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Water depth-dependent notonectid predatory impacts across larval mosquito ontogeny

A short running title: Notonectid predation against mosquito larvae

Arpita Dalal*¹, Ross N. Cuthbert², Jaimie T.A. Dick² and Susmita Gupta¹

¹Department of Ecology and Environmental Science, Assam University, Silchar- 788011, India

²Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, Northern Ireland, United Kingdom

*Correspondence to: Arpita Dalal, Department of Ecology and Environmental Science, Assam

University, Silchar, India, Email: rptdll4@gmail.com

Abstract

BACKGROUND: Context-dependencies can modulate the strength of predatory interactions and often remain unquantified. In particular, differences in water depth within aquatic systems could influence predator efficiencies towards prey which utilise 3D-space through the water column.

Differences in prey size could drive prey size-refuge effects, influencing the efficacy of natural enemies towards vector species. We thus quantify the predatory impact of two notonectid predators, *Anisops breddini* and *Anisops sardeus*, towards four different larval instars of *Culex quinquefasciatus* prey across a water depth gradient, using functional responses (FRs).

RESULTS: Consumption rates differed significantly between the predators, and interspecific differences in responses to variations in water depth were emergent. Both notonectids were able to handle *C. quinquefasciatus* prey across all instar stages, yet predation rates were generally

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higher towards early as opposed to late instar prey. *Anisops sardeus* was most voracious, and differential predation rates of this species were most pronounced in shallow waters. Type II FRs were displayed by notonectids in the majority of treatments; however, Type III FRs were emergent in specific treatment groups, with potential implications for prey population stability. Both capture rates and handling times were often greater at greater depths, and thus maximum feeding rates reduced as depth increased. Our results further demonstrate substantial predatory impacts of notonectid predators towards mosquito, and quantify biotic and abiotic context-dependencies which modulate their impact.

CONCLUSION: Given notonectids are capable of aerial dispersal between ephemeral aquatic habitats of varied volumes, their promotion in aquatic systems could help reduce proliferations of medically important mosquitoes.

Keywords: functional response, *Culex quiquefasciatus*, biological control, water depth, prey size

1 INTRODUCTION

Predator communities play a crucial role in the regulation of prey populations in natural systems,^{1,2} which, in turn, influences structuring and functioning at the ecosystem level.^{3,4} In ephemeral aquatic ecosystems especially, predaceous insects such as hemipterans, coleopteran larvae and odonate larvae are often top predators,⁵ and have the potential to structure communities *via* trait- and density-mediated interactions with their prey.^{6,7} However, both biotic and abiotic context dependencies can alter interaction strengths between trophic groups in aquatic ecosystems, and predictive understandings of how such contexts influence crucial top-down processes such as predation are lacking. In particular, within a biological control context,

environmental contexts could have important consequences for target prey population regulation, including towards medically important vector species.

Mosquitoes are vectors of deadly human diseases such as malaria, dengue, Zika, and yellow fever, especially in the tropics, with more than one billion people infected and over 700,000 people dying from vector-borne diseases annually.⁸ Mosquitoes successfully colonise a vast range of natural and artificial aquatic systems, including in urban areas where there is a high potential for encounters with humans.⁹ In particular, *Culex quinquefasciatus* Say, 1823 (Diptera: Culicidae) is a member of *Culex pipiens* complex and is a competent vector of bancroftian filariasis, West Nile virus, Sandbis virus, St Louis encephalitis, Rift Valley fever virus and lymphatic filariasis (see^{10, 11, 12}). Mosquitoes in the *C. pipiens* complex exhibit an abundant and worldwide distribution, and are known to colonise a broad range of aquatic environments, including wetlands, rice fields and artificial containers.^{13,14,15} To combat these vectors, predators such as aquatic insects, arachnids, crustaceans, fish, birds and bats have been identified.^{16, 17, 18} Biological control using predators has been effective in mosquito population management strategies (e.g.¹⁹), and particularly during efforts which target mosquitoes during their aquatic larval life history stage. However, context-dependencies, such as changes in water depth or mosquito prey size, could exert a significant influence on the efficacy of biocontrol agents, and should thus be integrated into considerations among candidate natural enemies.

Functional responses (FRs) are central to the derivation of density-dependent consumer-resource (e.g. predator-prey) interaction strengths.^{20, 21} The capture rate (i.e. attack rate, search efficiency), handling time and maximum feeding rate parameters are central components of FRs, and have been shown to be congruent with ecological impacts in biological control agent examinations.^{17, 18, 22} Indeed, comparative FRs can be integrated to examine relative efficacies

between biological control agents in vector control, and across multiple environmental contexts.^{17, 18} Three form of FRs types (shapes) have been described: Type I, II and III.^{20, 23, 24} In a predatory biocontrol context, the identification of agents which exhibit Type II FRs is desirable due to their potentially population destabilising effects, attributable to high rates of prey consumption at low densities that reduces the potential for prey refuge and thus persistence.²⁵ In the context of mosquitoes, Type II FRs may reduce the recruitment of mosquitoes through to the adult stage that is capable of vectoring disease. In contrast to Type II FRs, Type III FRs may impart stability to prey populations by providing refugia to low prey densities, owing to low proportional consumption rates where prey are scarce.²⁰

Notonectids (water boatmen) are voracious predators and are known to play a key role in the structuring of aquatic communities (e.g. ^{26, 27}). In the tropics, this group is often dominant in both natural and artificial ephemeral aquatic ecosystems, where mosquitoes successfully establish.^{28, 29} Whilst their diet is known to include all size classes of mosquito larvae, efficiencies can vary substantially according to the larval ontogenic stage of mosquito prey.³⁰ Although predatory efficacy of notonectids towards mosquito larvae has been well-described (e.g. ^{31, 32, 33}), it is unclear how variabilities in search area within 3D aquatic ecosystems influences the strength of offtake rates by such consumers. This is particularly important for mosquito prey, which forage throughout the water column. Indeed, examinations of biotic interactions often fail to address potential water depth variations, despite the dynamic and transient nature of small aquatic habitats where mosquitoes breed. Therefore, the present study employs comparative FRs (e.g. ^{17, 18, 27, 34}) to decipher the effects of water depth on the predatory impact of two widespread and abundant notonectid predators, *Anisops breddini* Kirkaldy, 1901

(Heteroptera: Notonectidae) and *Anisops sardeus* Herrich-Schaeffer, 1849 (Heteroptera: Notonectidae) towards four larval instar stages of *Culex quinquefasciatus* prey.

2 MATERIALS AND METHODS

2.1 Collection of predators and prey

The focal predators, size-matched adult *A. breddini* (7.04 ± 0.06 mm) and *A. sardeus* (7.21 ± 0.06 mm) were collected from fishery ponds (pond 1: 3800 sq. m, ~70 cm depth; pond 2: 3100 sq. m, ~ 62 cm depth) of Irongmara, Cachar, Assam, India ($24^{\circ}41'15.78''\text{N}$, $92^{\circ}45'12.21''\text{E}$) during the 2017 summer-monsoon season by trawling a kick net through the water column by hand. These notonectids were transported in source water to a laboratory in the Department of Ecology and Environmental Science, Assam University, Silchar. Both species were kept separately in a glass aquaria holding 25 L de-chlorinated tap water with continuous aeration. The laboratory was maintained at 30°C ($\pm 2^{\circ}\text{C}$) on a 12:12 light:dark photoperiod, and notonectids were acclimated to these conditions for at least one week prior to the experiment, with water changed every 10 days and fed *ad libitum* with mosquito and chironomid larvae.

The prey, larvae of *C. quinquefasciatus*, were collected from the Assam University campus by sampling egg rafts from artificial container that were filled with jaggery and water mixture to attract oviposition. The mosquito egg rafts were then transferred to the same laboratory as the notonectids, where, upon hatching, mosquito larvae were reared to the desired size class on a diet of crushed jaggery (Arya Farm Products Pvt. Ltd) *ad libitum*. Jaggery is a traditional non-centrifugal organic cane sugar lump made from date or palm tree.

2.2 Functional response experiment

Individuals of each predator species were starved in isolation for 24 h prior to experimentation to standardize hunger levels. We employed a factorial '3 × 2 × 4 × 6' experimental design to discern the effects of 'water depth', 'predator species', 'prey instar' and 'prey density', respectively, on notonectid-mosquito interaction strengths. Feeding trials were conducted in glass aquaria with a standard surface area of 18 cm². Aquaria were filled with dechlorinated tap water to three different depths: 5 cm (D1, ~ 1.44 L), 10 cm (D2, ~ 2.95 L) and 15 cm (D3, ~ 4.45 L). Both predator species (*A. breddini* and *A. sardeus*) were separately provided four different instar stages of *C. quinquefasciatus* (i.e. 1st instar, 1.1 ± 0.02 mm; 2nd instar, 2.21 ± 0.05 mm ; 3rd instar, 3.99 ± 0.03 mm; 4th instar, 4.9 ± 0.05 mm) under six prey densities (5, 10, 25, 50, 75, 100; $n = 3$ per experimental group). Following the addition of prey, notonectids were introduced to individual arenas and were allowed to feed for 24 h, after which the predators were removed and numbers of remaining live prey enumerated to quantify numbers killed. To eliminate time confounds, batches of experimental trials were fully randomised according to experimental treatment groups. Controls consisted of prey without predators at each water depth, prey instar stage and prey density ($n = 3$ per experimental group).

Statistical analyses were performed in R v3.3.2.³⁵ Raw consumption was analysed with respect to 'water depth', 'predator species' and 'prey instar', and their interactions, using generalised linear model (GLM) assuming a quasi-Poisson family, owing to residual overdispersion. 'Prey density' was included as an individual effect, owing to its importance in FR derivations. *F*-tests with Type III sums of squares were employed to derive main effects. Post hoc Tukey tests were conducted for pairwise comparisons of significant effects.³⁶

To discern FR types, logistic regression of the proportion of prey killed as a function of prey density was used for across each experimental group.^{24, 35, 37} Here, a significantly negative

first order term indicates a Type II FR, whilst a significantly positive first order term followed by a significantly negative second order term indicates a Type III FR. A generalised form of the FR was implemented that accounts for non-replacement of prey (i.e. prey depletion) over the allocated experimental time:^{37, 38, 39}

$$N_e = N_0(1 - \exp(bN_0^q(hN_e - T))) \quad (1)$$

where b is the attack or capture rate, which, combined with the scaling exponent q , gives the density-dependent capture rate (bN_0^q), h is the handling time, T is the total experimental time, N_0 is the initial prey density and N_e is the number of prey eaten. Here, q can be fixed at 0 (a Type II FR), 1 (a Type III FR) or instead optimized, providing a means to examine fits of categorical (i.e. Type II or III) or continuous (i.e. flexible) forms. We used maximum likelihood estimation for model fitting.⁴⁰ Where FR types were equivocal, we compared candidate models using Akaike's Information Criterion and fit models which minimised information loss (AIC; lower values indicate better fit). We then non-parametrically bootstrapped starting FR fits ($n = 2000$) to produce 95% confidence intervals (CIs) around FRs for each predator treatment combination, where significant differences were then deduced on the basis of CI overlaps.

3 RESULTS

Survival of prey was 100 % in the control groups across all treatments, and experimental mortality was thus attributed to predation by notonectids. Overall, consumption of *C. quinquefasciatus* larvae was not significantly affected by water depth ($F_{2, 403} = 1.391$, $P = 0.250$). *Anisops sardeus* consumed significantly more prey than did *A. breddini* overall ($F_{1, 403} = 16.516$, $P < 0.001$; Fig. 1). There were significant consumptive differences between prey instar stages by

notonectids ($F_{3, 403} = 138.336$, $P < 0.001$; Fig. 1). Significantly fewer 4th instar mosquito prey were consumed than earlier instars (all $P < 0.001$), whilst 2nd instar prey were consumed most (all $P < 0.001$). In turn, 3rd instar prey were consumed less than 1st instars overall ($P = 0.010$). However, the differential consumption of prey across instar stages was significantly altered by variations in water depth ($F_{3, 403} = 3.044$, $P = 0.006$; Fig. 1). Here, the effects of water depth were most pronounced for consumption of 1st instar prey, with significantly more prey killed at low and intermediate depths as compared to the greatest depth (both $P < 0.001$). Conversely, for 2nd – 4th instars prey, there were no overall significant consumptive differences across each water depth level (all $P > 0.05$). However, A significant ‘water depth \times predator species \times prey instar’ interaction term further indicated emergent complexities in feeding patterns between experimental treatments ($F_{6, 403} = 4.802$, $P < 0.001$). Here, consumption by *A. sardeus* towards 1st instar prey was most affected by water depth variations, and highest at intermediate depths as compared to either low or high depths (both $P < 0.01$; Fig. 1e). On the other hand, consumption by *A. breddini* was significantly greater at the lowest depth towards 1st instar prey, as compared to intermediate and high depths (both $P < 0.05$; Fig. 1a). Consumption of 2nd instar prey by *A. breddini* was, however, significantly greater at the intermediate as oppose to the low water depth ($P < 0.05$; Fig. 1b). Furthermore, significantly more prey were consumed as higher densities of prey were supplied overall ($F_{5, 403} = 317.798$, $P < 0.001$).

Type II FRs were most prevalent across experimental treatment groups, as indicated by significantly negative first order terms (Table 1). However, Type III FRs were evidenced towards 1st and 3rd instar prey at the shallowest depth by *A. breddini*, and towards 2nd instar prey at the greatest depth (Table 1; Fig. 2). Type III FRs were only evidenced at the intermediate depth towards 1st instar prey by *A. sardeus* (Table 1; Fig. 2). For *A. breddini*, capture rates were

significantly lower towards 1st instar prey at low water depths (Table 2; Fig 3a). Conversely, towards 2nd instars, *A. breddini* capture rates were significantly reduced at the high depth treatment as compared to low and intermediate groups (Table 2; Fig. 3b). For 3rd instar prey, capture rates increased with greater water depths, with rates significantly reduced at low water depths compared to intermediate and high groups (Table 2; Fig. 3c). Capture rates towards 4th instar prey groups were more similar (Table 2; Fig. 3d). Overall, *A. breddini* capture rates were relatively similar among instar stages (Fig. 3a–d).

For *A. sardeus*, capture rates were significantly lower towards 1st instar prey at intermediate water depths, as compared to low and high depths (Table 2; Fig. 3e). In turn, capture rates were significantly greater at the high compared to low depth here (Table 2; Fig. 3e). However, towards 2nd instar prey, capture rates were generally low and relatively unaffected by variations in water depth (Table 2; Fig. 3f). Capture rates of 3rd instar prey by *A. sardeus* were significantly reduced in the deepest treatment group, yet were more similar between intermediate and low depths (Table 2; Fig. 3g). Capture rates towards 4th instar prey were significantly reduced at the low as compared to intermediate and high depths (Table 2; Fig. 3h), and were significantly higher than towards all other prey instars (Fig. 3e–h). Capture rates of *A. sardeus* tended to be higher than *A. breddini* overall towards towards 1st, 3rd and 4th instar *C. quinquefasciatus* prey, whilst they were reduced toward 2nd instar groups (Fig. 3).

Overall, for *A. breddini*, handling times were similar across water depths for 1st, 2nd and 3rd instar prey, owing to overlapping of CIs (Fig. 4a–c). However, for these prey groups, handling times tended to be lowest at intermediate water depths (Table 2). For *A. breddini*, 4th instar handling times were also not significantly different according to water depth (Fig. 4d), yet tended to be reduced at low depths (Table 2). Overall, 4th instar prey handling times were

significantly longer than most other prey groups (Table 2; Fig. 4a–d). Accordingly, FR magnitudes were significantly reduced for this prey stage (Fig. 2a–d).

For *A. sardeus*, handling times were significantly shorter at the low and intermediate depths towards 1st instar prey, as compared to the high depth (Table 2; Fig. 4e). Towards 2nd instar prey, handling times were similar at intermediate and high depths (Table 2; Fig. 4f), but were significantly shorter at low compared to intermediate depths. For 3rd instar prey, handling times by *A. sardeus* were not significantly different across water depths (Fig. 4g), however were generally reduced at the highest depth treatment (Table 2). Handling times for 4th instar prey were not significantly different across depth treatments (Table 2; Fig. 4h), however 4th instar prey handling times were significantly greater than all other prey types (Fig. 4e–h). Functional response magnitudes were thus significantly reduced for 4th instar prey (Fig. 2e–h). Handling times of *C. quinquefasciatus* prey were generally similar between the two predators overall (Fig. 4).

4 DISCUSSION

Biological control of mosquitoes using natural enemies can be effective to mitigate the proliferation and emergence of medically important mosquito species (e.g. ^{19, 41}). However, the impacts of predators on prey have been shown to be highly context-dependent, with both biotic and abiotic factors potentially influencing the impact of consumers within ecosystems. ^{27, 32, 34, 42,}

⁴³ In this regard, FRs can act as a framework in deciphering the predatory potential of biological control agents in controlling larval disease vectors in aquatic ecosystems. ^{17, 18, 32, 34, 43} During the present study, *A. sardeus* was identified as a more voracious predator than *A. breddini*. Whilst potentially destabilising Type II FRs were most prevalent between the predators, the present

study also found emergent evidence for Type III FRs congruent with prey population stabilizing effects. Although interactive complexities were present in our results between water depths, predator species and prey sizes, consumptive differences between depths were particularly marked towards early mosquito instars, wherein predators consumed less in deeper waters. Moreover, earlier instar prey tended to be impacted more greatly than late instar mosquitoes, with handling times longer and thus maximum feeding rates lower for these groups. Second instar prey were most consumed overall.

Within laboratory settings, predator-prey interactions with invertebrate consumers typically conform to Type II FRs (e.g. ^{32, 33, 43}). In the present study, although both of the focal notonectids displayed Type II and Type III FRs, *A. sardeus* mostly showed Type II FRs, indicating that population-destabilising predatory impacts from this species upon low densities of mosquito larvae are particularly high, and robust to variations in water depth and prey size. The selection of biological control agents which are able to handle larval mosquitoes throughout their ontogeny is beneficial, given that size refuge effects in large mosquito prey could facilitate competition alleviation, in turn producing larger adults and that are better vectors.⁴⁴ Thus, the present study identifies notonectids as effective mosquito predators, and particularly *A. sardeus* which exhibits higher capture rates towards late instar prey, and so minimizes refuge effects.^{25, 34} Indeed, many predators are unable to handle resources relatively big or small, potentially reducing population-level offtake rates.^{45, 46} However, further research is required to quantify selectivity traits between multiple coexisting mosquito size classes. In contrast to Type II FRs, Type III FRs could have stabilising effects on prey that are associated with low-density prey refugia.^{25, 47} Therefore, whilst predation by *A. sardeus* has the potential for high impact on mosquito prey at low densities, *A. breddini* on the other hand, may better allow mosquito larvae

to persist *via* low density refugia in container-style habitats for specific water depths and prey sizes. However, empirically, Type III FRs could also be intertwined with switching behaviours, where the predator encounters alternative prey and exhibits frequency-dependent predation strategies (see ⁴⁸). Although, studies have repeatedly found notonectids to preferentially select mosquito prey, which may minimize such effects although; yet, this was not assessed in the present study. ^{49, 50, 51, 52}

In many cases, capture rates of *A. sardeus* surpassed those of *A. breddini*. The higher capture rates of *A. sardeus* compared to *A. breddini* further indicate higher predation pressure on mosquito larvae by this species at low prey densities. Capture rates of *A. breddini* were highest for 2nd followed by 3rd instars of mosquito prey. This suggests a unimodal response of capture rates to prey sizes, whilst handling time were generally shorter for small-sized prey, indicating greater maximum feeding rates. This result is consistent with previous work on other notonectids which displayed similar consumptive traits (see ³⁰). According to the optimal foraging theory, prey of intermediate relative size (i.e. 2nd and 3rd instars of mosquito larvae) are considered to be the most profitable prey for predators.⁵³ Given capture rates by *A. breddini* were greater mostly in higher water depths, destabilizing predatory impact on low prey densities can be deemed most profound in these water depths. On the other hand, handling times of *A. breddini* were typically longest in the lowest water depth and for the largest prey, indicating maximum feeding rates were generally decreased by reductions in water depth and greater prey size. For *A. sardeus*, capture rates were also mostly elevated in deeper waters, whilst, conversely, handling times were often reduced in shallow water. Further, both capture rates and handling times of *A. sardeus* generally increased with increasing prey size. This result corroborates with other predatory aquatic insects, such as odonates, belosomatids and other species of notonectids, which display

higher attack rates and handling times for larger instar stages of mosquito larvae.^{54, 55} This might be due to the increasing ability of the predator to detect larger prey, coupled with a longer time to subdue and consume such prey items (see ⁵⁶). Given both predators capture their prey by ambush, greater search areas may enhance capture efficiencies. Thus, the overall predatory impact of *A. breddini* and *A. sardeus* were dependent both on physical (water depth) and biotic (prey size and densities) factors.

Our findings corroborate with the results of previous work where notonectid predators were identified as potential biocontrol agents for mosquito larvae under laboratory conditions.^{32, 33, 54, 57, 58} Similar to our findings, the rate of consumption of mosquito larvae by a congeneric species of *Anisops* were higher in lower volumes of water.³² Previous studies have also shown that notonectid predators preferentially consume mosquito larvae over other alternative prey (e.g. ^{49, 50, 51, 52}). This further demonstrates the biocontrol efficacy of notonectid predators in controlling mosquito larvae in a container style habitat with multiple prey types. Considering the depth of water used in the present study experiment, notonectids may be effectively used in small natural or artificial aquatic bodies where mosquitoes successfully colonise. Unlike many other natural enemies of mosquitoes, notonectids carry an air bubble (atmospheric oxygen) on their abdomen, allowing them to be less susceptible to poor water qualities than other groups.⁵⁹ In particular, in the urban environment where aquatic systems are often highly polluted, notonectids are able to aeri ally colonise aquatic systems where other predators cannot persist.⁶⁰ Although the presence of notonectids in aquatic systems can lead to oviposition avoidance by mosquitoes, arrival of these predators after oviposition events *via* aerial dispersal could enable efficacious mosquito control across landscapes.⁶¹ Furthermore, Anisopinae (sub-family of Notonectidae) species can remain under water for much longer durations than Notonectinae sub-family species

as they can attain neutral buoyancy at water depth.⁵⁹ Therefore, given our results, both *A. breddini* and *A. sardeus*, have the potential to efficiently reduce populations of mosquitoes across varying levels of prey ontogeny, water depth and prey density. Further field studies and research are, however, required to further quantify the roles of notonectids and other invertebrate predators in regulating medically important mosquito species within aquatic habitats.

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Table 1. Functional response (FR) types resulting from logistic regression, with first and second order terms of *Ansiops breddini* and *Anisops sardeus* against four instars (1st – 4th) of *Culex quiquefasciatus* at three different level of water depth. First order terms are presented for Type II FRs, whilst first and second order terms are presented for Type III FRs.

| | | 1 st instar | | | 2 nd instar | | | 3 rd instar | | | 4 th instar | | |
|-------------------------|-------|------------------------|-----------------------|----------|------------------------|-----------------------|----------|------------------------|-----------------------|----------|------------------------|-----------------------|----------|
| Predators | Depth | 1 st order | 2 nd order | FR types | 1 st order | 2 nd order | FR types | 1 st order | 2 nd order | FR types | 1 st order | 2 nd order | FR types |
| <i>Anisops breddini</i> | D1 | -0.026 * | 0.0003 | III | -0.014 | - | II | -0.108 | 0.0009 | III | -0.013 | - | II |
| | | | ** | | *** | | | *** | *** | | *** | | |
| | D2 | -0.002 | - | II | -0.006 | - | II | -0.003 | - | II | -0.02 | - | II |
| | | | | | * | | | | | *** | | | |
| | D3 | -0.006 * | - | II | 0.013 | -0.00007 | III | -0.01 | - | II | -0.018 | - | II |
| | | | | | | | | *** | | | *** | | |
| <i>Anisops sardeus</i> | D1 | -0.012 *** | - | II | -0.016 | - | II | -0.024 | - | II | -0.019 | - | II |
| | | | | | *** | | | *** | | | *** | | |
| | D2 | 0.067 *** | -0.0005 | III | -0.054 | - | II | -0.026 | - | II | -0.033 | - | II |

| | | | | | | | | | | | | | |
|----|--------|-----|---|-----|--------|-----|----|--------|---|----|--------|---|----|
| | | *** | | *** | | *** | | *** | | | | | |
| D3 | -0.026 | *** | - | II | -0.046 | - | II | -0.002 | - | II | -0.018 | - | II |
| | | | | | *** | | | | | | *** | | |

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Table 2. Capture rate (b) and handling time (h) parameters of *Anisops breddini* and *Anisops sardeus* towards four instars (1st – 4th) of *Culex quiquefasciatus* larvae at three different water depth levels, alongside their significance.

| | | 1 st instar | | 2 nd instar | | 3 rd instar | | 4 th instar | |
|-----------------------------|-------|------------------------|-------|------------------------|-------|------------------------|-------|------------------------|-------|
| Predators | Depth | b | h | b | h | b | h | b | h |
| size | | | | | | | | | |
| <i>Anisops breddini</i> | D1 | 0.034 | 0.011 | 1.88 | 0.017 | 0.036 | 0.025 | 0.609 | 0.045 |
| | | *** | *** | *** | *** | * | *** | *** | *** |
| | D2 | 0.706 | 0.002 | 1.65 | 0.005 | 0.84 | 0.007 | 1.08 | 0.07 |
| | | *** | | *** | ** | *** | | *** | *** |
| | D3 | 0.838 | 0.007 | 0.043 | 0.011 | 1.365 | 0.015 | 0.967 | 0.062 |
| | | *** | * | *** | *** | *** | *** | *** | *** |
| <i>Anisops sardeus</i> | D1 | 1.449 | 0.009 | 0.155 | 0.014 | 2.694 | 0.024 | 3.776 | 0.057 |
| | | *** | *** | *** | *** | *** | *** | ** | *** |
| | D2 | 0.082 | 0.01 | 0.275 | 0.018 | 2.41 | 0.021 | 4.726 | 0.066 |
| | | *** | *** | *** | *** | *** | *** | *** | *** |
| | D3 | 2.37 | 0.025 | 0.258 | 0.016 | 0.881 | 0.004 | 5.02 | 0.082 |
| | | *** | *** | *** | *** | *** | | * | *** |

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$.

Figure legends

Figure 1. Mean raw consumption (\pm SE) of *Anisops breddini* (black coloured bar, a – d) and *Anisops sardeus* (grey coloured bar, e – h) across all four densities towards four prey instar stages (1st – 4th) of *Culex quinquefasciatus* at three increasing water depths (D1 – D3).

Figure 2. Functional responses of *Anisops breddini* (a – d) and *Anisops sardeus* (e – h) towards four prey instar stages (1st – 4th) of *Culex quinquefasciatus* larvae across low (D1, solid lines), medium (D2, dashed lines) and high (D3, dotted lines) water depths. Bootstrapped ($n = 2000$) 95% confidence intervals are the shaded areas. Points are raw residuals: D1 (squared points, \square), D2 (round shaped points, \circ) and D3 (triangle shaped points, \triangle).

Figure 3. Capture rates of *Anisops breddini* (round shaped dots, a – d) and *Anisops sardeus* (square shaped dots, e – h) against four different instar stages (1st – 4th) of *Culex quinquefasciatus* at three water depths (D1, D2, D3). Points are attack rate estimates and bars represent bootstrapped 95% CIs ($n = 2000$).

Figure 4. Handling times of *Anisops breddini* (round shapes, a – d) and *Anisops sardeus* (square shapes, e – h) against four different instar stages (1st – 4th) of *Culex quinquefasciatus* at three water depths (D1, D2, D3). Points are handling time estimates and bars represent bootstrapped 95% CIs ($n = 2000$).

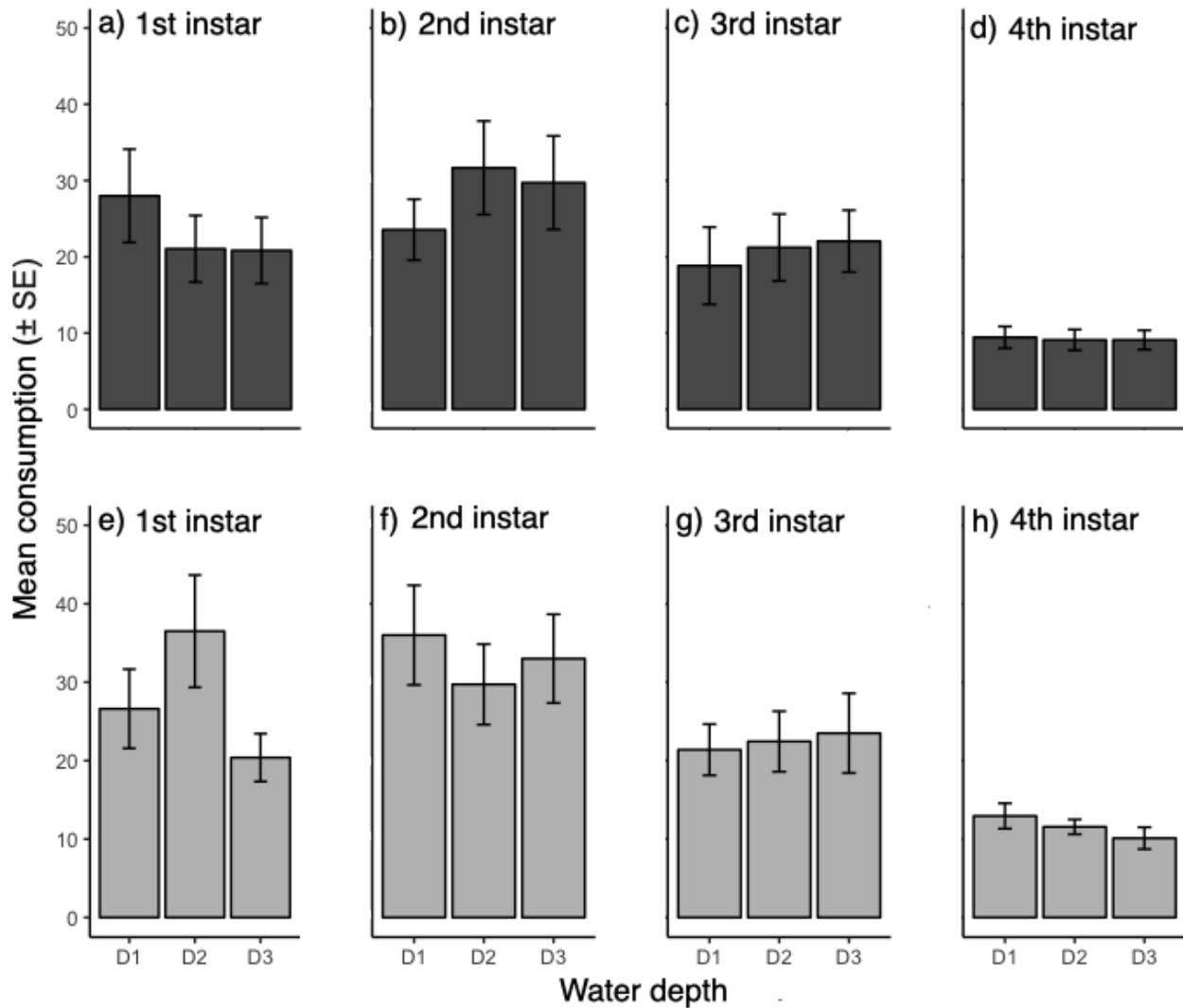


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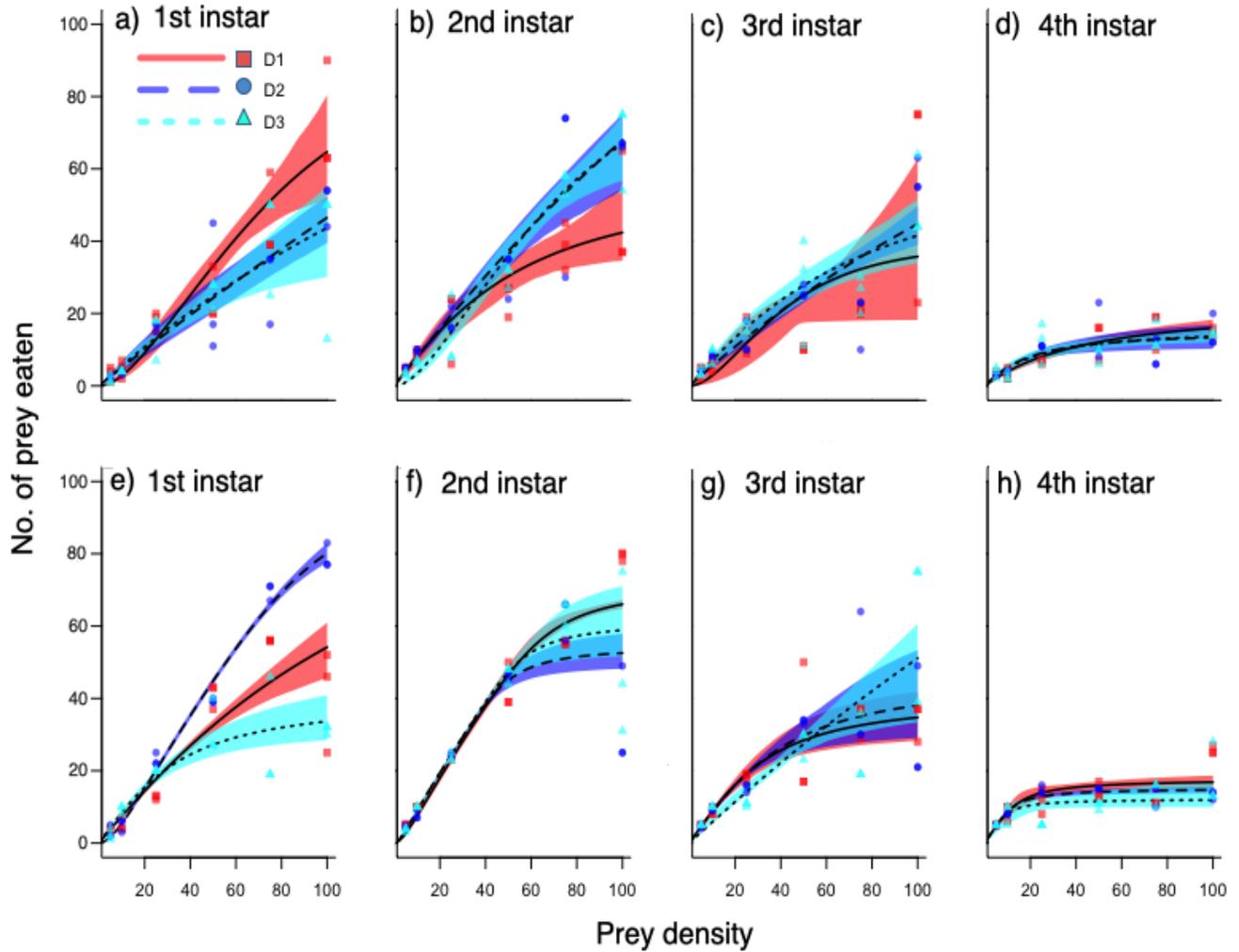


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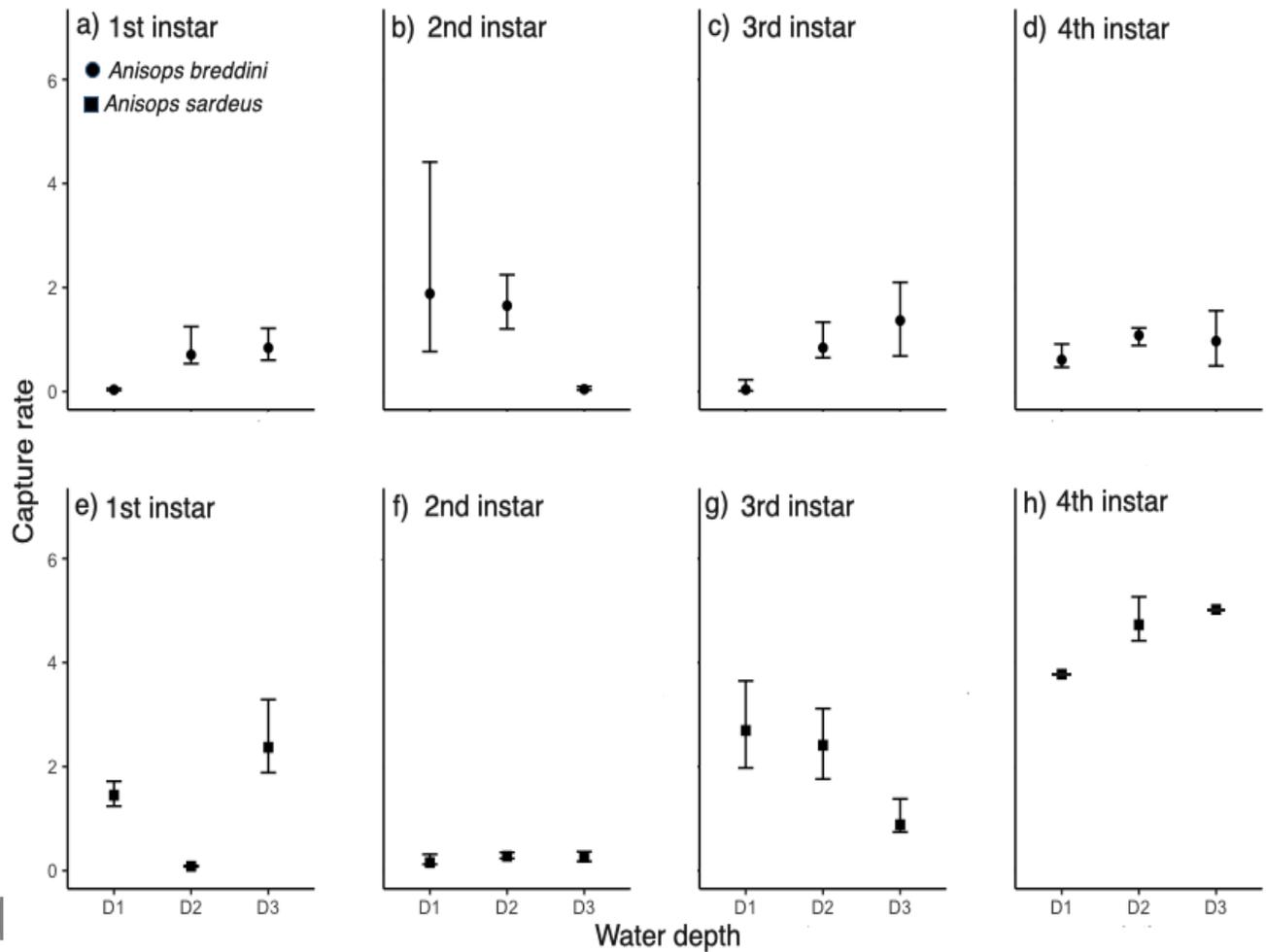


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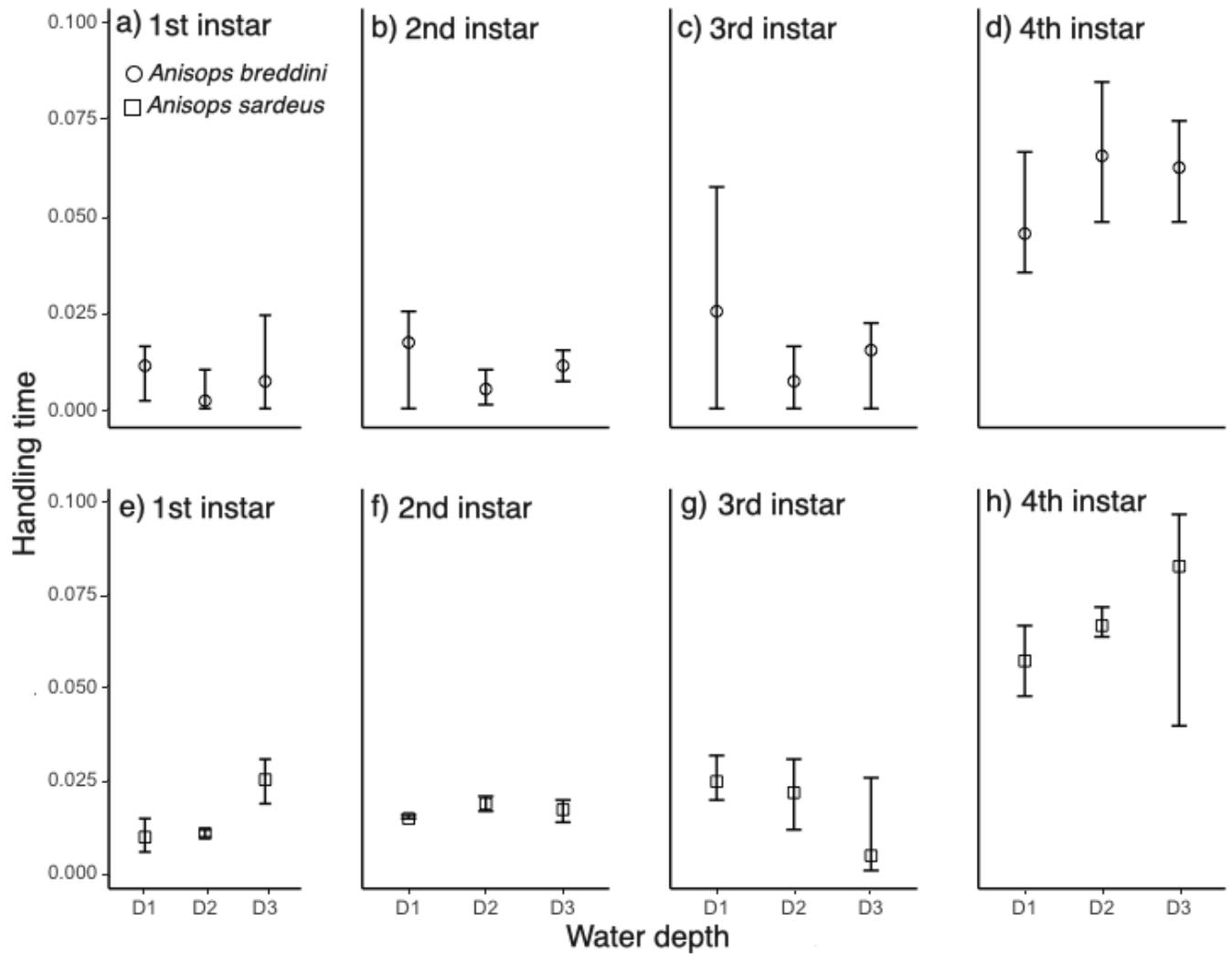


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