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Key-words:	Conspecific aggression, fish predators, heterospecific aggression, multiple predator effect, trait-based framework, predictive theory

1 **Using functional responses to quantify interaction effects among predators**

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21 **Running Head:** functional responses and multiple predator effects

22

23 **Summary**

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1. Predator diversity alterations have been observed in most ecosystems as a result of the loss and/ or addition of species. This has implications for predator-prey dynamics as non-trophic interactions among predators, so called multiple predator effects (MPE), are known to influence predation success. In addition, there is often a density-dependant relationship between prey availability and prey consumption (functional response). While MPE investigations are common in the literature, functional responses have rarely been incorporated into this field of predation ecology.
2. Here, we outline an experimental procedure that incorporates functional responses into multiple predator effect studies. Using three fish species with different functional traits as model predators (bluegill *Lepomis macrochirus*, southern mouthbrooder *Pseudocrenilabrus philander* and banded tilapia *Tilapia sparrmanii*), we assess intra- and inter-specific predator interaction outcomes on predator-prey dynamics. This was done by contrasting observed functional responses of heterospecific and conspecific combinations of predators with expected responses based on those of individual predators.
3. Multi-predator combinations produced variable results. Bluegill were the only species in which observed conspecific multi-predator functional responses matched those of expected based on individual performance (prey risk neutral effects). In contrast, prey risk reduction was observed for both mouthbrooder and tilapia conspecific multi-predator trials. Heterospecific combinations revealed strong prey risk reduction effects for mouthbrooder-tilapia and bluegill-tilapia trials, while mouthbrooder-bluegill multi-predator functional responses combined additively. These results are discussed within the context of behavioural traits of the species and the development of a trait-based predictive framework.
4. Using a functional response approach allowed for the assessment of multiple predator effects across a range of prey densities. We propose that the incorporation of within-guild predator combinations into classic functional response investigations will enhance predictive capacity development in competition and predation ecology.

Key-words: Conspecific aggression, fish predators, heterospecific aggression, multiple predator effect, trait-based framework, predictive theory

57 **Introduction**

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Across most ecosystems, biodiversity levels are changing as a result of human-mediated extinctions and introductions of species outside of their native ranges (Sala *et al.* 2000; Naeem, Duffy & Zavaleta 2012). These human-driven impacts often result in predator biodiversity alterations, which can involve guild homogenisation or increases in species richness (Simberloff & Von Holle 1999; Griffin, Byrnes & Cardinale 2013). Understanding predator diversity and its implications for trophic interactions is therefore important for

65 predictive theory (Schmitz 2007). Assessments of predator diversity effects represent an
66 important avenue of ecological research as predators provide valuable ecosystem services and
67 these services are likely to fluctuate when predator diversity levels are altered (Ives,
68 Cardinale & Snyder 2005; Dufy *et al.* 2005; Schmitz 2007). At the predator level,
69 considerations on the functional role of within guild interactions on prey consumption are
70 mixed, with implications for process level understanding of ecosystem functioning (Schmitz
71 2007; Griffin, Byrnes & Cardinale 2013).

72
73 Since virtually all ecological communities are comprised of multiple predators that utilise
74 mutual prey resources, the potential for competition is always present (Sih, Englund &
75 Wooster 1998; Barrios-O'Neill *et al.* 2014). In this way predators not only interact with their
76 prey, but they are often forced to interact with one another (Polis, Myers & Holt 1989;
77 Johnson *et al.* 2009). Considering the nature and strength of interactions among predators has
78 been identified as an important component of predation studies as predator-predator
79 exchanges can have implications for predator-prey interaction outcomes (Sih, Englund &
80 Wooster 1998; Bolker *et al.* 2003; Vonesh & Osenberg 2003). As such, it is now well
81 recognised that the effects of predators on prey do not necessarily combine additively as
82 multiple predators can modify predator-prey interactions (Soluk 1993; Sih, Englund &
83 Wooster 1998; Sokol-Hessner & Schmitz 2002; Barrios-O'Neill *et al.* 2014). Multiple
84 predator effects (MPE) resulting from non-additive competition among predators are
85 generally assessed across species, i.e. the outcome of interactions between two species
86 (predator and prey) as altered by an additional predatory species (Weigelt *et al.* 2007;
87 Barrios-O'Neill *et al.* 2104; Anderson & Whiteman 2015). However, studies contrasting
88 heterospecific and conspecific non-additive effects are less common (Young 2004; Forrester
89 *et al.* 2006; Anderson & Whiteman 2015). Conspecific non-additive effects are also relevant
90 within the context of biodiversity as introduced non-native predators can reduce within-guild
91 species richness while still functionally maintaining the trophic level in which they are
92 positioned, representing a type of predator homogenisation (Lohrer & Whitlatch 2002;
93 Griffin, Byrnes & Cardinale 2013). Additionally, while a wealth of information is available
94 regarding MPE on prey consumption, most of these studies assess these effects at single prey
95 densities (Griffen 2006; Harvey, White & Nakamoto 2004; Porter-Whitaker *et al.* 2012).
96 However, density-dependent prey acquisition is an important element of predator-prey
97 interactions and predation efficiency is known to vary with prey density (Abrams 1982;
98 Abrams 2000; Alexander *et al.* 2012). To this end, the present study proposes an application
99 of a classic ecological approach for the assessment of intra- and inter-specific predator
100 interaction outcomes on predator-prey dynamics.

101
102 The relationship between prey density and consumption rate is known as the predator
103 functional response (Holling 1959). Functional responses are extensively used by ecologists
104 and have helped gain insight into optimal foraging theory (Abrams 1982), invasion biology
105 (Dick *et al.* 2014) and the field of predation in general (Soluk 1993; Jeschke, Kopp &
106 Tollrian 2002). There is, however, a relatively small body of literature that has empirically
107 assessed the effects of interactions among predators on density-dependent prey acquisition
108 (Soluk 1993; Losey & Denno 1998; Barrios-O'Neill *et al.* 2014). **The little work that has**

109 been done in this regard has produced varied results. Firstly, it has been shown that when
110 combined, the overall functional response of heterospecific predator combinations may be
111 lower than one would expect based on single predator functional responses (Soluk 1993).
112 However, this is in contrast to heterospecific predator combinations that result in an overall
113 functional response that doubles the predicted functional response based on single predator
114 performance (Losey & Denno 1998). In addition, multiple predator effects at the conspecific
115 level have also shown that consumption can be additive and group consumption is, therefore,
116 predictable from individual-level consumption (Barrios-O'Neill *et al.* 2014). These
117 contrasting results highlight that multi-predator interactions are complex and that predator
118 combinations can result in prey risk reduction as a result of antagonism among predators, risk
119 enhancement as a result of synergism among predators or a risk neutral scenario whereby
120 predator effects simply combine additively. Here, we account on an experiment assessing the
121 effects of multiple fish predators on shared prey. In this experiment within the context of risk
122 reduction, risk enhancement and risk neutral multiple predator effects, we contrast the overall
123 functional responses of heterospecific and conspecific combinations of predators with
124 predicted responses based on individual predator performances.

125
126 Three predatory fish species were used in the experiment, bluegill *Lepomis macrochirus*
127 Rafinesque, 1819, southern mouthbrooder *Pseudocrenilabrus philander* (Weber, 1897) and
128 banded tilapia *Tilapia sparrmanii* A. Smith, 1840. Bluegill (Centrarchidae) are native to
129 North America, while southern mouthbrooder and banded tilapia (Cichlidae) are native to
130 parts of Africa. All three fishes have been distributed extensively outside of their native
131 ranges and at some localities in South Africa occur sympatrically as non-native predators
132 (Ellender & Weyl 2014; Muller, Weyl & Strydom 2015). Aside from the fact that the
133 presence of these fish species now represent a 'non-native species cocktail' in select regions,
134 these fishes have certain trait differences making for ideal combinations to highlight a suite of
135 possible MPE outcomes. Bluegill are a shoaling species (Colgan *et al.* 1979; Gross &
136 MacMillan 1981; Wahl & Stein 1988; Savino & Stein 1989; Dugatkin & Wilson 1992) and
137 are primarily predators incorporating mostly animal prey into their diets (Marshall 2011;
138 Taguchi *et al.* 2014) and while southern mouthbrooders (hereafter referred to as
139 mouthbrooders) are also primarily predatory, they are a non-schoaling species not found in
140 close association with conspecifics (Ribbink 1975; Polling, Schoonbee & Saayman 1995).
141 Banded tilapia (hereafter referred to as tilapia), however, occasionally occur in small shoals
142 or pairs, but are also often solitary (Skelton 2002; Marshall 2011). While this species readily
143 consumes animal prey, it is more omnivorous than the bluegill and mouthbrooder,
144 incorporating plant material into its diet (Zengeya & Marshall 2007; Marshall 2011). Bluegill
145 are considered a flexible species with proportions of populations often found in both open
146 waters and in close association with structure and littoral regions (Werner & Hall 1988;
147 Yonekura, Kohmatsu & Yuma 2007). While published information on the habitat preference
148 of the mouthbrooder and tilapia is limited, they are most commonly encountered in shallow
149 littoral habitats with the former often associated with sandy or rocky substrates and
150 vegetation structure and the latter more closely associated with vegetation structure (Bruton
151 1978; Khoza, Potgieter & Vlok 2012). The three species, therefore, overlap in habitat use in
152 environments in which they co-occur, as has been observed in impoundments and pools of

153 the Kariega River system, South Africa, where experimental animals were collected for the
154 present study (Table 1).

155

156 In this study, we firstly determined single species functional responses at the individual level
157 for bluegill, mouthbrooder and tilapia, towards small fish prey. We then use this information
158 to explore how individual performance translates to multiple predator overall performance
159 within the context of prey risk reduction, neutral or enhancement effects. This was achieved
160 by contrasting individual functional response information with that of both conspecific and
161 fully crossed mixed predatory fish species pairs (Fig. 1). We predicted that for conspecific
162 multi-predator pairs, prey risk reduction (rather than risk neutral or risk enhancement) would
163 be more prevalent for species that do not typically utilise habitats in close conspecific
164 association. For heterospecific combinations, however, we predicted that different multi-
165 species combinations would not result in similar prey risk scenarios and explored
166 mechanisms potentially driving any observed differences. In this way, the study highlights
167 the complexities of multiple predator effects and how prey consumption at different prey
168 densities is a potentially important addition warranting consideration in MPE studies.

169

170

171 **Materials and methods**

172

173 *Experimental design*

174

175 All predatory fish were collected in March 2015 by seine netting in Craig Doone Dam
176 (33°21'35.65" S; 26°28'41.29" E), near Grahamstown, South Africa. Prey used were
177 Mozambique tilapia *Oreochromis mossambicus* captive bred offspring, supplied by
178 AquaCulture Innovations, Grahamstown. All fish were transported to the Department of
179 Ichthyology and Fisheries Science, Rhodes University, Grahamstown and were housed in
180 separate 600 L tanks in a closed recirculating system (water flow to each tank 1 L min⁻¹; 18 ±
181 1°C). All fish were allowed to acclimate to the system for at least 72 hours prior to use in
182 feeding trials and were maintained on a diet of earthworms to standardise prior experience.

183

184 Experiments were conducted in individual 26 L cages constructed from 1.5mm mesh and
185 floated using buoyancy aids in 15 separate 300 L fibreglass tanks that were part of the same
186 flow-through system as the holding tanks so that each fibreglass tank held one mesh cage.
187 Predators were size-matched with respect to total length (TL): bluegill (mean ± SD) = 75.1 ±
188 1.9 mm TL; mouthbrooder = 76.4 ± 2.6 mm TL; and tilapia = 76.7 ± 2.2 mm TL. Prey were
189 selected from a common size class (10.0 ± 0.5 mm TL). Preliminary trials on prey in the
190 holding tanks indicated that in the absence of predators the prey used the entire tank with
191 individuals observed actively swimming throughout the water column. Fish predators were
192 randomly selected two hours prior to use and placed in the mesh cage to reacclimate, either
193 individually, with a conspecific individual, or in a mixed species pair depending on the
194 treatment. Single and mixed predator treatments were fully crossed so that all predator
195 combinations were trialled with fish only being used once for a single feeding trial. After the

196 reacclimation period, fish were presented with tilapia prey at six densities (2, 4, 8, 16, 32, 64)
197 with four replicates per density per treatment combination. Feeding trials were run for one
198 hour, after which prey consumption was examined. Controls were three replicates of each
199 prey density in the absence of predators. **Since all predators are active diurnally, feeding trials**
200 **were only conducted during daylight hours.**

201

202 As feeding behaviour was not continuously observed, it was not possible to know which
203 predator species was responsible for prey consumption in the heterospecific combinations.
204 For this reason, additional trials were run for all multi-species combinations at a single prey
205 density of 32 prey and filmed ($n = 3$ for each combination), which allowed the number of fish
206 consumed by the respective species during each combination to be determined. **For this**
207 **component of the study study, however, novel fish were not available and as such we used**
208 **fish that had been employed in the functional response component of the study.** The same
209 experimental procedures as outlined above were employed for this component and GoPro
210 cameras (Hero 3) were placed directly overhead the cages allowing for an aerial view of the
211 inside of the cages. Based on this footage the number of fish consumed by the **respective**
212 **species in each combination of predators was determined. In addition, interactions between**
213 **the predators could be quantified and related to interference. Behavioural traits were scored**
214 **for each species of fish from analysis of video footage that was gathered during filmed trials.**
215 **Four traits were selected for analysis that provided unambiguous assessment of behaviour: (1)**
216 **lateral displays, defined as the spreading of operculum and erecting of fins by an individual**
217 **when it was within one body length of the heterospecific; (2) lunging, defined as the quick**
218 **movement towards the heterospecific and included chasing (where one fish was chased in**
219 **short, rapid bursts by the other); (3) follow, defined as the slow following of a heterospecific**
220 **within a distance of one body length; (4) retreat, defined as the rapid swimming away from a**
221 **heterospecific in response to displays or any type of approach by the partner fish. These**
222 **behaviours were counted for both species in each filmed multi-predator trial.**

223

224 ***Data analysis***

225

226 To test whether functional responses of two fish predators (conspecifics and mixed species
227 pairs) could be predicted by summing individual responses, the predicted combined
228 consumption was calculated using the following multiplicative model (Soluk 1993);

229

$$230 C_{ab} = N_p (P_a + P_b - P_a P_b)$$

231

232 where C_{ab} is the predicted combined consumption for a particular initial prey density (N_p) and
233 P_a and P_b are the probabilities of being consumed by each predator present (fish a and fish b),
234 respectively, over a 1 h period of exposure. This multiplicative model calculates predicted
235 combined consumption that cannot exceed the total number of prey introduced. Data for
236 predator a and predator b were generated from single fish functional response experiments.
237 The predicted combined consumption was calculated as the expected data, while

238 consumption from the observed multipredator trials were modelled as the observed data
239 below.

240

241 Functional response type was first determined using logistic regression that tests for a
242 negative linear coefficient (fitted using the maximum likelihood procedure) in the
243 relationship between the proportion of prey eaten and prey density. This would indicate a
244 Type II functional response (Juliano 2001). We then modelled by maximum likelihood
245 estimation (Bolker 2008) Type II functional responses (see Results) using the ‘random
246 predator equation’ (Rogers 1972), which is appropriate where prey are not replaced as they
247 are consumed, as was the case here (Juliano 2001);

248

$$249 N_e = N_0 \{1 - \exp[a(N_e h - T)]\}$$

250

251 where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack constant,
252 h is the handling time and T is the experimental period. To compare the obtained functional
253 responses, data sets were non-parametrically bootstrapped ($n = 2000$) in order to construct
254 95% confidence intervals around functional response curves and their associated parameters.
255 The random predator equation was fitted to each bootstrapped data set using starting values
256 of a and h that were obtained from the original maximum likelihood estimates. As
257 bootstrapping allows data to be considered in terms of populations, if the confidence intervals
258 do not overlap we were able to deduce that the functional responses and/or their associated
259 parameters were different. Generalised Linear Models (GLMs) assuming quasi-poisson
260 distributions were used to compare overall prey consumption between appropriate predator
261 treatments.

262

263 Behavioural traits were scored and data arranged so that behaviour of the focal predator
264 species towards each of the other predator species could be compared. A mixed effects
265 ANOVA (between-factor = partner fish; within-factor = behavioural trait), which accounted
266 for non-independence of behavioural data, was used for each focal species. Data was log-
267 transformed prior to analyses to meet normality assumptions. Significant interactions were
268 investigated via post hoc t-tests that compared frequency of each of the behaviours performed
269 towards partner fish. All analyses were carried out in R v. 2.15.1 (R Development Core
270 Team, 2012) and functional response modelling was undertaken using the ‘frair’ package
271 (Pritchard 2014).

272

273 Results

274

275 In control trials, no prey deaths were recorded, therefore, prey mortality was attributed to
276 predation, which was also visually observed. First-order terms derived from logistic
277 regressions were all significantly negative, indicating that the functional responses obtained
278 were all Type II (Table 2; Figs. 2, 3 and 4), and the attack constant and handling time
279 parameters of the functional response models were also all significant at $p < 0.001$ (Table 2).

280

281 Overall consumption of prey by individual predators was dependent on predator identity ($F_{(2, 54)} = 6.66, p = 0.003$; Fig. 2), with tilapia consuming significantly fewer prey in comparison to
282 both bluegill ($z = 2.41, p < 0.05$) and mouthbrooder ($z = 3.48, p < 0.05$). This was further
283 evidenced in functional response curves where 95% confidence intervals did not overlap
284 across the full prey density range between mouthbrooders and tilapia, and only at prey
285 densities above 34 between bluegill and tilapia (Fig. 2).
286

287

288 Consumption by conspecific pairs was also dependent on the species identity ($F_{(2, 54)} = 9.34, p < 0.001$; Fig. 3) and bluegill pairs consumed significantly more prey than pairs of both
289 mouthbrooder ($z = 3.18, p = 0.004$) and tilapia ($z = 3.82, p < 0.001$). Differences in the
290 magnitude of the functional responses were also evident among the conspecific pairs, with
291 bluegill pairs producing heightened responses in comparison with the other two species (solid
292 lines in Fig. 3). Differences in these functional responses were also demonstrated in the
293 increased attack ($a = 4.931$) and decreased handling ($h = 0.047$) parameters in bluegill pairs
294 in comparison to pairs of mouthbrooders ($a = 1.530; h = 0.162$) and tilapia ($a = 0.922; h =$
295 0.093) (Table 2). Of the three species, the observed functional response of the bluegill pairs
296 overlapped with the predicted response across the full prey density range (Fig. 3a), however
297 for both mouthbrooder and tilapia conspecific pairs the observed functional responses were
298 significantly reduced in comparison to the predicted curves (Fig. 3b and c). This reduction
299 was greatest, however, in mouthbrooder comparisons and this was further evident in the
300 reduction in the observed attack and handling time values in comparison to the predicted
301 parameters (Table 2).
302

303

304 A significant difference in prey consumed by mixed pair treatments ($F_{(2, 54)} = 3.71, p = 0.03$;
305 Fig. 4) was driven by greater predation by bluegill and mouthbrooder in combination
306 compared to the mix of bluegill and tilapia ($z = 2.54, p = 0.02$; solid lines in Fig. 4a vs 4b).
307 The observed functional response of bluegill combined with mouthbrooder overlapped with
308 predicted values across the full prey density range and the response curves were overlaid on
309 each other (Fig. 4a). Observed responses of bluegill and tilapia in combination overlapped
310 with predicted responses for the most part, although there was separation in the 95%
311 confidence limits between prey densities of around 15 to 25 (Fig. 4b). Difference in
312 functional response between the observed and predicted responses of mouthbrooder and tilapia
313 were detected, however, and although the initial response was similar, responses diverged at
314 around prey densities just above 26 (Fig. 4c). This divergence at greater prey densities is also
315 evidenced in the two-fold increase in the handling parameter that was observed in
316 mouthbrooder and tilapia pairs ($h = 6.472$) compared to those that were expected ($h = 3.389$).
317

318

319 Video analyses revealed that when combined, bluegill and mouthbrooder consumed (mean \pm
320 SD) 13.33 ± 5.51 and 18.67 ± 5.51 prey items, respectively. However, the trials in which
321 tilapia was used in combination with another fish species, both bluegill and mouthbrooders
322 were responsible for 100% of prey consumption, eating all 32 prey. In behavioural
323 observations mouthbrooders failed to display retreating behaviour from either bluegill or
324 tilapia; thus, this was removed from the analysis of this species. There were no significant
main effects or interaction of partner fish and behavioural type on the frequency of

325 behaviours observed in mouthbrooders. The frequency of behaviours of bluegill varied
326 depending on behavioural type and the species of partnered fish ($F_{(3,12)} = 6.798, p < 0.01$).
327 This was driven by a significantly greater number of retreats from mouthbrooders compared
328 to tilapia ($t_4 = 3.069, p < 0.05$). In tilapia, frequencies of observed behaviours varied
329 depending on behavioural type and the species of partnered fish ($F_{(3,12)} = 22.448, p < 0.01$).
330 This was driven by a significantly greater number of chases of bluegills compared to
331 mouthbrooders ($t_4 = 10.128, p < 0.05$) and greater retreats from mouthbrooders compared to
332 bluegill ($t_4 = 4.247, p < 0.05$).

333

334 Discussion

335

336 In ecological communities prey are seldom exposed to single predators and, as predators
337 occur in a variety of combinations, varying degrees of impact on prey populations have been
338 observed (Schmitz 2007; Barrios-O'Neill *et al.* 2014). In this study we demonstrate that
339 predator-predator interactions can be important in determining prey consumption by
340 investigating functional responses in individual as well as conspecific and heterospecific
341 pairs of predatory fish. Specifically, we show that at both the conspecific and heterospecific
342 level, prey risk varies as a result of predator-predator effects. Differences in the functional
343 responses of individual species were found with mouthbrooders exhibiting heightened
344 responses compared to both bluegill and tilapia towards the prey Mozambique tilapia. In
345 conspecific pairs, however, this pattern did not hold as prey risk enhancement was observed
346 for bluegill pairs which exhibited heightened functional responses in comparison to pairs of
347 both mouthbrooder and tilapia where prey reduction was observed. Prey risk also differed
348 amongst mixed species pairs with combinations of bluegill and mouthbrooder producing a
349 risk neutral scenario whereby observed functional responses were congruent with expected,
350 based on individual predator performance. Often these results did not align with expected
351 outcomes and we show that differences in predator identity and diversity can have important
352 consequences for altering the outcome of what might be expected in density-dependent
353 relationships between predators and their prey.

354

355 In predator-prey interactions with single predators, the expectation that mouthbrooders, as a
356 solitary and predatory species (Polling, Schoonbee & Saayman 1995), would exhibit
357 increased resource use in comparison to the other species held true. Indeed, overall
358 consumption by this species was significantly greater than that of tilapia and a divergence in
359 functional responses with bluegill was evident from intermediate to high prey densities.
360 Bluegill is also predatory, however, this reduced consumption compared to the solitary
361 mouthbrooder predator may be reflective of a less efficient individual predator that most
362 commonly occurs in shoals with conspecifics (Smith & Warburton 1992). Tilapia exhibited
363 reduced functional responses in comparison to bluegill and mouthbrooders, and this was
364 similar across single fish and conspecific pair treatments. As an omnivorous species
365 (Zengeya & Marshall 2007; Marshall 2011) it is likely that tilapia is less motivated to feed on
366 this prey when it is rare, resulting in a reduction in the initial response, with increased
367 consumption occurring only at higher prey densities.

368

369 Observed and expected responses of conspecific pairs diverged among the species as was
370 evident via overlapping and separate confidence levels of the functional responses curves. Of
371 the three fish species investigated here, only the functional response of bluegill aligned with
372 what was expected. This suggests that although bluegill pairs do not facilitate feeding that
373 results in prey risk enhancement, or synergistic consumption, they may not act to aggravate
374 conspecifics. Conversely, a reduction in the responses of observed mouthbrooder pairs in
375 comparison to the expected response suggests that this non-schoaling fish responds to the
376 presence of conspecifics. Mouthbrooders are recognised as being an aggressive species
377 (Ribbink 1975; Polling, Schoonbee & Saayman 1995; Marshall 2011) and the results reported
378 here reflect this. In tilapia, observed responses for two individuals were comparable to that
379 for one individual. As a species that spends its time in loose association with conspecifics,
380 these results suggest that interference levels lie somewhere between the shoaling bluegill and
381 the solitary mouthbrooder. Additionally, tilapia are omnivorous and readily consume a broad
382 range of prey and so competition for live prey, even at the conspecific level, may not be as
383 fierce as in primarily predatory fish species.

384

385 All species exhibited or responded to aggression when in a heterospecific combination. These
386 combinations, revealed that there was strong interference competition between mouthbrooder
387 and tilapia, with mouthbrooder outcompeting the tilapia for prey resources at high prey
388 densities. The same was observed for bluegill-tilapia combinations, with tilapia not
389 consuming prey in the presence of bluegill. Video observations of heterospecific
390 combinations highlighted strong interference between the fish species, with mouthbrooder
391 and bluegill both reducing tilapia prey resource consumption. The mechanisms by which
392 mouthbrooder and bluegill reduced prey consumption by tilapia differed, however, as
393 indicated by the behavioural analyses. In the mouthbrooder-tilapia combinations,
394 mouthbrooder were highly aggressive toward tilapia and this resulted in tilapia spending
395 much time retreating and ultimately not feeding. In the bluegill-tilapia combinations, tilapia
396 were observed slowly following the bluegill rather than actively feeding. The combination of
397 bluegill and mouthbrooder functional responses combined additively, and video analyses
398 revealed that these species in combination each consumed prey at a similar rate as each did in
399 the single functional response trials. While this suggests that interference interactions
400 between solitary and shoaling species are less pronounced than between two solitary species,
401 aggressive behaviour was observed between mouthbrooder and bluegill. Indeed,
402 mouthbrooder were equally as aggressive toward bluegill as they were toward tilapia.
403 Bluegill also showed a high frequency of retreat behaviour toward mouthbrooder when in
404 combination. But unlike tilapia, bluegill returned non-contact aggression toward
405 mouthbrooder and spent time following the heterospecific, while repeatedly making
406 successful attempts at the prey between contact bouts.

407

408 These findings highlight subtle behavioural difference among the three predator species
409 which ultimately have implications for foraging. In addition, it is evident that similar
410 outcomes, such as the prey risk reduction observed in both the mouthbrooder-tilapia and
411 bluegill-tilapia combinations, can arise through alternate behaviourally mediated

412 mechanisms. The fish used for the behavioural component of the study had, however, already
413 been used once in functional response trials. It is therefore possible that there was an element
414 of learning associated with the behavioural component of the study, as suggested in the
415 overall higher consumption of prey in the behavioural experiment when compared to the
416 functional response experiment at the same density. Despite this consideration, the
417 behavioural results highlight key interactions between predator species in heterospecific trials
418 and provide mechanistic information into how multi-species observed- differ from expected-
419 functional responses based on individual performance.

420

421 Incorporating a simple approach contrasting functional responses of expected and observed
422 multiple predator combinations provides much information. For example, in addition to the
423 information acquired in classic MPE experiments, this approach can provide valuable
424 information on the importance of prey density dependence on predator-predator interactions.
425 This is highlighted by the conceptual model (Fig. 5) that exemplifies the ways in which
426 predator-prey dynamics can be altered. While both functional response and MPE
427 investigations are well established and independently highlight shortcomings of simple
428 pairwise predator-prey interaction experiments, the convergence of these two fields of study
429 is slowly beginning to be realised. In the present study, the incorporation of multiple prey
430 densities over a single prey density approach resulted in a more robust determination of
431 potential multiple predator effects. This was particularly evident in the multi-species
432 combinations whereby in contrast to the overall functional response data, at certain prey
433 densities no differences between observed and expected multiple-predator effects would have
434 been detected, despite the lack of 100% prey consumption at those densities. Similarly, under
435 certain single prey densities, differences were evident between observed and expected overall
436 consumption in multi-predator combinations, contrary to the overall functional response
437 derived outputs. The use of a functional response approach therefore provides an added
438 degree of confidence in results over the single prey density MPE approach, given that the
439 design requires the use of many predators thus minimising random effects. Furthermore, a
440 major advantage to using a functional response approach to assessing predator-prey
441 interactions is seen in the attack rate parameter, which provides information into prey
442 resource utilisation at lower prey densities and is important for the determination of
443 functional response type. While in the present study a type II functional response was
444 observed in all trials, other types of responses are possible. Functional response type is
445 thought to have implications for prey population persistence or the point at which prey-
446 switching by a predator occurs (Hassell 1978; Abrams 1982). The incorporation of this aspect
447 of predation into MPE studies is lacking and using a functional response approach in
448 combination with behavioural observations, as in the present study, could add insight into our
449 understanding of predator induced prey extinctions or allee effects.

450

451 The next step in integrating functional responses into the MPE literature would be to develop
452 a predictive framework whereby multiple species with various trait suites are trialled. To do
453 this, future studies will need to use conceptual models (Fig. 5) to identify the factors that may
454 alter predator-prey dynamics at each step. These factors can then be associated with species
455 traits. As such, species selection should be based on available autecological information in

456 order for traits such as habitat domain, territoriality, reliance on visual or olfactory cues and
457 foraging mode to be incorporated. In the present study we only used three species, and much
458 of this information is not available for tilapia and mouthbrooder. This makes it difficult to
459 develop such a framework using this data. The study does, however, provide a working
460 example of how such studies could be conducted to provide insight into how key traits can be
461 highlighted for predictive capacity regarding MPE outcomes. For example in the present
462 study, while maintenance of foraging area size was an unavoidable component of the design,
463 this experimental requirement may provide insight into how the importance of space depends
464 on functional traits of the test species. On the one hand, mouthbrooder are solitary and
465 potentially territorial and for this species, space may be important. Indeed mouthbrooder pairs
466 did not consume prey proportional to its availability, exhibiting overall attack rates and
467 handling times that were lower than even individual functional responses for the species. On
468 the other extreme are bluegill, a shoaling species. Shoaling species are less likely to be
469 threatened by conspecifics in close proximity as this is the mode in which they typically
470 forage (Wahl & Stein 1988; Savino & Stein 1989). Thus bluegill pairs exhibited functional
471 responses as expected based on individual performance. Space, therefore, seems to be less of
472 a factor affecting prey resource utilisation in bluegill. In the context of this study such
473 statements are largely speculative given the limited number of species tested and the lack of
474 sufficient species information such as mouthbrooder and tilapia habitat domains (Schmitz
475 2007). However, future studies on larger data sets incorporating more species could result in
476 the development of a trait-based framework with potential predictive capacity.

477
478 Within the context of developing a trait-based framework, prey species traits would also need
479 to be incorporated as prey are often not passive players in predator-prey interactions (Schmitz
480 2007; Dodd *et al.* 2014; Wasserman *et al.* 2016). In the present study, early life-history fish
481 prey were employed. These prey were active swimmers and would likely have been capable
482 of a degree of escape, more so than slower swimming prey such as certain species of tadpole,
483 for example. Prey selection is therefore an important consideration in any predator-prey
484 experimental study as prey species may respond differently to the same predator (Schmitz
485 2007). The development of a predictive framework using a functional response approach
486 would advance the field of trophic ecology within the context of predator diversity effects
487 given that functional responses, in theory, account for predator performance with and without
488 prey as a limited resource. Such information would have various ecological applications. One
489 example would be for the prioritisation of invasive species management. Functional response
490 studies have gained much momentum in the field of invasion biology, as it has been
491 highlighted that invasive species often have elevated and different functional responses to
492 similar native species (Dick *et al.* 2013a; Alexander *et al.* 2014; Dick *et al.* 2014; Barrios
493 O’Niell *et al.* 2015). As such, it has been proposed that functional responses could be used as
494 a tool to identify problematic invasive species or used in combination with other information
495 to potentially quantify impact (Alexander *et al.* 2014; Dick *et al.* 2014). There is, however,
496 limited information on the role of competition and predator-predator interactions within the
497 context of invasion biology for functional response studies. This is relevant as the invasion
498 meltdown theory predicts that one invasive species could facilitate a second invader
499 (Simberloff and Von Holle 1999; Jackson 2015), while biotic resistance theory specifies that

500 certain species facilitate immunity to further invasions (DeRivera *et al.* 2005; Dick *et al.*
501 2013b). The incorporation of functional responses into MPE studies therefore has much
502 potential for the field of invasion biology.

503

504 Simplification is key to the development of ecological theory and functional response
505 procedures offer relatively straightforward means of exploring aspects of optimal foraging
506 (Abrams 1982). While laboratory studies are often criticised for having limited field
507 relevance, they are still important for the development of ecological theory as they provide
508 insight into specific aspects of ecology (Lawton 1995; Chapman 2000; Barrios-O'Neill *et al.*
509 2015). Controlled experiments are often one of the only ways with which we can gain a
510 mechanistic understanding of how certain processes unfold (Benton *et al.* 2007; Alexander *et al.*
511 2012). We therefore propose that the incorporation of predator combinations into classic
512 functional response investigations would be useful for the development of competition and
513 predation ecology. Notably, this approach can be utilised in a comparative fashion in
514 regional, taxonomic and phenotypic, invasion or size structuring predation assessments with
515 the ultimate goal of enhancing predictive capacity development for multi-predator aspects of
516 predator-prey dynamics.

517

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525

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696

697

698 **Tables**699 **Table 1:** Summary of conspecific association level, predominant feeding mode and habitat

700 association of the three species employed in the multi-predator functional response

701 experiment

702

	<i>Lepomis macrochirus</i>	<i>Pseudocrenilabrus philander</i>	<i>Tilapia sparrmanii</i>
Common name	Bluegill	Mouthbrooder	Tilapia
Conspecific association	Shoaling	Solitary	Small shoals/ solitary
Feeding	Predator	Predator	Omnivore
Habitat association	Open water/ littoral	Rocky substrate/ vegetated littoral	Vegetated littoral

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707 **Table 2.** Parameter estimates and significance levels from first-order logistic regression
 708 analyses of the proportion of prey killed against initial prey density, with functional response
 709 parameters (a and h) and significance levels from the Rogers random predator equation. O =
 710 Observed, E = Expected.

Predators	Data	First-order term, p	a	p	h	p
1 Bluegill	O	-0.037, <0.001	4.517	0.008	0.087	<0.001
1 Mouthbrooder	O	-0.040, <0.001	3.099	<0.001	0.051	<0.001
1 Tilapia	O	-0.027, <0.001	0.765	<0.001	0.098	<0.001
2 Bluegill	O	-0.048, <0.001	4.931	<0.001	0.047	<0.001
2 Mouthbrooder	O	-0.025, <0.001	1.530	0.003	0.162	<0.001
2 Tilapia	O	-0.027, <0.001	0.922	<0.001	0.093	<0.001
2 Bluegill	E	-0.045, <0.001	3.758	<0.001	0.043	<0.001
2 Mouthbrooder	E	-0.038, <0.001	3.844	<0.001	0.028	<0.001
2 Tilapia	E	-0.028, <0.001	1.299	<0.001	0.051	<0.001
1 Bluegill, 1 Mouthbrooder	O	-0.047, <0.001	5.523	<0.001	0.035	<0.001
1 Bluegill, 1 Tilapia	O	-0.036, <0.001	2.183	<0.001	0.065	<0.001
1 Mouthbrooder, 1 Tilapia	O	-0.053, <0.001	6.472	<0.001	0.063	<0.001
1 Bluegill, 1 Mouthbrooder	E	-0.043, <0.001	4.654	<0.001	0.034	<0.001
1 Bluegill, 1 Tilapia	E	-0.037, <0.001	3.209	<0.001	0.048	<0.001
1 Mouthbrooder, 1 Tilapia	E	-0.039, <0.001	3.389	<0.001	0.037	<0.001

711

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714

715 **Figures**

716

717 **Fig. 1.** Experimental predator treatments comprising individual predator, conspecific and
718 heterospecific predator combinations of a) bluegill (*Lepomis macrochirus*), b) mouthbrooder
719 (*Pseudocrenilabrus philander*) and c) tilapia (*Tilapia sparrmanii*).

720

721 **Fig. 2.** Functional responses of individual bluegill (*Lepomis macrochirus*), mouthbrooder
722 (*Pseudocrenilabrus philander*) and tilapia (*Tilapia sparrmanii*) towards common fish prey.
723 Shaded areas are 95% confidence intervals.

724

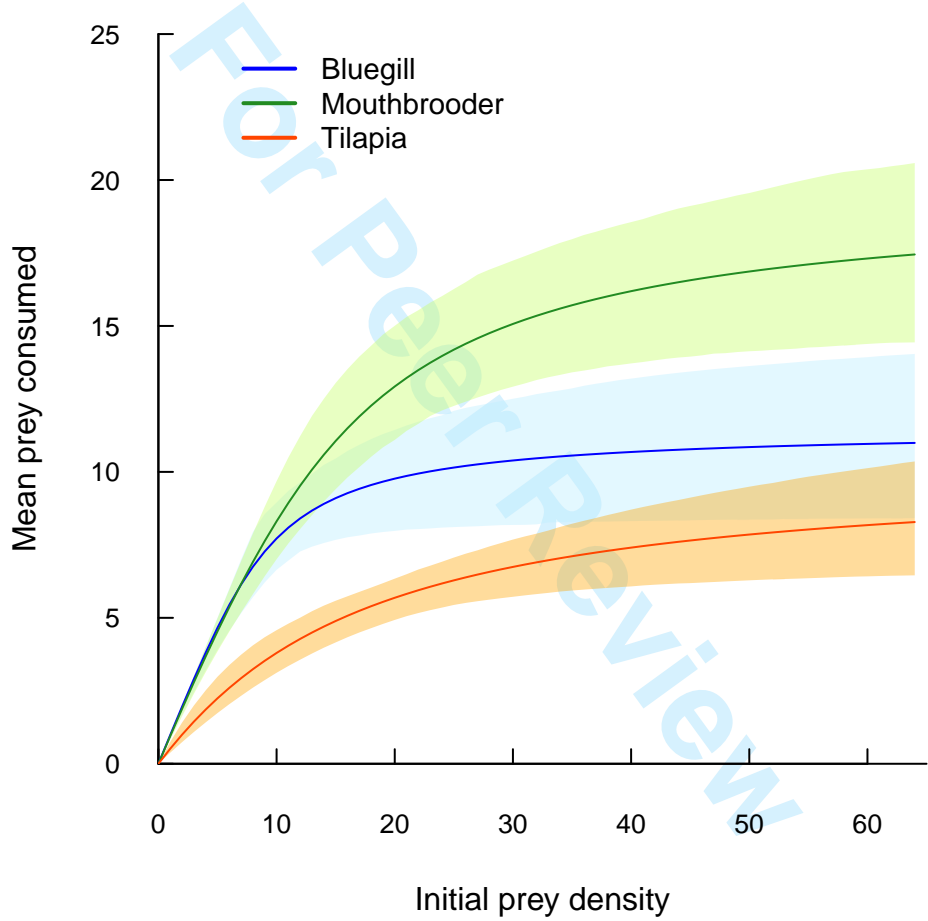
725 **Fig. 3.** Observed (solid) and expected (dashed) functional responses of conspecific pairs of a)
726 bluegill (*Lepomis macrochirus*), b) mouthbrooder (*Pseudocrenilabrus philander*) and c)
727 tilapia (*Tilapia sparrmanii*). Solid/dashed lines represent model curve, while shaded areas are
728 95% confidence intervals. Expected responses calculated from the performance of individuals
729 of each species (Fig. 2). **Note the different y-axes scales when comparing with Fig. 2.**

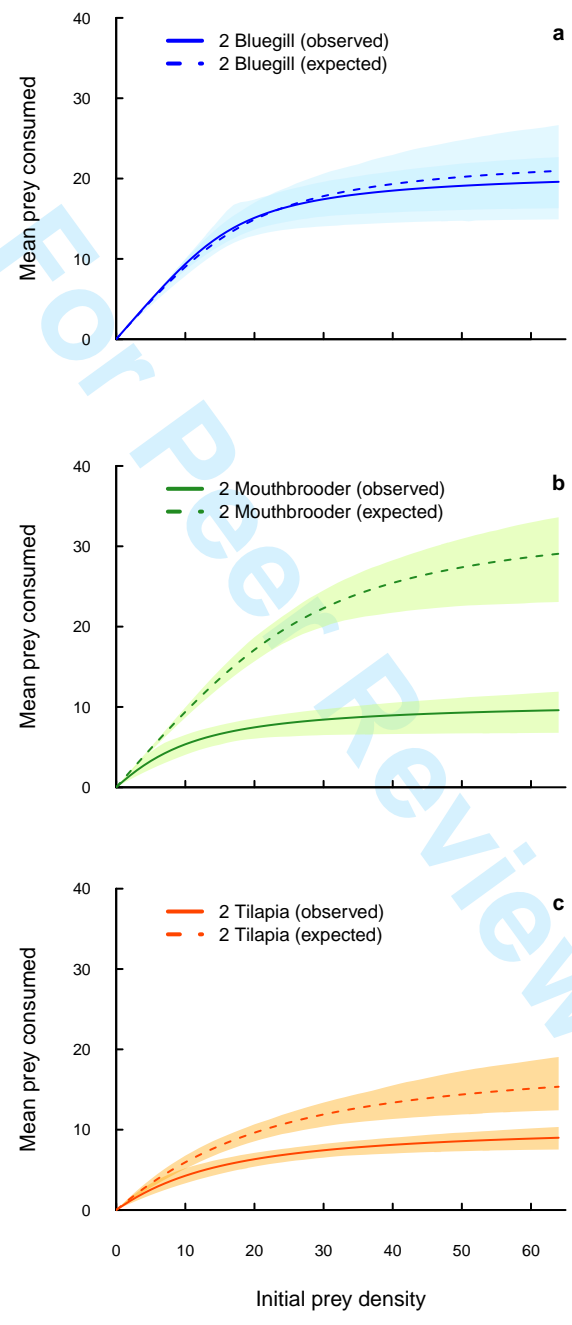
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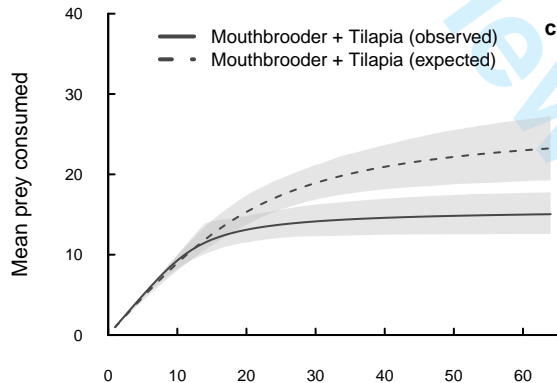
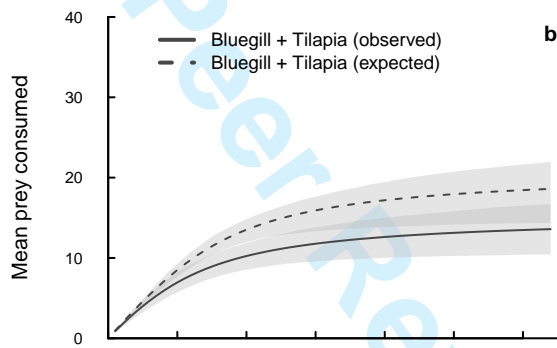
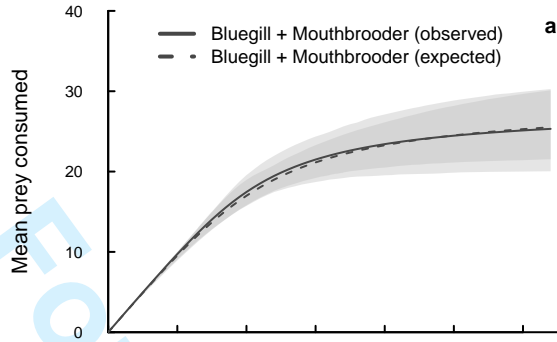
731 **Fig. 4.** Observed (solid line) and expected (dashed line) functional responses of
732 heterospecific pairs of a) bluegill (*Lepomis macrochirus*) and mouthbrooder
733 (*Pseudocrenilabrus philander*) b) bluegill and tilapia (*Tilapia sparrmanii*) and c)
734 mouthbrooder and tilapia. Expected responses calculated from the performance of individuals
735 of each species (Fig. 2). **Note the different y-axes scales when comparing with Fig. 2.**

736

737 **Fig. 5.** Conceptual models outlining example factors affecting predator-prey interactions
738 when considering (a) simple pairwise interaction between a single predator and prey (at fixed
739 density), (b) pairwise interactions between a single predator and prey at multiple prey
740 densities (functional response) (c) interaction between predator and prey as mediated by the
741 presence of an additional predator (multiple predator effect) and d.) interaction between
742 predator and prey at multiple prey densities as mediated by the presence of an additional
743 predator. **Bold solid lines represent interactions between predators and prey. Thin solid lines**
744 **represent factors that influence predator-prey interaction outcomes. Hashed lines represent**
745 **shifting predator-prey interaction outcomes in response to changes in prey densities. Note**
746 **that factor examples from model (a) (habitat domain, attack speed, naïveté) are still present in**
747 **models (b), (c) and (d) as are model (c) factors (facilitation, aggression, interference, distract/**
748 **court, naïveté) present in model (d). Models (c) and (d) are relevant at both the conspecific**
749 **and heterospecific multi-predator level and factors highlighted in (c) are contingent on**
750 **species traits.**







Initial prey density

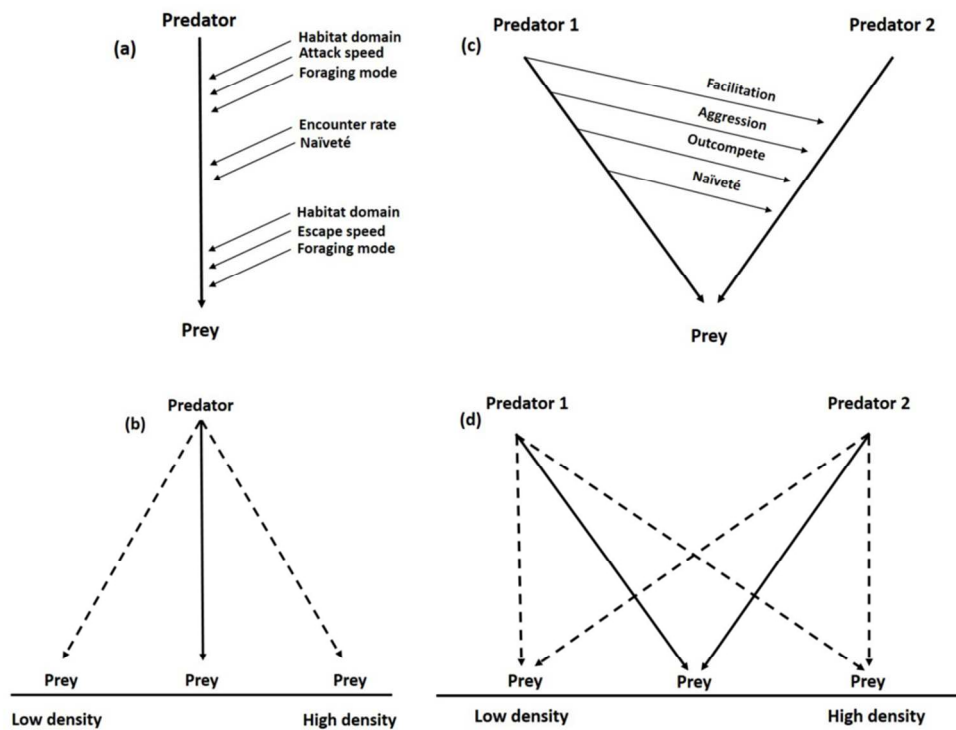


Fig. 5. Conceptual models outlining example factors affecting predator-prey interactions when considering (a) simple pairwise interaction between a single predator and prey (at fixed density), (b) pairwise interactions between a single predator and prey at multiple prey densities (functional response) (c) interaction between predator and prey as mediated by the presence of an additional predator (multiple predator effect) and d.) interaction between predator and prey at multiple prey densities as mediated by the presence of an additional predator. Bold solid lines represent interactions between predators and prey. Thin solid lines represent factors that influence predator-prey interaction outcomes. Hashed lines represent shifting predator-prey interaction outcomes in response to changes in prey densities. Note that factor examples from model (a) (habitat domain, attack speed, naïveté) are still present in models (b), (c) and (d) as are model (c) factors (facilitation, aggression, interference, distract/ court, naïveté) present in model (d). Models (c) and (d) are relevant at both the conspecific and heterospecific multi-predator level and factors highlighted in (c) are contingent on species traits.

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