**QUEEN'S
UNIVERSITY
BELFAST**

Inter-population similarities and differences in predation efficiency of a mosquito natural enemy

Cuthbert, R. N., Dalu, T., Wasserman, R. J., Weyl, O. L. F., William Froneman, P., Callaghan, A., & Dick, J. T. A. (2020). Inter-population similarities and differences in predation efficiency of a mosquito natural enemy. *Journal of Medical Entomology*, 57(6), 1983-1987. <https://doi.org/10.1093/jme/tjaa093>

Published in:
Journal of Medical Entomology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

Publisher rights
Copyright 2020 OUP. This work is made available online in accordance with the publisher's policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

1 *Short Communication*

2 **Inter-population similarities and differences in predation efficiency of a**
3 **mosquito natural enemy**

4 Ross N. Cuthbert^{1,2,3,*}, Tatenda Dalu^{4,3}, Ryan J. Wasserman^{5,3}, Olaf L. F. Weyl⁶, P. William
5 Froneman⁵, Amanda Callaghan⁷, Jaimie T. A. Dick²

6 ¹GEOMAR, Helmholtz-Zentrum für Ozeanforschung Kiel, 24105 Kiel, Germany

7 ²Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast,
8 Belfast BT9 5DL, Northern Ireland

9 ³South African Institute for Aquatic Biodiversity, Makhanda 6140, South Africa

10 ⁴Department of Ecology and Resource Management, University of Venda, Thohoyandou 0950,
11 South Africa

12 ⁵Department of Zoology and Entomology, Rhodes University, Makhanda 6140, South Africa

13 ⁶DSI/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute
14 for Aquatic Biodiversity, Makhanda 6140, South Africa

15 ⁷Ecology and Evolutionary Biology, School of Biological Sciences, University of Reading,
16 Harborne Building, Reading RG6 6AS, England

17 *Corresponding author: rossnoelcuthbert@gmail.com

18

19

Abstract

Predation is a critical factor that mediates population stability, community structure and ecosystem function. Predatory natural enemies can contribute to the regulation of disease vector groups such as mosquitoes, particularly where they naturally co-occur across landscapes. However, we must understand inter-population variation in predatory efficiency if we are to enhance vector control. The present study thus employs a functional response (resource use under different densities) approach to quantify and compare predatory interaction strengths among six populations of a predatory temporary pond specialist copepod, *Lovenula raynerae*, from the Eastern Cape of South Africa preying on second instar *Culex pipiens* complex mosquito larvae. All individuals from the sampled populations were predatory and drove significant mortality through *per capita* predation rates of 0.75 – 1.10 mosquitoes hour⁻¹ at maximum densities over a five hour feeding time. Individuals from all copepod populations exhibited Type II functional responses with no significant differences in attack rates. On the other hand, there were significant differences in handling times, and therefore also maximum feeding rates (maximum experimental prey density: 32), suggesting possible genetic differences among populations that influenced predation. Owing to a widespread distribution in arid landscapes, we propose that predatory calanoid copepods such as *L. raynerae* play a key regulatory role at the landscape scale in the control of disease vector mosquito populations. We propose that these ecosystems and their specialist biota should thus be conserved and enhanced (e.g., via selective breeding) owing to the ecosystem services they provide in the context of public health.

Key words: biological control, functional response, calanoid copepod, *Culex pipiens*, *Lovenula raynerae*

Introduction

Predation is a key biotic process that plays a pivotal role in the linking of food webs, stability of populations and the structuring and functioning of communities (Paine, 1980; Levi and Wilmers, 2012; Wasserman et al., 2013). The strength of such trophic interactions – hereafter interaction strength – facilitates quantification of a given predatory effect on prey (Gilbert et al. 2014). For natural enemies, interaction strength quantifications can allow for predictive inferences of regulatory impacts on target populations (Cuthbert et al. 2018a; Buxton et al. 2020). Mosquitoes comprise one such target species group, whereby predatory fish and invertebrate species have been examined in the context of population control (Roux and Robert, 2019). Owing to the enormous scale of public health risks driven by the vectoring of pathogens and parasites by mosquito species, this group has been the focus of much study (Benelli and Mehlhorn, 2016).

The functional response of consumers, i.e. the *per capita* rate of resource use by a consumer as a function of resource density per unit time, has been traditionally quantified to infer interaction strengths non-linearly between predators and prey (or in other consumer-resource systems) (Holling, 1959; Murdoch and Oaten, 1975). In this context, three common forms of functional response have been considered, with each pertaining, theoretically, to different outcomes for consumer-resource stability (Holling 1959): Types I (density-independent; linear), II (inversely density-dependent; hyperbolic) and III (positively density-dependent; sigmoidal). The functional response of consumers is often context-dependent owing to environmental parameters such as temperature (Englund et al. 2011) and biotic contexts such as non-trophic interactions (Sentis and Boukal, 2018). Recently, spatial context-dependencies of ecological impact have also been assessed at intra- and intercontinental scales (Howard et al. 2018; Boets et al. 2019), where differential interaction strength among regions can be paralleled with in-field predatory impacts.

However, whether such phenomena could alter natural enemy impacts among populations lacks examination for vector control.

Temporary ponds are excellent model systems to test ecological theory (De Meester et al. 2005). At the landscape scale, temporary pond ecosystems represent discrete habitat patches that often lack connectivity, with populations in individual ponds subject to different evolutionary lineages and genetic structuring (Jooste, 2018). Recently, calanoid copepods from temporary ponds have been proposed as important natural enemies of mosquitoes and potential biological control agents (Cuthbert et al. 2018b). However, whether the strength of their trophic interactions towards larval mosquito prey, such as species within the *Culex pipiens* complex which colonise temporary ponds (Ogbeibu, 2001), differs geographically among populations remains unexamined. The presence of population-level differences could allow for optimisation of biological control approaches through, for example, selective breeding of agents. The present study thus uses a functional response approach to quantify interaction strengths of a temporary pond specialist copepod towards larvae of mosquitoes which vector pathogens and parasites, with predator populations systematically sourced from multiple aquatic habitats across an arid, subtropical landscape.

Methods

Adult *Lovenula raynerae* Suárez-Morales, Wasserman, Dalu 2015 from its native range (Suárez-Morales et al., 2015) were collected from six ephemeral ponds in the Eastern Cape province [Peddie (Population 1: 33°12'46.9"S 27°20'11.2"E), Mosslands (Population 2: 33°24'37.3"S 26°24'56.9"E), Makhanda (Population 3: 33°16'48.1"S 26°35'39.8"E), Southwell (Population 4:

88 33°21'29.3"S 26°33'47.3"E), King William's Town (Population 5: 32°53'59.0"S 27°18'22.4"E),
89 Alice (Population 6: 32°48'35.5"S 26°45'53.1"E)] of South Africa using a 200 µm mesh net.
90 These systems ranged from 9 km to 100 km apart, within a total sampling region area of 3,400
91 km² (Table 1). All sampled ponds were in hydroperiod at the time of study, and we sampled at a
92 similar time (i.e., within one week of one another). Samples collected were transported in source
93 water to a controlled environment room at Rhodes University Department of Zoology and
94 Entomology, Makhanda (formerly Grahamstown). Adult males (total length range: 4 – 5 mm)
95 were maintained separately according to population at 25 ± 1 °C under a 14:10 light:dark regime
96 for 72 h, first fed *C. pipiens* complex larvae *ad libitum* for 48 h to standardise prey exposure, and
97 then starved for the final 24 h in continuously aerated 30 L tanks containing dechlorinated tap
98 water. Size-matched adult male copepods were selected for experimentation to standardise
99 predator characteristics as much as possible. As skewed sex ratios can develop in ephemeral
100 pond copepod populations (Wasserman et al. 2018), and gravid female copepods are more
101 voracious than males (Cuthbert et al. 2019), we opted to remove this natural variation from our
102 study. The focal prey, *C. pipiens* complex mosquitoes, were cultured using egg rafts collected
103 from artificial containers on the university campus, and reared to the desired size class in the
104 same controlled environment room (see before) using a diet of crushed rabbit pellets (Agricol,
105 Port Elizabeth), supplied *ad libitum*. Mosquitoes were identified following Jupp (1996). Second
106 instar *C. pipiens* complex (mean length ± SE: 2.58 ± 0.10 mm) were established at five prey
107 densities (2, 4, 8, 16, 32; *n* = 4 per density) in 80 mL arenas of 5.6 cm diameter containing
108 dechlorinated tap water from a continuously aerated source, two hours before the addition of
109 predators (one per experimental arena). Feeding experiments were undertaken using individuals
110 separately from each predator population across different prey densities (i.e., 6 populations × 5

densities $\times$ 4 replicates), resulting in 120 experimental units with predators present. Once added, the predators were allowed to feed undisturbed for 5 h during light conditions, after which time they were removed and remaining prey counted to derive numbers killed. Pilot studies indicated that this experimental duration was sufficient for asymptotic declines in feeding rates given the experimental prey densities used, thus allowing functional response models to be fit. Controls consisted of five replicates at each density without predators to quantify background prey mortality rates.

All statistical analyses were conducted in R v3.4.4 (R Core Team, 2018). Functional response (FR) analyses were undertaken using the ‘frair’ package in R (Pritchard et al. 2017). Logistic regression of proportion of prey consumed as a function of prey density was used to infer functional response types. A Type II functional response is characterised by a significantly negative first order term, whilst a Type III functional response is characterised by a significantly positive first order term followed by a significantly negative second order term (Juliano, 2001). As prey were not replaced as they were consumed, we fit Rogers’ random predator equation for depleting prey densities (Rogers, 1972):

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

Eqn. 1.

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant (volume per unit time), h is the handling time (time per prey item) and T is the total experimental period. The Lambert W function was used to solve the model (Bolker, 2008). Functional responses parameters (attack rate, handling time) were then non-parametrically bootstrapped ($n = 2000$ per parameter/group) and compared visually *via* 95 % confidence intervals, whereby

confidence interval convergence indicates non-significant differences among parameters, and divergence indicates significant differences.

Results

No prey died in controls and so prey mortality during the experiment was assumed to result from predation by calanoid copepods. Type II functional responses were evidenced from each population, owing to significantly negative first order terms (Table 2; Figure 1). Functional response attack rate and handling time parameters were estimated significantly from copepod predation within each population, with the borderline exceptions of attack rates from populations 3 and 5 (Table 2). Attack rates did not differ significantly among populations as confidence intervals always overlapped (Figure 2a). Handling times were also statistically similar among several populations owing to extensive confidence interval overlaps (Figure 2b). However, handling times from Population 3 (Makhanda) were significantly longer than Population 1 (Peddie) and 6 (Alice), owing to confidence interval divergence. Reciprocally, maximum feeding rates ($1/h$) by copepods were thus significantly higher for Populations 1 and 6 compared to Population 3 (Table 2; Figure 1).

Discussion

Predatory interaction strengths by an important mosquito natural enemy were shown in this study to be dependent on population-level context at the landscape scale. All populations consistently displayed hyperbolic Type II functional responses irrespective of their source, whereby predation rates were high at low resource densities. Accordingly, the efficacy of *L. raynerae* as a natural

enemy of larval *C. pipiens* complex mosquitoes, and probably other mosquito species, appears to be widespread geographically within its native and endemic range (southern Africa), where it imparts very little low-density refuge to larval mosquito prey. Whilst attack rates (initial functional response slope) were not significantly different among population, significant differences in handling times among predatory populations were emergent (inversely, functional response asymptotes). In turn, this mediated significant differences in maximum feeding rates, which were highest in Populations 1 (i.e. Peddie) and 6 (i.e. Alice). These inter-population differences could be harnessed to improve biological control of mosquitoes through, for example, population-specific applications, as well as through the development of breeding lineages from high impact populations. Whilst the specific drivers of these differences remain unclear, they could be caused by genetic differences between populations that influence predatory efficacies. In a study on the genetic connectivity of *L. raynerae* in the study region, Jooste (2018) showed that there was a connectivity break in the Makhanda region for the species, and that genetic structure of populations could vary between ponds separated by only a few metres. These results highlight the importance of considering different populations for species characterised by potentially isolated meta-communities. Nevertheless, various other factors may also be of importance in driving mosquito control at the population-level, such as predator abundance, fecundity, prey characteristics and wider community composition (Cuthbert et al. 2018a, 2019). Regardless, we propose that the regulatory efficacy of this calanoid copepod towards larval mosquitoes is geographically widespread in its native range, yet *per capita* interaction strengths may differ among populations.

Specialist temporary wetland organisms such as *L. raynerae* are adapted to withstand both wet and dry periods, with the ability to produce drought-resistant eggs which are dormant between

hydroperiods (Wasserman et al. 2016). Rapid maturation in these ecosystems could enable adult life stages of temporary pond copepods to be efficient predators for extended periods of time. Insect groups such as mosquitoes typically colonise ephemeral wetlands externally from other areas later in the hydroperiod, or can hatch directly from dormant eggs (e.g. *Aedes* spp.). Therefore, *in situ* mass-hatching events of predatory copepods could allow for rapid and efficient mosquito population regulation at the landscape level in ponds. In other areas of southern Africa where mosquito-borne disease risk is pronounced, predatory copepod presence may therefore provide an ecosystem service in a public health context, and populations should thus be conserved given known anthropogenic impacts that threaten these habitats (Dalu et al. 2017).

Whilst previous studies have examined differential predatory impacts of biota across more extensive spatial scales (i.e. continental) (Howard et al. 2018; Boets et al. 2019), we propose that the provincial scale is suitable here owing to the isolation of ponds across landscapes and their limited connectivity. Indeed, previous studies have indicated that *L. raynerae* can exhibit marked genetic divergence across very small geographic scales (Jooste, 2018). Owing to its size and predatory capacity, *L. raynerae* has been shown to be a top predator during early hydroperiod in temporary ponds within its native range (Dalu et al. 2016), and thus such paradiaptomids likely play an important role in environments that are dominated by ephemeral aquatic ecosystems (Cuthbert et al. 2018b). Whilst future work should further decipher the community composition and structure across spatial gradients in these ecosystems, including whether adult female copepods and juveniles show similar predation patterns, we propose that conservation could help to regulate disease vector species across arid landscapes. Further, inter-population predatory differences could potentially be harnessed to enhance biocontrol efforts towards disease vectors.

201 **Acknowledgements**

202 RNC is funded through a Postdoctoral Research Fellowship from the Alexander von Humboldt
203 Foundation. This study received funding from the Department for the Economy, Northern
204 Ireland. We thank Rhodes University for the provision of laboratory facilities. We acknowledge
205 use of infrastructure and equipment provided by the South African Institute for Aquatic
206 Biodiversity (SAIAB) Research Platform and the funding channelled through the National
207 Research Foundation – SAIAB Institutional Support system. This study was partly funded by the
208 National Research Foundation – South African Research Chairs Initiative of the Department of
209 Science and Innovation (Inland Fisheries and Freshwater Ecology, Grant No. 110507).

210

211 **References cited**

- 212 **Benelli, G., and Mehlhorn, H. 2016.** Declining malaria, rising of dengue and Zika virus:
213 insights for mosquito vector control. *Parasitol. Res.* 115: 1747–1754.
- 214 **Boets, P., C. Lavery, S. Fukuda, H. Verreycken, K. Green, R. J. Britton, J. Caffrey, P. L.**
215 **M. Goenthals, J. Pegg, V. Médoc, J. T. A. Dick. 2019.** Intra- and intercontinental
216 variation in the functional responses of a high impact alien invasive fish. *Biol. Invasions*
217 21: 1751–1762.
- 218 **Bolker, B. M. 2008.** *Ecological models and data in R.* Princeton University Press, Princeton,
219 United States.

220 **Buxton, M., R. N. Cuthbert, T. Dalu, C. Nyamukondiwa, and R. J. Wasserman. 2020.**
 221 Predator density modifies mosquito regulation in increasingly complex environments.
 222 Pest Manag. Sci. in press.

223 **Chandra, G., I. Bhattacharjee, S. N. Chatterjee, and A. Ghosh. 2008.** Mosquito control by
 224 larvivoracious fish. Indian J. Med. Res. 127: 13–27.

225 **Cuthbert, R. N., J. T. A. Dick, A. Callaghan, and J. W. E. Dickey. 2018a.** Biological control
 226 agent selection under environmental change using functional responses, abundances and
 227 fecundities; the Relative Control Potential (RCP) metric. Biol. Control 121: 50–57.

228 **Cuthbert, R. N., T. Dalu, R. J. Wasserman, A. Callaghan, O. L. F. Weyl, J. T. A. Dick.**
 229 **2018b.** Calanoid copepods: An overlooked tool in the control of disease vector
 230 mosquitoes. J. Med. Entomol. 55: 1656–1658.

231 **Cuthbert, R. N., Dalu, T., Wasserman, R. J., Weyl, O. L. F., Callaghan, A., Froneman, P.**
 232 **W., and Dick, J. T. A. 2019.** Sex-skewed trophic impacts in ephemeral wetlands.
 233 Freshw. Biol. 64: 359–366.

234 **Dalu, T., O. L. F. Weyl, P. W. Froneman, and R. J. Wasserman 2016.** Trophic interactions in
 235 an austral temperate ephemeral pond inferred using stable isotope analysis.
 236 Hydrobiologia 768: 81–94.

237 **Dalu, T., R. J. Wasserman, M. T. Dalu, 2017.** Agricultural intensification and drought
 238 frequency increases may have landscape level consequences for ephemeral ecosystems.
 239 Glob. Change Biol. 23: 983–985.

240 **De Meester, L., S. Declerck, R. Stoks, G. Louette, F. van de Meutter, T. De Bie, E. Michels,**
241 **L. Brendonck. 2005.** Ponds and pools as model systems in conservation biology,
242 ecology and evolutionary biology. *Aquat. Conserv. Mar. Freshw. Ecosys.* 15: 715–725.

243 **Englund, G., G. Öhlund, C. L. Hein, and S. Diehl. 2011.** Temperature dependence of the
244 functional response. *Ecol. Lett.* 14: 914–921.

245 **Gilbert, B., T. D. Tunney, K. S. McCann, J. P. DeLong, D. A. Vasseur, V. Savage, J. B.**
246 **Shurin, A. I. Dell, B. T. Barton, C. D. G. Harley, H. M. Kharouba, P. Kratina, J. L.**
247 **Blanchard, C. Clements, M. Winder, H. S. Greig, and M. I. O'Connor. 2014.** A
248 bioenergetic framework for the temperature dependence of trophic interactions. *Ecol.*
249 *Lett.* 17: 902–914.

250 **Holling, C. S. 1959.** Some characteristics of simple types of predation and parasitism. *Can.*
251 *Entomol.* 91: 385–398.

252 **Howard, B. R., D. Barrios-O'Neill, M. E. Alexander, J. T. A. Dick, T. W. Therriault, T. B.**
253 **Robinson, I. M. Côte. 2018.** Functional responses of a cosmopolitan invader
254 demonstrate intraspecific variability in consumer-resource dynamics. *PeerJ* 6: e5634.

255 **Jooste, C. M. 2018.** Genetic Connectivity in the World's Largest Freshwater Copepod. Master's
256 Thesis, University of Johannesburg.

257 **Juliano, S. A. 2001.** Non-linear curve fitting: predation and functional response curves, pp. 178–
258 196. In S. M. Scheiner and J. Gurevitch (eds.), *Design and analysis of ecological*
259 *experiments.* Oxford University Press, Oxford, United Kingdom.

260 **Jupp, P. G. 1996.** Mosquitoes of southern Africa: Culicinae and Toxorhynchitinae. Ekogilde
 261 Publishers, Hartebeespoort, South Africa.

262 **Levi, T., and C. C. Wilmers. 2012.** Wolves–coyotes–foxes: a cascade among carnivores.
 263 Ecology 93: 921–929.

264 **Murdoch, W. W., A. Oaten. 1975.** Predation and population stability. Adv. Ecol. Res. 9: 1–131

265 **Ogbeibu, A. E. 2001.** Composition and diversity of Diptera in temporary pond in southern
 266 Nigeria. Trop. Ecol. 42: 259–268.

267 **Paine, R. T. 1980.** Food webs: linkage, interaction strength and community infrastructure. J.
 268 Anim. Ecol. 49: 667–685.

269 **Pritchard, D. W., R. Paterson, H. C. Bovy, and D. Barrios-O’Neill. 2017.** Frair: an R package
 270 for fitting and comparing consumer functional responses. Methods Ecol. Evol. 8: 1528–
 271 1534.

272 **R Core Team. 2018.** R: a language and environment for statistical computing. R Foundation for
 273 Statistical Computing, Vienna, Austria.

274 **Rogers, D. 1972.** Random search and insect population models. J. Anim. Ecol. 41: 369–383.

275 **Roux, O., and Robert, V. 2019.** Larval predation in malaria vectors and its potential implication
 276 in malaria transmission: an overlooked ecosystem service? Parasites Vectors 12: 217.

277 **Sentis, A., and D. S. Boukal. 2018.** On the use of functional responses to quantify emergent
 278 multiple predator effects. Sci. Rep. 8: 1–12.

Suárez-Morales, E., R. J. Wasserman, and T. Dalu. 2015. A new species of *Lovenula* Schmeil (Copepoda, Calanoida, Diaptomidae) from the Eastern Cape Province of, South Africa. Crustaceana 81: 324–342.

Wasserman, R. J., M. Noyon, T. S. Avery, and P. W. Froneman. 2013. Trophic level stability inducing effects of predaceous early juvenile fish in an estuarine mesocosms study. PLoS One 8: e61019.

Wasserman, R. J., M. E. Alexander, D. Barrios-O'Neill, O. L. F. Weyl, and T. Dalu. 2016. Using functional responses to assess predator hatching phenology implications for pioneering prey in arid temporary pools. J. Plankt. Res. 38: 154–158.

Wasserman, R. J., Weston, M., Weyl, O. L. F., Froneman, P. W., Welch, R. J., Vink, T. J. F., and Dalu, T. 2018. Sacrificial males: the potential role of copulation and predation in contributing to copepod sex-skewed ratios. Oikos 127: 970–980.

World Health Organisation. 2017. Vector-borne diseases fact sheet. <https://www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases> (accessed 02 February 2020).

300 Table 1. Distance matrix (km) between ponds where individual copepod populations (1 – 6) were
301 sampled.

Pond	1	2	3	4	5	6
1	-	-	-	-	-	-
2	88.48	-	-	-	-	-
3	69.55	23.03	-	-	-	-
4	73.81	14.88	9.15	-	-	-
5	34.86	100.54	78.71	85.98	-	-
6	69.66	100.52	54.52	63.64	51.66	-

302

303

304

305

306

307

308

309

310

311

Table 2. First order terms generated from logistic regression of proportional predation as a function of prey density alongside parameter outputs from Rogers' random predator equation (a , attack rate; h , handling time; $1/h$, maximum feeding rate) across six temporary pond populations.

Population	First order term, p	a, p	h, p	$1/h$
1	-0.06, < 0.001	1.25, < 0.01	0.18, < 0.001	5.55
2	-0.05, < 0.001	0.98, < 0.05	0.20, < 0.001	5.00
3	-0.08, < 0.001	2.87, 0.07	0.29, < 0.001	3.45
4	-0.04, < 0.01	0.62, < 0.05	0.16, < 0.01	6.25
5	-0.07, < 0.001	2.89, 0.07	0.20, < 0.001	5.00
6	-0.06, < 0.001	1.26, < 0.01	0.15, < 0.001	6.67

325

326 Figure 1. Functional responses of adult male *Lovenula raynerae* towards larval mosquito prey
 327 from six different temporary pond populations. Points are means $\pm$ 1 SE ($n = 4$) per experimental
 328 group. Shaded areas are bootstrapped 95 % confidence intervals.

Figure 2. Attack rates (a) and handling times (b) of male *Lovenula raynerae* against mosquito larvae across six temporary pond populations with non-parametric 95 % confidence intervals ($n = 2000$). Points are starting parameter estimates generated from Rogers' random predator equation.