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Establishment and care of a colony of parthenogenetic marbled crayfish, Marmorkrebs

Article (Peer-reviewed)

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Abstract

Marmorkrebs are parthenogenetic marbled crayfish whose origins are unknown. They have potential to be a model organism for biological research because they are genetically uniform, and to be an invasive pest species. Maintaining self-sustaining breeding colonies is a key element of most successful model organisms. We tried to find the best conditions for establishing and maintaining a Marmorkrebs breeding colony for research. Marmorkrebs can be bred in a compact tank system originally designed for zebrafish. Occasional use of live food (*Artemia nauplii*) did not significantly enhance growth of Marmorkrebs compared to an *Artemia* replacement. The presence of shelters did not affect growth of Marmorkrebs. High juvenile mortality poses the most significant obstacle to establishing a self-sustaining research colony of Marmorkrebs, although relatively few adults would be needed to supply many viable embryos for developmental research.

Keywords: crayfish, parthenogenesis, Marmorkrebs

Introduction

Marmorkrebs are parthenogenetic marbled crayfish (Scholtz et al., 2003) in the genus *Procambarus* (Vogt, 2008). They currently have no formal species name. All Marmorkrebs individuals are genetically identical females (Martin et al., 2007) that reproduce without the need of males (Vogt et al., 2004). They first came to attention in the mid-1990s through German hobbyists who kept crayfish as pets (Scholtz et al., 2003; Vogt et al., 2004), but their origin remains unknown.

There are many reasons to conduct research on Marmorkrebs. First, they are genetically identical (Martin et al., 2007), a highly desirable feature for model organisms in basic research (Vogt, 2008). Second, they are an invasive species that have been introduced in Germany (Blanke and Schulz, 2003; Marten et al., 2004), the Netherlands (Holdich and Pöckl, 2007), Italy (Marzano et al., 2009), and Madagascar (Jones et al., 2009; Kawai et al., 2009), and have the potential to become a pest species. Marmorkrebs are especially problematic because one individual would be enough to produce a population. Third, because they are the only known parthenogenetic decapod crustacean (Scholtz et al., 2003), they are a potential model for testing the evolutionary puzzle of the relative benefits of sexual versus asexual reproduction (Otto, 2009).

Decapod crustaceans are important model organisms, particularly for neurobiology (Edwards et al., 1999; Harris-Warrick et al., 1992; Huber et al., 1997; Huxley, 1880; Kravitz and Huber, 2003), but our impression is that few researchers studying crustaceans in the lab maintain breeding colonies. More often, researchers take advantage of the ready availability of commercially fished adults, particularly crabs and lobsters. Given concerns about the ecological damage posed by, and the sustainability of, commercial fishing (Mora et al., 2009), it seems prudent for crustacean research to move towards using self-sustaining colonies of organisms rather than harvesting adults from the wild. A reason for experimenters opting to use fished adults may be due to the seasonal breeding variations within species, which would mean supplies of offspring would be intermittent. Marmorkrebs offer year round clutches which could make for a more productive breeding colony than other species.

The aims of this study were to test a housing system that was not designed for crayfish, and to test food and housing conditions for rearing Marmorkrebs. Some brief descriptions of animal care can be found in Vogt and Tolley (2004), Seitz et al. (2005) and Vogt (2008).

Live food often enhances survival and growth in crayfish. In particular, *Artemia* have been used due to their high nutritional content, but rearing nauplii requires more preparation and handling time than commercial foods. González and colleagues (2008) found that commercially available prepared food could substitute for live *Artemia* nauplii and not affect growth of juvenile signal crayfish (*Pacifastacus leniusculus*). This would eliminate the need of the extra processing time of the *Artemia* nauplii. We tested whether this would also be true for Marmorkrebs.

Habitat complexity could affect animal welfare. Crayfish frequently prefer having shelters and compete for access to them (Figler et al., 1999). At high densities, shelters can positively affect survival and reduce injuries with certain substrates (Savolainen et al., 2003) and increase reproductive output (Jones and Ruscoe, 2001). We tested whether the presence of shelters had any impact on growth.

Finally, we surveyed pet owners for information on what people have used successfully in keeping them as pets.

Portions of this work have appeared in abstract (Jimenez and Faulkes, 2009).

Methods

Animal care and housing

Four adult Marmorkrebs were provided from the lab of Steffen Harzsch in Germany in September 2007. An additional two adults and 70 juveniles – i.e., individuals past post-embryonic stage II (POII; Seitz et al., 2005), or what Vogt et al. (2004) termed “adolescents” – were purchased from a hobbyist in Canada in August 2008.

All animals were maintained in a room with windows that exposed animals to the local natural photoperiod. The water temperature of all aquaria was that of the ambient room temperature, ~21°C. Animals not used in the experiments described below were kept communally in standard aquaria of 35 litres or greater, lined with commercially available non-painted aquarium gravel and containing commercially available activated charcoal filters. Small lay pots and sections of polyvinylchloride (PVC) piper were included as shelters.

Water quality was recorded at least once a week using either API 5in1 Aquarium Strips or Jungle brand Quick Dip Aquarium Multi-Test Kit. Water quality remained stable through the experiments and within a range considered normal for healthy freshwater aquaria systems. This consisted of general hardness (GH) levels at 180 ppm, alkalinity (carbonate hardness; KH) between 40-80 ppm, pH of 7.2 to 7.5, nitrate (NO_3^-) between 0 and 20 pp, nitrite (NO_2^-) between 0 and 0.5 ppm, and temperature at an average of 21°C.

The “Canadian” juvenile Marmorkrebs used in experiments were housed in an AHAB 5-shelf medium stand-alone aquarium system (Figure 1: 1.52 m wide × 0.36 m deep × 2.13 m tall, capable of holding sixty 3 litre tanks; made by Aquatic Habitats) with recirculating aged tap water. All experimental animals were kept in 3 litre tanks, lined with commercially available non-painted aquarium gravel, with lids and a 750 µm baffle screen protecting the outflow. Water flowing out of each tank was treated by passing through coarse and 50 µm filters for mechanical filtration, Siporax® media (fine-pore, torus-shaped biofilter media that allows simultaneous nitrification and denitrification; Menoud et al., 1999) and Kaldnes filter media (wheel shaped, slightly buoyant media for coarse filtration; Ødegaard et al., 2003) for biological filtration, carbon filter, and passed by ultraviolet lamp for disinfection. Water flow rate averaged 18 L hr⁻¹. Some animals, not used in experiments, were kept in 10 litre tanks in the same system. The juveniles used in experiments were kept in the aquarium for three weeks to become accustomed to the conditions before the experiments began, Aquaria were monitored daily for animal deaths, moults, or any animals in berry. Carapace lengths (± 0.01 mm) were taken during the beginning of month and at end of testing using digital callipers.



Figure 1: Picture of the stand-alone aquarium system used for experiments. Pipes used as shelters in experiment 2 are visible in third row from top.

Marmorcrebs were fed a general diet four days a week (Monday, Tuesday, Thursday, Friday). The general diet included either 5-8 thawed Hikari Bloodworms Bio-Encapsulated with Multi-Vitamins, half of a thawed pea (shelled), or Proton #2 (an *Artemia* replacement; Inve Aquaculture). Proton #2 was administered in a 1.5 g Proton / 200 ml filtered water solution, the solution was stirred vigorously between animals and each animal received 1.5 ml. Animals were fed one crushed freshwater snail, *Planorbarius corneus*, during the entire test. Snails were readily consumed, but snail feeding was halted because snails can carry trematode parasites that in turn infect crayfish (Caveny and Etges, 1971).

Artemia nauplii were raised in salt water (~30 ppt) using a hatchery system in the lab. Nauplii were collected through a synthetic fabric mesh system, immediately rinsed with tap water, and fed to the crayfish. The same amount of food was given throughout both experiments.

Experiment 1: Does live food effect growth?

In the "Live food" group (n=18), one aquarium housed three animals, four aquaria housed two, and the remaining seven aquaria housed one individual; in the "Supplement" group (n=16), four aquaria housed two animals, and eight housed one individual each (mean carapace length of all animals at start of experiment = 14.78 mm, S.D. = 4.14). Each aquarium contained only gravel; no shelter was given to either of the rows. Both groups were fed the same general diet, except one day a week when the "Live food" group was fed live *Artemia* nauplii (about 100-200 nauplii per crayfish). The "Supplement" group was fed Proton #2. The experiment ran for four months, with carapace length measured on the first of each month and tanks checked for moults daily.

Test 2: Does the access to shelter effect growth?

The “Shelter” (n=11) and “No shelter” (n=12) groups were housed with one animal per aquarium (mean carapace length of all animals at start of experiment = 17.6 mm, S.D. = 3.55). The “Shelter” group were provided with gravel and a shelter 1” diameter PVC pipe about 4” in length, one pipe per aquarium (Figure 2). The “No shelter” group were kept in aquaria containing only gravel. Both groups were fed the same general diet. The experiment ran for four months, with carapace length measured on the first of each month and tanks checked for moults daily.

Pet owners’ survey

An electronic survey for Marmorkrebs pet owners in North America was created, then reviewed and approved by The University of Texas-Pan American’s Institutional Review Board for human subjects research. The survey was placed on the Marmorkrebs.org website from 20 January 2009, and responses were collected until 5 January 2010. The survey took about 10 minutes to complete, and included the following questions on animal care (among others):

- “Have any of your Marmorkrebs reproduced successfully?” (Options: No; Once; Multiple times).
- “What do you feed your Marmorkrebs?” (Open ended response)
- “What sort of physical conditions do you keep your Marmorkrebs in (i.e., water temperature, tank size, other plants and animals in tank, etc.)?” (Open ended response)

54 responses were received. Two respondents were removed from the analysis: one because the respondent was not over 18 and one because the respondent did not own Marmorkrebs, leaving 52 responses for analysis. Numbers reported do not always add up to 52, because of the open-ended nature of the questions. For example, some respondents reported keeping multiple tanks, with different conditions; these were counted as separate housing conditions. Only responses that specifically listed information were included in certain analyses. For example, in compiling whether Marmorkrebs were kept with other fish, only those that specifically mentioned the presence or absence of fish were counted. Some responses were compiled into “miscellaneous” categories.



Figure 2: Juvenile Marmorkrebs in 3 litre tank eating bloodworms, with pipe used for shelter visible in background.

Results

General observations

All adult “German” Marmorkrebs obtained in September 2007 generated multiple large batches of embryos. Three adults died over the course of the first year, and the fourth died during the second year. Given the large size of these adults, it is likely that these animals simply died of old age. After one year, the colony contained 14 descendants of the four original adults. After 28 months, three generations of Marmorkrebs descended from the original four “German” adult Marmorkrebs were successfully reared in the lab.

The 72 “Canadian” juveniles bought in August 2008 experienced 7% mortality in the first month in the colony; 41.7% mortality during the first nine months (42 survivors of original 72 in May 2009), and; 48% mortality after seventeen months (35 survivors original 72 in January 2010). In May 2009, the 42 surviving “Canadian” juveniles were given individual identification codes and placed in their own individual tanks in the AHAB system. 64% of these individuals (27/42) successfully reproduced in the 3 litre tanks of the AHAB system.

Our qualitative impression is that tank substrate is important to growth and health for Marmorkrebs. As part of experiments for another project, we kept several Marmorkrebs isolated in small plastic containers with no gravel, and found these animals tended to be much paler in colour, and grow more slowly, than animals in the AHAB system or in larger communal tanks. We also observed that adults in communal tanks would seek out shelters and spend long periods in them when they had eggs. Marmorkrebs appeared to prefer Proton #2, bloodworms (sold frozen, thawed for feeding), peas, and shrimp pellets as foods.

Live food and shelter experiments

The use of live food resulted in no significant differences in growth between the two test groups, measured both by size (repeated measures ANCOVA, $F_{1,29} = 1.08$, $p = 0.31$) and number of moults (repeated measures ANCOVA, $F_{1,29} = 0.41$, $p = 0.53$).

The presence of shelters were no significant differences in growth between the two test groups, measured both by size (repeated measures ANCOVA, $F_{1,20} = 3.46$, $p = 0.08$) and number of moults (repeated measures ANCOVA, $F_{1,20} = 0.26$, $p = 0.62$).

Survey results

38.5% of respondents reported their Marmorkrebs had not reproduced; 23% reported their animals reproduced once, and; 38.5% their animals had reproduced multiple times. Not surprisingly, owners were more likely to report their Marmorkrebs had reproduced multiple times if they got their animals in 2008 or earlier (~71%; 17 out of 24), compared to those who reported getting Marmorkrebs in 2009 (~11%; 3 out of 28).

Pet owners fed Marmorkrebs a wide range of foods (Table 1). Many used some manner of commercially prepared food. There were no readily apparent differences in the diet used by respondents who had their Marmorkrebs reproduce multiple times and the combined pool of all respondents; i.e., owners who were particularly successful in rearing animals did not feed their animals different diets than those that had not. Although we only asked what Marmorkrebs were fed and not what Marmorkrebs preferred, bloodworms and clams were specifically mentioned as being highly preferred by Marmorkrebs.

Pet owners kept Marmorkrebs in tanks less than 5 gallons to large outdoor ponds, with tanks in the 6-10 gallon range being most common. Marmorkrebs were maintained in water temperatures ranging from 15-30°C (mean and mode ~23°C), with some keeping unheated tanks (i.e., room temperature), and others specifically mentioning heating the tanks.

Table 1: Food fed to Marmorkrebs by pet owners.

Only items mentioned more than once are included.

Type	Food	All	Reproduced multiple times
Commercial	Flake food	21	7
	Algae wafers, tablets, pellets	17	6
	Commercial crab / shrimp / lobster food	9	2
	Shrimp pellets	8	4
	Sinking pellets or wafers	6	2
	Cichlid pellets or food	5	0
	Pellets, other	5	2
	Fish pellets	3	1
	Cyclop-eeze	2	0
	Krill-based foods	2	0
Plant	Vegetables, miscellaneous	10	3
	Leaves and plant, miscellaneous	8	2
	Elodea	5	2
	Beans	4	0
	Cucumber	4	1
	Zucchini	4	1
	Java moss	3	0
	Peas	3	0
	Spirulina pellets	3	1
	Algae (not pellets)	2	1
	Carrots	2	0
	Animal	Meat, miscellaneous	9
Fish		6	3
Snails		5	1
Blood worms		4	2
Worms, miscellaneous		3	2
Blackworms		2	0
Clams		2	2
Other	Homemade gel food	3	1
	Nothing (scavengers in group tank)	2	1

Most pet owners keep their crayfish in communal tanks with other fish, usually common tropical fish sold in pet stores (e.g., guppies, swordfish, etc.; 27 of 38 responses). A slight majority kept Marmorkrebs in tanks either with no plants or plastic plants (16 respondents with plastic or no plants compared to 14 reporting planted tanks), with four respondents specifically mentioned that Marmorkrebs ate plants in the tank.

A large fraction (46%; 24 of 52) of pet owners kept other kinds of crayfish, though for the remaining majority of respondents, Marmorkrebs were the only crayfish they kept.

Discussion

We were able to establish a self-sustaining colony of Marmorkrebs in about two years. Marmorkrebs grow and reproduce under a wide range of conditions, as evidenced both by our own experiments and the responses of pet owners. They grew and reproduced successfully despite variations in food and shelter. Notably, young Marmorkrebs were reared and reproduced successfully in small tanks (3 litre) in a system originally intended for zebrafish. We do not claim that such tanks provide optimal conditions for growth and reproduction, but such compact, high density systems are extremely convenient for monitoring animals for care and for experimental purposes.

The largest factor that researchers should consider in establishing a Marmorkrebs colony is high mortality, particularly for juveniles and adolescents beyond post-embryonic stage II (POII; Seitz et al., 2005; Vogt et al., 2004). Relatively few adults would be needed to supply embryos for developmental research, which has been a major focus of labs studying Marmorkrebs (Alwes and Scholtz, 2006; Fabritius-Vilpoux et al., 2008; Polanska et al., 2007; Schiewek et al., 2007; Seitz et al., 2005; Sintoni et al., 2007; Vilpoux et al., 2006; Vogt et al., 2004). If the planned research requires adults, however, a significantly larger number of breeding Marmorkrebs will be needed. The relatively long generation time, with individuals taking an average of seven months to become reproductive (Seitz et al., 2005), is also a consideration in planning colony establishment and use.

Although Marmorkrebs are parthenogenetic, and in theory should all have the potential to reproduce, we have found that not all individuals did so, in agreement with (Vogt et al., 2008). It is not clear how much of this lack of reproducing is due to subtle environmental stress compared to inherent individual variability in reproductive capacity. In the context of maintaining a research colony that sacrifices adults for research (e.g., for anatomy, etc.), it would be important to track which animals are reproductive, so that those that are highly reproductive can be used as breeders, and those that are not reproductive could be sacrificed for experimental purposes.

Crayfish have a deserving reputation as being robust and able to survive in a wide range of conditions. There is significant room to fine-tune their care to maximize the survival and reproductive rates of Marmorkrebs in laboratory settings, however, keeping in mind that the conditions for optimal rearing may not be identical to the conditions for optimal experimentation. For example, we found no effect of shelters on growth and survival of juvenile Marmorkrebs, but it is possible the presence or absence of shelters could affect the reproduction of larger adults, as suggested by informal observations that females use shelters extensively when they have eggs. Even if shelters did affect reproductive rates, experimenters might not want to put them in the tank to permit easier monitoring of the animals.

To develop Marmorkrebs to its full potential as a model organism, it will also be beneficial if labs working with these animals can start to develop similar methods of care for Marmorkrebs colonies, with the aim of standardizing care protocols as much as possible.

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