations of chlorophyll, which is an indicator of rich nutrient water where fish are likely to be abundant (Santos 2000; Zainuddin 2011).

## Conclusion

In conclusion, the order Perciformes dominated the area with the highest number of species of fish. Based on the IUCN red list status, 62% of the species of fish are of Least Concern, 35% Not Evaluated and 3% Data Deficient. Based on CITES the 71 species of fish in Pusing Bay are categorized as Not Evaluated. Data on these species of fish are important in terms of evaluating their status in the future.

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# ANTENNAL MORPHOLOGY AND SENSILLA OF THE PREDACEOUS LADYBIRDS, *MENOCHILUS SEXMACULATUS* AND *PROPYLEA DISSECTA*

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## ABSTRACT

*Menochilus sexmaculatus* and *Propylea dissecta* (Coleoptera: Coccinellidae) are predaceous ladybird beetles with immense biological control potential. The morphology of the antennae of the adults along with the diversity and distribution of sensilla were investigated using a scanning electron microscope as they are the main sensory organs involved in chemical communication, thermo-reception, mate-recognition, gustation, etc. The antennae of males and females in both species were clavate and consisted of three parts: scape, pedicel and a 9-segmented flagellum. The antennae of male and female *P. dissecta* were significantly longer than those of *M. sexmaculatus* despite their overall body size being smaller, probably due to the much longer F9 flagellomere in *P. dissecta*. Antennae of female ladybirds of both species exhibited sexual dimorphism in being longer than those of males. Scape was longer than other antennal parts in both species of ladybirds. There was a great diversity of sensilla with most of them on the ninth-flagellomere. We identified nine types of sensillum: chaetica, trichoidea, basiconica, Böhm bristles, campaniformia, placoidea, coeloconica, sporangia and styloconica. Coeloconica were restricted to flagellomere F8 in male *P. dissecta* and female *M. sexmaculatus*, respectively, indicating sexual dimorphism and male-related functions of this sensillum.

**Keywords:** antenna; aphid; Coccinellidae; ladybird; morphology; sensilla

## Introduction

Majority of predaceous ladybirds (Coleoptera: Coccinellidae) can be utilized for the biological control of numerous phytophagous insect-pests: aphids, scale-insects, mealybugs and whiteflies (Hodek et al. 2012; Omkar and Pervez 2016) infesting several economically important crops (Pervez et al. 2020). The optimization of their predation potential (Pervez and Yadav 2018; Pervez et al. 2018), prey consumption (Pervez and Kumar 2017) and mating behaviour (Pervez and Singh 2013) may enhance the biocontrol potential of ladybirds.

*Menochilus sexmaculatus* (Fabricius) is an Oriental generalist aphidophagous ladybird, which occurs abundantly in agricultural and horticultural fields on the Indian subcontinent (Poorani 2002; Omkar and Pervez 2004). Its reproductive and mating behaviour was recently studied by Chaudhary et al. (2016) and Dubey et al. (2018). Similarly, *Propylea dissecta* (Mulsant) is also an important Oriental aphidophagous ladybird (Omkar and Pervez 2011) with marked sexual dimorphism (Omkar and Pervez 2000), which have facilitated mating studies on this species (Pervez et al. 2004; Omkar and Pervez 2005). These species of ladybirds coexist in most agro-ecosystems. Scanning electron microscopy of the antennal sensilla may help in achieving a better understanding of the ecological interactions between prey-predator and predators sharing a common prey resource. It may also help in behavioural studies on mate-recognition, courtship and mating. Evidence indicates that adult males use their antennae to examine females, which may indicate that

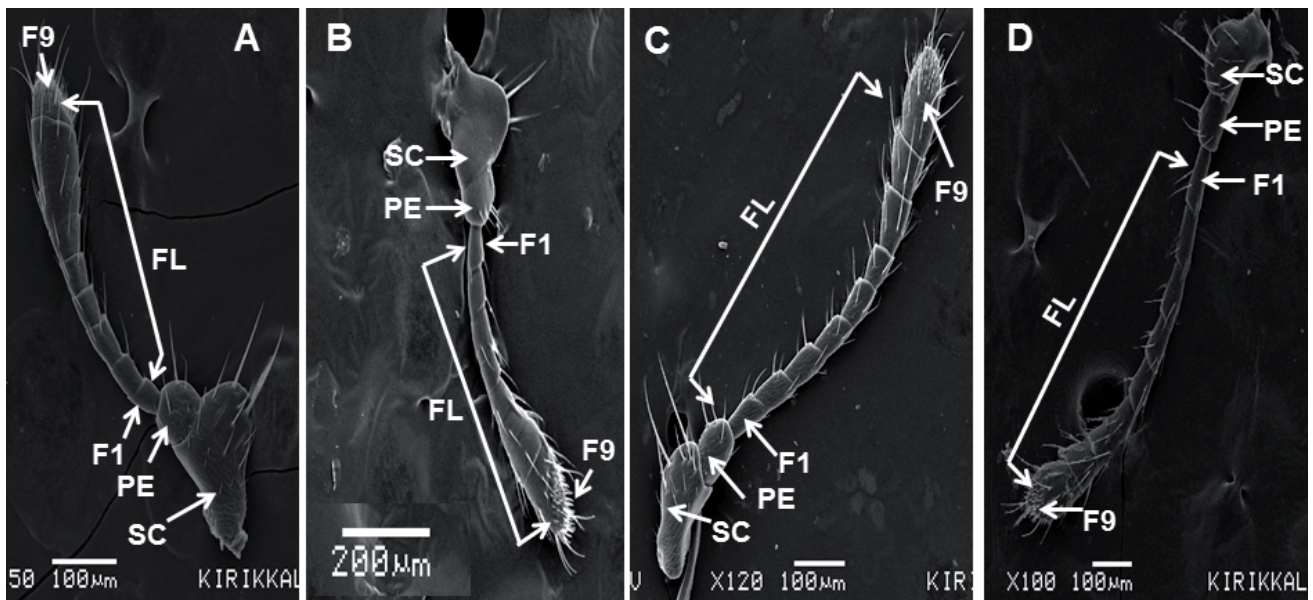
antennae are important in mate recognition (Srivastava and Omkar 2003; Omkar and Pervez 2005). In addition, there is a marked sexual dimorphism in the shape and size of their antennae and diversity of antennal sensilla (Jourdan et al. 1995; Srivastava and Omkar 2003; Omkar and Pervez 2008). Therefore, a scanning electron microscopy study of the antennal sensilla of two aphidophagous ladybirds, *M. sexmaculatus* and *P. dissecta*, was carried out in order to (i) determine the morphological differences in the male and female antennal sensilla (ii) identify the sensilla on the terminal segments that are involved in mate-recognition, and (iii) to identify the similarities and differences in the types and distribution of antennal sensilla in these two similar-sized, polymorphic and co-existing species of ladybird.

## Materials and Methods

### Insect culture

We collected adults of *M. sexmaculatus* and *P. dissecta* from agricultural fields near the city of Kashipur, India (29°21'04"N, 78°96'19"E) and in the laboratory they were sexually identified based on their genitalia viewed under a stereoscopic trinocular (*Lyzer*) at 40× and 100× magnifications, which was connected to a personal computer (DELL). Conspecific male and female beetles were paired in plastic Petri dishes (9.0 cm diameter × 2.0 cm deep) containing an *ad libitum* supply of the aphid *Aphis craccivora* (Koch) as food. Then, these Petri dishes were kept under constant conditions (27 ± 2 °C; 65 ± 5% RH;





**Fig. 1** Antennae of *P. dissecta* and *M. sexmaculatus*. A. Male (*M. sexmaculatus* *P. dissecta*); B. Female (*M. sexmaculatus*); C. Male (*P. dissecta*); D. Female (*P. dissecta*). SC – scape; PE – pedicel; F1–F9 – flagellomeres; FL – flagellum.

14L:10D) in an Environmental Test Chamber (REMI, Remi Instruments). The adults mated and the females laid eggs in clusters, which were collected daily and stored at the above temperature for experimental purpose. The larvae that hatched from these eggs were reared to the adult stage (space and food as above), which were then isolated in Petri dishes and kept for 10 days before being used in the following studies (space and food as above).

### Scanning electron microscopy

Antennectomy of freshly anaesthetized 10-day-old adult male and female *M. sexmaculatus* and *P. dissecta* was carried out under a Stereomicroscope (Lieca S8APO). The antennae were carefully excised using a micro-blade and then immersed in 10% KOH for 1 hour to remove unwanted tissues. The processed antennae were dehydrated in a graded series of ethanol (50%, 70%, 90% and 100%) for 10 minutes, air-dried for 30 minutes at 30 °C and coated with a thin layer of gold using a Polaron SC 502 sputter coater. Then, each antenna was mounted singly on a stub, using double-sided sticky tape and coated with gold-palladium. The stubs with the antennae were individually inserted into a scanning electron microscope (Jeol JSM 5600) and the morphological details recorded at different magnifications at 15 kV. This was replicated six times ( $n = 6$ ).

### Statistical analyses

The lengths of antennae and their different components for both the sexes were compared using a two-sample t-test and statistical software, SAS 9.0 (2002). The lengths of the antennal components were subjected to a two-way ANOVA using 'species' and 'sex' as independent variables and length of the different antennal components, *viz.* 'scape', 'pedicel', 'flagellum' and 'total length of antenna' as dependent variables using statistical software, SAS 9.0 (2002). Means were compared using

Tukey's HSD Test and SAS 9.0 (2002). Various sensilla were compared at the 'species' and 'gender' levels using 't' tests (SAS 9.0).

## Results

Antennae of the ladybirds, *M. sexmaculatus* and *P. dissecta* are located in front of their compound eyes. Each antenna consists of a scape, pedicel and flagellum consisting of nine flagellomeres (F1–F9) (Fig. 1).

### Morphology of the antennae of *M. sexmaculatus*

The scape of the antenna of adult males ( $200.17 \pm 5.31 \mu\text{m}$ ) is significantly ( $t = 17.08$ ;  $P < 0.0001$ ; d. f. = 1, 10) longer than that of females ( $150.00 \pm 4.86 \mu\text{m}$ ). The proximal and middle part of the scape is broader in the female and the distal part significantly broader in the male (Fig. 1A, B; Table 1). In females the pedicel of an antenna is longer ( $80.17 \pm 2.86 \mu\text{m}$ ) than that of males ( $60.00 \pm 3.41 \mu\text{m}$ ). In males, however, the pedicel is significantly broader in the distal region than in females (Table 1). The first flagellomere, F1, ( $t = -29.51$ ;  $P < 0.0001$ ; d. f. = 1, 10), F2 ( $t = -21.20$ ;  $P < 0.0001$ ; d. f. = 1, 10), F3 ( $t = -9.64$ ;  $P < 0.0001$ ; d. f. = 1, 10) and F9 ( $t = -3.39$ ;  $P < 0.01$ ; d. f. = 1, 10) are significantly shorter, and F8 significantly longer ( $t = 11.81$ ;  $P < 0.0001$ ; d. f. = 1, 10) in adult males than in females. The other flagellomeres (F4 to F7) are similar in size in both sexes (Table 1; N.S.). The overall lengths of the flagellum ( $t = -11.45$ ;  $P < 0.0001$ ; d. f. = 1, 10) and an antenna ( $t = -6.85$ ;  $P < 0.0001$ ; d. f. = 1, 10) are significantly shorter in adult males than females.

### Morphology of the antennae of *P. dissecta*

The scape ( $t = -2.91$ ;  $P < 0.05$ ; d. f. = 1, 10), pedicel ( $t = -4.09$ ;  $P < 0.01$ ; d. f. = 1, 10), F5 ( $t = -2.54$ ;  $P < 0.05$ ; d. f.

**Table 1** Lengths and widths of antennal segments of adult male and female *M. sexmaculatus*.

<i>Menochilus sexmaculatus</i>	Male			Female			T-test*
	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )		Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )		
		Proximal	Distal		Proximal	Distal	
Scapae	200.17 $\pm$ 5.31	57.5 $\pm$ 1.51	125.0 $\pm$ 5.20	150.00 $\pm$ 4.86	150.0 $\pm$ 4.52	80.0 $\pm$ 2.25	<b>t = 17.08; P &lt; 0.0001</b>
Pedicelel	60.00 $\pm$ 3.41	77.5 $\pm$ 2.50	70.0 $\pm$ 3.25	80.17 $\pm$ 2.86	75.0 $\pm$ 3.12	55.0 $\pm$ 3.25	<b>t = -11.11; P &lt; 0.0001</b>
F1	40.00 $\pm$ 3.22	30.0 $\pm$ 1.56	40.0 $\pm$ 1.76	95.17 $\pm$ 3.25	25.0 $\pm$ 1.25	35.0 $\pm$ 2.45	<b>t = -29.51; P &lt; 0.0001</b>
F2	50.00 $\pm$ 3.85	32.5 $\pm$ 1.95	35.0 $\pm$ 3.12	96.33 $\pm$ 3.72	35.0 $\pm$ 2.10	40.0 $\pm$ 2.20	<b>t = -21.20; P &lt; 0.0001</b>
F3	57.50 $\pm$ 4.59	25.0 $\pm$ 2.15	35.0 $\pm$ 3.45	80.00 $\pm$ 3.41	45.0 $\pm$ 2.30	40.0 $\pm$ 2.55	<b>t = -9.64; P &lt; 0.0001</b>
F4	50.17 $\pm$ 3.31	27.5 $\pm$ 3.10	35.0 $\pm$ 3.15	50.00 $\pm$ 3.16	40.0 $\pm$ 2.50	45.0 $\pm$ 2.40	<b>t = 0.06; N.S.</b>
F5	50.17 $\pm$ 3.31	30.0 $\pm$ 1.50	40.0 $\pm$ 2.20	50.00 $\pm$ 5.10	40.0 $\pm$ 2.25	55.0 $\pm$ 2.80	<b>t = 0.14; N.S.</b>
F6	50.00 $\pm$ 3.85	32.5 $\pm$ 2.25	52.5 $\pm$ 3.50	49.17 $\pm$ 3.31	50.0 $\pm$ 2.30	80.0 $\pm$ 2.90	<b>t = 0.37; N.S.</b>
F7	50.00 $\pm$ 3.41	55.0 $\pm$ 3.40	65.0 $\pm$ 3.35	50.83 $\pm$ 3.39	80.0 $\pm$ 2.85	90.0 $\pm$ 2.70	<b>t = -0.40; N.S.</b>
F8	80.00 $\pm$ 3.41	65.0 $\pm$ 1.52	65.0 $\pm$ 2.25	50.33 $\pm$ 5.13	87.5 $\pm$ 2.75	90.0 $\pm$ 3.30	<b>t = 11.81; P &lt; 0.0001</b>
F9	52.50 $\pm$ 4.93	65.0 $\pm$ 3.25	35.0 $\pm$ 2.54	62.50 $\pm$ 5.28	102.5 $\pm$ 2.95	37.5 $\pm$ 2.80	<b>t = -3.39; P &lt; 0.01</b>
Flagellum length	<b>480.30 <math>\pm</math> 16.60</b>			<b>584.50 <math>\pm</math> 14.8</b>			<b>t = -11.45; P &lt; 0.0001</b>
Total length of antenna	<b>740.50 <math>\pm</math> 18.60</b>			<b>814.70 <math>\pm</math> 18.9</b>			<b>t = -6.85; P &lt; 0.0001</b>

The data are means  $\pm$  S.D.

\* Only the lengths of antennal components of adult male and female, *M. sexmaculatus* were subjected to t-tests.

= 1, 10) and F6 ( $t = -4.45$ ;  $P < 0.05$ ; d. f. = 1, 10) of adult males are significantly shorter than those of adult females (Fig. 1C, D; Table 2). The flagellomeres are significantly broader in females than in males. The total lengths of the flagellum ( $719.50 \pm 22.40 \mu\text{m}$  in male and  $752.20 \pm 16.40 \mu\text{m}$  in female) and an antenna ( $1000.30 \pm 21.60 \mu\text{m}$  in male and  $1055.50 \pm 22.00 \mu\text{m}$  in females) are greater in females than in males.

Sensilla occurred mainly on F9 flagellomere in adult males and females in both species. Two-way ANOVA revealed significant effects of 'sex' ( $F = 70.80$ ;  $P < 0.0001$ ; d. f. = 1), 'species' ( $F = 473.62$ ;  $P < 0.0001$ ; d. f. = 1) and in the interaction between 'sex' and 'species' ( $F = 167.92$ ;  $P < 0.0001$ ; d. f. = 1) on the length of the 'scape' and significant effects of 'sex' ( $F = 87.86$ ;  $P < 0.0001$ ; d. f. = 1), 'species' ( $F = 5.96$ ;  $P < 0.05$ ; d. f. = 1) and 'interaction' (sex x species;  $F = 5.96$ ;  $P < 0.05$ ; d. f. = 1) on the length of the 'pedicel'. Similarly, 'sex' ( $F = 88.31$ ;  $P < 0.0001$ ; d. f. = 1), 'species' ( $F = 783.21$ ;  $P < 0.0001$ ; d. f. = 1) and 'interaction' (sex x species;  $F = 24.06$ ;  $P < 0.0001$ ; d. f. = 1) had a significant effect on the length of the pedicel. This cumulatively resulted in significant effects of 'sex' ( $F = 60.41$ ;  $P < 0.0001$ ; d. f. = 1), 'species' ( $F = 908.18$ ;  $P < 0.0001$ ; d. f. = 1) on the total length of an antenna. The

'interaction' between 'sex' and 'species', however was not statistically significant ( $F = 5.96$ ;  $P < 0.05$ ; d. f. = 1) for the total length of an antenna.

#### Types and distribution of sensilla on the antennae of *M. sexmaculatus* and *P. dissecta*

The scanning electron microscopy revealed nine types of sensilla in the two species of ladybird studied: sensilla chaetica (Sc1–Sc4), sensilla trichoidea (Tr1–Tr4), sensilla basiconica (Ba1–Ba3), Böhm bristles (BB), sensilla campaniformia (Ca), sensilla coeloconica (Co), sensilla styloconica (St), sensilla sporangia (Sp) and sensilla placoidea (Pl). Based on their lengths, these sensilla can be arranged in a descending order: Sc > Tr > Ba > BB > St > Ca > Pl.

#### Sensilla chaetica (Sc)

Sensillum chaetica is the longest type of sensilla present on the antennae of male and female ladybirds. There are four subtypes of sensilla chaetica (Sc1–Sc4). Sc1 is the longest, with a straight sensillum, which has grooved cuticular walls and a pointed tip. Sc2 is shorter than Sc1 with a blunt tip. It is present on the antenna at an angle of 50–70° to the antennal axis. Sc3 is straight with a slightly curved tip. Sc4 is the smallest of those with

**Table 2** Lengths and widths of antennal segments of adult male and female *P. dissecta*.

<i>Propylea dissecta</i>	Male			Female			T-test
	Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )		Length ( $\mu\text{m}$ )	Width ( $\mu\text{m}$ )		
		Proximal	Distal		Proximal	Distal	
Scape	220.83 $\pm$ 4.92	60.0 $\pm$ 3.15	100.0 $\pm$ 4.25	231.50 $\pm$ 7.50	102.56 $\pm$ 3.65	107.69 $\pm$ 4.50	<b>t = -2.91;</b> <b>P &lt; 0.05</b>
Pedicel	60.00 $\pm$ 5.83	55.0 $\pm$ 2.15	55.0 $\pm$ 3.75	71.83 $\pm$ 4.02	76.9 $\pm$ 3.20	71.79 $\pm$ 3.50	<b>t = -4.09;</b> <b>P &lt; 0.01</b>
F1	70.33 $\pm$ 4.55	40.0 $\pm$ 2.15	45.0 $\pm$ 2.35	71.83 $\pm$ 3.13	46.15 $\pm$ 2.30	56.0 $\pm$ 1.25	<b>t = -0.69; N.S.</b>
F2	65.00 $\pm$ 4.20	40.0 $\pm$ 1.65	45.0 $\pm$ 1.80	58.33 $\pm$ 8.16	56.41 $\pm$ 1.85	56.0 $\pm$ 1.45	<b>t = 1.78; N.S.</b>
F3	74.17 $\pm$ 3.82	40.0 $\pm$ 1.95	45.0 $\pm$ 1.25	72.17 $\pm$ 4.17	51.28 $\pm$ 1.85	46.15 $\pm$ 1.70	<b>t = 0.87; N.S.</b>
F4	70.00 $\pm$ 6.03	40.0 $\pm$ 1.45	45.0 $\pm$ 1.65	66.33 $\pm$ 2.16	41.02 $\pm$ 1.90	51.28 $\pm$ 1.45	<b>t = 1.40; N.S.</b>
F5	74.33 $\pm$ 5.89	35.0 $\pm$ 1.15	40.0 $\pm$ 1.20	82.50 $\pm$ 5.24	41.02 $\pm$ 1.65	46.15 $\pm$ 1.75	<b>t = -2.54;</b> <b>P &lt; 0.05</b>
F6	60.50 $\pm$ 4.37	30.0 $\pm$ 1.25	35.0 $\pm$ 1.60	71.83 $\pm$ 4.45	35.90 $\pm$ 1.50	46.15 $\pm$ 1.55	<b>t = -4.45;</b> <b>P &lt; 0.001</b>
F7	90.00 $\pm$ 4.47	30.0 $\pm$ 1.60	55.0 $\pm$ 1.85	92.33 $\pm$ 5.57	41.02 $\pm$ 2.15	66.66 $\pm$ 2.25	<b>t = -0.80; N.S.</b>
F8	50.17 $\pm$ 5.31	40.0 $\pm$ 2.15	65.0 $\pm$ 2.55	87.17 $\pm$ 6.59	66.66 $\pm$ 2.40	87.0 $\pm$ 2.65	<b>t = -10.52;</b> <b>P &lt; 0.0001</b>
F9	165.00 $\pm$ 9.49	55.0 $\pm$ 2.65	45.0 $\pm$ 1.75	149.67 $\pm$ 4.89	87.18 $\pm$ 2.95	92.3 $\pm$ 3.15	<b>t = 3.52;</b> <b>P &lt; 0.01</b>
Flagellum length	<b>719.50 <math>\pm</math> 22.40</b>			<b>752.20 <math>\pm</math> 16.40</b>			<b>t = -2.88;</b> <b>P &lt; 0.05</b>
Total length of antenna	<b>1000.30 <math>\pm</math> 21.60</b>			1055.50 $\pm$ 22.00			<b>t = -4.37;</b> <b>P &lt; 0.001</b>

The data are means  $\pm$  S.D.

\* Only the lengths of antennal components of adult male and female, *M. sexmaculatus* were subjected to t-tests.

a pointed tip. These sensilla occur mostly on the scape and ninth flagellomere (F9) of the antennae of adult males and females.

In *M. sexmaculatus*, three Sc1 (120–135  $\mu\text{m}$ ), two Sc2 (70–90  $\mu\text{m}$ ), five Sc3 (40–55  $\mu\text{m}$ ) and three Sc4 (10–20  $\mu\text{m}$ ) are present on the scape of males and three Sc1 (55–65  $\mu\text{m}$ ) and two Sc2 (20–30  $\mu\text{m}$ ) on those of females (Fig. 2). Two Sc2 and four Sc3 are present on the pedicel of males. Four Sc2 and two Sc4 are present on the pedicel of females (Fig. 2). On the flagellomeres, four and seven Sc1 (75–90  $\mu\text{m}$ ) are present on F8 and F9, respectively, and three Sc3 (50–80  $\mu\text{m}$ ) on F8 of male antennae. The females have five Sc1 (55–65  $\mu\text{m}$ ) and eight Sc2 (70–80  $\mu\text{m}$ ) on F9 and three Sc1 (55–65  $\mu\text{m}$ ) on F8 (Fig. 3).

There are three subtypes of sensilla chaetica: Sc1, Sc2 and Sc4, on the scape and pedicel of adult males of *P. dissecta*. There are two Sc1 (115–193  $\mu\text{m}$ ), one Sc2 (83–103  $\mu\text{m}$ ), three Sc3 (50–57  $\mu\text{m}$ ) and six Sc4 (26–53  $\mu\text{m}$ ) sensilla on the scape in males (Fig. 4A1) and one Sc1 (140  $\mu\text{m}$ ), three Sc2 (60–70  $\mu\text{m}$ ), four Sc3 (50–60  $\mu\text{m}$ ) and six Sc4 (26–30  $\mu\text{m}$ ) on the scape in females (Fig. 4A2). In addition, there are two Sc2, one Sc3 and two Sc4 sensilla on the pedicel in males and two each of Sc2, Sc3 and Sc4 in females (Fig. 4A1, A2). On male F8 flagellomere there are three Sc1 and two Sc2 and Sc3, and on F9 seven Sc1, three Sc2 and two Sc3 (Fig. 4B1). The Sc1 and Sc2 sensilla on F9 of females were signifi-

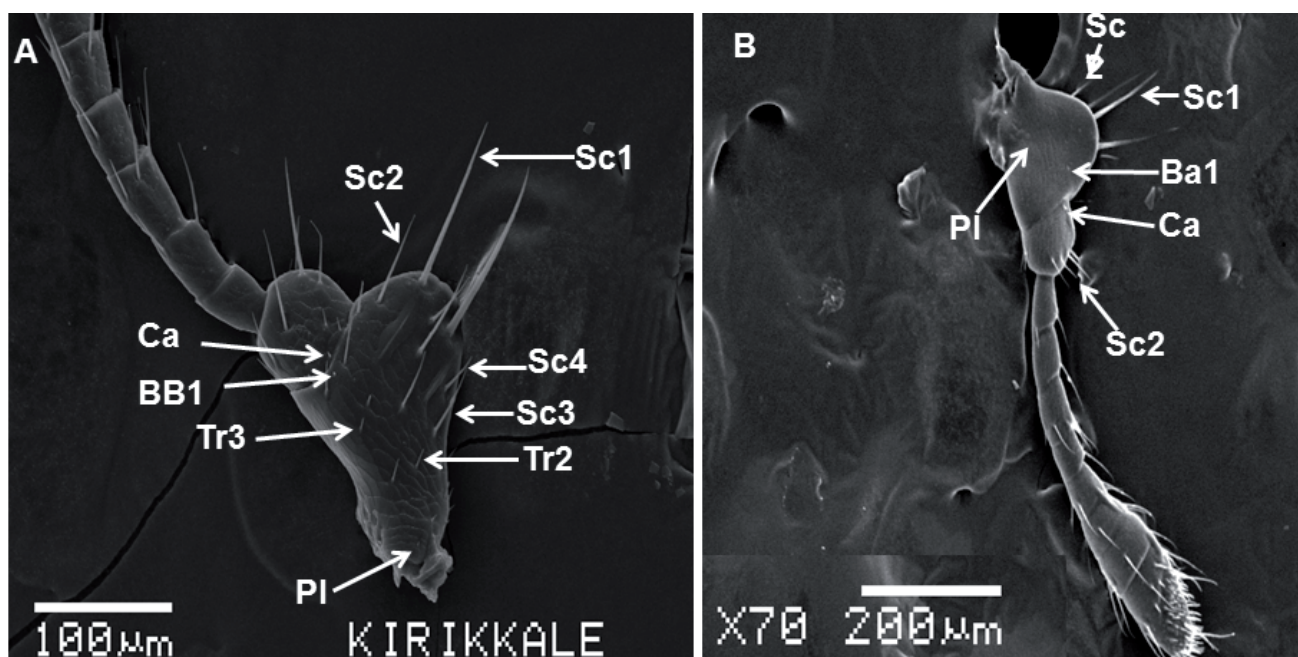
cantly shorter in length than those on males ( $t = -4.57$ ;  $P < 0.0001$ ; d.f. = 9).

### Sensilla trichoidea (Tr)

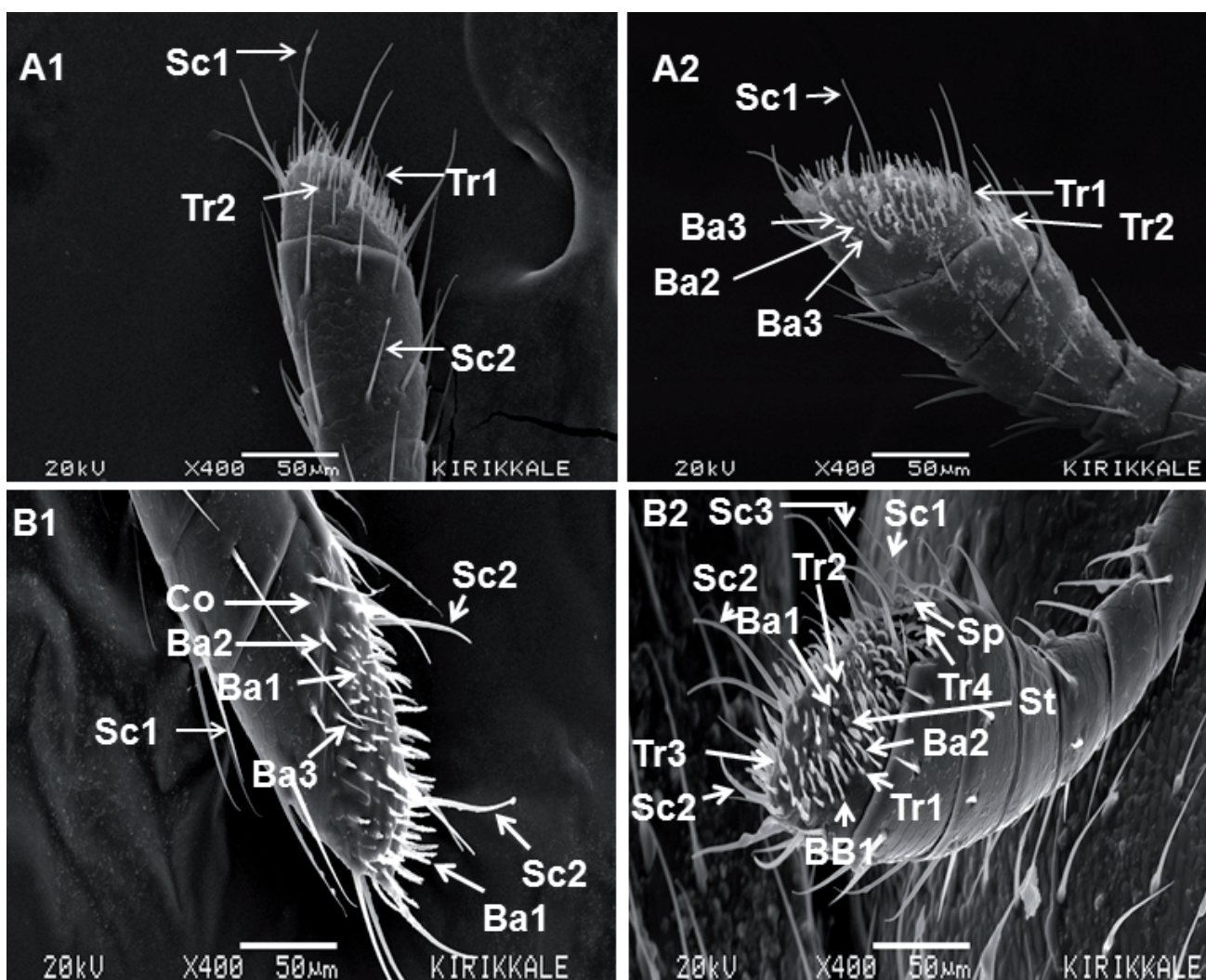
Trichoidea are more slender and the second-longest sensillum and are present on F9 flagellomere in both of the species and on the scape of female *M. sexmaculatus*. There are three subtypes of sensilla trichoidea (Tr1–Tr3). Tr1 has a pointed tip, Tr2 and Tr3 are shorter with pointed tip tapering from base to tip. In *M. sexmaculatus*, three subtypes of trichoidea (Tr1, Tr2 and Tr3) are present on the scape of females. There are two Tr1 and numerous Tr2 on F9 of males and 6–7 Tr1 and numerous Tr3 on that of females (Fig. 3). In *P. dissecta*, there are eight Tr1 (30–35  $\mu\text{m}$ ), two Tr2 (10–20  $\mu\text{m}$ ) and several Tr3 (6–12) on flagellomere F9 of males (Fig. 4B1) and four Tr1 (20–25  $\mu\text{m}$ ), one Tr2 (10–20  $\mu\text{m}$ ) and one Tr3 (4–6  $\mu\text{m}$ ) on that of females (Fig. 4B2).

### Sensilla basiconica (Ba)

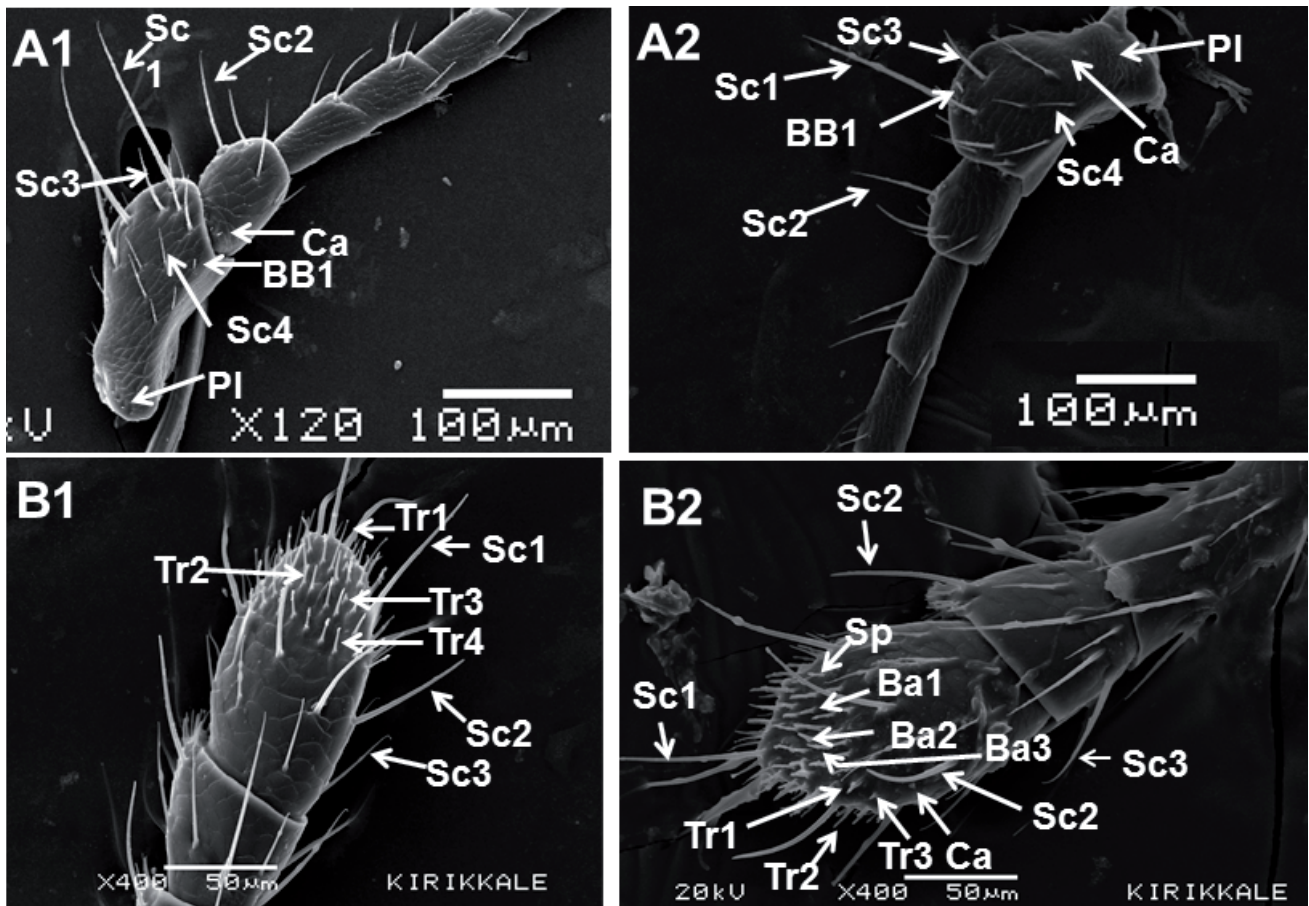
Sensilla basiconica are blunt tipped, slightly curved and stout pegs. There are three subtypes of Ba: Ba1, Ba2 and B3. Ba1 is the shortest and resembles Ba2 in having a blunt tip. Ba3 is longer than Ba1 and Ba2 with a pointed tip and only occurs on F9. In *M. sexmaculatus* there is one Ba1 sensilla on the scape (Fig. 2) and two Ba1 (6–9  $\mu\text{m}$ ), numerous Ba2 (8–13  $\mu\text{m}$ ) and Ba3 (12–15  $\mu\text{m}$ ) on apical region



**Fig. 2** Scape and pedicel of antenna of (A) male and (B) female *M. sexmaculatus*, showing sensilla chaetica (Sc1–Sc4), Ca (campaniformia), Böhm Bristles (BB1), basiconica (Ba1) and Placoidea (Pl).



**Fig. 3** Flagellomeres F8 and F9 of the antennae of male (A1, A2) and female (B1, B2) *M. sexmaculatus* showing different sensilla: chaetica (Sc), trichoidea (Tr), basiconica (Ba), sporangium (Sp), coeloconica (Co), styloconica (St), Böhm bristles (BB1).



**Fig. 4** Scape and pedicel of (A1) Male and (A2) Female *P. dissecta* showing sensilla: chaetica (Sc), placoidea (PI), campaniformia (Ca) and Böhm bristles (BB). Terminal flagellomere F9 of (B1) Male and (B2) Female *P. dissecta* showing sensilla: trichoidea (Tr), basiconica (Ba), campaniformia (Ca), sporangium (Sp) and chaetica (Sc).

of F9 in males, and four Ba3 (15–18  $\mu\text{m}$ ), numerous Ba1 (3–6  $\mu\text{m}$ ) and Ba2 (10–15  $\mu\text{m}$ ) on F9 in females (Fig. 3). In female *P. dissecta* there are three Ba1 (4–6  $\mu\text{m}$ ), five Ba3 (10–15  $\mu\text{m}$ ) and numerous Ba2 on F9 (Fig. 4B1, B2).

#### Böhm bristles (BB)

BB are small thorn-like smooth walled sensilla embedded in a circular pocket on the scape and pedicel of antennae. There are two subtypes of BB: BB1 and BB2. BB1 has a blunt tip and is shorter than Ba and thinner than Tr. BB2 has a 'V' shaped branched tip, which is blunt and short. One BB1 is present on the scapes of males and females of *P. dissecta* (Fig. 4A1, A2) and one BB1 (4–10  $\mu\text{m}$ ) on the scape of male *M. sexmaculatus*, but not on that of females (Fig. 2A, B).

#### Sensilla campaniformia (Ca)

They are small dome shaped structures in sockets and are present on the pedicel of male and female *M. sexmaculatus* (Fig. 2A, B). They are also present on the scape (Fig. 5A2) and F9 of female *P. dissecta* (Fig. 4B2).

#### Sensilla placoidea (PI)

They are elliptical sunken plates. There are three PI present on the scapes of both the sexes of *M. sexmaculatus* (Fig. 2A, B) and four and two present on the proximal

part of the scape in male and female *P. dissecta*, respectively (Fig. 4A1, A2).

#### Sensilla sporangium (Sp)

They are a pole-shaped and resemble sporangia, and have an apical protuberance. They are only present on F9 of females. There are three (7–18  $\mu\text{m}$ ) and four (10–25  $\mu\text{m}$ ), respectively, on F9 of *P. dissecta* (Fig. 4B2) and *M. sexmaculatus* (Fig. 3B2).

#### Sensilla coeloconica (Co)

They are small pits that resemble pores. One Co is present on F8 of male *M. sexmaculatus* (Fig. 3B1).

#### Sensilla styloconica (St)

They are smooth cylindrical structures with blunt tips and are present on the apical region of F9. There is one (5–8  $\mu\text{m}$ ) on the F9 of females of both *M. sexmaculatus* and *P. dissecta* (Fig. 3B2).

## Discussion

The antennae of females of *M. sexmaculatus* and *P. dissecta* are longer than those of the males, as is reported for *Coccinella septempunctata* L. (Srivastava and Omkar

2003). Adult female ladybirds are bigger, therefore, their appendages, like antennae, are also comparatively longer than those of males. Despite being smaller, the length of the antennae in *P. dissecta* are significantly longer than those of *M. sexmaculatus*. This difference was mainly due to length of *F9* flagellomere, which is almost three times longer in *P. dissecta* than in *M. sexmaculatus*. Even bigger ladybirds, like *H. axyridis*, have similar sized antenna (Chi et al. 2009).

Despite the morphological similarities in the shape, position and number of the antennal segments of the ladybirds studied with those of other species: *C. septempunctata* L. (Srivastava and Omkar 2003), *Harmonia axyridis* (Pallas) (Park et al. 2001) and *Cryptolaemus montrouzieri* (Mulsant) (Ping et al. 2013), there are subtle differences in the sizes of a few of the antennal segments. For instance, the size of the ninth flagellomere is significantly different from that of other flagellomeres in both male and female *P. dissecta*, which results in a great increase in the length of their antennae. The ninth flagellomere, however, is similar in size to other flagellomeres in *M. sexmaculatus*. Thus, the greater antennal length in *P. dissecta* is mainly due to a longer ninth flagellomere.

There are nine types of antennal sensilla in *M. sexmaculatus* and *P. dissecta* compared to five in *Semiadalia undecimnotata* Schn. (Jourdan et al. 1995), seven in *H. axyridis* (Chi et al. 2009) and *C. montrouzieri* (Ping et al. 2013) and eight in *C. septempunctata* (Srivastava and Omkar 2003). *Sc1* is the longest sensillum and its presence on all parts of an antenna indicates its importance in mechanoreception (Broeckling and Salom 2003). *Sc1* are significantly longer on *F8* of male antenna than that of the female in both species of ladybird, which might indicate it has secondary role in mate-recognition. Males of *P. dissecta* (Omkar and Pervez 2005) and *H. axyridis* (Obata 1987) examine female ladybirds by touching their body and antennae with their antennae. Presence of a large number of sensilla chaetica on male antennae further strengthens its probable role in mate-recognition.

Numerous sensilla trichoidea are present on *F9* of both species of ladybird. These sensilla are also probably involved in mate-recognition, as the *F9* of male ladybirds touch adult female ladybirds during courtship behaviour (Obata 1987; Omkar and Pervez 2005). Brožek and Chłond (2010) suggest that sensilla trichoidea are mechano-sensory and Chi et al. (2009) indicate that in the aggregative behaviour of *H. axyridis* they may act as pheromone receptors. Basiconica are the third-most abundant and conspicuous sensilla on the antennae of the two ladybirds studied. The presence of numerous *Ba* on the ninth flagellomere indicates a probable role in the chemoreception of female sex-pheromones. Thus, these sensilla might be involved in searching and recognition of mates. The large number of these sensilla on male antennae is in accord with Jourdan et al. (1995), who report

a large number of these sensilla on the antenna of male *S. undecimnotata*. Further, Zhang et al. (2011) report large numbers of sensilla basiconica on the antennae of males of the longicorn beetles *Leptura arcuata* Panzer and *Leptura aethiops* Poda (Coleoptera: Cerambycidae) and suggest they might have a role in the reception of sex-pheromones. Sensilla basiconica were initially thought to be thermo- and hygro-sensitive receptors (Steinbrecht 1989; Bartlett et al. 1999). However, they may also be chemoreceptors (Hu et al. 2009; Zheng et al. 2014; Yi et al. 2016). There are a few subtypes of sensillum basiconica, which vary in shape and size, which are present on *F9* of all ladybirds (Jourdan et al. 1995; Srivastava and Omkar 2003; Chi et al. 2009; Ping et al. 2013).

There are Böhm bristles on the scapes of male and female antennae of both species. In other insects these sensilla are usually on the scape and pedicel of antennae (Chi et al. 2009; Wei et al. 2015) and thought to be proprioceptors i.e. they perceive the movement and position of antenna (Ochieng et al. 2000; Onagbola and Fadamiro 2008). Böhm bristles sense mechanical stimulations and may induce cushioning action and control the speed of movement of antennae (Wei et al. 2015). These sensilla are named after L. K. Böhm (1911), who first described them in the order Lepidoptera. In the ladybirds studied, there was no sexual dimorphism in terms of the number, length or position of *BB*.

There are only a few sensilla campaniformia on the *F9* of male and female ladybirds. They are proprio-receptors that responds to stresses in the cuticle (Meng and Qin 2017), but may also have a gustatory function (Ochieng et al. 2000). Sensilla sporangium are present only on *F9* of females of both species of ladybirds. Sensillum ampullacea are only present on the *F9* of the antennae of males of *P. dissecta* and are thought to detect  $\text{CO}_2$  concentration (Kleineidam et al. 2000). Sensillum coeloconica are present on *F9* flagellomere of male *M. sexmaculatus* and are known to be thermo-receptors (Ruchty et al. 2009). Sensilla placoidea are more abundant on male antennae, which indicates they may have a role in courtship behaviour initiated by female pheromones (Yee 2014; Song et al. 2017). Thus, *P. dissecta* possibly benefits by having comparatively longer antenna and more sensilla basiconica than other coexisting ladybirds. This is the first report of an abundance of basiconica on their antennae other than those on *F9* and the first study on the antennal sensilla of these two species of ladybirds. However, the exact role of these sensilla will only be revealed by further morphological and physiological investigations.

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# GENERATION TIME RATIO, RATHER THAN VORACITY, DETERMINES POPULATION DYNAMICS OF INSECT – NATURAL ENEMY SYSTEMS, CONTRARY TO CLASSICAL LOTKA-VOLTERRA MODELS

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## ABSTRACT

Population dynamics of a predator-prey system is usually simulated by the classical Lotka-Volterra models, which were successfully applied to the population dynamics of snowshoe hare and lynx and many other predator-prey systems. Attempts were made to apply them also to insect predator-prey systems, but in terms of biological control, they did not reveal the features of the predators that control the abundance of their prey. The most conspicuous example of failure of Lotka-Volterra models applied to insect predator-prey systems are ladybird-aphid systems, in which these models usually fail to fit empirical data. Because of their practical importance and because they are very well studied, we have chosen aphid-ladybird systems as a model. We summarize the results published on various aspects of the population dynamics of aphid-ladybird systems and present them in the context of empirical data. Using new data, we more closely specify the existing metapopulation model of aphid-ladybird interactions.

Based on the arguments presented here, we conclude that the ladybird-aphid case can be generalized to insect (and maybe even other) predator-prey systems, where the ratio of the generation times of the predator to that of the prey (GTR) is large. In such systems, the main selection pressure on predators is choosing the best strategy to maximize survival of their offspring, rather than on maximization of the amount of prey eaten. Thus voracity, which is the main determinant of population dynamics in Lotka-Volterra models, loses its role and is replaced by optimization of the choice of oviposition sites in systems with large GTRs.

**Keywords:** aphids; ladybirds; Lotka-Volterra; metapopulation dynamics; predator-prey systems

## Introduction

Population dynamics of a predator-prey system is usually simulated by the classical Lotka-Volterra models (Lotka 1920, 1925; Volterra 1926, 1931). These models were successfully applied to the population dynamics of snowshoe hare and lynx (e.g., Hoppensteadt 2006; Carpenter 2018) and many other predator-prey systems. Attempts were made to apply them also to insect predator-prey systems (e.g., Varley et al. 1974; Hassell 1978), but in terms of biological control they did not reveal the features of the predators that control the abundance of their prey. The most conspicuous example of failure of Lotka-Volterra models applied to insect predator-prey systems are ladybird-aphid systems, in which these models usually fail to fit empirical data (Kindlmann et al. 2015). Understanding these systems, however, is very important in terms of classical biological control, as is outlined below.

The efficiency of ladybirds in aphid-ladybird systems is an important issue for biological control. Therefore, it has been the subject of many discussions and empirical studies, which attempt to evaluate the extent to which insect predators are able to suppress their aphid prey. The magnitude of the effect of predators on their prey is now questioned and evidence is increasing that pest regulation by predators is not as strong in nature (in contrast to confined habitats like glasshouses) as people would like to believe. Literature dealing with these complex problems, which touch on many different aspects, is scattered

and a comprehensive compendium, enabling a multifactorial view of the situation, is missing.

Because of their practical importance and because they were very well studied, we have chosen the aphid-ladybird system as a model system. We summarize the results published on various aspects of the population dynamics of aphid-ladybird systems and present them in the context of empirical data. Using new data, we show that the existing metapopulation model of aphid-ladybird interactions (Kindlmann and Dixon 2003; Houdková and Kindlmann 2006) that describes the within-season dynamics within one patch can work, if only the ladybird dynamics on trees and large stands of vascular plants is considered. Small solitary vascular plants probably do not have a significant effect on ladybird dynamics.

Then we present one of the few examples of other metapopulation models of insect predator-prey dynamics (the only one to our knowledge) and conclude with a generalization of the ladybird-aphid case to most insect predator-prey systems.

## Ladybird-Aphid System as an Example of an Insect Predator-Prey System

### Aphid dynamics

Aphid populations usually grow exponentially in the initial phase of their development and on reaching a certain population density, the aphids switch to alate pro-

duction and/or move to another part of the plant. The switch to migration can be seen as a response to deterioration in the quality of the host plant or severe intraspecific competition. The switch results in a rapid decline in colony size (Kindlmann and Dixon 1993). The adaptive significance of the response of aphids to their own density and deterioration in food quality, both of which result in migration, is discussed in Kindlmann and Dixon (1999a, 1999b, 2001, 2003).

### Optimal strategy of an aphid predator

From an evolutionary perspective, both predator and prey strive to maximize their reproductive potentials. However, whilst the existence of prey is not dependent on predators, the latter are dependent on prey. Therefore, it is advantageous for predators to conserve their prey. Thus, for predators, the optimal strategy involves counteracting pressures to maximize their own reproduction and survival while conserving enough prey to sustain a sufficient food supply for their offspring. This is very nicely exemplified by long-lived insect predators, such as ladybirds, feeding on short-lived prey, such as aphids (Dixon and Kindlmann 1998; Kindlmann and Dixon 1999a, 1999b, 2001; Dostálková et al. 2002; Dixon et al. 2015). As most of these predators suffer enormous egg and larval mortality due to cannibalism (Dixon and Kindlmann 2012) and intraguild predation (Mills 1982), selection acts mainly on optimizing their oviposition strategies in terms of maximizing the likelihood that their offspring will survive until reproductive age. The oviposition strategy of a predator with a long larval developmental time will depend on a longer projection of the future prey abundance in a patch, bearing in mind there are likely to be more bottlenecks or a higher probability of a bottleneck than for predators with short developmental times. Consequently, the former must be more conservative in terms of conserving their prey (the GTR hypothesis, Dixon and Kindlmann 1998; Kindlmann and Dixon 1999a, 2001; Kindlmann et al. 2015). This hypothesis is now beginning to be more widely accepted (e.g., Mills 2018).

### Effectiveness of aphid predators in regulating the abundance of their prey

Until recently, there was only one attempt to account for the low effectiveness of aphidophagous insect predators: Kindlmann and Dixon (1999a, 2001) propose that the ratio of the generation time of an insect predator to that of its prey (generation time ratio, GTR) determines their effectiveness in suppressing prey. Kindlmann and Dixon (1999a) assume that on a large spatial scale, at any instant, herbivore populations exist as patches of prey, associated with patches of good host plant quality. Predators exploit these patches, which vary greatly in number of prey both spatially and temporally (Kareiva 1990). GTR in insect predator-prey systems is often large: the developmental times of insect predators often span sev-

eral prey generations and are similar to the duration of a patch of prey (Dixon 2000).

Cannibalism is common in insect predators (Fox 1975; Agarwala and Dixon 1993; Kindlmann and Dixon 1999a; Dixon and Kindlmann 2012) and is adaptive, as eating conspecific competitors will increase their fitness (Dong and Polis 1992). Mortality during larval stages can reach 99% (Matsura 1976; Kirby and Ehler 1977; Wright and Laing 1982; Osawa 1993; Hironori and Katsuhiko 1997; Kindlmann et al. 2000). Because of the enormous larval mortality, the life history strategy of these predators is likely to be selected to maximize the probability of survival of their offspring, rather than maximize the number of eggs laid (Dixon et al. 1995, 1997; Kindlmann and Dixon 1999a).

In the majority of cases, the adult predators are winged and can easily move between patches, whereas the immature stages are confined to one patch throughout their development, and their survival is associated with the quality of the patch of prey in which they were born (Kindlmann and Dixon 1999a). Therefore, the fitness of most predators (such as aphidophagous ladybirds and hoverflies that feed on highly aggregated and ephemeral patches of prey, which is measured in terms of the number of offspring that survive to reproductive age), is likely to be more closely associated with their oviposition strategy (the choice of patch for laying eggs), than the trophic interactions commonly used in models of prey-predator population dynamics (Kindlmann and Dixon 1999a).

When GTR is large and cannibalism is common, eggs laid by predators late on in the existence of a patch of prey are highly likely to be eaten by larvae of predators that hatch from the first eggs to be laid (Kindlmann and Dixon 1999a). In addition, because of the large GTR, there is insufficient time for the larvae that hatch from late laid eggs to complete their development. Thus, cannibalism and the ephemeral existence of patches of prey pose constraints such that females that can assess the age of a patch of prey gain an advantage (Kindlmann and Dixon 1999a).

As a consequence, females oviposit in young patches (“egg window hypothesis”, Dixon 2000). The short “egg window” during which it is advantageous to lay eggs in a patch of prey in large-GTR systems reduces the number of eggs laid per patch. Incidence of cannibalism is likely to be proportional to the probability of encountering another predator, rather than prey, i.e., to the relative abundance of predators to prey (“meet and eat hypothesis”, Kindlmann and Dixon 2003). If this is true, then even if predators are abundant and many eggs are laid in a patch of prey during the egg window, strong density dependent cannibalism greatly reduces the abundance of the predators (Mills 1982).

There is a simple mechanism, which enables predatory females to oviposit only in young colonies. Ovipositing females strongly react to the tracks of coccinellid larvae, which indicate that predatory larvae are present

and might cannibalize their eggs. Thus, the presence of larval tracks is a good cue that it is time for the predatory females to leave a patch (Doubtina et al. 1998).

### Empirical verification of predator efficiency

The efficiency of predators in suppressing their prey is assessed mainly by indirect methods such as by using cages to exclude predators (Chambers et al. 1983; Elliott and Kieckhefer 2000; Michels et al. 2001; Basky 2003; Cardinale et al. 2003; Schmidt et al. 2003; Costamagna and Landis 2006, 2007). However, cages change the microenvironment (Hand and Keaster 1967), especially temperature, which is thought to be important in determining the outcome of predator-prey interactions (Frazer and Gilbert 1976; Frazer et al. 1981). Also, cages with a large (8 mm) mesh size, especially those used by Schmidt et al. (2003) do not prevent predators from entering cages (Ameixa and Kindlmann 2011). The most serious problem with exclusion cages is that they do not allow the aphids to leave a cage, which obscures the results, as it is not possible to distinguish, whether the larger number of aphids inside cages compared to their numbers outside cages, is caused by predators eating the aphids outside cages, or to the aphids being prevented from leaving the cage. For all these reasons, exclusion cages cannot be used for measuring the effect of predators on aphid numbers.

Also the positive correlations often reported between aphid and predator abundances (Rutledge et al. 2004; Nielsen and Hajek 2005; Rutledge and O'Neil 2005; Desneux and Joo 2006) do not indicate anything about predator efficiency, as they may only indicate that predators concentrate where aphids are abundant rather than predators determine the abundance of aphids.

Thus, other methods must be used for assessing predator efficiency in aphid – natural enemy systems. One such method is the daily manual removal of all stages of predators from some plants but not control plants and then the comparison of the numbers of aphids on both groups of plants, as in Kindlmann et al. (2015). In a three-trophic system consisting of *Hibiscus syriacus* shrubs, aphid *Aphis gossypii* and ladybirds *Coccinella septempunctata* and *Harmonia axyridis*, Kindlmann et al. (2015) have shown that daily hand-removal of all predators did not affect the peak numbers of aphids on *Hibiscus*. However, it did affect the speed of decline of the aphid population: aphid numbers declined more quickly when predators were not removed.

Also, when predation is assessed based on field observations, as in Costamagna and Landis (2007), one can easily calculate from their results that the effect of predators on the aphid population was minute.

All the above lead to the conclusion that predators have little effect on aphid population dynamics in the field (contrary to what occurs in confined situations, like glasshouses), exactly as predicted by the theory. Therefore, no matter whether abundant or not, insect predators have little effect on prey population dynamics, when

GTR is large (“GTR hypothesis”, Kindlmann and Dixon 1999a). The simple dynamic model published by Kindlmann and Dixon (1993) demonstrates why the verbal argument presented here is correct.

### Intraguild predation

Intraguild predation has become a major research topic in biological control and conservation ecology. It occurs when two species of predators compete for the same prey and one of them also feeds upon its competitor (Polis et al. 1989). It is assumed to occur widely in many, but not all, guilds of biological-control agents (Rosenheim et al. 1995; Holt and Polis 1997). As a consequence, intraguild predation combines two important structuring forces in ecological communities, competition and predation (Polis and Holt 1992; Polis and Winemiller 1996) and may generate a diversity of indirect effects among co-occurring species (Miller and Brodeur 2002). However, models of intraguild predation are usually based on Lotka–Volterra equations, which are inadequate for modeling population dynamics of aphidophagous insects and their prey (Kindlmann and Dixon 1993, 1999a, b, 2001). In addition, empirical data indicate that the incidence of intraguild predation is most likely much lower than usually assumed (Kindlmann and Houdková 2006).

### A metapopulation model

Based on the above biological assumptions, a model of aphid – natural enemy population dynamics was constructed. For the “tree-type” situation (which includes also large stands of small vascular plants and large solitary vascular plants that can support the complete development of predatory larvae), Kindlmann and Dixon (1993) and Kindlmann et al. (2002) show that the within-season dynamics of this predator-prey system can be described by the following set of differential equations (details described in Kindlmann and Dixon 2003):

$$gh/dt = ax, h(0) = 0 \quad (1a)$$

$$dx/dt = (r - h)x - vpxy/(b + px + y), x(0) = x_0 \quad (1b)$$

$$dy/dt = -vy^2/(b + px + y) \times y(0) = x_0 \quad (1c)$$

Where:

$h(t)$  – cumulative density of the prey at time  $t$ ,

$x(t)$  – density of prey at time  $t$ ,

$a$  – scaling constant relating prey cumulative density to its own dynamics,

$r$  – maximum potential growth rate of the prey,

$y(t)$  – density of predator at time  $t$ ,

$v$  – predator voracity,

$b$  – parameter of the functional response of the predator,

$p$  – predator's preference for prey,

$T$  – time when predator matures; coincides with the duration of a patch of prey, yielding initial values  $x(T)$  and  $y(T)$  for the next season.

Equation (1a) describes changes in cumulative density of prey, (1b) describes changes in prey density and (1c) describes the decrease in predator density due to cannibalism. The fractions in eqs. (1b) and (1c) represent Holling's functional response type II for predator eating prey (1b), and predators eating each other (1c). A typical trend in numbers in a patch predicted by model (1) is shown in Fig. 1. There is no predator reproduction in the patch; therefore, predator numbers monotonously decline. As a consequence, if prey abundance ( $x$ ) increases at the beginning (i.e., if  $y(0)$  is sufficiently small, so that  $(\lim_{t \rightarrow 0+}) \times dx/dt > 0$ ) then, as time proceeds, prey population dynamics is less and less influenced by the declining numbers of the predator. Because of the way the diet of the predator is defined (the fractions in (1b) and (1c)), the decline in predator numbers is more pronounced when there are few prey individuals relative to predator individuals. That occurs, when the ratio  $x/(x + y)$  is small at the beginning and when prey numbers have passed their peak and become small again due to the negative effect of cumulative density (Kindlmann and Dixon 2003).

The predicted trends in the abundance of predators and prey (Fig. 1) closely match those observed in nature for aphids (Kindlmann and Dixon 1996; Dixon and Kindlmann 1998; Kindlmann et al. 2002) and ladybird beetles (Osawa 1993; Hironori and Katsuhiko 1997; Yasuda and Ohnuma 1999; Kindlmann et al. 2000).

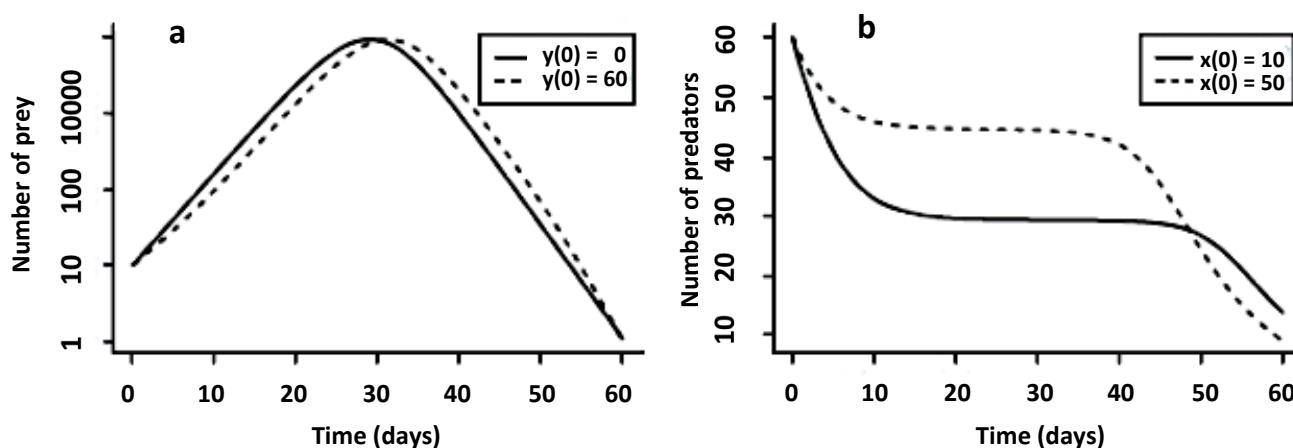
This model was then run for many seasons by Kindlmann and Dixon (2003) and Houdková and Kindlmann (2006), for a set of "patches" and the numbers of both prey and predator at the end of the season were lumped. This simulates both aphids and their natural enemies moving to overwintering sites. Then these individuals were subsequently distributed at random to patches, first aphids and then predators. For the predators, the above-mentioned biological restrictions were considered: they only lay eggs in patches of aphids not yet inhabited by predatory larvae. Then the within-season dynamics was repeated. In this way, an iterative simulation

of between-season dynamics was achieved. It is rather difficult to describe the whole set of results, therefore we refer the reader to the original papers in which there is a full description (Kindlmann and Dixon 2003; Houdková and Kindlmann 2006).

This leaves unanswered the question of whether the dynamics on small solitary vascular plants can affect the overall dynamics of the system. Our results indicate that they may not. As predators were observed only rarely on small solitary vascular plants and never repeatedly on the same plant on subsequent visits, these plants may just serve as stepping-stones for adult predators during their search for suitable aphid colonies. Therefore, metapopulation modelling of aphid – natural enemy systems may be justifiably restricted to those vascular plants occurring individually or in stands, which consist of a large area of leaves and stems on which aphids can feed and are also easily accessible to all stages of ladybirds. This, however, needs to be verified.

### The importance of vascular plant size in aphid – natural enemy systems

A 20 m sycamore tree has 116,000 leaves and can be infested with  $2.25 \times 10^5$  aphids at any one time during the vegetative season (Dixon 1971), whereas the total area of leaves and stems of small vascular plants is relatively minute compared to that of trees. Many small vascular plants, even the minute *Drosophila* of the plant world, *Arabidopsis thaliana*, however, are hosts of generally small specific or host alternating aphids, but the colonies, not surprisingly because space is limiting, tend to be very small and as a consequence rarely used by ladybirds for breeding. Adult aphid predators and sometimes even larvae freely move between aphid colonies/patches on both large vascular plants like trees and on stands of small vascular plants (nettles and cereals) and solitary large herbaceous plants (*Anthriscus*, *Rumex*, *Verbascum* etc.), and on these their immature stages complete their development. Thus, from the perspective of many aphidophagous predators, the aphid metapopulations they exploit consist of many



**Fig. 1** Trends over time of prey (left) and predator (right) abundances predicted by the within-season model when  $a = 0.000005$ ,  $r = 0.3$ ,  $v = 0.1$ ,  $b = 0$ ,  $e = 1$ , for  $y(0) = 0$  and  $y(0) = 60$  (left) and for  $x(0) = 10$  and  $x(0) = 50$  (right) – see insets for line codes.

**Table 1** Empirical data, collected in Greece and Czech Republic during 2017–2019: differences between aphid and predator dynamics on large vascular plants (trees) and small solitary vascular plants.

	Large vascular plants (trees, stands of small vascular plants)	Small solitary vascular plants		
	Greece 2017	Greece 2017	Greece 2018	Czech Republic 2019
Number of colonies	9	34	28	64
Number of visits per colony	24	24	22	15
Number of colonies with a “dent”	3	18	9	32
Number of adult ladybirds recorded	143	21	24	91
Percentage of visits, when adult ladybirds were recorded	66.2	2.6	3.9	9.5
Number of ladybird larvae recorded	187	39	5	86
Percentage of visits, when ladybird larvae were recorded	86.6	4.8	0.8	9.0
Number of ladybird pupae recorded	37	0	1	1
Percentage of visits, when ladybird pupae were recorded	17.1	0.0	0.2	0.1

populations, each of which consists of many colonies/patches of aphids. Migration of ladybirds between populations of aphids is mainly by adults that mature in the patches, many of which then leave the aphid population they matured in and reproduce in other suitable aphid populations.

Empirical data, collected in Greece and Czech Republic during 2017–2019, summarized in Table 1 (see Kindlmann et al. (2020) for details of sampling methods), illustrate the differences between the aphid and predator dynamics on large vascular plants and on small solitary vascular plants, which make up a large proportion of the vegetation in grassland landscapes.

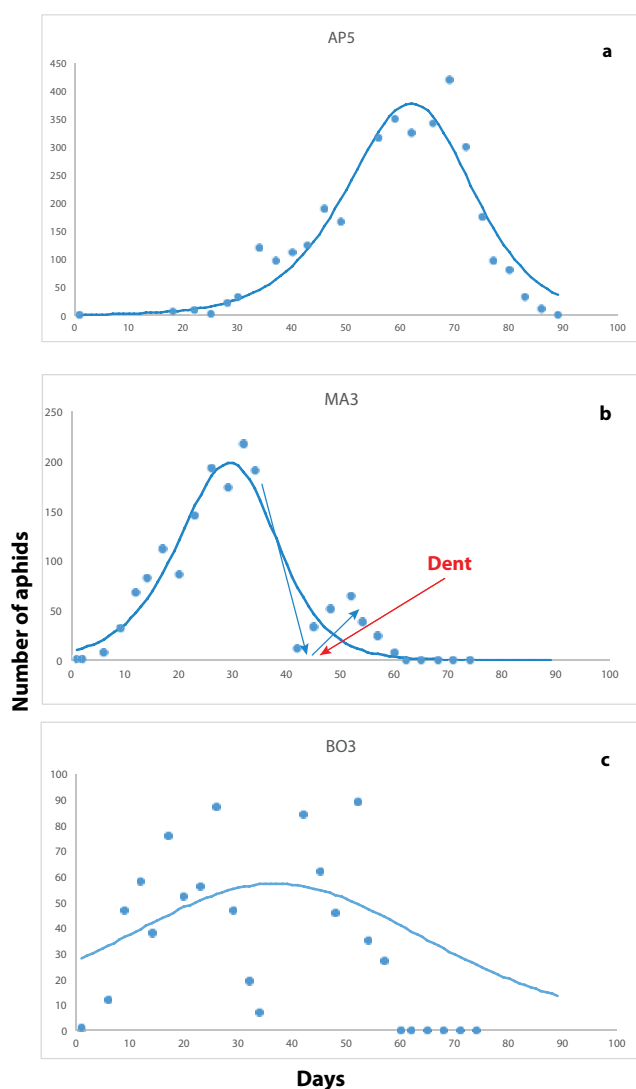
It is evident that population dynamics differ greatly between them. On small solitary vascular plants infested with aphids, predators were rarely observed (only about 1–9% of visits) and their larvae almost never recorded more than once on the same plant (Table 1). This is because it is likely that the larvae only survived for a short period of time and the effect of predatory larvae and adults on the aphid numbers in this colony is just incidental (the “dents” described below). Consequently, far fewer ladybird pupae were recorded on small solitary vascular plants than on large vascular plants. That means that small solitary vascular plants are unlikely to be suitable habitats for the larvae of ladybirds to complete their development, but serve as stepping-stones for adult predators to rest, occasionally lay a few eggs and most likely refuel during their search for patches of aphids suitable for reproduction.

On large vascular plants (trees) and stands of small vascular plants, predators were recorded much more frequently (more than 60% of visits, Table 1), which indicates that predators and aphids were present there con-

tinuously long enough for the dynamics described in model (1).

Three types of dynamics were recorded on small solitary vascular plants (Fig. 1). Most commonly, the aphid dynamics followed model (1) described above for when predators are absent (Fig. 1a). Sometimes there was a “dent” in the trend in the aphid population dynamics (Fig. 1b): a sudden decline in the numbers of aphids, followed by quick return to the standard trajectory in population dynamics (Fig. 1b). This could indicate the presence of a (probably adult) predator, which resulted in a significant reduction in the number of aphids, but was not recorded by the observer – most likely because the plant was used just for a short, probably refuelling stop: as a stepping-stone. The reason for the decline might have been different, however: for example, heavy rain, strong wind, or disturbance by large animals. Each of these disturbances might have caused a decline in aphid numbers on the plant, which was not possible to distinguish from the effect of predators. However, aphid numbers always returned to the standard trajectory very quickly. The rare third type of behaviour, illustrated in Fig. 1c, is typical of very small aphid colonies (see the numbers on the y-axis), where probably stochastic effects were more important.

In summary, two different habitats need to be considered, when modelling aphid – natural enemy systems: large vascular plants and stands of small vascular plants, and small solitary vascular plants. In the former, model (1) can be used, while the dynamics in the latter can be ignored, as they do not contribute to predator numbers. The approach used by Kindlmann and Dixon (2003) and Houdková and Kindlmann (2006) currently appears to be suitable for the analysis of predator metapopulation dynamics.



**Fig. 2** Three trends in aphid population dynamics were recorded on herbaceous plants growing individually: (a) aphid dynamics followed model (1) described above for when predators are absent; (b) the dynamics described as in (a) with a “dent” near the peak in the upward trend, followed by quick return to the standard population trajectory. The dent is most possibly due to the presence of a predator that reduced aphid numbers significantly; (c) chaotic dynamics, typical of very small aphid colonies, in which stochastic effects were probably the most important.

### Other Metapopulation Models for Predatory Insects

A metapopulation model for modelling predator-prey interactions was used by Nachman (2001) for a system consisting of the two-spotted spider mite (*Tetranychus urticae*) infesting cucumbers and the phytoseiid predatory mite *Phytoseiulus persimilis* in greenhouses. However, this system is characterized by a special type of dynamics, “hide-and-peek”, characterized by a high rate of turnover of local populations of prey and predators, because once the predators have found a patch of prey they quickly overexploit it, whereupon the starving predators either move to other plants or die (Nachman 2001). Continued persistence of prey and predators thus hinges on a long-term balance between local extinctions and founding of

new subpopulations (Nachman 2001). This is not the case for aphid – natural enemy systems, where no overexploitation occurs, except of small colonies on herbaceous plants early in the existence of an aphid colony. Therefore, for the latter system, a modelling approach other than that used by Nachman (2001) must be used. We are not aware of any other metapopulation model that would incorporate dynamics of both insect prey and predator.

### Conclusions for the Ladybird-Aphid System

Using new data, we more closely specify the existing metapopulation model of aphid-ladybird interactions. We have shown that population dynamics of aphid – natural enemy systems can be best modelled by model (1) run for many patches (large vascular plants and stands of small vascular plants). Then the final “autumn” numbers of both aphids and predators should be transferred to spring next year as in Kindlmann and Dixon (2003) and Houdková and Kindlmann (2006).

The biological traits (avoidance of colonies already occupied by predators, laying low numbers of eggs etc.) described here, together with the model predictions may have important consequences for biological control in the field as ladybirds and other insect predators are most unlikely to reduce abundance aphids.

This attempt to describe the aphid- natural enemy dynamics simply in terms of the structure of the plants on which aphids live, hopefully, will stimulate others to test this concept by collecting more data and concentrate on defining plants, not in terms of taxonomy, but in terms of features that are important in determining the fitness of the natural enemies of aphids.

### General Conclusions

The crucial assumptions of our aphid-ladybird metapopulation model that determine its behaviour are:

- (1) Prey live in discrete patches (in the case of aphids these are called colonies) regulated by strong intraspecific competition, which – when density is high – results in emigration from the patches; the amount of emigrating individuals is proportional to prey cumulative density.
- (2) Adult predators freely move between patches of prey, but immature predators are confined to one patch, because they cannot fly.
- (3) There is a strong cannibalism among the predators, if more of them live in the same patch
- (4) The ratio of the generation time of the predator to that of its prey (GTR) is large.

These assumptions lead to predictions of the metapopulation dynamics of the system, which coincide well with

reality. One very important prediction is that percentage mortality of immature predators is very high and therefore there is strong selection for maximising the survival of the offspring, which is much stronger than selection for an increase in voracity. These assumptions and predictions differ from those of classical Lotka-Volterra models, which explains, why these were not successful in modelling insect predator-prey dynamics. Thus voracity, which is the main determinant of population dynamics in Lotka-Volterra models, loses its role and is replaced by optimization of the choice of oviposition sites in systems with large GTRs.

As assumptions (1)–(5) seem to be quite commonly satisfied in many insect predator-prey systems, it seems that the conclusions presented here are quite general in insect predator-prey dynamics. Maybe only the inertia of many people prevents this model from replacing the classical Lotka-Volterra ones.

Finally, the GTR hypothesis can also account for one of the best examples of classical biological control involving a ladybird: that of *Rodolia cardinalis* controlling a scale insect infesting citrus trees in California (Dixon 2000). In this case the predator has a shorter developmental time than its prey, and as a consequence, as in the Nachman (2001) model, it overexploits its prey, which is then followed by hide-and-seek dynamics.

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