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Forty-year natural history study of *Bahalana geracei* Carpenter, 1981, an anchialine cave-dwelling isopod (Crustacea, Isopoda, Cirolanidae) from San Salvador Island, Bahamas: reproduction, growth, longevity, and population structure

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Abstract

Almost nothing has been reported on the natural history of any of the world's 92 species of cave cirolanids, including those from saltwater caves (anchialine). Over 1400 specimens of *Bahalana geracei* Carpenter, 1981 were collected in two caves from 1978–2018; size-frequency data provided insight into population structure. Some specimens were maintained alive over multiple years to study rarely reported activities for cave cirolanids: feeding, molting, growth, longevity, and reproduction. Photographs document these phenomena. Mating occurred after gravid females shed both halves of reproductive molts. Females can have multiple broods (iteroparous) with ~2.0–3.5 years per reproductive cycle: egg production (~9–24 months), mating, brooding (5–6 months), release of 6–55 mancas (2.3–3.3 mm long), and oostegite molt (~2–13 months after manca release). Estimated lifetime fecundity is 58 mancas per female; probable range is 20–120. In Lighthouse Cave, females outnumbered males (~4:1), grew larger (16.8 vs. 9.5 mm), and lived longer. Growth rates were slow: ~1–2 years for three instars of post-marsupial manca development (from ~2.3–4.0 mm); estimated adult growth rate was 0.8 mm/year (1.6 molts/year) for males, and 0.5 mm/year (1.5 molts/year) for females. Longevity estimates for females are 25–28 years with 23–30 instars, vs. 6–8 years for males with 13–15 instars. Males from Major's Cave were nearly as numerous and as large (14.8 mm) as females; estimated longevity for males is >20 years. Longevity estimates of >20 years appear to be the longest for any isopod species. Female longevity probably increased by being starvation resistant, surviving multiple broods, cannibalizing smaller *B. geracei*, and living in a low-stress environment. Populations appear to be stable, relatively large, and not currently threatened.

Keywords
Age compression, cannibalism, fecundity, gestation, iteroparous, mancas, molting, starvation resistance, stygobitic

Table of contents

Introduction

According to Bruce et al. (2017) the isopod family Cirolanidae Dana, 1852 is one of the largest families of free-living isopods, with more than 500 species in 62 genera, including about 91 described species that are stygobitic (aquatic and strictly subterranean). Messana (2020) recently described *Catailana whitteni*, a new genus and species of stygobiotic cirolanid from caves in China, making a new total of about 92 stygobitic species in 63 genera. Eight stygobitic species are from The Bahamas including six species of *Bahalana*. The type species *Bahalana geracei* Carpenter, 1981 is known with certainty only from caves on San Salvador Island. Almost nothing has been reported on feeding, growth, and reproduction of any of these 92 species. In discussing our growing need to protect cave invertebrates, Hutchins et al. (2010) noted that, "the basic ecology and life history of most subterranean species are unknown because access to their habitat is technically challenging, and especially because their lengthy life span and low population density render ecological studies difficult." Culver and Pipan (2019) pointed out the difficulties and rareness of breeding cave stygobites and, "Among crustaceans the only case of captive breeding known to us is that of Fong (1989) with the amphipod *G. minus*", in reference to *Gammarus minus* Say, 1818. In addition, Magniez (1975) reported success with the cave Stenasellid isopod *Stenasellus virei* Dollfus, 1897 that he, "bred in the laboratory for many years (1960–1974)."

Studies of almost all cave species naturally begin with collecting and preserving a few specimens for taxonomic descriptions and/or DNA studies. In most cases, additional specimens are never collected and kept alive for observation and attempted culturing. For example, Hutchins et al. (2010) collected 70 specimens of *Antrolana lira* Bowman, 1964 from nine sites for genetic data to analyze population structure, and "all specimens were preserved immediately in 95%-100% ethanol." Such studies are extremely valuable to support conservation initiatives, but immediate preservation obviously limits study of their natural history. As a result of these challenges, most published discussions on ecology of anchialine cave species are limited to salinity and a list of other animals found in the same caves.

The current long-term study of *B. geracei* was made possible by an extraordinary set of circumstances. First, because of my previous experience in culturing and describing new species of freshwater cave invertebrates (e.g., Carpenter 1970a, b), I was excited when the director of the Bahamian Field Station, Donald T. Gerace, agreed to lead my marine biology class to Lighthouse Cave in 1978. As soon as we started finding isopods and other unusual cave animals, the strong potential for new discoveries became apparent. I was fortunate to be able to teach marine biology courses on San Salvador Island almost every year for over 20 years (1977–2000), which gave us the opportunity to explore Lighthouse Cave as an example of an unusual marine habitat and for students to do research on several cave species. After my retirement in 2001, several former students and research colleagues kindly volunteered to help continue this cave research (2001–2020).

Fortunately, Lighthouse Cave is easily accessible from the Gerace Research Centre (formerly the Bahamian Field Station), the cave water is shallow enough to explore without scuba, and the population of *B. geracei* is usually moderately high and not endangered. Since specimens are relatively easy to maintain in the laboratory for long periods and are translucent enough to reveal sexual condition and digestive processes, they were ideal for students to use for research projects that have contributed to this study.

Although this study encompasses more than 40 years, collection data from several years are not included for a variety of reasons. Sometimes no specimens were found, or our research concentrated on other cave animals (e.g., other isopod species, remipedes, and brittle stars); some years I did not visit San Salvador Island because I taught courses in other locations (e.g., Australia or Ecuador), or my research associates or I had health issues or family obligations. Even when field studies were not carried out, laboratory culturing and research continued.

Three approaches were used in this study: (1) collecting over 1400 specimens (most were returned to the caves) during a 40-year period to provide insight into population structure based largely on size-frequency distributions, (2) maintaining some specimens over multiple years to learn about behavior, feeding, molting, growth, and longevity, and (3) observing life cycle stages and reproductive events: egg development, mating, gestation, release of mancas (offspring) from the marsupium, and development of post-marsupial mancas – all phenomena that have been rarely or never reported for cave cirolanids. These three approaches are covered in reverse order: first reproduction and life cycle development, then growth and longevity, and last population structure.

With 40 years of data and observations recorded in hundreds of pages of notes, it has been challenging to decide what to include in this paper. Some of my observations are of phenomena so rare that they may seem trivial, but they may also be the most interesting and valuable if they are the first times ever reported for this elusive group. Even with over 1400 specimens, several of the population phenomena examined (e.g., number of months between molts for specific sizes and reproductive conditions) do not have sufficient numbers to warrant traditional statistical tests, but they still provide evidence to support growth and longevity patterns. The section on "Growth rates and longevity" is one of the longest because it has so many components and because it is important to explain how my longevity estimates were calculated, since any claims of extreme longevity will likely be scrutinized and questioned. Results of this unusually extensive long-term study are presented with the hopes that it will also provide insight into the lives of other cave cirolanids and other crustaceans.

Materials and methods

Study areas

San Salvador Island is a small island (about 16 km by 8 km) in the eastern part of The Bahamas archipelago, 24°06'N, 74°29'W (Fig. 1A). It sits atop a shallow-water carbon-

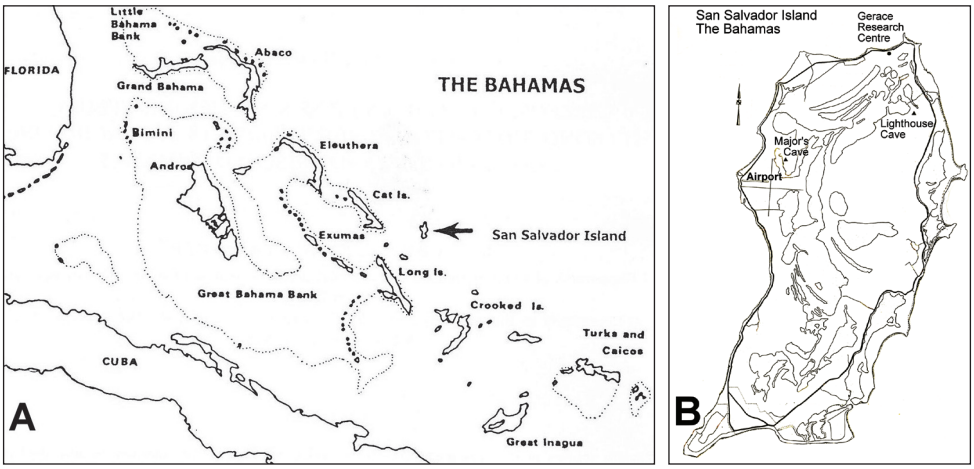

Figure 1. Maps showing **A** location of San Salvador Island in The Bahamas and **B** San Salvador Island with locations of Lighthouse Cave and Major's Cave.

ate bank isolated from other banks, like the Grand Bahama Bank, by deep water (Yager and Carpenter 1999). Many caves in The Bahamas are close to the ocean, so they contain salt water. They fit the definition of anchialine, which was originally described as a habitat consisting of "pools with no surface connection with the sea, containing salt or brackish water, which fluctuates with the tides" (Holthuis 1973). Due to the use of the restrictive word "pools", this definition was modified by Stock et al. (1986) as, "Anchialine habitats consist of bodies of haline waters, usually with a restricted exposure to open air, always with more or less extensive subterranean connections to the sea, and showing noticeable marine as well as terrestrial influences." Bishop et al. (2015), proposed a broader definition of anchialine as, "a tidally-influenced subterranean estuary located within crevicular and cavernous karst and volcanic terrains that extends inland to the limit of seawater penetration."

Many anchialine caves in The Bahamas and other locations around the world have a saltwater layer below a substantial freshwater layer, so cave divers need to dive through the freshwater layer and halocline to study the marine waters and its inhabitants below. In contrast, the anchialine caves on San Salvador Island do not have this stratification; instead, they have salt water or brackish water all the way to the surface. As such, these caves do not conform well with the new definition by Bishop et al. (2015) because they do not have the influence of a freshwater stream or river that is typical of estuaries. However, they may still be influenced by the mixing of oceanic salt water with meteoric fresh water that penetrates through the soil and/or with underground freshwater aquifers, even if the freshwater habitats are not readily accessible for humans to explore. The anchialine caves on San Salvador Island might best be described by combining the first two definitions: Anchialine habitats consist of salt or brackish bodies of water which fluctuate with the tides, and have subterranean connections to the sea, but no surface connection.

Two caves were used in this long-term study (Fig. 1B): Lighthouse Cave and Major's Cave. The more important one is Lighthouse Cave where we collected *B. geracei* in at least 23 of the years from 1978 to 2018. This cave is located about ½ km from the Dixon Hill Lighthouse (northeastern side of the island) and about 1 km from the ocean (Carpenter 1981); it is about 3 km from the Gerace Research Centre and is a popular field trip for many courses taught at GRC. The geology of Lighthouse Cave was described by Mylroie (1980) and the hydrology by Davis and Johnson (1988). According to Carpenter (1981), "The cave consists mainly of one room about 40 m in diameter with a large pile of breakdown rocks in the middle mostly surrounded by quiet water up to 1 m deep. Close to the entrance, which is a narrow hole near the roof, is a small room about 10 m in diameter where the isopods were found." Since then, specimens of *B. geracei* have been found throughout the cave's rooms and channels. Tidal fluctuations of about 0.5 to 1 m occur through complex conduit systems; tides are delayed (compared to ocean tides) by ~45 minutes. Surprisingly, Davis and Johnson (1988) reported that "the tidal range is greater (sometimes by as much as 0.5 m) than that in the ocean." We found it much easier to collect isopods and other marine animals near low tides. "The water in Lighthouse Cave is usually near full ocean salinity (~35 ppt), but sometimes it drops to ~20 ppt or lower after heavy rains (my samples on 18 June 2013 varied from 20–32 ppt depending on location in the cave)", Carpenter (2016). In laboratory experiments, *B. geracei* easily tolerated salinities down to 15 ppt and survived for several days when near 11 ppt.

Besides *B. geracei*, other aquatic life in Lighthouse Cave includes: the asellote gnathostenetroid isopod *Neostenetroides stocki* Carpenter & Magniez, 1982, the red shrimp *Barbouria cubensis* (Von Martens, 1872), several copepod and ostracod species, several sponge species (see van Soest and Sass 1981), tube worms (*Spirorbis* sp.), the mangrove rivulus fish *Kryptolebias marmoratus* (Poey, 1880) and occasional microbial colonies (probably a species of *Beggiatoa* or *Thiothrix*). Terrestrial life includes numerous bats and cockroaches (*Periplaneta americana* Linnaeus, 1758), evaniid wasps that parasitize cockroach egg cases, four isopod species, the pseudoscorpion *Paraliochthonius carpenteri* Muchmore, 1984, two snail species, land crabs and rats.

Major's Cave is on the northwest side of the island near the San Salvador Island International Airport, about 6 km southwest of Lighthouse Cave, and is considerably more challenging to access. It was discovered in 1997 by men working on the runway extension. A faunal survey was conducted by professors and students from Siena College (Loudonville, NY) and Le Moyne College (Syracuse, NY) on 15 June 1997, during which Dr. Nancy Elliott (Siena College) collected and preserved two remipedes from the surface of a pool. Every year from 1997 to 2004 my marine biology classes and research associates visited Major's Cave, primarily to search for and study the remipedes. Jill Yager and I described the remipede as a new species, *Speleonectes epilimnius* Yager & Carpenter, 1999. In the same issue of Crustaceana, I published a companion paper on the behavior and ecology of this species; this is the only species ever found at the surface of anchialine waters (rather than below a halocline), which allowed me to keep a few specimens alive long enough to study feeding, grooming, and resting

behaviors; a description of Major's Cave is included in the section on "Habitat and fauna" (Carpenter 1999). Salinity is usually about 24 ppt (~70% of ocean salinity). Other marine animals found in this cave include copepods, marsh crabs *Armases miersii* (Rathburn, 1897), and *B. geracei*. Between 1997–2004 we collected enough *B. geracei* specimens to determine that the populations in Major's Cave and Lighthouse Cave are remarkably different from each other (especially regarding sizes and numbers of males), which are described in the section on Life cycle and population structure.

Sampling

Collections were almost always made in June or July, but also in January 1980, 1999, and 2013.Collecting and export permits were required (requested and granted) starting around 2007. My marine biology classes and research groups usually collected in at least one cave once or twice during each trip to San Salvador Island; we spent about one hour collecting, usually at low tides when most collecting areas were less than 1 m deep. Most collecting in Lighthouse Cave was done in a small side room near the entrance; this left the population in the remainder of the cave relatively unaffected. There were usually 6–10 collectors, but this varied from 2–20, which strongly affected the number of specimens collected; previous experience and natural collecting skills also contributed to success.

Several collecting techniques were tried over the years. Baited traps tended to catch other animals such as red shrimps (*B. cubensis*) and ostracods. Black aquarium nets with long handles were most effective in collecting the white *B. geracei* that were easily seen either swimming or resting on the dark silt-covered substrate or rocks. A variety of flashlights were used; strong underwater flashlights increased chances of finding small specimens. Specimens were transferred to individual containers (usually 35 mm film cannisters) to avoid cannibalism that often occurred when two specimens were put together. Containers were nearly filled with cave water to reduce turbulence during transport to the field station. They were then kept in my laboratory/bedroom, where air conditioning was maintained near cave temperature (~25–26 °C). Each specimen was examined alive under a dissecting microscope (7–40×) to determine size, gender, manca stage, and sexual condition for females (bearing eggs or oostegites or neither). Oostegites, visible as shiny plates (Figs 3D, 4A–E), are flexible flaps that extend from the coxae (first segments) of pereopods (legs) 1–5 to form the marsupium or brood pouch; if oostegites were present, but no eggs or developing embryos or mancas, these females had released their mancas within the previous few months. Males were easily identified in dorsal view by white sperm-packed sperm ducts (vasa deferentia) extending from pereonites 5–7 (Figs 3A, B, 7C, D) to ventrally located penes (Figs 3B, 5F); sometimes males needed to be confirmed by finding the clear penes and/or an appendix masculina (Fig. 3B) on a pleopod 2. Each specimen was numbered in the order in which it was examined, often starting with the largest ones. Measurements of body length (front of head to tip of telson) were made to the nearest 0.1 mm using an ocular micrometer and/or ruler or grid beneath specimens (Fig. 5B).

Collection data are summarized in Table 1 and Fig. 2. Most specimens were returned to the caves after examination to reduce our effect on the populations. Some were kept for long-term observation in Kentucky, especially if they were likely to provide additional information on growth or reproduction.

Culture methods

Specimens kept for long-term observation and experimentation were maintained in clear plastic jars or translucent food storage containers with tight fitting lids and 20–100 ml of salt water (near 35 ppt) at a depth of only 1–2 cm. This shallow depth provided a high surface area to volume ratio for better oxygen exchange, since no extra aeration or filtration was used. Small rocks or pieces of dry wall sanding screens were used as substrate to facilitate molting. When females were releasing their mancas, they were housed in jars with a horizontal sanding screen held 1–2 cm above the substrate so mancas could crawl through and avoid being trampled (Fig. 4F). Mud from the caves was sometimes used as substrate, but specimens did well in containers without supplemental substrates. Although *B. geracei* do not seem to be light sensitive, they were stored in a dark room or incubator. Sometimes they were kept at ambient room temperatures ~20–27 °C, but usually close to cave temperatures of 25–26 °C with supplemental heating. From 1979 to 2001 specimens were kept in a research laboratory at Northern Kentucky University where students could learn maintenance techniques and perform experiments on *B. geracei* and other cave species. Since retiring from NKU in 2001, it has been convenient to keep specimens at my home.

Each animal was kept in a separate container to avoid cannibalism and to provide data on individual feeding, molting, growth, and egg production; of course, breeding experiments required short-term exceptions to this practice of separation. Jars were labeled with each adult specimen's collection number and year (e.g., female #5, 2018) to facilitate multi-year tracking. Mancas were kept in jars labeled with the date of birth (release from marsupium), plus a letter if more than one was released on that date (e.g., 7-27-20A). Adults were routinely offered food every 3–6 weeks, which was the typical time for digestion. Mancas were offered food every 1–3 weeks. After each feeding, containers were cleaned with a paper towel, and newly aerated water was added. Many different food items were eaten including brine shrimp, ghost shrimp, crab, crayfish, California black worms, earthworms, cockroaches, dragonfly nymphs, mayfly nymphs, mosquitoes (larvae, pupae, and adults), centipedes, spiders, terrestrial isopods, asellote isopods from Lighthouse Cave, frog tadpoles, and cooked meat (shrimp, lobster, fish, chicken, and turkey).

All photographs of *B. geracei* in this paper are of live specimens (Figs 3A–7E) except for the shed exuvium (Fig. 7F) using various Nikon cameras with built-in flashes and a 60 mm micro-Nikkor lens, either shot through a dissecting microscope or directly. An accessory flash helped illuminate microscope photographs. Each figure is labeled with the isopod's orientation (dorsal, ventral, or lateral), size (body length in mm), identification number, year of collection, and date photograph was taken.

Table 1. Numbers and sizes of *Bahalana geracei* from Lighthouse Cave (1978–2018).

Year	Mancas / Sizes [mm]	Males / Sizes [mm]	Females / Sizes [mm]	Total
1978	0 / 0–0	1 / 8.0	4 / 13.6–15.0	5
1979	1 / 4.0	2 / 8.0	4 / 12.0–14.0	7
1992	0 / 0–0	1 / 6.0	27 / 5.0–16.0	28
1993	3 / 3.0–3.3	4 / 5.8–7.9	31 / 4.5–16.0	38
1994	10 / 2.5–3.8	9 / 4.5–8.3	60 / 4.8–16.0	79
1995	3 / 3.3–3.9	7 / 4.4–7.5	48 / 4.7–16.5	58
1996	7 / 2.5–3.3	19 / 4.6–7.1	62 / 4.2–16.8	88
1997	2 / 3.7–3.8	27 / 4.0–8.3	61 / 3.8–13.3	90
1999	10 / 2.6–4.2	32 / 3.6–8.0	80 / 4.0–15.5	122
2000	7 / 2.6–3.9	22 / 4.2–9.5	68 / 5.0–16.2	97
2001	5 / 2.3–3.8	21 / 4.5–7.0	112 / 4.5–16.0	138
2002	6 / 2.5–3.5	15 / 4.8–8.0	63 / 4.5–14.8	84
2003	11 / 2.5–3.8	24 / 4.0–8.5	72 / 4.0–14.7	107
2004	1 / 3.2	4 / 3.5–7.0	58 / 4.5–16.5	63
2005	3 / 2.4–3.9	3 / 6.0–8.2	55 / 4.0–16.0	61
2006	4 / 2.6–4.0	8 / 5.0–8.0	60 / 4.0–13.0	72
2007	8 / 2.8–4.0	17 / 3.5–7.0	70 / 4.0–16.5	95
2008	8 / 2.8–4.0	3 / 5.5–7.3	19 / 4.3–14.5	30
2011	0 / 0–0	6 / 4.0–7.0	12 / 6.0–16.0	18
2013	0 / 0–0	0 / 0–0	6 / 6.8–9.0	6
2013	0 / 0–0	0 / 0–0	7 / 5.0–13.0	7
2014	0 / 0–0	0 / 0–0	10 / 8.8–13.8	10
2016	0 / 0–0	7 / 5.0–7.5	12 / 4.0–14.0	19
2018	3 / 3.2–4.0	12 / 3.5–7.0	46 / 4.5–16.0	61
Totals	92 / 2.3–4.0	244 / 3.5–9.5	1047 / 3.8–16.8	1383

Results

Reproduction and development

Until this study, little has been reported on any aspect of reproduction in cave cirolanids. Fortunately, I was able to observe all stages of the life cycle of *B. geracei*. These include: mancas (with three stages: M1, M2, and M3) that had recently been released from brooding females; males that were young pre-reproductive juveniles and older mature breeders; and females that were pre-reproductive juveniles, egg-bearers, brooders, oostegite-bearers, inter-cycle females, and post-reproductive females. Numbers and sizes of specimens in these stages that were collected in Lighthouse Cave from 1978–2018 are summarized in Table 1 and Fig. 2. Details of these data are discussed later in the sections on "Growth rates and longevity" and "Population structure." However, first I will describe details of the reproduction and development observed in the laboratory, accompanied by photos (Figs 3A–6F), to give visual images of the life cycle stages.

Although the description of the life cycle could start at any stage, I decided to start with: (1) egg-bearers that had not yet undergone reproductive molts to produce marsupia with oostegites, followed by (2) breeding procedures that led to mating and fertilization of eggs, (3) brooding of embryos and mancas inside marsupia, (4) release of mancas, (5) post-marsupial manca development, (6) oostegite-bearing females, and (7) females that were collected with eggs or mancas still in their marsupia.

Size ranges in mm	3.0-3.9	4.0-4.9	5.0-5.9	6.0-6.9	7.0-7.9	8.0-8.9	9.0-9.9	10.0-10.9	11.0-11.9	12.0-12.9	13.0-13.9	14.0-14.9	15.0-15.9	16.0-16.9	Totals and %
Males	4	45	87	66	30	11	1								244 = 19%
Egg-bearers	0	2	44	137	99	41	13	7	4	2	2	2	0	1	354 = 27%
Oost.-bearers	0	0	6	49	47	18	3	5	1	4	13	11	8	2	167 = 13%
Non-breeders	2	55	73	80	61	54	28	27	19	21	29	37	21	19	526 = 41%
All females	2	57	123	266	207	113	44	39	24	27	44	50	29	22	1047 = 81%
															M+F = 1291

Figure 2. Post-manca specimens of *Bahalana geracei* from Lighthouse Cave (1978-2028), color coded for quantities within reproductive conditions, **Blue** = males in top row: 2 smallest size classes (3 & 4 mm) were pre-reproductive (light blue), males peaked in 5 mm class then declined rapidly in next 4 size classes, **Green** = smallest egg-bearers (4 mm class) and oostegite-bearers (5 mm class), **Pink** = smallest non-breeders (3, 4, & 5 mm classes) were presumed to be pre-reproductive, **Yellow** = peak numbers for females were in 6 mm class, **Red** = lowest numbers for females were in 11 mm class, **Gray** = females persisted in largest classes (13, 14, 15, & 16 mm) in all stages.

Egg-bearers

As seen in Fig. 2, 354 egg-bearing females (without oostegites) were collected in this study. Egg size for each female was estimated as small, medium, or large. Some of these females were kept for long-term study to determine length of time for egg production and for possible breeding (Fig. 3A, C–F). It took about 9–24 months for females to grow eggs to maturity.

The two smallest egg-bearing females (Fig. 2, green) were 4.5 and 4.8 mm (probably 2nd juvenile instars) with many more (n = 44) in the 5 mm class and the most egg-bearers (137 of 266 females = 52%) in the 6 mm class, followed by gradual declines over the next 10 classes. Egg-bearing females (n = 354) represented ~27% of all the 1047 females collected. This is an extraordinarily high number, since collections of most cave cirolanid species have never included any specimens reported as "ovigerous", and this certainly contributed to the relatively high population in *B. geracei*.

Incidentally, for this study I avoid using the vague term "ovigerous", which has been applied to females with either eggs or embryos inside ovaries, or pereon, or marsupium.

Breeding procedures and mating

Isopods typically molt in two stages, including the reproductive molt (parturial molt). According to Wilson (1991), the gravid female "first molts the exoskeleton posterior to the fifth thoracic segment", mates when her exoskeleton is soft, and "the female will then molt the anterior half of the body and deploy the oostegites that form the brood pouch." But the process is different in *B. geracei*. Several times captive females were observed to have had molted only their posterior halves, males were put with them for

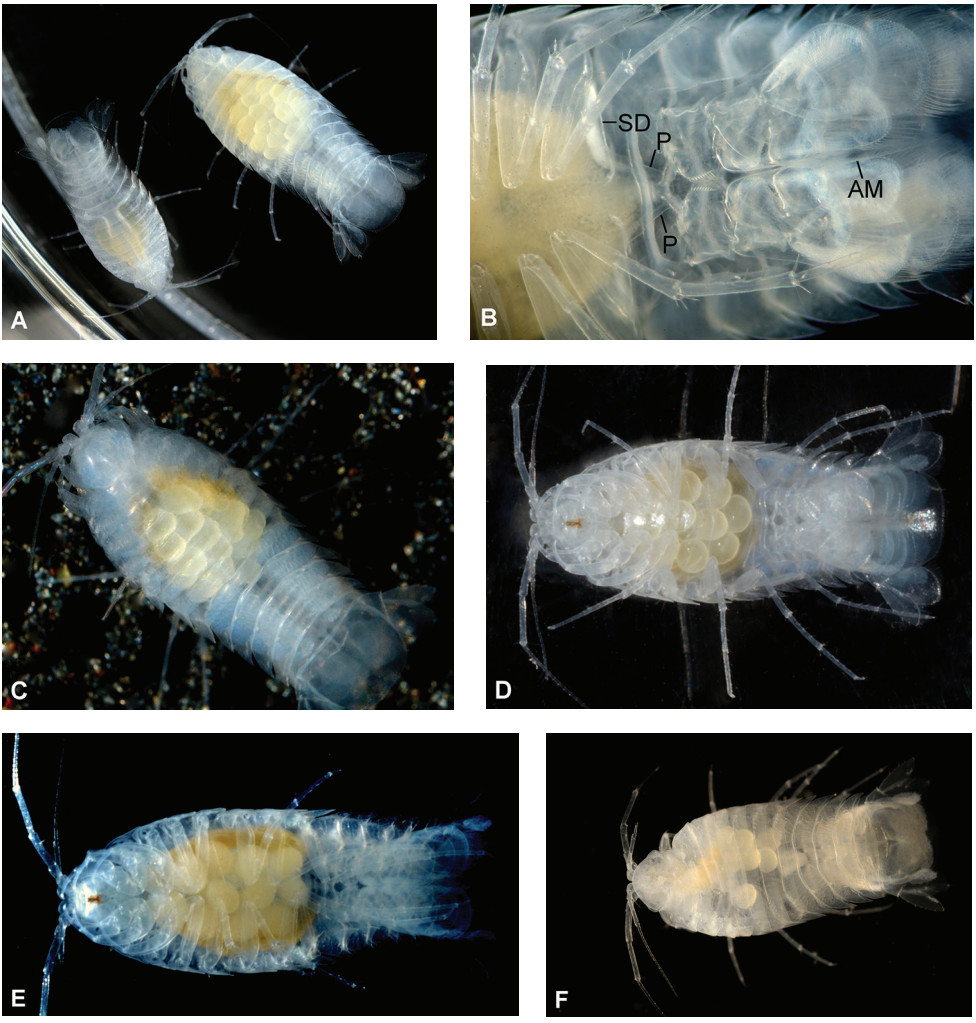

Figure 3. Male and female *Bahalana geracei* **A** dorsal view; 5.8 mm ♂ #5 (2016) on left with white sperm ducts; 8.0 mm ♀ #6 (2016) on right with ~20 eggs ~0.5 mm diameter; she was later cannibalized by 6.0 mm ♂ #11 (2016); 1 Oct. 2016 **B** ventral view; 8.5 mm ♂ #5 (2016) with white sperm-filled ducts (SD), penes (P), and appendix masculina (AM); 24 August 2020 **C** dorsal view; 7.5 mm ♀ #33 (2018) with ~12 eggs ~0.6 mm diameter, after reproductive molt and before mating; 20 May 2019 **D** ventral view; 7.5 mm ♀ #33 (2018) with ~12 round eggs ~0.65 mm diameter, in marsupium 3 weeks after mating; 13 June 2019 **E** ventral view; 7.5 mm ♀ #33 (2016) with ~12 elongated embryos in marsupium, 6 weeks after mating; 6 July 2019 **F** dorsal view; 8.0 mm ♀ #6 (2016) with a few eggs after being cannibalized by 6.0 mm ♂ #11 (2016); 1 Oct. 2016.

possible mating, but neither the males nor females showed interest in mating; the male was then removed. This was my first indication that female *B. geracei* mate only after molting the anterior half (Fig. 7F), which is usually ~4 days after the posterior half. Sometimes this led to successful mating, and sometimes not. If mating did not occur

and eggs were not fertilized, eggs inside her pereon gradually deteriorated into two white masses and were reabsorbed. Such white masses were never observed in freshly collected females, which indicates females in the caves probably had little trouble attracting mates at the appropriate time.

It was challenging to breed *B. geracei* since all adult males and females were normally kept in individual containers to avoid cannibalism. To reduce the chances of cannibalism during a mating encounter, males were fed before being placed with a female. On one occasion, 6.0 mm male #11 (2016) was placed with 8.0 mm female #6 (2016) when timing seemed to be right for mating (after molting posterior and anterior halves), but he unexpectedly attacked her and ate some of her eggs before he could be removed (Fig. 3F). Even though this male was smaller, and he had eaten 13 days before their encounter, he was apparently more interested in feeding than mating; in subsequent attempts, even smaller males were paired with females whenever possible, and food was offered to them within 5 days of pairing.

Sometimes actual mating was not observed, but the pair was left unattended for several hours or days after her anterior molt, and females subsequently produced successful broods. These successes allowed for observation and photography of females incubating eggs and embryos during their incubation periods of 5.5–6.0 months, release of mancas, and their subsequent development.

According to Wilson's (1991) report on isopod genitalia, "The details of copulation are generally unclear because it occurs so quickly (Ridley 1983)." Apparently, there are no published records of copulation in any cave cirolanids. As mentioned above, many isopod species copulate during the female's biphasic reproductive molt, but several times male *B. geracei* were unsuccessfully paired with females before her anterior half was molted; all successful matings occurred after the anterior half was molted (up to 8 days afterwards) and oostegites were deployed. This provided a narrow window of opportunity for me to find females when they might be receptive. Fortunately, this also provided an opportunity for me to control the circumstances for mating, to observe the actual mating activity at least three times, and to record it on film once.

The first successful captive breeding event for *B. geracei* was with 6.2 mm female #49 (1995) collected with large eggs in Lighthouse Cave, 4 July 1995. By 15 November 1995 she had molted both halves, so 6.6 mm male #27 (1995) was put into her container. Six months later, on 12 May 1996 (Mother's Day in the U.S.), #49 released 3 mancas, 2 more the next day, and 4 more on 15 May 1996; development of these 9 mancas are described at the end of the section on Post-marsupial manca development. During #49's 6-month gestation (described in next section on Gestation) her activity level decreased; she remained stationary on a vertical screen for 18 days straight. But she was active enough to eat four small meals and grew to 7.0 mm. She molted 51 days after manca release, then again 4 months after that.

The second successful breeder was #88 (1996), a 13.2 mm female with no discernible eggs when collected in Lighthouse Cave, 15 July 1996. By 30 July 1997 (1 year after collection) she had molted both halves, now 13.8 mm with oostegites and

~14–16 eggs on each side. Two days later I added 6.3 mm male #72 (1997) and filmed his mating behavior. He swam past her twice then climbed onto her right side, rapidly tapped her antennae while his head was near the top of her head, moved to her left side and tucked his abdomen near her 5th pereopod for about 3 seconds, moved back to her right side and pushed her 6th and 7th pereopods posteriorly, mated for about 10 seconds with his abdomen tucked under her while thrusting his pleon and rapidly beating his pleopods. After mating, he rested on her side nearly 10 minutes; at one time he put his head near the ventral part of pereopod 5 for about 30 seconds, possibly to check sperm. After dismounting, he rested near her side for a few minutes until she slowly moved away. Under a dissecting microscope, sperm were visible inside his sperm ducts and on her 5th pereonite. Microscopic examination the next day revealed sperm inside her spermathecae and eggs inside her marsupium (in contrast to #49 described above). For the next three weeks she periodically pushed against her oostegites with her "elbows" of pereopods 1 to move ~18 eggs forward and backward inside her marsupium, while rapidly ventilating with her maxillipeds (~50 times/15 seconds); then she flexed her body to remove some excess water from the marsupium. Small sperm packets remained visible in her spermathecae for the next three weeks. Surprisingly, her movement of eggs back and forth gradually pushed all of them out the posterior end of her marsupium, and no embryos or mancas were produced. Some of these mating behaviors are compared to other crustaceans in the discussion section.

Gestation

The successful mating of female #49 (1995) described above resulted in an unusual gestation. During incubation, I examined her marsupium using mirrors, fiber optic lights and a microscope. Side views showed the marsupium expanding and contracting with fluid (aiding the circulation created by beating maxillae), but eggs, embryos or mancas were never visible inside her marsupium (Fig. 4A). Instead, they appeared to be retained inside her pereon (Fig. 4C). This was totally unexpected and did not match the normal incubation inside the marsupium of most other isopods (Johnson et al. 2001; Wilson 1991), nor of my later successful *B. geracei* brooders described below (more on this in the discussion section).

On 6 July 2018, 61 specimens of *B. geracei* were collected in Lighthouse Cave; 17 females were egg-bearers; eight were retained for further study. During the next 17 months, three completed their reproductive cycles. Males were added to each of the females after their reproductive molts were completed; no mating was observed, but a male was left with each one for several days. Photos (Figs 4B, D–F, 5A) document these reproductive events over 12–17 months. Additional photos document growth and development of mancas (Fig. 5A–F). Here are timelines for these reproductive events (egg development through manca release) for these three females:

1. #5 (2018) 11.0 mm with medium eggs, ate 5 times, reproductive molt on 10 February 2019 (7 months after collection), ate 4 times during gestation (5.5 months), 55 mancas released in 11 days (27 July to 6 August 2019), 2.3–2.8 mm (Figs 4B, D, E, 5A, B).

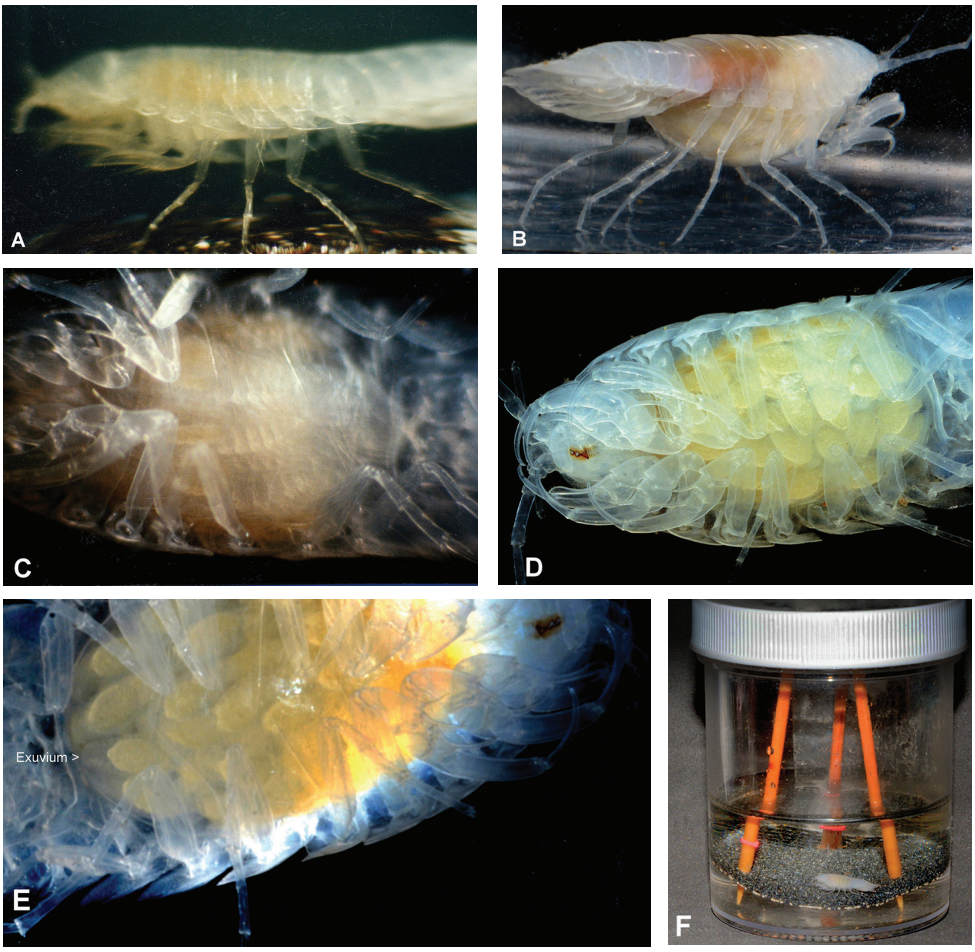

Figure 4. *Bahalana geracei* gestation **A** lateral view; 6.2 mm ♀ #49 (1995), marsupium with water partly expelled and no mancas; May 1996 **B** lateral view; 13.0 mm ♀ #5 (2018) with mancas in marsupium, brown gut 14 days after eating worm; 31 July 2019 **C** ventral view; 6.2 mm ♀ #49 (1995) with mancas inside perion; May 1996 **D** ventral view; 13.0 mm ♀ #5 (2018) with developing embryos inside marsupium, 3 months after mating; 11 May 2019 **E** ventral view; 13.0 mm ♀ #5 (2018) with developing embryos, some at posterior end with exuvia, 4 months after mating; 8 June 2019 **F** maternity jar with screen holding 13.0 mm ♀ #5 (2018) 1–2 cm above bottom of jar; 3 August 2019.

2. #33 (2018) 7.5 mm with large eggs, ate 5 times, reproductive molt 20 May 2019 (10 months after collection), ate 4 times during gestation (5.5 months), 13 mancas released in 18 days (2 November to 19 November 2019), 2.3–3.0 mm (Fig. 3C–E).

3. #35 (2018) 7.0 mm with large eggs, ate 10 times, reproductive molt 29 June 2019 (11.5 months after collection), ate 2 times during gestation (5.7 months), 6 mancas released in 8 days (19 December to 26 December 2019), 3.0–3.3 mm.

The number of mancas released each day ranged from 0 to as many as 17 for #5, 4 for #33, and 2 for #35. Since #5 had so many growing eggs and embryos, her length

increased ~18% from 11.0 mm when caught to 13.0 mm before releasing mancas; #33 increased ~20% from 7.5 to 9.0 mm, and #35 increased only ~3% from 7.0 to 7.2 mm.

As is typical of isopods (Johnson et al. 2001), the overall trend for the six females with reliable records was for smaller females to have much smaller broods than larger females. The four smallest females in the 6–9 mm range had 6–13 mancas: 6 for 7.2 mm #35 (2018), 9 for 7.0 mm #49 (1965), 12 for 7.2 mm #92 (2000), and 13 for 9.0 mm #33 (2018). The two largest females in the 13–15 mm range had 32–55 mancas: 32 for 14.8 mm #1 (2002) and 55 for 13.0 mm #5 (2018). For details on 7.2 mm #92 (2002) and 14.8 mm #1 (2002) see section below on Brooders with eggs or mancas.

Here are additional details regarding eggs and brood sizes for various females, including #5, #33, and #35. Eggs were usually round while developing inside the pereon (Fig. 3C) and when first placed in the marsupium (Fig. 3D); within three weeks they became elliptical embryos (Fig. 3E). Egg diameters were ~0.5 mm for large females such as 8.0 mm #6 (2016) (Fig. 3A) and 13 mm #5 (2018). For two smaller females, 7.5 mm #33 (Fig. 3C, D) and 7.0 mm #35, eggs were slightly larger (~0.60 to 0.65 mm).

Egg sizes and brood sizes of *B. geracei* were compared to marine cirolanids with comparable body lengths (5–16 mm) found in table 3 in Johnson et al. (2001). Only two such species were listed with egg diameters: *Cirolana carinata* (0.43 mm) and *Excirolana chiltoni* (0.6–0.9 mm); so, *B. geracei*'s egg diameters of ~0.5–0.65 mm were not unusual for cirolanids of this size.

Johnson et al. (2001) listed the following brood sizes for ten comparable species: *Cirolana harfordi* (18–68), *Cirolana imposita* (15–33), *Cirolana parva* (11–28), *Cirolana carinata* (14–45), *Eurydice longicornis* (34–59), *Eurydice natalensis* (13–25), *Excirolana chiltoni* (10–55), *Excirolana japonica* (17–68), *Pseudolana cocinna* (7–45), and *Pseudolana towrae* (18–24). The lower number $\bar{X}$ for these 10 is 15.7; the higher number $\bar{X}$ is 45.0. So, *B. geracei*'s brood sizes of 6–55 ($\bar{X}$ = 21.2, n = 6) appear to be in the lower end of the range for cirolanids of this size, although my sample size is small.

According to Johnson et al. (2001), "Three molts occur while the embryos are still in the brood pouch in isopods. The three molts include hatching from the egg membranes, a postnaupliar molt, and a larval ecdysis just prior to release from the brood pouch."

Apparently, mancas from female #5 (2018) molted late in development since shed exuvia could be seen inside her marsupium (Fig. 4E), and she released remains of exuvia into the water along with mancas; some of these exuvia were shed in one piece (i.e., monophasic). The marsupial molts seen in Fig. 4E were photographed ~6 weeks before release, which suggests they were probably postnaupliar molts, although they could also have been larval ecdyses.

Post-marsupial manca development

Most isopod species, including *B. geracei*, go through three instars or manca stages (M1, M2, and M3) before the 7th pair of pereopods becomes formed and functional (Wilson 1981); then I call them "juveniles." Manca 1 (M1) is the first instar upon release from the marsupium; there was no sign of 7th pereopods or external male genitalia. After their first molt they were in the manca 2 stage (M2); they still had no clear

sign of 7th pereopods, but males had a pair of tiny penes. Since M1's varied in size from 2.3–3.3 mm, and molts resulted in a size increase of 0.3–0.5 mm, M1 and M2 individuals overlapped in size and there was no way to distinguish between M1 and M2 females in the overlapping size range; M2's were identified as male M2's if penes were visible (but no 7th pereopod). In manca 3 stage (M3), 7th pereopods were partially developed, non-functional, and held across the body beneath pereopods 6 (Fig. 5E, F). Some M3 males collected in Lighthouse Cave had sperm in their sperm ducts.

Because manca stages are difficult to tell apart, published reports on field collections (including type series for descriptions of new species) often recognize all post-marsupial instars simply as "mancas" or "immatures;" (e.g., Botosaneanu and Illife 1997, 2003a; Bruce 2008). Fortunately, two *B. geracei* mancas from female #5 (2018) survived long enough to go through stages M1, M2, and M3 to develop into juveniles. Here are more details of the three manca stages in this species, based on mancas from #49 (1995), #5 (2018), #33 (2018), and #35 (2018).

In the above section on Gestation, the unusual 6-month incubation for #49 (1995) was described. When she released her 9 mancas, body lengths were 2.5–2.7mm. They began eating at 12 days and ate regularly every 1–3 weeks until fasting for 1–2 months before molting. Five mancas survived long enough to molt from M1 to M2 in 111–296 days old ($\bar{X}$ =169 days); these molts increased body lengths by 0.3–0.5 mm; they lived another 105–300 days without molting to M3, eventually dying at 10–20 months old.

Large 13 mm female #5 (2018) released her first 5 mancas on 27 July 2019 while being prepared for photographs under a dissecting microscope, so she and 3 mancas were photographed together (Fig. 5A). She released 50 more mancas over the next 10 days. Some were kept in individual containers; sometimes all from one day were kept together in one jar to observe interactions and to reduce maintenance activities. Newly released mancas held their antennae and pereopods straight against their bodies (Fig. 5B); several were dead when released, or so weak that they moved little and died in a few days. Each had a white hepatopancreas (gastric caeca or midgut gland) that contained enough nourishment for several days (Fig. 5B–D). First meals began at 6–15 days old (Fig. 5C, D). Food was offered at intervals of 1–3 weeks and included California black worms (Figs 6A, B), pieces of shrimp (Fig. 5C), brine shrimp (Figs 5D, 6C, D), centipedes, spiders, and live 1–2 mm long *N. stocki* isopods from Lighthouse Cave; there was one case of cannibalism. The clear exoskeleton allowed for observation of food intake, including the eyes of brine shrimp clearly visible in the stomach of one (Fig. 6D). Shrimp pieces and brine shrimp often turned red inside their stomachs (Fig. 6E); later the hepatopancreas turned red and remained red for several days (Figs 5E, F, 6F). Feces appeared in hind gut within 1 day after eating; a fecal string was sometimes passed a few days later.

Nine mancas from #5 (2018) survived long enough to molt to M2; most of them died shortly after molting. One healthy survivor molted from M2 to M3 in 59 days, then from M3 to juvenile (J1) in another 58 days. Another one molted from M2 to M3 in 84 days, then from M3 to J1 in 65 days. Thus, the time spent in each stage for

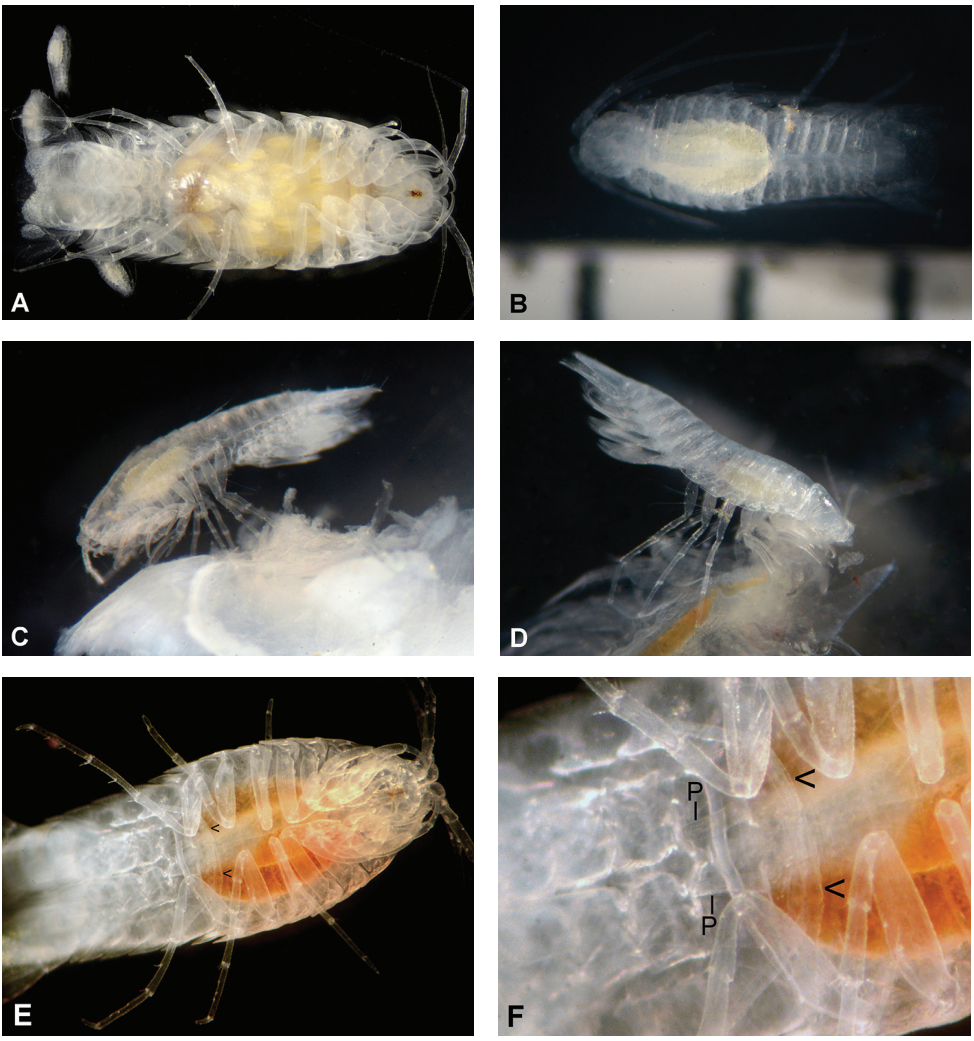

Figure 5. *Bahalana geracei* manca development **A** ventral view; 13.0 mm ♀ #5 (2018) releasing first mancas 2.3 mm long; 27 July 2019 **B** dorsal view; manca (M1) 2.3 mm long, just released from female #5 (2018); note white hepatopancreas and most appendages held along sides; 27 July 2019 **C** lateral view; 2.5 mm manca (M1) #7-31A (2019) eating first meal (shrimp); 5 August 2019 **D** lateral view; manca (M1) eating brine shrimp; 11 August 2019 **E** ventral view; 3.5 mm ♂ manca (M3) #8-6 (2019) with red hepatopancreas from eating shrimp; arrows at developing 7th pereopods crossed under 6th pereopods; 18 April 2020 **F** ventral view; 3.5 mm ♂ manca (M3) #8-6 (2019) with red hepatopancreas; arrows at developing 7th pereopods, P's point to penes; 18 April 2020.

these 9 mancas were: M1 65–123 days ($\bar{X}$ = 103.2 days, n = 9), M2 59–84 days ($\bar{X}$ = 71.5 days, n = 2), M3 58–65 days ($\bar{X}$ = 61.5 days, n = 2), total 254–268 days ($\bar{X}$ = 261 days, n = 2). This is a long time for isopod manca development and is compared to other species in the section on Life cycle stages.

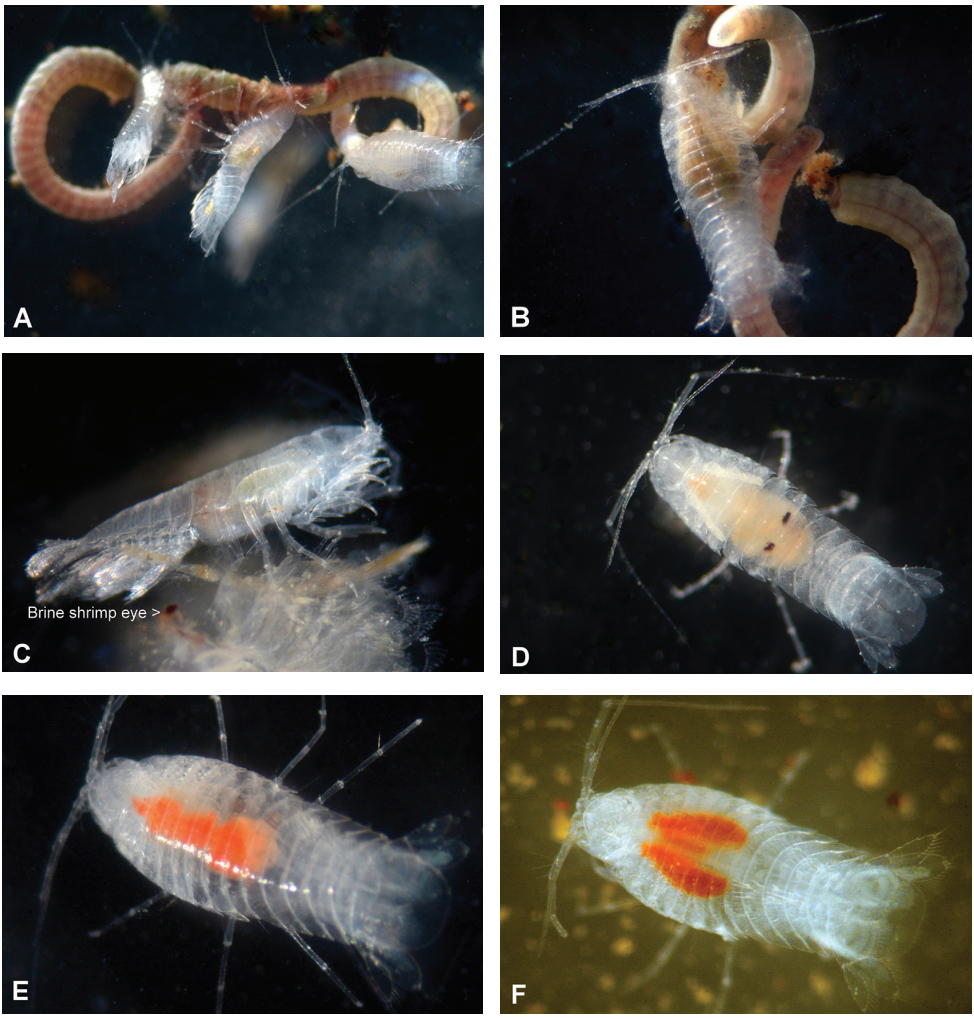

Figure 6. *Bahalana geracei* mancas feeding **A** lateral and dorsal views; ~2.5 mm mancas (M1) eating California black worm; 27 August 2019 **B** dorsal view; ~2.5 mm manca (M1) with full gut from eating worm; 27 August 2019 **C** lateral view; ~2.5 mm manca (M1) #7-30 (2019) eating brine shrimp; eye at arrow was eaten and shows in next photo; 11 August 2019 **D** dorsal view; ~2.5 mm manca (M1) #7-30 (2019) with dark brine shrimp eyes in stomach; 11 August 2019 **E** dorsal view; ~2.5 mm manca (M1) #7-27A (2019) with red gut after eating cooked shrimp; 21 October 2019 **F** dorsal view; ~2.5 mm manca (M1) #7-27A (2019) with red hepatopancreas 10 days after eating cooked shrimp; 31 October 2019.

Unfortunately, many mancas refused to eat anything, and others stopped eating after a few meals. Fasting was often related to preparation for molting; mancas usually fasted for 1–5 weeks before molting and 1–2 weeks afterwards. It took 1–4 days ($\bar{X}$ ~ 2.0, n = 9) between molting posterior and anterior halves; once molting was monophasic to leave a complete exuvium. First molts occurred after eating 3–8 meals. Molting seems to be a challenging process required for isopod growth, especially for mancas. Of the 13 mancas from #33 and 6 mancas from #35, none lived long enough to complete their first molts.

Even mancas released on the same day varied in size from 2.3 to 3.3 mm, which provided opportunities for cannibalism. Molts resulted in size increases of 0.3–0.5 mm.

Irregular molting and fasting created additional cannibalism opportunities for mancas that completed their molts to become larger than their smaller (and sometimes fasting) siblings housed with them. Cannibalism was observed only three times for mancas from #5, #33, and #35. After three months all surviving mancas were housed separately.

Oostegite-bearers

After mancas are released from a female's marsupium, she retains her oostegites for several months until her next molt, which I call an "oostegite molt." Oostegite-bearers have rarely been observed in other cave cirolanids. Botosaneanu et al. (1986) pointed out that, "Concerning the reproduction, it is interesting to note that ovigerous females or females with brood plates or pouches, were apparently never found in the subterranean species (this was expressly noted, for instance, for *Antrolana*, *Bahalana*, some *Typhlocirolana*…); this phenomenon still awaits explanation (one published explanation being that ovigerous females are very rare and secretive, rarely foraging in areas accessible to sampling)." In their description of a single oostegite-bearing specimen of their new species *Zulialana coalescens* Botosaneanu & Viloria, 1993, the authors re-emphasized the rareness of this phenomenon by noting that, "this is one of the very few known cases of subterranean cirolanids where specimens with oostegites were found (to the best of our knowledge the only already known case being that of *Skotobaena*)."

In this study of *B. geracei* it was surprisingly common to find females with oostegites. In fact, out of 1047 adult females collected in Lighthouse Cave, 167 (= 16.0%) were oostegite-bearers (Fig. 2). These females had released mancas from marsupia within the previous few months, and some molted their oostegites 3–13 months later in culture (Table 5). The six smallest oostegite-bearers (green) were in the 5 mm class with many more (n = 49) in the 6 mm class, followed by declines to a low of one in the 11 mm class, then a surprising increase in the last 5 size classes. Since oostegite-bearers were found in all size classes larger than 5.9 mm, this is a strong indication that females were capable of having multiple broods over their long lifetimes. More individuals probably stayed in their larger instars longer (including oostegite-bearing) because of slower molt cycles.

Non-breeders

About 50% of all females collected (526 of 1047) did not have detectable eggs or oostegites, so they were considered non-breeders. This group included: (1) pre-reproductive females that were too young and small to produce detectable eggs, (2) inter-cycle females that had completed a reproductive cycle (including release of mancas and shedding of oostegites) and had not yet produced a new set of detectable eggs, and (3) post-reproductive females that were larger/older and seemed to have stopped reproducing. The smallest females in the 3, 4, & 5 mm classes (pink) were presumed to be pre-reproductive,

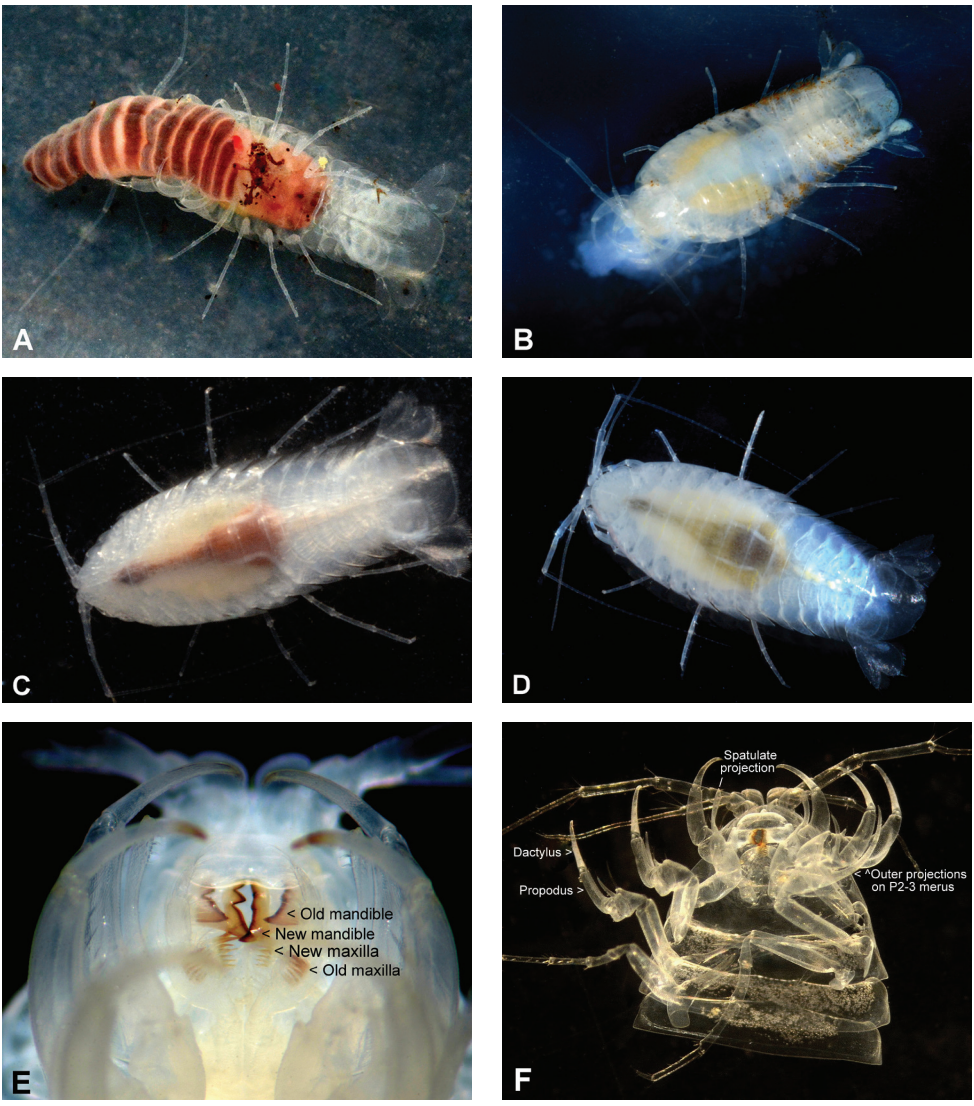

Figure 7. *Bahalana geracei* adults, feeding and molting **A** ventral view; 6.0 mm ♀ #7 (2016), pereopods 1–3 holding earthworm, worm in gut; 7 January 2017 **B** dorsal view; 9.0 mm ♀ #1 (2013), pereopods 1–3 holding shrimp piece forward to eat; shrimp in gut; 21 January 2013 **C** dorsal view; 5.5 mm ♂ #18 (2016), 4 weeks after eating earthworm, visible in gut; 26 November 2017 **D** dorsal view; 8.7 mm ♂ #37 (2018), 4 weeks after eating centipede; white fecal pellets forming in hind gut; 30 May 2020 **E** ventral view; 9.5 mm ♀ #10 (2018), double mandibles and maxillae before molting; 10 July 2020 **F** ventral view; anterior exuvium from 6.3 mm ♀ #23 (2018); 24 September 2019.

although some might have been producing eggs that were too small to be detected. The 6 mm class (yellow) had the greatest number of non-breeders (80) and likely consisted of a mix of pre-reproductive females and inter-cycle females. The largest non-breeders likely

consisted of inter-cycle and post-reproductive stages; the number of large inter-cycle females may have increased with size partly because this recovery stage should require more energy and time after larger broods. Non-breeders and other females gradually declined to lows in the 11 mm range (red), presumably because of mortality.

Brooders from caves

There have been few reports of cave cirolanid females brooding eggs or mancas within their marsupia. In their description of *Yucatalana robustispina* (Botosaneanu & Iliffe, 1999) the authors mentioned that a "female allotype has a well-developed marsupium in which 3 very large eggs were found." Botosaneanu and Iliffe (2000) later remarked that one additional female specimen of *Y. robustispina* was caught and "deserves a special mention, because it has 10 pulli in its marsupium–a remarkably high number for a stygobitic cirolanid." Messana (2020) reported that one female in his type series of *Catailana whitteni* was "ovigerous, 15.4 × 4.6 mm, bearing 9 eggs in brood pouch."

Brooding female *B. geracei* were also rare in our cave collections, so they are not shown in Fig. 2. Out of 1047 females collected in Lighthouse Cave, only four were incubating eggs or mancas. Here are notes regarding each of them, recorded soon after they were collected:

1. 17 July 2000, #92. 7.2 mm ♀ with ~12 large eggs in marsupium; 6 Nov. 2000, #92 in mud-bottomed container had 11 mancas, most are healthy and active; mother appears to have 2 mancas inside (consistent with original estimate of ~12 large eggs on 17 July); mother was never observed to dig into mud and remained moderately active, so the hypothesis of pregnant ♀♀ being rare due to hiding in substrate still needs confirmation.
2. 22 July 2002, #1. 14.8 mm ♀ with 11 mancas in film can; had 21 more in next 4 days.
3. 16 July 2003, #67. 7.0 mm ♀ with oostegites & 2 mancas (2.5 mm) in film can.
4. 11 July 2006, #41. 8.4 mm ♀ with 1 manca in marsupium & 2 (2.6 mm) in film can.

Probable explanations for why so few brooders were collected are covered in the discussion section on Gestation. Fortunately, considerable information about brooders was obtained from successful breeding in captivity, described above in Gestation. They are also included later in Table 7 Life cycle stages.

Feeding behaviors

Feeding in culture and in caves

Individual records were kept for dozens of *B. geracei* specimens that were collected in the caves, then raised under laboratory conditions for up to seven years. They were measured

approximately every six months and/or after a molt. Measurements were made before feeding because a full meal could increase length by ~20%. Food was offered every ~3–6 weeks even when food was still visible inside them, and they often still accepted the food.

As mentioned in culture methods and in descriptions of manca feeding, *B. geracei* ate a large variety of foods. Live food such as California black worms, earthworms, ghost shrimp, and brine shrimp were attacked and eaten while still alive. Species in the genus *Bahalana* can be distinguished from all others in the family Cirolanidae because pereopods 1–3 (P1–3) are prehensile with the two distal segments (dactylus and propodus) elongated and with long projections on several segments (especially the merus) (Fig. 7F). Photographs showed that P1–3 were used to grasp and manipulate prey (Fig. 7A, B), while mandibles pulled food into the mouth. Pointed tips of P1–3 often penetrated prey tissue, but projections on the outer side of P2–3 were often held away from prey (see lower side of worm in Fig. 7A). Other possible functions for lateral projections are described in the Discussion section.

When dead food, such as a piece of shrimp, was placed close to a *B. geracei*'s head it was often attacked right away. If food was placed further away, isopods usually increased searching activity until the food was found, sometimes while "dancing" rapidly with head down near substrate and tail up, apparently following a scent trail. However, it was not unusual for them to wait 30 minutes or more before eating. Individuals took about 1–30 minutes to complete their meals, which roughly corresponded to the amount of food ingested. Food intake and the digestive processes were easily monitored since *B. geracei* exoskeletons are relatively clear. Dark food such as earthworms, spiders, and centipedes could be seen in enlarged digestive tracts, sometimes for several weeks (Fig. 7C, D). When shrimp of any kind (commercial, brine, ghost, or *B. cubensis*) was eaten, the hepatopancreas often turned red or orange as digestion proceeded (Fig. 6F).

Digestion time varied widely depending on size and type of meal. If a small liquid meal was eaten (e.g., body fluids from prey) it was processed as quickly as 2–4 days, and fecal pellets were not formed. More often the food consumed consisted of muscle (e.g., cooked shrimp) or other internal and external body parts (Fig. 7C, D), which often took 3–8 weeks to digest and for the gut to clear; fecal pellets started forming in a few days, and fecal pellets or strings were passed about 2–8 weeks after eating.

Experiments were performed to observe interactions between *B. geracei* and other crustaceans that live in Lighthouse Cave. When 14.3 mm female #39 (1995) was placed in a bucket with a 6 cm red shrimp *B. cubensis*, within 3 minutes the shrimp grabbed the isopod, but a few seconds later the isopod pulled off the shrimp's leg and ate on it for 15 minutes, which turned its gut red. However, when 7.2 mm female #53 (1996) was left overnight with a 5 cm *B. cubensis*, the shrimp ate the inside of the isopod, leaving an empty exoskeleton. In other trials, live small (1–2 mm) *N. stocki* isopods from Lighthouse Cave were readily eaten by *B. geracei* adults and mancas, which turned the gut white or gray.

In some years (n = 13) I made notes when freshly collected specimens clearly had food in their guts. In 7 years, only 2–7% of specimens had food; in 6 years, 17–50% had food. Three days after hurricane Bertha hit in 1996, salinity in Lighthouse Cave dropped to ~25 ppt, and 37 of 88 specimens (= 42%) had food in gut, possibly from

food that washed in. The color of the gut hinted at probable food consumed: white for another *B. geracei* or a *N. stocki* isopod; red, pink, or orange for *B. cubensis*; brown or black from terrestrial arthropods (e.g., insects or spiders). A few specimens were dissected to examine gut contents, but this usually revealed nothing identifiable. However, specimens raised in the laboratory that ate pieces of arthropods (e.g., brine shrimp and centipedes) sometimes passed feces with remains of exoskeletons.

Cannibalism

Cannibalism has often been reported in isopods. Wong and Moore (1995) described the cirolanid *Natatolana borealis* (Lilljeborg, 1851) as a voracious omnivorous scavenger, and "Cannibalism of damaged or moulting individuals was observed frequently in the laboratory." Jormalainen and Shuster (1997) studied cannibalism in the freshwater sphaeromatid Socorro isopod *Thermosphaeroma thermophilum* (Richardson, 1897) and found that, "In laboratory containers without refuges, males cannibalized females, males and females cannibalized mancas, and mancas cannibalized each other, even in the presence of alternative food." Studies performed by one of my students, Ron Bitner, showed similar cannibalistic behavior for *B. geracei*; when 2–4 individuals were housed together, all sizes and genders were susceptible to cannibalism by individuals of the same size or larger (n = 6). However, in 10 other trials, 2–4 specimens of various sizes and genders were together >1 month without cannibalism (unpublished observations presented at 1997 Kentucky Academy of Science meeting).

Only once was cannibalism observed directly in Lighthouse Cave. On 4 July 1995, I saw a large *B. geracei* on a rock, but it did not start swimming when touched with an aquarium net. When it was maneuvered into the net, I saw it was holding and eating another *B. geracei*. Later examination showed the cannibal was an 11.0 mm female, while the victim was a 6.5 mm female (still barely alive). On another occasion (27 July 1999), while 14.0 mm female #102 (1999) was being measured soon after capture, I noticed her gut was full and white; dissection revealed the remains of a small *B. geracei* inside her stomach. Large female isopods could be more important predators than mangrove rivulus fish. A broader perspective is covered in the discussion section on Cannibalism.

Molting, fasting and starvation resistance

Most laboratory-raised *B. geracei* adults fed regularly, usually every month, but some refused food for several consecutive months, then started eating again. Others died after several months of fasting, probably because they had trouble with some aspect of molting; this was especially true for mancas, juveniles, and large adults. This emphasizes a common problem in keeping isopods and other crustaceans alive for long periods–they often have problems molting. This has been noted by other researchers such as Vogt (2018), who stated that in his "laboratory population of marbled crayfish, more than 85% of the adults died during ecdysis." Molting problems may be compounded for *B. geracei* by the long extensions on pereopods 1–3.

It was routine for adult *B. geracei* to fast for 1–3 months before a molt, when feeding structures could not function; for instance, several days before a molt, double mandibles and maxillae appeared, as seen in Fig. 7E. The time between molting posterior and anterior halves was ~2–7 days. Fasting persisted for 1–3 weeks after molting while feeding structures hardened. The fasting routine associated with molting gives crustaceans some natural resistance to starvation.

A dramatic example of starvation resistance in *B. geracei* came in June 2015 as I was cleaning film cannisters for my 2015 cave trip; I found one cannister that still had a 7.3 mm female isopod in it from a field trip at least two years before. This cannister had the usual 35 ml of saltwater and had had no water changes or aeration. This female later ate and seemed to have no negative effects from this extended fasting experience. Another extreme example is the deep-sea isopod that fasted for >5 years in a Japanese aquarium (for details, see Growth rates and longevity, below). In general, older/larger individuals have more reserves so they can probably survive pre-molt fasts much longer. Starvation resistance is particularly important for brooding females so they can apparently remain safely hidden as they fast for six months. The broad impact of this phenomenon on crustaceans in general, and especially on cave crustaceans, is described in the discussion section on Starvation resistance.

Growth rates and longevity

Growth rates in general

For many years people have asked me, "How long do your isopods live?" My reply has been, "I estimate they could live as long as 20–35 years, since the growth rates in all stages of their life cycle are extremely slow." But several variables make it difficult to accurately determine growth rates and longevity for long-lived crustaceans like *B. geracei*. These variables include: (1) higher temperatures usually create faster growth, which is probably not a major variable in this study, since lab temperatures were usually close to cave temperatures at ~25 °C, (2) food is in low supply in caves, but abundant in culture, (3) length of female molt cycles vary with their reproductive condition and age, (4) multiple broods allow for longer life spans, (5) young isopods molt much more often than older ones that may go more than a year without molting, and (6) starvation resistance permits some older slow-growing individuals to appear to be young because they remain small.

This last variable can create misleading estimates of age because a large range of ages can be in the same size class due to variations in molt and growth rates. I call this phenomenon "age compression." It can have the strongest effects in larger size ranges because growth and molt cycles become progressively slower due to reproductive costs and age, and at variable rates. Smaller size ranges were probably affected by age compression, too. For instance, "all females" in Fig. 2 increased from 123 in the 5 mm class to 266 in the 6 mm class, probably because the age range for 5.0–5.9 mm females was ~2–3 years, while the age range for 6.0–6.9 females was probably ~3–6 years.

According to Vogt (2018), "Precise data on longevity can be obtained only by rearing in captivity from hatching to death and by long-term marking with internal tags. In practice, most life span data are calculated from growth models based on length-frequency distribution, mark and recapture, and the analysis of molt increment, intermolt duration, and reproduction parameters." Vogt (2018) also stated that, "These indirect aging techniques have a small probability of error at younger ages but a large one at older ages. Therefore, in long-lived species, they give only a rough estimate of life span (Hartnoll 2001; Vogt 2012a)." This is largely due to age compression.

All the above methods were used in this study except for long-term marking with internal tags, which were not used because of the small size of *B. geracei*. I was not able to keep any specimens alive for an entire life span of >20 years, but many individuals of different sizes and reproductive conditions survived for several years to give a reasonably accurate picture of their lives as presented in Tables 1–7. Vogt (2018) pointed out that longevity can be expressed in several ways: age of oldest specimen, age of oldest cohort, mean age of oldest 10% of population, or maximum age estimated by growth models. The last method was used for this study.

Determining precise longevity in *B. geracei* was complicated because body length measurements varied considerably depending on when measurements were taken relative to feeding, molting, and stage of reproduction. A large meal could increase body size by 20%, followed by gradual return to normal over 1–2 months of digestion (so, specimens measured upon capture sometimes shrank over the next few weeks). Size sometimes increased by ~20% immediately after molting as water was absorbed to expand the new exoskeleton, then part of that gain was lost over the next few days. Females also increased length by ~10–20% while growing eggs and embryos, then lost some of that increase when mancas were released. All these variables were considered in developing and analyzing the following estimates of growth and longevity.

In the early years of this study (1993–1996) estimates of longevity were based on morphometrics: observed changes with each molt (n = 44) and length of intermolt periods ($\bar{X}$~12 months). For instance, the number of telson setae on the posterior end ranged from 11 in mancas (M1) to 60 in large females, increasing by 1–5/molt ($\bar{X}$~2); with an average of 1 molt/year, the increase of 49 telson setae (60–11 = 49) from smallest to largest individuals, divided by 2 setae/molt, gave an estimate of 49/2 = 24.5 years to develop 49 additional setae. A similar estimate of 28 years longevity was based on increases in the number of flagellar articles in antenna 1: 0–2 articles/molt ($\bar{X}$~0.5), range of 9–23 (increase of 14 articles/life), so 14 articles/0.5 articles/molt = 28 molts = 28 years. A third estimate of 35 years longevity was based on increases in the number of flagellar articles in antenna 2: 0–5/molt ($\bar{X}$~1), range of 15–50 (increase of 35/life), so 35 articles/1 article/molt per year = 35 years to produce 35 additional articles. It now appears that these estimates of ~24.5 to 35 years are more reasonable for females, rather than males and females combined, because my sample population had a slightly disproportionate number of females which had longer intermolt periods.

Table 2. Molt and growth records for male *Bahalana geracei* from Lighthouse Cave arranged by size.

Size [mm]	Specimen, year	No. of molts	Months between each molt	Total months	Months/ molt	Molts/ year	Total size increase [mm]	Increase/ molt [mm]	Increase/ year [mm]
5.0	#32, 2018	3	6.0, 5.0, 8.0	19 mo. = 1.6 yr.	6.3	1.9	5.0–6.4=1.4	0.47	0.9
5.5	#4, 2016	5	8.0, 6.0, 4.5, 5.0, 5.0	28.5 mo = 2.4 yr.	5.7	2.1	5.5–8.3=2.8	0.56	1.2
5.5	#30, 2018	4	7.0, 5.0, 5.0, 6.0	23 mo. = 1.9 yr.	5.8	2.1	5.5–7.0=1.5	0.38	0.8
5.5	#58, 2018	3	6.0, 5.0, 8.0	19 mo. = 1.6 yr.	6.3	1.9	5.5–6.5=1.0	0.33	0.6
5.8	#5, 2016	5	5.0, 14.0, 9.0, 9.0, 12.0	49 mo. = 4.1 yr.	9.8	1.2	5.8–8.5=2.7	0.54	0.7
6.0	#21, 2018	2	6.0, 13.0	19 mo. = 1.6 yr.	9.5	1.3	6.0–8.0=2.0	1.00	1.3
6.0	#61, 2018	2	5.0, 15.0	20 mo. = 1.7 yr.	10	1.2	6.0–7.2=1.2	0.60	0.7
6.3	#52, 1995	3	3.0, 6.0, 4.5	13.5 mo = 1.1 yr.	4.5	2.7	6.3–8.0=1.7	0.57	1.5
6.5	#54, 1994	2	14.0, 8.0	22 mo. = 1.8 yr.	11	1.1	6.5–7.7=1.2	0.60	0.7
7.0	#3, 2016	7	8.0, 8.0, 6.0, 4.0, 5.0, 6.0, 7.0	44 mo. = 3.7 yr.	6.3	1.9	7.0–10.0=3.0	0.43	0.8
7.0	#28, 2018	2	12.0, 8.0	20 mo. = 1.7 yr.	10	1.2	7.0–7.5=0.5	0.25	0.3
7.0	#37, 2018	2	9.0, 12.0	21 mo. = 1.8 yr.	10.5	1.1	7.0–8.7=1.7	0.85	0.9
7.5	#12, 2016	4	12.0, 11.0, 13.0, 8.0	44 mo. = 3.7 yr.	11	1.1	7.5–9.0=1.5	0.38	0.4
Totals	n = 13	n= 44	Avg = 7.8				Avg = 1.6	Avg=0.54	Avg=0.8

Growth rates and longevity for males

Many more molt and growth data are now available to provide better analyses, including separate growth rates and longevity estimates for males and females. Table 2 shows molt and growth records for 13 adult males (5.0–7.5 mm long when collected), and each had 2–7 molts (total n = 44); these males were arranged by size to examine the effect of size on molt rates. Months between molts (column 4) were 3–15; the average months/molt (column 6) increased with size: 6.8 months for 5.0–5.8 mm males (n = 5), 8.8 months for 6.0–6.5 mm males (n = 4), and 9.5 months for 7.0–7.5 mm (n = 4). Months/molt were converted to molts/year ($\overline{X}$ = 1.6) in column 7. Total size increases (column 8) were from times of collection to last molts. Average size increases/molt (column 9) ranged from 0.25 to 1.0 mm/molt ($\overline{X}$ = 0.54 mm). Size increases/year (column 10) ranged from 0.3 to 1.5 mm ($\overline{X}$ = 0.83 mm) and were less ($\overline{X}$ = 0.60 mm) for the four largest males (7.0–7.5 mm).

"Increased size/molt" multiplied by "molts/year" yields "increased size/year", which is a logical way to express growth rates. So, how does this relate to longevity? Although 7.0 mm #3 (2016) in Table 2 grew to 10.0 mm in captivity, the size range of adult males collected from Lighthouse Cave was 5.0–9.5 mm; at an average size increase of 0.8 mm/year (column 10), this 4.5 mm growth could occur in ~5–6 years, with ~8–9 molts (4.5 mm growth/0.54 mm/molt = 8.3 molts). Note that fast growers like 6.3 mm #52 (1995) might grow 4.5 mm in only 3 years at his rate of 1.5 mm increase/year, while slow growers like 7.0 mm #28 (2018) might take 15 years to grow 4.5 mm at the rate of 0.3 mm/year. The average length of mancas released from marsupia (instar M1) was ~2.5 mm; length increased by ~0.3–0.5 mm/molt in the next four molts (to instars M2, M3, J1, J2) to approach the 5.0 mm size in Table 2; this early growth occurred as fast as 1–2 years in culture. So, it appears that male *B. geracei* from Lighthouse Cave (at least in culture) would likely live a total of ~6–8 years (probable range is ~4–17 years) with ~13–15 instars (5–6 pre-adult, plus 8–9 adult).

Table 3. Molt records for egg-bearing female *Bahalana geracei* from Lighthouse Cave arranged by size.

Size [mm]	Specimen, year	Reproductive condition	Months to reproductive molt
6.0	#23, 2018	Egg-Bearer	4
6.0	#76, 1994	Egg-Bearer	14
6.0	#47, 1995	Egg-Bearer	3
6.2	#49, 1995	Egg-Bearer	4
7.0	#35, 2018	Egg-Bearer	11.5
7.1	#15, 1993	Egg-Bearer	12
7.1	#60, 1996	Egg-Bearer	8
7.2	#59, 1996	Egg-Bearer	15
7.5	#33, 2018	Egg-Bearer	10
7.7	#18, 1993	Egg-Bearer	24
8.1	#20, 1996	Egg-Bearer	11
9.0	#28, 1993	Egg-Bearer	15
11.0	#5, 2018	Egg-Bearer	7
13.2	#88, 1996	Egg-Bearer	12
15.7	#69, 1996	Egg-Bearer	15
Totals			n = 15, Avg = 11.0

To support this probable range of ~4–17 years, please note three males in Table 2: 5.8 mm #5, 7.0 mm #3, and 7.5 mm #12. These three were retained (along with four other males) from our 30 June 2016 collection in Lighthouse Cave. All three were still alive in October 2020 (= 4.3 years in captivity) after 5–7 molts. Based on above growth rates, they were probably 3–10 years old (with 5–10 instars) when collected, which would now make them 7–14 years old, with ~10–15 instars. Some males probably live even longer in the caves, with irregular food availability resulting in longer molt cycles.

In the next section on Life cycle and population structure, I point out that several male *B. geracei* from Major's Cave grew much larger than those in Lighthouse Cave; the largest was 14.8 mm. If growth rates for males are the same for both caves, and if males in Major's Cave grow an additional 5.3 mm (to 14.8 mm in Major's Cave vs. 9.5 mm in Lighthouse Cave), this might take another 6.6 years at an average increase of 0.8 mm/year. That would give a truly extraordinary longevity for males in Major's Cave of ~12–15 years (probable range is ~10–24 years) with ~23–25 instars. However, molt intervals increased with size and age (typical of crustaceans, as noted by Gilligan et al. 2007), resulting in gradual decreases in annual growth rates/year from an $\bar{X}$ of 0.8 for all Lighthouse Cave males to an $\bar{X}$ of 0.6 for the four largest Lighthouse Cave males (Table 2); so, growth rates for large males in Major's Cave was probably slower and the resulting longevity longer than the above estimates. However, whatever allowed these males to grow larger (e.g., a better food supply) might also have allowed them to grow faster, so the longevity estimate for Major's Cave males is still open.

Growth rates and longevity for females

Determining molt and growth rates for female *B. geracei* was more complicated than for males because of longer life spans and long reproductive cycles with various stages and types of molts. Females had three types of molts. They began life the same as

Table 4. Molt records for oostegite-bearing *Bahalana geracei* from Lighthouse Cave arranged by size.

Size	Specimen, year	Reproductive condition	Months to oostegite molt
6.2	#28, 1995	Oostegite-Bearer	3.5
6.5	#15, 2016	Oostegite-Bearer	8
7.0	#15, 1999	Oostegite-Bearer	7
7.5	#86, 1996	Oostegite-Bearer	3
12.0	#31, 1993	Oostegite-Bearer	13
15.6	#35, 1995	Oostegite-Bearer	9
15.7	#37, 1996	Oostegite-Bearer	13
			n = 7, Avg = 8.07
6.0	#76, 1994	Egg-Bearer->Oost-Bearer	10
6.0	#47, 1995	Egg-Bearer->Oost-Bearer	6
6.0	#7, 2016	Egg-Bearer->Oost-Bearer	6
6.9	#52, 1996	Egg-Bearer->Oost-Bearer	5
7.1	#60, 1996	Egg-Bearer->Oost-Bearer	11
7.7	#18, 1993	Egg-Bearer->Oost-Bearer	11
8.5	#8, 1992	Egg-Bearer->Oost-Bearer	6
9.0	#28, 1993	Egg-Bearer->Oost-Bearer	8
16.3	#37, 1995	Egg-Bearer->Oost-Bearer	12
			n = 9, Avg = 8.33

males, starting with manca 1 (M1) (~2.5 mm) and increasing by ~0.3–0.5 mm/molt with regular growth molts to the next four instars (M2, M3, J1, J2) to approach the 5.0 mm size. They started producing eggs at ~4.0–4.9 mm (see Fig. 2), which took ~9–24 months before their reproductive (parturial) molts that produced oostegites forming the marsupia. This was sometimes followed by mating, then brooding for 5.5–6.0 months. After mancas were released, oostegite molts produced new oostegite-free exoskeletons; this happened ~2–16 months after brooding (longer for larger females). A few oostegite-bearers also had eggs, indicating they had started producing eggs for the next reproductive cycle before their oostegite molts.

Data to show these complex molt and reproductive cycles are presented in Tables 3–5. The summary at the end of this section gives an estimated life span for females of ~25–28 years, with a total of ~23–30 instars. Although the following details are somewhat tedious, it is important for me to present my data, methods, and rationale for these extraordinary estimates for future discussions and comparisons.

Table 3 shows the number of months after 15 egg-bearers completed egg production and had reproductive molts ($\overline{X}$ = 11 months). These females had been bearing eggs for several months (exact number undetermined) before being collected, which partly explains the wide range of 3–24 months (plus, the trend of more months for larger females), so the actual time spent in this stage is likely to be ~9–24 months, with 16 months as a possible average.

Table 4 shows two sets of oostegite-bearers; the first seven specimens had oostegites when collected (having released mancas an undetermined number of months before), then underwent oostegite molts 3–13 months later in captivity ($\overline{X}$ = 8.07 months). The next nine specimens were egg-bearers when collected, they had reproductive molts, did not mate, and then had their oostegite molts 5–12 months later ($\overline{X}$ = 8.33). In both sets the number of months increased with size. The oostegite molts for those

Table 5. Molt and growth records for non-breeding *Bahalana geracei* from Lighthouse Cave arranged by size; G = growth molts, R = reproductive molts, O = oostegite molts.

Size [mm]	Specimen, year	Molt no.	Months between molts	Total months	Mo./ Molt	Molts/ Year	Total size increase [mm]	Increase/Molt [mm]	Increase/year [mm]
			Pre-reproductive						
3.9	#57, 1995	4	3(G), 6(G), 8(G), 5G)	22 mo = 1.8 yr	5.5	2.2	3.9–5.6 = 1.7	0.4	0.80
4.2	#3, 1996	1	11(G)	11 mo = 0.9 yr	11	1.1	4.2–4.4 = 0.2	0.2	0.20
4.5	#35, 1993	1	13(G)	13 mo = 1.1 yr	13	0.9	4.5–4.8 = 0.3	0.3	0.25
5.8	#36, 1993	1	13(G)	13 mo = 1.1 yr	13	0.9	5.8–6.5 = 0.7	0.7	0.70
	n = 4	n = 7	**Avg. for 7 growth molts = 8.4 mo.**					Avg = 0.4 mm	Avg = 0.49
			Inter-cycle						
6.5	#15, 2016	4	8(O), 15(R), 7(O), 7(G)	37 mo = 3.1 yr	9.2	1.3	6.5–9.0 = 2.5	0.6	0.80
8.8	#50, 1995	3	4(G), 7(G), 9(R)	20 mo = 1.7 yr	6.7	0.6	8.8–9.4 = 0.6	0.2	0.35
	n = 2	n = 7	**Avg for 3 growth molts = 6.0 mo.**					Avg = 0.4 mm	Avg = 0.58
Totals			**Avg for all 10 growth molts = 7.7 mo.**						
			Post-reproductive						
11.0	#21, 1995	0	10 meals, no molts	23 mo = 1.9 yr					0.0
14.3	#39, 1995	0	12 meals, no molts	14 mo = 1.2 yr					0.0
15.3	#38, 1995	0	12 meals, no molts	14 mo = 1.2 yr					0.0
16.5	#22, 1995	0	15 meals, no molts	24 mo = 2.0 yr					0.0
16.8	#71, 1996	0	10 meals, no molts	17 mo = 1.4 yr					0.0
	n = 5	n = 0							

that did not mate (second set) probably occurred sooner after reproductive molts than might be expected because they did not spend 5–6 months brooding, and because they could reabsorb nutrients from their unfertilized eggs, rather than spending more energy brooding.

Table 5 shows molt and growth records for a few females designated as non-breeders, since they were not egg-bearers, oostegite-bearers, or brooders. This table includes: (1) pre-reproductive females that were too young and small to produce detectable eggs, (2) inter-cycle females that had completed a reproductive cycle (including release of mancas and shedding of oostegites) and had not yet produced a new set of detectable eggs, and (3) post-reproductive females that were larger/older and probably had stopped reproducing.

The first set in Table 5 shows four small (3.9–5.8 mm) pre-reproductive females that had a combined total of seven growth molts (no reproductive or oostegite molts). The smallest one (3.9 mm #57, 1995) was collected as a manca (M3), went through four more instars (J1, J2, J3, J4) in 22 months and grew 1.7 mm to yield a growth rate of 0.8 mm/year (surprisingly similar to growth rates cited above for males and oostegite-bearers). The other three pre-reproductive females each molted only once in 11–13 months, with size increases of only 0.2–0.7 mm/year.

The second set in Table 5 shows molt and growth records for two mid-sized inter-cycle females that had a mix of molts (G = growth molts, R = reproductive molts, and O = oostegite molts). The first one is 6.5 mm #15 (2016), which is very important

because it accurately shows the time for several stages of the life cycle. She was collected with oostegites, which she shed in 8 months (so she is also listed in Table 4 Oostegite-bearers). Within 6 months, she produced eggs that were clearly visible; she had her reproductive molt 9 months later (15 months after her oostegite molt), so she was recognizable as an egg-bearer for only 9 months. Note that there were no growth molts before more eggs were produced, so she did not enter an inter-cycle stage. She had a second oostegite molt 7 months after her reproductive molt, followed by a growth molt after another 7 months; she then produced more eggs that were visible in 5 months and later deteriorated. So, this last time she did have an inter-cycle stage of 7 months. She grew 2.5 mm during this 3.5-year process (0.7 mm/year). So, except for brooding, this female went through two complete reproductive cycles in 3.5 years, which is strong evidence that females are capable of multiple broods (iteroparous), one right after the other without any growth molts in between.

The other inter-cycle female was 8.8 mm #50 (1995), collected without eggs or oostegites; she had two consecutive growth molts (at 4 and 7 months), followed by egg production and a reproductive molt after 9 months; she grew only 0.6 mm in 1.7 years (0.35 mm/year). This 8.8 mm inter-cycle female is probably the best representative of non-breeders in the 8 mm size range, and the 11 months (4 + 7) preparing for her two growth molts may be a good estimate of the time inter-cycle females often spend recovering from brooding, at least near the 8 mm range.

The third set in Table 5 lists five relatively large females (11.0–16.8 mm) that never had eggs, oostegites, or molts of any kind during their 14–24 months in captivity. These were active females that fed regularly (10–15 meals apiece). I use them as examples of post-reproductive females because it seems unlikely that they would have produced more eggs (based on the low percentage of egg-bearers in these size ranges), and it shows that some large/old females may have extremely long intermolt periods that strongly extend their life spans.

Figure 2 (see section on Reproduction and development) provides a way to support the above estimates for how much time females of a specific size (e.g., the prime breeding ranges) were likely to spend in each reproductive condition, since the times should be roughly proportional to the numbers collected in each condition. For instance, if a female spends 16 months as an egg-bearer and 8 months as an oostegite-bearer, she is twice as likely to be captured as an egg-bearer vs. an oostegite-bearer. In the three size ranges with the most egg-bearers and oostegite-bearers (6.0, 7.0, 8.0 mm), 586 females were collected: 277 egg-bearers (137 + 99 + 41 in the 6, 7, and 8 mm size ranges, respectively) (277/586 = 47%), 114 oostegite-bearers (= 19%), and 195 non-breeders (= 33%). These numbers are roughly proportional to the average times estimated for each reproductive condition in Tables 3–5 (with a total of 16 + 8 + 11 months = 35 months): 16 months for egg-bearing (16/35 = 46%), 8 months for oostegite-bearing (8/35 = 23%), and 11 months for recovering inter-cycle females (11/35 = 31%).

If we add 6 months for brooding (after 16 egg-bearing months), that should give a reasonable estimate for an entire reproductive cycle: 16 + 6 + 8 + 11 = 41 months, or nearly 3.5 years! However, it would likely be considerably shorter in younger/smaller

reproductive females that tend to have shorter intermolt periods. For instance, 6.2 mm female #49 (1995) (described above in the section on Mating) molted 51 days after releasing mancas (oostegite molt) and again 4 months after that (growth molt), so her cycle could have been: 16 (egg-bearing) + 6 (brooding) + 2 (oostegite-bearing) + 4 (inter-cycle recovery) = 28 months. Thus, a range of ~2.0–3.5 years seems to be a reasonable estimate for female *B. geracei* reproductive cycles.

If we can determine the growth rate during a reproductive cycle, that should tell us how many broods are likely in a long-lived female and ultimately provide insight into longevity. If the average increase/molt during an entire reproductive cycle was near the average for males, females would average ~0.5 mm/molt X 3 molts/cycle = 1.5 mm in 2.0–3.5 years. However, it is likely that growth during a female's reproductive cycle would be slower than growth for males since brooders fast for ~6 months, and a major portion of food consumed during the cycle would go to egg and embryo development. Gilligan et al. (2007) noted that in most crayfish, "mature females divert energy to egg production as opposed to growth and therefore grow more slowly than mature males." Overall, it seems reasonable to estimate ~1.0 mm growth for a reproductive cycle lasting ~2.0 years (= 0.5 mm/year; 1.5 molts/year) for smaller *B. geracei* females.

In the above description of Table 5 and growth rates I described the important sequence for 6.5 mm #15 (2016) that was oostegite-bearing when collected, so she had already had one brood; she then produced another set of eggs (without an inter-cycle growth molt), had a reproductive molt, followed by an oostegite molt and a growth molt, before producing more eggs. This tells us that oostegite-bearers in the 5–6 mm size ranges can have at least two consecutive broods, with the second brood being released by oostegite-bearers in the 7 mm range. It is likely that these reproductive cycles each take ~2.0–3.5 years.

In Fig. 2 we can see that the next size range (8.0–8.9 mm) had only 18 oostegite-bearers, compared to 49 in the 6 mm range and 47 in the 7 mm range; this was a major decline of 62%. There was also a 45% decline in the entire female population (only 113 in the 8 mm class compared to 207 in the 7 mm class, presumably from increased mortality). This may indicate that only about half the females had a third consecutive brood soon after their first two, possibly due to the physical toll of producing two broods, including two six-month long fasts.

The eight size ranges >8.9 mm continued to show decreases in the percentage of egg-bearers, as females either died or spent more time as oostegite-bearers, or in the inter-cycle recovery stage, or eventually as post-reproductive. So, most females probably had one reproductive cycle in the 6 mm range, one in the 7 mm range, about half probably had a 3rd brood in the 8 mm range, and some had additional broods in the 9–17 mm ranges as indicated by the 47 large oostegite-bearers.

So, if most females produced 2–3 broods while ~6.0–8.9 mm long, what was the probable growth rate and longevity for the remainder of their lives at 9.0–16.9 mm? This is an important part of the life cycle, since it represents a substantial part of the population (out of 1047 females collected in Lighthouse Cave, 279 were in the 9.0–16.9 mm ranges = 27%); it is also where many females spent the longest parts of their lives, since growing and molting processes are slowed. But it was also difficult to

determine growth rates in these size ranges because large females had lower survival rates in captivity and molts were less common.

Tables 3–5 show the few molt records available for large females, along with those of smaller females. The four large egg-bearers (9.0, 11.0, 13.2, & 15.7 mm) in Table 3 had reproductive molts at 15, 7, 12, and 15 months ($\bar{X}$ = 12.2 months). In Table 4 the three large females collected as oostegite-bearers (12.0, 15.6, & 15.7 mm) had oostegite molts at 13, 9, and 13 months ($\bar{X}$ = 11.7 months); the two egg-bearers that had oostegite molts (9.0 & 16.3 mm) molted 8 and 12 months after their reproductive molts ($\bar{X}$ = 10.0 months). And the five large females (11.0 to 16.8 mm) in Table 5 never molted while in captivity for 23, 14, 14, 24, and 17 months ($\bar{X}$ = 18.4 months). This mix of 14 intermolt periods probably gives a good overall picture of instar lengths for large females, with an average of 14.0 months: (15 +7 + 12 + 15) + (13 + 9 + 13) + (8 + 12) + (23 + 14 + 14 + 24 + 17) = 196; 196/14 = 14.0 months).

This average instar length of 14.0 months is nearly twice (actually 1.8 times) the average instar length of 7.8 months for males (Table 2, column 4 = months between molts); males had an average increase/molt of 0.54 mm (Table 2, column 9) and an average increase of 0.8 mm/year (Table 2, column 10). So, the average increase/year for large females would likely be ~1/2 of 0.8 mm/year = ~0.4 mm/year. That would mean that if large females grew 7.9 mm (from 9.0 to 16.9 mm) that would take 7.9/0.4 = 19.75 years, and 7.9 mm of growth at the rate of 0.54 mm/molt = 14.6 molts.

To summarize, longevity estimates for female *B. geracei* are exceptional. Longevity is estimated to be 25–28 years: 2–3 years pre-reproductive (2.5 mm-6.0 mm) + 4–6 years producing 2–3 broods (6.0–8.5 mm) + up to 19 years mostly post-reproductive. Females could probably have a total of 23–30 instars: 6–8 pre-adult + 5–7 for 2–3 reproductive cycles + 12–15 while mostly post-reproductive. These are extraordinary estimates for any isopod species, but especially for one living in warm water (25–26 °C). However, growth rates for *B. geracei* maintained in captivity and fed regularly were probably faster than for those animals living in the caves with low food supply, so the life span could be even longer than the above estimates. Possible explanations for such long life spans are analyzed in the discussion section on Growth rates and longevity.

Life cycle and population structure

Many aspects of the *B. geracei* life cycle have been covered in preceding sections. Now I want to further compare the numbers for each stage and give an overview of the population. These are best covered by elaborating on Table 1 (introduced after Methods and materials), on new Tables 6, 7, and on Fig. 8.

Table 1 Numbers and sizes from Lighthouse Cave

This table summarizes 23 years of collections of *B. geracei* in Lighthouse Cave from 1978–2018, with numbers and sizes of mancas, males, and females. (There are two

entries for 2013 because collections were made in January and June.) Totals for each year are shown on the right side. In most years we were able to collect >50 specimens, which is unusually high for stygobitic cirolanids; possible explanations for this are in the discussion on Population size. The population appeared to be reasonably stable in most year, with similar proportions from year to year for mancas, males, and females.

Reproduction appeared to be continuous and probably not seasonal, based on the nearly constant presence of females in all stages of the reproductive cycle, except for brooders that stay hidden. Even though the isopods tended to not swim very often or very far, the population was not confined to the room where we typically collected them. When we collected in that same room a second or third time within a few days, sizeable samples were still collected, indicating considerable movement of isopods from other parts of the cave. Also, we usually saw many when we explored other parts of the cave. Although Lighthouse Cave is relatively confining for us as collectors, isopods can probably move freely through the water table and porous limestone to other parts of the island, including other caves.

Fluctuations in numbers of specimens collected each year seemed to be due mostly to the number of collectors and our proficiency, rather than to large changes in the population size. There are good reasons why fewer than 11 specimens were collected in four years. In our first visit to Lighthouse Cave in 1978 we did not have proper collecting equipment, and the five specimens (used for the type series) were caught with our hands (without nets) as they swam toward the surface. In 1979 we had collecting equipment, but our flashlights were relatively weak. In 2013 and 2014 specimens were unusually difficult to find; in June 2013 there were so many white microbial clumps and strands growing on almost everything (rocks, dirt, and sponges) and floating free in the water, that it was hard to identify the white *B. geracei* unless they were swimming. The deteriorated water quality was a concern for many of us at the Gerace Research Centre. It was thought that it may have been associated with too many visitors with sunscreens or insect repellants, so cave explorers were advised to refrain from using these chemicals in the future. Fortunately, water quality and *B. geracei* populations returned to normal by 2016.

Table 1 includes all manca stages combined (M1, M2, and M3) in the second column. They ranged in size from 2.3–4.0 mm, which were similar to sizes of mancas raised from laboratory broods; size ranges for the three manca stages are shown in Table 7. The total number of mancas collected was 92 = 6.6% of all 1383 Lighthouse Cave specimens. Since mancas are the smallest stages, they are much harder to find, and the numbers collected are not a good measure of birth rates.

One of the most striking patterns shown in Table 1 is that every year females were larger and more numerous than males. The percentage of males in the total adult population ranged from 0% (2013 and 2014) to 36.8% (7 males + 12 females in 2016). The total for all years was 1291 adults with 244 males (= 19%). Only one of these 244 males was >8.5 mm; that was 9.5 mm #19 (2000). The consistency of these

female-biased ratios suggested that some basic biological phenomena were at work. However, it eventually became clear that the size of males was not strictly limited by an inherent biological phenomenon (e.g., genetics), since a few males kept in captivity for several years grew to >9.5 mm.

Furthermore, samples of *B. geracei* from Major's Cave showed that males can be nearly as numerous and grow to be nearly as large as females. On 28–29 July 1999 we collected 35 *B. geracei* in Major's Cave: 10 mancas (3 M1-M2, 7 M3), 10 adult males, and 15 adult females (10 males out of 25 adults = 40%); this was a higher percentage than in any collection in Lighthouse Cave. Even more dramatic were the sizes of these 10 males (4.2, 7.0, 7.0, 8.0, 9.6, 10.6, 11.0, 12.2, 12.5, and 14.8 mm; $\bar{X}$ = 9.7 mm); that is, 6 of these 10 males were larger than any ever found in Lighthouse Cave! The 15 females ranged in size from 4.8–16.0 mm, $\bar{X}$ = 10.2 mm.

Table 6 Major's Cave males and females

This table combines data of *B. geracei* collected in Major's Cave from 1999, with collections from 2000–2004, making a total of 21 males of 52 adults = 40%. Figure 8, Graph A (Lighthouse Cave) and Graph B (Major's Cave) compare the dramatic differences in the two populations, indicating the strong influence the environment can have. One key difference in the two caves is that mangrove rivulus fish are known predators of isopods in Lighthouse Cave, while these fish have not been found in Major's Cave. Other possible explanations for these differences are found in the discussion section on Life cycle and population structure.

Figure 8 A Lighthouse Cave males and females

Another interesting pattern for the Lighthouse Cave population is shown in this graph. The size distribution follows a normal distribution by size (bell-shaped curve) until the dip at 11.0–11.9 mm, which is then followed by increases in the largest sizes. This puzzling pattern has been shown in some shrimp species (see Conides et al. 1994, Relini and Relini 1998), but researchers did not give adequate explanations. I hypothesize that four factors may be responsible for this pattern in *B. geracei*: (1) larger females should have a survival advantage by cannibalizing smaller *B. geracei*, (2) larger females may have less stress from brooding if they are post-reproductive, (3) mangrove rivulus

Table 6. Number of post-manca specimens of *Bahalana geracei* from Major's Cave (1999–2004) by 1 mm size ranges and sex.

Sex	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	8.0–8.9	9.0–9.9	10.0–10.9	11.0–11.9	12.0–12.9	13.0–13.9	14.0–14.9	15.0–15.9	16.0–16.9	Total & %
Males	1	2	2		4	2	1	2	4	2		1			21=40%
Females		1		2	4	9	4	4	2	2	2			1	31=60%
Total M+F	1	3	2	2	8	11	5	6	6	4	2	1		1	52=100%

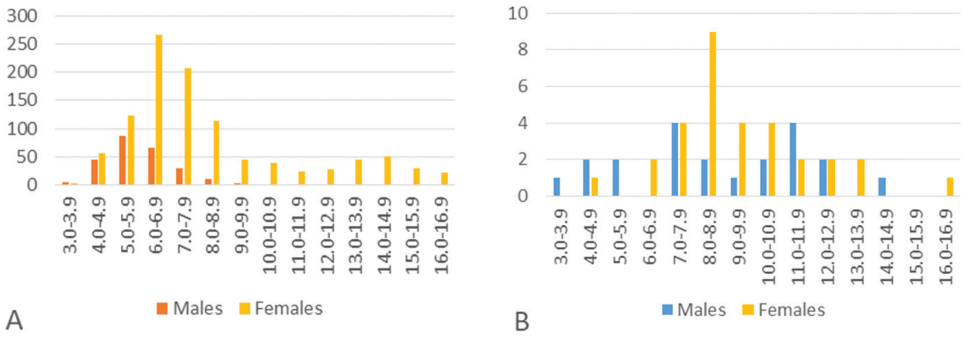

Figure 8. Size distribution of male and female *Bahalana geracei* in **A** Lighthouse Cave (1978–2018) and **B** Major's Cave (1999–2004) with more and larger males (blue).

Table 7. Life cycle stages of *Bahalana geracei* from Lighthouse Cave, 1978–2018.

Stage	Manca 1	Manca 2	Manca 3	Juv. 1	Juv. 2	Male breeders	Egg-bearers	Brooders	Oost.-bearers	Inter-cycles	Post-repro.
Size range [mm]	2.3–3.3	2.6–3.8	3.0–4.3	3.5–4.8	4.0–5.3	4.5–9.5	4.5–16.5	5.8–16.5	5.8–16.5	5.8–16.5	9.0–16.8
Instar no.	1	2	3	4	5	6–15	6–30	7–30	7–30	8–30	14–30
Time in stage	2–10 mo.	2–10 mo.	2–10 mo.	3–10 mo.	3–12 mo.	4–14 mo/ instar	6–24 mo.	5.5–6 mo.	2–13 mo.	7–18 mo/ instar	7–24 mo/ instar
Approx. age	0–10 mo.	2–20 mo.	4–24 mo.	6–30 mo.	9–36 mo.	1–17 yrs.	2–26 yrs.	3–26 yrs.	3–26 yrs.	3–26 yrs.	16–26 yrs.

fish, which appear to be a major predator of the isopods in Lighthouse Cave, may be gape-limited and have difficulty eating larger isopods, and (4) age compression has the strongest effect in the largest size ranges (see Growth rates in general).

Table 7 Life cycle stages

This table summarizes data for all life cycle stages for *B. geracei* from Lighthouse Cave (1978–2018); for each stage it includes estimates for size range, instar number(s), time in stage, and approximate age based on hundreds of observations of live laboratory specimens. It should be stressed that the time spent in each stage varied widely from a few weeks in the first few instars to years in the oldest/largest instars. So, estimates of minimum ages (bottom lines) were mostly determined by minimum times it took to go through all instars to that point.

One important point is that all life cycle stages for *B. geracei* took longer than in other isopods. For instance, in most terrestrial isopods all three manca stages are completed in a few days (compared to >6 months for *B. geracei*); Zecchini and Montesanto (2019) reported mean duration for mancas of *Armadillidium granulatum* Brandt, 1833 as M1 = 6 hours, M2 = 15 days, and M3 = 32 days. Koop (1979) reported that *Ligia dilatata* Brandt, 1833 produce their first brood at ~11 months (vs.2–3 yrs. in *B. geracei*). Johnson (1976) reported that in the intertidal isopod

Cirolana harfordi (Lockington, 1877), "females produce 1 or 2 broods of 18–68 young during their 2-year life-span" and "marsupial incubation lasts 3 or 4 months." Having longer durations for every stage for *B. geracei* (plus having multiple broods) results in a much longer life span than for all other isopods reported, as seen in Table 8 in the discussion section on Growth rates and longevity. Females may also live longer than males because they apparently spend six months of each reproductive cycle brooding in isolation, so they are not susceptible to predation (including cannibalism). On the other hand, the physical stresses of brooding probably cause post-brooding impairment in some brooders, leading to the strong declines in "all females" from 113 in the 8 mm class to only 44 in the 9 mm class (Fig. 2).

Estimates for the total number of instars in Table 7 (up to 30 for females) are quite large for any isopod species and are directly related to *B. geracei*'s extreme longevity. It appears that most cirolanid species have fewer than 12 post-marsupial instars; for example, *Natatolana borealis* (Lilljeborg, 1851) has up to 11 instars, 2–3 broods, and a life span of 2.5 years (Johansen 1996, Wong and Moore 1996). However, some large crustaceans have even more instars. According to Vogt (2018), "Many decapods molt more than 20 times in their lifetime, and the Murray crayfish *Euastacus armatus* even molts up to 80 times in its 28 years of life (Gilligan et al. 2007)."

Fecundity

The numbers of egg-bearers and oostegite-bearers (and presumed brooders) for *B. geracei* appear to be very high for a stygobite, so fecundity should also be high. Rockwood (2015) described fecundity as "the mean number of offspring produced per individual (usually female) in the population, per unit time." According to Vogt (2018), "Most crustacea reproduce throughout their entire adult life span, and there is a positive correlation between body size (which itself is positively correlated with age) and clutch size." These traits seem to be held by *B. geracei*.

Most researchers estimate fecundity by counting eggs or mancas per brood, as indicated in my earlier section on Gestation. These are usually based on many females bearing eggs or mancas; instead, I will use oostegite-bearers. For *B. geracei*, I think it is best to base fecundity on the mean number of mancas produced in a female's lifetime, rather than per brood or per year (since cycles take ~2–3.5 years), and rather than egg number because eggs are difficult to count accurately in live animals. According to Johnson et al. (2001), "Within a species, the general trend for brood size to increase with the size of females is almost universal." Thus, it should be expected that both brood size and fecundity should be highly variable for *B. geracei*, since females can have two or more broods that vary greatly in size.

Using the distribution of oostegite-bearing females in Fig. 2 and data for my six brooders (see Gestation) the number of mancas/brood can be estimated for females of various sizes and the probabilities of producing 2, 3, or 4 broods. During this study we collected 167 oostegite-bearers (5.8–16.9 mm) that had released mancas in the

previous few months. Most of them (120 of 167 = 72%) were in the four small size ranges (5, 6, 7, & 8 mm) (Fig. 2). From the methods explained above, it seems likely that most females produced a total of ~20 mancas in their two broods combined during their prime breeding ages (3–8 years) and sizes (5.0–7.9 mm). About half of them probably produced a 3rd brood in the 8.0–9.9 ranges. The mean manca number was 10 for my four brooders in the 6–9 mm ranges (#'s 49, 35, 92, & 33; details in Gestation).

This species seems to be unusual because 44 of 167 oostegite-bearers (= 26%) survived into the upper half of the size ranges (10.0–16.9 mm), with moderately high numbers even in the last four size ranges of Fig. 2. So, there is a 26% probability that adult females produced a 4th additional brood (and possibly a 5th or 6th) sometime later in life when they are 10.0–16.9 mm. I did not have any brooders in the mid-size ranges (10, 11, & 12 mm); my two largest brooders (13.0 mm #5 of 2018, and 14.8 mm #1 of 2002) are in the larger sizes. Brood size for #5 was 55. Brood size for #1 was uncertain because she was collected in the cave, she had 11 mancas in her film can when examined and 21 more in the next 4 days, but she had probably released some before being collected; since she was larger than #5, her brood size was probably as large or larger, so a conservative average for these 2 broods is ~50, which I will use as an estimate for all broods from oostegite-bearers 10.0–16.9 mm. If these are somewhat reasonable assumptions, we can estimate the number of mancas/brood and the number of broods for all 167 as follows:

1. 167 females would probably produce 10 mancas × 2 broods = 167 × 20 = 3340.
2. 83 females (= 167 × 1/2) would probably produce a 3rd brood of 50 = 4150.
3. 43 (= 167 × 0.26) would probably produce a 4th brood of 50 = 2150.

The total for all these is 3340 + 4150 + 2150 = 9640 mancas. 9640 mancas/167 = 58 mancas/oostegite-bearer. It is probably reasonable to think similar fecundity would come to fruition for the other non-oostegite-bearers, including egg-bearers and non-breeders. So, a probable range of fecundity for *B. geracei* is 20–120 mancas per female per lifetime, with a mean of 58. The significance of this surprisingly high fecundity is found in the discussion section on Fecundity.

Discussion

Reproduction and development

There are few reports on marine isopods that have studied the complete reproductive sequence of egg production (time and numbers), breeding, incubation time, and manca development. However, combining data from a variety of reports such as Johnson et al. (2001) can provide information on individual aspects of reproduction to compare to my observations on *B. geracei*.

Mating

It is particularly interesting that *B. geracei* had successful matings only after both the posterior and anterior halves were molted, instead of after the posterior half and before the anterior half as described by Wilson (1991), Johnson et al. (2001), and Wilson and Humphrey (2020). However, Wilson (1991) also pointed out several variations in mating patterns (e.g., precopula or mate pairing, and long-term retention of sperm in spermathecae); the copulatory behavior is best known for the Oniscidea (terrestrial isopods) and Asellota. With so much diversity in isopods, and so few observations of actual mating, I wonder if the pattern of mating after the anterior molt that I observed in *B. geracei* might be common in some groups (e.g., Cirolanids in caves and in other habitats).

The specific mating behaviors observed in *B. geracei* (described earlier in Breeding procedures and mating) appear to be similar to those described by Johnson et al. (2001) for several other pericaridan crustaceans: "Increased activity or directional orientation in males when in close proximity of females nearing their ovigerous molt has been reported in gammarids . . . , mysids . . ., and tanaids." The reason for such directional orientation in males, which I have observed several times in *B. geracei* pairing events, is probably related to exchange of pheromones. Johnson et al. (2001) noted that, "Lyes (1979) showed that female *Gammarus duebeni* release a pheromone in their urine that is received by the male second antennae." Johnson et al. (2001) also noted that, "Other structures on the antennae including male-specific sensory aesthetascs on some isopods . . . may be used to detect female pheromones, but experimental confirmation is needed." It is worth noting that in large *B. geracei* males (~7.5–8.0 mm), antenna 1 is ~40% longer, with ~40% more articles, than in females of comparable size, and they have more and larger aesthetascs than females.

One other note on mating behavior is that palpation with antennae that I observed has also been observed in other crustaceans, such as the amphipod *Eogammarus confervicolus* (Stimpson, 1856). Johnson et al. (2001) described the behavior as, "Once the female has been located, she is usually grasped by the male and then examined by repeated contact or palpation with antennae and other appendages (Heinz 1932; Dunham et al. 1986). . . .The stimulus involved may be a 'contact pheromone' (Michel 1986; Borowsky and Borowsky 1987) where the chemical is on the surface of the female rather than in solution."

Gestation

While most isopods use marsupial brooding, several groups developed internal brooding inside the female's pereon (Johnson et al. 2001). Thompson (2014) reported that "*Cirolana harfordi* individuals from New South Wales, Australia were found to incubate embryos and mancas inside the pereon (thoracic) cavity." Thompson (2014) also pointed out that Johnson (1976) described the reproduction of *C. harfordi* in American specimens as "marsupial incubation of eggs and later stages but did not provide any evidence to support that description." In addition, Klapow (1970) found that 7 species of *Excirolana* also carry embryos and mancas inside the pereon. Thompson (2014) and Klapow (1970) imply that incubating inside the pereon is characteristic of the species they examined (or

the entire genus). Brooding inside the pereon of *Annina lacustris* Budde-Lund, 1908 was also observed by Messana (1990). So, my observation of #49 (1995) incubating inside her pereon now seems plausible, and the definite marsupial incubation of my other specimens implies that the location of incubation may be flexible in some species such as *B. geracei*.

It is rare to find or collect brooding females of any cirolanid isopod species, and the favored explanation is that they hide in the sediment to protect themselves and their brood. One bit of supporting evidence is that, of the thousands of giant *Bathynomus giganteus* Milne-Edwards, 1879 isopods collected by researchers, Barradas-Ortiz et al. (2003) noted that only three brooders have ever been collected, and all were collected from sediment with trawl dredges or nets, rather than by attraction to baited traps. According to Johnson et al. (2001), "Brooding female isopods and tanaids may feed little if at all. The volume of the growing embryos compresses the female's internal organs, including the gut, which would hinder food intake. In addition, mouthparts are so reduced or modified in some brooding females that they cannot feed." Johnson et al. (2001) described the highly modified maxillipeds of brooding females in *B. giganteus* as "oostegites" that help keep embryos inside the marsupium, which may make feeding difficult or impossible. In *B. geracei*, the maxillipeds are slightly modified to circulate water in the marsupium, but all the brooding females I observed in culture (n = 4) fed more than once during incubation (see Fig. 3B). Since we seldom collected brooders, it seems likely that they do not actively hunt for food in the caves. My laboratory observations of brooders indicate that they don't bury into the substrate to hide, although they might still do that in the caves. They might also hide under rock ledges or inside crevices or go to inaccessible deeper areas of the cave, but where they stay when brooding is still a mystery. Fasting for 6 months or more during incubation probably takes a toll on the long-term health for brooders, even though some survive to produce more broods during their long lives.

Feeding behaviors

Feeding behaviors and structures

As mentioned earlier, species in the genus *Bahalana* can be distinguished from all others in the family Cirolanidae because pereopods 1–3 (P1–3) are prehensile with the two distal segments (dactylus and propodus) elongated and with long projections on several segments (especially the merus) (Fig. 7F). Many cirolanid isopod species have substantial spines and projections, especially on the palmar side of feeding pereopods to help hold and manipulate food. In *B. geracei* the spatulate projection on the palmar side of P1 (Fig. 7E, F) bear 4–7 teeth and is used to position food near the mouth. However, the long projections on the outer margins of P2–3 on *Bahalana* species are the most extreme of any cirolanid, which elicits the question, "Why are they so well developed in this particular group?" As mentioned in the earlier section on Feeding behaviors (in Results section), photographs showed that the pointed tips of P1–3 often penetrated prey tissue, but the projections on the outer side of P2–3 were often held away from prey (visible on lower side of worm in Fig. 7A). So, if these lateral projections are used only secondarily in handling prey, might they also have other functions?

In the section describing the increase in the proportion of larger females (>11.9 mm) shown in Fig. 8, I suggested that one possible explanation was that mangrove rivulus fish may be gape-limited predators that have difficulty eating larger isopods. It follows then that having long lateral extensions on P2–3 might help protect *B. geracei* of all sizes from potential predators, including fish, larger cannibalistic *B. geracei*, *B. cubensis* shrimp, and possibly even remipedes, which are known to occur in some of the same caves as species of *Bahalana*. Messana (1990) also hypothesized a correlation between fish predation and the stygobitic stenasellid *Acanthastenasellus forficuloides* Chelazzi and Messana,1985, which is extremely spiny "due to lateral expansion of the tergites." Messana (1990) suggested these isopods evolved their fearful armor "after the arrival of the ancestors of modern stygobitic fish in Somalian underground waters" as a means of protection from them. So, I speculate that the prominent lateral projections on P2–3 of *Bahalana* species may have evolved as a defense against predators (including cannibals), as well as being useful for feeding and possibly grooming.

Cannibalism

Cannibalism is common in carnivores, and especially in the young of precocial species in which parents provide no food or protection for them. As noted by Elgar and Crespi (1992), "The parent that produces a clutch which is partly consumed by her offspring is providing nutrition." This is a common and effective life strategy that provides survival assistance to the most vulnerable stages of a life cycle. The most commonly available food sources for offspring are usually siblings and other members of their cohort because they are the right size and are abundant in their surroundings, relative to other animals; this appears to be the case for *B. geracei* mancas. Elgar and Crespi (1992) suggested that cannibalism is responsible for a significant proportion of mortality in many species. Fox (1975), in describing two-year old pike and four-year old pike, said LeCren (1965) "calculated that cannibalism could account for all mortality among the younger class." Two *B. geracei* mancas that I raised from birth ate 21 and 26 meals in their first year. If they had similar eating frequencies in the caves, and if most of their meals were other mancas, that would certainly have a huge impact on the mortality of an average brood of ~20 (calculated in Gestation section of Results). Perhaps this strong potential impact of cannibalism may have promoted a predator-prey arms race to produce the prominent lateral projections on P2–3 that could be used for both attacks by cannibals and defense against them.

Starvation resistance

Since fasting before and after each molt is routine for crustaceans, they could be considered naturally resistant to starvation. This may help explain why crustaceans are the most abundant group of anchialine animals, although Pérez-Moreno et al. 2016 suggested that, "The reason for the high diversity of crustaceans, the endemism of higher taxa to anchialine systems, and their preponderance over other higher taxa is unknown (Stock, 1995; Sket, 1999)." Enhanced starvation resistance is a general characteristic of

Table 8. Longevity in cave and surface animals.

Longevity	Species	Taxon	Habitat	References
>20 years	*Bahalana geracei* Carpenter, 1981	Isopoda, Cirolanidae	SW cave	This study
>10 years	*Aega antarctica* Hodgson, 1910	Isopoda, Aegidae	SW fish parasite	Wägele 1990
>6 years	*Bathynomus* sp. Milne-Edwards, 1879	Isopoda, Cirolanidae	SW deep sea	Krulwich 2014
3 years	*Mesidotea entomon* Richardson, 1905	Isopoda, Chaetiliidae	SW brackish	Leonardsson 1986
2.5 years	*Natatolana borealis* (Lilljeborg, 1851)	Isopoda, Cirolanidae	SW sea loch	Wong and Moore 1996
2 years	*Cirolana harfordi* (Lockington, 1877)	Isopoda, Cirolanidae	SW beach	Johnson 1976
<2 years	*Cyathura carinata* (Kroyer, 1847)	Isopoda, Anthuridae	SW estuary	Marques et al. 1994
15 years	*Stenasellus virei* Dolfus, 1897	Isopoda, Stenasellidae	FW cave	Magniez 1975
2 years	Asellus aquaticus (Linnaeus, 1758)	Isopoda, Asellidae	FW surface	Magniez 1975
8 years	*Venezillo tenerifensis* Dalens, 1984	Isopoda, Oniscidea	Terrestrial cave	Zimmer and Topp 1999
5–10 years	*Armadillo officinalis* Dumeril, 1816	Isopoda, Oniscidea	Terrest. Surface	Warburg and Cohen 1992
3–4 years	*Porcellio dilatatus* Brandt, 1833	Isopoda, Oniscidea	Terrest. Surface	Heeley 1941
1–2 years	*Porcellio laevis* Latreille, 1804	Isopoda, Oniscidea	Terrest. Surface	Nair 1978
38 years	*Orconectes australis australis* (Rhoades, 1941)	Decapoda, Cambaridae	FW cave	Vogt 2018
22+ years	*Orconectes australis* (Rhoades, 1941)	Decapoda, Cambaridae	FW cave	Venarsky et al. 2012
2–3 years	*Orconectes placidus* (Hagen, 1970)	Decapoda, Cambaridae	FW surface	Taylor 2003
16 years	*Procambarus erythrops* Relyea & Sutton, 1975	Decapoda, Cambaridae	FW cave	Streever 1996
<2 years	*Procambarus clarkii* (Girard, 1852)	Decapoda, Cambaridae	FW surface	Huner 2002
1.6 years	*Bryocamptus pyronaicus* (Chappuis, 1923)	Copepoda, Harpacticodida	FW cave	Rouch 1968
0.7 years	*Bryocamptus zschokkei* (Schmeil, 1893)	Copepoda, Harpacticodida	FW surface	Rouch 1968
7 years	*Amblyopsis spelaea* DeKay, 1842	Osteichthyes, Amblyopsidae	FW cave	Poulson 1963
1.3 years	Chologaster cornuta Agassiz, 1853	Osteichthyes, Amblyopsidae	FW surface	Poulson 1963

cave animals, apparently as an adaptation to food supplies that are low or periodically absent (Culver and Pipan 2019). Hervant and Renault (2002) compared long-term fasting effects on a hypogean isopod species, *Stenasellus virei* Dolfus, 1897, to an epigean species, *Asellus aquaticus* (Linnaeus, 1758), and found that the hypogean species "showed lower magnitudes of response to long-term fasting than the surface-dwelling *A. aquaticus*, with a 7.3-fold slower rate of relative mass loss."

Growth rates and longevity

Apparently, my estimates of >20 years longevity for *B. geracei* are the longest for any isopod species in any habitat, so it is important to compare them to estimates for other species of isopods and for non-isopod taxa. Table 8 lists longevity estimates for eight cave species compared to surface species in the same or similar taxa. In every case, the cave species have much greater longevity than their surface counterparts. This table is divided into six sections based on taxa and habitats.

The first section compares seven saltwater (SW) isopod species from a variety of habitats. *Bahalana geracei* is the only SW cave isopod known to have longevity estimates, and these estimates are at least twice as long as for other isopods living in SW surface habitats. Curiously, the next longest longevity record I could find for a SW isopod was for the Antarctic fish parasite *Aega antarctica* Hodgson, 1910; Wägele (1990)

reported keeping it "in aquaria for more than 2 years", and that "females spawn at an age of more than 10 years." Since the host fish provides protection from predators, perhaps that is a key to this isopod's longevity. Table 8 includes five other SW isopod species from three different families and five different habitats for comparison.

Giant deep-sea isopods like *Bathynomus giganteus* (or other *Bathynomus* species) should be prime candidates for longevity records because they live in cold water, and it should take a long time to grow to 17–50 cm. Unfortunately, there are few records on growth, molting, or longevity for this group. According to an NPR blog report by Krulwich (2014), a giant deep-sea isopod like *Bathynomus giganteus* lived at Japan's Toba Aquarium where it apparently fed regularly for over a year, then fasted for 1868 days (>5.1 years) before dying. This is my basis for including it in Table 8 with a life expectancy of >6 years; however, since Krulwich (2014) described it as "big, almost a foot long, weighing over 2 pounds", it may have been several years old when it arrived at the aquarium. It is also another extreme example of starvation resistance that relates to longevity.

The second section compares two freshwater (FW) isopod species. As noted in my introduction, Magniez (1975) reported his successful breeding of the Stenasellid isopod, *Stenasellus virei*. He estimated a life span of 15 years for *S. virei*, which he said is 10–20 times longer than for an epigean Asellid of the same size, *Asellus aquaticus* (Linnaeus, 1758). The estimated lifespan of 15 years for *S. virei* is the next longest for any isopod after *B. geracei*.

The third section compares terrestrial isopods. Apparently, longevity has been studied much more in terrestrial isopods than in aquatic species because they are easier to maintain over long periods. Vogt (2018) reported on life spans of various groups of crustacea and said, "Isopods have life spans between one and ten years", and cited Warburg (2011) who "compiled longevity data for 14 terrestrial species and concluded that most live less than three years; only three species exceeded an age of five years." The longest-lived species (*Armadillo officinalis* Duméril, 1816) and two other species with more typical life spans are included in Table 8 for comparison to the terrestrial cave isopod *Venezillo tenerifensis* Dalens, 1984. Zimmer and Topp (1999) used growth curves of two adults (kept for four years) and their 12 offspring (from four consecutive broods) to calculate longevity of this cave isopod at ~8 years.

The fourth section compares longevity for five species of freshwater (FW) crayfish: two long-lived FW cave species of *Orconectes* (longevities of 38 and 22+ years) to a surface species of *Orconectes* (2–3 years), and a long-lived *Procambarus* cave species (16 years) to a surface *Procambarus* (<2 years). Venarsky et al. (2012) pointed out in their analysis of longevity of *Orconectes australis* that this cave species lives "4 to 20× longer than any other crayfish within the same genus." The cave *Procambarus* species appears to live several times longer than the surface *Procambarus* as well.

The fifth section shows that the FW cave copepod *Bryocamptus* appears to live 2–3 times longer than the surface species. And the sixth section compares longevity for two amblyopsid fish. According to Culver and Pipan (2019), Poulson (1963) found in his comparison of three stygobiont vs. two non-stygobiont amblyopsid fish that the three cave species had a doubling of life span and "at least a 50% increase in the maximum number of broods as a result of increased longevity, among other traits."

This pattern of greater longevity for cave species appears to be consistent across various taxa and habitats: FW isopods, terrestrial isopods, FW crayfish, FW copepods, and FW fish. So, it is not surprising that *B. geracei* would have greater longevity than saltwater isopods in various surface habitats. The longevity of this stygobitic isopod species is probably not unique among anchialine isopods. It just happens to be the only one seriously studied so far.

So, why do cave species tend to have greater longevity? Vogt (2018) pointed out that, "Cave animals usually experience low and erratic food supplies, as well as constantly low temperature and low oxygen. These factors were shown to result in reduction of metabolism, motility and growth rate, later onset of maturity, and irregular reproduction when compared to epigean relatives (Streever 1996, Venarsky et al. 2012)." However, this does not fully explain greater longevity.

The cave environment is often considered to be very harsh. Benvenuto et al. (2015) in their paper on crustaceans of extreme environments said, "Crustaceans have colonized and filled almost every type of niche available, including the most inhospitable places on our planet, such as Antarctic lakes, subterranean waters, hydrothermal vents, xeric deserts, hypersaline lakes, and highly acidic habitats." We are so dependent on our own sight that subterranean habitats may seem extreme and inhospitable due to the lack of light, but many species have adapted to the absence of light and primary production with slow metabolic rates, starvation resistance, and enhanced chemoreception to find food and mates. For those species that have adapted, the cave environment is not at all inhospitable, but is instead relatively stress free compared to most surface environments.

Cave animals are fortunate that they don't have to respond to the stresses of extreme weather conditions (heat, cold, storms, wind, drought), annual migrations, daily searches for food, nearly constant noise, and the social stresses of courtship, caring for offspring, competing within social hierarchies, defending territories to protect food and mating opportunities, and being constantly alert for predators. It appears that this concept of a low-stress environment as a major factor in increasing longevity for cave animals has been largely overlooked. Stress may also help explain the difficulties in keeping long-lived cave animals alive in captivity for long periods; our laboratory environments and maintenance practices probably add considerable stresses to our captive animals, even though we provide adequate food and protection from predators.

Life cycle and population structure

Males vs. females

The preponderance of female to male *B. geracei* in Lighthouse Cave collections has long been a mystery, with several possible explanations. One that I have long favored is that males are more active since they have to search for receptive females, so they are more likely to be eaten by other *B. geracei* or other predators that live in Lighthouse Cave such as the mangrove rivulus, *K. marmoratus*; rivulus prey on *B. geracei* in laboratory experiments, and the feces of rivulus caught in Lighthouse Cave frequently have remains of *B. geracei*. In contrast, we never found mangrove rivulus in Major's Cave, which has more

and larger males. Also, the number of mancas in Major's Cave was considerably higher (14 of 66 = 21%) than in Lighthouse Cave (92 of 1383 = 6.7%), which may indicate less predation pressure. It is worth noting that collections of almost all populations of cave cirolanids have more females, sometimes many more; e.g., Bruce et al. (2017) reported collecting 37 females and only one male *Lucayalana troglexuma* (Botosaneanu & Iliffe), 1997 in Hatchet Bay Cave on Eleuthera Island, Bahamas. Whatever causes the imbalance in *B. geracei* is probably causing similar imbalances in other species, and selective predation and cannibalism on males seems most likely.

As noted earlier in Table 6 and Fig. 8, females in Major's Cave outnumbered males, but only 3:2. Major's Cave has a different set of potential predators including: remipedes (*Speleonectes epilimnius*) and marsh crabs (*Armases miersii*). Our laboratory experiments showed that marsh crabs are effective predators on *B. geracei*, including large females. Another possible explanation (besides differences in predation) is differences in types or quantities of prey for the isopods; more food could be available for isopods in Major's Cave, which might reduce cannibalism and allow males to survive longer. Another explanation could be simply that male *B. geracei* survive better with the lower salinity of Major's Cave. Vogt (2018) gives examples of other species where, "differences in longevity were also found between populations of the same species in the same geographical area or even in the same water body."

Population size

The population of *B. geracei* in Lighthouse Cave appears to be remarkably high compared to those of most other stygobitic cirolanids, many of which have been collected by cave divers; several of these species have been so sparse, they resulted in type series having <5 specimens (e.g.,1 ♂ *Bahalana exumina* Botosaneanu & Iliffe, 2002; 1 ♀ *Exumalana reptans* Botosaneanu & Iliffe, 2003b; 1 ♂ *Bahalana abacoana* Botosaneanu & Iliffe, 2006). There are several possible reasons for such differences in population sizes, including the great variation in habitats within anchialine environments. Cave divers who explore anchialine habitats usually swim mid-water to avoid hitting stalactites on the ceiling or stirring up the bottom substrate, both areas that may be preferred by some anchialine animals. Areas of scuba exploration are often long distances from access to the surface where food might be brought in by bats and rainwater.

In contrast, the populations of *B. geracei* in Lighthouse Cave and Major's Cave may be relatively large because the caves have entrances large enough to allow substantial populations of bats. Evidence is lacking that *B. geracei* eat bat guano directly, but guano certainly supports large populations of terrestrial animals that probably fall into the water as food for isopods. In addition, *B. geracei* eagerly consumed small asellote isopods, *N. stocki*, which eat Lighthouse Cave detritus almost continuously (personal observation). Thus, they appear to provide an important link between nutrients in the detritus-based food chain and *B. geracei* and other carnivores.

A few other large populations of cave cirolanids have been reported. As mentioned above in Males vs. females, Bruce et al. (2017) reported that 38 specimens were collect-

ed from Hatchet Bay Cave, Eleuthera Island, Bahamas, for their taxonomic study of *L. troglexuma*; 1 male (6.9 mm) and 37 females (sizes and reproductive conditions not mentioned) were collected from baited traps set at 1–3 m for two hours, then preserved in 95% ethanol for DNA studies. Hatchet Bay Cave is similar to Lighthouse Cave, in that it is a walk-in cave (rather than mostly submerged) with an entrance large enough for colonies of bats, and it has pools of water open to the air (Bruce et al. 2017).

Romero (2009) pointed out that, "A popular misconception about cave biodiversity and biomass is that such environments are always poor in both. Although it is true that many hypogean environments are small, lack primary producers, and have a depauperate fauna when compared with the epigean environment, it is not uncommon to find tropical caves with ceilings literally covered by bats, the soil covered by myriads of invertebrates, and water teeming with aquatic life, including hundreds if not thousands of fish in a single pool. The origin of this misconception stems from the fact that most cave research has been conducted in temperate caves (USA, Europe) where biodiversity and biomass are rather poor."

It is probably significant that most anchialine cirolanids in the Western Hemisphere are from tropical and semi-tropical environments, so the food supply provided by the surrounding terrestrial environment should be more substantial and more reliable than in temperate locations. Iliffe and Botosaneanu (2006) provided valuable insight into the distribution patterns of subterranean Cirolanidae, including the high biodiversity of stygobitic cirolanids in the peri-Caribbean and Mexican Realm.

Low oxygen levels may be an even more important factor for population size (and for the evolution of low metabolic rates for cave animals) than low food supply. As pointed out by Culver and Pipan (2019), "Aquifers isolated from the surface tend to have low-oxygen concentration because there is no way to replenish oxygen used up by aerobic respiration of the organisms living in the aquifer and relatively little food unless chemoautotrophy occurs." Bishop et al. (2004) measured the extremely low oxygen levels in the lower levels of two stratified anchialine caves and presented strong evidence that "a unique community of macrofauna has adapted to cope with constant low oxygen conditions and a depauperate food supply." In contrast, the caves on San Salvador Island do not have this stratification, and the substantial entrances to the two caves in this study provide access to oxygen exchange with the surface as water moves back and forth twice daily with the tides. Oxygen levels in Lighthouse Cave and Major's Cave are moderately high at ~2–6 mg/l. This, along with a better food supply, may help explain the relatively high populations, compared to those of anchialine cirolanids that live in stratified anchialine caves and are accessed only by cave divers swimming considerable distances from entrances.

Differences in water chemistry with depth may partially explain which animals live at various depths within anchialine habitats. Furthermore, water samples change markedly when they are brought to the surface from depth. While diving in Mexican caves in 1992, I observed that when a collecting bottle was filled with water at 20 m, then opened later at the surface, compressed gasses (including carbon dioxide) escaped, which markedly changed the pH and allowed calcium carbonate to precipitate. These changes in water chemistry created problems in keeping delicate animals such as remipedes alive for long-term observation.

Fecundity

It appears that *B. geracei*'s fecundity is surprisingly high, especially for a cave isopod. Culver et al. (1995) listed "lowered fecundity" as one of several traits (troglomorphisms) commonly found in cave organisms when compared to surface-dwelling organisms. On the other hand, when Poulson (1963) compared stygobitic and non-stygobitic amblyopsid fish, he found that stygobionts have "a doubling of life span, at least a 40% increase in egg size, and at least a 50% increase in the maximum number of broods as a result of increased longevity." Female *B. geracei* follow this pattern of high number of broods, which greatly increases fecundity.

Unfortunately, it is difficult to make good comparisons of fecundity in other species since there are few reports on cirolanids that include data on mancas per brood in females of different sizes, along with probabilities of having 2, 3, or 4 broods. Hopefully, these estimates and the ways I arrived at them will be useful for others.

Population studies (including fecundity) are often done to provide guidance for conservation work. Fortunately, *B. geracei* populations seem to be relatively large and stable. The Gerace Research Centre has done a good job restricting collecting in sensitive areas on San Salvador Island, including Lighthouse Cave. Optimistically, their conservation initiatives will continue to protect all the island's anchialine habitats. It is hoped that this information about *B. geracei* can provide a basis for conservation work on other cave crustaceans, but considerable caution should be used because population dynamics can vary considerably from species to species and cave to cave as illustrated by the remarkable differences between Lighthouse Cave and Major's Cave populations.

Conclusions

The study of the natural history of cave cirolanids and many other animals has largely been eclipsed by the large number of taxonomic descriptions of fascinating species, including *Bahalana geracei*. It is certainly important to study the taxonomic diversity of various groups and the relationships within them, partly to develop strategies to help them survive threats to their environments. One other way to help protect them is to learn about their behaviors, reproduction, and population dynamics. I feel very fortunate that I have had the opportunity to make dozens of trips to The Bahamas and to study the lives of several species of anchialine animals. Hopefully, this first extensive natural history study of a cave cirolanid will encourage other researchers to study the lives of other cave isopods for extended periods to compare to *B. geracei*. To study reproduction in any long-lived cave species, it may be easier to obtain females with eggs by keeping them alive and feeding them over long periods, compared to trying to find egg-bearing females in the caves where food is less available. It may be wise to carry out such long-term studies as side projects, along with shorter-term ones that students can participate in during their relatively short college careers. Understandably, the first goal of many invertebrate zoologists and entomologists is to identify and classify their animals. However, it is easy to underestimate the excitement and importance of studying live animals.

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