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Title: Exceptional color preferences for flying adult aquatic insects

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Abstract

This study tested the hypothesis that color affects the behavior of Ephemeroptera, Plecoptera, and Trichoptera (EPT) adults in the riparian zone of a gravel-bed river in northern Japan. EPT abundance was measured using plot-scale surveys and a color-choice experiment that utilized non-shiny sticky traps in two contrasting colors, yellow and blue. Chloroperlidae and Hydrobiosidae were caught more abundantly in yellow and blue traps, respectively whereas other taxa exhibited little or no color-affected responses. We proposed that Chloroperlidae responses were driven by relatively strong diurnal activity compared with those of other taxa. Hydrobiosidae's preference of blue remained unknown. Understanding the evolutionary background of color preferences in relation to other possibly interfering factors, such as reflection–polarization characteristics, at the species level will help advance the visual sensory ecology of aquatic insects.

Keywords: dispersal, EPT, gravel-bed river, riparian zone, sticky traps

Introduction

Understanding of habitat through organisms' life stages is important for full appreciation of insect ecology and their conservation. Macroinvertebrates including aquatic insects are a ubiquitous and diverse group that form a vital part of the aquatic ecosystem as can they constitute intermediate and upper trophic levels (Wallace & Webster, 1996; Rosi-Marshall & Wallace, 2002; Negishi *et al*., 2019). In rivers, their importance in food-web extends to riparian zones adjacent to the rivers via flight dispersals of aquatic insects (Baxter *et al*., 2005). These functions require sustained populations of insects with successful reproduction at their adult stage in the terrestrial zone. Although abundant knowledge on habitat requirements of larval aquatic stage exists, adult habitat is less known with previous studies focusing largely on environmental factors such as wind, humidity, vegetation and artificial barriers (Collier & Smith, 2000; Blakely *et al.*, 2006; Carlson *et al.*, 2016).

The visual characteristics of objects, such as color and reflectivity, are among the important cues (Kevan & Baker, 1983). In pollination ecology, pollinator insects are attracted to yellow and white colors (Vrdoljak & Samways, 2012). Color preferences of insects have also been described in agricultural pest (e.g., aphid) control studies in relation to the effective use of traps with specific colors (Döring & Chittka, 2007; Shimoda & Honda, 2013). Furthermore, the polarization of light affects the navigation behavior of insects (Weir & Dickinson, 2012). Reflection–polarization characteristics of object surfaces also attract some aquatic insects (Kriska *et al*., 2006), which is interpreted as the utilization of this attribute in optimizing the location of oviposition sites (Horváth *et al*., 2011). However, whether color affects the flight behavior of adult aquatic insects is scarcely known.

Subsets of aquatic insects, Ephemeroptera, Plecoptera, and Trichoptera (EPT) are useful indicators of river conditions and are often used in bio-assessment programs (Bonada *et al.*, 2006; Beyene *et al.*, 2009). They spend several weeks to years in water and a day to few weeks on the land where they mate, after which the females return and oviposit in rivers (Huryn & Wallace, 2000). Several taxa, including Plecoptera species, feed at their adult stages (Wesner, 2012; Tierno *et al*., 2019). During these adult stages, they disperse some distance over and along the water or within riparian forests (Petersen *et al*., 2004; Muehlbauer *et al*., 2014). Thus, adult EPTs encounter various objects with different colors after emerging from the water, and colors may be used as cues for their behavior.

This study examined the hypothesis that color affects the behavior of EPT taxa, with some taxa with higher daytime activity, such as Plecoptera species (Hynes, 1976), predicted to be disproportionately reactive to color. Sticky traps in blue and yellow were selected because they are among the most commonly tested colors and the only traps that differed in color that were readily available.

Materials and Methods

The field study was conducted during the summer (June and July) of 2018 and 2020 in the riparian zones of the Satusnai River in Northern Japan (**Figure 1**). The Satsunai River is a regulated gravel-bed river with multiple channels interspersed with both exposed and forested gravel bars. Riparian vegetation commonly comprises willows such as *Salix rorida* and *Populus suaveolens*, with understory vegetation dominated by *Fallopia sachalinensis*, *Carex* spp., and *Urtica platyphylla*. During summer, the daily mean air temperature ranged between 15 and 20 °C and the daily mean flow rate of the river was approximately 4–13 m^3/sec (Negishi *et al*., 2019).

The color-choice experiment was conducted in 2018 at six sites (**Figure 1**). Sticky traps in two colors (yellow and blue, 26 × 10 cm, total sticky surface on both sides of 520 cm^2; Horiver, Arysta LifeScience Co., Tokyo, Japan) were tied to trees at the boundary of the riparian forest and active channel (five sites) or around 50 m away from the channel in the riparian forest (one site) (**Figure 2**). Traps were hung vertically in the tree shade and suspended >1-m above the ground, with the relative position of two colors being randomly assigned (closest edge-to-edge distance between two colors was 5 cm). Additionally, the preference was tested also in a plot-scale survey in 2020 (**Figure 1, S1**). Four plots were set, with two each on the riparian forests on the right and left sides. One plot on each side was provided with yellow or blue traps, and 10 traps were set up across the plots with at least 10-m distance between them (40 traps in total). Traps were maintained in the shade at a height of approximately 160-cm above the ground (**Figure 2**). The traps were replaced at intervals of 3–5 days (2018) or 7–10 days (2020). At each replacement, in 2018, the EPTs were *in situ* counted for the order level whereas the traps were preserved in 70% ethanol, and family-level identification was later performed for EPTs in 2020. Species-level identifications were performed only for the family Chloroperlidae because the swift identification in the field was established for this taxon in a parallel study (Rahman *et al.*, 2021). Species-level identification for other families was not possible even in the preserved samples because of difficulty in reliable morphological identifications of trapped individuals entangled with the trap glue. The surface of the traps was neither shiny nor smooth because of the adhesive surface layer.

The insect responses to color were tested by developing generalized linear mixed models (GLMMs) with abundance as a response variable and trap color, taxa (four or six taxa), and their interactions as main factors, adopting a negative binomial distribution. The date of sampling and site (in the color-choice experiment) or bank location (left or right bank in the plot-scale survey) were included as random factors. When an interaction term was significant at p<0.05, multiple comparisons were performed between color types within each taxon by rerunning GLMMs after removing the effects of the taxa. Statistical significance was corrected using the Bonferroni method for multiple comparisons.

Results

A total of 255 EPTs in four taxa (Ephemeroptera, Plecoptera excluding Chloroperlidae, Chloroperlidae, and Trichiptera) were caught in the color-choice experiment. A total of 4,339 EPTs were caught and six numerically dominated families (Heptageniidae, Baetidae, Nemouridae, Chloroperlidae, Philopotamidae, and Hydrobiosidae; 95.6%) were further analyzed in the plot-scale survey.

In both cases, there were significant interactions between color and taxa when compared with the model without the interaction term (p<0.001, likelihood ratio tests). The yellow traps caught more Chloroperlidae in both experiments with Hydrobiosidae caught in more blue traps than in yellow traps in the plot scale survey (**Figure 3**). *Alloperla ishikariana* dominated Chloroperlidae in both choice experiment cases (>98%), followed by *Sweltsa abdominalis* and *Suwallia thoracica*.

Discussion

To our knowledge, this study is the first report on color-related behavioral responses of adult aquatic insects. Consistent with our predictions, the behavior of diurnal Chloroperlidae aquatic insects was affected by color. However, Hydrobiosidae, was positively responsive to blue color, indicating that the responses to color differed among taxa with complex taxon-specific preferences in exceptional taxa. Different colored traps were the same in terms of material, direction, and light conditions without high reflection of light, suggesting that reflection–polarization did not confound the results.

The color preferences of flying insects have been determined using traps in non-aquatic environments (Broughton & Harrison, 2012). Several flower-visiting insects express an innate color preference, with many insects being attracted to yellow (wavelength: 560–590 nm) (Prokopy & Owens, 1983), including Diptera (flies) and Lepidoptera (butterflies and moths)

(Kevan, 1983). Blue (400–500 nm) flowers have been observed to be attractive to Hymenoptera (bees), whereas pink and red (650–700 nm) flowers are frequently visited by Lepidoptera (Kevan, 1983). Although the evolutionary mechanisms underlying these color preferences remain equivocal, the attractiveness of different colors may be partially related to taxon-specific differences in the diurnal cycles of their flight activities.

Adult aquatic insects are mainly crepuscular or nocturnal (Brakel *et al*., 2018; Shimoda & Honda, 2013). Thus, the absence of color preferences for most Trichoptera and Plecoptera was possibly related to their nocturnal flight activities. The attraction of insects to colors that differs in relation to the time of the sampling has been shown, with yellow being more attractive for flying insects during the day than at night (Long *et al*., 2011). Briers *et al*. (2003) suggested that some plecopterans were more active during the day. At our study site, *A. ishikariana* were observed to be largely diurnal, with large numbers of individuals being spotted resting and occasionally mating on plant leaves in the riparian zones during the day (personal observations). Therefore, the higher abundance of Chloroperlidae in the yellow traps could be attributed to an increased distinguishability of colors during the day. An intriguing exception in Trichoptera was the preference of blue by Hydrobiosidae. Nocturnal insects can be sensitive to light (Shimada & Honda, 2013), and thus this taxon might have a relatively high ability of sensing color differences at night. Future studies should determine circadian rhythm in their flight activities in relation to color preferences at species-level identification. This species-level understanding is needed for other taxa because the color-related behavior might have been blurred by coarse taxonomic identifications at the order or family levels.

In conclusion, we showed that some taxa of adult aquatic insects could exceptionally distinguish between at least two contrasting colors. This points to the possibility that visual appearance of objects in riparian zone may affect terrestrial habitat use of aquatic insects. However, ecological reasons behind color preference remains unclear. The interference effects of polarization also need to be further examined. Regarding the preference for yellow, one explanation is that the color acts as a cue for the insects to locate food resources. Yellowish resources, such as pollen, are utilized as food items by some taxa, including Chloroperlidae (Tierno De Figueroa & López-Rodríguez, 2019). They may also benefit from color cues in increasing the probability of encountering mates and reaching forested riparian zones. Future studies on the mechanistic understanding of the importance of colors in Chloroperlidae and Hydrobiosidae will help advance adult habitat ecology as well as the visual sensory ecology of aquatic insects. In such efforts, potential artifacts in this study such as hormone effects of trapped individuals and the presence of attractant ingredient in the trap glue need to be carefully controlled.

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Declarations

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Conflicts of interest/Competing interests

No conflict of interest exists

Availability of data and material

Available from authors upon reasonable requests

Code availability

Not applicable

Authors' contributions

Project design: JNN, TN, FN, data collection and analysis: JNN and TN, and paper writing: JNN, TN, FN

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Figure captions

Figure 1: The location of the study area in the Tokachi River in Hokkaido (a), and the study site in the Satsunai River (b). In (b), the point source of nutrient inputs from waste water treatment plant (WWTP) in a red circle, the location of the study sites in the color-choice experiment in the gray-filled circles, and the location of study plots in the plot-scale survey in a shaded gray box.

Figure 2: Sticky traps used in the color choice experiment (a), a yellow trap used in the plot-scale survey (b) and a blue trap used in the plot-scale survey. Ziplock bags were set above the upper end of the trap to prevent rainfall from reducing glue stickiness of the traps.

Figure 3: Number of individuals caught per day by blue or yellow sticky traps in the color-choice experiment (a) and plot-scale survey (b). ***: $p<0.001$ in multiple comparisons between colors for respective taxa after Bonferroni correction for statistical significance. Outliers were shown in dots.

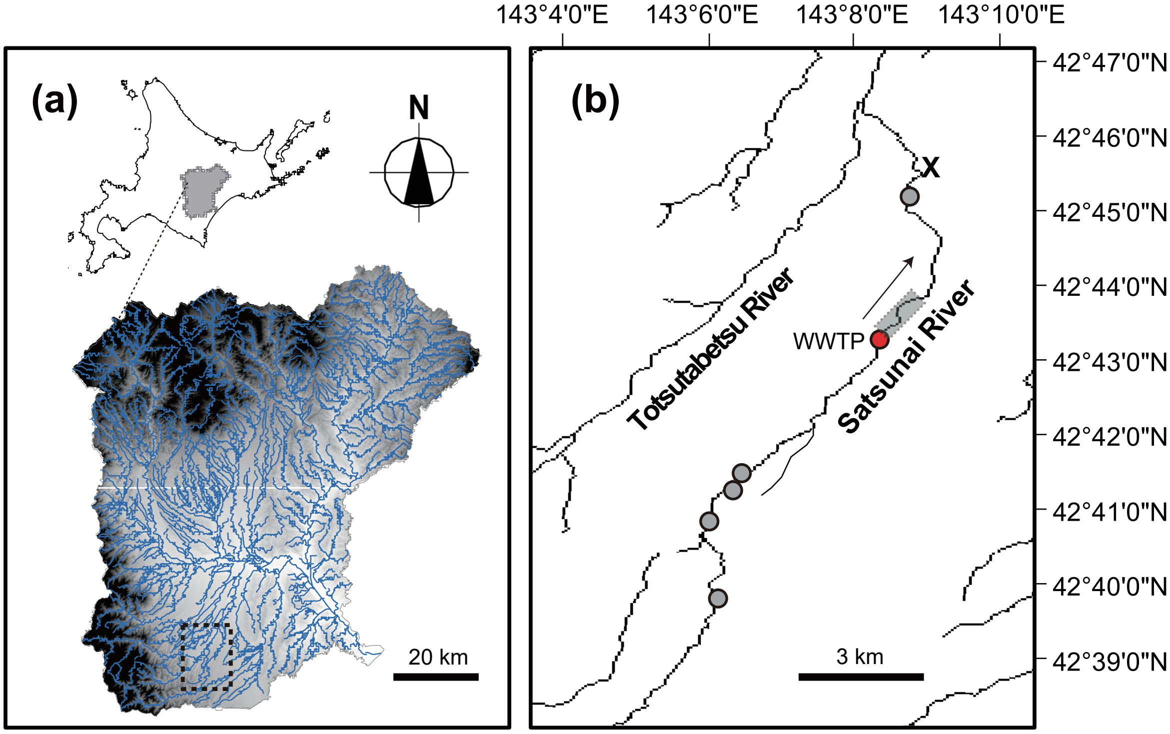

Figure 1 Negishi et al.

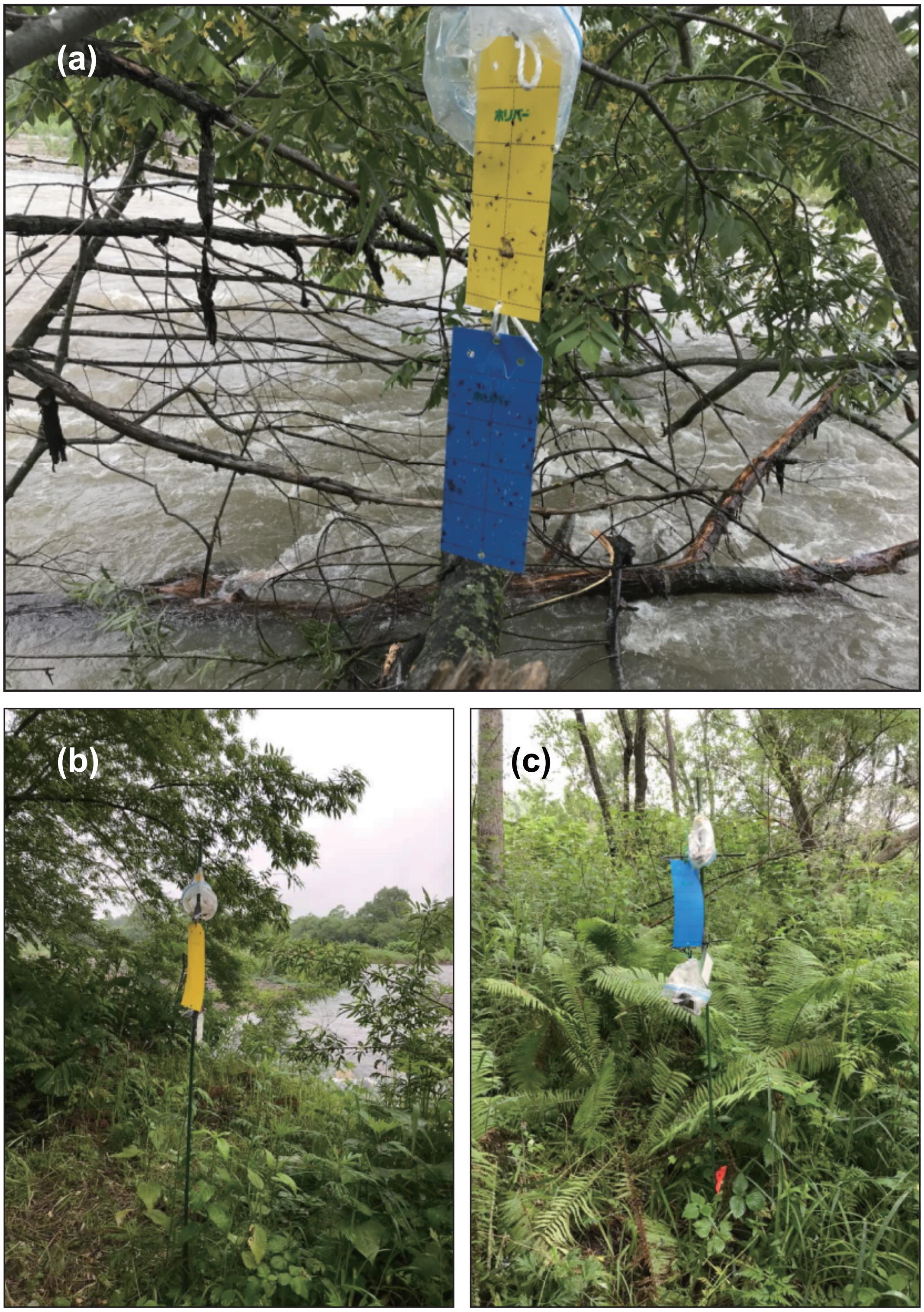

Figure 2 Negishi et al.

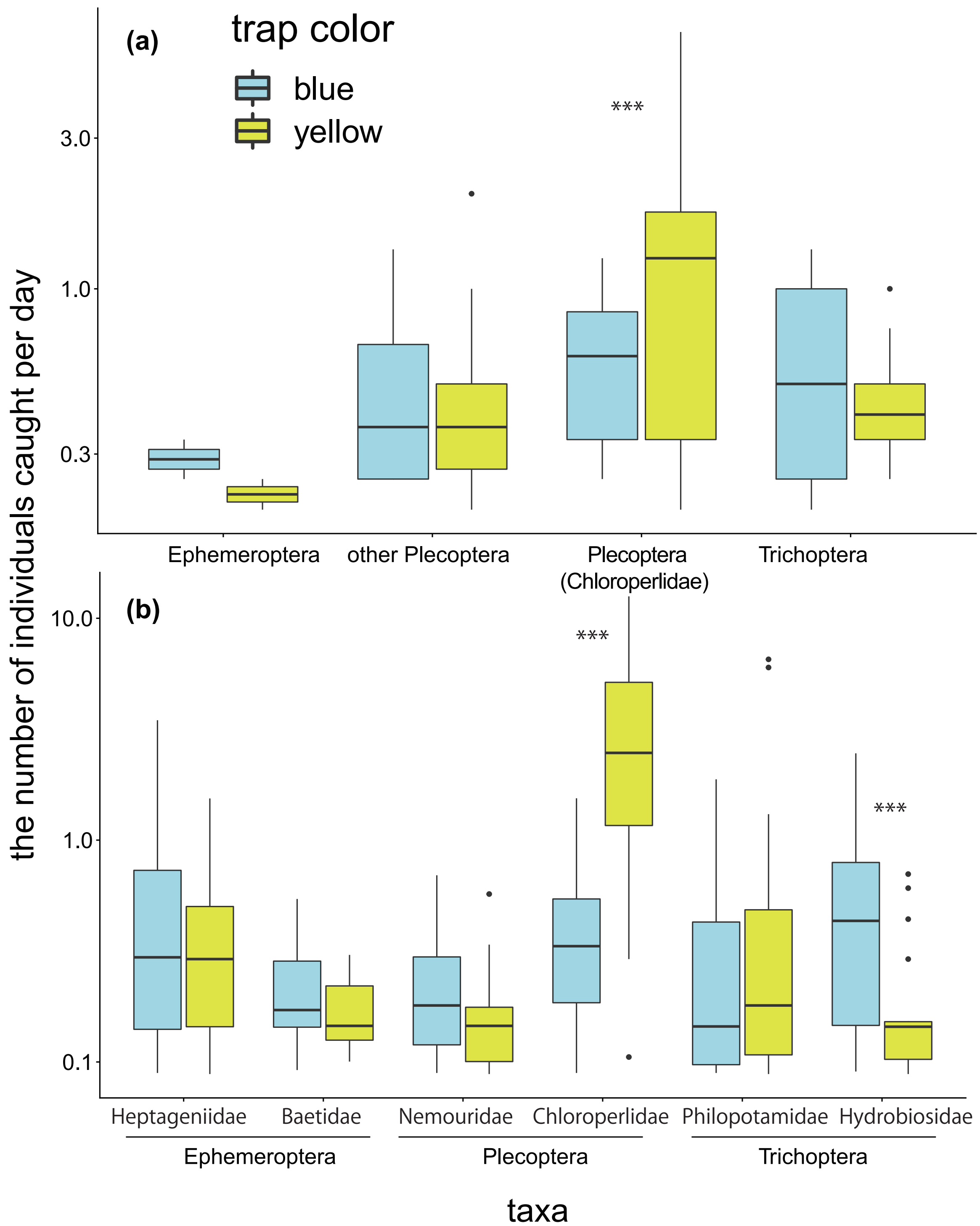

Figure 3 Negishi et al.