2-2009

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Recommended Citation

Poster Abstracts, Integrative and Comparative Biology, Volume 49, Issue suppl_1, February 2009, Pages e191–e332, https://doi.org/10.1093/icb/icp003

Establishment of a research colony of Marmorkrebs, a parthenogenetic crayfish species

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Abstract

Marmorkrebs, or marbled crayfish, are recently discovered parthenogenetic crayfish. Its origins are unknown, but it has been introduced into natural ecosystems and could become a highly invasive pest species. Marmorkrebs have potential as a model organism for research in many biological fields because they are genetically uniform. A key element of most successful model organisms is maintaining self-sustaining breeding colonies. We report on our efforts find the best conditions for establishing and maintaining a Marmorkrebs breeding colony for research. The colony was founded by four adults of unknown age (P generation). These foundresses were housed communally in standard aquaria and mainly fed vegetables (mostly peas), which were readily eaten. All adults generated multiple large batches of embryos, although three adults died over the course of a year. One daughter of the original adults (F_1 generation) had her own offspring (F_2 generation). The colony contained 14 descendants of the original adults after one year. The colony was later supplemented with additional 72 juveniles, which experienced 7% mortality in the first month in the colony. High juvenile mortality poses the most significant obstacle to establishing a research colony of Marmorkrebs, although relatively few adults would be needed to supply many viable embryos for developmental research.

Figure 1. A. Adult Marmorkrebs in berry. Photo by Fabritius-Vilpoux & Harzsch. B, C. Juvenile Marmorkrebs.

Introduction

Marmorkrebs are parthenogenetic crayfish (Scholtz et al. 2003) that appear to belong to the genus *Procambarus* (Vogt, 2008). All individuals are genetically identical females that reproduce without the need of males. Their origin is unknown, but they first came to attention in the mid 1990s by German hobbyists who kept crayfish as pets.

Marmorkrebs are interesting because:

- They are **genetically identical**. This makes them highly desirable as lab animals to control for individual genetic variation.
- They have the potential to be **invasive pests**. They have been introduced in Madagascar and could pose a threat to endemic crayfish there (Jones et al., in press).
- Their **origin is unknown**. This poses an interesting evolutionary puzzle as to how this asexually reproducing species evolved.

The aim of this study was to determine optimum food and housing conditions to rear Marmorkrebs to reproductive age and establish a stable breeding colony.

Methods

Animals

Four adult Marmorkrebs were provided from the lab of Steffen Harzsch in September 2007. An additional 70 juveniles and two adults were purchased from a Canadian hobbyist in August 2008.

Housing

All animals used in experiments were housed in an AHAB 5-shelf medium stand-alone aquarium system (60” wide × 14” deep × 84” tall by Aquatic Habitats) with recirculating aged tap water. This system included mechanical filtration (filter pads and filter cartridge), biological filtration (siporax and kaldness), and ultraviolet light disinfection. All experimental animals were kept in 3 litre tanks with lids and a baffle with a 750 µm screen protecting the outflow. Water flowing out of each tank was treated by passing through a coarse filter pad, followed by 50 µm filter, carbon filter, and passed by ultraviolet lamp. Water flow rate averaged 18 L/hr. Some animals, not used in experiments, were kept in 10 litre tanks in the same system.

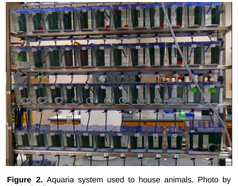

Figure 2. Aquaria system used to house animals. Photo by Kevin Faulkes.

Water quality was recorded at least once a week using either 5 in 1 Aquarium Strips (API brand) or Quick Dip Aquarium Multi-Test Kit (Jungle brand). Water quality remained stable through the experiments and within a range considered normal for healthy freshwater aquaria systems (Table 1).

Table 1. Average Water Quality of Housing

General hardness (ppm)	Carbonate hardness (ppm)	pH	Temp. (°C)	Nitrite (ppm)	Nitrate (ppm)
180	180	7.5	21	0.5	0-20

Methods (continued)

Experiment 1: Does live food affect growth?

Live food often enhances survival and growth in crayfish, but may be time-consuming to provide to all animals. 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*). We tested whether this would also be true for Marmorkrebs.

The general diet of the animals consisted of feedings on all days of the week except Wednesday, Saturday, and Sunday. The general diet included either 5-8 thawed Hikari Bloodworms Bio-Encapsulated with Multi-Vitamins, half of a thawed pea, or Proton #2 (an *Artemia* replacement; Inve Aquaculture). Proton was given in a mixture of 1.5 g Proton / 200 ml filtered water. Each animal received 1.5 ml, and the mixture was stirred vigorously between each feeding. Animals were fed one crushed freshwater snail, *Planorbarius corneus*, during the test. Snail feeding was ceased due to concern about possible trematode infection.

Figure 3. Marmorkrebs in shelter eating a thawed bloodworm. Photo by Karen Faulkes.

Testing included Row A and Row B. Row A initially housed a total of 18 animals. Four aquaria housed two animals each and one aquarium housing three animals; all other aquaria housed only one animal. Row B began testing with a total of 16 animals. Four aquaria housed two animals each and all others had one animal each. Each aquarium contained only gravel; no shelter was given to either of the rows.

Rows A and B were fed the same general diet, except one day a week when Row A was fed live *Artemia* nauplii while Row B was fed Proton #2. Row A received between 100-200 nauplii per crayfish during these feedings. *Artemia* 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 the experiment.

Test 2: Does the access to shelter affect growth?

Habitat complexity could affect animal welfare. Crayfish frequently prefer having shelters, particularly when ovigerous. We tested whether the presence of shelters had any impact on growth.

Both Row C and Row D housed 12 animals each, one animal per aquarium. Both rows were fed the same general diet as stated previously. Row C aquaria were provided with gravel and a shelter 1” diameter PVC pipe about 4” in length, one pipe per aquarium. Row D was not provided with a shelter and each aquarium contained only gravel.

Results

General observations

All adults generated multiple large batches of embryos, although three adults died over the course of a year. One daughter of the original adults (F_1 generation) had her own offspring (F_2 generation). To date, this one F_1 female has had two batches of eggs. The colony contained 14 descendants of the original adults after one year. In August 2008, the colony was supplemented with additional 72 individuals, which experienced 7% mortality in the first month in the colony. Nine of these individuals generated eggs in late December.

Marmorkrebs ate a variety of foods, but they appear to prefer food in the following rank:

1. Proton #2
2. Thawed bloodworms
3. Peas
4. *Artemia* nauplii
5. Carrots

It appeared that behaviour differed with food type. Proton #2 generated the strongest reaction, which included rapid movement towards the feeding hole and begging behaviour.

Experiment 1: Live food

There was no noticeable difference between Marmorkrebs fed live food versus those that did not receive live food. In both conditions, two animals became ovigerous.

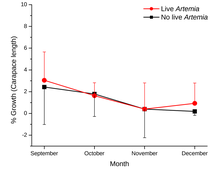

Figure 4. Effect of live food on growth.

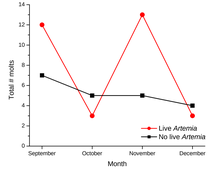

Figure 5. Effect of live food on molting.

Experiment 2: Shelter

There was no noticeable effect of providing a shelter on growth. Two individuals without shelter became ovigerous, compared to three individuals with shelter.

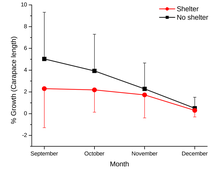

Figure 6. Effect of shelter on growth.

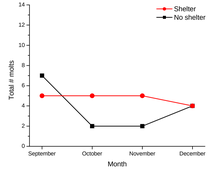

Figure 7. Effect of shelter on molting.

Discussion

After 15 months, we are near to establishing a viable research colony. We estimate a lab could have a self-sustaining working research colony in about 2 years. High juvenile mortality poses the most significant obstacle to establishing a research colony of Marmorkrebs, although relatively few adults would be needed to supply many viable embryos for developmental research.

The future

Future experiments include:

- Characterization of neural anatomy.
- Preliminary research on genetics and molecular biology of Marmorkrebs.
- Behavioural experiments on social interactions, such as aggression, within and between species.

Marmorkrebs.org

Resources

Marmorkrebs.org (also known as MarbledCrayfish.org) is a website devoted to advancing research on Marmorkrebs. It provides a complete list of publications on Marmorkrebs, a blog that is regularly updated (usually every Tuesday), and will provide more information and resources to the research community as they become available.

If you are interested in working with Marmorkrebs in your lab, please feel free to contact us to request animals or ask questions.

Acknowledgements

We thank Steffen Harzsch and Silvia Sintoni for providing the original Marmorkrebs used in this study. This research is supported by a National Science Foundation small grant for exploratory research (award 0813581). Stephanie Jimenez is supported by a Research Experiences for Undergraduates (REU) grant (award 064927). We also thank the Charlotte Mangum Student Support Program and SICB for providing and coordinating Stephanie’s housing scholarship for the conference.

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