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Examining intraspecific multiple predator effects across shifting predator sex ratios

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24 **Abstract**

25 Predator-predator interactions, or “multiple predator effects” (MPEs), are pervasive in the
26 structuring of communities and complicate predictive quantifications of ecosystem dynamics.
27 The nature of MPEs is also context-dependent, manifesting differently among species, prey
28 densities and habitat structures. However, there has hitherto been a lack of consideration for the
29 implications of intraspecific demographic variation within populations for the strength of MPEs.
30 The present study extends MPE concepts to examine intraspecific interactions among male and
31 female predators across differences in prey densities using a functional response approach.
32 Focusing on a copepod-mosquito model predator-prey system, interaction strengths of different
33 sex ratio pairs of *Lovenula raynerae* were quantified towards larval *Culex pipiens* complex prey,
34 with observations compared to both additive and substitutive model predictions. Copepods
35 exhibited destabilising Type II functional responses in all treatments, with female copepods
36 significantly more voracious than males under multiple predator groups. *Lovenula raynerae*
37 exhibited significantly negative MPEs overall which resulted in prey risk reductions. However,
38 whilst not statistically clear, the magnitude of antagonistic interactions subtly differed among
39 predator-predator compositions and prey densities, with female-female antagonisms generally
40 prevalent at low prey densities, and male-male negative interactions greater under high prey
41 densities. Mixed-sex copepod group predation was predicted by both additive and substitutive
42 models, and additive models generated significantly higher consumption estimates than
43 substitutive equivalents given that their predictions were based on the absence of antagonistic
44 non-trophic interactions. We propose the importance of internal population sex demographics as

a further context-dependency which influences the nature of MPEs, with demographic implications requiring investigation across other taxonomic and trophic groups.

Keywords:

Temporary pond; functional response; predator-predator interaction; sex demographic; *Lovenula raynerae*; *Culex pipiens*

Introduction

Predation has a pervasive impact on community dynamics, with implications for structuring and functioning at the ecosystem-level (Hairston et al. 1960; Sih et al. 1985; Estes et al. 2011; Waterkeyn et al. 2016). Quantifications of trophic dynamics are, however, often based on paired consumer-resource interaction strengths, which ignore context-dependencies that influence predatory impacts (Bolker et al. 2003; Werner & Peacor 2003; Schmitz 2007). In particular, the assumption that predators act independently ignores “multiple predator effects” (MPEs) which may alter prey risks (Soluk 1993). Indeed, multiple predator species which share resources are commonplace in ecosystems, resulting in interactions such as interference and intraguild predation which may alter predatory efficiencies (Sih et al. 1998). In this context, predators are assumed to act non-independently when observed prey consumption levels differ from those predicted from models. Multiple predator effects may manifest in three broad ways: (1) antagonistically, resulting in prey risk reductions (Rosenheim et al. 1995; Wasserman et al. 2016b); (2) additively, where prey risk is well-predicted from individual predators (Chang 1996; Barrios-O’Neill et al. 2014), or; (3) synergistically, resulting in prey risk enhancements (Soluk 1993; Losey & Denno 1998). Accordingly, quantitative understandings of the nature of MPEs are integral to robust community- and ecosystem-level models (Sih et al. 1998; McCoy et al. 2012; Sentis & Boukal 2018), and may, for example, elucidate the implications of predator extirpation within communities.

Whilst the strength of MPEs has been examined under single, or just a few, prey densities (Harvey et al. 2004; Griffen 2006; Porter-Whitaker et al. 2012), it is well-established that predation is also density-dependent, with efficiencies differing across levels of prey availability (Abrams 1982, 2000; Cuthbert et al. 2019b). Fundamentally, the shape of resource use (e.g.

predation) rates across resource (e.g. prey) densities is known as the functional response (Holling 1959), and different functional response forms have important implications for prey population stabilities (Hassell 1978). Whilst the functional response is a classical ecological concept, regularly applied to quantify interaction strengths in the context of biological control (Cuthbert et al. 2018b), invasion science (Dick et al. 2014) and optimal foraging theory (Abrams 1982), relatively few studies have employed a functional response approach to quantify emergent MPEs, with contrasting results reported (Soluk 1993; Losey & Denno 1998; Barrios-O'Neill et al. 2014; Wasserman et al. 2016b). Centrally, the attack rate and handling time (inverse maximum feeding rate) parameters of functional response curves offer insights into the strength of MPEs across a spectrum of prey densities. Accordingly, the comparative functional response approach lends itself to quantifications of multiple predator effects.

Previously, MPE studies have considered predator-predator interactions at the interspecific level (Soluk 1993; Sih et al., 1998; Vance-Chalcraft & Soluk 2005). However, more recently, the nature of MPEs has been examined for both heterospecific and conspecific predator combinations (Lampropoulos et al. 2013; Barrios-O'Neill et al. 2014; Wasserman et al. 2016b). Nevertheless, there has been a distinct lack of consideration for differences in conspecific interactions associated with population demographics. This is despite sex and reproductive status having been shown to significantly alter interaction strengths by individual predators across multiple prey densities (Cuthbert et al. 2019b). Accordingly, we thus propose intraspecific interactions based on key demographic characteristics to be an appropriate context within the MPE framework. Therefore, given the lack of consideration for emergent MPEs across demographic variations, we here examine the implications of compositional sex ratio change for the nature of MPEs, using the numerically abundant and predatory paradiaptomid copepod

Lovenula raynerae Suárez-Morales, Wasserman & Dalu 2015 (Suárez-Morales et al. 2015) feeding on larval mosquito prey as a model predator-prey system. *Lovenula raynerae* is a temporary pond specialist copepod, which hatches from egg banks within substrate following inundation with water. Mass-hatching events exhibited in these aquatic systems make intraspecific interactions pervasive, and perhaps more common than those at the interspecific level. Further, this copepod species occupies top trophic levels in temporary pond ecosystems for much of the hydroperiod (Dalu et al. 2016), exerts substantial predatory impact on lower trophic groups (Wasserman et al. 2016a; Cuthbert et al. 2018a) and is widespread and often dominant in temporary pond systems in its native range (Wasserman et al. 2018). Sex-skewed demographics of this predator are known to develop over the hydroperiod due to selective process such as predation (Wasserman et al. 2018). Therefore, the implications of MPEs may vary over the course of hydroperiod in temporary ponds, owing to shifting population demographics over relatively rapid timescales.

We firstly determine the functional responses of male and female *L. raynerae* when present singularly with larval mosquito prey. We then quantify multiple predator functional responses in mixtures of conspecific pairs (i.e. female-female, male-male and female-male). We use these observations as a framework to decipher whether MPEs are emergent in this study system, and whether the strength of MPEs is modulated by variations in sex ratio. To do this, we employ both additive and substitutive models to predictively quantify MPEs, before comparing the estimates generated by both modelling techniques across prey densities. The relative appropriateness of additive and substitutive experimental designs has been debated in the context of ecological community dynamics (Griffen 2006; O’Gorman et al. 2008; Lampropoulos et al. 2013). Whilst additive designs compare the observed predation rate of individuals to the consumption of

predators in combination, substitutive designs alter the diversity of predator combinations, in turn fixing total predator abundance. As such, inferences from additive designs are made on the basis of experimental increases in predator density, whilst substitutive designs alter the proportional composition of predator groupings to decipher the relative effect of interactions among different predator types. Our experimental design enables explicit comparison of predictions from additive and substitutive models in the mixed-sex predator treatment. Ultimately, our study aims to examine the potential importance of intraspecific sex demographics for the strength of MPEs in temporary ponds and other ecosystems.

Materials and methods

Animal collection and experimental design

Adult *L. raynerae* were collected from ephemeral ponds in Bathurst, Eastern Cape, South Africa (33°29'21.7"S 26°49'48.2"E) using a 200 µm mesh zooplankton net and transported in source water to a controlled environment room at Rhodes University, Makhanda (Grahamstown). Adult males and females (male, 4.71 mm ± 0.11; female, 4.88 mm ± 0.08) were maintained at 25 ± 1 °C under a 12:12 light:dark regime for 48 h and starved in continuously aerated 30 L tanks containing dechlorinated tap water. The focal prey, *Culex pipiens* complex larvae (4.33 mm ± 0.18) were cultured using egg rafts collected from artificial containers on the University campus, and reared to the desired size class in the same laboratory using a diet of crushed rabbit pellets (Agricol, Port Elizabeth), supplied *ad libitum*.

Five predator treatments were designated using adult male and ovigerous female *L. raynerae*: (1) female; (2) male; (3) female + female; (4) male + male, and; (5) female + male. Larval *C. pipiens*

were established at five prey densities (2, 4, 8, 16, 32; $n = 4$ per density) in 80 mL arenas of 5.6 cm diameter containing dechlorinated tap water from a continuously aerated source, two hours before the addition of predators in the laboratory (25 ± 1 °C). Once predators were added they were allowed to feed undisturbed for five hours during light conditions, after which they were removed and remaining live prey counted to derive those killed. Controls consisted of four replicates at each density in the absence of predators to quantify background mortality rates.

Data analyses

All statistical analyses were performed in R v3.5.1 (R Core Team 2018). Generalised linear models (GLMs) assuming a Poisson error distribution were used to test for differences in overall prey consumption among all observed predator groups (i.e. 5 levels including single predator treatments) and prey supplies (i.e. 5 levels). We did not find evidence of residual overdispersion in relation to degrees of freedom in GLMs. Non-significant terms and interactions were removed stepwise from the model to facilitate parsimony, with χ^2 used for model simplification *via* deviance analyses (Crawley 2007). Tukey's *post-hoc* tests were employed for multiple pairwise comparisons using the 'emmeans' package in R (Lenth 2019).

We generated predictions of multiple predator consumption based on single predator treatments to examine emergent MPEs between conspecifics using the multiplicative risk model (Soluk 1993):

$$C_{ab} = N_p (P_a + P_b - P_a P_b)$$

Eqn. 1.

where C_{ab} is the predicted consumption under each prey density (N_p) and P represents the probabilities of being consumed by predators a and b . To capture variability in underlying

consumption data, we paired single predator replicates (i.e., $n = 4$ per predicted group). This model is applicable to additive experimental designs and accounts for prey removal by individual predators, given a prey item eaten by one predator is no longer available for others. Three-way beta regression *via* the ‘betareg’ package was used to examine observed and predicted proportional consumption with respect to the multiple predator groups (i.e. 3 levels), prey supplies (i.e. 5 levels) and a model evaluation factor (i.e. 2 levels; observed and predicted). Proportions were transformed prior to analyses to remove 0 and 1 values and satisfy model assumptions (Smithson & Verkuilen 2006; Cribari-Neto & Zeileis 2010):

$$y_t = (y(n - 1) + 0.5)/n$$

Eqn. 2.

where n is the sample size. Whilst the pooling of ‘observed’ and ‘predicted’ consumption rates renders the single ‘predator’ and ‘prey density’ terms meaningless (see Vance-Chalcraft & Soluk 2005), their two- or three-way interaction with the model evaluation factor indicates whether MPEs are different among predator pairings and prey densities. Nested likelihood ratio tests were employed to quantify the significance of main effects using the ‘lmttest’ package (Zeileis & Hothorn 2002), with Tukey’s post-hoc comparisons used for pairwise comparisons (as above).

To further examine the intensity of intraspecific interactions between copepod sexes, a substitutive model was used to predict prey survival rates in the mixed female-male predator pairs, based on paired observations of survival rates in female-female and male-male pairs (Griffen 2006):

$$E_{(1,1)} = \sqrt{F_{(1,1)} \times M_{(1,1)}}$$

199 where $F_{(1,1)}$ and $M_{(1,1)}$ are the observed survival rates in conspecific pairs of female and male
 200 copepods, respectively. Two-way beta regression on proportioned survival (transformed as per
 201 Eqn. 2) was then used to detect emergent MPEs with respect to prey density (5 levels) and the
 202 model evaluation factor considering observations and predictions (2 levels). As before, the
 203 pooling of ‘observed’ and ‘predicted’ levels rendered the single ‘prey density’ term
 204 uninformative, however the presence of an interaction would signal MPE differences with
 205 respect to survival rates among prey densities. Nested likelihood ratio tests were used as before
 206 to infer significance.

207 We next compared transformed proportional consumption predictions between the additive (Eqn.
 208 1) and substitutive (Eqn. 3) models for the mixed-sex treatment group using two-way beta
 209 regression, with prey density (5 levels) and model type (2 levels) as predictor variables. Nested
 210 likelihood ratio tests were again used for significance inference.

211 Functional response analyses were undertaken phenomenologically using the ‘frair’ package in R
 212 (Pritchard et al. 2017). Logistic regression of the proportion of prey consumed as a function of
 213 prey density was used to deduce functional response types. A Type II functional response is
 214 characterised by a significantly negative first order term, whilst a Type III functional response is
 215 characterised by a significantly positive first order term followed by a significantly negative
 216 second order term (Solomon 1949; Holling 1959). As prey were not replaced as they were
 217 consumed, we fit Rogers’ random predator equation for depleting prey densities (Rogers 1972;
 218 Trexler et al. 1998; Juliano, 2001):

$$219 \quad N_e = N_0(1 - \exp(a(N_e h - T)))$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total experimental period. The Lambert W function was used to allow model fitting (Bolker 2008). Predicted consumption estimates (see Eqn. 1) were rounded as fitting Eqn. 4 using maximum likelihood estimation requires integer data. We used the difference method, based on indicator variables (see Juliano, 2001), to undertake pairwise comparisons of functional responses with respect to the attack rate and handling time parameters. Here, we did not adjust p -values for multiplicity as not all treatment pairs were logically comparable. Significance was inferred at the 95% confidence interval in all analyses.

Results

Observed consumption

Prey survival in controls exceeded 99%, and so all experimental deaths were attributed to predation by *L. raynerae*. For observed predator groups, overall, consumption was significantly affected by the ‘predator’ treatment (Table 1). There was no significant difference in consumption between male and females when present singularly ($p = 0.40$), yet two females consumed significantly more than two males ($p = 0.04$). However, no significant difference was detected between the mixed multiple predator treatment and either single-sex multiple predator treatments (two males, $p = 0.25$; two females, $p = 0.92$). Predation increased significantly overall with greater prey densities (Table 1). Consumption at prey densities of 32 was significantly higher than under prey densities of 2, 4 and 8 (all $p < 0.05$), whilst consumption at densities of 16 was significantly greater than at prey densities of 2 and 4 (both $p < 0.05$). Similarly,

consumption at densities of 8 significantly exceeded that under densities of 2 ($p = 0.03$). The non-significant ‘predator $\times$ prey density’ interaction was removed from the final model (Table 1), indicating that differences among predator groups were consistent across prey density levels.

Additive model

The observed proportions of prey eaten were significantly lower than those predicted by the multiplicative risk model overall, indicating antagonistic interactions between intraspecific predator pairs (Table 2; Fig. 1). This is congruent with overall prey risk reductions. There were no significant two- or three-way interaction terms considering the multiplicative risk model (Table 2). Accordingly, MPEs resulting in prey risk reductions were consistent across predator pairings and prey densities. Nevertheless, the strength of male-male prey risk reductions tended to be higher than either the female-female or mixed groupings at the highest prey densities, whilst female-female risk reductions were strongest at lower densities (Fig. 1).

Substitutive model

The predictions from the substitutive model of prey survival for mixed-sex copepod treatment groups did not differ significantly from survival rates observed overall, and there was no significant ‘prey density $\times$ model’ interaction term (Table 3). Therefore, survivorship under mixed copepod groups was well-predicted from single-sex conspecific pairs irrespective of prey density (Fig. 2).

Model comparisons

Overall, the substitutive model predicted significantly lower estimates of prey mortality as compared to the additive model for the mixed-sex copepod groups (Table 4; Fig. 3). This effect was consistent across prey densities, owing to a non-significant interaction term (Table 4).

Accordingly, this aligns with higher predictability of multiple predator consumption by the substitutive as compared to additive model (see above).

Functional response

Type II functional responses were detected across all observed and predicted predator treatments (Table 5; Fig. 4). There was no significant difference between attack rates ($z = 0.72, p = 0.48$) or handling times ($z = 0.88, p = 0.38$) between single male and single female treatments (Fig. 4A). For multiple predator groups, predicted functional responses tended to be lower than those observed, indicating antagonistic interactions in multiple predator treatment groups (Fig. 4B, C, D). However, predicted functional response parameters were not significantly different from those observed in the double female treatment ($a, z = 1.78, p = 0.08$; $h, z = 0.56, p = 0.57$) (Fig. 4B). Further, observed attack rates were not significantly different from those predicted in the double male treatment ($z = 0.66, p = 0.51$), however, observed handling times were significantly longer than those predicted here ($z = 2.75, p = 0.01$), indicating antagonistic male-male interactions which significantly reduced maximum feeding rates (Fig. 4C). Predicted functional response parameters were not significantly different from those observed in the mixed female-male predator treatment groups ($a, z = 1.14, p = 0.26$; $h, z = 0.59, p = 0.56$) (Fig. 4D).

Discussion

In this study, we determine the effects of sex demographics for the quantification of intraspecific MPEs. Conspecific pairs of *L. raynerae* exhibited significant antagonistic interactions overall, culminating in prey risk reductions for *C. pipiens* larval prey. Whilst MPE differences among predator groupings lacked statistical clarity, antagonisms tended to be strongest for male-male

interacting copepods, whereby observed predation rates were substantially reduced under higher prey densities as compared to model predictions. Evidenced by functional response analyses, this manifested in significant increases in observed handling times for prey relative to those predicted under male copepod pairs, indicating antagonisms which reduced maximum feeding rates. Conversely, female-female conspecific pairs tended to reduce prey risk under lower prey densities, in turn driving reductions in attack rates compared to predictions, yet these effects were not statistically clear. Mixed female-male predator treatment observations were relatively closely predicted under both additive and substitutive models. These findings are important, given the distinct lack of experimental consideration for demographic effects on MPEs across resource densities.

Ovigerous female copepods were significantly more voracious than male *L. raynerae* in the present study when comparing female-female and male-male units, yet not for single predator treatments. Cuthbert et al. (2019b) found higher energetic demands for progeny development to drive greater interaction strengths by gravid female calanoid copepods, corroborating evidence from other copepod groups (Laybourn-Parry et al. 1988). Accordingly, overall predatory impact in temporary pond ecosystems is likely to be exacerbated as predator sex demographics transition to female-skewed ratios, which have been shown to develop over time (Wasserman et al. 2018). Owing to the numerical dominance of *L. raynerae* in temporary pond ecosystems resulting from internal recruitment dynamics (Wasserman et al. 2018), alongside high *per capita* offtake rates and high trophic positioning (Dalu et al. 2016; Wasserman et al. 2016b; Cuthbert et al. 2018a), such quantifications of sex demographic implications for conspecific interactions provide important insights in a temporal prey risk context within these understudied systems in arid environments.

309 Consumptive predictions from the multiplicative risk model were significantly greater than
310 predation rates experimentally observed for multiple predator groups. Accordingly, antagonistic
311 interactions between copepods resulted in prey risk reductions overall, with a lack of significant
312 evidence for negative MPEs being dependent on the composition of predator pairs or prey
313 densities. Nevertheless, subtle differences were emergent among predator treatment pairs and
314 prey densities, with the magnitude of antagonisms by female-female pairs most pronounced at
315 low prey densities. This, in turn, resulted in substantial reductions in attack rates for female-
316 female treatments towards larval mosquito prey, although non-significantly. Conversely, male-
317 male pair predation was more evidently reduced under high prey densities, with significant
318 increases in prey handling times. In turn, this interference resulted in reductions in maximum
319 feeding rates. Whilst the specific behavioural mechanisms for these differences require further
320 elucidation, it is possible that differences relate to mate searching processes. In certain
321 zooplankton species, greater activity levels by males compared to females has been shown to
322 make males more vulnerable to predation (Kiørboe 2006, Gusmão et al. 2013), and male *L.*
323 *raynerae* have been shown to be significantly more motile than females (Cuthbert et al. 2019a).
324 The elevated motility rates exhibited by males may therefore increase the potential for male-male
325 encounter rates which could elicit interference and drive prey risk reductions, and particularly
326 under higher prey densities. Nevertheless, whilst differences in predicted and observed
327 functional response parameters were observed in the present study, Type II functional responses
328 were displayed by copepods across all treatment groups. Therefore, MPEs did not impart low
329 density refuge effects for prey, with the potential to accordingly drive stabilising effects (Hassell
330 1978; Dick et al. 2014). For medically important prey such as mosquitoes, Type II functional

responses may facilitate population extirpations under certain conditions, in turn benefitting public health.

A broad range of statistical approaches have been proposed to quantify the nature of multiple predator interactions (Abrams & Ginzburg 2000), and debate exists regarding the choice between prey-dependent and predator-dependent models. Several predator-dependent functional response models which directly account for predator density in parameterisation are available (Beddington 1975; Crowley & Martin 1989; DeAngelis et al. 1975), and their use may provide a better fit to a given dataset than a prey-dependent model (Médoc et al. 2013), particularly where prey densities are high and predator density does not affect predation rates (Skalski & Gilliam 2001).

Nonetheless, in the present study, predictions were based on consumption rates from single predator groups using the multiplicative risk model. This follows a similar approach to many other studies where the multiplicative risk model is commonly used based on *per capita* consumption rates alone (e.g., Vance-Chalcraft & Soluk 2005; Wasserman et al. 2016b; but see also Sentis & Boukal 2018), and provides useful comparative insights into the strength of non-trophic interactions across a range of factorial experimental conditions.

In the present study, predictions for mixed female-male treatment groups were derived and compared across both additive and substitutive models. Prey survival rates predicted from the substitutive model were closer to empirical observations, and were significantly lower than those predicted from the additive multiplicative risk model. The significant difference between models is unsurprising, with the substitutive model inferences based on predation rates from the single-sex predator pairs, where antagonisms were already prevalent. Conversely, the additive model is based on predators singularly, whereby *per capita* interaction strengths were intensified in the present study. These results corroborate with those from Lampropoulos et al. (2013), where the

substitutive model was more closely aligned with observed predation rates. Additive designs have been criticised due to reduced explanatory power given the inherent difference in predator numbers (Sih et al. 1998; Griffen 2006), whilst mechanistic interpretation of substitutive designs is made difficult by differences in proportional densities between predators (Inouye 2001; Griffen 2006). Therefore, comparisons of these modelling approaches across experimental contexts is of importance to gain more holistic understandings of predator-predator interactions. Furthermore, Sentis and Boukal (2018) found the multiplicative risk model to underestimate predation rates when prey densities are high or prey depletion is substantial, and therefore the magnitude of antagonistic MPEs here may be considered conservative estimates with regards to risk reduction.

Whilst the use of simplified experimental arenas has the potential to enhance the prevalence of negative MPEs and limits mechanistic interpretations (Schmitz 2007), applications of comparative functional responses can still yield powerful predictions of interaction strengths in a multiple predator context. Temporary pond ecosystems in arid regions are poorly understood at present, with a relatively small body of literature examining trophic dynamics within these systems (O'Neill & Thorp 2014; Dalu et al. 2016; Wasserman et al. 2016a; Cuthbert et al. 2019b). The present study demonstrated significant overall antagonism in interacting conspecific copepods and, although lacking statistical clarity, identified sex demographics as a potential mediator of MPEs. Future studies should further integrate differences in population demographics, such as sex ratios and life history stages, into quantifications of community dynamics across multiple habitat types and taxonomic groups.

Author contributions

RC, TD, RW, OW and JD conceived and designed the experiments. RC and TD performed the experiments. RC analysed the data. RC, TD, OW, WF and AC contributed materials and analysis tools. RC wrote the first draft of paper. All authors prepared revised versions

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Table 1. Main effects resulting from deviance analysis of Poisson generalised linear model considering raw consumption counts according to the predator (P) and prey density (D) treatment terms.

Source	χ^2	df	<i>p</i>
P	15.16	4	0.004
D	44.56	4	< 0.001
P $\times$ D	10.51	16	0.839

Table 2. Main effects resulting from likelihood ratio tests of beta regression analysis considering observed and predicted multiple predator predation rates from the multiplicative risk model (M) among predator pairs (P) and prey densities (D).

Source	χ^2	df	<i>p</i>
P	4.46	2	0.108
D	94.91	4	< 0.001
M	4.50	1	0.034
P $\times$ D	2.22	8	0.973
P $\times$ M	0.35	2	0.838
D $\times$ M	0.66	4	0.957
P $\times$ D $\times$ M	7.68	8	0.466

Table 3. Main effects resulting from likelihood ratio tests of beta regression analysis considering observed and predicted survival rates from the substitutive model (M) for mixed female-male intraspecific predator pairs across prey densities (D).

Source	χ^2	df	<i>p</i>
D	31.98	4	< 0.001
M	0.38	1	0.540
D × M	5.61	4	0.230

Table 4. Main effects resulting from likelihood ratio tests of beta regression analysis considering predicted mortality rates between the additive and substitutive models (M) for mixed intraspecific predator pairs across prey densities (D).

Source	χ^2	df	<i>p</i>
D	28.99	4	< 0.001
M	4.97	1	0.026
D × M	0.31	4	0.989

Table 5. First order terms and significance levels resulting from logistic regression of the proportion of prey eaten as a function of prey density across differing predator treatments (female, F; male, M) and models (observed, O; predicted, P). Functional response parameter estimates (attack rate, a ; handling time, h) are shown alongside significance levels resulting from Rogers' random predator equation.

Predator	Observed/Predicted	First order term, p	a	p	h	p
F	O	-0.07, < 0.001	1.85	0.04	0.22	< 0.001
M	P	-0.06, < 0.001	1.07	0.10	0.30	< 0.001
F + F	O	-0.04, < 0.001	1.00	0.002	0.10	< 0.001
F + F	P	-0.07, < 0.001	3.37	0.01	0.12	< 0.001
M + M	O	-0.11, < 0.001	4.18	0.11	0.35	< 0.001
M + M	P	-0.06, < 0.001	2.34	0.03	0.17	< 0.001
F + M	O	-0.06, < 0.001	1.39	0.01	0.16	< 0.001
F + M	P	-0.07, < 0.001	2.88	0.02	0.14	< 0.001

Fig. 1. Observed and predicted proportional prey consumption under different intraspecific predator pairings and prey densities. Predictions are based on the multiplicative risk model. Means are $\pm$ SE ($n = 4$ per experimental group). Predicted predator performance was calculated from individuals (Eqn. 1).

Fig. 2. Observed and predicted proportional prey survivability in the mixed female-male predator pairing. Predictions are based on the substitutive model. Means are $\pm$ SE ($n = 4$ per experimental group). Predicted prey survival was calculated from predation by single-sex conspecific pairs (Eqn. 3).

Fig. 3. Predictions of proportional prey consumption in the mixed female-male predator pairing, between the additive and substitutive models across prey densities. Means are $\pm$ SE ($n = 4$ per experimental group).

Fig. 4. Observed functional responses of single predators, and observed and predicted multiple predator functional responses. Means are ± 1 SE ($n = 4$ per experimental group). Predicted predator performance was calculated from individuals using the rounded multiplicative risk model (Eqn. 1).