Comparing the ecological consequences of globally invasive fishes versus their F1 hybrids in recreational fisheries

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2   **in recreational fisheries**

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16   **Abstract**

17   Recreational angling is a major introduction pathway for non-native fish into freshwaters,

18   where multiple non-native fishes are often released into waterbodies to diversify the angling

19   opportunities. When these non-native fishes are taxonomically similar, then there is concern

20   that their hybridisation will result in F1 generations comprising of novel phenotypes that

21   outperform their parental species, resulting in the impacts of these ecological engineering

22   species being accelerated. Across two water temperatures (18°C, 26°C), comparative functional

23   response analyses (CFR) quantified the consumption patterns of the globally invasive

24   freshwater fish carp *Cyprinus carpio* and goldfish *Carassius auratus*, plus their F1 hybrids,

25   before then testing differences in their specific growth rates (SGRs). In CFRs, carp consumed

26   significantly more prey at 18°C than the other fishes, and with no differences between any of

27   the fishes at 26°C. SGRs also did not differ substantially between the fishes at either

28   temperature. These results suggest that hybridisation between the high impacting parental

29   species did not produce novel phenotypes of high ecological performance that could accelerate

30   their ecological impacts in invaded ecosystems. Accordingly, the ecological risks of their use

31   in recreational angling remain an issue that is primarily associated with the parent populations,

32   and this can be reflected in their invasion management.

33

34   **Key words:** Common carp, goldfish, heterosis, comparative functional response.

Introduction

Recreational angling remains an important introduction pathway for alien freshwater fishes, where the aims of introductions include diversification of target species and to increase angler satisfaction with their catch related experiences (Carpio et al. 2019; Hirsch et al. 2021). However, these introduced fishes often develop invasive populations, with biological invasions recognised as a major driver of biodiversity loss (Britton 2023). Given fishes released for freshwater angling enhancement are generally large bodied and of high trophic position then their invasive populations can have strong top-down effects that disrupt ecosystem functioning (Eby et al. 2006; Britton 2023).

Introductions of freshwater fishes for angling has resulted in a relatively small number of non-native fishes developing invasive populations globally, such as the North American largemouth bass *Micropterus salmoides* (Pereira and Vitule 2019). Ecological concerns on their invasive populations relate to the interactions with native prey fishes, where their increased predation pressure can lead to substantial declines in prey population abundances (Gratwicke and Marshall 2001). However, species that concomitantly have both top-down and bottom-up effects (i.e. middle-out effects) are arguably even more damaging ecologically, given their impacts across multiple trophic levels are through both direct and indirect processes (Weber and Brown 2009; Vilizzi et al. 2015). Species such as common carp *Cyprinus carpio* and brown goldfish *Carassius auratus* have been used to enhance recreational fisheries across much of Europe, with releases of both species being commonplace in many waters, despite high ecological concerns through middle-put effects (Britton et al. 2010).

Where introductions of multiple non-native species are released into novel communities that are taxonomically similar, such as carp and goldfish, then this raises additional concerns over

60   their hybridisation, as this can alter the functional traits and ecological interactions of the

61   hybridised progeny versus their parental species (Horvick and Whitney 2014; Selz and

62   Seehausen 2019). This results from the generation of novel phenotypes arising from the

63   combining of alleles that have not segregated before in the same population (Selz and

64   Seehausen 2019). Although hybridisation results in a general surge of genetic variation from

65   the admixed divergent genomes and the acquisition of specific adaptive traits through lateral

66   gene transfer and introgression (Seehausen 2004), its effects on ecological performance are

67   unpredictable. For example, providing that the interactions between the alleles do not result in

68   intrinsic incompatibilities then transgressive segregation can result in the hybrids

69   outperforming parental taxa via adaptive diversification from the novel combination of parental

70   traits and/ or expression of new traits (Kagawa and Takimoto 2018; Nieto Feliner et al. 2020).

71   Conversely, introgression can result in the performance and fitness of hybrids being lower than

72   their parental species through outbreeding depression (Pregler et al. 2023). In hybrids of the

73   F1 generation, the general patterns tend to be an expression of high hybrid vigour through

74   heterosis, where their expressed traits are superior in performance to those of their parents

75   (Šimková et al. 2021; Dong et al. 2022).

76

77   Heterosis in the F1 generation is especially important to consider when the parental species are

78   both high impacting non-native species of global concern, such as in carp and goldfish. Both

79   species are highly invasive globally with foraging behaviours that drive dietary overlaps with

80   native fishes and strongly modify ecosystem functioning (Britton et al. 2010; Britton 2023). In

81   recreational freshwater fisheries where these fishes co-exist, fertile hybrids are often produced

82   (Hänfling et al., 2005). In the England and Wales, hybrids between these species are produced

83   in hatcheries and are then frequently released into lentic catch-and-release fisheries to enhance

84   angling performance. However, the ecological performance of these hybrids and the outcomes

85   for invaded ecosystems remain untested, including across a range of environmental contexts.

86   Accordingly, to provide the evidence base for the risk analysis of hybrids arising from the

87   introgression of genes between taxonomically similar species, we use carp and goldfish here

88   as model species to experimentally test their foraging behaviours (as comparative functional

89   responses, CFR) and growth performance (as specific growth rate, SGR) versus their first-

90   generation (F1) hybrids in contexts of two contrasting temperatures. We predict that the

91   ecological performance of the F1 fish will be superior to both non-hybrid carp and goldfish,

92   with this heterosis being independent of temperature.

93

94   **Materials and Methods**

95   *Experimental Fish*

96   The experimental carp, goldfish and F1 hybrids were from the same hatchery in Southern

97   England, where the fish were produced from the same parental lines, and with experimental

98   fish exposed to the identical rearing conditions. Both parental species have thermal optima

99   >20°C and critical thermal maxima >30°C (Britton et al. 2010; Ferreira and Farrell 2014). All

100   fish were age 0+ years and of initial body mass 2.0 to 6.0 g on arrival in the laboratory, where

101   they were then individually tagged (7 mm passive integrated transponder tag), and acclimated

102   for 20 days (18°C; 16:8 h light: dark cycle). Outside of experiments, the fish were fed a

103   maintenance diet of crushed pelletized fishmeal.

104

105   *Comparative Functional Response Experiments*

106   For CFR experiments, individual fish were exposed to Chironomid larvae as prey resources in

107   10 L tanks at 18°C following a 24-hour starvation period and a 4 hour acclimation period to

108   their experimental tank. Food densities were 4, 8, 16, 32 and 64 larvae (and 128 for 26°C

109   experiment). Food exposure was for one hour, after which the number of larvae consumed was

110   quantified, with three replicates per prey density per species. When all replicates were

111   completed, the water temperatures were increased to 26°C over 8 days and, following a 5-day

112   acclimation period, the CFR experimental process was repeated. With the fish being PIT

113   tagged, no individual fish was used more than twice in CFRs, with a minimum of five days

114   between use.

115

116   Values of the CFR parameters attack rate (a) and handling time (h) were calculated for each

117   species and temperature using maximum likelihood estimation (MLE) in the Random Predator

118   Equation [13], completed in the R package '*Frair*' (Pritchard et al. 2017). The equation

119   assumes a Type II functional response and the non-replacement of prey, where $N_e = N_0$ (1 −

120   exp($a(N_e h$-T))), with N_e = number of prey eaten, N_0 = initial density of prey, a = attack rate, h

121   = handling time and T = total time. Analyses also provided the significance of differences in a

122   and h between the species (Pritchard et al. 2017; Cuthbert et al. 2019). To visualise uncertainty,

123   2000 non-parametric bootstraps enabled empirical 95% confidence intervals to be fitted around

124   the functional responses, which were used to provide CFR plots between the parental species/

125   F1s, and water temperature.

126

127   *Specific Growth Rate Experiments*

128   Following completion of CFRs, water temperatures were returned to 18°C and the fish

129   acclimated for 10 days. The growth performance of the fishes was then tested in co-habitation

130   experiments completed in tanks of 25L. Experimental treatments used controls (species/hybrid

131   in allopatry; n=6) and treatments (combinations of two species in sympatry; n=3+3; and all

132   species in sympatry; n=2+2+2), each replicated three times. Each species per experimental

133   treatment was batch weighed (to 0.01 g) before released into their tanks, where they were held

134   at 18°C and fed a daily food ration (crushed pelletised fishmeal) at a mean of 2% starting body

135   mass. After 15 days, the fish were removed from their tanks, re-weighed, returned to their tanks

136   and the water temperature increased to 26°C over five days before the experimental process

137   was repeated. For each species/hybrid, control and treatment, and water temperature, SGR was

138   determined from ([(lnW_{t+1}) lnW_t)⁄$_t$]/n) x 100 (Equation 1), where W_t = total starting weight of

139   the species in the tank, W_{t+1} = total finishing weight, n = number of fish, and $_t$ = number of

140   days between W_t and W_{t+1}. A generalised linear model (GLM) tested the differences in SGR

141   between treatments for each species, where SGR was the dependent variable, treatment was

142   the independent variable, and total starting mass of fish per replicate used as an initial covariate

143   and retained in final models when its effect was significant. Model outputs were the overall

144   significance of the model and the mean SGR values ($\pm$ 95 % CI) according to species and

145   treatment. All analyses were performed in R (version 4.2.3; R Core Team 2023).

146

147   **Results**

148   *Comparative functional responses*

149   The functional responses of all species at all temperatures were Type II and significant (Fig.

150   1). The 95% confidence limits of consumption rates at 18°C suggested carp had significantly

151   higher rates than goldfish and hybrids, which had similar values to each other (Fig. 1). All

152   species revealed higher consumption rates at 26°C versus 18°C, with no significant differences

153   between them. Handling times and attack rates of carp and hybrids were also significantly

154   higher at 26°C versus 18°C, but not for goldfish. There were also significant differences in

155   these metrics between the species at both temperatures, with the attack rate of goldfish being

156   lower than the other fishes at both temperatures (Table 1).
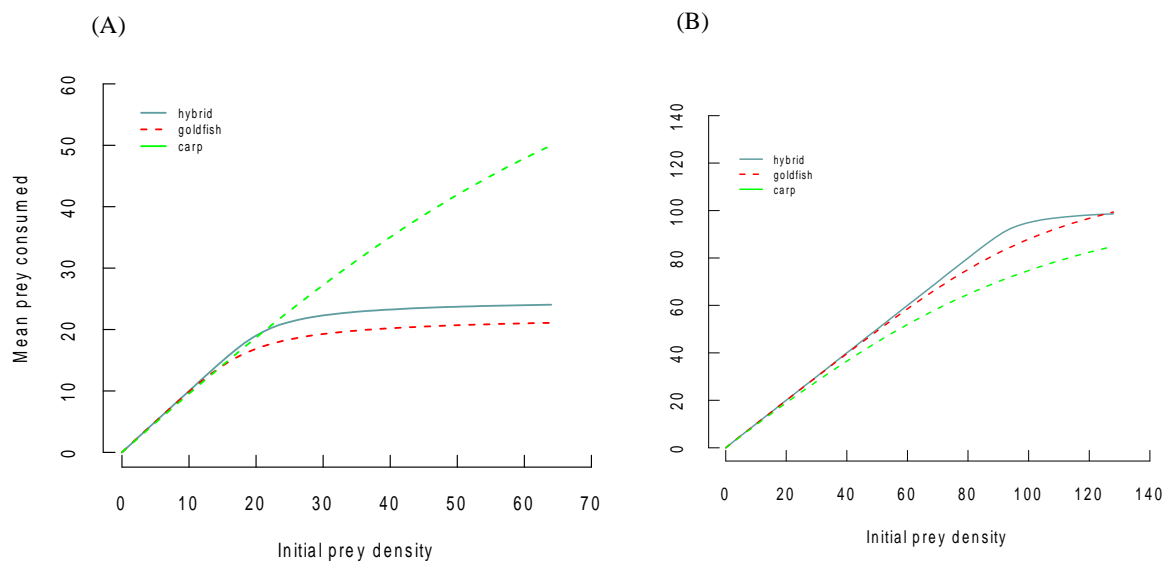
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Figure 1. Comparative functional response curves for carp (green), goldfish (red) and their F1 hybrids (blue) at 18ºC (left) and 26ºC (right). Shaded areas around the curves represent 95 % confidence intervals generated by boot-strapping. Note differences in values on both axes between the plots.

Table 1. (A) First order linear coefficient results from logistic regressions for the predator and prey combinations. All values indicate a Type II functional response. (B) Parameters of the comparative functional responses, with statistically significant differences in the parameters between species (α = 0.05) in bold. a = attack rate, h = handling time. Z and P values are statistical outputs from regression that indicate whether a and h differ significantly between the comparator species.

(A)

Temperature	Species	Linear coefficient
18ºC	F1	-0.09
	Goldfish	-0.06
	Carp	-0.03
26 ºC	F1	-0.06
	Goldfish	-0.05
	Carp	-0.02

(B)

18 ºC	F1/Goldfish	F1/Carp	Goldfish/Carp
a	12.72/7.67	12.72/3.43	7.67/3.43
Z	-1.15	2.34	2.11
P	0.25	**0.02**	**0.04**

h	0.04/0.05	0.04/0.01	0.05/0.01
Z	1.10	8.11	8.77
P	0.27	**< 0.001**	**< 0.001**
26 °C			
a	42.18/6.89	42.18/3.46	6.89/3.46
Z	-8.55e7	-19.59e7	3.28
P	**< 0.001**	**< 0.001**	**0.001**
h	0.010/0.008	0.010/0.008	0.008/0.008
Z	-3.39	-12.54	-0.19
P	**< 0.001**	**< 0.001**	0.87

158

Specific Growth Rates

Increased fish mass occurred in all SGR treatments at both temperatures (Fig. 2). The effect of treatment on SGR was significant for each species (GLM: carp: Wald χ^2 = 452.39, df = 22, p < 0.0001; goldfish: Wald χ^2 = 130.91, df = 23, p < 0.001; hybrid: Wald χ^2 = 128.92, df = 22, p = 0.002), where starting mass as a covariate was significant for carp (p = 0.01) and hybrids (p = 0.03) (retained in final models), but not goldfish (p = 0.16; removed from final model). At both temperatures and all species, SGRs in the allopatric controls were generally lower than in the sympatric treatments, but with the effect of temperature on SGR being minor; where elevated SGRs were apparent then this was at 18°C rather than 26°C (Fig. 2).
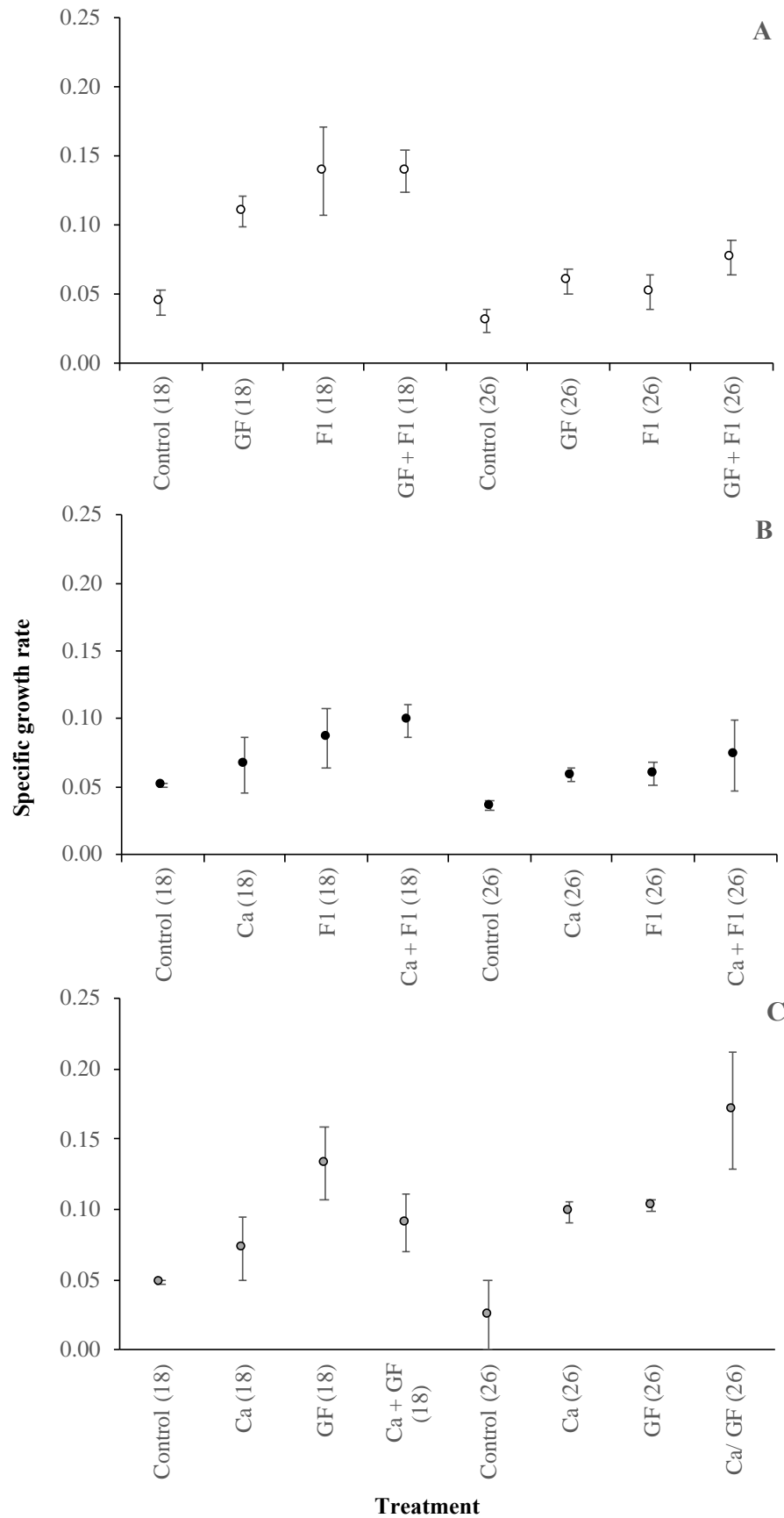
168

Figure 2. Mean specific growth rates of cohabitation experiments for (A) carp ('Ca'), (B) goldfish ('GF'), and (C) their F1 hybrids ('F1') at 18ºC ('18') and 26ºC ('26').

169

170   **Discussion**

171   There was no evidence to suggest heterosis was apparent in the performance of the F1 hybrids

172   across both experiments, with their performance in CFRs being weak versus carp at 18°C and

173   differences in consumption rates not being significant at 26°C. Their CFR metrics were

174   significantly higher at the elevated water temperature, but this was also apparent in goldfish,

175   with the maximum consumption rates of all of the fishes being similar at this elevated

176   temperature. The SGR experiment also did not indicate any substantially enhanced

177   performance in the F1 fish versus the other fishes, nor was there a strong effect of temperature

178   on SGR, most likely due to the feed rations being maintained at a constant level across both

179   temperatures.

180

181   Heterosis is a common outcome of hybridisation in early generations, as observed in invasive

182   plants (Hahn and Riesberg 2017), where admixture can increase performance across multiple

183   generations (Li and van Kleunen 2018), and in fishes, where similar patterns of heterosis were

184   detected in crosses of three-spine stickleback *Gasterosteus aculeatus* from different lake

185   populations (Thompson and Schluter 2022). Interspecific hybridisation is common in

186   freshwater fishes (Bolnick 2009), with the few performance studies on inter-specific

187   hybridisation involving invasive fishes in the wild being equivocal in their results. For

188   example, low larval mortality in the F1 generation of non-native red shiner *Cyprinella lutrensis*

189   and native blacktail shiner *Cyprinella venusta stigmatura* was suggested as being evidence of

190   heterosis (Blum et al. 2010). However, in early generation hybrids of Asian bighead carp

191   species (*Hypophthalmichthys* spp.), nutritional performance was intermediate between the

192   parental lines, with advanced generations then becoming increasingly similar to parental

193   species (Liss et al. 2016). Heterosis in the performance of F1 generations of fishes mixed in

194   aquaculture is more evident (Šimková et al. 2022) where advantages can be through decreased

195   viral susceptibility and faster growth rates (Bryden et al. 2004). We nevertheless argue that our

196   results, generated using hybrids and parental species in controlled conditions, represent novel

197   outcomes in that we could find no similar studies comparing the ecological performance of

198   such high impacting alien species versus their F1 generation.

199

200   This absence of transgressive segregation and heterosis in the hybrids of these globally invasive

201   pest fishes is then important for their risk screening within management frameworks regulating

202   the release of non-native fishes in recreational fisheries (Vilizzi et al. 2019; Copp et al. 2021).

203   Both parental species have been assessed in risk screening as being of high ecological risk

204   where, for example, they were assessed as representing the highest ecological risk of all non-

205   native fishes introduced into freshwaters in England (Britton et al. 2010). Consequently, there

206   was high concern that their hybrids would be composed of novel phenotypes that result from

207   combinations of alleles that were previously always segregated (Selz and Seehausen, 2019).

208   Given there was no evidence that the F1 generation had superior performance in their foraging

209   and growth rate, especially at 18°C where carp were superior, but also at 26°C where

210   differences between the species were generally not significant, then this represents an

211   important outcome for their scientifically informed management. Accordingly, the ecological

212   concern with introductions of these species remain with their parental populations and so where

213   risk-based invasion management programmes are implemented to minimise the impact and

214   dispersal of these species, they do not need to account for the possibility of these species

215   producing hybrids that will accelerate their ecological impacts. This means that where invasive

216   populations develop from fishes initially introduced for recreational angling then control and

217   containment programmes could be effective in reducing the impacts of their populations

218   (Britton et al. 2023), with the evidence base for such management decisions enhanced by the

219   results presented here.

220

221   **References**

222   Blum MJ, Walters DM, Burkhead NM, Freeman BJ, Porter BA (2010) Reproductive isolation
223   and the expansion of an invasive hybrid swarm. Biological Invasions 12: 2825–2836.
224
225   Bolnick DI (2009) Hybridization and speciation in centrarchids. In: Cooke SJ, Philipp D (eds)
226   Centrarchid fishes: diversity, biology, and conservation: 39–69.
227
228   Britton JR (2023) Contemporary perspectives on the ecological impacts of invasive freshwater
229   fishes. Journal of Fish Biology 103: 752–764.
230
231   Britton JR, Cucherousset J, Davies GD, Godard MJ, Copp GH (2010) Non-native fishes and
232   climate change: predicting species responses to warming temperatures in a temperate
233   region. Freshwater Biology 55: 1130–1141.
234
235   Britton JR, Lynch AJ, Bardal H, Bradbeer SJ, Coetzee JA, Coughlan NE, Dalu T, Tricarico E,
236   Gallardo B, Lintermans M, Lucy F, Olden J, Pritchard E (2023) Preventing and controlling
237   non-native species invasions to bend the curve of global freshwater biodiversity
238   loss. Environmental Reviews 31: 310–326.
239
240   Bryden CA, Heath JW, Heath DD (2004) Performance and heterosis in farmed and wild
241   Chinook   salmon   (*Oncorhynchus   tshawytscha*)   hybrid   and   purebred
242   crosses. Aquaculture 235: 249–261.
243
244   Carpio AJ, De Miguel RJ, Oteros J, Hillström L, Tortosa FS (2019) Angling as a source of
245   non-native freshwater fish: a European review. Biological Invasions 21: 3233–3248.
246
247   Copp GH, Vilizzi L, Wei H, Li S, Piria M, Al-Faisal AJ, Almeida D, Atique U, Al-Wazzan Z,
248   Bakiu R, Bašić T (2021) Speaking their language–development of a multilingual decision-
249   support   tool   for   communicating   invasive   species   risks   to   decision   makers   and
250   stakeholders. Environmental Modelling & Software 135: 104900.
251
252   Cuthbert RN, Dickey JWE, Coughlan NE, Joyce PWS, Dick JTA (2019) The Functional
253   Response Ratio (FRR): Advancing comparative metrics for predicting the ecological
254   impacts of invasive alien species. Biological Invasions 21: 2543–2547.
255
256   Dong X, Yan Y, Wang Y, Ren L, Zhang C, Tao M, Luo K, Zeng Y, Liu S (2022) Network-
257   based quantitative proteomics identified significant proteins associated with growth
258   heterosis in triploid fish. Aquaculture Research 53: 5063–5074.
259
260   Ferreira EO, Anttila K, Farrell AP (2014) Thermal optima and tolerance in the eurythermic
261   goldfish (*Carassius auratus*): relationships between whole-animal aerobic capacity and
262   maximum heart rate. Physiological and Biochemical Zoology 87: 599–611.
263

264　Gratwicke B, Marshall BE (2001) The relationship between the exotic predators *Microopterus*
265　　　*salmoides* and *Serranochromis robustus* and native stream fishes in Zimbabwe. Journal of
266　　　Fish Biology 58: 68–75.
267
268　Hahn MA, Rieseberg LH (2017) Genetic admixture and heterosis may enhance the
269　　　invasiveness of common ragweed. Evolutionary Applications 10: 241–250.
270
271　Hänfling B, Bolton P, Harley M, Carvalho GR (2005) A molecular approach to detect
272　　　hybridisation between crucian carp (*Carassius carassius*) and non-indigenous carp species
273　　　(*Carassius* spp. and *Cyprinus carpio*). Freshwater Biology 50: 403–417.
274
275　Hirsch PE, N'Guyen A, Burkhardt-Holm P (2021) Hobbyists acting simultaneously as anglers
276　　　and aquarists: Novel pathways for non-native fish and impacts on native fish. Aquatic
277　　　Conservation: Marine and Freshwater Ecosystems 31: 1285–1296.
278
279　Hovick SM, Whitney KD (2014) Hybridisation is associated with increased fecundity and size
280　　　in invasive taxa: meta-analytic support for the hybridisation-invasion hypothesis. Ecology
281　　　Letters 17: 1464–1477.
282
283　Kagawa K, Takimoto G (2018) Hybridization can promote adaptive radiation by means of
284　　　transgressive segregation. Ecology Letters 21: 264–274.
285
286　Li Y, Stift M, van Kleunen M (2018) Admixture increases performance of an invasive plant
287　　　beyond first-generation heterosis. Journal of Ecology 106: 1595–1606.
288
289　Liss SA, Lamer JT, Sass GG, Suski CD (2016) Physiological consequences of hybridization:
290　　　early generation backcrossing decreases performance in invasive bigheaded carps. Journal
291　　　of Freshwater Ecology 31: 543–554.
292
293　Nieto Feliner G, Casacuberta J, Wendel JF (2020) Genomics of evolutionary novelty in hybrids
294　　　and polyploids. Frontiers in Genetics 11: 792.
295
296　Pereira FW, Vitule JRS (2019) The largemouth bass *Micropterus salmoides* (Lacepède, 1802):
297　　　impacts of a powerful freshwater fish predator outside of its native range. Reviews in Fish
298　　　Biology and Fisheries 29: 639–652.
299
300　Pregler KC, Obedzinski M, Gilbert-Horvath EA, White B, Carlson SM, Garza JC (2023)
301　　　Assisted gene flow from outcrossing shows the potential for genetic rescue in an endangered
302　　　salmon population. Conservation Letters 16: e12934.
303
304　Pritchard DW, Paterson RA, Bovy HC, Barrios-O'Neill D (2017) Frair: an R package for fitting
305　　　and comparing consumer functional responses. Methods in Ecology and Evolution 8: 1528-
306　　　1534.
307
308　R Core Team 2023. R: A language and environment for statistical computing. R Foundation
309　　　for Statistical Computing, Vienna, Austria.
310
311　Seehausen O (2004) Hybridization and adaptive radiation. Trends in Ecology and Evolution
312　　　19: 198–207.
313

314 Selz OM, Seehausen O (2019) Interspecific hybridization can generate functional novelty in
315   cichlid fish. Proceedings of the Royal Society B 286: 20191621.
316
317 Šimková A, Civáňová K, Vetešník L (2022) Heterosis versus breakdown in fish hybrids
318   revealed by one-parental species-associated viral infection. Aquaculture 546: 737406.
319
320 Šimková A, Janáč M, Hyršl P, Krasnovyd V, Vetešník L (2021) Vigour-related traits and
321   immunity in hybrids of evolutionary divergent cyprinoid species: advantages of hybrid
322   heterosis? Journal of Fish Biology 98: 1155–1171.
323
324 Thompson KA, Schluter D (2022) Heterosis counteracts hybrid breakdown to forestall
325   speciation by parallel natural selection. Proceedings of the Royal Society B 289: 20220422.
326
327 Vilizzi L, Copp GH, Adamovich B, Almeida D, Chan J, Davison, PI, Dembski S, Ekmekçi FG,
328   Ferincz Á, Forneck SC, Hill JE (2019) A global review and meta-analysis of applications
329   of the freshwater Fish Invasiveness Screening Kit. Reviews in Fish Biology and Fisheries
330   29: 529–568.
331
332 Vilizzi L, Tarkan AS, Copp GH (2015) Experimental evidence from causal criteria analysis for
333   the effects of common carp *Cyprinus carpio* on freshwater ecosystems: a global
334   perspective. Reviews in Fish Biology and Fisheries 23: 253–290.
335
336 Weber, M.J. and Brown, M.L. 2009. Effects of common carp on aquatic ecosystems 80 years
337   after "carp as a dominant": ecological insights for fisheries management. Rev. Fish. Sci.
338   17, 524-537.