The Scientific Naturalist

Co-occurrence of large branchiopods and killifish in African savannah ponds

Tom Pinceel [1,2,7] Birgit Vanden Berghen,[1,3]
Falko Buschke,[1,2] Aline Waterkeyn,[1] Ivan da Costa
Nerantzoulis,[4] Mery Rodrigues,[4] Eli Thoré,[1]
Rick Burrough,[5] Ellery Worth[5] and
Luc Brendonck[1,6]

[1]Animal Ecology, Global Change and Sustainable Development, KU Leuven, Leuven, Belgium.
[2]Centre for Environmental Management, University of the Free State, P.O. Box 339, Bloemfontein, 9300 South Africa.
[3]Society-Environment Interactions, Division of Bio-economics, KU Leuven, Leuven, Belgium.
[4]Department of Biological Sciences, Eduardo Mondlane University, P.O. Box 257, Maputo, Mozambique.
[5]Karingani Game Reserve, Massingir, Mozambique.
[6]Water Research Group, Unit for Environmental Sciences and Management, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom, South Africa.
[7]E-mail: tom.pinceel@kuleuven.be

It is conventionally understood that large branchiopod crustaceans are limited to temporary waters because they are sensitive to fish predation in permanent habitats (Kerfoot and Lynch 1987, Brendonck et al. 2008). However, our field observations in February and March 2018, 2019, and 2020 showed that all major groups of large branchiopods (i.e., fairy, clam, and tadpole shrimps) co-occur with killifish in temporary ponds across the savannah of southern Mozambique (Fig. 1A–D; Appendix S1: Fig. S1). Remarkably, this region, part of the international Maputo-Albany-Pondoland Biodiversity Hotspot (Brooks et al. 2006), appears to also be a center for exceptionally high large branchiopod diversity, with >15 species and several recently discovered species awaiting formal description. Some of the ponds were inhabited by at least six sympatric species during a single inundation during our visits (L. Brendonck, *personal observation*). To further explore predatory interactions between killifish and the two most common branchiopod groups in the region, fairy shrimps (Anostraca) and clam shrimps (Spinicaudata), we carried out a feeding experiment in Karingani Game Reserve (KGR; Maputo Province Mozambique).

Branchiopod crustaceans are an evolutionary ancient group, which adapted to life in temporary waters after the rise of fish predators during the Mesozoic (Kerfoot and Lynch 1987). These temporary habitats are usually rain fed, vary widely in size from just a couple of square meters to several square kilometers in surface area and are inundated during the rainy season for a few days to several months. Ponds remain dry for the rest of the year, but branchiopods have evolved the ability to produce drought-resistant eggs that lay dormant in the sediment until the next wet season (Brendonck et al. 2017). Most fish species are unable to survive the dry phases and are restricted to permanent waters. However, similar to large branchiopods, several killifish genera including *Austrofundulus* (South America), *Aphyosemion*, and *Nothobranchius* (Africa) evolved the ability to produce drought-resistant eggs (Fishbase; Froese and Pauly 2000). At the onset of rain, fairy shrimps, clam shrimps, and killifish enter a race against time to mature and reproduce before the next dry period (Brendonck et al. 2017). *Nothobranchius* killifish from Zimbabwe and Mozambique have particularly fast life cycles, with maturation in three to six weeks and a maximum average lifespan of around 4–12 months to maximize their chances of successful reproduction (Watters 2009, Polačik et al. 2016).

After observing sympatric populations of killifish, fairy, clam, and tadpole shrimps during consecutive wet seasons (2018 and 2019), we returned in February–March 2020 to perform feeding trials in the field laboratory at KGR. We subjected field-collected populations of a common species of fairy shrimp (*Streptocephalus bidentatus* Hamer and Appleton 1993; Fig. 1B) and clam shrimp (*Cyzicus* sp.; Fig. 1C) to predation by the co-occurring killifish (*Nothobranchius furzeri* Jubb 1971; Fig. 1D). Tadpole shrimps (cf. *Triops granarius* Lucas 1864) were not included in feeding trials because they also prey on large branchiopods (Brendonck et al. 2017), so including them in feeding trials would have confounded the effects of killifish predation. The overall goal was to assess killifish predation rates and prey preference (fairy vs. clam shrimps) and to distinguish further whether predation is affected by predator body size or the sex and body size of prey.

After approximately four weeks of inundation, we sampled both killifish predators and large branchiopod
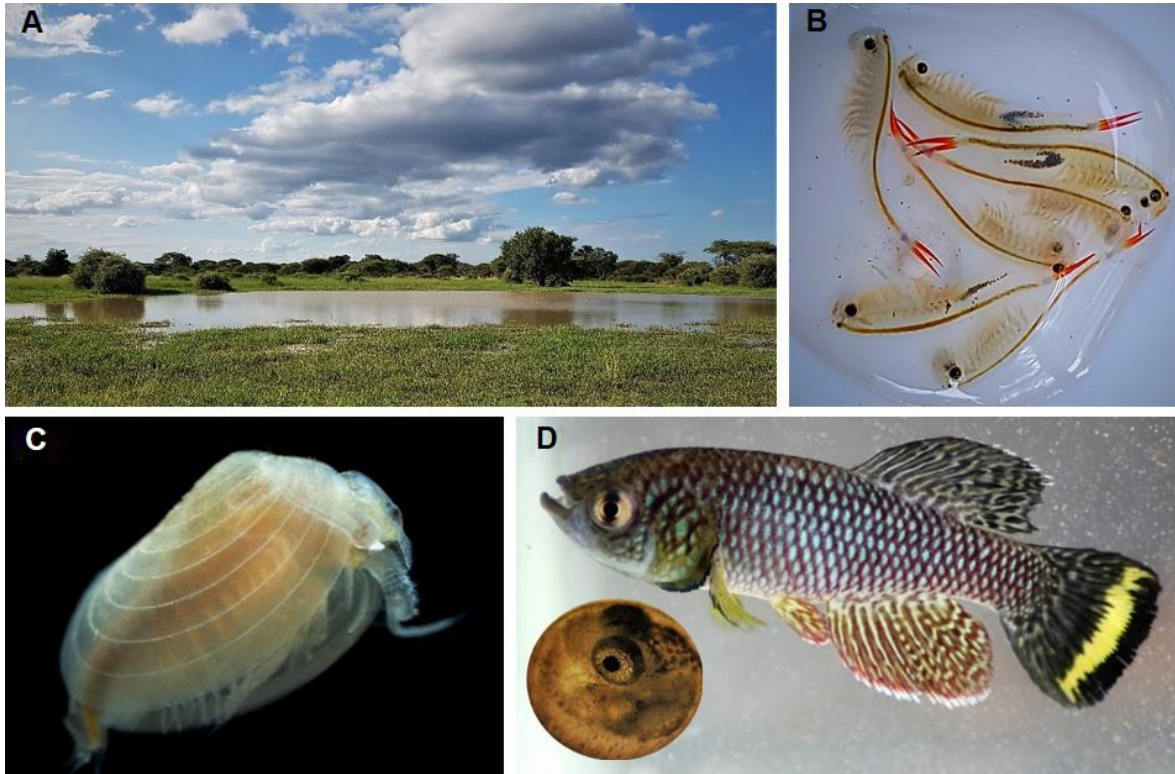
Fig. 1. During the rainy season, (A) temporary ponds tend to fill across savannah areas in Southern Africa. Although many of these systems typically only hold water for a number of weeks (photo by T. Pinceel), they are home to a unique aquatic flora and fauna including (B) fairy shrimps (photo by R. Burrough; size: 19–25 mm), (C) clam shrimps (photo by Jean-François Cart; size: 7 mm), and (D) *Nothobranchius* killifish (photo by A. Waterkeyn; size 53 mm). These organisms mature rapidly and produce drought-resistant stages (killifish egg shown bottom left in panel D; size: 1.2 mm) that bridge the dry phase in a state of dormancy in the sediment.

prey from three ponds using a 500-µm D-frame kick-net until a total of ±120 killifish, ±400 fairy, and ±250 clam shrimps were collected. Furthermore, we collected ±1,000 mL of wet sediment from five random points in each of the sampled ponds using a plastic measuring beaker. All sediment samples were mixed and used as a substrate for the experimental tanks. Standard water quality variables were measured in situ using a handheld multi-meter (Hanna Instruments HI9828; Temse, Belgium; pH and conductivity) and fluorometer (Aquafluor, Turner Designs Model 8000-010; San Jose, CA, USA; turbidity and chlorophyll *a*). Water variables were comparable among ponds (pH 6.14–6.47, conductivity 45–67 µs/cm, turbidity 207–570 NTU, and chlorophyll *a* 198–293 µg/mL). Since the ponds are rain-fed, we captured rainwater (pH 6.5, conductivity 0 µs/cm) during preceding weeks to fill the experimental tanks.

We performed two separate feeding trials, one with small (13–25 mm, mean 19 mm, SD 2.8) immature and another with large (28–54 mm, mean 39 mm, SD 6.65) sexually mature killifish. While it would have been ideal to include a third size cohort of recently hatched killifish (e.g., 8–13 mm), fish of this size were no longer present in any of the ponds by the time of sampling. We set up 10 opaque cylindrical 10-L tanks (radius 16.5 cm; height 12 cm) and filled each with 750 mL of natural pond sediment and 8 L of aerated rainwater to approximate natural pond conditions. Fairy shrimps and clam shrimps, randomly selected from the naturally occurring body-size distribution, were simultaneously introduced as prey in both trials. Prey were added first and allowed to acclimate for approximately 1 h before killifish were added (Fig. 2). To each of the tanks, 5 small killifish, 20 fairy shrimp, and 11 clam shrimps were added during the first trial and 4 large killifish, 15 fairy shrimp, and 10 clam shrimps in the second trial. All prey were measured to the nearest millimeter before being introduced, and the sex of mature fairy shrimps was recorded. A total of 350 fairy shrimps were used across both trials of which 41 were mature males and 77 mature females, all with viable eggs in their brood pouch. Predation trials ran for 24 h under a natural light/dark cycle, after which all
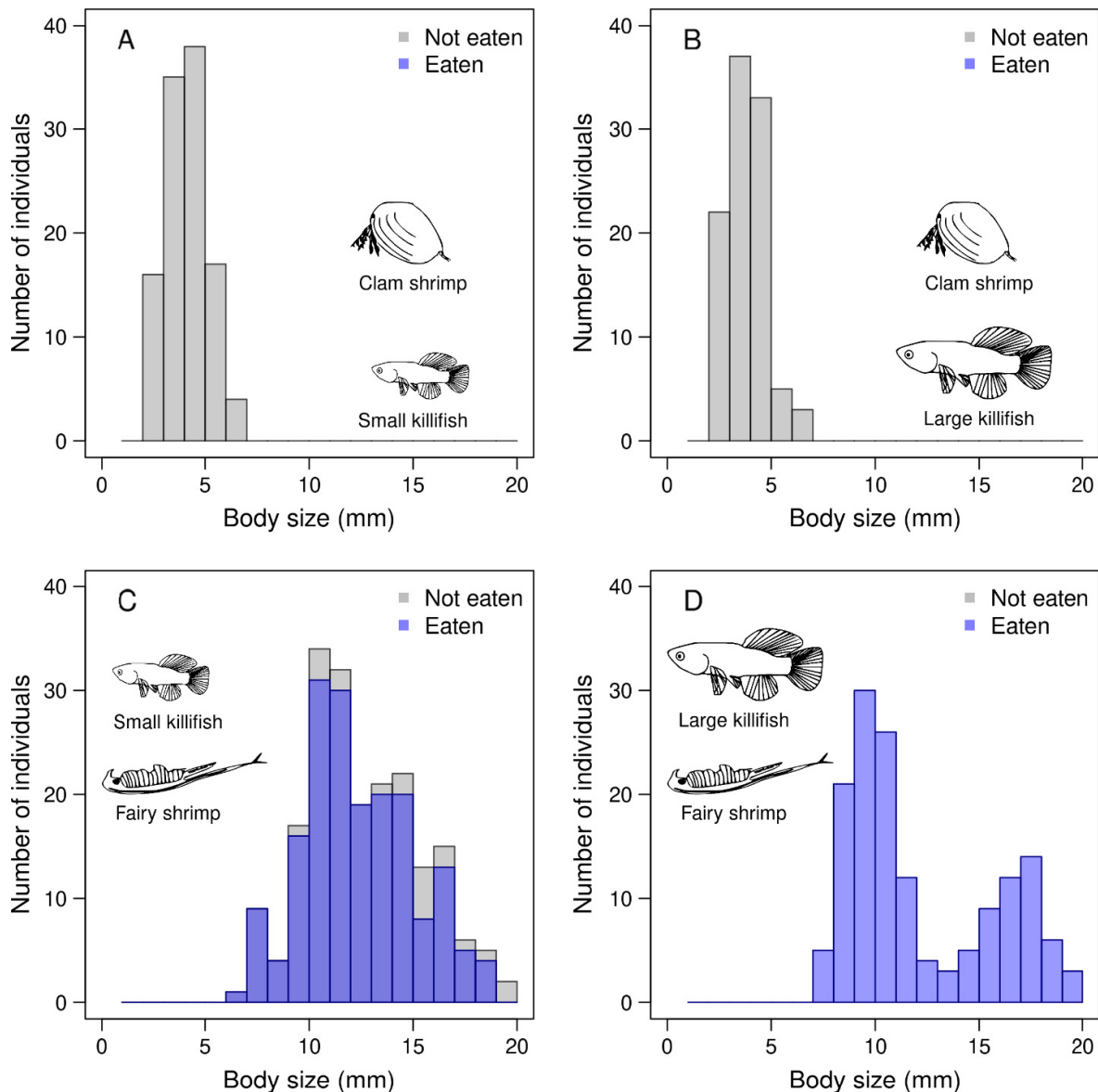
FIG. 2. We conducted two predation trials (Trial 1, A and C; Trial 2, B and D) to assess predation rates and prey preference (fairy vs. clam shrimps) of killifish. We simultaneously subjected field-collected populations of the commonly occurring fairy shrimp *Streptocephalus bidentatus* and the clam shrimp *Cyzicus* sp. to predation by the co-occurring killifish *Nothobranchius furzeri*. Prey individuals were randomly selected from the naturally occurring body-size distribution. The bin width of all histograms was chosen at 1 mm, since all specimens were measured up to the nearest 1 mm.

remaining specimens in each tank were caught, counted, sexed, and measured again. This research was approved by ANAC (Administracao Nacional das Areas de Concervacao; Ref. nr. 246/ANAC/MITADER/2018-2020) and conducted in accordance with their guidelines and ethical standards.

One of our goals was to investigate if killifish prefer fairy shrimps over clam shrimps as prey. Clam shrimps are typically cited to be much less prone to invertebrate predation than fairy shrimps (Jocque et al. 2007, Brendonck et al. 2017) and we wanted to assess if the same holds true for fish predation. Strikingly, not a single clam shrimp was eaten during our experiment by small or large killifish, even though clam shrimps of only 3 mm were housed in tanks with much larger killifish of up to 54 mm (Fig. 2A, B) during the trials. This observation can be explained in several ways. First, clam shrimps may be unsuitable as prey because of their rigid

bivalved carapace (Brendonck et al. 2008). Even though the clam shrimps were overall smaller than the fairy shrimps, their carapace would require that fish ingest them in one bite, so killifish may have been gape-limited. Second, clam shrimps could have avoided predation through their benthic and often burrowing lifestyle (Brendonck 1999). Such behavior was observed both in the field and during our experiment, with the majority of clam shrimps being covered by a layer of sediment at the end of each trial.

Our trials showed a strong predatory impact of killifish on fairy shrimps. Small killifish ate 90% of the offered fairy shrimps (Fig. 2C), while large killifish consumed all fairy shrimp prey (Fig. 2D). We tested if small killifish favored smaller fairy shrimps using a generalized linear mixed effect model (GLMM) with a binomial error distribution. Fairy shrimp body size was included as a continuous fixed factor and the experimental tanks as a random effect (*lme4* package in R version 4.0.0: R Development Core Team). This confirmed that small killifish were statistically more likely to eat smaller fairy shrimps ($\chi^2 = 5.187$; $P = 0.023$). In order to further explore size-mediated predation risk, we calculated mean predator–prey size ratios for all of the experimental tanks in our first predation trial and related them to predation risk (Appendix S2: Fig. S1). This exploratory analysis supported the view that 100% prey consumption only occurs when the average size-ratio between predator and prey exceeds 1.5 and is consistent with the notion that fairy shrimps can (partially) withstand predation while killifish are still small. We used a similar GLMM as described above on a subset of mature fairy shrimps that could reliably be sexed ($n = 64$), but found no evidence of sex-specific predation ($\chi^2 = 0.714$; $P = 0.398$).

Our natural history observations illustrate long-term co-occurrence of fish and large branchiopod crustaceans, something that is commonly cited as highly unlikely (e.g., Kerfoot and Lynch 1987, Bohonak and Whiteman 1999, Brendonck et al. 2008). Such co-occurrence is probably only possible because killifish have evolved a similar life-history to large branchiopods and active populations need to hatch again from dormant eggs during every wet season (Watters 2009, Pinceel et al. 2015). Newly hatched fish larvae are typically small (±8 mm) and gape-limited toward some invertebrate prey, whereas fish in permanent ponds have overlapping generations and can continuously feed on large branchiopods until prey populations are depleted.

Fairy shrimps from short-lived habitats, such as the studied ponds, can mature, reproduce, and reach a body size of up to 20 mm in less than one week (Vanschoenwinkel et al. 2011, Brendonck et al. 2017). Since, at this stage of the inundation, killifish are typically only around 8–10 mm, predator–prey size ratios would be much smaller (˜0.4–0.5) than those associated with complete predation (˜1.5) in our trials and predation rates would be correspondingly low (Appendix S2: Fig. S1). Therefore, some fairy shrimps could produce dormant eggs before predation becomes too intense, as has been described elsewhere for fairy shrimps that co-occur with predators such as salamanders (Bohonak and Whiteman 1999, Messerman et al. 2021). Selection apparently mediates the "three-way race" between the life cycles of fairy shrimps, killifish and the drying of the ponds by driving rapid growth and maturation of the shrimps so that they are not eaten before killifish grow larger and have almost 100% success preying on the shrimps and also before the ponds dry up. The fairy shrimps that cannot reproduce fast enough might withstand predation temporarily by finding refuge in highly turbid waters or among submerged vegetation. Such behavioral adaptations could not be tested in our feeding trials, but are testable in a subsequent study linking community composition to the environmental characteristic of ponds.

Clam shrimps tend to occur later than fairy shrimps in the sequence of ecological succession (Jocque et al. 2007, Brendonck et al. 2017), and our results show that they are less susceptible to killifish predation, at least when more vulnerable fairy shrimp prey are present. This raises an intriguing hypothesis that selective predation by killifish refines the sequence of ecological succession by limiting the temporal overlap, and hence the interspecific competition, between fairy and clam shrimps. In addition to the temporal changes in abiotic conditions (e.g., water quality), resource availability (e.g., phytoplankton production and composition), and interspecific competition (Jocque et al. 2007, Brendonck et al. 2017), killifish predation might add an extra niche axis to the succession sequence.

The evolution of dormant eggs in killifish might have modulated the interplay of selective predation and ecological succession to the extent that it affects species diversity. Although this hypothesis remains speculative, our observations inform the broader ecological and evolutionary perspective of the processes that maintain diversity in temporary ponds. This will fuel subsequent work on the structure of temporary pond communities, which places killifish predation in the context of the environmental conditions and the spatial configuration of ponds in the landscape. Moreover, future studies should explore how the presence of killifish might affect the ecological functioning of these habitats, in particular the suppression of algal blooms, which is often mediated by large branchiopod grazing (Horváth et al. 2013, Yang and Park 2017). Ultimately, our observations should encourage aquatic ecologists to reconsider the generalization that large branchiopod crustaceans cannot co-occur with fish. Instead, the interplay between selective predation and ecological succession may sometimes actually be a driver of branchiopod species diversity.

Literature Cited

Bohonak, A. J., and H. H. Whiteman. 1999. Dispersal of the fairy shrimp *Branchinecta coloradensis* (Anostraca): Effects of hydroperiod and salamanders. Limnology and Oceanography 44:487–493.

Brendonck, L. 1999. Conchostraca. Freshwater invertebrates of Southern Africa. Crustacea I: Notostraca, Anostraca, Conchostraca and Cladocera. Report TT 121/00. Water Research Commission, Pretoria, South Africa.

Brendonck, L., T. Pinceel, and R. Ortells. 2017. Dormancy and dispersal as mediators of zooplankton population and community dynamics along a hydrological disturbance gradient in inland temporary pools. Hydrobiologia 796:201–222.

Brendonck, L., D. C. Rogers, J. Olesen, S. Weeks, and W. R. Hoeh. 2008. Global diversity of large branchiopods (Crustacea: Branchiopoda) in freshwater. Hydrobiologia 595:167–176.

Brooks, T. M., R. A. Mittermeier, G. A. da Fonseca, J. Gerlach, M. Hoffmann, J. F. Lamoreux, C. G. Mittermeier, J. D. Pilgrim, and A. S. Rodrigues. 2006. Global biodiversity conservation priorities. Science 313:58–61.

Froese, R., and D. Pauly, editors. 2000. Page 344. FishBase 2000: concepts, design and data sources. ICLARM, Los Baños, Laguna, Philippines.

Horváth, Z., C. F. Vad, L. Voeroes, and E. Boros. 2013. The keystone role of anostracans and copepods in European soda pans during the spring migration of water birds. Freshwater Biology 58:430–440.

Jocque, M., B. J. Riddoch, and L. Brendonck. 2007. Successional phases and species replacements in freshwater rock pools: towards a biological definition of ephemeral systems. Freshwater Biology 52:1734–1744.

Kerfoot, W. C., and M. Lynch. 1987. Branchiopod communities: associations with planktivorous fish in space and time. Pages 376–382 *in* W. C. Kerfoot, and A. Sih, editors. Predation: Direct and indirect impacts on aquatic communities. University Press, New England, UK.

Messerman, A. F., A. G. Clause, S. V. L. Catania, H. B. Shaffer, and C. A. Searcy. 2021. Coexistence within an endangered predator–prey community in California vernal pools. Freshwater Biology 66:1296–1310.

Pinceel, T., et al. 2021. Co-occurrence of large branchiopods and killifish in African savannah ponds. Figshare, data set. https://doi.org/10.6084/m9.figshare.13751974.v1

Pinceel, T., B. Vanschoenwinkel, P. Deckers, A. Grégoir, T. VerEecke, and L. Brendonck. 2015. Early and late developmental arrest as complementary embryonic bet-hedging strategies in African killifish. Biological Journal of the Linnean Society 114:941–948.

Polačik, M., R. Blažek, and M. Reichard. 2016. Laboratory breeding of the short-lived annual killifish *Nothobranchius furzeri*. Nature Protocols 11:1396–1413.

Vanschoenwinkel, B., A. Waterkeyn, T. Nhiwatiwa, T. Pinceel, E. Spooren, A. Geerts, B. Clegg, and L. Brendonck. 2011. Passive external transport of freshwater invertebrates by elephant and other mud-wallowing mammals in an African savannah habitat. Freshwater Biology 56:1606–1619.

Watters, B. R. 2009. The ecology and distribution of *Nothobranchius* fishes. Journal of the American Killifish Association 42:37–76.

Yang, D., and S. Park. 2017. Freshwater anostracan, *Branchinella kugenumaensis*, as a potential controlling consumer species on toxic cyanobacteria *Microcystis aeruginosa*. Aquatic Ecology 51:449–461.

Supporting Information

Additional supporting information may be found in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ecy.3505/suppinfo

Open Research

Data (Pinceel et al. 2021) are publicly available in Figshare: https://doi.org/10.6084/m9.figshare.13751974