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Short Communication

Using functional responses to quantify notonectid predatory impacts across

increasingly complex environments

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24 **Abstract**

25 Predation is a key biotic interaction that influences both the structure and functioning of

26 ecosystems, and is relevant in the biological control context. Levels of habitat complexity in

27 aquatic ecosystems are highly variable and can profoundly affect predator-prey interactions

28 through the presence of prey refugia, which can in turn reduce predatory efficacy. Here, we

29 use functional responses (FRs, resource use under different resource densities) to quantify the

30 predatory impact of the notonectid *Anisops debilis* towards larvae of the mosquito *Culex*

31 *pipiens* under a habitat complexity gradient. *Anisops debilis* displayed a potentially

32 population-destabilising Type II FR towards larval *C. pipiens* prey across the habitat

33 complexity gradient. Attack rates were highest in simple environments, however handling

34 times were not significantly affected by habitat complexity. Maximum feeding rates of *A.*

35 *debilis* towards *C. pipiens* larvae were thus robust to habitat complexity variations. Our

36 results demonstrate the substantial predatory impacts of notonectids towards larval mosquito

37 prey irrespective of habitat complexities, which may assist in the biological control of pests

38 and vectors in aquatic systems.

39 **Keywords**

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1. Introduction

47

48   Biotic interactions such as predation play a central role in ecosystem structuring and

49   functioning (Brooks and Dodson 1965; Carpenter et al. 1975; Wasserman and Froneman

50   2013). Regulatory impacts of natural enemies towards pests, vectors and invasive species are

51   in turn reliant on processes such as predation (Solomon 1949; O'Neil 1990; Golding et al.

52   2015). As environmental context-dependencies can have a significant influence on predatory

53   impacts (e.g. South et al. 2017), considering predator-prey interaction strengths across these

54   contexts is important for robust impact quantifications towards target prey (e.g. Cuthbert et

55   al. 2018a, b, c). Functional responses have been central to the development of consumer-

56   resource (e.g. predator-prey) theories (Solomon 1949; Holling 1959), and have been regularly

57   applied in examining environmental context-dependencies of biotic interaction strengths (e.g.

58   Wasserman et al. 2016; Cuthbert et al. 2018a, b, c). Three FR forms have been broadly

59   described: a linear Type I, hyperbolic Type II and sigmoidal Type III (Holling 1959), with

60   each pertaining to different consumer-resource interaction outcomes. For instance, Type II

61   FRs are considered to be destabilising to populations under certain conditions due to high

62   resource (e.g. prey) acquisition at low prey densities by predators (Dick et al. 2014). Thus, in

63   the context of biological control, such destabilising FRs can elicit eradications of target

64   species.

65   Mosquitoes comprise an important trophic stage in many ecosystems, and biotic

66   interactions strengths towards their larvae by predators are known to affect overall mosquito

67   species distributions (Golding et al. 2015). Indeed, mosquitoes colonise a vast range of

68   natural and artificial aquatic environments, including within urban areas (Townroe and

69   Callaghan 2014). The physical structure of these habitats is inherently varied, with features

70   such as submerged plants often constituting the main components of habitat complexity in

71   aquatic environments (Tokeshi and Arakaki 2012). Such complexity can modulate the

72   interaction strengths between mosquitoes and their predators (e.g. Cuthbert et al. 2018b),

73   which in turn could result in the emergence of reduced predatory success due to physical

74   refugia of prey (e.g. Alexander et al. 2013), and thus has implications for vector population

75   control.

76   *Culex pipiens* complex mosquitoes are competent vectors of diseases such as West

77   Nile Virus (WNV) (Fonseca et al. 2004) and exhibit particularly marked ecological plasticity

78   with respect to habitat selection, colonising a range of habitats across rural-urban gradients

79   (Townroe and Callaghan 2014). Given that these habitats are highly varied in terms of their

80   physical structuring, understanding the implications of habitat complexity for the predatory

81   impact of natural enemies of mosquitoes is of great importance. Notonectids are voracious,

82   carnivorous insects, and can play a key role in the structuring of communities (e.g. Blaustein

83   1998; Wasserman et al. 2016, 2017), including within transient, ephemeral aquatic systems

84   where mosquitoes frequently proliferate. Indeed, notonectids have been shown to occupy the

85   highest trophic level in ephemeral aquatic ecosystems (e.g. Dalu et al. 2016). Although

86   notonectid predatory efficacy towards mosquito larvae has been described (e.g. Fischer et al.

87   2013; Silberbush et al. 2014), effects of habitat complexity on these biotic interactions have

88   not been fully constrained. Therefore, here we use a comparative, phenomenological FR

89   approach (e.g. Dick et al. 2014; Wasserman et al. 2016; Cuthbert et al. 2018a, b, c) to

90   decipher the effects of habitat complexity on the predatory impact of the notonectid *Anisops*

91   *debilis* Gersaeker 1873 towards larvae of the mosquito complex *C. pipiens*.

92   **2. Materials and Methods**

93   Adult *A. debilis* (6.7 – 7.1 mm body length) were collected from an impounded

94   stream in Grahamstown, Eastern Cape, South Africa (33° 19' 00.1" S 26° 31' 21.2" E) during

95   the 2017 – 2018 austral summer by trawling a kick net through the water column.

96   Notonectids were transported in source water to a controlled environment (CE) room at

97   Rhodes University, Grahamstown and maintained at 25 ± 1 °C under a 14:10 light:dark

98   photoperiod. The prey, larvae of the *C. pipiens* mosquito complex, originated from egg rafts

99   sampled from artificial container-style habitats situated around the Rhodes University

100   campus. Larvae were reared to the desired size class (see below) in the CE room on a diet of

101   crushed rabbit food pellets (Agricol, Port Elizabeth). Notonectids were housed in

102   continuously aerated aquaria with 25 L dechlorinated tap water for six days prior to

103   experimentation, and fed *ad libitum* on larvae of the mosquito *C. pipiens*. Excellent

104   survivability of notonectids (> 95 %) was observed during housing (Cuthbert pers. obs.). On

105   the seventh day, notonectids were separately starved in glass experimental arenas of 5.6 cm

106   diameter, containing 80 mL dechlorinated tap water from a continuously aerated source.

107   We employed a '3 × 7' experimental design with respect to 'habitat complexity' and

108   'prey supply'. We used stalks of the bulrush *Schoenoplectus brachyceras* (4 – 6 mm

109   diameter), collected from the notonectid sampling site, to generate three levels of habitat

110   complexity (i.e. low, medium, high). Low complexity arenas held zero stalks, medium

111   complexity arenas contained two stalks and high complexity arenas had four stalks, arranged

112   in a uniform array (*c*. Wasserman et al. 2016), broadly representing the range of habitat

113   complexities encountered at the notonectid collection site (Cuthbert pers. obs.). *Culex pipiens*

114   larvae (2.4 – 3.2 mm length incl. respiratory siphon) were supplied at seven densities for each

115   complexity level (2, 4, 6, 8, 16, 32, 64; $n = 4$ per experimental group) in glass 80 mL

116   experimental arenas of 5.6 cm diameter holding dechlorinated tap water from a continuously

117   aerated source, and allowed to settle for 2 h. Individual notonectid predators were then

118   carefully transferred to arenas containing the allocated prey supply and complexity treatment

119   and allowed to feed for 1 h during light conditions, before being removed. Then, remaining

120   live larval mosquito prey were counted. Controls consisted of a replicate at each prey supply

121   and complexity level in the absence of notonectid predators.

122   Statistical analyses were conducted in R v.3.4.2 (R Core Team 2017). The present

123   study applies FR modelling to address the phenomenological implications of habitat

124   complexity variabilities for predator-prey interactions. That is, our experiment is comparative

125   and does not seek to offer mechanistic insights into predator behaviour (see Jeschke et al.

126   2002; Dick et al. 2014). Functional responses were thus modelled using the 'frair' package

127   (Pritchard et al. 2017). Functional response types were inferred through the use of logistic

128   regression of the proportion of prey consumed as a function of the prey density supplied,

129   wherein a Type II FR is characterised by a significantly negative linear coefficient (Juliano

130   2001). The Rogers' random predator equation was fit as prey were not replaced as they were

131   consumed:

132 $$N_e = N_0(1 - \exp(a(N_e h - T)))$$

133   Eqn. 1.

134   where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant,

135   h is the handling time and T is the total experimental period. We fit the Lambert W function

136   to further enable FR model fitting (Bolker, 2008). We then bootstrapped ($n = 2000$) FRs to

137   infer 95 % confidence intervals (CIs) using original maximum likelihood estimations (MLEs)

138   (e.g. Wasserman et al. 2016). Overall differences in attack rate and handling time parameters

139   were derived using the difference method outlined in Juliano (2001). We applied Bonferroni

140   corrections for multiple comparisons between the three habitat complexity levels (i.e. critical

141    $p = 0.017$).

3. Results

142

143   All 396 mosquito prey survived in controls, and so prey deaths were attributed to

144   predation by notonectids, which we also directly observed. Type II FRs were found

145   irrespective of habitat complexity treatments, and evidenced by significantly negative first

146   order terms (Table 1). Functional response magnitudes trended towards being highest at the

147   low complexity treatment (Figure 1), driven by higher attack rates (initial FR slope) and

148   marginally lower handling times (FR asymptote) (Table 1). Whilst CIs were divergent at only

149   intermediate prey supplies between low and medium habitat treatments, CIs were divergent at

150   all except the lowest prey supplies between low and high habitat complexities (Figure 1). On

151   the other hand, between medium and high habitat complexities, CIs overlapped across all

152   prey densities and thus FRs were not significantly different here. Significantly greater attack

153   rates were found in the low compared to medium complexity treatment ($z = 2.585$, $p = 0.01$),

154   and in the low compared to high complexity treatment overall ($z = 2.592$, $p = 0.01$). Attack

155   rates did not vary significantly between the medium and high complexity treatments ($z =$

156   0.102, $p = 0.92$). However, significant differences in handling times were not detected

157   between any levels of habitat complexity (low − medium, $z = 0.546$, $p = 0.59$; low − high, $z =$

158   1.405, $p = 0.16$; medium − high, $z = 0.836$, $p = 0.40$).

159   **4. Discussion**

160   Our results demonstrate consistency of Type II FRs of *A. debilis* towards larvae of the

161   disease vector *C. pipiens* irrespective of habitat complexities. Thus, destabilising predatory

162   impacts of this notonectid are unaffected by the presence of habitat complexity, with Type II

163   FRs characteristically associable with localised prey extinctions under certain conditions due

164   to high resource (e.g. prey) utilisation at low prey densities (Dick et al. 2014). These results

165   corroborate with the recorded ability of notonectids to exert marked impacts upon the biotic

166   structure of communities within varied aquatic ecosystems (e.g. Blaustein 1998; Wasserman

167   et al. 2016, 2017). Indeed, Type II FR forms have been previously recorded with congenerics

168   of *A. debilis* (e.g. Zuhurah and Lester 2011). However, as FRs only examine *per capita*

169   effects of consumers, further investigation of population-level responses (e.g. abundance or

170   fecundity) would be of value in constraining comprehensive ecological impacts (see Dick et

171   al. 2017; Cuthbert et al. 2018a).

172   As attack rates of *A. debilis* were highest under low complexities, destabilising

173   predatory impacts at low prey densities can be deemed more profound in simplified habitats.

174   However, as handling times were similar across habitat complexities, maximum feeding rates

175   were relatively unaffected by the habitat complexity gradient. Thus, as maximum feeding

176   rates have proven robust in derivations of consumer-resource impact (Dick et al. 2017), the

177   overall predatory impact of *A. debilis* towards mosquito prey can in turn be deemed similar

178   across habitat complexities here. Previous research has shown *A. debilis* to be tolerant to

179   ranging environmental conditions, however predatory efficiency has been shown to be

180   affected by salinity gradients (Silberbush et al. 2014). Furthermore, other effective predatory

181   biological control agents of mosquitoes, such as cyclopoid copepods, have shown similar

182   responses to habitat complexity as those demonstrated in the present study (e.g. Cuthbert et

183   al. 2018b). Although implications of habitat complexity are constrained, biotic contexts such

184   as alternative prey may induce further implications for predator-prey interactions through

185   processes such as prey preferences and switching (e.g. Cuthbert et al. 2018d). Alternative

186   prey has been shown to influence predatory impacts, and studies have shown that notonectid

187   impacts on larval mosquito prey remain high in the presence of certain alternative prey (e.g.

188   Saha et al. 2010; Fischer et al. 2013; Saha et al. 2014). In particular, Saha et al. (2010, 2014)

189   demonstrate that a congeneric *Anisops* will preferentially consume mosquitoes under many

190   circumstances, whilst generalist feeding traits are advantageous as notonectids can forage on

191   alternative prey when mosquito abundances are low.

192   Given the range of forms of aquatic habitats which mosquitoes colonise across rural-

193   urban gradients, our results indicate that certain natural enemies of mosquitoes, such as *A.*

194   *debilis*, may retain strong predatory efficacy irrespective of habitat variations. Aerial adult

195   mosquito life-history stages ensure efficient colonisation of aquatic systems across the

196   landscape. Unlike other aquatic mosquito predators such as copepods (Cuthbert et al. 2018a,

197   b, c), notonectids can efficiently and naturally colonise aquatic habitats given that they too

198   are capable of aerial dispersal (McCauley and Row 2010). Thus, encouraging the

199   proliferation of such natural enemies may assist with population-level control of vectorially-

200   important mosquitoes across a broad range of aquatic systems with different environmental

201   characteristics. Further research should seek to examine the impacts of additional

202   environmental contexts on biotic processes, particularly in terms of their effects on the

203   efficacy of natural enemies of pests, vectors and invasive species.

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309   Tables and Figures

310   Table 1. First order terms derived from logistic regression of proportional prey consumption

311   as a function of prey supply across all habitat complexity levels, alongside original attack rate

312   and handling time parameter estimates from Rogers' random predator equation.

Habitat complexity	First order term, p	Attack rate, p	Handling time, p
Low	-0.036, < 0.001	2.490, < 0.001	0.040, < 0.001
Medium	-0.030, < 0.001	1.387, < 0.001	0.044, < 0.001
High	-0.028, < 0.001	1.356, < 0.001	0.052, < 0.001

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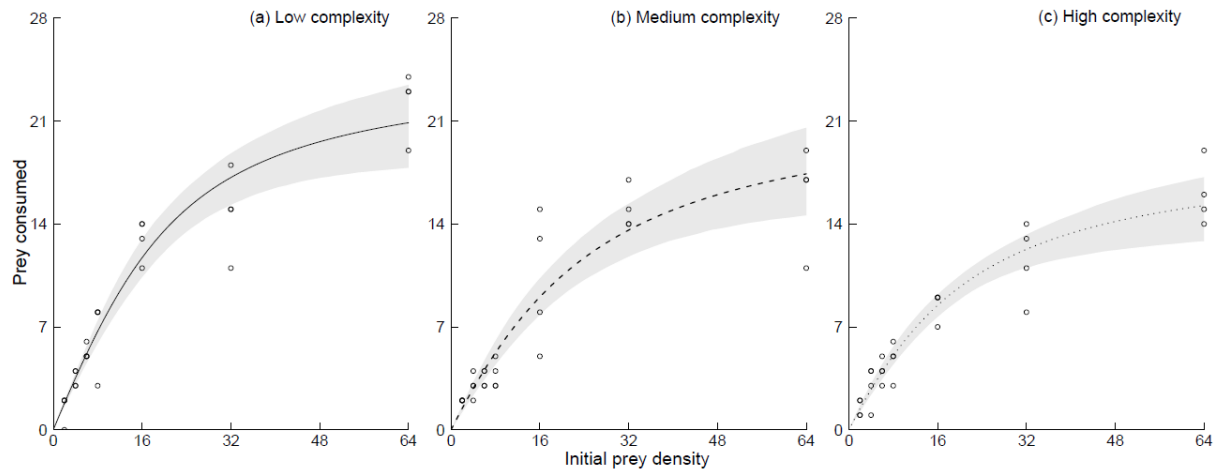
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Figure 1. Type II functional responses of *Anisops debilis* towards *Culex pipiens* across low (a, solid line), medium (b, dashed line) and high (c, dotted line) habitat complexities. Shaded areas represent bootstrapped 95% confidence intervals (*n* = 2000) and open circles represent raw residual points (*n* = 4 replicates per density).